Proceedings of the Fifth Annual Rutgers Turfgrass Symposium.

Abstracts of platform presentations and poster presentations are grouped under the following catagories:

- Turfgrass Pathology
- Entomology
- Germplasm Enhancements
- Endophytes
- Turfgrass Management
- Biotechnology

1. Turfgrass Pathology

- Summer Patch Disease Development as Influencedby Pathogen Inoculum Density, Pathogen Inoculum Placement, Seed Density, and Repeated Applications of the Biocontrol Bacterium,Xanthomonas maltophilia (Donald Y. Kobayashi, Nour El-Barrad and Gabrielle MacDonald)
- Assessing the Role of the Serine Protease from Serratia marcescens in the Biological Control of Summer Patch Disease on Kentucky Bluegrass (Hsiao-Wang Chen and Donald Y.Kobayashi)

Summer Patch Disease Development as Influenced by Pathogen Inoculum Density, Pathogen Inoculum Placement,

Seed Density, and Repeated Applications of the Biocontrol Bacterium, Xanthomonas maltophilia

(Donald Y. Kobayashi, Nour El-Barrad and Gabrielle MacDonald)

Understanding disease cycles is critical to the development and implementation of integrated strategies for the management of plant pathogens. However, despite their destructive nature and economic importance, little in known about the epidemiology of turfgrass diseases caused by ectotrophic, root-infecting fungi such as Magnaporthe poae, the causal agent of summer patch disease. Summer patch, which affects cool-season turfgrass varieties, is one of the most important diseases in the northeastern United States. Symptoms of the disease are observed as necrotic foliar areas that occur in circular patches, resulting from extensive colonization of the vascular tissue of roots by the pathogen that causes root dysfunction.

Actual infection and pathogenesis by M. poae occurs on roots of host plants. However, since summer patch disease is observed as foliar symptoms and root ratings for infection are difficult, most disease studies are conducted by rating leaf necrosis. As a consequence, disease measurements by foliar symptoms do not directly quantify pathogen levels or infection. In efforts to better correlate foliar symptoms with root infection, disease development was studied in greenhouse/growth chamber assays. In these studies, a direct correlation between reduced root mass resulting from M. poae infection and the percentage of foliar necrosis was established. A study on the development of foliar symptoms of summer patch indicated that percent necrotic area increased exponentially over time, as indicated by a sigmoidal-shaped curve when percent foliar necrosis of summer patch that developed within a confined area of turf was plotted over time.

Summer patch disease development was further studied under conditions of altered inoculum density, placement of inoculum and plant seed density. The rate of summer patch disease development remained constant when the density of M. poae inoculum was varied. However, symptom development was delayed in plants inoculated with lower densities of M. poae. As a result, the shapes of disease progress curves were not changed, but instead were shifted over time. Similar results were observed when the distance between M. poae inoculum and Kentucky bluegrass seeds were varied. Rates for summer patch disease development did not change; however, disease onset was increasingly delayed as the distance between seeds and inoculum was increased. Reduced seed density also delayed symptom development. For all three experiments, disease onset occurred more rapidly under conditions that favored rapid colonization of the roots by the pathogen. These results provide strong evidence for the importance of ectotrophic colonization of Kentucky bluegrass roots by M. poae for disease development.

Based on information generated from epidemiology studies, strategies for biological control of summer patch center around preventing ectotrophic colonization of turfgrass roots by M. poae. In growth chamber assays, standard applications of the biocontrol bacterium Xanthomonas maltophilia to M. poae-inoculated Kentucky bluegrass resulted in a delay of summer patch disease onset but no reduction in the rate of disease development, compared to disease development in untreated controls. This delay was interpreted to be a reflection of initial root colonization by M. poae by X. maltophilia. Repeated application of X. maltophilia on a 2 week basis to pathogen-inoculated turfgrass continuously shifted disease progress curves, resulting in high level disease suppression for extended periods of time.

Studies on the population dynamics of X. maltophilia in the turfgrass rhizosphere indicated that the bacterium was established at 107 colony forming units (cfu)/g rhizosphere sample after initial inoculations. Populations declined between 1 to 2 log units as rapidly as 1 week after application of bacteria and appeared to stabilize at 105 cfu/g sample in standard application treatments. In repeated treatments, populations of X. maltophilia were restored to 107 cfu/g rhizosphere sample upon each application of bacteria. These data suggest that the establishment of X. maltophilia at 107 cfu/g rhizosphere sample on a two week basis is critical to the suppression of summer patch disease.

Assessing the Role of the Serine Protease from Serratia marcescens in the Biological Control of Summer Patch Disease on Kentucky Bluegrass

(Hsiao-Wang Chen and Donald Y. Kobayashi,
Department of Plant Pathology, Rutgers University)

Serratia marcescens 9M5 is an effective biocontrol agent for summer patch disease caused by Magnaporthe poae. The bacterium produces several extracellular enzymes that are thought to contribute to its antagonistic behavior. These include several forms of chitinases, which have been cloned, characterized and demonstrated to play important roles in biocontrol by S. marcescens strains. S. marcescens also produces several proteases, of which the serine protease is known to have major enzymatic activity. The serine protease has been previously cloned and characterized; however, its role in biocontrol by S. marcescens has not been established. To assess a role for the serine protease in the biocontrol of summer patch, the gene was cloned from S. marcescens 9M5. The gene was transferred and expressed in Citrobacter strain BF14, which was previously identified as a summer patch biocontrol strain with good rhizospere colonization ability on Kentucky bluegrass. Using this strain, the effect of protease activity on the biocontrol of summer patch and rhizosphere colonization ability was tested.

In summer patch biocontrol experiments conducted in greenhouse/growth chamber assays, although no differences were detected in plants treated with BF14 compared to the BF14 transconjugant (BF14-Prt+) that carried the serine protease gene and 9M5 at a significant level, however, plants treated with BF14 gave a better disease suppression than BF14-Prt+ and 9M5. Furthermore, better rhizosphere colonization ability of BF14 on Kentucky bluegrass was detected compared to strain BF14-Prt+ although not at a significant level. These differences suggest that serine protease activity in BF14 may negatively affect rhizosphere colonization and the disease suppression ability but not at a significant level.

2. Entomology

Biocontrol of Japanese Beetle Larvae with Entomopathogenic Nematodes

(Randy Gaugler, Department of Entomology, Rutgers University)

Host Affiliations

The consequences of founder effect and inadvertent selection are being studied in H. bacteriophora and s. glaseri. The purpose of this study is to test for the effects of different culturing methods and genetic bottlenecks on the infectivity and reproductive potential of these species of entomopathogenic nematodes commonly used for biological control of P. japonica in turf. Current tests concentrate on S. glaseri lines reared for over ten infection cycles on two different insect hosts (G. mellonella and P. japonica), and which were either subjected to or not subjected to an initial severe genetic bottleneck. Few differences have been found among the lines selected for maximum or minimum genetic diversity, or different rearing regimes. An important exception is that two of the three lines subjected to genetic bottlenecks exhibit significantly lower reproductive potential than most of the other lines. Moreover, there are also significant differences in infectivity, sex ratio, and reproductive potential between most of the current lines and the original data collected for the base strain at the beginning of the study. Most selected lines show higher levels of infectivity and reproductive potential, and a more male biased sex ratio than the original base strain. Similar tests with H. bacteriophora are underway.

Based on previous work, we designed an assay of host recognition by exposing infective juvenile S. carpocapsae to the cuticle of candidate hosts, then to a source of volatile host cues. Recognition was assessed by measuring the percent response to the volatile cues. Twelve potential hosts were tested;

- 3 Lepidoptera,
- 4 Coleoptera,
- 1 Orthoptera,
- 1 Blattodea,
- 1 Diptera,
- 2 non-insect arthropods.

Reproductive potential of nematodes in each host, and nematode-induced mortality were also correlated with response to volatile cues. There are definite differences in the level of attraction stimulated by the cuticle of various arthropod species. Further, these differences correlate with the differences found among the hosts with respect to reproductive potential and nematode-induced mortality. This test has provided further evidence that S. carpocapsae is not an appropriate pathogen or P. japonica control measures.

Another line of research in this area examines the relationship between nematode infectivity and host defenses. The defenses tested so far include morphological, behaviorial and immunity of P. japonica. White grub larvae demonstrate remarkable tactile sensitivity to nematodes, reacting with stereotyped grooming behaviors when a single infected juvenile is applied to the cuticle. We further found these grooming behaviors to protect the grubs from infection. Another defensive measure of P. japonica grubs is fluid in their gut that is toxic to some species of penetrating entomopathogenic nematodes. We found S. glaseri to be significantly more resistant to this measuer that H. bacteriophora. The cellular and humoral immune responses of P. japonica to entomopathogenic nematodes also varies with nematode species. S. carpocapsae and H. bacteriophora stimulate a strong immune response from grubs, whereas S. glaseri seemingly avoids non-self recognition by the grub. Despite the differences in effectiveness of host defenses, S. glaseri and H. bacteriophora caused similar host mortality in lab assays. We continue to probe the interactions between entomopathogenic nematodes and host immunity to better explain host affinities of nematodes.

Field Population Biology

The interaction of natural populations of S. carpocapsae and H. bacteriophora with P. japonica was studied. Spatial heterogeneity is probably a fundamental aspect of the population biology of these species of nematodes and has important ramifications for their population dynamics, population genetics, and community structure. Both species of nematodes were recovered from field plots from April to December, although S. carpocapsae tended to be the more prevalent. However, H. bacteriophora had a higher density than S. carpocapsae when only plots with nematodes were compared. Plot sections with H. bacteriophora has significantly lower P. japonica densities compared to sections without nematodes. It may be possible to manipulate H. bacteriophora populations to increase the spatial scale over which they can reduce P. japonica populations.

We evaluated the efficacy of three strains of S. glaseri (NC, NJ-43, and a strain genetically selected for improved efficacy, SI-12), Steinernema anomoli and Steinernema sp. for the control of P. japonica grubs in the lab and the field. Virulence of nematodes was assessed by the mortality of the host larvae and by the number of nematodes established per host. In laboratory tests, NJ-43 and SI-12 strains of S. glaseri were significantly more virulent to larvae than the NC strain. S. anomoli and Steinernema sp. were as effective as the NC strain of S. glaseri. Similar results were found with the field tests.

3. Germplasm Enhancement

- Priorities for Breeding Improved Disease and Pest Resistant Turfgrasses for the Northeastern United States (William A. Meyer)
- Recent Advances in Control of Take-All Patch (Bruce B. Clarke, James A. Murphy, Tracy E. Bunting, and Pradip Majumdar)
- Developing Turfgrasses with Enhanced Resistance to Insect Pests (Jennifer Johnson-Cicalese)
- Breeding and Evaluation of Kentucky Bluegrass, Perennial Ryegrass, Tall Fescue, Fine Fescues, and Bentgrass for Turf (Reed Funk et al)
- Greenhouse Screening of Turfgrasses for Aluminum Tolerance (Haibo Liu, Joseph R. Heckman, and James A. Murphy)

- Evaluation of Kentucky Bluegrass Cultivars and Selections in Response to Summer Stress (S.A. Bonos, J.A. Murphy, and P. Perdomo)
- Identification of Turfgrass Cultivars Using Reversed-Phase Hi-Performance Liquid Chromatography (Glenn W. Freeman, Charles A. Wagg and Mayia M. Mileva)
- Differences in Aluminum Tolerance Among Creeping Bentgrass and Colonial Bentgrass Genotype (Haibo Liu, Joseph R. Heckman, and James A. Murphy)

Priorities for Breeding Improved Disease and Pest Resistant Turfgrasses for the Northeastern United States

William A. Meyer, Pure Seed Testing, Inc.

In the past three decades, there have been many cool-season turfgrass cultivars released with improvements in resistance to disease and insect pests. However, there are still major disease, pest and environmental stresses that can effect the quality of turf in the northeastern United States. This paper will focus on some of the breeding challenges in Kentucky bluegrass, perennial ryegrass, creeping bentgrass, colonial bentgrass, tall fescue, hard fescue, and strong creeping red fescue.

Kentucky Bluegrass

Kentucky bluegrass can form a hardy, attractive turf and is the premiere lawn species in the United States. It has good sod strength and cold hardiness. There is a need to develop cultivars with the ability to tolerate cutting heights of 1.9 cm or less for fairways and that will not be crowded out by annual bluegrass. There is also a demand for cultivars with improved shade tolerance, the ability to remain dense under low fertility and persist under severe heat and drought stress.

Many of the present cultivars have good resistance to leaf spot. This must be maintained in new cultivar developments along with improved resistance to stripe smut, leaf and stem rust, dollar spot, necrotic ringspot, summer patch, and red thread. Under summer stress, bill bugs, chinch bugs and grubs can seriously damage Kentucky bluegrass turf. Resistance to these insects from new endophytes or genetic sources is a high priority.

Consistent seed production is the primary weakness in many present day Kentucky bluegrass cultivars. The bluegrass types with the most consistent seed production are usually not the top turf formers. Ergot is another serious problem that can greatly reduce seed yields. No sources of genetic resistance to ergot have been reported.

Perennial Ryegrass

The production of improved, turf-type perennial ryegrass has increased at a faster rate (140 million pounds in 1995) than any of the other turf species in the past 25 years. This species has excellent establishment characteristics and traffic tolerance. The leaf spot resistance in some of the present cultivars is improved and has enabled them to establish under severe traffic conditions. There is a need for new germplasm sources with improved cold hardiness. This is a species well adapted to maritime climates and their active growth under cool conditions can lead to severe winter injury.

Perennial ryegrass has good resistance to the summer patch diseases and when used in mixtures with Kentucky bluegrass or hard fescues they can reduce the severity of these diseases. There is a need to develop cultivars with improved resistance to brown patch, Pythium blight, stem and crown rust and gray leaf spot. Gray leaf spot is a relatively new disease appearing under warm, wet summer conditions. Since this disease has recently become quite severe on the East coast, there is need to determine if resistance is present in any of the present adapted germplasm.

Red thread is a severe disease on perennial ryegrass with little resistance identified to date. Finding sources of resistance to incorporate in new cultivars is a very high priority.

Fungal endophytes have proven to provide good resistance to above ground feeding insects in the present commercial cultivars. New sources of fungal endophytes that have less toxic effects on livestock that graze or eat seed production residues would be a major breakthrough if the current level of insect resistance can be maintained.

Creeping and Colonial Bentgrass

The trend toward reduced cutting heights (1 cm to 1.3 cm) on golf course fairways has resulted in an expanded market for creeping bentgrass. This market has doubled to three million pounds per year in the past 10 years. Several creeping bentgrass cultivars with the ability to tolerate the shorter mowing heights on putting greens (0.3 cm) have been released in the past few years.

Bentgrasses used for fairways or putting greens require improved resistance to dollar spot, brown patch, leaf spot, take-all patch, and winter snow molds. Some of the recently released cultivars with better dollar spot, brown patch and leaf spot resistance have been encouraging to turfgrass breeders. Further improvements in disease resistance could reduce the fungicide requirements on golf courses. Genetic resistance or fungal endophytes are also needed in bentgrasses to provide resistance to insects which can inflict severe damage on golf turf.

Colonial bentgrasses with improved brown patch and yellow tuft resistance are needed for golf course fairways. The rhizome development of colonial and dryland bentgrasses along with improved disease resistance should allow better recovery from divots.

Tall Fescue

Tall fescue is a deep rooted species that has shown good drought avoidance and heat tolerance. A major weakness of this species has been its inability to establish under traffic and poor competitive abilities with other turfgrass species and annual bluegrass. This species will not persist well under cutting heights of 3.2 cm or less.

Leaf spot (net blotch) and gray leaf spot are two important diseases in newly seeded tall fescue turf. Improved resistance to these two diseases should result in cultivars with improved establishment characteristics.

Brown patch is a severe disease of tall fescues under hot, humid summer conditions. New sources of resistance are needed to this disease and Pythium blight.

Strong Creeping Red Fescue

The strong creeping red fescues have a lot of potential to be used in mixtures of cool-season turf. Their extensive rhizome system helps them recover effectively from summer stress conditions. The present commercial cultivars of this species has poor resistance to red thread. New germplasm sources or fungal endophytes that could reduce red thread in creeping red fescues are needed.

Hard Fescues

With the exception of summer patch disease, which can be a serious problem of the hard fescues have shown improved disease resistance compared with the other fine fescue species. Finding sources of resistance to summer patch in hard fescue germplasm is a high priority.

Recent Advances in Control of Take-All Patch

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Take-all patch, caused by the ectotrophic root-infecting fungus Gaeumannomyces graminis var. avenae, is an extremely destructive disease of turfgrasses in Australia, Europe, and North America. Although the disease has been reported on annual bluegrass (Poa annua), rough bluegrass (P.trivialis), Kentucky bluegrass (P. pratensis), and velvetgrass (Holcus lanatus), it is most troublesome on bentgrass (Agrostis) species. Take-all is most severe on bentgrass growing under conditions of cool temperatures (5-15 C), ample soil moisture, and high soil and rhizosphere pH. Several researchers have reported dramatic reductions in the incidence and severity of this disease in the field through the use of acidifying fertilizers or the application of sterol inhibiting or mercury-based fungicides. However, little is known about the factors that govern fungicide efficacy (i.e., chemical mode of action, optimum rates, timing, methods of application) or the effect of nitrogen sources and pH on disease development and reduced fungicide usage.

To evaluate the impact of selected fungicides and nitrogen sources on take-all in the field, two bentgrass fairways naturally infested with G. graminis var. avenae were utilized from 1993 to 1995 at the Metedeconk National Golf Course in Jackson, NJ. Fungicides were applied as either surface or subsurface (0 to 7.6 cm depth) treatments using a commercial Chem Pro (1000 L H2O ha-1 @ 0.3 MPa) or Toro Hydroject (4500 L H2O ha-1 @ 34.5 MPa) sprayer, respectively. Six timing regimes (1 to 4 applications yr-1) were compared utilizing fenarimol, the only fungicide currently labeled for the control of take-all. Nine additional fungicides, representing six distinct chemical groups, were evaluated on an Apr., May, Sept., and Oct. spray schedule. Strongly acidifying and weakly acidifying nitrogen sources, ammonium sulfate (AS) and milorganite (M), respectively, were applied to turf as split-plot treatments.

Over the three year study, AS and M treatments reduced soil pH from 6.7 to 5.6 and 6.1, respectively. Compared to M treated turf, AS reduced disease severity 33% in 1994 and 42% in 1995. After one year of application, only phenyl mercury acetate (PMA), strobilurin (Heritage), triadimefon (Bayleton, 3.0 kg ai ha-1), and tebuconazole (Lynx, 2.3 kg ai ha-1) provided an acceptable level of control (82 to 97%). At the end of the second season, however, cyproconazole (Sentinel) and propiconazole (Banner) were also as effective as PMA in suppressing take-all. Although fenarimol (Rubigan) provided a fair to good level of disease control, thiophanate methyl (Cleary 3336) and fluazinam were not effective in reducing the incidence and severity of this disease.

In comparison to untreated controls, subsurface applications of fenarimol were 23 to 36% more effective in suppressing take-all than surface applications. Throughout the study, fenarimol was most effective when applied in Apr. and Sept. (3.0 kg ai ha-1) or in Apr., Sept., and Oct. (1.5 kg ai ha-1). The relationship between soil pH, reduced fungicide rates, and disease severity will be examined more closely in the future.

Developing Turfgrasses with Enhanced Resistance to Insect Pests

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Numerous opportunities exist to develop turfgrasses with enhanced resistance to insect pests. Although these efforts are critical to reducing reliance on pesticides, relatively little work has been done in this area. The discovery and utilization of endophyte-enhanced insect resistance has been an important development in turfgrass science. Many cultivars of endophyte-enhanced ryegrass and fescue are currently available, yet there is still much to learn about fully utilizing these fungi. Several cases of genetic resistance have also been identified; such as billbug resistance in Kentucky bluegrasses, chinch bug resistance in St. Augustine grass and Kentucky bluegrass, and mealybug resistance in buffalo grass. However, little is known about the mechanisms or inheritance of this resistance.

The objectives of my work here at Rutgers will be to investigate both genetic and endophytic sources of insect resistance. I will concentrate on three important turf pests: chinch bugs, white grubs and billbugs. The turfgrass breeding project's extensive germplasm collection and many established breeding trials will greatly facilitate the screening of plant material. Current projects include: evaluating leaf scarring and discoloration in Kentucky bluegrass, thought to be caused by thrips, and the possible tolerance of some bluegrasses to this insect; screening Kentucky bluegrass for resistance to white grubs; and evaluating chinch bug reaction to several sources of fine fescue endophytes. In the future I hope to develop a rearing technique for billbugs which would facilitate rapid and large-scale screening of bluegrasses for resistance. Trials to study the mechanisms of resistance will also be conducted for billbugs and white grubs.

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

**Reed Funk, James Murphy, Bruce Clarke, James White,
Joseph Heckman, Lisa Lee, Haibo Liu, Jennifer Johnson-Cicalese,
Ronald Bara, Dirk Smith, William Dickson, Joseph Clark,
Raymond Schaaf, George Ziemienski, Michael Reynolds,
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1. Over 7,000 new turfgrass evaluation plots and over eight acres of spaced-plant nurseries were established during 1995. Turfgrass evaluation tests included 3,305 plots of Kentucky bluegrass, 1,470 plots of perennial ryegrass, 1500 plots of tall fescue, 850 plots of fine fescues, and 305 plots of creeping, colonial, dryland, and velvet bentgrasses. A total of over 50,000 plots of turfgrass cultivars, experimental selections, and germplasm sources are under observation and evaluation in field trials at Adelphia, North Brunswick, and Pittstown, New Jersey.

2. Intraspecific and interspecific hybridization programs are being expanded in Poa. Many of the interspecific crosses are directed to the transfer of a useful endophyte into Poa pratensis as well as increasing our pool of genetic diversity. Many of our intraspecific crosses are directed to the development of improved mid-Atlantic type bluegrasses with enhanced tolerance of heat and drought, improved resistance to insect pests, and economical seed production. Current mid-Atlantic type Kentucky bluegrasses such as Wabash, Bel 21, Vantage, and Eagleton are not widely used because of low seed yields. Improved resistance to and/or tolerance of billbugs is of vital importance to the summer performance and survival of many non-irrigated, medium-to-low maintenance turfs. Thinning of turf by billbugs creates conditions favorable to additional damage by chinch bugs and grubs. Weed invasion follows, restricting recovery of the Kentucky bluegrass turf. We need improved mid-Atlantic types of Kentucky bluegrass with excellent disease and insect resistance, deep roots and rhizomes, increased tolerance of heat and drought, and high seed yields. Useful endophytes might enhance many of these characteristics.

3. Significant differences in damage by, tolerance of, and recovery from white grubs were observed in an older Kentucky bluegrass test growing under conditions of reduced air circulation which resulted in periods of severe heat stress. Kentucky bluegrasses showed striking differences in their ability to maintain an active, deep root system and to regenerate roots severed by grubs under these conditions. Differences in the ability of various Kentucky bluegrasses to maintain an adequate rate of net photosynthesis is suggested as a partial explanation of these differences. Tolerance of high soil temperatures could also be involved. Mid-Atlantic ecotypes generally showed the best performance.

4. Germplasm developed at the New Jersey Agricultural Experiment Station contributed to a number of new turfgrass cultivars including Calypso II, Citation III, Manhattan III, Windstar and RPBD perennial ryegrasses; Titan II, Coronado, Finelawn Petite, and Coyote tall fescues; Southport Chewings fescue; Warwick hard fescue; Princeton 105 and Eagleton Kentucky bluegrasses; and L-93 creeping bentgrass.

5. Improved cultivars of Koeleria macrantha show promise as an attractive, fine-leaved, low-growing turfgrass for low-maintenance turfs. The cultivar Barkoel has performed well in turf trials receiving little or no fertilizer. However, it becomes excessively dense and is damaged by Rhizoctonia brown patch and other diseases when given too much fertilizer. Hard fescues and blue fescues also perform best in lower maintenance trials receiving limited fertilizer. Hard and blue fescues frequently show serious damage from summer patch and subsequent chinch bug feeding when over fertilized, mowed close, and subjected to soil compaction. Acremonium endophytes frequently enhance performance of all fine fescues in New Jersey turf trials. This includes improved resistance to the dollar spot disease and perhaps summer patch.

6. Most improved turf-type cultivars of perennial ryegrass were developed from a very limited genetic base. Extensive population improvement programs using phenotypic and genotypic recurrent selection and occasional backcrossing has been very effective over thirty years in developing ryegrasses with striking improvements in persistence, attractiveness and overall turf performance. Dramatic progress has been made in developing ryegrasses with a darker color, greater density, finer leaves, a lower growth profile, improved mowing quality, and endophyte-enhanced insect resistance. Significant advances have also been made in seed yield potential as well as in seed production technology. Moderate progress has been made in improving tolerance of heat, cold, drought, shade, and wear. However, only limited progress has been made in developing stable resistance to crown rust, red thread and dollarspot. Turfgrass breeders have a great challenge and opportunity to find new sources of genetic resistance and better selection techniques to make improvements in these characteristics.

7. Most improved turf-type tall fescues were developed from a very narrow genetic base. Recurrent phenotypic assortive mating frequently combined with clonal and/or progeny trials

conducted under frequent close mowing has been used over a 34 year period. A few dozen plants surviving in old turfs of the United States were the primary parental germplasm. Substantial improvements have been made in developing tall fescues with darker color, finer leaves, greater density, a lower growth profile, greater persistence under close mowing, and high seed yields. Kentucky 31 and most improved turf-type tall fescues show much better resistance to the Rhizoctonia brown patch disease compared to unadapted tall fescue accessions from the cool-moist summer or hot-dry summer climates of Europe. However, further genetic improvements in resistance to Rhizoctonia brown patch are needed. In warm, humid environments, the currently available resistance is not sufficient to overcome the conditions favorable for disease development created by the dense, lush turfs that are frequently developed using high fertility, frequent close mowing and the new turf-type varieties. Pythium blight can also be severe in dense, lush, highly fertilized turfs in hot, humid environments.

8. Most of our major cool-season turfgrasses evolved in the cool-moist, or hot-dry regions of Europe and are not really well-adapted to warm-humid environments of much of the United States. This helps explain the reason why most of the germplasm used in the improved turf-type perennial ryegrasses and tall fescues originated from a few plants which had survived in old naturalized turfs in warm humid areas of the United States. Much of the germplasm used in the development of the best performing Chewings fescues, strong creeping red fescues, blue fescues, Kentucky bluegrasses, creeping bentgrasses, and rough bluegrasses was also collected from old turfs surviving in stressful environments of the United States. There is an urgent need for additional collection efforts in the United States as well as in the regions of origin of all these species.

9. We have much greater genetic diversity in our germplasm collections of Kentucky bluegrass than any other turfgrass species. We have most characteristics desired in a cool-season, lawn-type turfgrass within this pool of germplasm. Unfortunately, we currently lack the population improvement techniques that have been so successful in breeding the turf-type perennial ryegrasses and tall fescues. Because of the apomictic reproductive behavior of Kentucky bluegrass, we have been unable to use recurrent selection and backcross breeding techniques to concentrate all of these useful characteristics into a single cultivar or interbreeding population. The development of successful efficient methods of population improvement in Kentucky bluegrass could lead to great progress in developing better turfgrasses. It might also contribute to more effective breeding methods in other apomictic species.

10. Kentucky bluegrass (Poa pratensis L.) is a facultative apomictic with complex cytological and embryological characteristics. The basic chromosome number of the genus is $x = 7$ with chromosome numbers of Kentucky bluegrass ranging from $2n = 28$ to $2n = 153$. Most Kentucky bluegrass cultivars range from $2n = 39$ to $2n = 93$ with many successful bluegrass cultivars being aneuploids. Apomictic reproduction overcomes much of the sterility that would normally occur

with the irregular meiosis and imbalanced gametes associated with aneuploids. Highly sexual Kentucky bluegrass plants that have been studied in greatest detail include Warren's A20, A25, and A26. All three have poor floret fertility and very low seed yields. Hybrids of these highly sexual Kentucky bluegrasses with apomictic strains of P. compressa L. give vigorous but sterile hybrids whereas hybrids of P. compressa with highly apomictic Kentucky bluegrass were frequently moderately to highly apomictic with moderate to good floret fertility.

Interestingly, aneuploid Kentucky bluegrasses produce adequate amounts of viable pollen. It appears that most pollen contains nuclei with approximately one-half the chromosome numbers of the parent plant as a result of meiotic division. Is this a result of a genetic mechanism for fertility? Alternatively, could this be a result of a high level of polyploidy where there is sufficient buffering to allow unbalanced gametes to function effectively. A third possibility might be that many chromosomes are not really vital to important physiological processes and are somewhat redundant. It is often observed that different chromosome numbers can be observed in different tissues of the same Kentucky bluegrass plant.

The observation that many aneuploid Kentucky bluegrasses produce ample amounts of viable reduced pollen suggests that we should be able to find Kentucky bluegrasses with the ability to produce a high number of reduced eggs even though the mother plants are aneuploid with irregular meiosis.

Turfgrass breeders at Rutgers have observed some highly sexual Kentucky bluegrass plants with good seed yields and high floret fertility. A more intensive search might identify highly sexual plants with excellent floret fertility and excellent seed yield. We would also like these plants to have excellent turf quality, stress tolerance, and pest resistance and to also show excellent general combining ability for these characteristics.

Greenhouse Screening of Turfgrasses for Aluminum Tolerance

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Greenhouse screening can be used to identify the genetic diversity of turfgrass cultivars to Al tolerance within a relatively short period of time. Four genotypes of each Kentucky bluegrass (Poa pratensis L.), perennial ryegrass (Lolium perenne L.), tall fescue (Festuca arundinacea

Schreb.) and fine fescue (Festuca spp.) were evaluated at different Al concentrations using solution culture under greenhouse conditions. In solution culture, fine fescue was the most Al tolerant of the four turfgrasses at different Al concentrations. Eight genotypes of Kentucky bluegrass, fine fescues, and bentgrasses (Agrostis spp.) were selected based on previous screening studies to represent a wide range in Al tolerance and compared under greenhouse conditions using solution culture, sand culture and acid Tatum soil. The grasses were seeded and grown for a short term (four weeks) before harvesting. Then the cultivars were seeded and grown in acid Tatum soil for a longer term (10 weeks). The correlation coefficients were significant and the results indicate that short term (four weeks) screening is a useful approach. Significant correlations were also found between relative root growth and relative shoot growth among the three turfgrass species when they were grown in acid Tatum soil for 10 weeks. This result suggests that clipping yield may be a useful measurement for field screening of turfgrasses for Al tolerance.

Evaluation of Kentucky Bluegrass Cultivars and Selections in Response to Summer Stress

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Kentucky bluegrasses (Poa pratensis L.) have been shown to differ in heat and drought stress tolerance. The objective of this study was to evaluate the ability of a growth chamber screening technique to predict stress tolerance of Kentucky bluegrass cultivars and selections in the field. Tolerant and intolerant bluegrasses were identified based on performance in field plots. Bluegrasses grown under field conditions were evaluated for soil moisture use, leaf canopy temperature, and shoot and root growth. Shoot and root growth of bluegrass seedlings were evaluated at 12 and 30°C in a growth chamber with 16h day length. Tolerant bluegrasses had significantly greater turf quality and lower leaf canopy temperatures than intolerant bluegrasses during stress periods in the field. Gravimetric soil moisture content under the tolerant bluegrasses was lower than the intolerant bluegrasses suggesting that tolerant bluegrasses had greater ability to extract soil moisture. Initial growth chamber results indicated that some intolerant cultivars have lower root dry weight and subsequently a lower root-to-shoot ratio than tolerant cultivars when grown at higher temperature. Root and shoot growth under field conditions are being monitored for comparison with growth dynamics under heat stress in the growth chamber.

Identification of Turfgrass Cultivars Using Reversed-Phase Hi-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 tall fescue (Festuca arundinacea Schreber), Kentucky bluegrass (Poa pratensis L.), and creeping bentgrass (Agrostis stolonifera var. palustris (Hudson) Farwell) was undertaken to determine if RP-HPLC could be adapted to the cultivar identification of turfgrass species. Chromatograms of the different cultivars of the seed kinds studied were all different and were used to characterize those cultivars.

HPLC has also been shown to be applicable to the analysis of turfgrass blends and mixtures. Chromatograms of bentgrass blends and mixtures of hard fescue (Festuca longifolia Thuill) and creeping red fescue (Festuca rubra L. subsp. rubra) illustrate that changes in component percentages of seed mixtures may be reflected in changes of the characteristic peak sizes and shapes. Commercial mixtures of turfgrasses supervised by agricultural inspectors were compared with mixtures of the same components made in the laboratory. The chromatograms of these samples were identical, indicating that laboratory produced blends and mixtures can be used for regulatory purposes by comparing them to the products offered for sale.

RP-HPLC was shown to be a repeatable and reliable method of turfgrass cultivar identification and turfgrass blend analysis.

Differences in Aluminum Tolerance Among Creeping Bentgrass and Colonial Bentgrass Genotypes

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Fifteen creeping bentgrass (Agrostis palustris Huds.) cultivars and three colonial bentgrass (A. capillaris L.) cultivars selected from the 1993 National Bentgrass Test were screened for aluminum tolerance under greenhouse conditions using solution culture, sand culture, and an acid Tatum soil (Clayey, mixed, thermic, typic, Hapludult). The acid Tatum soil had 69% exchangeable Al and a pH of 4.4. An Al concentration of 640 mM and a pH 4.0 were used in solution and sand cultures. All grasses were seeded and grown for four weeks before harvesting. Al tolerance was evaluated by measuring relative length and weight of shoots and roots. A separated experiment of comparing clipping yield among cultivars grown in acid Tatum soil for seven months was conducted under the same greenhouse conditions. Differences in Al tolerance were identified at both the species and cultivars levels based on relative growth. Colonial bentgrasses 'SR 7100' and 'Exeter' were more Al tolerant than most creeping bentgrass cultivars. 'Regent' and 'Seaside' were the two most Al tolerant creeping bentgrass cultivars studied. The results indicate that genetic improvements in Al tolerance of bentgrass are possible.

4. Endophytes

- Endophyte-Mediated Stress Tolerance of Turfgrasses (Michael D. Richardson)
- Chitinase Activity from Endophyte-Infected and Endophyte-Free Poa ampla (Mi Feng and Thomas Gianfagna)
- Turfgrass Endophyte Systematics (James F. White, Jr. and Ponaka N. Reddy)
- Cloning of an Abundant Proteinase from the Fungal Endophyte Acremonium typhinum (Ponaka N. Reddy, Cuong K. Lam, Faith C. Belanger)

Endophyte-Mediated Stress Tolerance of Turfgrasses

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During any given year, most turfgrass sites are challenged by biotic and abiotic stresses. While damage from these stresses can be minimized through intensified management, many management practices are not environmentally benign and are inefficient over extended periods. If turfgrasses of the future are to be maintained under conditions of limited inputs, the development of varieties that are more efficient under stress conditions is of primary importance. Endophytes of the tribe Balansieae form symbiotic associations with many common cool-season turfgrasses and are known to improve host plant fitness, especially in harsh environments. While it has been known that endophytes are important to Turfgrass culture, the true potential of these fungi in Turfgrass development has never been realized. This primarily reflects a poor understanding of most facets of the symbiosis, including variation in endophyte-induced traits, basic endophyte biology, physiology of host-plant interactions, and general agronomic features of endophytic grasses maintained as turf. The proposed research will seek to expand our basic knowledge about endophytes of turf, especially those endophytes of the fine fescues (Festuca spp.), bluegrasses (Poa spp.), and bentgrasses (Agrostis spp). The long-term goal of this project is to develop elite grass-endophyte associations that increase Turfgrass performance in stressful environments.

Chitinase Activity from Endophyte-Infected and Endophyte-Free Poa ampla

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Chitinase activity was extracted from endophyte-infected and endophyte-free leaves of Poa ampla after induction with ethylene or mercuric chloride. Chitinase activity was measured 1-4 days after treatment. Neither ethylene or mercuric chloride stimulated chitinase activity in Poa ampla. Nevertheless, there was measurable chitinase in all samples tested. Endophyte-infected plants had about 20% greater chitinase activity than endophyte-free leaves. The greater chitinase activity may contribute to the increased insect and disease resistance of endophyte-infected grasses.

Turfgrass Endophyte Systematics

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The members of the fungal tribe Balansieae (Clavicipitaceae; Ascomycotina) are common endophytic associates of grasses. Any given natural grass community is likely to contain at least some individuals that are infected by some member of the Balansieae, and many communities in Europe, North America, and South America are made up of predominantly Balansieae-infected individuals. Many of the impacts of the Balansieae on fitness of grass populations are now well documented. They have been shown to enhance insect resistance, drought tolerance, fungus disease resistance, and in some cases vegetative growth of host plants. Endophytic Balansieae are now being employed in turfgrass breeding programs to improve hardiness of commercial varieties and reduce turf maintenance requirements for water and pesticide applications. In this respect endophytic Balansieae continue to hold promise to alleviate environmental problems caused by heavy pesticide and water applications to maintain turf in the urban environment. Balansieae also have major impacts on the livestock industry worldwide. Toxic syndromes affecting livestock grazed on Balansieae-infected tall fescue, ryegrass, needle grass, and numerous other forage grasses frequently occur in this country and throughout the world.

Knowledge of the systematics and biology of the graminicolous Clavicipitaceae is very rudimentary. Most of the taxonomic work was conducted in the early half of this century. Recent work on these fungi indicate that many genera represent heterogenous assemblages of fungi that are only superficially similar. In other instances species concepts are too broad with several species being lumped under a species category. Very little information is available on the life cycles of the majority of the Clavicipitaceae. Because of the importance of the graminicolous Clavicipitaceae, it is imperative that we develop a complete knowledge of the systematics of this group of fungi.

At Rutgers University we are attempting to employ grass endophytes to improve turfgrass quality. Because endophytes have been found to differ with respect to the degree of benefit that they confer on hosts, it is important that we develop methods to distinguish individual strains of turfgrass endophytes. We are beginning to employ rDNA ITS (internal transcribed spacer) sequences for this purpose. Using the ITS sequences, we will be able to distinguish strains, species, and phylogenetic relationships between the endophytes. The goal of this research project is to fill in the large gaps in our knowledge of the systematics and biology of the graminicolous

Clavicipitaceae.

Cloning of an Abundant Proteinase from the Fungal Endophyte Acremonium typhinum

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Many cultivated and wild grass species are hosts to fungal endophytes. In the Poa ampla (big bluegrass)/Acremonium typhinum interaction, a fungal proteinase is surprisingly abundant and may constitute 1-2% of the total leaf sheath protein (Lindstrom and Belanger, Plant Physiol. 1994, 106:7-16). We have obtained N-terminal and internal amino acid sequence of the purified protein. Degenerate oligonucleotides based on the amino acid sequence were used to obtain a 300 bp PCR clone which was then used to screen a cDNA library prepared from endophyte-infected leaf sheath tissue. Sequence analysis indicates this proteinase is highly homologous to fungal proteinases produced by insect and nematode pathogens and to the well known fungal proteinase, proteinase K. DNA blot analysis indicates this proteinase is probably encoded by a single gene.

5. Turf Management

Turfgrass Management Research (J.A. Murphy, G. Gentilucci, and M.E. Secks)

Establishment of Kentucky Bluegrass Affected by Amendment of Soil(Gary Gentilucci, James A Murphy, and Haibo Liu)

Performance of Commercial Fertilizers on Kentucky Bluegrass at New Brunswick, New Jersey (Joseph R. Heckman, Wendy Hill, and Haibo Liu)

Turf sod as a Base for Ornamental Sod(Dan B. Strombom1 and James A. Murphy)

Turfgrass Management Research

J.A. Murphy, G. Gentilucci, and M.E. Secks

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Water injection cultivation was developed to cultivate compacted turf without excessive disruption of the playing surface. The objective of this study was to evaluate the effect of water injection cultivation on the frequency of hollow tine core cultivation of a compacted putting green. The study was initiated August 1993 on a 8-yr old 'Penncross' creeping bentgrass green grown on a native soil. A 3 x 3 factorially arranged randomized complete block design with 4 replications was used. Hollow tine cultivation was applied at levels of none, October, or June and October. Water injection cultivation was applied at levels of none, weekly, or triweekly from June through September. Soil physical properties evaluated in September 1994 indicated that water injection applied weekly and triweekly increased water conductivity in the 0- to 76-mm soil depth zone compared to soil receiving no water injection cultivation. Weekly application of water injection cultivation reduced turf quality of the creeping bentgrass green, whereas water injection at a triweekly frequency caused no reduction in quality during 1994. Hollow tine cultivation increased rooting in the 20- to 30-cm soil depth zone in September 1994. Weekly water injection reduced rooting in the 10- to 20-cm and 20- to 30-cm depth zones in September 1994. Longer term evaluation of these cultivation regimes will be continued.

Establishment of Kentucky Bluegrass Affected by Amendment of Soil

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The incorporation of composted waste into a soil can modify physical and chemical properties which affect turfgrass growth. A field study was conducted to evaluate the influence of soil amending on the establishment of Kentucky bluegrass (Poa pratensis L.). Three composted waste materials were incorporated to a depth of 15-cm at rates of 0.05 and 0.10 m3 m-2 into a sandy loam. The compost sources were food waste, leaf waste, and a co-composted municipal solid waste having C:N ratios of 29, 35, and 42, respectively. A non-amended check plot was included for comparison purposes. The compost amendments were applied 19 August and the plot area was seeded 20 Sept., 1993. Nitrogen was applied in split plots to each amended main plot at 0, 2.4, 4.9, 7.3, and 9.8 g N m-2 using ammonium nitrate. The development of turf cover on 4 May 1994 for the 0.10 m3 m-2 amendment rate and 0 g N m-2 were 81, 76, and 33% for the leaf, food, and co-compost materials, respectively; the non-amended soil at 0 g N m-2 produced 53% turf cover. Nitrogen applied at 7.3 and 9.8 g N m-2 raised turf cover on the co-compost plots to 55 and 53%, respectively. Composts can be useful for improving soil nutrition and turfgrass establishment; the detrimental effects of a compost having a high C:N ratio can be managed with nitrogen fertilization.

Performance of Commercial Fertilizers on Kentucky Bluegrass at New Brunswick, New Jersey

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Turf quality is dependent on the type and degree of management. Fertilization, especially nitrogen (N), is one of the most important management factors influencing turf quality. A fertilizer trial was conducted to compare turfgrass color ratings from single applications of various soluble and slowly available nitrogen sources. A single application of 1.5 lbs. N/1000 sq. ft. was made in spring 1995 on a recently established Kentucky bluegrass (50:50 blend of 'Eclipse' and 'Flyking') sod. The experimental site was irrigated weekly as needed during the duration of the trials (May - October) to maintain vigorous turfgrass growth. The unfertilized control had the lowest turf color ratings for the eight week period following application of the fertilizer treatments. The rapid green-up response to the fertilizers, indicates that the soil at the field site was nitrogen deficient and quite responsive to the addition of nitrogen. Ammonium sulfate, which is a completely soluble nitrogen source caused the most rapid green-up. Turf color ratings were highest with ammonium sulfate for the initial 3 weeks after application, but the color ratings declined rapidly over the next 8 weeks until color ratings fell below all other treatments including the control turf plots. This rapid rise and fall of turf color illustrates the typical response of turfgrass to soluble nitrogen sources and provides an interesting contrast to compare with controlled release nitrogen sources. IBDU is a slow release nitrogen that contains 4.6% water soluble nitrogen and 26.4% water insoluble nitrogen. This fertilizer material initially had the lowest turf color rating of the various fertilizer products that were compared. The green-up with IBDU was very slow, but color continued to improve over the 20 weeks of color ratings. At about mid-season, the steady climb in turf color ratings with IBDU overtook all other fertilizer materials and it maintained this position until late October. The various commercial fertilizers that contained more balanced mixtures of water soluble and water insoluble N provided for good early season green-up and maintained good to fair color ratings throughout the season. Results indicate that a single application of a fertilizer that contains a balanced mixture of soluble and slow release sources of N can provide for acceptable turf color for most of the growing season.

Turf Sod as a Base for Ornamental Sod

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Turf sod producers can benefit from an expansion of their product line made possible by a newly invented process utilizing harvested sod as a base for the propagation of ornamental and edible plants. The versatility of this process will allow sod producers to enter new markets as well as to increase sales to their existing customer base. Rutgers University is pursuing a patent on this process with licensing available in 1996. Field and greenhouse studies were conducted in 1995 with a farmer cooperator in Cape May County, New Jersey. Waste bluegrass sods left after commercial harvesting was collected from two sod producers. The sods were cut to a length of thirty inches for ease of handling and then laid in double rows on six mil black plastic. The grass was killed by an application of glyphosate at labeled rates. Five to seven days after the herbicide application transplant plugs of seventeen varieties of annual and six varieties perennial bedding plants and herbs were set into slits made in the sod at spacings recommended for each variety. The sod was fertilized once at week 3 with a 20-20-20 soluble fertilizer at recommended rates. Overhead irrigation was required at a frequency of twice to four times daily depending on ambient temperature to maintain adequate moisture levels. Selected varieties were then tested for establishment by laying the ornamental sod on bare ground. All variety trials grew successfully in the sod base. Growth rates of the plants were comparable to rates for the same varieties grown in 6-inch pots. It was found, however, that top-heavy varieties, such as taller cultivars of marigolds, tended to lodge. These varieties could be grown, but only if dwarf cultivars were used or tall cultivars were harvested before reaching mature height. Plant roots grew into and down through the sod mat. The majority of root masses were found under but attached to the mat, being constrained by the black plastic underlayer. The sod retained considerable knitting strength for 15 weeks, tested by lifting selected sods while gripping the ends. Thereafter, approximately one out of ten sods failed this test. It is not known to what degree the breaking may have been due to the use of waste sods.

These field trials have demonstrated that turf sod can serve as a base for growing other plants. Being freed from dependence on the root knitting of desired varieties opens up a considerable diversity of products for turf farmers with the numerous and continually growing number of varieties and cultivars of ornamental and edible plants. This development will particularly benefit producers who can take advantage of the landscape design potential of ornamental sod and thereby avoid commodity-type competition.

Steps in Producing Ornamental Sod

Harvested turf sod, which may or may not include plastic mesh reinforcement, is treated with herbicide to kill the grass and any existing weeds. Grass sod which has died due to natural causes also may be used as long as root knitting has not been compromised to the point that the sod does not hold together. The sod is laid upon plastic sheeting or other material which will restrict root growth downward. This underlayer should be perforated or sloped to allow for drainage. After the herbicide is no longer active, bedding plants can be established in the sod by seeding on top of the sod or implanting transplants. Seeding may require spreading a top layer of growth medium depending on the needs of particular plant varieties. Plants are grown to market requirements using standard horticultural practices. A wide variety of plant varieties can be incorporated into this product, including ornamental bedding plants, and edible plants such as herbs or miniature peppers which are commonly grown in closely spaced block plantings. The resulting product can be lifted, transported, and reestablished as a sod. The product may be established permanently or laid temporarily at a purchaser's site. In addition to reestablishing the sod on the ground, the product can be attached or laid as a cover for surfaces of structures such as pillars, roofs, etc. The sod layer acts not only as a medium for holding the plants together, but, also, as a mulch layer which will help discourage weed growth and conserve soil moisture at establishment sites.

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6. Biotechnology.

- Development of Enhanced Disease Resistance in Turfgrass by Transformation (Lisa Lee, Cynthia Laramore, Inna Zimmerling, Nilgun Tumer and Peter Day)
- Molecular and Physiological Basis for Enhanced Drought Resistance in Turf (Nourredine Hadjeb and Gerald Berkowitz)
- Cloning and Characterization of Extracellular Lytic Enzymes from Bacterial Isolate N4-7, a Biocontrol Agent of Summer Patch (M.A. Holtman and D.Y. Kobayashi)

Development of Enhanced Disease Resistance in Turfgrass by Transformation

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Development of disease resistant turfgrass is one of our objectives in turfgrass improvement through transformation. Creeping bentgrass and Kentucky bluegrass are target species for transformation. We have developed a creeping bentgrass tissue culture, regeneration, and transformation system using the herbicide bialaphos for selection. Several other genes including the bO gene, three chitinase genes, and two pokeweed antiviral protein mutant genes are being introduced into creeping bentgrass to improve disease control.

Kentucky bluegrass tissue culture, regeneration, and transformation systems are being developed. The program goal is to obtain disease resistant Kentucky bluegrass. Several other genes are also being introduced into Kentucky bluegrass cultures to test their effects on enhancement of disease resistance

Molecular and Physiological Basis for Enhanced Drought

Resistance in Turf

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Current studies with commercial fine turf cultivars indicate that genotypes which maintain leafmconductance (i.e., avoidance of stomatal closure) as leaf water potential declines demonstrate superior performance during imposed plant water deficits. Reduction in the extent of stomatal closure during drought periods is facilitated by the maintenance of leaf turgor (pressure potential). Leaf turgor maintenance during water stress can lead to the following; a) enhanced transpirational cooling, resulting in relatively lower leaf temperatures, b) enhaced photosynthetic capacity (due to the maintenance of carbon dioxide supply to the leaf interior), and c) maintenance of minimum turgor thresholds necessary for continued cell expansion (i.e., leaf growth). Anecdotal evidence, and a few published studies have indicated that endophyte infection of grass species (specifically turf plants) can lead to enhanced performance under water deficit conditions. Our research has focused on delineating the physiological, and molecular basis for this endophyte effect. We have used isogenic Poa ampla plants which are either infected with the endophytic fungus Acremonium typhinum, or are endophyte-free, as a model system to identify plant physiological parameters which are associated with enhaced performance under drought. We are also conducting studies which probe the molecular basis for enhanced drought resistance in endophyte-infected plants.

Greenhouse studies with container-grown P. ampla plants have demonstrated that endophyte infection alters the leaf water potential:turgor pressure relationship in plants subjected to water stress. This leads to the avoidance of stomatal closure as leaf water potential declines. We have used subtractive cDNA library generation to identify the specific gene expression associated with this endophyte-induced drought resistance. The critical technical hurdle which had to be overcome to facilitate these molecular studies was the generation of high quality mRNA pools from the turf plants. Standard RNA isolation protocols are either aurintricarboxycilic acid-, guanidinium isothiocyanate-, or phenol-based (with respect to RNAase inhibition). We have evaluated the different protocols, and have identified the phenol-based procedure as the best choice when working with tough, lignifiedtissue such as turfgrass leaf blades. We have also identified modifications in the phenol extractionprotocol which enhance the quality, and quantity of mRNA which can be isolated from turfgrass leafblades. Specifically, additional centrifugation steps after the chloroform extraction remove interfering substances from the purified mRNA.

We have successfully purified mRNA from water-stressed and control non-infected plants, and from water-stressed and control endophyte-infected plants. Subtractive cDNA libraries have been generated from these mRNA pools by synthesizing immobilized cDNAs, and hybridizing respective mRNAs from the nucleotide pools isolated from plants grown under the different conditions. To date we have identified pools of about 500 genes which are expressed specifically under water stressedconditions in both endophyte-infected, and non-infected plants. Subtractive hybridization between these mRNA pools have led to the identification of about 50 genes which are water stress-associated, and expressed only in endophyte-infected plants. Southern analysis will identify these genes as endophyte-, or plant-derived. Sequence analysis should lead to the identification of specific genes which are responsible for the altered physiological response to water deficits, and enhanced performance under drought, of endophyte-infected turfgrass plants.

Cloning and Characterization of Extracellular Lytic Enzymes from Bacterial Isolate N4-7, a Biocontrol Agent of Summer Patch

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Long standing interest in signal recognition by a biocontrol agent in response to host-pathogen interaction as well as regulation of bacterial biocontrol genes has prompted our study of the gram-negative soil bacterium, N4-7. N4-7 is an effective control agent of summer patch disease caused by the turf pathogen Magnaporthe poae. The organism is rhizosphere competent on Kentucky bluegrass and is closely related to Xylella spp., as determined by 16s DNA sequence analysis. The mechanism of control by N4-7 is thought to be through production of inhibitory compound(s) and extracellular lytic enzymes. N4-7 is unique from other biocontrol bacteria in that it produces several extracellular degradative enzymes, including chitinase, lipase, protease and glucanase making it a novel model system of study.

Lipase, protease and glucanase activities have been cloned from N4-7. Six cosmid clones producing b-1,3-endoglucanase activities were isolated from a wild type genomic library of N4-7 that was expressed in a Citrobacter sp. The clones shared several common restriction fragments but differed in substrate specificity for laminarin (soluble b-1,3-glucans), zymosan (insoluble b-

1,3-glucans), cellobiose (soluble b-1,3-1,4-glucans) and 4-methylumbelliferyl-b-glucoside (MUG). Two clones, 15(6-2) and 15(3-2) were highly active against laminarin, cellobiose and MUG and slightly active against zymosan and contained both 3 and 8 kb BamHI fragments. Further subcloning of clone 15 (6-2) by partial digests with EcoR1 indicate that at least three of these activities are linked within an 11.5 kb region.