Turfgrass Breeding

Introduction

The quality and stress tolerance of a turf is the product of the environment, management practices, and genetic potential of the grass plant. In many cases, various stress effects are the major causes of poor quality turf.

The turfgrass breeding projects are directed at reducing water and pesticide use through the development of resistance to several stress and pest problems. The most desirable characteristics of potential new turfgrasses include:

• drought tolerance high and low temperature tolerance
• tolerance of non-potable water
• tolerance of acid, alkaline or saline soils
• reduced mowing requirements
• efficient use of fertilizers
• traffic tolerance
• genetic stability of characters
• disease, insect and nematode resistance
• competitiveness against weeds
• tolerance to smog and other pollutants
• shade tolerance

The USGA turfgrass breeding programs have focused on the improvement of zoysiagrass, native grasses, Poa annua, bermudagrass and bentgrass. The turfgrasses resulting from the sponsored research must meet the needs of golf courses.

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

Dr. C. Reed Funk

Rutgers University

Goals:

• Collect and evaluate potentially useful turfgrass germplasm.

• Collect and evaluate endophytes associated with cool-season turfgrass species.

• Continue the breeding and development of new cool-season turfgrasses.

Cooperators:

James Murphy
Bruce Clarke
James White
Joseph Heckman
Lisa Lee
Haibo Lui
Jennifer Johnson-Cicalese
Ronald Bara
Dirk Smith
William Dickson
Joseph Clark
Raymond Schaaf
George Ziemienski
Michael Reynolds
Pedro Perdomo
Michael Ventold
Margaret Secks
Stacy Bonos
Barbara Smith

The USGA has enjoyed a very long and productive relationship with Dr. Reed Funk at Rutgers University. Today, the financial contribution of the USGA to his breeding program is small compared to the progress made each year. 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.
More than 7,000 new turfgrass evaluation plots and 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, 1,500 plots of tall fescue, 850 plots of fine fescues, and 305 plots of creeping, colonial, dryland, and velvet bentgrasses.

Intraspecific and interspecific hybridization programs are being expanded in Poa. Many of the interspecific crosses are directed to transfer a useful endophyte into Poa pratensis, as well as increasing the pool of genetic diversity. Intraspecific crosses are directed to develop improved mid-Atlantic type bluegrasses with enhanced tolerance of heat and drought, deep roots and rhizomes, improved disease and insect resistance, and economical seed production. Current midAtlantic type Kentucky bluegrasses such as WABASH, BEL 21, VANTAGE, and EAGLETON are not widely used because of low seed yields.

Improved resistance or tolerance to 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.

Significant differences in damage by, tolerance of, and recovery from white grubs were observed in an older Kentucky bluegrass test. This test was 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.

One partial explanation for the differences observed among the Kentucky bluegrasses was the varying rate of net photosynthesis. Tolerance of high soil temperatures also could be involved. Mid-Atlantic ecotypes generally showed the best performance.

Germplasm developed at the New Jersey Agricultural Experiment Station contributed to a number of new turfgrass cultivars, including CALYPSO 11, CITATION III, MANHATTAN III, WINDSTAR and RPBD perennial ryegrasses; TITAN 11, CORONADO, FINELAWN PETITE, and COYOTE tall fescues; SOUTHPORT Chewings Fescue; WARWICK hard fescue; PRINCETON 105 and EAGLETON Kentucky bluegrasses; and L-93 creeping bentgrass.

Improved cultivars of Koelaria 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.

Most improved turf-type cultivars of perennial ryegrass were developed from a very limited genetic base. For more than thirty years, extensive population improvement programs using phenotypic and genotypic recurrent selection, and occasional backcrossing, has been very effective in developing improved perennial ryegrasses. Dramatic progress has been made developing ryegrasses with a darker color, greater density, finer leaves, a lower growth profile, improved mowing quality, and endophyte-enhanced insect resistance. Significant advances also have 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 dollar spot. 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.

Most improved turf-type tall fescues were developed from a very narrow genetic base. Recurrent phenotypic assortive mating, combined with clonal and/or progeny trials conducted under frequent close mowing, has been used for a 34-year period. A few dozen plants surviving in old turfs in 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 accessions from the cool-moist or hot-dry summer climates of Europe. However, genetic improvements in turf-type varieties for resistance to *Rhizoctonia* brown patch have not been sufficient to adequately overcome the more favorable conditions for this disease. A dense, lush turf that develops under high fertility and frequent close mowing remains very susceptible to *Rhizoctonia* brown patch in warm humid environments. Pythium blight also can be severe in turf-type tall fescues maintained in this manner.
Most of our major cool-season turfgrasses evolved in the cool-moist or hot-dry regions of Europe. This is the major reason why these cool-season species are not naturally well-adapted to warm-humid environments of much of the United States. It also helps explain 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 bluegrass, creeping bentgrasses, and rough bluegrasses also was 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 for all these species.

Among the extensive cool-season germplasm collections at Rutgers University, Kentucky bluegrass has the greatest amount of genetic diversity. Most of the characteristics desired in a cool-season, lawn-type turfgrass occur within this pool of Kentucky bluegrass germplasm. Unfortunately, recurrent selection and backcross breeding techniques to concentrate all of these useful characteristics into a single cultivar or interbreeding population cannot be used because of the apomorphic reproductive behavior of Kentucky bluegrass. Efforts are underway to develop successful and efficient methods of population improvement in Kentucky bluegrass that will lead to faster progress in developing better turfgrasses.

**Breeding and Development of Bentgrass**

**Dr. Milt Engelke**

**Texas A&M University**

**Goals:**

- Develop stress tolerant bentgrass cultivars with specific emphasis on heat tolerance, root growth characters, turf quality, and resistance to natural disease and insect pests.

- Continue genetic studies involving heritability and stability of biological traits associated with stress tolerance.

**Cooperators:**

* I. Yamamoto
* J. M. Mills M. H. Delambre
CRENSHAW and CATO were released in April 1993. Considerable success has been realized in the performance and utility of both grasses, especially throughout the southern United States. CATO was licensed to Pickseed West, Tangent, Oregon and was commercially available in the fall of 1994. CRENSHAW was licensed to Lofts Seed, Inc., Somerset, New Jersey and was available commercially with limited supplies in 1993. The demand for CRENSHAW increased steadily in 1994, and despite increased production, seed was sold out for the year.

Syn 1-88 is a reselection from SEASIDE and has been optioned to Pick Seed West for increase. It will be commercially available in 1996. Syn 1-88 has been tentatively named MARINER and is recognized for its low maintenance requirements and excellent salt tolerance. This variety was released as a utility bentgrass targeted to replace SEASIDE creeping bentgrass with quality similar to the standard PENNCROSS.

A series of four synthetic creeping bentgrass populations were developed in 1992 and designated as the Syn 92 series. These populations (varieties) incorporate stronger physiological and disease resistance traits to produce better stress tolerant grasses. Each of these populations was comprised of four to ten parental lines which underwent further selection and breeding for floral initiation, leaf texture, color and field performance under close mowing and cultivation.

Burlingham & Sons have an option agreement with Texas A&M University for testing and evaluation rights on Syn92-1 (CENTURY) and Syn92-5 (IMPERIAL) creeping bentgrasses. Turf Merchant Inc. and Scotts have a joint option for testing and evaluation of Syn92-2. These three experimental varieties were entered into the National Turfgrass Evaluation Program's 1993 Creeping Bentgrass Trials and are performing well. Sufficient seed stocks of Syn92-4 has allowed for limited testing and evaluation. In 1996, larger quantities of seed will permit more extensive testing and evaluation of Syn92-4.

Vegetative selections of creeping bentgrasses were identified in the Oregon production fields among the progeny of the advanced lines. These selections will be included in field and greenhouse performance tests at TAES-Dallas to evaluate vegetative growth characters, turf quality, disease resistance, insect resistance, traffic and salinity tolerance, heat tolerance and root growth characters.

Breeding and Evaluation of Cold-tolerant Bermudagrass Varieties Golf Courses

Dr. Charles Taliaferro
Oklahoma State University

Goals:
• Assemble, evaluate, and maintain Cynodon germplasm with potential for contributing to the genetic improvement of the species for turf.

• Improve bermudagrass germplasm populations for seed production potential, cold tolerance, and other traits conditioning turf performance.

• Develop, evaluate and release superior seed-propagated, cold-tolerant, finetextured, turf bermudagrass varieties for the U.S. transition zone and similar climates.

• Develop, evaluate and release improved vegetatively propagated bermudagrass varieties with specific adaptations and uses in the southern U.S., e.g. varieties for golf course putting greens in the deep South.

Cooperators:

James Baird
Dennis Martin
Jeffery Anderson
Michael Anderson

The turf bermudagrass breeding program was initiated in 1986 at Oklahoma State University. The initial broad objective was to develop fine-textured, winter hardy, seedpropagated varieties for the U.S. transition zone. The program was expanded in 1990 to include the development of superior vegetatively-propagated varieties.

Important ongoing activities supporting the breeding effort include: 1) the development, improvement and use of techniques to measure physiological and morphological parameters related to environmental stresses; 2) the procurement, evaluation, and use of new turf bermudagrass germplasm in the breeding effort; 3) the use of tissue culture in generating genetic variation and screening for desirable traits at the cellular level; and 4) the evaluation of bermudagrass varieties and breeding lines for turf performance.

Two broad genetic-base C. dactylon populations, one derived from cold-tolerant relatively infertile germplasm, the other from cold-sensitive highly fertile germplasm have been developed using phenotypic recurrent selection. Selection within the cold-tolerant population, \( C_{3fer3tex} \) has been for increased seed production potential and finer texture. Selection within the cold-sensitive population \( C_{2u} \) has been for increased freeze tolerance. Cumulative performance data indicate synthetic varieties from the cold-hardy breeding population to be well-adapted to the U.S. transition zone with turf
quality competitive with other seeded bermudagrasses. Commercial release of one or more synthetic varieties is planned for 1996. Field evaluations are underway to define optimum cultural management strategies for the varieties.

African bermudagrass, *C. transvaalensis*, has extensive phenotypic variation within the species for many traits influencing adaptation and turf quality. A population is now being studied that will permit estimation of genetic parameters for traits of interest.

Field evaluation of selected African genotypes indicate their major weaknesses to be instability of turf quality and lightgreen color. In tropical environments, the African selections maintain good to excellent putting-green turf in winter, but dramatically decline in quality during summer. Variation in summer performance of genotypes has been documented, indicating potential for improvement.

As a result of its weaknesses, African bermudagrass is now being evaluated for potential use on tees and/or fairways. Furthermore, African genotypes having demonstrated the best overall performance are being used extensively as parents in crosses with *C. dactylon* to produce large F^1^ progeny populations. Several selections from these populations are performing well in field tests.

In 1995, African bermudagrass tolerance to herbicides and response to fertility were more clearly defined. The phenoxy class of herbicides caused some phytotoxicity. Most of the other herbicides commonly used for bermudagrass did not cause any problems. Generally, annual nitrogen fertilization rates of 6 to 12 lbs/1000 ft^2^ provided the highest turfgrass quality.

Alterations in protein synthesis associated with cold acclimation have been documented in MIDIRON and TIFGREEN bermudagrasses. Acidic proteins were diminished in crowns of both varieties following cold acclimation. Both varieties synthesized cold-regulated (COR) proteins of several sizes in association with cold acclimation. MIDIRON crowns synthesized low molecular weight basic cold-regulated COR proteins in greater numbers and amounts, and intermediate molecular weight acidic COR proteins in greater amounts than TIFGREEN crowns. Peptide sequence analysis of a prominent low molecular weight protein from MIDIRON crowns indicates it to likely be a chitinase.

New germplasm from the Peoples Republic of China that has demonstrated good turf quality plus good seed production potential in initial evaluations and will be advanced to intensive replicated testing for adaptation, turf quality, and seed production capability. This germplasm is being used to formulate new breeding populations, create narrow-base synthetic varieties, and may be incorporated into the two existing breeding populations.
Breeding, Evaluation and Culture of Buffalograss for Golf Course Turf

Dr. Terrance Riordan

University of Nebraska

Goals:

- Develop vegetative and seeded turf-type buffalograsses which conserve energy and water.

- Develop buffalograss establishment protocols and management systems to provide acceptable golf course rough and fairway turf which significantly reduced cultural inputs.

- Determine the range of adaptation of turf-type buffalograss.

- Evaluate potential insect and disease pests of buffalograss.

- Evaluate physiological and biochemical principles of environmental stress and nutrient utilization in buffalograss.

Cooperators:

Paul Johnson
Fred Baxendale
Roch Gaussoin
Leonare Wit
Charles Rodgers
Kevin Frank
Shuizhang Fei
TM Heng-Moss
Jennifer Johnson-Cicalese

Through September 1995, sales of '609' were approximately $1,125,000. This is slightly better than in 1994. Total production is now 440 acres at four locations in Texas. In September, a royalty check for $48,642.86 was received from Crenshaw & Doguet for '609' sales during the first half of 1995. Sales of '378' have been fairly good ($53,424), although the weather in Nebraska this spring and summer was poor, having a significant effect on sales. Figures for '315' have not been released.
Three new vegetative buffalograsses are targeted for release in 1996. Those are NE 86-61, NE 86-120 and NE 91-118. Crenshaw & Doguet continue to expand production through sub-licensees. So far this has been a small part of their business, but there is potential for growth in Arizona, Missouri, and Colorado.

Native Turf Group and the University of Nebraska cooperatively released CODY and TATANKA seeded buffalograsses in 1995. There was only a limited amount of CODY available this year, and it was sold in small (2 lb.) lots. However, interest was very strong, and fair supplies of seed will be available in 1996. Only research supplies of TATANKA were available this year, but it should be commercially available in 1996. Sharp Brothers Seed has planted production fields of their UNL derived synthetics, but these seeded buffalograsses will not be commercially available until 1997.

The hot and dry summer experienced in Nebraska this year was ideal for evaluating buffalograss selections for tolerance to heat and drought. However, the best performing genotypes in previous years have been very consistent. This is evident in the National Turfgrass Evaluation Program's buffalograss trial where '3 7 8', '609', and '3 15' were the top vegetative materials in the test. Top experimentals and cultivars in a low cutting height evaluation were put into a crossing block for the development of a buffalograss variety appropriate for fairway use. In other evaluation trials, several entries repeatedly show up at the top of the rankings, even ahead of currently released '3 15' and '3 7 8'. These include the potential new releases 86-61 and 86-120, but also the experimentals: 91-181, 92-116, 92-135, and 93-181.

Real improvement in the seeded varieties also is evident. The experimental synthetics 90-504-JK and 90-503-JK show very good quality and color ratings, better than the standard seeded varieties. Four synthetic populations are undergoing the third cycle of recurrent selection to improve each population farther for turfgrass quality and disease resistance characteristics. After further testing, one of these populations may be released from the University of Nebraska breeding program.

Divergent selection for caryopsis size on two synthetic populations has shown inheritance and potential breeding progress. Realized heritabilities for caryopsis size in both populations are low (0.08 to 0.26) indicating that multiple cycles of selection will be necessary to improve this trait. In separate work, we are selecting for increased germination percentage to improve establishment.

We also are using laboratory techniques for buffalograss improvement through tissue culture and transformation procedures. Thus far, plants of two female clones and a male clone were regenerated through immature inflorescence culture. Callus induction was promoted 'in the male by an ethylene antagonist (AgNO₃), but not in the female. Efforts will continue in refining the cellculture techniques.
Initial establishment research at two locations indicates significant differences for percent cover of buffalograss and percent weed cover. These preliminary results indicate the best planting date is between May 15 and July 15 for the Nebraska location, and August 15 for Logan, Utah. Final recommendations for planting dates will be made after determining winter survival and the effectiveness of the fall planting dates. Mowing and fertilization studies will be initiated in 1996.

Scanning electron microscope observations of mealybugs on buffalograss leaves suggest that pubescence may facilitate oviposition by providing a framework for the waxy filamentous ovisac, and/or provide a foothold for mealybugs. Evidence suggests that mealybug resistance mechanisms may operate primarily on a whole plant basis or may involve interactions with parasitoid wasps. Heritability estimates for mealybug resistance using maternal half-sib analysis ($h^2_m$) and offspring-parent regression ($h^2_{OP}$) were 0.87 and 0.56, respectively. These relatively high heritabilities suggest that improvements in resistance should be possible.

**Improvement of *Poa annua* var *reptans* for Golf Turf**

**Dr. Donald White**

**University of Minnesota**

**Goals:**

- Expand the evaluation and development of the advanced selections for turf quality, seed production, and seeding recommendations.

- Continue and expand seed production evaluations in Oregon.

- Continue and expand the development of a breeder's seed supply.

- Expand seeded evaluation plantings at selected golf course and university locations.

Three selections (MN#42, MN# 184, and MN#208) were approved for release by the Minnesota Agricultural Experiment Station Horticultural Plant Release Committee in 1994. An exclusive agreement was subsequently executed by the University of Minnesota Office of Research and Technology Transfer (Patents and Licensing) and Peterson Seed Company, Savage, Minnesota.
One-acre seed production fields for MN#42 and MN# 184; and 2 acres of MN #208 have been monitored for seed production since their establishment on November 12, 1993 at a site in Oregon. Most of the seed has been successfully harvested with standard windrow and combine equipment during the early summer.

MN#42 had the most uniform heading and exhibited the least amount of shattering. NW#184 and MN#208 exhibited some uneven ripening and shattering. There are some indications that two harvests per season may be possible. In 1994, MN#42, MN#184, and NIN#208 produced 291, 170, and 208 total pounds of seed per acre, respectively. However, each selection produced sufficient seed to warrant continued production and evaluation.

On the basis of second year seed production and performance under mowing trials, it was decided that the first introduction will be MN# 184 instead of MN#42 as originally indicated. Fifteen acres was seeded to MN# 184 in Oregon during October 1995 for production of breeder's seed in 1996. An additional planting of 6 acres is planned for the spring of 1996. All three selections under consideration for introduction maintained trueness to type in the seed field.

Selection MN# 184 performed exceptionally well during the 1994-95 growing season. All three selections responded equally to a series of herbicide treatments. No phytotoxicity was observed for Poast (herbicide) treated Poa reptans selections in the seed field. This will be very beneficial for keeping the seed fields free of bentgrass and weeds. Poast will be the herbicide of choice for seed production.

Vernalization and photoperiod requirements are major determinants to perenniality. Vernalization requirements are met between 4C and 8C after 10 to 12 weeks exposure. Some perennials are induced to flower under short days while others are induced under long days and some are day neutral but require vernalization. Plants require no fewer than 4 to 6 leaves in order to be receptive to these flower induction stimuli.

Observations of crosses between annual (continuous flowering) and perennial (seasonal flowering) type of Poa reptans indicate a 3:1 ratio of continuous flowering to seasonal flowering types. These inheritance investigations indicate that flowering pattern fits a genetic model involving one locus with continual flowering being dominant to seasonal.

The cytology and flow cytometry research was completed and submitted for publication. Earlier studies documented the occurrence of diploid (2N = 2X = 14) and tetraploid (2N = 4X = 28) Poa annua in our breeding populations. Subsequent field sampling on one golf green revealed the presence of diploids to a level of approximately 24% of the population on greens. Interestingly, no 2N = 14 types have been found in either the fairway or the rough Poa populations. The diminutive stature, fine texture, slow
growth, and persistence appear to be clear indicators of the $2N = 14$ types. All of the $2N = 14$ types observed to date have been sterile.

Plants resulting from seed of interspecific crosses and reciprocals, between *Poa supina*, *Poa infirma* and *Poa reptans* exhibit some unique plant types. These materials will be observed for several generations, and they will be evaluated as parents.

Research plans for 1996 include expanding the number of evaluation sites in the cool season turfgrass area; continuing efforts into improving seed production; and establishing replicated plantings to evaluate potential use for winter overseeding in the Southern U.S. Seed blends of MN#42, MN# 184, and MN#208 continue to be evaluated for compatibility and performance under putting green conditions.

### Colonial Bentgrass, (*Agrostis tenuis*), Breeding and Cultivar Development

**Dr. Bridget Ruemmele**

**University of Rhode Island**

**Goals:**

- Develop resource-efficient, improved colonial bentgrasses for use individually, in blends, or in mixtures with fine fescues.

- Improvements desired for colonial bentgrass include: brown patch resistance, increased cold hardiness, dark green color, close mowing tolerance, recuperative ability and wear tolerance, tolerance to reduced cultural inputs, retention of desired turf-type characters.

**Cooperators:**

*Dr. Joel Chandlee*
*Ms. Pei-Yu Zeng*
*Ms. Stephanie Legare*

The colonial bentgrass breeding and cultivar development program was initiated by Emeritus Professor C. R. Skogley. Dr. Skogley received partial support for his breeding efforts from the USGA prior to the arrival of Dr. Bridget Ruemmele. After Dr. Ruemmele's arrival, the USGA increased its financial support beginning February 1, 1993 to encourage greater efforts in developing new colonial bentgrass cultivars.
The USGA was instrumental in providing access to germplasm from Dr. William Rumball’s breeding program in New Zealand. Seed was sent directly from New Zealand and collections were made among nursery plantings maintained at Rutgers University by Dr. C. Reed Funk.

The primary emphasis of the project is to improve *Rhizoctonia* brown patch resistance. Additional goals include the development of cultivars with improved cold hardiness, darker green leaf color, low maintenance requirements, close mowing tolerance, recuperative ability, and wear tolerance. Hybridization with related species to improve the characteristics noted above also will be investigated.

Accessions continue to be added to the germplasm assembled at the University of Rhode Island. Four private companies have been actively involved with cooperative acquisition and assessment of materials introduced from this program. A fifth company is in negotiation for cooperative efforts with the intention of marketing into Europe as well as the United States.

Two collection trips were taken in 1995. The major collection area included sites in Georgia, Kentucky, Tennessee, and Virginia. A second effort involved previously unexplored areas of New England. Numerous opportunities, in conjunction with other events, permitted collection during single day travel.

Progeny from 140 additional collections were planted in Rhode Island in 1995 for turf trial evaluation. Earlier genotype collections determined to be superior in turf trials and *Rhizoctonia* brown patch resistance screening in Rhode Island have been planted into space plantings for seed production in both Oregon and Rhode Island. Additional polycross plantings were established in Rhode Island in Fall, 1995, including materials screened for brown patch resistance in greenhouse trials.

Ms. Pei-Yu Zeng, an M.S. degree student, completed advanced greenhouse screening trials for *Rhizoctonia* brown patch resistance in 277 Colonial bentgrasses. Follow-up screening for brown patch resistance was performed by students enrolled in the plant breeding and genetics course. The USGA had previously sponsored a colonial bentgrass breeding project in New Zealand under the direction of Dr. William Rumball. Some of Dr. Rumball’s material acquired from plots established at Rutgers University have shown superior brown patch resistance. Other accessions from various collection efforts have also demonstrated resistance to brown patch.

Molecular efforts have included successful preparation of both creeping and colonial bentgrass in tissue culture suitable for gene transfer. We are seeking chitinase genes from multiple sources to introduce into these cultures using a newly-acquired gene gun.
Development of Stress Tolerant Seashore Paspalum for Golf Course Usage

Dr. Ron Duncan

University of Georgia

Goals:

- Establish an extensive collection of genetic material.
- Improve the adaptability of the species with special emphasis on: acid soil stress tolerance with deep rooting and root plasticity in high bulk density (compacted) soil, winter hardiness to expand its adaptation zone, and wear resistance that will meet or surpass golf course requirements.

A total of 5,660 plots of Paspalum have been established in Georgia ranging from mother nursery and increase plots of about 500 ft' each to 2 ft x 2 ft spaced plants. Of this total, 1,134 plots are planted at Blairsville (altitude 1,530 ft - 34' 50.44N, 83' 55.80W) for the ultimate field evaluation for cold hardiness testing.

Collection trips were made to Southern California, Alabama, and Florida to obtain additional Paspalum. Up to 15 possible ecotypes have been added to the 300-ecotype collection of Paspalums.

Mowing height ranges from 5/32 of an inch for putting green tests to 1/2 an inch for fairway evaluation (most plots). Mowing heights were raised to one inch going into the winter months. Ongoing studies involve greens evaluation, establishment from sod or stolons utilizing mycorrhizal enhancement, herbicide management, traffic-wear-fertility interactions, overseeding, and insect resistance for mole cricket and fall armyworm.

Eighteen Paspalums and TIFGREEN were evaluated on 10 ft. by 10 ft. plots throughout the growing season. Mowing height was reduced from 3/4 in. to 5/32 in. gradually over the summer. The close mowing was instrumental in identifying potential candidates for additional putting green evaluations as well as fairway types.

Dollar spot appeared on the putting green test after mowing heights reached a 1/4 of an inch. Natural infection in combination with additional inoculations provided constant pressure on the green throughout the summer. No fungicides were applied during the year. With the exception of a selection from Argentina, all plots suffered dollar spot infestations ranging from 10 to 90 percent of the plot area. From this test, two
ecotypes (Argentina selection and an Aldine Pines selection) will be increased for testing on golf courses in the Caribbean region and southern U.S.

Approximately 775 hybrid seedlings were space-planted during the summer of 1995 for evaluation. Seed from 61 polycross combinations and other field plots were collected in November 1995. Somaclonal variation resulting from tissue culture is being utilized as a selection tool, especially for the finer-textured Paspalums. A total of 3,640 regenerated plants have been planted in the field and are maintained at a 1/2 inch mowing height. Forty finer-textured selections have been selected from tissue culture regenerants. Somaclonal variation also exists for genetic color, growth rate, and winter hardiness.

A cold temperature chamber has been used to induce cold shock (-9C) and recovery tests on several Paspalum ecotypes. In conjunction with electrolyte leakage tests, the relative cold threshold of 170* F (-8C) has been verified. Field evaluations at Blairsville, Georgia have indicated that some Paspalums survived -30 F (-18C) during the 1993-1994 winter and -2*F (-17C) during the 1994-1995 winter. The cold chamber and field evaluations suggest sufficient variability exists among the ecotypes in the current collection for improvement in winter hardiness.

Breeding and Development of Zoysiagrass

Dr. Milt Engelke
Texas A&M University

Goals:

- Develop improved zoysiagrass cultivars with multiple character performance involving low water-use, persistence under drought and temperature stress, and tolerance to poor water quality.

- Develop seeded zoysiagrasses that are genetically stable, with improved turf quality, persistence, and competitive ability.

- Continue genetic studies involving the heritability and stability of biological traits.

Cooperators:

Dr. Ikuko Yamamoto
Mr. Samuel K. Riffell
Marking the 11th year of this USGAsponsored research project, more than $500,000 has been directed toward the breeding and improvement of zoysiagrass. Germplasm acquisition and maintenance continue, and TAES-Dallas is serving as a remote quarantine site for zoysiagrasses introduced from other countries, primarily China and Japan.

The NTEP zoysiagrass trials established in 1991 include nine TAES entries. DALZ8507, a fine-textured, cold hardy Zoysia matrella, topped the trials since 1993. Other varieties that are doing well include DALZ8512, DALZ8514, DALZ9006.

The zoysiagrass breeding program has expanded its personnel with the addition of Dr. Ikuko Yamamoto (Pennsylvania State University, 1994) as an Assistant Research Scientist. Dr. Yamamoto is working closely with Drs. Colbaugh and Reinert on the heritability of host-plant resistance mechanisms. This research employs both conventional and biotechnical approaches to genetic recombination, with additional collaboration with Dr. Andrew Patterson (Soil and Crop Science Dept., Texas A&M University, College Station, TX).

Additional germplasm has been introduced to the program from Japan and China. Several progeny populations of previous hybrids and recent introductions are under evaluation. Over the past several years, greater emphasis has been directed to salinity tolerance (initiated by Dr. Ken Marcum, now at University of Arizona.), shade tolerance (Ms. Sharon Morton, now a Pott's Fellow in Ph.D. Program, Texas A&M University), water-use requirements (Dr. Richard White, now Associate Professor, Texas A&M University), and establishment technology (Dr. Bridget Ruemmele, now Assistant Professor, University of Rhode Island).

Five zoysiagrasses, DALZ8502 (Z. matrella: fine texture), DALZ8507 (Z. matrella: medium-fine texture), DALZ8512 (Z japonica” medium-coarse texture), DALZ8514 (Z japonica: medium-coarse texture), and DALZ9006 (Z. matrella: medium-fine texture) will be submitted for release in the spring 1996. Three of them are presently under foundation increase. High productivity is one of the superior characteristics of these new lines. In contrast to MEYER, which requires 12 to 24 months between crop cycles, DALZ8512 and DALZ8514 can be harvested two to three times every 24 months. DALZ8502 also produces two to three crops every 24 months at Dallas; however, this selection may not provide sufficient winter hardiness in the transition zone climates of the U.S. DALZ8507 and DALZ9006 produce a crop on a 9 to 12-month cycle.

**Cultural Practices**
Introduction

A series of research projects with the aim of substantial reduction in water use, pesticide use and maintenance costs have been and continue to be conducted on a regional basis. This is necessary because of regional differences in climate, soil, and stress conditions. The objectives of these studies have focused on the following:

- Range of adaptation and stress tolerance of new grasses resulting from the breeding projects
- Evaluation of direct and interacting effects of two or more cultural practices
- Management of native and low maintenance grasses
- Development of cultural programs that substantially reduce weedy species in golf turf
- Development of cultural practices that allow efficient turf management under conditions of poor quality soils or severe air pollution, or that permit the use of effluent or other marginal quality waters
- New research techniques that reduce pesticide and other chemical usage

The results of these studies have led to the development of maintenance programs that conserve substantial quantities of water, reduce fertilizer needs, and decrease mowing frequency; all without impairment of functional quality or aesthetic appeal.

Physiological Basis for Selection of Bentgrasses With Superior Drought Resistance

Dr. Richard White

Texas A&M University

Goals:

- Determine the water balance in creeping bentgrasses with performance in adverse environments.
• Assess management systems for the incorporation of diverse bentgrass germplasm into existing bentgrass putting greens to improve their stress resistance and functional quality.

Cooperators:

Mr. Dave Gilbert  
Mr. Gene Taylor

Creeping bentgrass provides a premier surface for golf course putting greens. A preference for this species and increasing demands by the public for quality sports turf surfaces have fueled the expansion of bentgrass use throughout the deep South, well beyond the area of adaptation for this species. The expansion of bentgrasses throughout this environmentally stressful area has out-paced development of stress tolerant bentgrass cultivars.

Irrigation and syringing are used on bentgrasses throughout the South to prevent moisture and heat stress. Golf course superintendents pay close attention to soil conditions to ensure adequate soil moisture levels. However, shallow root systems and high evaporative demand frequently expose bentgrass putting greens to physiological drought when atmospheric demand exceeds the turgor maintenance capability of bentgrass. This in turn predisposes bentgrass to heat stress by limiting or even terminating the normal dissipation of thermal energy by evapotranspirational cooling.

Selection of maternal clones and populations to assess mechanisms of stress resistance has progressed with the assistance and close cooperation of Dr. Milt Engelke. Initial stress tolerance and stress tolerance mechanisms are being assessed in maternal clones. Progeny were obtained and increased to assess progeny response. The population(s) used will allow determination of the heritability of the various mechanisms of turgor maintenance or drought resistance and should provide insight into several mechanisms of bentgrass summer stress tolerance.

Three experimental sites were selected for interseeding CRENSHAW creeping bentgrass into existing PENNCROSS putting greens including the Texas A&M University Research and Extension Center at Dallas, Brookhaven Country Club, and Dallas Country Club. Mechanical disruption and chemical suppression treatments were employed in a multiple strip-split plot design. The most vigorous chemical suppressant was glyphosate, which was used to allow easy visual determination of seedling emergence in dead bentgrass sod.

Cimectacarb (Primo) and none chemical suppression treatments were also used. Mechanical disruption treatments were none, vertical mowing, core aerification, and startine aerification. Interseeding was accomplished during April 1 through 15, 1995.
Visual observations within the glyphosate treatments indicate that vertical mowing may be the most effective means of mechanical disruption. However, overall seedling emergence was less than expected at all sites. This experiment was planted again in October 1995 at the Dallas Country Club.

Electrophoretic analysis of isozyme banding patterns from samples collected from the TAMU-REC at Dallas location is providing, through close cooperation with Drs. M. C. Engelke and Ikuko Yamamoto, the necessary information to determine population changes. Current estimates of 10 to 20% contribution of CRENSHAW produced from a single spring interseeding of this PENNCROSS putting green provides the first quantified documentation that a new bentgrass cultivar can be successfully incorporated in an existing bentgrass putting green.

Studies to determine the effects of blending bentgrass cultivars on bentgrass putting green turf quality, disease resistance, and performance were established at the Turfgrass Field Laboratory in College Station, Texas. These blending experiments will provide insight into the effects of creeping bentgrass blends on overall putting green quality. This concept is extensively used for other cool-season grasses, but has not been reported for creeping bentgrasses.

Evaluating Bermudagrass for Putting Greens

Dr. Elizabeth Guertal

Auburn University

Goals:

- Evaluate new bermudagrass cultivars in comparison with TIFGREEN and TIFD WARF on both a USGA green and a native sandy loam soil.

- Evaluate management practices including mowing height, irrigation and topdressing frequency.

Cooperators:

Dr. Coleman Ward
Dr. Ray Dickens
Bermudagrass (*Cynodon dactylon*) is the best adapted species for putting greens in the warm humid regions of the United States due to its superior heat tolerance and recuperative ability under low mowing heights. To date, limited effort and resources have been expended to identify or develop bermudagrass cultivars with the quality of creeping bentgrass (*Agrostis palustris*).

Soon after TIFGREEN was released, distinct off-types appeared in greens throughout the Southeast. Although TIFDWARF was the dominant cultivar released, others, such as PEE-DEE and TIF TURF were said to be distinctly different from TIFDWARF. Although TIFDWARF was the only one of the off-types to become established in the trade, there is considerable evidence that it is not the only variant existing originally, or at the present time.

Turf managers have continually reported the occurrence of variants within TIFGREEN and TIFDWARF greens. In many cases attempts have been made to interest researchers in testing these strains of grasses, which are said to exhibit superior performance under conditions of excess moisture, heavy traffic, or poor soil structure common to the Southeast. To date, there has been little or no evaluation of these unique ecotypes to determine their value. Thus, the objective of this research was to evaluate bermudagrass cultivars or TIFGREEN or TIFDWARF ecotypes on both a USGA green and a native sandy loam soil.

A 5,000 ft² USGA-type golf putting green was constructed in August of 1993 at the Auburn University Turfgrass Research Unit. This putting green, along with a similarly sized native soil putting green, were used for evaluation of 12 bermudagrass cultivars. These cultivars consisted of 8 selected ecotypes of TIFDWARF or TIFGREEN, plus four experimental lines: T596 and TW72, from GA, and 2747-OK and 2474-OK from Oklahoma. Oklahoma State grasses were African bermudagrasses, *Cynodon transvaalensis*.

TIFGREEN or TIFDWARF ecotypes were collected from various sources, including selected golf course greens. Bermudagrass cultivars were sprigged into native soil and USGA putting greens on April 14-15, 1994, using a Ryan aerifier at a 6-inch plug spacing. Twelve cultivars were planted in 4 blocks, with cultivars arranged in a completely random design within each block in each putting green. Each cultivar main block was 3 feet wide and 25 feet long.

All plots were irrigated and mowed uniformly. Sand topdressing was initiated in the summer of 1994 with monthly topdress applications of 1/3 yard/1000 ft² per month. Plots were evaluated for percent ground cover during grow-in, fall color (Nov), seedhead production (fall and spring) and spring greenup.
Putting green type affected percent cover at both evaluation dates (19 May and 21
June). There was no difference in percent cover among bermudagrass cultivars grown on
the same type of putting green. Percent cover was greater in the native (96%) than USGA
(85%) putting green, when measured on June 21. It may be that ecotypes selected from
native putting greens were more adapted to rapid growth on native soil rather than USGA
putting greens.

 Bermudagrass cultivars grown on the USGA putting green were usually darker green
than when grown on the native putting green. The two Oklahoma C. transvaalensis
bermudagrasses were significantly paler than any of the other cultivars. TIFDWARF,
T596 and a TIFDWARF ecotype (from green #10, Mobile) were greener than other
cultivars.

 The only bermudagrass ecotype to produce fall seedheads was the selection from
green #10 at Mobile. This grass produced seedheads on both the USGA and native
putting greens. Turf quality was severely impacted. In the spring, seedhead production
was affected by both green type and bermudagrass selection. When averaged over type of
green, the Lakewood and 2747-OK cultivars produced significantly more seedheads than
any other grass. Only the Mobile #10 and TW72 selections did not produce spring
seedheads. When averaged over all grasses seedhead production rating on the USGA
putting green was 4.7 and seedhead production rating on the native putting green was 3.5.

 Both putting green type and bermudagrass selection affected spring greenup. Greenup
ratings on the USGA putting green were higher, with an average greenup rating of 5.2.
Grasses on the native putting green had an average greenup rating of 4.4. When averaged
over green type, the 2747-OK selection had the significantly lowest greenup (rating of
2.6) of any of the grasses.

Low Temperature and Drought Regulated Gene Expression in
Bermudagrass

Dr. Wm. Vance Baird

Clemson University

Goals:

- Isolate cDNA clones of genes preferentially transcribed under
  conditions of low temperature or related water stress.

- Characterize their stress-specific expression.
• Determine the primary molecular structure of these clones.

• Isolate the corresponding genomic clones that contain the inducible response elements(s).

Cellular membranes have been considered a primary site of freezing injury, and alterations of membrane composition correlate with cold acclimation processes that allow plants to tolerate freezing temperatures. As major components in membrane bilayers, the polar lipid fatty acids could directly regulate membrane structure, and therefore membrane function through the alterations of acyl chain length (number of carbon atoms) and/or unsaturation (number of double bonds). Alterations in plant membrane lipid fatty acids can be induced by many physiological and environmental factors, and these changes could play an important role in adaptation to low temperature.

Bermudagrass, *Cynodon dactylon*, shows an increased tolerance to cold after a period of exposure to moderately low temperatures. However, whether this cold acclimation correlates with cell membrane alterations, and how the membrane lipid fatty acids respond to low temperature, are unknown for bermudagrass.

*MIDIRON* and *U3* bermudagrass were exposed to conditions known to induce cold acclimation [e.g., 8/30°C (day/night) temperature, 10 hr photoperiod, 250 umol/m²/s photosynthetic photon flux density] for one or four week time periods. Tissues (leaves, crowns and roots) were harvested and total lipids isolated by organic extraction. The polar fraction was purified by thin layer chromatography. The fatty acids (FA) were released from the polar lipids by saponification, and then converted to FA-methyl esters. Separation and identification of individual FAs within the mixture was accomplished by gas chromatography, with the aid of an automated, computer-based identification system.

Different organs of the same plant responded differentially to low temperature. Crowns showed the most dramatic and sustained increase in total FA content. Overall, significantly greater than 95% of the polar FA content was accounted for by four FA species: palmitic acid (16:0), stearic acid (18:0), linoleic acid (18:2) and linolenic acid (18:3).

*MIDIRON* (relatively cold-tolerant) responded more rapidly and to a greater extent than did *U3* (relatively cold-sensitive) for the changes in FA composition documented in this study. This was illustrated by the nearly four-fold increase of unsaturated: saturated FA ratio for N11DIRON over U3, and by the significant difference between the double bond index of the two genotypes, at the end of the cold acclimation treatment. Our results point to specific desaturase enzymes (e.g., W-3 and A-12) as being of primary importance in controlling membrane lipid-FA composition in response to low
temperature, and ultimately in avoiding the winter damage suffered by bermudagrass along its northern zone of adaptation.

Differential display/RNA profiling has been used to identify genes expressed in bermudagrass in response to low temperature (i.e., during the process of cold acclimation). This procedure uses total or poly(A) + RNA as a substrate for the synthesis of cDNA molecules (i.e., partial gene clones) expressed in the particular organ, developmental stage or treatment from which the RNA was isolated. Additionally, this method uses a set of random anchor and 10-mer oligonucleotide primers to initiate clone synthesis.

By comparing gel displays from 0, 24 and 48 hrs post-low temperature exposure to that from control non-acclimating tissue over the same time frame, four sequences putatively expressed in response to low temperature were identified. These sequences have been subcloned for further analysis and characterization; such as DNA sequence determination and their use as probes on Northern blots to confirm their differential, cold-specific expression.

In addition to continuing this avenue of investigation, we are designing degenerate (sequence variable) oligonucleotide primers to conserved sequence regions of fatty acid biosynthesis genes for use in differential display experiments and in reverse transcriptase-polymerase chain reaction (RT-PCR) analysis.

Seeded Bermudagrass Water Use, Root and Shoot Growth Under Soil Stresses

Dr. Robert Carrow

University of Georgia

Goals:

• Evapotranspiration (ET), drought resistance, rooting/water extraction patterns and shoot responses were determined under field conditions.

• Basic cultural programs (fertility, disease/insect, traffic tolerance) were defined.

• The genetic stability of these grasses with respect to environment, disease, and insect pressures was determined.
Bermudagrasses, *Cynodon* spp., are drought resistant grasses in many areas of the southern United States. In the Piedmont region, as well as Utisol and Oxisol soils worldwide, turfgrass root growth can be inhibited by soil stresses: a) high soil strength, and b) acid soil complex, a combination of element toxicities with nutrient deficiencies. Genotypes of bermudagrass may differ in tolerance to these stresses. This project evaluated the water use, rooting patterns, and best-suited cultural programs for eight seeded bermudagrass genotypes from the USGA-supported breeding program at Oklahoma State University versus two commercial cultivars (Arizona common and PRIMAVERA) under three traffic levels and three nitrogen fertilizer regimes. A summary of the results to date include:

1. The most rapid establishment was observed for PRIMAVERA, 91-2, 91-1, and Arizona (AZ) common, while least was for 91-14, 91-12, and 91-3.

2. AZ common and PRIMAVERA exhibited some winterkill (i.e., 5 to 10%), while no winter injury was noted on the OSU experimentals.

3. Cultivars with consistently higher visual quality and shoot density than AZ common across all N levels (2, 4, and 6 lb. N per 1000 ft² per year), and at no traffic or soil compaction were 91-3, 91-15 and 91-4.

4. Under the most severe traffic regime (soil compaction with a power roller plus pressure/tearing on shoot tissues), 91-3 and 91-4 demonstrated improved traffic tolerance, regardless of N level.

5. Evapotranspiration averaged across 39 days in 1994 and 1995 revealed that cultivar differences were present. PRIMAVERA and 91-15 had 27% higher ET values than Arizona common.

6. Cultivars which extracted significantly greater water from the 21 to 60 cm soil zone than Arizona common during drydown periods were 91-1, 91-15, and PRIMAVERA.

**Characterization of Water Use Requirements and Gas Exchange of Buffalograss Turf**

**Dr. Daniel Bowman**

**University of Nevada**

**Goals:**
• To determine water use requirements of buffalograss.

• To examine genotypic variation in water use.

• To determine the effect of nitrogen fertilization on water use.

• To determine the relationship between photosynthesis and growth of buffalograss under drought stress.

Buffalograss may be the ideal species for both water savings and aesthetics in the central and southwestern U.S., but water use data are scarce and one can only speculate on water requirements. This two-year study generated crop coefficients for buffalograss and identified intraspecific water use differences among a diverse selection of genotypes.

The field project was installed at the University of Nevada - Reno Valley Road Field Station to determine water use requirements of seventeen buffalograss genotypes representing a diverse genetic background. This project utilized a line source water gradient in which buffalograss varieties were planted in strips along the gradient. Turf performance could be measured at any given irrigation amount, and minimum irrigation requirements were indicated the point in the gradient beyond which the turf goes dormant or cannot survive.

Mini-lysimeters (15 cm diameter, 30 cm depth) were planted, four per genotype, and established in the greenhouse. Cores for the lysimeters were drilled in each plot 2 meters from the main irrigation line. These were used to determine evapotranspiration (ET) gravimetrically under non-limiting conditions.

These lysimeters were used in a previous greenhouse experiment to determine intraspecific differences in water use rates. The results indicate that significant differences do exist in water use between buffalograss varieties, but the differences are relatively small. However, average water use rates are quite low (approximately half) in comparison to a similar experiment with tall fescue.

The line source irrigation gradient was established in July 1994 and June 1995, with the irrigation schedule for both years based on ET (modified Penman) as determined with weather station data. Data on ET under nonlimiting conditions, turf quality, canopy temperature, soil moisture, minimum water requirements, and plant water status were collected both years.

The data demonstrate significant differences among genotypes for water use and turf quality. Crop coefficients ranged from 0.76 to 1.02 in 1994 and from 0.60 to 0.92 in 1995. For both years, canopy temperatures were relatively unaffected by drought until the end of the experiment, and then only increased at the very outer edge of
the plots.

Over the course of the 31 day experiment in 1994, the point demarcating the minimum irrigation required to prevent total dormancy corresponded to approximately 10 to 20% of ET. During the 70 day experiment in 1995, the point demarcating the minimum irrigation required to prevent dormancy was approximately 40% of ET.

It is apparent from the two-year study that some genotypes of buffalograss can produce an acceptable turf with deficit irrigation of 50 to 60% of ET. Compared to a cool-season turfgrass species, one of the better performing genotypes of buffalograss used on golf course roughs could significantly reduce the irrigation needs of a golf course.

Turfgrass Irrigation with Municipal Effluent: Nitrogen Fate, Turf Kc Values and Water Requirements

Dr. Paul Brown

University of Arizona

Goals:

• Determine the potential movement of nitrogen contained in municipal secondarily treated wastewater used to irrigate turf.

• Determine how effluent irrigation influences the water and nitrogen requirements of turf.

• Evaluate five evapotranspiration equations currently used in the United States to predict actual turfgrass water use.

• Accumulate an atmospheric database and turfgrass water use database that can be used by the public and private sector to develop and test the accuracy of evapotranspiration equations.

Cooperators:

Dr. Charles Mancino
Dr. Thomas Thompson
Dr. Peter Wierenga
Two large weighing lysimeters located at the University of Arizona Karsten Turfgrass Research Center are being used to evaluate water use and movement of nitrogen (N) fertilizer under turfgrass irrigated with potable and effluent irrigation water.

Each lysimeter is 13 feet deep and 8 feet in diameter and weighs approximately 100,000 pounds. Truck scales are used to measure changes in lysimeter weight, thus allowing measurement of evapotranspiration (evaporation from vegetation). Sampling ports, located at a depth of 3.3 ft. and then every additional 1.6 ft. to a depth of 11.6 ft., provide access to the lysimeter soil for extraction of soil water and measurement of soil water status.

Turf water use is determined from daily changes in lysimeter weight and related to reference evapotranspiration (ETo) as computed by automated weather stations. This relationship between actual turf water use and ETo is known as a crop coefficient (Kc) and is required to convert ETo to turf water use for irrigation purposes.

Five popular methods of estimating ETo are presently under evaluation - the Penman Equations used by the four regional public weather networks (Arizona, California, New Mexico and Southern Nevada) and the Penman Montieth Equation. Results from the first year of study show the five methods of estimating ETo differ by as much as 20%, showing a clear need to match Kc with the method of ETo estimation. Appropriate Kc values for the five methods of estimating ETo varied from 0.74 to 0.91 during the bermudagrass season and from 0.72 to 0.90 for the winter ryegrass season.

A second portion of this study involves development of a database containing turf water use and meteorological data for use by public and private entities involved in providing turf water management information to the turf industry. Such a database would allow companies providing weather stations and/or irrigation scheduling software to develop Kc values and/or calibrate their procedures for estimating ETo. This database is presently in development and will include turf water use and most meteorological data used to estimate ETo.

Work on movement of N under the two irrigation regimes began with the bermudagrass season in April. One lysimeter was irrigated with effluent and the other with potable water. Nitrogen, applied as labeled (N\textsuperscript{15}O) ammonium sulfate, is applied to both lysimeters every two weeks. The rate of N applied to the lysimeter receiving effluent is adjusted downward to ensure both lysimeters receive similar levels of N.
Results from the first 40 days of evaluation reveal no movement of labeled N below the bermudagrass root zone. Low N concentrations in soil solution extracted from 3.3 ft. suggest very efficient N uptake by the bermudagrass. Higher N concentrations were observed in soil solution samples extracted from 6.6 ft. and likely reflect residual N from the previous winter turf season.

Work on both turfgrass water use and N movement will continue during calendar year 1996.

**Alternative Pest Management**

**Introduction**
The purpose of these research projects is to evaluate alternative methods of pest control for use in integrated turf management systems. Projects investigate alternative pest control methods that include:

- Biological control
- Non-chemical control, including cultural and mechanical practices
- Allelopathy
- Selection and breeding for pest resistance
- Ecological balance of plant species in turfgrass swards
- Application of integrated turf management practices utilizing IPM and low cultural inputs

**Recovery of*Rhizoctonia solani* Resistant Creeping Bentgrass Using the Host-Pathogen Interaction System**

**Dr. Jeffrey Krans Maria Tomaso-Peterson**

**Mississippi State University**

**Goals:**
• Recover Rhizoctonia solani selected variants of creeping bentgrass using the Host-Pathogen Interaction System (HPIS).

• Screen and grade recovered R. solani selected creeping bentgrass variants using an in vitro whole plant disease screening system.

• Establish a clonal repository of in vitro screened R. solani resistant creeping bentgrass variants.

• Verify whole plant resistance of in vitro screened variants using greenhouse studies for determining R. solani resistance.

• Select parents that exhibit resistance to R. solani in conjunction with other desirable turf characteristics.

• Evaluate progeny for resistance to R. solani.

The Host-Pathogen Interaction System (HPIS) is an in vitro cell selection system developed in conjunction with efforts to obtain creeping bentgrass with resistance to Rhizoctonia solani.

The primary objective throughout our USGA research projects has been to verify HPIS as a valid in vitro cell selection system. With this objective achieved, we can recover bentgrass germplasm from HPIS selections and evaluate those genotypes in the field, with confidence that some will segregate from the population and exhibit enhanced disease resistance.

The first step in achieving our primary objective was to obtain disease resistant bentgrass callus via HPIS selection. HPIS refinement studies associated with our initial USGA research project confirmed selection of resistant callus. Callus mortality increased significantly as PENNCROSS calli were cocultured in HPIS with a virulent isolate of R. solani. Small numbers of plantlets were regenerated from resistant callus compared to high numbers of plantlets recovered from control populations.

With bentgrass germplasm successfully regenerated from resistant calli, our USGA research project progressed from there to determine whether enhanced resistance could be exhibited at plantlet and whole plant levels. Plantlets were evaluated for tolerance to R. solani using two in vitro screening techniques:

1) HPIS Chamber - Plantlets, were placed in an HPIS Chamber, exposing them to R. solani for two weeks. More than 33% of the plantlets did not survive.
2) **Leaf Bioassy** - Leaves of plantlets were exposed to exudate produced by *R. solani*. Plantlets recovered from resistant callus displayed significantly less leaf necrosis as compared to control plantlets. Plantlets surviving both screening techniques were subsequently transferred to soil (whole plant) and maintained in a greenhouse.

Selected bentgrass plants [variant(s)] were inoculated with *R. solani* using growth chamber techniques and evaluated for disease response. Preliminary results indicated that some variants expressed enhanced resistance to *R. solani*. Based on these findings, variants were established in the field under putting green conditions. Establishment and turf quality data indicate the majority of variants are similar to or better than PENNCROSS. Concurrent with establishment, variants were rated for brown patch resistance based on natural infection. Preliminary observations indicate enhanced resistance may exist among some variants. Several plots displayed brown patch symptoms while adjacent plots had no symptoms. Bentgrass variants will be evaluated under various stress and environmental conditions through two successive brown patch seasons (2 years). Natural infection and field inoculations will occur under natural putting green conditions. Results from these evaluations will provide us the opportunity to

Callus is cultured on regeneration medium under continuous low light to induce plantlet regeneration. Bentgrass germplasm is recovered from callus that survived co-culture with confidently identify variants exhibiting enhanced resistance to brown patch. Selected variants will be used to improve existing creeping bentgrass gene pools by incorporating genes with enhanced *R. solani* resistance. A clonal repository of BPIS germplasm is maintained in an environmentally controlled greenhouse at Mississippi State University. To date, almost 200 genotypes are established under putting green conditions at locations in Mississippi and North Carolina.

HPIS refinement studies have confirmed HPIS as a valid *in vitro* cell selection tool, giving us confidence that some bentgrass variants will be identified as having enhanced resistance brown patch.

**Development of Genetically Engineered Creeping Bentgrass Resistant to Fungal Diseases**

**Dr. Sam Ha**

**Virginia Polytechnic Institute and State University**

**Goals:**
• Improve disease resistance of creeping bentgrass using a new genetic engineering approach.

• Introduce the chitinase gene into creeping bentgrass to develop varieties resistant to fungal diseases.

Chitinase is one of several anti-fungal proteins produced in plants upon fungal infection. This enzyme catalyzes the hydrolysis of chitin, a cell wall component of many fungal pathogens. It was shown that constitutive over expression of the chitinase gene in transgenic tobacco plants resulted in enhanced resistance to fungal diseases.

This project is designed to improve disease resistance of creeping bentgrass using genetic engineering. The objectives are 1) to develop an efficient gene transfer system in creeping bentgrass and 2) to develop genetically engineered creeping bentgrass that is resistant to fungal diseases through over expression of chitinase genes.

An efficient gene delivery system for creeping bentgrass was developed during this research project. A hygromycin resistance gene was transferred into embryogenic creeping bentgrass cells by particle bombardment, and transformed cells were selected on the medium containing 150 or 200 mg/L of hygromycin. A total of 124 transformed calli were obtained from 27 bombarded plates, with an average of 4.6 hygromycin-resistant colonies per bombardment. Thirteen transgenic plants were regenerated from the resistant colonies. Southern blot analysis confirmed the integration of the transgene into the genome of the transgenic plants.

The research program successfully isolated three genomic clones of chitinase genes (chi1, chi2, chi3) from Kentucky bluegrass using adaptor-ligation polymerase chain reaction (PCR). The chi1 and chi2 genes encode full length chitinases of 340 and 320 amino acids, respectively. The chi3 gene appears to encode a truncated chitinase (49 amino acids) due to the presence of a stop codon in the coding region.

Using reverse transcription and PCR, they found that both chi1 and chi2 were induced by ethylene, strongly indicating both genes were involved in plant defense responses.

Each of these two genes (chi1 and chi2) were subcloned into the expression vector (plasmid). The plasmids containing the Kentucky bluegrass chitinase genes were then transferred into creeping bentgrass calli by particle bombardment. Transformed calli have been selected on the medium containing hygromycin. Transgenic plants are now being regenerated from these calli. Once transgenic plants are developed, those which exhibit a high level of chitinase expression will be screened and tested for resistance to fungal pathogens.
Development of Improved Turfgrass with Herbicide Resistance and Disease Resistance Through Transformation

Dr. Peter Day

Rutgers University

Goals:

- Establish a transformation system for creeping bentgrass.
- Improve the utility of creeping bentgrass by incorporating genes to confer herbicide resistance or enhanced resistance to fungal pathogens.

Cooperators:

Dr. Lisa Lee
Dr. C. L. Hartman
Dr. N.E. Tumer
Dr. C. Laramore

This project seeks to improve creeping bentgrass through genetic transformation to provide golf course managers with more effective and selective weed control with herbicides and more environmentally sound and cost-effective control of plant diseases with reduced use of fungicides. We have accomplished several major goals:

1) Development of a creeping bentgrass tissue culture and regeneration system
2) Successful biolistic and protoplast transformations of creeping bentgrass
3) Recovery of several cultivars of creeping bentgrass with resistance to two different herbicides
4) Field tests of clones of a herbicide resistant creeping bentgrass
5) Turf quality (including disease resistance) study of herbicide-resistant transgenic creeping bentgrass
6) Progeny analysis of Finale-resistant creeping bentgrass is underway.

Good progress was made incorporating single gene traits for herbicide resistance and enhanced disease resistance in turfgrass. We have expanded the creeping bentgrass tissue culture and regeneration system from nine creeping bentgrass cultivars to several other elite cultivars including PENNCROSS, PENNEAGLE, CRENSHAW, and A-1. Transgenic creeping bentgrass clones of COBRA, EMERALD, and SOUTHSHORE have been obtained from particle and protoplast transformation with resistance to Finale
herbicide (bialaphos). Greenhouse and field tests of herbicide-resistant creeping bentgrass were conducted in 1994 and 1995. The screening tests indicate that the transgenic bentgrasses are resistant to five times the field rate in greenhouse herbicide spray tests and up to three times the recommended rate in field herbicide applications of Finale.

Transgenic creeping bentgrasses from the 1994 field test were vernalized in the field over the winter and returned to a containment greenhouse in the spring before flowering. At flowering, they were cross-pollinated with wild type plants. Seed was harvested and progeny analysis was performed in the fall of 1995 to determine the heritability of the transgene.

Four field tests of herbicide resistant creeping bentgrasses were conducted in 1995. The COBRA transgenic plants obtained from protoplast transformation showed a high level of herbicide tolerance, up to three times the recommended field rate of Finale, like the transgenic plants tested in the 1994 field test.

To enhance fungal disease resistance, we have performed greenhouse herbicide tests with putative transgenic plants that carry genes expressing bean chitinase, tobacco chitinase B and maize chitinase. These transgenic plants are now being tested for resistance to *Rhizoctonia solani* brown patch.

**Control of Bentgrass Pathogenic Fungi Dollar Spot, Brown Patch and Pythium Blight Using Chitinase**

**Dr. Joseph Vargas, Jr.**

**Michigan State University**

**Goals:**

- Express our cloned chitinase gene in coli, and purify and collect chitinase protein.
- Identify the level of chitinase required to control three major turfgrass pathogens.
- Transform bentgrass with plasmids containing the chitinase gene.
- Evaluate the transgenic plants for resistance to major turfgrass pathogenic fungi.

**Cooperators:**
Dr. Mariam Sticklen  
Dr. Bruce Branham

Dollar spot (*Sclerotinia homeocarpa*), brown patch (*Rhizoctonia solani*) and pythium blight (*Pythium aphanidermatum*) are major pathogenic diseases of turfgrass. All of these pathogens contain chitin in their cell walls, and therefore may be susceptible to chitinases. Also, all of these pathogens contain proteinases which are essential for the survival of the pathogenic fungi.

The research project has cloned and characterized a full length chitinase gene which contains the necessary chitin-binding domain. Several plasmids were constructed that contain a potato proteinase inhibitor 11 controlled by different (monocot-specific, wound-inducible, and constitutive) promoters.

During the first year of the project, the chitinase gene was successfully expressed in *E. coli*. The expression of this plant gene in *E. coli* was confirmed by extracting a chitinase-containing slurry. Dr. Vargas's laboratory examined the effect of the recombinant chitinase on turfgrass pathogenic fungus, *R. solani*. Due to the hydrophobic (water insoluble) nature of the expressed chitinase, the bioassay did not provide accurate results. Work is in progress to remove the hydrophobic portion of this gene, and express the modified gene in *E. coli* again.

The chitinase gene also was successfully expressed, in a homozygous state, in second generation tobacco plants. Tobacco is relatively quick and easy to transform. This transformation provides information about the plant's ability to produce active chitinase from this gene. If tobacco can successfully produce the chitinase with antifungal activity, it would indicate that bentgrass plants will likely do the same. Dr. Vargas's laboratory is testing these plants against *R. solani*. Work is in progress to express this chitinase gene in creeping bentgrass.

During the first year of the project, the potato proteinase inhibitor 11 and the bar (bialaphos -Ignite or Finale" herbicide resistance) genes were successfully expressed in creeping bentgrass. To date, thirty-seven independent groups of transgenic creeping bentgrass (with different sites of gene insertion) have been identified. Professor Donald Penner, the MSU herbicide physiologist, has confirmed the degree of resistance of these transgenic plants to bialaphos. Several of these independent transgenic plants have been transferred to the field. Further analysis against both Finale herbicide and turfgrass pathogenic fungi will be performed on these plants during Spring and Summer of 1996.
Identification of Parasitic Bacteria as Biological Control Agents Against Summer Patch Disease

Dr. Donald Kobayashi

Rutgers University

Goals:

- Isolate and identify bacteria which can colonize and parasitize the "mycelia" of Magnaporthe poae, the causal agent of summer patch disease.
- Screen isolated bacteria for disease control potential using controlled growth chamber and field studies.

Cooperator:

Dr. Bruce Clarke

A fungal trapping method and an enrichment culture method were used to isolate several hundred bacteria from turf and soil sources. These isolates were screened in a greenhouse/growth chamber assay for the suppression of summer patch disease, caused by Magnaporthe poae, on Kentucky bluegrass. At least eight bacterial isolates were identified that were capable of consistently suppressing summer patch symptom development by greater than 50% compared to ftmgal-inoculated control plants. All eight isolates expressed activity of one or more of the extracellular enzymes chitinase, glucanase, protease and lipase. In addition, all eight isolates were capable of colonizing and persisting in the rhizosphere of Kentucky bluegrass at significant populations.

Two bacterial strains, Xanthomonas maltophilia 34S I (Xm34SI) and Serratia marcescens 9M5, were capable of suppressing summer patch by greater than 70% and 50%, respectively, compared to disease in untreated control plants over a 3 week period. The rates at which disease progressed in plants treated with bacteria were not different compared to untreated plants; however, disease onset was significantly delayed in bacteria-treated plants. These results were interpreted to reflect the activity of antagonistic bacteria in reducing pathogen colonization of turfgrass roots. Disease onset was delayed for an extended period when Xm34SI was applied to plants on a repeated schedule. Rhizosphere populations of Xm34SI indicated that disease suppression was associated with populations between $> 10^5$ and $> 10^7$ cfu/g rhizosphere sample.
Xm34 S I was applied to pathogeninoculated field plots in 1994 and 1995. Summer patch suppression was not observed in field trials in either year. High disease pressures and insufficient population establishment of Xm34S I were attributed to the lack of performance in the field. Extensive field population studies in 1995 indicated that populations of Ym34S I were maintained above \(10^5\) cfu/g rhizosphere sample throughout the entire summer season, but did not reach populations above values of \(10^7\) cfu/g rhizosphere sample. These populations were clearly 10-fold lower than the critical population values established in growth chamber studies.

Genetic Basis of Biological Control in a Bacterium Antagonistic to Turfgrass Pathogens

Dr. Eric Nelson
Cornell University

Goals:

- Identify and clone DNA sequences that encode pathogen-suppressive properties in *Enterobacter cloacae*.

- Determine the nucleotide sequence of *E. cloacae* DNA encoding pathogen-suppressive properties and tentatively establish a function for the gene product.

- Evaluate, infield studies, the expression of the biocontrol-related gene, *pspl*, under typical turfgrass management conditions.

- Identify nucleotide sequences of *E. cloacae* DNA encoding for pathogen suppression.

The main goal of our project on *Enterobacter cloacae* genetics has been to identify the genetic determinants for biocontrol traits in *Enterobacter cloacae* so that their role in the suppression of Pythium incited diseases of turfgrasses can be specifically elucidated. Even though our focus has been on *Pythium-incited* diseases of creeping bentgrass, we believe our studies will have broad applicability to other bacterium-pathogen interactions.

Our studies in 1995 focussed primarily on the first two objectives. During work in 1994, we spent considerable time studying mutant V58, which was a biocontrol negative mutant deficient in malate dehydrogenase activity. We further isolated other mutants lacking significant levels of biological control activity. One such mutant, 21 - 1, was the
focus of our studies in 1995. These studies were concerned with establishing the role of fatty acid metabolism in biological control.

The parent strain of Enterobacter cloacae, strain EcCT-501, suppresses several different Pythium species, including P. ultimum, P. aphanidermatum, and P. graminicola, on creeping bentgrass. Furthermore, this strain inactivates the stimulatory activity of creeping bentgrass seed exudate, as well as the exudate of many other crop plants, thus preventing responses of these Pythitim species to plants. With P. ultimum in particular, sporangium germination is greatly reduced in the presence of strain EXT-501. As a result, many of our studies focussed on interactions with P. ultimum on creeping bentgrass.

![Graph showing the inactivation of creeping bentgrass seed exudate and linoleic acid solutions by Enterobacter cloacae strain 1.](image)

From among all exudate components, long chain fatty acids (LCFA) are important stimulants of sporangium germination. Our work in 1995 centered on initial attempts to examine the role of LCFA catabolism in the expression of biological control properties in E. cloacae. Strain EcCt501 reduced the stimulatory activity of the LCFA, linoleic acid., the most abundant LCFA found in creeping bentgrass and other plant seed exudates. A series of TnphoA mutants (Kan') were screened for growth on linoleic acid as a sole carbon and energy source. One out of 5000 Kan' colonies was deficient in the ability to inactivate the stimulatory activity of creeping bentgrass seed exudate and linoleic acid to P. ultimum sporangium germination. Furthermore, this mutant, 21-1, no longer protected creeping bentgrass from Pythium seed and seedling disease. A cosmid, PKVI, mobilized into mutant 21-1, complemented the linoleic acid catabolic deficiencies and restored the ability to inactivate creeping bentgrass seed exudate stimulatory activity. Furthermore,
this clone fully restored biological control properties to wild-type levels. Current evidence suggests a role of fatty acid metabolism in biological control properties in *Enterobacter cloacae*.

Cultural Control, Risk Assessment, and Environmentally Responsible Management of White Grubs and Cutworms

Dr. Daniel Potter

University of Kentucky

Goals:

- Determine factors that affect the distribution and abundance of white grubs and cutworms on golf courses.

- Reduce the use of insecticides by identifying methods to reduce white grub and cutworm insects through modified cultural practices.

- Provide better information on the effects of pesticides on natural enemies of turfgrass pests and other beneficial species that live in golf course turf.

Cooperators:

*Dr. A.J Powell*

*Dr. K.F. Haynes*

*B.A. Crutchfield*

*R. C. Williamson*

Cultural practices were manipulated to determine effects on densities of Japanese beetle and masked chafer grubs. High mowing throughout the summer, or application of aluminum sulfate just before beetle flights reduced subsequent densities of grubs by as much as 48 and 77%, respectively. Beetles were attracted to irrigated turf for egg-laying, resulting in 2- to 4-fold increases in grub densities in irrigated plots. Liming, fertilization with urea, heavy rolling, and aerification had no effect on white grubs during this 4-year study.

Fertilization with composted cow manure or activated sewage sludge [Milorganite] may result in higher populations of green June beetle grubs. Eggs of black cutworms were laid singly on the tips of bentgrass leaf blades. Mowing at 1/8” or 3/16” was shown
to remove nearly all of the eggs laid on bentgrass greens. The mower roller itself did not
dislodge eggs from grass blades. This suggests that cutworm infestations may originate
from larger larvae that migrate onto greens from aprons or roughs. Cutworm larvae were
observed to crawl as far as 75 feet in one night. More than half of the eggs on clippings
collected from mower baskets hatched into healthy larvae. These tests suggest that
disposal of clippings away from greens or tees may eliminate one source of infestation.
Most cutworm activity on golf greens occurred from midnight until just before dawn,
suggesting that control measures would be most effective if applied in the early evening
or at night. Young larvae tended to feed on the turf surface, while older larvae fed mostly
from burrows. About 13% of the cutworms collected in late July were fatally infected
with parasitic flies or wasps. This is the first documentation of parasitism of cutworms on
golf courses. Cutworms showed no preference between aerified and nonaerified areas,
but our results suggest that they may be repelled by sand topdressing. Female black
cutworm moths preferred creeping bentgrass over other grasses for egg laying.

Research continued on how long it takes for populations of predators, earthworms,
and other beneficial species to return to normal levels following an insecticide treatment.
Ethoprop (Mocap) applied in April resulted in 100% kill of earthworms. Populations had
still not fully recovered after 30 weeks. Several important groups of predators were
unaffected, while others were more sensitive to the insecticide. Comparative work on
effects of two important new insecticides (imidacloprid [Merit], and RH-0345 [an insect
growth regulator]) on the turfgrass ecosystem was begun in 1995.

The fraction containing the chemical sex pheromone of masked chafers was
pinpointed by gas chromatography and electroantennogram/behavioral analysis. The
active compound was characterized by infrared and mass spectroscopy. Identification of
the pheromone is expected soon. Synthesis of this attractant will provide means for
monitoring these pests on golf courses and home lawns.

Behavioral Studies of the Southern and Tawny Mole Cricket

Dr. Rick L. Brandenburg
North Carolina State University

Dr. Michael G. Villani
NY State Agricultural Experiment Station
Geneva, NY

Goals:
• Isolate and determine the activity of sex, aggregation and alarm pheromones of the tawny and Southern mole crickets.

• Improve our understanding of environmental conditions that affect tawny and southern mole cricket behavior.

• Compare the activity of healthy crickets to those infected with microbial control agents.

Previous studies funded by the USGA suggested the possible existence of a sex attractant in tawny mole crickets. This information has not been published in the scientific literature to date. To explore this possibility, tawny mole cricket adults and nymphs were collected in North Carolina and transported to the NYSAES, Geneva, New York for laboratory analysis.

At the present time, air-borne samples are collected from isolated virgin male, virgin female, mated male and mated female tawny mole crickets. These various samples are being used to determine if specific compounds are being released into the air that cause attraction in crickets of the opposite sex.

If active crude pheromones are detected through behavioral and electroantennagram assays, then active fractionation and synthesis will proceed through the winter and spring of 1996. Field testing is anticipated in North Carolina during the fall of 1996.

The biological activity of alarm pheromone in southern mole cricket is under evaluation. It was noted in the first year progress report that when disturbed, both mole cricket species discharged an oily, highly odorous substance from their abdomen. Discharges were collected for biological and chemical assays in our laboratory.

A small discharge sample from each cricket species was prepared for analysis through the use of gas chromatography. There were also clear differences in the Southern and tawny mole cricket discharges. This could indicate the existence of unique compound constituents in the discharges for these two species.

During the second year, radiographic bioassays were conducted that indicated that tawny mole crickets would avoid southern mole cricket discharge incorporated into soil. Radiographic studies also determined that when two highly predacious southern mole crickets were placed in large soil arenas, the crickets would space themselves in the arenas to allow no contact between them. This further suggests a chemically mediated alarm or identification pheromone that reduces chance encounters in complex soil systems, thereby reducing aggression between conspecific predators. Continued research will focus on the isolation and identification of these compounds.
The effect of the soil environment on mole cricket behavior also is under investigation. Extensive studies outlined in the previous progress report detailed the typical foraging behavior of tawny mole crickets and host-finding behavior of the southern mole cricket. We suggested that the "Y" shaped burrow of the tawny mole cricket aided in predation avoidance and water and temperature regulation.

Studies in 1995 focused on the impact of soil stratification and compaction on mole cricket construction. These studies indicated that soil texture and stratification can significantly modify tunnel construction, thereby affecting on the ability of these mole crickets to escape adverse environmental conditions. These studies will be continued and expanded during 1996.

The impact of biological and chemical control agents on mole cricket behavior is being evaluated. Field studies conducted during 1995 by Dr. Brandenburg suggested that biological and chemical insecticides may alter the behavior of mole crickets, thereby affecting the performance of these agents in the field. Preliminary radiographic assays with one synthetic insecticide suggests that tawny mole crickets can sense and avoid high concentrations of the product in the soil. This behavior may ultimately reduce the overall effectiveness of the insecticide.

Radiographic experimental designs, where crickets could not escape the insecticide, suggested a decline in burrow construction and maintenance. Although interesting, this work must be expanded and verified in 1996. Additional studies on the effects of fungal pathogens and entomogenous nematodes are in progress and will be continued during 1996.

Allelopathy vs. Acremonium Endophytes vs. Competition Effect On Crabgrass Suppression by 12 Perennial Ryegrasses

Dr. John King

University of Arkansas

Goals:

- Conduct Lemna bioassays for allelopathic effects from leaf-stem and root tissue extracts from field grown plants.

- Conduct crabgrass seedling bioassays by overseeding crabgrass into the field plots.
• Evaluate crabgrass suppression by overseeding the perennial ryegrass cultivars into a common bermudagrass lawn area and overseeding with crabgrass.

• Conduct crabgrass seedling bioassays by overseeding crabgrass into petri dishes containing the surface 1 cm of soil from a 5 cm diameter plug.

• Determine Acremonium endophyte content of field grown plant stems.

• Determine Acremonium endophyte contribution to allelopathy in the cultivar(s) showing strong allelopathic effects in the bioassays.

Twelve (12) perennial ryegrasses, which range from moderate to high stand density and zero to 95 percent endophyte infection, were selected and six replications of field plots were planted in late October, 1993. The cultivars and their expected percent endophyte infection are LORETTA (0), GATOR (0), DERBY (5-10), DERBY SUPREME (40-45), ENVY (40), OMEGA11 (76), MANHATTAN 11 (50-90), SATURN (80), SR4200 (80-85), BRIGHTSTAR (90), ASSURE (95), and YORKTOWN 111 (97).

Our basic laboratory evaluation for allelopathy is the *Lemna minor* L. (duckweed) bioassay. The *Lemna* bioassay measures allelopathic effects of extracts of plant tissues against the growth rate of duckweed fronds. Extracts from shoots are applied to duckweed cell plates at three concentrations. The amount of allelopathic inhibition (or stimulation) of duckweed varies with season of shoot tissue sample collection and extract concentration. All cultivars have affected duckweed growth.

We are still working to refine a ryegrass extract-agar crabgrass seed bioassay. Tissue extracts are added to agar in the cell plates; then crabgrass seeds are placed on the agar and seedling germination and development are measured. Procedures to stabilize crabgrass germination rate in controls are still being worked on. Early results suggest that extracts can inhibit germination directly and/or cause yellowish seedlings that don't live long. The perennial ryegrass overseeding into common bermudagrass fairway test was initiated in the summer of 1994 by preparing an area with weed control, fertilizing and mowing at 3/4 inch height. The 12 perennial ryegrasses were overseeded at 60 g/1.5 x 1.5m plot after vertical grooving on October 25. Crabgrass was overseeded into the east half of each plot after spiking on March 30, 1995, and benefin pre-emergence was applied to the west half of each plot.

Visual estimates of percent winter broadleaf weeds were taken in February, March, April and May. The mean broadleaf weed cover increased to only 2.4 percent, with a range of 0 to 5 percent in April and decreasing to nearly zero in May. The differences were not statistically significant. Adjacent non-overseeded bermudagrass plots had 23 percent broadleaf weeds in May. Overseeding, of course, reduced winter weeds.
Visual estimates of percent ryegrass cover were made in February (82%), March (86%), April (98%), May (97%), June (59%) and in July in the east (16%) and west (23%) halves of the plots (mean percentage in parentheses). The differences among cultivars were significant only in March. LORETTA and MANHATTAN 11 were highest with 91 percent and BRIGHTSTAR was lowest with 76 percent cover and statistical overlap was abundant. By May it was possible to distinguish bermuda by making estimates in the morning when dew was on the turf. The mean percentage of bermudagrass cover was 3 percent in May, 41 percent June, and, in July, 63 percent in the east half and 76 percent in the west half.

Crabgrass seedlings were not discernable in May or early June. The east half of the plots where crabgrass had been overseeded had a mean of 21 percent crabgrass with a range of 5 to 40 percent in mid-July. The west half of the plots where pre-emergence herbicide had been applied did not have crabgrass.

Differences due to ryegrass cultivars in crabgrass by percent cover estimates and stem counts per 4-inch diameter plug were not statistically significant. Thus any differences in allelochemical content of the 12 perennial ryegrass cultivars selected for this investigation were not great enough to produce practical field differences in crabgrass suppression.

The NTEP 1994 Perennial Ryegrass Test was undertaken as an adjunct to our allelopathy studies. It was planted in the fall of 1994, fertilized well *in the fall and late winter and mowed at a 3 1/2 inch height. On April 1, 1995 a 21-inch strip on the western edge of the plots was spiked, overseeded with crabgrass and kept mowed at a 3/4 inch height.

The visual estimate of percent crabgrass cover in the 99 perennial ryegrass cultivars was taken on July 24. Although statistical overlap was abundant, APM and TOPHAT plots had only 8 percent crabgrass while Linn had 45 percent. Whether these differences are due to allelopathy and/or density cannot be determined by this test, but clearly Linn was the least dense and APM and TOPHAT were among the more dense cultivars.

_Pasteuria_ sp. for Biological Control of the Sting Nematode, _Belonolaimus longicaudatus_), in Turfgrass

Dr. Robin Giblin-Davis

University of Florida


**Goals:**

- Examine bacteria ultrastructure with transmission electron microscopy and begin describing a new species of Pasteuria that we have discovered parasitizing the sting nematode, Belonolaimus longicaudatus.

- Perform host range studies on this new Pasteuria sp.

- Begin studies to elucidate the population dynamics of this new Pasteuria sp. on sting nematode grown on St. Augustinegrass in laboratory pot cultures under controlled conditions.

This research project is describing a new species of bacterium in the genus Pasteuria that was discovered parasitizing the sting nematode, Belonolaimus longicaudatus in Florida. They are hopeful that this obligate bacterial parasite of nematodes [Pasteuria n. sp. (S-1)] will have some potential for inoculative biological control in golf course greens against the sting nematode; a destructive ectoparasite that can reduce the root dry weight of turfgrasses and other crops in sandy soils by as much as 30 to 50 percent.

In 1995, ultrastructural studies of the bacterium were completed with transmission electron microscopy (TEM) and lowtemperature scanning electron microscopy (SEM) that show Pasteuria n. sp. (S-1) is a new species. These studies have helped to finish elucidating the development and life cycle of this bacterium. Excellent photomicrographs illustrating all aspects of the biology of Pasteuria n. sp. (S-1) have been produced by the project. Use of the new technique of low temperature SEM has helped to visualize spore morphology outside and inside the infected nematodes without the usual artifacts associated with TEM.

A second population of this bacterium was isolated from a golf course in Gainesville, FL. TEM is being used to confirm that it is ultrastructurally similar to Pasteuria n. sp. (S-1). This will give Dr. Giblin-Davis a better idea of the possible distribution of this bacterium within Florida.

A population dynamics study (390 days long) was completed on Pasteuria n. sp. (S-1) in laboratory pot cultures of the sting nematode on the model turfgrass host (FX313 St. Augustinegrass) under controlled conditions. There were four treatments: 1) no sting nematodes with no bacteria, 2) sting nematodes (99 + 10) with no bacteria, 3) sting nematodes (99 + 10) plus 10 sting nematodes encumbered with 8 ± 6 spores of Pasteuria n. sp. (S-1), and 4) sting nematodes (99 + 10) plus 25 sting nematodes encumbered with 8 ± 6 spores of Pasteuria n. sp. (S-1). The assumption was that inoculated sick nematodes would not add to the population growth of the healthy sting nematodes but would die and release bacteria that would negatively affect the healthy population. Unfortunately, the results demonstrate that this was not the case.
The population of the healthy sting nematodes were increased by the addition of "sick" nematodes, suggesting that spore encumbrance is not a good indicator of spore production or nematode health. Root dry weights for the different treatments confirmed that the greatest root loss occurred in the treatments with the most nematodes. Although spore encumbered sting nematodes were recovered throughout most of the 390-day study, the levels were never greater than one percent from treatments receiving spores. This suggests that inoculative release of "sick" nematodes will be an unacceptable method for establishment and population suppression work.

In 1995, a monthly survey of 6 different sites of hybrid bermudagrass (fairway conditions) at the Ft. Lauderdale Research and Education Center where Pasteuria n. sp. (S-1) occurs naturally at different levels was initiated to monitor its suppressive effects on sting nematodes at three different soil depths. Soil temperature was also monitored at these different depths. After 6 months of sampling, locations that started with low levels of spore encumbrance had higher numbers of sting nematodes than areas that started with high encumbrance levels. These results suggest that Pasteuria n. sp. (S-1) might help produce suppressive soil for the sting nematode. This new information is encouraging but the survey work will require at least one year before any conclusions can be made.

**Best Management Practices**

**Introduction**

Golf courses provide beautiful green areas within our urban and suburban landscapes. However, there is public concern about the possible effects of golf courses on the environment. In response to this concern, the USGA completed a three-year research program in 1994 that examined the degradation and fate of turfgrass chemicals, as well as the development of alternative pest control methods and documentation of the turfgrass and golf courses benefits to humans, wildlife and the environment.

As a continuation of a responsible and scientifically-based investigation of the environmental impact of golf courses, the USGA is sponsoring further research to understand the effects of turfgrass pest management and fertilization on water quality and the environment. To achieve this goal, three-year research projects were initiated in 1995 to focus on *Best Management Practices* and *Pesticide and Nutrient Fate*.

The purpose of the Best Management Practices research is to demonstrate that pesticides and fertilizers can be applied to golf course turfs while having a negligible effect on environmental quality. The current research projects focus on:
1. Evaluating the effects of specific pesticides and nutrients that have a perceived environmental problem; and

2. Identifying cultural practice systems that minimize pesticide and nutrient volatilization, surface runoff, and groundwater contamination.

Evaluation of Best Management Practices to Protect Surface Water Quality from Pesticides and Fertilizer Applied to Bermudagrass Fairways

Dr. James H. Baird

Oklahoma State University

Goals:

• Develop effective and practical management practices that protect surface water from runoff of pesticides and fertilizer applied to golf course fairways and other turf areas

Cooperators:

James Cole
Dr. Raymond Huhnke
Dr. Nicholas Basta
Dr. Gordon Johnson
Dr. Daniel Storm
Dr. Mark Payton
Dr. Michael Smolen
Dr. Dennis Martin

A portable rainfall simulator is being used to simulate heavy precipitation events that may occur shortly after the application of pesticides and fertilizer, thus increasing the likelihood of water contamination from surface runoff. The simulator is capable of applying rainfall intensities of up to 5 inches per hour onto four plots, each measuring 6 ft by 32 ft. In 1995, a preliminary study was conducted to evaluate the effectiveness of various combinations of buffer-strip: 1) length (0 vs. 8 ft. vs. 16 ft); 2) mowing height (0.5 inches vs. 1.5 inches); and 3) aerification (solid-tine aerification vs. no aerification) in reducing pesticide and nutrient runoff.

In July, an experiment was conducted at a location in Stillwater, OK consisting of common bermudagrass maintained under golf course fairway conditions. Within 24 hours
prior to a simulated rainfall event, 2,4-D, mecoprop, and dicamba (formulated as Trimec Classic), chlorpyrifos (0.5G), nitrogen (urea) and phosphorus (triple superphosphate) were applied at normal rates recommended for fairway turf to designated areas on plots containing the buffer treatments. One of the treatments, containing no buffer-strip, was left untreated to determine the amount, if any, of pesticides and nutrients already present in the turf environment.

The experimental design was an unbalanced, randomized incomplete block with eight treatments and four replications. The design insured that important treatment comparisons showed up in the same simulator set-up (block) at least twice.

The experiment was repeated in August, whereupon the untreated control was substituted with a treatment consisting of no buffer-strip and application of identical rates of the 50WP formulation of chlorpyrifos and the sulfur-coated-urea form of nitrogen fertilizer, in addition to identical rates and formulations of the herbicides and phosphorus.

Soil moisture conditions prior to the July and August simulated rainfall events were significantly different and affected the volume of runoff from plots and the total amount of pesticides and nutrients recovered. In the July run, no natural precipitation was detected within 12 days of simulated rainfall; by contrast, 6.5 inches of natural precipitation fell on the runoff site within 6 days of the simulated rainfall in August.

In July, percent recovery of pesticides and nutrients was less than 3% and 2%, respectively, based upon the total amount applied. The highest levels of nutrients and pesticides were recovered from the treatment containing no buffer-strip. In August, percent recovery of pesticides and nutrients was as great as 15% and 11%, respectively.

Results from the July run indicated that buffer-strips were very effective in reducing pesticide and nutrient runoff. Although few treatment comparisons were statistically significant, numerical trends from the July data showed reduced pesticide and nutrient runoff from the 16-ft buffer length compared to the 8-ft buffer length, the 1.5-in mowing height compared to the 0.5-in mowing height, and solid-tine aerification compared to no aerification at the 0.5-in mowing height. At the 1.5-in mowing height, aerification resulted in greater pesticide and nutrient runoff. It is possible that the aerification process created channels in the higher-cut turf canopy, thus expediting movement of the chemicals in surface runoff.

In August, several of the trends observed in July were reversed, possibly indicating that the effectiveness of the buffer-strip treatments was overcome by the increased volume of surface runoff. Reduced pesticide and nutrient runoff occurred from the wettable powder formulation of chlorpyrifos compared to the granular formulation, and from the sulfur-coated urea form of nitrogen compared to urea. The correlation between
the physical and chemical properties of pesticides and nutrients and their relative runoff potential was substantiated by this investigation.

Based upon the 1995 preliminary study, the following management practices are recommended to reduce pesticide and nutrient runoff. 1) incorporate a buffer-strip between surface water features and treated areas; 2) avoid application of pesticides and fertilizer when high soil moisture conditions exist; and 3) develop pest and nutrient management programs that utilize pesticide and fertilizer formulations with low runoff potential.

Evaluation of Management Factors Affecting Volatile Loss and Dislodgeable Foliar Residues

Dr. John M. Clark

University of Massachusetts

Goals:

- The role of vapor pressure and temperature will be evaluated in terms of developing a screening system for turfgrass pesticides
- Pesticides with possible safety concerns will be further evaluated in the context of best management practices, including the role of spray volume and adjuvants.
- The role of thatch accumulation on the dissipation of volatile and dislodgeable residues will be assessed.

Cooperators:

Dr. R. Cooper, NC State University
Dr. D. Haith, Cornell University

Volatile and dislodgeable samples and weather data have been collected for the completion of the first objective, "Development of a best management system for screening turfgrass pesticides for potential volatiles and dislodgeable foliar residues." To date, 450 samples and weather data were collected from June to November, 1995. Samples have been concentrated and stored in a freezer for analysis that is currently being carried out.
Analytical methods have been developed that allow multiple residues to be
determined in each of three groups of pesticides that are applied together: Group I
(Diaxion, Ethoprop, Chlorpyrifos, Isazofos, Isofenphos); Group 2 (Trichofon, DDVP,
Carbaryl, Bendiocarb, Cyfluthrin); Group 3 (Chlorothalonil, Propiconizol, Iprodione,
Thiophanate methyl).

Two additional 10-meter radius turfgrass plots were established in September in order
to complete Objective 3 in the third year of funding of the current proposal.

Dr. D. Haith, Cornell University, has been brought onto the project to provide his
expertise in developing an algorithm relating HQs, volatility, temperature, and relative
humidity for use as a "best management" tool for superintendents in the proper selection
of pesticides to avoid golfer exposure.

**Pesticide and Nutrient Fate**

**Introduction**

Understanding and quantifying the fate of applied turfgrass pesticides and fertilizers
are required for accurate prediction or simulation of the environmental impacts of golf
courses. From 1991 through 1994, USGA-sponsored research demonstrated that
measured nitrogen and pesticide leaching generally is minimal, the turf/soil ecosystem
enhances pesticide degradation, and the current agricultural models are inadequate for
predicting the fate of pesticides and fertilizers applied to turfgrasses grown under golf
course conditions. The purpose of these projects is to further evaluate the mobility and
persistence of parent compounds and transformation products of commonly applied
pesticides and fertilizers. Research results from these projects will provide information
on:

- Degradation and volatilization rates for commonly used pesticides in several
  important turfgrass environments

- Identification of conditions that enhance microbial degradation

- Adsorption coefficients for organic and inorganic materials as a function of residence
time in the turfgrass environment
A mass balance assessment of the fate of applied pesticides that takes into account the initial distribution among turfgrass plant, volatilization, soil, water, runoff, and leachate.

Nitrogen fertilizer studies include research to assess the importance of factors which influence volatilization, denitrification, mobilization, immobilization, adsorption, plant uptake, and fixation, as well as assess loss by surface runoff and leaching. Similar experimental conditions and research techniques are being used to determine the fate of phosphorous in the turfgrass environment.

Modeling Pesticide Transport in Turfgrass Thatch and Foliage

Dr. Mark Carroll
University of Maryland

Goals:

- To quantify the washoff of pesticides from bentgrass foliage as a function of time after application and pesticide formulation.
- To determine the effect of solution residence time on the sorption of pesticides to turfgrass thatch.
- To determine if the linear equilibrium form of convection/dispersion equation is able to provide accurate estimates of pesticide transport in turf.

Pesticides applied to mature turf move into the soil only after being washed off foliage and moving through turfgrass thatch. Attempts to predict the movement of pesticides applied to turf require that the retention characteristics of the pesticide to foliage and the sorptive properties of the pesticide to thatch be known.

Pesticide movement from foliage to underlying porus media layers is usually modeled using foliar washoff algorithms. The use of foliar washoff algorithms requires accurate estimates of the fraction of applied pesticide that is deposited on the foliage, and of the fraction of pesticide that is removed from the foliage as a function of rainfall amount. In the case of the latter, the amount of time elapsed between pesticide application and the first rainfall event can significantly affect the fraction of pesticide removed from the foliage.
In the summer of 1995, dicamba, carbaryl and three formulations of chlorothalonil were applied to creeping bentgrass maintained at a cutting height of 5/8 inches. One, 8, 24, or 72 hours after pesticide application, 1.2 to 1.3 inches of rainfall was applied using a rainfall simulator. Foliage samples were collected immediately before and after simulated rainfall. The foliage samples are currently being analyzed to determine the effect of pesticide formulation and residence time for the washoff of pesticides from creeping bentgrass foliage.

Many pesticide transport models, such as PRZM2 and LEACHM, use the linear equilibrium form of the convection-dispersion equation to predict pesticide movement in porous media. A major assumption inherent in the use of this form of the convection-dispersion equation is that the residence time of solution containing the pesticide is of sufficient duration that sorption equilibrium between the solution and porous media is achieved. It has been hypothesized that turfgrass thatch differs from soil in that it exhibits non-equilibrium pesticide sorption. In such cases, pesticide movement within the media may be predicted with greater accuracy when a nonequilibrium form of the convection-dispersion equation is used to model pesticide transport.

In the summer of 1995, Ms. Sanju Raturi, a Ph.D candidate in the Agronomy Department, began to conduct a series of studies to determine the sorption kinetics of 2,4-D, carbaryl and chlorothalonil to creeping bentgrass and zoysiagrass thatch. Ms. Raturi will use the sorption information to evaluate the performance of linear equilibrium, one-site kinetic non-equilibrium, and two-site kinetic non-equilibrium solute transport models to predict pesticide movement in thatch/soil profiles.

Measurement and Model Prediction of Pesticide Partitioning in Field-Scale Turfgrass

Dr. Marylynn Yates
Dr. Robert Green

University of California

Goals:

- Determine the partitioning of commonly-used turfgrass pesticides among the components of a turfgrass system including the atmosphere, soil, soil-water, leachate, thatch, verdure, and clippings.

- Assess the ability of mathematical models, such as CHAIN 2D and PRZM2, to accurately predict pesticide movement in a field-plot-scale turfgrass system.
• Modify the mathematical model and/or change tile data collection protocol as necessary to improve the accuracy of model predictions.

• Test the model using independently-derived data to assess further its predictive capabilities.

• Conduct a sensitivity analysis of the mathematical model to determine which input parameters have the greatest effect on tile model predictions and therefore should be known to the highest degree of accuracy.

Concern over environmental contamination by pesticides has become widespread during the last several years. The United States Environmental Protection Agency has established mandatory standards for several pesticides, including 2,4-D, glyphosate, and atrazine, in drinking water. In addition, several states have established regulations to limit further environmental contamination by pesticides.

In California, pesticides that are detected in groundwater or have potential to leach to groundwater are regulated by the Department of Pesticide Regulations (DPR). Recently, the California DPR proposed that several pesticides be designated as toxic air contaminants. This list includes several compounds commonly used on turfgrass, including carbaryl (Sevin), 2,4-D, mancozeb, maneb, and trifluralin (Treflan).

Previous USGA-funded research at the University of California, Riverside (UCR) indicated that less than 0.1% of the applied carbaryl was lost by volatilization and leaching through the putting green plots. More of the applied 2,4-D could be accounted for: approximately 1% volatilized into the atmosphere, and approximately 5% leached through the soil. However, in both cases, more than 90% of the applied compound was not accounted for. In this project, we are performing more detailed analysis of the fate of pesticides in field plots to enable a determination of the mass balance.

A second area of concern after the first three-year cycle of environmental fate research was the need to be able to predict ground-water concentrations of pesticides. It is usually not feasible to monitor ground water for the pesticides of concern, so measurements of pesticide concentrations in the near-surface soil and soil water are made. Mathematical models are then used to predict the concentration of pesticides that one might expect at deeper points in the subsurface. Smith and Bridges (1993) attempted to predict pesticide movement through their greenhouse lysimeters using the GLEAMS (Groundwater Loading Effects of Agricultural Management Systems). They found that the model significantly overpredicted the amount pesticides that would leach through the soil even when a thatch layer was included in the model.
There are several possible explanations for the model's predictions failing to match experimental data. One is that this model is very simple from a hydrologic standpoint: it assumes that when water is applied to the soil surface, it uniformly displaces an equal volume of water from the underlying soil (the so-called "tipping bucket" model). It is also a one-dimensional model in that it assumes that the water and pesticides are moving in one dimension. This model is classified as a functional, management-level model because it incorporates certain simplifications in the subsurface processes that reduce the requirement for input data. One advantage of a model such as GLEAMS is that it does not require massive amounts of difficult-to-acquire input data. The subsurface processes, and their ability to accurately predict chemical movement may be decreased. There is a need to investigate the ability of other more sophisticated, albeit more data-intensive models, to predict chemical movement through turfgrass-soil systems.

### Mobility and Persistence of Turfgrass Pesticides in a USGA Green

**Dr. George H. Snyder**  
**Dr. John L. Cisar**  
**University of Florida, WAS**

**Goals:**

- Conduct mobility (leaching and dislodgeability) and persistence studies on pesticides not examined in previous work.

- Monitor percolate collected on a golf course site for applied pesticides

- Quantify volatilization of certain pesticides applied to golf turf.

- Develop and document the results of using best management practices (BMPs) for fenamiphos and other pesticides that appear to have appreciable mobility, including evaluation of pesticide adsorbing amendments.

The mobility and persistence of the phenoxy-acid herbicides dicamba and 2,4-D were investigated in two studies conducted on a USGA green at the Fort Lauderdale Research and Education Center that is outfitted with lysimeters for collecting percolate water.

In each study, the herbicides were applied twice at one-week intervals at 58 and 6 mg active ingredient (a.i.) per square meter for 2,4-D and dicamba, respectively. Although
the dicamba application rate was only 10% that of 2,4-D, the recovery of these materials (expressed as mass) in percolate water was of the same order of magnitude, being approximately 10% of that applied for dicamba and 1% for 2,4-D. Detectable levels of both herbicides were observed in thatch and soil for several months. Very little (≤ 0.25%) was recovered in clippings.

In previous studies, fenamiphos, and especially its sulfoxide and sulfone metabolites, were sufficiently mobile to be observed in percolate waters. Three approaches were investigated to reduce leaching and/or improve efficacy of fenamiphos: 1) irrigation management, 2) surfactants, and 3) adsorbents.

Fenamiphos leaching in plots receiving twice-weekly irrigations during the week following application was less than 2% over a three-week period of that observed in plots receiving daily irrigations. Fenamiphos metabolite leaching also was dramatically reduced by restricting irrigation, except in the samples collected following a 72 min rainfall. This event occurred two weeks after the fenamiphos application and the metabolite found in leachate from reduced-irrigation plots exceeded that found in plots receiving daily irrigation. Over a three week period, metabolite leaching was approximately the same in the two irrigation treatments.

Thatch strongly adsorbs fenamiphos, but nematodes are most prevalent in the underlying soil. In field studies, surfactant applied to the soil one month before fenamiphos, the same day as fenamiphos, or with the fenamiphos did not greatly increase the movement of fenamiphos through the thatch into the soil. None of the surfactant treatments enhanced the control nematodes.

A method was developed for rapidly screening surfactants in the laboratory for their ability to reduce fenamiphos adsorption by thatch in order to identify candidate surfactants for future field trials. A zeolite soil amendment was evaluated for reducing fenamiphos and fenamiphos metabolite leaching in sand columns. The zeolite reduced saturated hydraulic conductivity by 94% and allowed 42 and 75% of the fenamiphos and metabolite to leach.

Potential Movement of Certain Pesticides Following Application to Golf Courses

Dr. Albert E. Smith
Dr. David C. Bridges

University of Georgia
Goals:

- To obtain and develop mathematical equations for predicting the potential movement of pesticides through golf course greens constructed according to USGA specifications.

- To determine the potential runoff movement of pesticides from golf course fairways on Piedmont soils and to develop management strategies for reducing the movement.

Funding provided by USGA for the previous project (1991-1994) resulted in the development of facilities at the Georgia Experiment Station and the initiation of a research program to determine the potential movement of pesticides following application to golf courses.

Data indicated that only small quantities (<1%) of the applied 2,4-D DMA, dicamba DMA, mecoprop DMA, dithiopyr, chlorpyrifos, and chlorothalonil are transported through the sod and the lysimeters. In summary, it appeared that the dynamics of a well maintained sod contribute to a high adsorption and decomposition rate for these pesticides, and the GLEAMS model, developed for agricultural row-crops, underestimates the dynamics of the ecosystem around the sod.

Research has been continued to document movement of additional pesticides through the lysimeters and to resolve the differences between the GLEAMS model prediction of analyte transport and data obtained from the lysimeters.

Results of recent studies indicated that only small quantities of methyl bromide and bromide ion were transported through the outside lysimeters following treatment with methyl bromide. It can be concluded that the small quantities transported would be of minor importance compared to the quantities released as a gas following fumigation.

Plots to determine the potential transport of pesticides in runoff water from treated plots during storm events were developed on soils typical of the Piedmont region. As much as 40 to 70% of the rainfall left the plots as runoff during simulated storm events. The collected surface water contained moderately high concentrations of treatment pesticides having a high water solubility.

Data for nine analytes resulted in a high correlation $r^2 = 0.91$) between the fraction of analyte transported and the water solubility for the analyte when fit to a quadratic equation. Less than 1% of the applied chlorothalonil, chlorpyrifos, benefin, and pendimethalin was transported from the plots in the runoff water. On the other hand, as much as 9 to 16% of the 2,4-D, dicamba, mecoprop, and nitrate were transported in the surface water from the first two simulated storm events.
Compared to broadcast application, pressure injection decreased the fraction of 2,4-D (7.4 times) and trichlorfon (5.2 times) transported, and the inclusion of a buffer strip between the points of treatment and water collection did not significantly reduce the fraction of analyte transported under saturated soil conditions. Research on reducing the potential movement of pesticides in surface water will be continued.

Leachate collected from lysimeters under practice greens at the Town and Country Club golf course contained only trace quantities of chlorothalonil, chlorpyrifos, and OH-chlorpyrifos. Slightly more OHchlorothalonil and nitrate were determined 'in the leachate in response to treatments with chlorothalonil and fertilizer.

Results of a project designed to determine the potential herbicide exposure from kneeling on a treated golf course green indicate that an average sized golfer can kneel as many as 20,000 times on greens, at 6 hours after a treatment with 2,4-D, mecoprop, and dicamba, before receiving an exposure equal to the NOEL (No Observed Effect Level).

Degradation of Fungicides in Turfgrass Systems

Dr. Ron Turco
Dr. Clark Throssell

Purdue University

Goals:

• Determine the character of the turfgrass leaf as a sorption material for fungicide.

• Determine the importance of microbial populations in controlling the degradation of fungicides on the turfgrass leaf surface.

• Investigate the significance of time on the fate of fungicides introduced into turfgrass ecosystems.

Increasing public concern over the fate of organic pesticides has necessitated research describing the degradation and potential accumulation of these compounds. This USGA-funded study strives to increase understanding of the fate of fungicides that reach the turf canopy, thatch and soil.

This project is broken into three tasks with specific objectives of describing sorption coefficients for fungicides in the turfgrass canopy, determining the importance of
microbial populations in controlling the degradation of fungicides on turfgrass leaves, and characterizing the long term fate of fungicides that reach the thatch and soil environment.

The work completed the first year of the three-year project has focused on the field portion of task one, which investigates the hypothesis that the chemical/physical structure of the leaf controls initial sorption of fungicides. Pesticide which is tightly sorbed to the turfgrass leaf may not be available for microbial degradation or movement into the thatch and soil.

Concentrations of fungicides on PENNCROSS creeping bentgrass clippings were measured experimentally over a period of four weeks, allowing determination of half-lives and dissipation patterns of parent compounds. Data was averaged from two identical experiments initiated on May 12 and August 25, 1995.

Other areas of progress include developing laboratory procedures for all tasks and obtaining three of the four radiolabeled fungicides needed to meet our objectives. To extend the applicability of our work, four fungicides which represent four families of chemicals are being used in this project: chlorothalonil (Daconil 2787), metalaxyl (Subdue), triadimefon (Bayleton) and vinclozolin (Curalan).

Loss of fungicide was clearly shown in turfgrass clippings from creeping bentgrass plots. Concentrations of metalaxyl, triadimefon, and vinclozolin fell below detectable limits within 13, 10, and 17 days respectively. In both experiments, chlorothalonil was detectable at low levels from clippings collected at the termination of the experiment on day 27.

The average peak fungicide concentrations extracted from turfgrass clippings were 38.16 g g^{-1} (dry clipping weight) for metalaxyl, 112.46 g g^{-1} for triadimefon, 549.65 g g^{-1} for chlorothalonil, and 370.35 kg g^{-1} for VM clozolin. The half-lives of parent fungicides were 4.4 days, 1.1 days, 3.2 days and 2.6 days for metalaxyl, triadimefon, chlorothalonil, and vinclozolin respectively.

High initial concentrations of fungicides coupled with rapid loss from turfgrass clippings indicates that dissipation in the field is not limited by sorption. In conclusion, when fungicides are applied at recommended rates, pesticide loading is unlikely on the live turfgrass plant.

**Construction and Maintenance of Greens**

**Introduction**
After years of investigation, the USGA Green Section introduced its Specifications for a Method of Putting Green Construction in 1960. The method utilized sand as the principal component of the root zone mix to provide adequate drainage and resistance to compaction, and incorporated a perched water table in the profile to provide a reservoir of moisture for use by the turf. When built and maintained properly, USGA greens have provided good results over a period of many years for golf courses in most regions of the United States and the world. During the past 10 to 15 years, changes have occurred in the way greens are maintained and in the number of products and technologies that have been developed. Play has increased, golfers have demanded closer mowing and perfection in maintenance, new grasses have been developed that have different maintenance requirements, and many more golf courses are using recycled water or poor quality water sources for irrigation. A wide array of organic and inorganic soil amendments have been introduced, and ideas for new green construction methods have been proposed. In addition to agronomic changes, the cost of golf course construction has increased dramatically, threatening to limit the growth of the game.

To take advantage of new ideas and technologies, and to address the environmental and economic challenges of the coming decades, the USGA will sponsor research studies on construction and maintenance of golf course greens. The goal of this research will be to:

*Identify the best combinations of construction, grow-in procedures and post-construction maintenance practices that prevent long-term problems, reduce environmental impacts, and produce high quality playing surfaces.*

The reduction of maintenance costs and resource inputs, and the simplification of construction procedures were included among the research project objectives addressing this goal. During 1995, the USGA Turfgrass and Environmental Research Committee selected ten putting green construction and maintenance projects which will be conducted over the next five years at a cost of $870,000. The Golf Course Superintendents Association of America (GCSAA) will co-fund five of the projects with the USGA. The following is a brief summary of the ten projects.

**Engineering Characteristics and Maintenance of Golf Putting Greens**

**Michigan State University**

*Dr. James Crum and Dr. John Rogers, III*

Why are some sands more stable than others? This project will investigate the physical properties of sands and establish relationships between strength and stability. The secondary objective is to evaluate the short and long term effects of post-grow-in
maintenance practices on putting greens constructed by three different methods: USGA recommendations, a modified loamy sand over gravel, and an unamended loam soil.

Methods for Classifying Sand Shape and the Effects of Sand Shape on USGA Specification Rootzone Physical Properties

Pennsylvania State University
Dr. Charles Mancino

How does the shape (i.e., angular or round) of the sand affect green performance? The project will first develop a simple, inexpensive and quantitative procedure to give a reliable estimate of sand shape without having to examine individual sand grains. The effect of sand shape on the physical properties of rootzone sands and whether the particle size distribution needs modification due to differences in sand shape will then be examined.

Layers in Golf Green Construction

Sports Turf Research Institute
Dr. Stephen Baker

Can the conditions for the elimination of the intermediate layer be less stringent? The migration of particles and water retention will be closely examined where the rootzone layer directly overlies the gravel drainage layer. Profiles of the rootzone and gravel layer will be established with different combinations of gravel size, gravel shape, rootzone mix and initial moisture content. Water retention in the rootzone layer also will be examined when it is placed over intermediate layers of varying size and composition.

Understanding the Hydrology of Modern Putting Green Construction Methods

The Ohio State University-OARDC
Dr. Edward McCoy

How does the profile design, root zone composition, slope of the green, drain spacing, profile depth, and irrigation protocol impact water movement and the extent of water perching in a USGA green? This research project will focus on water drainage, redistribution and use by turfgrass as influenced by a variety of factors related to modem putting green construction methods.
Assessing Differential Root Zone Mixes for Putting Greens Over Time Under Two Environmental Conditions

Rutgers University/Cook College
Dr. James Murphy

How do alternative putting green construction methods stack up to the USGA Green Section recommendations? Over a five-year period, recommendations for sand particle size distribution and the depth of the root zone mix in response to the microenvironment will be evaluated. A variety of organic composts and inorganic additives for root zone mixed will be compared to commonly used peat sources. The physical, chemical, and biological changes that occur as root zones mature, and the factors that contribute to the success or failure of greens, will be determined.

Evaluation of New Technologies in Construction and Maintenance of Golf Course Greens

North Carolina State University
Dr. Daniel Bowman

This research is designed to characterize the physical, chemical and biological changes that occur in a sand-based golf course green over the first five years. It proposes a novel two-phase rootzone mix as an alternative to existing sand:organic matter mixes and questions whether the incorporation of stabilized organic material (i.e., sphagnum peat) is warranted over the long term. The research also will address the question of the perched water table, specifically regarding changes over time, and the effects by air injection and water evacuation.

Grow-in and Cultural Practice Inputs on USGA Putting Greens and Their Microbial Communities

University of Nebraska
Dr. Roch Gaussoin

Beyond the questions dealing with the chemical and physical properties of putting green root zone mixes, how should they be grown in and made ready for play? Are the high rates of nitrogen used to accelerate growth a short term solution to meet opening day requirements, but a path to long term failures? What are the criteria for determining when play should be allowed on new greens?
This project will evaluate grow-in and post-grow-in cultural practices and procedures and readiness-for-play criteria. The long-term effect of these parameters on putting green performance, depth and extent of turfgrass rooting, and root zone hydrological, physical and chemical characteristics will be determined. The project also will assess the influence of these procedures on the microbes found in the root zone.

**Organic Matter Dynamics in the Surface Zone of a USGA Green: Practices to Alleviate Problems**

**University of Georgia**  
*Dr. Robert Carrow*

The primary objective of this project is to determine the effectiveness of selected fall/spring-applied cultivation practices on the enhancement of bentgrass root development, water infiltration, and soil oxygen. The effectiveness of selected summer-applied cultivation, topdressing and wetting agent practices on bentgrass root growth, water infiltration, and soil oxygen status during the summer months when root decline occurs will be examined.

**Nontarget Effects of Turfgrass Fungicides on Microbial Communities in Putting Green Profiles**

**Cornell University**  
*Dr. Gary Harmon and Dr. Eric Nelson*

This research effort will investigate the nontarget effects of fungicides used for disease control on golf course putting greens. The nontarget effects on greens treated with fungicides potentially include substantial changes in the soil ecosystem, possibly increasing disease susceptibility and affecting nitrogen cycling and the health of the turf.

**Bacterial Populations and Diversity within New USGA Putting Greens**

**University of Florida** - *Dr. Monica Elliott*  
**Auburn University** - *Dr. Elizabeth Guertal*  
**Clemson University** - *Dr. Howard Skipper*

What species of bacteria are found in new greens? Where do they come from? How do microbial populations change over time? This project will monitor the microorganisms in newly constructed bermudagrass and bentgrass greens on golf courses in South
Carolina, Alabama, and Florida. Effects on bacterial populations will be examined based on differences among organic material, fumigation, nitrogen fertility, and clay minerals.