1994 TURFGRASS RESEARCH SUMMARY

THE USGA

1994
Turfgrass
Research Summary

SUBMITTED BY:

United States Golf Association
Golf House
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Statement of Intent

It is the intent of the United States Golf Association (USGA) Executive Committee, through the USGA Foundation, to collect and disseminate substantial amounts of money for support of research to: 1) produce improved turfgrasses which substantially reduce water use, pesticide use, and maintenance costs; 2) develop management practices for new and established turf which protect the environment while providing quality playing surfaces; and 3) encourage young scientists to become leaders in turfgrass research.

It is anticipated that funds for this purpose will be derived, in major part, from contributions to the USGA Foundation. Additional funds may be derived in the future from royalties attributed to marketable discoveries. The USGA intends to return any income received from royalties to the support of turfgrass research.

Historically, the sport of golf has maintained a leadership role in the development of improved turfgrasses through the activities of the USGA Green Section. While those developments have provided better playing areas for golf, they also have had a far-reaching impact on turfgrass improvement for other uses. Home lawns, parks, school grounds, highway rights-of-way and all other turfgrass areas have been improved by developments pioneered by the USGA.

The USGA expects to support research at numerous institutions. In some cases, several institutions will be involved with the development of grasses and maintenance practices where the research may interact and overlap.

In view of this Statement of Intent, it is expected that recipients of grants will embrace a spirit of cooperation and engage in a free exchange of information with other investigators.

USGA Turfgrass Research 1993 - 1997
Conserving Natural Resources

Project/Subproject	University/Investigator	Actual 1993	Actual 1994	Budget 1995	Budget 1996	Budget 1997	Total
Turfgrass Breeding:							
Bentgrass	TAMU/Engelke	60,000	63,000	66,150	69,458	72,930	331,538
Cool Season	Rutgers/Funk	8,000	8,000	8,000	8,000	8,000	40,000
Bermudagrass	USDA/Burton	8,000	8,000	8,000	8,000	8,000	40,000
Bermudagrass	OSU/Talifaferro	60,000	63,000	66,150	69,458	72,930	331,538
Buffalograss	Univ. of Nebraska/Riordan	60,000	63,000	66,150	69,458	72,930	331,538
Colonial Bentgrass	Rhode Island/Ruemmele	20,000	21,000	22,050			63,050
Seashore Paspalum	GA Exp. Station/Duncan	20,000	21,000	22,050			63,050
Poa annua	Univ. of Minnesota/White	40,000	20,000	10,000			70,000
Zoysiagrass	TAMU/Engelke	60,000	63,000	66,150	69,458	72,930	331,538
	Subtotal	336,000	330,000	334,700	293,832	307,720	1,602,252
Alternative Pest Management:							
Mole Cricket	NC State Univ./Brandenburg		20,000	20,000	20,000		60,000
Black Turfgrass Ataenius	Univ. of California/Cowles		10,000				10,000
Black Turfgrass Ataenius	Cornell Univ./Villani		10,000				10,000
Sting Nematode Control	Univ. of Florida/Giblin-Davis		20,000	20,000	20,000		60,000
Allelopathy	Univ. of Arkansas/King		10,000	10,000	10,000		30,000
Dollar Spot	Michigan State Univ./Vargas		20,000	20,000	20,000		60,000
White Grubs	Univ. of Kentucky/Potter		20,000	20,000	20,000		60,000
Disease Suppression	Cornell Univ./Nelson		20,000	20,000	20,000		60,000
Summer Patch	Rutgers/Kobayashi	20,000	21,000	22,050			63,050
Rhizoctonia solani	Mississippi State/Krans	25,000	26,031	27,333			78,364
Disease Resistance	Virginai PolyTech/Ha	25,000	26,250	27,563			78,813
Herbicides Restance	Rutgers/Day	45,000	47,252	49,615			141,867
	Subtotal	115,000	250,533	236,561	110,000	0	712,094
Cultural Practices:							
Water Use/Buffalograss	Univ. of Nevada/Bowman	15,000	15,750	16,538			47,288
Water Use/Bermudagrass	GA Exp. Station/Carrow	12,273	12,360	13,488			38,121
Water Use/Zoysiagrass	GA Exp. Station/Carrow	21,500					21,500
Water Use/Bentgrass	GA Exp. Station/Carrow	6,000					6,000
Effluent Water	Univ. of Arizona/Mancino	25,000	26,250	27,563			78,813
Low Temperature/Drought	Clemson/Barid	20,000	21,000	22,050			63,050
Drought Stress/Bentgrass	TAMU/White		22,453	23,576	24,754		70,783
Putting Green/Bermuda	Auburn Univ./Dickens			10,000	10,000	10,000	30,000
Mycorrhizae	Rhode Island/Jackson	40,000					40,000
	Subtotal	139,773	97,813	113,215	34,754	10,000	395,555
Other:							
International Turfgrass Conf.	ITS/Watson	5,000					
	Subtotal	5,000					
	TOTAL	595,773	678,346	684,476	438,586	317,720	2,709,901
	BUDGET	650,000	682,500	716,625	752,456	790,079	3,591,660
	DIFFERENCE	54,227	4,154	32,149	313,870	472,359	881,759

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Executive Summary

Overall Goals:

- Reduce turfgrass water requirements, pesticide use, and maintenance costs.
- Protect the environment while providing good quality playing surfaces.
- Encourage young scientists to become leaders in turfgrass research.

The Turfgrass Research Program sponsored by the United States Golf Association, in cooperation with the Golf Course Superintendents Association of America, has three primary goals:

First, develop turfgrasses for golf courses that substantially reduce water use, pesticide use and maintenance costs;

Second, develop management practices for new and established turf that protect the environment while providing quality playing surfaces;

Third, encourage young scientists to become leaders in turfgrass research through the USGA's direct involvement in and financial support of higher education in the United States.

This annual research summary reviews the progress made by sponsored researchers in projects directed toward: 1) plant breeding for the development of turfgrasses with better resistance to stress and pest problems, 2) evaluation of cultural practices with potential to improve the ability of golf course turf to tolerate stress, and 3) evaluation of alternative methods of pest control for use in integrated turf management systems.

Highlights of 1994

- Promising new endophytes were collected by Rutgers University. Endophytes are fungi which help protect some turfgrass species from insect pests and may play a role in improved stress tolerance.

- CRENSHAW (Lofts Seed) and CATO (Pickseed West) creeping bentgrasses continue to perform well in the southern United States.

Highlights of 1994 (continuted)

- Syn1-88, a re-selection from SEASIDE, was released to Pickseed West for low-maintenance areas using poor quality irrigation water.
- Syn92-1 and Syn92-5, selected for improved heat tolerance and rooting, were released to Burlingham & Sons.
- PENNLINKS (Tee-2-Green) and PROVIDENCE (Seed Research) creeping bentgrasses are still used very successfully throughout the United States.
- OKS 91-11 has demonstrated superior cold tolerance among seeded bermudagrasses and will be released in 1995.
- MI-40, a mutant of vegetatively propagated MIDIRON bermudagrass, was released by Dr. Wayne Hanna from the USDA-ARS at Tifton, Georgia, for use on golf course fairways.
- Several promising creeping bluegrasses (*Poa annua* var *reptans*) were released to Peterson Seed by the University of Minnesota.
- Several of the vegetatively propagated zoysiagrasses developed by the Texas A&M University Agriculture Experiment Station at Dallas have performed well and will be released in late 1995 or early 1996.
- CODY and TATANKA seeded buffalograss were released by the University of Nebraska to the Native Turf Group. Seed will be available in 1995.
- The vegetative buffalograss varieties '609', '315', and '378' all continue to perform well on golf course roughs.
- Results from University of Nevada indicate that buffalograss can provide adequate turf for roughs with deficit irrigation of 50 to 60 percent of evapotranspiration (ET).
- Molecular genetics techniques continue to provide information on how some bermudagrass plants tolerate lower cold temperatures.
- Creeping bentgrass plants resistant to glufosinate ammonium herbicide (Finale or Ignite) were successfully produced using genetic engineering technology.
- Biological technology is being used to produce disease resistant bentgrasses by introducing genes which produce the chitinase protein.
- Bentgrasses resistant to brown patch (*Rhizoctonia solani*) were successfully selected using the Host Plant Interaction System (HPIS) developed at Mississippi State University.
- Several bacteria are under evaluation for their disease suppressive characteristics on summer patch (*Magnaporthe poae*) and *Pythium*-incited diseases of creeping bentgrass.
- Results at the University of Kentucky suggest that the number of grubs required to cause noticeable injury was much higher than prevailing rule-of-thumb estimates used by the turf industry.
- A new species of bacteria which parasitizes the sting nematode (*Belonolaimus longicaudatus*) is under evaluation at the University of Florida.

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. In Table I, the breeding projects, species, and status of varieties are summarized.

Summary of USGA Turfgrass Breeding Projects

Turfgrass	University	Status of Varieties
Creeping Bentgrass *Agrostis palustris*	Texas A&M University	CRENSHAW (Syn3-88), CATO (Syn4-88) and Syn1-88 were released. Syn92-1, Syn92-2, and Syn92-5 are under evaluation for release. All are entered in 1993 NTEP trials.[1]
	University of Rhode Island	PROVIDENCE was released.
	Pennsylvania State University	PENNLINKS was released
Colonial Bentgrass *Agrostis tenuis*	DSIR-New Zealand and University of Rhode Island	A preliminary line, BR-1518, was entered in the NTEP trials. A new line is being evaluated at the University of Rhode Island.
Bermudagrass *Cynodon dactylon*	New Mexico State University	NuMex SAHARA, and several other seed propagated varieties were developed from this program.
	Oklahoma State University	Two seeded types, OKS 91-11, and OKS 91-1 were entered in the 1992 NTEP trials. OKS 91-11 will be released in 1995.
C. transvaalensis	Oklahoma State University	Thirty experimental cultivars are in initial turfgrass evaluations.
C. dactylon X C. transvaalensis	University of Georgia	TIFTON 10 and MI-40 were released; other MIDIRON and TIFWAY mutants are under evaluation for release.
Buffalograss *Buchloe dactyloides*	University of Nebraska	Several varieties are entered in the 1991 NTEP trial. Vegetative: NE 84-315, NE 84-378, NE 84-436, NE 84-453, and NE 84-609. Seeded: NTG-1, NTG-2, NTG-3, NTG-4, and NTG-5. Vegetative varieties 609, 315, and 378 were released. Seeded varieties CODY and TATANKA were released.
Alkaligrass *Puccinellia* spp.	Colorado State University	Ten improved families are under evaluation and have been released.
Blue grama *Bouteloua gracilis*	Colorado State University	ELITE, NICE, PLUS and NARROW are under evaluation in anticipation of release.
Fairway Crested Wheatgrass *Agropyron cristatum*	Colorado State University	Narrow leaved and rhizomatous populations are entering preliminary turfgrass trials and a second cycle of selection.
Curly Mesquitegrass *Hilaria belangeri*	University of Arizona	Seed increases of 'fine' and 'roadside' populations are available for germplasm release and further improvement.
Annual bluegrass *Poa annua* var *reptans*	University of Minnesota	Selections #42, #117, #184, #208, and #234 have been released and are under evaluation for seed production.
Zoysiagrass *Zoysia japonica* and *Z. matrella*	Texas A&M University	Several vegetative selections are entered in 1991 NTEP trial: DALZ8501, DALZ8502, DALZ8507, DALZ8508, DALZ85012, DALZ85014, DALZ85016, DALZ8701, and DALZ9006. DALZ8507, DALZ8512, and DALZ8514 will be released in 1995.
Seashore Paspalum *Paspalum vaginatum*	University of Georgia	Germplasm has been assembled and is under evaluation.

[1]National Turfgrass Evaluation Program, Beltsville Agricultural Research Center, Beltsville, MD 20705

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

Dr. C. Reed Funk

University Rutgers

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.

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 during the last year. Promising new turfgrass germplasm and associated endophytes were collected from old turfs in Argentina, Oregon, Wyoming, Utah, and New Jersey. Herbarium studies showed that a number of *Poa* and *Festuca* species native to South America contain endophytes. More than 5,000 new turfgrass plots and more than six acres of spaced-plant nurseries were established in 1994.

Germplasm developed at the New Jersey Agricultural Experiment Station contributed to a number of new turfgrass cultivars including PRIZM, ADVANTAGE, and TOPHAT perennial ryegrasses; HOUNDOG V and JAGUAR III tall fescues; WINTERPLAY rough bluegrass; BRITTANY Chewings fescue; and PREAKNESS Kentucky bluegrass. Other turfgrasses being evaluated for commercial use include LASER II rough bluegrass, AZURE blue fescue, TREAZURE Chewings fescue, NORDIC hard fescue, and GEN 91 tall fescue.

Considerable winter kill was observed on closely mowed, highly fertilized perennial ryegrasses, tall fescues, and annual bluegrasses, which were covered with ice for nearly three months. Nearby plantings of Kentucky bluegrasses, fine fescues, and bentgrasses showed little or no damage except for some pink and grey snow mold. Adjacent plantings of tall fescues maintained at a higher cut and lower fertility showed little damage from ice sheet cover. Selection of attractive plants surviving prolonged ice cover were made. Turf trials have been seeded with progenies of the most promising selections. Ice sheet damage on perennial ryegrasses and tall fescues illustrate the need to obtain new sources of germplasm.

Considerable progress continues to be made in developing populations of tall fescues, perennial ryegrasses, Chewings fescues, strong creeping red fescues, and rough bluegrasses with a darker green color, a lower growth profile, more tillers, greater density, and a slower rate of leaf elongation. Many of these characteristics are noted in ecotypes collected from the far northern latitudes. They are also observed when local ecotypes are growing under the short daylengths and cool temperatures of late fall and early winter. Many of these lower growing populations show other short daylength effects such as prolonged winter dormancy. Increased efforts are being made to identify and utilize lower growing plants that are independent of daylength effects.

Kentucky bluegrass cultivars and selections are being screened for resistance to a race of stripe smut that is highly virulent on many widely-used cultivars, including BARON, VICTA, MARQUIS, MERIT, GNOME, KELLY, and VIVA.

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.

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 regardless of increased production, seed was sold out for the year.

Syn1-88 is a re-selection from Seaside, and Pickseed West will have seed available commercially in 1995. Syn1-88 is recognized for its low maintenance requirements and excellent salt tolerance. Syn1-88 is being evaluated extensively in California and West Texas for salinity tolerance under golf course conditions.

Burlingham & Sons have negotiated an option agreement with Texas A&M for the testing and evaluation rights on Syn92-1 and Syn92-5 creeping bentgrasses. Four elite bentgrasses lines were increased in 1993, and three of them were entered into the 1993 NTEP bentgrass trials. These new varieties were developed specifically for improvements in heat tolerance, deep root growth characters, disease resistance, persistence, and competitive ability.

Progeny of advanced lines were selected from Oregon production fields and will be included in tests to evaluate total plant performance at TAES-Dallas, including vegetative growth characters, turf quality, disease resistance, insect resistance, traffic and salinity tolerance, heat tolerance, and root growth characters.

Mean Turfgrass Quality Ratings of Bentgrass Cultivars for Each Month Grown on a Fairway or Tee at Twenty-One Locations in the United States and Canada. 1994 Data.[2]

NAME	Turfgrass Quality Ratings 1 - 9; 9 = Ideal Turf: Months[1]											
	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	MEAN
PROVIDENCE	5.3	5.8	6.1	6.6	6.7	6.6	6.6	7.1	7.0	6.5	6.7	6.7
CATO	4.0	4.9	6.3	6.4	6.8	6.8	6.6	7.0	6.9	5.8	6.0	6.6
CRENSHAW	2.3	5.3	6.2	6.4	6.9	6.9	6.6	6.8	6.5	5.7	6.0	6.6
PENNEAGLE	4.7	6.0	6.3	6.6	6.4	6.8	6.6	6.9	6.7	5.9	6.0	6.6
SOUTHSHORE	4.3	5.1	6.1	6.5	6.5	6.6	6.5	6.7	6.5	5.9	6.3	6.4
G-6	2.7	5.0	5.9	6.1	6.3	6.5	6.4	6.9	6.8	6.0	6.8	6.4
PRO/CUP	3.3	5.6	6.0	6.4	6.4	6.4	6.3	6.4	6.2	6.3	6.3	6.3
G-2	5.3	4.3	5.6	6.0	6.2	6.4	6.2	6.8	6.7	5.9	7.3	6.3
PENNCROSS	4.0	6.2	6.2	6.5	6.4	6.2	6.2	6.2	6.1	5.8	4.8	6.2
BAR WS 42102	2.3	4.3	5.8	6.2	6.4	6.3	6.1	6.4	6.2	5.3	5.0	6.2
TRUELINE	4.0	4.8	5.5	5.9	6.2	6.3	6.3	6.5	6.2	5.8	5.8	6.1
LOPEZ	4.3	4.7	5.5	5.9	6.1	6.2	6.3	6.4	6.2	5.8	5.8	6.1
18TH GREEN	2.7	4.7	5.7	6.1	6.2	6.4	6.3	6.2	5.9	5.1	3.3	6.1
DF-1	5.0	5.3	5.7	5.8	5.9	6.2	6.0	6.5	6.2	6.0	6.0	6.1
ISI-AT-90162	5.3	5.6	5.8	5.6	5.9	5.4	5.8	6.1	6.2	5.5	6.2	5.8
SR 7100	6.0	5.7	6.1	5.9	6.2	5.6	5.7	5.8	5.9	5.9	4.7	5.8
OM-AT-90163	4.0	5.4	6.1	5.8	5.7	5.2	5.6	5.8	5.9	5.4	5.8	5.7
TENDENZ	3.3	4.8	6.1	5.8	5.7	4.8	5.5	5.4	5.6	5.1	4.0	5.4
BAR AS 492	6.0	4.4	4.8	5.4	5.2	5.1	5.5	5.9	5.6	6.1	6.2	5.4
SEASIDE	5.3	5.2	5.3	5.2	4.8	4.7	4.9	4.9	5.1	5.3	4.8	5.1
EXETER	5.0	5.8	5.1	4.9	4.5	4.4	4.6	4.9	5.0	4.9	4.8	4.8
LSD VALUE	0.9	1.0	0.8	0.6	0.5	0.6	0.5	0.5	0.5	1.0	1.1	0.4

[1] To determine statistical differences among entries, subtract one entry's mean from another entry's mean. Statistical differences occur when this value is larger than the corresponding LSD Value (LSD 0.05).

[2] Source: National Turfgrass Evaluation Program. National Bentgrass Test - 1993 (Fairways/Tee)

Mean Turfgrass Quality Ratings of Bentgrass Cultivars for Each Month Grown on a Green at Twenty-Six Locations in the United States. 1994 Data.[2]

	Turfgrass Quality Ratings 1 - 9; 9 = Ideal Turf: Months[1]												
NAME	**JAN**	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**	**MEAN**
A-4	5.2	6.2	5.6	5.7	6.2	6.6	6.6	6.5	6.5	6.4	5.9	4.9	6.5
L-93	3.8	5.2	5.3	5.1	5.8	6.1	6.4	6.6	6.7	6.6	5.8	4.5	6.4
PROVIDENCE	4.8	5.8	5.5	5.6	6.1	6.4	6.3	6.4	6.4	6.3	5.8	4.4	6.3
A-1	5.0	5.4	5.4	5.3	5.9	6.1	6.4	6.4	6.6	6.5	5.9	4.8	6.3
CRENSHAW	5.7	5.4	5.6	5.3	6.1	6.1	6.4	6.5	6.2	6.1	5.5	3.9	6.2
CATO	4.8	5.0	5.3	5.3	6.1	6.3	6.4	6.3	6.2	6.2	5.7	4.4	6.2
G-6	4.3	5.2	4.7	4.9	5.6	6.0	6.2	6.3	6.3	6.4	5.8	4.4	6.1
G-2	4.2	4.7	4.8	4.9	5.6	5.9	6.0	6.2	6.4	6.4	5.8	5.0	6.1
SOUTHSHORE	5.0	5.9	5.4	5.4	5.8	6.1	6.1	6.2	6.3	5.9	5.5	4.4	6.1
SYN 92-1	4.5	5.4	5.3	5.3	6.2	6.3	6.3	6.3	6.1	5.9	5.3	3.8	6.0
SYN 92-5	4.3	6.1	5.2	5.5	5.9	6.0	6.1	5.9	6.1	6.0	5.3	4.2	6.0
SYN 92-2	4.2	5.7	5.6	5.2	6.0	6.0	6.0	6.1	6.0	5.8	5.1	3.9	5.9
SR 1020	5.2	5.1	5.3	5.2	5.8	5.9	6.0	6.0	5.9	5.9	5.6	4.4	5.9
PENNLINKS	4.0	4.8	5.3	5.4	5.7	5.8	5.8	5.9	6.0	5.9	5.3	4.1	5.8
REGENT	5.5	5.6	5.6	4.8	5.5	5.7	5.8	5.9	6.0	5.8	5.3	4.5	5.8
BAR WS 42102	3.8	4.6	5.2	5.1	5.7	5.8	6.0	5.8	6.0	5.9	4.6	3.5	5.8
MSUEB	4.3	4.6	5.5	5.3	5.6	5.6	5.7	5.8	5.9	5.8	5.2	4.1	5.7
ISI-AP-89150	4.3	5.2	4.8	5.0	5.7	5.6	5.6	5.8	5.9	5.8	5.3	3.8	5.7
18TH GREEN	4.2	3.8	4.8	4.9	5.6	6.0	5.9	6.0	5.6	5.5	4.7	3.1	5.7
LOPEZ	3.5	4.4	4.6	4.9	5.1	5.4	5.8	5.9	5.9	5.9	5.1	4.2	5.6
PRO/CUP	4.5	5.0	5.3	4.9	5.6	5.6	5.5	5.8	5.7	5.7	4.8	3.8	5.6
DG-P	4.0	4.8	4.4	4.4	5.1	5.4	5.6	5.9	5.8	5.8	5.1	3.9	5.6
PENNCROSS	5.0	5.1	5.5	5.4	5.5	5.7	5.6	5.5	5.5	5.6	4.8	3.8	5.5
TURELINE	3.5	4.8	5.0	4.5	5.2	5.4	5.6	5.8	5.7	5.7	5.0	3.8	5.5
SYN-1-88	3.8	4.7	5.6	4.7	5.2	5.5	5.2	5.4	5.6	5.3	5.1	4.2	5.4
TENDENZ	3.0	4.9	5.1	5.0	4.9	5.2	4.7	4.7	4.9	4.9	4.2	3.4	4.8
BAR AS 492	2.8	3.9	4.0	4.1	4.5	4.3	4.5	4.5	5.0	4.9	4.4	4.1	4.6
SEASIDE	4.5	4.8	5.8	5.0	4.9	4.5	4.4	4.4	4.6	4.3	4.2	3.2	4.5
LSD VALUE	2.1	1.5	1.1	0.8	0.7	0.5	0.5	0.5	0.5	0.6	0.9	1.1	0.4

[1] To determine statistical differences among entries, subtract one entry's mean from another entry's mean. Statistical differences occur when this value is larger than the corresponding LSD Value (LSD 0.05).

[2] Source: National Turfgrass Evaluation Program. National Bentgrass Test - 1993 (Putting Greens)

Bermudagrass Breeding - Vegetative

Dr. Glenn Burton

USDA and University of Georgia

Goals:

- To develop improved, fine-textured bermudagrass for golf course putting greens, tees and fairways.
- Develop and refine efficient screening techniques for evaluating cold-hardiness and energy reserves.

Winter injury and survival continues to be one of the major problems of bermudagrass in the transition zone. Bermudagrass has winter survival problems during some years, and bentgrass has summer survival problems in other years. Careful management, daily vigilance and use of fungicides can help bentgrass survive disease attacks associated with warm, muggy summers.

The lost of bermudagrass turf during an occasional winter is not so predictable. A weather front that drops night temperatures below zero, but allows day temperatures of 45° when the sun shines, will have little effect on soil temperature unless repeated for several days. Most arctic fronts in the South last less than a week, and they usually allow no time for plants and grass to harden off before they arrive.

For years, we have been trying to breed more freeze tolerant turf and forage bermudagrasses. Lack of an effective screening method has kept us from making the progress that should be possible. We are trying to freeze plugs as one approach that others have tried with little success. In our last report, we described our freeze procedures. Since then we replaced the seed germinator with a small freezer unit that can maintain 10°F below zero.

We are still inverting the plugs, exposing them to the two bottom coils, and insulating the bottom of the plugs from the freezing effect of the top coil. We have found that the management of the genotypes to be screened must be uniform, that the moisture in the soil must be uniform, and that it is still difficult to get consistent results. We plan to continue to try to improve our freezing efforts as time permits.

We are convinced that the concentration of reserves in a grass sod will influence its winter hardiness. In a grass sod, these reserves, non-structural carbohydrates, proteins and other growth promoting compounds, may be in crowns, corms, rhizomes, and roots. In year-old sod, some of the crowns, corms, rhizomes, and roots will be dead or so nearly dead that they cannot generate a plant, but many will contain non-structural carbohydrates. In grass sod, it is impossible to separate those organs capable of generating new growth from those that are not. Therefore, we believe that growing plugs in the dark and measuring the etiolated growth produced is the only way to measure significant reserves in a sod.

Winter survival in plants has been associated with reserves stored in their roots and underground parts. In 1962 we described "A Method for Measuring Sod Reserves," *Agronomy Journal* 54:53-55. The method involved cutting 6-inch plugs of sod, putting them in empty No. 10 cans from a cafeteria, letting them develop etiolated stems in the dark, and measuring the dry matter so produced. We have modified this method, since used by others, by inverting another can over the one containing the plug. A small black opening is left on the north side for air exchange and adding water. The cans are attached to each other with electricians black plastic tape which excludes the light. We have then been able to grow them out in the lighted greenhouse and separate the cans to measure the etiolated growth. More detailed information on our method to evaluate carbohydrate reserves can be found in the manuscript that has been accepted for publication as a note in *Crop Science* in 1995.

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, fine-textured, 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.

The objectives of the Oklahoma State University bermudagrass breeding program are to develop improved seed and vegetatively propagated varieties for use in the transition zone and southern states.

Phenotypic recurrent selection (PRS) is continuing in two broad genetic base *C. dactylon* populations, one derived from cold-tolerant, relatively infertile germplasm, the other from cold-sensitive, highly fertile germplasm. Selection within the cold-tolerant populations, $C_{3fer3tex}$, has been for increased seed production potential and finer texture. Selection within the cold-sensitive population (C_{2ct}) has been for increased freeze tolerance. An additional cycle of selection was completed within each of the populations over the past year.

The $C_{3fer3tex}$ population and synthetic varieties derived from it have demonstrated good cold tolerance and turf quality in multi-environment tests. The seeded experimental OKS 91-11 has performed well in the National Turfgrass Evaluation Program bermudagrass test. Scale-up production has been initiated for two synthetic varieties in preparation for commercialization.

Research with African bermudagrass, *C. transvaalensis*, has demonstrated extensive phenotypic variation within the species for many traits influencing adaptation and turf quality. Development of a genetic population was completed that will permit estimation of genetic parameters within the species. Field evaluation of selected African genotypes indicate their major weaknesses to be instability of turf quality and light-green color. In tropical environments, the African selections maintain good to excellent putting-green turf in winter, but dramatically decline in quality during summer.

The decline is less severe in temperate environments.

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

Mean Turfgrass Quality Ratings of Bermudagrass Cultivars for Each Month Grown at Twenty-Three Locations in the United States. 1994 Data.[2]

	Turfgrass Quality Ratings 1 - 9; 9 = Ideal Turf: Months[1]												
NAME	**JAN**	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**	**MEAN**
TDS-BM1	5.0	4.4	5.5	5.6	6.1	6.0	6.4	6.3	6.5	6.6	6.3	4.1	6.0
MIDLAWN	4.9	4.6	4.9	5.0	6.0	5.6	5.9	6.2	5.9	5.8	5.5	3.4	5.9
MIDFIELD	5.0	4.4	5.1	4.7	6.1	5.8	5.9	6.0	6.0	5.8	5.4	3.6	5.8
MIDIRON	5.1	4.2	5.3	5.0	5.8	5.6	6.1	6.3	5.9	5.5	6.0	4.3	5.8
TIFGREEN	5.2	4.8	5.2	5.1	5.7	5.6	6.0	6.0	6.2	6.4	6.3	4.2	5.8
TIFWAY	5.4	4.3	5.4	5.0	5.6	5.7	6.2	6.2	6.2	6.4	6.4	4.4	5.7
MIRAGE (90173)	5.3	4.1	4.3	4.4	5.3	4.9	5.5	5.7	5.5	5.8	5.6	3.7	5.3
TEXTURF 10	5.1	4.2	5.1	5.0	5.1	5.4	5.7	5.7	5.8	5.9	5.9	3.8	5.3
OKS 91-11	5.1	4.4	4.5	4.5	5.5	5.0	5.4	5.7	5.3	5.5	5.3	3.7	5.3
STF-1	4.9	4.3	5.0	4.7	5.2	5.1	5.5	5.6	5.5	5.6	5.5	4.1	5.3
J-27	5.0	4.2	4.1	4.1	5.4	5.1	5.4	5.4	5.1	5.3	5.2	3.4	5.1
GUYMON	5.2	3.9	4.5	4.3	5.4	5.0	5.1	5.4	5.2	5.3	5.1	3.4	5.1
JACKPOT (J-912)	4.2	4.1	4.4	4.0	4.6	4.6	5.2	5.3	5.2	5.4	5.4	3.6	4.8
SUNDEVIL	4.9	3.7	3.9	3.7	4.5	4.2	4.8	5.0	5.0	5.4	5.4	3.7	4.7
FMC 5-91	5.0	4.0	4.1	3.9	4.4	4.2	4.9	4.9	5.0	5.4	5.3	3.7	4.6
FMC 6-91	5.1	4.1	4.0	3.9	4.3	4.2	4.8	5.0	4.9	5.4	5.5	3.7	4.5
OKS 91-1	5.3	4.1	3.8	3.6	4.2	4.0	4.5	4.8	4.8	5.2	5.3	3.5	4.4
FHB-135	4.6	3.9	4.7	4.2	4.1	4.4	4.7	4.5	4.5	5.1	6.1	4.2	4.3
FMC 2-90	4.9	3.7	4.1	4.1	4.2	4.1	4.5	4.7	4.8	5.2	5.3	3.8	4.3
FMC 3-91	4.9	4.1	4.1	3.9	4.1	4.0	4.4	4.7	4.7	5.2	5.5	3.7	4.3
SAHARA	5.1	4.1	4.3	4.0	4.2	4.0	4.5	4.7	4.8	5.2	5.3	3.5	4.3
CHEYENNE	5.0	3.7	3.9	3.8	4.0	4.0	4.4	4.7	4.5	5.0	5.1	3.6	4.2
SONESTA	5.3	4.1	4.1	3.9	4.1	3.8	4.3	4.4	4.3	5.0	5.4	3.4	4.1
PRIMAVERA (FMC 1-90)	5.0	3.6	3.9	3.7	3.9	3.7	4.1	4.3	4.3	5.0	5.3	3.5	3.9
AZ. COMMON -SEED	5.1	3.6	3.7	3.6	4.0	3.7	4.0	4.2	4.4	4.8	5.1	3.4	3.9
AZ. COMMON-VEG.	4.6	3.8	3.3	3.2	3.3	3.6	3.9	4.2	4.2	4.6	4.8	3.3	3.8
LSD VALUE	1.1	0.6	1.4	1.1	0.7	0.8	0.7	0.7	0.7	0.7	0.7	1.1	0.6

[1] To determine statistical differences among entries, subtract one entry's mean from another entry's mean. Statistical differences occur when this value is larger than the corresponding LSD Value (LSD 0.05).

[2] Source: National Turfgrass Evaluation Program. National Bermudagrass Test - 1993.

Breeding, Evaluation and Culture of Buffalograss for Golf Course Turf

Dr. Terrance Riordan

University of Nebraska

Goals:

- Develop vegetative and seeded turf-type buffalo-grasses which conserve energy and water.
- Develop buffalograss establishment protocols and management systems to provide acceptable golf course rough and fairway turf at significantly reduced levels of energy input.
- Determine 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.

Sales of '609' are expected to meet Crenshaw & Doguet projections of $1.5 million for 1994. Sales are still predominately from their original farm at Bastrop, Texas. Three new farms in Bay City, Poteet, and Dallas, will bring total production in Texas to over 400 acres. Performance of '609' has been excellent. The '609' planted in the rough at the Boulders Golf Course in Lake Acworth, Georgia seems to be doing well. This course was rated the top new golf course in Georgia in 1994.

Approximately $60,000 of '378' plugs were sold by Todd Valley Farms, Inc., Mead, Nebraska and $10,000 of '315' plugs were sold by Oak Point Sod, Nickerson, Nebraska during 1994.

In 1995, Native Turf Group will have seed available from two new varieties named CODY and TATANKA. Poor weather and adjustments in planting procedures have resulted in delays in obtaining their first commercial harvest. Sharps Brothers provided seed from six experimentals for our 1994 Evaluation Trial.

During 1994, the project focused on improving tolerance to wear and low mowing, insect resistance, and seedling vigor. To evaluate low mowing tolerance, the mowing height of the 1990 evaluation trial was lowered to 5/8 inches in 1993. Significant differences in turf quality were found among entries, indicating that some genotypes were able to better tolerate low mowing. The top ten entries, four male and six female, were selected from this trial and established in a polycross in May 1994 to allow for recombination and the development of a buffalograss variety for fairway use.

In order to develop a seeded cultivar with improved seedling vigor, divergent phenotypic recurrent selection for caryopsis size is being performed with two synthetic populations. Larger caryopsis size has been shown to increase seedling vigor in buffalograss. Realized heritability estimates will be calculated for determining selection for large and small caryopsis size.

Traffic treatments were applied to two evaluation trials with a traffic simulator. Treatments were applied to half of each plot during June through August 1993. Significant differences were found among selections in their traffic tolerance, and '315' and a number of experimentals were among the top performers. For some cultivars, the difference between trafficked and untrafficked halves was minimal. In a trial containing 2000 plants, severe traffic pressure was applied during May through July 1994. At the end of treatments, 81 of the most traffic-tolerant plants were selected for further evaluation.

Results from the 1993 variety trial indicate that two synthetic populations, 90-503 and 90-504, are performing well. It is hoped that one of these will soon be released as a seeded cultivar. Nine vegetative selections were increased in 1994 for possible commercialization. The three with the most potential appear to be 86-61, 91-118, and 86-120. A new replicated evaluation trial, consisting of 48 entries, was also planted in 1994 and a total of 132 new selections were made from our nurseries, native stands or old turfs.

Weed pressure continues to be a major problem during buffalograss establishment. Research was initiated in 1994 to investigate registered and unregistered herbicides for use during seeded buffalograss establishment at two locations in Nebraska and one in Kansas. Plots treated with herbicides of the imidazolinone family (Pursuit, Cadre) produced significantly higher establishment rates than plots treated with other herbicides tested. Additionally, herbicides currently registered for use on established buffalograss (i.e., Dimension, Ronstar G, Surflan, and Dacthal) severely retarded seed establishment. Pursuit and Image are also being evaluated in a replicated trial on a Crenshaw & Doguet sod farm.

Sod strength of 22 entries in the National Buffalograss Trial were evaluated at two locations using an S-beam load cell connected to a digital read-out. PRAIRIE and '609' exhibited superior sod strength, while seeded and diploid entries exhibited unacceptable sod strengths. Root regrowth was also measured on National Turfgrass Evaluation Program entries and 609 had superior performance. Transplant shock was evaluated for 315 and 378. 378 exhibited superior recovery characteristics over 315, and sod replanted immediately recovered quicker than sod replanted at 48 hours after harvest. Three anti-transpirants tested had no effect on sod recovery.

A highly significant positive correlation was found between the amount of pubescence and susceptibility to mealybugs ($r = 0.78$). Scanning electron microscopy was used to investigate this possible mechanism of resistance. Results suggest that pubescence may provide a framework for the attachment of waxy ovisacs and a foothold for the mobile first instar. The inheritance of mealybug resistance and the development of a seeded, resistant cultivar is underway. Seed has been harvested from a crossing block containing mealybug resistant plants, and seedlings from this crossing block are currently being evaluated forresistance.

A study was conducted to develop an effective, non-destructive way to monitor mealybug populations on buffalograss plants. Adhesive-covered "sticky stakes" were placed in pots of mealybug-infested buffalograss to determine if the stakes would trap mealybugs. Large numbers of mealybugs were captured on the sticky stakes, indicating that they can be used to detect mealybugs. Work is underway to evaluate the biology and life cycle of these mealybug pests and to determine the role several parasitic wasp species play in reducing mealybug population levels.

Two studies were conducted to evaluate control of buffalograss chinch bugs using *Beauvaria bassiana*, entomopathogenic nematodes, and combinations of insecticidal soap.

Mean Turfgrass Quality Ratings of Buffalograss Cultivars for Each Month Grown at Nineteen Locations in the United States. 1994 Data.[2]

	Turfgrass Quality Ratings 1 - 9; 9 = Ideal Turf: Months[1]												
NAME	**JAN**	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**	**MEAN**
NE 85-378	5.2	4.5	4.3	5.9	5.8	6.6	6.2	5.8	5.7	4.8	4.3	4.3	5.8
609 (NE 84-609)	5.0	4.7	4.6	4.6	4.9	6.0	6.1	5.6	6.1	5.4	5.8	5.1	5.6
NTG-4	4.8	4.5	4.7	6.1	5.2	6.1	5.9	5.7	5.6	4.9	4.8	4.1	5.6
NTG-5	5.3	5.0	4.2	6.3	5.2	6.1	5.9	5.6	5.3	4.9	4.9	3.6	5.5
315 (NE 84-315)	5.2	4.7	4.7	4.8	5.9	6.4	5.7	5.5	5.5	4.7	4.2	3.4	5.5
NTG-2	5.5	4.2	4.6	6.0	5.1	5.9	5.7	5.6	5.5	4.9	4.8	3.6	5.5
NE 84-436	5.0	5.2	4.7	4.8	4.8	6.1	5.8	5.7	5.6	4.7	5.1	3.4	5.4
NTG-3	5.0	5.0	4.2	4.3	5.1	6.0	5.9	5.6	5.5	4.9	5.2	3.6	5.4
AZ 143	5.5	4.7	4.4	4.9	4.9	6.1	5.6	5.6	5.4	4.7	4.4	3.9	5.4
TATANKA (NTG-1)	4.8	4.8	4.2	4.8	4.9	6.0	5.8	5.1	5.3	4.7	4.9	3.9	5.3
TEXOKA	5.2	4.3	4.3	4.8	4.6	5.7	5.6	5.2	5.2	4.8	4.8	3.8	5.2
BISON	5.2	4.3	4.8	5.4	4.8	5.1	5.4	5.0	5.5	4.8	5.3	3.6	5.1
SHARPS IMPROVED	4.8	4.7	4.8	4.2	4.6	5.7	5.4	5.0	5.3	4.8	5.0	3.7	5.1
TOP GUN (BAM 101)	5.0	4.7	4.3	5.4	4.6	5.6	5.5	5.0	5.1	4.6	4.9	3.3	5.0
PLAINS (BAM 202)	4.8	4.3	4.7	4.8	4.4	5.5	5.4	4.8	5.1	4.8	5.2	3.8	5.0
PRAIRIE	5.2	5.0	4.3	4.3	4.1	5.6	5.3	4.9	5.5	5.1	5.2	4.4	5.0
BUFFALAWN	5.0	5.0	4.1	4.1	3.7	5.5	5.4	5.4	5.7	5.0	5.3	4.2	4.9
NE 84-45-3	5.0	4.2	4.1	4.1	4.3	5.3	5.2	4.6	4.4	4.0	4.2	3.1	4.6
HIGHLIGHT 25	5.2	4.8	3.9	3.9	3.6	5.3	4.7	4.9	5.1	5.7	5.2	4.1	4.5
HIGHLIGHT 4	5.2	4.7	3.8	4.0	3.5	5.0	4.9	4.8	5.1	5.0	5.4	4.1	4.5
HIGHLIGHT 15	5.0	4.2	4.0	4.1	3.4	4.8	4.6	4.4	4.9	5.1	5.3	4.3	4.4
RUTGERS	5.3	4.2	3.4	3.8	3.3	4.8	4.7	4.5	5.0	5.1	5.0	4.0	4.3
LSD VALUE	1.6	1.1	1.9	1.7	0.9	0.8	0.7	0.8	0.7	0.9	1.3	1.9	0.6

[1] To determine statistical differences among entries, subtract one entry's mean from another entry's mean. Statistical differences occur when this value is larger than the corresponding LSD Value (LSD 0.05).

[2] Source: National Turfgrass Evaluation Program. National Buffalograss Test - 1993

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 (Minnesota #'s 42, 184, & 208) were approved for release by the Minnesota Agricultural Experiment Station - Horticultural Plant Release Committee on 14 February 1994. Materials were approved for release under exclusive agreement to Peterson Seed Co., Savage, Minnesota. An exclusive agreement was subsequently executed by the University of Minnesota Office of Research and Technology Transfer (Patents and Licensing) and Peterson Seed Company.

One-acre seed production fields for numbered selections MN#42 and MN#184; and 2 acres of MN #208 were seeded on November 12, 1993 at a site in Oregon under agreement with the Peterson Seed Company. Some of the seed was harvested with a John Deere lawnmower with a grass catching attachment and spread in windrows on paper to cure. However, most of the seed heads were cut with a standard grass seed windrower, which worked well. The grass was cut on June 20, 1994.

MN#42 had the most uniform heading and exhibited the least amount of shattering. MN#184 and MN#208 exhibited some uneven ripening and shattering. There are some indications that two harvests per season may be possible. MN#42 produced 291 total pounds of seed; MN#184 produced 170 total pounds and MN#208 produced 305 total pounds of uncleaned seed.

Three important conclusions from this experimental planting are: 1) the late (November) planting was far more successful than expected; 2) harvesting can be accomplished with standard equipment and practices; and 3) each of the selections produced sufficient seed to warrant continued seed production and introduction.

On the basis of the seed harvest and other factors, we decided, in consultation with Peterson

Seed Company, to concentrate on MN#42 as the first introduction from the program.

On the basis of the decision to concentrate on the introduction of MN#42, a new 5-acre planting of MN#42 was seeded in Oregon about November 1, 1994 for the production of breeder's seed.

The cytology and flow cytometry research has been essentially completed and is currently being written up. 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.

The inheritance of flowering habit and expression of characteristics, under our observations, is influenced by environmental conditions. Observations of crosses between annual (continuous flowering) and perennial (seasonal flowering) type of *Poa* indicate a 3:1 ratio of continuous flowering to seasonal flowering types. If the model holds, it could indicate a single gene difference between continual and seasonal flowering perennial types.

Numerous investigations continue to determine critical photoperiod and vernalization requirements of flower induction in our four genotypes. Preliminary observations indicate that 39 to 46^{0}F is the optimal vernalization temperature and that vernalization is required for 10 to 12 weeks. Plants require no fewer than 4 to 6 leaves in order to be receptive to these flower induction stimuli.

For at least 2 of our genotypes, the critical photoperiod appears to be between 10 and 12 hours.

Other Highlights

- Seed mixtures of MN#42, MN#184, and MN#208 were sown for evaluation for compatibility and performance under putting green conditions.

- Crossing blocks have been constructed to maximize natural crossing and to develop populations for future selection.

- The top 2 percent of new progeny materials were identified for further evaluation. Plants were identified based on superior growth habit, color, disease resistance, vigor and density. These plants will be selfed to observe uniformity and stability of the characteristics.

- Plants resulting from seed of interspecific crosses between *Poa supina* and *Poa annua* have produced some unique plant types which exhibit dark color and vigorous growth habits. These materials will be observed for several generations, and they will be evaluated as parents.

- Preliminary experiments were conducted to investigate the potential for using gibberellic acid to aid in the removal of flowers on *Poa annua* turf. Some concentrations of GA induced sufficient culm elongation to allow removal of substantial portions of flowers under normal mowing conditions.

- Seed has been furnished to the University of Nebraska for a replicated evaluation planting and to the University of North Carolina, Raleigh, for an experiment in overseeding bermudagrass.

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.

New accessions continue to be added to the current germplasm collection at the University of Rhode Island. Four private companies have been actively involved during the acquisition and assessment of materials introduced into this program.

Up to five clones of more than 600 plants were established in the field during fall 1993. A cold, snowy winter and cold spring stressed young plants set in the field in late fall. Most of these plants survived the winter stress, despite their late establishment. Additional material was propagated for greenhouse assessment and planting in other field plots during 1994.

Ms. Pei-Yu Zeng, an M.S. degree student, conducted preliminary and advanced greenhouse screening trials for *Rhizoctonia* sp. (brown patch) resistance in bentgrasses. From eight strains of this fungus, the three most virulent strains were identified for use in the screening of the entire germplasm collection later in the year. Differences among the fungal strains were confirmed by polymerase chain reaction (PCR) technology.

Progeny from 69 accessions were planted last fall in Rhode Island and varied in establishment and survival this spring, as well as possible resistance to weed invasion. The highest-rated material was planted in a polycross this past fall for seed production next spring in Oregon. This material was also included in the brown patch screening at Rhode Island.

Molecular genetics techniques have included successful preparation of both creeping and Colonial bentgrass in tissue culture suitable for gene transfer. Genes that are available and useful for this process are being identified.

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, winterhardiness to expand its adaptation zone, and wear resistance that will meet or surpass golf course requirements.

A 274-ecotype collection of *Paspalum vaginatum* Swartz has been assembled during the last three years. During 1994, collection trips were made to Hawaii (34 ecotypes), Sea Island, GA (133 ecotypes), Jekyll Island, GA (2 ecotypes), Fort Pulaski, GA near Savannah (30 ecotypes), and Tybee Island, GA (6 ecotypes). Four accessions from Brazil and two from Israel cleared quarantine. The oldest known native seashore paspalums located in the U.S., thus far, are those from Sea Island, GA. The golf course on the island was built in 1925 and the grass was already established at that time. Possible collection sites for 1995 may include coastal regions in Florida, Alabama, Mississippi, Louisiana, Texas and southern California.

By October 1994, more than 3,000 plots of seashore *Paspalum* had been planted in Georgia, with about 2,800 plots located in the Griffin area and 350 plots at Blairsville, in North Georgia. More than 2,200 tissue culture regenerated *Paspalums* from six diverse ecotypes were planted in the field. We have collected data on a number of these somaclonal variants and will expose them to winter cold temperatures to assess, and hopefully select, improved winterhardy genotypes.

The winter hardiness (field survival) of seashore *Paspalum* is apparently much greater than what is published in the literature, at least for some ecotypes. ADALAYD supposedly is killed at 17° F, but some of our ADALAYD derivatives (EXCALIBRE, ADALAYD, FIDALAYEL), SIPV-2 (from Sea Island), and three accessions from Argentina (PI509018-1, PI509020, PI-509022) survived -3° F at Blairsville during the 1993-1994 winter. In general, the finer-textured turf types survived better than the coarse-textured types at both Griffin (+3^0 F) and Blairsville. An additional 124 ecotypes are being evaluated at Blairsville (1,500 feet elevation) during 1994-1995. Additional winter hardiness evaluation and improvement was initiated with calli and tissue culture regenerated plants using a cold chamber and modified bermudagrass cold shock-recovery protocol (Taliaferro, Oklahoma State).

Eighteen paspalum ecotypes are being evaluated on a USGA-specification green. In 1994, the grass was mowed at a ½ inch during establishment (from sprigs) and will be maintained at 5/32 inches with a greens mower during 1995. Additional plots will be established during 1995 for herbicide management and fairway moisture utilization studies.

Several genetic analysis techniques have been employed on seashore *Paspalum*, including flow cytometry, random amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphisms (RFLPs), and microsatellites. The techniques are useful in assessing the diversity or relatedness within the collection, in definitively fingerprinting ecotypes, and in genome mapping. Seashore *Paspalum* is a sexual diploid (2N = 20), but sexual incompatibility reduces viable seed production to 5% or less with most ecotypes. Some ecotypes have photoperiod or cool temperature ($\leq 60^0$F) requirements to initiate flowering. Several breeding techniques have been employed to enhance the production of viable seed.

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.

Marking the 10th year of this USGA-sponsored 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. Plant materials are manipulated to produce self- and cross-pollinated seed to expedite their removal from quarantine. Seed are not subjected to the severe testing period or procedures that are required for vegetative material.

The NTEP zoysiagrass trials established in 1991 include nine TAES entries. DALZ8507, a fine-textured, cold hardy *Zoysia matrella*, topped the trials in both 1992 and 1993. Other varieties that are doing well include DALZ8512, DALZ8514, DALZ9006, and DALZ8508.

Breeder fields of DALZ8502, DALZ8507, DALZ8512 and DALZ8514 (15,000 ft^2 0.139 ha each) were planted in July 1992. Considerable planting stock for each of these varieties was harvested and entered into advance testing at ten golf courses throughout the United States.

Foundation fields of DALZ8507, DALZ8512 and DALZ8514 were established vegetatively in June 1994. Planting stock can be harvested by spring 1995 to coincide with the anticipated release of these varieties for commercial production.

The DALZ8502 putting greens at TAES-Dallas continue to perform well. The chipping green established at Colonial Country Club is reported to be doing well. The shaded tee box evaluation supports the potential use of this grass under low light conditions. Rapid regrowth of DALZ8502 occurs due to its extensive rhizome system.

The Linear Gradient Irrigation System (LGIS) has been reestablished with 12 *Zoysia* experimentals, three bermudagrasses, a buffalograss, a St. Augustinegrass and a Texas bluegrass in order to provide extensive inter- and intra-species water-use and cultural input comparisons. The influence of fertility and moisture levels on turfgrass performance is of particular interest.

Mean Turfgrass Quality Ratings of Zoysiagrass Cultivars for Each Month Grown at Twenty-Three Locations in the United States. 1994 Data.[2]

	Turfgrass Quality Ratings 1 - 9; 9 = Ideal Turf: Months[1]												
NAME	**JAN**	**FEB**	**MAR**	**APR**	**MAY**	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**DEC**	**MEAN**
TC 2033	5.7	4.7	5.2	4.8	5.4	6.2	5.9	6.7	6.6	6.5	6.1	4.9	6.1
CD 2013	5.4	4.3	4.7	4.9	5.2	5.8	6.2	6.7	6.2	6.6	6.1	4.3	6.1
DALZ 8507	5.2	5.0	5.3	5.3	5.0	5.8	6.3	6.7	6.4	6.3	5.8	4.4	6.0
EMERALD	5.3	4.6	5.1	5.2	5.4	6.2	6.2	6.4	6.1	6.2	5.6	4.5	6.0
TC 5018	5.4	4.0	4.8	4.7	5.6	6.1	5.9	6.5	6.1	5.9	5.7	4.1	5.9
QT 2004	5.3	4.4	4.6	4.6	5.1	5.5	6.0	6.4	6.2	6.3	6.1	4.2	5.9
SUNBURST	5.0	4.3	4.7	4.8	5.4	5.8	5.9	6.3	6.0	6.3	6.1	4.4	5.8
DALZ 8508	5.2	4.8	5.6	5.3	4.8	5.8	6.0	6.6	6.1	6.1	5.3	4.0	5.7
CD 259-13	5.2	4.2	4.3	4.0	5.6	6.1	6.1	6.1	5.8	5.7	4.9	3.5	5.7
MEYER	5.0	4.3	4.1	4.6	5.2	5.7	5.9	6.2	6.0	6.0	5.5	3.7	5.7
BELAIR	4.8	4.3	3.9	4.1	5.2	5.7	5.6	6.2	5.6	5.6	5.5	3.9	5.6
DALZ 9006	5.9	5.0	5.3	5.4	4.8	5.9	5.9	6.3	6.1	5.9	5.3	4.1	5.6
DALZ 8514	5.7	4.9	4.9	4.8	4.7	5.6	5.6	6.1	6.0	6.0	6.0	4.3	5.5
DALZ 8512	5.7	4.4	4.9	5.0	4.8	5.8	5.7	6.2	5.9	6.2	6.4	4.8	5.5
TGS-W10	5.2	4.0	4.0	4.6	5.2	5.7	5.5	6.0	5.4	5.7	5.3	3.8	5.4
EL TORO	5.3	4.7	4.9	4.8	4.5	5.5	5.5	6.0	5.8	6.1	6.3	4.3	5.3
TGS-B10	5.3	4.2	4.2	4.1	5.1	5.5	5.5	5.9	5.5	5.5	5.1	3.7	5.3
QT 2047	4.8	4.0	3.8	4.2	5.1	5.4	5.4	5.7	5.5	5.4	4.7	3.5	5.3
DALZ 8516	5.3	4.7	4.9	5.3	4.6	5.1	5.1	5.4	5.4	5.9	5.7	4.2	5.0
KOREAN COMMON	4.9	4.1	4.1	4.1	4.9	5.1	5.1	5.4	5.1	5.2	5.0	3.5	5.0
JZ-1	4.9	4.2	4.1	4.1	4.8	5.0	5.1	5.4	5.1	5.5	5.2	3.2	5.0
DALZ 8502	6.0	5.2	4.8	5.0	4.2	4.7	4.8	4.8	5.2	5.4	5.7	4.7	4.6
DALZ 8501	4.8	4.7	3.9	4.4	3.9	3.8	4.2	4.2	4.4	4.9	5.1	3.6	4.0
DALZ 8701	5.6	5.2	3.9	4.3	3.4	4.1	4.0	4.2	4.2	4.9	5.6	3.6	3.8
LSD VALUE	1.3	0.8	1.0	0.9	0.7	0.6	0.6	0.6	0.6	0.7	0.9	1.2	0.5

[1] To determine statistical differences among entries, subtract one entry's mean from another entry's mean. Statistical differences occur when this value is larger than the corresponding LSD Value (LSD 0.05).

[2] Source: National Turfgrass Evaluation Program. National Zoysiagrass Test - 1993.

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.

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, but water use data are scarce and one can only speculate on water requirements. This study is generating crop coefficients for buffalograss and identifies intraspecific water use differences among a diverse selection of genotypes.

A 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 utilizes a line source water gradient in which buffalograss varieties are planted in strips along the gradient. Turf performance can be measured at any given irrigation amount, and minimum irrigation requirements are indicated by that 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 will be 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 fesuce.

The line source gradient was established in July, 1994, with the irrigation scheduled based on ET (modified Penman) as determined with weather station data. Data on ET under non-limiting conditions, turf quality, canopy temperature, soil moisture, minimum water requirements, and plant water status were collected during 1994.

The data demonstrate significant differences among genotypes for water use (crop coefficients ranged from 0.76 to 1.02) and turf quality. 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 this experiment (31 days), the point demarcating the minimum irrigation required to prevent total dormancy corresponded to approximately 10 to 20% of ET. It is apparent from this preliminary data that buffalograss can produce an acceptable turf with deficit irrigation of 50 to 60% ET, at least for a relatively short period of time. The experimental period will be extended to at least 10 weeks in 1995 to stress the turf more severely.

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

Dr. Robert Carrow

University of Georgia

Goals:

- ET, drought resistance, rooting/water extraction patterns and shoot responses will be determined under field conditions.
- Basic cultural programs (fertility, disease/insect, traffic tolerance) will be defined.
- Determine genetic stability of these grasses with respect to environment, disease, and insect pressures.

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 world-wide, 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 is evaluating 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 (AZ common and Primavera) under three traffic levels and three nitrogen fertilizer regimes.

Results to date:

1. The most rapid establishment was observed for Primavera, 91-2, 91-1, and 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 experimentals.

Data was obtained in 1994 on genotype responses under the traffic and N treatments for shoot aspects, rooting, water use, water extraction by root depth, and rhizome production. Further data will be obtained in 1995 before conclusions are developed.

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.

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.

Two key individuals were added to this project since July. Mr. David Gilbert, a Master's Degree candidate in the Soil & Crop Sciences Department, will evaluate the water balance in 20 cultivars and experimental lines of creeping bentgrass. The second key individual is Mr. Gene Taylor, a Ph.D. candidate in the Soil & Crop Sciences Department. His research responsibilities will emphasize the genetic links to water stress resistance mechanisms in an elite population of creeping bentgrass. Mr. Taylor will conduct allied studies to assess the response of creeping bentgrass root development to temperature.

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 (MLFA) respond to low temperature are unknown for bermudagrass.

Bermudagrass total MLFA (ug), per unit of total lipids (mg), increased in crown tissues, but not in roots or leaves, over the four week exposure to moderately low temperatures (46°F day /37°F night, 14 hour photoperiod). The major fatty acids in bemudagrass were determined to be palmitic acid, stearic acid, linoleic acid, and linolenic acid. These four made up 95% of the total MLFA.

In bermudagrass crown tissues, the concentration of shorter chain and saturated fatty acids declined significantly during the cold treatment, while the concentration of the longer chain, unsaturated fatty acids increased. As a result, the double bond index increased in crown tissues over this same four-week period. These changes increase the fluidity of membranes, and, therefore, could reduce cold-induced membrane leakage and freezing injury.

Messenger mRNA profiling and differential display techniques are being refined and employed in our efforts to characterize genetic polymorphisms between bermudagrass cultivars differing in levels of cold tolerance. These PCR-based methods allow for the relatively rapid identification and cloning of gene sequences expressed in response to a particular environmental stimulus such as low temperature, drought or chemical applications. The reproducibility of this technique and the identification of the appropriate mRNA or DNA primers has been the focus of our current efforts.

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

Dr. Charles Mancino

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.

Two weighing lysimeters were installed at the University of Arizona Karsten Turfgrass Research Center for researching the consumptive water use of turf, nitrogen fertilizer loss through leaching, and solute transport through a fine sand soil profile.

Each tank is 13 feet deep and 8 feet in diameter, and has a soil-filled weight of approximately 99,120 lbs. Each tank has sampling ports in groups of five, which are spaced at 120° intervals around the tank. These ports begin at the 3.3 foot depth (level) and are then positioned every 1.6 feet down to a depth of 11.5 feet. Tensiometers with pressure transducers, Time Domain Reflectometry (TDR) probes, and ceramic and stainless steel solution samplers have been installed, three at each level.

A single neutron probe access tube was installed in the center of each lysimeter. These devices are used for sampling soil water and monitoring soil moisture content. A Cardinal scale with an electronic loadcell measures changes in tank weight to ± 0.44 lbs (0.002" water loss or gain).

Atmospheric and turfgrass water use data collection began in 1995 following about eight months of data collection with bare soil. The data continue to show that the two giant weighing lysimeters are behaving similarly and accurately.

Results show that the various Penman equations being tested in this project respond similarly to changes in atmospheric conditions. Some variation does exist in how well these equations predict actual turf water use, as measured by the lysimeters. All of the equations overestimate turf water use. The Kc values (ratio of actual turf water use to predicted water use) of this bermudagrass turf appear to be 86% to >100% depending on the particular equation. This is higher than currently reported in the literature, but it may be due to the immaturity of the bermudagrass turf stand in 1994.

Next year's data should clarify this issue. An atmospheric and turfgrass water use database is being collected and will become available to the private and public sector for evapotranspiration (ET) equation development and testing after completion of this project. It will provide more atmospheric data than is usually needed for calculating ETo. This additional information may be useful in determining why predicted water use values differ from actual water use.

Data collection is completed for the 1994 bermudagrass growing season and has begun on winter overseeded ryegrass. These data will shed light on the water requirements of winter turf, particularly in the spring when air and soil temperatures increase. The fate of nitrogen applied to turf in the lysimeters will be investigated in summer 1995 after the bermudagrass is fully mature.

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

Development of Improved Turfgrass with Herbicide Resistance and Enhanced Disease Resistance Through Transformation

Dr. Peter Day

Rutgers University

Goals:

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

Through genetic engineering, effective and selective weed control with herbicides and more environmentally sound and cost-effective control of plant diseases with reduced use of fungicides is possible. We have reached several milestones: successful turfgrass transformation, efficient tissue culture and regeneration systems, recovery of several cultivars of creeping bentgrass with resistance to two different herbicides, and field tests of clones of Ignite-resistant creeping bentgrass. Transgenic bentgrass, a product of laboratory experimentation, shows promise as a useful tool for golf course management.

We are making good progress in incorporating single gene traits for herbicide resistance and enhanced disease resistance in turfgrass. We now have embryogenic callus lines and suspension cultures derived from them with high regeneration potential from nine creeping bentgrass cultivars. We have established both particle gun and protoplast transformation systems for creeping bentgrass and have obtained first-generation stable transformants with resistance to the herbicide Ignite (bialaphos).

In tests of more than one thousand regenerants from transformed tissues for herbicide sensitivity in the greenhouse, we have obtained more than one hundred herbicide-resistant transgenic plants of COBRA, EMERALD and SOUTHSHORE. This summer, we conducted the first field test of herbicide-resistant creeping bentgrass in the USA, and showed that the transgenic plants were resistant to up to three times (2.25 lb AI/A) the label rate (1.5 to 4 fluid ounces per gallon of water).

To enhance fungal disease resistance in turfgrass, three chitinase gene constructs were obtained, adapted to our transformation vectors, and introduced into creeping bentgrass through particle gun and protoplast transformation. We have begun greenhouse herbicide tests with putative transgenic plants that carry genes expressing bean chitinase or tobacco chitinase B, and are preparing to test transgenic plants for disease resistance.

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 *E. 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.

More than one thousand creeping bentgrass plants have been putatively transformed for herbicide and insect resistance with the *bar* and *pinII* genes. They exhibit resistance to Ignite herbicide (1.2% commercial product containing 200g/L active ingredient of glufosinate ammonium) foliar spray. Molecular analysis of these plants is in progress.

A chitinase gene cloned from elm trees has been successfully manipulated to express in *E. coli*. The *E. coli* cells with the gene produced the GST-chitinase fusion protein. Attempts to purify the GST-chitinase fusion protein were not successful. Therefore, experiments were set up to collect fractions of the bacterial extract at several steps during the purification process. These experiments resulted in discovering that the GST-chitinase fusion protein was not soluble in the buffer used.

In an effort to remedy this situation, two steps have been taken. The first step is to remove the signal peptide currently located at the amino terminal of the protein. This amino acid sequence is 16 amino acids in length and is highly hydrophobic, which may be contributing to the insolubility of the GST-chitinase fusion protein. A PCR primer has been designed and will be used to produce a PCR product containing the chitinase gene without the hydrophobic signal peptide. This PCR product will be cloned into a bacterial expression vector. The construct will be used to transform *E. coli* and will be induced to determine if they contain soluble chitinase with antifungal activity.

The second step underway is transformation of tobacco with the chitinase gene isolated from elm. Tobacco is relatively quick and easy to transform via *Agrobacterium*. This successful transformation will give us information about the plant's ability to produce active chitinase from this gene. If tobacco can successfully produce elm chitinase with anti-fungal activity, it would indicate that bentgrass plants will likely do the same. At that point it is expected that no further manipulation of the elm chitinase gene will be necessary before the transformation of bentgrass plants. Upon successful transformation of tobacco, leaf extracts will be used in bioassays to test for antifungal activity.

Antifungal bioassays have been attempted. However, due to the insolubility of the GST-chitinase fusion protein produced in bacteria, no activity has yet been observed.

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 overexpression 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 via genetic engineering. The objectives are 1) to develop efficient gene transfer systems in creeping bentgrass and 2) to develop transgenic creeping bentgrass that is resistant to fungal diseases by overexpression of a chitinase gene.

For the second year of this project we focused our research efforts on developing a gene transfer system for creeping bentgrass and isolating a chitinase gene from Kentucky bluegrass.

We have developed an efficient gene transfer system for creeping bentgrass using particle bombardment. 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.

Using a PCR *in vitro* cloning method, we have isolated a chitinase gene from Kentucky bluegrass. We are currently determining the sequence of this gene.

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

Dr. Jeffrey Krans

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.*

Research efforts in 1994 focused on developing a valid, quantifiable procedure at the whole plant level to verify resistance to *Rhizoctonia solani* exhibited by Host Pathogen Interaction System (HPIS) derived creeping bentgrass variants. Several studies were conducted to address inoculation techniques, optimum environmental factors for disease, and evaluation of the pathogenicity of *R. solani* isolates.

Four creeping bentgrass lines have been identified with enhanced resistance to *R. solani*. This study was conducted using HPIS derived bentgrass variants maintained in pots in an environmentally controlled greenhouse. Under ideal disease conditions for brown patch, HPIS variants were inoculated with a highly pathogenic isolate of *R. solani*. Average diameter of diseased turf for lines displaying enhanced resistance were 21.6, 25, 27.5, and 28.3 mm. These values represent a significant improvement compared to PENNCROSS, with an average 50.0 mm of diseased turf.

This phase of research has shown that the HPIS is a valid *in vitro* cell selection technique for selecting creeping bentgrass germplasm with enhanced resistance to *Rhizoctonia solani*. Prior to field evaluations, selected creeping bentgrass lines have undergone three levels of exposure to *R. solani*. A cycle for selecting germplasm with enhanced resistant has been accomplished beginning at the cellular level, affirmed at the plantlet level (*in vitro*), and confirmed at the whole plant level.

Selected lines will be evaluated in the field under golf course conditions. Continued progress will lead to parental lines of creeping bentgrass that exhibit enhanced resistance to *Rhizoctonia solani*.

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.

Summer patch, caused by the ectotrophic, root-infecting fungus, *Magnaporthe poae*, is a devastating disease of cool season turfgrasses. Current control methods for summer patch, as well as other patch diseases caused by root-infecting fungi, rely heavily on the use of fungicides. In efforts to reduce the amount of fungicides used to control turfgrass diseases such as summer patch, we are investigating the potential use of beneficial bacteria as biological control agents for the disease.

In previous studies, we isolated several bacteria by a fungal trapping method and by enrichment procedures that were capable of suppressing summer patch symptom development at significant levels under controlled environmental conditions. Characterization of these bacteria indicated that several isolates shared common features, including the expression of extracellular enzymes such as chitinases, glucanases, lipases or proteases.

In addition, all bacteria identified as good suppressors were capable of colonizing the turfgrass rhizosphere at high concentrations. A few isolates were observed to produce antibiotic-like activity against *M. poae* in *in vitro* assays. Two bacteria, *Xanthomonas maltophilia* and *Serratia marcescens*, were further characterized for their suppressive abilities. These bacteria were found to consistently suppress summer patch symptoms at greater than 50 percent compared to untreated control plants over a three week period.

Further characterization of these bacteria indicate that application timing is important relative to the level of disease suppression that is achieved. In general, when bacteria were applied prior to fungal colonization of plant roots, less disease suppression was achieved.

Dose level of bacteria also affected the level of disease suppression. A slight but significant difference was observed for the lowest and highest doses of *X. maltophilia*, ranging from 10^8 to 10^{10} cells/ml, in which disease suppression was greatest for plants treated with the highest dose. However, plants treated with similar doses of *S. marcescens* responded in drastically different fashion. Optimal suppression was observed with 10^9 cell/ml. The level of disease suppression decreased with either increasing or decreasing doses from this cell concentration.

This data, correlated with root and soil populations of both bacteria, suggest that bacteria had a direct effect on the fungal pathogen inoculum density in the soil.

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, in field studies, the expression of the biocontrol-related gene, *psp1*, under typical turfgrass management conditions.
- Identify nucleotide sequences of *E. cloacae* DNA encoding for pathogen suppression.

The purpose of this project on *Enterobacter cloacae* genetics is to determine the array of bacterial traits responsible for biological control activity in bacteria. Our focus has been on *Pythium*-incited disease of creeping bentgrass, but we believe our studies will have broad applicability to other bacterium-pathogen interactions.

Our studies in 1994 focussed primarily on the identification of nucleotide sequences of *E. cloacae* DNA encoding for pathogen suppression. Prior to the initiation of the work, we had isolated mutant V58 which was unable to suppress *Pythium ultimum* seed rot of cucumber. We were able to verify that, whereas the wild-type strain, EcCT-501, was an effective biological control agent of *Pythium graminicola* on creeping bentgrass, mutant V58 was an ineffective biological control agent against this pathogen. Our work subsequently was focussed on the molecular and physiological characterization of mutant V58.

We have inserted the wild-type DNA into the mutant strain V58 and effectively restored all of the biological phenotype of the wild-type strain. Using conventional molecular techniques, we have been able to isolate and sequence portions of the disrupted gene in mutant V58. From sequence analyses, we have discovered that the gene shares high homologies, both at the nucleotide and amino acid levels, with malate dehydrogenase from both *Escherichia coli* and *Salmonella typhymurium*. We performed a series of enzyme assays to verify that the gene we had cloned was actually a malate dehydrogenase (Mdh) gene. Clearly, the wild-type EcCT-501 possessed high levels of Mdh activity. This activity was totally absent in mutant V58.

Furthermore, upon complementation with the putative Mdh gene, Mdh activity was restored. Therefore, we feel confident that we have discovered a malate dehydrogenase gene with a major influence on the biological control of *P. graminicola* on creeping bentgrass and *P. ultimum* on cucumber.

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

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.

Fertilization, watering, mowing height, soil compaction, soil pH, and aerification were manipulated in large field plots to determine how these factors affect choice of egg-laying sites, and subsequent population of Japanese beetle, masked chafer, and green June beetle grubs. Soil moisture was the most important factor determining abundance of white grubs; infestation levels were 2- to 4-fold higher in irrigated plots. In contrast, grubs were less abundant in high-mown turf, and in plots treated with sulfur to lower soil pH. In 1994, for example, total biomass of grubs was reduced by 55 percent and 77 percent, respectively, in high-mown and sulfur-treated turf. Liming, fertilization with urea, heavy rolling, and aerification had no effect on white grub population during this four-year study.

The number of grubs required to cause noticeable injury was found to be much higher in all common turfgrasses than suggested by prevailing rule-of-thumb estimates used by the industry. Irrigation and fertilization encouraged regrowth of foliage and enhanced appearance and rooting strength of grub-damaged turfgrasses.

Most 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 80-91 percent of black cutworm eggs laid on bentgrass greens. This suggests that cutworm infestations may originate from larger larvae that migrate onto greens from aprons or roughs. Cutworms showed no preference between aerified and non-aerified areas, but our results suggest that they may be repelled by sand topdressing. When aerification holes were available, about 60 percent of cutworms used them as a refuge. Cutworm larvae were most active on greens from midnight until just before dawn. Most larvae were observed grazing, i.e., feeding on the turf surface, suggesting that control measures would be most effective if applied in the early evening or at night.

A study was conducted in 1994 to clarify how long it takes for the population of predators, earthworms, and other beneficial species to return to normal level following use of broad-spectrum insecticides. Ethoprop (Mocap) applied in April resulted in 100 percent kill of earthworms. Populations were still reduced by 70 to > 90 percent in both small and large plots at the end of the growing season. Samples containing predators and other beneficials are presently being sorted and analyzed.

Analysis of female extracts by electrophysiology and gas chromatography pinpointed the fraction containing the sex pheromone of masked chafer beetles. 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 Brandenburg
North Carolina State University

Dr. Michael Villani
New York State Agricultural Experiment Station/Cornell University

Goals:

- Isolate and determine the activity of sex, aggregation and alarm pheromones of the tawny mole cricket.
- Improve our understanding of environmental conditions that affect tawny mole cricket and southern mole cricket behavior.
- Compare the activity of healty mole crickets to crickets infected with microbial control agents.

Tawny and southern mole crickets were collected in North Carolina and transported to the New York State Agricultural Experiment Station, Geneva, New York for laboratory analysis. It was noted 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 (GC). Although there appeared to be basic similarities in the discharges from the two species, as indicated by overlapping peaks in parts of the GC detection strip charts, there were also clear differences in the tawny and southern mole crickets discharges (Figure 1a and 1b), indicating unique compound constituents in the discharges for these two species. We are currently working in concert with electro-antennogram analysis to determine which peaks are bioactive and therefore should be analyzed further.

The purpose of this procedure was to determine whether the volatiles emitted from the crude discharges of the tawny and southern mole crickets caused a neurological response from the ablated antenna of either mole cricket species. The antenna of either cricket was attached through a saline-electrode system to an oscilloscope chart recorder; volatiles were then puffed over the antenna. There was no measurable response from the southern mole cricket antenna (a predator of TMC) suggesting that they cannot detect discharge volatiles from either species through sensory organs in their antenna. However, tawny mole cricket antenna reacted positively to volatiles from either species discharges. Each time volatiles were puffed over tawny mole cricket's antenna, the antenna discharged a pulse on the oscillascope, indicating the sensory organs on the antenna were sensing compounds from both species. Tawny mole crickets are prey for the southern mole cricket, suggesting that these discharges may serve as alarm and warning pheromones for the tawny mole cricket.

Tawny mole cricket discharge was collected on absorbent cotton and placed in soil arenas along with several tawny nymphs. Radiographic analysis showed a clear avoidance of tawny mole cricket to areas near the cotton with the alarm discharge, further suggesting the biological activity of the discharge.

Studies were begun using radiographic technology (x-rays) to visualize the movement and feeding patterns of both TMC and SMC in the soil matrix. Through the placement of a small lead tag on each cricket, tunnel construction and cricket movement in the tunnel could be monitored over time. These studies indicate: a) TMC produce a characteristic 'Y' shaped tunnel that allows two escape routes to the surface, and a long tunnel into the soil profile that most likely aides in thermal and water regulation; b) each TMC builds its own tunnel system that it maintain over time; c) as TMC grow, their burrows widen and extend further into the soil profile, suggesting a possible cause for the difficulty in bringing older crickets to the surface through soap flushes and baits; and d) SMC appear to create less extensive burrows than do TMC.

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 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 perennial ryegrasses, which range from moderate to high stand density and 0 to 95 percent endophyte infection, were established into six replications 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%), Omega II (76%), Manhattan II (50-90%), Saturn (80%), SR 4200 (80-85%), Brightstar (90%), Assure (95%), and Yorktown III (97%). The plots are maintained with good fertilizer, weed control, irrigation, and 2 cm mowing practices.

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. Loretta, Derby Supreme, Envy and Brightstar inhibited duckweed at certain concentrations. Stimulation of duckweed occurred from most other concentrations and cultivars. Root extracts from Gator, Derby, Saturn, SR 4200, Brightstar and Assure were tested at three concentrations. Full strength extracts from Gator, Saturn and Brightstar stimulated duckweed, but no effects were found from other concentrations.

We have developed a crabgrass bioassay which uses a ryegrass extract-agar. Ryegrass 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. Problems with procedures to improve crabgrass germination and surface sterilize the seed to prevent fungal contamination have been largely overcome.

Determination of *Acremonium* endophyte content of stem samples from field plots showed actual infection levels different from those expected, so monitoring endophytes is important.

One half of each field was overseeded with crabgrass in March 1994. Crabgrass stands ranged from 16 to 23 percent of plot cover by late June, but the correlation between perennial ryegrass density in May and crabgrass cover was not statistically significant.

The original field plots were overseeded with small amounts of seed from the original seed lots in mid-October 1994. Bermudagrass "fairway" plots were overseeded with new seed lots of the 12 cultivars on October 25, 1994. Half of all plots will be overseeded with crabgrass early next spring and evaluated for crabgrass suppression.

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.

We are describing a new species of bacterium in the genus *Pasteuria* that we discovered parasitizing the sting nematode, *Belonolaimus longicaudatus* in Florida. We 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 30-50 percent.

In 1994, we completed ultrastructural studies with transmission electron microscopy (TEM) that showed that *Pasteuria* n. sp. (S-1) is a new species and helped to elucidate its life cycle. The sporangium and endospore diameters of *Pasteuria* n. sp. (S-1), are on the average at least 1.0 and 0.5 μm wider than these respective measurement for the other described species of *Pasteuria*. The outer cortical wall thickness at its thickest point is ⅓ the endospore diameter for *Pasteuria* n. sp. (S-1), compared with 1/4-1/15 for the other described species of *Pasteuria*.

A brief description of the life cycle follows. After attachment of a mature endospore to the cuticle of the host, penetration ensues via a germ tube through the cuticle into the pseudocoelom of the nematode. A mycelial microcolony is formed, which eventually breaks up and is distributed throughout the pseudocoelom (fragmentation). Mycelial filaments are divided by septa and possess double-layered cell walls. Endospores are produced endogenously, and the formation sequence (sporogenesis) for *Pasteuria* n. sp. (S-1) is similar to the three other described species of *Pasteuria*. A septum is formed within the sporangium, the sporangium cytoplast condenses to form a forespore, the endospore walls form, the endospore matures, and areas adjacent to the endospore give rise to perisporal "attachment" fibers.

Laboratory host attachment studies and field observations completed in 1994 on *Pasteuria* n. sp. (S-1) demonstrate that it is highly host specific and attacks only nematodes in the genus *Belonolaimus* or within the species *B. longicaudatus*. We have initiated population dynamic studies on *Pasteuria* n. sp. (S-1) in laboratory pot cultures of the sting nematode on the model turfgrass host (FX-313 St. Augustinegrass) under controlled conditions. After 84 days, sting nematode cultures which were inoculated with 10 or 25 sting nematodes with *Pasteuria* n. sp. (S-1) have not shown suppression or a disease epizootic. The experiment will run for at least 210 days and it may take at least this long for establishment of the bacteria under these conditions.

In 1995, we propose to do studies on the effects of temperature on development of *Pasteuria* n. sp. (S-1), and conduct monthly survey work in golf course areas where this bacterium occurs naturally, to assess its suppressive effects on sting nematodes. We also will begin sampling golf course areas where sting nematode is no longer a problem, to try and isolate different species or isolates of antagonists to nematodes of turfgrass.