1996 ANNUAL RESEARCH PROGRESS REPORT

of

PHYSIOLOGICAL BASIS FOR SELECTION OF BENTGRASSES WITH SUPERIOR DROUGHT RESISTANCE

Submitted by:

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Executive Summary

Principle Investigator:	Dr. Richard H. White
Graduate Research Assistant:	Mr. Gene Taylor and Mr. Scott Abernathy
Research Period:	1 November 1995 through 1 November 1996

Electrophoretic analysis of isozyme banding patterns from samples collected from two inter-seeding locations is providing, through close cooperation with Drs. M. C. Engelke and Ikuko Yamamoto, the necessary information to determine population changes. Analysis of samples collected from the TAMU-REC at Dallas location 6 and 14 months after inter-seeding indicate that Crenshaw contributed from about 10 to 95% of the plant population.

Analysis of samples collected from the Dallas Country Club location 4 weeks after inter-seeding indicated, based on plant density counts, successful emergence of Crenshaw in an existing Penncross bentgrass putting green and was superior to emergence observed in spring 1995 inter-seedings at the TAMU-REC at Dallas location. Mechanical treatments had minimal effect on seedling emergence. Isozyme analysis of samples collected from the Dallas Country Club location 6 months after inter-seeding indicate that, overall, Crenshaw contributed less than 10% of the plant population. Water management during the extremely dry fall and winter of 1995 and 1996 probably contributed to very low percentages of Crenshaw in the population at the Dallas Country Club location.

Selection of maternal clones and populations to assess mechanisms of stress resistance has progressed with the assistance and close cooperation of Dr. Milt Engelke. Initial stress tolerance of maternal clones was completed in fall 1995. Progeny were obtained and increased to assess progeny response and established in parent/progent tests in January 1996. Disease problems occurred in the initial parent/progeny plantings and through the summer of 1996. Parent progeny tests were reestablished during fall 1996 and are progressing but behind schedule.

Studies to determine the effects of blending bentgrass cultivars on bentgrass putting green turf quality, disease resistance, and performance were established at the Turfgrass Field Laboratory in College Station, Texas during the fall 1995. A severe thunderstorm caused soil and seed movement and cross-contamination of treatments. These blending experiments are being reestablished on a newly constructed 25,000 square foot golf green in College Station. This concept is extensively use for other cool-season grasses, but has not been reported for creeping bentgrasses.

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PHYSIOLOGICAL BASIS FOR SELECTION OF BENTGRASSES WITH SUPERIOR DROUGHT RESISTANCE

Richard H. White

I. I. INTRODUCTION

This program is a cooperative research project funded jointly by the Texas Agricultural Experiment Station (TAES) and the United States Golf Association (USGA). This project was initiated in August 1994. Annual progress reports are submitted 1 November each year and semi-annual progress reports are submitted in 1 May. This report constitutes the 1996 annual progress report for the project and highlights activities between 1 November 1995 and 1 November 1996.

II. PROFESSIONAL AND TECHNICAL SUPPORT

Mr. David Gilbert was a Graduate Research Assistant and an M. S. Degree candidate in the Soil and Crop Sciences Department at Texas A&M University. David lived in Dallas and conducted his research at the TAMU Research and Extension Center at Dallas. Unfortunately, Mr. Gilbert changed his career and educational objectives during the winter of 1995 and 1996 and entered medical school during the summer of 1996. Dave's contributions to this project will be missed.

Mr. Gene Taylor joined the project in August 1994. Gene is a Ph. D. candidate and Graduate Research Assistant funded by this USGA grant. Gene holds a B.S. degree from East Carolina University and an M. S. degree from North Carolina State University. Dr. Milt Engelke is co-chair of Gene's graduate committee. Mr. Taylor is preparing his Dissertation Research Project to address mechanisms of summer stress tolerance in creeping bentgrass with emphasis on water balance.

Mr. Scott Abernathy joined the project in August 1996. Scott received his B. S. in Biomedical Sciences in December 1995. Following graduation Scott worked in Dallas, Texas at BentTree Country Club for 9 months. He returned to the Soil and Crop Sciences Department at Texas A&M University to pursue a M. S. degree in Agronomy in August 1996. Scott will provide support for the bentgrass blending and bentgrass inter-seeding portions of this project.

III. BENTGRASS INTERSEEDING

A. Bentgrass Inter-seeding - Spring 1995 and Fall 1995

Objectives: The objectives of this study are to determine the effects of mechanical and chemical treatments on the establishment of Crenshaw creeping bentgrass interseeded into existing Penncross creeping bentgrass putting green turf.

Materials and Methods: Two primary locations are used for this study. An Penncross green at the Texas A&M University Research and Extension Center at Dallas (TAMU-REC at Dallas) was inter-seeded in April 1995. The second location is a Penncross nursery green at Dallas Country Club in Dallas, Texas.

The experimental design used in all locations was a multiple-strip-split plot design. Treatments were seeding, mechanical disruption, and chemical suppression. Main plots were seeding treatments consisting of seeding or no seed application. Sub-plots were chemical suppression treatments consisting of none, cimectacarb (Primo), and glyphosate (Roundup). Sub-sub plots were mechanical disruption treatments consisting of none, vertical mowing, star-tine aerification, and core aerification. Chemical and mechanical treatments were systematically arranged in row and column strips to include all possible combinations of chemical with mechanical treatments. Chemical treatments were applied about 72 hr prior to mechanical treatment aerification. Mechanical treatments were applied followed by seed treatments and the area uniformly topdressed. Seed were applied at 1.0 lb per 1000 square feet. Experimental areas were maintained as a putting green throughout the study period. Daily mowing was applied continuously and immediately after seeding. Random plant density counts within each plot were taken about 4 weeks after planting to determine seeding emergence. Data were analyzed by appropriate statistical models to determine treatment effects.

The primary focus since the last report was the use of electrophorectic identification of populations in inter-seeded Penncross experimental areas to assess the success of establishing the inter-seeded Crenshaw. This technology proposed by Dr. Yamamoto-Tucker, Post-doctoral Fellow working under the direction of Dr. M. C. Engelke, is applied through close collaboration with Dr. Engelke's research group. This technology allows the determination of the percentage of a bentgrass turf populated by the germplasm used for inter-seeding compared with the percentage of the stand populated by the original or existing bentgrass. In this technique, the green's composition is estimated by comparing the isoenzyme banding patterns from the inter-seeded green with those of standard blends (proportional blends produced under laboratory conditions). Dr. Yamamoto presented the technique and prelimary results at the 1995 American Society of Agronomy, Crop Science Society of America annual meeting in St. Louis, MO during the week of October 29, 1995.

Results: Seedling counts indicated greater plant density in the seeded than no seed treatment the TAMU-REC at Dallas and the Dallas Country Club (Table 1). Vertical mowing was superior to other mechanical treatments in aiding initial seedling establishment at the TAMU-REC at Dallas location but mechanical treatments were similar at the Dallas Country Club. Absolute plant density was greater for no mechanical treatment at both locations. Topdressing following inter-seeding may be a reasonably good way to provide good germination without other means of mechanical incorporation.

Table 1. Seedling emergence 4 weeks after inter-seeding at the Texas A&M University Research and Extension Center at Dallas, Texas in April and the Dallas Country Club in October 1995.

Seeding treatment	Mechanical treatment	May[H] 1995	Nov 1995
		------------- plants m^{-2}	--------------
No seed	Core tine	237 c	581 c
No seed	Star tine	161 c	560 c
No seed	Verticut	194 c	484 c
No seed	None	194 c	312 c
Seeded	Core tine	538 b	1378 ab
Seeded	Star tine	624 b	2110 a
Seeded	Verticut	1894 a	1238 ab
Seeded	None	926 b	2282 a

[H]Means within a column followed by the same lower case letter are not different at the 0.05% level of probability. Plant density was determined only for the glyphosate treatment.

Isozyme analysis allows for the genetic identification of Crenshaw and Penncross as well as some other creeping bentgrass genotypes. Because of unique band markers possessed by Crenshaw, a determination of the percentage Crenshaw and Penncross can be made for a blend of these two cultivars. When samples with unknown populations of Crenshaw and Penncross are compared with standard blends, the isozyme analysis technique can determine the percentage ("5%) of each cultivar in the blend. The technique is invaluable to the assessment of the success of inter-seeding programs and to this research project.

Isozyme analysis is currently being used to identify changes in the population caused by inter-seeding at both locations. Isozyme analysis was conducted 6 months after establishing the inter-seeding study at the TAMU-REC at Dallas and the Dallas Country Club. Across all treatments, the single inter-seeding of Crenshaw into the existing Penncross putting green at the TAMU-REC at Dallas caused an overall shift to about 25% Crenshaw in the bentgrass population 6 months after inter-seeding (Table 2). The percentage increased to 35% at about 14 months after inter-seeding. However, Crenshaw percentages ranged from about 7 to 33 at 6 months after inter-seeding for the no chemical treatment. At about 14 months after inter-seeding, the percentage of Crenshaw ranged from about 8 to 16 for the no chemical treatment.

Severe growth suppression of the existing Penncross by glyphosate allowed more rapid conversion to Crenshaw. Glyphosate in combination with all mechanical treatments except core tine cultivation resulted in 50% or more Crenshaw in the population 6 months after inter-seeding and as much as 95% at about 14 months after inter-seeding. Suppression of existing bentgrass is too aggressive to be of much practical importance on most golf course putting greens. Turf quality evaluations indicate that the inter-seeded cultivar and incompletely glyphosate killed existing cultivar provide recovery that is too slow for this to be pragmatic.

Table 2. Percentage of Crenshaw creeping bentgrass identified by isozyme analysis 6 and 14 months after inter-seeding into an existing Penncross putting green at the Texas A&M University Research and Extension Center at Dallas, Texas.

Seeding	Percentage Crenshaw			
treatment	glyphosate†	cimectacarb	none	Mean
	6 months			
No seed	3	3	5	4
Seeded	51*	11	14*	26*
	14 months			
No seed	3	3	3	3
Seeded	83*	10	11	35*

†Chemical treatments were applied 7 days before mechanical and seeding treatments. Glyphosate did not cause complete kill of the existing Penncross.

*Significantly different within a sampling date and chemical treatment at the 0.05 level of probability.

Seedling emergence (Table 1) at the Dallas Country Club location 4 weeks after inter-seeding indicated, based on plant density counts, successful emergence of Crenshaw in an existing Penncross bentgrass putting green and was superior to emergence observed in spring 1995 at the TAMU-REC at Dallas location. Mechanical treatments had minimal effect on seedling emergence. Isozyme analysis of samples collected from the Dallas Country Club location 6 months after inter-seeding indicate that, overall, Crenshaw contributed less than 10% of the plant population. Water management during the extremely dry fall and winter of 1995 and 1996 probably contributed to very low

IV. BENTGRASS SUMMER STRESS TOLERANCE

Objectives: Determine the genetic variability and heritability of water deficit tolerance among creeping bentgrass germplasm and cultivars.

Materials and Methods: This study is proceeding in three stages: first, determine if there are significant differences in the ability to tolerate low soil water potentials between a group of 28 maternal bentgrass varieties; Second, determine if observed levels of high, medium and low water stress tolerance is passed on to progeny plants from a select group of maternal plants; Third, determine major physiological and morphological characteristics which influence water deficit stress tolerance in the creeping bentgrass plants.

Stage one of this study was initiated in June of 1995 when samples of 28 different maternal creeping bentgrasses were collected from the creeping bentgrass breeding program field evaluation plots, at the TAMU-REC at Dallas under the direction of Dr. Milton Engelke. The maternal plants were transported to the Texas A&M Turfgrass Field Laboratory in College Station, Texas where they were planted in flats for propagation. The plants were allowed to grow in the greenhouse for eight weeks to allow them to reach maturity. On August 28, 1995 eight 5 cm^2 sod plugs were cut from each flat. The plugs were washed to remove soil from the roots and planted in 90 x 60 x 25 cm pots filled with fritted clay. A total of eight pots were planted with one complete set of the maternal plants in random order per pot. The plants were allowed to grow in the fritted clay for six weeks until the plants were well established. Plants were fertilized biweekly with a 21-17-19 water soluble fertilizer providing an equivalent of .45 kg (1 lb) N per month. Timing of the initiation of the water deficit stress period was set so as to minimize high temperature stress and provide for optimum creeping bentgrass growing conditions. This choice of timing was selected to reduce the influence of high temperature stress, thus allowing water deficit stress to be the major environmental stress factor placed on the plants.

On October 19, 1995 watering of four of the pots was ceased, to begin the water deficit study. At which time a fully emerged leaf sample was collected from each plant and immediately placed into a thermocouple psychrometer chamber for water potential measurement. Pots were weighed to facilitate gravimetric measurements of soil water content. Visual ratings of plant color, quality, and percent cover were taken. Leaf water potential measurements and pot weights were made on a weekly basis through out the experimental period. Visual ratings were made every two weeks. Currently, plants have experienced 3 weeks of water stress and are just beginning to show signs of water deficit stress. Completion of stage one of this project will be on November 28, 1995. At which time plants under water deficit stress will be watered and rated for recovery. Statistical analysis will then be used to determine if there are significant differences in water deficit tolerance based on leaf water potentials, turgor potential, quality ratings and recovery of percentages of Crenshaw in the population at the Dallas Country Club location.

Based on these data, the percentage population change that one could expect following a single seeding operation would range from about 5 up to 30% at 6 to 12 months after inter-seeding. More rapid population changes may be achieved by multiple inter-seedings.

Table 3. Percentage of Crenshaw creeping bentgrass identified by isozyme analysis at 6 and 14 months after inter-seeding into an existing Penncross putting green at the Texas A&M University Research and Extension Center at Dallas, Texas.

Mechanical treatment	Percentage Crenshaw† glyphosate	cimectacarb	none	Mean
		6 months		
Core tine	12	8	7	9
Star tine	53	10	7	23
Verticut	70	12	10	31
None	70	15	33	39
Mean	51	11	14	26
$LSD_{0.05M}$§	29	NS	NS	25
		14 months		
Core tine	61	10	10	27
Star tine	89	8	8	35
Verticut	85	11	16	37
None	95	10	11	39
Mean	83	10	11	35
$LSD_{0.05M}$	24	NS	NS	NS

†Chemical treatments were applied about 7 days before mechanical and seeding treatment. Glyphosate did not cause complete kill of the existing Penncross.

§ $LSD_{0.05M}$, Least significant difference for comparison of mechanical treatment means within dates; NS, not significantly different at the 0.05 level of probability.

Future: Isozyme analysis will be conducted at approximately 6-month intervals to determine population changes with time. We plan to establish blends of Crenshaw and Penncross and conduct multiple inter-seeding studies at College Station during the next 12 months.

the plants after long-term water deficit stress. If significant variability is observed in the maternal plants, they will be separated into groups of low, medium and high tolerance. Representative maternal plants and six progeny plants from each tolerance level group will then undergo water deficit stress tests as explained in step one of this experiment. Results from the second stage of this experiment will allow determination of the heritability of water deficit tolerance in specific creeping bentgrass varieties. Progeny plants for stage two of this experiment are presently being propagated in the greenhouse and will be ready for the start of stage two by the completion of stage one.

The third stage of this experiment will evaluate, for maternal plants which have different levels of water deficit tolerance, physiological and morphological characteristics that may contribute to stress tolerance. Characteristics to be quantified will include: root length density, rooting depth, leaf area, osmotic adjustment, carbon allocation and partitioning, and stomatal closure at specific temperatures and soil water potentials.

Selection of maternal clones and progeny has progressed through close cooperation and assistance of Dr. Milt Engelke. The population(s) with which we are working was provided by Dr. Engelke and selected based on past performance and disease resistance. Screening will be conducted on maternal clones to assess competitive performance and turgor maintenance under high evaporative demand and low to moderate water stress. Several turgor maintenance mechanisms including physiological and morphological are being explored. Progeny are being obtained and increased to assess progeny response. The population(s) used will allow determination of the heritability of the various mechanisms of turgor maintenance or drought resistance.

Results: Phase one of this study was completed during the fall 1995. Maternal clones having high, intermediate, and low resistance to water stress were identified for further testing (Figures 1 and 2). Maternal clone 2833 is representative of high, 9999 low, and 3106 and 3153 intermediate water stress tolerance groups. These data are in agreement with previous field evaluations of parent/progeny performance at the TAMU-REC at Dallas. Water relations characteristics were assessed for the maternal clones and preliminary assessments indicate that turgor maintenance may explain the superior performance of specific clones such as 2833. A definitive mechanism for turgor maintenance in these maternal clones has not been established but basal osmotic potential and osmotic adjustment appear to contribute to the response.

Parent/progeny tests were established during January 1996 based on tests of maternal clones. A stress cycle was being imposed for the first parent/progeny disease problems occurred in the initial parent/progeny plantings and through the summer of 1996 and the initial plantings were abandoned. Parent progeny tests were reestablished during fall 1996 and are progressing but behind schedule.

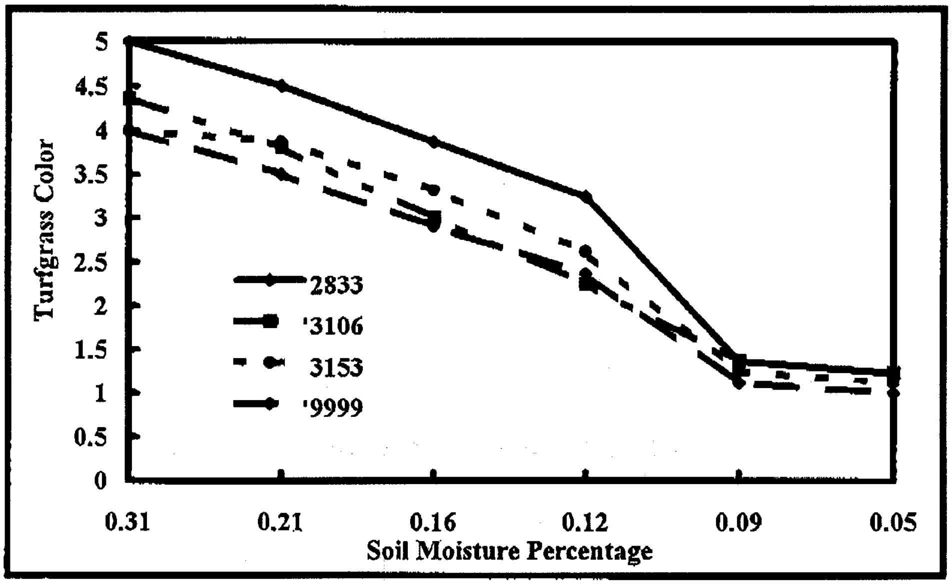

Figure 1. Turfgrass color response of selected maternal clones during soil drying. A total of 28 maternal clones were examined. The clones presented here represent those with high, intermediate and low drought tolerance.

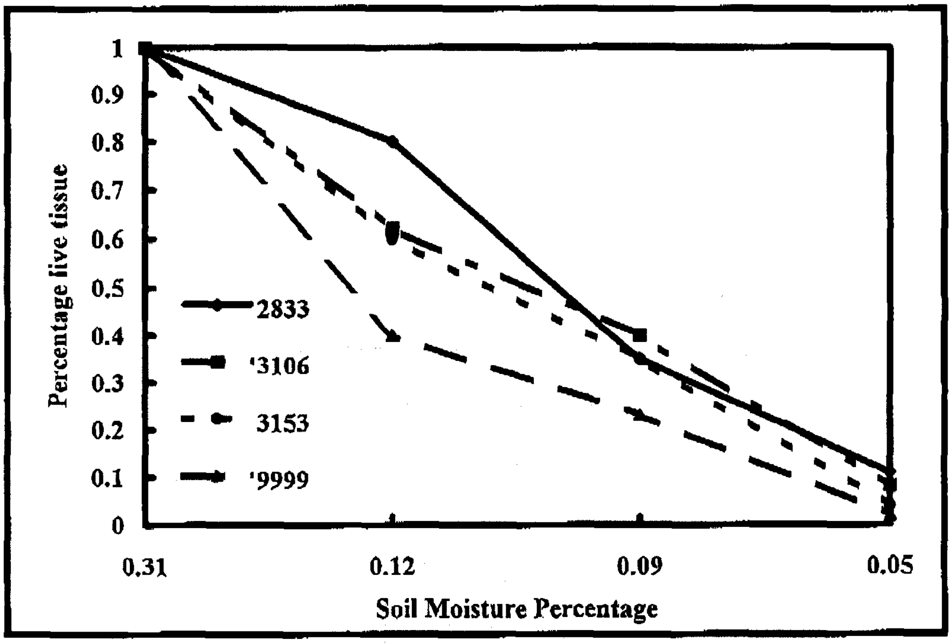

Figure 2. Percentage live tissue of selected maternal clones during soil drying. A total of 28 maternal clones were examined. The clones presented here represent those with high, intermediate and low drought tolerance.

V. BENTGRASS BLENDS

Objectives: Determine the effects of blending bentgrass cultivars on bentgrass putting green turf quality, disease resistance, and performance.

Materials and Methods: Three bentgrass cultivars including Penncross, SR1020, and Crenshaw were planted the last week of October 1995 in a multiple split-plot design. The experimental design provided main plots of Penncross and consisted of Penncross and no Penncross. Sub-plots consisted of Crenshaw and no Crenshaw, and sub-sub plots consisted of SR1020 and no SR1020. This design allows a comparison of the main effects of each cultivar and two- and three-way interaction effects. Similar plantings including Penncross, National, and Crenshaw, and Penncross, Crenshaw, and Putter were also established.

The study was established on a high-sand content root zone with subsurface drainage and surface irrigation. The area will be maintained as a putting green throughout the conduct of the study. Pesticides will not be applied unless total plot devastation is anticipated. Visual ratings of turfgrass quality, density, color, weed infestation, and disease will be used to assess overall performance.

A new 25,000 square foot experimental green was constructed in September and October 1996. A new bentgrass blending study will be planted in November 1996. Five bentgrasses selected for performance based on National Turfgrass Evaluation Program trials and disease resistance will be planted. These five grasses, including Penncross, L-93, A-1, Crenshaw, and Mariner, will be planted to 6 foot by 6 foot plots in all possible combinations of single, two-way, and three-way blends. A completely random design with three replications will be used. Planned comparisons among single, two-way, and three-way blends will be accomplished for turf quality, disease, and putting quality. Other performance characteristics, such as plant density, thatch development, and rooting characteristics, will also be assessed.

RESULTS: An excessively hard rain following planting of this test caused significant movement of seed and cross-contamination of plots in 1995. The site will require planting again during the fall 1997.

FUTURE WORK: Assessments will be made on establishment, disease, turf quality, and putting quality during the 1996 through 1997 period.