1995 ANNUAL RESEARCH PROGRESS REPORT

of

PHYSIOLOGICAL BASIS FOR SELECTION OF BENTGRASSES WITH SUPERIOR DROUGHT RESISTANCE

Submitted by:

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PHYSIOLOGICAL BASIS FOR SELECTION OF BENTGRASSES WITH SUPERIOR DROUGHT RESISTANCE

Executive Summary

Principle Investigator: Dr. Richard H. White
Graduate Research Assistants: Mr. Dave Gilbert and Mr. Gene Taylor
Research Period: 1 November 1994 through 31 October 1995

Three experimental sites were selected for interseeding Crenshaw creeping bentgrass into existing Penncross putting greens including the Texas A&M University Research and Extension Center at Dallas, Brookhaven Country Club, and Dallas Country Club. Mechanical disruption and chemical suppression treatments were employed in a multiple-strip-split plot design. The most vigorous chemical suppressant was glyphosate and was used to allow easy visual determination of seedling emergence in dead bentgrass sod. Cimectacarb (Primo) and none chemical suppression treatments were also used. Mechanical disruption treatments were none, vertical mowing, core aerification, and star-tine aerification. Interseeding was accomplished during 1 through 15 April 1995. Visual observations within the glyphosate treatments indicate that vertical mowing may be the most effect means of mechanical disruption. However, overall seedling emergence was less than expected at all sites. This experiment was planted again in October 1995 at the Dallas Country Club. Electrophoretic analysis of isozyme banding patterns from samples collected from the TAMU-REC at Dallas location is providing, through close cooperation with Drs. M. C. Engelke and Ikuko Yamamoto, the necessary information to determine population changes. Current estimates of 10 to 20% contribution of Crenshaw produced from a single spring interseeding of this Penncross putting green provides the first quantified documentation that a new bentgrass cultivar can be successfully incorporated in an existing bentgrass putting green.

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

Studies to determine the effects of blending bentgrass cultivars on bentgrass putting green turf quality, disease resistance, and performance were established at the Turfgrass Field Laboratory in College Station, Texas. These blending experiments will provide insight into the effects of creeping bentgrass blends on overall putting green quality. This concept is extensively 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. 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 1995 annual progress report for the project and highlights activities between 1 November 1994 and 31 October 1995.

II. PROFESSIONAL AND TECHNICAL SUPPORT

Mr. David Gilbert is a Graduate Research Assistant and an M. S. Degree candidate. David lives in Dallas and will conduct his research at the TAMU Dallas Research and Extension Center and complete course work for his degree at the University of Texas in Arlington and at Texas A&M in College Station. Dr. Milt Engelke is co-chair of David's graduate committee.

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.

III. BENTGRASS INTERSEEDING

A. Bentgrass Interseeding - Spring 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: Three test sites were identified during winter 1995 to test the effects of interseeding Crenshaw creeping bentgrass in existing Penncross creeping bentgrass. Site one is at the Texas A&M University Research and Extension Center at Dallas (TAMU-REC at Dallas). Site two is a Penncross nursery at Brookhaven Country Club in Dallas, Texas and site three is 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.

The experiment was established at the three sites during 1 through 15 April 1995. Weekly monitoring visits to each site are made. Random counts within each plot were taken about 4 weeks after planting to determine seeding emergence. Bimonthly visual assessments were taken to determine treatment effects on turf quality. Data were analyzed by appropriate statistical models to determine treatment effects.

Experimental areas were maintained as a putting green throughout the study period. Daily mowing was applied continuously and immediately after seeding. Irrigation was applied as needed to prevent water stress. No fertilization was applied to the TAMU-REC at Dallas location during the months after seeding.

We are currently utilizing electrophorectic identification of populations in interseeded Penncross experimental areas to assess the success of seedling establishment. This technology proposed by Dr. Yamamoto, 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 interseeding 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 interseeded 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: The Dallas Country Club location was abandoned because of poor seed germination and establishment. Watering practices at the experimental location were inadequate to meet the needs of germinating bentgrass. Data from the Dallas Country Club location will not be reported. Data from the TAMU-REC at Dallas location are reported in this progress report.

Overall seedling emergence was below expected at all sites. Emerged seedlings were about 1 per square inch or less for treatments that received the glyphosate treatments. The glyphosate treatment was used to provide an area that would allow easy assessment of seedling germination yet provide non-living plant material competition, primarily for light.

Statistical differences were determined among the no seed and seeded treatments for seedling emergence. Mechanical treatments were statistically similar for seedling density. However, within the seeded treatment, vertical mowing produced about twice the seedling density as no mechanical treatment and a three-fold increase in plant density compared to core and star tine treatments. Seedlings are reported for the no seed treatments because of incomplete death of existing Penncross in the glyphosate treatments. Visual observations within the glyphosate treatments indicate that vertical mowing may be the most effect means of mechanical disruption for introducing new bentgrass germplasm into an existing bentgrass putting green. Seedling emergence in the core and star tine treatments were primarilly confined to holes made by the tines, whereas, seedling emergence for the none and vertical mowing treatments was more uniform. We are currently recommending seeding followed by topdressing or vertical mowing, seeding, followed by topdressing to superintendents attempting interseeding.

Table 1. Crenshaw creeping bentgrass seedling emergence four weeks after interseeding into a Penncross Creeping bentgrass putting green on 1 April 1995 at the TAMU-Dallas Research and Extension Center. Data include glyphosate treatments only.

Seeding treatment	Mechanical Treatment	Plants foot$^{-2\dagger}$
No seed	Core tines	21.6
No seed	Star tines	14.4
No seed	Vertical mowing	18.0
No seed	None	18.0
Seeded	Core tines	50.4
Seeded	Star tines	57.6
Seeded	Vertical mowing	176.4
Seeded	None	86.4

† Values were determined from three random subsamples from each plot of bentgrass plants possessing a single crown (i.e. plants were without stolons).

Turf quality was similar for most treatments during the post-seeding period. Seeding treatments did not effect differences in turfgrass quality (Tables 2 and 3). During the first 5 months after planting, no difference in quality resulted for the seeded compared to non-seeded areas. However, differences in turfgrass quality were evident among chemical treatments. Turf quality for the Primo treatment was similar to none chemical treatment and is not presented. Primary differences were noted in turfgrass quality among the none and glyphosate treatments. However, by September 1995 chemical by seeding treatment interaction effects were evident. Within the glyphosate treatments, seeding affected turfgrass quality on 13 September, indicating that Crenshaw was successfully established from the spring planting in this treatment combination. The glyphosate treatments that received the seeded treatment were 100% covered by creeping bentgrass in August compared with glyphosate plots that were not seeded that were only about 50% covered by the September observation date (data not presented).

Table 2. Turf quality during 1995 of a Penncross creeping bentgrass putting green interseeded with Crenshaw creeping bentgrass on 1 April 1995 at the TAMU-Dallas Research and Extension Center.

Seeding treatment	Chemical treatment	Turf Quality†				
		6 June	26 June	16 July	15 Aug	13 Sept
No seed	None	4.6	6.0	6.5	5.6	5.5
Seeded	None	4.2	5.4	6.3	5.5	5.4
No seed	Glyphosate	1.2	1.3	1.7	3.1	3.7
Seeded	Glyphosate	1.6	2.2	3.3	4.6	5.4
Source of variation						
Seeding (S)		NS	NS	NS	NS	NS
Chemical (C)		***	***	***	*	*
Seeding by chemical		NS	NS	NS	NS	*
Mechanical (M)		*	NS	NS	NS	NS
Seeding by mechanical		NS	NS	NS	NS	NS
Mechanical by chemical		NS	*	NS	NS	NS
S by M by C		NS	NS	NS	NS	NS

† Turf quality based on a 1 to 9 scale with 9 equal best density, color, uniformity, and texture.
*,**,***,NS Significant at the 0.05, 0.01, and 0.001 levels of probability; NS, not significant.

Mechanical treatments did not effect major differences in turfgrass quality during the initial 5 months after interseeding (Table 3). A general trend towards superior turf quality was noted for the seeded compared to the no seed treatments. In general, the seeded treatments produced better turf quality than the no seed treatments. The none mechanical treatment by seeded treatment combination produced numerically better turf quality than the other mechanical treatments in combination with seeding.

These developments are probably realistic expectations for interseeding of the Penncross putting green at the TAMU-REC at Dallas. The area was of resonably good turf quality prior to interseeding and the location does not suffer from poor air movement, drainage, or management. The area would not be typical of putting greens requiring interseeding with superior bentgrass varieties for major improvements in green performance.

A primary concern during the conduct of this work has been how to determine whether the interseeded bentgrass actually germinated and established within the existing bentgrass stand.

Glyphosate treatments provide a base line for potential germination and establishment. Electrophoretic analysis of isozyme banding patterns from samples collected from the TAMU-REC at Dallas location is providing the necessary information to determine population changes. Although turf quality is the ultimate indicator of success, a golf course superintendent practicing interseeding desires to know whether he or she has caused a shift in the putting green grass population. The isozyme analysis of all plots is not complete but preliminary data indicate that the glyphosate by seeded treatments are about 80% Crenshaw and 20% Penncross. Treatments that did not receive glyphosate contain substantially less Crenshaw within the population. The isozyme banding patterns indicate that Crenshaw comprises 10 to 20% of the bentgrass plants in the population for all other seeded treatment combinations.

Table 3. Turf quality during 1995 of a Penncross creeping bentgrass putting green interseeded with Crenshaw creeping bentgrass on 1 April 1995 at the TAMU-Dallas Research and Extension Center.

Seeding treatment	Mechanical treatment	Turfgrass Quality† 6 June	26 June	16 July	15 Aug	13 Sept
No seed	Core tine	2.7	3.5	3.7	4.8	5.2
No seed	Star tine	3.2	4.2	4.3	4.5	4.8
No seed	Vertical mow	2.8	3.8	4.3	3.5	3.7
No seed	None	2.8	3.2	4.0	4.5	4.7
Mean		2.9	3.7	4.1	4.3	4.6
Seeded	Core tine	2.5	3.7	4.5	4.5	4.3
Seeded	Star tine	3.2	4.0	4.5	5.0	5.8
Seeded	Vertical mow	3.2	3.8	4.8	5.0	5.3
Seeded	None	2.7	3.7	5.2	5.7	6.2
Mean		2.9	3.8	4.8	5.0	5.4
$LSD_{0.05}$§		0.4	NS	NS	NS	NS
Source of variation						
Seeding (S)		NS	NS	NS	NS	NS
Chemical (C)		***	***	***	*	*
Seeding by chemical		NS	NS	NS	NS	*
Mechanical (M)		*	NS	NS	NS	NS
Seeding by mechanical		NS	NS	NS	NS	NS
Mechanical by chemical		NS	*	NS	NS	NS
S by M by C		NS	NS	NS	NS	NS

† Turf quality based on a 1 to 9 scale with 9 equal best density, color, uniformity, and texture. §$LSD_{0.05}$, Least Significant difference for comparison of mechanical treatment means within columns and seeding treatments. *,**,***,NS Significant at the 0.05, 0.01, and 0.001 levels of probability; NS, not significant.

The isozyme banding technique will provide considerable insight into the population shifts caused by interseeding. Again, the long-term success of interseeding will be measured by changes in putting green quality and may require more than a single seeding and season. Our approach was to seed once and monitor changes with time. More complete sampling for isozyme analysis was accomplished at 6 months after interseeding. Samples are currently being processed. Current estimates of 10 to 20% contribution of Crenshaw produced from a single spring interseeding of this Penncross putting green provides the first documentation that a new bentgrass cultivar can be successfully incorporated in an existing bentgrass putting green. These data do not indicate that 10 to 20% of the planted seed survived. In fact much less than 10% seedling survival may have occurred. The technique does not provide an indication of seedling survival, but the isozyme technique indicates, that from a single spring interseeding, the population of bentgrass 6 months after seeding was a blend of about 80 to 90% Penncross and 10 to 20% Crenshaw. Whether this is an acceptable change or rate of change in the bentgrass population can only be determined through time and evaluation of putting green quality and performance changes. Based on observations during 1995, a trend toward improved turfgrass quality appears to have occurred for the seeded treatment.

Based on this work, golf course superintendents should not expect instantaneous, positive changes in putting green performance from interseeding. The process can be successful, although changes in the bentgrass population may be far below current expectations. Many turf managers have preconceived notions that interseeding bentgrass with bentgrass will produce results similar to overseeding bermudagrass with perennial ryegrass or *Poa trivialis*. Competitiveness of the existing bentgrass stand will no doubt play a role in bentgrass interseeding success. Fall interseeding of summer stressed bentgrass greens may produce more visible changes in turfgrass quality. Overall performance from fall interseedings will require assessment the following summer. The existing bentgrass at the TAMU-REC at Dallas location planted in spring 1995 was healthy and probably very competitive. Yet, Crenshaw creeping bentgrass was successfully interseeded. More rapid and higher magnitude poplulation shifts may likely be caused by fall interseeding bentgrass putting greens severely injured by summer stresses. The use of electrophorectic identification isozymes from interseeded bentgrass greens will provide the technology needed to develop the methodology to most rapidly incorporate new germplasm into existing bentgrass putting greens.

Future Plans: This experiment is currently being repeated at the Dallas Country Club through cooperation with Mr. Scott Parker, Golf Course Superintendent. The design and treatment combination is comparable to that used for the spring interseeding. Interseeding was accomplished in mid-October on a bentgrass nursery at the Dallas Country Club. Visual observations will be made on a bimonthly basis for turf quality. Isozyme analysis will be completed for the spring planting and conducted for both spring and fall plantings at 6-month intervals after planting. Additional work is needed to assess the effects of multiple seedings and seeding amounts on bentgrass putting green population shifts.

B. Potential Germination Inhibition by Bentgrass Plant Residues

Germination and establishment of bentgrass interseeded into existing bentgrass putting greens can be inhibited by environment, existing plant competition, and potentially by degradation products of plant residues. A study was designed to address the potential for germination in bentgrass plant residues to determine if potentially inhibitory effects were present.

Objectives: Determine the effects on bentgrass plant residue (thatch) on germination of creeping bentgrass.

Materials and Methods: The study was initiated in late April to test the effects of creeping bentgrass plant residue on Crenshaw creeping bentgrass germination and seedling emergence. Crenshaw was seeded to pots containing a high-sand rootzone mix or the same high-sand rootzone mix with creeping bentgrass plant residue. Creeping bentgrass plant residue consisted of 2-cm thick sod cut from an experimental Penncross creeping bentgrass putting green in College Station, Texas allowed to die from severe drought stress. The plant residue was placed on the surface of a high-sand rootzone mix within pots. Seedling emergence was determined by seedling counts and appropriate statistical comparisons. Similar studies were established in late-April in petri-dish tests to compare germination and seedling emergence in two treatments. The treatments consisted of a high-sand rootzone mix and creeping bentgrass thatch obtained from an existing Penncross bentgrass green.

Results: Germination was similar for Crenshaw creeping bentgrass on a high-sand rootzone and on the same high-sand rootzone with bentgrass plant residue (data not presented). Plant residue did not affect seed germination in the petri-dish test either. The results of this study indicate that Penncross creeping bentgrass plant residue (thatch) does not inhibit germination of Crenshaw creeping bentgrass.

IV. BENTGRASS SUMMER STRESS TOLERANCE

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

Materials and Methods: This study will proceed 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 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: Results from this study are forthcoming. Considerable progress has been made toward identification of maternal clones and progeny for future testing. Water deficit stress is underway for 28 maternal clones with detailed measurements of water relations characteristics being conducted. Determination of the water relations characteristics of such a large number of genotypes is a monumental task and the results from the first phase will provide one of the largest data bases on water relations characteristics of cool-season turfgrasses to date.

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.

Results: Data are not available for this study at this time but will be included in the semi-annual report.

Future Work: Establishment characteristics, performance, quality, and disease resistance will be monitored bimonthly for these studies during the next year. Summer performance will be of special interest in the hot-humid climate of College Station.