FIRST ANNUAL
PROGRESS REPORT

of

PHYSIOLOGICAL BASIS FOR SELECTION OF BENTGRASSES WITH SUPERIOR DROUGHT RESISTANCE

Submitted by:

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Executive Summary
First Annual Report

PHYSIOLOGICAL BASIS FOR SELECTION OF BENTGRASSES WITH SUPERIOR DROUGHT RESISTANCE

Principal Investigator:	Richard H. White
Research Assistants:	Mr. David Gilbert (M.S. Degree Candidate) Mr. Gene Taylor (Ph. D. Degree Candidate)
Research Reporting Period:	1 August through 1 November, 1994

The cost, availability, and environmental impact of resources utilized in turfgrass management including water, energy, fertilizers, and pesticides will continue to have a major impact on the turf industry. The intensity of culture required to maintain acceptable turf is dependent on the cultivar in use and on local climatic conditions. 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 this species area of adaptation. 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.

Physiological Basis for Selection of Bentgrasses with Superior Drought Resistance was initiated to address the relationship of *i*) water balance in creeping bentgrasses with performance in adverse environments and to *ii*) assess management systems for the incorporation of diverse bentgrass germplasm into existing bentgrass stands for rapid and efficient improvement of putting green stress resistance and functional quality.

Two key individuals were added to this project since 1 July. Mr. David Gilbert, a Master's Degree candidate in the Soil & Crop Sciences Department, joined the project as a Research Assistant. Mr. Gilbert's research responsibilities will emphasize water balance in 20 cultivars and experimental lines of creeping bentgrass. The design phase of his project is complete and data collection will begin during the coming weeks. The second key individual is Mr. Gene Taylor, a Doctor of Philosophy candidate in the Soil & Crop Sciences Department. Mr. Taylor joined the project in late August. 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.

A third individual was scheduled to begin a Master's program within this project during August 1994. His responsibilites were to be the assessment of management systems for the incorporation of diverse bentgrass gemplasm into existing bentgrass stands. This individual located three cooperator sites in the Dallas/Fort Worth area to conduct this work. At the last minute, the student quit the program and this phase of the program did not move forward this fall. Cooperating golf courses and superintendents appear willing to move ahead with a spring and fall 1995 program.

First Annual Report 1994

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. This report constitutes the Annual Progress Report for the project.

II. PROFESSIONAL AND TECHNICAL SUPPORT

Two key individuals were assigned to this project during July and August 1994. Mr. David Gilbert joined the project as a Graduate Research Assistant and a 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 a M. S. degree from North Carolina State University.

III. OVERVIEW

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 this species area of adaptation. The expansion of bentgrasses throughout this environmentally stressful area has out-paced development of stress tolerant bentgrass cultivars. Irrigation and syringing have been 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. On bentgrass greens in the South, maintenance of evapotranspirational water loss is desirable because of the cooling effects of this process. Turgor maintenance and efficient, rather than low water consumption should be the primary objective in screening for stress resistant bentgrass.

While survival of climatic drought is not a priority on golf course putting greens *per se*, the ability of a bentgrass to survive summer stress periods with a minimum of resource and pesticide inputs is of paramount importance. The impact of temperature and other environmental factors on bentgrass rooting have been explored in Texas and lead to the development of bentgrass lines with superior root extension rates and tolerance of high soil temperatures. Root characteristics are but one facet of drought resistance. Plant physiological adaptation to water deficits has been shown to be associated with superior plant response to stress and survival of drought and is likely of equal or greater importance than rooting characteristics. However, superior bentgrasses should be able to both avoid and tolerate drought, thus a better understanding of all primary mechanisms of drought resistance is required for rapid germplasm improvement.

Progress in breeding plant's for drought resistance is profoundly slow and measured in years or decades. This is not due to inactivity, but to 1) conflicting definitions of drought resistance, 2) a poor understanding of turf drought resistance mechanisms, 3) an inadequate knowledge of which mechanisms are most important for drought resistance in grasses, and 4) a general lack of screening procedures for the rapid selection of germplasm with superior mechanisms of drought resistance. Drought resistance and plant response to drought is complex. Rapid development of stress resistant bentgrass cultivars will depend on a fundamental understanding of drought resistance mechanisms. Secondly, a rapid, economical screen for such mechanisms will be required. The selection tool must be relevant to field performance.

A determination of the effects of drought resistance mechanisms on plant response to short- and long-term soil moisture deficit is necessary to understand the biological significance of such mechanisms to plant persistence and survival. This will be a key to rapid breeding success. Secondly, the relationship of such mechanisms to turf performance in terms of aesthetic and functional quality must be assessed. Such an approach should provide a balance between quantifiable and qualitative attributes applied to germplasm evaluation and selection. We propose an in-depth evaluation of both morphological and physiological mechanisms of drought resistance in commercially available bentgrass cultivars and the development of a physiologically based procedure for selection of bentgrasses with superior drought tolerance. Future development and culture of turfgrass cultivars will depend on rapid drought resistance assessment techniques based on physiological mechanisms of stress resistance. The proposed project will explore a rapid, economical, and physiologically based drought resistance screening procedure for greenhouse use that is relevant to field performance of bentgrass germplasm. Additionally, the work will determine the relationship between mechanisms of drought tolerance to the performance, drought resistance, and *in situ* water requirements of bentgrass germplasm.

This proposal has been augmented to include the evaluation of blending bentgrass cultivars for use on golf course putting greens and to include the evaluation of overseeding technologies for incorporation of new bentgrass germplasm into existing putting greens. Such information should be of primary benefit to superintendents desiring to increase the genetic diversity of bentgrass plants within putting greens to capitalize on pest and stress resistance. Limited information is currently available on appropriate cultivar blends and overseeding methods for development of superior performing putting green surfaces.

IV. OBJECTIVES:

A. Develop a rapid, economical, physiologically based drought resistance screening procedure for greenhouse use that is relevant to field performance of bentgrass germplasm.

B. Determine the relationship between mechanisms of drought tolerance to the performance, drought resistance, and *in situ* water requirements of commercial and experimental bentgrass germplasm under field conditions.

C. Assess management systems for the incorporation of diverse bentgrass germplasm into existing bentgrass stands for rapid and efficient improvement of putting green stress resistance and functional quality.

Implementation

Objective A

A Competitive Soil Moisture Extraction Technique (CSMET) that simulates drought shows considerable promise for selecting turfgrass germplasm with superior drought tolerance. This procedure has recently been used to determine that tall fescue genotypes differ in basal osmotic potential and osmotic adjustment. Through use of this physiologically based screen, it was determined for the first time that tall fescue genotypes with the potential to maintain turgor through low basal osmotic potential and by osmotic adjustment are also better able to survive extended soil drying. Zoysiagrasses have also been evaluated with this technique and a strong relationship between drought tolerance mechanisms, survival and water use was observed. Low basal osmotic potential and substantial osmotic adjustment was associated with increased drought survival and a low irrigation water requirement in zoysiagrasses. Our intent is to apply this technique to the evaluation of selected bentgrass germplasm. The primary focus will be to determine the primary mechanism of drought tolerance in diverse bentgrass genotypes.

Plant Culture. Entries in this first phase will include parents and progeny of one to three advanced creeping bentgrass breeding lines selected through collaboration with Dr. M. C. Engelke. Parent(s) and progeny will be propagated vegetatively and planted to 100 cm^2 containers filled to within 1 cm of the top with fritted clay and with a second set in similar containers filled with an 80% sand:20% peat:10% soil medium. A balanced fertilizer will be applied weekly to provide the equivalent of 0.25 kg N are^{-1} $month^{-1}$. After a 2-month establishment phase, entries will be transplanted to CSMET using a randomized complete block experimental design with five replications. Root zones of fritted clay and 80% sand:20% peat will be used in separate CSMET analyses. A border of Penncross is to be planted to reduce edge effects. A two-month growth period in CSMET will be used prior to initiating stress. Plants will be maintained with adequate nutrition and irrigation and mowed at a 1 cm height. Mowing will be terminated at the beginning of a stress preconditioning cycle, a necessary step to allow sufficient foliar growth for sampling.

Water-release Curves. Immediately before preconditioning to water stress (before water stress), vertical stem segments will be detached from each entry by replication, recut under water, and hydrated in a vial of distilled water for 16 h in darkness at 5°C. Entries will be sampled again in a similar manner 7 to 14 d after initiating the stress cycle at which time leaf lamina of all plants should be severely rolled and soil water potentials should be between 0.05 and 0.1 MPa. The preconditioning cycle will consist of withholding irrigation until soil water potential at the 10 cm depth is between 0.05 and 0.075 MPa.

After rehydration, most recently, fully expanded leaves of vertical stem segments will be detached, quickly blotted dry, and weighed (TW). The leaves will be placed on filter paper within a hydraulic press and preselected, incremental pressures applied for 30 s. Pressure will be relieved slowly and the leaves quickly weighed again (FW). Pressures of 0, 0.41, 0.62, 0.83, 1.04, 1.24, 1.45, 1.66, 1.86, 2.07, 2.28, 2.48, 2.69, 2.90, 3.11, 3.45, and 3.80 MPa will be applied. Osmotic potential at full turgor ($\psi_{\pi100}$), water potential at zero turgor (ψ_{L0}), relative water content at zero turgor (RWC_0), and the proportion of cell wall or bound water (β) will be derived from each curve. Bulk modulus of leaf tissue elasticity (ϵ) will be determined from the relationship between ψ_p and RWC. Turgid weight:dry weight ratios will be determined from initial weight after hydration and oven dry weight for each selection. Osmotic adjustment will be determined as the difference between osmotic potential measured before and after stress. This data will allow determination of physiological mechanisms of drought resistance.

Visually Assessed Response to Stress. During the stress cycle, entries will be visually scored daily for wilt and leaf firing. Comparisons will be made among entries for days without irrigation for wilting and firing to appear and for point-in-time wilting and firing severity.

Recovery from Stress. After a sufficient period of stress (3 to 4 weeks), the herbage will be cut at 1 cm above the growth medium surface and plants rewatered and fertilized. Survival of water stress will be determined by visually scoring entries for percent green cover and by comparing top growth mass produced during a 4 to 6 wk recovery period.

Rooting Response. The plant characteristic most often associated with drought resistance is maximum root depth, root extension rate, and root length density. These characteristics will be determined for all entries in 4.75 cm diameter by 60 cm long butyrate tubes. Entries will be propagated in 4.5 cm diameter by 5 cm deep containers filled with washed sand. Following a sufficient establishment period, entries will be transplanted to tubes. Nutrients and irrigation will be non-limiting. Temperatures in the greenhouse will be maintained at 25°C at night and 32°C during the day. Maximum root depth will be marked twice a week on the outside of the clear butyrate tube. After six weeks, entries with intact root systems will be removed from tubes, washed free of sand, and root numbers at 10 cm increments, root length densities, maximum rooting depth, and dry root mass will be determined. A completely random experimental design with six replications will be used.

Progeny Testing. Following characterization of parent(s) and progeny a determination of the heretibility of drought tolerance mechanisms will be made. Visual assessments of stress response will be weighed more heavily in intial stages. Water stress tolerance mechanisms will be quantified in progeny groups representing, based on parental performance, the range of tolerance for the cultivar. Heritibility estimates for specific traits will be determined.

Timing and Expected Results. The initial experiments in this phase will begin in late 1994 and during 1995. However, the CSMET procedure will be repeated during the course of funding to determine variation in time as well as space. Information from this initial phase will be used to select germplasm that differs widely in drought resistance. Differences in water relations characteristics, response and survival to simulated drought, and rooting characteristics are expected to occur. Mr. Gene Taylor is currently leading this objective of the project. Conferences with Dr. M. C. Engleke and the project leader have narrowed the selection of bentgrass germplasm for use in this phase of the project. Propagation of plant material and experimental facility design will be completed during the next few months.

Objective B

A Competitive Soil Moisture Extraction Technique (CSMET) as described under Objective A will also be used to accomplish this objective. Twenty cultivars of bentgrass including Cato, Crenshaw, Penncross, Pennlinks, SR1020, National, Providence, Putter, MSCB-8, Emerald, SYN1-88, Seaside, Cobra, Southshore, PSU-126, BR1518, Carmen, Forbes 89, Lopez, and Regent will be used.

Plant Culture. Cultivars will be obtained from established field experiments at the TAMU Dallas Research and Extension Center. Entries will be transplanted to CSMET containers. A cylinder of dialysis membrane will be positioned below each entry to permit free water flow to entry root systems and to physically isolate a representative intact portion of the root system for discrete growth analysis. A balanced fertilizer will be applied weekly to provide the equivalent of 0.25 kg N are^{-1} month^{-1}. Plants will be maintained with adequate nutrition and irrigation and mowed at a 1 cm height. Mowing will be terminated at the beginning of the stress preconditioning cycle, a necessary step to allow sufficient foliar growth for sampling. Two water stress treatments will be imposed and consist of well watered and stressed. The water stress treatment will be achieved by withholding irrigation for 3 weeks.

Water-release Curves. After a 4-week establishment period, the water stress treatment will be preconditioned to water stress by withholding irrigation for 7 days then rewatered. Irrigation will then be withheld from the water stress group for 3 weeks. After the preconditioning period, segments will be detached from each entry and stress treatment by replication, recut under water, and hydrated in a vial of distilled water for 16 h in darkness at 5°C. Entries will be sampled again in a similar manner 7 to 14 d after initiating the stress cycle at which time leaf lamina of all plants should be severely rolled and soil water potentials should be between 0.05 and 0.1 MPa. The preconditioning cycle will consist of withholding irrigation until soil water potential at the 10 cm depth is between 0.05 and 0.075 MPa. Leaf, osmotic, and turgor potential will be determined every other day during the stress cycle.

After rehydration, most recently, fully expanded leaves of vertical stem segments will be detached, quickly blotted dry, and weighed (TW). The leaves will be placed on filter paper within a hydraulic press and preselected, incremental pressures applied for 30 s. Pressure will be relieved slowly and the leaves quickly weighed again (FW). Pressures of 0, 0.41, 0.62, 0.83, 1.04, 1.24, 1.45, 1.66, 1.86, 2.07, 2.28, 2.48, 2.69, 2.90, 3.11, 3.45, and 3.80 MPa will be applied. Osmotic potential at full turgor ($\psi_{\pi 100}$), water potential at zero turgor (ψ_{L0}), relative water content at zero turgor (RWC_0), and the proportion of cell wall or bound water (β) will be derived from each curve. Bulk modulus of leaf tissue elasticity (ϵ) will be determined from the relationship between ψ_p and RWC. Turgid weight:dry weight ratios will be determined from initial weight after hydration and oven dry weight for each selection. Osmotic adjustment will be determined as the difference between osmotic potential measured before and after stress. These data will allow determination of physiological mechanisms of drought resistance.

Visually Assessed Response to Stress. During the stress cycle, entries will be visually scored daily for wilt and leaf firing. Comparisons will be made among entries for days without irrigation for wilting and firing to appear and for point-in-time wilting and firing severity.

Recovery from Stress. After a sufficient period of stress (3 to 4 weeks), the herbage will be cut at 1 cm above the growth medium surface and plants rewatered and fertilized. Survival of water stress will be determined by visually scoring entries for percent green cover and by comparing top growth mass produced during a 4 to 6 wk recovery period.

Rooting Response. The plant characteristic most often associated with drought resistance is maximum root depth and root length density. These characteristics will be determined for all entries from roots growing within the dialysis membrane. At the termination of the study, maximum root length and root length density at 5-cm increments will be determined.

Timing and Expected Results. The experiment was started during mid-September and will be completed during 1995. Initial data will be reported in the semi-annual report. However, the CSMET procedure will be repeated during the course of funding to determine variation in time as well as space. Information from this study will be used to identify germplasm that differs widely in drought resistance. Differences in water relations characteristics, response and survival to simulated drought, and rooting characteristics are expected to occur. Mr. David Gilbert is currently leading this objective of the project.

Objective C

Turfgrass blends and mixtures are frequently used when planting turfgrass stands to greatly increase the genetic diversity and thus pest and abiotic stress resistance of turf. However, this has not been used extensively on bentgrass putting greens. Rather, reliance on the diversity of bentgrass populations created by polycross or synthetic breeding technologies has been the norm. Limited efforts have been made to explore the use of bentgrass blends on putting greens. This may, in part, be due to difficulties some turfgrass managers have reported with overseeding existing stands of creeping bentgrass. However, technologies exist that should make this practice routine in the future. Routine seeding of putting surfaces with new germplasm should 1) increase the desirable plant to weed seed propagule ratio, 2) increase the genetic biotic and abiotic stress resistance within the new bentgrass population with subsequent reductions of management inputs required, and 3) improve the aesthetic and functional qualities of the putting surface.

Bentgrasses to be used in these tests will include cultivars such as 'Regent', 'SR1020', 'Putter', 'Providence', 'Penncross', 'Penneagle', 'Pennlinks', 'Cobra', 'National', 'Emerald', 'Seaside', 'PRO/CUP', 'Carmen', 'Biska', 'Cato', 'Crenshaw' and the colonial bentgrasses 'Bardot' and 'Dutchess'. Experimental protocols will provide two and three way blends as well as plantings of single cultivars for comparison.

In addition to evaluations of single cultivars and blends, trials to assess overseeding methodologies will be used on existing Penncross and/or Seaside putting greens. Crenshaw will be used as the overseeding grass. Methods to be tested will include verticutting, spiking, aerifying, topdressing, slit-seeding, and growth regulator. Verticutting treatments will consist of none, light, and severe. Spiking treatments will consist of none, once, and twice. Aerification treatments will consist of none and once. Topdressing treatments will consist of none and topdressing with a suitable mix. Slit-seeding treatments will consist of none and slit-seeding. Growth regulator treatments will consist of two materials, paclobutrazol and cimectacarb, and including 0, 0.5X and 1X of label recommended application rates. The experimental design used will attempt to incorporate treatments in all possible combinations and to include three replications. Visual evaluations will include uniformity, disease incidence, and overall turfgrass quality. Quantitative assessments will include evaluations such as rooting depth and distribution, plant density, and where appropriate greens speed.

<u>Timing and Expected Results</u>. Initiation of this phase of the project was delayed until spring 1995. Spring plantings are not anticipated to produce positive results, but should be tried to determine optimum timing of seeding. Cooperators have been identified and are anxious to begin on-site trials.

V. SUMMARY

The major accomplishment for this project during 1994 was the identification and recruitment of two Graduate Research Assistants to participate in the project. These two individuals will contribute directly to the knowledge of turfgrass science during the conduct of this project. One of these individuals receives direct educational funding support through this United States Golf Association - Green Section grant. Planning and development phases of two major research projects associated with this grant are complete and data collection phases will begin during the near future. A third Graduate Research Assistant is currently being recruited to participate in this project and will be funded from University, Department of Soil and Crop Sciences, and/or grant and gift funds.