# ANNUAL PROGRESS REPORT

**Concerning**

**Breeding and Evaluation of Cold-Tolerant Bermudagrass Varieties
and
Bermudagrass Varieties for Golf Course Putting Greens**

**For the Period**

**1 November 1989 - 31 October 1990**

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**Jointly Sponsored by:**

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**and**

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**EXECUTIVE SUMMARY**

Present objectives of the bermudagrass breeding program are to: 1) develop seed-propagated, cold-tolerant, finer-textured varieties for the transition zone; and 2) develop improved varieties for golf course putting greens, with emphasis on adaptation to southern coastal states.

Significant progress was made last year in development of cold-tolerant, seed-propagated bermudagrass varieties for the U.S. transition zone. Four experimental synthetic varieties were produced in 1989 and established in turf evaluation trials in Colorado, Iowa, Missouri, and Oklahoma this spring (1990). These experimental varieties will be tested for cold tolerance and suitability for use in fairways and roughs.

To further improve turf quality, bermudagrass populations bred for increased basic fertility (seed-set) were subjected to intense greenhouse screening for finer texture and increased density. In 1989, 162 of 10,000 plants were selected (1.6%) and established in spring 90 in Oklahoma and Arizona nurseries. Inter-crossed seed from these plants were used to establish an additional 10,000 greenhouse plants for selection in late summer, 1990. From this population 328 selections were established in field polycross nurseries in September, 1990. Continued selection in these breeding populations for turf quality, cold-tolerance, and seed yield should refine them to a level permitting development of high-quality seed-propagated, cold-tolerant varieties.

Development of new bermudagrass varieties for putting greens is proceeding on schedule. Thirty-three hundred *C. transvaalensis* progeny plant selections were established at Stillwater in early June for evaluation under putting green maintenance. In late October 1990, the 500 best appearing plants from this nursery were planted in groups of 100 on five Florida golf courses. Evaluations over the next year at Stillwater and the five Florida locations will be used to select 25-30 plants which be subjected to more intensive evaluation.

An experiment was completed to assess the effects of temperature and duration of exposure on cold acclimation of bermudagrass plants in the growth chamber. The information allows refinement of a mass screening procedure for cold hardiness whereby we grow plants in cone-tainers in the greenhouse, acclimate them in a growth chamber, and then subject to freezing temperatures as a screen for cold tolerance. Results to date suggest the procedure to be a feasible means of mass screening bermudagrass plants for cold tolerance.

Tissue cultures of several turf bermudagrass cultivars, including Tifgreen, Tifgreen II, Tifway, Tifdwarf, and NoMow, are being used in experiments to develop efficient regeneration systems and cellular/tissue screening procedures. We will also ascertain extent of somaclonal variation among regenerated plants.

## I. INTRODUCTION

The turf bermudagrass breeding program initiated in 1986 under the joint sponsorship of the United States Golf Association and the Oklahoma Agricultural Experiment Station has as its basic objective the development of fine-textured, winter-hardy, seed-propagated varieties for use in the transition zone of the USA. The program was expanded in 1990 to include the development of superior vegetatively propagated bermudagrass varieties, particularly for use on putting greens in the deep south where bentgrass is poorly adapted.

The "phenotypic recurrent selection" breeding procedure is being used to develop cold-tolerant, fine-textured, seed-propagated varieties. The premise of this procedure is ongoing population improvement via increase in the frequency of desirable alleles (genes) for the traits under selection. Available evidence indicates that the major traits for which we are breeding (seed yield, cold tolerance and fine texture) are quantitatively inherited with heritabilities sufficiently high to ensure response to selection. Also, because bermudagrass is easily propagated vegetatively, any superior plant may be extracted from a breeding population and maintained indefinitely as a variety or breeding line.

Initially phenotypic recurrent selection was applied to basic fertility (% seed set) in cold hardy germplasm populations. Presently, it is being used to refine the texture in these cold hardy populations initially subjected to selection for increased seed set, and to further increase seed production potential in breeding populations by improving seed yield components including % of florets setting seed, prolificacy of seed head production, and flowering date.

An important part of the overall project has been the development of cold-tolerance evaluation techniques for bermudagrass. Such techniques are needed to enable the quantification of relative and/or actual cold-hardiness of individual plants or populations of plants. We have developed such techniques and are now refining and applying them to mass screening for increased cold tolerance.

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

## II. RESEARCH PROGRESS

Breeding Seed-Propagated Varieties. Significant progress has been made toward the development of seed-propagated, cold-tolerant, fine-textured bermudagrass varieties for the U.S. transition zone. As outlined in previous reports, three cycles of recurrent selection for increased basic fertility in cold hardy germplasm increased by three-fold the percentage of florets setting seed. Parental plants extracted from these breeding populations in late 1987 were used in 1988 and 1989 to synthesize four experimental varieties (OKS 90-1, OKS 90-2, OKS 90-3 and OKS 90-4) which were planted in evaluation tests in 1989 at Stillwater, OK (Fig. 1), and this spring (1990) in tests at Fort Collins, CO (Dr. Tony Koski), Ames, IA (Dr. Nick Christians), and Columbia, MO (Dr. John Dunn). These experimental strains should be considered as intermediate products which haven't the degree of textural fineness that we ultimately desire, but which have potential present value. Visual ratings of several performance characteristics for entries in the Stillwater test are given in Table 1.

Fixation of fine texture and other turf quality indices in breeding populations is now a major thrust of our program. During the summer and fall of 1989, approximately 10,000 plants from the breeding populations were screened in the greenhouse for fine texture and density. One hundred and sixty-two of the 10,000 plants (1.6%) were selected for further evaluation and inter-crossing. These plants were planted this spring at Yuma, Arizona, and at Stillwater, OK. From these plantings sufficient seed was obtained in summer 1990 to grow out an additional 10,000 greenhouse plants for a second round of selection. Three hundred twenty-nine of the 10,000 plants were selected and planted in a Stillwater nursery September 10, 1990. Five of the best appearing of the 329 plants were also planted in an isolated polycross block to produce an experimental synthetic for testing. Additional polycrosses will be established next spring based on winter recovery and appearance of the 329 plants.

Six hundred fifty plants additional to the 162 mentioned above were planted at Yuma, Arizona, in mid-March. These materials were planted for the purpose of selecting plant types with superior seed yield potential under the desert environment. The principle characters of interest are early and prolific seed head production and good seed set. Early seed head production appears to be a necessary characteristic of varieties adapted to that environment, allowing them to mature a seed crop before the onset of high summer temperatures. Selections from this nursery will be made in spring 1991.

Breeding Vegetatively Propagated Varieties. Approximately 4,000 *C. transvaalensis* progeny plants grown in the field in 1989 showed wide variation in morphological traits, vigor and habit of growth, and in cold tolerance. Approximately 25% of the plants winterkilled during the unusually severe winter of 89-90. The remaining 75% varied greatly in earliness, uniformity and vigor of regrowth, and morphological features. The variation observed among these progenies suggests significant potential for selection. Figure 2 shows two unmowed plants depicting difference in appearance as seen in the breeding nurseries. Thirty-three hundred of the *C. transvaalensis* progeny plants from the breeding nurseries were established on 32 inch centers during the first week of June in a putting green maintenance evaluation test at Stillwater. Plants were not replicated in this test. Mowing height was reduced from 1/2 inch to 1/4 inch as the plants established during the summer and fall. Mowing height will be further reduced in 1991 to as low as 1/8 inch. Figures 3 and 4 show, respectively, an overview of one block of the putting green test and a close up of plants as they appeared in September. Differences in spread, texture, density, and color were evident among the 3,300 plants.

Five-hundred of the 3,300 _C_. _transvaalensis_ plants in the putting green test were selected in late October, 1990 on the basis of apparent putting green quality and planted in groups of 100 plants on five different Florida golf courses. Names and addresses of the golf courses are given in Table 2. This is pursuant to our original strategy and time line (Table 3) for evaluating and selecting the best of these 3,300 plants.

Fifteen vegetatively propagated plants with good turf potential were established in a replicated turf evaluation test in August 1990. These included 13 _C_. _dactylon_ x _C_. _transvaalensis_ hybrids from our breeding nurseries and two plants received from the Virginia Country Club and the Quicksand SCS Plant Materials Center, respectively. Evaluation for winter survival and turf performance will begin next spring.

_Cold Acclimation of Bermudagrass in a Controlled Environment_. We have detailed the development of the "electrolyte-leakage" and "freeze-regrowth" laboratory techniques for measuring relative or actual cold hardiness of bermudagrass plants in previous reports and in a scientific publication (Anderson, J.A., M. P. Kenna, and C. M. Taliaferro. 1988. Cold Hardiness of 'Midiron' and 'Tifgreen' Bermudagrass. HortScience 23(4):748-750). The benefits of laboratory procedures to evaluate plant material from breeding programs for superior cold hardiness have been recognized. Exposing samples from field plots to controlled freezing and viability testing can indicate relative hardiness levels and seasonal patterns of cold tolerance. However, information on the effect of temperature on cold acclimation is lacking. The objective of research conducted over the past year was to determine the effects of acclimation temperature and time on cold hardiness levels of bermudagrass genotypes known to differ in cold tolerance.

Three genotypes, 'Midiron', 'Tifgreen', and '40-3', were grown in cone-tainers in the greenhouse for at least 6 weeks. Plants were transferred to a growth chamber set at progressively lower temperatures as outlined in Figure 1. After the appropriate acclimation treatment, samples were brought to the laboratory and watered to field capacity. Top growth was trimmed to about 2 cm, and a thermocouple was inserted to a depth of 2 cm into the soil in the center of the cone-tainer. Two replicate samples for each genotype-treatment temperature combination were placed in a 2 cm thick aluminum plate with holes drilled to accommodate the cone-tainers. Up to 168 samples were placed in a low temperature chamber and equilibrated at -3C. Crushed ice was sprinkled over the tops of each cone-tainer, and samples were held overnight at -3C. Time-temperature records were evaluated graphically for exotherms to ensure that none of the samples had avoided freezing. Chamber temperature was lowered 1C per hour with samples removed at 1C intervals spanning the anticipated killing temperature. Cone-tainers removed from the low-temperature freezer were held overnight at 5C to permit slow thawing. Grasses were evaluated for regrowth after at least 4 weeks in the greenhouse.

Little or no acclimation was observed in 'Tifgreen' or '40-3' following exposure to 25/15C day/night temperatures for 4 weeks, while 'Midiron' increased in hardiness by about 2C (Figure 6). 'Midiron' continued to acclimate nearly linearly with increasing time in the growth chamber at progressively lower temperatures. '40-3' exhibited a substantial increase in hardiness following exposure to 7/0C with little or no further benefit from 7/-3C. 'Tifgreen' responded similarly to '40-3' but with acclimation delayed and to a reduced extent.

Additional samples placed into the chamber after 4, 8, and 12 weeks indicated that exposing the grasses to 13/8C directly resulted in equal or greater hardiness compared with prior exposure to 25/15C (data not presented). Plants exposed directly to 7/-3C increased in hardiness, but not as extensively as plants with prior exposure to warmer temperatures. The effects of holding plants at the same acclimating temperatures for periods longer than 4 weeks is not known.

Screening bermudagrass genotypes for cold hardiness in controlled environment chambers appears to have potential. Evaluations can be conducted at any time, rather than over the winter, as is the case for materials hardened in the field. Using cone-tainers for growing, acclimating, and treating samples reduces labor and space requirements and minimizes disturbances to the plants. Bermudagrasses readily acclimate in a growth chamber and exhibit relative hardiness patterns consistent with previous work.

The above described procedure is now being used to evaluate bermudagrasses from the breeding program. Plants are grown in "cone-tainers" in the greenhouse, then transferred to a growth chamber for acclimation. After four weeks at 7 / 0C (day / night) temperatures, plants are subjected to low temperature stress in a laboratory freeze-chamber. Plant response is evaluated by regrowth after at least four weeks in a greenhouse. This procedure is being used with two variations. Seeded materials, with one plant per genotype, are exposed to one sub-freezing temperature expected to result in strong selection pressure. In the other series of experiments, survivors from low temperature exposure are clonally propagated. Each genotype is then acclimated and exposed to a range of sub-freezing temperatures spanning the anticipated killing temperature. As a result, freeze-killing temperatures can be determined, facilitating comparisons between genotypes.

<u>New Germplasm Procurement</u>. Sixteen new turf bermudagrass accessions were acquired this year for evaluation and inclusion in the breeding program. Eleven of these were turf bermudagrasses found growing on golf courses in Zimbabwe by Taliaferro during a visit to that country August 6-18, 1990. These were variant types found on fairways and greens of the Royal Harare, Chapman and Enterprise Country Clubs in Zimbabwe. These collections are now at the National Plant Quarantine Center in Glen Dale, Maryland. New accessions were also obtained from the SCS Plant Materials Center at

Quicksand, KY; the Country Club of Virginia, Richmond; North Hempstead Country Club, Port Washington, NY; North Shore Country Club, Glenn Head, NY; and Plymouth Country Club, Norristown, PA.

Tissue Culture Research. The emphases on tissue culture research in the past year have been the development of improved techniques for regeneration, use of seed explants (as opposed to use of vegetative parts), and identification of responsive genotypes. A successful procedure was developed for using seed explants. This procedure involves surface sterilizing the seed and germinating them on a callus inducing medium. Callus tissue develops from the emerging shoots of the seed and ultimately produces regenerated plants from somatic embryoids. Some genotypes respond much better than others in producing callus and/or in developing embryogenic callus. Large numbers of regenerated plants have been regenerated from responsive genotypes.

Calli cultures from the varieties 'Tifgreen', 'Tifgreen II', 'Tifdwarf' and 'Tifway' and 'NoMow' were established from young inflorescence explants during the summer. Of these, Tifgreen appears to be most responsive in terms of developing embryogenic callus. About two dozen plants have been regenerated to date from Tifgreen and many more are expected. Regenerated plants are now growing in the greenhouse and will be field grown next season. A common feature of tissue culture is the production of mutant plants (somaclonal variants) in low frequency. We are interested in identifying such variants as a means of broadening the germplasm base of turf bermudagrasses.

## RESEARCH PLANNED

Development of Seed-Propagated Varieties:

1. Proceed with determination of performance of experimental synthetic varieties planted in tests in Colorado, Iowa, Missouri, and Oklahoma. These evaluations should provide data indicating whether or not any of these strains have potential for commercial release.

2. Continue greenhouse screening for fine-texture in breeding populations. This includes identification of superior plants from nurseries in Arizona and Oklahoma to intercross to produce progeny populations for subsequent cycles of greenhouse selection for finer plant texture. Selection in the Arizona nursery will be of the basis on turf-type morphology and seed production potential.

3. Develop several experimental synthetic varieties from the greenhouse selected fine- textured material currently in Arizona and Oklahoma nurseries. Individual experimental synthetic varieties will be developed by planting selected parent plants in isolated polycross nurseries.

4. Continue with refinement of techniques for laboratory evaluation of cold tolerance and mass screening of populations for cold-tolerant plants.

Development of Vegetatively-Propagated Varieties:

1. The 3,300 C. transvaalensis progeny plants planted in early June 1990 will continue to be evaluated according to the schedule in Table _. The 500 plants established in the five Florida test sites this fall will be evaluated in the greenhouse at Stillwater during the winter and spring of 1990/91 and at the five Florida sites during the 1991 growing season.

2. Selected C. transvaalensis plants will be established on a putting green surface and subjected to replicated cultural/management treatments to better define their requirements for maintenance of high turf quality. Treatments will include fertility, cutting height, thatch management, etc.

3. Initiate experiments to determine the basis of differences in winter survival of bermudagrass plants, especially C. transvaalensis plants. Differences in winter response of C. transvaalensis plants in our breeding nurseries has ranged from complete winterkill to excellent survival and spring green-up. We hypothesize that such differences may be due to genotypic differences in tissue cold tolerance, or depth of crowns and/or rhizomes, or a combination of the factors. Experiments will be performed to test the hypothesis.

4. Continue evaluating the C. transvaalensis X C. dactylon hybrids selected from breeding nurseries in spring 1990 and established in a replicated evaluation test in summer 1990 on the Turfgrass Research Center at Stillwater.

5. The approximately 3,000 C. transvaalensis progeny growing as spaced plants in field nurseries that survived the 89-90 winter will be further evaluated for seed production and morphological characters and breeding potential. Additional inter- and intra-specific crosses will be made between selected plants of C. dactylon and C. transvaalensis.

Tissue Culture Research:

1. Tissue culture research is continuing, with emphasis on improving regeneration efficiency for bermudagrass and developing screening procedures at the cellular level. Cell cultures of several turf bermudagrass varieties are established and being used in those experiments. Varieties include Tifgreen, Tifgreen II, Tifdwarf, and NoMow. Cultures of additional varieties will be established.

Table 1. Visual ratings of performance characteristics of bermudagrasses seeded June 18, 1989. Turfgrass Research Center, Stillwater, OK. Values are means of 3 replicate plots.

| | 1989 | | | | | | | | | | 1990 | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % Cover | | Turf Quality | Color Retention | | | | | | | Spring Greenup | | | | | | | | Turf Quality | | | | | Color | Density | Texture | Color Retention | | | | | |
| Entry | 8/8 | 9/19 | 9/19 | 10/2 | 10/12 | 10/20 | 10/23 | 11/3 | 11/6 | 11/13 | 3/16 | 3/23 | 4/4 | 4/9 | 4/24 | 5/9 | 5/25 | 5/30 | 6/22 | 7/24 | 8/22 | 9/25 | 10/2 | 6/28 | 6/28 | 6/28 | 10/1 | 10/9 | 10/17 | 10/22 | 10/26 | 10/29 |
| Numex Sahara | 97 | 100 | 5.7 | 6.7 | 7.7 | 6.3 | 2.3 | 1.7 | 1.0 | 0.7 | 0.0 | 0.3 | 0.3 | 1.0 | 6.7 | 6.7 | 15.0 | 16.7 | 2.0 | 4.3 | 4.0 | 5.3 | 5.7 | 5.0 | 1.7 | 2.0 | 8.0 | 8.3 | 7.7 | 7.3 | 5.3 | 5.7 |
| Common | 87 | 100 | 5.3 | 6.7 | 6.7 | 6.3 | 3.3 | 1.7 | 1.7 | 1.0 | 3.7 | 6.7 | 15.0 | 10.0 | 45.0 | 75.0 | 91.7 | 95.0 | 5.3 | 6.0 | 5.7 | 6.3 | 6.7 | 8.0 | 4.3 | 3.0 | 8.0 | 8.3 | 7.3 | 7.3 | 5.7 | 5.0 |
| Guymon | 57 | 100 | 4.7 | 6.7 | 6.7 | 6.0 | 3.7 | 2.0 | 1.7 | 1.3 | 3.7 | 5.0 | 13.3 | 6.7 | 38.3 | 71.7 | 86.7 | 93.3 | 6.0 | 6.3 | 6.3 | 6.3 | 7.3 | 7.7 | 6.0 | 3.0 | 8.7 | 8.3 | 8.0 | 8.0 | 6.0 | 5.3 |
| OKS-1 | 60 | 100 | 5.3 | 6.3 | 6.3 | 5.7 | 3.0 | 1.3 | 1.0 | 1.0 | 3.7 | 6.7 | 20.0 | 10.0 | 60.0 | 86.7 | 96.7 | 100.0 | 7.3 | * | 3.3 | 4.0 | 5.3 | 6.0 | 8.0 | 8.0 | 8.3 | 8.3 | 8.0 | 8.0 | 6.0 | 6 |
| OKS-2 | 65 | 95 | 6.0 | 6.3 | 7.3 | 6.7 | 4.0 | 2.3 | 2.0 | 1.3 | 2.3 | 5.0 | 8.3 | 5.0 | 45.0 | 61.7 | 91.7 | 98.3 | 6.3 | 6.0 | 6.7 | 7.0 | 7.7 | 8.0 | 5.7 | 3.3 | 9.0 | 8.7 | 7.7 | 7.3 | 5.3 | 4 |
| OKS-3 | 60 | 93 | 6.3 | 5.7 | 7.0 | 6.0 | 3.7 | 3.0 | 2.7 | 1.3 | 1.0 | 3.7 | 6.7 | 5.0 | 21.7 | 41.7 | 83.3 | 95.0 | 6.0 | 5.7 | 6.0 | 6.7 | 7.3 | 8.0 | 6.0 | 3.0 | 8.0 | 8.0 | 7.7 | 7.0 | 5.3 | 4.. |
| OKS-4 | 55 | 83 | 5.0 | 6.0 | 7.7 | 7.0 | 4.3 | 4.0 | 2.7 | 1.3 | 1.7 | 5.3 | 12.0 | 5.3 | 30.0 | 56.7 | 76.7 | 88.3 | 5.0 | 5.7 | 5.7 | 5.7 | 7.0 | 8.0 | 6.0 | 3.0 | 8.7 | 8.7 | 8.0 | 8.0 | 5.0 | 5.3 |
| OKS-5 | 67 | 93 | 6.0 | 6.3 | 7.0 | 6.0 | 4.0 | 2.7 | 2.7 | 1.3 | 2.3 | 5.0 | 16.7 | 5.0 | 51.7 | 58.3 | 91.7 | 98.3 | 5.7 | 6.0 | 6.3 | 6.7 | 7.7 | 8.0 | 7.0 | 3.0 | 8.7 | 8.7 | 7.7 | 7.0 | 5.0 | 4.3 |

Ratings for turf quality, color retention, spring greenup, color, density, and texture are on a scale of 0-9, with 9 being best.

Table 2. Florida golf courses participating in the evaluation of *C. transvaalensis* selections as potential putting green varieties.

Deerwood Country Club
Tom Cowan, CGCS
10239 Golf Club Drive
Jacksonville, Fl 32256

Country Club of Orlando
Cary Lewis, CGCS
1601 Country Club Drive
Orlando, FL 32804

Fiddlesticks Country Club
Lou Conzelmann, Superintendent
15527 Fiddlesticks Blvd.
Fort Meyers, FL 33912

High Ridge Country Club
David Bailey, CGCS
2400 Hypoluxo Road
Lantana, FL 33462

Palm Beach Country Club
Paul Crawford, Superintendent
P.O. Box 997
Palm Beach, FL 33480

## Table 3

**Research Timetable For Developing New Putting Green Varieties**

| Year | | Season | Activity |
|---|---|---|---|
| 89/90 | | W | * Selections from breeding nurseries- 3000 |
| | | Sp | * Establish and evaluate 3000 selections under putting green maintenance. |
| | Yr 1 | Su | * Field selection - 500 best plants. Establish and evaluate in Florida nurseries. |
| | | F | |
| 1991 | | W | * Greenhouse evaluation (Rooting and Drought). |
| | | Sp | |
| | Yr 2 | Su | * Field and greenhouse evaluation (Flowering and breeding behavior). |
| | | F | * Greenhouse evaluations (Disease and Drought). |
| 1992 | | W | * Increase best selections (30 selections). |
| | | Sp | |
| | Yr 3 | Su | * Establish and evaluate in 8-10 preliminary variety trials in Florida and Oklahoma. |
| | | F | |
| 1993 | | W | * Increase best selections (3-5 Selections). |
| | | Sp | * Establish and evaluate final variety trial (30 environments = 15 loc X 2 yr). |
| | Yr 4 | Su | |
| | | F | * Evaluate Sod Production Potential. |
| 1994 | | W | * Greenhouse evaluation (Nutrition). |
| | | Sp | * Establish field increase blocks. |
| | Yr 5 | Su | * Cultural practice studies. |
| | | F | * Establish entire green on golf course in south Florida. |
| 1995 | | W | * Compile five year history of best selection and release as a named vegetative variety. |
| | | Sp | |

Fig. 1. Experimental seed-propagated bermudagrasses in a test planted June 18, 1989.

Fig. 2. Two C. transvaalensis plants in the breeding nursery that depict visual differences observed among more than 4,000 such progeny plants.

Fig. 3. An overview of a portion of the putting green nurseries containing the 3,300 C. transvaalensis plants established during the first week of June. Photograph was made in early September.

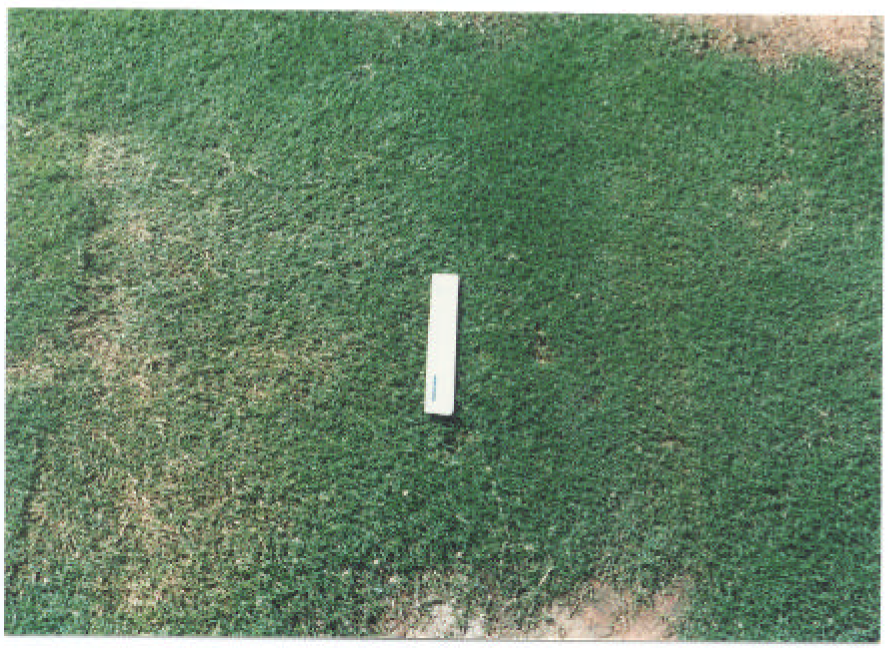

Fig. 4. Close-up of C. transvaalensis plants in the putting green evaluation trial mowed at 1/4". Differences in speed of establishment and morphological features i.e. color, texture, density, etc. were evident.

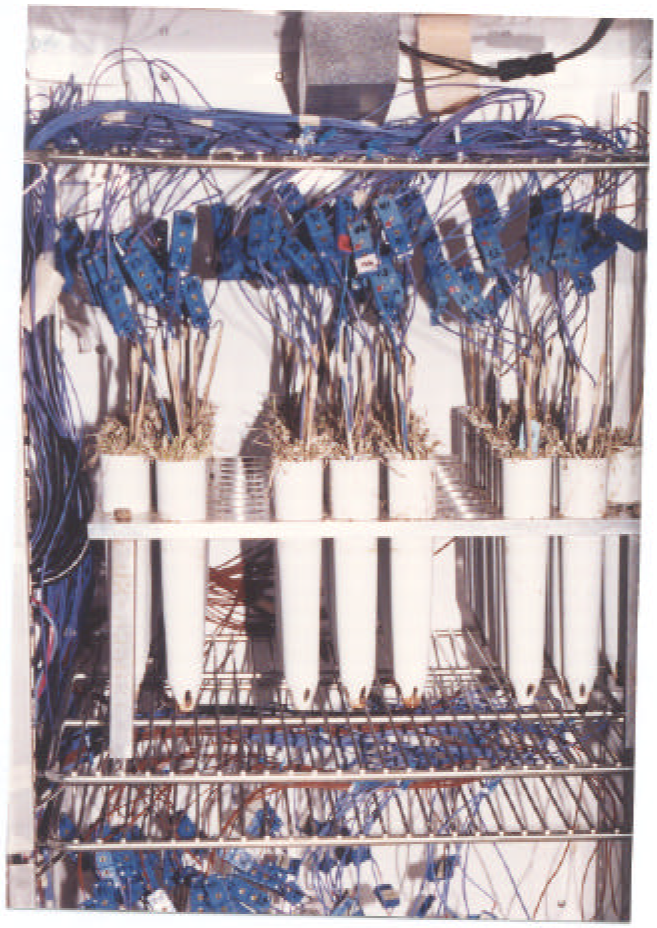

Fig. 5. Bermudagrass plants in a freezing chamber. Mass screening for cold hardy plants is accomplished by growing plants in cone-tainers, acclimating them in a growth chamber and then subjecting to freezing temperatures.

Fig. 6. Cold acclimation of 'Midiron', 'Tifgreen', and '40-3' bermudagrasses in a controlled environment chamber. Coldest survival temperatures were determined at 2 week intervals for greenhouse-grown plants in cone-tainers sequentially exposed to 4 weeks at day/night regimes of 25/15 (A), 13/8 (B), 7/0 (C), and 7/-3 °C (D). Additional samples were placed into the chamber at 4 week intervals to determine the effect of prior conditioning at warmer temperatures.