ANNUAL PROGRESS REPORT

CONCERNING

BREEDING AND EVALUATION OF COLD-TOLERANT BERMUDAGRASS VARIETIES

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EXECUTIVE SUMMARY

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BREEDING AND EVALUATION OF COLD TOLERANT BERMUDAGRASS CULTIVARS

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RESEARCH PROGRESS:

Mean fertility (seed set) has been tripled in a cold-tolerant broad gene-base bermudagrass population using phenotypic recurrent selection. Progress has also been made in selecting for finer texture within this population. The premise of the recurrent selection breeding procedure is ongoing population improvement via increase in the frequency of favorable quantitative genes controlling the characters under selection. Experimental strains derived from this population are now being tested for turf performance, and new strains will be available on a regular basis for evaluation.

Plants of the very fine textured _C_. _transvaalensis_ having excellent seed head production and seed set were found in our germplasm nurseries. The four best of these plants had seed set percentages of 83, 77, 73, and 72. These plants will be further assessed for seed yield and genetic improvement potential. Because this material already possesses extremely fine texture, sod density, and cold tolerance, the development of strains with economic seed yield potential would be of major importance.

Research to date has demonstrated the reliability and feasibility of two laboratory techniques for measuring cold tolerance of bermudagrass plants. Further assessment of the electrolyte leakage and freeze/regrowth techniques during the winter of 1987-88 confirmed earlier results indicating good agreement between the techniques, with both accurately ranking varieties of known relative cold tolerance.

Tissue culture techniques have been developed permitting regeneration of large numbers of bermudagrass plants from embryogenic callus derived from somatic (diploid meristematic) tissue explants. Although regeneration of plants from cultured bermudagrass anthers has not been achieved to date, some anther callus from some bermudagrass plants has been produced. This and the fact that haploid plants have been regenerated in several grass species via anther culture gives hope that continued refinement of technique will result in eventual success with bermudagrass.

I. INTRODUCTION

The turf bermudagrass breeding effort jointly sponsored by the United States Golf Association and the Oklahoma Agricultural Experiment Station has as its basic goal the development of seed-propagated, fine-textured, winter hardy varieties suitable for use in the northern sections of the bermudagrass belt of the USA. Phenotypic recurrent selection is being used to effect improvement in basic fertility and morphology (texture) within an initial broad gene base, cold-tolerant, bermudagrass (Cynodon dactylon) breeding population. Selection for the different characters is by independent truncation (i.e., select first for basic fertility, secondly for morphological form). The premise of the recurrent selection breeding procedure is ongoing population improvement via increase in the frequency of favorable quantitative genes controlling the selected characters. Desirable plants can be extracted from the population whenever found for use as parents in the synthesis of a variety; or any individual plant may be propagated vegetatively as a cultivar, if desired. Additionally, evaluation of other Cynodon germplasm and inclusion of desirable types into the breeding effort is standard and ongoing. Development and/or improvement of techniques to measure physiological parameters related to stress reaction and water use, and the measurement of those parameters in advanced breeding selections, are important parts of this program. Finally, we are attempting to develop a technique for regenerating plants from tissue cultured anthers of Cynodon, as a means of obtaining haploid plants, which after chromosome doubling will be genetically homozygous.

II. RESEARCH PROGRESS

A. Breeding and Development

As indicated in previous reports, two cycles of recurrent selection in a broad gene base, cold tolerant, tetraploid population has resulted in more than a three-fold increase in basic fertility (% of florets setting seed) as indicated by mean seed-set percentages for the two populations. Seed set percentages are now being determined for the third cycle population. Seed set in many of the plants in the third cycle population for which a determination has been made is 60% or higher. Selection has also resulted in changes in growth habit or morphology of plants for traits like height, density, and texture. The principle changes in growth habit appear to be in height (shorter internodes) and greater sod density. Additionally, there is variation among plants in size of leaves and stems, which should permit an overall improvement in texture for this population via recurrent selection. Figure 1 shows a nursery of third cycle population plants.

Several bermudagrass plants from the recurrent selection populations were planted in polycross and/or singlecross nurseries under isolation for production of adequate seed to allow field evaluation of turf performance (Figure 2). Additionally, nineteen bermudagrass clones or synthetic populations were established in a replicated test in Arizona in September for seed yield evaluation. This is cooperative with the Farmers Marketing Corporation. A copy of the Memorandum of Agreement for this test is included in the appendix.

Sixty-four of about 2500 space-planted progeny plants from matings of selected parent plants were advanced to preliminary evaluation under turf mowing conditions. Some of these do not have sufficient fertility for use as parents in seed propagated varieties, but may have potential as vegetatively propagated varieties. Additionally, 900 progeny plants from crosses of selected parental plants were established in a nursery for preliminary evaluation and selection.

As part of our continuing effort in evaluation of new germplasm for inclusion in the breeding effort, diploid (2n=2x=18) C. Transvaalensis plants with good seed head production and good seed set were identified. The C. transvaalensis species has the finest texture and greatest density of the Cynodon species. It has served as the traditional parent in crosses with C. dactylon parents to produce the triploid (2n=3x=36) turf bermudagrass varieties, e.g., Tifgreen, Tifway, etc. The four best of these plants had seed set percentages of 83, 77, 73, and 72. Figures 3 and 4 show a field plot and seed of one of these plants. The four best plants, referred to above, were established in an isolated field crossing block near El Reno, Oklahoma (Fig. 5); and 780 seedlings from the open-pollinated seed obtained from the four plants were space planted in a field nursery (Fig. 6). Over 90% of these plants have the phenotype of true C. transvaalensis, indicating only slight outcrossing with adjacent C. dactylon plants having coarser texture and the tetraploid (2n=4x=36) chromosome number. Because of the much greater textural fineness and density of C. transvaalensis compared to C. dactylon, and its inherent cold tolerance, we view the discovery of these plants as being potentially very important. Conceivably, they could shave years off the time necessary to develop a really fine-textured cold-tolerant seed-propagated bermudagrass cultivar. In the coming months we will continue evaluation of these materials for seed production, progeny performance, and genetic variation and breeding potential.

B. COLD TOLERANCE

An experiment evaluating two laboratory methods (electrolyte leakage and regrowth) measuring cold tolerance in bermudagrass was completed and the results published. A reprint of the paper entitled "Cold tolerance of 'Midiron' and 'Tifgreen' bermudagrass" is included in the appendix. The USGA is credited with partial support of this study. Details concerning the two methods are given in last year's report and in the journal article reprint. Briefly, there was good agreement between the two methods, indicating both give acceptably accurate measures of relative or absolute cold tolerance of bermudagrass plants. Each method has its assets and liabilities. The regrowth test may be slightly more accurate, but requires more time (3-4 weeks more) than the electrolyte leakage test. It also requires considerable greenhouse space. The electrolyte leakage test requires only two days to complete exclusive of time required to prepare the tissue samples. However, preparation of the tissue samples is labor intensive, negating handling of large numbers of plant samples without considerable manpower.

Cold tolerance analysis of two groups of bermudagrass plants was completed in 1988. The first group constituted 40 selections from the breeding nurseries in which sampling was done in February, a time of maximum acclimation and cold tolerance according to previous study. Evaluation was only by the regrowth test. The results separated the 40 plants into three groups, i.e., those killed by a freezing temperature of -6°C, those surviving -6°C but killed by -8°C, and those surviving -8°C. This type of testing appears to be most feasible for preliminary evaluation of large numbers of plants in breeding nurseries. More comprehensive evaluation was conducted on 13 bermudagrass plants included in an advanced turf performance field test. Regrowth tests were conducted on plant samples collected about every two weeks beginning 9/14/87 and ending 6/8/88. Figures 7-19 show the results of these tests for the respective plants tested. The data demonstrate differences in cold tolerance among the plants and enable assessment of relative cold tolerance of the plants at the respective sampling dates. Of the 13 entries in the test, the Midiron cultivar attained the greatest degree of cold tolerance (Fig. 19). Maximum cold tolerance of Midiron was attained in late December, January, and February. Other entries in the test appear to have good to excellent cold tolerance. For example, OK78 1-8 (Fig. 8) had cold tolerance not materially different from the Midiron cultivar. The ability to accurately assess the cold tolerance of plants with these laboratory methods represents an important advance. This allows information to be incorporated into decision making regarding release of plants as new cultivars, or their use in breeding programs. Use of these techniques in characterizing cold tolerance of breeding lines and advanced selections in our program will continue, and improvements in the techniques will be sought.

C. VARIETY TRIALS

Three field evaluation trials were conducted in 1988. These included the National Bermudagrass Variety Trial, established in 1986. This test contains 28 varieties formally entered in the nationwide test, plus other strains from our program. In 1987, the test was fertilized with 1 lb. actual N/1000 ft.^{2}, and mowed at a height of 1.5 inches. In 1988, 6 lb. actual N/1000 ft.^{2} was applied and the mowing height was 0.75 inch. Data are given for leaf width and turfgrass quality in 1987 (Table 1), and spring greenup, turf quality, and texture in 1988 (Table 2). Among the vegetatively propagated, medium textured varieties, Midiron, A-29, Tufcote, and A-22 were the best performers for spring greenup in 1988. Following the relatively mild winter at Stillwater, the Sahara cultivar had slightly faster greenup than Arizona common, and both greened up faster than the strongly dormant Guymon variety.

A second test comprised of 10 experimental strains from the OSU breeding program plus four control varieties was established in July, 1987. Data are presented for establishment rate, turfgrass quality, color, texture, and fall dormancy in 1987 (Table 3), and spring greenup (Table 4), turfgrass quality, texture, and percent cover (Table 5) at various dates in 1988. The rate of establishment of all experimental strains was superior to the four control varieties. Some of the experimental strains have texture and turfgrass quality approaching Midiron and U-3, but still considerably more coarse that Tifgreen or Tifway. Most of the experimental strains had faster greenup in spring 1988 than Tifway or Tifgreen. One of the experimental strains, OK87 47-3, had faster greenup than Midiron. Spring greenup is, however, not a reliable indicator of cold tolerance. Examination of the cold tolerance data for OK87 47-3 and Midiron in Figures 14 and 19, respectively, demonstrate the latter variety to be substantially superior in winterhardiness.

A preliminary nonreplicated field evaluation trial, containing 52 plants from the breeding nurseries, was established in spring 1988. These selections will be evaluated under mowing for turf attributes. Any plants judged superior on the basis of these preliminary evaluations will be advanced to more comprehensive replicated evaluation trials.

D. TISSUE CULTURE

Tissue culture is being pursued as an aid to the genetic improvement of turf bermudagrasses. As indicated in previous reports, regeneration of plants from young inflorescence explants via somatic embryogenesis, has been achieved in our laboratory and by workers at the University of Arkansas. This achievement makes possible the application of recombinant DNA technology to the genetic improvement of turf bermudagrasses, and also allows the development of screening procedures at the cellular level. Such screening procedures might be directed toward selection for traits such as increased tolerance to herbicides, environmental stresses, or salt. In the coming months we will attempt to screen bermudagrass cell cultures for increased tolerance to selected herbicides.

A priority in our bermudagrass tissue culture work during the past year has been the development of a successful anther culture procedure. The objective of this work is the regeneration of haploid plants which, following chromosome doubling with chemical agents, would constitute genetically homozygous plants. Unfortunately, we have yet to attain this goal. Several experiments were conducted during the spring and summer of 1988 testing genotypes, different media, and medium components, but no treatment has to date resulted in regenerate plants. Some calli masses have been produced, but they are slow growing and nonembryogenic. We will continue trying different genotypes and media treatments in an effort to hit upon the correct combination that permits haploid regeneration. We have succeeded in regenerating haploid plants of a forage grass (_Bothriochloa ischaemum_) via anther culture, but bermudagrass is proving to be much more intractable.

III. RESEARCH PLANNED

Recurrent selection for increased fertility, seed yield, and turf quality will continue. Selected plants from the recurrent selection nurseries will be intercrossed to provide seed for turf performance evaluation, and to test combining ability. Several selected plants were intercrossed under isolation this year (1988). Testing of these synthetic strains, where seed quantity is adequate, can begin next year. Data collection on bermudagrass strains established in a seed production test in Arizona in cooperation with Farmers Marketing Cooperation will begin in 1989. Screening of plants for drought tolerance by growing on a deep sandy soil will be undertaken. Plants from the recurrent selection breeding population and the _C. transvaalensis_ germplasm will be included in this effort. Also, experiments will be undertaken to further assess the seed yield potential and genetic improvement potential of the _C. transvaalensis_ germplasm. The turfgrass field evaluation trials will continue. Evaluation of materials by cooperators at other institutions has been initiated. Trials have been initiated in California and Colorado. Selected plants from the breeding nurseries will be evaluated for cold tolerance using one or both laboratory methods. Work will continue on refinement of the cold tolerance determination techniques. A study is also planned on the relationship between water stress and cold tolerance. New tissue culture research will be initiated relating to the development of techniques for screening for herbicide resistance. Efforts will continue on the development of a successful technique for bermudagrass anther culture.

Fig. 1. *Space-planted bermudagrass plants in the Cycle-3 (C-3) population.*

Fig. 2. Isolated crossing blocks in which selected plants from the breeding program are being intercrossed to provide seed for field evaluation of turf performance.

Fig. 3. One of four *Cynodon transvaalensis* accessions with good seed head production and seed-set at Stillwater, OK, in 1988.

Fig. 4. Individual seeds from the plant in Fig. 3.

Fig. 5. Increase plantings of the four C. transvaalensis accessions were made at the S.W. Forage & Livestock Station near El Reno, OK, in late July 1988. These plantings will be used in further assessing the potential of this germplasm for cultivar development.

Fig. 6. Spaced plants derived from open-pollinated seed from the four C. transvaalensis accessions. This nursery was established August 1, 1988, on the Agronomy Res. Sta., Stillwater.

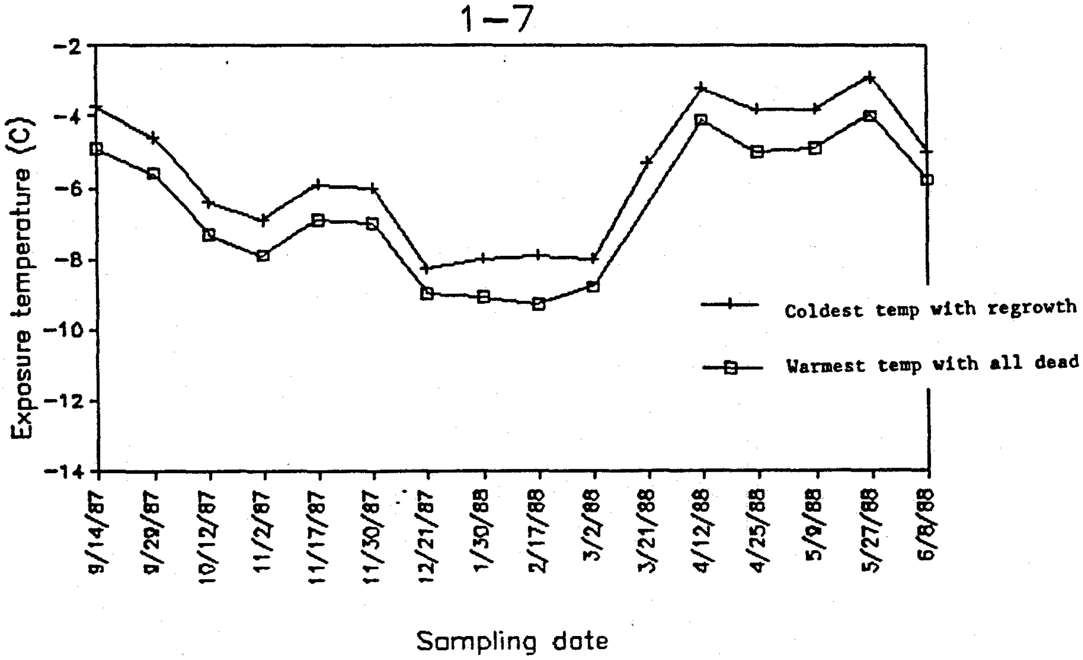

Figure 7. Cold tolerance of the bermudagrass strain OK87 1-7 at several sampling dates during 1987-88.

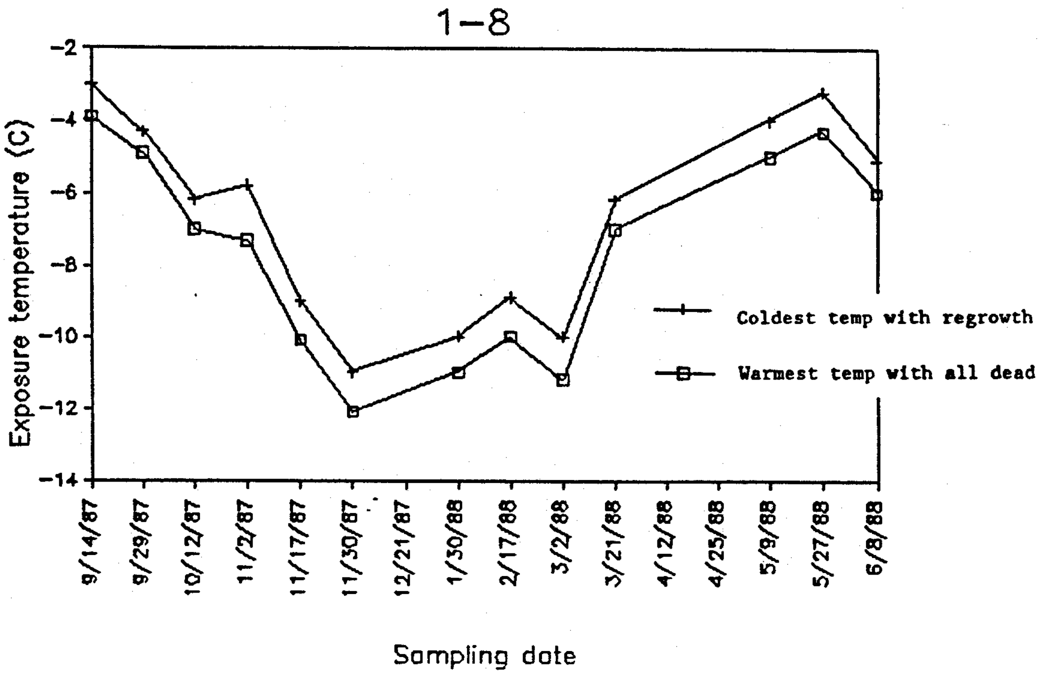

Figure 8. Cold tolerance of the bermudagrass strain OK87 1-8 at several sampling dates during 1987-88.

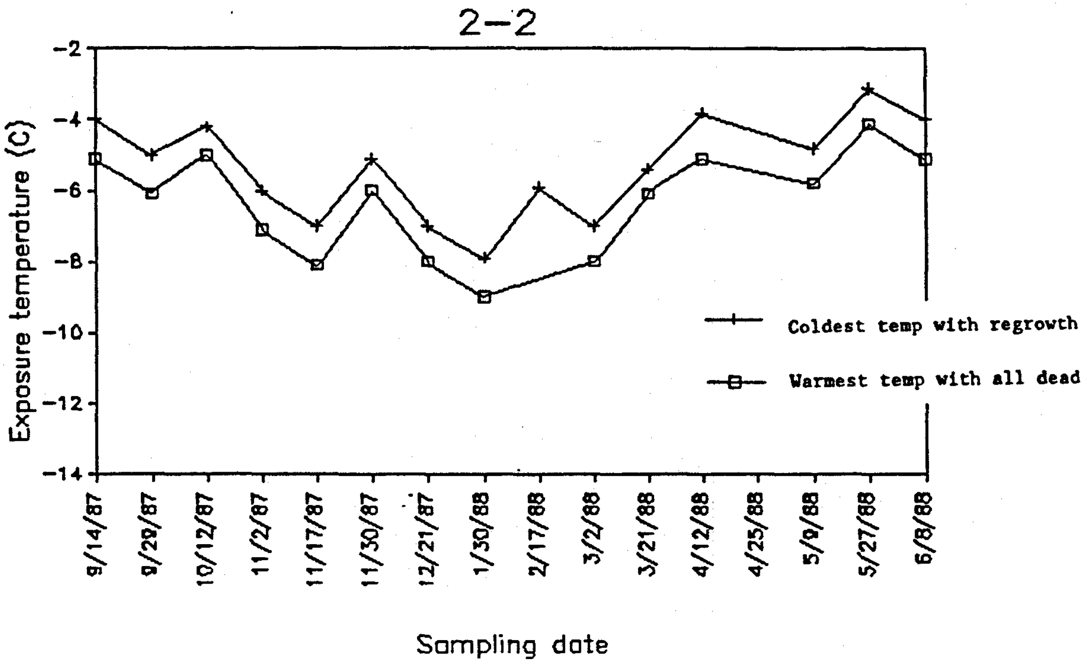

Figure 9. Cold tolerance of the bermudagrass strain OK87 2-2 at several sampling dates during 1987-88.

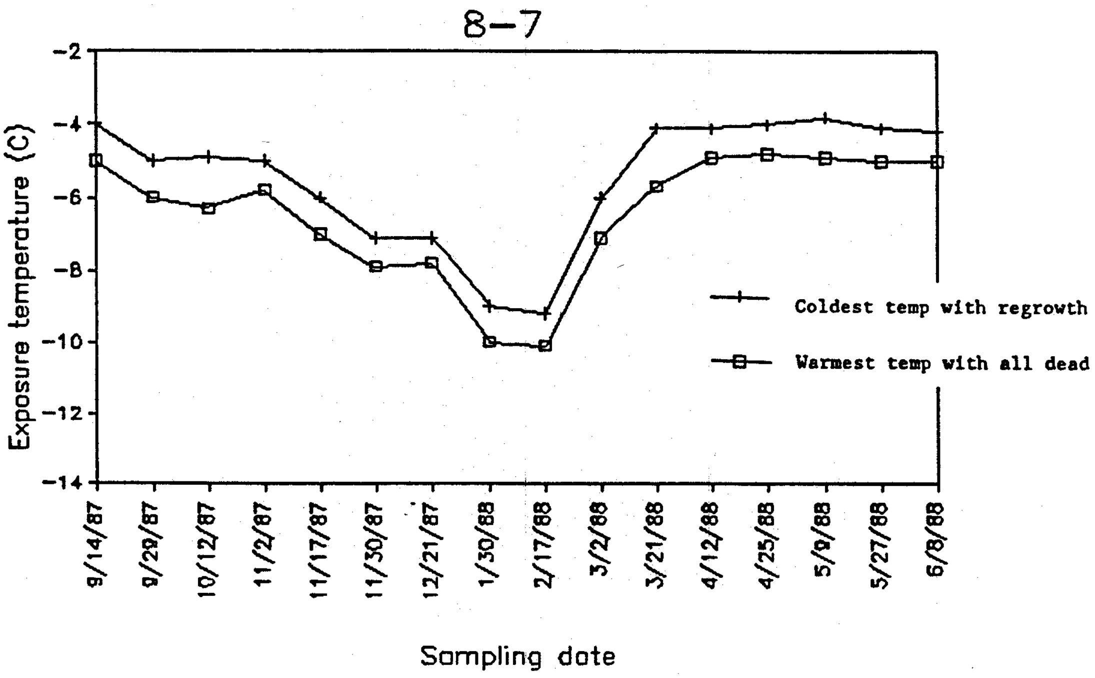

Figure 10. Cold tolerance of the bermudagrass strain OK87 8-7 at several sampling dates during 1987-88.

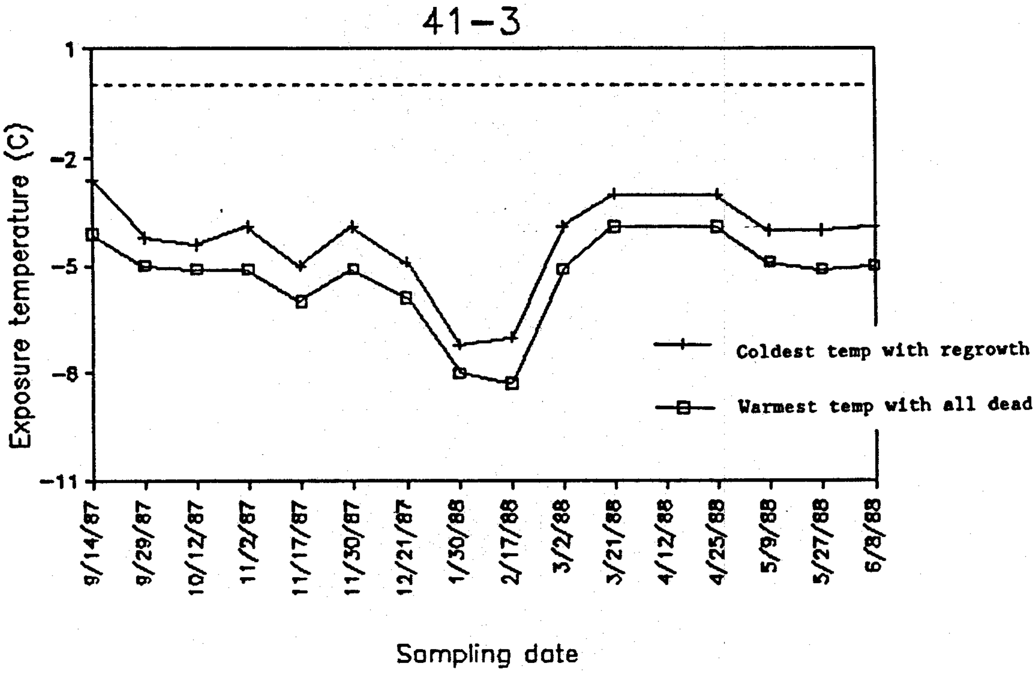

Figure 11. Cold tolerance of the bermudagrass strain OK87 41-3 at several sampling dates during 1987-88.

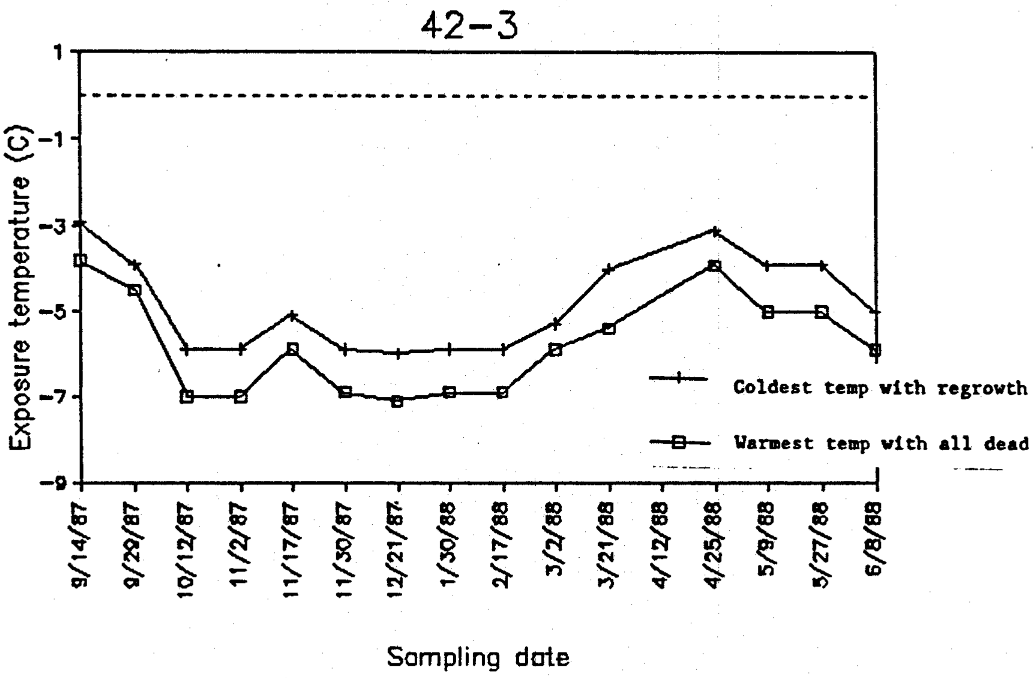

Figure 12. Cold tolerance of the bermudagrass strain OK87 42-3 at several sampling dates during 1987-88.

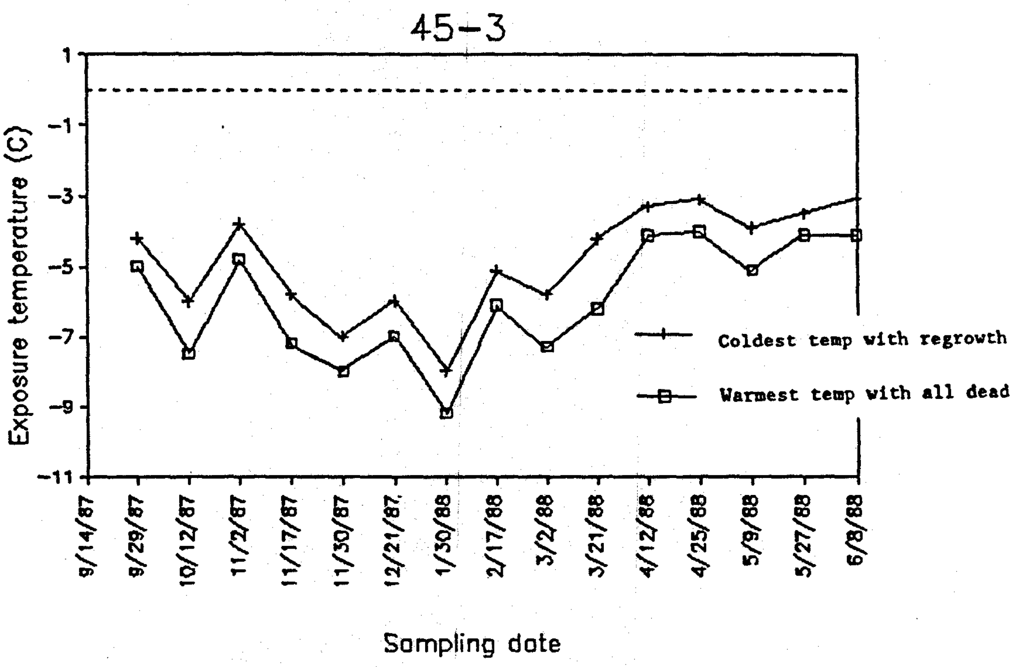

Figure 13. Cold tolerance of the bermudagrass strain OK87 45-3 at several sampling dates during 1987-88.

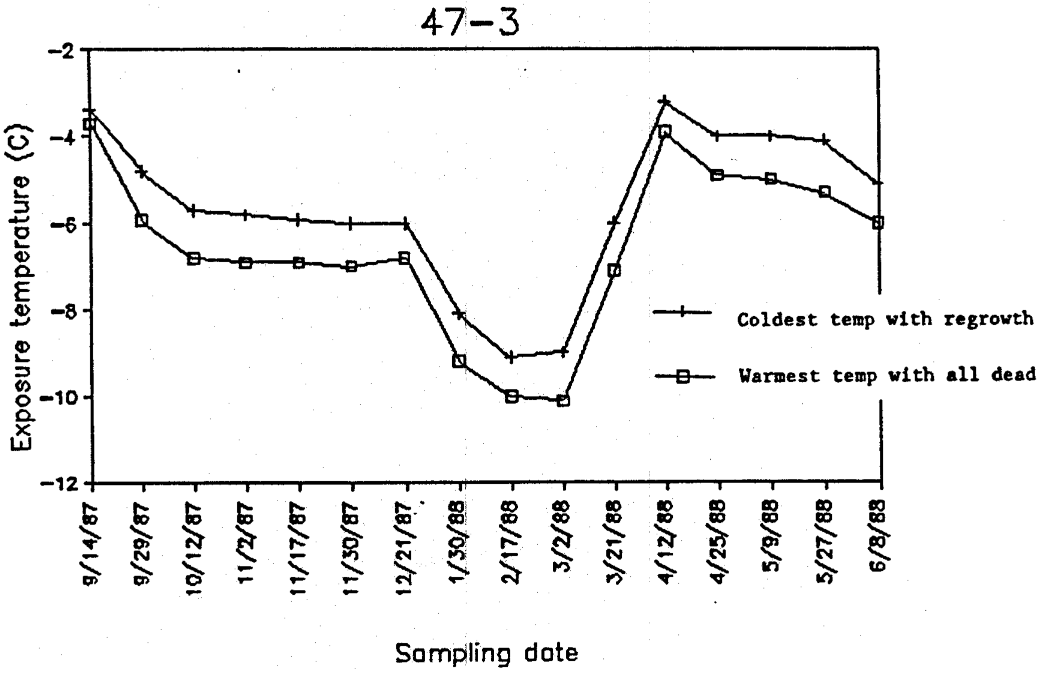

Figure 14. Cold tolerance of the bermudagrass strain OK87 47-3 at several sampling dates during 1987-88.

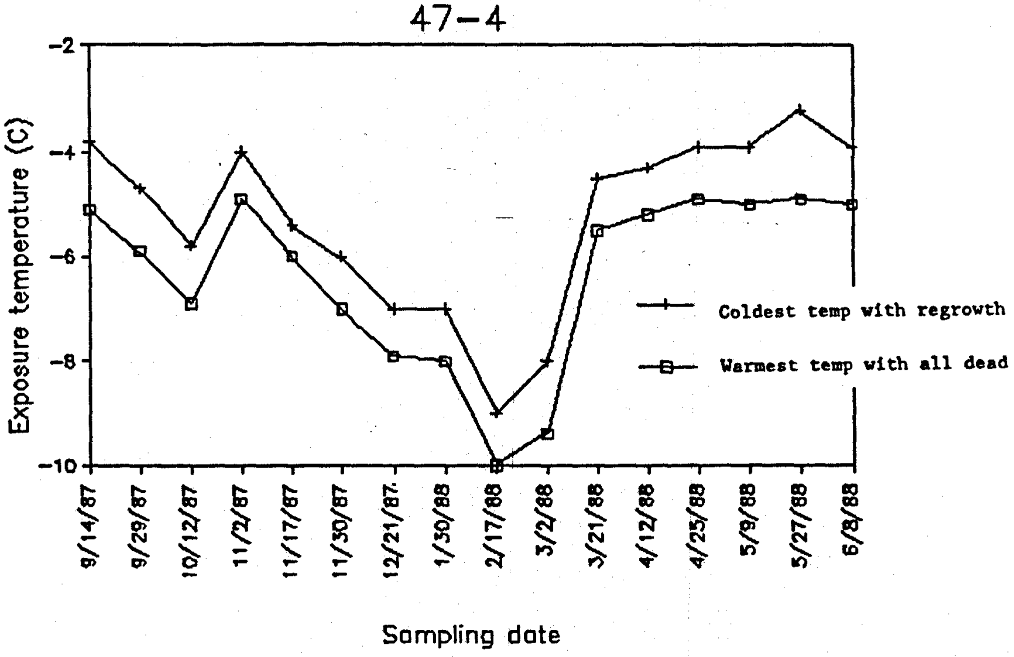

Figure 15. Cold tolerance of the bermudagrass strain OK87 47-4 at several sampling dates during 1987-88.

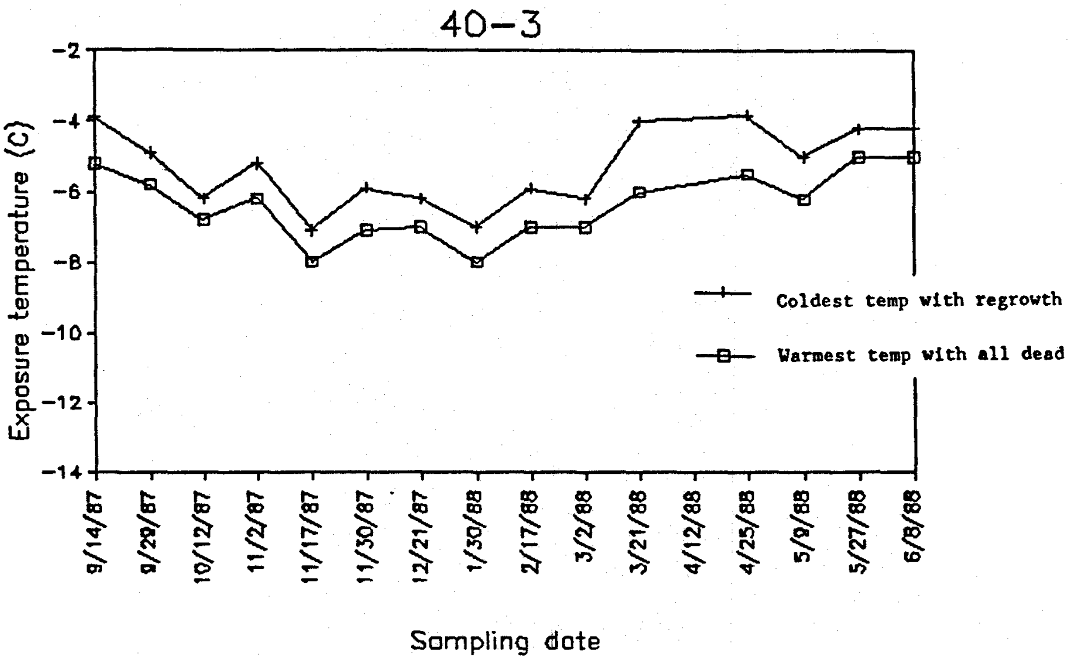

Figure 16. Cold tolerance of the bermudagrass strain OK87 40-3 at several sampling dates during 1987-88.

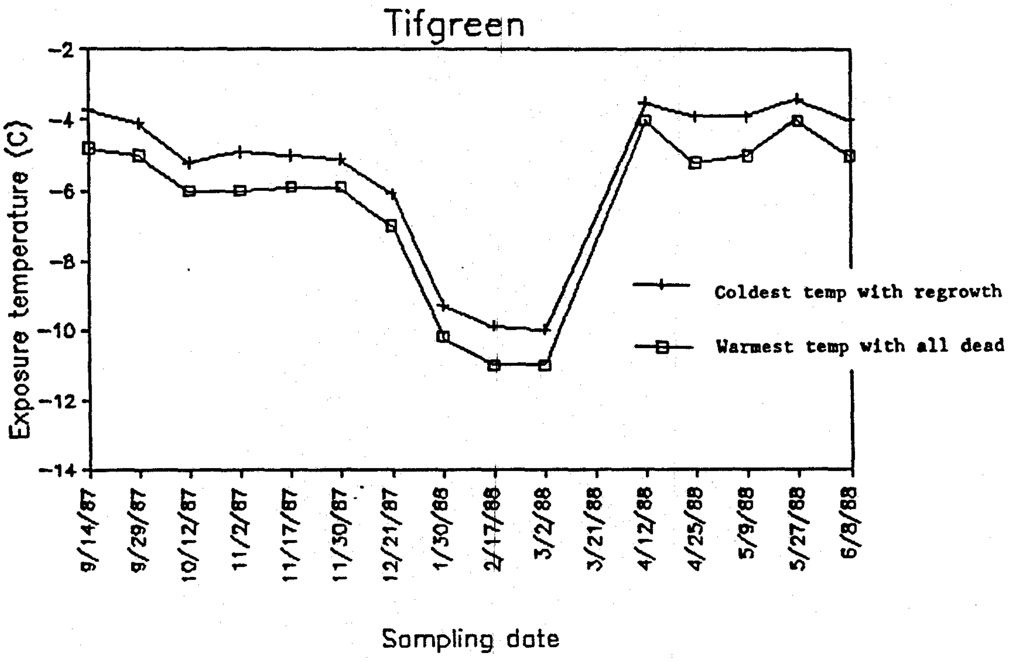

Figure 17. Cold tolerance of Tifgreen bermudagrass at several sampling dates during 1987-88.

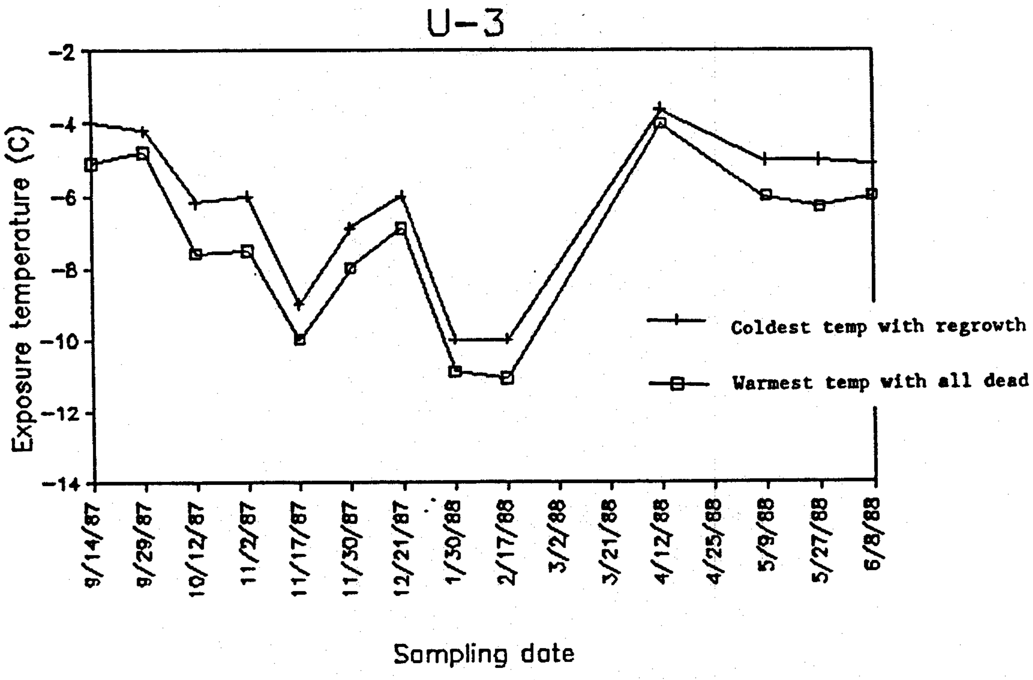

Figure 18. Cold tolerance of U-3 bermudagrass at several sampling dates during 1987-88.

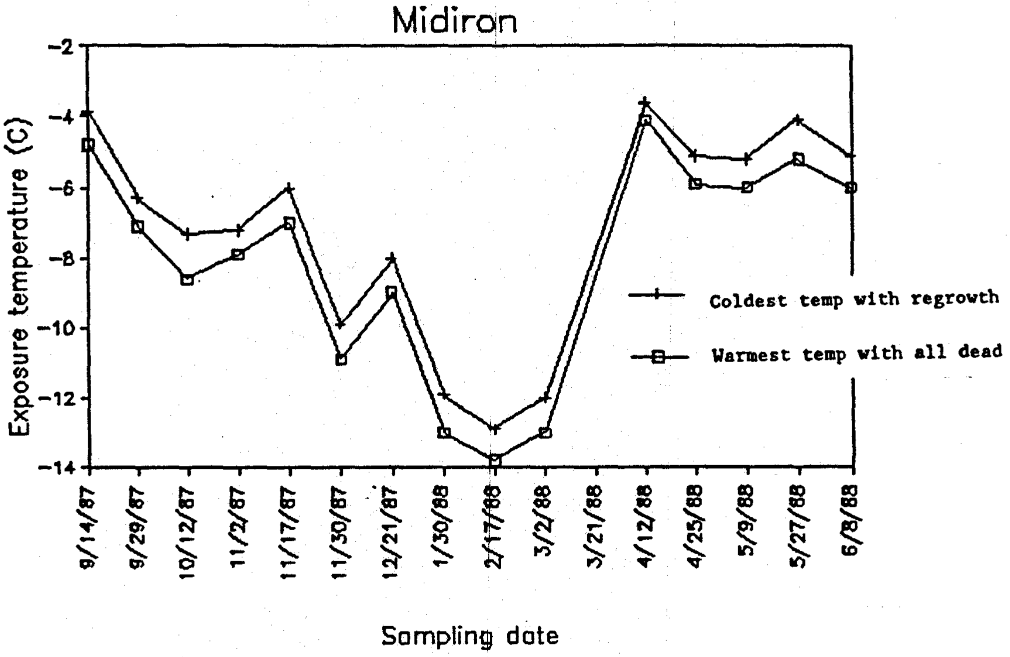

Figure 19. Cold tolerance of Midiron bermudagrass at several sampling dates during 1987-88.

Table 1. Bermudagrass Variety Trial. Spring and Summer 1987.
Oklahoma Turfgrass Research Center, Stillwater, OK.

			Turfgrass Quality[y]				
No.	Entry	Leaf width[z]	April	May	June	August	Mean
		-- mm --					
28	Hilltop	3.1	5.3	6.0	5.3	7.0	5.9
36	Texturf	3.8	5.3	6.3	5.3	6.3	5.8
2	MSB-20	2.1	5.3	5.7	4.7	7.3	5.8
23	A-29	2.7	5.0	6.0	5.3	6.7	5.8
37	Tifgreen	2.0	4.7	6.3	3.3	8.0	5.6
15	Vamont	3.9	5.7	4.7	4.7	6.7	5.4
6	NM-43	2.2	4.7	6.0	4.0	7.0	5.4
16	RS-1	3.3	5.7	5.0	4.7	6.3	5.4
42	TUFCOTE	2.7	5.0	6.0	5.7	5.0	5.4
44	E-29	2.9	5.3	6.0	4.3	6.0	5.4
11	NMS-1 (Sahara)	3.7	5.0	4.3	5.3	6.3	5.2
1	Tifway	2.3	4.7	5.7	3.7	6.7	5.2
25	NM-507	3.2	4.0	4.7	4.7	7.3	5.2
40	OK 86-12	2.7	4.7	5.0	4.7	6.3	5.2
24	NM-72	2.7	4.3	3.7	5.3	7.3	5.2
27	NMS-2	3.7	4.7	4.0	4.7	7.0	5.1
8	FB-119	2.7	5.0	4.7	4.3	6.0	5.0
20	NMS-14	3.2	5.0	4.3	5.0	5.7	5.0
45	A-22	2.4	5.0	5.0	4.3	5.7	5.0
47	OK 86-11	4.3	4.0	4.7	5.0	5.7	4.8
39	MSB-30	3.9	4.0	4.7	5.0	5.3	4.8
26	NMS-4	3.7	4.0	4.3	4.7	5.7	4.7
3	OK 86-3	3.0	4.3	4.0	4.3	6.0	4.7
34	Tifway II	2.2	4.0	5.3	3.3	6.0	4.7
21	OK 86-4	3.6	5.0	4.3	4.0	5.0	4.6
12	MSB-10	3.0	4.0	4.7	3.3	6.0	4.5
43	CT-23	2.4	4.3	5.0	3.7	5.0	4.5
5	Midiron	3.2	4.0	4.7	3.7	5.3	4.4
7	OK 86-9	3.2	3.7	4.0	4.7	5.3	4.4
30	OK 86-2	4.4	3.7	4.0	4.0	6.0	4.4
35	U-3	2.6	3.7	4.7	4.0	5.3	4.4
32	OK 86-5	3.6	3.7	3.7	4.3	5.7	4.3
38	NM-471	2.8	3.7	4.0	3.7	6.0	4.3
9	NM-375	3.7	3.3	3.3	4.0	6.7	4.3
46	Midway	3.6	4.0	4.3	4.0	5.0	4.3
13	Sunturf	2.6	4.3	3.7	3.3	5.7	4.3
31	OK 86-8	3.9	3.3	3.7	4.0	5.7	4.2
29	OK 86-1	4.3	3.7	3.7	3.7	5.3	4.1
41	NMS-3	3.3	3.7	3.3	3.7	5.0	3.9
14	Guymon	4.6	3.0	3.3	2.7	5.3	3.6
17	OK 86-6	4.6	3.0	3.3	3.3	4.7	3.6
19	OK 86-10	3.9	3.3	3.0	3.3	4.7	3.6
33	Arizona Common	3.7	3.3	3.0	3.0	5.0	3.6
48	OK 86-7	4.0	2.7	3.3	3.0	5.3	3.6
	LSD (0.05)		1.3	1.4	1.5	1.4	
	CV (%)		19.2	19.5	23.3	15.7	

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[z] Leaf width was measured on the fourth leaf from newest leaf. Means are for three samples from each plot averaged over three replications.

[y] Turfgrass quality where 1 = poor to 9 = excellent.

Table 2. National Bermudagrass Variety Trial. Spring and Summer 1988.
Oklahoma Turfgrass Research Center, Stillwater, OK.

	Spring Greenup[z]								Turf Quality[y]			Texture[x]
Entry	4/05	4/08	4/12	4/19	4/26	5/03	5/10	5/13	6/22	7/13	8/23	6/22
	%								rating			
Tifway	5	7	13	35	68	78	95	100	6	8	7	6
MSB-20	3	5	8	30	53	82	95	100	7	6	6	8
OK 86-3	2	1	3	17	30	60	78	88	6	6	6	5
Midiron	10	15	17	57	68	82	92	100	5	6	6	5
NM-43	5	6	8	35	63	80	92	97	6	7	7	8
OK 86-9	13	18	22	60	70	83	95	100	5	6	6	5
FB-119	8	10	10	30	58	63	82	88	6	6	7	6
NM-375	8	10	13	40	50	73	87	97	7	6	6	6
NMS-1 (Sahara)	17	18	20	57	50	82	92	97	5	7	7	4
MSB-10	5	7	10	28	63	72	88	97	6	7	7	6
Sunturf	3	7	10	28	45	67	90	98	7	7	6	8
Guymon	8	8	13	42	50	67	88	97	6	6	6	3
Vamont	12	13	18	60	73	92	100	100	7	7	7	4
RS-1	8	10	17	50	77	90	97	100	7	7	6	5
OK 86-6	6	8	13	42	67	85	93	98	6	6	6	3
OK 86-10	3	3	5	23	40	75	90	97	7	7	6	3
NMS-14	13	13	20	47	65	75	87	93	5	6	6	4
OK 86-4	5	5	8	25	53	62	80	87	7	7	7	3
A-29	4	6	8	37	45	85	97	100	7	7	7	6
NM-72	2	2	5	12	40	45	68	78	6	6	6	6
NM-507	2	2	3	10	35	42	63	78	7	7	7	8
NMS-4	5	5	5	17	25	57	77	82	7	6	6	5
NMS-2	7	10	13	43	40	70	83	87	6	6	6	5
Hilltop	7	8	12	42	67	87	98	100	6	6	6	5
OK 86-1	8	8	10	28	48	72	87	92	6	6	6	2
OK 86-2	5	5	8	32	60	78	93	98	6	6	6	3
OK 86-8	2	2	2	5	28	42	72	90	6	6	6	4
OK 86-5	4	7	10	33	40	73	90	97	7	6	6	4
Arizona common	13	15	20	52	55	67	80	85	5	6	6	5
Tifway II	3	6	7	23	67	78	92	100	6	7	6	7
U-3	7	8	10	27	53	72	90	93	6	7	7	5
Texturf	4	5	7	33	58	87	97	98	7	6	7	6
Tifgreen	3	4	7	32	58	82	95	98	6	7	7	8
NM-471	3	3	3	15	42	47	70	77	6	7	7	7
MSB-30	3	3	3	8	22	38	67	80	8	8	8	6
OK 86-12	8	13	17	43	55	82	93	100	6	7	7	7
NMS-3	2	2	4	12	18	38	65	77	6	6	6	5
Tufcote	17	20	30	65	80	87	97	97	7	7	6	7
CT-23	17	20	23	53	63	78	93	95	5	6	6	7
E-29	12	12	17	47	80	92	100	100	6	6	6	5
A-22	17	18	23	60	77	87	97	100	7	7	7	7
Midway	12	12	17	53	77	88	98	98	7	7	7	7
OK 86-11	4	5	10	50	80	95	100	100	5	6	6	4
OK 86-7	3	4	8	32	65	80	92	98	8	6	7	4
LSD(0.05)	5	6	6	18	34	18	17	16	2	2	2	2
CV (%)	43	43	34	32	38	16	12	11	21	17	16	24

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[z] Spring greenup was estimated by the percent of the plot area that was green.

[y] Quality rated on a scale of 1 to 9 where 1 = poor and 9 = excellent.

[x] Texture rated on a scale of 1 to 9 where 1 = coarse and 9 = fine.

Table 3. Experimental Bermudagrass Variety Trial.
Spring, Summer, and Fall 1987.
Oklahoma Turfgrass Research Center, Stillwater, OK.

	Coverage				Color[z]			
Entry	Aug.	Sep.	Oct.	Dormancy	Sep.	Oct.	Texture[y]	Quality[x]
	%				rating			
OK 1-7	89	92	99	25	5.2	5.2	4.3	6.7
OK 1-8	29	51	84	14	3.5	3.2	4.0	5.5
OK 2-2	69	84	94	30	4.2	4.0	3.8	5.7
OK 40-3	78	85	93	28	5.5	5.5	3.8	6.0
OK 41-3	70	75	91	28	5.2	6.2	4.8	6.0
OK 42-3	75	81	91	27	4.5	7.2	4.5	4.5
OK 45-3	80	89	98	38	4.7	6.0	4.3	7.0
OK 47-3	84	88	94	24	5.0	4.0	3.8	5.5
OK 47-4	74	84	93	15	2.2	3.5	3.0	6.2
OK 8-7	65	76	90	18	4.0	3.5	2.7	5.0
Tifgreen	34	48	64	17	4.5	2.7	8.0	6.0
Tifway	15	30	40	9	4.7	2.0	7.5	5.7
U-3	45	58	78	9	4.5	4.2	6.0	6.5
Midiron	31	40	55	30	3.7	5.2	6.0	5.0
LSD(0.05)	16	14	12	17	2.5	2.2	1.3	2.1
CV(%)	20	16	11	15	41.5	35.1	20.0	26.5

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[y] Color rated on a scale of 1 to 9 where 1 = dark green and 9 = yellow green.

[y] Texture rated on a scale of 1 to 9 where 1 = coarse and 9 = fine.

[x] Quality rated on a scale of 1 to 9 where 1 = poor and 9 = excellent.

Table 4. Experimental Bermudagrass Variety Spring Greenup.
Spring 1988.
Oklahoma Turfgrass Research Center, Stillwater, OK.

	Spring Greenup[z]								
Entry	4/05	4/08	4/12	4/15	4/19	4/26	4/29	5/03	5/13
OK87 47-3	15	18	19	30	58	80	81	85	99
OK87 2-2	14	19	20	31	61	78	78	79	100
OK87 1-7	12	16	18	28	56	75	78	80	95
OK87 41-3	10	11	12	22	40	62	66	68	90
OK87 42-3	10	12	14	22	42	62	66	71	91
OK87 1-8	9	12	15	28	44	64	69	70	86
OK87 40-3	9	10	11	19	35	51	58	58	78
U-3	9	12	12	25	55	74	79	79	95
OK87 8-7	9	11	15	26	52	71	75	75	94
OK87 47-4	8	11	12	24	46	74	78	80	98
OK87 45-3	8	11	12	21	36	64	65	66	88
Tifgreen	8	10	11	22	42	56	62	62	71
Midiron	6	10	10	27	48	70	72	74	99
Tifway	4	4	8	15	35	36	40	42	59
LSD (0.05)	4	6	6	10	18	15	16	15	13
CV %	34	33	31	28	28	16	16	15	16

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[z] Spring greenup was estimated by the percent of the plot area that was green.

Table 5. Experimental Bermudagrass Variety Trial.
Spring and Summer 1988.
Oklahoma Turfgrass Research Center, Stillwater, OK.

	Rank[z]		Texture[y]	Coverage		Quality[x]		
Entry	4/08	4/19	6/16	6/22	7/15	6/16	7/15	8/23
OK87 47-3	1.8	2.5	5.2	92	96	6.2	7.0	7.0
OK87 2-2	1.8	2.5	5.2	96	100	5.8	6.4	6.6
OK87 1-7	4.5	3.2	5.0	96	99	6.5	7.6	7.2
OK87 41-3	7.5	9.2	5.0	98	99	5.0	6.9	6.1
OK87 42-3	6.2	7.2	5.8	92	95	5.5	6.5	5.9
OK87 1-8	8.5	9.2	5.2	84	96	4.5	6.9	6.9
OK87 40-3	11.5	11.2	4.8	89	94	6.2	6.6	6.2
U-3	8.8	6.5	5.8	86	90	6.0	6.8	6.4
OK87 8-7	6.8	5.5	3.8	94	72	5.8	6.6	6.6
OK87 47-4	7.0	6.2	3.8	94	99	6.2	7.6	7.2
OK87 45-3	9.0	9.8	5.2	92	96	7.2	7.2	7.5
Tifgreen	10.0	10.5	8.0	76	85	5.2	6.4	6.2
Midiron	9.0	8.8	5.2	92	96	6.2	7.2	7.5
Tifway	12.8	13.8	7.2	65	76	4.2	7.0	7.8
LSD (0.05)	4.4	4.2	0.7	12	25	1.4	1.0	0.7
CV %	41.2	38.9	8.8	9	19	16.4	11.7	7.0

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[z] Ranked from 1 to 14 for each plot in all four replications where 1 = best and 14 = worst.

[y] Texture rated on a scale of 1 to 9 where 1 = coarse and 9 = fine.

[x] Quality rated on a scale of 1 to 9 where 1 = poor and 9 = excellent.

APPENDIX

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Cold Hardiness of 'Midiron' and 'Tifgreen' Bermudagrass

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Additional index words. cold acclimation, *Cynodon*, freeze tolerance, winter hardiness

Abstract. **Electrolyte leakage and regrowth tests were used to estimate cold hardiness levels of field-grown 'Midiron' and 'Tifgreen' bermudagrass (*Cynodon dactylon* x *C. transvaalensis* crowns. The two procedures were in close agreement. 'Midiron' was hardier than 'Tifgreen' on all sampling dates. Greatest levels of freeze tolerance were –11°C for 'Midiron' and –7° for 'Tifgreen' during December and January. 'Midiron' was killed at –5° in early June while 'Tifgreen' had lost all freeze tolerance by this date. Although the electrolyte leakage procedure was rapid and required no greenhouse space, it was relatively difficult to set up and evaluate.**

Bermudagrass cultivars currently used throughout the northern boundaries of adaptation periodically sustain winter damage. As a result, selection of new cultivars with superior cold hardiness is an important breeding objective. The classical approach to selection for cold hardiness has been to evaluate field plots in the spring following a severe winter. Although this procedure probably gives the best indication of field response to low temperature stress, it can be very time-consuming. Extended evaluation periods become necessary when many years elapse between test winters. In addition, results are not reproducible, either in time or in regard to location due to the unpredictability of test winters.

Many workers have developed laboratory procedures to evaluate cold hardiness levels of turfgrasses and cereals. However, most of the early techniques provide only relative hardiness estimates, take considerable time and greenhouse space, or have limited application to crowns of warm-season grasses. Tests involving exposure to one treatment temperature in a freezer can provide information on relative hardiness if an appropriate temperature is chosen. Determination of absolute hardiness levels requires removing samples sequentially from a series of temperatures. This procedure does not lend itself to small tissue samples in an air-cooled chamber, since large temperature fluctuations can occur when the chamber is opened to remove samples. Refrigerated baths using ethylene glycol or alcohol vary less in temperature than air-cooled chambers. In addition, samples can be removed without disturbing experimental units of other treatment levels. This approach was used to evaluate freezing resistance of a variety of cool-season grasses (6, 8). The latter study also used the electrolyte leakage test to quantify plant responses. The turn-around time of the electrolyte leakage test was 2 days, compared to many weeks for regrowth tests.

Our objective was to determine whether the electrolyte leakage test could discern cold hardiness levels of bermudagrass crowns following exposure to freezing temperatures in a refrigerated bath. A procedure similar to one used by Rajashekar, et al. (8) was employed except the sensitivity of the test was increased by reducing the treatment temperature interval from 5° to 2°C. Results were compared on certain dates with regrowth tests of intact plants. Development of a rapid, reproducible testing procedure would facilitate evaluation of cold hardiness levels of several bermudagrass cultivars on an annual basis.

Two independent procedures were evaluated for determining cold hardiness levels of 'Midiron' and 'Tifgreen' bermudagrass (*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy) from research plots in Stillwater, Okla. In the electrolyte leakage test, crowns were removed from the soil, thoroughly washed and placed in 25 × 150

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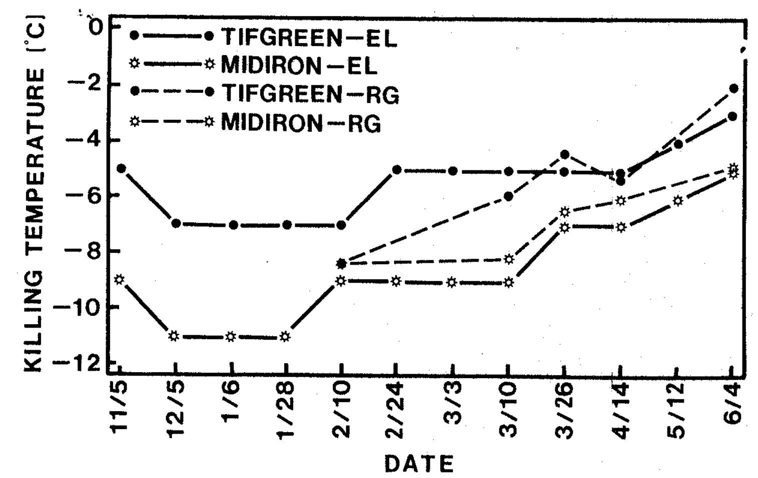

Fig. 1. Seasonal cold hardiness levels, expressed as killing temperature of 'Tifgreen' and 'Midiron' bermudagrass. Evaluations were made with electrolyte leakage (EL) and regrowth (RG) tests.

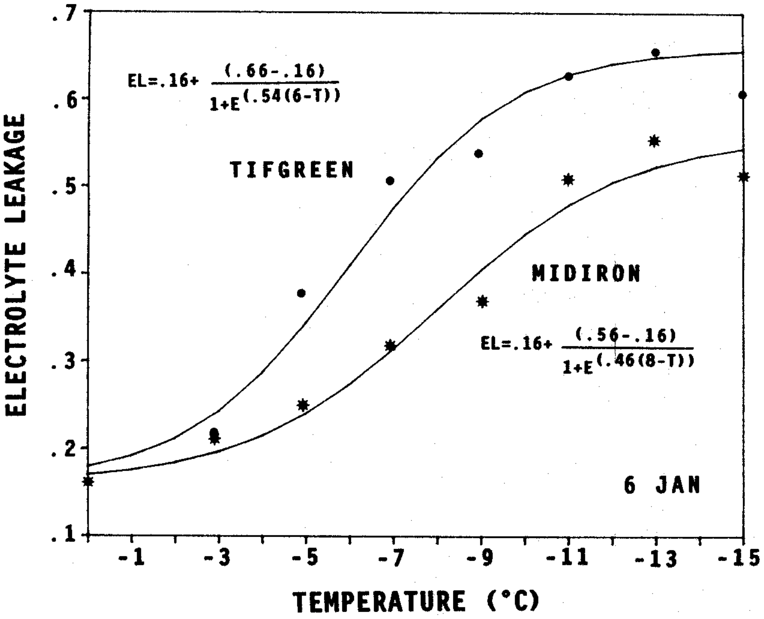

Fig. 2. Electrolyte leakage (EL) from 'Midiron' and 'Tifgreen' bermudagrass crowns following low temperature stress on 6 Jan. (E = 2.718; T = absolute value of treatment temperature).

mm test tubes. Tubes containing the crowns were submerged in a refrigerated bath with three replicates per treatment level. After equilibration at −3°C, chips of ice were dropped into the tubes to prevent excessive supercooling, and to ensure evaluation of freeze tolerance rather than avoidance. After being held overnight at −3°, samples were removed at 2° intervals while the bath was cooled at a rate of 2°/hr. Following slow thawing at 0°, 20 ml of distilled water was added to the tubes containing the crowns. The electrical conductivity of the water was measured 24 hr later (Model 35 conductance meter, Yellow Springs Instrument Co., Yellow Springs, Ohio). Samples were then heat-killed in an autoclave, and conductivity measurements taken following an additional 24 hr at room temperature. Response data were expressed as the ratio of the conductivity reading following exposure to freezing to the value after being heat-killed, thereby accounting for variation in crown mass. The killing temperature was determined as the warmest treatment level resulting in ≥40% loss of total electrolytes. This value was found to correspond most closely to the value with the greatest slope in a plot of electrolyte leakage vs. treatment temperature.

Response curves were fitted using the following model developed for electrolyte leakage data from heat stress studies (7): EL = $Y_{min} + (Y_{max} - Y_{min})/(1 + e^{k(Tm-T)})$ where EL = electrolyte leakage, Y_{min} = lower bound of EL, Y_{max} = upper bound of EL, Tm = temperature at the inflection point, k is a function of slope at Tm, and T = absolute value of treatment temperature. Parameters were estimated by the Gauss-Newton method of nonlinear regression (10).

In the second procedure, soil cylinders 60 mm in diameter and 80 mm in height containing intact plants were cooled at ≈1.5°C/hr in a low-temperature chamber capable of linear cooling rates (Model CEC 23, Rheem Scientific, Asheville, N.C.). Samples were removed at selected temperatures as measured by thermocouples inserted into each soil cylinder. The mass of the soil cylinders relative to isolated crowns reduced temperature disturbances of other experimental units when the chamber was opened to remove samples. The use of detachable plugs (Omega Scientific, Stamford, Conn.) in the thermocouple wires facilitated rapid removal of soil cylinders from the chamber since the thermocouple junctions typically froze into the soil. Samples were thawed at room temperature, then placed in 150-mm pots containing commercial soil mix and transferred to a greenhouse. Survival was judged by regrowth as determined visually 3 to 4 weeks later. The critical temperature in this test was taken as the coldest treatment temperature that retained at least one viable shoot.

Results of electrolyte leakage and regrowth tests were in close agreement, differing in hardiness estimates by 0.1° to 1.4°C (Figure 1). However, actual differences could have been somewhat larger since 2° temperature intervals were used. One-degree intervals should improve the accuracy of hardiness level estimates and may be more appropriate when comparing cultivars with small differences in hardiness.

'Midiron' was found to be hardier than 'Tifgreen' on all dates (except for the regrowth test on 10 Feb.), which is in agreement with previous observations of the two cultivars (1). The hardiness level of 'Tifgreen' in November and March was similar to values reported for 'U-3', 'Midway', and 'Westwood' in Missouri (3). Differences in killing temperatures of 2° to 4°C were estimated by the electrolyte leakage test while regrowth indicated hardiness differences of 0° to 2.3° between cultivars. 'Tifgreen' did not permit this comparison on 4 June because the warmest subfreezing temperature treatment from both tests killed all of the samples.

Both 'Midiron' and 'Tifgreen' were within 2°C of their maximum hardiness on 5 Nov., the first sampling date. The period of greatest freeze tolerance was December and January for both cultivars. 'Tifgreen' retained hardiness to about −5° through mid-April, losing all freeze tolerance by 4 June. 'Midiron' was killed at about −7° in mid-April and retained freeze tolerance to −5° on 4 June. We do not know if or when 'Midiron' loses the capacity to tolerate ice formation within the tissues. Freeze tolerance was observed subsequent to growth of shoots in the spring.

These results are valid only for the acclimating conditions to which the plants were exposed. Other locations and/or years may result in different hardiness levels. These experiments were designed to detect differences in freeze tolerance. Samples were nucleated with ice to prevent supercooling, since damage in winter wheat was increased when crowns supercooled to temperatures

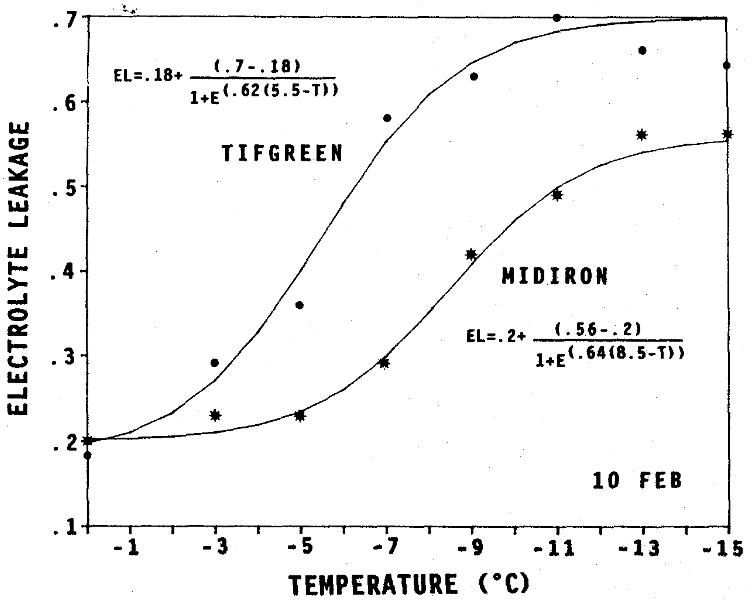

Fig. 3. Electrolyte leakage (EL) from 'Midiron' and 'Tifgreen' bermudagrass crowns following low temperature stress on 10 Feb. (E = 2.718; T = absolute value of treatment temperature).

≤3°C before freezing (5). Long equilibration periods for initial freezing and slow cooling rates allowed tissues to come to equilibrium with the stress. Any survival features in the field, such as deeply buried organs, that would promote alleviation of low temperature stress by temperature buffering, would not be detected.

The relationship between electrolyte leakage and treatment temperature was sigmoidal in Kentucky bluegrass leaves, while crowns yielded a nearly linear relationship (8). Similarly, a one-third reduction in percentage survival of 'Meyer' zoysia occurred over a span of 5.5°C (9). Our results generally suggest a gradual transition from undamaged to killed tissues over the span of several degrees (Figs. 2 and 3). Undoubtedly, a study with a broad range of treatment temperatures with large intervals between treatment levels favors sigmoidal curves at the expense of differentiating small differences in cold hardiness. The procedure could be improved if the moisture status of the crowns were determined. Extremes in tissue hydration have been shown to affect the hardiness level of dogwood stems (2), and crowns of Kentucky bluegrass (6). Gusta and Fowler (4) found a close correlation between water content and killing temperature in deacclimating cereal crowns. Since plant material for our study was gathered from plots a few meters apart, it was likely that crowns were exposed to similar soil moisture contents.

The electrolyte leakage test was able to measure cold hardiness levels of 'Tifgreen' and 'Midiron' bermudagrass crowns on a seasonal basis. The relative hardiness of the two cultivars agreed with previous field observations. In addition, the test corresponded closely to another independent estimate of freeze tolerance. The short turn-around time and lack of need for greenhouse space are advantages of the electrolyte leakage procedure. The major drawback may be the intensive labor necessary to excise and thoroughly wash crowns from a large number of cultivars.

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MEMORANDUM OF AGREEMENT

between

Oklahoma Agriculture Experiment Station
Oklahoma State University
Stillwater, OK 74078

and

Farmers Marketing Corporation
P.O. Box 1231
Yuma, Arizona 85364

The purpose of this agreement is to provide for the cooperative evaluation of advanced and potentially new cultivars of bermudagrass from the Turfgrass Genetic and Breeding Research Program of the Agronomy Department, Oklahoma Agricultural Experiment Station, Oklahoma State University (hereafter referred to as "University"), and relates specifically to further procedures for orderly, effective release of University originated cultivars to seed and sod growers, seedsmen, and thence to planters and other cooperators of the testing and evaluation program.

The purpose of the cooperative evaluation is to determine the performance potential of the new cultivars when established under the desired conditions, both of which are listed and defined in the schedule attached hereto.

The University, through its duly authorized representative, Charles M. Taliaferro, at Stillwater, Oklahoma, herewithin makes available to the Cooperator the materials (sprigs and/or seeds) to be evaluated; as listed and defined in the schedule attached hereto.

Cooperator certifies that it has under its direct control and operation trial grounds maintained at Yuma, Arizona, and has in its employ individuals qualified to grow and evaluate the listed material.

Cooperator further certifies that it will:

1. Accept the plant materials (vegetative sprigs or seed) of the above described strains and protect them from unauthorized distribution and propagation.
2. Propagate the material under such field, greenhouse, or laboratory techniques as required to compare their performance characteristics with such commercial varieties and strains as may be mutually determined by the University and the Cooperator.
3. Record and provide test results to the University as set forth in the attached schedule.
4. Cooperator will not retain any seed or propagating materials from these strains, and dispose of materials as outlined or authorized by the University; Cooperator will destroy all plants produced from these strains at the end of the evaluation period.

It is mutually agreed:

1. That decisions relative to the further propagation or the release of any of these strains shall be the sole responsibility of the University.

2. That all evaluation data obtained shall be confidential and subject to analysis and publication by the University and that its subsequent use in advertising or sales promotion literature will require the expressed permission of the University.

3. That this agreement shall become effective upon approval by all parties concerned and shall be terminated at the end of 1991.

AGREEMENT:

Charles M Taliaferro 9/6/88
Charles M. Taliaferro, Project Leader Date

Ronald D Johnson Assoc Dir 8/23/88
for C. B. Browning/Director of Experiment Station Date

Dick Cooley Cooley 9/8/88
Dick Cooley, Manager Date
Farmers Marketing Corporation

SCHEDULE 1

I. Propagating materials (sprigs or seed) of each of the following cultivars will be supplied to Farmers Marketing Corporation in sufficient quantity to establish four plots, each 6'x 15'.

1. OKLA 42-4, 3200W
2. " 32-4, 3200E
3. " 34-4, "
4. " 53-3, "
5. " 53-7, "
6. " 53-9, "
7. " 53-11, "
8. " 3-6, 5200W
9. " 5-1, "
10. " 5-7, "
11. " 6-1, "
12. " 50-2, "
13. " 53-1, "
14. " 3-1, Turf Nursery
15. " 3-3, "
16. " 4-4, "
17. " PC85-1
18. " 10978b x 12156
19. " Ft. Reno Advance

II. Purpose of the test is to evaluate the seed production of the respective cultivars at Yuma, Arizona under cultural and management conditions normally used for commercial production of bermudagrass seed in that area. The 'Arizona Common' and 'Numex Sahara' varieties will be included in the test as "check" varieties.

III. Farmers Marketing Corporation will furnish to the University data in the form of weight of pure seed and harvested area for each plot. Other measurements or subjective ratings of performance which help characterize overall adaptation and seed production potential of the cultivars should also be taken.