BREEDING, EVALUATION, AND CULTURE OF BUFFALOGRASS FOR GOLF COURSE TURF

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Paul G. Johnson	Post Doctoral Associate, Project Coordinator
Terrance P. Riordan	Professor, Horticulture, Turfgrass Breeding
Roch Gaussoin	Asst. Professor, Horticulture, Ext. Turfgrass Specialist
Fred Baxendale	Professor, Entomology
Leonard A. Wit	Supervisor, JSA Turfgrass Research Facility
Charles A. Rodgers	Graduate Student
Kevin Frank	Graduate Student
Shuizhang Fei	Graduate Student
Tiffany M. Moss	Graduate Student, Entomology

Cooperators:

Garald L. Horst, Associate Professor, Turfgrass Physiology
Robert D. Grisso, Asst. Professor, Biological Systems Engineering
John E. Watkins, Professor, Plant Pathology
Gary Y. Yuen, Assistant Professor, Plant Pathology
Robert V. Klucas, Professor, Biochemistry
Robert C. Shearman, Professor, Horticulture
Tony P. Weinhold, Technician, Entomology
Jeff W. Witkowski, Technician, Horticulture
Steve R. Westerholt, Technician, Horticulture
Andrew Kramer, Undergraduate Student, Horticulture
Eric D. Miltner, Utah State University
Jack D. Fry, Kansas State University

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EXECUTIVE SUMMARY

Through October, sales of buffalograss by Crenshaw & Doguet Turfgrasses, Inc. has been at or above last year's level (approximately $1.5 million for '609'). A/G Sod of Phoenix, AZ, currently has 90 acres of '609' and Prairie under production. This region has great market potential because of water issues in Arizona and the interest in using buffalograsses on golf courses. Three selections: NE 86-61, NE 86-120 and NE 91-118, are currently being processed for release, protection, and commercialization. These genotypes have excellent quality, density, low mowing tolerance, and excellent sod production characteristics. Disclosures are being developed for these three genotypes, and they were entered into the 1996 NTEP Buffalograss Trial. Two of these selections have been vegetatively increased at Todd Valley Sod Farm at Mead, NE. The third will be planted next spring.

The older top selections in the program ('315', '378', 86-61, 86-120, 91-118) continued to perform well, but due to the mild conditions, fewer differences were seen this year. Additional vegetative selections, identified from nurseries and progeny rows, will be the subjects of further evaluation. Experimental seeded varieties show good performance compared to the vegetative varieties and better than standards. Single cross varieties were first evaluated in the field in 1996. Single crosses may provide improved uniformity compared to the more typical synthetic crosses. Inheritance studies continue to yield useful information. Selection for seed weight increased means by 13.7 and 25.6 mg per 100 caryopses in two populations. These gains from selection suggest increases in seedling vigor with additional cycles.

Buffalograss managment research centered around planting date, mowing height, and nitrogen fertilization programs. The optimal seeding dates for buffalograss at Nebraska are April through June and at Utah, April through July. Late season plantings suffered winter kill or had inadequate buffalograss cover the next year. Weed competition is the most important factor restricting buffalograss establishment. A major study was started in 1996 to determine best management practices for buffalograss. Mowing heights of 1, 2, and 3 in., and nitrogen levels of 0, 0.5, 1.0, 2.0, and 4.0 lb. N/1000ft^2 were applied.

The lab component of this project includes tissue culture, DNA content measurement, and molecular marker research. Cell suspension cultures of buffalograss have been established from '609', and 84-45-3. Callus can also be initiated from unexpanded leaf material of 84-45-3 and '315', however no organogenesis has been observed so far. This research is essential for future transformation of buffalograss with genes, such as those conferring resistance to herbicides. Flow cytometry has enabled differentiation of three ploidy levels among buffalograss accessions. It has identified a trend of tetraploids in southern Great Plains and hexaploids in the northern Plains.

Our expenditures for 1996 through October 15 include approximately $41,000 in salaries for a post-doctoral associate, graduate assistant support, and undergraduate student labor. Costs for laboratory, greenhouse, and field research are approximately $10,000. $3,500 was needed for travel to research sites and meetings to report on research from this project. $10,000 was allocated for university overhead. If this is extrapolated to the end of the year, the total expenditures for 1996 sum to approximately $70,000. Currently on the project we have one professor-level faculty member devoting 40% time, one post-doctoral associate at 100%, three graduate assistants at 100%, and one graduate assistant at 50%. This does not include temporary help and technical assistance at the field research facility.

STATUS OF NEW RELEASES

Seeded Releases

Dr. Jeff Klingenberg of Seeds West, Inc., Phoenix, AZ, is working with Nebraska germplasm with the objective to develop new proprietary cultivars. These cultivars would be released through UNL, and a royalty would be paid as with all USGA-sponsored research. He continues to work on material that will have improved quality, better seed production and characteristics, such as low mowing tolerance, that would make them better grasses for use on golf courses.

Charlie Rodgers, who has completed his Ph.D., is working on improving the germination and establishment of buffalograss through selecting and intercrossing germplasm with increased seeding vigor. This advanced generation material will be used to develop new cultivars, or it will be used to improve the vigor of other advanced synthetics, Dr. Rodgers has also made some F_1 hybrids with his best germplasm, with the objective to develop a cultivar with excellent quality and seedling vigor. This material is being evaluated in turf plots at this time.

A planting of two experimental selections from NE 85-436 has produced excellent seed yields and progeny with uniformity and high quality. This hybrid produced as an F_1 or F_2 may provide a vigorous, dense cultivar for use on golf course roughs. Plans to possibly develop a new cultivar will be made this winter.

Vegetative Releases

Three selections - NE 86-61, NE 86-120 and NE 91-118 - are currently being processed for release, protection and commercialization. These genotypes have excellent quality, density, low mowing tolerance, and excellent sod production characteristics. Disclosures are being developed for these three genotypes, and they were entered into the 1996 NTEP Buffalograss Trial. Two of these three selections have been vegetatively increased at Todd Valley Sod Farm at Mead, NE. The third selection, NE 86-120, will be planted next spring.

Other plant materials are in the evaluation process and included in advanced trials at our research facility.

Crenshaw & Doguet Turfgrass, Inc. Update

This has been an interesting year for our national licensee. Late last year they brought in new investors and became a larger company with high expectations for increased sales. When this did not occur, significant changes were made in the company. David Doguet and Arthur Milberger, who were managing the company, are no longer involved in any way. Bob Kay who had been CEO, has returned as interim CEO, and the company seems to be doing fairly well with sales at or above last year's level (approximately $1.5 million for '609').

Todd Valley Turf Farms

Sales have continued to increase for '378' buffalograss, and the cultivar is now available both as plugs and as sod. However, because of a problem with the '378' patent and a problem with off-type plants, Todd Valley Turf Farms will be gradually converting their production to one or more of the new experimentals.

Oak Point Farms

Bill Steavenson, the owner of Oak Point Farms, died this fall, and his wife is trying to sell the farm and license to someone else. Because of Bill Steavenson's health problems, little progress was made in 1996 in selling '315' buffalograss. Time will tell what will happen with this cultivar, since other new and better grasses are becoming available.

A/G Sod Farms

A/G Sod of Phoenix, AZ, currently has 90 acres of '609' and 'Prairie' under production. Because of water issues in Arizona, sales are starting to increase. There is interest in using buffalograsses on golf courses. In October, Terry Riordan visited several of these courses to observe and discuss management and use of buffalograss in Arizona. This will be an area to watch over the next few years.

Licensing

The worldwide license to produce, market and sub-license buffalograss by Crenshaw & Doguet Turfgrass, Inc. is up for renewal in 1997. Currently, this is being discussed, and it is likely that it will be renewed with some minor changes. The license to produce and market seeded buffalograsses is being negotiated with NTG, and progress should be made over the winter in developing a license similar to that for the vegetative buffalograsses.

National Turfgrass Evaluation Program

A new buffalograss evaluation was initiated by NTEP during 1996. Nine vegetative selections and five seeded varieties were planted at 12 sites around the country. Because these are in their establishment year, no cumulative data is available. Table 1 shows the results of the Nebraska trial during the latter half of 1996. All varieties were similar in establishment rate and should be fully covered by July, 1997.

BREEDING WORK

The current breeding objectives of the Univ. of Nebraska buffalograss breeding project are to improve various traits relating to golf course turf. These include overall turfgrass quality, tolerance to low-mowing (use in golf course fairways), insect resistance, and seedling vigor. We have initated efforts to evaluate our germplasm through molecular techniques including random amplified polymorphic DNA (RAPD) markers and flow cytometry. We plan to use this technology in developing improved cultivars, but also for needed germplasm enhancement.

The growing season of 1996 was in sharp contrast 1995. Summer temperatures were below normal, with only a couple of days above 90°F, and none above 100°F. The best selections continued to perform well, but due to the mild conditions, fewer differences were seen among breeding materials.

Table 1. Percent cover ratings of the NTEP Nebraska trial location. Planting was established July 10, 1996.

Selection	Establishment 8/28/96	Establishment 9/27/96
Vegetative Cultivars	------ Percent cover ------	
Stampede	36.67	56.67
91-118	36.67	56.67
UCR-95	36.67	43.33
86-61	36.67	43.33
'378'	43.33	43.33
Bonnie Brae	33.33	33.33
86-120	33.33	30.00
'609'	33.33	30.00
Midget	30.00	26.67
Seeded Cultivars		
BAM-1000	40.00	70.00
Texoka	43.33	63.33
Cody	33.33	56.67
Tatanka	26.67	40.00
Bison	23.33	33.33
LSD(.05)	15.73	25.84

Evaluation for Golf Course Fairway Mowing Heights

Buffalograss is most commonly used as a low maintenance turf species, typically mowed at or above 2.5 inches. The goal of this project is to select for genotypes that are tolerant to mowing heights of golf course fairways. Buffalograsses tolerant to low mowing would be suited to dryland areas and where irrigation is not, or will not, be available.

One plot area has been mowed at 5/8 in. since 1993 and has shown significant differences in tolerance. Selections having very good turf quality include 86-61, 87-24, 86-120, 87-86, and 87-76 (Table 2). Standard varieties, such as 'Texoka', do not show tolerance to the low mowing (Table 2). Similar quality ratings were observed among many of these materials established in 1995 and also mowed at 5/8 in. (Table 3). These top performing genotypes were used in a crossing block in 1994 and 1995 giving rise to new populations with more potential for low-mowing tolerance. A number of outstanding plants were identified from progeny rows to be further evaluated in 1997. An additional planting established this year (1996) included more of these progeny and seeded plots originating from each maternal parent with potential for a low mowing tolerant seeded variety. Another plot area (est. 1993) will be managed at the 5/8 in. height. During 1996, the mowing height was gradually lowered to one inch and will be further reduced to 5/8 in. for evaluation in 1997. In 1996, we started evaluation of wear tolerance (mowing height 5/8 in.) on the oldest low-mow plot area. The wear treatments were apparent, but little difference or damage was seen among the selected genotypes (Table 2). We plan to repeat and improve these tests in 1997.

Table 2. Low mowing evaluation (Plots established in 1990).

Selection	Worn	Unworn	Turf Qual 7/26/96	Turf Color 7/26/96
	------ Percent cover ------		----- Rating (1-9) -----	
86-61	48.33	79.17	7.67	7.67
85-633	49.17	73.33	7.00	6.33
87-86	54.17	75.00	6.67	6.33
87-76	40.00	75.83	6.33	6.33
86-120	40.83	74.33	6.00	6.00
84-315	46.67	77.50	5.67	5.67
84-45-3	43.33	76.67	5.67	5.67
87-25	42.50	81.67	5.33	5.67
85-378	36.67	78.33	5.33	6.00
85-443	40.00	73.33	5.33	5.67
84-609	45.83	75.00	5.00	5.50
Texoka	35.00	62.83	4.33	5.00
Prairie	38.00	77.50	3.33	5.00
LSD(.05)	29.23	15.83	1.36	1.44

Table 3. Low mowing evaluation (Established 1995).

SELECT	TurfQual. 1996	Color 1996	FallColor 10/96
	--------------- Rating (1-9) ---------------		
86-61	8.00	7.83	6.67
87-76	6.83	6.50	4.33
86-120	6.67	6.17	4.67
91-118	6.67	6.00	6.33
94-542-LM*	6.50	6.50	5.33
'315'	6.50	6.33	5.33
85-443	6.33	5.83	3.67
86-23	6.33	5.67	6.00
85-648	6.33	5.50	4.00
87-93	6.00	5.50	5.33
87-80	6.00	4.33	6.00
84-45-3	5.33	5.33	3.67
LSD(.05)	1.32	1.20	1.27

* Seeded Population

Performance of Buffalograsses in Replicated Trials

The replicated trials are managed similar to a low maintenance situation including 2 lbs N/1000 ft^2/year, mowing every other week, and no supplemental irrigation. In the 1994 trial, we saw 86-61, 86-120, '315', and '378' performing well (Table 4). These have performed well in both warm, dry years, as well as cool, moist years. Additional vegetative selections are also doing well: 93-170 and 93-176. 91-248, 93-173, and 93-181 show good fall color, and may be suited to southern regions to take advantage of the long green period. The seeded varieties (92-501-JK1, etc.) show good performance compared to the vegetative varieties, but most importantly, better than standards (Table 4).

Table 4. 1994 Replicated Trial- Low maintenance.

Selection	TurfQual. 1996	Color 1996	FallCol Oct. 1996
	--------------- Rating (1-9) ---------------		
86-61	7.11	6.50	4.67
93-170	7.11	5.92	2.00
93-185	6.66	6.50	3.33
378	6.56	6.17	2.33
315	6.33	5.92	5.33
86-120	6.22	5.67	3.00
93-181	5.78	3.67	7.33
93-173	5.67	4.83	7.67
92-501-JK1	5.67	5.25	4.67
92-502-JK1	5.67	4.83	5.00
92-503-JK1	5.56	5.25	5.00
92-504-JK1	5.55	4.83	5.33
91-248	5.44	4.33	7.33
84-45-3	5.11	4.83	2.33
Topgun	4.89	4.50	7.00
91-118	4.89	4.42	5.67
Texoka	4.56	4.67	7.00
SharpsImprov	4.11	4.75	7.00
LSD(.05)	1.21	1.00	1.00

Similar statements could be made about materials in the 1995 planting. This is the first full year of turf evalutions with '315', '378', and 86-61 continuing to perform well (Table 5). Others will be followed closely in future years, based on good performance this year. Two seeded varieties, 93-540-mb, and 93-539-mb show promise in the seeded varieties (Table 5).

Table 5. 1995 Replicated Trial- Low maintenance.

Selection	TurfQual 1996	Color 1996	FallColor Oct. 10
	------------Rating (1-9) ------------		
'315'	6.22	5.67	3.67
'378'	5.22	6.11	3.00
86-61	6.55	7.22	4.00
90-164	5.33	4.89	7.33
91-118	6.55	5.11	5.10
93-539-mb	5.78	5.00	4.67
93-540-mb	5.89	5.33	5.00
94-100	7.33	7.33	3.67
94-51	6.11	6.22	6.67
94-55	6.78	6.56	3.67
94-82	6.33	7.11	4.33
94-99	5.89	4.55	6.67
Cody	5.34	5.22	5.33
Tatanka	5.22	5.00	5.00
Texoka	4.89	4.44	5.33
LSD(.05)	1.03	0.96	1.42

One of the newest plantings, includes a wide variety of materials. Selections from the nursery were noted for their superior color and growth during the drought of 1995. These were put in a replicated trial. Most showed good establishment vigor (Table 6), and were nearly filled in by the end of September. Experimental 91-118 shows the most rapid establishment, characteristic of this good sod producing variety. A number of plant mesurements made in the greenhouse were done to compare establishment rate in the field. Unfortunately none of these were useful as a predictor. The 1996 trial also includes seeded populations from single crosses. Twenty-one females crossed with 84-45-3 were planted out and many show good seedling vigor and establishment. These are denoted by 'AreaY' in Table 6. Single crosses may provide improved uniformity compared to more typical synthetic crosses.

Table 6. 1996 Replicated Trial- Low maintenance.

Selection	Est. August	Est. Sept.
Vegetative	------ Percent Cover ------	
91-118	63.33	86.67
95-36	56.67	70.00
86-61	53.33	70.00
95-37	50.00	70.00
95-3	56.67	66.67
85-378	53.33	66.67
86-120	43.33	63.33
84-609	40.00	63.33
84-315	50.00	56.67
Seeded Populations		
Texoka	80.00	90.00
AreaY 93-185	70.00	90.00
Tatanka	66.67	83.33
504-JK2	70.00	80.00
AreaY 93-176	65.00	80.00
AreaY 93-191	60.00	80.00
AreaY 91-275	65.00	75.00
AreaY 93-171	65.00	75.00
AreaY 93-187	65.00	75.00
AreaY 93-186	55.00	75.00
Cody	70.00	73.33
AreaY 315	55.00	70.00
AreaY 378	45.00	50.00
LSD(.05)	21.16	22.30

Breeding for Caryopsis Weight in Two Nebraska Seeded Turf-Type Buffalograss Populations (Charlie Rodgers)

Heavy caryopses have been shown to produce more vigorous seedlings in buffalograss. In field studies, seedlings from heavier caryopses have more vigorous growth during the year of establishment, but not during subsequent years. This would be desirable for turf-type buffalograsses since high clipping yield after establishment is not desirable. The objective of this experiment was to determine the potential for selection for caryopsis weight in two Nebraska synthetic populations.

Heritability estimates for heavy and light caryopsis weight were 0.55 and 0.44 in NE-501, and 0.59 and 0.28 in NE-503. Gain from selection for heavy caryopsis weight was 13.7 and 25.6 mg per 100 caryopses in NE-501 and NE-503, respectively. Intermediate heritability estimates as well as moderate gains from selection suggest that after another cycle of selection a significant increase in caryopsis weight will have been achieved, resulting in increased seedling vigor for these synthetics.

Development of an F_1 Hybrid Seeded Turf-Type Buffalograss with Increased Germination (Charlie Rodgers)

Buffalograss is suited for the development of F_1 hybrid (single cross) varieties. A critical component of hybrid development is the selection of a female parent for seed production, as well as seed quality characteristics. A desirable female plant would be high yielding, and produce seed that has high germination rates. This project was initiated to identify female plants to use in F_1 crosses, which produce a large amount of seed with superior germination, yet desirable turfgrass quality.

A single selection, 91-154, exceeded the mean of all thirty genotypes for four traits measured, and shows potential for use as a parent for the production of hybrid seed. Two other genotypes were identified that exceeded the mean of all genotypes for three of the four traits measured, and was near the mean for two other traits. Progeny will be evaluated in a replicated field trial, and used as the final criteria for selection of which parent to use in the development of F_1 seed.

BUFFALOGRASS MANAGMENT RESEARCH

Buffalograss Date of Planting (Kevin Frank)

Research was initiated in 1995 to determine date of planting effects on seeded buffalograss. Seven planting dates were investigated at the JSA Turfgrass Research Facility near Ithaca, Nebraska and at the Greenville Research Farm at Logan, Utah. The objectives of this research were to 1) identify the optimal seeding date(s) for buffalograss, 2) determine the relationship between growing degree days (GDD) and buffalograss establishment, and 3) determine the effects of weed competition on buffalograss establishment.

Two seeded buffalograss cultivars, 'Cody' and 'Texoka', were seeded at 9.8 g burrs/m^2 on seven dates in 1995 at Nebraska and Utah. The planting dates were April through October with plantings as close to the 15th of each month as possible. Percent buffalograss and weed cover were rated visually on a 0 to 100% scale. GDD were calculated using the mean-minus-base method. The base temperature used for buffalograss was 5° C.

$$GDD = [(Min. + Max.)/2] - 5° C$$

There were no significant differences among planting dates for percent buffalograss cover at Nebraska in 1995. At Nebraska, the planting dates of April through June had the highest percent buffalograss cover early in the second season. At Utah, the August planting date had the highest percent buffalograss cover the first season, but by the second season the April through July planting dates had the greatest percent buffalograss cover. The optimal seeding dates for buffalograss at Nebraska are April through June and at Utah, April through July. Regardless of location, none of the 1995 planting dates used in this research were able to reach 70% buffalograss cover by 20 June 1996. There were no significant differences between Texoka and Cody for either percent buffalograss or weed cover at Nebraska and Utah in 1995 and 1996.

This research also determined that to ensure adequate coverage and survival of late summer plantings at least 1000 post-planting GDD need to be accumulated before temperatures fall below 5° C. The plantings in this research that did not accumulate at least 1000 GDD suffered winter kill or had inadequate buffalograss cover the season after establishment.

Weed competition during buffalograss establishment is consistently cited as a major factor limiting successful cover of an area in one season. Significant negative correlation between percent buffalograss and weed cover was reported at both Nebraska and Utah for several planting dates in both 1995 and 1996. The data from this research reenforce the contention that weed competition is the number one factor restricting buffalograss establishment.

Buffalograss Establishment and Management (Kevin Frank)

In 1995 four buffalograss cultivars were planted for a management study to be conducted in 1996. Two vegetatively established cultivars, '378' and experimental selection 91-118, and two seeded cultivars, 'Cody' and 'Texoka', were planted the JSA Turfgrass Research Facility located near Ithaca, Nebraska (UNL), the Rocky Ford Turfgrass Research Farm at Manhattan, Kansas (Kansas State University), and the Greenville Farm at Logan, Utah (Utah State University).

Vegetative buffalograss plugs were not successfully established at Utah in 1995. The transport of plugs to Utah, which included washing of soil from the plugs, was believed to be a factor inhibiting establishment. 'Cody', 'Texoka', and 91-118 had the highest percent buffalograss cover eight weeks after planting (WAP) at Nebraska and Kansas. By 12 WAP there were no significant differences for percent buffalograss cover among the four cultivars at Nebraska and Kansas. Stolon number and vegetative plug diameter were significantly different among cultivars 4 WAP at Nebraska and Kansas. At both locations 91-118 had greater stolon number and plug diameter at 4 WAP.

In 1996 management treatments were applied to determine best management practices for buffalograss. Mowing heights of 2.54 cm, 5.08 cm, and 7.62 cm and nitrogen levels of 0, 2.4, 4.9, 9.8, and 19.5 g N/m2 were applied. Nitrogen treatments were applied as a split-application with the first application in the first week in June and the second application six weeks after the first. Buffalograss quality, color, density, and uniformity were rated throughout 1996. Buffalograss clippings were harvested and weighed at 4 weeks after the first and second nitrogen applications. Determination of carbohydrate reserves was made six weeks after the second nitrogen application. Data collection from this research recently concluded and data are presently being analyzed.

Fine Fescue - Buffalograss Interseeding

A pilot study was started in 1996 to investigate whether fine fescue can be grown as a companion with buffalograss to extend the growing season of the turf. We overseeded into an established buffalograss turf at 1, 2, & 4 lbs hard fescue per 1000 ft^2. If this study yields promising results, we will expand this experiment.

Relationship between Trichome Density and Geographic Distribution in Buffalograss (Andrew Kramer)[1]

Recent investigations in our project found a correlation between buffalograss selections with glabrous leaves and resistance to mealybug damage. These glabrous plants may have a lower ploidy level and exhibited poor winter hardiness in Nebraska. The objective of this study was to examine a large number of buffalograss accessions to determine if a relationship may exist between pubescence on the leaves and geographic distribution. Information obtained in this study could be helpful to plant breeders when selecting buffalograss germplasm for regions of adaptation.

Our data suggests that glabrous plants are more common in the southern Great Plains (Texas and Mexico) and pubescent plants in the central and northern Great Plains (Kansas and Nebraska). Rainfall or humidity may also influence leaf pubescence. Pubescence may adversely affect turf color, since a densely pubescent leaf surface appears lighter in color. The Nebraska release '609' buffalograss, for example, is glabrous, and has very good color and turf quality. '609' is also resistant to mealybugs. However, it does not perform well in the colder winters of the central and northern Great Plains. Pubescence likely does not influence winter hardiness so breeding efforts may be succeed to develop winter hardy germplasm that also exhibits glabrous leaves and their resulting benefits.

BIOTECHNOLOGY

Tissue Culture and Transformation of Buffalograss (Shuizhang Fei)

Tissue culture and transformation of buffalograss lines will provide novel sources of variation that can be used in the breeding program. The goals of this work are to develop cell - culture and regeneration methods, and transformation procedures using biolistic techniques.

In 1996, we achieved plant regeneration of another cultivar, 'Texoka'. We further demonstrated that even within the same cultivar, 'Texoka', male inflorescences gave a better *in vitro* response than female inflorescences. Somatic embryogenesis, which is better than organogenesis, was observed on cultures of '315' and '609'. Fully developed somatic embryos have been documented using scanning electron microscope. Anther culture may provide an efficient way to generate inbreeding lines. Extensive work had been done to manipulate various components of culture medium for haploid production, but limited progress had been made. Multicelluar structures, which may be considered as a proembryogenic complex, was observed on medium containing 2 mg 2,4-D/l, 1 mg NAA/l and 0.5 mg Kinetin/l. Maltose (10%) is better than sucrose for pollen survival and division.

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[1]Andrew was a student employee on the project for two years. He prepared a paper for a senior ecology course at UNL on this topic. This is a summary of part of that paper.

Efforts will continue in refining the tissue culture techniques in hopes of increasing regeneration frequency. Finally, when possible, we will begin transformation of buffalograss cell cultures with genes including those conferring resistance to herbicides.

Identification of ploidy level of buffalograss accessions using flow cytometry

The analysis of DNA using flow cytometry has proven useful in determining ploidy level of buffalograss accessions and characterizing germplasm. The technique measures DNA content using a dye which, when excited by a laser, floresces with an intensity proportional to the amount of DNA in the cell. This has enabled the differentiation of the three ploidy levels commonly found among buffalograss accessions. Diploid buffalograss (2n=2x=20) has 0.93 pg DNA/nucleus; tetraploid accessions (2n=4x=40) have 1.78 pg DNA/nucleus; and hexaploids (2n=6x=60) have 2.59 pg DNA/nucleus. Comparison of these data with actual chromosome counts have shown a direct correlation with DNA content. DNA contents are more variable among seeded populations. The data indicate a trend for tetraploids in southern Great Plains and hexaploids in the central and northern Great Plains. Higher ploidy levels may more prevalent in colder, more stressful environments of the northern Great Plains. This is a typical trend of polyploid evolution. Some DNA content values fall between the discrete categories and could be due to intermediate chromosome numbers or variation within the ploidy level. When used to determine base pair ratios, all buffalograsses were measured as having 41-44% G:C base pairs. No differences were observed between genders or ploidy levels. The application of these techniques allow fast screening of buffalograsses for their ploidy level, will improve our hybridization program, and enable the study of accessions and populations having chromosome numbers other than the common ploidy levels.

Use of Randomly Amplified Polymorphic DNA to Identify and Characterize Buffalograss Clones and Populations.

Randomly amplified polymorphic DNA (RAPDs) can be a useful tool for identifying individual clones of buffalograss. Individual genotypes display unique banding patterns based on their genomic DNA. RAPDs can be used to 'finger print' buffalograss clones for purposes of plant patent, and quality control in turfgrass sod production. The advantage of using RAPD markers are that they are based on genomic DNA, and not influenced by environment, whereby cultivars can be accurately and efficiently identified. Three potential vegetative clones 86-61, 86-120, and 91-118 are currently being finger printed using a set of 20 decamer primers (OPA 1-20, Operon Technologies, Alameda, CA).

Similarly, RAPDs can be used to characterize seeded populations of buffalograss. Ten buffalograss populations will be characterized using a set of the most informative primers from the previous study. Thirty individuals per population have been started from seed in the greenhouse. RAPD patterns will be used to: determine the number of individuals needed to identify a seeded heterogenous buffalograss population, look at relatedness among and within populations, and characterize the diversity that exists in seeded buffalograss germplasm based on genomic DNA.

ENTOMOLOGY RESEARCH

Biological Control of Buffalograss Insects (Tiffany Heng-Moss & Dr. Frederick Baxendale)

The goal of this research is to gather information about potentially serious pests of buffalograss, identifying the beneficial arthropod community associated with buffalograss, and developing effective and environmentally sound pest management alternatives.

The beneficial arthropod community associated with buffalograss was investigated during the 1995 season by collecting and examining samples from buffalograss evaluation plots at the JSA Turfgrass and Ornamental Research Facility and from vegatatively established lawns at the University of Nebraska East Campus during the 1995 season. Sod plugs were placed in Berlese funnels for 48 hours to extract beneficial arthropods. Several beneficial arthropods were collected on buffalograss including: big-eyed bugs (Lygaeidae), ground beetles (Carabidae), rove beetles (Staphylindae), ants (Formicidae), spiders (Araneae), and numerous parasitoid (Hymenoptera) wasps. Ants and spiders were the most abundant natural enemies collected throughout the 1995 season for both sod plugs and pitfall samples (Figure 1 and 2).

Samples were again collected in 1996 from the various buffalograss locations to identify additional natural enemies and collect seasonal abundance information. Beneficial arthropods were also surveyed on high maintenance and low maintenance sites at the University of Nebraska East Campus and the JSA Research Facility to determine if management practices influenced the composition and relative seasonal abundance of these beneficial natural enemies. High maintenance sites were mowed every two weeks at 6.25cm, received three applications of .75 lbs. N/1000 ft^2, and were irrigated as needed to maintain optimal growing conditions. The low maintenance sites were mowed once in the spring and fall, received no nitrogen applications, and were not irrigated. Results from this study have not been processed at this time.

The second important objective of this research was to identify hymenopterous parasitoids of buffalograss mealybugs. A series of rearing and dissection studies were conducted in the laboratory to identify the parasitoid complex associated with buffalograss mealybugs. Parasitoid wasps identified as Rhopus sp. (Encyrtidae) were reared and dissected from buffalograss mealybugs which suggests their potential regulating effect on mealybug populations. The seasonal abundance of Rhopus sp. and other hymenopterous parasitoids was monitored in the field using adhesive covered yellow sticky traps. Late in the summer of 1994, large numbers of mealybugs were collected from a 1990 evaluation plot at the JSA Research Facility. By the spring of 1995 however very few mealybugs could be detected in the same plots. A possible explanation for this decline may be the presence of relatively high levels of Rhopus sp. Large number of these parasitoid wasps were collected on sticky traps in these plots during the 1995 growing season.

Paired comparison tests are currently being conducted in the greenhouse to evaluate the effectiveness of Rhopus sp. as a biological control agent for buffalograss mealybugs. An initial experiment indicated that cages containing both mealybugs and parasitoids sustained minimal plant damage and had high levels of mealybug parasitism.

Choice and no-choice studies also are being conducted in the laboratory to determine if Rhopus sp. prefers certain age classes of mealybugs. In preliminary experiments, Rhopus sp. attempted to oviposit in all mealybug age classes, but seemed to prefer female and third instar mealybugs. Additional studies are being conducted to learn more about mealybug/parasitoid interactions.

In a third study, resistance screening trials are being carried out in the greenhouse to identify buffalograss selections that exhibit resistance to the buffalograss mealybug and/or chinch bug. Preliminary screenings have indicated 91-118, 86-120, 86-61, 'Cody', and 'Tatanka' are susceptible to buffalograss mealybugs. Additional trials will determine the level of susceptibility.

This research is vital to the overall buffalograss project. Although relatively few pests have been associated with buffalograss, the two grass-feeding mealybugs and the buffalograss chinch bug continue to pose a threat to buffalograss stands. While considerable progress has been made toward the goal of developing effective and environmentally responsible pest management alternatives for the insect and mite pest affecting buffalograss there is a continuing need for additional research.

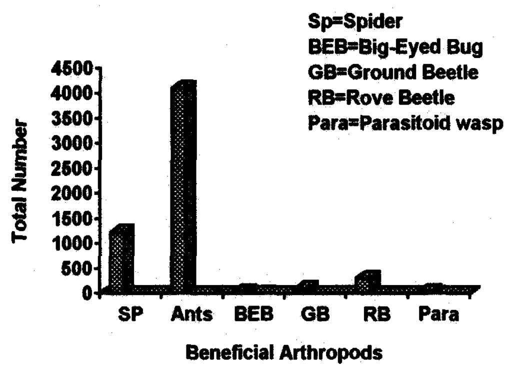

Figure 1. Beneficial arthropods collected from sod plugs for May-November 1995.

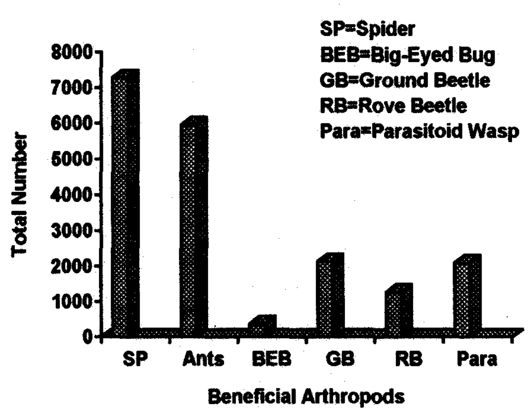

Figure 2. Beneficial arthropods collected from pitfall samples for May-November 1995.

Student Progress

Dr. Charles Rodgers, whose research has been on seed germination and establishment, finished his Ph.D. this past summer. Kevin Frank will be finishing his M.S degree in November, and will continue towards a Ph.D. His work has been on buffalograss management and is co-advised by Terry Riordan and Roch Gaussoin. Tiffany Moss is making good progress in entomology research and in her masters program. She is co-advised by Fred Baxendale and Terry Riordan. Shuizhang Fei continues to make excellent progress in his Ph.D. program in the area of tissue culture and biotechnology.

The following papers will be presented at the 8th International Turfgrass Conference in Sydney, New South Wales, Australia in July 1997:

Non-destructive monitoring of mealybugs (Homoptera-pseudococcideae) on buffalograss. T.M. Moss, F.P. Baxendale* J.M. Johnson-Cicalese , and T.P. Riordan.

Seedling germination and establishment of buffalograss caryopses vs. burs. T.P. Riordan*, P.G. Johnson, R.E. Gaussoin, and J.F. Svoboda.

Sod production characteristics of turf-type buffalograss. M.S. Giese, R.E. Gaussoin*, R.C. Shearman, and T.P. Riordan.

Vegetative establishment of buffalograss with plugs: Method and management. P.G. Johnson*, T.P. Riordan, R.E. Gaussoin, D. Schwarze, and K. Kerner.

Multiple trait selection for the improvement of turf-type, seeded buffalograss *Buchloe dactyloides* (Nutt.) Englem. J.P. Klingenberg* and T.P. Riordan.

In vitro regeneration of buffalograss *Buchloe dactyloides* (Nutt.) Englem. through immature inflorescence culture. S. Fei*, P.E. Read, and T.P. Riordan.

Inheritance of seed weight in two Nebraska synthetic buffalograss populations. C.A. Rodgers*, T.P. Riordan, and B.E. Johnson.

The following titles were presented at the American Society of Agronomy meetings in November, 1996 in Indianapolis:

Date of planting effects on seeded buffalograss. K. Frank*, R.E. Gaussoin, T.P. Riordan, and E.O. Miltner.

Analysis of ploidy level and DNA base-pair composition in buffalograss. P.G. Johnson*, T.P. Riordan, and K. Arumuganathan.

The following titles were presented at the American Society of Horticultural Science meetings:

$AgNO_3$ promotes callus production and regeneration efficiency of immature buffalograss inflorescence culture. S. Fei*, P.E. Read, and T.P. Riordan

The following title was presented at the workshop, *Recent Cellular and Molecular Approaches to Turfgrass Improvement*:

Plant breeding, plant regeneration, and flow cytometry in buffalograss. T.P. Riordan*, S. Fei, P.G. Johnson, and P.E. Read.

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* Presenting author