United States Golf Association Research Summary and Annual Report: 1998

A. Germplasm Development for Buffalograss Varieties

B. Management Practices for Golf Course Roughs, Fairways, and Tees Using Buffalograss

USGA Progress Report – 1998
University of Nebraska-Lincoln

Steve Westerholt	Project Coordinator
Terrance P. Riordan	Professor, Horticulture, Turfgrass Breeding
Roch Gaussoin	Assoc. Professor, Horticulture, Ext. Turfgrass Specialist
Fred Baxendale	Professor, Entomology
Shuizhang Fei	Ph.D. Post-Doctoral Associate
Leonard A. Wit	Supervisor, JSA Turfgrass Research Facility
Kevin Frank	Ph.D. Graduate Student, Horticulture
Taotao Yu	Ph.D. Graduate Student, Horticulture
Tiffany M. Moss	Ph.D. Graduate Student, Entomology

Cooperators:

Robert C. Shearman	Professor, Horticulture
Robert V. Klucas	Professor, Biochemistry
Garald L. Horst	Professor, Turfgrass Physiology
John E. Watkins	Professor, Plant Pathology
Gary Y. Yuen	Associate Professor, Plant Pathology
Tony P. Weinhold	Technician, Entomology
Jeff W. Witkowski	Technician, Horticulture
Jack D. Fry	Associate Professor, Kansas State University
Paul G. Johnson	Assistant Professor, Utah State University

A USGA/University of Nebraska Research Project Initiated February, 1993

United States Golf Association

Executive Summary – 1998

Seeded Releases: NTG is considering the possibility of selling NTG-5, which was included in the 1991 NTEP Trial, and they are looking at NTG-7 and FW-3 (a low mowing tolerant experimental) for future release and production.

Vegetative Releases: Patents have been filed for new releases NE 86-61, NE 86-120 and NE 91-118, but have not been granted. Official UNL release statements have been completed and these cultivars are included in the 1996 NTEP Buffalograss Trial. NE 91-118 has been vegetatively increased at Crenshaw Turf (CT) located at Poteet, Texas. Todd Valley Farms located at Mead, NE, bought a new farm and planted 35 acres of NE 86-61.

Crenshaw Turf, Inc. Update: Crenshaw Turf (CT) has purchased Ellsberry Sod in Florida and Milberger Sod in Bay City, Texas. They continue to grow and have positive growth plans for production of buffalograss and other southern turf species. Todd Valley Farms: Sales have continued to increase for `378' from Todd Valley Farms (TVF), but TVF now has a greater role in developing the buffalograss market in the Northern United States. UNL, CT and TVF are working cooperatively on the development of our new releases.

Summary of Breeding Work: Overall, the levels of performance continue to improve with each generation of selection. Newly release cultivars continue to show their superiority over older varieties with improved sod strength, color, quality, and density. Accessions from fairway maintained areas look very promising and show continuing improvements towards a high quality, low maintenance fairway turf. The top performers in the Nebraska National Buffalograss trial were 91-118 and 86-61, which are being commercialized. The seeded varieties Cody and Tatanka showed little differentiation during the first year of this study. However, in 1998 the advance-seeded types began to show better performance than the common types like Texoka.

Evaluation for Low-mowing and Wear tolerance: Under low mowing and no wear the female clone 92-135, which outperformed all other entries in 1997, performed very well again in 1998 along with female clone 92-31. However, two male clones, 92-141 and 92-116, had the best overall performance in 1998. All seed established experimentals exhibited average color and quality characteristics. The trial did have a number of promising male and female clones. Under wear results indicated that male and monoecious clones exhibited the most damage and wear tolerance of female cultivars was significantly better than males, but not as good as for mixed seeded types.

Fertility and Mowing Effects on Buffalograss: At the Nebraska site, NE 91-118 and 378 had the highest quality ratings at the 2.5 cm mowing heights for years 1996-1998. Cody and Texoka had poor quality ratings at the 2.5 cm mowing height for all years. In 1998, NE 91-118, 378, and Cody had the highest quality ratings at the 5.1 cm mowing height. At the 7.6 cm mowing height Cody and Texoka had the highest quality rating in 1997 but Cody and 378 had the highest quality ratings in 1998.

From 1997 to 1998 several trends are evident. First, turfgrass quality decreased from 1997 to 1998 for all cultivars at the 0, 2.4, and 5.0 g N m^{-2} rates. At 10 g N m^{-2}, NE 91-118 and 378 had higher quality in 1998 than in 1997. All cultivars had improved quality ratings in 1998 at the 20 g N m^{-2} rate. Quality ratings in 1998 were poor (ie. < 6) for all cultivars at 0, 2.4, and 5.0 g N m^{-2} rates. At 10 g N m^{-2} NE 91-118, 378, and Cody had good turfgrass quality. Management recommendations for 378 and NE 91-118 are 2.5 or 5.1 cm mowing heights and a nitrogen rate of 10 g N m^{-2} $year^{-1}$. Recommendations for Cody and Texoka are 5.1 or 7.6 cm mowing heights and a nitrogen rate of 10 g N m^{-2} $year^{-1}$.

Nitrogen Partitioning in Turfgrasses: Field experiments to determine the fate of nitrogen fertilizer applied to three turfgrass species were initiated in 1997 at the John Seaton Anderson Turfgrass Research Facility near Mead, Nebraska. Fate of fertilizer nitrogen will be followed in buffalograss, Kentucky bluegrass, and tall fescue. Established turfgrass plots of two cultivars of buffalograss, NE 91-118 and NE 86-120, a blend of Kentucky bluegrass and a blend of tall fescue. The total amount of actual nitrogen that will be applied each year to a 9 m^2 plot is 0, 10, and 20 g N m^{-2}. For Kentucky bluegrass and tall fescue 80% of evapotranspiration will be returned every four days and for buffalograss 60% of evapotranspiration will be returned weekly. Plots will be randomly sampled prior to each fertilizer application to analyze for nitrogen content in plant and soil fractions. A Giddeon Soil Probe will be used to extract six cores (5 cm diameter) to a depth of 62 cm. Cores will be divided into thatch, verdure, roots, and soil components. The soil cores will be partitioned to four depths: 0 to 8, 8 to 16, 16 to 32, and 32 to 62 cm. After partitioning the cores by depth, the six samples will be composited, mixed thoroughly, and analyzed for total N, NH_4^+-N, NO_3^--N, and N-isotope ratio.

Buffalograss Resistance to Chinch Bugs: The initial phase of this research involved developing screening methods and evaluating selected buffalograss germplasm for resistance to *B. occiduus*. Eleven buffalograss cultivars/selections ('Cody', 'Bonnie Brae', 'Tatanka', 'Texoka', NE 91-118, NE 86-120, NE 86-61, '315', '378', '609', and NE 84-45-3) were screened for resistance to *B. occiduus* in 2 greenhouse trials. Using chinch bug numbers and plant damage ratings to assess levels of resistance, the 11 buffalograss cultivars/selections were separated into categories of resistance. 'Cody' and 'Tatanka' consistently exhibited high levels of resistance to chinch bug feeding, while 'Bonnie Brae' and NE 91-118 showed high to moderate levels of resistance. Other cultivars/selections, including '378', '315', NE 84-45-3, and NE 86-61, were moderately to highly susceptible. 'Cody' and 'Tatanka' maintained acceptable turf quality although both became heavily infested with chinch bugs. This suggests tolerance may be a mechanism of the resistance. Studies designed to characterize the mechanisms of resistance are currently underway. Antixenosis experiments have revealed chinch bug preference for 'Texoka', NE 86-120, and 'Bonnie Brae'. Other cultivars/selections such as, '609' and NE 91-118 are rarely preferred.

Table of Contents

Status of New Releases

Seeded Releases

Dr. Charlie Rodgers, who replaced Dr. Jeff Klingenberg at Seeds West, Phoenix, Arizona, continues to work with our germplasm and their derived germplasm to develop new seeded cultivars. A new agreement is in place between UNL and Native Turf Group (NTG) which gives them the proprietary rights to all our germplasm to develop seeded buffalograsses.

NTG is considering the possibility of selling NTG-5, which was included in the 1991 NTEP Trial, and they are looking at NTG-7 and FW-3 (a low mowing tolerant experimental) for future release and production. Their material is being included in our evaluations.

Vegetative Releases

Patents have been filed for new releases NE 86-61, NE 86-120 and NE 91-118, but have not been granted. Official UNL release statements have been completed and these cultivars are included in the 1996 NTEP Buffalograss Trial. NE 91-118 has been vegetatively increased at Crenshaw Turf (CT) located at Poteet, Texas. Todd Valley Farms located at Mead, NE, bought a new farm and planted 35 acres of NE 86-61.

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

Crenshaw Turf, Inc. Update

Crenshaw Turf (CT) has purchased Ellsberry Sod in Florida and Milberger Sod in Bay City, Texas. They continue to grow and have positive growth plans for production of buffalograss and other southern turf species.

CT has had a fairly good year considering the drought in Texas. Management of the company seems to be very good, and the principals, Jimmy Raines, a venture capitalist from San Antonio, Texas, and Arthur Milberger of Milberger Sod, are providing vision and stability that were lacking in the past. Sales for 1998 of `609' will be the 1997 level, or $1.5 million dollars.

CT has signed a five-year agreement to work with the University of Nebraska.

Todd Valley Farms

Sales have continued to increase for `378' from Todd Valley Farms (TVF), but TVF now has a greater role in developing the buffalograss market in the Northern United States. UNL, CT and TVF are working cooperatively on the development of our new releases.

Summary of Breeding Work

Steve Westerholt, Terrance P. Riordan and Paul Johnson

The current goals of the University of Nebraska buffalograss project are to improve germplasm and develop management systems for use of buffalograss in golf course turf, as well as for other turf uses. Specifically, our objectives include selecting for exceptional turfgrass quality and color, heat and drought resistance, tolerance to low-mowing (for use in golf course fairways), insect resistance, and establishment vigor. We also use molecular breeding techniques, such as flow cytometry and random amplified polymorphic DNA (RAPD) markers to better characterize germplasm.

The 1998 growing season consisted of relatively mild, wet weather early in the summer turning to very hot and dry conditions in the second half of the summer. Early precipitation in May and June delayed the establishment of a number of projects, but should not effect data collection in 1999. High temperatures, humidity and heat in the later part of the summer did provide good conditions for screening buffalograss cultivars and collecting data on wear recovery.

Overall, the levels of performance continue to improve with each generation of selection. Newly release cultivars continue to show their superiority over older varieties with improved sod strength, color, quality, and density. Accessions from fairway maintained areas look very promising and show continuing improvements towards a high quality, low maintenance fairway turf.

National Turfgrass Evaluation Program (NTEP) 1996 Buffalograss Test

The 1996 buffalograss test was planted at eleven sites around the country including at our research facility near Mead, Nebraska. National results can be viewed on the World Wide Web at "http://hort.unl.edu/NTEP/".

The top performers in the Nebraska trial were 91-118 and 86-61, which are being commercialized. Bonnie-Brae was the best overall of the non-Nebraska cultivars in this test. The southern adapted types, especially diploid varieties like Stampede and UCR-95, did not survive the 1997 winter in Nebraska and most of theses plots were replanted to Texoka to maintain turf uniformity. Cultivar 609 was also adversely effected by the winter of 1997 and was re-established from plugs, therefore, its ratings are a result of this re-establishment. (Table 1.)

The seeded varieties showed little differentiation during the first year of this study. However, in 1998 the advance-seeded types began to show better performance than the common types like Texoka. Although Tatanka and Bison showed slower establishment than the other varieties in 1996, their performance was not affected. Both cultivars exhibited good color and quality characteristics. (Table 1.)

Table 1. National Turfgrass Evaluation Program 1996 Buffalograss Trial results from 1998.

Selection Name	Estab.	Spring Color	Fall Color	Color Average	Spring Quality	Fall Quality	Quality Average
91-118	Veg	6.0	5.7	5.8	7.0	7.0	7.0
86-61	Veg	7.3	7.3	7.3	7.0	7.0	7.0
'378'	Veg	7.0	7.0	7.0	7.0	6.7	6.8
91-181	Veg	7.3	7.0	7.2	7.0	6.7	6.8
Bonnie-Brae	Veg	6.7	6.0	6.3	6.3	7.0	6.7
86-120	Veg	6.3	6.3	6.3	6.3	6.3	6.3
93-181	Veg	6.3	5.7	6.0	6.3	6.0	6.2
Midget	Veg	6.0	5.0	5.5	5.7	6.0	5.8
93-170	Veg	5.0	5.0	5.0	1.3	3.3	2.3
Stampede	Veg	-	-	-	-	-	-
'609'	Veg	-	3.0	2.0	1.0	2.7	1.8
UCR-95	Veg	-	-	-	-	-	-
Mean		5.2	5.3	5.3	4.9	5.3	5.1
LSD		2.2	1.0	.48	1.1	.84	.51
Cody	Seed	6.0	6.0	6.0	6.3	5.7	6.0
Tatanka	Seed	6.0	5.7	5.9	6.3	5.3	5.9
BAM-1000	Seed	5.3	5.7	5.5	5.3	6.0	5.7
Bison	Seed	6.3	6.7	6.5	5.0	6.0	5.5
Texoka	Seed	5.0	5.3	5.2	4.7	5.7	5.2
Mean		5.7	5.9	5.8	5.5	5.7	5.6
LSD		.59	1.5	2.1	2.6	1.9	1.4

† Plot maintained as a low maintenance turf: (2.5 inch mowing height, 1-2 times per month, 2 lb. N/1000 sq. ft./year, no supplemental irrigation)

Evaluation for Low-mowing and Wear tolerance

Buffalograss is most commonly grown at high mowing heights and low maintenance situations. However in recent years, we have evaluated the species for potential use on golf course fairways in arid regions where superintendents need to make drastic cuts in their water use. We have observed that buffalograss does quite well at mowing heights even as low as 1.4 cm (5/8"). Our oldest plot in this evaluation has been mowed at 1.4 cm for 5 years and continued to provide high quality low maintenance fairway-type turf.

Since the initiation of the buffalograss program at Nebraska, buffalograss has also been considered tolerant to traffic and wear. However, very little information exists on the tolerance of buffalograss to wear under golf course fairway conditions. A simulated wear study was initiated on this area to evaluate buffalograss performance under three levels of wear using a wear simulation machine. The use of the wear simulator will provide information on the levels of tolerance we may expect, potential cultivar variation, and expected wear recovery.

Area 23: 1993 Advanced Evaluation (Table 2)

This plot area was mowed at 6.3 cm (2.5") (until 1996, when it was then gradually lowered to 1.4 cm. The plot was still exhibiting unevenness in 1997, but it still provided good evaluations. In 1998, spring color and quality data were collected prior to the introduction of wear treatments of 2, 4, and 8 passes with the wear simulator.

The female clone 92-135, which outperformed all other entries in 1997, performed very well again in 1998 along with female clone 92-31 (see Table 2). However, two male clones, 92-141 and 92-116, had the best overall performance in 1998. All seed established experimentals exhibited average color and quality characteristics. The trial did have a number of promising male and female clones.

Wear tolerance evaluations

Wear tolerance treatments with the wear simulation machine began the last week in June and continued weekly until the end of summer. Results of these treatments provided some valuable insight on buffalograss wear tolerance and potential acceptance as a fairway turf. Medium and low traffic conditions showed very little to no effect on the overall performance of the buffalograss cultivars. The high traffic treatment did exhibit significant damage to some individual plots, but overall did not destroy the turf, and recovery from injury was complete within 7 days after treatments. More importantly, wear treatment recovery was just as rapid under high temperatures and low moisture conditions, as under less stressful conditions.

When individual turf plots began to show varying amounts of damage, wear damage data was collected and analyzed. Results indicated that male and monoecious clones exhibited the most damage (It should be noted that only three cultivars in this trial were monoecious, and therefore, did not provide a representative sample). Wear tolerance of female cultivars was significantly better than males, but not as good as for mixed seeded types. (Table 3.)

Table 2. Low mowing† evaluation established 1993 (Area 23).

Selection Name	Gender	Spring Color 5/14/98	Spring Quality 5/14/98
90-504-jk	Seed	5.33	6.67
90-501-jk	Seed	4.33	5.67
90-503-jk	Seed	4.67	5.67
90-502-jk	Seed	5.00	5.67
93-537-97	Seed	5.33	5.67
92-116	Male	6.00	6.00
92-137	Male	6.33	6.00
92-141	Male	7.00	6.00
90-157	Male	5.00	5.67
92-103	Female	4.67	6.33
92-125	Female	6.33	6.33
91-116-tr	Female	3.33	6.00
91-114-dd	Female	3.67	6.00
92-104	Female	6.00	6.00
92-115	Female	4.67	5.67
92-135	Female	6.67	5.33
92-31	Female	6.67	5.33
Mean		4.93	4.71
LSD (.05)		1.17	1.66

† Plot maintained at 5/8 inch mowing height, 2 times per week, 3 lb. N/1000 sq. ft./year, no supplemental irrigation).

Table 3. Wear tolerance of buffalograss cultivars as related to gender.

Gender	Adjusted Means **	Probability table †			
		Monoecious	Female	Male	Seeded
Monoecious	3.56	-	.7227	.4110	.2268
Female	3.39		-	.0214	.0481
Male	4.00			-	.0002
Seeded/Mixed Sex	2.91				-
Wear Simulation Machine Treatments*		Monoecious	Female	Male	Seeded
8 Passes		6.3 A*	5.3 A	6.7 A	4.8 A
4 Passes		2.3 B	2.7 B	3.6 B	2.7 B
2 Passes		2.0 B	2.0 C	2.3 B	1.3 C

† Probability of > |T| to test the hypothesis LSMeans (I) = LSMeans (j).
* Means within the same column with the same letter are not significantly different.
** Ratings 1-9 scale 1=no damage – 9=heavy turf damage.

Area 27: 1995 Preliminary Evaluation

In 1998 wear treatments were also applied in area 27. This trial did not exhibit as severe of as damage as area 23. This is possibly due to the number of selections included that were made based on wear tolerance and aggressiveness. Selections from this area will be used for advance testing and hybridization with other wear tolerant material.

Area 31: 1996 Preliminary Evaluation

This plot area was established with progeny from a crossing block of six parents that showed tolerance to low mowing. This plot has allowed study of parental effects (maternal only) on low mowing tolerance. A number of quantitative measurements were taken on these plants to differentiate between the families and individual progeny, and to study traits important to turfgrass quality.

Selections were made from progeny plantings. These selections will be used for advance testing and hybridization with fairway type buffalograss accessions in 1999.

Genetic Transformation of Buffalograss

(Non-USGA Buffalograss Research Project)
Shuizhang Fei, Taotao Yu, Terrance P. Riordan and Thomas E. Clemente

A significant problem of buffalograss that limits its use and success is its lack of competitiveness against weeds, especially bermudagrass in the south. We have obtained rights from Scotts and Monsanto that allow us to incorporate the gene for glyphosate resistance into buffalograss.

The objectives of this project were to use the biolistic procedure (gene gun) to introduce the glyphosate resistant gene into several buffalograss cultivars. This project started in December 1997. Our research has been focused on: (1) developing an efficient regeneration protocol through organogenesis; (2) optimizing bombarding parameters based on transient expression; and (3) choosing a proper selection scheme.

Regeneration: Buffalograss regeneration through immature inflorescence culture has been accomplished, however, its low efficiency and limited availability of explants prevented this protocol from being used efficiently for genetic transformation. Male immature inflorescences exhibited a much higher embryogenic callus induction frequency than female immature inflorescences, but it would also take much more time to get a stable cultivar by the required conventional crossing. Node sections with preexisting axillary buds were exploited as an explant source for regeneration through organogenesis. Plants regenerated through organogenesis are less likely to develop somaclonal variants and the process takes less time compared to somatic embryogenesis.

Buffalograss stolons taken from greenhouse were disinfected with 70% ethanol for 1 minute followed by 20% commercial bleach for 15 to 30 minutes. Stolons were then cut into single segments each with one or two preexisting buds. The tops of the primary shoots (if any) and preexisting axillary buds (upper half) were removed before culture to stimulate new shoot formation on MS basal medium containing cytokinin (BA). An average of more than four shoots per explant were induced on MS basal medium plus 1 mg BA l^{-1} after 2 weeks of culture. As culture continued, secondary and tertiary branching occurred, adding more shoots to each explant. Among the cultivars tested, '609' performed better than '91-118' regarding the number of shoots regenerated. This efficient regeneration protocol makes it feasible to use the biolistic device to deliver the gene of interest into the target regions of the explants, and then recover transformed plants from bombarded explants under a certain period of selection. Meanwhile, limited efforts were also taken to regenerate buffalograss through immature inflorescence cultures. Embryogenic calli were obtained from and maintained for female genotype 91-118, but growth was slow.

Optimization of bombarding parameters: PDS/He-1000 gene gun was used for all experiments involving bombardment. In order to maximize the gene expression level while minimizing tissue damage, several factors that are considered responsible for particle penetration and coverage, tissue damage and gene expression were evaluated based on the number of blue spots resulted from the GUS gene expression. These factors include:

Rupture disk pressure: 650psi - 2200 psi (pound per square inch);

Particle travel distance: from the top shelf to the shelf next to the bottom shelf;

Different DNA precipitation procedure;

Different particles (Gold and Tungsten) with different sizes;

Length of preculture of explants;

Supercoiled DNA vs Linear DNA;

In all experiments, node segments with preexisting axillary buds were cut back to expose the meristematic regions after 0-10 days of preculture. About 35-40 explants were placed vertically in the center of a petri plate for bombardment. The vacuum reading was fixed at 27 inch Hg and the gap distance was maintained at 1/4 inch. Preliminary results indicated that 650-900psi, using either 1.6μm or 1.0μm gold particles, DNA precipitation adopted from the Scotts Company, plant tissues that were precultured for seven days and then cut back, and putting the plate on the top shelf resulted in better transient expressions. The number of GUS spots varied from 0 to around 50 per explant. This large variation indicated that there is still room to improve transient expression. Regardless the size of the particles, gold particles showed higher transient expression than tungsten particles. Contrary to our expectation, the supercoiled DNA had higher transient expression than the linearized DNA.

Several different promoters (regulatory sequence that regulate gene expression) were also evaluated based on transient evaluation, it was found that the *ubiquitin* promoter has the most intensive expression in terms of the number of gus spots and the sizes of the spots.

Selection scheme: Glyphosate levels used in our experiments ranged from 0.025 to 0.1mM. Explants that were put into selection 24 hours after receiving bombardment died within two weeks. When explants were put into selection medium 72 hours after receiving bombardment (three days of delay), survival rates were improved and with a delay of 5 days, the survival rate was significantly improved. These survived explants continued to proliferate in the presence of glyphosate, forming clusters of shoots, though none of them elongated significantly. In order to have minimum delay while maintaining a reasonable survival rate, a compromise has been made in one of our experiment, explants were put into selection medium containing low concentration of glyphosate (0.025mM) 24 hours after receiving bombardment. Explants were then transferred to a higher glyphosate level (0.075) after being on 0.025mM glyphosate for various days. Results have yet to be surveyed. Choosing the right selection scheme is a challenge because no measurements are available before transformed plants are recovered, often in small numbers.

In our recent rooting experiment, survived shoots were place on the rooting medium after 3 months of selection at 0.05mM glyphosate. About 80% of the shoots started to form roots on rooting medium without glyphosate, and the above medium plant parts also started to elongate. On rooting medium containing 0.025mM glyphosate, however, only 5.7% of the shoot clusters formed short thin roots and the above medium plant parts did not elongate. The rooted plants were then transferred to soil in the greenhouse, and more than 90% of the plants survived. A PCR test will be performed to determine if the glyphosate resistance gene has been integrated.

***Agrobacterium* infection experiments:** Three different strains of *Agrobacterium* EHA101, C58C1, and LBA4404 that carries a super binary vector have been used to infect buffalograss node segments. Strains C58C1 and LBA4404 also carry the antibiotic resistant gene, NPT II. Explants that were cocultured with these two strains were cultured and subcultured on the selection medium containing 20 mg Geneticin (G418 sulfate) l^{-1}. After three 2-week transfers, some explants are still growing on the selection medium. More research will be conducted. Monocot crops are not considered a natural host for *Agrobacteria*, however, successful transformation of important crops such as rice and wheat using *Agrobacteria* have been recently reported, though mostly through somatic embryogenesis. More research is still required to apply this technique routinely into other crops or regeneration systems. We believe our research on buffalograss using *Agrobacteria* will add to the body of knowledge in this arena.

In conclusion, we have achieved a reasonably high regeneration efficiency for genetic transformation of buffalograss, and obtained first-hand knowledge of particle bombardment and subsequent selection. So far, no report of successful genetic transformation of monocot crops through particle bombardment of meristematic tissue is available, therefore our project, if successful, will contribute significantly to research that involves transformation of the grass family including most turfgrasses and important cereal crops.

Buffalograss Management Research

Fertility and Mowing Effects on Buffalograss

Kevin Frank, Roch Gaussoin, Terrance Riordan, Eric Miltner, Jack Fry, and Paul Johnson

'Cody', 'Texoka', '378', and NE 91-118 buffalograss genotypes were planted at locations in Nebraska, Utah, and Kansas in 1995 to determine the effect of nitrogen fertilization and mowing height on turf-type buffalograsses. The three locations were the John Seaton Anderson Turfgrass Research Facility near Mead, Nebraska, the Rocky Ford Turfgrass Research Center at Manhattan, Kansas, and the Greenville Research Farm at Logan, Utah. In 1996 mowing heights of 2.5, 5.1, and 7.6 cm and nitrogen treatments of 0, 2.4. 5, 10, and 20 g N m^{-2} $year^{-1}$ were imposed to identify best management practices for turf-type buffalograss. Nitrogen rates are applied as a split application with the first application in early June and the second application in mid-July, 6 weeks after the first application. The nitrogen source is a polymer coat, Scotts® Poly-S 36N-3P-7K. Immediately following nitrogen applications, plots are irrigated with 1.3 cm water.

At the Nebraska site, NE 91-118 and 378 had the highest quality ratings at the 2.5 cm mowing heights for years 1996-1998 (Table 4). Cody and Texoka had poor quality ratings at the 2.5 cm mowing height for all years. In 1998, NE 91-118, 378, and Cody had the highest quality ratings at the 5.1 cm mowing height. At the 7.6 cm mowing height Cody and Texoka had the highest quality rating in 1997 but Cody and 378 had the highest quality ratings in 1998.

There was a significant entry by nitrogen rate interaction at the Nebraska site at two weeks after the second nitrogen application in both 1997 and 1998. From 1997 to 1998 several trends are evident. First, turfgrass quality decreased from 1997 to 1998 for all cultivars at the 0, 2.4, and 5.0 g N m^{-2} rates (Table 5). At 10 g N m^{-2}, NE 91-118 and 378 had higher quality in 1998 than in 1997. All cultivars had improved quality ratings in 1998 at the 20 g N m^{-2} rate. Quality ratings in 1998 were poor (ie. < 6) for all cultivars at 0, 2.4, and 5.0 g N m^{-2} rates. At 10 g N m^{-2} NE 91-118, 378, and Cody had good turfgrass quality.

Management recommendations for 378 and NE 91-118 are 2.5 or 5.1 cm mowing heights and a nitrogen rate of 10 g N m^{-2} $year^{-1}$. Recommendations for Cody and Texoka are 5.1 or 7.6 cm mowing heights and a nitrogen rate of 10 g N m^{-2} $year^{-1}$.

Table 4. Quality ratings for Entry X Mowing Height at the Nebraska site at two weeks

	Mowing Height		
1996 Entry	2.5 cm	5.1 cm	7.6 cm
NE 91-118	5.85[†] Ab	6.19 Aa	5.97 Bb
378	5.84 Ab	6.19 Aa	6.16 Aa
Cody	5.53 Bb	6.17 Aa	6.15 Aa
Texoka	5.16 Cc	5.87 Bb	6.09 Aa
1997			
NE 91-118	6.04 Aa	5.96 Aa	5.60 Bb
378	6.04 Aa	6.21 Aa	5.63 Bb
Cody	5.63 Ba	6.03 Aa	5.92 Aa
Texoka	5.16 Cb	6.12 Aa	6.09 Aa
1998			
NE 91-118	5.30 Ab	5.87 Aa	5.23 Bb
378	5.40 Ab	5.93 Aa	5.83 Aa
Cody	4.80 Bb	5.60 Aba	5.37 Aba
Texoka	4.03 Cb	5.33 Ba	5.33 Ba

Means within columns followed by the same capital letter are not significantly different (p=0.05; LSD).
Means within rows followed by the same small letter are not significantly different (p=0.05; LSD).
[†] Buffalograss quality rated from 1-9, with 9 = excellent, 6 = acceptable, and 1 = poor.

Table 5. Quality ratings for Entry X Nitrogen rate at the Nebraska site at two weeks after the second nitrogen application in 1997 & 1998

	Nitrogen Rate				
Entry	0†	2.4	5.0	10	20
Year 1997					
NE 91-118	4.56‡ Ce	5.49 Bd	5.96 ABc	6.51 Ab	6.82 Aa
378	5.36 Ae	5.73 Ad	6.07 Ac	6.24 Bb	6.40 Ca
Cody	4.69 Be	5.60 Bd	5.84 Cc	6.47 Ab	6.69 Ba
Texoka	4.47 Ce	5.58 Bd	5.91 BCc	6.40 Ab	6.60 Ba
Year 1998					
NE 91-118	3.00 Bce	4.44 Bd	5.39 ABc	7.00 Ab	7.50 Aa
378	4.00 Ae	4.89 Ad	5.61 Ac	6.78 Ab	7.33 Aba
Cody	3.22 Be	4.67 ABd	5.06 BCc	6.11 Bb	7.22 Ba
Texoka	2.78 Ce	3.89 Cd	4.83 Cc	5.89 Bb	7.11 Ba

Means within columns followed by the same capital letter are not significantly different (p=0.05; LSD).
Means within rows followed by the same small letter are not significantly different (p=0.05; LSD).
† Nitrogen rates - g N m^{-2} $year^{-1}$
‡ Buffalograss quality rated from 1-9, with 9 = excellent, 6 = acceptable, and 1 = poor.

Nitrogen Partitioning in Turfgrasses

Kevin Frank, Roch Gaussoin, Terrance Riordan

Buffalograss is cited in numerous sources as having minimal response to fertilizer nitrogen applications but to date no research has investigated the fate of fertilizer nitrogen applied to buffalograss. Information on fertilizer nitrogen fate in turf-type buffalograss is not available and comparisons with nitrogen fate in other turfgrass species would be valuable to identify differences in nitrogen use. The objectives of this research will be to determine the quantity and turnover rate of soil and fertilizer nitrogen in aboveground vegetation, thatch, roots, and soil for three turfgrass species.

Field experiments to determine the fate of nitrogen fertilizer applied to three turfgrass species were initiated in 1997 at the John Seaton Anderson Turfgrass Research Facility near Mead, Nebraska. Fate of fertilizer nitrogen will be followed in buffalograss, Kentucky bluegrass, and tall fescue. Established turfgrass plots of two cultivars of buffalograss, NE 91-118 and NE 86-120, a blend of Kentucky bluegrass (cv. 'Merit', 'Baron', 'Touchdown', 'Adelphi') and a blend of tall fescue (cv. 'Arid', 'Mustang', 'Olympic') will be used. The soil type is a Sharpsburg silty clay loam (fine montmorillonitic, mesic Typic Argiudoll).

The total amount of actual nitrogen that will be applied each year to a 9 m^2 plot is 0, 10, and 20 g N m^{-2}. The nitrogen amounts will be split among four applications for Kentucky bluegrass and tall fescue and over two equal applications for buffalograss. The approximate timings for the nitrogen applications to Kentucky bluegrass and tall fescue are: 1 May, 28 May, 3 September, and 4 November. The approximate timings for the nitrogen applications to buffalograss are: 4 June and 16 July. Immediately following nitrogen application, plots will be irrigated with 1.3 cm water. The first year, 5% ^{15}N enriched NH_4NO_3 will be applied for the 28 May nitrogen application for Kentucky bluegrass and tall fescue and for the 4 June nitrogen application for buffalograss. All other nitrogen applications in the first and second year will use an unlabeled NH_4NO_3 fertilizer. Plots will be mowed weekly at 5 cm and clippings returned. Irrigation will be applied according to individual species need. For Kentucky bluegrass and tall fescue 80% of evapotranspiration will be returned every four days and for buffalograss 60% of evapotranspiration will be returned weekly. Dithiopyr pre-emergence herbicide will be applied in April and fungicides will be applied if necessary.

Plots will be randomly sampled prior to each fertilizer application to analyze for nitrogen content in plant and soil fractions. In addition to sampling before each fertilizer application, the buffalograss plots will be sampled once in late August. A Giddeon Soil Probe will be used to extract six cores (5 cm diameter) to a depth of 62 cm. Cores will be divided into thatch, verdure, roots, and soil components. Because buffalograss has no thatch layer, all aboveground vegetation will be analyzed together. The soil cores will be partitioned to four depths: 0 to 8, 8 to 16, 16 to 32, and 32 to 62 cm. After partitioning the cores by depth, the six samples will be composited, mixed thoroughly, and analyzed for total N, NH_4^+-N, NO_3^--N, and N-isotope ratio. Roots will be washed from the remaining composited soil samples by depth, dried at 60°C, weighed, and total N and N-isotope ratio determined. NH_4^+-N and NO_3^--N of soil samples will be determined via automated colorimetric procedures. Total N of plant and soil samples will be determined via combustion. N-isotope ratio of samples will be determined using a mass spectrometer. The experimental design is a randomized incomplete block design and the treatment design is a factorial with grass species/cultivar and nitrogen as treatment factors.

Results are not yet available as samples from 1997 and 1998 are currently being analyzed.

Areas 15 and 19: Buffalograss Fairway Management Evaluation

The management recommendations of buffalograss as a fairway grass are relatively unknown. This study was initiated to evaluate buffalograss under fairway conditions using various rates of nitrogen, aerification, irrigation, mowing and wear will be included to simulate a normal fairway. Four seeded and vegetative cultivars were selected and planted on June 23, 1998. Initial treatments will be applied to the areas in 1999.

Buffalograss Entomology Research

Buffalograss Resistance to Chinch Bugs: Germplasm Evaluation, Mechanisms of the Resistance, Physiological Mechanisms Underlying Plant Tolerance to Chinch Bug Injury

Tiffany M. Heng-Moss and Frederick P. Baxendale

The development of turfgrass species requiring less water, mowing, and fertilization has received considerable attention over the last decade. Buffalograss, *Buchloë dactyloides* (Nuttall) Engelmann, is an appealing, alternative turfgrass species because of its low maintenance requirements and relative freedom from diseases and arthropod pests. Recently, however, the chinch bug, *Blissus occiduus* Barber has emerged as an important insect pest of buffalograss in Nebraska. The objectives of this research are to identify buffalograss germplasm resistant to *B. occiduus*, characterize the mechanisms of this resistance, identify antixenotic and antibiotic traits associated with buffalograss selections, and understand the physiological mechanisms underlying buffalograss tolerance to chinch bug injury. This information will be used to screen buffalograss germplasm and develop chinch bug resistant buffalograss cultivars.

The initial phase of this research involved developing screening methods and evaluating selected buffalograss germplasm for resistance to *B. occiduus*. Eleven buffalograss cultivars/selections ('Cody', 'Bonnie Brae', 'Tatanka', 'Texoka', NE 91-118, NE 86-120, NE 86-61, '315', '378', '609', and NE 84-45-3) were screened for resistance to *B. occiduus* in 2 greenhouse trials. Using chinch bug numbers and plant damage ratings to assess levels of resistance, the 11 buffalograss cultivars/selections were separated into categories of resistance. 'Cody' and 'Tatanka' consistently exhibited high levels of resistance to chinch bug feeding, while 'Bonnie Brae' and NE 91-118 showed high to moderate levels of resistance. Other cultivars/selections, including '378', '315', NE 84-45-3, and NE 86-61, were moderately to highly susceptible. 'Cody' and 'Tatanka' maintained acceptable turf quality although both became heavily infested with chinch bugs. This suggests tolerance may be a mechanism of the resistance.

Studies designed to characterize the mechanisms of resistance are currently underway. Antixenosis experiments have revealed chinch bug preference for 'Texoka', NE 86-120, and 'Bonnie Brae'. Other cultivars/selections such as, '609' and NE 91-118 are rarely preferred. Experiments quantifying levels of chinch bug tolerance among the 11 buffalograss cultivars/selections found no statistical differences in turf quality between infested and non-infested 'Cody' and 'Tatanka' plants. These experiments served to develop and evaluate several tolerance indices based on chinch bug damage ratings, plant height, and plant biomass. Antibiosis studies evaluating differences in chinch bug fecundity and biomass among the 11 buffalograss cultivars/selections are planned for next season.

In addition to these resistance mechanism experiments, research will investigate antibiotic, antixenotic, and tolerance traits of buffalograss by: (1) characterizing chinch bug probing behavior on resistant and susceptible plants using an electronic insect feeding monitor; (2) examining leaf trichome density and epicuticular leaf structures of buffalograss germplasm under a scanning electron microscope (SEM); and (3) investigating the physiological differences between susceptible and tolerant plants to chinch bug injury by measuring rates of photosynthesis, light and A-Ci curves, transpiration and conductance, water potential, leaf chlorophyll content, and leaf flourescence.

Planned Evaluations for 1999

Fairway Buffalograss Evaluation Project

Objective: To directly compare and evaluate buffalograss and other species under fairway conditions. This study will evaluate currently used fairway species and buffalograss. Recommended management programs will be used for each species and wear treatments will be imposed. Upon completion, this area could also be used to evaluate methods of fairway conversion to buffalograss. Some of the information that will be obtained:

- The study will compare bentgrass, perennial ryegrass, and Kentucky bluegrass versus buffalograss under fairway conditions.
- Compare establishment rates of the various species.
- Compare mowing, fertilizer values, and water use rates.
- Evaluate turf recovery from wear and divots.
- Evaluate winter playability, recovery, and spring greenup.
- Evaluate fairway conversion (to evaluate the rate and methods to convert various turfgrass species to buffalograss).

Buffalograss Physiology Evaluations

Very little is known about the physiology of buffalograss and what effect growth hormones play in the plant's development. Evaluation could provide information on the effect on sex ratios, increase establishment rates and increase densities of turf. This study could provide a better understanding of how the environment influences buffalograss.

Sex Ratio Evaluation

Observations in trials at Mead, NE have indicated that predominately female cultivars will at various times produce an occasional male inflorescence. To better evaluate this phenomenon, dioecious and monoecious accessions of buffalograss will be established in geographically diverse areas to determine female and male expression. This test should provide information on the environment influence on sex expression.

Evaluation of Rooting Compounds

Pre-rooting of plugs has shown to increase the establishment rate of buffalograss. One possible explanation for this is that the initial root pruning during plugging disrupts growth until root development has been reestablished. Studies have shown that many species produce growth hormones in the meristematic tissue in the roots. If buffalograss responds in this way, applications of a rooting compound could potentially increase its establishment rate.

Objectives: To evaluate the effect of different growth and rooting compounds on Buffalograss. This study will be conducted in the greenhouse in the fall of 1998. It will determine:

- If plant growth hormone can be used to stimulate buffalograss establishment.
- If rooting hormone can enhance plug development and spread, compared to pre-rooted plugs.
- Determine general effects of various plant growth hormone on buffalograss.
- Evaluate effective application techniques.

Establishment of buffalograss turf using sprigs

Objectives: To study the establishment of buffalograss using sprigs. The recommended time frame for this study will be in the spring of 1999. This study will determine:

- The effectiveness of sprigging on the establishment of buffalograss
- The comparison of sprigging versus plugging.
- Determine the effects of rooting hormones to increase sprig establishment.

Student Progress

- Kevin Frank received a Watson Fellowship at the 1998 GCSAA Conference and Show. He also received one of 16 Widaman Trust Awards during the year 1998.

- Tiffany Moss continues to make excellent progress toward her Ph.D. Her MS dissertation was selected as the most outstanding M.S. dissertation at UNL this last year.

- Shuizhang Fei successfully defended his Ph.D. thesis in December 1997 and became project leader/Post Doc on our plant transformation team.

- Taotao Yu, began work on a Ph.D. in August of 1997 and is making excellent progress in the area of plant transformation.