Sporobolus airoides and *Muhlenbergia asperifolia:* population developments for southern Nevada

Midterm Report of 2005 Activities

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Introduction

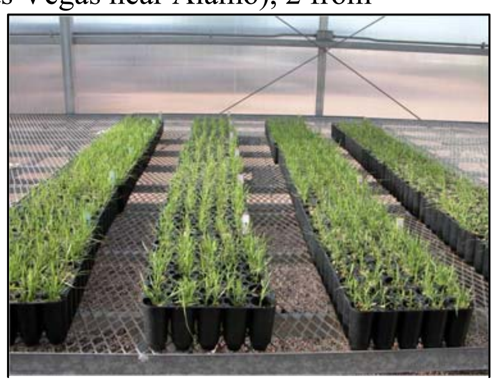
As part of a collaborative project with the Southern Nevada Restoration Team (SNRT), represented by the four major federal agencies (and land holders) of southern Nevada, the NRCS Tucson Plant Materials Center (PMC) has completed the first phase in the development of genetically diverse, regionally adapted ecotypes of two native grasses, alkali sacaton (*Sporobolus airoides*) and alkali muhly (*Muhlenbergia asperifolia*). This seed will be used in 2006 to establish local seed growers in southen Nevada. The release of ecotypes of these species provides material commercially, making them available for use in large-scale restoration of Mojave desert riparian areas.

Materials & Methods

Due to differences in the amount of seed available, different methods were used for the two species, particularly in timing of activities, field plot design, and the opportunity to evaluate accession differences in the year of establishment. The step-by-step process taken to develop these two populations is explained below.

1. Alkali sacation population

Alkali sacaton seed were supplied to us from collections made at nine distinct sites within Clark, Lincoln and Nye Counties in southern Nevada (Appendix A): 3 from Moapa National Wildlife Refuge (40 mi northeast of Las Vegas near the town of Moapa), 3 from Pahranagat National Wildlife Refuge (100 mi north of Las Vegas near Alamo), 2 from Ash Meadows National Wildlife Refuge (60 mi west of Las Vegas near the town of Pahrump), and one from Sacaton Canyon, Lake Mead National Recreation Area (70 mi southeast of Las Vegas near the Arizona border).



Alkali sacaton plugs in the PMC greenhouse (April 2005)

Upon arrival, visible differences in seed color and size were apparent. Certain accessions were twice the size of others, some collected within the same vicinity. Most of the seed arrived already processed, and percent purity and percent fill were determined with x-ray. These values were used to calculate and combine equal amounts of pure live seed to create the four accessions of the four sites named. (Appendix B).

With adequate seed to work with, seed from the four accessions were directly sewn into plugs in March 2005. Three accessions germinated in the greenhouse within 5 days at 70 deg F (day)/60 deg F (night), but the accession from Sacaton Canyon did not germinate until the temperature was raised to 80 deg F (day)/70 deg F (night). Plugs were fertilized and clipped weekly, and moved to the shade house in May. In June the plugs were planted in a 0.5 ac field at the PMC. Plugs were planted by hand 1 foot apart in four rows in a latin square design to maximize hybridization between accessions. An

Alkali sacaton field, early (right) and mid-growing season (July and September 2005)

experimental unit consisted of 10 plants. (Appendix C). The field was watered as necessary for vigorous establishment (more often in the year of establishment than in successive years). Observations were made for size and initiation of inflorescence 2 weeks after planting and again at 6 weeks after planting.

Seed were harvested 3 times during the growing season with the Woodward Flail vac seed stripper. The flail vac works by brushing, or stripping, only ripened seed without damage to the inflorescence or foliage. For species like alkali sacaton with indeterminate flowering, this process allows for multiple harvests throughout the growing season. This also allows for continued data collection on plant growth. In October, additional observations were made to compare inflorescence maturity and stage of flowering.

In November a more formal and quantitative evaluation was conducted to compare forage and reproductive characeristics. The second plant of each experimental unit of the center two rows was clipped at the base approximately 3 inches off the ground and data collected for six measurements: foliar height, reproductive height, weight, diameter, level of rust and number of inflorescence stalks. Clipped plants were bundled individually for measurement. Plants were weighed, measured for foliar height (length of the bulk of the leaves before tapering off) and reproductive height (length of the tallest inflorescence). Each bundle was opened to count total number of inflorescences and to measure rust along a scale of 1-6. After clipping the diameter of each plant clump was measured. Data were analyzed using Statistix (Version 8.1). Inferential statistics were used to compare these variables using Analysis of Variance and LSD All-Pairwise comparisons tests.

Alkali sacaton field and harvest (October 2005)

One plant from each accession unit was clipped and measured for the evaluation (November 2005)

In February 2006 hay bales were harvested from the alkali sacaton field. Five hay bales were cut weighing a total of 300 lb. Based on these values we expect a minimum of 1200 lb biomass/ac to be produced per cutting.

2. Alkali muhly population

Layering of alkali muhly for cloning

Three collections of alkali muhly (scratchgrass) were made in Clark County and sent to the PMC, 2 from Moapa National Wildlife Refuge and one from Pot-O-Gold Spring in Rainbow Gardens of Lake Mead National Recreation Area. The seed were combined to form three accessions. Because insufficient seed were available, extra steps were needed to have sufficient plugs to fill a PMC field.

To maximize seed, they were sown directly into flats, and the germinated seedlings were individually transplanted into plugs. Approximately 6 weeks later, when the plugs were large enough, they were periodically cloned using a clipping and layering process. The rooted cuttings were then planted into plugs. The extraordinary vegetative reproduction capabilities of this species, through rhizomes and stolons, lends itself well to this layering process as well as, presumably, asexual establishment in its native environment.

Alkali muhly planting at the PMC (Sept. 2005)

The extra steps required to increase the number of alkali muhly plugs postponed the planting of the field by several months. By late September sufficient plugs to fill an approximately 0.2 ac field were cloned and grown to adequate size. Plugs were randomly mixed and planted into a PMC field using a mechanical transplanter.

Rhizomes emerge from alkali muhly 2 ½ months after planting (December 2005)

Due to the late planting, seed from this field were not harvested in the initial year of establishment. However, we were pleased to see that in this short time the plants had already begun to produce seed and rhizomes. Unequal proportions of the three accessions did not lend this population to the evaluation process that was conducted for the Alkali sacaton.

Observations & results from first growing season

Although visual observations were made on both species, data were collected from only the Alkali sacaton throughout the growing season of 2005.

1. Preliminary observations

By June, 2 weeks after planting, distinct differences in size were becoming apparent between the accessions of alkali sacaton. On June 29 and July 29, visual observations were made on plant size and measurements were taken on initiation of inflorescence.

Size differences between accessions (July 2005)

Two weeks after planting, few plants were initiating inflorescence, but Pahranagat accession had the greatest margin (Table 1). A month later, Moapa was the only accession with a majority in flower. Ash Meadows and Sacaton Canyon were not yet in reproductive mode.

Table 1. Mean presence of inflorescence in plants

Accession	June (%) n=80	July (%) n=80
Moapa	4 [a†]	68 [a]
Pahranagat	14 [b]	23 [b]
Ash Meadows	7 [bc]	3 [c]
Sacaton Canyon	2 [c]	0 [c]

[†] Subscripts without common letters are significantly different ($\alpha=0.05$). Randomized complete block AOV and LSD All-Pairwise Comparisons Tests were conducted.

Inflorescence differences across the four accessions (July 2005)

Two and a half months later, on October 12, only two plants had not grown seed stalks yet (Table 2). Maturity of inflorescence and stage of flowering were measured between the four accessions. Maturity of inflorescence compares initiation of inflorescence, and flowering represents potential for hybridization. Inflorescence initiation is best compared by looking at the presence of immature seed stalks on the plants. While the majority of Sacaton Canyon had only recently initiated inflorescence, Ash Meadows had gone through this stage long before (98% of the inflorescence had desiccated). In contrast, Moapa and Pahranagat had relatively equal proportions of immature and mature seed stalks. Plants from all four accessions were flowering simultaneously, however Moapa and Pahranagat had a majority in flower. Ash meadows flowered earlier in the season, and Sacaton canyon was just entering its flowering stage.

Table 2. October measurements: mean inflorescence maturity and flowering

Accession	Inflorescence maturity (%) n=100			Flowering (%) n=100
	Immature	Mature	Both	
Moapa	23	24	53	80
Pahranagat	31	22	47	60
Ash Meadows	2	45	54	40
Sacaton Canyon*	80	8	10	10

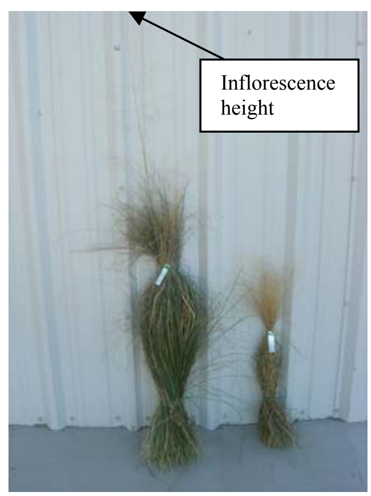
*Two plants had no seed stalks

2. Evaluation

Data were collected at the end of the growing season to evaluate the four accessions systematically and quantitatively. Six growth characteristics representing size and reproductive ability were measured: two heights, weight, diameter, number of inflorescences (Table 3). Significant differences were observed for all characteristics except reproductive height. Plants were also evaluated for rust, a pathogen. Although less of an issue in its natural environment, rust may potentially affect production rates in agricultural conditions with higher moisture levels and close proximity of plants. Rust was moderate in all four accessions, although the rates were significantly higher in the smallest accession. We will monitor rust over the next two years to address potential production problems by altering irrigation rates and use of fungicides.

Collecting data for the final evaluation (Nov. 2005)

In all variables describing size, the Sacaton canyon accession was the largest and Ash Meadows was the smallest. Sacaton canyon was the tallest in both foliar height (the length of the bulk of leaves before tapering off) and reproductive height (length of the tallest inflorescence). Differences in reproductive height were visually apparent but not statistically significant, perhaps due to the fact that most inflorescences were dry and brittle by the time of measurement, and many of the tallest had broken off. Foliar estimates of height revealed that Sacaton canyon and Moapa plants were significantly taller than Pahranagat, which was significantly taller than Ash Meadows. Sacaton canyon was approximately 1.5 times heavier than the Moapa and Pahranagat plants, and almost three and a half times heavier than Ash Meadows.



Size differences were most visible between Sacaton Canyon and Ash Meadows

Width and reproductive potential of the plant revealed surprising contrasts with the above measurements of size. Although taller and heavier, Sacaton Canyon did not differ in width from Pahranagat or Moapa, although it was greater than Ash Meadows. Even more interesting was the difference in reproductive potential, represented by total number of inflorescences present at the end of the growing season. Pahranagat, although only of moderate height and mass, had more inflorescences than the three other accessions. This variable was also the only one in which the smaller accession, Ash Meadows, did not rank last. In fact, foliar height was negatively correlated with number of inflorescences. Shorter plants may invest more in reproductive ability.

Table 3. November evaluation of size and reproductive ability

Accession	Foliar height (m) n=38	Reproductive height (m) n=34	Weight (g) n=39	Diameter (m) n=39	Inflorescence (#) n=39
Moapa	0.63 [a†]	1.10 [ab]	471 [b]	0.22 [bc]	55 [b]
Pahranagat	0.54 [b]	1.06 [ab]	451 [b]	0.26 [a]	121 [a]
Ash Meadows	0.40 [c]	0.91 [b]	200 [c]	0.20 [c]	61 [b]
Sacaton Canyon	0.69 [a]	1.23 [a]	694 [a]	0.25 [ab]	74 [b]

[†] Subscripts without common letters are significantly different (α=0.05). Randomized complete block AOV and LSD All-Pairwise Comparisons Tests were conducted.

Conclusions

The establishment year for two southern Nevada species population developments has revealed interesting findings. The large quantity of alkali sacaton seed allowed for planting early in the season and evaluation of the four accessions provided to us from distinct locations in southern Nevada. Unfortunately, due to the extra steps required for alkali muhly, conditions did not allow for the same evaluative process. Although different procedures were undertaken for alkali sacaton and alkali muhly, we expect the same final product in the coming year: a bag of seed. The seed developed from these populations will provide for restoration projects in southern Nevada into the future.

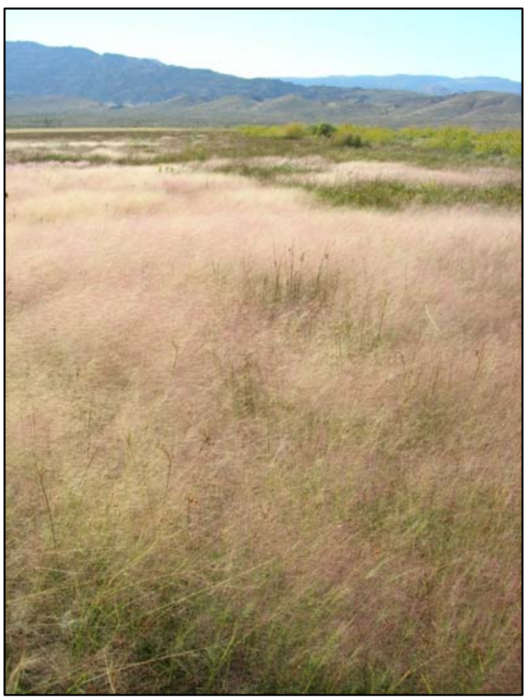
Alkali sacaton growing at Ash Meadows NWR, Nevada

The differences observed between the four accessions of alkali sacaton exemplifies that large variability can exist within species— even within small regions such as southern Nevada. Distance may not be as important as other factors in determining variability in populations. From early observations on seed variation and germination requirements to differences in foliage development and timing of inflorescence, this species reveals remarkable genetic variation within its range in the Mojave Desert of Nevada. While the accession from Sacaton canyon was the tallest and heaviest, its mass was invested in vegetation, whereas the smaller accessions invested more in inflorescence development and reproduction.

These differences observed at the PMC suggest variable tolerances and resource abundance in the accessions' respective origin locations. The exceptionally harsh climate of the Mojave may allow for particularly distinct divergences in a species within short distances. The observations made on alkali sacaton at the PMC suggest the importance of additional research to determine the value of adapted ecotypes and populations for successful germination and persistence in the landscape. With parent material collected from the peripheries of the area of proposed use (in this case southern Nevada), the progeny germplasm should be well adapted across that area. It may be less desirable to use this material outside that area of origin. We will continue to observe these accessions at the PMC over the next two years for any changes or synchronization in plant growth patterns such as flowering. Following hybridization, it will also be interesting to observe diversity in the progeny, both at the PMC and in field plantings across southern Nevada.

In the coming year, we will continue to observe the alkali sacaton and the alkali muhly fields for time of flowering and other potential differences. We also plan to periodically harvest both fields for maximum production and diversity of seed. We expect the following products from the two population developments:

- Alkali sacaton and alkali muhly seed to plant F1 generation fields at the PMC (spring 2006)
- Alkali sacaton and alkali muhly plugs to establish at least 0.5 ac fields for 2 seed growers in Nevada (fall 2006)
- Hay bales of alkali sacaton from the 2005 growing season (spring 2006)
- Hay bales of both alkali sacaton and alkali muhly from the 2006 growing season (winter 2006)



Alkali muhly growing at Pahranagat NWR, Nevada

This fall we plan to assist two seed growers in southern Nevada establish alkali sacaton and alkali muhly fields. We will grow approximately 7,000 plugs of each species/ac and deliver them to the two seed growers. We will help them design and plant the field, and provide information on production, including watering rates, fertilization rates, fungicides and herbicides, and be available to answer or assist with problems they encounter. The continued observations at the PMC on the population development fields, as well as the progeny fields, should help us iron out any potential problems before they occur in production fields in Nevada.

Appendix A. Map of alkali sacaton and alkali muhly collection sites in southern Nevada

Appendix B. Percent purity and percent fill (100 seed X-ray) used to calculate 10 g of pure seed.

Collection Name	Accession Number	Seed/pound	%Purity	% Fill	Quantity Received (g)	Quantity Used (g)
MOAPA	9092497	1,374,545	92	93	12.26	11.44
MOAPA	9092498	1,680,000	92	73	213.30	13.21
MOAPA	9092499	1,463,225	94	93	69.46	11.26
PAHRANAGAT	9092501	1,417,000	94	78	15.89	12.67
PAHRANAGAT	9092502	2,160,000	91	73	34.50	13.36
PAHRANAGAT	9092508	2,268,000	87	83	2117.00	12.78
ASH MEADOW	9092504	N/A	N/A	N/A	76.29*	11.45
ASH MEADOW	9092505	1,744,600	99	94	368.65	10.70
SACATON CANYON	9092507	2,268,000	87	61	59.02	14.69

Appendix C. Alkali sacaton plot plan in Latin Square design with four accessions and four replications.