THESIS

TRAIT EVALUATION OF SECOND GENERATION LINES OF *DISTICHLIS SPICATA*

Submitted by

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ABSTRACT

TRAIT EVALUATION OF SECOND GENERATION LINES OF *DISTICHLIS SPICATA*

Converting to more drought-tolerant, low-input turfgrass varieties that can help conserve water in the landscape is critical for the future of turf in the arid portions of the western United States. This study attempts to address the growing demand for native, low-input turfgrass by breeding a turf-type variety of inland saltgrass (*Distichlis spicata*) that is well adapted to grow in arid and salty sites while maintaining acceptable quality. Two breeding cycles have been completed for improving the turf quality of inland saltgrass and the current elite lines were selected out of the second-generation nursery to initiate Cycle 3. The goal of this thesis is to evaluate turf-type traits in all second-generation lines. Objectives of this research are three-fold: (1) document, analyze, and report the second-generation nursery of 2,933 saltgrass plots grown at the Horticulture Research Center between 2006-2009, (2) compare improvements of saltgrass through cycles of selections, and (3) maintain and evaluate Cycle 3 crossing blocks for survival, seed yield, and spread.

Seed yield increased through cycles of selection and over half the flowering females in the second-generation elite population showed the ability to produce commercially acceptable levels of seed (448-673 kg/ha). Selecting for short canopy height and greater spread/fill was effective and second-generation lines were unique from the wild types and first-generation breeding populations in both of these traits. Nearly 50% of second-generation elite lines showed no signs of leaf rust (*Puccinia aristidae*) infection in 2008 and roughly 38% showed no signs of leaf shredding and/or browning after mowing. The top 5 elite lines recommended for potential vegetative variety releases from the second-generation were: A37-15x84-6 (M), A37-15xA50-

20-1 (F), 84-8x84-6-1B (F), 84-8x84-6-2A (M) and A37-28xA34-18-4B (M). The variability observed in leaf shredding (relating to mowing quality) suggests that more work needs to be done before a uniform, seeded turf-type may be released; however, there is potential to release improved seed for native revegetation projects owing to the increased seed yield and spread in the second-generation lines. Third generation seed was harvested in 2013 and is available for future progeny evaluations.

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CHAPTER 1: INTRODUCTION

Use of saltgrass in the landscape

Water scarcity is an important issue in Colorado and much of the arid western United States. Finding ways to conserve water in the landscape, such as through reducing landscape irrigation requirements and irrigating with non-potable water sources, are critical to meeting the water supply demands of our growing population. Converting to native and drought tolerant landscape plants is encouraged as a way to help reduce outdoor water use, however, when it comes to our lawns and golf courses options are limited for drought-tolerant turfgrass varieties that maintain acceptable visual quality at low mowing heights. Another increasingly important issue in the arid West is the build-up of salts in the soil having a negative impact on turfgrass growth and plant community structure. This study attempts to address the growing demand for attractive, mowable, drought and salt tolerant turfgrass by developing a turf-type variety of inland saltgrass (*Distichlis spicata*).

Inland saltgrass is indigenous to western North America and Australia where it has adapted to grow in specific niches of wet, alkaline, and saline soils, but is also found on drier and less salty sites. It is not uncommon for saltgrass to dominate saline sites that exclude other plants (Cluff, 1983); saltgrass is classified as a halophyte and has shown to tolerate full strength seawater soil salinity under dry salt playa conditions (Kemp and Cunningham, 1981). Further, many of the selections under observation at Colorado State University remained green under levels of drought stress that induced dormancy in blue grama, buffalograss, and bermudagrass, other known drought tolerant grasses (Hughes, 2002). These stress adaptations inherent in saltgrass contribute to its high potential value for use in the water smart landscape. The planting of saltgrass on fairways and roughs could help golf courses conserve potable water because of its tolerance to lesser quality (reclaimed water, saline ground and surface waters) water resources while maintaining attractive turf and still providing excellent playing conditions (Qian, 2007). On a broader scale, inland saltgrass has value for use in re-vegetation projects and can act as a buffer in areas such as roadsides that commonly have high salinity levels. Plus, it could have an advantage in home lawns as an option for an attractive, drought tolerant turf that doesn't require as much water as a typical cool season lawn to stay green.

Challenges in breeding saltgrass

Saltgrass is a perennial, warm season grass that is dioecious with separate male and female plants, but mainly reproduces vegetatively through vigorous rhizomes in the wild (Fults, 1959). The main challenges faced with reproduction are that native saltgrass stands generally produce little seed, and that seed usually exhibits low germination rates (Cluff, 1983). On the positive side, techniques have been found to enhance germination, and breeding efforts appear to have yielded lines that produce more seed. Development of a seeded variety is important for keeping cost down and because deep rhizome growth makes sod production difficult (Qian, 2006, Harrington, 2000, and Shahba, 2008). Even still, it is possible to establish vegetatively propagated saltgrass varieties through the use of both sprigs and plugs, as shown in CSU research plots and large-scale native restoration projects in California, although these techniques can be more labor and time intensive than direct seeding. Another finding that has not been fully explored is that saltgrass' relatively deep rhizome mass may be moving closer to the surface due to breeding for turf-type traits. This could have implications for sod production in the future; however, this characteristic may also lessen drought tolerance. Nonetheless, both seeded and vegetatively propagated varieties can be successfully established in the field and many elite females have shown to produce commercially adequate levels of seed.

Acceptable visual quality is of major concern for a high quality turfgrass variety, however it is not easy to achieve in a seeded saltgrass line due to saltgrass' plasticity and diverse genome (Vogel and Pederson, 1993). A plant's visual trait expression, or phenotype, is a product of its genotype, environment and genotype by environment interaction, with each of the three factors having varying degrees of influence on final appearance for each species. Phenotypic plasticity in saltgrass means that visual appearance is highly dependent on the environment. In addition, saltgrass is heterozygous for most loci and populations can easily change to adapt to growing conditions (Castler, 2003). Evaluations need to be performed over multiple environments and years to help ensure acceptable visual quality across conditions. Moreover, relating to its diverse genome, when insufficient genetic variation is maintained during sexual reproduction saltgrass will suffer from inbreeding depression, or a reduction in performance associated with an increase in homozygous recessive loci (Nielson, 1956). Maintaining a breeding population of at least 25 individuals is recommended to avoid negative genetic effects and as a result, producing a hybrid saltgrass cultivar is not a viable option. Consequently, breeding is designed to improve the population through increasing the frequency of desired alleles for quantitative turf-type traits while maintaining sufficient genetic variability.

Breeding for saltgrass follows the phenotypic recurrent selection procedure. Male and female plants showing the best trait values are selected from the nursery and duplicated for isolated crossing blocks. Open pollination is allowed among these top lines and the new gene combinations produce a greater proportion of seed with desired trait values compared to the previous population, hence improving mean population values. Seed is collected from mother plants, space planted in the field, then evaluated to determine which parental combinations produce offspring with the best trait values and which lines should be carried on for further cycles of population improvement. These repeated cycles of selection, crossing and evaluation continue until enough progeny appear with satisfactory quality and uniformity (Falkoner, 1996, and Poehlman and Sleper, 1995). As a positive for the saltgrass breeding program, high narrow sense heritability estimates for turf-type traits, such as canopy height and shoot density, indicate that phenotypic recurrent selection is an effective means of improving these traits (Christensen, 2009). On the downside, however, this is a long process since one cycle of selection alone for saltgrass takes at least three years to allow plants to reach adequate sexual maturity for seed production (Heide, 1994).

In order to speed the improvement process, single crosses were also made during breeding cycles to evaluate combining ability of specific pairs of plants and take advantage of potential hybrid vigor. Hybrid vigor is the functional opposite of inbreeding depression, resulting in progeny with superior trait values compared to their parents and it was found in shoot density and rate of spread (Falkoner, 1996 and Christensen, 2009). Making single crosses does entail the risk of uncovering deleterious alleles for other traits of interest; however, all intermittent hybrids were subsequently placed in open pollination crossing blocks as a way to re-diversify the population genome in general and hopefully avoid inbreeding. Full effects of mating closely related saltgrass individuals are still unknown and it is possible that select crosses have resulted in a decrease in performance for some progeny.

Evaluation of saltgrass at Colorado State University

The turf-type saltgrass breeding program was conceived during a collection trip made in 1995. Wild accessions were observed to form low growing, high-density stands with attractive color under high traffic and compacted soil conditions, indicating turf potential. Additional studies in Arizona reaffirmed saltgrass' turf potential and shortly after the breeding program began as a collaborative effort between Colorado State University and the University of Arizona with support provided by the United States Golf Association (Kopec and Marcum, 2001). The program was unique in that it was the first attempt to breed for turf-type traits in the non-domesticated grass. The long-term goal was to achieve golf course quality of a seeded turf-type saltgrass variety. Colorado State University has completed two breeding cycles improving the turf quality of inland saltgrass and turf quality is now high in several improved accessions.

The inland saltgrass source nursery was constructed out of 158 accessions collected from four regions, the Front Range of Colorado, the Great Basin, South Dakota and Nebraska, during 1999-2001. The first two breeding cycles took place from 2001 to 2009 and Cycle 3 began in 2010. Cycle 1 is described below, while background for Cycles 2 and 3 are covered in the introductions for subsequent chapters to go along with the data analysis for those breeding cycles.

Cycle 1:

In 2001, the top 26 turf-type individuals out of the source nursery were selected to cross for Cycle 1 seed (Christensen, 2009). Inspection of the original source nursery warranted that the four traits most in need of improvement for achieving higher turf quality in saltgrass were resistance to rust (*Puccinia aristidae*), short height, shoot density and seed yield. A high incidence of rust greatly detracts from turf health and quality, short height is beneficial for reducing mowing requirements, high shoot density enhances turf appeal, and high seed yield is important for commercial production. Cycle 1 selections were made based on a selection index ranking values for these four primary traits of interest.

The top 26 accessions selected for Cycle 1:

Females: A53, A138, A126, A24, A35, A61, A50, A34, A18, A21, A97, 84, A123, A37

Males: 92, A48, A107, A73, A124, 86, A136, A137, A60, A39, A51, A41

All top lines selected for further improvement originated along the Front Range of Colorado. Front Range accessions showed the most desirable trait values for turf-type traits of interest, plus they were most cold hardy to Colorado winters, making them ideal for a turf-type saltgrass breeding program in Fort Collins, CO. Restricting selection to Front Range accessions also helped ensure matching chromosome numbers, a concern in breeding because cytological work showed evidence that saltgrass lines in the U.S. are delineated by chromosome count, with coastal types having 40 chromosomes and inland types having 38 chromosomes (Reid, 2001). Mismatching chromosome numbers in crosses could result in infertile or no seed production. Therefore, it was decided to maintain a breeding population with 38 chromosomes, originating from along the Front Range of Colorado.

Breeding the top individuals for Cycle 1 involved establishing open pollination crossing blocks, as well as making single crosses. Seed was harvested from mothers in both crossing techniques and transplanted into the first generation nursery in 2003 (this nursery contained a total of about 17,000 genotypes). Clonal material from the 26 parents, as well as 26 random or ‘native’ checks, was also included in the nursery in order to make heritability estimates. Results from the first cycle of selection were published in a dissertation, “Heritability Estimates, Accession Evaluation, and Digital Imaging in *Distichlis spicata*” (Christensen, 2009). Overall, the first breeding cycle resulted in an improved population with average increase in rust resistance of about 20 percent, a decrease in height of 3.3 cm, an increase in shoot density by 0.78 shoots per cm^2, and an increase in seed yield by 30 percent to a hand harvest yield of 194.8 grams per square meter.

Single crosses made in Cycle 1:

A138xA86, A138x72, A138xA107, A126xA60, A126xA51, A126xA48, A126xA107, A126xA49, A126xA86, A126x72, A50xA49, A50xA60, A50xA51, A50xA48, A50xA107, A61xA51, A61xA48, A61xA107, A61xA49, A61xA60, A53xA60, A53xA51, A53xA48, A53xA107, A53xA49

Information was also collected on other turf-type traits of interest. Data was recorded on sex and flowering time to ensure pollen and seed production are in sync when crossing elite varieties. The ratio of seed head height over canopy height was recorded to assist in selecting females with taller seed heads for ease of harvest; however, only source nursery data exists for this trait and it may need to be reconsidered in the future. In addition, saltgrass lines appeared that were slow to spread vegetatively, or otherwise not fill in completely, which is not desirable. Accordingly, data was collected on gap and rate of spread as measures of how completely and quickly accessions fill in after planting. These characteristics are important for establishment and visual appeal of the turf variety. Significance of each recorded characteristic will vary depending on what type of variety release is made. Moreover, the lineage and selection of the saltgrass germplasm makes it particularly well adapted to mountain desert conditions found along the Front Range of Colorado and it can be marketed accordingly.

Improved saltgrass germplasm left remaining for further study and distribution included the elite parents in the Cycle 3 crossing blocks, as well as bulk seed collected from the second-generation nursery. The program has yet to release a variety, and resources are now too limited to perform further cycles of genetic advancement at the CSU Horticulture Research Center. A plan to distribute the improved germplasm is needed: both seeded and vegetative releases will be considered.

Challenges in varietal release

In terms of a variety release, a turf-type saltgrass cultivar can be the progeny of a cycle or progeny from a smaller combination of parents in the case of a seeded line, or a clone for a vegetative variety. If quality of Cycle 3 seed is deemed acceptable following future progeny evaluations, parents from the current crossing blocks may potentially be released as a synthetic (seeded) variety. Golf course quality in a seeded line will likely require more cycles of selection, however, due to the challenges in achieving acceptable visual quality discussed above. A common practice in breeding perennial grasses, such as saltgrass, is to release a clonal variety in the short-term while selection cycles for improved traits and uniformity continue. If an individual accession is identified with acceptable quality it is easier to ensure uniformity through vegetative propagation. A final consideration is releasing a seeded variety for native re-vegetation projects. Visual quality of the current germplasm should be acceptable for this market since uniformity is not a major concern, thus traits of higher importance will be seed yield and origin. In any case, evaluating the remaining improved germplasm and describing its unique turf-type characteristics will assist in preparing for a potential variety release, or distribution for further breeding work. The goal of this study is to provide these analyses so the benefits of turf-type saltgrass may be taken advantage of in the landscape.

Objectives of this study

This study began in Fall 2012. The objectives of the study are to:

1) Document, analyze, and report the second-generation nursery of 2,933 saltgrass plots grown at the Horticulture Research Center between 2006-2009;

2) Compare improvements of saltgrass through cycles of selections;

3) Maintain and evaluate Cycle 3 crossing blocks for survival, seed yield and spread;

CHAPTER 2: TURF-TYPE TRAIT DESCRIPTIONS FROM THE SECOND GENERATION NURSERY

The grass breeding program at CSU, under the direction of Dr. Christensen, has completed 2 cycles for improving the turf-quality of inland saltgrass (*Distichlis spicata*) compared to wild stands.   As discussed in the history of the breeding program in the first Chapter, traits of interest from Cycle 1 were recorded in 2004 and 2005 then analyzed and published in "Heritability Estimates, Accession Evaluation and Digital Imaging, in *Distichlis spicata*" (Cristensen, 2009). Results comparing mean trait values of first-generation accessions and wild types were encouraging, showing promise for improving the turf-quality of saltgrass through continued recurrent selection breeding cycles. Consequently, a second breeding cycle ensued and turf-type traits of interest were recorded from second-generation accessions in the 2008 nursery. These traits were never documented, systemically analyzed, and published however, and the current elite lines were selected out of the second-generation nursery based on overall visual turf-quality without needing to analyze specific trait values. Hence, since it has yet to be done, this chapter will document and report on second-generation nursery data from 2008 as a means to evaluate change in trait values through the most recent breeding cycle and identify uniqueness of turf-type traits in elite saltgrass lines.

Cycle 2 selections and crossing:

The second breeding cycle began in 2005. In similarity to Cycle 1, a selection index ranking the 4 primary traits of interest (rust resistance, canopy height, shoot density and seed yield) was used to determine top individuals in the first-generation nursery. For Cycle 2, however, additional top lines were also selected based on turf appearance under mowed, drought, and saline conditions to incorporate desirable phenotypes under these conditions into the improved gene pool. Mowing quality is a concern for visual quality and ball playability in the new turf variety, while drought and salt tolerance are the important physiological traits that drew us to this grass for use as a turf in the arid west in the first place. The additional top lines for Cycle 2 were identified visually from field conditions, without using a selection index, to speed the process and because maintaining an overall acceptable appearance under stress is what is most important for the improved turf variety. In total, 35 high quality turf-type individuals (the 21 females and 14 males listed below) were chosen out of the first-generation nursery to advance the breeding population. When interpreting the names, the number after the dash indicates which number progeny it was from the mother listed before the dash. Single crosses were made among these lines in order to produce Cycle 2 seed, thus all second-generation lines are the result of hybrid crosses.

The top 35 accessions selected for Cycle 2:

Females: A53-14, A53-3, A53xA48-11, A37-28, 84-19, A126-27, 84-2, A126-46, A50-9, A50-4, A61-4, A37-25, A37-15, A126xA107-6, 84-5, 84-8, A50-19, A138-31, A50xA107-13, A138-16, A50-1

Males: 84-6, 84-12, 84-21, A126-5, A24-32, A35-6, A137-25, A34-18, A61-32, A126xA49-19, A53-5, A138-29, A50-7, A50-20

Second-generation traits of interest:

For clarity in discussion, generations in terms of breeding cycles are defined as follows: Cycle 0 includes the native ecotypes that were collected from the wild from 1999-2001 (or wild types) and established in the source nursery. In Cycle 1 top quality wild types were selected and crossed to produce first-generation improved progeny, which were then space planted in the first-generation nursery for trait analysis in 2004 and 2005. Wild types were also included in the first-generation nursery for calculating heritability estimates, thus Cycle 1 data from 2004 and 2005 includes wild type and first-generation trait values evaluated under similar growing condition. Cycle 2 consisted of selecting and crossing the top quality first-generation lines, establishing their progeny in the second-generation nursery and recording second-generation turf-type traits in 2008. Finally, the elite saltgrass lines described are the breeding population selected out of the second-generation nursery to commence Cycle 3.

Each second-generation turf-type trait will compare the elite lines selected for Cycle 3 to the rest of the second-generation nursery. Then when previous data is available, trait values for second-generation lines will be compared to the first-generation and wild types. Finally, any significant differences in trait values will be evaluated among elite lines.

Turf-type traits recorded from the second-generation nursery in 2008 include: sex, flowering date, number of seed heads, canopy height, percent spread, leaf rust and leaf shredding. Shoot density and gap were not measured during Cycle 2 due to time and resource constraints; as a way to compensate, however, visual estimates of percent spread in this cycle accounted for not only area of coverage, but overall fill and density of turf cover. Leaf shredding was a new trait added in Cycle 2 as a measure of mowing quality.

Materials and Methods:

Seeds for the 2nd generation nursery were germinated in the greenhouse in 2005, and in summer 2006, nearly 3,000 saltgrass plugs of 10 x 10 cm were randomly transplanted into the field. This total number of plots accounts for roughly 1,500 different saltgrass accessions in the nursery since two replicates of each accession were included. In some instances, however, there were multiple clones next to each other labeled with the same name, resulting in more than two clones for an accession. Two elite lines had more than 2 clones: 84-2xA126-5-2 (F) had 4 clones and A126-27xA35-6-3B (M) had 5 clones. In other cases, there were accessions where only 1 clone was planted in the nursery. Three elite females had 1 clone: 84-19xA50-7-11, 84-2xA126-49-19 and 84-2xA35-6-3A. All clones (replications) of elite lines were included in the analysis to account for variation in trait expression across environments. Variability is unknown for the three accessions with 1 clone.

The second-generation nursery was located at the CSU Horticulture Research Center (HRC) in Fort Collins, CO. The field site received an average 18 cm of rain per year, from 1999 to 2006. The minimum winter temperature is -32 degrees C and the frost-free growing season lasts from May 20 to September 20. The soil is a uniform, deep Nunn clay loam (Aridic Argiustolls). Well water for irrigation has salinity levels approximately 2-3 dS/m and soil salinity was between 3-5 dS/m. Nursery management was minimal, including irrigating plugs with an overhead linear irrigation system after planting to assist in establishment, after which irrigation was only applied once yearly before flowering in mid-May. Soil nitrogen tested at 180 kg per hectare and no additional fertilizer was applied (Christensen, 2009).

Turf-type traits of all second-generation saltgrass nursery accessions were recorded throughout the 2008-growing season. Sex was determined along with date of first flower.

Number of seed heads per plant was approximated in multiples of 5. On July 23 and 24, un-mowed canopy height was measured with a yardstick. Spread was measured by imagining a 1.5 x 1.5 meter frame, and accounting for turfgrass ground cover and gap to estimate percent fill of the ‘box’; measurements were taken on August 29, accounting for growth since establishment in 2006. Leaf rust was rated throughout October on a scale of 0,1,5,10,15,20 or 25; scale values represent percent leaf area affected by uredia and telia, where 0= no rust, 1= minimum rust present and 25= maximum rust present. Leaf rust (*Puccinia aristidae*) is naturally present on site therefore no artificial inoculation was used. Throughout the summer, half of each plot was mowed to a height of 7.5 cm when 12 cm height was reached. Leaf shredding was measured after mowing on October 15 and 16 on a scale of 1 to 3, where 1= no shredding, 2= 1.3 cm shredding, and 3= 2.5+ cm shredding/browning of grass blades.

SAS version 9.3 was used to perform the statistical analysis. Review of each turf-type trait provides general descriptive statistics comparing the elite lines selected for Cycle 3 (n=56 total surviving plots including all replications) to the rest of the second-generation nursery (n=2933 total surviving plots including all replications). The PROC UNIVARIATE procedure was used to generate means and standard deviations and create histograms for comparing elite and general second-generation populations. The PROC MIXED procedure was used to test significant differences in population least squares means at a 0.05 level; however, the large difference in population sizes made significant differences hard to detect.

Contrasts of mean trait values are then made to the first-generation and wild types to evaluate turf-type saltgrass improvement over time. Previous data on first-generation and wild type accessions was taken from “Heritability Estimates, Accession Evaluation and Digital Imaging, in *Distichlis spicata*” (Cristensen, 2009).

Finally, LS means estimates for individual accessions within the elite population are generated and compared with the MIXED procedure. Owing to the large number of comparisons made among accessions, Tukey-Kramer adjustments were applied to control maximum experiment-wise error rate. Confidence intervals (95%) were included in the results to assist with interpreting LS means estimates.

Results and Discussion:

Sex:

Sex was recorded to assist in selecting females and males for future breeding cycles. The second-generation nursery contained 2,933 total inland saltgrass plots after accounting for death since establishment. Nursery population proportions broken down by sex are 50.2% female (1472 total plots), 45.2% male (1326 total plots) and 4.6% unknown (didn't flower). Previously over 7 years, 22 accessions (14%) never formed head spikes; not all males and females will flower and when these reproductive organs are missing it is hard to identify the sex of the plant. Moreover, in the second-generation nursery there were 5 flowering accessions marked as both male and female, further showing how complicated identification may be. It should be noted that Eppley et al. (1998) found that gender in saltgrass is genetically determined and individual accessions will remain male or female.

The 14 females and 13 males still alive in the current Cycle 3 crossing blocks, discussed in more detail in the next chapter, are the elite accessions being described out of the second-generation nursery. After accounting for death since establishment there are 56 total plots among all replications of elite lines. In total, there are 27 female (48.2%) and 29 male (51.8%) elite plots.

Days to first flower:

First flowering date was recorded to assist in synchronizing seed and pollen production for crossing. Average first flowering dates for the general nursery population fell in the range of May 23 to July 27, 2008, while the range in dates for the elite lines lasted from May 23 until June 21, 2008. First flowering date was converted to number of days until first flower by counting consecutive days from January 1, 2008 until the flower date recorded in order to provide a numerical value for statistical analysis.

As shown in Figure 2.1, the general nursery population has a similar distribution in number of days to first flower as the elite lines. Rounded to the nearest whole number, mean number of days to first flower in 2008 was 159 for the general population versus 158 for elite lines and both populations had a standard deviation of 7 days. The similarity in distributions is not surprising since first flower dates were not considered in the breeding selection criteria. Hence, selection pressure was not directly applied for this trait and first flower dates remained relatively random in the population. Correlation to another trait under selection such as seed yield is possible however and could be the reason why elite lines fall in the range of earlier flowering times (i.e. relating to higher seed production). Nonetheless, the means of second-generation populations remain nearly equal due to the much smaller population size of elite lines. In addition to genetics, environmental effects on floral development also play a role in determining first flower timing and they could contribute to the distinct peaks (around 160 days) observed in all second-generation accessions.

The environmental effect on floral development complicates comparisons of first flower dates to past generations. Different climates experienced across years will result in changes in mean number of days to first flower due to the temperature, light and water effect on reproductive development, which is beyond the scope of this chapter. Nonetheless, past trait values are presented here for reference. Evaluation of mean number of days to first flower in the original saltgrass source nursery (Cycle 0) in 2000 showed Front Range accessions had 147 mean days, with no significant difference in accessions collected from Nebraska or the Great Basin (Christensen, 2009). This trait was not recorded in Cycle 1 due to lack of time so no other prior data exists for comparison. Results from Front Range accessions in Cycles 0 and 2 do appear different (147 vs. 159 mean days); however, the increase in mean number of days to first flower in 2008 compared to 2000 most likely indicates a colder spring in 2008.

More informatively, individuals in the second-generation elite population were further compared amongst each other since they were all measured in 2008 under similar environmental conditions. Differences of least squares means with Tukey-Kramer adjustments showed 3 groups in first flower timing between elite lines (see Table 2.4). Two accessions 84-2xA126-49-19 (F) and 84-2xA50-7-1A (M) stood out for fewest days until first flower since they only belonged to group C with the smallest mean, and they were significantly different from accession A61-4x84-21-1 (M) with most days until first flower in group A. These 3 accessions with means furthest apart, however, were not significantly different from other accessions in the elite population and the remaining elite accessions were similarly grouped. Comparisons of elite accessions by replication showed variability of up to 2 weeks difference in observed first flower timing, to first flowering between replications occurring on the same day. The 2-week variability between replications is similar to the deviation around the estimated mean, and a likely reason for the overall similarity in groupings.

In terms of potentially correlating first flower date to seed production, data on male first flower dates is of less use since the origin of the male pollen contribution is unknown in most crosses. Looking at female data, 84-2xA126-49-19 in Group C was the first to flower in 2008 and the best seed producer in 2013 (discussed in Chapter 3). Again, comparisons of results are hard to make across years but it is possible that the early first flowering time for female 84-2xA126-49-19 correlates to higher seed yield through a longer overall flowering period and increased chance of receiving pollen. If this correlation can be confirmed, females with fewer days to first flower can be preferentially selected for as another means to improve seed yield.

Additionally, the exact range in number of days to first flower allowing successful pollination has yet to be studied, thus values are presented here for reference for future breeding efforts. Certain males and females at the extremes of the range might be preferentially crossing without our knowledge. The link between first flower timing, seed and pollen production, and population diversity could be the topic of future study.

Number of seed heads:

Number of seed heads was recorded to provide an idea of seed and pollen production, since measuring seed yield directly is very time consuming. It was measured by approximating the total number of heads per accession (each whole plot) in multiples of 5. Data on number of seed heads presented below was already multiplied by 5 to represent an estimation of total number of seed heads per accession.

Distribution in number of seed heads between the second-generation general and elite populations was not significantly different (see Figure 2.2). The general population produced an average 120 seed heads per accession, while the elite lines (B) produced an average 122 seed heads per accession. The lack of difference is likely due to the small sample size of the elite population and the large peak in distribution observed around 120 seed heads for all accessions.

In fact, over 70% of accessions in both populations produced roughly 120 seed heads per accession in 2008.

Despite the significant mode of 120 seed heads per accession, when examining the extreme accessions, the observed range was large for both populations: 25 to 625 seed heads per accession for the general nursery and 25 to 300 seed heads per accession in elite lines. These ranges indicate that higher seed yielding types remain in the general population over what was selected for in the elite lines and potential remains to improve seed yield through further breeding and selection of high yielding types. On the other hand, because the elite lines were selected based on overall visual turf-quality it is not surprising that the very high seed yielding types were not included; production of too many seed heads may detract from visual turf-quality. Thus, lower seed yielding types might be preferable for a vegetative variety release where seed production is not of concern. In consequence, maintaining large ranges (extreme observations) in the populations is beneficial for accommodating both ends of the breeding spectrum.

Mean seed yield can also be compared from past generations. Results from Cycle 1, evaluated in 2005 and 2006, showed an increase in seed yield from wild types to their first-generation progeny, in addition to a very significant year effect on seed yield. LS means estimates presented only include accessions with greater than 0 kg/ha seed yield (see Table 2.1 below). In 2005, mean seed yield for first-generation accessions was 513 kg/ha, with a standard deviation of 32 kg/ha, whereas their wild type parents had an estimated yield of 373 kg/ha and standard deviation of 62 kg/ha. The first-generation progeny outperformed their wild type parents again in 2006, plus overall seed yield greatly increased in both populations. In 2006, the first-generation accessions had a mean 1966 kg/ha seed yield and 45 kg/ha standard deviation, compared to their parental wild types with a mean 1603 kg/ha yield and 128 kg/ha standard deviation. The large difference in yield between years is likely due to the sexual maturity level of the perennial saltgrass plants since not all plants had time to reach maturity by 2005, in addition to possible environmental effects on seed production.

Table 2.1: Cycle 1 mean seed yield (kg/ha)

Population	Year	Estimate	St. Error
Wild-type parents	2005	373	62
1st generation	2005	513	32
Wild-type parents	2006	1603	128
1st generation	2006	1966	45

The primary interest in measuring seed yield is to answer whether or not improved turf-type saltgrass accessions produce commercially acceptable levels of seed. Seed production in the range of 400 to 600 pounds per acre is generally considered acceptable, equivalent to roughly 448 to 673 kilograms per hectare. The high seed yield observed in 2006 among all accessions surpasses commercially acceptable production levels. In 2005, no wild types achieved acceptable seed production levels however several first-generation accessions did. First-generation accessions producing at least 448 kg/ha of seed in 2005 include: A97, A24, A37, A138, A61, 84, A126, A50, A35 and A21. Thus, it was concluded selecting for high seed yield in wild types improved seed production in first-generation progeny. Furthermore, the ability to produce commercially levels of seed, as witnessed in 2006, should pass on to the second-generation and future generations. As of yet, it is hard to make direct comparisons to the 2008 measurements because more data is needed to link average number of seed heads to seed yield. Once this done, however, a more accurate idea of change in seed yield throughout the breeding generations can be gathered from the 2008 data presented here.

Differences among elite lines were also evaluated by generating LS means estimates for seed head production in 2008 (see Table 2.5). The large span in estimated means (25-300) shows high variability among elite accessions in number of seed heads. After applying Tukey-Kramer adjustments, 3 different groups appeared (A, B and C); however, the majority of accessions (21 out of 27) belonged to all 3 groups with no significant difference between them. Two males, A37-28xA34-18-4B and A61-4R7-1B, stood out at the high end of the groupings with male A37-28xA34-18-4B producing significantly more seed heads than any other accession. Lineage from the high performing males can be incorporated in future populations for increasing pollen, and thus seed production. In addition, large 95% confidence intervals for accessions indicate that a lot of variability was present between replications, and a likely reason for the overall similarity in significant groupings. Two males and two females stood out at the low end of the groupings (in groups BC and C). Nonetheless, accessions with low LS means estimates for number of heads produced should not be ruled out as potentially adequate seed producers due to environmental and sexual maturity effects on seed yield. It is recommended this trait be recorded again over multiple years in order to better explain the variability.

Canopy height:

The second-generation nursery had a 16.5 cm mean natural canopy height in 2008, with a standard deviation of 4.1 cm. In contrast, the elite lines were 4.2 cm shorter: elites had a 12.3 cm mean canopy height and 3.0 cm standard deviation. Figure 2.3 depicts how the proportion in height varied throughout both second-generation populations in 2008. The general nursery ranged in height from 3.8 to 35.6 cm, while elite lines ranged in height from 6.4 to 21.6 cm. It can be seen in the Figure that elite lines were selected from the lower end of the general nursery height distribution. This was done to apply selection pressure on low canopy height as a turf-type trait and hopefully lower the mean height of the third generation. Low canopy height is beneficial in a maintained turfgrass variety for reducing mowing requirements.

In order to evaluate change over time, mean canopy height estimates were compared to past generations. Cycle 1 results varied by year based on precipitation, thus results are presented by year (see Table 2.2). Precipitation received at the field site (from March 1 until August 23) was 18.6 cm in 2004 versus 23.7 cm in 2005, and the greater precipitation in 2005 resulted in taller canopy heights across populations. Even still, a similar relationship among the populations existed in both years. In 2004 and 2005, parental wild types and their first-generation progeny had significantly shorter mean canopy heights than the general wild-type population, as a response to selection. An unexpected result appeared however when comparing the wild type parents to their first-generation progeny. Short height was being selected for in the parents yet in both years progeny were taller than their parents. Thus, hybrid vigor was observed in canopy height but in this case it is not beneficial since low canopy height is preferred. Dr. Christensen posited that future gene recombination could moderate hybrid vigor owing to the desired response in trait change from all wild types to first-generation progeny. A comparison to Cycle 2 is made below to see what effect hybrid crosses had on canopy height in the second-generation.

Table 2.2: Cycle 1 results for canopy height (in cm)

Population	**Year**	**Estimate**	**St. Error**
All wild types	2004	20.6	0.4
Parental wild types	2004	16.8	0.3
1st. generation	2004	18.1	0.1
All wild types	2005	23.1	0.4
Parental wild types	2005	19.5	0.3
1st. generation	2005	20.0	0.1

When comparing Cycle 2 results to Cycle 1, the 16.5 cm mean height of the second-generation nursery in 2008 is shorter than any previous breeding population. Precipitation must also be considered for a more reliable comparison since the environment can play a role in trait expression. A nearby CoAgMet weather station at AERC measured 23.8 cm of precipitation from March 1 to September 1, 2008, which is nearly equal to 2005 levels (The ARDEC station is not referenced due to incomplete data in 2008). The second-generation height, at 16.5 cm, is significantly shorter (by at least 3 cm) than all wild types and first generation progeny measured in 2005 under similar levels of moisture. These results indicate that further gene recombination has proven effective in lowering mean saltgrass canopy height in the second-generation. Moreover, the selection pressure for low height in the elite lines could decrease mean canopy height of the third generation compared to the second generation. Genetic gain between generations is also related to the amount of variability present for selection however, and variability of height within populations also appears to be decreasing over time. Trait improvement will be less distinct as there is less variability for selection and populations become more uniform.

Differences in height between the elite lines were also examined more closely. Untransformed results were provided above for ease of interpretation, but height was log transformed to correct unequal variances for further statistical analysis between elite lines. After making Tukey-Kramer adjustments, no significant differences appeared in log height between elite accessions (see Table 2.6). This lack of difference is probably due to the fact there were only 2 replications per elite accession; the large confidence intervals show the variation between replications was similar to that between accession means. Certain patches in the nursery, for instance, could receive different amounts of precipitation, resulting in varying canopy heights among the 2 replications and a lack of significant difference among the elite population. As a result, log height of elite clones in 2008 may be described by the estimated mean of 2.48 cm. When back transformed this value roughly equals 12.0 cm, with a 95% confidence interval of 11.2 to 12.8 cm that coincides with our unadjusted mean estimate of 12.3 cm. In summation, the naturally low canopy height of all elite lines in 2008 is a unique characteristic distinguishing them from wild types and first-generation accessions in a potential variety release. Due to the plasticity of canopy height as discussed in Chapter 1, future trials testing potential vegetative varieties should be performed across multiple environments. Then the most desirable individual under all conditions can be selected and a better estimate of canopy height can be provided.

Spread:

Spread is accounting for how much ground area a turfgrass accession covers, in addition to how well it fills in without gaps. More rapid spread and complete fill assist in establishment, weed control and overall turf appeal. As shown in Figure 2.4, the distributions for spread in both populations are similar. The second-generation nursery population and elite lines both had a 75% mean spread in 2008, with standard deviations of 11.6% and 11.4%, respectively. Measurements were taken in a 1.5 by 1.5 meter frame and accounted for 2 years growth (since establishment in 2006). Therefore, mean 75% spread equates to an approximate coverage of 1.74 square meters per accession over 2 years. The similar distribution of spread in elite lines and the general population can be contributed to the limited variability of the trait in the general population and the small sample size of the elite population. Variability of the trait is discussed more below, in reference to past generations for comparison.

Spread estimates were compared to previous generations in order to evaluate change in trait expression over time, although different measurement techniques were used in Cycle 1. In 2004, digital imaging analysis was used to measure percent spread in order to minimize possible human error when calculating heritability estimates. In addition, measurements were based on percent cover of a 1.8 by 1.8 meter plot after only one year's growth. Results showed a significant increase in spread from wild type parents to their first-generation progeny (see Table 2.3 below). First-generation accessions had a mean 18.0% spread versus their parental wild types with a 10.9% spread. When comparing accessions within a generation, however, little difference in spread was observed. The general wild type population was similar to the parental wild type population (selected for Cycle 1 crossing), with an 11.8 mean percent spread compared to 10.9%. Thus, the limited variability in spread among accessions within the same generation is found throughout the saltgrass breeding cycles. Even still, as a positive for the breeding program the mean trait value does appear to be improving over time.

Table 2.3: Cycle 1 results for percent spread (in 1.8x1.8 m plot, over 1 year's growth)

Population	**Year**	**Estimate (%)**	**St. Error**
All wild types	2004	11.8	0.7
Parental wild types	2004	10.9	0.6
1st. generation	2004	18.0	0.4

The different measurement techniques used in 2004 versus 2008 make direct comparisons difficult, however, a general idea of improvement is provided. Percent spread estimates from 2004 equate to an area of coverage of roughly 0.35 square meters for parental wild types versus 0.58 square meters observed in the first-generation. Cycle 1 measurements were all taken after one year's growth since establishment, under similar growing conditions.

Thus, it can be concluded that the first-generation had 1.7 times greater spread than their wild type parents in 2004 after 1 year of growth. Comparisons to Cycle 2 are more difficult because measurements in 2008 accounted for two year's growth since establishment. The environment can influence trait expression, plus the change in growth rate over time is unknown, therefore it is hard to extrapolate differences in growth in one versus two years. As mentioned previously, the second-generation accessions covered roughly 1.74 square meters in 2008 after two years growth, which is more than twice the growth observed in first-generation accessions in 2004 after 1 years growth. Future studies including multiple generations in the same nursery will help compare change in spread over time with more confidence.

Nonetheless, significant increase in spread in first-generation accessions beyond what was observed in their parental wild types indicates heterosis in this trait. Heterosis, or hybrid vigor, in spread means that by making hybrid (single) crosses between accessions, progeny with even greater spread will appear. All Cycle 2 seed was the result of hybrid crosses, thus it is likely second-generation accessions do have increased spread over the first-generation. What is more, spread can continue to improve in future generations beyond what is currently observed as a result of breeding. Too rapid of a spread may become undesirable if the grass gains a tendency to invade, therefore rapid spread should also be evaluated for its potential negative consequences and an upper bound on rate of spread may be determined.

The elite lines were compared more closely to examine any uniqueness among the existing vegetative germplasm. After applying Tukey-Kramer adjustments, no significant difference appeared in mean percent spread among elite accessions (see Table 2.7). This is not surprising considering the limited number of replications within the elite population and overall low variation within second-generation accessions. Even still, spread in all second-generation accessions can be considered unique from wild types in a potential variety release. Furthermore, when considering a potential vegetative variety release from the current elite population, the lack of significant difference in spread means selecting top accessions visually based on cover and fill might be preferred.

Leaf rust:

Upon evaluation of the inland saltgrass source nursery from 1999-2001, it became apparent that presence of the leaf rust *Puccinia aristidae* detracts from the health and quality of certain saltgrass accessions. Therefore, resistance to leaf rust was measured during Cycle 1 as an important trait for breeding a turf-type saltgrass variety. *Puccinia aristidae* is naturally found on field site and it was prevalent again in 2004 allowing for field evaluations to be made. Comparing resistant versus susceptible accessions from the wild type parent population and the first-generation progeny showed a dominant gene is controlling resistance (Christensen, 2009). Thus, it was determined if homozygous dominant accessions are identified they can be crossed into the desired population to confer resistance. As an additional consideration however there are over a hundred different known races of leaf rust and specific resistance must consider the local race present plus its potential to evolve. Not to mention, breeding for general resistance takes a long time. Consequently, even though detecting the dominant gene is beyond the scope of this project, visual leaf rust ratings from 2008 are presented here in order to give an idea of resistance in the second-generation nursery and provide reference for potential future work.

Leaf rust was rated in 2008 on a scale of 0, or 1 to 25 in increments of 5, with the rating representing percent cover of telia and uridea on the grass blades. Refer to Figure 2.5 for a visual representation of rust rating scores 1 to 25. Generally, a score of 20 or 25 will be detrimental to the grass plant, as depicted by the two farthest right grass blades in the Figure.

Population proportions of rust rating in the second-generation nursery (n=2882) versus the elite population (n=56) are shown in Figure 2.6. The majority of accessions had minimal signs of infection and there were individuals in both populations displaying zero signs of infection. These results are promising for breeding for rust resistance in saltgrass, although as mentioned general resistance to the rust remains unstudied and it is possible a less virulent race of *Puccinia aristidae* was present in 2008. On the other end of the spectrum, 2.4% of the entire nursery population and 3.6% of elite lines displayed high rates of infection with a rating of 20 or 25, indicating that homozygous recessive individuals are likely present. Accessions also fell in the middle ratings hence a range of rust resistance exists in the second-generation population due to the out crossing nature of the species.

Ratings from the elite lines were examined more closely since they are the parents for the third-generation and therefore the contributors of resistance genes to future breeding populations. As a result of selection the majority of elite lines show minimal signs of infection, however, there were two plots in the elite population that got a rating of 20. It turns out they were from the same male accession A126-27xA35-6-3B (see Table 2.8). This accession had 5 clones in the nursery and since the other 3 clones with the same name have minimal infection rates, it is possible there was a labeling error. LS means are not given due to non-normality of data, thus ratings are provided for each plot. Plots with a rating of 0, indicating no sign of infection to the leaf rust race present in 2008, are good candidates to test for finding homozygous dominant genes. What is more, crossing the elite lines should increase the proportion of accessions with dominant resistance genes in the third-generation.

Leaf shredding:

Leaf shredding was measured to evaluate mowing quality. Mowing quality in turn relates to visual quality and ball playability, and thus is an important characteristic for a maintained turfgrass variety. Since leaf shredding was rated in three categories, population proportions for the categories are presented rather than histograms (see Figure 2.7). Proportions for the general nursery population are out of 2853 total plots, while proportions for the elite population are out of 55 total plots. A rating of 1 represents no shredding and is the most desired result. A rating of 1 was assigned to 12.2% of the second-generation nursery, compared to 38.2% of the elites. A significantly greater proportion of elites do not show signs of leaf shredding after mowing because the elite lines were selected out of the second-generation nursery based on their high visual quality. The majority of accessions in both populations got a rating of 2, representing 1.3 cm leaf shredding and/or browning. This category included 79.5% of second-generation nursery accessions and 56.4% of elites. A rating of 3 represents 2.5+ cm of leaf shredding and/or browning, which would significantly detract from visual quality and hence is undesirable. Luckily, these proportions remained small; 8.3% of the second-generation nursery and 5.5% of elite parents got a rating of 3. The different shred ratings observed among second-generation accessions indicates that variability is present for selection and most accessions do shred/brown after mowing, although not much more than 1.3 cm.

As mentioned, leaf shredding was not recorded in previous saltgrass breeding cycles therefore data is not available for comparison. Furthermore, since heritability estimates for mowing quality are unknown, it is hard to predict how selecting for the trait will influence population proportions in future generations. Nonetheless, due to the presence of many elite accessions with good mowing quality, a vegetative variety may still be considered for releasing a high-quality turf type saltgrass cultivar. Leaf shred scores for all elite parents are listed in Table 2.9; LS means were not calculated due to non-normal data. Seven elite lines had a shred rating of 1 in all replications, indicating good mowing quality (females 84-19xA50-7-11, 84-5x84-6-5, 84-8x84-6-1B and A37-15xA50-20-1, and males 84-8x84-6-2A, A37-15x84-6 and A37-28xA34-18-4B).

Conclusion:

The second-generation turf-type inland saltgrass traits are documented and reported on in this Chapter in order to evaluate breeding progress and provide reference for potential future study and/or a variety release. Turf-type traits evaluated in 2008 included: sex, flowering date, number of seed heads, canopy height, percent spread, leaf rust and leaf shredding.

The sex ratio of the second-generation nursery was roughly 50:50 and 4.6% of accessions did not flower in 2008. The range in days to first flower was 1 month shorter for the elites than the general population; however, mean days to first flower were similar for both populations peaking around 160 days. Number of seed heads was counted in 2008 as a measure of seed production and over 70% of all second-generation accessions produced on average 120-122 seeds heads per plot. In 2008, the mean canopy height of elite lines (12.3 cm) was significantly shorter than the general second-generation nursery population (16.5 cm) as a result of selection. Spread of saltgrass accessions is another trait that has been improving throughout breeding cycles, yet it remains fairly uniform within accessions from the same generation. All second-generation accessions covered an area roughly equal to 1.74 square meters after 2 years growth since establishment. Nearly 50% of second-generation elite lines showed zero signs of rust infection in 2008. Additionally, 38.2% of elite plots had a leaf shred rating of 1, which signifies no leaf shredding or browning after mowing.

Although more work needs to be done to improve uniformity before a seeded turf-type variety of inland saltgrass can be released, the most desirable elite lines from the second-generation may be tested for a potential vegetative variety release. Considering all the traits, the 5 most promising elite accessions for a vegetative variety release are (starting with the top 3): A37-15x84-6 (M), A37-15xA50-20-1 (F) and 84-8x84-6-1B (F) because they had the most desirable ratings for rust resistance and mowing quality, plus they were on the low end of the spectrum for canopy height. The other 2 males with good mowing quality, 84-8x84-6-2A and A37-28xA34-18-4B, may also be considered for future variety trials since they had decent rust ratings and high values for spread/density, plus releasing a male is preferred to protect the rights of the improved germplasm. These selections did not include seed yield because that is not an important trait for a vegetative variety; however, all elite lines are further examined in the Cycle 3 crossing blocks and their progeny (third-generation accessions) are now available for continued analysis of the progress toward a uniform seeded turf-type saltgrass variety.

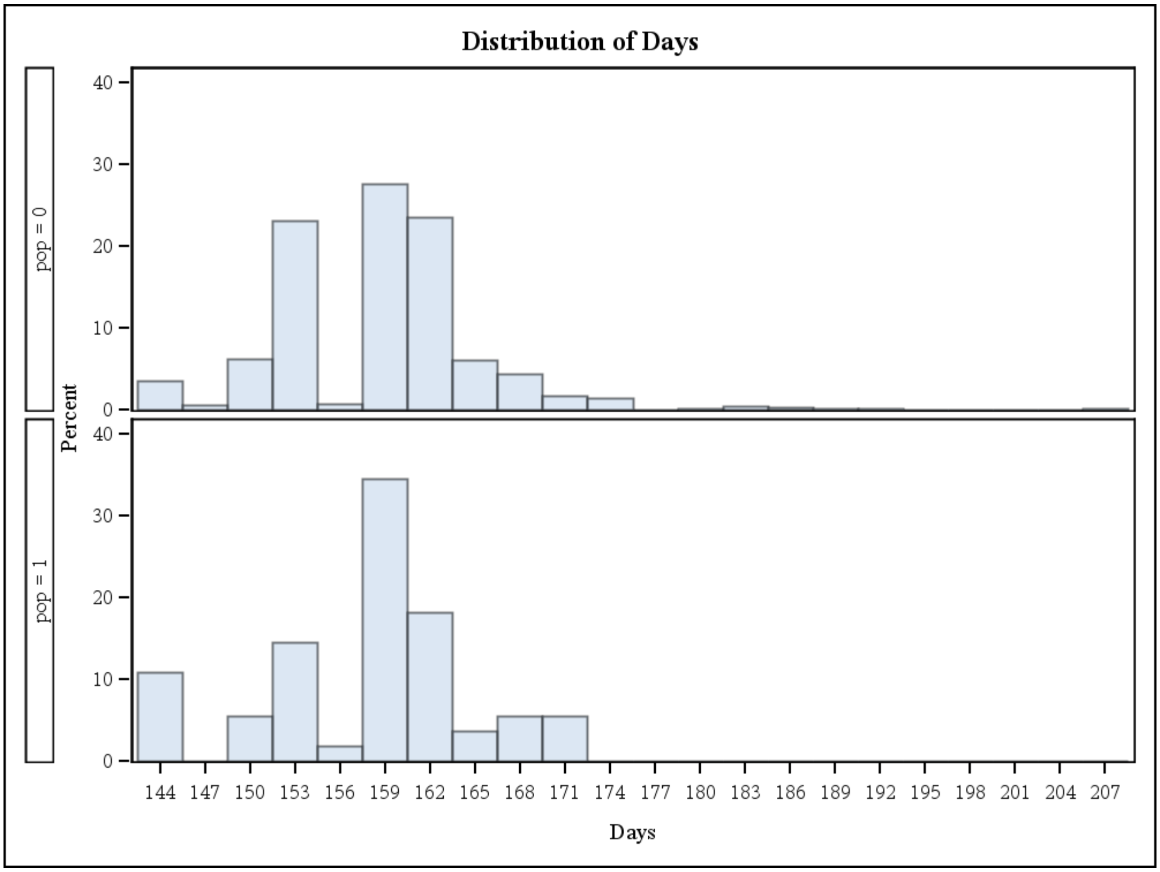

Figure 2.1: Distribution of number of days to first flower for second-generation populations. Pop=0 (above) includes all flowering plots in second-generation nursery minus elite lines (n=2727). Pop=1 (below) includes flowering elite plots (elite lines selected out of 2nd gen. nursery) (n=55).

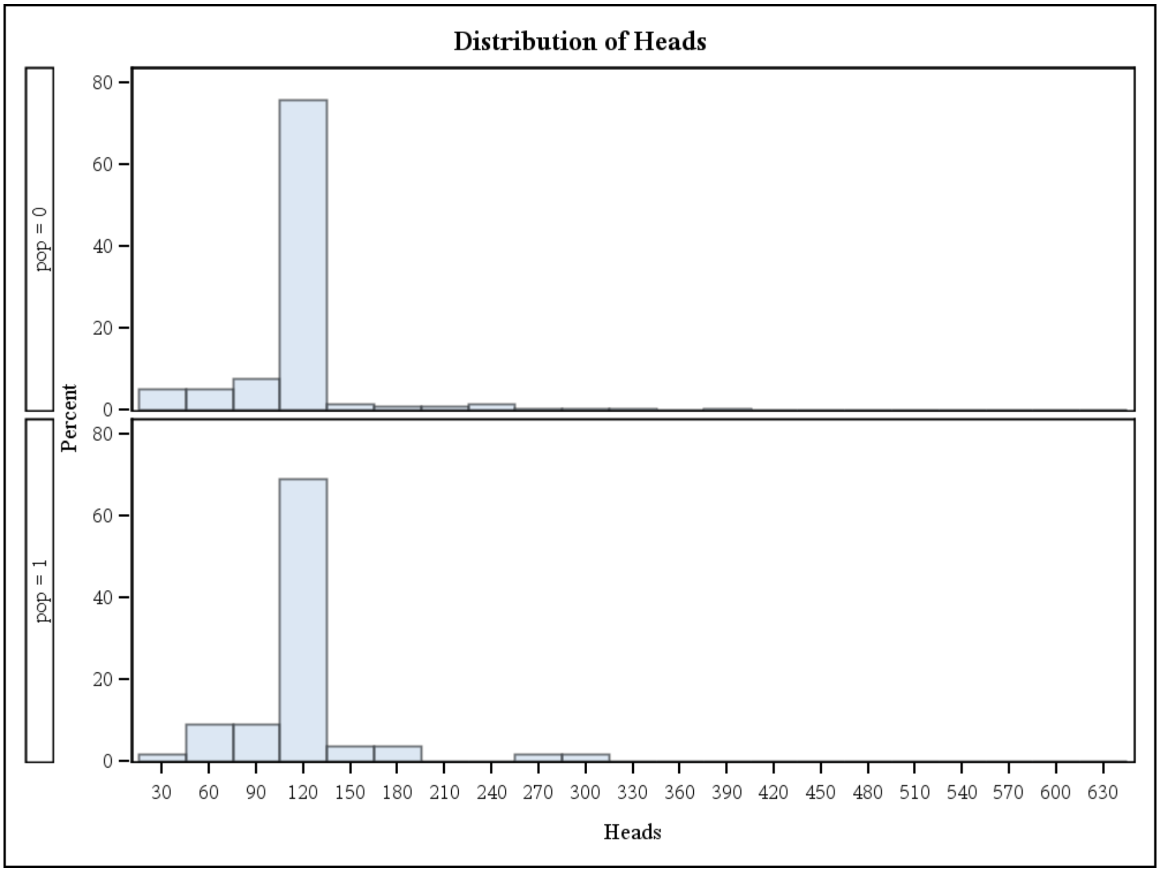

Figure 2.2: Distribution of number of seed heads per plot for second-generation populations. Pop=0 (above) includes all plots in second-generation nursery minus elite lines (n=2838). Pop=1 (below) includes elite plots (elite lines selected out of 2nd gen. nursery) (n=55).

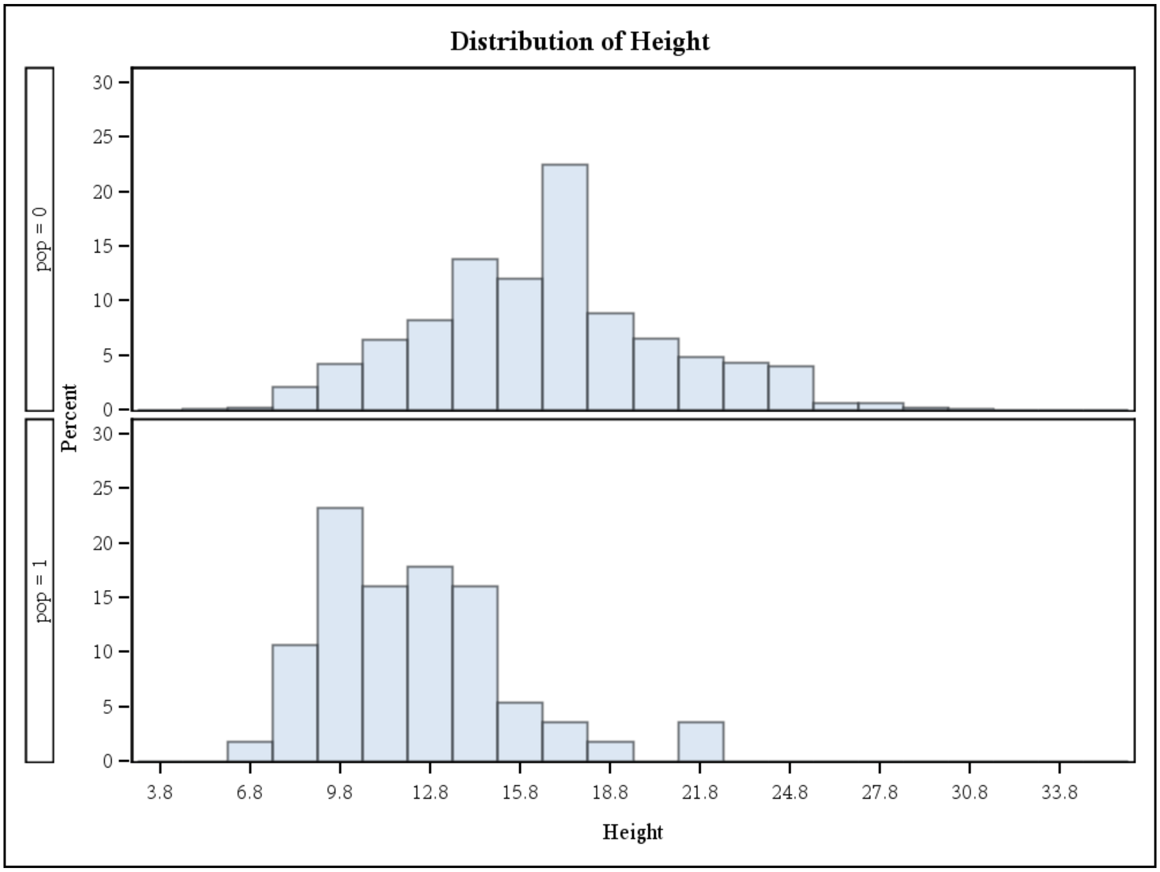

Figure 2.3: Distribution of height (in cm) for second-generation populations. Pop=0 (above) includes all plots in second-generation nursery minus elite lines (n=2933). Pop=1 (below) includes elite plots (elite lines selected out of 2nd gen. nursery) (n=56).

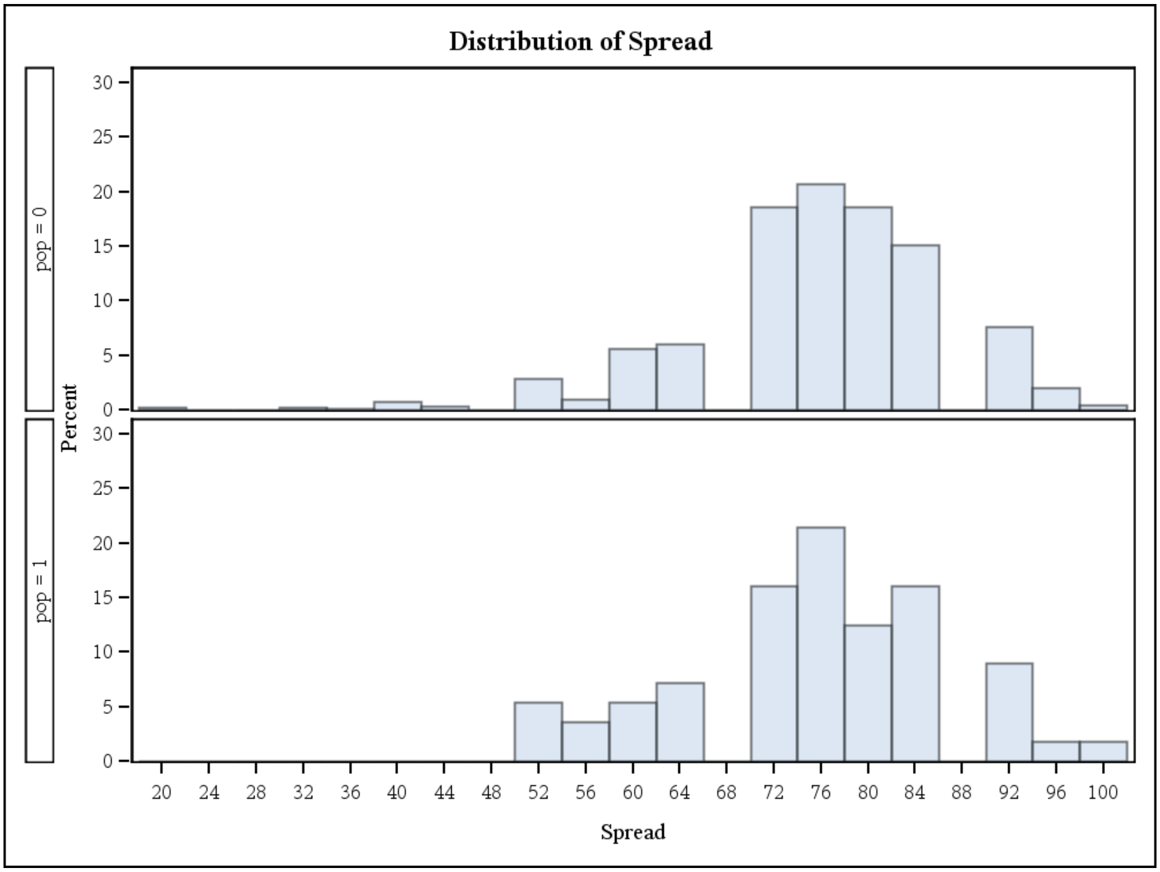

Figure 2.4: Distribution of percent spread for second-generation accessions in 1.5x1.5m frame. Pop=0 (above) includes all plots in second-generation nursery minus elite lines (n=2882). Pop=1 (below) includes elite plots (elite lines selected out of 2nd gen. nursery) (n=56).

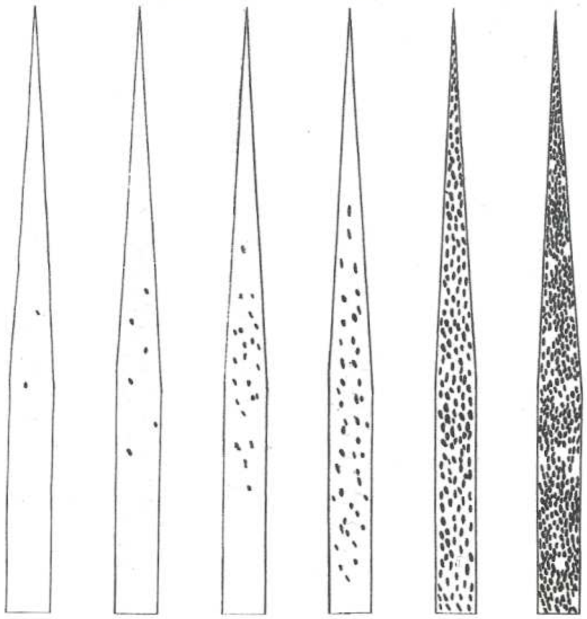

Figure 2.5: Drawing showing the disease classes as defined by percent leaf area affected by uredia and telia on *Distichlis spicata* var. *stricta*. The causal organism is *Puccinia aristidae*. Adapted from the key “Leaf Rust of Cereals’ by James (1971).

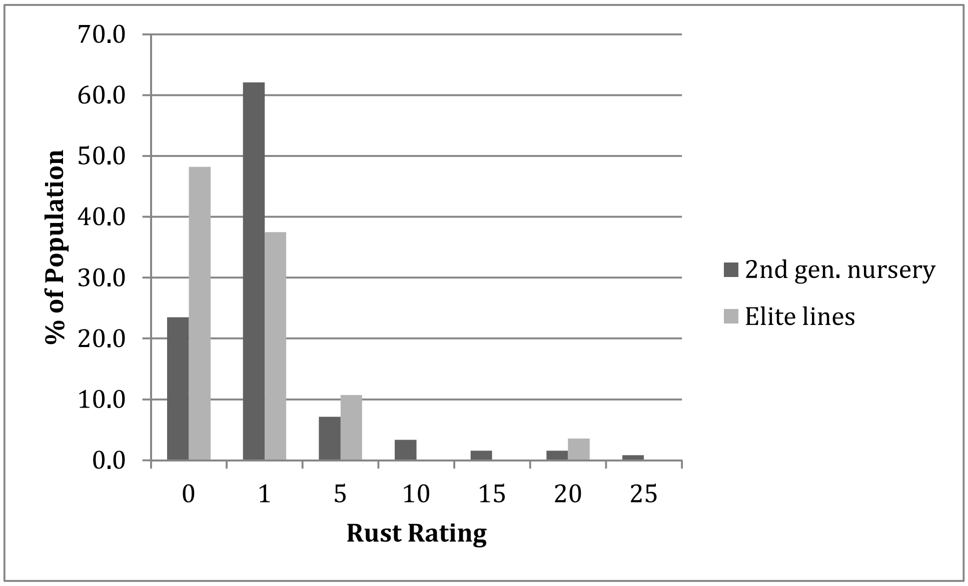

Figure 2.6: Leaf rust rating for infection by *Puccinia aristidae.* Rated on a scale of 0,1,5,10,15,20 or 25; scale values represent percent leaf area affected by uredia and telia, where 0= no rust, 1= minimum rust present and 25= maximum rust present.

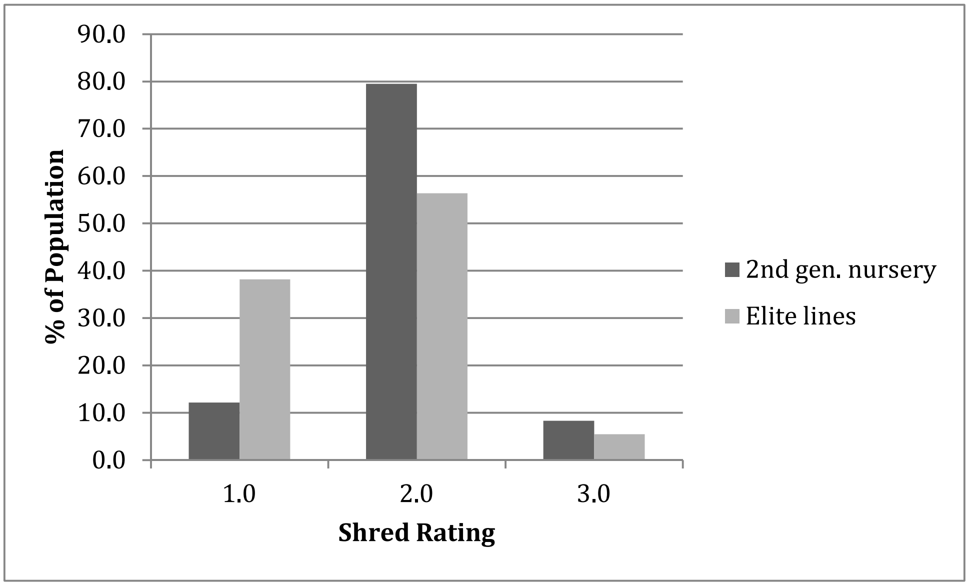

Figure 2.7: Leaf shred rating after mowing. Measured on a scale of 1 to 3, where 1= no shredding, 2= 1.3 cm shredding, and 3= 2.5+ cm shredding/browning of grass blades.

Table 2.4 LS means days to first flower for second-generation elite accessions with Tukey-Kramer adjustments (α=0.05)

Accession	Sex	Mean	95% Confidence Limits		Group
84-2xA126-49-19	Female	145	135.0	155.0	C
84-2xA50-7-1A	Male	145	135.0	155.0	C
A37-28xA34-18-3B	Female	149	141.9	156.1	BC
84-19xA35-6-13	Male	149	141.9	156.1	BC
84-2xA35-6-3A	Female	150	140.0	160.0	ABC
84-5xA35-6-3A	Female	151.5	144.4	158.6	ABC
A37-28xA34-18-4B	Male	151.5	144.4	158.6	ABC
A37-28x?-7A	Male	152.5	145.4	159.6	ABC
A61-4R7-1B	Male	154.5	147.4	161.6	ABC
A126-27xA50-7-11	Male	156	148.9	163.1	ABC
84-5x84-6-5	Female	156	148.9	163.1	ABC
84-19xA126-5-1	Male	158.5	151.4	165.6	ABC
84-2xA126-5-2	Female	159	154.0	164.0	ABC
A126-27xA35-6-3B	Male	159.2	154.7	163.7	ABC
A37-15x84-6	Male	159.5	152.4	166.6	ABC
A37-25xA126-5-2A	Female	160	152.9	167.1	ABC
84-8x84-6-1B	Female	160	152.9	167.1	ABC
A37-25xA126-5-5	Female	160.5	153.4	167.6	ABC
84-8x84-6-2A	Male	160.5	153.4	167.6	ABC
A50-4R9-3A	Male	160.5	153.4	167.6	ABC
84-2xA61-32-3	Female	160.5	153.4	167.6	ABC
A126-27x?-4	Male	161	151.0	171.0	ABC
84-19xA50-7-11	Female	161	153.9	168.1	ABC
A37-15xA50-20-1	Female	163	155.9	170.1	ABC
A50-4R8-2	Female	164.5	157.4	171.6	ABC
A37-15xA50-20-7	Female	168.5	161.4	175.6	AB
A61-4x84-21-1	Male	171	163.9	178.1	A

A,B,C grouping indicates significant differences among LS-means (accessions with the same letter are not significantly different).

Table 2.5 LS means estimates for number of seed heads per second-generation elite accession, with Tukey-Kramer adjustments (α=0.05)

Name	Sex	# Heads LSMEAN	95% Confidence Limits		Group
A37-28xA34-18-4B	Male	300	223	377	A
A61-4R7-1B	Male	263	185	340	AB
A50-4R9-3A	Male	200	123	277	ABC
84-5xA35-6-3A	Female	200	123	277	ABC
A37-15x84-6	Male	150	73	227	ABC
84-19xA126-5-1	Male	150	73	227	ABC
84-2xA126-49-19	Female	150	41	259	ABC
84-19xA35-6-13	Male	138	60	215	ABC
84-2xA50-7-1A	Male	125	16	234	ABC
84-2xA61-32-3	Female	125	48	202	ABC
A37-25xA126-5-2A	Female	125	48	202	ABC
A37-25xA126-5-5	Female	125	48	202	ABC
A50-4R8-2	Female	125	48	202	ABC
84-2xA126-5-2	Female	119	64	173	ABC
84-5x84-6-5	Female	113	35	190	ABC
A126-27xA50-7-11	Male	100	23	177	ABC
A37-15xA50-20-1	Female	100	23	177	ABC
A126-27x?-4	Male	88	10	165	ABC
84-8x84-6-1B	Female	88	10	165	ABC
A37-15xA50-20-7	Female	88	10	165	ABC
A37-28xA34-18-3B	Female	88	10	165	ABC
84-8x84-6-2A	Male	75	-2	152	ABC
A126-27xA35-6-3B	Male	75	26	124	ABC
A37-28x?-7A	Male	63	-15	140	BC
84-19xA50-7-11	Female	50	-59	159	BC
84-2xA35-6-3A	Female	50	-59	159	BC
A61-4x84-21-1	Male	25	-52	102	C

A,B,C grouping indicates significant differences among LS-means (accessions with the same letter are not significantly different).

Table 2.6 LS means log canopy height of second-generation elite accessions, with Tukey-Kramer adjustments (α=0.05)

Accession	Sex	LogHeight LSMean	95% Confidence Limits	
84-8x84-6-1B	Female	2.0	1.7	2.3
A37-28x?-7A	Male	2.1	1.8	2.4
A126-27x?-4	Male	2.3	2.0	2.5
84-2xA50-7-1A	Male	2.3	2.0	2.5
84-19xA50-7-11	Female	2.3	1.9	2.7
84-2xA35-6-3A	Female	2.3	1.9	2.7
84-5x84-6-5	Female	2.3	2.0	2.6
A37-15x84-6	Male	2.4	2.1	2.6
84-5xA35-6-3A	Female	2.4	2.1	2.7
84-19xA126-5-1	Male	2.4	2.1	2.7
84-19xA35-6-13	Male	2.5	2.2	2.8
A37-15xA50-20-1	Female	2.5	2.2	2.8
A61-4x84-21-1	Male	2.5	2.2	2.8
A61-4R7-1B	Male	2.5	2.2	2.8
A37-25xA126-5-5	Female	2.5	2.2	2.8
A50-4R8-2	Female	2.5	2.2	2.8
84-8x84-6-2A	Male	2.5	2.2	2.8
84-2xA61-32-3	Female	2.5	2.2	2.8
A126-27xA50-7-11	Male	2.5	2.3	2.8
A37-15xA50-20-7	Female	2.5	2.3	2.8
A37-25xA126-5-2A	Female	2.5	2.3	2.8
84-2xA126-49-19	Female	2.5	2.1	2.9
A37-28xA34-18-3B	Female	2.6	2.3	2.9
A50-4R9-3A	Male	2.6	2.4	2.9
84-2xA126-5-2	Female	2.6	2.4	2.8
A37-28xA34-18-4B	Male	2.7	2.4	3.0
A126-27xA35-6-3B	Male	2.8	2.6	3.0

All accessions were in 1 significant group

Table 2.7 LS means % spread of second-generation elite accessions in 1.5x1.5m frame, with Tukey-Kramer adjustments (α=0.05)

Accession	Sex	% Spread LSMEAN	95% Confidence Limits	
A37-28xA34-18-4B	Male	87.5	73.3	101.7
84-5x84-6-5	Female	87.5	73.3	101.7
A37-15xA50-20-7	Female	87.5	73.3	101.7
A37-25xA126-5-2A	Female	85	70.8	99.2
84-8x84-6-2A	Male	82.5	68.3	96.7
A37-15xA50-20-1	Female	82.5	68.3	96.7
A37-28xA34-18-3B	Female	82.5	68.3	96.7
A61-4x84-21-1	Male	80	65.8	94.2
84-2xA61-32-3	Female	80	65.8	94.2
A37-15x84-6	Male	77.5	63.3	91.7
A50-4R9-3A	Male	77.5	63.3	91.7
A126-27x?-4	Male	77.5	63.3	91.7
A37-25xA126-5-5	Female	77.5	63.3	91.7
A126-27xA35-6-3B	Male	77	68.0	86.0
84-19xA35-6-13	Male	75	60.8	89.2
84-2xA126-49-19	Female	75	54.9	95.1
84-5xA35-6-3A	Female	75	60.8	89.2
A50-4R8-2	Female	75	60.8	89.2
A126-27xA50-7-11	Male	72.5	58.3	86.7
84-19xA126-5-1	Male	70	55.8	84.2
84-19xA50-7-11	Female	70	49.9	90.1
84-2xA35-6-3A	Female	70	49.9	90.1
A37-28x?-7A	Male	67.5	53.3	81.7
84-2xA126-5-2	Female	65	54.9	75.1
A61-4R7-1B	Male	62.5	48.3	76.7
84-2xA50-7-1A	Male	55	40.8	69.2
84-8x84-6-1B	Female	52.5	38.3	66.7

All accessions were in 1 significant group

Table 2.8 Leaf rust rating of second-generation elite plots

Accession	Sex	Rust Rating	Accession	Sex	Rust Rating
84-19xA126-5-1	Male	0	A126-27xA35-6-3B	Male	20
84-19xA126-5-1	Male	0	A126-27xA35-6-3B	Male	20
84-19xA35-6-13	Male	0	A126-27xA50-7-11	Male	1
84-19xA35-6-13	Male	0	A126-27xA50-7-11	Male	5
84-19xA50-7-11	Female	1	A37-15x84-6	Male	0
84-2xA126-49-19	Female	1	A37-15x84-6	Male	0
84-2xA126-5-2	Female	0	A37-15xA50-20-1	Female	0
84-2xA126-5-2	Female	0	A37-15xA50-20-1	Female	0
84-2xA126-5-2	Female	0	A37-15xA50-20-7	Female	0
84-2xA126-5-2	Female	1	A37-15xA50-20-7	Female	5
84-2xA35-6-3A	Female	0	A37-25xA126-5-2A	Female	0
84-2xA50-7-1A	.	1	A37-25xA126-5-2A	Female	0
84-2xA50-7-1A	Male	1	A37-25xA126-5-5	Female	0
84-2xA61-32-3	Female	1	A37-25xA126-5-5	Female	1
84-2xA61-32-3	Female	1	A37-28x?-7A	Male	0
84-5x84-6-5	Female	1	A37-28x?-7A	Male	1
84-5x84-6-5	Female	1	A37-28xA34-18-3B	Female	0
84-5xA35-6-3A	Female	0	A37-28xA34-18-3B	Female	1
84-5xA35-6-3A	Female	1	A37-28xA34-18-4B	Male	0
84-8x84-6-1B	Female	0	A37-28xA34-18-4B	Male	1
84-8x84-6-1B	Female	0	A50-4R8-2	Female	1
84-8x84-6-2A	Male	0	A50-4R8-2	Female	1
84-8x84-6-2A	Male	1	A50-4R9-3A	Male	0
A126-27x?-4	Male	5	A50-4R9-3A	Male	0
A126-27x?-4	Male	5	A61-4R7-1B	Male	1
A126-27xA35-6-3B	Male	1	A61-4R7-1B	Male	5
A126-27xA35-6-3B	Male	1	A61-4x84-21-1	Male	0
A126-27xA35-6-3B	Male	5	A61-4x84-21-1	Male	0

Ratings are given for all 56 elite clones, ordered by accession name

Table 2.9 Leaf shred rating of second-generation elite plots

Accession	Sex	Shred Rating	Accession	Sex	Shred Rating
84-19xA126-5-1	Male	1	A126-27xA35-6-3B	Male	2
84-19xA126-5-1	Male	2	A126-27xA35-6-3B	Male	2
84-19xA35-6-13	Male	1	A126-27xA50-7-11	Male	1
84-19xA35-6-13	Male	2	A126-27xA50-7-11	Male	2
84-19xA50-7-11	Female	1	A37-15x84-6	Male	1
84-2xA126-49-19	Female	2	A37-15x84-6	Male	1
84-2xA126-5-2	Female	1	A37-15xA50-20-1	Female	1
84-2xA126-5-2	Female	2	A37-15xA50-20-1	Female	1
84-2xA126-5-2	Female	2	A37-15xA50-20-7	Female	2
84-2xA126-5-2	Female	2	A37-15xA50-20-7	Female	2
84-2xA35-6-3A	Female	2	A37-25xA126-5-2A	Female	2
84-2xA50-7-1A	Male	2	A37-25xA126-5-2A	Female	3
84-2xA50-7-1A	Male	3	A37-25xA126-5-5	Female	1
84-2xA61-32-3	Female	2	A37-25xA126-5-5	Female	3
84-2xA61-32-3	Female	2	A37-28x?-7A	Male	1
84-5x84-6-5	Female	1	A37-28x?-7A	Male	2
84-5x84-6-5	Female	1	A37-28xA34-18-3B	Female	2
84-5xA35-6-3A	Female	2	A37-28xA34-18-3B	Female	2
84-5xA35-6-3A	Female	2	A37-28xA34-18-4B	Male	1
84-8x84-6-1B	Female	1	A37-28xA34-18-4B	Male	1
84-8x84-6-1B	Female	1	A50-4R8-2	Female	2
84-8x84-6-2A	Male	1	A50-4R8-2	Female	2
84-8x84-6-2A	Male	1	A50-4R9-3A	Male	2
A126-27x?-4	Male	2	A50-4R9-3A	Male	2
A126-27x?-4	Male	2	A61-4R7-1B	Male	1
A126-27xA35-6-3B	Male	1	A61-4R7-1B	Male	2
A126-27xA35-6-3B	Male	1	A61-4x84-21-1	Male	2
A126-27xA35-6-3B	Male	2	A61-4x84-21-1	Male	2

Ratings are given for all 56 elite clones, ordered by accession name

CHAPTER 3: EVALUATING THE CURRENT CYCLE 3 CROSSING BLOCKS

Cycle 3 began in 2009 by visually selecting the top 31 individuals (18 females and 13 males) out of the second-generation nursery based on verdure. After two breeding cycles, desired trait values were appearing in greater abundance, thus it was decided a visual determination of which types overall looked the best as a turfgrass was sufficient for Cycle 3 selection. Names of the elite individuals are listed below. When interpreting the names, the mother's name is listed before the "x" and the father is listed after (named from his mother). For instance with female 84-2xA126-49-19, the mother is 84-2 and the father is A126-49-19 from female A126. The number after the dash indicates which number progeny it was in the space planting.

The top 31 saltgrass accessions selected for Cycle 3:

Females: 84-2xA126-49-19, 84-2xA35-6, 84-2xA61-32-3, 84-2xA126-5-2, 84-2xA50-7-1A, 84-5xA35-6-3A, 84-5x84-6-5, 84-8x84-6-1B, 84-8x84-6-3, A61-4R3-6, A37-15xA50-20-1, A37-15xA50-20-7, A37-25xA35-6-2, A37-25xA126-5-5, A37-25xA126-5-2, A37-28xA34-18-3B, A50-4R8-2, A53-14xA124-32-6

Males: 84-8x84-6-2A, 84-19xA35-6-13, 84-19xA50-7-11, 84-19xA126-5-1, A37-15x84-6, A37-28xA34-18-4B, A37-28x?-7A, A50-4R9-3A, A61-4x84-21-1, A61-4R7-1B, A126-27xA50-7-11, A126-27xA35-6-3B, A126-27x?-4

The 31 elite saltgrass accessions selected out of the second-generation nursery represent the best turf-type individuals produced to date. They were cloned and planted in open pollination crossing blocks in 2010 in order to produce third generation (Cycle 3) seed, and then the saltgrass breeding program was put on hold until 2012. In 2012 when this project was initiated, not all the elite clones were still alive in the crossing blocks and those that did remain were small in size. Therefore, objectives of this chapter are to record the survival and spread of the remaining elite individuals as well as to report on seed production. A small amount of third generation seed was produced and harvested in 2012, however any yield was too insignificant to report. On the plus side, by 2013 clones filled in and seed production was more substantial. Seed was harvested, weighed and analyzed for yield in 2013, then stored to preserve third generation progeny. Additionally, vegetative material from the best representative of each surviving accession is currently being duplicated in the greenhouse as a backup source of elite germplasm and to have material available for companies interested in a potential variety release.

Materials and methods:

On June 20, 2010 the second-generation elite turf-type saltgrass accessions were planted in 4 replicated crossing blocks at the CSU Horticulture Research Center in Fort Collins, CO. Two rows of 8 females (16 total) were surrounded by two outer rows of 13 total males in the first and fourth replications, while a total of 17 females and 12 males were used in the second and third replications. Field conditions were similar to the 2nd generation nursery described in Chapter 2. The Cycle 3 crossing blocks received minimal maintenance between 2010 and 2012. Plants were mowed once yearly in spring to remove dead growth and control weeds. Weeds were hand pulled for the rest of the growing season in 2012 and 2013 after the first mowing.

Seed yield was determined from hand harvest on a 30.5 cm square from August 29 to September 1, 2013. Seed heads were placed in a paper bag, air dried at ambient temperature and weighed for seed head weight. Seed heads were then hand threshed and weighed to get seed yield for each mother clone. Spread was determined by estimating percent grass cover in a 46 x 46 cm square frame on September 12, 2013. Clones larger than the frame were marked as 100% without being measured further. Clones with less than 5% fill of the frame were marked as not surviving, while all clones filling over 5% of the frame were recorded as surviving.

Microsoft Excel was used to construct data tables and calculate percent survival rates. SAS 9.3 was used to perform additional statistical analysis. Seed yield by mother was compared with PROC MIXED and a fitted regression line for seed head weight and seed yield was formed using PROC REG. Spread of the clones was analyzed by making a histogram with PROC UNIVARIATE.

Results and Discussion:

Survival:

As of September 2013, 27 elite accessions (14 females and 13 males) remain in at least one replication in the Cycle 3 crossing blocks. The 4 females 84-8x84-6-3, A37-25xA35-6-2, A53-14xA124-32-6, and A61-4R3-6 no longer exist in any replication, whereas there remains at least one clone of every male. Among all replications there were a total of 58 surviving clones out of 116 originally planted, for an overall survival rate of 50%. Roughly 44% of the female clones survived (29 out of 66), while roughly 58% of the males survived (29 out of 50). Coincidently, both females and males ended with an equal number of 29 surviving clones, since they started with different population sizes. Table 3.1 shows which female and male clones survived in which replications. Rep 1 had 6 females and 8 males remaining, rep 2 had 7 females and 7 males remaining, rep 3 had 7 females and 6 males remaining, and finally rep 4 had the most survivors with 9 females and 8 males.

The mortality observed in elite clones between 2010 and 2012 is thought to be due to lack of irrigation. The crossing blocks are located along the fence at the edge of the Research Center. An overhead irrigation system is set up to water the adjacent corn crop and only the last sprinkler head on the line reaches the crossing block site. Normally saltgrass would be tolerant of dry conditions; however, the crossing block clones were never able to fully establish and grow deep roots to access alternate water sources. As mentioned above, many clones were small in size in 2012 even though they were planted two years earlier. Adding to the lack of irrigation were record-breaking heat waves. The summer of 2012 was particularly hot with several days reaching over 37 degrees Celsius. The soil in the crossing block replications was so dry it was visibly cracking open. These conditions may have led to death of clones before they could establish. Significantly more males survived than females, which could be the subject of further study: whether males are better able to establish/survive under drought conditions.

Seed yield:

Sorting by mother shows that 9 different elite female accessions produced seed in 2013. 5 out of 14 females never produced seed heads in the Cycle 3 crossing blocks (38%). Table 3.2 shows all seed yielding female clones. Different females were assigned numbers to assist in graphing and analysis since their names are so long.

The primary research question is whether or not any of the females were able to produce commercially acceptable levels of seed (equivalent to roughly 448 to 673 kilograms per hectare)? Seed production in four different females, numbers 2,3,4 and 6, was within these levels, producing around 450 to 500 kg/ha. Female number 5 yielded even more seed at roughly 740 kg/ha, while number 2 had the highest yielding clone at nearly 1,045 kg/ha. Numbers 2 and 3 were the only two females that produced seed in all four replications. In summation, as can be seen in Figure 3.1, females numbered 2 through 6 all reached or surpassed commercially acceptable levels of seed production, for a total of 5 different females with adequate seed production. Considering the fact that the crossing blocks were only planted three years earlier and they were under visible drought stress, 5 out of 9 females reaching commercial seed production levels shows promise for commercial production of a seeded saltgrass cultivar.

One-way ANOVA, comparing difference in mean seed yield by mother, gave a non-significant p-value (p=0.4453), so we cannot conclude that means are different between females. Moreover, the sample size is small and uneven between replications. These complications, in addition to the uneven environmental conditions, led to the decision to not perform any further statistical analysis comparing mean seed yield by mother.

Pollen is also an important component of seed production and should be considered in the analysis. As mentioned in Chapter 1, any of the pollen-producing males in the crossing blocks can potentially be fathers due to the unknown distance pollen will travel. Therefore, the exact location of the males in relation to females is not a major concern. There were 11 different males (see list below) that flowered in 2013. Since there were 13 surviving males total, roughly 85% flowered in 2013. The 2 males that did not produce pollen were A37-15x84-6 and A61-4x84-21-1. Maintaining a breeding population of at least 25 individuals is recommended to avoid the negative effects of inbreeding. The 11 males and 9 females producing pollen and seed respectively, gives a total of 20 individuals, falling short of the recommended requirement. Inbreeding effects should be considered in future progeny analysis.

Males with pollen (in at least one replication):

84-2xA126 84-2xA50-7-1A, 84-8x84-6-2A, 84-19xA35-6-13, 84-19xA126-5-1, A37-28xA34-18-4B, A37-28x?-7A, A50-4R9-3A, A61-4R7-1B, A126-27xA50-7-11, A126-27xA35-6-3B, A126-27x?-4

Flowering females and males were also sorted by replication to evaluate field variability (see Table 3.3). With the females, replication 4 had noticeably higher number of flowering females than the others; 8 compared to 3 and 4 found in reps 1-3. Males overall performed better in total number of pollinators, however, replication 3 was noticeably low. Replications 1,2 and 4 each had 7 pollinating male clones, while replication 3 only had 4 pollinating males.

There is a possibility that replication 4 had more favorable growing conditions for the females. The highest seed-yielding female, however, was from replication 1 on the opposite end of the crossing blocks. One commonality is that both replications 1 and 4 were on the edges of the crossing blocks and thus bordered by other vegetation (mostly weeds). Soil samples were not taken, so it is unknown exactly what variability existed throughout the plots and only conjectures can be made. As discussed previously, based on visual evaluations, the variability in survival and subsequent seed production is most likely due to lack of water and dry soil conditions during establishment. It is possible that due to water stress not all female clones were well enough established to reach maturity for adequate seed production. Therefore, conclusions about which mothers did not reach commercial levels should be made with caution.

Seed head weight by seed yield:

On a different note, inland saltgrass produces small seed that is tedious to clean after harvest. Thus, seed heads were weighed before hand threshing in order to answer the question of whether seed head weight is a good predictor for estimating seed yield. Results showed 90% of the variability in seed yield is explained by the linear regression on seed-head weight, making seed-head weight a good predictor of seed yield. Predicted average yield increases by 0.28 grams for every 1-gram increase in average seed head weight, as shown in Figure 3.2. Due to the high correlation, this relationship can be used to predict seed yield from seed head weight without threshing the seed. Future studies might confirm this relationship however owing to the relatively small sample size of seed yielding accessions (n=30).

Spread:

Spread was recorded to give an idea of the size of the surviving crossing block clones. The distribution of spread can be seen in Figure 3.3; percent cover is on the x-axis. Out of the 58 total male and female clones recorded, the median percent cover was 70%. Seventy percent spread equates to coverage of a 0.15 square meter area. The largest quarter of the population covered 100% of the frame or more, while the smallest quarter of the population covered 25% of the frame or less.

Conclusion:

In conclusion, the variation observed in environmental conditions throughout the crossing block replications and the uneven numbers of surviving clones makes current visual analysis of traits less reliable, which is the reason why more traits were not characterized in this section.

Thus, observations of the Cycle 3 elite lines taken from the second-generation nursery are a more reliable source for trait descriptions. The elite second-generation population is being propagated vegetatively in the greenhouse and is available for further study and/or a variety release.

Females 84-2xA126-49-19 and 84-5x84-6-5 show the most promise for improving the seed yield of inland saltgrass and should be utilized in future crosses. Third generation seed saved from the crossing blocks may be used for conducting potential future progeny evaluations.

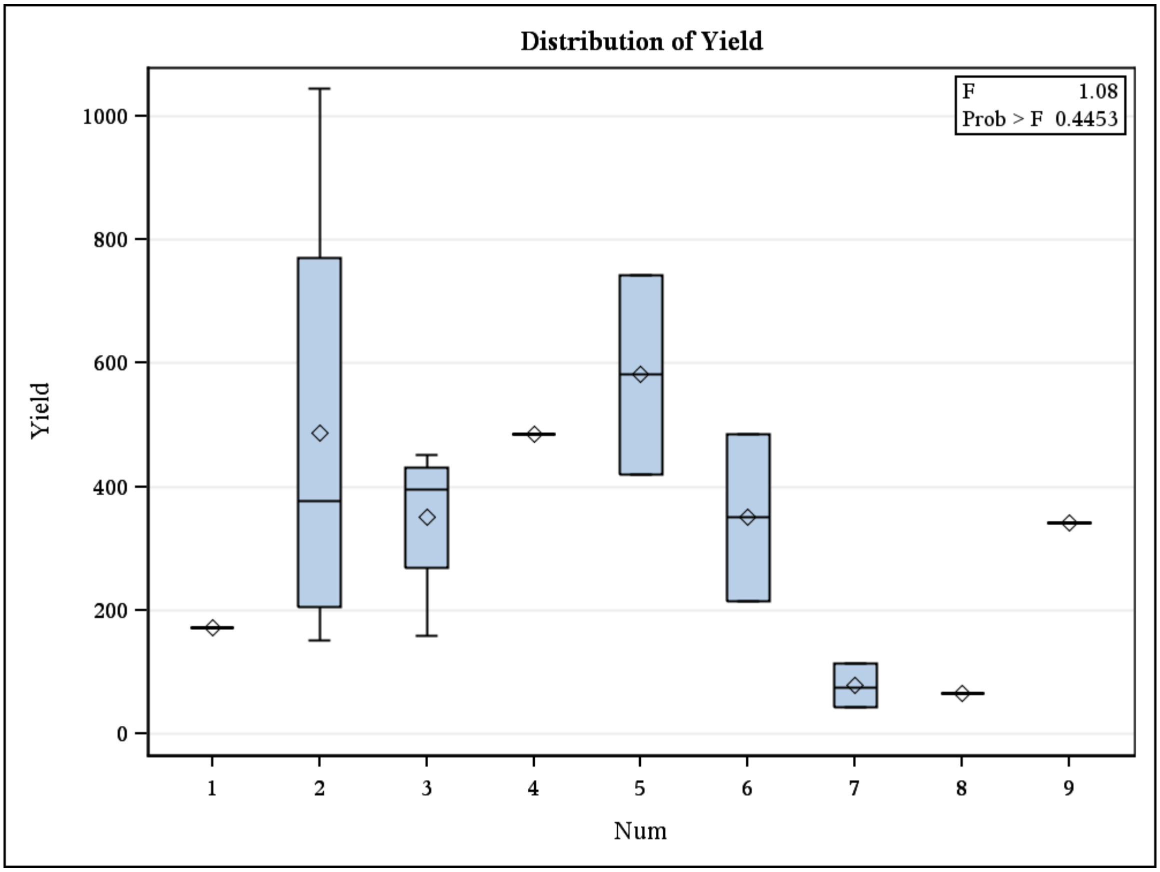

Figure 3.1: Seed yield (kg/ha) of flowering females in Cycle 3 crossing blocks. Num on x-axis corresponds to female names listed below and includes all clones producing seed for that female (ranging from 1-4 clones/female).

Name	Num
84-19xA50-7-11	1
84-2xA126-49-19	2
84-2xA126-5-2	3
84-2xA35-6	4
84-5x84-6-5	5
84-5xA35-6-3A	6
84-8x84-6-1B	7
A37-15xA50-20-7	8
A37-28xA34-18-3B	9

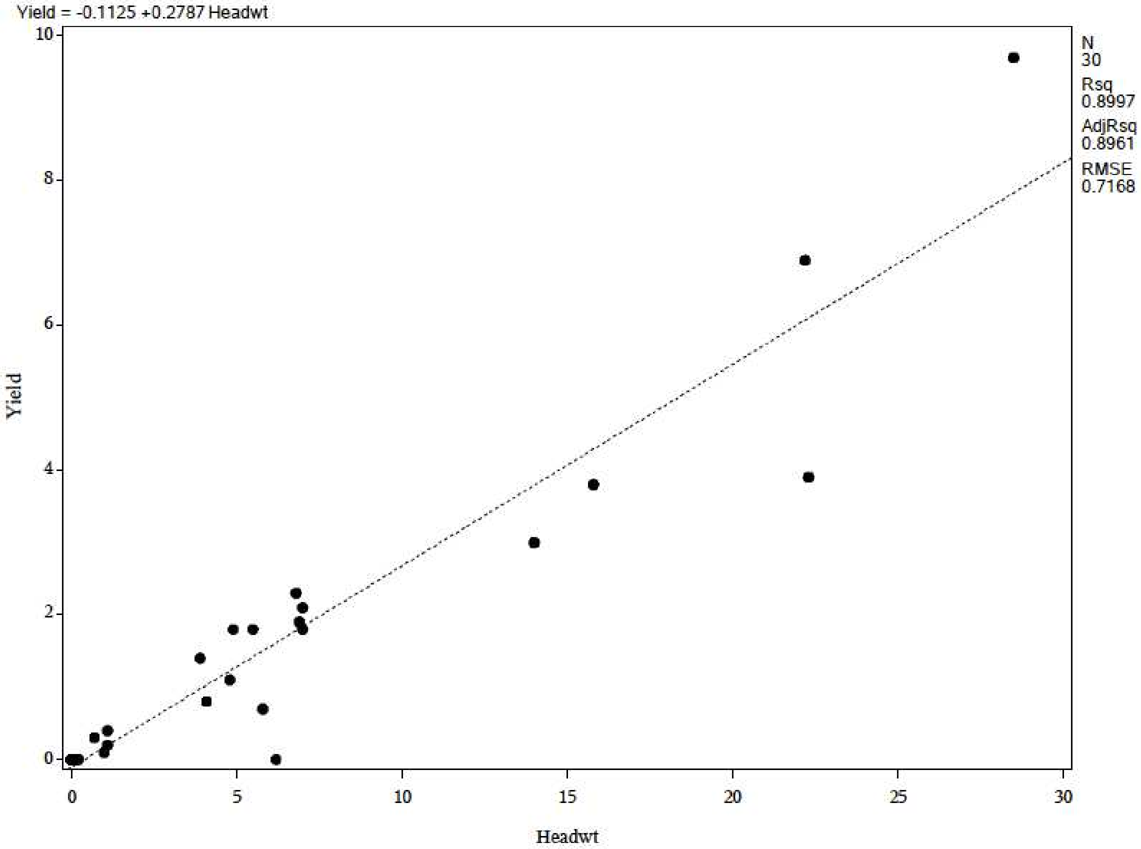

Figure 3.2: Seed head weight by seed yield. Seed head weight (x-axis) and seed yield (y-axis) are both measured in grams. Dots in figure represent female clones in Cycle 3 crossing blocks.

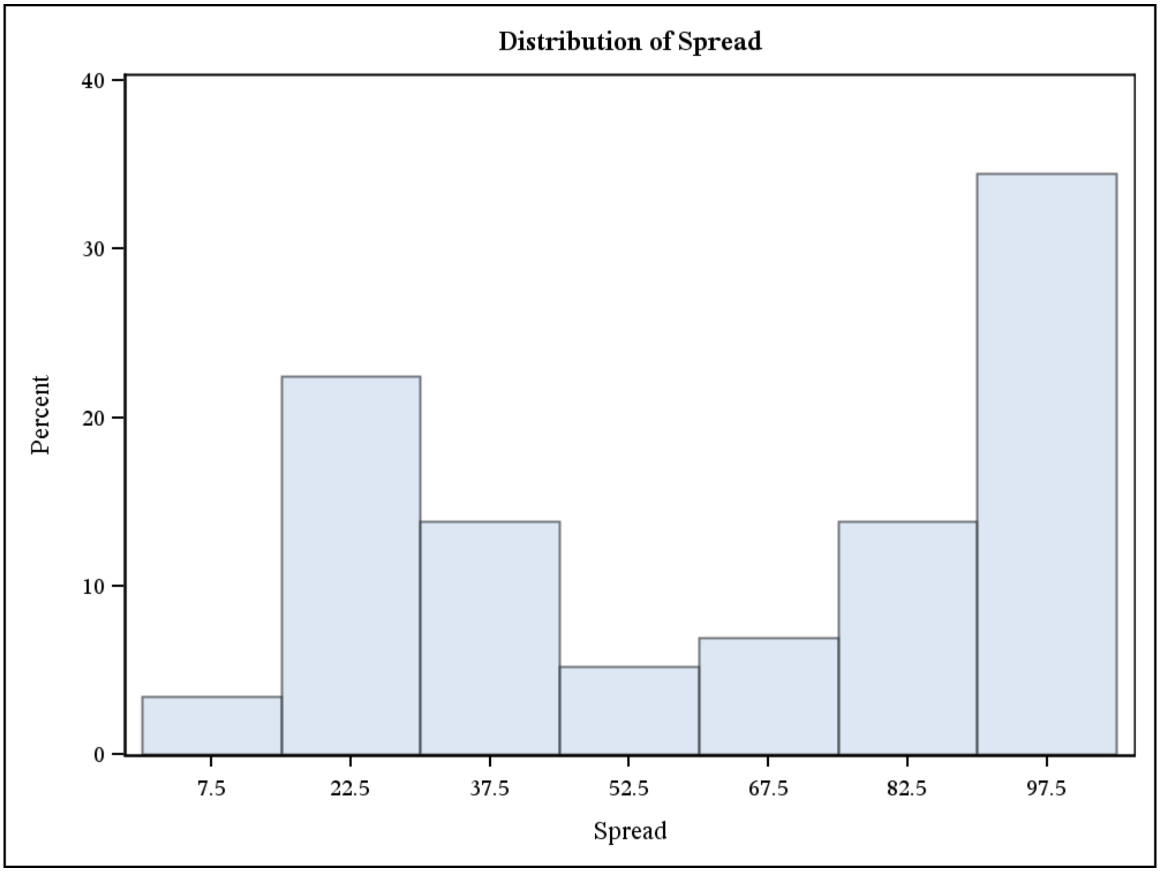

Figure 3.3: Distribution of percent spread for Cycle 3 crossing block clones (n=58). X-axis represents % grass cover in a 45.7 x 45.7 cm square frame on September 12, 2013.

Table 3.1: Survival of Cycle 3 crossing block clones by sex

Crossing Block Survival: Females

Name	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
84-2xA126-49-19	x	x	x	x
84-2xA35-6		x		x
84-2xA61-32-3	x		x	x
84-2xA126-5-2	x	x	x	x
84-5xA35-6-3A			x	x
84-5x84-6-5		x		x
84-8x84-6-1B	x	x	x	
84-19xA50-7-11				x
A37-15xA50-20-1	x			
A37-15xA50-20-7				x
A37-25xA126-5-5	x			
A37-25xA126-5-2A		x	x	
A37-28xA34-18-3B			x	x
A50-4R8-2		x		
Survivors:	6	7	7	9
Original clones:	16	17	17	16
% Survival:	37.5	41.2	41.2	56.3

Crossing Block Survival: Males

Name	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**
84-2xA50-7-1A	x		x	x
84-8x84-6-2A	x		x	x
84-19xA35-6-13				x
84-19xA126-5-1	x	x		x
A37-15x84-6			x	
A37-28xA34-18-4B	x	x		x
A37-28x?-7A			x	
A50-4R9-3A	x	x		x
A61-4x84-21-1			x	x
A61-4R7-1B		x		
A126-27xA50-7-11	x	x	x	
A126-27xA35-6-3B	x	x		
A126-27x?-4	x	x		x
Survivors:	8	7	6	8
Original clones:	13	12	12	13
% Survival:	61.5	58.3	50.0	61.5

Table 3.2: Seed yielding females in Cycle 3 crossing blocks

Name	Num	Rep	Seed Yield (kg/ha)
84-19xA50-7-11	1	4	172.2
84-2xA126-49-19	2	1	1044.1
84-2xA126-49-19	2	2	150.7
84-2xA126-49-19	2	3	258.3
84-2xA126-49-19	2	4	495.1
84-2xA126-5-2	3	2	157.9
84-2xA126-5-2	3	1	409
84-2xA126-5-2	3	3	379.9
84-2xA126-5-2	3	4	452.1
84-2xA35-6	4	4	484.4
84-5x84-6-5	5	2	742.7
84-5x84-6-5	5	4	419.8
84-5xA35-6-3A	6	3	484.4
84-5xA35-6-3A	6	4	215.3
84-8x84-6-1B	7	1	75.3
84-8x84-6-1B	7	2	43.1
84-8x84-6-1B	7	3	114.8
A37-15xA50-20-7	8	4	64.6
A37-28xA34-18-3B	9	4	340.9

Table 3.3: Number of flowering females and males by replication in Cycle 3 crossing blocks

	Rep 1	**Rep 2**	**Rep 3**	**Rep 4**
Females	3	4	4	8
Males	7	7	4	7
Total	10	11	8	15

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