DISSERTATION

HERITABILITY ESTIMATES, ACCESSION EVALUATION, AND DIGITAL IMAGING, IN *DISTICHLIS SPICATA*

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY DANA K. CHRISTENSEN ENTITLED HERITABILITY ESTIMATES, ACCESSION EVALUATION, AND DIGITAL IMAGING BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

HERITABILITY ESTIMATES, ACCESSION EVALUATION, AND DIGITAL IMAGING IN *DISTICHILIS SPICATA*

Conserving water in the landscape is critical to inhabiting the arid portions of the western United States. Native accessions of the inland form of saltgrass [*Distichlis spicata* var *stricta* (Torr.) Beetle] remained green, while turfgrass lines of blue grama, buffalograss, crested wheatgrass, and bermudagrass went dormant from lack of rainfall during the drought of 2000 and 2001in Colorado. Since saltgrass is non-domesticated, this research selected plants for four traits needing improvement to make saltgrass more turf like. Resistance to leaf rust (*Puccinia aristidae* Tracy), short height, high shoot density, and high seed yield were traits that made up a selection index which ranked all 158 accessions collected from the Front Range of Colorado, the Great Basin, South Dakota, and Nebraska. The top 14 females and 12 males of these were topcrossed, and progenies were evaluated. Response to selection was recorded for all four traits. Realized heritability, narrow sense heritability from half-sib analysis, narrow sense heritability from parent-offspring regression, and broad sense heritability were very high for height and shoot density. Broad sense heritability and narrow sense heritability from half-sib analysis were high for seed yield, but narrow sense heritability from parent-offspring regression and realized heritability were moderate. A major gene for rust resistance was inferred. Negative heterosis measured on the midparent for height and seed yield were noted. Positive heterosis occurred for shoot density.

Accessions were grouped by their region of origin (four), and analyzed for the above four traits, as well as, days to flower, spread, gap, seed length, and a measure of the

female head height. There were significant differences among regions for most traits. Arid region plants tended to have different values than humid region plants.

Digital imaging was used to measure spread of plants in one year's time. Correlations between camera scan and visual estimates of spread were high. Calibration of equipment is important in digital imaging, but digital imaging appeared accurate at medium levels of cover. The high rate of spread in progeny was postulated to be due to heterosis, and not a correlated response from selecting the four traits.

The indication of a major gene for rust resistance suggests ease of incorporating resistance, although durability may be reduced with a single gene. Collecting from the Front Range rather than the Great Basin and Central Plains, would be more effective in developing a turf variety because this area contains accessions with better values for turf traits. Digital imaging can discriminate plant spread when differences are so small they are indiscernible by visual estimates. Significant responses to selection and/or very high heritability estimates indicate breeding to change these traits will not be difficult.

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Dr. Pat Byrne came onto the committee late. A special thanks for his critical review of the quantitative genetics. And finally, thanks to Dr. Tony Koski, who helped me navigate the bureaucracy that graduate students must endure. Once, I asked Dr. Koski what I should do with the plants after they were in the ground. He said "mow 'em!". This was something not obvious to a person who worked with forage grasses much of his life.

The United States Golf Association, through Dr. Mike Kenna and Dr. Jeff Nus of the Research Program, provided funding over a nine year period for the study. Grants supported over twenty undergraduate students while they worked on the project and three people with degrees (2 who later went on to graduate school). Thanks to Fernanda Powers and Betts Miller for their considerable time. This is the third dissertation on the germplasm. Without the USGA, all of this would not have been possible.

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CHAPTER I

Introduction

This research is about the improvement of desert saltgrass [*Distichlis spicata* var *stricta* (Torr.) Beetle] for use as a turfgrass in salty and arid areas. The study uses conventional statistical analyses of plant traits and is unique in that this is the first attempt to breed traits in saltgrass, a non-domesticated grass.

Climate in the Western U. S. and Water Shortage

The recent drought that started in North America in 1999 increased awareness again, that the arid portions in western North America are very vulnerable to water shortages. Shortages were seen in precipitation, surface waters and reservoirs, and ground water aquifers. A survey of the newspapers during this time contain articles ranging from municipal restrictions on watering lawns to introduced legislation for bonding immense water supply projects to Federal intervention on interstate river compacts. Previously, the western U. S. has recorded droughts 3 times, around 1990, 1950, and during the Dust Bowl of the 1930s (NOAA, 2003). The impact of the severe drought of the 30s resulted in millions of people leaving the Great Plains and migrating westward in search of jobs and better living conditions (NOAA, 2003). Droughts are not uncommon in the western U. S. when based on Paleolithic time.

Grissino-Mayer (1996) has estimated more severe droughts lasting centuries in the past 2000 years (Figure 1). Tree ring width from live and fossilized wood from northwestern New Mexico was analyzed extensively (Figure 1A and 1B) to predict past annual rainfall and compared with another complex model (Figure 1C) which was made

up of tree ring data, ^{14}C, and archeologically dated alluvial units (type of soil and deposition thickness).

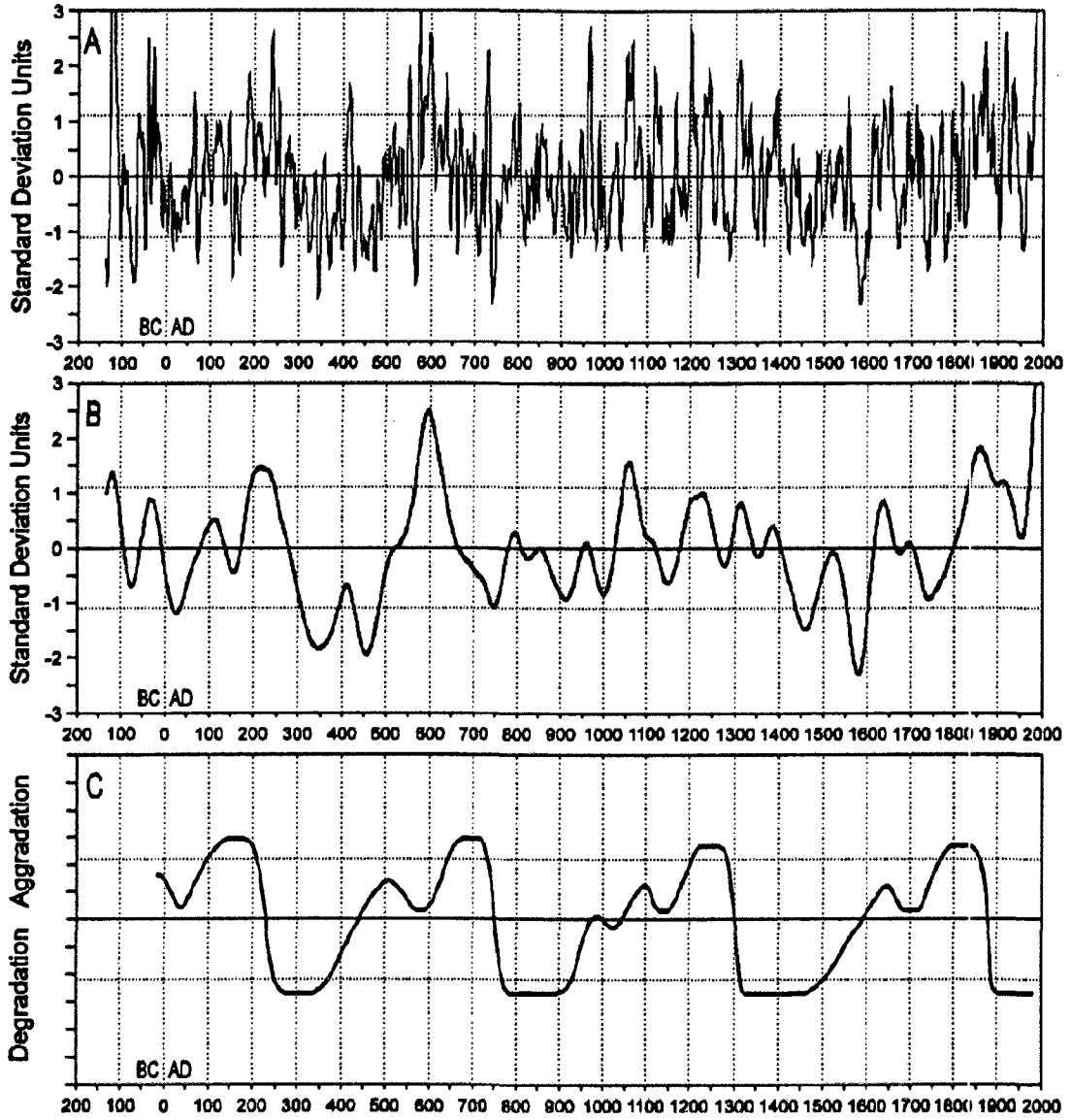

Figure 1. Paleolithic drought graphs: A. Reconstruction of annual rainfall (in standard deviation (SD) units from mean) from analyzed tree ring data in New Mexico. The curve represents a 10-yr smoothing spline fit through the reconstruction to accentuate short term (<50 yr) climate episodes. Dashed horizontal lines indicate ± SD 1.1 thresholds. B. A 100-yr smoothing spline fit through the reconstruction to accentuate long term(>100 yr) trends in climate. C. Primary aggradation-degradation curve developed by Euler et al. (1979) and Karlstrom (1988) for the Black Mesa area of Arizona. The curve is relative and therefore dimensionless (Grissino-Mayer, 1996).

Population Influx and Water shortage

Although drought raises awareness of water shortage, it is the recent influx of people into the arid west and their increased use of an already limited water supply which is creating a water crisis. The mountain West and its wealth of unspoiled vistas and beautiful landscapes invites all that would live here, creating a serious environmental change in tandem with the economic development (Booth, 2002).

The West leads the nation in net migration from 1990-2003 by a large amount (Figure 2).

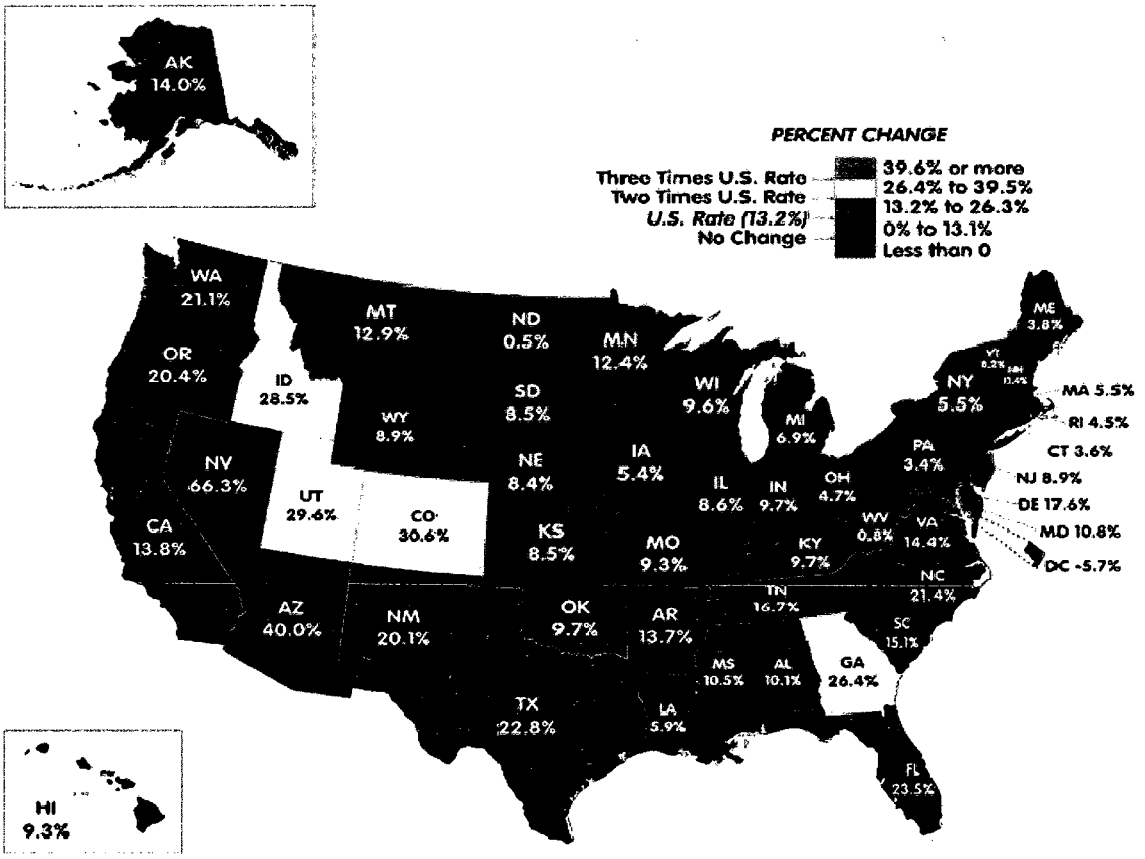

Figure 2. Net migration from 1990-2003 (Water 2025, 2003)

Counties in mountain states show increased population by migration and birth rates. Counties in red also represent metropolitan areas (Figure 3).

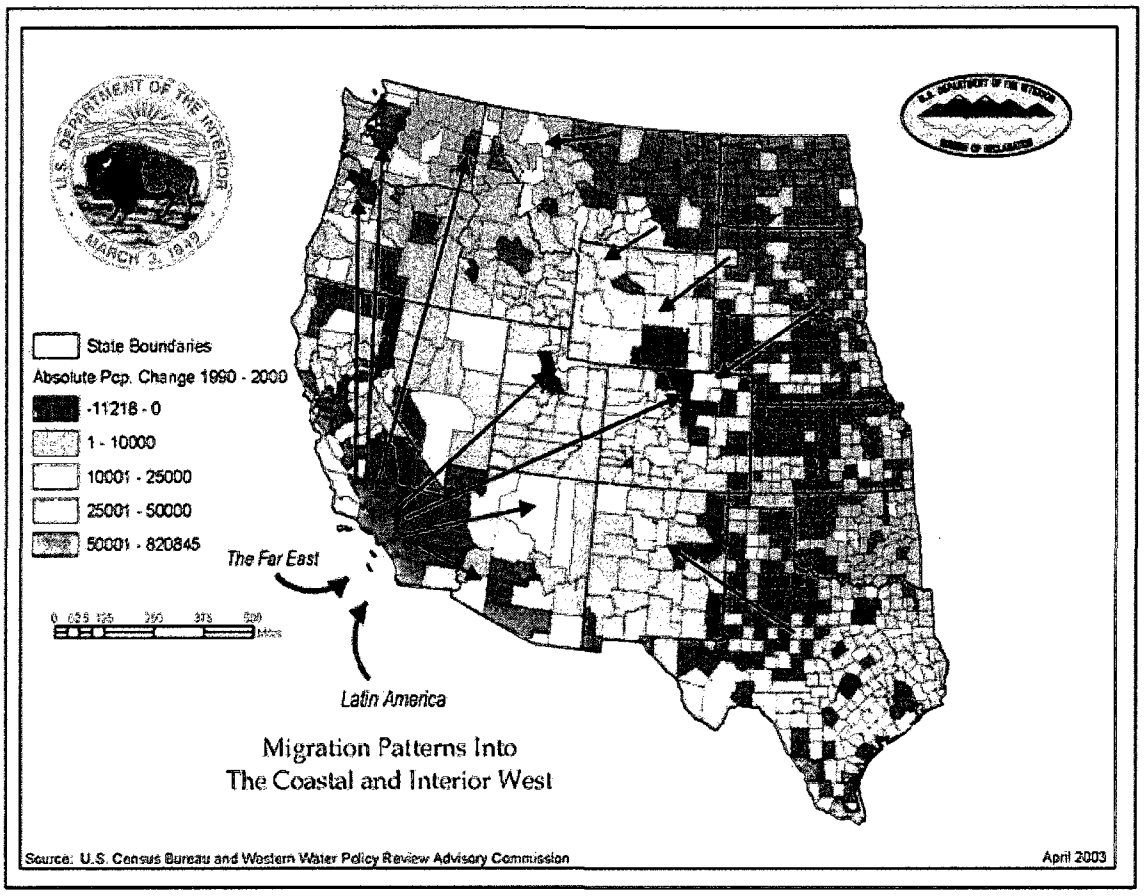

Figure 3. Migration patterns into the coastal and interior west. Arrows show general movement of people. (Department of Interior, 2003)

Native Grass to Reduce Water Use

The U.S. Department of the Interior proposal, *Water 2025: Preventing Crises and Conflict in the West* (2003), emphasizes the critical water shortage. It lists conservation as the number one tool in alleviating water shortage. One method of water conservation in landscaping uses plants that need little water (Grisham and Fleming, 1989; Mintenko et al., 2002). The Green Industries of Colorado (nurseries, landscape industries, turfgrass industries, greenhouses, and florists) (2003), Colorado State University (1999), and Denver Water (2003), support the use of native and drought tolerant plants. Lawn care accounts for 32% of the total residential outdoor water use nationally (EPA, 1995). This percentage is higher in the western U.S., where evapotranspiration is much greater. Over 50% of annual water consumption that a Denver residential customer uses, goes to sustaining the landscape. Planting a native grass lawn can reduce this by half (Denver Water, 2003).

CHAPTER II
Literature Review

Saltgrass as a Turfgrass

Native grasses are adapted to dry, hot conditions of the west, having evolved through natural selection in an arid climate over millions of years. Desert saltgrass has potential as a turfgrass (Fults, 1959; Cuany, 1987). During the heat and drought in 2000 and 2001, a large collection of desert saltgrass remained green at the same time lines of blue grama, buffalograss, crested wheatgrass and bermudagrass had turned brown from lack of rainfall (Hughes et al., 2002). In comparison to other native grasses, desert saltgrass exhibited good wear tolerance to traffic under drought (Fraser and Anderson, 1980). Saltgrass is adapted to compacted soils (Hansen et al., 1976), and is found in heavily trafficked areas. Effluent and many ground water sources are too salty to be used as potable water sources, but can be used instead on salinity tolerant turfgrass cultivars. Desert saltgrass can tolerate salinity levels of 60,000 ppm NaCl (Kopec and Marcum, 2001), a concentration exceeding that of seawater at 35,000 ppm. Many of the adaptions found in saltgrass make it a desirable turf species for arid parts of western North America.

Species Description

Desert saltgrass is a warm season C4 perennial grass native to western North America, from Saskatchewan into Mexico, and from California into Iowa (Hitchcock and Chase, 1950). From recent collections used in the Colorado State University breeding

program, the U.S. east-west major distribution follows the inside of the 61 cm precipitation line (Figure 4).

The species occupies specific niches of wet, alkaline, and saline soils, but is also found on drier less salty sites. It is dioecious, but mainly reproduces by vigorous rhizomes in the wild (Freas, 1987). Desert saltgrass is present on 500,000 hectares in Colorado and Wyoming and serves as a low value forage (Mueller et al., 1985). It has potential as a forage, but large amounts of seed are unavailable. Clones of wild types are used infrequently in small revegetation projects (personal communication, Dr. Nicholas Yensen, NYPA, Tucson, AZ).

Saltgrass is in the subfamily Chloridoideae, which represents pioneer species that evolved in stressful, arid environments (Loch, 1995). *Distichlis spicata* is often divided into 2 variants in the U.S., a coastal type subsp. *spicata* and the desert type subsp. *stricta* (Gould and Shaw, 1983). Cytological work by Reid (2001) presents evidence in delineating the types based on finding 40 chromosome plants mainly on the coasts, and 38 chromosome plants inland. *D. spicata* is also found in South America, and 21 homonyms exist for the species (Soreng et al., 2005). Other members of the genus are *D. palmeri, D. scoparia* (S. America), and *D. australis* (Australia). These form a species complex and also intermate with *Aeluropus* species (eastern hemisphere, personal communication, Dr. Nicholas Yensen). Wild grasses tend to form species complexes (Smith, 1995).

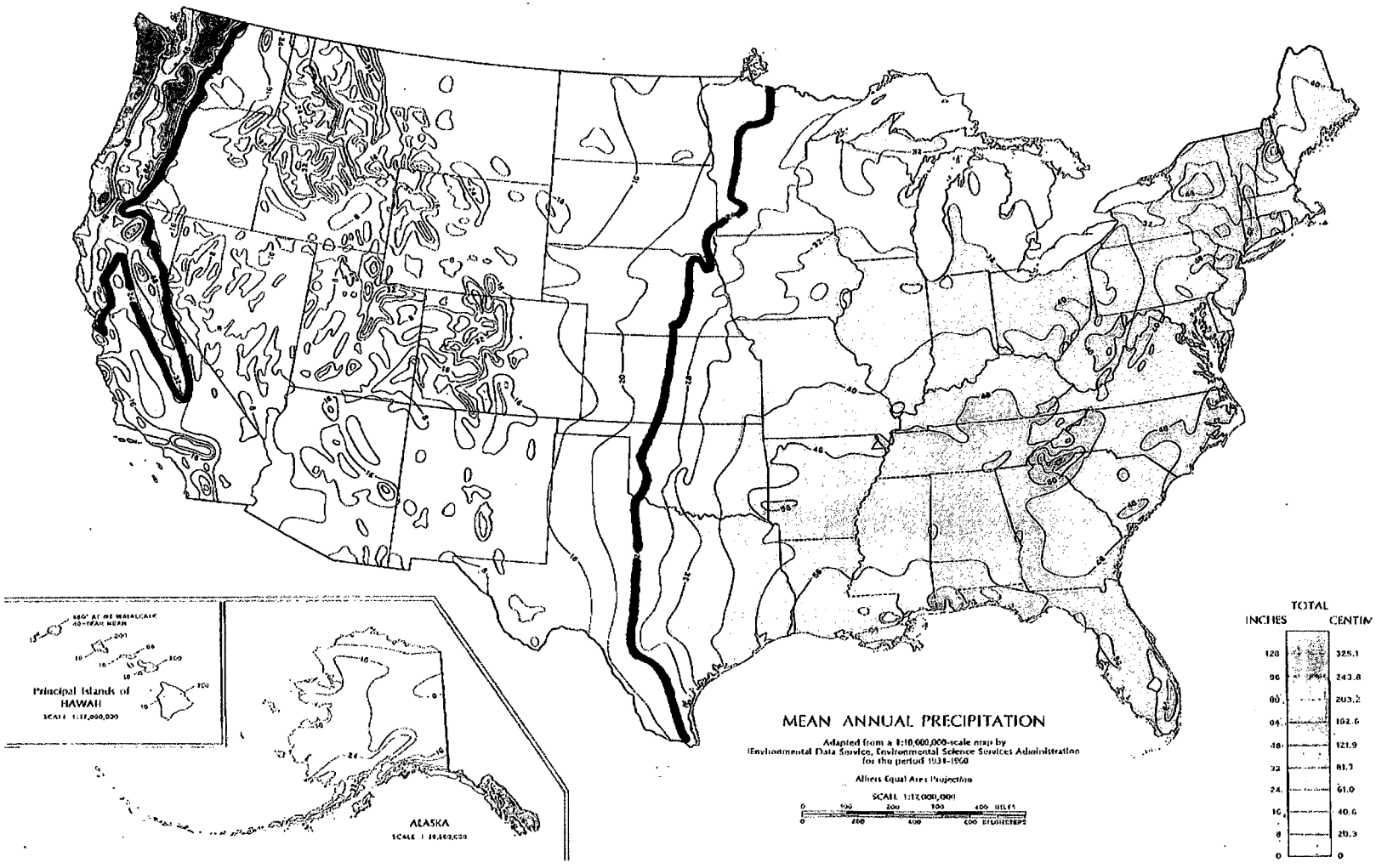

Figure 4. Major desert saltgrass distribution between the 61 cm precipitation lines (bold). From collecting trips made 1990-2000 (adapted, Geological Survey, 1970).

Domesticating Saltgrass

Problems in domesticating saltgrass as a crop were twofold. The first of these is low seed production. Earlier work described saltgrass as a poor and infrequent seed producer (Cluff et al., 1983; Freas, 1987). However, Nielson (1956) did not mention problems in obtaining collections of wild seed for his work. Personal observation based on extensive collecting is that wild stands appear to be variable in number of genotypes, based on morphology. Stands with little flowering generally consist of few genotypes, while those that produce flowers appear to consist of many (≥20) genotypes. The frequency of stands with many individuals is low. In addition, wild seed production is higher in wet years (personal communication, Julie Etre, Western Botanical Services, Reno, NV; Rick Storre, Freshwater Farms, Eureka, CA). Nevertheless, in the nursery collection at the Horticulture Field Center, Colorado State University, Fort Collins, over half of the females produce commercially acceptable levels of seed. Based on unpublished data at the Horticulture Field Center, seed production is influenced strongly by genetic and environmental factors.

The second obstacle to domestication is that seed dormancy was high in saltgrass, but was partially overcome with a day to night temperature difference of about 16 deg C. (Nielson,1956; Sabo et al.,1979; Cluff et al., 1983). Harrington (2000) reviews the literature on germinating saltgrass seed, and examined seed scarification as the key to overcoming dormancy. Granite Seed, Lehi, UT, presently uses a deburrring machine to roughen the seedcoat on a wild race of saltgrass. This treated seed line germinated successfully in plots at the Horticulture Field Center, Fort Collins, CO, in August of 1999, with day temperatures of 35^0 C, and night temperatures of 18^0C.

Domesticating a wild species occurs because some trait(s) of a plant provides a human benefit, thereby ensuring the plant is reproduced with the aid of man. Turfgrasses provide erosion control, natural outdoor carpets, sports surfaces, cooling from transpiraton, and visual aesthetics. The visual determinants of turf quality are described by Turgeon (1985) as:

1. Density is a measure of the number of aerial shoots per unit area.
2. Texture is a measure of the width of the leaf blades.
3. Uniformity is an estimate of the even appearance of a turf, divided into the mass of aerial shoots and the evenness of the turf's surface.
4. Color is the measure of the light reflected by the turf.
5. Growth habit describes the type of shoot growth: bunch type, rhizomatous, and stoloniferous.
6. Smoothness is a surface feature defined by visual quality and playability. Mowing injury can disrupt the surface with ragged leaves or dieback. A rough surface can affect playability by slowing ball roll.

Meyer and Funk (1989) describe other turfgrass attributes:

1. Ease and economy of establishment; with the emphasis on seed propagation as opposed to vegetative, even in vegetative type species such as bermudagrass [genus *Cynodon* (L.)]. High seed production is demanded by seed growers, even though it is not a trait for eventual use.
2. Dependability; such as consistency in spring green up, or lack of pest problems.

3. Persistence; whether plants are long lived or not; many turfs are decades old.
4. Durability; mainly, how well plants hold up to traffic.
5. Reduced maintenance requirements; minimal top growth, low fertilizer and irrigation requirements.
6. Attractive appearance; fine leaved, high density, and uniformity.

Meyer and Funk (1989) proceed with listing present breeding objectives for turfgrass species:

1. Resistance to pests.
2. Lower growing grasses with better turf properties.
3. Increased tolerance to environmental stresses such as heat, cold and drought.

The above attributes were considered in evaluating each of the 158 accessions in the nursery. The importance of several of these became apparent early on, and it was decided that the traits in a breeding program to improve the turf quality of saltgrass would be, in priority: (1) resistance to rust (*Puccinia aristidae*); (2) short height; (3) Shoot density; (4) seed yield.

Breeding Perennial Grasses

Casler and Duncan (2003), Burton (1992), Poehlman and Sleper (1995), Sleper (1987), and Briggs and Knowles (1967), discuss breeding theory and method for forage species and turfgrass species (outcrossing polyploids). These species differ from major agronomic crops (where plant breeding theory and method have been developed), in that in addition to seed yield, other traits are as important, and the need for perenniality

requires performance data over different annual environments. Casler and Duncan (2003) further distinguish turfgrass screening from forage screening as mainly visually subjective and arbitrary, yet gains in response to selection have been extraordinary. While forages are selected for sward yield, a complex quantitative trait, turfgrasses are selected for a combination of more qualitative traits.

Perennial turfgrass cultivars originate from populations with a high degree of genetic variability. Individual plants are highly heterozygous for any gene (generally), and the populations are heterogenous (each plant different than another) (Vogel and Pedersen, 1993; Casler and Duncan, 2003). Plants are self-incompatible (with exception of the facultative apomicts), and the resulting outcrossing maintains heterozygosity and heterogeneity. In addition, most are polyploidy (and form polyploid series) allowing more allelic interaction than diploids, and also cross with closely related species, which results in more variation. Most present day grass species arose out of wide crosses, and a concurrent doubling of the chromosomes in the hybrid; i.e., most grasses are amphidiploids (allopolyploids). Their perennial nature indicates that they have to be plastic in adapting to climatic variation year to year, and at least somewhat resistant to pests and other stressors. Having evolved generation-wise through millions of years of stressors, they carry a large number of adaptive genes (Casler, 2003). Being polyploid (essentially duplication of genes) also allows some repetitive genes to mutate, adding more potential for genetic variability.

Grasses suffer from inbreeding depression, which occurs from crossing closely related types, resulting in a loss of vigor, sterility, or death. Therefore, turfgrass breeding programs carry working populations into future years by making a sufficient number of

crosses among different overall types to prevent inbreeding, while at the same time, selecting for plants that have traits listed by Turgeon (1985) and Meyer and Funk (1989). At the point in the program when desirable individuals start to appear (a point when desirable alleles have increased in number), a cultivar can be selected as:

1. a clone (reproduced by plugs or sod)
2. apomicts, as in the case of Kentucky bluegrass (*Poa pratensis* L.).
3. a narrow based population (such as Penncross creeping bentgrass (*Agrostis stolonifera* L.), in which seed is produced from vegetatively planted fields of three parents, and that seed is never advanced to produce more seed because of inbreeding depression).
4. a broad based population (which maintains variability similar to native populations, with the exception of turf desirable traits, whose genes approach homozygosity in individuals, resulting in homogeneity (uniform looking individuals) in the cultivar.

Generational advance of the main breeding population is termed recurrent selection because the improvement process is repeated again and again through several to many cycles of selection. Most turfgrass cultivars have been developed through recurrent selection (Casler and Duncan, 2003). Hallauer and Miranda (1981) present several recurrent selection schemes dependent on the breeding structure of the species.

Research Objectives

Hallauer (1992) defines plant breeding as the science and art of effective management of genetic variability to attain desired breeding goals. The potential goal of

developing saltgrass being a drought resistant turfgrass was made by Fults (1970) several decades ago when he observed its carpet like growth in its natural habitat. Significant variability exists for many turf traits, such as height, shoot density, and color.

The objectives of this research were to 1) initiate a turfgrass breeding program and note its early success by measuring response to selection and calculating heritabilities. 2) analyze the accessions for turf traits by geographic origin, and 3) determine if digital image analysis is an efficient method in selecting plants.

1. Measuring the success of the breeding program

Most plant breeding programs are concerned with improving more than one trait. Resistance to rust, short height, seed yield, and shoot density appear to be the traits in need of improvement in the saltgrass accession population. All of these traits showed significant differences in the accession nursery (Chapter IV).

Since this is multitrait selection, a selection index can be constructed that strongly weights rust resistance over short height, shoot density and seed yield. Rust, at higher levels, causes top growth to die. Saltgrass also needs improvement in other traits, however, as more traits are considered in a selection scheme, fewer plants exist with all desirable traits. Falconer and Mackay (1996) state index selection as the better type of multitrait selection over independent culling, and, over tandem selection, to give the most rapid improvement of economic value.

The Smith-Hazel index considers the phenotypic and genetic variances (heritability) and covariances of traits as well as an economic weight for each trait, but the need for

mating designs (to calculate heritability) and large sampling errors for variances and covariances, have generally limited the use of this optimum index (Bernardo, 2002).

Baker (1986) presents a base index which uses relative economic values (a_i) of the traits as index coefficients:

$$I = a_1P_1 + a_2P_2 + \ldots\ldots a_nP_n.$$

I = index value for accession

P_i = phenotypic value for the *i*th trait

Elston (1963) proposed a multiplicative or economic weight-free index which uses a minimum trait level for a desired trait:

$$I = (P_1 - min_1)\,(P_2 - min_2)\ \ldots\ldots(P_n - min_n)$$

I = index value for individual accession

P_i = phenotypic value for the *i*th trait

min_i = minimum value.

The phenotypic value in selection indices is often standardized due to differences in scale and range among the traits:

$$\text{Standardized variable} = \frac{\text{(Phenotypic value - mean value)}}{\text{Standard error}}$$

Expected gain (response to selection) for k traits = (1/ %k) (Baker, 1986), so as the number of traits increases, the expected gain for each trait decreases. This relationship assumes no correlation between traits.

Charmet et al. (1993) used Smith-Hazel multitrait selection in perennial ryegrass (*Lolium perenne* L.) to predict changes in crown rust, spring growth, aftermath heading,

summer regrowth, and autumn regowth. Phenotypic and genetic variances and covariances were calculated by taking seed from the 43 parent plants (the C_0), and growing them out as 43 half-sib families. Economic weights were actually adjustments to scale, and, how important the breeders viewed the trait. Predicted genetic gain (response to selection) of the composite trait value of C_i, decreased from the C_0 to the C_5, because predicted genetic variance decreased over cycles of selection. They cited other researchers that showed variable effects from recurrent selection on realized (not predicted) genetic variance.

Humphreys (1995) examined multitrait response (10 traits) in perennial ryegrass, also using Smith-Hazel index, but compared predicted to actual response from selection, from the C_0 to the C_1. The author concluded good correspondence between predicted and observed selection responses, both in direction and magnitude, although some exceptions were apparent among different source populations (the C_0's used).

Simmonds (1979) shows the general response to selection (R), over long term based on the accumulative information in plant breeding (Figure 5).

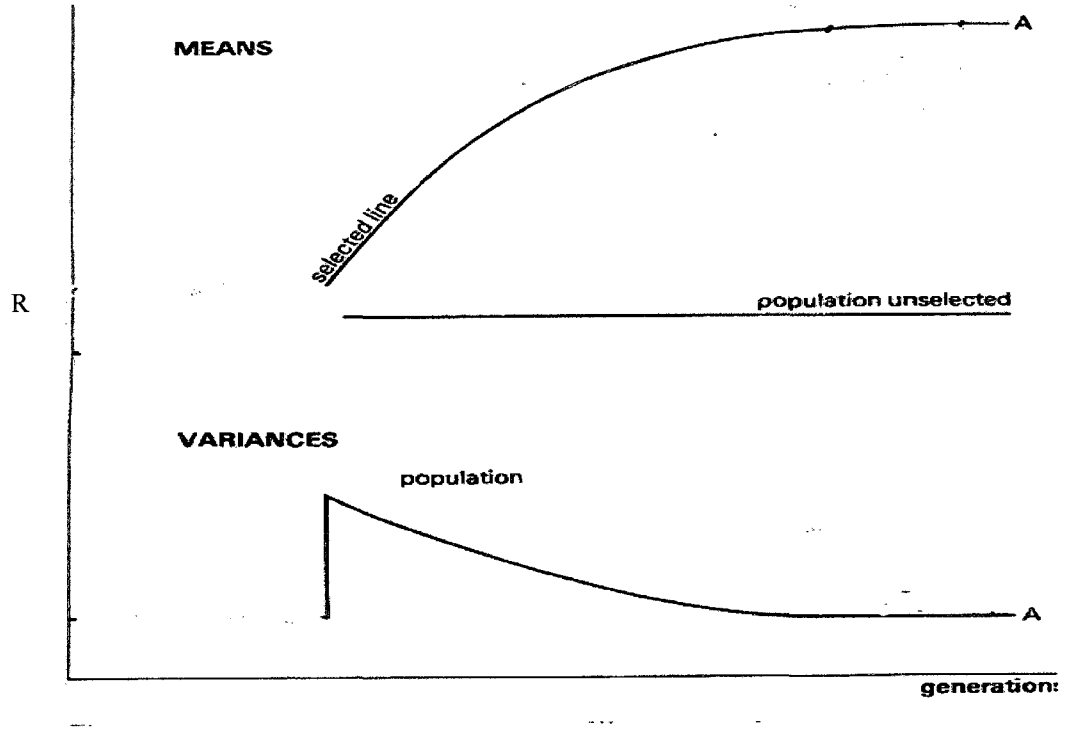

Figure 5. Trait response to selection, R, (y axis) over generations (x axis) under constant selection pressure, and, representation of the associated decline in genetic variability (adapted from Simmonds, 1979).

The response to selection, R, is equal to:

R = mean of progeny of selected parents - mean of population

Measuring R is straightforward in the type of recurrent selection program in breeding turfgrasses. The breeding population is advanced yearly, and selected parents from the previous population are usually carried on as clonal checks. From R, *realized* heritability can be calculated (Lynch and Walsh, 1998):

$$h^2 = R/S$$

S = mean of selected parents-mean of population

But highly heritable characters rapidly decrease their genetic variation from the base population, leading to underestimation when extrapolating realized heritability backwards, and overestimation when projecting forward. Also, problems may occur with environmental effects, and inbreeding depression unless checks are included. There is also the problem of genetic drift. Therefore, Falconer and Mackay (1996) advocate using mating designs to calculate genetic variances of relatives within and across generations.

Genetic variance is estimated from an analysis of variance of random genotypes in multiple environments. This variance contains additive, dominance, and epistatic variance. Additive genetic variance is the variability associated with the average effect of substituting one allele (one form of the gene) for another at a locus. Additive genetic effects are transferred from one generation to the next. Dominance is the interaction of alleles at one locus, and epistasis the interaction of alleles at different loci. The interactions can be important for the performance of an individual and contribute to the total genetic variance. However, interactions are not transmitted from one generation to the next. Breeders are interested in determining additive genetic variance, because it is the variance that individual plants can be selected from and pass on their traits to progeny. The additive genetic variance is related to heritability (h^2) in the following way (Fehr, 1991):

$$h^2 = \sigma^2_A / \sigma^2_{Ph}$$

σ^2_A = additive genetic variance

σ^2_{Ph} = phenotypic variance, which includes variation due to experimental error, genotype X environment interaction, and total genetic variation (additive, dominance, epistasis).

The heritability used above is termed *narrow sense* heritability and is the preferred type,

however, sometimes total genetic variance is used in the numerator, changing heritability to the *broad sense*. Heritability is a general value referring to a specific trait, population and environment, but its components are used to calculate breeding values (the mean value of a parent's progeny). When genetic variance is presented, it is important to define how it was obtained.

Browning et al. (1994) used progeny of half- sib families and their parental buffalograss [*Buchloë dactyloides* (Nutt.) Engelm] lines to calculate narrow sense heritability of turfgrass traits. Traits measured were roots per node, regrowth, uniformity, stolon count, color, quality, and diameter of spread. Treatment effects were the female parent lines and heritability was :

$$h^2 = 4\sigma^2_T/\sigma^2_P$$

σ^2_T = maternal variance

σ^2_P = total variance

The authors also calculated heritability using offspring- parent regression:

$$h^2 = 2bo.p$$

$b_{o.p}$ = the regression of offspring values on the maternal values

Maternal half-sib analysis gave the higher heritability, with heritability estimates for spring color = 0.62 ±0.26 SE, and quality= 0.44 ±0.19 SE. They suggest evaluation of traits they measured should be made when buffalograss was actively growing and not under environmental stress.

Bonos et al. (2004) reported increased resistance to gray leaf spot [*Pyricularia grisea* (Cooke)] in several perennial ryegrass populations developed by selecting resistant

parents. They calculated S, the selection differential, R, response to selection, and realized heritability ≃ 1.0. Broad sense heritability, using variances of genotypic lines, ≃ 0.92, with a 95% confidence interval (CI) = 0.98 to 0.71. The authors also used the equation by Fain (1978) to determine if there was a major gene responsible for resistance:

$$\text{Var}(O_l) = a + b_1\bar{O}_i + b_2\bar{O}_i^2$$

Var (O_i) = phenotypic variance within the *i*th sibship

$\bar{O}_i$ = midparent value for the sibship

b_2 = when significant, is an indication of a major gene

The significance of a single or several major genes indicates possible susceptibility to new virulent races. This research concluded minor and major genes were segregating for resistance. Rose-Fricker et al. (1986) found frequency distributions of reaction patterns to stem rust (*Puccinia graminis* subsp. *graminicola*) in perennial ryegrass indicated predominantly quantitative inheritance of minor and major genes.

Ashraf et al. (1986) screened and selected seedlings of several perennial grasses for salt tolerance, and reported progeny with greater dry matter yield than unselected progeny. Edwards and Cooper (1963) reported an increase in leaf size from 9.4 cm^2 to 12.6 cm^2 in 3 cycles of selection in perennial ryegrass, with realized heritability ≃ 0.61. Faulkner et al. (1982) selected for seedling vigor in several lines of tall fescue (*Festuca arundinacea* Schreb.) under hydroponic and compost conditions and found speed of emergence had the highest response to selection of 5 seedling traits. Reeder et al. (1984)

selected divergently over several cycles for leaf area expansion rate in tall fescue and found the high population increased expansion at13.3 mm^2/day per cycle and the low population decreased expansion at11 mm^2/day per cycle. They concluded gene action was additive and sufficient variation still existed after 5 cycles. Carlson et al. (1983) selected divergently for specific leaf weight (SLW) in reed canarygrass (*Phalaris arundanacea* L.) with 11% and 10% gain for high and low SLW, respectively; the second cycle resulted in 12% and 9% additional gain.

The preceding paragraphs discuss physiological, morphological, and resistance traits which have shown a response to selection in perennial grasses. In addition, some of these studies have calculated heritability for traits in order to make general inference about expected progess in a breeding program. Since saltgrass needs improvement for several turfgrass traits, and such a breeding program entails a considerable amount of work, measuring current progress, and more importantly, estimating future progress, would help to direct resources. In reviewing the literature, realized heritability can be calculated to estimate response to selection in generations adjacent to the calculation. In addition half-sib family analysis, and offspring-parent regression can be used to calculate additive genetic variances which would give a better estimate of response to selection in distant cycles. Resistance to leaf rust could be ascertained as to whether it is polygenic or controlled by a major gene.

2. Accession evaluation

Collection of the accessions was purposefully divided into 4 geographic locations to determine if accessions differed by their location for turf traits. The practical reason for

this is, if one of the locations showed more, or specific turf traits, then resources could be directed to that location for future collection. In observing the overall collection, it seemed anecdotally, that collections from the Colorado Front Range, and the lower river system from the Front Range, had plants that were shorter, which is a turf trait. There is some speculation that plants growing in the Great Plains regions were subject to grazing over millions of years and they adapted to a more cespitose (dense, matlike) form over this time (Smith,1995).

Studies of variation within species have been ongoing for over 200 years, and is reviewed by Langlet (1971). Nicholas Vavilov (1926) was the first to state the necessity of collections to form the broad genetic base for crop improvement. Vavilov's work led him to postulate "centers of diversity" (centers of origin) wherein traits were highly differentiated among plants. Additionally, traits varied because of natural selection operating through biotic and abiotic factors. As time went on, studies on intraspecific variation occurred on smaller and smaller geographic scales, to a point where significant variation due to natural selection is now recognized in distances as small as 1 meter (Linhart and Grant, 1996).

Much of the recent literature on germplasm resources for crop development focuses on the variability still available in cultivars (Hallauer, 1992; Baenziger and Peterson 1992; Rasmussen and Phillips, 1997) , the small amount of variability in plant collections relative to the wild (Teshome, 2001), the large variability associated with crosses of wild types in primitive farming systems (Teshome, 2001), needed evaluation of large present collections (Chang, 1992), and the loss of wild populations (Maunder et al., 2004).

Allard's 1970 *Population Structure and Sampling Methods* is still often cited in regards to strategies for plant collecting in the wild. Wild populations contain huge amounts of variability. A map which shows the species populations and their variability for the specific traits is necessary to be objective in collecting, but this map is arrived at only after extensive surveying, collecting and evaluation of the species. When this information is not available, guidance is found from extrapolating case studies. Unfortunately, there are few case studies. Pest resistance often arises in certain geographic populations, and not others, exhibiting an overall mosaic pattern. Qualitative morphological and physiological traits can identify variants from certain geographic areas. The same identification can be done with quantitative measurable traits. Quantitative variation is often associated with clinal patterns, such as tall plants from temperate humid regions to shorter plants as the climate gets warmer and arid. However, quantitative variation is also observed at sub-geographic levels, attributed to strong biotic and abiotic selectors, and at very short distances, ruling out buffering effects from gene flow. The net effect is also a mosaic pattern. Variability is easily promoted in outcrossing species, but large amounts of variability also occur within populations of selfing species.

The general consensus is to sample the entire range of the species, and not just centers of variability, but also including the extreme edges of the ecological range, which will result in collecting the maximum amount of variability (Allard, 1970; Hawkes,1981; Brown and Marshall, 1995). The division of the entire range into the next level of sampling is generally termed geographic areas, and they are further divided into regions, then populations or sites, although there appears to be no set nomenclature (Allard, 1970; Guerrant et al., 2004; Walters, 2004; Brown and Marshall, 1995). Imam and Allard

(1964), after considerable surveying, proposed sampling wild oats (*Avena fatua*) in Central California based on statistically significant changes in traits. This resulted in 200km grids on the north-south cline, a uniform cline. The east-west cline represented diverse topography and climates, and sampling was proposed at 50 km intervals. The grid intersects were termed regions. They proposed collecting in 100 regions, 5 populations per region, 200 plants per population, and 10 seeds per plant. This represents a 600 km X 200 km area.

Oka (1969) described total variability (G) as:

$$G = 1 - \{1 - P + P(1-p)^n\}^N$$

Where P = proportion of total variation represented by one population

p = proportion of one population's variation represented by one plant

N = number of populations sampled

n = number of plants sampled per population

Brown and Marshall (1995), substituting to maximize G , and based on assumptions, recommend 50 individuals per population in each of 50 populations per ecogeographic region (clusters, not grids) for a given taxon. This would result in one copy of 95% of the alleles that occur in a population at frequencies greater than 0.05. To pick up rare alleles, or to increase the probability that alleles are not missed, greatly increases sample size. Many modifications to the 50:50 rule are presented, with location of variability taking priority.

For vegetatively reproduced species, Hamilton and Chorlton (1995), radically downsize Brown and Marshall's within population sample towards 2 or several. Numbers of individuals in vegetative populations have been measured to be much lower than outcrossing seeded species, and, fewer individuals show lower variability.

Elzinga et al. (1998) emphasizes that measuring and monitoring wild plant populations is often done at too detailed of a level, that more effort should be made on the setting of priorities, and, that limited valuable resources should be focused in obtaining results that are broader in scope. The final size of the plant collection is determined by available resources (Allard, 1970; Brown and Marshall, 1995; Guerrant et al., 2004; Hamilton and Chorlton, 1995). Since samples are restricted by resources, then $Nn = k$, where k is the number of samples that can be handled. G is maximal at $n = 1$, so that $N = k$ regardless of the values of p and P (Hamilton and Chorlton, 1995).

Selective sampling is discouraged because of missing potentially desirable alleles (Allard, 1970; Hawkes, 1981; Brown and Marshall, 1995). An exception to this may be in collecting vegetative species because of the handling of propagules causing constraints. Sometimes coarse grid sampling is practiced to provide a survey for future selective sampling.

Walters (2004), in discussing germplasm in gene banks, states the number of accessions for a particular species is often a matter of convenience or opportunity rather than a science-based study of genetic constitution. Evaluation studies of potential turfgrass germplasm were done on USDA collections which had no consistent objective in uniform sampling (Casler, 1995; Casler and Van Saten, 2000).

Casler and Santen (2000) analyzed genotypic variation of collections of meadow fescue (*Festuca pratensis*) for 15 agronomic and turf traits and related that variation to geographic source of the accessions. Variation among accessions was significant for all traits at all locations. Accessions from similar climatic areas as the test sites usually performed well, but there were unexplained exceptions. Leaf blade length and plant height rankings, and differences, significantly changed between two diverse test environments. Cluster analysis was then performed to describe an arbitrary 70% of the variation to be among clusters. The variation is from a composite of the standardization of 15 traits. Five countries with single accessions were included in the analysis. A dendrogram represented the stepwise analysis in which minimized pooled variances grouped accessions into clusters. Adjacent clusters on the same branch were more similar. The 35 resulting clusters formed, were an attempt to group phenotypically similar and adaptively similar accessions. Most clusters were geographically diverse. Wide crowns, narrow stems, short leaves and low dry matter yield were desirable turf traits found in Moroccan and Yugoslavian collections. These were in adjacent clusters.

Casler (1995) analyzed genotypic variation of collections of perennial ryegrass for agronomic and turf traits and attempted to relate the variation to geographic areas. Some material had been genetically contaminated with Italian ryegrass(*L. multiflorum* Lam.) and was discarded. Accessions-within-countries was the largest source of variation for all 4 traits: seedling vigor, leaf width, crown rust resistance, forage yield. Country means-within-regions differed for all traits except leaf width of cultivated accessions. Geographic areas did not differ for the traits. Naturalized germplasm from areas where perennial ryegrass is thought to have evolved showed the highest standardized variances.

Cluster analysis was performed to describe an arbitrary 80% of the variation to be among clusters. Countries with single accessions were omitted citing lack of representation. The analysis of the three phenotypic traits and yield resulted in 17 clusters. Five of the clusters represented a large proportion of naturalized accessions from the Mediterranean area with undesirable traits. Using the results of this study, germplasm utilization, or plant collecting, based on seeking out countries with variability would result in few desirable traits. Casler recommended additional plant exploration with specific objectives if future breeding progress is to occur.

Both of the above experiments used an entire species gene bank that had little documentation of the collecting, nor consistent sampling based on the species range. Some countries had 49 accessions, and others 1. Core subsets for breeder distribution were formed representing potentially useful, adapted germplasm based on cluster analysis. Clearly, more uniform sampling needs to be done, with consideration towards the species range and shared objectives among individual collections.

Even though modern molecular techniques can reveal the effects of population size, coancestry, migration patterns, and breeding system, they do not directly evaluate variation patterns in adaptation (Teshome, 2001). For that, morphological, edaphic, climatic, and pest resistance traits must be measured in plot trials. As an example, low variability at the DNA level in landraces of barley did not associate with high levels of variability of useful phenotypic traits (Bjornstad et al., 1997).

Actual saltgrass collecting entails considerable costs, time and effort. The only known germplasm collection is being made by Colorado State University in cooperation with the University of Arizona. The only maps are taxonomic references to the western

states it inhabits (Hitchcock and Chase, 1950). It occupies niche habitats, so it is not uniformly dispersed across a region, and populations are isolated from each other. More efficient use of resources would be targeted at a broad survey of the species range since little information exists on its distribution. In addition, vegetative samples are necessary since the species is a poor seed producer. Vegetative samples take more effort to handle, than available seed. A collecting day typically results in about 5 sites and 10 accessions. Since vegetative reproduction is the primary form of reproduction in the wild, the resulting number of genotypes at a site can be very low, making the genetic variability low. Therefore, sampling should focus more among areas than within areas. The geographic area was intentionally restricted by latitude and climatic zone in order that plants collected would hopefully have similar anthesis dates to facilitate crosses.

3. Digital imaging

Most breeding efforts in perennial grasses start out with a spaced plant nursery of many genotypes, followed by evaluation with subjective ratings because of the high numbers of individuals, then crossing of selected plants in various combinations, and finally, evaluation of the progenies for the best combination (Allard, 1960; Vogel and Pedersen,1993)

Lack of rapid establishment by a turfgrass can lead to problems for the duration of the planting (Richardson et al., 2001). Weed invasion, or recovery from damage, such as from divots, can affect turfgrass cultivar performance. Studies have demonstrated not only variability between human assessors in visual estimates (Shelton, 1988; Richardson et al., 2001), but also estimates by a single experienced assessor (Olmstead et al., 2004). Differences in cover are easily detected at small amounts of cover, but at high amounts of

cover, visual estimates may not detect the same difference (Coren and Girgus, 1978). The Weber-Fechner law approximates visual estimates being the least accurate at around 50% cover, with accuracy increasing at the extremes (Lindow and Webb, 1983).

Quantitative measurements in turfgrass cover estimates are limited because of the large amounts of time and labor, therefore, subjective visual ratings are used instead (Richardson et al., 2001).

Advances in digital imaging offers an alternative to subjective ratings and costly quantitative data. Digital cameras have dropped in price in the last decade at the same time their technology has advanced. Software has become readily available for analyzing digital images. An instantly stored, retrievable image can be quantitatively analyzed for color and area (Richardson et al., 2001; Karcher and Richardson, 2003; Olmstead et al., 2004). Plant traits that are discerned by color and area can be measured for variation. Automation packages in the software allow rapid analysis of large numbers of images. Conversion of digital images into numbered data allows determination of significant differences among treatments or individuals.

Recent studies of the digital imaging of plants have found very high correlation with subjective rating(Richardson, 2001; Karcher and Richardson, 2003; Olmstead, 2004). Often, the ranking in treatments has been the same, but the experimental error is higher in the subjective rating. A digital image can provide multiple trait recording, and an image can be recalled for further analysis. Digital images are accurate, and overall, less costly and time consuming. Digital image analysis (DIA) has been used to measure herbaceous cover (including turfgrass), forest canopies, leaf diseases, sized leaf shapes, and different colored vegetation (Olmstead et al., 2004).

Spaced plants are an ideal setup for using DIA. The traits of plot fill-in, gaps, and shoot density, which are difficult to measure traits, lend themselves to DIA. Use of DIA for in-the-field genetic plant screening is absent in the literature, even though it has great potential. Cover measurements are differentiated by specific colored pixels making up the total pixel image. Shoot density can be determined by early emergence when color contrast is strong enough, or with the technique of Yonekawa et al. (1996), using simple dimensionless shape factors. Shape factors in digital analysis are an evolving area, and emerging shoots, because of their initial simple shape, lend themselves to this research. A commercially available and versatile software, SigmaScan Pro 5.0 (SPSS, Chicago,IL), is already being used broadly in the scientific community, and has been used successfully in measuring turfgrass cover (Richardson, et al, 2001; Karcher and Richardson, 2003).

CHAPTER III

Material and Methods

Accession Nursery

"A" lines were obtained by Dr. David Kopec, School of Plant Sciences, University of Arizona, and Dr. Tony Koski, Department of Horticulture and Landscape Architecture, Colorado State University, along the Front Range. Non-lettered lines were collected by Dana Christensen, Colorado State University, in 1998. List of accessions and coordinates are in Appendix I. Maps are in Appendix II. All materials were vegetative and from 4 different geographic regions:

Great Basin- low desert of northern Utah and Nevada	(20 accessions)
South Dakota-east of the Black Hills of South Dakota	(22 accessions)
Nebraska-central Nebraska	(16 accessions)
Front Range-east of the Front Range in Colorado	(100 accessions)

In the first week of August of 1998, 10 x 10 cm plugs were planted on 4.6 meter centers in a 2 replicate nursery at the Horticulture Field Research Center, Fort Collins, Colorado. The frost-free growing season lasts from May 20 to September 20. Maximum winter lows at the site are -32^0 C (Center data). The soil is a Nunn clay loam, which is very uniform and deep. The water table is at 4 to 7 meters.

Plugs were furrow irrigated immediately after planting. After establishment, irrigation was applied once annually in mid-May, before flowering. Soil nitrogen tested at 180 kg per ha, coming out of alfalfa (*Medicago sativa*, L.). Length of rhizomes from the center of the plug was measured on north-south and east-west axes on August 15, 1999; area of spread was calculated as an ellipse (area=π(axis a)(axis b) since axes tended to be

unequal. In 2000, flower head spikes were counted in a 30.5 x 30.5 cm square on June 19, 20, and 21. Over 7 years, 22 accessions (14%) never formed head spikes.

In 2000 and 2001, half of each plant was mowed to a height of 7.5 cm with a rotary mower whenever the canopy reached approximately 12 cm. Days from 1 January 2000 to first flower was noted from mid May through mid June. Seed was hand-harvested on a 30.5 cm square, from July 28 to August 4, 2000. After harvest, seed heads were placed in paper sacks, air dried at ambient temperature, hand threshed, weighed, and seed lengths were measured. Leaf canopy height was measured with a height board on September 10, 2000 and July 23, 2001. Several (3-4) of the tallest female spikes were measured with a ruler and averaged for height on July 23, 2001. Gap was estimated on September 7, 2000 as percent area of plot that never filled in with shoots or rhizomes. Shoot density was calculated from number of shoots counted within a 5 x 10 cm grid from November 6 through November 13, 2000 on the mowed half of the plot.

Plants were evaluated for leaf rust using a modified leaf rust rating of 0, 1, 5, 10, 15, 20, 25 percent leaf area affected (see Figure 6). Leaf rust (*Puccinia aristidae* Tracy) was identified by Dr. Bill Brown, Department of Biological Sciences and Pest Management, CSU, and confirmed by Dr. S. Singh, Department of Plant Pathology, Kansas State University. Ratings were made on September 7, 2000, and September 17, 2001. Natural rust pressure appeared high, with susceptible plants uniformly scattered throughout the nursery.

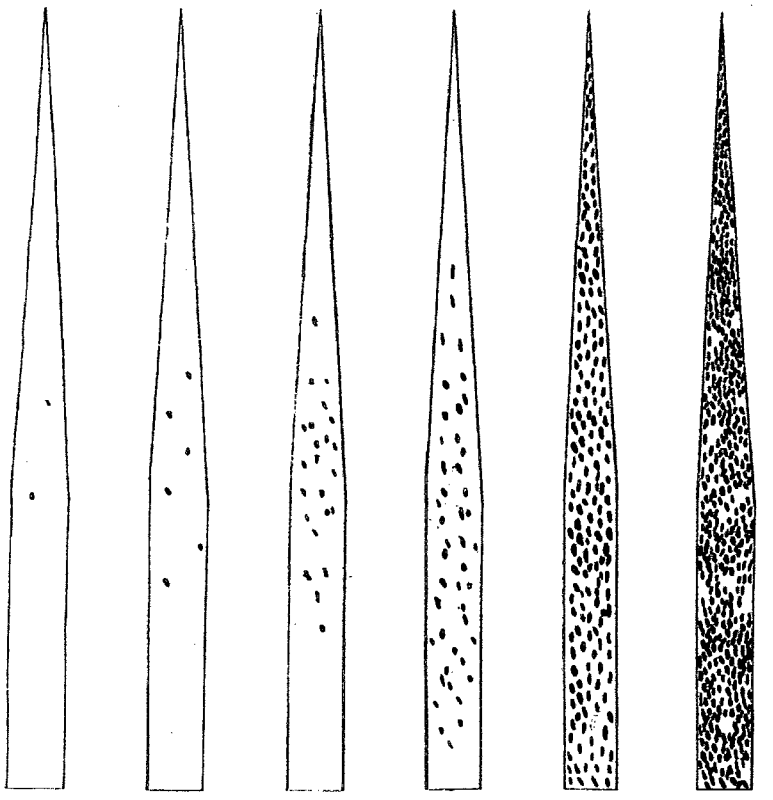

Figure 6. Drawing showing the disease classes as defined by percent leaf area affected by uredia and telia on *Distichlis spicata* var. *stricta*. The causal organism is *Puccinia aristidae.* Adapted from the key ' Leaf Rust of Cereals' by James (1971).

Progeny Nursery

Parents were selected from the accession nursery based on the highest values of each individual phenotype from the selection index = (standardized spike numbers) + (standardized shoot density) - (standardized height) - (standardized rust rating). Standardization = (Y- trait mean)/ standard error. Y is the individual plant trait value. Selection based on the selection index resulted in choosing a subset of the top 26 out of 158 accessions which consisted of Front Range types collected in an area with less than 64 km in separation between the most distant accessions. The subset was considered a random mating population for genetic interpretation. The subset represents turf-type saltgrass plants.

The Front Range as a region had favored values for the 4 traits over the other regions, hence all parents came from the Front Range (see Table 44). The parents had consistently high values for spike numbers and shoot density in the selection index.

The number of parents, 26, was advocated (actually 25) by M. D. Hayward, Welsh Plant Breeding Station, in a discussion I had with him in 1975 about the number of parents to include in a long term recurrent selection program. His work with breeding forages resulted in empirical evidence that little inbreeding occurred with 25 or greater number of parents. Theoretical calculations showed higher rates of inbreeding, but Hayward believed the number of chiasmata formed explained the maintenance or release of variability and lack of inbreeding in ongoing populations that he and other researchers have reported in forage species (Melton et al., 1969).

Half-sib progeny (termed 'improved' hereon) were formed under random mating in an isolated polycross (2 replicates of 1 meter^2 plots of 14 females each surrounded by 2 replicates of .3 meter^2 plugs of 12 males) and seed harvested to form 14 maternal half-sib families. Seed was germinated in December, 2002, and grown on in a greenhouse to ensure survivability as a transplant. Plugs of 10 x10 cm were planted into the field in the first week of August of 2003 into a Nunn clay loam. The soil is deep and the water table is at 7 meters. Nitrogen was applied annually in mid May at a rate of 90 kg/ha for 3 years. Plants were sprinkler irrigated at flowering with 13 cm water, annually. The progeny test was a randomized complete block design with 6 replications, and 14 maternal half-sib families, with 10 plants per plot, and plants on 1.8 meter centers. Clonal material of the 26 parents and 26 random checks (checks termed 'native' hereon) from the Front Range accession group was replicated 6 times throughout the nursery; these were also on 1.5 meter centers; each replicate had 52 plants The native group included the parents A123, A137, and A60 as random checks. All measurements were on an individual plant basis. Each plant was burned the first week of April 2004 with a handheld propane weed burner, to remove the previous year's dead growth. Thereafter, the previous year's growth was removed by rotary mowing at 2.5 cm the first week of April. During the growing seasons after 2004, half of each plant was mowed to maintain a height of 7.5 cm in order to count shoot density (and record shred and regrowth that are not part of this study). Data for rust was taken October 30 to November 10, 2004, and again September 1 to September 20, 2005. Canopy height was measured October 11 to October 12, 2004, and again August 23 to August 24, 2005. Seed yield was determined from a hand harvest area of 30.5 x 30.5 cm from August 1 to August 26, 2005, and again from July 31 to

August 30, 2006. Seed heads were placed in paper bags, air dried at ambient temperature, hand threshed and weighed. Shoot density was calculated by counts within a 5 x 20 cm grid from July 17 to July 27, 2005, and from July 10 to July 21, 2006, on the mowed portion of the plant.

Digital Imaging

Spread of all plants in the progeny nursery (including parents and natives) was measured digitally from September 14 to September 17, 2004. Project personnel visually estimated spread during the same time, based on percent area covered in the 1.5 meter square plot. An Olympus C-5000 digital camera (Olympus America Inc., Melville, NY), mounted 2.44 meters above isolated spaced plants and 2.29 meters on center (Figure 7 and Figure 8), was used. Bubble levels on the camera and rig kept the camera plane parallel to the soil surface. The camera was centered by measuring off the rig and the original plug. An infrared remote triggered the camera. Photographs were made on cloudless days between 10 am and 3 pm to avoid shadows. Image size was 1600 x 1200 pixels. Shutter speed was 1/400 second with an aperture of F 2.8. Sigma Scan Pro 5.0 (Systat Software, Inc., Point Richmond, CA) was used to analyze photos. Hue was set 30-100 (of 0-255) and saturation 22-100 (of 0-100). These values were adjusted ± 4 if it was obvious leaves were not being picked up in specific shots. Isolated weeds were taken out of the image with Adobe Photoshop CS2 (Adobe Systems Inc., San Jose, CA). The image database was analyzed at one time, using a macro obtained from Dr. Douglas Karcher, Department of Horticulture, University of Arkansas.

Figure 7. Portable rig for overhead digital photographs. Measurements were made on unmowed, first year growth of spaced plants.

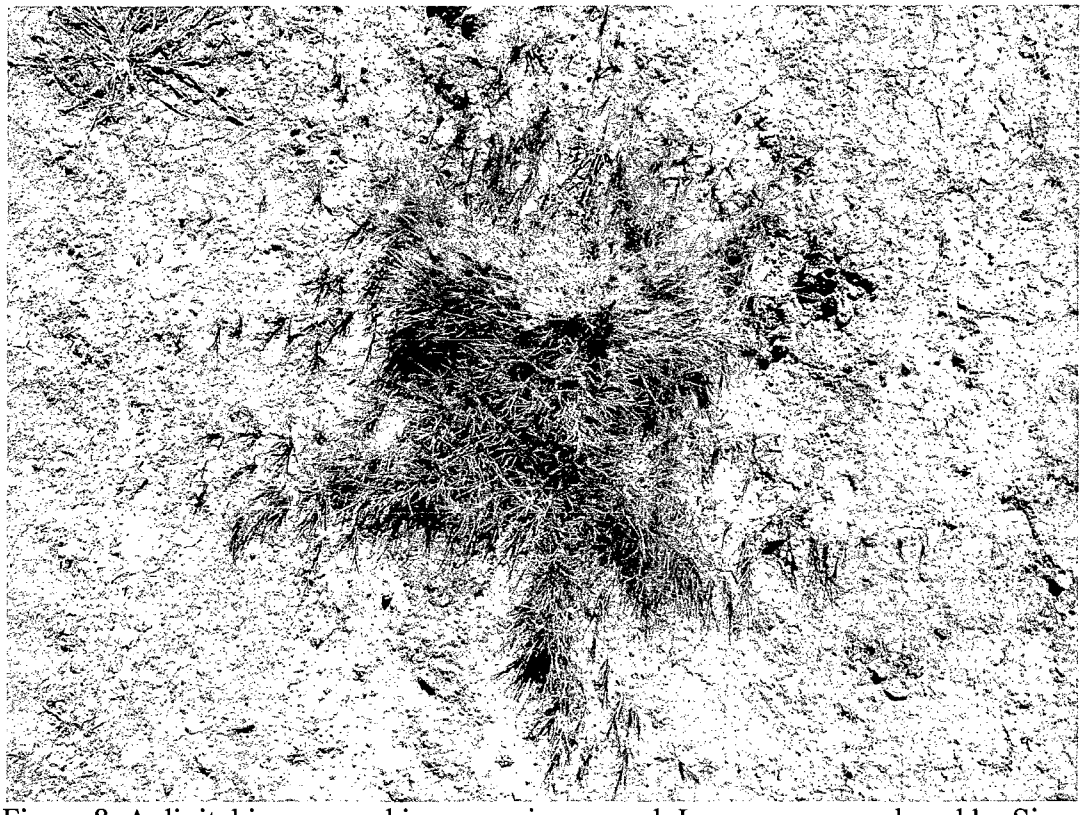

Figure 8. A digital image used in measuring spread. Images were analyzed by Sigma Scan Pro 5.0.

Statistical Analysis

Statistical Analysis Systems version 9.2, SAS Institute, Cary, NC, USA, was used to perform the analyses. Several SAS related references were used to construct code and graphs, and for interpretation (Cartier, www.sas.com/rnd/datavisualization/papers; Littell et al., 2006; and Schlotzhauer and Littell, 1997).

1. Response to Selection and Heritability Estimates

Response to selection was calculated as the difference in mean trait value between native and improved populations. Proc Mixed REML with population as a fixed effect, was used to obtain tests of significance between the 2 populations (native, improved), estimates of means, and confidence intervals. Levene's test showed non-homogeneity of variances for height and shoot density (α=.05). Height was transformed by $\log_n$ (log e^x=x). Shoot density was transformed by the square root transformation. Transformations corrected problems with variance differences.

Rust data residuals did not show normality, and transformations with log, square root, or quasi likelihood (examples pg 207, Proc GLIMMIX, SAS v 9.1), nor their modifications, nor a suite of transformations (SAS Stat Studio 3.1) corrected normality. The Kruskal-Wallis test was performed on the 14 family data due to non-normality.

For heritability estimates, the models were balanced with all effects random (Nguyen and Sleper, 1983; Nyquist, 1991). Confidence intervals of heritability estimates require balanced data (Knapp and Bridges, 1987). Proc Mixed, Method = Type 3 was used to determine positive and negative variances, covariances, and F tests of significance, and to confirm proper variances (print out of theoretical expected mean

squares) and divisors (print out of the theoretical error term as divisor in F-test) were used by SAS. Best Linear Unbiased Predictions (BLUPs) estimated female performance. REML covariances and BLUPs were identical to Method = Type3. Broad sense heritability was calculated from the clonal parent phenotypes included in the progeny nursery. Heritability calculated by parent-offspring regression used parental phenotypes in the progeny nursery. Heritability and confidence intervals for half-sib family analysis in a split block in time were calculated according to Knapp and Bridges (1987). No correlation matrix was used, since there were only 2 points in time. Heritability and confidence intervals for half-sib family analysis in a randomized complete block were calculated according to Knapp et al. (1985).

When the effect of years was insignificant ($p>0.05$), or when the analysis was done within a year, then expected mean squares are :

Source of variation	df	Expected mean square
Replications	r-1	
Families	f-1	$\sigma_w^2 + n\sigma^2 + rn\,\sigma_f^2$
Families x Rep	(r-1)(f-1)	$\sigma_w^2 + n\sigma^2$
Individuals within plots	rf(n-1)	σ_w^2

Where:

r = number of replications
f = number of families
σ_f^2 = family variance
σ^2 = error variance (plot to plot environmental variance)
σ_w^2 = within plot variance

and heritability on a family selection basis:

$$h_f^2 \simeq \frac{\sigma_f^2}{\sigma_f^2 + \sigma^2 \text{ /r} + \sigma_w^2 \text{ /rn}}$$

(above section adjusted for one year, one location; adapted from Nyquist, 1991)

When years are significant ($p<0.05$):

Source of variation	df	Expected mean square
Replications	r-1	
Families	f-1	$\sigma_d^2 + \text{n } \sigma_c^2 + \text{y}\sigma_w^2 + \text{ny } \sigma_a^2 + \text{rn } \sigma_{fy}^2 + \text{rny } \sigma_f^2$
Families x Rep	(r-1)(f-1)	$\sigma_d^2 + \text{n } \sigma_c^2 + \text{y}\sigma_w^2 + \text{ny } \sigma_a^2$
Individuals within plots	rf(n-1)	$\sigma_d^2 + \text{y } \sigma_w^2$
Years	y-1	
Families x Years	(f-1)(y-1)	$\sigma_d^2 + \text{n } \sigma_c^2 + \text{rn } \sigma_{fy}^2$
Reps x Family x Years	(r-1)(f-1)(y-1)	$\sigma_d^2 + \text{n } \sigma_c^2$
Residual	rf(n-1)(y-1)	σ_d^2

Where:

r = number of replications
f = number of families
y = number of years
σ_a^2 = whole plot effect in space(families)
σ_c^2 = subunit effect with respect to space and time
σ_d^2 = random error effect within plot and time

and heritability on a family selection basis:

$$h_f^2 \simeq \frac{\sigma_f^2}{\sigma_f^2 + \sigma_{fy}^2/\text{ y} + \sigma_a^2 \text{ /r} + \sigma_w^2 \text{ /rn} + \sigma_c^2 \text{ /yr} + \sigma_d^2/\text{ yrn}}$$

(above section adjusted for one location; adapted from Nyquist, 1991)

Heritability from parent-offspring regression using only maternal parents is defined as:

$$h^2_{PO} \simeq 2\ b_{PO} = 2\ COV(P,O) / \sigma^2_P$$

Where:

b_{PO} = slope of the regression line

$COV(P,O)$ = covariance between parent and offspring

σ^2_P = phenotypic maternal parental variance

Phenotypic means were used in regression from alternate generation-year when possible. (above section from Nyquist, 1991).

2. Detection of Major Locus

Fain (1978) examined the relationship between the phenotypic mean of a sibship and the within-sibship variance. Segregating sibships are, in general, characterized by intermediate means and higher variance among sibs when a major locus is present (segregating). The relationship for several genetic models is defined by the regression model:

$$Y = A + B_1 X + B_2 X^2 + B_3 X^3$$

Where Y is the within-sibship variance (or $\log_n$ of for variance and normality transformation), and X is the within sibship mean. The regression weights (B_i) are not significantly different from 0 in a random sample of sibships under polygenic inheritance. However, a single gene with major effect will inflate any one or more B_i depending on

dominance, gene frequency, and/or sex linkage. This study uses the full quadratic model (i.e. absent the cubic term), which is sensitive to unequal gene frequencies and/or dominance.

3. Heterosis

Heterosis was calculated as described by Hallauer and Miranda (1988) when means between parents and progeny were significantly different:

$$H = 100[(Mean_{progeny} - Mean_{parents}) / Mean_{parents}]$$

4. Realized Heritability

Realized heritability was calculated according to Lynch and Walsh, 1998:

$$h^2 = R / S = \frac{\text{progeny mean-native mean}}{\text{parent mean-native mean}}$$

5. Accession Evaluation

Accessions A1-A10, A13, A32, A34, A39, A43-A61, A65, A75, A137, and A138 were removed from accession evaluation since they were selected in the wild for short height or under mowed conditions. The remaining lines were considered random selections.

Kruskal-Wallis and Wilcoxon rank sum were non-parametric tests used on rust data due to non-normality of original rust data and their residuals. Kruskal-Wallis tested for significant difference over all regions if rust scores differed among regions. Wilcoxon tested paired comparisons of rust scores from 2 regions.

Proc Glimmix was used for analyzing the remaining traits in the accession study to determine differences in trait values based on region of origin of accessions. A benefit of

Proc Glimmix is the display of mean-mean scatter plots to graphically interpret differences in confidence intervals among region of origin effect on accession traits.

6. Digital Imaging

The native, parent and progeny populations had different sample sizes for camera scan spread data. Levene's test showed different variances (α=.05). Square root transformation corrected variance differences.

SAS code to estimate genotypic correlations and their standard errors from a single environment experiment with a randomized complete block design and a one-way classification of genotypes was taken from Supplement 3 to "Estimating Genotypic Correlations and Their Standard Errors Using Multivariate Restricted Likelihood Estimation with SAS Proc MIXED" (Holland, 2006).

CHAPTER IV

Response to Selection and Heritability Estimates

Leaf Rust (*Puccinia aristidae* Tracy)

The difference of the improved population mean-native population mean was -1.2% in leaf rust area. This seems a small response to selection after breeding resistant parents. The realized heritability calculated from means was very low, 0.19.

Figure 9 and Figure 10 graph the response to selection for rust resistance in 2004 and 2005, respectively, for the native population (25 random accessions from the Front Range) and improved population (topcross progeny of 26 parents selected for rust and other traits). The important classes are the 20% and 25% leaf area affected, as these are visually unaesthetic in the field, turning the turf brownish or blackish as early as the first week in August. The graphs are similar for both years with the exception of 20% and 25% classes. In 2004, the 25% class for the native population had a higher percent of its population in this class than the improved population. In 2005, with overall fewer plants observed infected in the nursery, the 20% class for the native population had a higher percent of its population in this class than the improved population. The higher percentage of the native population in the 20% and 25% classes compared to the improved population in these classes may indicate selection for rust resistance was effective. The shift in the high number of plants in the native 25% class in 2004, to the native 20% class in 2005 could be attributed to lower rust pressure in 2005 that came on later, and was less severe (personal observation).

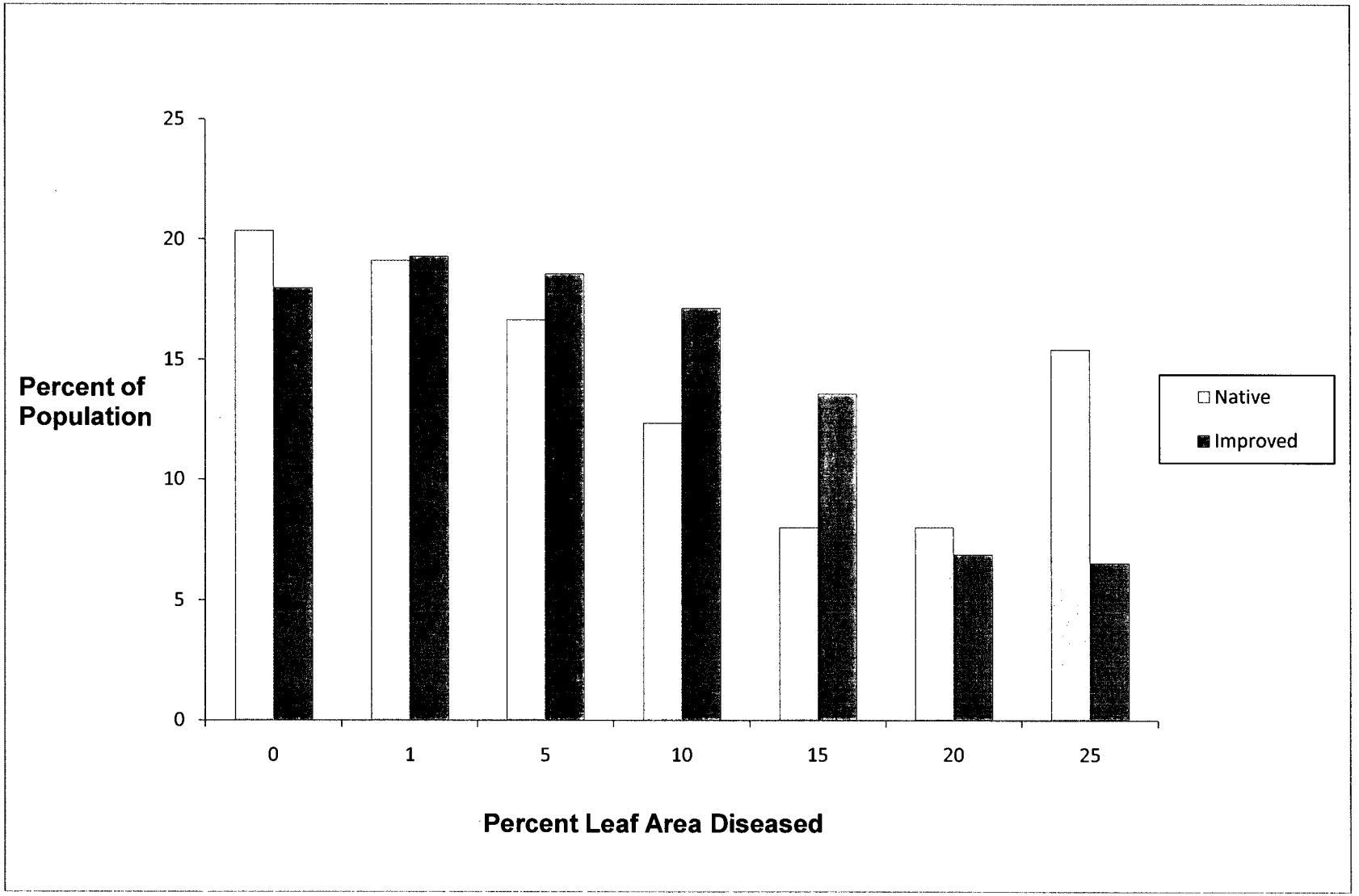

Figure 9. Percent leaf area diseased in 2004 at the Horticulture Field Research Center. Native population = 156 plants, improved population = 840 plants.

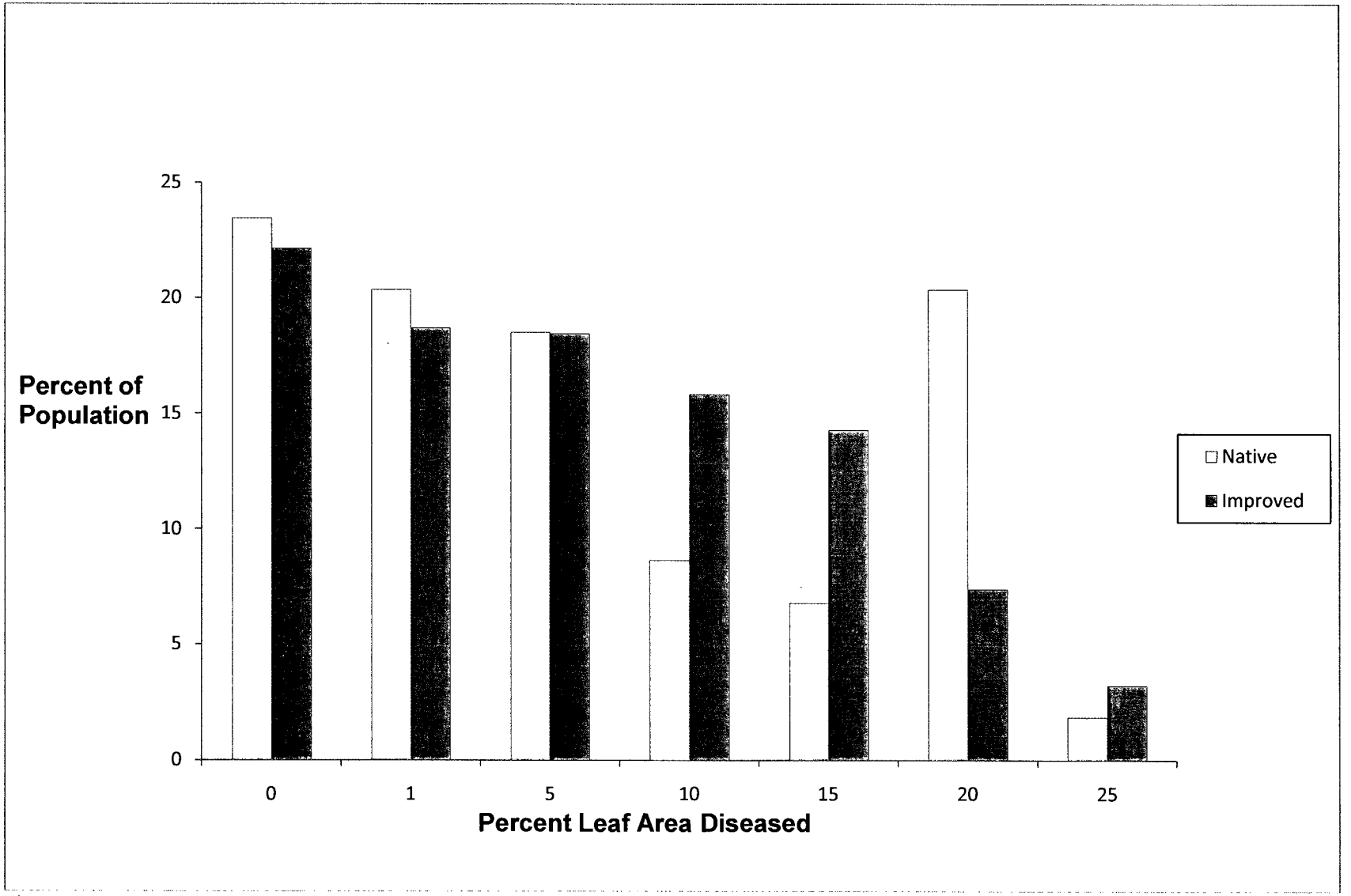

Figure 10. Percent leaf area diseased in 2005 at the Horticulture Field Research Center. Native population = 156 plants, improved population = 840 plants.

Rose-Fricker et al. (1986), found a normal distribution for stem rust susceptibility in a population of perennial ryegrass. However, in the present study, the rust data did not show normality, but more importantly, the studentized residuals did not show a normal distribution (Figure 11). Data were run through a suite of transformations (SAS Stat Studio 3.1) for normality. Square root gave the best visual transformation for residuals distribution (Figure 12). Skewness of the original data changed from 0.77 to 0.29, but kurtosis decreased from -0.70 to -1.26. However, the Kolmogorov-Smirnov test showed the original or any transformed data did not fit normality. Resistance to transformation

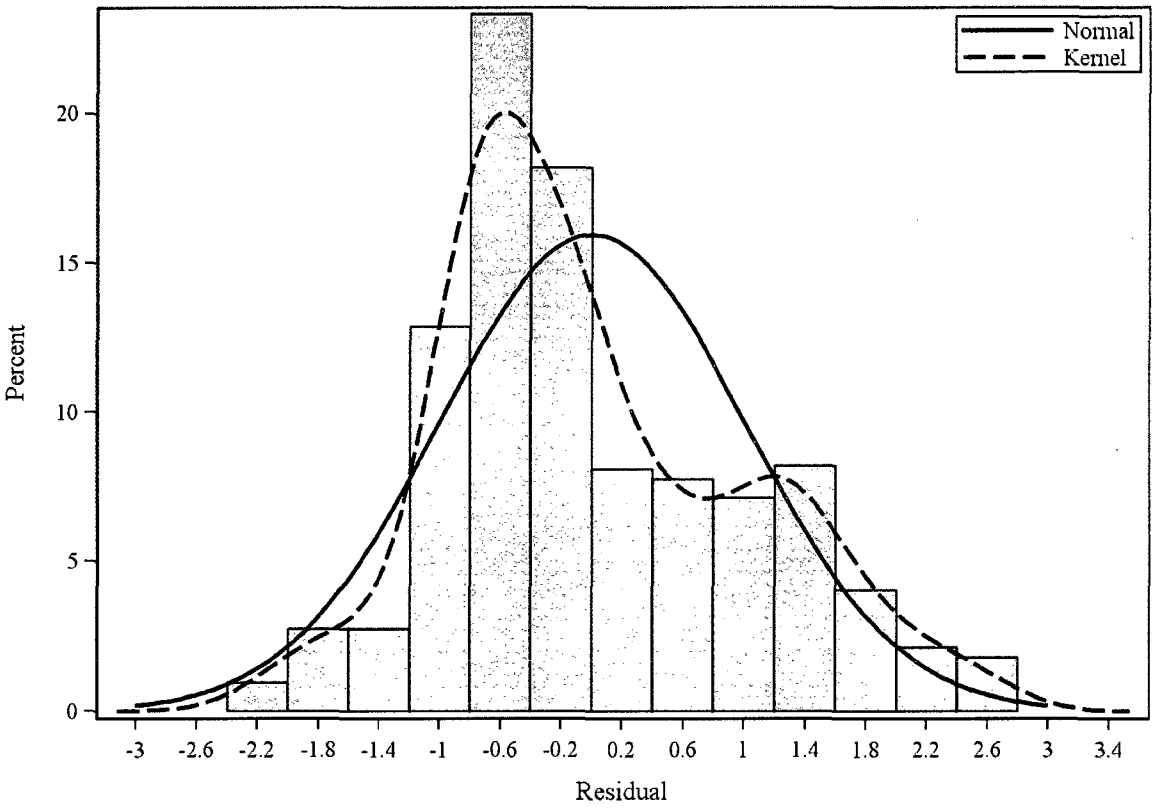

Figure 11. Distribution of studentized residuals of progeny rust data of 2004. Data of 2005 were similar. Native population showed similar distribution. Kernel smoothness is estimated based on mean square error using generalized cross validation. Cross validation leaves out points (x_i, y_i) one at a time and computes the kernel regression at x_i based on the remaining n-1 observations. The fitted normal curve is the probability density function..

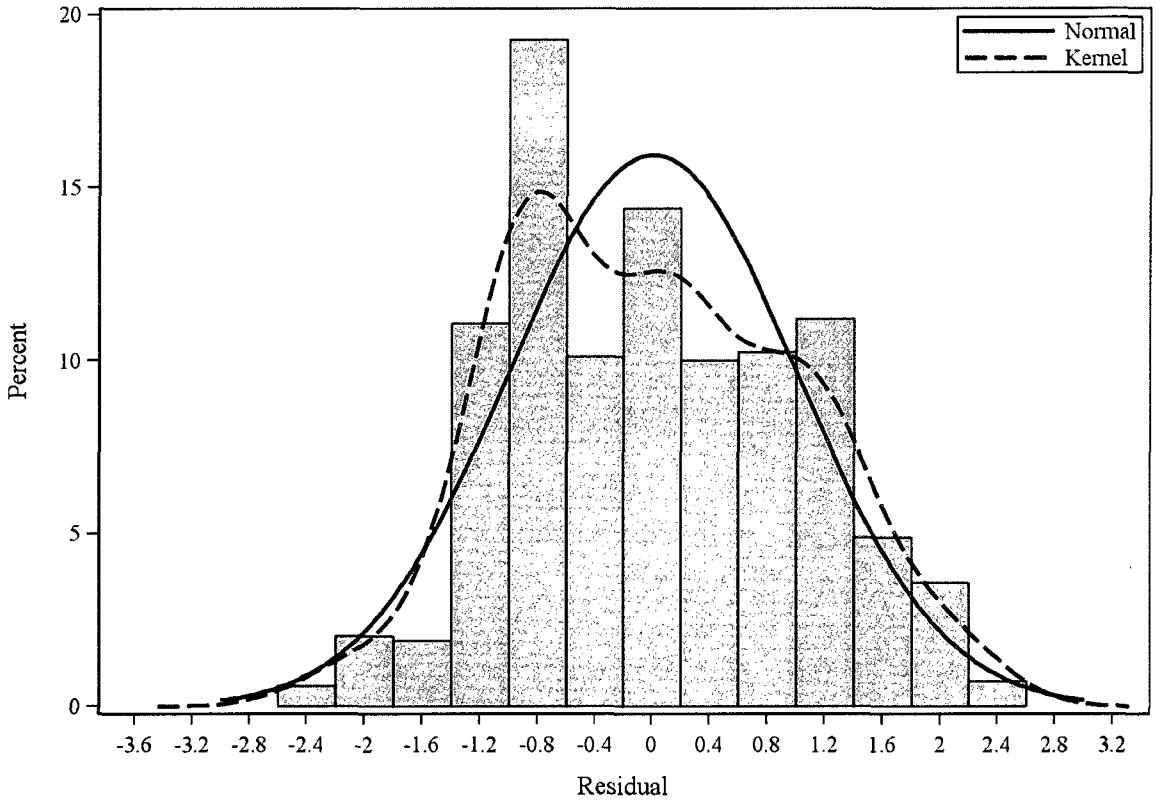

Figure 12. Distribution of studentized residuals of square root transformed rust data from progeny 2004. Kernel smoothness is estimated based on mean square error using generalized cross validation. Cross validation leaves out points (x_i, y_i) one at a time and computes the kernel regression at x_i based on the remaining n-1 observations. The fitted normal curve is the probability density function.

can be attributed to major locus effect (Fain, 1978).

Fain's test was run with the transformed data, presuming distortion to normality may be due to a major locus segregating and not to polygenic (many small effects) or environmental effect. The quadratic regression of the offspring variance on sibship mean phenotypic value and its square was highly significant, with F= 13.51, Pr >F =.0011 (Appendix III). The t test for the coefficient of the quadratic term, b_2, was significant with Pr >t = .0014. These results indicate a major gene was segregating.

When applying this method to data, the cause of heterogeneity of variance, other than a major locus, must be thoroughly examined. In this study, the expression of the phenotype in the populations (progeny or native) is so strong that visual confirmation in plots of more than a single distribution is convenient.

The 2 modes appearing in the distributions are similar to major gene segregation found in other traits in other plant species (Allard, 1960, Briggs and Knowles, 1967, Kearsey and Pooni, 1998). Supposing there are 2 distinct visual plant responses to leaf rust rather than a single normal distribution, a logical separation of the data would be to lump phenotypic frequencies of the 15% leaf area class and lower percent classes together as 'resistant', and the 20 and 25% classes together as 'susceptible'. This corresponds to a visual partitioning discernible in the field (see Materials and Methods), and also is representative of the bimodal distribution in the bar charts. The blending of the peaks in the graphs can be due to environmental effects (Figure 13).

The frequencies of resistant to susceptible then become, in 2004, 77:23, and in 2005, 78:22. These ratios are very close to 3:1 segregation expected in a single gene pair with complete dominance (Allard, 1960, Kearsey and Pooni, 1998).

To test this theory, Pearson's Chi-square Goodness of Fit Test was performed on the native population (unselected for resistance) for each year of data (less disease in 2005). The null hypothesis is the research hypothesis that data conform to a 3:1

Table 1. Chi-square for testing a 3:1 segregation of resistant to susceptible. The probability, p, of seeing a greater chi square is high indicating the data does not reject a 3:1 ratio. Type I error is set at $\alpha = 0.05$

Year	Observed χ^2	Critical χ^2	p for observed χ^2
2004	0.13	3.84	0 .72
2005	0.53	3.84	0.46

segregation. The results in Table 1 indicate that a 3:1 segregation expected with a single gene pair with dominance is not rejected.

Type II error is controlled by the low number of different segregant classes (2) and the high number of plants used in the test (Ott and Longnecker, 2001).

Although testing of a major gene is done in experimental populations, the consistency of the bimodal distribution in progeny and accession nurseries (see Chapter 4, Accession Evaluation) across years warrants further examination of the segregation ratio.

If a single gene pair with complete dominance is the case, then parents that were selected as resistant will either be AA or Aa. If the group they were selected from (Front Range population) was random mating, with respect to rust genes, then the percent AA and Aa types in the parents are derived as:

Front Range population genotypic frequencies	0.25 AA	:	0.50 Aa	:	0.25 aa
	⇩		⇩		X selected against
Parent population	0.33 AA		0.67 Aa		

Families should segregate out with 0.33(14 females) ≈ 5 families which show all progenies as having resistance, with the remaining families segregating out with:

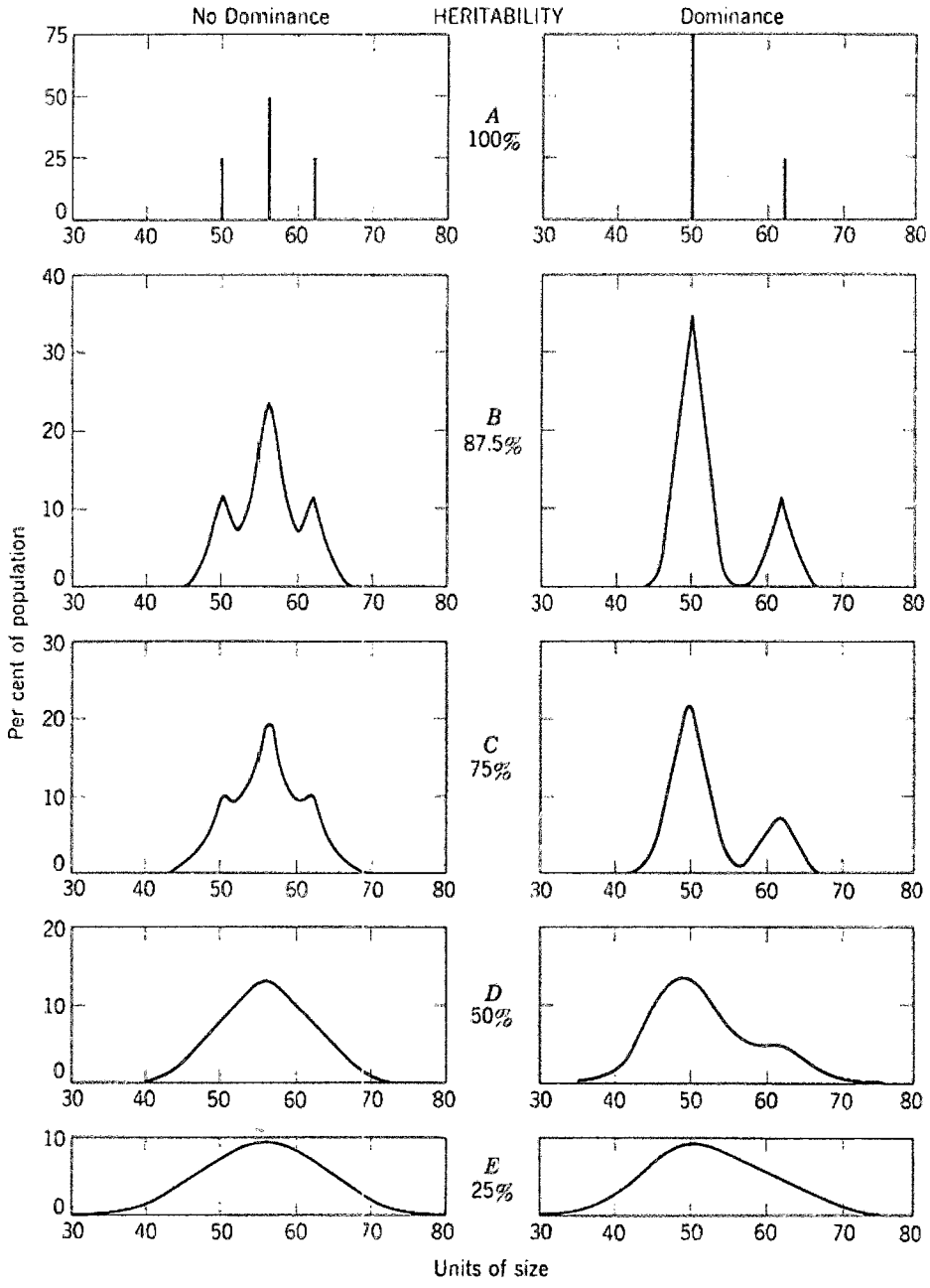

Figure 13. Single gene inheritance. Left graphs : no dominance, right graphs: full dominance. Descending graphs represent increasing environmental effect, where the bottom graphs depict 75% environmental effects, 25% heritability (Allard, 1960, permission for reproduction by Gillian Allard).

Polycross heterozygous females: gametes and frequencies

A	a
0.5	0.5

Polycross heterozygous and homozygous males: gametes and frequencies

Progeny genotypes and their frequencies

		AA	Aa
A	0.67	0.34	0.33
a	0.33	Aa 0.16	aa 0.17

Therefore the progeny in segregating families should show phenotypic frequency ≈ 83% resistant to 17% susceptible.

To substantiate this, counts in the 20% and 25% classes for families were added to get the genotype aa frequency % and presented in Figure 14 A. All families show some segregation. The mean across yearly data (not shown) for families A61-1, A50-1, A53-1, and A24-1 is 63% resistant to 37% susceptible. The mean across yearly data (not shown) for A138-1, A18-1, A34-1, 84-1, A37-1, A35-1, and A97-1 is 93 % resistant to 7% susceptible. A123-1, A126-1, and A21-1 fall in between the other two parent groups. Segregation frequencies do not follow those postulated.

Average rust rating for maternal parents grown alongside progeny was graphed in Figure 14B. In comparing Figure 14A and Figure 14B, the association in diseased

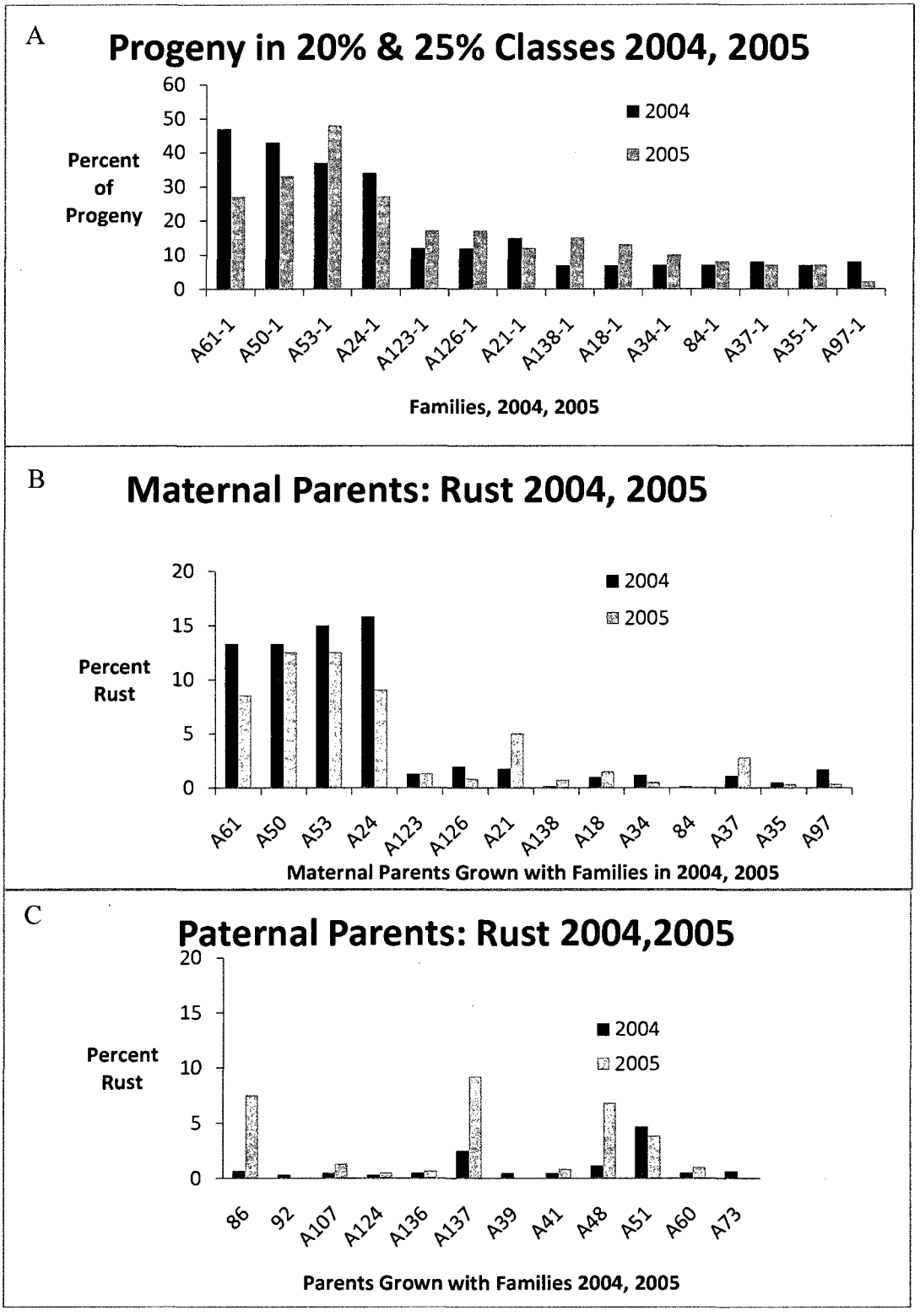

Figure 14. Leaf rust in A. progeny, B. maternal parents, and C. paternal parents in 2004 and 2005 at the Horticulture Field Research Center.

families with their diseased maternal parents is striking. Families showing high amount of disease come from maternal parents showing relative high amount of disease in 2004 and 2005. Large loss in resistance did not appear in any of the paternal parents as seen in Figure 14C. Since each female parent was randomly pollinated with a composite of resistant males, the disease pattern expression in the progeny is attributed to the female contribution.

Going back to the accession nursery, from which the parents were first screened for rust in 1999, 2000, and 2001, average rust was graphed in Figure 15. During these years the same maternal parents average leaf rust scores were 0% or under 2% under heavy rust pressure .

A possible explanation is that infection was not complete in the accession nursery (where screening took place) and A-61, A50, A53 and A24 were misses, but they were susceptible. Alternatively, the prevalent rust race(s) had changed from the early years, and its virulence gene(s) was able to infect and produce rust pustules in A-61, A50, A53, and A24 (and their progeny), since the plants would not have resistant genes to the new race(s). The remaining parents appear to have resistance to the new race(s), and the presence of low levels of pustules would indicate horizontal resistance, that is, infection occurs, but pustule formation is suppressed (Agrios, 1997). A third alternative is new race(s) may not be prevalent, and instead, horizontal resistance may be breaking down in the 4 families due to change in environment (Agrios, 1997).

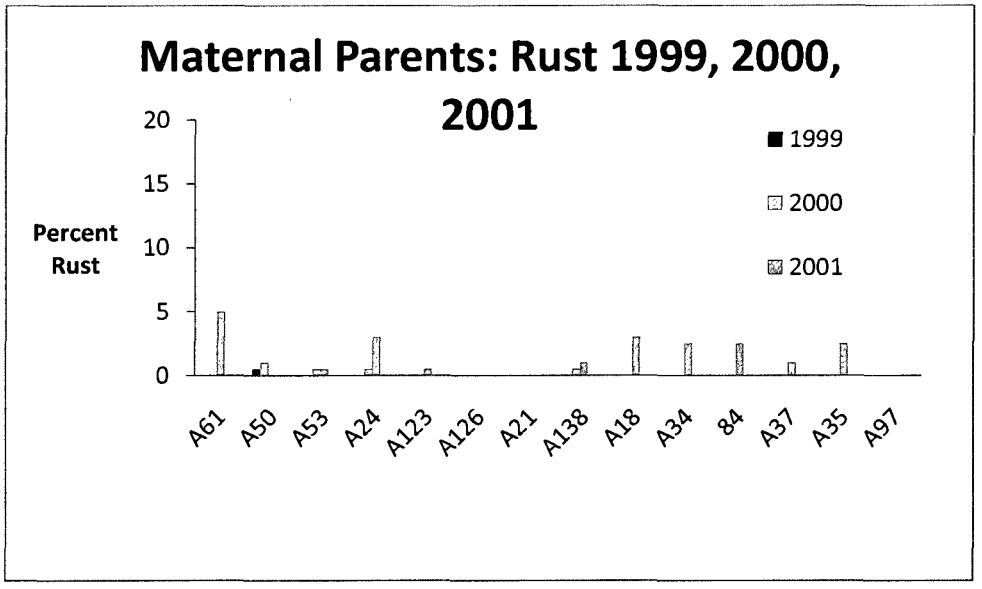

Figure 15. Leaf rust in maternal parents in 1999, 2000, and 2001 at the Horticulture Field Research Center.

It is still possible that the dominant single gene model could apply. Progeny in susceptible families segregate at 63:37, which is close to 67:33 if the male parent genotypes are represented as expected, 33 AA: 67 Aa. The chi square on the observed response is 3.02, and does not exceed the critical value of 3.81 when $\alpha = 0.05$ for Type I error. Here, $p = 0.082$. On this basis, the model fits. Confirmation would come by crosses of known genotypes. For now, unknown plant genotypes, prevalence of different rust races and their genotypes, and escapes from relying on natural infection can create error in calculated frequencies. The large association between females and their progeny is evidence in favor of a major gene effect.

Once genotypes are known through crossing and segregation studies based on accurate inoculation, they can be used in breeding resistance. Known resistant and susceptible genotypes can also be crossed to develop a segregating population, which can be analyzed through the use of molecular markers to find a correlation with

electrophoretic gel bands of the amplified sections of DNA. The markers should be tightly linked to the rust gene. The markers must be polymorphic, i.e. the band pattern should change when comparing homozygote resistant to homozygote susceptible. In order to detect the heterozygote carrying the recessive in this study, a codominant marker should be used such as simple sequence repeat (SSR). Sampling of successfully inoculated plants with the prevalent rust population(s) must be accurate. An inoculation chamber and a developed inoculation procedure can aid this. Once the markers have been determined, DNA analysis can proceed on the breeding population. Culling plants carrying the recessive for susceptibility would allow space in the greenhouse and nurseries for many more homozygote resistant types. However, it takes much time and resources to identify the SSRs, and designing and evaluating the primers. The costs should be weighed against the benefits for marker assisted selection.

Monitoring rust races could also be accomplished through the use of molecular markers. Such studies also can determine whether the variety of *Puccinia aristidae* in saltgrass, and its prevalent races, are the ones whose basidiospores infect and cause red rust in spinach. Just because saltgrass and spinach are known alternate hosts to *Puccinia aristidae* does not preclude other hosts causing infection in spinach (Milgroom and Peever, 2003). In this study, for a point in time, the maternal parents are an accurate predictor of progeny response to virulence.

Fungi which have sexual reproduction, such as *Puccinia*, produce an abundance of genetic diversity (Milgroom and Peever, 2003). Several hundred races of stem rust of cereals (*Puccinia graminis*) are known to date, and new ones appear every year (Agrios, 1997). It is assumed this happens in other rusts (Agrios, 1997). Several or more races can

be causing the pattern of disease seen in Figures 14 and 15. In saltgrass, continued selection for rust resistance will possibly contribute to durability of resistance across time, by incorporating additional host resistant genes, if available, as new rust races appear. Durability also is increased by retaining older lines whose genes are still effective against new races.

Since the data were not normal, Kruskal-Wallis Tests were performed by year, to see if families had a similar reaction to disease. In 2004, chi square was 183.71, 13 df, and $p = < 0.0001$. In 2005, chi square was 118.07, 13 df, and $p < 0.0001$. This indicates some families were resistant, and some families were susceptible. A24-1 was the only family that was different across years with a $p = 0.01$. The means by year are in Table 2.

Table 2. Rank of families for 2004 and 2005 for mean percent leaf area rust.

2004		2005	
family	%leaf rust	family	%leaf rust
84-1	3.27	A35-1	4.18
A35-1	4.56	84-1	4.52
A138-1	4.73	A34-1	5.08
A34-1	5.63	A97-1	5.10
A97-1	5.93	A37-1	6.00
A18-1	5.95	A21-1	6.45
A37-1	6.07	A138-1	7.38
A126-1	6.60	A18-1	7.92
A21-1	6.95	A24-1	8.83
A123-1	7.82	A126-1	9.37
A24-1	12.38	A123-1	9.58
A50-1	14.17	A61-1	12.08
A53-1	14.90	A50-1	12.21
A61-1	16.18	A53-1	15.05

Canopy Height

The difference of the improved population mean – the native population mean was -2.8 cm in height. This response to selection from breeding short parents is moderate. The realized heritability calculated from means was 0.77, which is high.

Response to selection is graphed by years (Figures 16 and 17) because of significant year differences.. Comparisons between native and improved populations were analyzed in $\log_n$ scale because of non-homogeneity of variance in original scale (cm). Years showed significant effect ($F=59.9$, $p<0.0001$) so analysis was done by years. Actual means and standard errors for populations, with $\log_n$ conversions, are shown in Tables 3 and 4.

Table 3. Analysis of canopy height between native and improved populations in 2004. Actual least squares means (cm) and log transformation with least squares means. Analysis is in the log scale because variances between actual population data are significantly different.

Actual least squares means (cm) and standard errors

2004		
pop	**Estimate**	**Standard Error**
native	20.6	0.3
improved	18.1	0.2

Least squares means of log transformation and analysis

pop	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
native	3.00	0.02	5	150.50	<0.0001	0.05	2.95	3.06
improved	2.86	0.01	5	249.81	<0.0001	0.05	2.84	2.90

Table 4. Analysis of canopy height between native and improved populations in 2005. Actual least squares means (cm) and log transformation and least squares means. Analysis is in the log scale because variances between transformed populations are not significantly different.

Actual least squares means (cm) and standard errors

2005		
pop	**Estimate**	**Standard Error**
native	23.1	0.4
improved	20.0	0.2

Least squares means of log transformation and analysis

pop	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
native	3.11	0.02	5	165.07	<0.0001	0.05	3.06	3.16
improved	2.97	0.01	5	286.87	<0.0001	0.05	2.94	3.00

Even though 2004 heights were taken 49 days later in the growing season they showed shorter height, possibly due to differences in precipitation from March 1 to August 23 (2005 height date), where 2004=18.6 cm precipitation and 2005=23.7 cm precipitation. Growth slows down after September 1, with saltgrass turning color mid-September. Warm season grasses grow best between 27^0 to 35^0 C (Turgeon, 1985) and 15 days after August 23, 2004 the average daytime high was 25^0 at the site. Therefore, difference in height between years is attributed to precipitation differences.

Type 3 analysis of variance was performed to obtain estimates of variance components (Table 5). Families (which make up the improved population) were significantly different from each other. Plants within plots were significantly

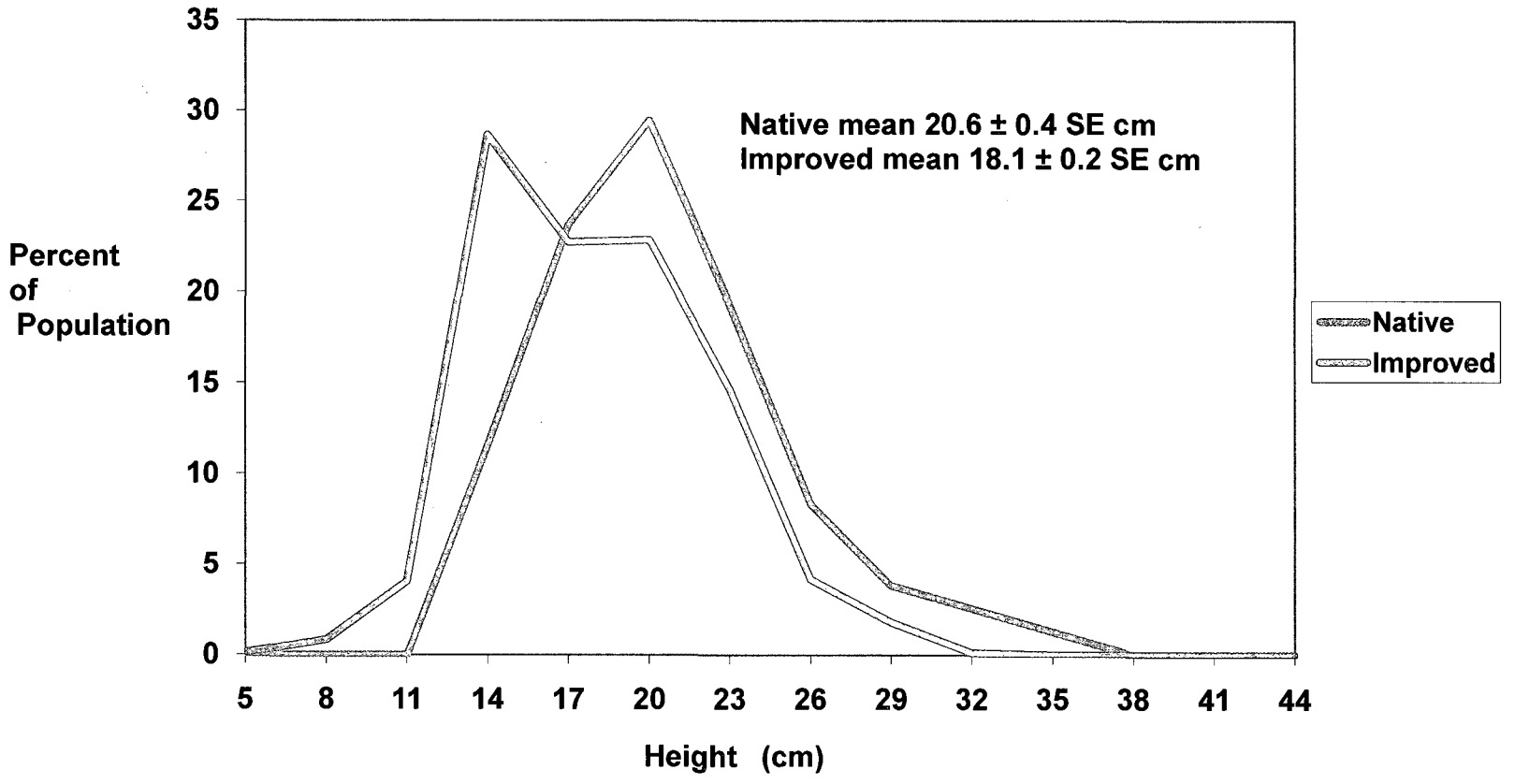

Figure 16. Canopy height (cm) on October 12, 2004 for native and improved populations at the Horticulture Field Research Center. Native population size = 156, improved population size = 840.

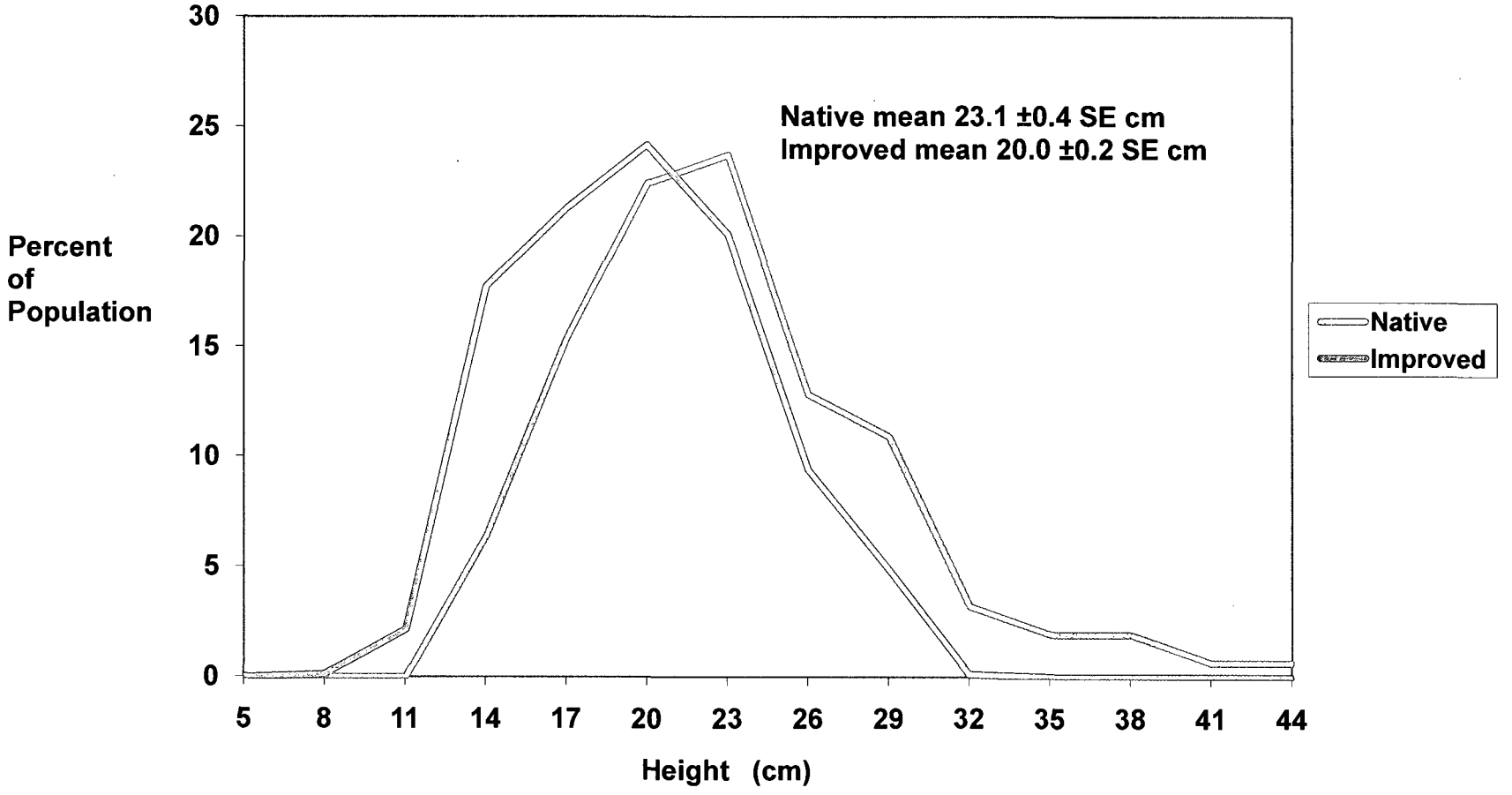

Figure 17. Canopy height (cm) on August 23, 2005 for native and improved populations at the Horticulture Field Research Center. Native population size = 156, improved population size = 840.

Table 5. Analysis of variance for height (cm) for families, combined 2004 and 2005 years, using a random effects model to obtain estimates of variance components.

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
rep	5	257.60	51.52	Var(Residual)+10 Var(rep*family*year) + 140 Var(rep*year) +2 Var(plant(rep*family)) +20 Var(rep*family) +280 Var(rep)	MS(rep*family) + MS(rep*year) - MS(rep*family*year)	17.01	1.69	0.1918
family	13	7977.39	613.64	Var(Residual) +10 Var(rep*family*year) + 60 Var(family*year) +2 Var(plant(rep*family)) +20 Var(rep*family) + 120Var(family)	MS(rep*family) + MS(family*year) - MS(rep*family*year)	29.45	15.44	<0.0001
rep*family	65	1310.03	20.15	Var(Residual) +10 Var(rep*family*year) + 2 Var(plant(rep*family)) +20 Var(rep*family)	MS(plant(rep*family)) + MS(rep*family*year) - MS(Residual)	448.87	0.99	0.5098
plant(rep*family)	756	14736	19.49	Var(Residual) +2 Var(plant(rep*family))	MS(Residual)	756	4.65	<0.0001
year	1	1483.33	1483.33	Var(Residual) +10 Var(rep*family*year) + 60 Var(family*year) +140 Var(rep*year) + 840 Var(year)	MS(rep*year) + MS(family*year) - MS(rep*family*year)	12.89	42.24	<0.0001
rep*year	5	77.63	15.52	Var(Residual) +10 Var(rep*family*year) + 140 Var(rep*year)	MS(rep*family*year)	65	3.03	0.0161
family*year	13	321.24	24.71	Var(Residual) +10 Var(rep*family*year) + 60 Var(family*year)	MS(rep*family*year)	65	4.82	<0.0001
rep*family*year	65	333.02	5.12	Var(Residual) +10 Var(rep*family*year)	MS(Residual)	756	1.22	0.1181
Residual	756	3166.20	4.18	Var(Residual)	.	.	.	.

different. Year effect was large, as it was in the fixed model. Interactions with year were significant, so that ranking within a source may have changed over other sources of variation, for example, significance in the source family*year indicates family rankings may have changed from one year to the next.

Half-sib family analysis can be used to estimate predominantly additive gene action. From the variance components the narrow sense heritability can be calculated:

Phenotypic $\sigma^2 \simeq$ MS(family)/120

HS family $\sigma^2 \simeq \frac{[\text{MS(family)-MS(family*year)-MS(family*rep)+MS(rep*family*year)}]}{120}$

Heritability, $h_f^2 =$ HS family σ^2/Phenotypic $\sigma^2 \simeq 4.78/5.11 = 0.94$
Upper 90% CI limit = 0.97
Lower 90% CI limit = 0.85

This heritability value is very high, indicating selection for plant height is likely to be effective.

Best linear unbiased predictions (BLUPs) were made for the families. These predict the performance of the maternal parent when crossed to turf type males from the same region of collection. These are ranked from lowest height to highest, with a 95% confidence interval for each prediction (Table 6)

Table 6. Best linear unbiased predictions (BLUPs) for height (cm) for combined years.

Estimates								
Family	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
A138-1	15.8	1.1	6.48	14.53	<0.0001	0.05	13.2	18.4
A53-1	16.3	1.1	6.48	14.98	<0.0001	0.05	13.7	18.9
A50-1	16.5	1.1	6.48	15.12	<0.0001	0.05	13.8	19.1
A61-1	17.2	1.1	6.48	15.81	<0.0001	0.05	14.6	19.8
84-1	17.6	1.1	6.48	16.14	<0.0001	0.05	14.9	20.2
A126-1	18.0	1.1	6.48	16.54	<0.0001	0.05	15.4	20.6
A21-1	19.2	1.1	6.48	17.60	<0.0001	0.05	16.5	21.8
A34-1	19.4	1.1	6.48	17.77	<0.0001	0.05	16.7	22.0
A123-1	20.0	1.1	6.48	18.39	<0.0001	0.05	17.4	22.7
A24-1	20.1	1.1	6.48	18.49	<0.0001	0.05	17.5	22.8
A97-1	20.2	1.1	6.48	18.56	<0.0001	0.05	17.6	22.8
A37-1	21.7	1.1	6.48	19.9	<0.0001	0.05	19.1	24.4
A137-1	21.8	1.1	6.48	20.04	<0.0001	0.05	19.2	24.5
A35-1	21.9	1.1	6.48	20.13	<0.0001	0.05	19.3	24.6

Based on the estimates, maternal parent A138 would give the shortest progeny in crosses, and A35 would give the tallest progeny in crosses, although overlap of the confidence intervals would suggest several parents would give similar short types or tall types.

Since the source family*years was significant, analysis was run for each year (Table 7).

Table 7. Analysis of variance for height (cm) calculated separately using a random effects model to obtain estimates of variance components.

Type 3 Analysis of Variance 2004								
Source	**DF**	**Sum of Squares**	**Mean Square**	**Expected Mean Square**	**Error Term**	**Error DF**	**F Value**	**Pr > F**
rep	5	151.69	30.33	Var(Residual)+10 Var(rep*family)+140 Var(rep)	MS(rep*family)	65	2.71	0.0274
family	13	4174.30	321.10	Var(Residual)+10 Var(rep*family)+60 Var(family)	MS(rep*family)	65	28.72	<0.0001
rep*family	65	726.71	11.18	Var(Residual)+10 Var(rep*family)	MS(Residual)	756	0.97	0.5435
Residual	756	8705.30	11.51	Var(Residual)	.	.	.	.

Type 3 Analysis of Variance 2005								
Source	**DF**	**Sum of Squares**	**Mean Square**	**Expected Mean Square**	**Error Term**	**Error DF**	**F Value**	**Pr > F**
rep	5	183.54	36.70	Var(Residual)+10 Var(rep*family) + 140 Var(rep)	MS(rep*family)	65	2.60	0.0330
family	13	4124.33	317.25	Var(Residual)+10 Var(rep*family) + 60 Var(family)	MS(rep*family)	65	22.50	<0.0001
rep*family	65	916.34	14.09	Var(Residual)+10 Var(rep*family)	MS(Residual)	756	1.16	0.1911
Residual	756	9196.75	12.16	Var(Residual)	.	.	.	.

For each year, replication was significant, and families were significant, showing differences in height. Heritability estimates for each year would indicate the response to selection for that particular environment. Narrow sense heritability in this case is calculated as:

Phenotypic $\sigma^2 \simeq$ MS(family)/60

HS family $\sigma^2 \simeq \frac{\text{MS(family)-MS(family*rep)}}{60}$

For year 2004, h_f^2 = HS family σ^2/Phenotypic $\sigma^2 \simeq 5.16/5.35 = 0.97$

Upper 90% CI limit = 0.98,
Lower 90% CI limit = 0.92

For year 2005, h_f^2 = HS family σ^2/ Phenotypic $\sigma^2 \simeq 5.05/5.29 = 0.96$

Upper 90% CI limit= 0.98
Lower 90% CI limit=0.90

Again the heritability is very high, indicating that selection for plant height is likely to be effective.

BLUPs were made for the families for each year (Tables 8 and 9). These predict the performance of the maternal parent when crossed with turf-type males from the same region of collection. Family A97-1 (all progeny derived from female A97) shows the largest change in rank and contributes the most to the source variation family*year (Table 5). Family A97-1 changes from a rank of 7 in the drier year of 2004 to a rank of 12 in the wetter year of 2005. A97-1 may be more sensitive to moisture than other families, and may be expressing this in height differences.

A breeding objective could be to develop lines for each environment (if rainfall is the cause, target a dry type saltgrass and a wet type saltgrass), since an interaction exists. However, in a start-up program, the limited resources should focus on a variety that has acceptable performance across environments.

Table 8. Best linear unbiased predictions (BLUPs) for height (cm) for 2004.

Estimates								
Family	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Alpha**	**Lower**	**Upper**
A138-1	14.9	0.4	839	33.22	<0.0001	0.05	14.0	15.8
A53-1	15.4	0.4	839	34.21	<0.0001	0.05	14.5	16.3
A50-1	15.7	0.4	839	34.89	<0.0001	0.05	14.8	16.6
A61-1	15.9	0.4	839	35.35	<0.0001	0.05	15.0	16.8
84-1	16.6	0.4	839	36.84	<0.0001	0.05	15.7	17.4
A126-1	16.8	0.4	839	37.48	<0.0001	0.05	16.0	17.7
A97-1	18.0	0.4	839	40.06	<0.0001	0.05	17.1	18.9
A34-1	18.5	0.4	839	41.10	<0.0001	0.05	17.6	19.4
A21-1	18.5	0.4	839	41.24	<0.0001	0.05	17.7	19.4
A24-1	19.2	0.4	839	42.78	<0.0001	0.05	18.3	20.1
A123-1	19.5	0.4	839	43.46	<0.0001	0.05	18.7	20.4
A37-1	20.8	0.4	839	46.36	<0.0001	0.05	20.0	21.7
A18-1	21.1	0.4	839	47.00	<0.0001	0.05	20.2	22.0
A35-1	21.6	0.4	839	47.99	<0.0001	0.05	20.7	22.5

Table 9. Best linear unbiased predictors (BLUPs) for height (cm) for 2005.

Estimates								
Family	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
A138-1	16.5	0.5	63.8	32.79	<0.0001	0.05	15.5	17.5
A50-1	17.1	0.5	63.8	33.91	<0.0001	0.05	16.1	18.1
A53-1	17.1	0.5	63.8	33.91	<0.0001	0.05	16.1	18.1
A61-1	18.4	0.5	63.8	36.55	<0.0001	0.05	17.4	19.4
84-1	18.5	0.5	63.8	36.71	<0.0001	0.05	17.5	19.5
A126-1	19.1	0.5	63.8	37.91	<0.0001	0.05	18.1	20.1
A21-1	19.8	0.5	63.8	39.27	<0.0001	0.05	18.8	20.8
A34-1	20.3	0.5	63.8	40.15	<0.0001	0.05	19.2	21.3
A123-1	20.6	0.5	63.8	40.79	<0.0001	0.05	19.6	21.6
A24-1	21.1	0.5	63.8	41.83	<0.0001	0.05	20.1	22.1
A35-1	22.4	0.5	63.8	44.47	<0.0001	0.05	21.4	23.4
A97-1	22.5	0.5	63.8	44.55	<0.0001	0.05	21.5	23.5
A18-1	22.7	0.5	63.8	44.95	<0.0001	0.05	21.7	23.7
A37-1	22.8	0.5	63.8	45.11	<0.0001	0.05	21.8	23.8

From a breeding standpoint, A97 could be culled since its progeny does not have a stable height across environments. The BLUPs for the six shortest potential crosses did not change ranks.

Parent-offspring regression is a different method to determine narrow sense heritability. A family by year interaction is known from the previous analysis, therefore, separate regressions for each year were carried out. Since 2 years of data had been obtained, progeny was regressed onto parents of the alternate year (Figure 18).

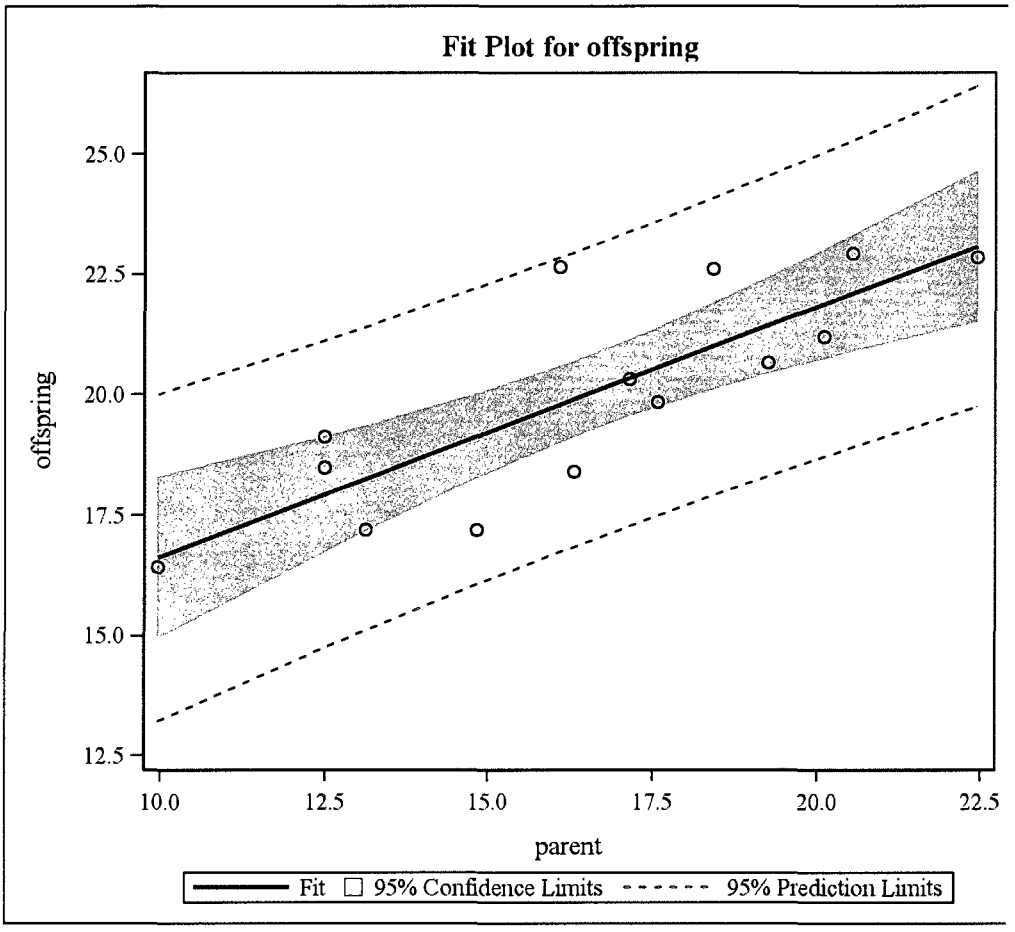

Figure 18. Height (cm) regression of offspring in 2005 on parents in 2004.

Regressing with different years removes some of the environmental covariance between parents and offspring grown in the same years. However, different environments introduces scaling in the size of variances, so *b* is multiplied by the ratio of the standard deviation of parents in regression / standard deviation of parents in the alternate year, as suggested by Nyquist (1991).

The analyses of variance of the regressions are contained in Appendix III. Heritability is twice *b*, since *b* is the COV(PO)/Var Par using only the maternal parent . With the adjustments mentioned above, for the parents in 2004 and progeny in 2005, $h^2 \simeq$

0.98 ±0.20 SE Regression using progeny in 2004, and parents in 2005, is graphed in Figure 19.

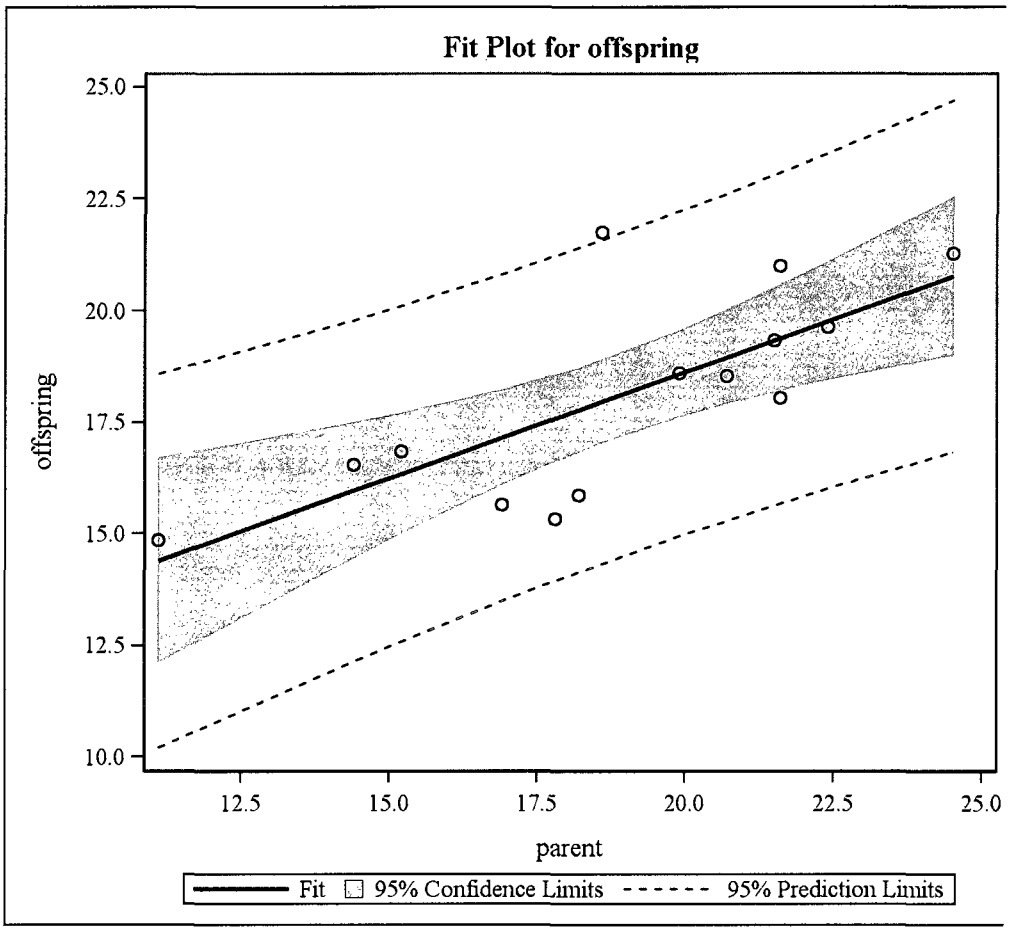

Figure 19. Height (cm) regression of offspring in 2004 on parents in 2005.

With adjustments mentioned previously, for the 2005 parents, and 2004 progeny regression, $h^2 \simeq 1.00 \pm 0.25$ SE. Both of these values, 0.98 and 1.00 are very high, but they are also in the range of heritability estimates obtained in the half-sib family analysis.

Broad sense heritability is of interest because in clonal or hybrid cultivars one can utilize all the genotypic variance in a population. It was calculated from the parent analysis of variance (AppendixIII). This was equal to 0.94 for 2004, and to 0.90 in 2005.

These values are slightly lower than the narrow sense heritability estimates from half-sib analysis and parent-offspring regression, and the difference between the estimates may be attributable to error.

Wofford and Baltensperger (1985), reported on stem internode length in a turfgrass type, broad based germplasm source in bermudagrass. For one year, broad sense heritability was 0.96, narrow sense heritability from half-sib analysis was 0.92, but heritability from parent-offspring regression was only 0.14. Stolon internode length had high heritability in all 3 calculations. The genetic variance was significant for each of these traits.

Nguyen and Sleper (1983), reported on height in a forage type, broad based germplasm source in tall fescue. For two years and two locations, broad sense heritability was 0.66 ±0.37 SE, narrow sense heritability was 0.00 because of lack of differences in half-sib families, and heritability from parent-offspring regression was 0.21.

de Araújo and Coulman (2002) reported on height in a forage type, narrow based germplasm source in meadow bromegrass and calculated heritability of 0.60 ±0.12 SE, calculated from half-sib family analysis.

The heritability for height in this experiment is high, and similar to that found in an initial breeding program for turf traits in bermudagrass (Wofford and Baltensperger, 1985). The bermudagrass germplasm consisted of accessions from 10 Agricultural Experiment Stations, representing a large geographic area. The saltgrass germplasm was collected from a small area in Colorado.

An analysis for major gene segregation (Appendix III) was performed by quadratic regression of offspring variance on sibship mean phenotypic value, as proposed by Fain (1978). When the parameter estimate, b_2, is significant, it indicates a major gene. The estimate for height in either year was not significant ($p = 0.05$), and a major gene for height was not detected.

The means in 2004 show midparent heterosis = 8% (Table 10), in which their 95% confidence intervals do not overlap. The range in midparent heterosis in the 14 crosses is -11% to 30%. Since short height is being selected for, and the progeny are taller, this should be thought of as a negative heterosis for the trait being inherited. This was in a drier year than 2005. The means in 2005 had overlapping confidence intervals. If the significance is related to less moisture in 2004, the progeny are able to put on more biomass with less water.

Table 10. Analysis of height (cm): progeny versus parents.

2004								
pop	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Alpha**	**Lower**	**Upper**
parent	16.8	0.3	989	52.55	<.0001	0.05	16.2	17.4
progeny	18.1	0.1	989	131.12	<.0001	0.05	17.8	18.4
2005								
pop	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Alpha**	**Lower**	**Upper**
parent	19.5	0.3	989	58.54	<.0001	0.05	18.9	20.2
progeny	20.0	0.1	989	138.97	<.0001	0.05	19.7	20.3

Shoot Density

The difference of the improved population mean–the native population mean was 0.7 shoots/cm^2. This response to selection is a large increase considering the small area. The realized heritability calculated from means is 1.77, an anomaly of the calculation since heritability cannot be greater than 1.0. Nevertheless it indicates heterosis from crosses.

Since original data showed non-homogeneity of variances, comparisons between the native and improved population were done after a square root transformation of data. Analysis showed no year effect and the data are combined over years, showing non-overlapping 95% confidence intervals between native and improved (Table 11).

Table 11. Analysis of shoot density between native and improved populations in 2005 and 2006, combined years. Actual least squares means (no./cm^2) and square root transformation and least squares means analysis.

Actual least square means in numbers/cm^2 and standard errors

pop	Estimate	Standard Error
native	1.14	0.03
improved	1.82	0.02

Least squares means of square root transformation and analysis

pop	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
native	1.050	0.0138	5	75.91	<0.0001	0.05	1.0150	1.0862
improved	1.336	0.0096	5	138.23	<0.0001	0.05	1.3115	1.3612

Improved population and the native population are compared in Figure 20.

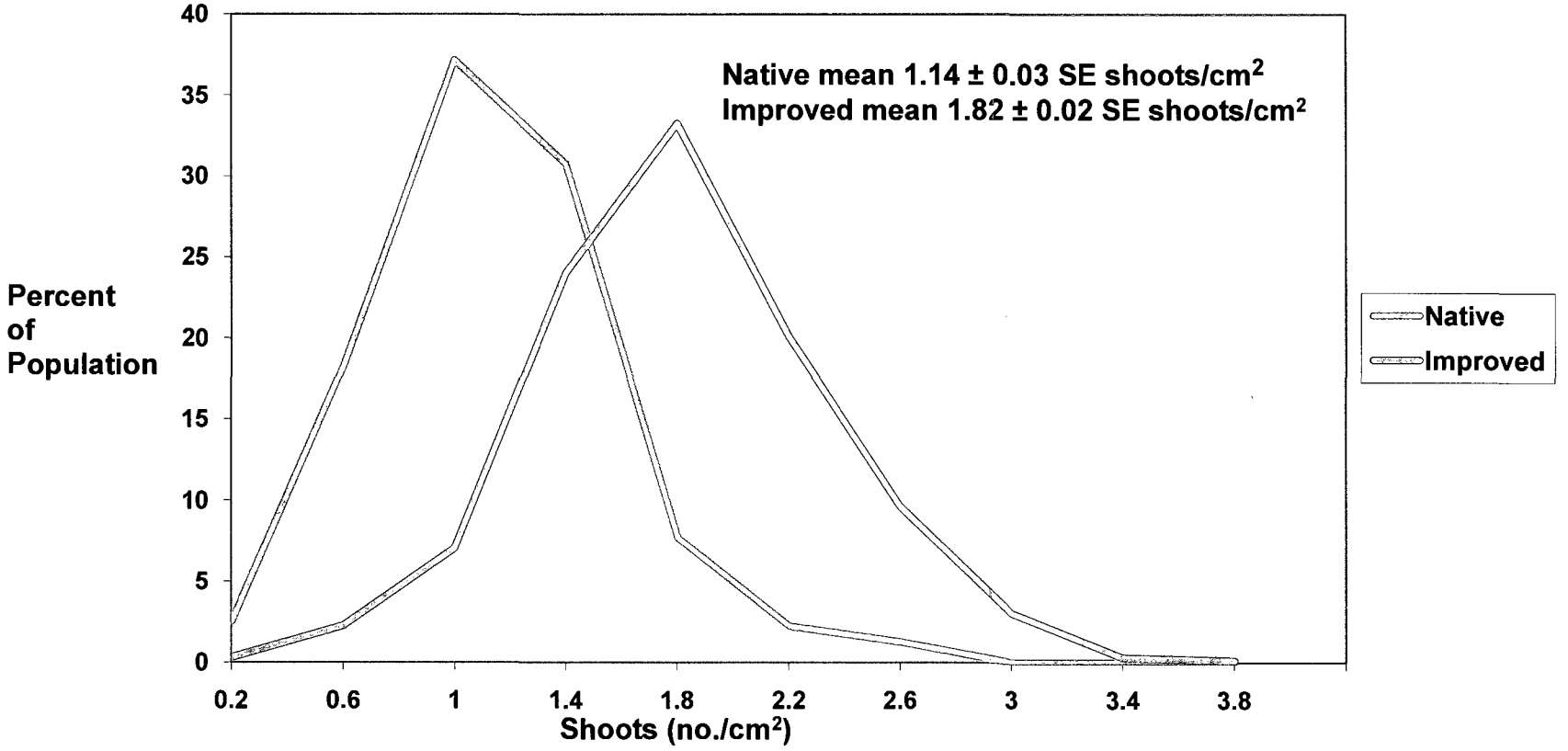

Figure 20. Shoot density (no./cm^2) for July 17-27, 2005 and July 10-21, 2006 combined, at the Horticulture Field Research Center. Native population size = 312, improved population size = 1680.

Table 12. Analysis of variance for shoot density (no./cm^2) for families in 2005 and 2006, combined years, using a random effects model to obtain estimates of variance components.

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
rep	5	2.57	0.515	Var(Residual) +10 Var(rep*family*year) + 140 Var(rep*year) +2 Var(plant(rep*family)) +20 Var(rep*family) +280 Var(rep)	MS(rep*family) +MS(rep*year) - MS(rep*family*year)	40.668	1.39	0.2488
family	13	77.69	5.976	Var(Residual) +10 Var(rep*family*year) + 60 Var(family*year) +2 Var(plant(rep*family)) +20 Var(rep*family) +120 Var(family)	MS(rep*family) + MS(family*year) - MS(rep*family*year)	63.19	18.65	<0.0001
rep*family	65	19.84	0.305	Var(Residual) +10 Var(rep*family*year) + 2 Var(plant(rep*family)) +20 Var(rep*family)	MS(plant(rep*family)) + MS(rep*family*year) - MS(Residual)	702.56	0.76	0.9171
plant(rep*family)	756	302.57	0.400	Var(Residual) +2 Var(plant(rep*family))	MS(Residual)	756	12.81	<0.0001
year	1	0.02	0.022	Var(Residual) +10 Var(rep*family*year) + 60 Var(family*year) +140 Var(rep*year) + 840 Var(year)	MS(rep*year) +MS(family*year) - MS(rep*family*year)	6.0552	0.20	0.6735
rep*year	5	0.49	0.098	Var(Residual) +10 Var(rep*family*year) + 140 Var(rep*year)	MS(rep*family*year)	65	3.03	0.0161
family*year	13	0.61	0.047	Var(Residual) +10 Var(rep*family*year) + 60 Var(family*year)	MS(rep*family*year)	65	1.46	0.1555
rep*family*year	65	2.11	0.032	Var(Residual) +10 Var(rep*family*year)	MS(Residual)	756	1.04	0.3945
Residual	756	23.62	0.031	Var(Residual)	.	.	.	.

Analyses of variance were performed on families (Table12). The analysis shows families are different, and that plants within plots are different. Year effects are not significant. There is a rep*year interaction that is difficult to explain. Narrow sense heritability is calculated as:

Phenotypic $\sigma^2 \simeq$ MS(family)/120

HS family $\sigma^2 \simeq \frac{[\text{MS(family)-MS(family*year)-MS(family*rep)+MS(rep*family*year)}]}{120}$

Heritability, $h_f^2 =$ HS family σ^2 / Phenotypic $\sigma^2 \simeq 0.3032/0.3203 = 0.94$

Upper 90% CI limit = 0.97
Lower 90% CI limit = 0.88

This is a high value for heritability and indicates selection for shoot density is likely to be effective.

BLUPs were calculated for the families (Table 13). These predict the performance of the maternal parent when crossed with turf-type paternal males obtained from the same region of collection. These are ranked from highest density to lowest, with a 95% confidence interval for each prediction.

Table 13. Best linear unbiased predictions (BLUPs) for shoot density (no. /cm^2) for 2005 and 2006, combined.

Estimates								
Label	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
A50-1	2.23	0.05	1679	43.80	<0.0001	0.05	2.13	2.33
A34-1	2.05	0.05	1679	40.20	<0.0001	0.05	1.95	2.15
A138-1	2.05	0.05	1679	40.20	<0.0001	0.05	1.95	2.15
84-1	2.01	0.05	1679	39.40	<0.0001	0.05	1.91	2.11
A53-1	1.91	0.05	1679	37.57	<0.0001	0.05	1.81	2.01
A61-1	1.84	0.05	1679	36.15	<0.0001	0.05	1.74	1.94
A24-1	1.81	0.05	1679	35.58	<0.0001	0.05	1.71	1.91
A126-1	1.78	0.05	1679	34.96	<0.0001	0.05	1.68	1.88
A37-1	1.74	0.05	1679	34.18	<0.0001	0.05	1.64	1.84
A35-1	1.71	0.05	1679	33.50	<0.0001	0.05	1.61	1.81
A123-1	1.64	0.05	1679	32.20	<0.0001	0.05	1.54	1.74
A137-1	1.64	0.05	1679	32.14	<0.0001	0.05	1.54	1.74
A97-1	1.57	0.05	1679	30.90	<0.0001	0.05	1.47	1.67
A21-1	1.48	0.05	1679	29.12	<0.0001	0.05	1.38	1.58

A50 would produce progeny with the highest shoot density, along with A34 and A138; likewise, A21 would produce progeny with the lowest shoot density, along with A97, A137, and A123.

Parent-offspring regression was conducted (Appendix III) to calculate heritability estimates and compare them to those obtained under half-sib analysis. Even though the analysis of variance showed no year effect, nor family*year interaction, regressing with different years removes some of the environmental covariance between parents and offspring grown in the same years. However, different environments introduces scaling in the size of variances, so *b* is multiplied by the ratio of the standard deviation of parents in

regression/standard deviation of parents in the alternate year, as suggested by Nyquist (1991).

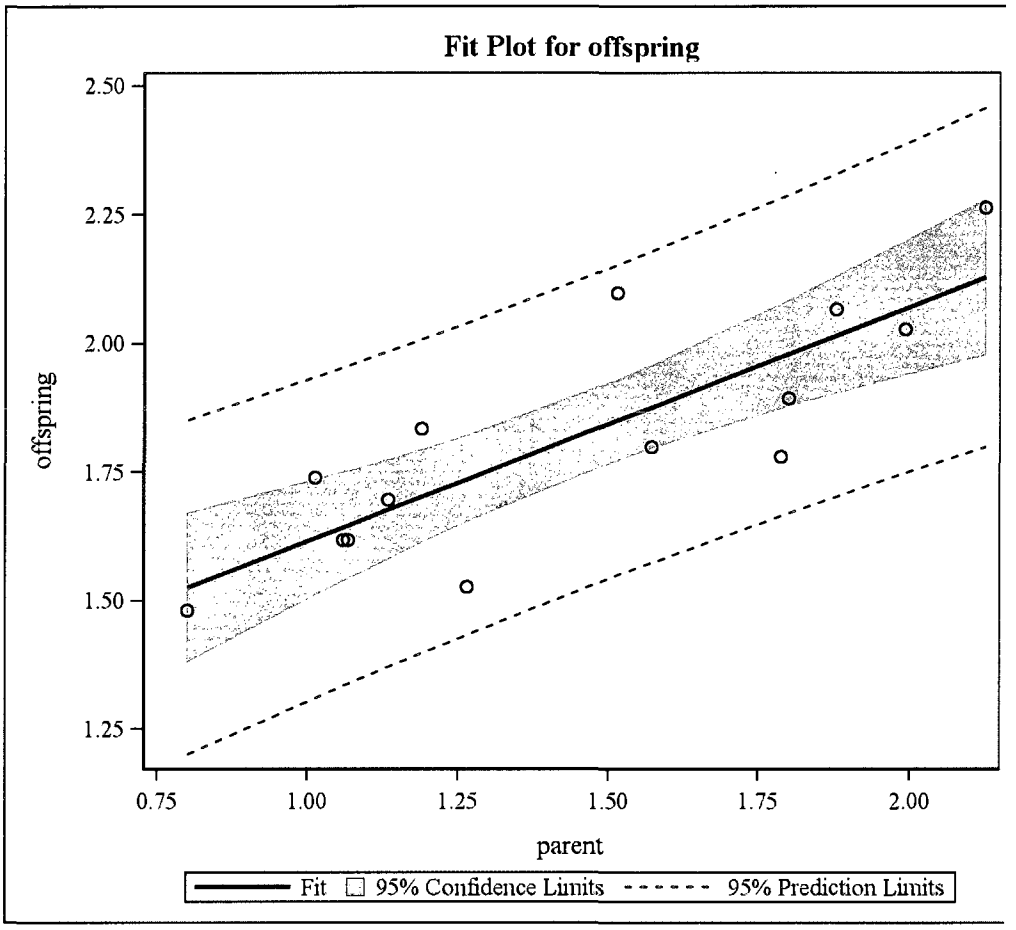

Figure 21. Density (no./cm^2) regression of offspring in 2006 onto parents in 2005.

Narrow sense heritability using 2005 parental data becomes 0.96 ±0.18 SE and using 2006 parental data, heritability is 0.89 ±0.19 SE.

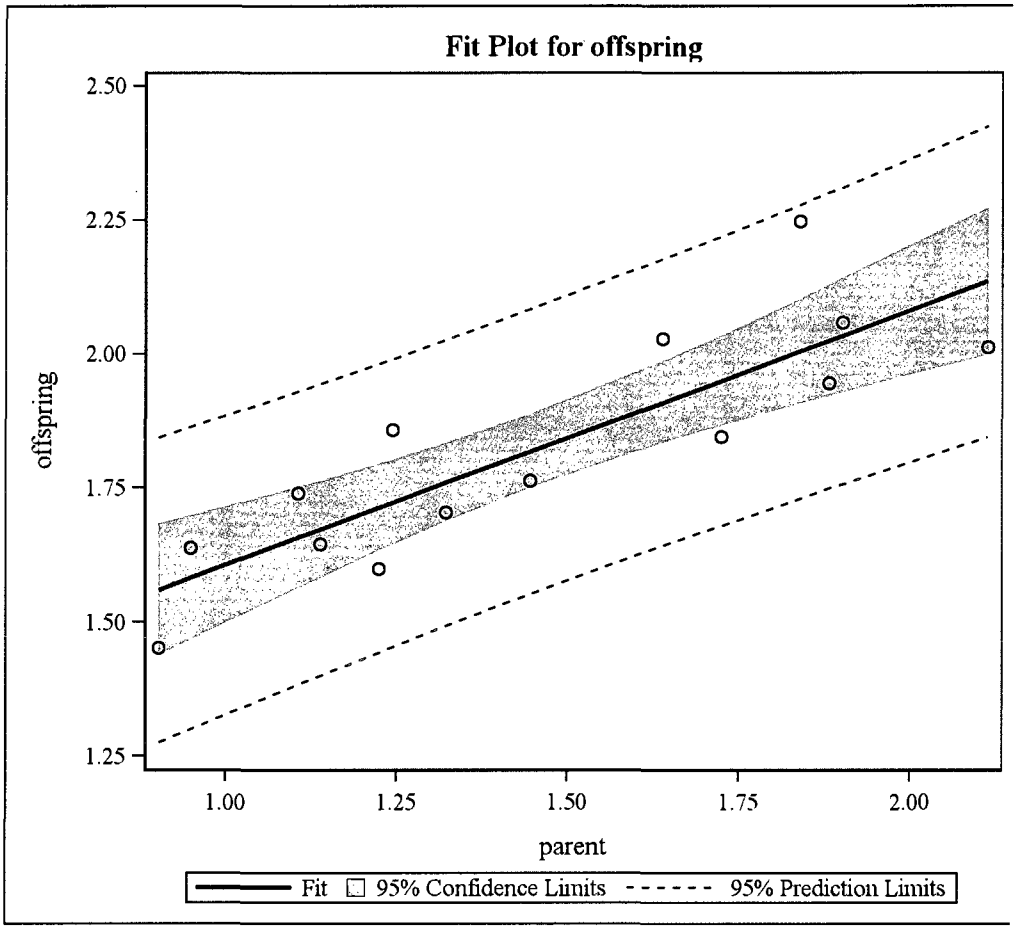

Figure 22. Density (no/cm^2) regression of offspring in 2005 onto parents in 2006

These values are in the range of that derived from half-sib family analysis, and all are very high.

Broad sense heritability is of interest because in clonal or hybrid cultivars one can utilize all the genotypic variance in a population. It was calculated from the parent analysis of variance (Appendix III). This was equal to 0.96 for combined years and is in the range for narrow sense heritability.

In sod forming grasses, in a broad based germplasm in reed canarygrass, Casler (1981), found high narrow sense heritability for tiller density of 0.68 ±0.36 SE. In a narrow sampling of several germplasms of smooth bromegrass (*Bromus inermis*, Leyss), narrow sense heritability for tiller density was 0.55 (Tan et al., 1977).

In bunch grasses, heritability from parent-offspring regression was 0.60 for tiller number in a localized population of perennial ryegrass (Wedderburn et al., 1992). Jaferi and Naseri (2007) calculated parent-offspring heritabilities for tiller number in a broad based orchardgrass (*Dactylis glomerata*, L.) germplasm of 0.79 ±0.13 SE.

An analysis for major gene segregation was performed by quadratic regression of offspring variance on sibship mean phenotypic value, as proposed by Fain (1978). When the parameter estimate, b_2 is significant, it indicates a major gene. The estimate for density was not significant, and a major gene was not detected.

Means of the parents were compared to means of the progeny (Table14).

Table 14. Analysis of variance of shoot density (no./cm^2) : progeny versus parents, 2005 and 2006.

Least Squares Means								
pop	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Alpha**	**Lower**	**Upper**
parent	1.53	0.03	1985	53.03	<.0001	0.05	1.47	1.59
progeny	1.82	0.01	1985	146.21	<.0001	0.05	1.79	1.84

Progeny had significantly higher shoot density above the midparent, based on non-overlapping confidence intervals, with heterosis = 18%. The range in midparent heterosis in the 14 crosses was from -3% to 46%.

Seed Yield

In comparing the native and improved population, there was a very large significant difference in yield due to years (Table 15). In 2005, the yield was 412 kg/ha, and in 2006 the yield was 1730 kg/ha, across populations. However, the difference is more likely due to sexual maturity of perennial grass. Perennial grasses need to mature in order to produce seed (Heide, 1994). Commercial type seed production of perennial grass species rises quickly in the first several years, peaks, and then declines (Canode, 1968; Van Keuren and Canode, 1963).

Table 15. Least squares means estimate for seed yield (kg/ha) between 2005 and 2006 across native and improved improved populations at the Horticulture Field Research Center.

Least Squares Means								
year	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
2005	412	52	719	7.79	<.0001	0.05	308	516
2006	1730	52	719	32.65	<.0001	0.05	1626	1834

Seed yield was significantly improved by selecting parents with high seed yield from the native population. The significance is not present in 2005 (Table 16) which may be due to plants not reaching seed bearing age, but significant differences are evident in 2006 (Table 17) under high seed production. Results are presented by years because of the previous significant year differences. The tables are split to demonstrate the effect of excluding lines that do not produce seed. Excluding these non-bearing lines demonstrates selection for seed production is less effective than with these lines. Including lines with 0

seed production, results in a difference between native and improved (progeny of selected parents) of 428 kg/ha (response to selection). Excluding these lines results in a difference of only 363 kg/ha (response to selection). Response to selection in advanced generations would be represented more by the latter, since lines with 0 seed yield would be absent.

Table 16. Analysis of seed yield (kg/ha) between native and improved populations in 2005 at the Horticulture Field Research Center. The table is split to show analysis of all entries and of only entries producing seed.

Least Squares Means of entries with seed yield ≥0								
pop	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Alpha**	**Lower**	**Upper**
native	361	62	43.5	5.79	<.0001	0.05	235	487
improved	505	34	5.01	14.54	<.0001	0.05	416	594
Least Squares Means of entries with seed yield >0								
pop	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Alpha**	**Lower**	**Upper**
native	373	62	54.9	6.00	<.0001	0.05	248	498
improved	513	32	5.03	15.94	<.0001	0.05	430	596

Table 17. Analysis of seed yield (kg/ha) between native and improved populations in 2006 at the Horticulture Field Center, Ft. Collins, Colorado. The table is split to show analysis of all entries and of only entries producing seed.

Least Squares Means of entries with seed yield ≥0								
pop	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Alpha**	**Lower**	**Upper**
native	1516	126	473	11.96	<.0001	0.05	1266	1765
improved	1944	45	473	42.36	<.0001	0.05	1854	2034.
Least Squares Means of entries with seed yield >0								
pop	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Alpha**	**Lower**	**Upper**
native	1603	128	463	12.46	<.0001	0.05	1350	1856
improved	1966	45	463	43.04	<.0001	0.05	1876	2055

Realized heritability was 0.42 using all entries, and 0.36 using only entries that produced seed. The following analyses included entries with ≥ 0 seed yield since progeny also had entries producing 0 seed yield.

A comparison of populations was plotted for each year because of the large yearly differences in seed yield (Figures 23 and 24).

Analysis of variance for combined years based on random effects was run to obtain estimated variances for heritability calculations (Table 18).

Replications did not significantly reduce experimental variability. Seed yield is a highly quantitative trait which shows a high amount of variability (Hallauer and Miranda, 1988). Family effects were significant at a $p = 0.0493$. Family*year effect was significant, meaning the ranks of families ability to produce seed may have changed from year to year. Years were highly significant, which does not necessarily indicate a year effect due to weather conditions, but most likely reflects sexual maturity of the plants (Heide,1994), with fewer flower heads in 2005, and many more in 2006. The validity of the split block model under juvenile versus mature traits is unknown, and maturity effects confound combined year effects (Nyquist, 1991). This differs from annual agronomic crops where maturity is reached every season, and confounding is not present in combined year analysis. BLUPs were calculated for years and the difference in seed yield is very large, but the 95% confidence intervals overlap and contains 0.0 for 2005 (Table 19).

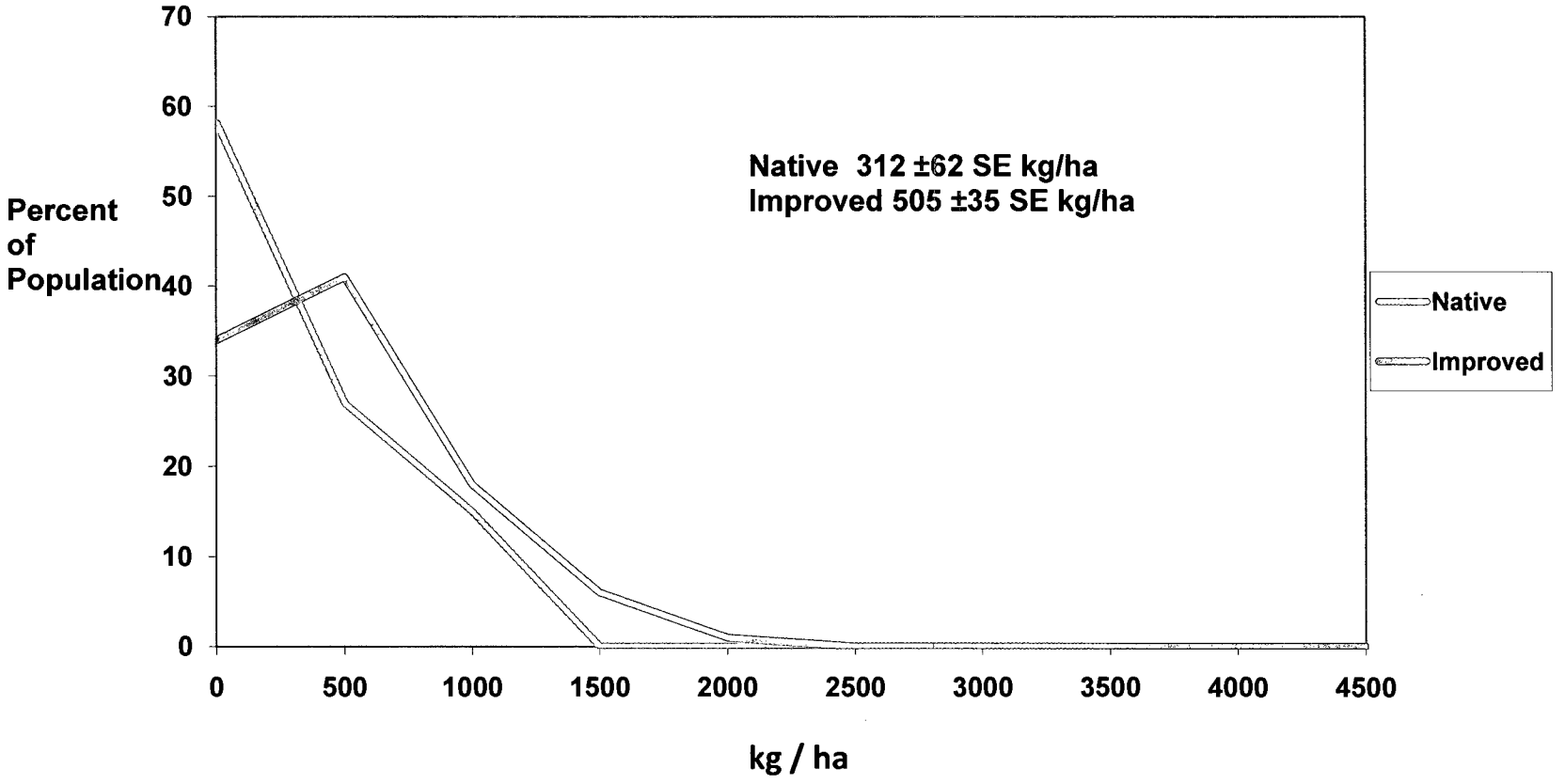

Figure 23. Seed yield (kg/ha) in 2005 at the Horticulture Field Research Center. Native population size = 55 plants, improved population size = 420 plants.

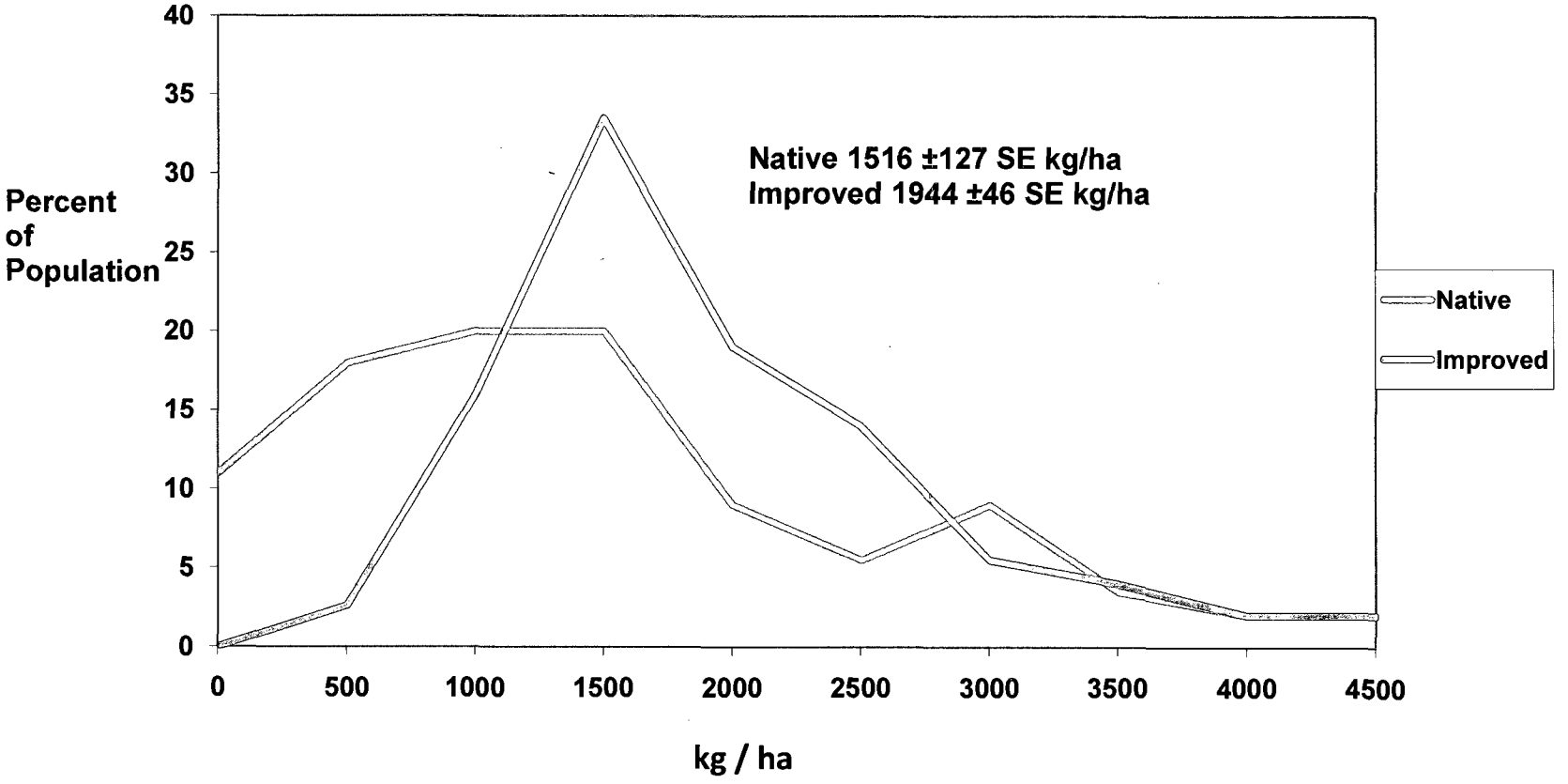

Figure 24. Seed yield (kg/ha) in 2006 at the Horticulture Field Research Center. Native population size = 55 plants, improved population size = 420 plants.

Table 18. Analysis of variance for seed yield (kg/ha) for 2005 and 2006, combined years. Random effects model to obtain estimates of variance components.

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
rep	5	1622411	324482	Var(Residual)+5Var(rep*family*year) + 70 Var(rep*year) Var(plant(rep*family)) +10 Var(rep*family) +140 Var(rep)	MS(rep*family) + MS(rep*year) - MS(rep*family*year)	17.105	0.62	0.6881
family	13	36338086	2795237	Var(Residual) +5 Var(rep*family*year) +30 Var(family*year) +2Var(plant(rep*family)) + 10 Var(rep*family) + 60 Var(family)	MS(rep*family) + MS(family*year) - MS(rep*family*year)	20.36	2.25	0.0493
rep*family	65	27956923	430107	Var(Residual) +5Var(rep*family*year) + 2 Var(plant(rep*family)) +10Var(rep*family)	MS(plant(rep*family)) + MS(rep*family*year) - MS(Residual)	216.76	0.62	0.9881
plant(rep*family)	336	254261184	756730	Var(Residual) +2Var(plant(rep*family))	MS(Residual)	336	3.45	<0.0001
year	1	434634939	434634939	Var(Residual) +5Var(rep*family*year) +70 Var(rep*year) +30Var(family*year) +420 Var(year)	MS(family*year) + MS(rep*year) - MS(rep*family*year)	13.249	407.03	<0.0001
family*year	13	12644741	972672	Var(Residual) +5Var(rep*family*year) +30 Var(family*year)	MS(rep*family*year)	65	6.13	<0.0001
rep*year	5	1269599	253920	Var(Residual) +5Var(rep*family*year) +70 Var(rep*year)	MS(rep*family*year)	65	1.60	0.1728
rep*family*year	65	10320397	158775	Var(Residual) +5Var(rep*family*year)	MS(Residual)	336	0.72	0.9430
Residual	336	73721135	219408	Var(Residual)	.	.	.	.

Table 19. Best linear unbiased prediction (BLUPs) for seed yield (kg/ha) for 2005 and 2006, improved population.

Estimates								
Label	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
2005	507	66	1	7.59	0.0834	0.05	-342	1356
2006	1942	66	1	29.05	0.0219	0.05	1092	2791

Narrow sense heritability estimates for combined years:

Phenotypic variance= MS(family)/60
HS family variance= [MS(family)-MS(family*year)-MS(family*rep)+MS(rep*family*year)]

60

Heritability, h_f^2 = HS family σ^2/Phenotypic $\sigma^2 \simeq$ 2.24/ (241.88/60) = 0.56
Upper 90% CI limit = 0.80
Lower 90% CI limit = -0.08

The lower CI limit goes through 0.0. BLUPs for seed yield by families for combined years had overlapping CIs, and not presented.

Because of the possible confounding due to maturity effects, the analysis was run separately for 2005, and, 2006 (Table 20), and represents seed yield at 2 different sexual maturities. The F value is more than twice as large as the previous analysis.

Narrow sense heritability calculations for 2005 and 2006:

Phenotypic variance=MS(family)/30
HS family variance = (MS(family)-MS(rep*family))/30

2005 Heritability, h_f^2 = HS family σ^2/ Phenotypic $\sigma^2 \simeq 1.95 / 2.27 = 0.86$
Upper 90% CI limit = 0.92
Lower 90% CI limit = 0.63

2006 Heritability, h_f^2 = HS family σ^2/ Phenotypic $\sigma^2 \simeq 7.22 / 8.59 = 0.84$
Upper 90% CI limit = 0.91
Lower 90% CI limit = 0.63

Heritability estimates are higher for the combined year analysis, and the confidence intervals are narrower. However, heritability is now inflated by genotype x environment interaction with only one year of data at one location. Highly quantitative traits such as seed yield are affected greatly by environment (Bernardo, 2002; Hallauer and Miranda, 1988). Nevertheless, plots were provided ideal growing conditions, and future seed production is anticipated in the same environment, so that heritability estimates can be interpreted to apply to this germplasm in the specific situation.

Table 20. Analysis of variance for seed yield (kg/ha) for 2005 and 2006, separate years. Random effects model to obtain estimates of variance components.

Type 3 Analysis of Variance 2005 seed weight kg/ha								
Source	**DF**	**Sum of Squares**	**Mean Square**	**Expected Mean Square**	**Error Term**	**Error DF**	**F Value**	**Pr > F**
rep	5	2594127	518825	Var(Residual) +5Var(rep*line)+70Var(rep)	MS(rep*line)	65	4.60	0.0012
line	13	10247159	788243	Var(Residual) +5Var(rep*line)+30Var(line)	MS(rep*line)	65	6.98	<0.0001
rep*line	65	7337067	112878	Var(Residual) +5Var(rep*line)	MS(Residual)	336	0.76	0.9124
Residual	336	50039714	148928	Var(Residual)	.	.	.	.

Type 3 Analysis of Variance 2006 seed weight kg/ha								
Source	**DF**	**Sum of Squares**	**Mean Square**	**Expected Mean Square**	**Error Term**	**Error DF**	**F Value**	**Pr > F**
rep	5	297882	59576	Var(Residual) +5Var(rep*line)+70Var(rep)	MS(rep*line)	65	0.13	0.9862
line	13	38735669	2979667	Var(Residual) +5Var(rep*line)+30Var(line)	MS(rep*line)	65	6.26	<0.0001
rep*line	65	30940253	476004	Var(Residual) +5Var(rep*line)	MS(Residual)	336	0.58	0.9961
Residual	336	277942605	827210	Var(Residual)	.	.	.	.

BLUPs were calculated for 2005 and 2006, and families ranked highest to lowest for seed production (Tables 20 and 21). These predict the performance of the maternal parent when crossed to turf-type males collected from the same region of origin. The ranks are similar, with the exception of family 84-1, which jumped from rank 6 in 2005 to rank 1 in 2006. Because of family*year interaction, 2005 data would not accurately predict seed yields in 2006. Plants need to be maintained over sufficient years until they reach sexual maturity and can be evaluated for maximum seed production. The 2006 data represent commercial seed yields and are the data of greater importance.

Table 21. Best linear unbiased predictions (BLUPs) for 2005 seed yield (kg/ha).

Estimates								
Label	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Alpha**	**Lower**	**Upper**
A97-1	831	65	295	12.77	<0.0001	0.05	702	959
A24-1	737	65	295	11.33	<0.0001	0.05	609	865
A37-1	555	65	295	8.54	<0.0001	0.05	427	683
A138-1	542	65	295	8.34	<0.0001	0.05	414	670
A61-1	529	65	295	8.14	<0.0001	0.05	401	657
84-1	510	65	295	7.85	<0.0001	0.05	382	638
A126-1	500	65	295	7.70	<0.0001	0.05	372	629
A50-1	477	65	295	7.34	<0.0001	0.05	349	606
A35-1	469	65	295	7.21	<0.0001	0.05	341	597
A21-1	451	65	295	6.94	<0.0001	0.05	323	579
A137-1	423	65	295	6.51	<0.0001	0.05	295	552
A34-1	361	65	295	5.56	<0.0001	0.05	233	489
A53-1	360	65	295	5.54	<0.0001	0.05	232	488
A123-1	323	65	295	4.98	<0.0001	0.05	195	451

Table 22. Best linear unbiased predictions (BLUPs) for 2006 seed yield (kg/ha).

Estimates								
Label	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
84-1	2612	112	131	23.29	<0.0001	0.05	2390	2834
A97-1	2192	112	131	19.54	<0.0001	0.05	1970	2413
A24-1	2147	112	131	19.15	<0.0001	0.05	1925	2369
A126-1	2030	112	131	18.10	<0.0001	0.05	1808	2252
A37-1	1990	112	131	17.74	<0.0001	0.05	1768	2212
A61-1	1983	112	131	17.69	<0.0001	0.05	1761	2205
A138-1	1968	112	131	17.55	<0.0001	0.05	1746	2189
A35-1	1894	112	131	16.89	<0.0001	0.05	1672	2116
A21-1	1888	112	131	16.83	<0.0001	0.05	1666	2110
A50-1	1794	112	131	16.00	<0.0001	0.05	1572	2016
A34-1	1789	112	131	15.95	<0.0001	0.05	1567	2011
A53-1	1763	112	131	15.72	<0.0001	0.05	1541	1984
A123-1	1620	112	131	14.45	<0.0001	0.05	1398	1842
A137-1	1543	112	131	13.76	<0.0001	0.05	1321	1765

Parent-offspring regression was used to calculate narrow sense heritability, using 2006 offspring data regressed against 2005 parent data, and, 2005 offspring data regressed against 2006 parent data (Appendix III). The first regression $b = 0.02 \pm 0.06$ SE. The second regression, $b = -0.12 \pm 0.22$ SE. Confidence intervals contain 0.

Because of the poor results with regression using alternate year data, 2006 offspring were regressed on 2006 parents. This represents both generations at sexual maturity. Now $b = 0.22 \pm 0.09$ SE. (see Figure 25). The narrow sense heritability is 0.45 ± 0.18 SE. The estimate may be inflated from environmental covariance between parent and offspring. This heritability is much lower than that from half-sib analysis. If linkage were present, it

inflates the covariance between relatives, as in the case of half-sibs, but does not inflate parent-offspring relationships (Lynch and Walsh, 1998).

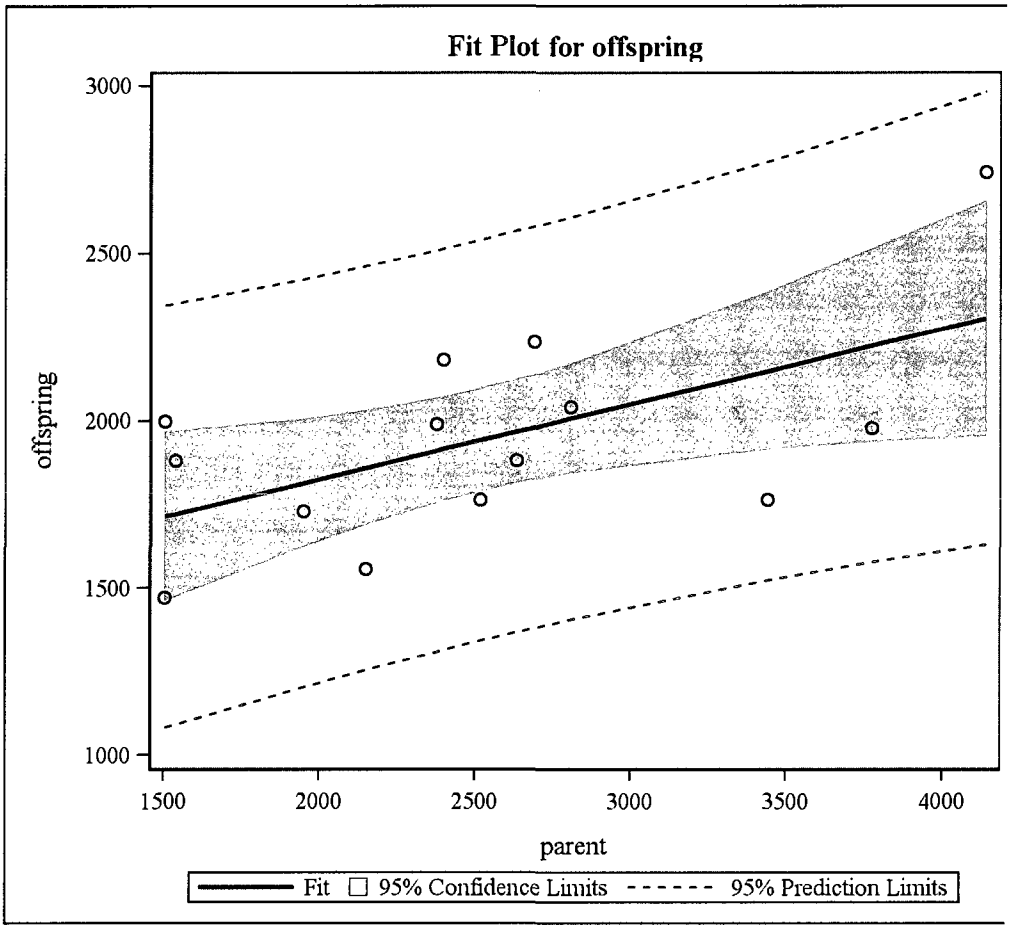

Figure 25. Seed yield (kg/ha) regression of 2006 offspring on 2006 parents.

Broad sense heritability was calculated using 2006 parent data (Appendix III). Broad sense heritability for seed weight is 0.80, and includes, besides additive, dominant and epistatic gene action.

In bermudagrass (Wofford and Baltensperger, 1985), broad sense heritability for seed head production was 0.98, narrow sense heritability from half-sib analysis was 0.84, and narrow sense heritability from parent-offspring regression was 0.11.

In tall fescue (Nguyen and Sleper, 1983), broad sense heritability for seed yield was 0.43 ±0.41 SE. Narrow sense heritability from half-sib analysis was 0.67 ±0.34 SE. Narrow sense heritability from parent-offspring regression was 1.20.

In meadow bromegrass (de Araújo and Coulman, 2002), narrow sense heritability for seed yield calculated from half-sib analysis was 0.30 ±0.19 SE.

A test for major gene segregation (Fain, 1978) for seed yield was run in SAS. The test was not significant and a major gene was not detected (Appendix III).

Table 23. Analysis of seed yield in kg/ha: progeny versus parents 2006.

Least Squares Means									
Effect	**pop**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Alpha**	**Lower**	**Upper**
pop	parent	2527	129	469	19.64	<0.0001	0.05	2274	2779
pop	progeny	1944	45	469	42.74	<0.0001	0.05	1854	2033

Mean of the parents was compared to mean of the progeny (Table 23). The progeny population mean is significantly less than the mid-parent value and results in -23% heterosis. The range of heterosis for the 14 crosses was -42% to 8%.

Summary and discussion

The fact that the selection index selected individual phenotypes coming from a localized area relative to the area sampled seems unusual. It would indicate natural selection pressures were similar to turgrass selection criteria for this area. Some bias occurs because the Fort Collins research site is in the Front Range collection site, sharing a similar environment. However, many plants in the Front Range area were excluded by the selection index.

The traits and their response to selection, heritability, major gene detection, and heterosis are summarized in Table 22.

Realized heritability used the native population as the reference population. Realized heritability is usually calculated from several years' data (Nyquist, 1991). Narrow sense and broad sense heritability use the parents and/ or turf types that the selection index identifies, as the reference population.

Falconer and Mackay (1996) state heritability would be theoretically lower in the advanced generation, but Nyquist (1991) states, that in cross fertilizing species, heritability estimates across generations are in practice, not different.

Shoot density shows realized heritability > 1.0 and high midparent heterosis, due to high average density measured in the progeny exceeding that of the parents. Subhanij (1974) found heterosis for tiller number in wide crosses in tall fescue.

Seed yield parent-offspring regression with alternate year data had standard errors that allowed the coefficient to pass through 0.0, so regression was performed using same

year data. This biases the heritability estimate upwards due to environmental correlation among relatives.

Rust resistance is most likely due to a single gene with dominance, based on regression of the sibship variance with the quadratic expression of the sibship mean, chi-square analysis of segregation ratios, and visual examination of distributions. For the remaining three traits none showed evidence of a major gene. Fain's test (Fain, 1978) has power in detecting the absence of a major gene, but not in detecting a major gene (Lynch and Walsh, 1998). A normal distribution is expected under polygenic inheritance, and the segregation of individual genes with small effects, generally cannot be distinguished. A major gene should show a combination of distributions, with inheritance in a Mendelian fashion. In order to distinguish between the two genetic models, the phenotypic mean of a major locus must be large enough to detect from deviations due to other genetic and/or environmental factors. Skewness in distributions is not uncommon and should be thoroughly examined as to cause.

Breeding systems that can be used effectively to improve a species are determined more by a species' mode of reproduction than by any other factor (Allard, 1960). Most perennial grasses reproduce predominantly or exclusively sexually via cross pollination, and a few by apomixis (Poehlman and Sleper 1995). Saltgrass is cross pollinated.

Recurrent restricted phenotypic selection, which is a form of mass selection, is used most often in cross pollinated grasses, because of significant additive genetic variance present in these species. Mass selection operates on additive genetic variance, meaning that traits which are selected for visually in the parents are passed onto the progeny.

Table 24. Response to selection, heritability estimates, heterosis, and major gene detection for 4 traits. Realized heritability uses Front Range population for reference. Other heritabilities use parents as reference population. *single year data . NA=not applicable.

	Response To Selection	Realized heritability	Narrow sense heritability half-sib families	Narrow sense heritability parent-offspring	Broad sense heritability from parents	Heterosis against midparent	Major gene
Rust resistance (%leaf area in pustules)	-1.2%	0.19	NA	NA	NA	NA	Yes
Height in cm	-2.8 cm	0.77	0.94	0.99	0.92	-8%	No
Shoots/cm^2	1.8/cm^2	1.74	0.94	0.93	0.96	18%	No
Seed yield kg/ha	428 kg *	0.42 *	0.84 *	0.45 *	0.80 *	-23%*	No

Heritability estimates are made to determine which traits have large additive effects and which do not. Narrow sense heritability is the ratio of estimated additive genetic variance over the estimated phenotypic variance. Quantitative traits which do not have large additive variance need either more replication or complex testing in order for selection to be effective.

When breeding a non-domesticated species, it is advantageous to start with a population having a high mean value and genetic variance for the traits of interest, in order to obtain a higher mean through selection (Bernardo, 2002). In the case of turfgrass, a high mean is a composite mean of those traits proposed by Turgeon (1985), and, Meyer and Funk (1989) and listed in the Introduction. In this experiment, for saltgrass, it is the composite mean of rust resistance, short height, shoot density, and seed yield. Means are easier to calculate than variances since they are first order statistics, whereas variances are more difficult to estimate because they are second order statistics, whose significance is based on variance of a variance. It takes more resources to estimate variances through more sampling and the use of mating designs (Bernardo, 2002). Breeders would much rather spend resources on the actual breeding and advancing the mean of the material than calculating variances (Bernardo, 2002).

Often, from a practical standpoint of resource allocation, the accuracy of variance estimates may be sacrificed. Throughout this chapter, comparisons are made of heritability estimates from other research, but all of these works, including this one, have sacrificed some of the requisite of random mating and sufficient environments in determining the variance components.

Major requirements for valid heritability estimates from half-sib family analysis are (Nguyen and Sleper, 1983):

1. parents are from a random mating population (in Hardy-Weinberg equilibrium).
2. population is at linkage equilibrium.
3. no linkage between all pairs of loci.
4. parents are selected at random.
5. disomic inheritance (saltgrass behaves as diploid; Reid, 2001)

The major requirements for valid heritability estimates from parent-offspring regression are 1. and 2. above, and additionally, no environmental covariance between parents and progeny.

In saltgrass, a minimum of 3 years of establishment and growth are necessary before all plants reach sexual maturity and could adequately mate randomly. It would take 6 years to plant the required generation from random mating suggested by Hallauer and Miranda (1988), and another 3 years before the first reliable data is obtained on rust resistance and seed yield. Estimates should be based on a minimum of 2 years, so the data is obtained after 10 years. If the experiment is duplicated at another location, the resource cost has doubled. It is easier to ask, how reliable are the estimates?

Hallauer and Miranda (1988) recommend 2 generations of random mating in maize before crosses to determine variance components. Nyquist (1991) recommends several. However, Bingham (1998) states linkage equilibrium doesn't reach a practical state until the F_8, so plants before those generations contain linkage groups that could inflate additive variance. These references are made in regard to crossing inbreds to produce F_2

populations and not in regard to cross fertilizing natural populations. Nguyen and Sleper (1983), in discussing forage breeding heritability estimates, recommend a single generation of random mating of germplasm. Vogel recommends a minimum of 2 generations of random mating. Vogel (1993) states that if perennial grass germplasm has not been randomly mated, then linkages have not been broken up, and means are also being represented by heterosis, and not solely additive gene action. Upon recombination, favorable linkage blocks would be broken up, and realized gain would be lower in successive generations. However, Vogel is referencing the bringing together of extensive collections separated by thousands of kilometers from diverse environments in which there would be greater expectation of diverse haplotypes.

Saltgrass used in the analysis is a very small subset of the four geographic regions sampled, with the furthest distance between parents of 64 km. The parents were considered a random mating population because of proximity in the upper South Platte watershed.

Midparent heterosis was found for seed yield (-23%), shoot density (18%), and short height (-8%). Falconer and Mackay (1996) define heterosis of a cross as:

$$\text{Heterosis} = dy^2$$

where d = dominance effect
y = difference in gene frequencies between the 2 parents;
parents represent populations with different gene frequencies.

Heterosis in this study is due to dominance effects (directional dominance so the sum of effects is not equal to zero) and unequal gene frequencies across parents. However, half-

sib and parent-offspring heritability estimates require equal gene frequencies across parents for precise estimates; i. e. parents should represent gene frequencies from a single random mating population.

Heterosis at a single locus with 2 alleles is a complementary effect of the 2 different alleles, A and a. The genotype Aa has a greater trait value than the mean of AA + aa (the parents of Aa). In this study, the progeny have a greater frequency of the heterozygote than the parents, with a parent in a cross, on average, having a greater frequency of homozygote (AA) of an allele than expected in a random mating population, with the other parent having a greater frequency of homozygote (aa) of the complementary allele than expected.

Females which produce half-sib families will have more homozygotes for the locus than females from a random mating population. However, the 14 females used in the study will vary in the number of homozygous loci, and their family trait means will vary by a similar amount. Since the difference between half-sib families is expected to measure additive variance, the additional variance from heterosis inflates the heritability estimate.

Parent-offspring regression is based on how offspring resemble the parent. Heterosis will change the mean value of progenies and the average effect across female parents will be to shift the regression line in the direction of trait value due to heterosis but parallel to the line without heterosis. If heterosis is higher in female parents with a high trait value than females with a lower trait value, then heritability will be inflated by heterosis. In corn (*Zea mays* L.), a few researchers have reported higher trait valued parents

demonstrating more heterosis than low valued parents, although other researchers have seen no difference (Hallauer and Miranda, 1988). The difference in results may have been due to culling in the parent population of inferior types before crosses.

Realized heritability will also be affected upwards since it is calculated by means of parents and progeny trait values, similar to the calculation for heterosis.

Broad sense heritability is not affected since it estimates total genetic variance.

The narrow sense heritabilitiy for short height, shoot density, and seed yield calculated by half-sib analysis are as high or higher than the broad sense estimate and may be inflated. Narrow sense heritability for short height and shoot density calculated by parent-offspring are as high as or higher than broad sense estimates and may be inflated.

If there is limited mating in this native saltgrass population, Hardy-Weinberg genotypic frequencies are not achieved and the average effect of each allele will have a slightly different value (Nyquist, 1991). Kearsey and Pooni (1998) state that natural mating is ill-defined, and that mixed inbreeding and random mating would be very difficult to model in genetic variance analysis. Interpretation of genetic variance estimates from such populations in terms of additive variance should be made cautiously (Dudley and Moll, 1969).

The heritability estimates, BLUPs, rust resistance pattern, major genes, and heterosis results and discussion should be confined to turf types selected by the selection index and their progenies. Heritability estimates would most likely fall in the next generation due to

attaining Hardy-Weinberg equilibrium. Generations after this would see a slight decrease due to attaining linkage equilibrium.

Linkages would inflate heritability estimates using half-sib family analysis, but not parent-offspring analysis (Lynch and Walsh, 1998; Nguyen and Sleper, 1983). Here, the only large difference seen in estimates between the 2 analyses is in seed yield heritability.

According to Falconer and Mackay (1996), when the parents are selected, parent-offspring regression is still a valid measure of ½ h^2. As stated above, the reference population would be turf-types defined by the selection index.

The common estimators for additive genetic variance usually contain additive x additive epistatic variance (1/16 among half sib families and 1/4 in COV(P,O)), but these are also selected for in recurrent phenotypic selection. In corn, estimates of epistatic variance were considered small (Hallauer and Miranda, 1988).

Bias due to environmental factors is considered more important, and can be more easily controlled (Nyquist, 1991). A variety, as well as its heritability estimates, is targeted for specified space and time for a geographic region. With too few experimental sites, the heritability estimate in half-sib family analysis, broad sense heritability calculation, and parent-offspring regression becomes biased upwards as the numerator is inflated (Nyquist, 1991; Casler, 1982; see Nyquist for corrections to Casler). In the saltgrass experiment, with a single location for density and height, the numerator actually becomes:

for half-sib and broad sense calculations σ_f^2 becomes: $\sigma_f^2 + \sigma_{fl}^2 /l$

for parent-offspring $2\ COV(P,O)$ becomes: $2\ [COV(P,O) + COV_{FL}\ (P,O) /l]$

Where:

σ^2_{fl} /l = family x location variance
COV_{FL} (P,O) /l = covariance between parent and offspring due to family x location

Only one year of data was used for seed yield because of maturity effect. This biases the heritability estimate additionally by inflating the numerator:

for half-sib and broad sense calculations σ^2_f becomes :

$$\sigma^2_f + \sigma^2_{fl} / l + \sigma^2_{fy} /y + \sigma^2_{fly} /ly$$

for parent-offspring 2 COV(P,O) becomes :

$$2[COV(P,O) + COV_{FY} (P,O) / l + COV_{FL} (P,O) /y + COV_{FLY}(P,O) / ly]$$

Where:
σ^2_{fy} /y = family x year variance
σ^2_{fly} /l y = family x location x year variance
COV_{FL} (P,O) /y = covariance between parent and offspring due to family x year
COV_{FLY}(P,O) /ly = covariance between parent and offspring due to family x location x year

The heritability estimates in this experiment are biased upwards because of genetic and environmental factors. However, there is agreement in the relative magnitude of the estimates with other research. The heritability estimates are high for density and height, and moderate for seed yield (presuming the half-sib estimate for seed yield as distorted from linkage). Corn has the most extensive genetic record, and estimates of heritability

(Hallauer and Miranda 1988) based on many years and locations are presented for comparison:

Heritability estimates, %	Traits
$h^2 > 70$	number of tillers
$50 < h^2 < 70$	plant height, days to flower, ear height
$30 < h^2 < 50$	number of ears, ear length, ear diameter, kernel weight
$h^2 < 30$	seed yield

Heritability estimates in the literature are usually upwardly biased because of genetic or environmental factors (Nyquist 1991). In presenting estimates, they should be described how they were obtained and where bias occurs. Biased estimates inflate expected response to selection, R, through the equation (Simmonds, 1979):

$$R=ih^2\sigma$$

Where:

i = intensity of selection

σ = phenotypic standard deviation of the parental population

Expected response from biased estimates will not be realized. However, in this experiment, realized significant response to selection for height and seed yield was effective and acting on additive genetic variance. The single location biases all these estimates upwards. Nevertheless, as preliminary estimates, these give the relative magnitudes for the traits under study.

The results indicate that successful breeding for rust would be to identify genotypes by segregation ratios in their pedigrees to determine homozygote resistant types. These can be used as parents, and in test crosses with phenotypic resistant plants to examine progeny for segregation. No segregation from the cross would also indicate homozygosity for resistance. If the program resources allow, DNA analysis and marker assisted selection should be started as described, as it would be more efficient over time.

Even though most of the narrow sense heritability estimates (possible exception is parent-offspring for seed yield) are most likely biased upwards, their very high values suggest breeding for the turf traits would be effective. Recombination from advancing the breeding population would most likely give lower and truer narrow sense heritability estimates for the traits. Recombination in later generations also would probably moderate the negative heterosis seen in seed yield and short height and make selection more effective in these traits. Heterosis in shoot density could be taken advantage of in specific crosses, and, more importantly, the dioecism of the species should be taken advantage of, mainly as a method of protecting the effort that goes into the breeding for a turf variety. Recent releases of buffalograss and bermudagrass have very low numbers of parents, and unscrupulous propagation of these varieties from varietal seed would result in extreme inbreeding and poor plant performance.

Future research is needed in elucidating the major gene for rust resistance; whether or not marker assisted selection would be cost effective in the program; and monitoring the rust races that occur in the natural environment. Leaf shredding from mowing, phenotypic plasticity of traits (ability of trait value to change due to environment), and

longevity of individual plants (some apparently healthy plants died in nurseries for unknown reasons) are just a few of the other traits that should be studied. Correlations and correlated response from selection should be examined for all traits. Unpublished data showed a movement of the relatively deep horizontal rhizome mass in saltgrass closer to the surface due to breeding for the 4 turf traits. Negative correlated responses may also be expected and these would be important from the standpoint they would hinder advance to a turf type.

The high heritability estimates and the demonstrated response to selection for turf traits in this study indicate saltgrass can be bred to a desirable turfgrass and provide benefits to the landscape.

CHAPTER V

Accession Evaluation

The four traits considered in need of improvement (percent leaf rust, height, shoot density, and seed yield) are graphed from the 1999-2001 accession nursery data (Figure 26, Figure 27, Figure 28, and Figure 29.). These graphs are very similar to the native population graphs for the same traits from the 2004-2006 progeny nursery data in Chapter III. Note the latter are composed only of Front Range random checks, while the accession nursery includes plants from the Great Basin, Nebraska, South Dakota, and the Front Range.

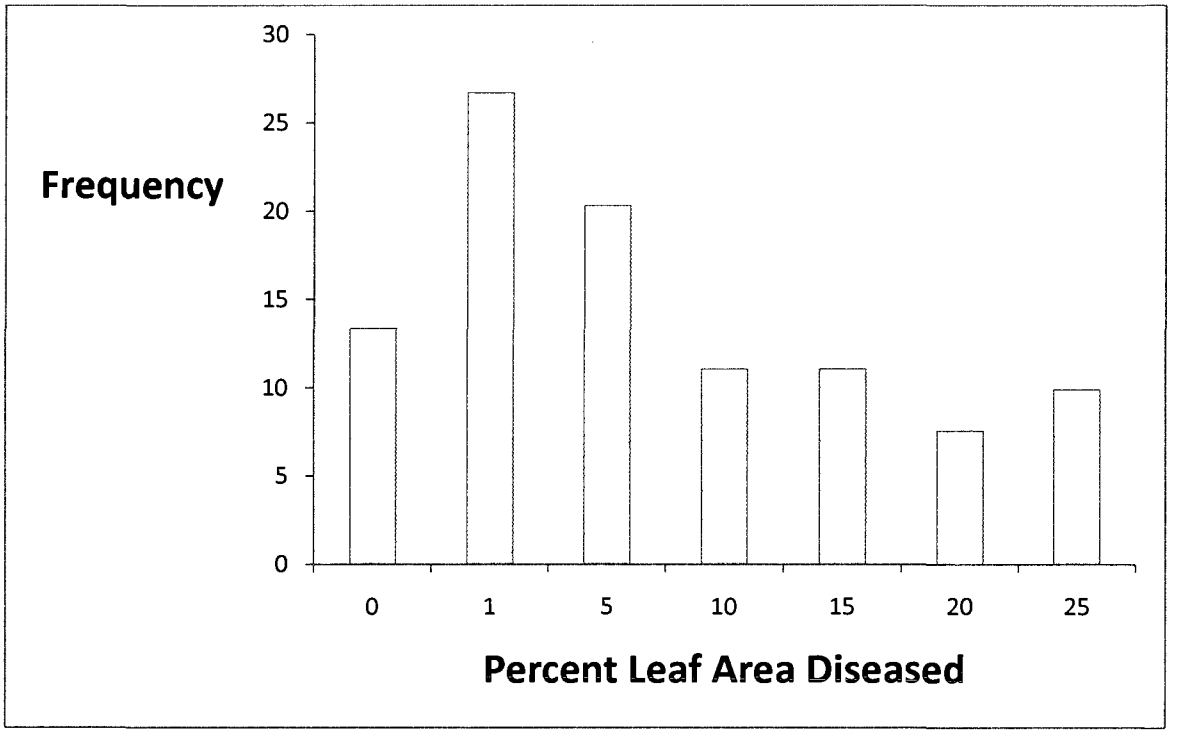

Figure 26. Male and female accessions (79) falling into 0, 1, 5, 10, 15, 20, and 25 percent leaf area diseased classes in 2001, at the Horticulture Field Research Center.

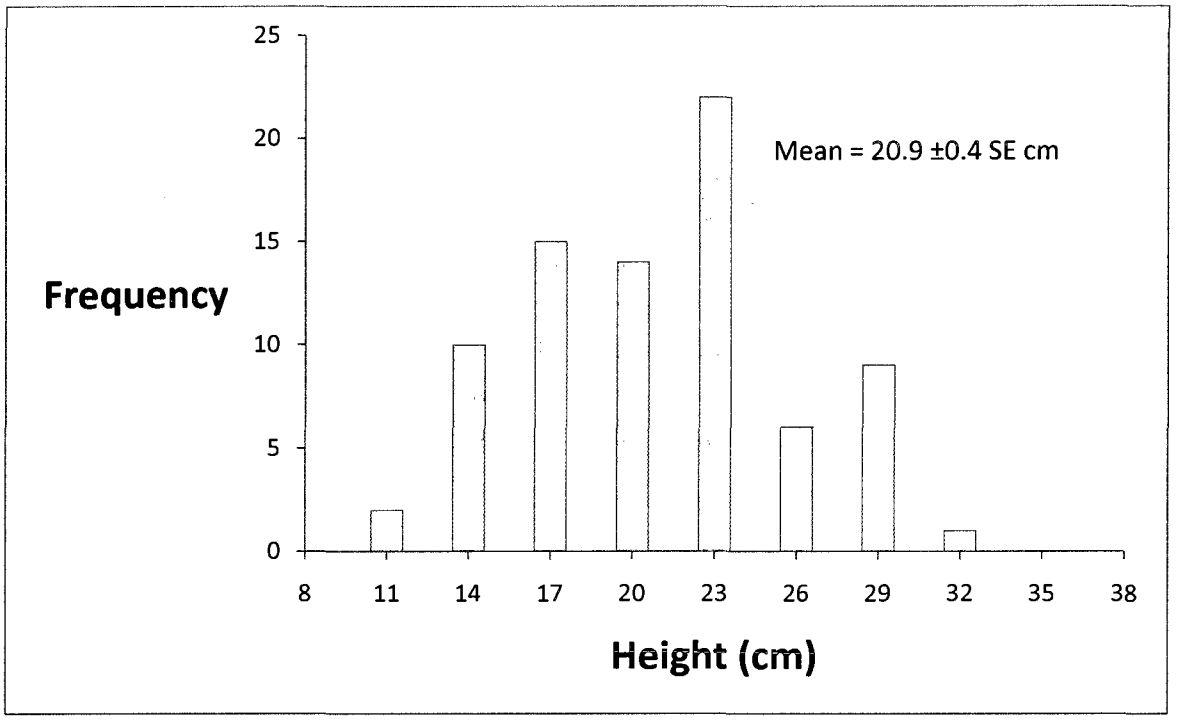

Figure 27. Male and female accessions (79) and their heights (cm) in 2000 and 2001at the Horticulture Field Research Center.

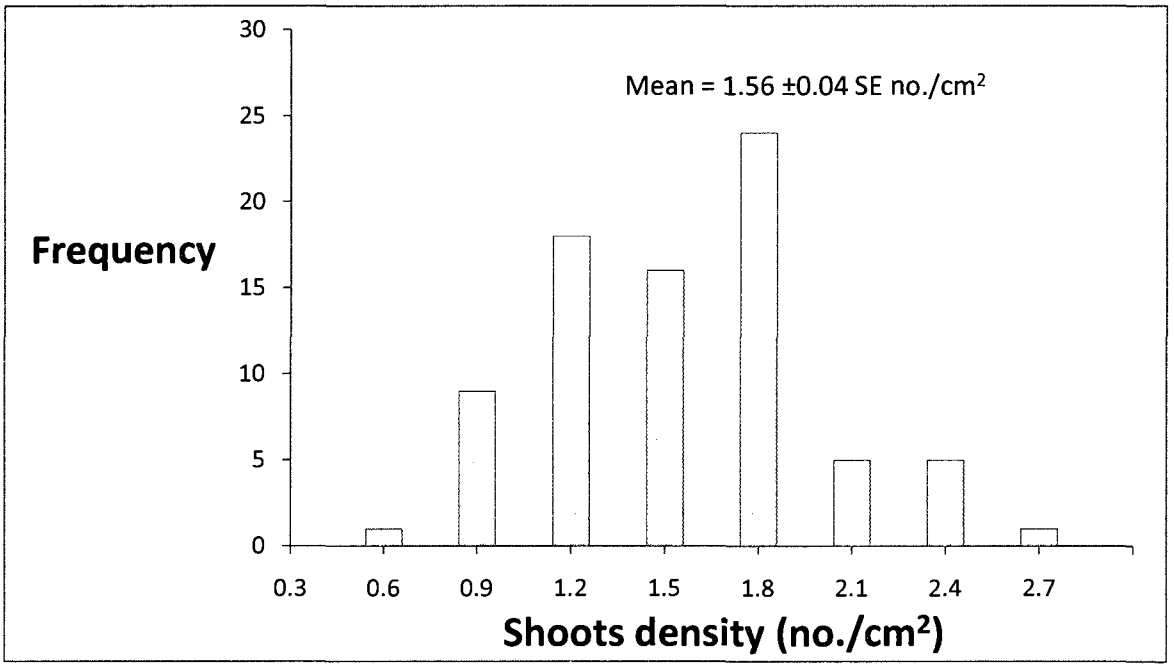

Figure 28. Male and female accessions (79) and their number of shoots per square centimeter in 2005 and 2006 combined at the Horticulture Field Research Center.

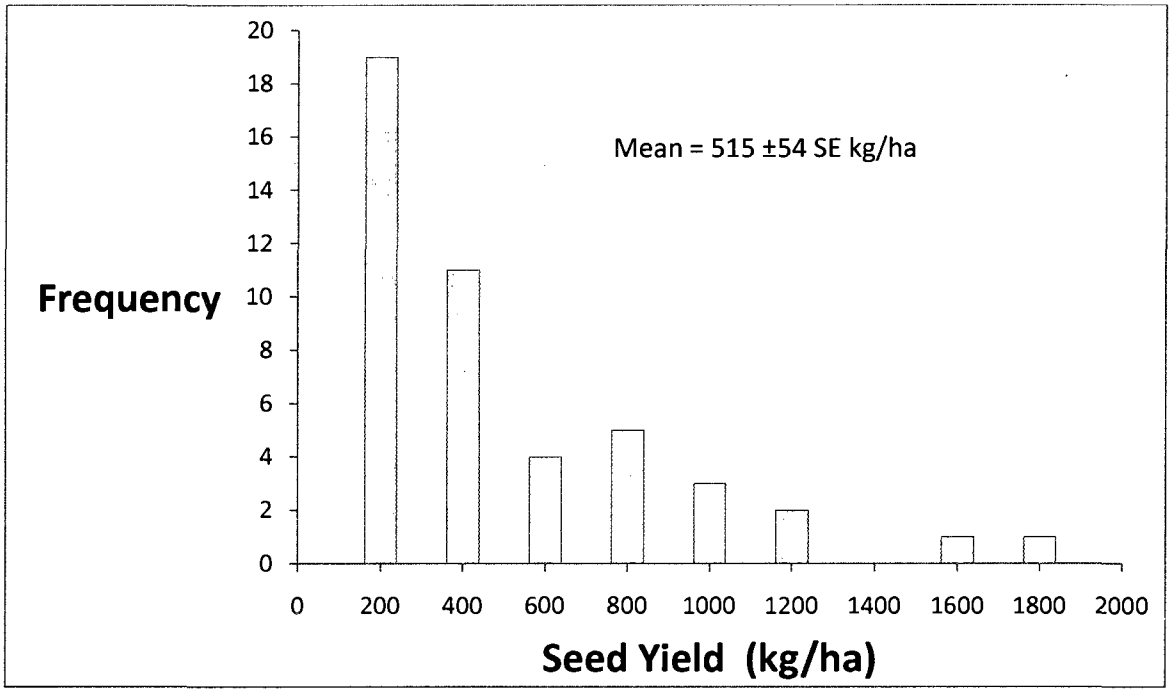

Figure 29. Female accessions (47) and seed yield (kg/ha) at the Horticulture Field Research Center.

The following traits (their relevance in parentheses) were analyzed to see if there were differences between the four geographic regions from which collections were made:

1. Average percent leaf rust (leaf rust causes leaves to die in August)
2. Canopy height (short height for less mowing)
3. Shoot density (high density for uniform surface)
4. Seed yield (high seed yield for propagating a variety)
5. Days to first flower (to determine synchrony so crosses can be made)
6. Spread (measure of vigor)
7. Gap (estimate of percent ground never filled in after rhizomes reach plot edge)
8. Seed length (estimate of endosperm available for seed germination)
9. Height of female head / height of leaf canopy (high ratio makes harvesting easier)

Average Percent Leaf Rust

Since the residuals of percent leaf rust data were not normal for severe rust pressure in 2001, the Kruskal-Wallace test was run on Wilcoxon scores (Table 25). The probability of observing a greater chi-square is <0.0001, which indicates region of origin had an effect on accessions' susceptibility to leaf rust.

Table 25. Mean percent leaf rust and Kruskal-Wallis test for origin of accession effect on leaf rust susceptibility. Probability that plants from four different regions of origin have the same reaction to leaf rust is <0.0001

Region-accessions	Mean percent leaf rust	Wilcoxon Scores (Rank Sums) for percent leaf rust 2001			
		Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Great Basin- 21	14.46%	4381	3239	244.2	106.8
S. Dakota-21	2.64%	2274	3318	246.1	54.1
Nebraska-16	4.00%	2301	2528	224.0	71.9
Front Range-21	8.67%	3446	3318	246.1	82.0

Kruskal-Wallis Test	
Chi-Square	34.9
DF	3
Pr > Chi-Square	<0.0001

All two region comparisons were made using Wilcoxon Two-Sample Test (Table 26). A Bonferroni adjustment to the probability values (division by the number of comparisons=6) was used to control the maximum experiment wise error rate (MEER, Schlotzhauer and Littell, 1997)

Table 26. Region to region comparisons of effect of origin of accession on susceptibility to leaf rust in the 2001. Asterisk denotes significant difference between the regions in susceptibility to leaf rust. Controlled for multiple comparisons.

Region to Region Comparison	**Wilcoxon Two Sample Test with Bonferroni Adjustment for MEER, α=.05, then a comparison is significant when p<0.0083**
Great Basin to S. Dakota	p<0.0001 *
Great Basin to Nebraska	p=0.0001 *
Great Basin to Front Range	p=0.0058 *
S. Dakota to Nebraska	p=0.0132
S. Dakota to Front Range	p=0.0045 *
Nebraska to Front Range	p=0.1888

Canopy Height

Canopy height showed significant differences by region, and by years (Table 27).

Table 27. Tests of fixed effects for canopy height (cm) in 2000 and 2001(n=21 for regions, except Nebraska, n=16).

Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
region	3	151.1	8.01	<.0001
year	1	1.843	51.14	0.0236
region*year	3	149.8	0.56	0.6407

Differences between year means was ≈ 8.8 cm (Table 28), and most likely due to different dates for measurements, September 10, 2000, and July 23, 2001. Both tests received approximately 16 cm of irrigation during the first 2 weeks of May. Precipitation from the previous October 1 up until height measurement was 8.9 cm in 2000 and 20 cm in 2001. The longer time for growth in 2000 may have caused the difference in measured height between 2000 and 2001.

Table 28. Estimate of height (cm) by years in the accession nursery.

year	Estimate	Standard Error	DF	t Value	Pr > |t|
2000	25.1	0.9	2.02	29.51	0.0011
2001	16.3	0.8	1.98	19.27	0.0028

Tukey-Kramer grouping showed Front Range and Great Basin accessions had significantly shorter height than accessions from South Dakota and Nebraska (Table 29).

Table 29. Tukey-Kramer grouping for region of origin effect on accessions' height (cm) (α=0.05). LS-means with the same letter are not significantly different.

Region-accessions	Estimate	
S. Dakota-21	22.6	A
Nebraska-16	22.5	A
Great Basin-21	19.5	B
Front Range-21	18.5	B

Differences of least squares means are shown in Table 30 and these pair wise differences are graphed in a diffogram immediately below (Figure 30).

"The Diffogram, also known as a mean-mean scatter plot, is a graphical display of all pairwise differences. The 45° reference line indicates whether two least-squares means are significantly different at a given significance level. Vertical and horizontal reference (grid) lines are drawn at the values of the least-squares means. A line is drawn at the intersection of the grids lines that corresponds to the (1-α) x 100% confidence interval of the difference of the two least-squares means in the comparison." (Littell et al., 2006).

The diffogram (Figure 30) shows that Front Range--Nebraska, Front Range--South Dakota, and Great Basin-- South Dakota differences are significant.

Table 30. Differences of region of origin effect on least squares means for height (cm). and Tukey-Kramer adjustment for multiple comparisons.

region	_region	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P
Front Range	Great Basin	-1.0	0.8	298	-1.26	0.2081	0.5881
Front Range	Nebraska	-4.1	0.9	298	-4.66	<0.0001	<0.0001
Front Range	S. Dakota	-4.1	0.8	298	-5.10	<0.0001	<0.0001
Great Basin	Nebraska	-3.1	0.9	298	-3.51	0.0005	0.0029
Great Basin	S. Dakota	-3.1	0.8	298	-3.85	0.0001	0.0008
Nebraska	S. Dakota	-0.1	0.9	298	-0.04	0.9649	1.0000

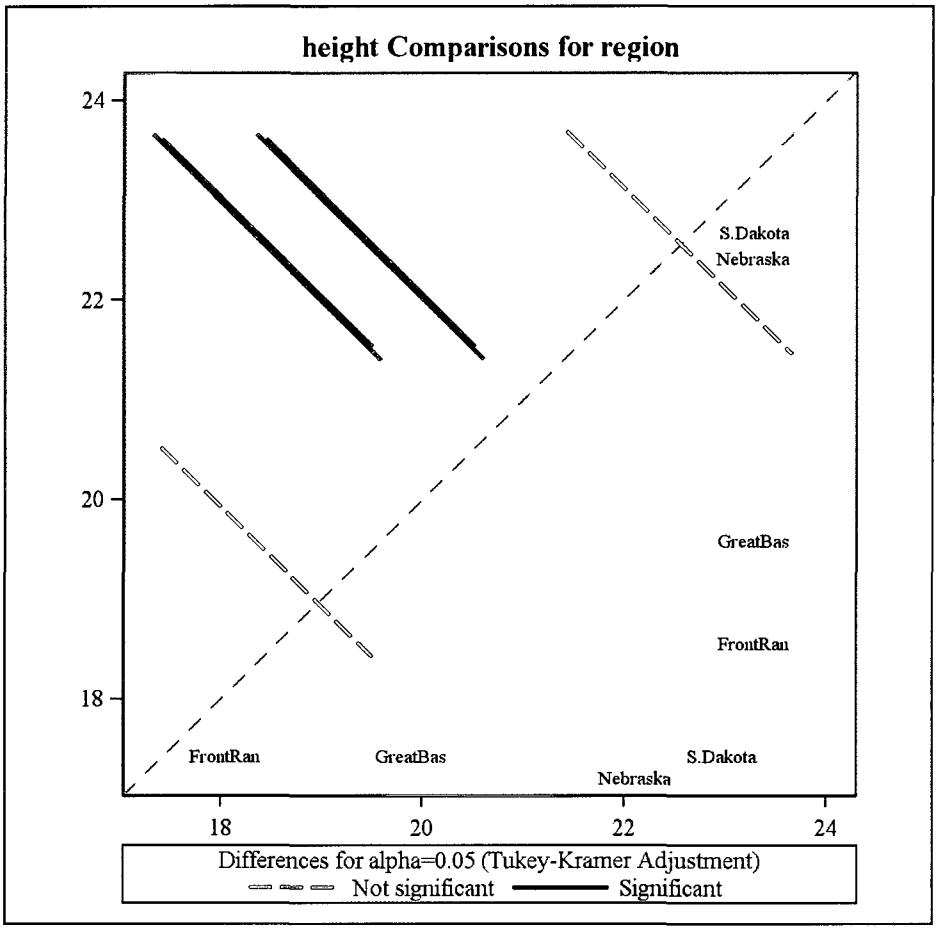

Figure 30. Diffogram showing pairwise differences in height (cm) due to origin of accession. Confidence intervals are always in the upper part of the square. If they intersect the 45° line, the differences are not significant.

Shoot Density

Tukey-Kramer grouping showed that the only difference in shoot density was between Front Range and South Dakota regions (Table 31).

Table 31. Tukey-Kramer grouping for region of origin effect on accessions' shoot density (no./cm^2) (α=0.05). LS-means with the same letter are not significantly different.

Region-accessions	Estimate		
Front Range-21	1.84		A
Great Basin-21	1.59	B	A
Nebraska-16	1.50	B	A
S. Dakota-21	1.30	B	

Differences of least squares means are shown in Table 32 and these pairwise differences are graphed in a diffogram in Figure 31.

Table 32. Differences of region of origin effect on least squares means for shoot density (no./ cm^2) and Tukey-Kramer adjustment for multiple comparisons.

region	_region	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P
Front Range	Great Basin	0.25	0.11	3	2.25	0.1097	0.2877
Front Range	Nebraska	0.34	0.12	3	2.91	0.0622	0.1723
Front Range	S. Dakota	0.54	0.11	3	4.96	0.0157	0.0464
Great Basin	Nebraska	0.09	0.12	3	0.79	0.4848	0.8538
Great Basin	S. Dakota	0.30	0.11	3	2.68	0.0749	0.2043
Nebraska	S. Dakota	0.20	0.12	3	1.71	0.1856	0.4486

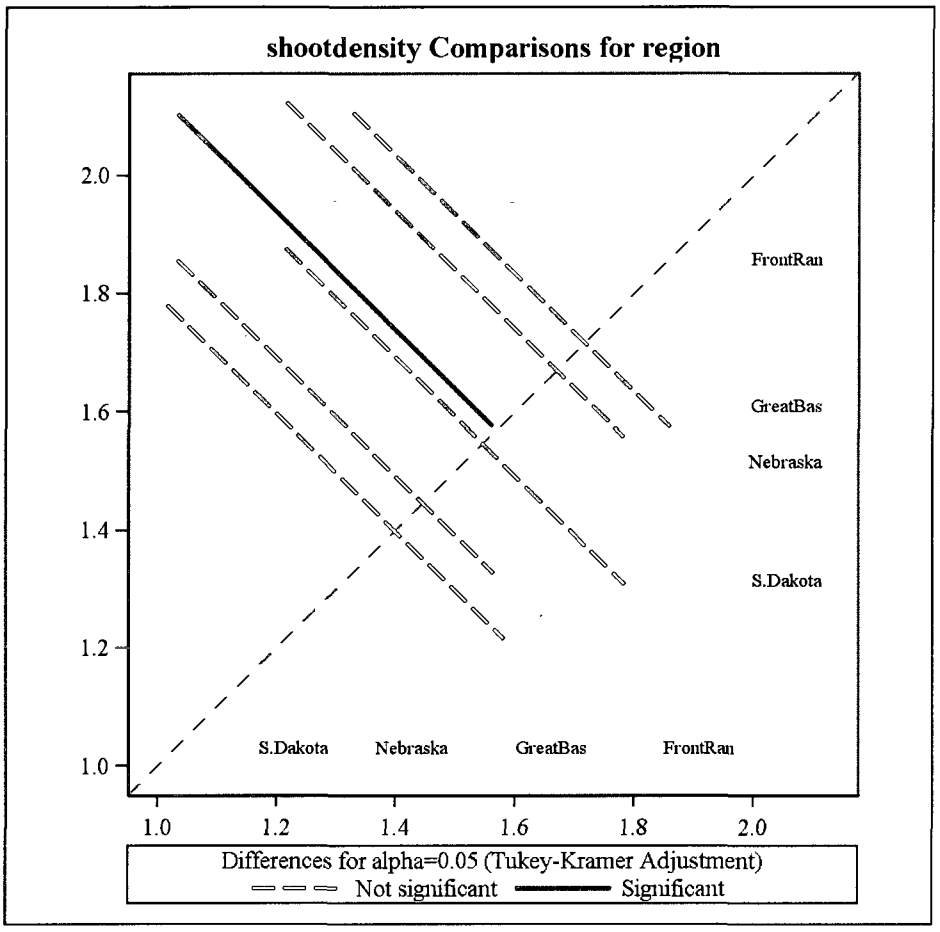

Figure 31. Diffogram showing pairwise differences in shoot density (no. /cm^2) due to origin of accession. Confidence intervals are always in the upper part of the square. If they intersect the 45° line, the differences are not significant.

Seed Yield

There was no region effect on seed yield (Table 33 and Appendix III). LS means for seed yield in kg/ha by region are shown in Table 34. The Front Range mean is more than twice the Nebraska mean.

Table 33. Tests of fixed effects for seed yield in 2000 (α=0.05).

Effect	Num DF	Den DF	F Value	Pr > F
region	3	3	2.75	0.2139

Table 34. Estimates of seed yield in kg/ha by region of origin in 2000.

Region-accessions	Estimate	Standard Error	DF	t Value	Pr > |t|
Front Range - 12	711	104	3	6.79	0.0065
Great Basin - 10	593	112	3	5.28	0.0132
Nebraska - 13	315	98	3	3.20	0.0494
S. Dakota - 12	475	104	3	4.54	0.0200

Days to First Flower

Tukey-Kramer grouping showed South Dakota accessions had significantly shorter time to flower than accessions from other regions (Table 35).

Table 35. Tukey-Kramer grouping for region of origin effect on accessions' days to first flower from Jan 1, 2000 (α=0.05). LS-means with the same letter are not significantly different.

Region-accessions	Estimate	
Front Range-21	147.00	A
Nebraska-16	146.38	A
Great Basin-21	146.27	A
S .Dakota-21	143.12	B

Differences of least squares means are shown in Table 36 and these pairwise differences are graphed in a diffogram in Figure 32.

Table 36. Differences of region of origin effect on least squares means for first flower in days from January 1, and Tukey-Kramer adjustment for multiple comparisons.

region	_region	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P
Front Range	Great Basin	0.7	0.7	3	1.10	0.3512	0.7138
Front Range	Nebraska	0.6	0.7	3	0.92	0.4239	0.7973
Front Range	S. Dakota	3.9	0.6	3	6.34	0.0079	0.0238
Great Basin	Nebraska	-0.1	0.7	3	-0.16	0.8826	0.9982
Great Basin	S. Dakota	3.1	0.6	3	4.89	0.0164	0.0483
Nebraska	S. Dakota	3.3	0.7	3	5.01	0.0153	0.0452

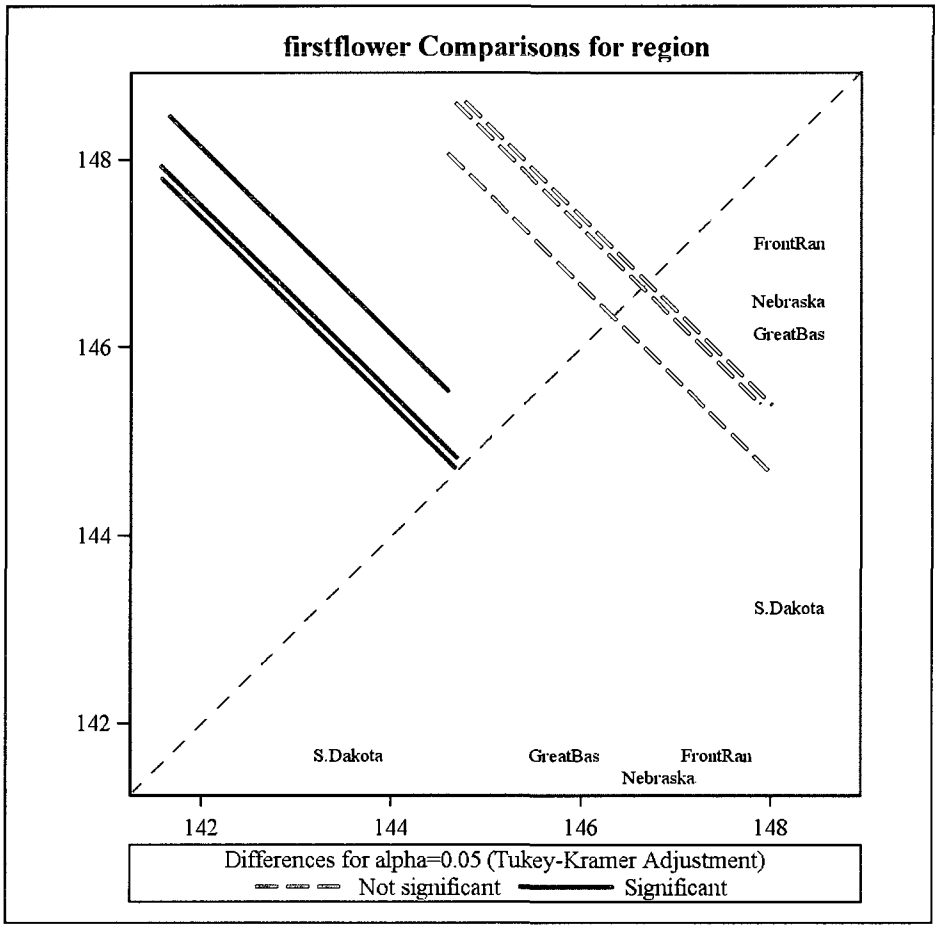

Figure 32. Diffogram showing pairwise differences in days to first flower from January 1 due to origin of accession. Confidence intervals are always in the upper part of the square. If they intersect the 45° line, the differences are not significant.

Spread

Spread from a 10 x 10 cm plug in one year's time was measured as an elliptical area calculated by rhizome length on north-south and east-west axes and expressed as a percentage of 4.6 X 4.6 meter plot. Front Range accessions showed a significantly higher percent fill in of the plot in one year than other regions' accessions (Table 37).

Table 37. Tukey-Kramer grouping for region of origin effect on accessions' spread (percent cover of plot) in one year (α=0.05). LS-means with the same letter are not significantly different

Region-accessions	Estimate	
Front Range-21	50.6180	A
Great Basin-21	33.7912	B
S. Dakota-21	21.9543	B
Nebraska-16	21.5906	B

Differences of least squares means are shown in Table 38 and these pairwise differences are graphed in a diffogram in Figure 33.

Table 38. Differences of region of origin effect on least squares means for spread (percent cover of plot), and Tukey-Kramer adjustment for multiple comparisons.

region	_region	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P
Front Range	Great Basin	16.8	3.4	3	4.90	0.0163	0.0481
Front Range	Nebraska	29.0	3.6	3	7.96	0.0041	0.0125
Front Range	S. Dakota	28.7	3.4	3	8.45	0.0035	0.0105
Great Basin	Nebraska	12.2	3.7	3	3.31	0.0455	0.1286
Great Basin	S. Dakota	11.8	3.4	3	3.45	0.0411	0.1169
Nebraska	S. Dakota	-0.4	3.6	3	-0.10	0.9269	0.9996

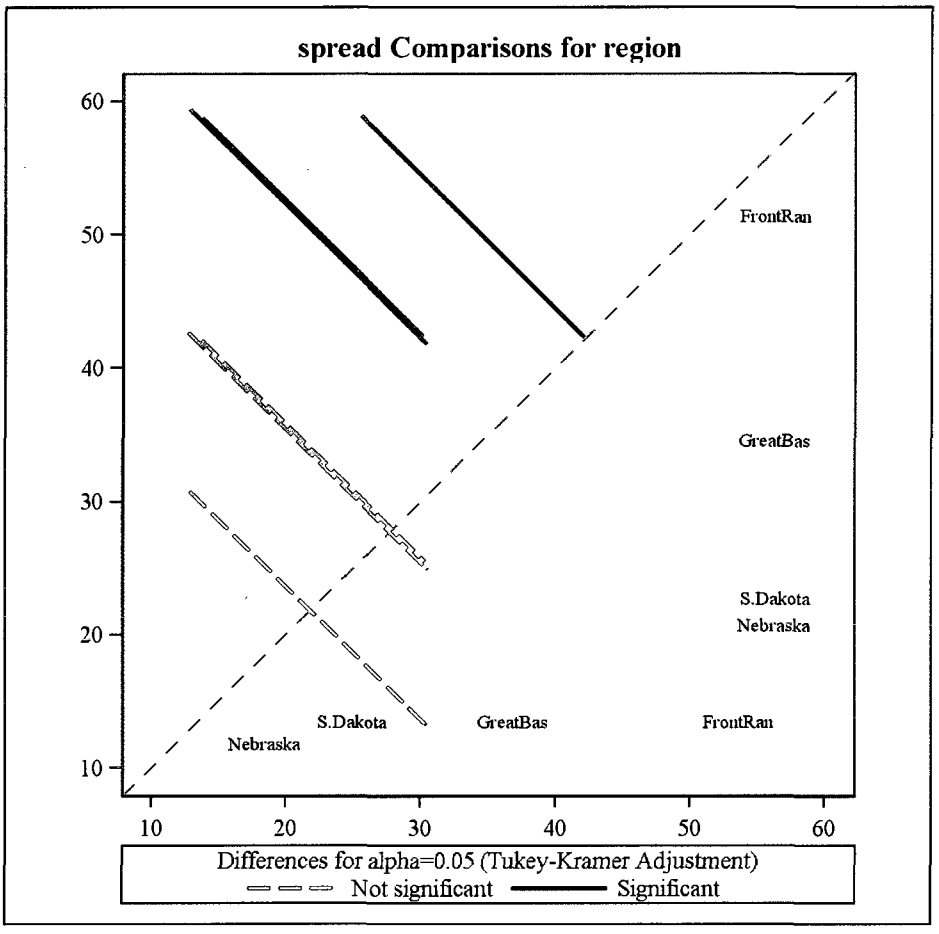

Figure 33. Diffogram showing pairwise differences in spread (percent fill of 4.6 x 4.6 meter plot in one year) due to origin of accession. Confidence intervals are always in the upper part of the square. If they intersect the 45° line, the differences are not significant.

Gap

Some accessions exhibited a trait termed gap, in which, even though rhizomes had reached the edge of the 4.6 x 4.6 meter plot rapidly, there was barren ground within the plot that did not fill in. Other accessions filled in the plot uniformly without exhibiting gap. Gap is a visual estimate of percent barren ground in the plot after two years' growth from a 10 x 10 cm plug.

Tukey-Kramer grouping showed significant differences in gap among regions (Table 39). The Front Range accessions averaged significantly lower for bare ground after 2 years growth.

Table 39. Tukey-Kramer grouping for region of origin effect on accessions' gap trait (percent barren ground after two years in a 4.6 x 4.6 meter plot (α=0.05). LS-means with the same letter are not significantly different.

Region-accessions	Estimate		
S. Dakota-21	37.0		A
Nebraska-16	29.7		A
Great Basin-21	21.9	B	A
Front Range-21	9.7	B	

Differences of least squares means are shown in Table 40 and these pairwise differences are graphed in a diffogram in Figure 34.

Table 40. Differences of region of origin effect on least squares means for gap (percent barren ground in a 4.6 x 4.6 meter plot after two years) and Tukey-Kramer adjustment for multiple comparisons

region	_region	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P
Front Range	Great Basin	-12.2	3.3	3	-3.65	0.0356	0.1020
Front Range	Nebraska	-20.0	3.6	3	-5.60	0.0112	0.0335
Front Range	S. Dakota	-27.3	3.3	3	-8.20	0.0038	0.0115
Great Basin	Nebraska	-7.8	3.6	3	-2.17	0.1188	0.3084
Great Basin	S. Dakota	-15.1	3.3	3	-4.51	0.0204	0.0597
Nebraska	S. Dakota	-7.3	3.6	3	-2.06	0.1318	0.3372

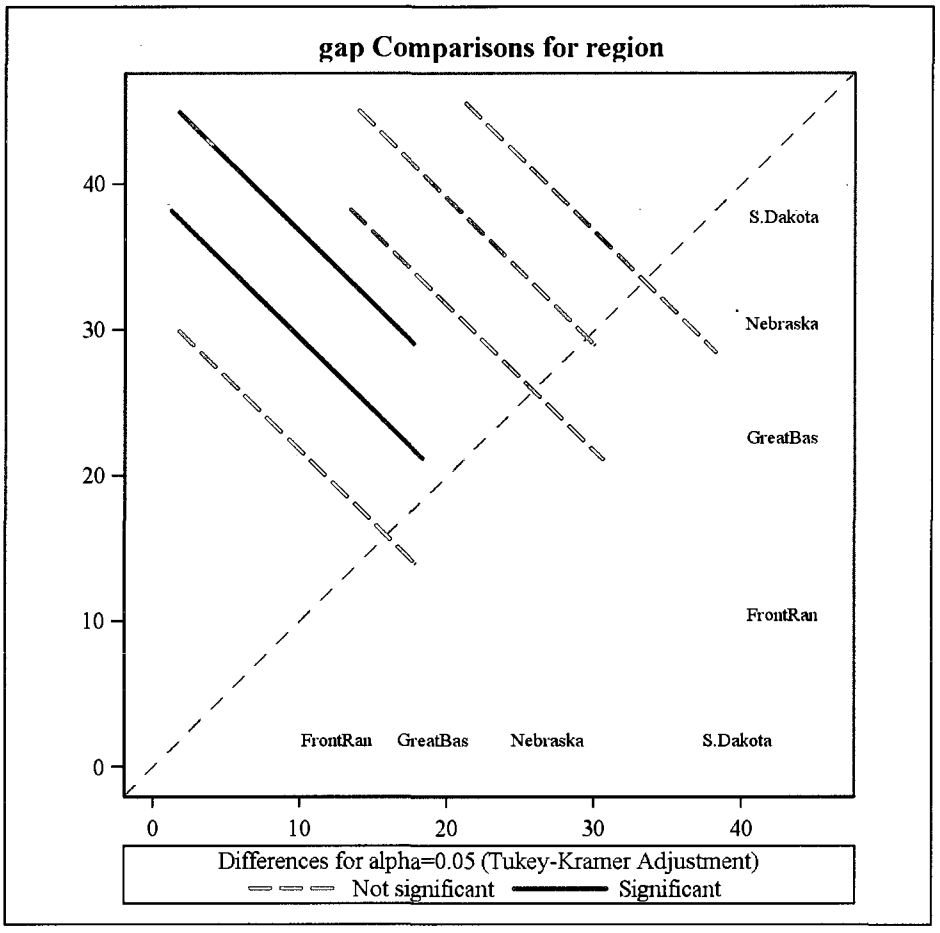

Figure 34. Diffogram showing pairwise differences in gap (percent barren ground in a 4.6 x 4.6 plot after two years growth) due to origin of accession. Confidence intervals are always in the upper part of the square. If they intersect the 45° line, the differences are not significant.

Seed Length

Seed length was used as an estimate of seed endosperm size. Great Basin area accessions showed much smaller seed length than other regions (Table 41).

Table 41. Tukey-Kramer grouping for region of origin effect on accessions' seed length (mm) (α=0.05). LS-means with the same letter are not significantly different.

Region-accessions	Estimate		
Nebraska-13	4.3		A
S. Dakota-12	4.2		A
Front Range-12	3.7	B	A
Great Basin-10	3.0	B	

Differences of least squares means are shown in Table 42 and these pairwise differences are graphed in a diffogram in Figure 35.

Table 42. Differences of region of origin effect on least squares means for seed length (mm) and Tukey-Kramer adjustment for multiple comparisons.

region	_region	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P
Front Range	Great Basin	0.7	0.2	3	4.26	0.0237	0.0692
Front Range	Nebraska	-0.7	0.2	3	-4.26	0.0237	0.0691
Front Range	S. Dakota	-0.5	0.2	3	-3.09	0.0537	0.1502
Great Basin	Nebraska	-1.4	0.2	3	-8.69	0.0032	0.0097
Great Basin	S. Dakota	-1.2	0.2	3	-7.33	0.0052	0.0158
Nebraska	S. Dakota	0.2	0.2	3	1.04	0.3760	0.7444

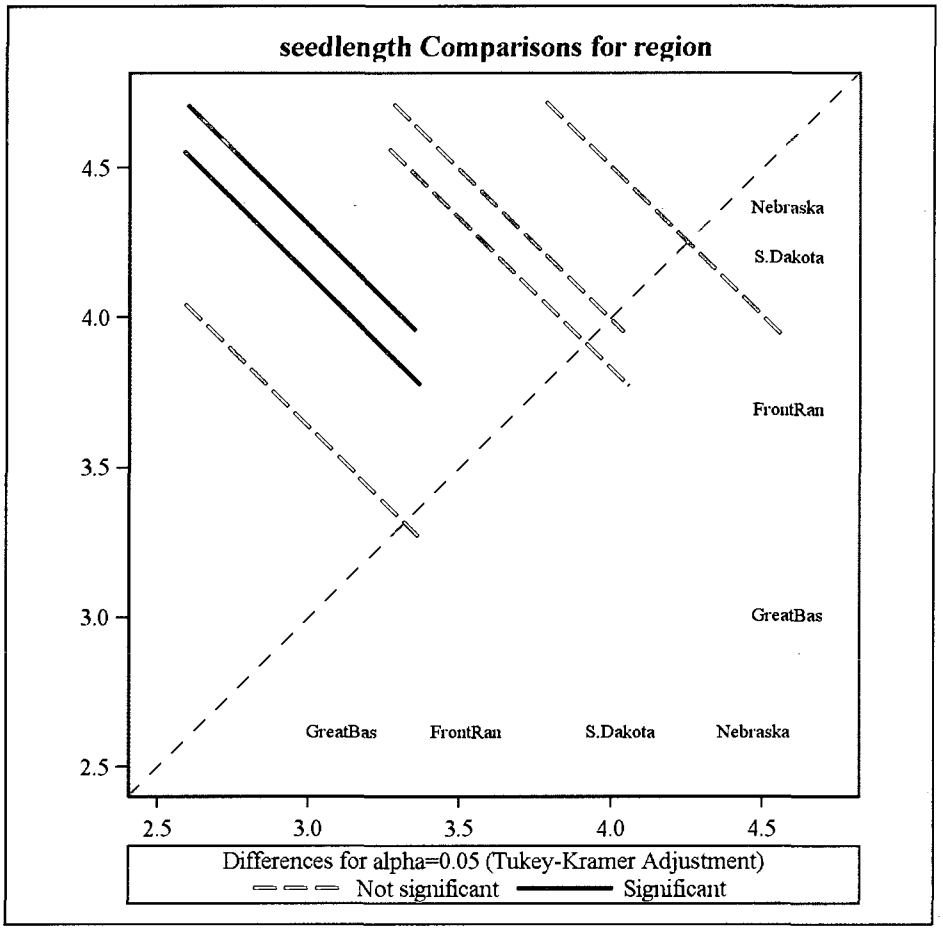

Figure 35. Diffogram showing pairwise differences in seed length (mm) due to origin of accession. Confidence intervals are always in the upper part of the square. If they intersect the 45° line, the differences are not significant.

Female head height / leaf canopy height

Desert saltgrass female heads are below the leaf canopy, while the male heads are above. Most accessions have the female heads within several centimeters of the ground, even with 20+ cm leaf canopy. A tall female head relative to leaf canopy height is advantageous to mechanical harvest. The height ratio = height of female head/height of leaf canopy. Great Basin accession female heads were very close to the top of their leaf canopies compared to the accessions from the other three regions (Table 43).

Table 43. Tukey-Kramer grouping for region of origin effect on accessions' height ratio (female head height / leaf canopy height) (α=0.05). LS-means with the same letter are not significantly different

Region-accessions	Estimate	
Great Basin-10	0.90	A
S. Dakota-12	0.46	B
Front Range-12	0.40	B
Nebraska-13	0.43	B

Differences of least squares means are shown in Table 44 and these pairwise differences are graphed in a diffogram in Figure 36.

Table 44. Differences of region of origin effect on least squares means for height ratio (female head height / leaf canopy height) and Tukey-Kramer adjustment for multiple comparisons.

region	_region	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P
Front Range	Great Basin	-0.50	0.03	3	-13.91	0.0008	0.0025
Front Range	Nebraska	0.03	0.03	3	0.03	0.9803	1.0000
Front Range	S. Dakota	-0.06	0.03	3	-1.00	0.3929	0.7639
Great Basin	Nebraska	0.47	0.03	3	13.01	0.0010	0.0030
Great Basin	S. Dakota	0.44	0.03	3	12.34	0.0011	0.0035
Nebraska	S. Dakota	-0.04	0.03	3	-0.95	0.4128	0.7858

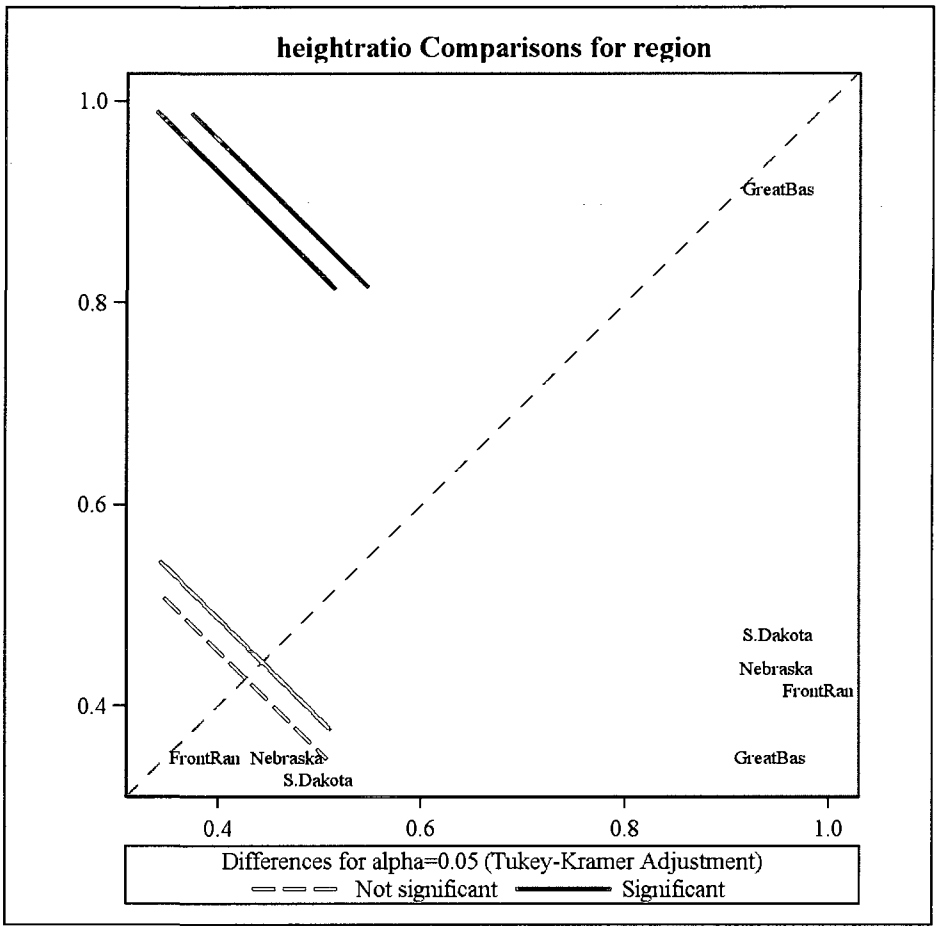

Figure 36. Diffogram showing pairwise differences in height ratio (female head height / leaf canopy height) due to origin of accession. Confidence intervals are always in the upper part of the square. If they intersect the 45° line, the differences are not significant.

Summary and Discussion

Table 45 shows trait means by region.

Table 45. Trait means by region. Asterisk denotes significant differences from other regions for a trait towards the favored value for selection as a turf trait.

Region / Trait	Front Range	South Dakota	Nebraska	Great Basin
Percent leaf rust average	8.76 *	2.64 *	4.00 *	14.46
Canopy height cm	18.5 *	22.6	22.5	19.5 *
Shoot density no. / cm^2	1.84 *	1.30	1.50	1.59
Seed yield kg / ha	711	473	315	593
Days to first flower	147.0 *	143.1	146.4 *	146.3 *
Spread %	50.6 *	22.0	21.6	33.8
Gap %	9.7 *	37.0	29.7	21.9
Seed length mm	3.7	4.2 *	4.3 *	3.0
Height head / Height canopy	0.43	0.46	0.43	0.90 *

South Dakota accessions had the least infection from leaf rust, followed by the Nebraska, and the Front Range accessions; the Great Basin accessions appeared to be most susceptible. Since significant differences in leaf rust susceptibility occurred (Table 26), either different allelic frequencies, or different alleles are present for resistance among the populations. Rusts in grasses generally evolve into many different races with differing virulence, and given the distance and climatic differences among regions selecting for different host genetic backgrounds and different pathogen genetic

backgrounds, genetic interactions between host and pathogen are probably complex among regions (Agrios, 1997). A saltgrass population from the San Joaquin Valley in California grown along-side these four groups never showed rust pustules over three years. Crosses between resistant and susceptible host types would provide segregation ratios to determine major gene action. DNA analysis on both the host and pathogen could provide rapid screening for present resistance and identify rust races (Chapter IV, p. 55).

The Front Range group and the Great Basin group had significantly shorter height, and also the highest shoot density. These two traits are related by Grafius's corollary that number and size tend to have an inverse relationship (Grafius, 1978). The Great Basin group showed the same inverse relationship, though density was not significantly different from that of other groups (although the confidence intervals are skewed in the diffogram indicating a difference at a higher probability threshold).

Canopy height may be correlated with precipitation. The Front Range and Great Basin regions' plant ancestry evolved in areas receiving less than 40 cm of precipitation per year, compared to Nebraska and South Dakota regions whose plant ancestry evolved in areas receiving 51 cm per year. Plants in arid areas tend to be shorter than plants in humid areas (Allard, 1970). Also, since the Front Range area is heavily populated by humans, mowing and traffic may have increased alleles in the population for short height, as has occurred in other species in human-dominated habitats (Linhart and Grant, 1996).

Seed yield was not significantly different among regions of origin, even though the largest mean (from the Front Range region), was over twice that of the smallest mean (from Nebraska region). The standard errors of the estimates of differences were high, sometimes higher than the estimates themselves (Appendix IV). This indicates data were

quite variable. About 14 % of the accessions never flowered over 5 years, and many produced little seed. Also, some variability can be attributed to a smaller sample size. The sample size for this trait was around 10 accessions per region, about half the size for measuring the other traits (because only females were used). Though there was only one location for this study, seed yield interactions with different environments (here, regions) are the norm in most species, and any significance among the four groups would be expected to be different in other regions (Bernardo, 2002).

Regions had similar accumulated days from January 1 to first flower, with the exception of South Dakota which was earlier by about three days. This indicates that crosses with South Dakota accessions may be more difficult because anthesis is not in synchrony with the accessions from the other three locations. MacMillan (1959) found that northern ecotypes of prairie grasses flowered earlier when transplanted at a central test site; the date of flowering was similar to the ecotypes' native habitat. In the saltgrass recurrent breeding population, flower induction can be manipulated so that elite plants flower in synchrony.

Front Range types had a significantly higher percentage of ground area covered in one year's growth. At 50.6% of plot fill-in, it was twice the average of the other three groups. Spread is a measure of vegetative reproduction (Freas, 1987), and a measure of fitness of saltgrass to its regional environment (Falconer and Mackay, 1996). Front Range types may have been favored for spread, since the study site was in the same region in which Front Range types evolved in. Transplanted ecotypes of four prairie grasses from over twenty-five states established over three years in Austin, Texas showed surviving

types after five years were mainly from local populations, or from nearby regions, and survivability was indicated by similar environments (McMillan, 1969).

Gap is a trait some phenotypes express in which rhizomes and shoots do not grow into bare ground, creating an asymmetric mosaic pattern. Front Range types were significantly lower in this trait. Great Basin types would have been significant at a higher probability threshold (Figure 34). Many turfgrass diseases show distinct boundaries between healthy sod and dead sod (Shurtleff et al, 1987). Dieback in potted saltgrass plants has been noted without any shoot signs of disease (personal observation). Avoidance of rhizome growth into soil areas by some accessions may be due to presence of soil borne pathogens which possibly could elicit an antagonist response in rhizomes. However, an extensive search of the literature revealed no research addressing antagonistic responses of roots or rhizomes to soil borne plant disease.

The South Dakota and Nebraska groups had significantly larger seed size than the groups from the Front Range or Great Basin. Though seed yield was not significantly different among groups, an inverse relationship exists between seed yield and seed size (Table 45). Turnbull et al. (1999) determined that species producing larger seeds suffer reduced fecundity. However, large seeded species won their establishment site when in direct competition with other species, while small seeded species won many sites by forfeit because large seeded species are recruitment limited. During accession collection it appeared that the South Dakota and Nebraska sites had greater species diversity and more vegetative cover than the Front Range and Great Basin sites. In order to compete for sites, based on results of Turnbull et al. (1999), South Dakota and Nebraska accessions would have larger seeds, whereas with the Front Range and Great Basin sites,

with fewer species and less vegetation (allowing more sites for establishment), smaller seed and greater seed number would be an advantage.

The height of female head / height of the leaf canopy ratio is significant and large in the Great Basin accessions, when compared to the other three groups. This is an important finding, since other regions had female heads close to the ground, making mechanical harvest difficult. Strong selection pressures resulted in the differences between the Great Basin accessions and other regions'accessions for this trait. The Front Range, South Dakota, and Nebraska region are within the Great Plains. The Great Plains contained the highest densities of bison (*Bison bison*) in North America starting 300,000 to 600,000 years ago, and only when the white man colonized the west were populations decimated (Lott, 2002). The primitive Great Plains bison population from southern Saskatchewan to northern Texas was estimated at 24 -27 million. Bison were rare to nonexistent in the Great Basin. Since bison need water, they would have followed the river valleys while grazing, the major environment where saltgrass grows. Seed survivability would be largely based on avoidance of grazing, and those seeds in heads close to the ground would avoid the mastication and ruminant digestion of the large herbivores. Short plant types, in the case of saltgrass, plant types with short female heads, would have a selection advantage under grazing, with seed surviving and passing on their alleles for short female heads.

Short plant types due to grazing have been demonstrated (Painter et al, 1993). *In situ* plants of four North American grasses under several grazing regimes by prairie dogs (*Cynomys ludovicianus*) were removed to a protected common environment. After several growing seasons, when phenotypic plasticity should have been negligible, height

differences were still observed among the grazing pressures. This may indicate past genetic selection from grazing pressures, in which short plants had higher survivability, and were able to produce the progeny carrying alleles for short height.

The Front Range and Great Basin regions shared similar precipitation rates year (40 cm annually) and soil pH levels (7.8 to 8.0) , while the South Dakota and Nebraska regions were similar in terms of precipitation (51 cm annually) and soil pH (6.2 to 7.0) (Soil Survey Staff, 2009). Likewise, the Front Range and Great Basin areas had higher means for leaf rust, shoot density, seed yield and spread; means for canopy height, gap, and seed length, were lower compared to the accessions from the more eastern regions.

Populations, sets of individuals that form a local breeding group, are often defined by physical location. Though they share many genes through ancestry, differences arise due to response from environmental parameters, competition with other species, and tolerance to predators, all specific to their habitat (Volk et al., 2007). Mutation, genetic drift, and founder effects (establishment of a new population from a few individuals) also contribute to differentiation. The large distance between collecting sites prevented seed dispersal and pollen movement, so that gene flow between regions was minimal. The large distance between the four collecting regions contributed to the differences in trait values.

Accessions from the Front Range area exhibited favorable combinations of rust resistance, short height, high shoot density, and high seed yield. These traits are attributes found in turfgrasses (Meyer and Funk, 1989), and determine saltgrass as a suitable turfgrass. Qian (2006) found only a slight decrease in turf quality of some accessions at salinity levels of 36 mmhos/cm. In addition, saltgrass accessions were tolerant to traffic

and resistant to drought compared to nearby plots of other native grass turf lines. Quality of some accessions is high, but seed production of parents yielding uniform types needs further work. Further work on inheritance of rust resistance needs to be carried out, as well as other characters, such as leaf shredding and perenniality. In addition, cultural practices in turf management need to be studied.

CHAPTER VI

Digital Imaging

A nursery of individual spaced plants allows observations in order to rank plants for selection in breeding (Poehlman and Sleper, 1995; Vogel and Pedersen, 1993). Digital imaging could be a tool in a nursery by measuring the spread of each plant. Spread here is defined as lateral growth in time and is interpreted as plant vigor. The distribution curves show an increase in spreading rate in progeny (improved), over the native and parent populations (Figure 37). The three populations showed non-homogeneity of variance. The progeny spread was significantly different from the parents selected from the native population and the native population based on a square root transformation (Table 46). The parents had similar spreading rate as the native population.

Table 46. Analysis of plant spread from 2004 digital images. Actual least squares means in percent fill of a 1.8 x 1.8 meter plot in one year's time and differences of square root transformation of population effect on spread. Adjusted for multiple comparisons with Tukey-Kramer.

Actual least square means and standard errors

pop	Estimate	Standard Error
native	11.8	0.6
parent	10.9	0.7
progeny	18.0	0.4

Least square means difference of square root transformation and analysis

pop	_pop	Estimate	Standar Error	DF	t Value	Pr > |t|	Adj P
native	parent	0.134	0.102	10	1.31	0.2195	0.4216
native	progeny	-0.798	0.078	10	-10.18	<0.0001	<0.0001
parent	progeny	-0.932	0.081	10	-11.39	<0.0001	<0.0001

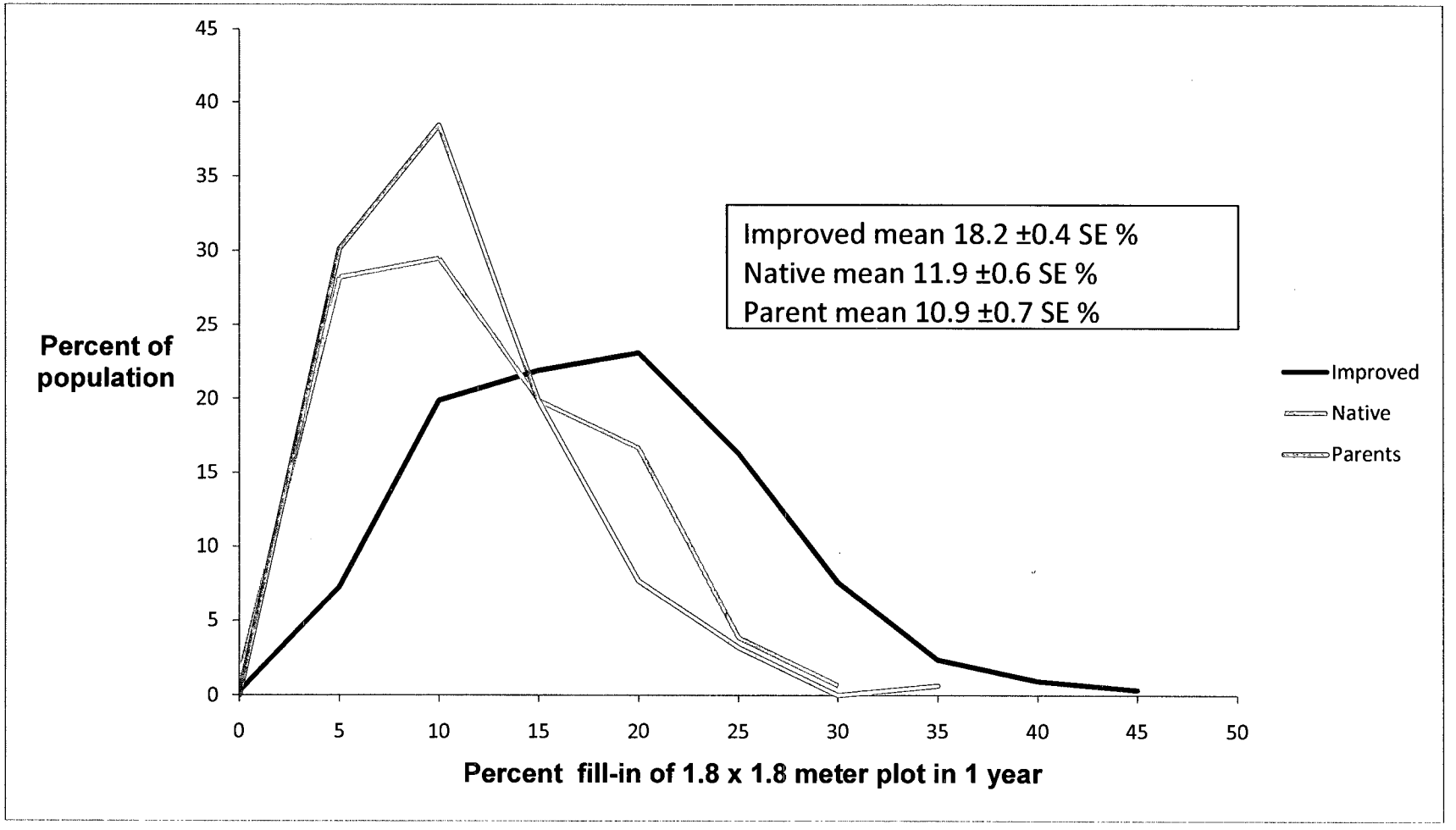

Figure 37. Difference in percent digital spread between native, improved, and parent populations. Based on percent fill of a1.8 x 1.8 meter plot in one year at the Horticulture Field Research Center.

Increased Spread in Progeny

Since there is no significant difference between the parent and native populations in spread, but there is significant spread in the progeny (improved), then the increase in spread is either a correlated response from selecting the four traits, or, a heterotic response from crossing heterogenous plants.

Genotypic correlations in the native population were low between 2004 spread and other traits (Table 47). The standard errors were as large as or larger than the correlation coefficients. (along with phenotypic correlations, Appendix IV).

Table 47. Genotypic correlations of height, density, and seed yield with 2004 spread.

Trait	2004	2005	2006
height	0.17	-0.08	
density		-0.06	0.30
seed yield			0.27

Since the rust data is not normal, means for average spread for rust classes for 2004 are presented instead, in Table 48. The spread increases with increased rust, so selecting for rust resistance would indicate spread may be lower in rust resistant progeny. This could be due to linkage if the genes for rust resistance are linked to genes which account for a slow spreading rate. If linkage were tight, it may be very difficult to to breed resistance and and have plants which have a high spreading rate. Both traits are desirable, with resistance keeping the turf green and disease free throughout the summer, and spread contributing to establishment and an indication of plant vigor.

Table 48. Rust classes and their associated average plant spread in a 1.8 meter square plot in 2004.

Rust	0%	1%	5%	10%	15%	20%	25%
Spread	8.8 %	8.2%	9.4%	12.7%	15.5%	15.1%	16.5%

The above correlations would seem to indicate there is little association between the 4 traits and spread. Crossing heterogenous plants may be a more likely explanation for increased spread in the progeny.

Saltgrass grows in isolated colonies of limited numbers. Isolation can cause genetic drift and founder effect (establishment of a new population from a few individuals) (Falconer and Mackay, 1996). Also, mutation in an isolated colony would contribute to genetic differences between colonies. If too few plants are in a colony, inbreeding would also occur, fixing some alleles, losing others, but within a colony. Isolation causes heterogenous plants between colonies. Artificially increasing the opportunity for recombination of isolated germplasms could lead to new gene combinations, and a possible increase in vigor beyond the parents, as measured by spreading rate. Crossing selected saltgrass parents provided an opportunity for new gene combinations that may have been superior to the parents, with a resulting increase in spread in the progeny. Subhanij (1974) found heterosis for spread in wide tall fescue crosses. Segovia-Lerma et al. (2004) found heterosis for forage yield in some crosses of 9 distinct alfalfa germplasms. Latta et al. (2007) found heterosis for seed yield in xeric-mesic ecotype crosses within the California ecotype of *Avena barbata.*

Visual Estimates versus Camera Scan

A correlation matrix was plotted for visual estimates and camera scan (Figure 38). Grouping of data points into lines is a consequence of visual estimates being categories of 5 % increments. Human visual rating was restricted to 5% class increments because of the difficulty distinguishing between plants that had less than 5% difference. Visual estimates overestimated spread at all levels. This is seen in the upper plot, by the line

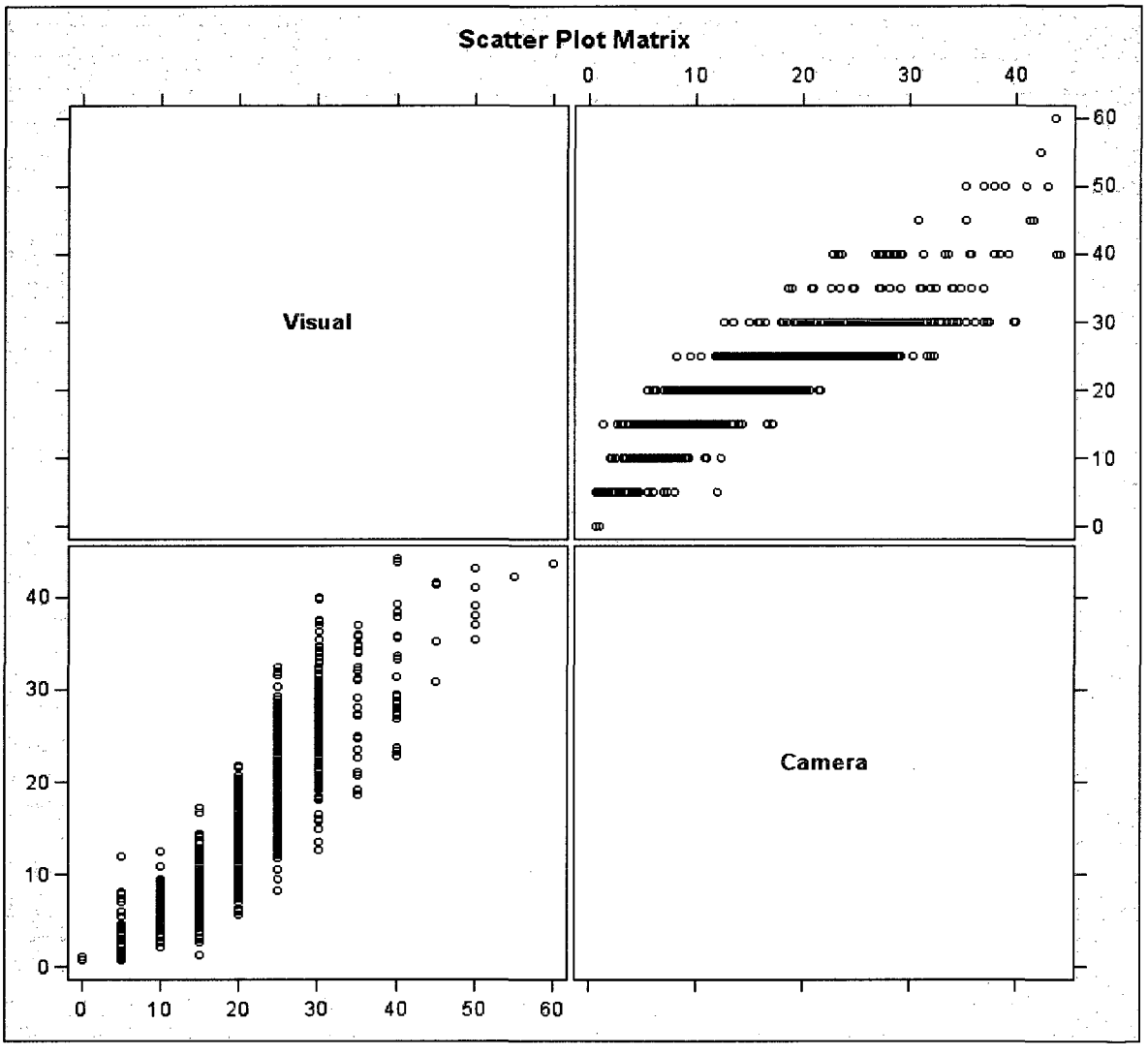

Figure 38. Scatter plot of visual estimate versus camera scan of percent fill in by plants in a 1.8 x 1.8 meter plot in one year at the Horticulture Field Research Center.

groupings being skewed to the left of the intersect of the same values for each axis.

Some of the scan values seem questionable. For example, the visual 30% category shows some camera scan values under 15%. A 15% value is easily discernable from a 30% value by visual estimate. Data like this needs further examination to answer why a large difference exists between the camera scan and visual estimate. Using the camera and setting up the parameters for scanning requires expertise, and is affected by many decisions. For example, even though photographs were taken in the middle of the day to avoid shadows, shadows were apparent in tall plants and lacking in short plants. Even though SigmaScan Pro 5.0 can adjust for these, the settings for adjustment are somewhat subjective.

Visual rating was done by the saltgrass project personnel, and an Olympus C5000 camera took digital images, which were later processed in SigmaScan Pro 5.0. Plants in middle categories for spread were difficult to visually estimate. The horizontal lines in these categories are longer indicating increased variance in the relation of visual estimate with camera scan. These results agree with other work (Coren and Girgus, 1978; Lindow and Webb, 1983) that differences at middle amounts of cover are difficult to distinguish by human vision.

Plants which were small and plants which had grown large have a great effect on increasing the correlation coefficient (Ott and Longnecker, 2001). The correlation coefficient is $r = 0.86$, with $p<0.0001$ under the null hypothesis that the correlation is 0. This indicates visual rating is efficient in determining the rate of spread. Visual rating in early cycles of selection would be sufficient to select individuals with high spreading ability, but in later cycles of recurrent selection, when variability for the trait decreases,

digital imaging would prove more useful in distinguishing differences between plants. For example, subjectively, the majority of plants fell into the 15% to 25% range. If this range represented an advanced population with reduced variability, its correlation coefficient between visual estimates and camera scans is $r = 0.66$, $p<0.0001$. At this value, digital imaging may be a better measure in selecting individual plants.

Only slight differences existed in correlation coefficients among 3 evaluators: 0.86, 0.86, and 0.81, with $p<0.0001$ for each correlation. The most pronounced differences were time for visually rating the 1284 plants, with the most experienced person at 6 hours, the next person at 9 hours and the least experienced at 12 hours. This compared to 2 people taking digital photographs over three 6 hour days, and considerable time scanning. Much of the time spent on scanning was a learning process, and later scans took little time to analyze.

Heritability of Spread

Parent-offspring regression was used to determine narrow sense heritability for spread (Figure 39). Camera scan data was used. Heritability was 0.60 with a standard error of 0.28 (Appendix IV), but the 95% confidence limits are -0.01 to 1.20, both beyond the range of limits for heritability values. Since only one year and one location of data were used, this estimate is biased upwards by genotype x environment interaction in the numerator.

Spread is vegetative reproduction. As rhizomes elongate they form new roots and shoots capable of independence from the mother plant. Freas (1987) concluded that vegetative reproduction was the main form of reproduction in saltgrass in the wild, rather

than seed production. Spread can be considered a major fitness trait. Fitness traits have low heritability because natural selection reduces additive variance (see Falconer and Mackay, 1996 for a detailed discussion). For this reason, inflation of the additive variance for genetic reasons (Chapter IV), and the lack of study environments, the true heritability is probably much lower than reported.

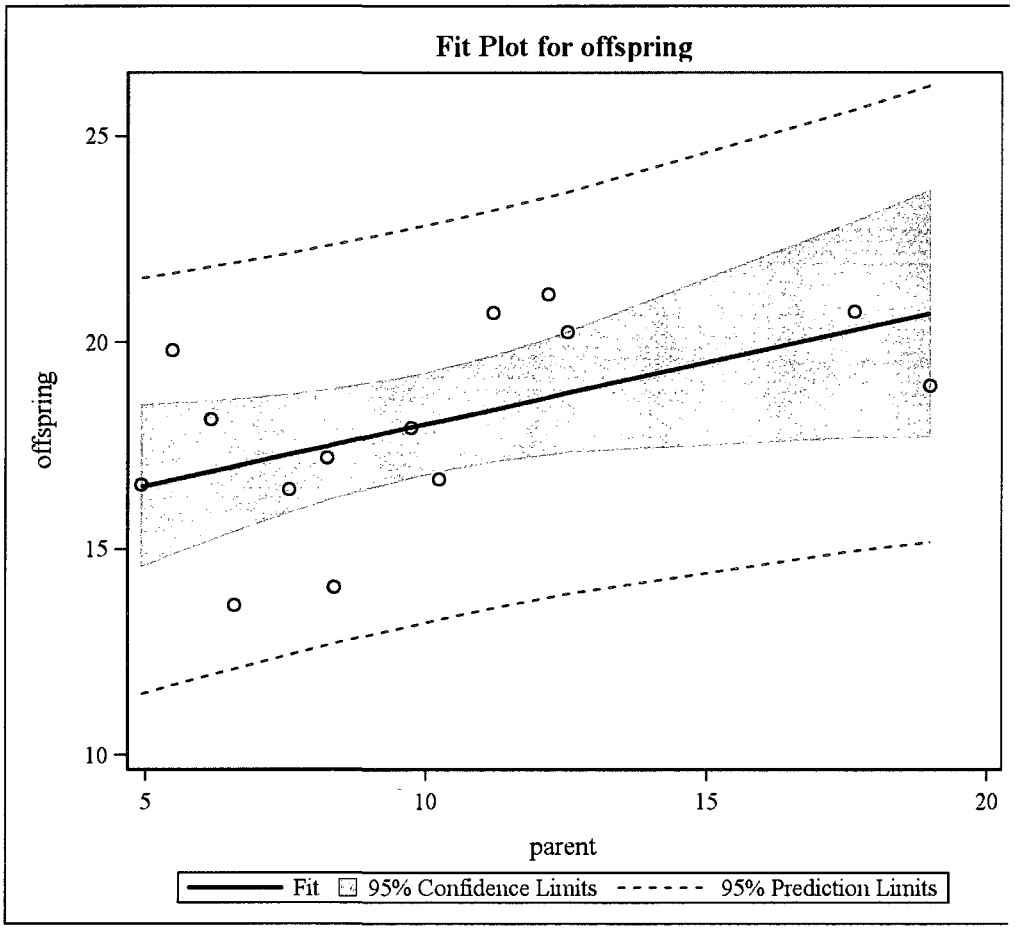

Figure 39. Offspring regressed onto parents for percent spread in 2004.

Analysis of variance was run to note significant differences among female parents and calculations of BLUPs (Tables 49 and 50, respectively). Replication was effective in taking out variability, and may be due to the lighter soils to the east. Family differences were highly significant.

BLUPs showed some family estimated predictors had confidence limits that did not overlap, although multiple comparisons will result in a Type I error. Nevertheless, family A21-1 would have the lowest rate of spread, with 14.5% of fill in of the plot, and family A37-1 would have the highest rate of spread, with 20.6% of fill in of the plot.

Digital imaging is useful in turf breeding. Since selection is on individual spaced plants, a digital image can record a plant for immediate or future analysis. Spread was measured in this study and the progeny (improved) population showed increased spread over the native and parent populations. The reason for this may be increased vigor from crossing heterogenous plants. Differences in spread were also noted for families. Heritability for spread was 0.60, but may be biased upwards from lack of environments. Correlation of camera scan with visual estimates was 0.86. However, more work in calculating the scan settings needs to be done to perfect the pixel counts so that they reflect the actual plant phenotype. Imagery could be used for leaf rust analysis, shoot density, injury from mowing, and many other turf traits which exhibit differences in color

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
rep	5	1009	202	Var(Residual) +10Var(rep*line)+140 Var(rep)	MS(rep*line)	65	2.99	0.0172
line	13	4524	348	Var(Residual) +10Var(rep*line)+60 Var(line)	MS(rep*line)	65	5.15	<0.0001
rep*line	65	4390	68	Var(Residual) +10Var(rep*line)	MS(Residual)	756	1.26	0.0888
Residual	756	40577	54	Var(Residual)	.	.	.	.

Table 49. Analysis of variance for spread in a 1.8 x 1.8 meter plot in one year, using a random effects model to obtain estimates of variance components.

Estimates								
Label	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
A21-1	14.5	1.1	55.8	13.47	<0.0001	0.05	12.3	16.6
A53-1	14.8	1.1	55.8	13.80	<0.0001	0.05	12.7	17.0
A126-1	16.8	1.1	55.8	15.57	<0.0001	0.05	14.6	19.0
A123-1	16.9	1.1	55.8	15.66	<0.0001	0.05	14.7	19.0
A138-1	17.0	1.1	55.8	15.77	<0.0001	0.05	14.8	19.1
A61-1	17.4	1.1	55.8	16.15	<0.0001	0.05	15.2	19.5
84-1	18.0	1.1	55.8	16.69	<0.0001	0.05	15.8	20.1
A97-1	18.1	1.1	55.8	16.85	<0.0001	0.05	16.0	20.3
A24-1	18.8	1.1	55.8	17.45	<0.0001	0.05	16.6	20.9
A35-1	19.5	1.1	55.8	18.09	<0.0001	0.05	17.3	21.6
A137-1	19.8	1.1	55.8	18.42	<0.0001	0.05	17.7	22.0
A34-1	20.2	1.1	55.8	18.77	<0.0001	0.05	18.0	22.4
A50-1	20.2	1.1	55.8	18.79	<0.0001	0.05	18.1	22.4
A37-1	20.6	1.1	55.8	19.11	<0.0001	0.05	18.4	22.7

Table 50. Best linear unbiased predictors (BLUPs) for spread in a 1.8 x 1.8 meter plot in one year.

CHAPTER VII

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APPENDICES

Appendix I.

Table A.1. List of accessions and their region of origin.

Region	Accession	Selected as parent	Latitude (^{0}N)	Longitude (^{0}W)
Great Basin	20		40° 42'	115° 41'
Great Basin	21		40° 42'	115° 41'
Great Basin	22		40° 38'	115° 42'
Great Basin	23		40° 23'	115° 39'
Great Basin	25		40° 41'	116° 6'
Great Basin	27		40° 37'	116° 56'
Great Basin	28		40° 55'	115° 39'
Great Basin	29		40° 36'	116° 58'
Great Basin	30		40° 55'	115° 38'
Great Basin	32		40° 49'	111° 25'
Great Basin	33		40° 47'	113° 59'
Great Basin	34		40° 54'	115° 38'
Great Basin	35		40° 45'	114° 1'
Great Basin	36		40° 42'	112° 9'
Great Basin	37		41° 3'	111° 40'
Great Basin	38		41° 4'	111° 19'
Great Basin	39		40° 42'	113° 22'
Great Basin	40		40° 42'	113° 19'
Great Basin	43		39° 30'	117° 4'
Great Basin	44		39° 30'	117° 4'
South Dakota	45		44° 24'	103° 15'
South Dakota	46		44° 24'	103° 15'
South Dakota	47		43° 56'	102° 40'
South Dakota	48		43° 56'	102° 40'
South Dakota	49		43° 48'	99° 22'
South Dakota	50		43° 48'	99° 22'
South Dakota	51		43° 46'	99° 22'
South Dakota	52		43° 46'	99° 19'
South Dakota	53		44° 24'	103° 4'
South Dakota	54		44° 24'	103° 4'
South Dakota	55		44° 24'	102° 57'
South Dakota	57		44° 24'	102° 57'
South Dakota	58		43° 48'	99° 22'
South Dakota	59		43° 48'	99° 22'
South Dakota	60		44° 21'	102° 49'
South Dakota	61		44° 20'	102° 50'
South Dakota	62		44° 11'	102° 49'
South Dakota	63		44° 11'	102° 49'
South Dakota	64		44° 4'	101° 9'
South Dakota	65		44° 4'	101° 8'

Table A.1. Continued.

Region	Accession	Selected as parent	Latitude (^{0}N)	Longitude (^{0}W)
South Dakota	66		43° 43'	101° 58'
South Dakota	67		43° 43'	101° 58'
Nebraska	68		41° 46'	99° 22'
Nebraska	69		41° 45'	99° 22'
Nebraska	70		41° 38'	99° 22'
Nebraska	71		41° 38'	99° 22'
Nebraska	72		41° 17'	99° 22'
Nebraska	73		41° 17'	99° 22'
Nebraska	74		40° 6'	99° 25'
Nebraska	75		40° 6'	99° 25'
Nebraska	76		40° 6'	99° 26'
Nebraska	77		40° 41'	99° 25'
Nebraska	78		40° 40'	99° 25'
Nebraska	79		40° 43'	99° 22'
Nebraska	80		40° 43'	99° 22'
Nebraska	81		40° 45'	99° 44'
Nebraska	82		41° 9'	100° 46'
Nebraska	83		41° 10'	100° 46'
Front Range	84	Parent-F	40° 9'	104° 58'
Front Range	85		40° 9'	104° 58'
Front Range	86	Parent-M	40° 9'	104° 58'
Front Range	87		40° 9'	104° 58'
Front Range	92	Parent-M	40° 9'	104° 58'
Front Range- Denver	A1		39° 42'	104° 51'
Front Range- Denver	A2		39° 42'	105° 3'
Front Range- Denver	A6		39° 42'	105° 3'
Front Range- Denver	A7		39° 42'	105° 3'
Front Range- Denver	A8		39° 42'	105° 3'
Front Range- Denver	A10		39° 43'	105° 3'
Front Range- Denver	A11		39° 43'	105° 3'
Front Range- Denver	A12		39° 43'	105° 3'
Front Range- Denver	A13		39° 43'	105° 1'
Front Range- Denver	A14		39° 43'	105° 1'
Front Range- Denver	A15		39° 43'	105° 1'
Front Range- Denver	A18	Parent-F	39° 43'	105° 1'
Front Range- Denver	A19		39° 43'	105° 1'
Front Range- Denver	A20		39° 44'	105° 0'
Front Range- Denver	A21	Parent-F	39° 44'	105° 0'
Front Range- Denver	A22		39° 44'	105° 0'
Front Range- Denver	A23		39° 44'	105° 0'

Table A.1. Continued.

Region	Accession	Selected as parent	Latitude (^{0}N)	Longitude (^{0}W)
Front Range- Denver	A24	Parent-F	39° 45'	105° 0'
Front Range- Denver	A26		39° 45'	105° 0'
Front Range- Denver	A27		39° 45'	105° 0'
Front Range- Denver	A28		39° 45'	105° 1'
Front Range- Denver	A29		39° 45'	105° 0'
Front Range- Denver	A30		39° 45'	105° 0'
Front Range- Denver	A31		39° 45'	105° 0'
Front Range- Denver	A32		39° 45'	105° 1'
Front Range- Denver	A34	Parent-F	39° 45'	105° 1'
Front Range- Denver	A35	Parent-F	39° 44'	104° 58'
Front Range- Denver	A36		39° 44'	104° 58'
Front Range- Denver	A37	Parent-F	39° 44'	104° 57'
Front Range- Denver	A38		39° 44'	104° 57'
Front Range- Denver	A39	Parent-M	39° 46'	104° 57'
Front Range- Denver	A40		39° 47'	104° 54'
Front Range- Denver	A41	Parent-M	39° 47'	104° 54'
Front Range- Denver	A42		39° 47'	104° 54'
Front Range- Denver	A43		39° 43'	104° 53'
Front Range- Denver	A44		39° 43'	104° 53'
Front Range- Denver	A45		39° 43'	104° 52'
Front Range- Denver	A46		39° 43'	104° 52'
Front Range- Denver	A47		39° 43'	104° 52'
Front Range- Denver	A48	Parent-M	39° 43'	104° 52'
Front Range- Denver	A49		39° 43'	104° 52'
Front Range- Denver	A50	Parent-F	39° 43'	104° 52'
Front Range- Denver	A51	Parent-M	39° 43'	104° 52'
Front Range- Denver	A53	Parent-F	39° 43'	104° 52'
Front Range- Denver	A54		39° 43'	104° 52'
Front Range- Denver	A55		39° 43'	104° 52'
Front Range- Denver	A56		39° 43'	104° 52'
Front Range- Denver	A59		39° 43'	104° 52'
Front Range- Denver	A60	Parent-M	39° 43'	104° 52'
Front Range- Denver	A61	Parent-F	39° 43'	104° 52'
Front Range- Denver	A62		39° 43'	104° 52'
Front Range- Denver	A64		39° 43'	104° 52'
Front Range- Denver	A65		39° 43'	104° 52'
Front Range- Denver	A67		39° 43'	104° 52'
Front Range- Denver	A68		39° 42'	104° 56'
Front Range- Denver	A70		39° 43'	104° 57'
Front Range- Denver	A71		39° 43'	104° 57'

Table A.1. Continued.

Region	Accession	Selected as parent	Latitude (^{0}N)	Longitude (^{0}W)
Front Range- Denver	A72		39° 43'	104° 57'
Front Range- Denver	A73	Parent-M	39° 45'	105° 2'
Front Range- Denver	A75		39° 45'	105° 0'
Front Range- Denver	A77		39° 43'	105° 0'
Front Range- Denver	A78		39° 43'	105° 0'
Front Range- Denver	A79		39° 43'	105° 0'
Front Range- Denver	A83		39° 43'	105° 0'
Front Range- Denver	A85		39° 43'	105° 0'
Front Range- Denver	A86		39° 43'	105° 0'
Front Range- Denver	A89		39° 43'	105° 0'
Front Range	A93		40° 9'	104° 58'
Front Range	A94		40° 9'	104° 58'
Front Range	A97	Parent-F	40° 9'	104° 59'
Front Range	A101		40° 9'	104° 59'
Front Range	A103		40° 12'	104° 58'
Front Range	A104		40° 12'	104° 58'
Front Range	A105		40° 12'	104° 58'
Front Range	A107	Parent-M	40° 12'	104° 58'
Front Range	A108		40° 34'	105° 0'
Front Range	A109		40° 36'	105° 0'
Front Range	A111		40° 37'	105° 1'
Front Range	A112		40° 37'	105° 1'
Front Range	A114		40° 39'	105° 1'
Front Range	A116		40° 40'	105° 1'
Front Range	A119		40° 44'	105° 0'
Front Range	A120		40° 44'	105° 1'
Front Range	A122		40° 44'	105° 0'
Front Range	A123	Parent-F	40° 21'	104° 54'
Front Range	A124	Parent-M	40° 21'	104° 54'
Front Range	A126	Parent-F	40° 21'	104° 54'
Front Range	A127		40° 25'	104° 54'
Front Range	A128		40° 25'	104° 54'
Front Range	A129		40° 21'	104° 54'
Front Range	A131		40° 22'	104° 56'
Front Range	A135		40° 18'	104° 58'
Front Range	A136	Parent-M	40° 18'	104° 59'
Front Range	A137	Parent-M	40° 15'	104° 59'
Front Range	A138	Parent-F	40° 14'	104° 54' [1]

[1] F = female, M = male. Accessions labels are not continuous because collections were made by different groups and/or accessions were lost by the time of establishment (death, mislabeling, unaccounted for).

Appendix II.
Maps of accessions.

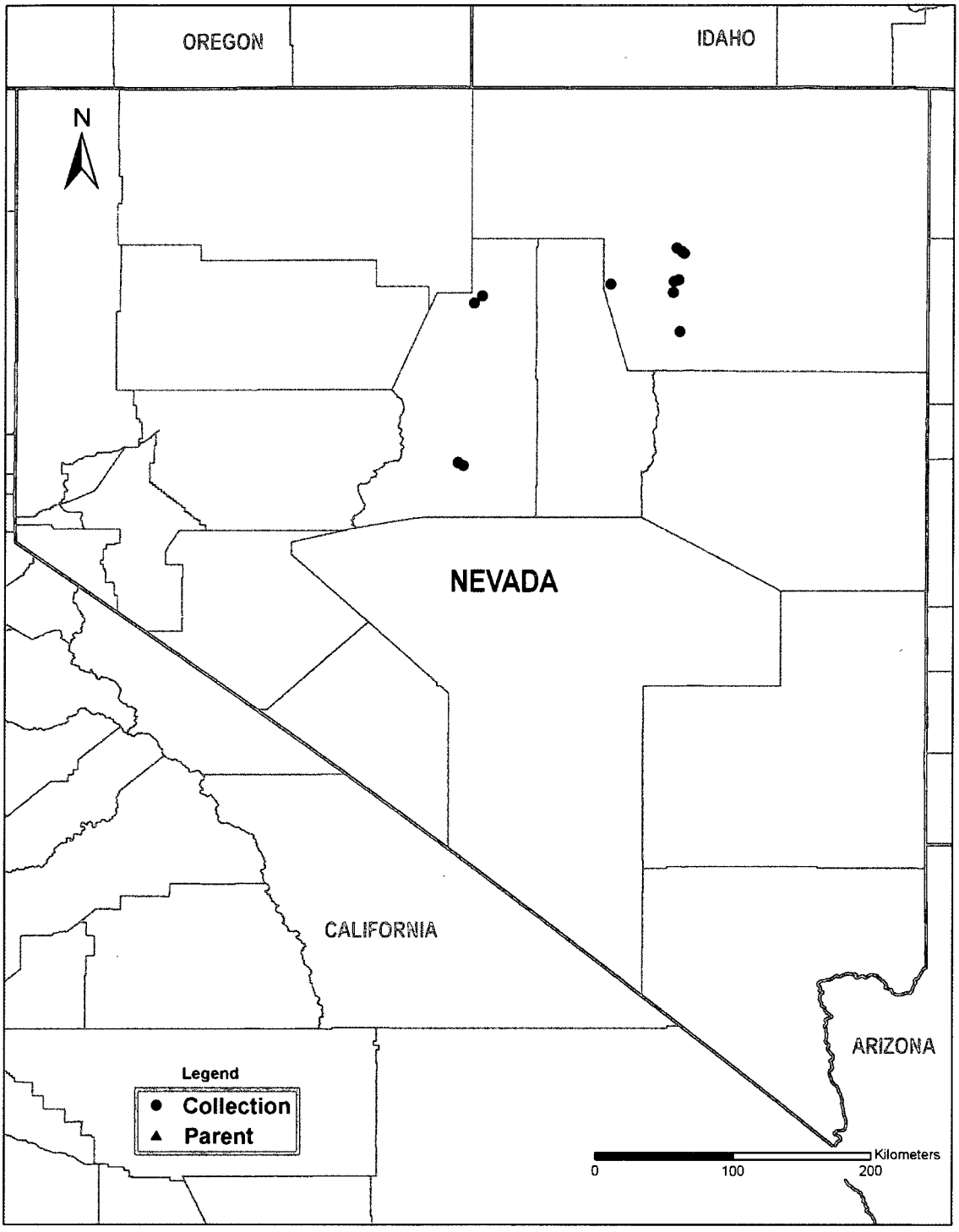

Figure A.1. Relative location of accessions collected in Nevada in 1998. See coordinates in Appendix I for a more detailed location.

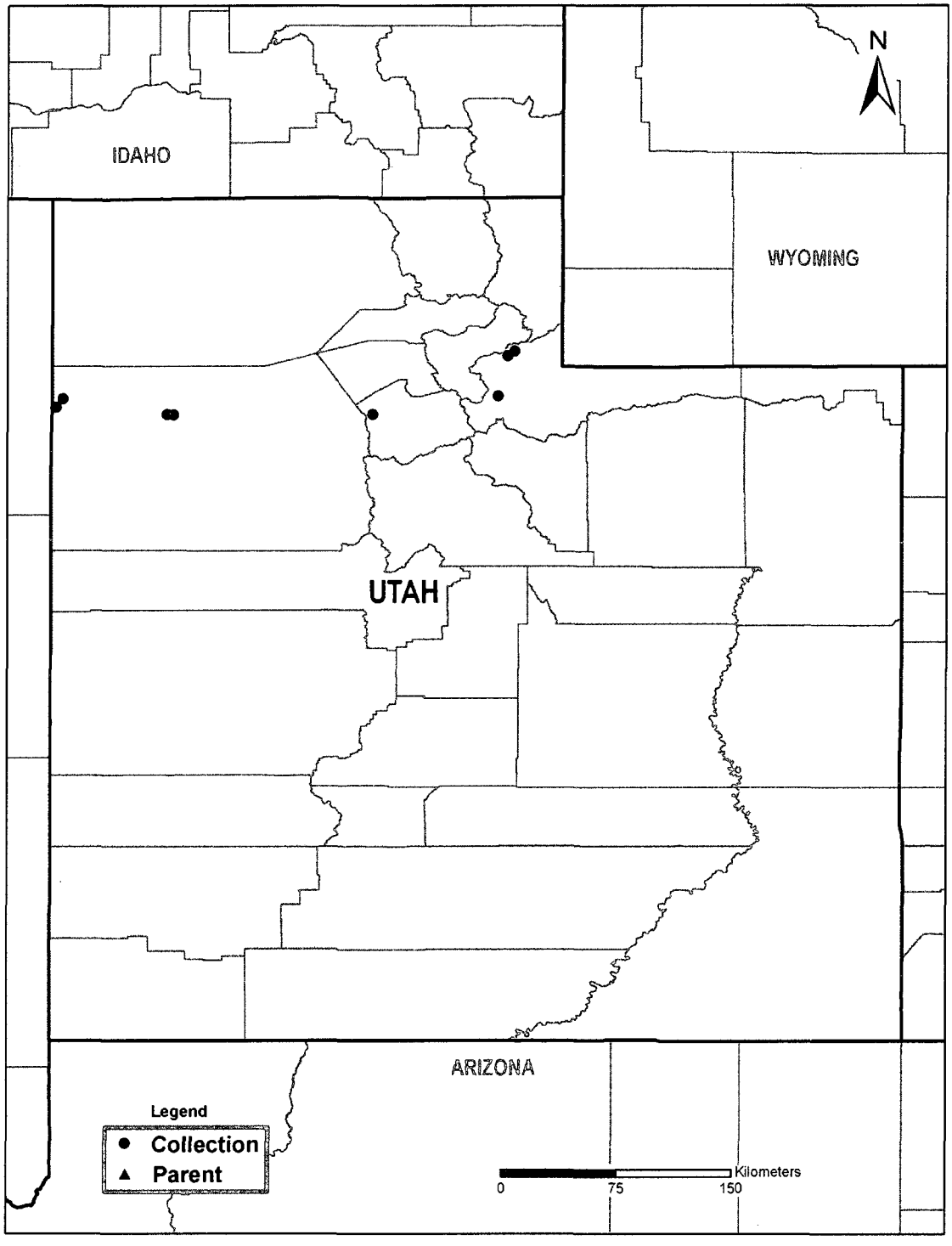

Figure A.2. Relative location of accessions collected in Utah in 1998. See coordinates in Appendix I for a more detailed location.

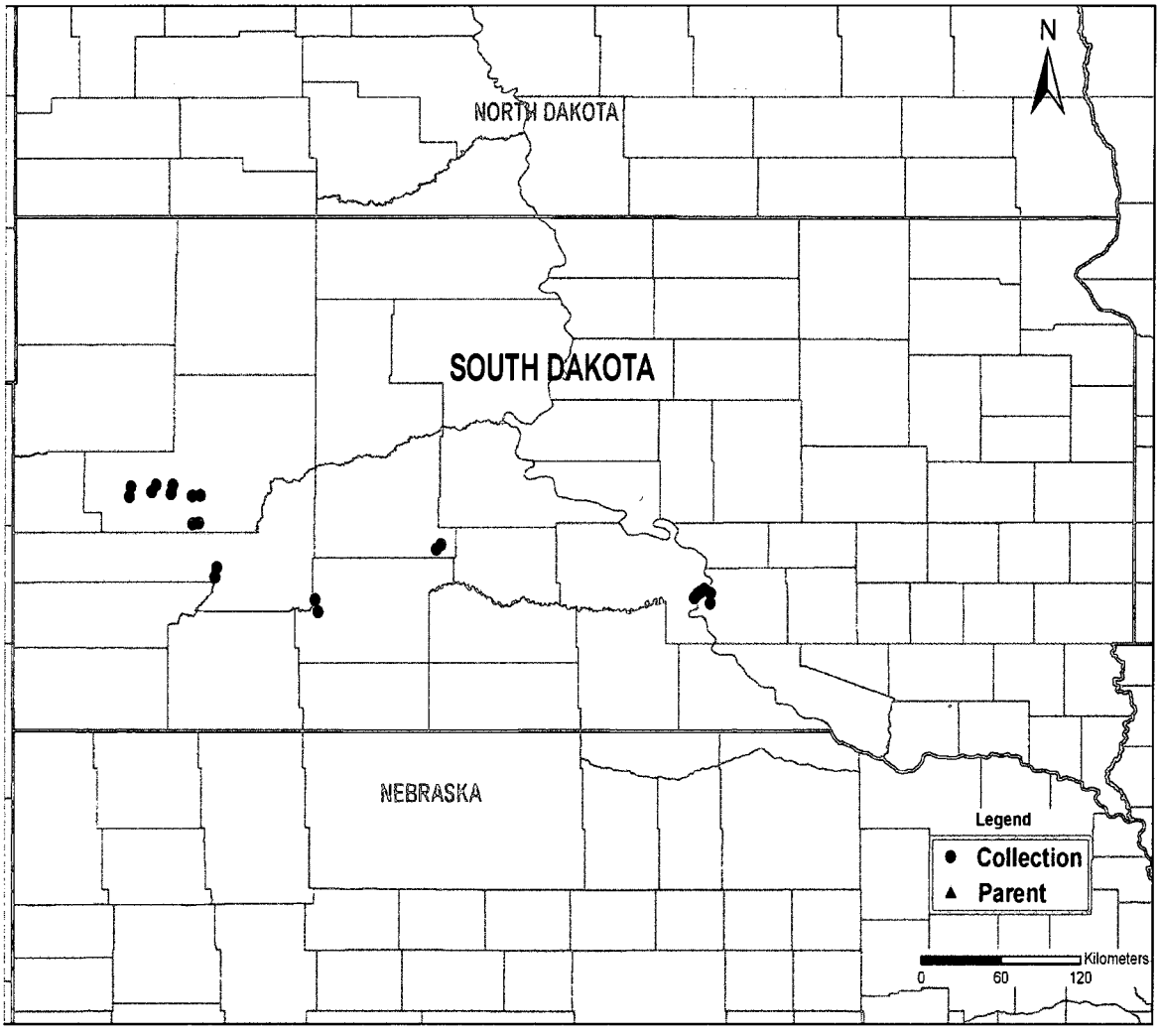

Figure A.3. Relative location of accessions collected in South Dakota in 1998. See coordinates in Appendix I for a more detailed location.

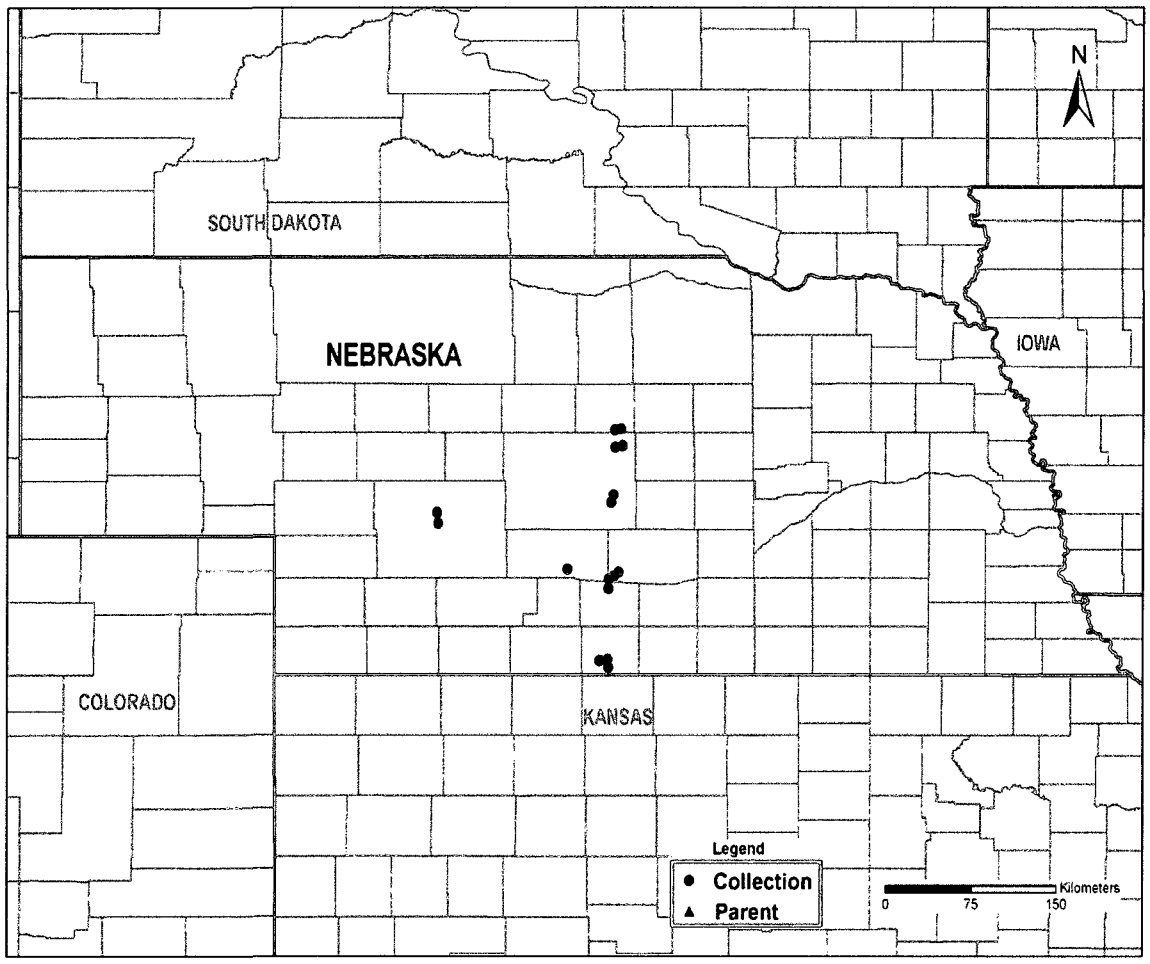

Figure A.4. Relative location of accessions collected in Nebraska in 1998. See coordinates in Appendix I for a more detailed location.

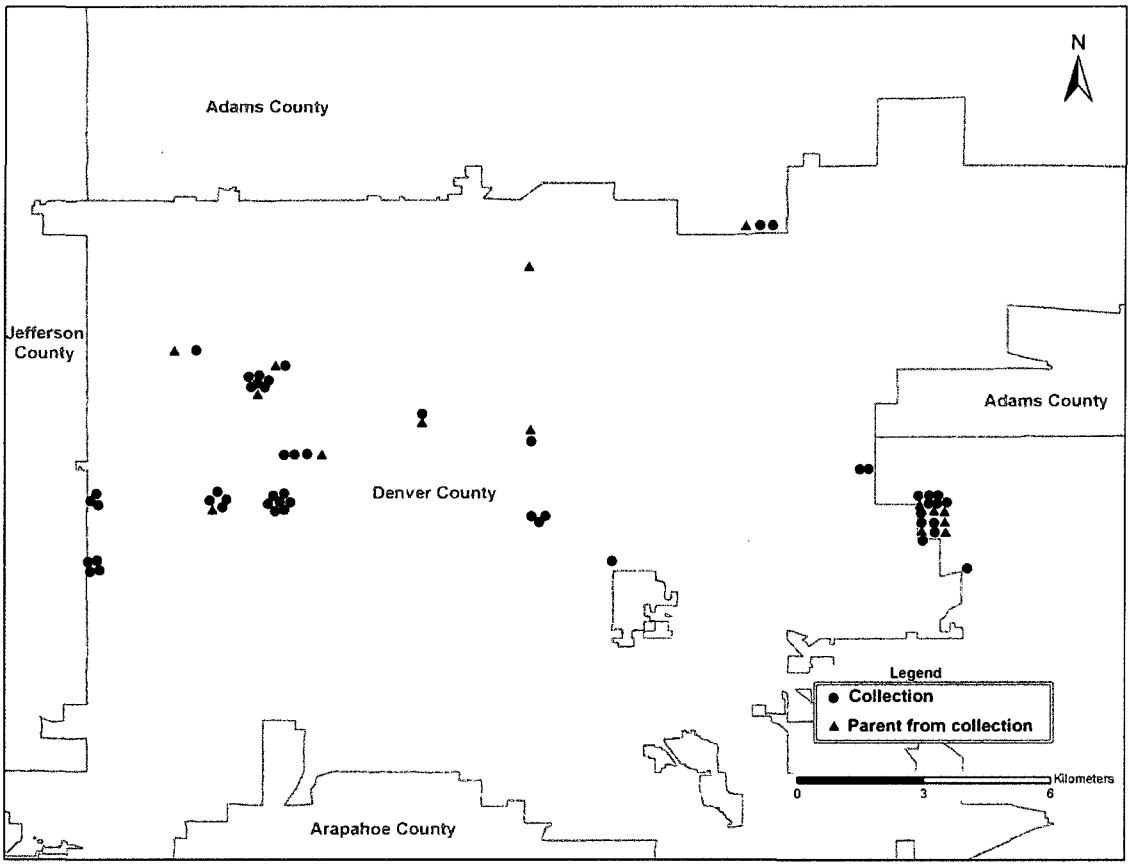

Figure A.5. Relative location of accessions collected in 1995 in the Front Range-Denver area. See coordinates in Appendix I for a more detailed location.

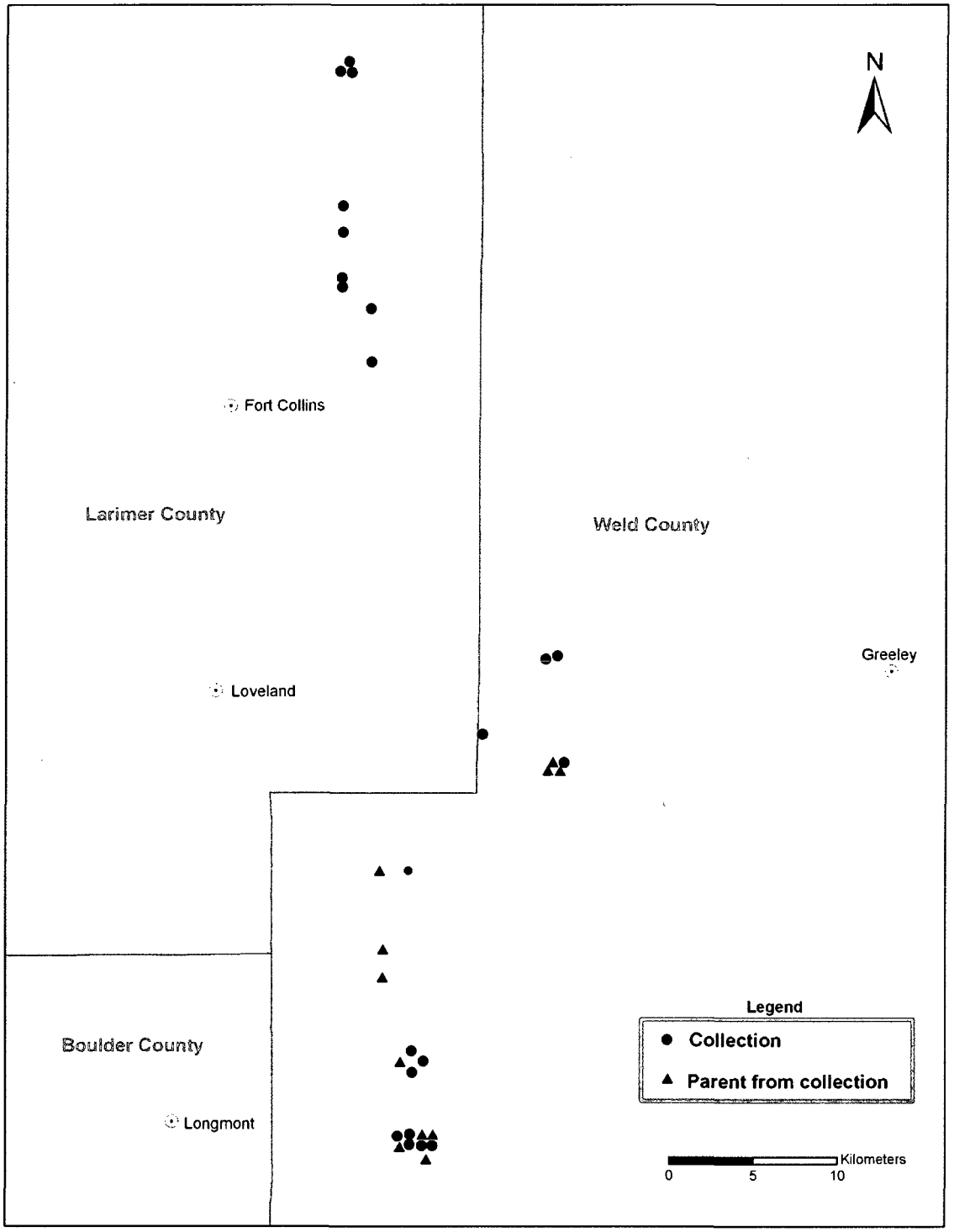

Figure A.6. Relative location of accessions collected along the Front Range, Colorado, in 1995 and 1998. See coordinates in Appendix I for a more detailed location.

Appendix III. Heritability estimates and variance regression on sibship means- analyses.

Table A.2. Statistical analysis of the $\log_n$ sibship phenotypic variance regression on the quadratic equation of the sibship mean for the trait square root of percent leaf area affected by rust.

Analysis of variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	2	0.289	0.145	13.51	0.0011
Error	11	0.118	0.011		
Corrected Total	13	0.408			

Root MSE	0.104	**R-Square**	0.710
Dependent Mean	0.414	**Adj R-Sq**	0.658
Coeff Var	25.04		

Parameter Estimates						
Variable	**Label**	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	Intercept	1	-2.66	0.65	-4.06	0.0019
mean	the mean, sqrts	1	2.11	0.47	4.49	0.0009
Sibs m sq		1	-0.33	0.07	-4.25	0.0014

Table A.3. Statistical analysis of 2005 progeny height (cm) regression on the 2004 parent height.

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	1	44.66	44.68	24.42	0.0003
Error	12	21.95	1.82		
Corrected Total	13	66.64			

Root MSE	1.35	**R-Square**	0.670
Dependent Mean	19.99	**Adj R-Sq**	0.643
Coeff Var	6.76		

Parameter Estimates							
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**	**95% Confidence Limits**	
Intercept	1	11.47	1.76	6.51	<0.0001	7.62	15.31
parent	1	0.51	0.10	4.94	0.0003	0.28	0.74

Table A.4. Statistical analysis of 2004 progeny height (cm) regression on the 2005 parent height.

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	1	38.43	38.43	14.79	0.0023
Error	12	31.19	2.59		
Corrected Total	13	69.62			

Root MSE	1.61	**R-Square**	0.552
Dependent Mean	18.09	**Adj R-Sq**	0.514
Coeff Var	8.91		

Parameter Estimates							
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**	**95% Confidence Limits**	
Intercept	1	9.10	2.37	3.83	0.0024	3.92	14.28
parent	1	0.47	0.12	3.85	0.0023	0.20	0.74

Table A.5. Analysis of variance for 2004 height (cm) for broadsense heritability estimate and Nyquist adjustment for environmental covariance.

Type 3 Analysis of Variance							
Source	**DF**	**Sum of Squares**	**Mean Square**	**Error Term**	**Error DF**	**F Value**	**Pr > F**
rep	5	96.61	19.32	MS(Residual)	125	4.83	0.0004
family	25	1634.53	65.38	MS(Residual)	125	16.35	<0.0001
Residual	125	499.88	3.99	.	.	.	.

Covariance Parameter Estimates	
Cov Parm	**Estimate**
rep	0.58
family	10.23
Residual	3.99

Table A.6. Analysis of variance for 2005 height (cm) for broadsense heritability estimate and Nyquist adjustment for environmental covariance.

Type 3 Analysis of Variance							
Source	**DF**	**Sum of Squares**	**Mean Square**	**Error Term**	**Error DF**	**F Value**	**Pr > F**
rep	5	190.28	38.05	MS(Residual)	125	5.28	0.0002
family	25	1796.39	71.85	MS(Residual)	125	9.97	<0.0001
Residual	125	901.11	7.20	.	.	.	.

Covariance Parameter Estimates	
Cov Parm	**Estimate**
rep	1.18
family	10.77
Residual	7.20

Table A.7. Statistical analysis of $\log_n$ sibship phenotypic variance regression on the quadratic equation of sibship mean for the trait height (cm) in 2004.

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	2	0.295	0.147	2.29	0.1472
Error	11	0.708	0.064		
Corrected Total	13	1.004			

Root MSE	0.253	**R-Square**	0.294
Dependent Mean	2.41	**Adj R-Sq**	0.165
Coeff Var	10.5		

Parameter Estimates					
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	1	0.340	5.334	0.06	0.9503
sibsm	1	0.165	0.590	0.28	0.7847
sibsmsq	1	-0.002	0.016	-0.17	0.8679

Table A.8. Statistical analysis of $\log_n$ sibship phenotypic variance regression on the quadratic equation of sibship mean for the trait height (cm) in 2005.

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	2	0.109	0.054	0.68	0.5286
Error	11	0.891	0.081		
Corrected Total	13	1.000			

Root MSE	0.284	**R-Square**	0.109
Dependent Mean	2.48	**Adj R-Sq**	-0.052
Coeff Var	11.4		

Parameter Estimates						
Variable	**Label**	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	Intercept	1	3.139	7.336	0.43	0.6770
mean1	the mean, height5	1	-0.107	0.745	-0.14	0.8883
sibsmsq		1	0.003	0.018	0.20	0.8476

Table A.9. Analysis of variance of 2005 parents for shoot density (no./cm^2) for Nyquist adjustment to environmental covariance.

Type 3 Analysis of Variance							
Source	**DF**	**Sum of Squares**	**Mean Square**	**Error Term**	**Error DF**	**F Value**	**Pr > F**
rep	5	0.26	0.0539	MS(Residual)	125	0.74	0.5970
family	25	23.63	0.9455	MS(Residual)	125	12.92	<0.0001
Residual	125	9.14	0.0731	.	.	.	.

Covariance Parameter Estimates	
Cov Parm	**Estimate**
rep	-0.0007
family	0.1454
Residual	0.0731

Table A.10. Analysis of variance for 2006 parents for shoot density (no./cm^2) for Nyquist adjustment to environmental covariance.

Type 3 Analysis of Variance							
Source	**DF**	**Sum of Squares**	**Mean Square**	**Error Term**	**Error DF**	**F Value**	**Pr > F**
rep	5	0.17	0.0352	MS(Residual)	125	0.48	0.7877
family	25	21.04	0.8416	MS(Residual)	125	11.55	<0.0001
Residual	125	9.10	0.0728	.	.	.	.

Covariance Parameter Estimates	
Cov Parm	**Estimate**
rep	-0.0014
family	0.1281
Residual	0.0728

Table A.11. Statistical analysis of 2006 offspring shoot density (no./cm^2) regressed on 2005 parent shoot density.

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	1	0.476	0.476	26.68	0.0002
Error	12	0.214	0.017		
Corrected Total	13	0.690			

Root MSE	0.133	**R-Square**	0.689
Dependent Mean	1.81	**Adj R-Sq**	0.663
Coeff Var	7.34		

Parameter Estimates							
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**	**95% Confidence Limits**	
Intercept	1	1.162	0.131	8.82	<0.0001	0.874	1.449
parent	1	0.454	0.087	5.17	0.0002	0.262	0.646

Table A.12. Analysis of variance of 2005 offspring shoot density (no./cm^2) regressed on 2006 parent shoot density.

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	1	0.442	0.442	31.94	0.0001
Error	12	0.166	0.013		
Corrected Total	13	0.608			

Root MSE	0.117	**R-Square**	0.726
Dependent Mean	1.82	**Adj R-Sq**	0.704
Coeff Var	6.44		

Parameter Estimates							
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**	**95% Confidence Limits**	
Intercept	1	1.13	0.12	8.97	<0.0001	0.85	1.40
parent	1	0.47	0.08	5.65	0.0001	0.29	0.65

Table A.13. Analysis of variance for 2005 and 2006 shoot density (no./cm^2) for broadsense heritability estimate.

Source	DF	Sum of Squares	Mean Square	Error Term	Error DF	F Value	Pr > F
rep	5	0.38	0.076	MS(Residual)	281	1.14	0.3418
family	25	44.01	1.760	MS(Residual)	281	26.06	<0.0001
Residual	281	18.98	0.067	.	.	.	.

Covariance Parameter Estimates	
Cov Parm	**Estimate**
rep	0.0002
family	0.1411
Residual	0.0676

Table A.14. Statistical analysis of $\log_n$ sibship phenotypic variance regression on the quadratic equation of sibship mean for the trait shoot density (no./cm^2).

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	2	0.02	0.009	0.06	0.9378
Error	11	1.54	0.140		
Corrected Total	13	1.56			

Root MSE	0.373	**R-Square**	0.012
Dependent Mean	-1.68	**Adj R-Sq**	-0.168
Coeff Var	-22.23		

Parameter Estimates						
Variable	**Label**	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	Intercept	1	-1.28	6.78	-0.19	0.85
mean1	the mean, density	1	-0.600	7.37	-0.08	0.93
sibsmsq		1	0.205	1.98	0.10	0.92

Table A.15. Statistical analysis of 2006 offspring seed weight (kg/ha) regressed on 2005 parent seed weight.

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	1	30285	30285	0.29	0.6000
Error	12	1252657	104388		
Corrected Total	13	1282942			

Root MSE	323.09	**R-Square**	0.023
Dependent Mean	1944.21	**Adj R-Sq**	-0.057
Coeff Var	16.61		

Parameter Estimates							
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**	**95% Confidence Limits**	
Intercept	1	2031.38	183.43	11.07	<0.0001	1631.71	2431.06
parent	1	-0.11	0.21	-0.54	0.6000	-0.59	0.35

Table A.16. Statistical analysis of 2005 offspring seed weight (kg/ha) regressed on 2006 parent seed weight.

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	1	4527	4527	0.16	0.6952
Error	12	337231	28103		
Corrected Total	13	341758			

Root MSE	167.63	**R-Square**	0.013
Dependent Mean	505.48	**Adj R-Sq**	-0.069
Coeff Var	33.16		

Parameter Estimates							
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**	**95% Confidence Limits**	
Intercept	1	448.42	149.05	3.01	0.0109	123.66	773.17
parents	1	0.02	0.05	0.40	0.6952	-0.10	0.14

Table A.17. Analysis of variance of 2006 offspring seed weight (kg/ha) regressed on 2006 parent seed weight.

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	1	437826	437826	6.22	0.0283
Error	12	845116	70426		
Corrected Total	13	1282942			

Root MSE	265.37	**R-Square**	0.341
Dependent Mean	1944.21	**Adj R-Sq**	0.286
Coeff Var	13.64		

Parameter Estimates							
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**	**95% Confidence Limits**	
Intercept	1	1376.40	238.51	5.77	<0.0001	856.71	1896.09
parent	1	0.22	0.09	2.49	0.0283	0.028	0.42

Table A.18. Statistical analysis of $\log_n$ of sibship phenotypic variance regression on the quadratic equation of the sibship mean for the trait seed weight (kg/ha).

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	2	6.78	3.39	2.79	0.1047
Error	11	13.38	1.21		
Corrected Total	13	20.16			

Root MSE	1.10	**R-Square**	0.336
Dependent Mean	12.67	**Adj R-Sq**	0.215
Coeff Var	8.70		

Parameter Estimates						
Variable	**Label**	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	Intercept	1	8.04622	2.96446	2.71	0.0201
mean1	the mean, seedweight	1	0.00291	0.00230	1.27	0.2319
sibsmsq		1	-3.899E-7	4.196861E-7	-0.93	0.3728

Table A.19. Statistical analysis of 2006 parent seed weight (kg/ha) for broadsense heritability estimates.

Type 3 Analysis of Variance							
Source	**DF**	**Sum of Squares**	**Mean Square**	**Error Term**	**Error DF**	**F Value**	**Pr > F**
rep	3	2300115	766705	MS(Residual)	39	1.37	0.2658
family	13	35636115	2741240	MS(Residual)	39	4.90	<0.0001
Residual	39	21806569	559143	.	.	.	.

Covariance Parameter Estimates	
Cov Parm	**Estimate**
rep	14826
family	545524
Residual	559143

Appendix IV- miscellaneous

Table A.20. Differences of region of origin effect on least squares means for seed yield (kg/ha). Tukey-Kramer adjustment for multiple comparisons.

region	_region	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P
FrontRan	GreatBas	1.10	1.42	3	0.77	0.49	0.86
FrontRan	Nebraska	3.68	1.36	3	2.70	0.07	0.20
FrontRan	S.Dakota	2.24	1.35	3	1.66	0.19	0.46
GreatBas	Nebraska	2.58	1.41	3	1.83	0.16	0.40
GreatBas	S.Dakota	1.14	1.40	3	0.82	0.47	0.84
Nebraska	S.Dakota	-1.44	1.31	3	-1.08	0.35	0.72

Table A.21. Genotypic and phenotypic correlations of traits with 2004 spread (% digital plant cover in one year in 1.8 x 1.8 meter plot) and ±SE.

	2004		2005		2006	
	Genotypic	Phenotypic	Genotypic	Phenotypic	Genotypic	Phenotypic
Height (cm)	0.17 ± 0.27	0.12 ± 0.22	-0.08 ±0.28	0.00 ±0.22		
Shoot Density (no./cm^2)			-0.06 ±0.40	-0.05 ±0.17	0.30 ±0.30	0.15 ±0.19
Seed yield (kg/ha)					0.27 ±0.27	0.21 ±0.20

Table A.22. Statistical analysis of 2004 offspring camera scan (percent digital plant cover in one year in a 1.8 x 1.8 meter plot) regressed on 2004 parent camera scan.

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	1	20.86	20.86	4.59	0.0533
Error	12	54.50	4.54		
Corrected Total	13	75.36			

Root MSE	2.13	**R-Square**	0.276
Dependent Mean	18.03	**Adj R-Sq**	0.216
Coeff Var	11.81		

Parameter Estimates							
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**	**95% Confidence Limits**	
Intercept	1	15.06	1.49	10.08	<0.0001	11.81	18.32
parent	1	0.21	0.13	2.14	0.0533	-0.004	0.59