Selection of Turf Type and Seed Production of Inland Saltgrass (*Distichlis spicata*)

Colorado State University & University of Arizona

Harrison Hughes; Dana Christianson; Scott Reid; Judy Harrington; Tony Koski
David Kopec; Ken Marcum

Objectives:

1. *Determine turf performance of 7 elite CSU-USGA lines, 7 elite University of Arizona lines, 7 Great Basin lines (check lines from the University of Arizona).*
2. *Determining the range of stress tolerance (drought, salinity) present in inland saltgrass.*
3. *Determine seed production of 7 elite CSU-USGA lines.*
4. *Evaluate Kopec collection and Northern Great Plains collection.*
5. *Evaluate seed germination and seedling vigor of all crosses.*
6. *Evaluate RAPD as a means of identifying unique genotypes of saltgrass.*
7. *Determine the relative chromosome number of elite clones.*
8. *Study the viability and germination requirements of inland saltgrass seed.*
9. *Evaluate seed priming as a possible method by which germination can be improved.*

A total of 7 elite CSU-USGA clones, 13 University of Arizona clones and a Salt Lake seed source were subjected to standard turf practices relative to mowing and weed control. Observations of vigor, shoot density and fill-in as of May 21 and color, regrowth and shoot density on October 14 were made. The turf from the Salt Lake seed source showed considerable winter damage. No apparent damage when herbicides at standard rates were used to control weeds. Data collected from next year will be tabulated with this years data to determine relative potential for use of elite lines for turf.

The collection of 190 clones were observed in fall 1998 and spring/summer 1999 for an array of characteristics including fall color, densities, sex, flowering and many other turf and seed characters. The 7 CSU-USGA clones showed the highest vigor while the other Colorado and Nebraska types tended to be short and dense which the Utah and Nevada types tended to have taller female racemes. The South Dakota types were intermediate. Hand crosses were made among those showing the best turf charactheristics.

Chromosome counts and intercrossing among the various chromosome accessions continue. A total of 71 accessions have had chromosome counts made. The most common chromosome number is $2n=38$ (42 accessions) while $2n=40$ (7accessions), $2n=74$(8), $2n=38+1B$(1), $2n=38+2B$(1), and $2n=40+2B$(2) chromosome numbers have been observed. The most common chromosome type (38) is geographically widespread in California, Nevada, Idaho, Colorado, and Nebraska. Crosses among these genotypes have been made and seed produced with some of these. These are presently being evaluated as to their chromosome number. Meiosis of these accessions are also under study.

Seed germination studies continue to indicate that hand scarification of seed increases or at least speeds germination in laboratory experiments. The use of me mechanical means of scarification appears to result in similar rates of germination. Field comparisons of scarified seed (hand and mechanical) and nonscarifed seed gave mixed results depending on relative viability of seed. However, excess moisture and associated disease problems may have influenced these results. At the end of the season the fill-in of the plots were similar in all treatments.

Annual Report
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Harrison Hughes
Dana Christianson
Scott Reid
Judy Harrington
Tony Koski
Colorado State University

David Kopec
Ken Marcum
University of Arizona

The report will present progress according to the objectives as presented in the original proposal.

Objective 1:Determining turf performances of elite lines.

1999 research initiated and results.

A total of 7 elite CSU-USGA clones, 13 University of Arizona clones and a Salt Lake seed source were established in 1998 and have shown somewhat slow growth in the 1999 growing season in Colorado. The Salt Lake seed source showed evidence of winterkill in Ft. Collins. Slow growth of these plots as compared to the nursery was probably due to nitrogen deficiency. Plots have received over 20 inches of rainfall as compared to 11 inches in a normal year. As a result of this rainfall they were irrigated less frequently from a well containing high nitrates. Since nitrogen was only applied in this study at 1 lb/1000 sq ft on July 29th the plants likely suffered from nitrogen deficiency. In the first week of August, a 50 cm twice weekly reel cut, and a 72 cm, weekly rotary cut were initiated. Higher shoot densities were observed by the middle of September in the elite CSU-USGA clones, excluding C10 and C8 which had low densities. C92 still has some green as of October 26th after several -5o C freezes. No apparent herbicide injury resulted form application of Buctril at 2 pts/A mixed with 2,4-D 4 lb amine at ½ pt/A on May 22cd, or with an atrazine application at 1 lb/A on July 29th. An Access database was developed which includes observations of vigor, shoot density and fill-in as of May 21st and color, regrowth and shoot density as noted on October 14th

A summary of results from Arizona will be forth coming from Arizona.

2000 proposed research and anticipated results.

A northern California seed accession will replace the winter kill susceptible Salt Lake seed accession in the spring. The summer growing season of 2000 will be the first full year of turf management on these plots due to the slow fill-in this past year. Nitrogen will be applied at a seasonal total of 3 lbs/1000 sq ft. Poorer performing clones will be replaced in August 2000. Observation data will be collected as in the previous season and comparisons will be made among selections.

Objective 2: Determining the range of stress tolerance (drought and salinity) present in inland saltgrass.

1999 research initiated and results.

Data on relative drought and salt tolerance of selected clones were made in the greenhouse in hydroponic culture during this past season in Arizona. A summary of results will be forth coming from Arizona.

2000 proposed research and anticipated results.

See forthcoming report from Arizona on plans for the coming year. We recently have accepted the transfer of an international student, supported by his government, from the Dept. of Range Science who will do some stress tolerance studies. These will involve salt and cold temperature tolerances. The cold temperature tolerances will involve both field observations of greening in fall and in early spring. This data will be correlated with freezing tests made in the laboratory. We hope that in this way we may be able to select those lines with the greatest cold temperature tolerance as well understand some of the mechanisms. We will also initiate salt tolerance studies using the information developed by Dr. Marcum in Arizona to select 2-3 of the most salt tolerant as well as 2-3 of the most salt sensitive lines to study the mechanisms associated with the tolerance. This will involve both greenhouse and laboratory studies. It will be done in cooperation with Dr. Yaling Qian in our department.

Objective 3: Determining seed production of 7 elite CSU-USGA clones.

1999 research initiated and results.

Plant cover was removed on half of this experiment on March 18th to determine if cool, spring temperatures would effect floral initiation during rapid vegetative growth. No differences were observed. However, racemes numbered on in the tens per square foot as compared to hundreds per square foot in 1998. One noted variable was that in 1997 the plots were subjected to fall burning which was followed by profuse flowering in 1998. Since this is a standard practice in areas where turf seed is produced there may be a stimulative effect of burning. We fall burned these plots this year for evaluation in 2000. In addition, C66 at this site had the least number of shoots as compared to the nursery trial where it had the highest number of shoots. This might be related to higher soil nitrogen in the nursery.

2000 proposed research and anticipated results.

The plots fall burned will be observed for relative flowering in 2000. In addition, a group of clones are presently undergoing 4.5° C and 10° C treatments with 12 hour days in growth chambers to evaluate temperature on floral induction. A second study using a clone exhibiting year to year spike number differences will be subjected to varying lengths of vernalization and nitrogen fertilizer treatments. We hope this leads to a better understanding of these influences on flowering and hence a means of inducing greater flowering.

Objective 4:Evaluate Colorado (includes Kopec), Northern Great Plains, Great Basin and Northern California collections.

1999 research initiated and results.

A germplasm nursery of 190 clones, replicated twice, was established in 1998 at the HRC, Fort Collins. An additional 15 clones from a northern California seed accession were added in August, 1999. The 1999 growth was vigorous for greenhouse-acclimated material transplanted in 1998 as compared to material field collected and directly transplanted in 1998. The recently added northern California clones have only spread 6 inches to date. High growth rates in the nursery as compared to the boxes (plants established for objective 1) are probably due to the application of 3lbs/1000 sq ft of available N to the nursery.

Leaf rust appeared in the nursery at the end of July and will be evaluated in 2000.

An Access database is being built which will allow us to retrieve or rank accessions based on specific queries. The following is listed for 380 plants:

1998 fall color, fall spread, presence of flowers.

1999 spring rhizome length, flowering date, spring turf density rating, spring flower density rating, spring height rating, sex, total female raceme weight, total female raceme number, seed yield, area spread, female spike density, height, shoot density, rust rating, and fall color.

A digital photograph is tied to each plant in the database, as well as original location.

It is interesting to note that C66 had the greatest vigor (and highest number of racemes), in the nursery, spreading to a 5 meter circle by August 1, 1999 from a 10 cm clump planted June 23, 1998. It had the lowest vigor (and relatively low number of racemes) of the 7 CSU-USGA clones in Objective 3. As a group, the 7 CSU-USGA clones have the highest vigor in the nursery. This group also tends toward tallness, lower density, and greater number of spikes. This group represents selection by Dr. Robin Cuany out of 100 collected plants from the western U.S. The remaining Colorado and Nebraska types tend to be short and dense while the Utah and Nevada types tend to have taller female racemes. The South Dakota types are intermediate.

Seed yield was limited and may be due to distance from the pollen source. The wet spring kept us out of the field for much of the flowering peak, although some hand crosses were made. During hand harvesting, visual inspection showed little seed as compared to Objective 3 plots in 1998.

Criteria for selecting parents will be seed yield, low height, high shoot density, and absence of rust based on 2 years data. Next year will complete this selection. However, this fall roughly 20 potential parents will be brought into the greenhouse (selection in part based on spike number) in order to induce flowering for use in winter crosses.

2000 proposed research and anticipated results.

With the aid of Dr. Bill Brown, winter strains of spinach will be set among the nursery plots to substantiate (confirm) the presence of the rust organism Puccinia aristideae. This is a standard technique to confirm that the rust observed is this particular species since spinach is an alternate hose for this rust.

Observation and data collection of the nursery planting will continue in 2000 as in 1999. A summary of this data will then be used to select superior lines for further evaluation.

Crosses between phenotypically similar male and female plants. These crosses will be used to identify parents that readily produce seed which are easily germinated. Selected parents will be brought into CSU greenhouses for winter crosses in fall of 2000 and their summer progeny A limited number of crosses were made in 1999 and potential parents are being brought into the greenhouse this year in order to initiate will be tested for emergence in mineral soils. In addition, 20 randomly selected female plants will form a population to compare genetic gain for seed production. They will be pollinated along with superior females by a collection of male tester pollen with the half-sib progeny seed collected for a progeny evaluation trial.

Objective 5:Evaluation of seed germination and seedling vigor of all crosses.

1999 initiated research and results.

In a preliminary trial a limited number of crosses were made this year and potential parents have been brought into the greenhouse.

2000 proposed research and anticipated results.

Seed from the initial crosses will be treated for optimum germination, germinated and vigor assessed. Information from this trial will form the basis for the following years work.

Objective 6:Evaluate RAPD as a means of identifying unique genotypes of saltgrass.

1999 initiated research and results.

See midyear report for these results.

2000 proposed research and anticipated results.

We anticipate applying the RAPD procedures developed in 1999 to the fingerprinting of superior clones of saltgrass.

Objective 7:Determine the relative chromosome number of saltgrass.

1999 initiated research and results.

Chromosome numbers have been determined for 71 Distichlis spicata clones from several regions. Sixyt-three of these accessions are clonal collections, six are individuals established form seed originating from a commercially harvested field near Salt Lake City, Utah (referred to as the Granite population or Granite seed source), and two are individuals established form seed collected in Modoc County, California (referred to as the Modoc population or seed source). Three classes of 2n individuals have been observed: 2n=38, 2n=40, and 2n=74. In addition to these major variations in chromosome number, one or two additional small, morphologically different chromosomes believed to be B chromosomes have been observed in two 2n=38 accessions and one 2n=40 accession. Although the basic chromosome number of the genus is presumed to be x=10 (Gould, 1968; Reeder, 1971), no true diploids (2n=2x=20) have been observed in our collections or reported in previous, more limited investigations. Analysis of chromosome pairing in meiosis I of 2n=4x=38, 2n=4x=40 individuals and the 2n=4x=40 +2B clone suggest the tetraploid genome is highly diploidized. The 38 chromosome individuals observed have 19 bivalent associations during meiosis I, and although we are still accumulating data, bivalent pairing appears to predominate in the 40 chromosome individuals. Assuming the same evolutionary origin, these observations suggest the 2n=38 chromosome individuals are nullisomic. The 38-chromosome type, however, is geographically widespread and apparently persists as a distinct chromosome race, being ovserved in accessions originating in northern California, Nevada, Idaho, Colorado, and Nebraska. Within the most extensively sampled region, the northeastern plains near the front range of Colorado, it is the exclusive representative of the tetraploid A chromosome complement in all 43 accessions examined. Table 1 summarizes the data for our survey of chromosome numbers to date.

Table 1. Number of Distichlis spicata accessions observed from our collection in each chromosome class according to the general division of geographical varieties as described Gould (1975, 1968).

	Somatic chromosome number					
Type	**2n=38**	**2n=40**	**2n=74**	**2n=38+1B**	**2n=38+2B**	**2n=40+2B**
Inland	42	7	8	1	1	2
Coastal	0	10	1	0	0	0
Total	42	17	9	1	1	2

The 38-chromosome saltgrass do not seem to represent a distinct separation of the coastal and inland types as described by Gould (1975, 1968). Gould's description of the distribution of geographical varieties is simplified and somewhat modified from that of Beetle (1943). Individuals from the Granite population, accession AZ 203 from Holbrook, Arizona, and accession C-12 (considering the A set) collected from Colorado's western slope near Delta are 2n=40 as are all of our tetraploid accessions originating in or regionally near coastal habits. The 40-chromosome accessions from Arizona, Utah and western Colorado are more similar morphologically to the 38-chromosome inland accessions than the coastal saltgrass. Reeder's (1971) reports of 2n=40 for saltgrass collected north of Fort Stockton, Texas and near San Lorenzo, Mexico, combined with our observations suggest there may be a more southern distribution of inland saltgrass with 40 chromosomes and a generally more northern 38-chromosome race. There is, however, one report of 2n=40 for saltgrass at the extreme northern range of the species from a collection near Fort Smith, Northwest Territories, Canada (Bowden, 1960). Additional collections are required to understand the limits of the distribution of the two inland types observed. Figure 1 illustrates the geographic distribution and chromosome number for the accessions in our collection surveyed to date.

Over 12% of our accessions for which the chromosome number has been determined are hypo-octaploid. This estimate may be subject to some sample bias due to possible sampling of the same genet more than once when collecting within a particular area (the result of extensive clonal spread), but the frequency is still about 10% when all possible duplicate samples are eliminated. We are currently analyzing additional replicates to confirm our initial observations that all of the hypo-octaploids have 74 chromosomes. Reeder (1967) had earlier reported two counts of "about 72" chromosomes for saltgrass. Individuals with 74 chromosomes have been identified in our collections as a variation associated with both 38- and 40-chromosomes types. We have not yet had adequate flowering in these plants to observe meiosis. The relatively frequent occurrence of these polyploids underscores the need to screen the parents selected for inclusion in the breeding program for compatible chromosome numbers.

Seed from 22 different hand crosses made in the greenhouse during June and July 1999 are currently being germinated. Eighteen additional crosses will be ready for harvest beginning in mid=November. The specific crosses were made primarily to develop additional material for chromosome research, but include parents varying in traits associated with turf quality such as height, density, and color. Progeny from these crosses can be used for establishment of a random mated population from which estimates of heritability may be more reliably made. Seventeen of the crosses include the 40-chromosome males 9032708 and 9032694 and the 40+2B-chromosome male C-12 pollinating 8, 4 and 5 different 38-chromosome females, respectively. Although the number of replicate crosses per pair was too small and pollination conditions were too variable to allow statistical analysis, seed set in these crosses was similar to that in the 38- by 38-chromosome crosses. Average seed yield was 42.6 seeds/inflorescence (an average of about 33% of the available florets developed mature seeds) for all crosses from one hand pollination. In addition to cytological observation of the expected (hypothetically) monosomic progeny, we plan to cross the monosomics with 40-chromosome parents to provide material to complete the classical cytological studies. Root-tip chromosomes of four progeny of a 1998 cross of C-12 (40+2B chromosomes) and AZ 126 (38 chromosomes) have been examined and all have 41 chromosomes (39+2B). Our hypothesis based on the meiotic behavior we have observed to date in C-12 is that gametes would have equal probability of having 20, 21, or 22 chromosomes, but these limited results suggest random inclusion or exclusion of one or both B chromosomes may not explain the mechanism. Although the 38- and 40-chromosome types are cross fertile and the simplest hypothesis is the 39-chromosome individuals would have essentially regular meiosis forming either 19- or 20-chromosome gametes, it is an important possibility that substantial rearrangements have occurred which may promote multivalent associations and possibly interfere with regular meiosis. We will attempt further study of this as the progeny from these crosses reach sexual maturity. Additional progeny from this and crosses to other 38-chromosome females are being grown for the study.

2000 proposed research and anticipated results.

Our immediate goal for this phase of the project is to determine the chromosome number of additional selections to be used as parents in the breeding program. Additional accessions from areas currently not well represented in our collection will also be classified with the goal of better defining the geographic distribution of the chromosome races. Germplasm collected from the different geographic regions and potentially the inter-regional crosses that have been made are being made available for the study of cold hardiness and environmental stress responses in saltgrass. We are continuing to study meiosis in the various materials described above as well as working to develop karyotypes using both computer image analysis of our existing data and additional staining methods. Our goal is to develop a theory of how the 38-chromosome race may have originated and how it is different form the 40-chromosome individuals. Although traditional banding techniques often yield little information in plant species with very small chromosomes, large rearrangements or enough additional resolution to discriminate similar homologous pairs may be possible. Ultimately, more information, but unfortunately, the cost of developing probes and time and labor constraints will postpone this research into the future. Understanding the mechanism of formation and chromosome relationships in the 74-chromosome individuals is also essential to complete understanding of the breeding system of this species, and may provide valuable evidence to support developing theories of the evolution of changes in the tetraploids.

Literature cited:

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Objective 8:Study the viability and germination requirements of inland saltgrass seed.

1999 initiated research and results.

The study of temperature and scarification influence on seed germination is nearly complete. Additional replications are being completed for the temperature treatments which should finish in November. Statistical analysis completed thus far indicates that four replications provide a fairly narrow 95% confidence interval around mean germinability at the tested temperatures. Increasing the number of replications from 3 to 4 has resulted in improvements in the confidence intervals. With completion of four replications, it appears that two of the tested temperature regimes, 25 C constant and 28C/18C alternating, which appeared after one replication to be somewhat different in their ability to induce germination in scarified seeds, are not significantly different. The seed lot designated "66" in the 1998 year-end report has been dropped from the testing schedule as having a viability too low for its response to that various temperature regimes to be of interest to commercial producers. Testing continues on the seed lots designated "Granite" and "Modoc" in the 1998 year-end report. Scarified seeds continue to germinate at a higher rate than intact seeds in all replications at all temperature regimes tested.

The results of the seed germination studies by Ms. Harrington suggest that scarification of seed is critical to speed germination. We therefore initiated studies using a mechanical scarification procedure. We compared nonscarified, handscarified and machine scarified seed from "Granite" and "Modoc" seed lots in the laboratory at 25 C constant temperature. Figures 2 & 3 illustrate total germination of 100 seed of the two seed lots. It is evident from this data that machine and hand scarified treatments are similar although the numbers germinated were slightly higher with machine scarification. Nonscarifed seed had a much lower germination. Further replications of these treatments are underway.

A field treatment was also conducted to compare the scarification treatments (hand and machine) with nonscarified seed relative to germination. Figures 5 & 6 illustrate an average of numbers of seed germinated per square inch for 3 observations for each of 4 replications of the last three days of data collection. The results indicate differences between the seed lots. The scarified seed of "Granite", the seed lot with greater viability, germinated much better with scarification as compared to nonscarified seed. However, machine scarified seed germinated better than the hand scarified seed. "Modoc" seed, the seed lot with lower viability, germinated better without scarification. We feel the differences may reflect the observation of disease in the plots associated with excess moisture during initial germination. Statistical analysis is underway and will likely give us a clearer picture of this.

It is interesting to note that there was no apparent differences among treatments in the field by the end of September. Although initial differences were readily apparent with treatment, by the end of the growing season nonscarified and scarifed seed treatments had similar stand establishment. Therefore if the grower is welling to wait scarification is not needed for grow in and may even be detrimental for low vigor (low viability) seed.

2000 proposed research and anticipated results.

We will complete the laboratory experiments on scarification and temperature influences on germination this year. Data will be analyzed and a paper will be prepared for submission for publication.

We plan to repeat the field experiment with particular attention to moisture levels during germination. We anticipate that with lower moisture levels during initial germination that we will have lower levels of disease incidence and therefore greater differences (in favor of scarification) in initial germination. We also plan to do some laboratory studies on the assessment of seed vigor relative to the seed lots. We hypothesis that relative vigor may influence some of the results observed in the field as well, ie the susceptibility of "Modoc", low viability and likely low vigor, to disease during initial germination.

Objective 9:Evaluate seed priming as a possible method by which germination can be improved.

1999 initiated research and results.

None were initiated during 1999.

2000 proposed research and anticipated results.

We propose to initiate this research in preliminary experiments to test various levels of inorganic salts such as KNO_3 and K_3PO_4 and polyethylene glycol on scarified and nonscarified seed relative to seed priming. These experiments will be designed to determine relative perimeters to begin more detailed studies in 2001.

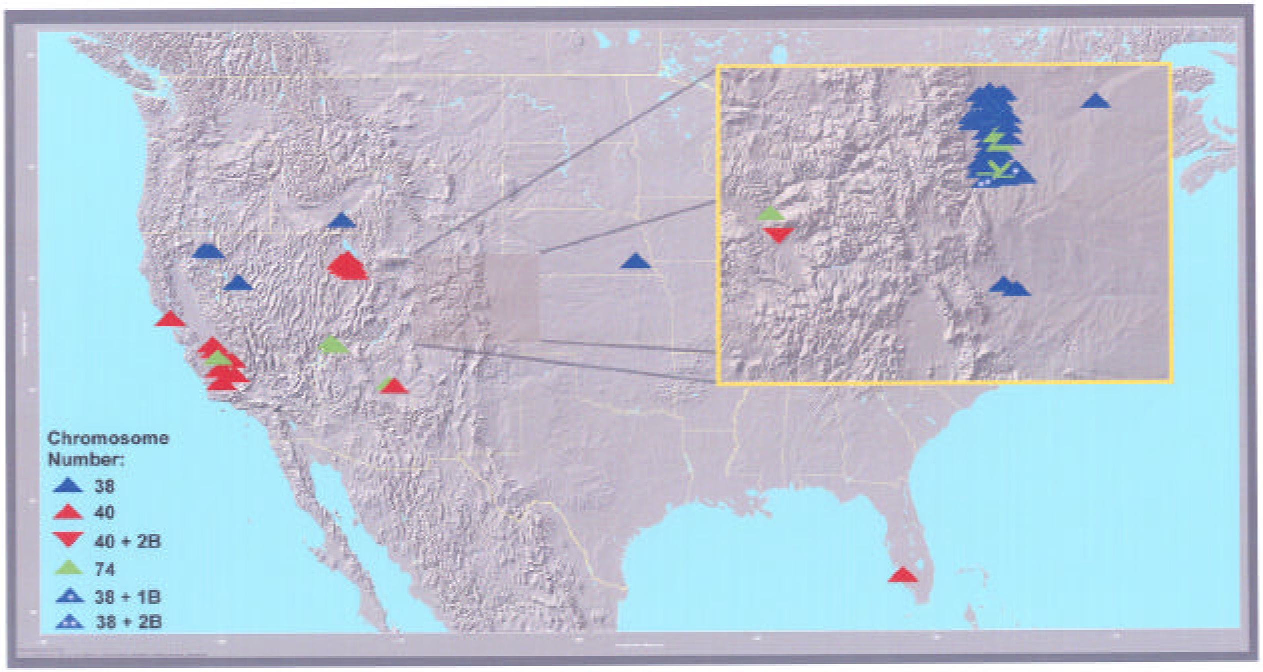

figure 1. Geographic origin of *Distichlis spicata* accessions classified by chromosome number.

Figure 3 Granite Lab Data

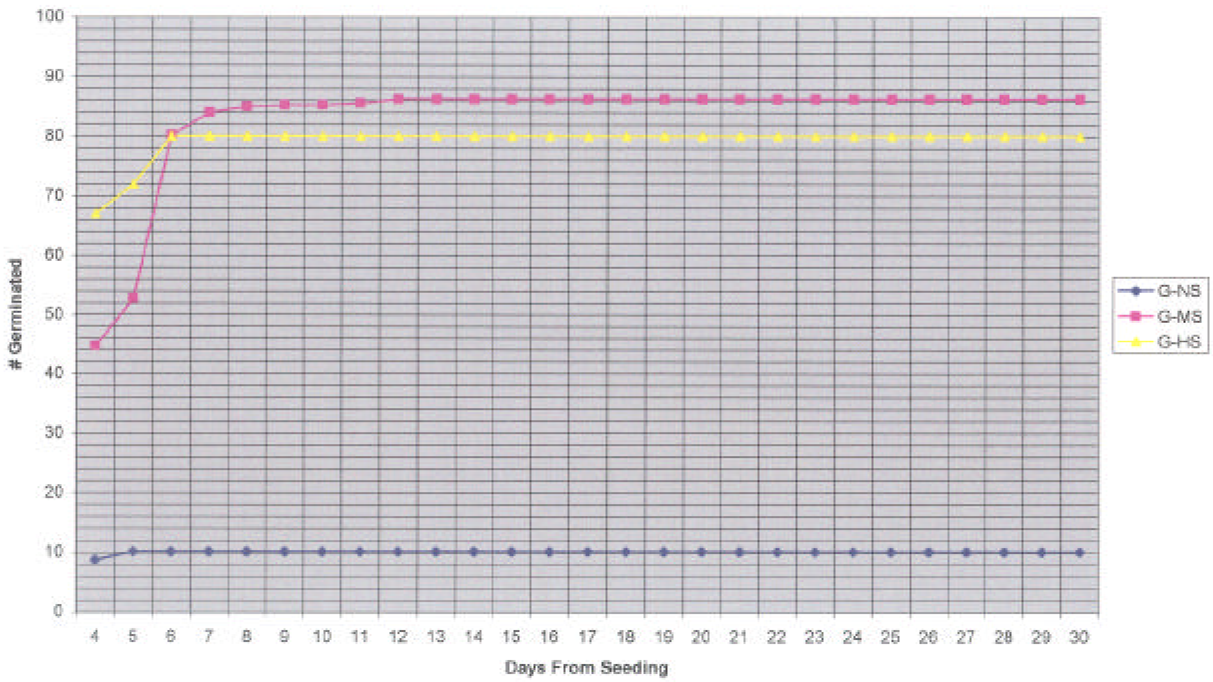

Figure 3. Modoc Lab Data

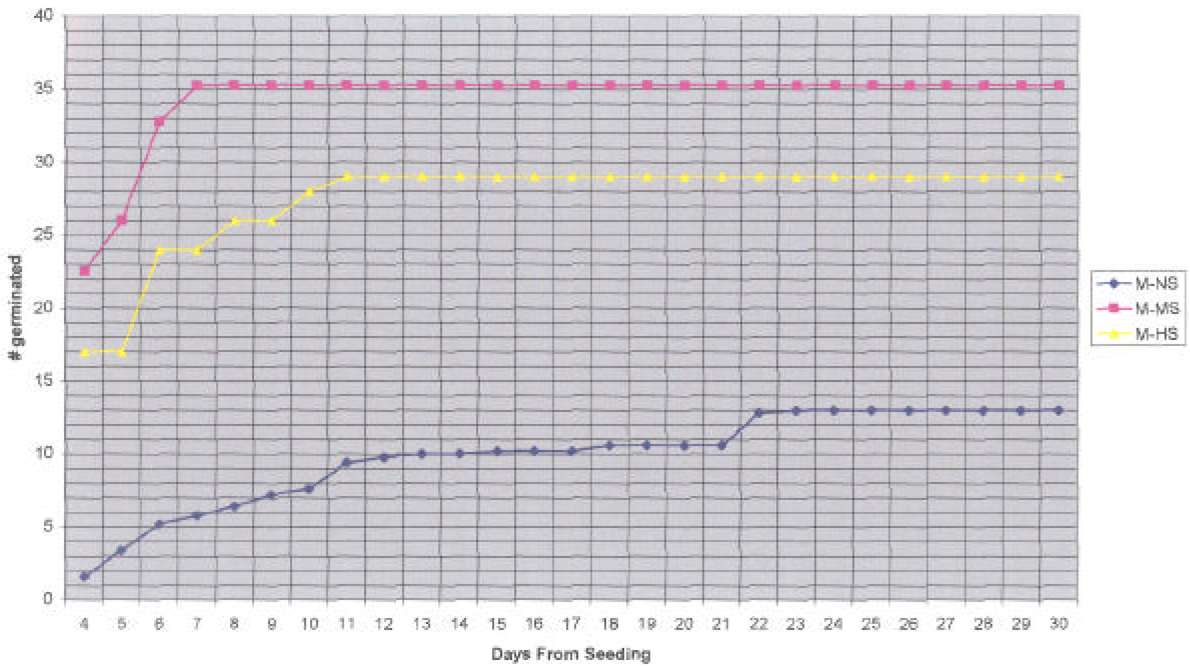

Figure 4 Granite Field Data (Last Three Days)

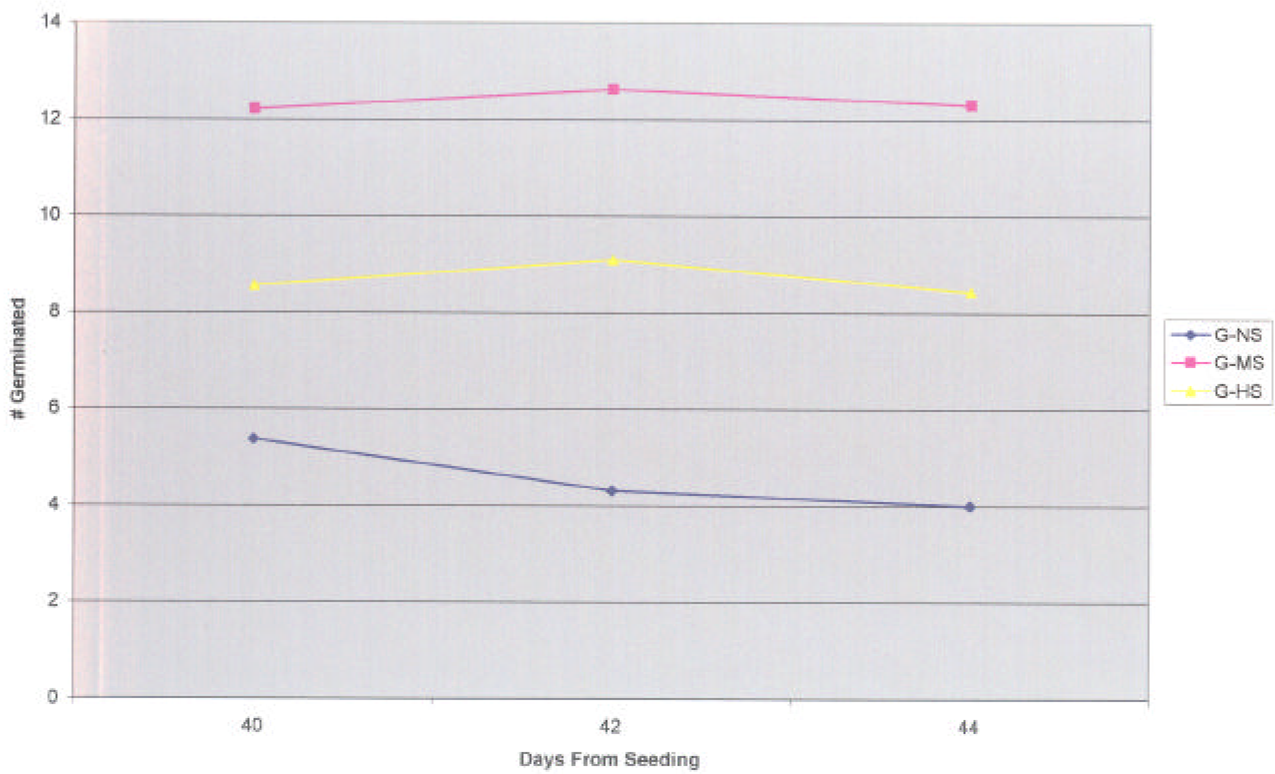

Figure 5, Modoc Field Data (Last Three Days)