Selection of Turf Type and Seed Production in Inland Saltgrass

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Goals:

- *Determine turf performance of elite lines.*
- *Determine range of stress tolerance.*
- *Determine relative seed production of elite lines.*
- *Establish a germplasm nursery and evaluate lines for turf and seed production.*
- *Determine chromosome numbers of genotypes from diverse origins.*
- *Evaluate the viability and germination requirements of seeds.*

The goal of this project is to develop turf type inland saltgrass with good seed set and germinability.

Initially, elite lines from the University of Arizona collection and the CSU-USGA lines were established with 3 replications in both Arizona and Colorado. This initial year was a grow-in year with data on turf quality and seed production to be observed in future years. The material in Arizona will be used for drought studies in the field as well.

CSU-USGA elite lines previously established in Colorado were observed for flowering and seed set. Seed production was evident but shattering was a problem.

An extensive nursery consisting of the Arizona lines as well as additional lines from a collecting trip to Utah, Nevada, South Dakota and Nebraska with approximately 200 accessions replicated twice was established at the CSU Horticulture Research Center outside of Fort Collins.

In order to better understand seed production of inland saltgrass a study of chromosome numbers of genotypes found throughout the region was initiated. Variation in chromosome number may lead to low viability of pollen and egg resulting in poor seed set. Root tip smears of 40 genotypes were observed with the most common chromosome number being 2n=38. However, 39, 42, 40, and 74 chromosome counts were observed. Coastal saltgrass has been determined to have 40

chromosomes as previously published and in our observations as well. This would indicate that our most commonly observed chromosome number of 38 is likely an aneuploid, probably a nullisomic.

Studies to determine pollen viability via examination of pistils demonstrated that pollen readily germinated at least in those clones examined. This was seen via microscopic examination of pistil structure. Furthermore, pollen tube growth reached the egg sac as well. Therefore, pollen viability is not apparently a problem in those clones observed herein. However, crossing among clones of different chromosome number may still influence successful seed production. In crosses among plants with 38 chromosomes and between 38 and 42 successful seed set was apparent. These seed have been harvested and germinated and will be examined in future studies. Due to high pollen viability and observable seed set, we believe that poor seed set as reported elsewhere is probably due to pollen availability rather than pollen quality or genetic deficiencies in most cases.

Three seed lots of inland saltgrass were examine for viability and germination. Viability of seed of these seed lots were 15, 62 and 92% as determined by TZ test. This low viability in some cases was probably due to a combination of extreme age, early harvest and poor storage conditions.

Inland saltgrass seed at maturity appears to be dormant and this dormancy is apparently due to the seed coat. Recently harvested seed lots scarified readily germinated in excess of 90% of viable seed. For unscarified seed, alternating extremes of temperature prompted greater germination than moderate temperatures. Old seed exhibited low viability but those seeds that were determined to be alive via TZ test germinated without scarification.

Establishment of Inland Saltgrass elite lines and Nursery Evaluation Plots as well as Field Seed Production

OBJECTIVE 1: Determining turf performance of 7 elite CSU-USGA lines, 13 University of Arizona lines, and a seed accession.
OBJECTIVE 3: Determining seed production of 7 elite CSU-USGA lines.
OBJECTIVE 4: Evaluate Kopec, Northern Great Plains, and Great Basin collections.

By Dana Christensen

Research Initiated, Progress, and Results

SUMMARY: We accomplished all of the objectives for this year as outlined in the initial proposal, which essentially was the establishment of all accessions in all field plots. We had some difficulty harvesting seed in OBJ 3, due to shattering in August at a time when we had no labor. Some greenhouse spike counts are being summarized.

OBJECTIVE 1: Determining turf performance of 7 elite CSU-USGA lines, and 13 University of Arizona lines, and a Salt Lake seed source. This has been modified to include more elite material from Arizona, deleting A107, A 89(because of poor vigor), and adding a seed source from Salt Lake. There are 21 lines blocked 3 times. We buried 63 4x4x2-foot plywood boxes to serve as root barriers to contain accessions. Clonal lines were planted on July 13 with the seed lot planted on July 17 with excellent emergence in 5 days under ideal maximum temperatures of 100 degrees F. Sprigs showed no signs of stress and established well, even though they were pot bound. Establishment data is being summarized. Plot plan is in the appendix. Tuscon has a similar experiment

OBJECTIVE 3: Determining seed production of 7 elite CSU-USGA lines. We are still harvesting seed off of this material. Shattering is a problem, and data has to be taken in August.

Flowering dates of clones with May 31=151 days:

Males: C8=136 C12=174 C56=152 C92=157
Females: C10=144 C11=140 C66=159

Head counts/sq. ft of clones:

Males: C8=184 C12=127 C56=3* C92=7*
Females: C10=265 C11=228 C66=80

*poor establishment

There are differences in establishment which affect seed production data. Also, C66 has a low shoot density growth habit.

OBJECTIVE 4: Evaluate Kopec , Northern Great Plains, and Great Basin collections. A germplasm nursery was established this year at the HRC, Fort Collins, which includes the Kopec collection(plains of the Front Range). Additionally, Dr. Tony Koski provided funds for travel to Utah and Nevada to collect 24 accessions from the Great Basin, and to travel to South Dakota and Nebraska to collect 43 accessions.

All plants survived transplanting, although material from the collecting trips had low vigor as compared to the greenhouse grown Kopec material. Transplanting conditions were harsh, with above 90 degree days and moderate winds, but saltgrass survived and some plants have put on several 3 foot runners from a 3in x 3in clump planted at the end of June.

The nursery has close to 200 accessions replicated twice and takes up 2 acres since individual plants are spaced 15 feet to isolate vigorous vegetative growth.. Seed accessions had not developed enough (due to non-mineral soil media?) to warrant planting in the nursery this year, so there are some blanks in the nursery plan in the appendix.

Proposed Research Schedule and Anticipated Results for the Coming Year

SUMMARY: This follows the original proposal. Two additional experiments are planned. The first is a greenhouse study to induce winter flowering in order to determine parameters for winter crosses. The second, imitating commercial seed production will be planted at the HRC involving a row of a female in between rows of a male. This will allow us to start looking at combine harvests. We are looking at the possibility of another collecting trip, again exclusive of USGA funds. From the plot maintenance viewpoint, herbicides will be used more extensively, and adequate labor will be hired to fulfill the research schedule.

OBJECTIVE 1: Determining turf performance of 7 elite CSU-USGA lines, 13 elite University of Arizona lines, and one seed accession. These will be rated for establishment, shoot density, seasonal color, mowed color, and flowering dates. These 2nd year results will be combined with a 3rd year of data to start making selections based on additional evaluations in the OBJ3 crossing blocks, and OBJ4 nursery. Warm season pre-emergence herbicide will be selected for weed control, while minimizing turf effects.

OBJECTIVE 3: Determining seed production of 7 elite CSU-USGA lines. A 2nd year of data will target the matching of crosses. Flowering dates and seed production will again be taken. An additional experiment will be set up at the HRC to test combine harvests.

OBJECTIVE 4: Evaluate Kopec, Northern Great Plains, and Great Basin collection. Record establishment vigor, seasonal color, mowed color, shoot density, flowering dates, pollen shed, and head density. Hand crosses will be made between phenotypically similar male and female plants on flowers with the same flowering dates. Flowering starts with the original sprig in late May, and continues on older rhizomes through August. Controlled crosses would identify parents that 1. Produce seed, and 2. Produce seed capable of rapid establishment. Any parents exhibiting seed potential are brought into the CSU greenhouses for winter crosses, and their summer progeny will be greenhouse tested for emergence in mineral soils. The protocols for winter flowering are being determined from a fall 1998 initiated study.

In addition, 20 randomly selected female plants will be selected for determining general combining ability / heritability for seed production. They will be pollinated with a collective male tester pollen, and half-sib progeny seed will be collected for a progeny evaluation trial.

Distichlis spicata Box Study 1998
Horticulture Research Center

	Row 1	Row 2	Row 3	Row 4	Row 5
Range 1	138	137	119	65	10
Range 2	56	86	seed	51	40
Range 3	11	8	48	61	12
Range 4	66	72	55	41	77
Range 5	92	65	40	92	10
Range 6	12	8	48	seed	119
Range 7	61	72	56	41	66
Range 8	137	77	55	11	138
Range 9	51	86	66	92	12
Range 10	55	48	41	56	8
Range 11	72	137	61	65	10
Range 12	138	119	51	40	11
Range 13		86	seed	77	

Distichlis spicata Nursery 1998
Horticulture Research Center

← N

East Rep.

	Row 1	Row 2	Row 3	Row 4	Row 5	Row 6	Row 7	Row 8	Row 9	Row 10	Row 11
Range 1	A124	21	59	A34	44	70	A32	C56 mutant	A86		
Range 2	46	45	A135	60	A56	C8	22	83	A128	A7	
Range 3	20	47	61	A122	72	71	A132	A30		A42	
Range 4	A65	A86	62	A15	A44	A31	84	A97	A70		
Range 5	48	A23A	25	63	A19	A77	26	85	A104	A109	
Range 6	A136	49	A13	A28	73	74	A49	A8	A43	A62	
Range 7	42	A71	28	A46	23	A55	86	A94	A112		
Range 8	50	A26C	64	65	A50	A75	29	87		A101	
Range 9	A35	51	A67	A12	75	C10	A105	A37	A93	A73	
Range 10	27	A127	30	A16	A114	76	31		A38		
Range 11	52	A137	A47	66	24	A48		A125	A6	A2	
Range 12	A60	53	A59	A10	A78	77	A21		A19	A68	
Range 13	34	A39	32	A53	C66	A18	33	A107	A14	A20	
Range 14	54	A61	A1	67	78	C56		A36		A64	
Range 15	A79	55	36	A83	A45	79	38		A27	A103	
Range 16	A51	A24	A129	A89	A120	A54	A123		A108	A36	
Range 17	57	A72	A111	68	80	C11		A27	A21	A123	
Range 18	A126	58	39	A85	C92	81	40		A107		
Range 19	A11	A138	A116	A41	82	A40	A103	A20	A125		
Range 20	35	A29	41	69	37	C12	43			A105	

Distichlis spicata Nursery 1998
Horticulture Research Center

West Rep.

← N

	Row 1	Row 2	Row 3	Row 4	Row 5	Row 6	Row 7	Row 8	Row 9	Row 10	Row 11
Range 1	A95	45	A30	59	A10	35	85	A108	A72		
Range 2	46	A119	21	A120	72	A67	22	A85		A126	
Range 3	20	A125	60	A132	C8	73	A15	A111	A51	A79	
Range 4	A78	47	A65	61	A83	A47	86	41	A125		
Range 5	48	A45	25	A136	74	C12	26	A16		A39	
Range 6	A48	A54	62	A26	A120	34	87	A46	A24	A14	
Range 7	A49	49	A86	63	A1	75	A34	44		A138	
Range 8	50	A18	28	A124	76	A59	29		A29		
Range 9	23	A75	64	A23	A135	77		A122	A11	A95	
Range 10	A77	51	30	65	78	C10	31		A104	A62	
Range 11	52	A97	A127	A71	A89	27		A70		A101	
Range 12	A55	A40	66	A61	79	A129	A28		A73		
Range 13	A50	53	32	67	C11	80	33	A86	A109	A119	
Range 14	24	A31	A35	A60	A135	81		A128		A43	
Range 15	54	A19	36	A137	82	42	38		A64	A6	
Range 16	A32	55	68	69	C66	A41		A42	A22		
Range 17	57	A86	A114	A37	A116	83	A7	43	A93	A38	
Range 18	A44	A56	39	70	C56	A53	40		A112	A68	
Range 19	37	58	71	A94	84	C92		A2			

Saltgrass Seed Germination Project, 1998 Year-End Report

Saltgrass seeds are dormant at maturity. The objective of this facet of the project was to determine the type of dormancy and to explore ways to overcome it. The three seed lots that have been tested are of three different ages and come from three different geographic areas. Not all treatments have been replicated four times for statistical reliability. Thus, the findings are preliminary. The major findings are that dormancy seems to be mostly, perhaps entirely, coat-imposed, and that dormancy can be overcome by scarification in two young seed lots (Fig. 1). For young seed lots, alternating extremes of temperature prompt some increased germination of intact seed, but not as much as can be achieved by scarification. Germination of a 13-year-old seed lot seems to be lowered by both scarification and alternating extremes of temperature. Proper temperature is sufficient to achieve maximum germination of this old seed lot, although the viability may be too low to permit statistically reliable conclusions to be drawn.

Pending the results of further replications, the highest germination seems to be achieved at a temperature of about 30C with scarification for young seed lots (Figs. 2 and 3), and about 30C without scarification for older seed lots (Fig. 4).

The three seed lots were designated 'Granite' (commercially collected in the summer of 1997 near Salt Lake City, Utah, TZ = 92%), 'Modoc' (commercially collected on July 11, 1996, at Goose Lake on the Modoc Plateau in California, TZ = 62%), and '66' (collected by CSU researchers as part of a previous USGA grant on June 17, 1985, near Lovelock, Nevada, TZ = 15%). The viability of this last seed lot was questioned at the time of collection because of the early harvest date, so it is unknown whether harvest date or extreme age is the main factor in its performance. Seeds (scarified or left intact according to the treatment) were briefly surface-sterilized with alcohol and bleach, and were placed on moistened germination blotters in petri plates. The covered plates were sealed with parafilm to reduce loss of moisture and were placed in germinators at the test temperature. Germinated seeds were counted daily and water was added as needed to keep the blotters moist.

Table 1 lists the treatment conditions. In several of the experiments, conditions were altered after 10 days or 15 days to show whether seeds that failed to germinate under the original conditions would germinate under other conditions. Thus, seeds in the cool (18C/15C) treatment were later moved to a warm germinator (28C/18C), seeds in the dark treatment were later moved to the light, and some seeds that began a treatment intact were later scarified. Results are shown in Table 2. Treatments involving altered conditions are shown in italics immediately following the original conditions, so that final germination can be compared with germination under the original conditions. Cool temperatures inhibited germination (Fig. 5), while light had no effect (Fig. 6). Scarification frequently prompted additional germination in young seed lots.

Because some previous researchers (Sabo *et al.*, 1979; Cluff *et al.*, 1983; Cluff and Roundy, 1988) reported that extreme alternating temperatures (40C/10C) or very high temperatures were helpful or required to prompt germination, experiments were conducted at several temperatures, both constant and alternating. An alternating temperature of 40C/10C increased the germination of unscarified seeds, but not as much

as scarification did.

About 2% of unscarified seeds germinated under many of the laboratory test conditions. This result was confirmed under field conditions at the Horticulture Research Center, where, as part of another facet of the saltgrass project, unscarified Granite seeds were broadcast by Dana Christensen in three plots. Seedlings in these plots were counted by Judy Harrington and the number of seeds planted was estimated based on the measured weight of seed. Germination was calculated to be about 2%.

Because researchers have reported (Bewley and Black, 1994) that the palea and/or lemma of *Avena fatua* contain germination inhibitors, the Granite and Modoc seed lots were tested for germination with the palea and lemma remaining on the seeds. The 66 seed lot had been cleaned too thoroughly to use for this treatment. There was no statistical difference between intact seeds with the palea and lemma still on and intact seeds with the palea and lemma removed. Seeds that were scarified while still inside the palea and lemma achieved a lower germination percentage than scarified seeds with the palea and lemma removed, so perhaps additional tests are needed to clarify possible effects of these enclosing structures.

For most of these experiments, mechanical scarification was accomplished by slicing off part of the seed coat with a scalpel. This is impractical on a large scale, but commercial seed producers have long used scarifying equipment to process seeds. Acid scarification is effective (Fig. 7), but in these experiments an acid treatment that was sufficient to cause substantial scarification also damaged some of the embryos. Acid scarification would require close supervision if used commercially.

Tetrazolium (TZ) viability tests were done on each seed lot and the results were compared to the germination percentages resulting from the experimental treatments. The percentage of completely white embryos in tetrazolium tests is very close to the percentage of ungerminated seeds in the most favorable treatments, suggesting that the presence of even faint pink color in tetrazolium-treated saltgrass embryos indicates germinability under suitable conditions.

Activities planned for the coming year include completion of four replications of each treatment and statistical analysis of the results. Additional seed lots will be tested. Thickness of the seed coat will be measured on all seed lots and the possibility of having a commercial seed processor run scarification trials will be explored. If such trials can be arranged, commercially scarified seeds will be germinated in the laboratory and sown in the field, and manually-scarified seeds will be sown in the field for comparison.

Literature Cited

Bewley, J.D., and Black, M. 1994. Seeds: physiology of development and germination. Plenum Press, New York.

Cluff, C.J., Evans, R.A., and Young, J.A. 1983. Desert saltgrass seed germination and seedbed ecology. Journal of Range Management 36(4):419-422.

Cluff, C.J., and Roundy, B.A. 1987. Germination responses of desert saltgrass to temperature and osmotic potential. Journal of Range Management 41(2):150-153.

Sabo, D.G., Johnson, G.V., Martin, W.C., and Aldon, E.F. 1979. Germination requirements of 19 species of arid land plants. USDA Forest Service Research Paper RM-210.

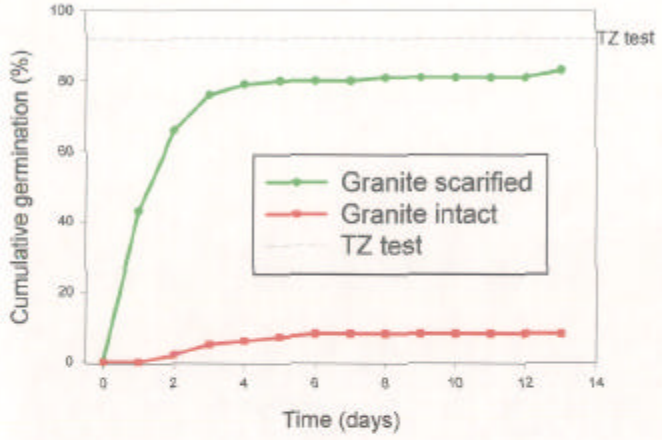
Table 1

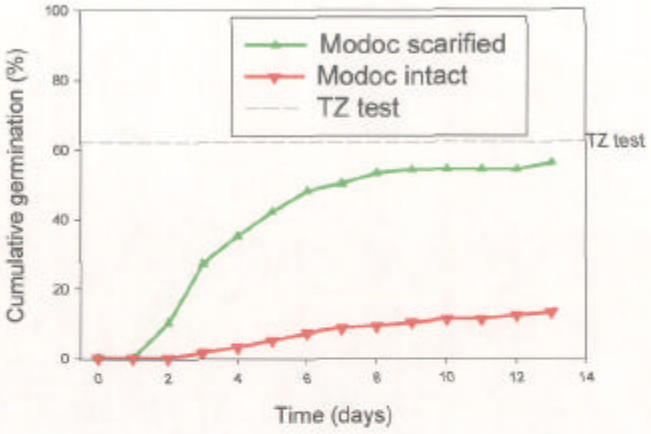
Germination was tested under the following conditions (boldface indicates variables of major interest for that treatment):

Temperature	Day/Night	Seed Lots	Reps	Other
18C/15C	varying time, lab bench	all 3 seed lots	2 reps of 25 seeds each	scarified
25C constant	16 hr day/8 hr night	all 3 seed lots	1 rep of 50 seeds each	**scarified and intact**
28C constant	16 hr day/8 hr night	all 3 seed lots	1 rep of 50 seeds each	**scarified and intact**
28C/18C	**16 hr day/8 hr night**	all 3 seed lots	4 reps of 50 seeds each	**scarified and intact**
28C/18C	16 hr day/8 hr night	Granite and Modoc	4 reps of 50 seeds each	**palea and lemma intact, seed scarified and intact**
30C constant	16 hr day/8 hr night	all 3 seed lots	1 rep of 50 seeds each	**scarified and intact**
40C constant	16 hr day/8 hr night	all 3 seed lots	1 rep of 50 seeds each	**scarified and intact**
40C/10C	**8 hr day/16 hr night**	all 3 seed lots	4 reps of 50 seeds each	**scarified and intact**
28C/18C	**16 hr day/8 hr night**	all 3 seed lots	2 reps of 25 seeds each	scarified, **dark**
28C/18C	16 hr day/8 hr night	Granite only	1 rep of 25 seeds	intact, **bleach 15 min**
28C/18C	16 hr day/8 hr night	Granite only	1 rep of 25 seeds	intact, **bleach 30 min**
28C/18C	16 hr day/8 hr night	Granite only	1 rep of 25 seeds	intact, **H_2SO_4 5 min**
28C/18C	16 hr day/8 hr night	Granite only	1 rep of 25 seeds	intact, **H_2SO_4 10 min**

Table 2
Germination percentages for each treatment, averaged if multiple reps were done:

	Granite: TZ 92%	Modoc: TZ 62%	66: TZ 15%
18C/15C scarified	28	4	0
18C/15C scarified, later moved to 28C/18C	92	54	0
25C constant scarified	78	52	2
25C constant intact	4	2	8
28C/18C scarified	85.5	62.5	1.5
28C/18C intact	3	4.5	16
28C constant scarified	82	68	2
28C constant intact	2	8	6
30C constant scarified	90	70	0
30C constant intact	10	6	16
40C constant scarified	76	40	0
40C constant intact	2	8	0
40C/10C scarified	88	46	0
40C/10C intact	34	60	4
dark 28C/18C	90	46	0
dark 28C/18C, later moved to light	90	46	0
bleach 15 min	4		
bleach 30 min	4		
H_2SO_4 5 min	36		
H_2SO_4 10 min	76		
palea-lemma present, intact	0	2	
palea-lemma present, intact, later seed was cut	58		





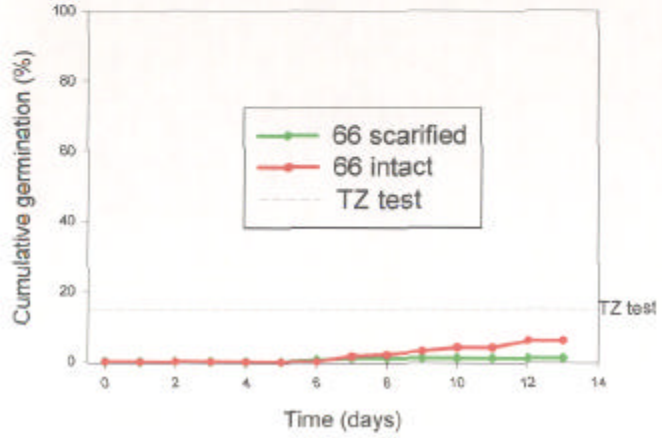


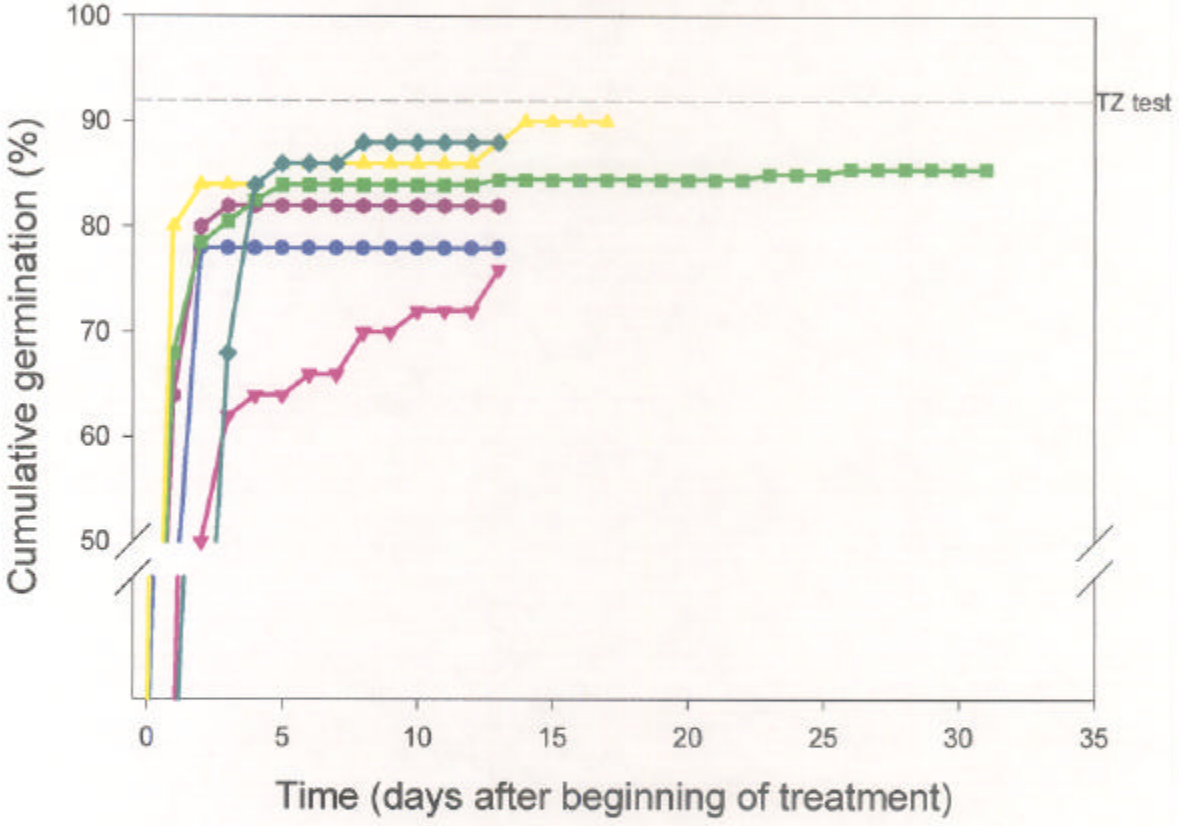
Figure 1

Cumulative percent germination, averaged over all temperatures, of scarified versus intact seeds from three seed lots

Each line is averaged over 25C constant, 28C constant, 28C/18C, 30C constant, 40C constant, and 40C/10C.

Figure 2

Effect of various temperature treatments on germination of scarified Granite seed



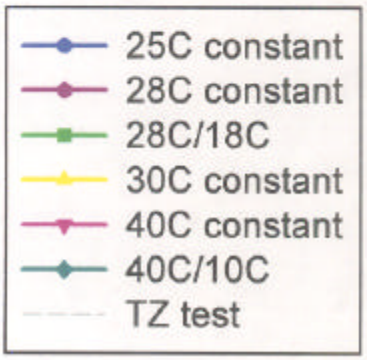
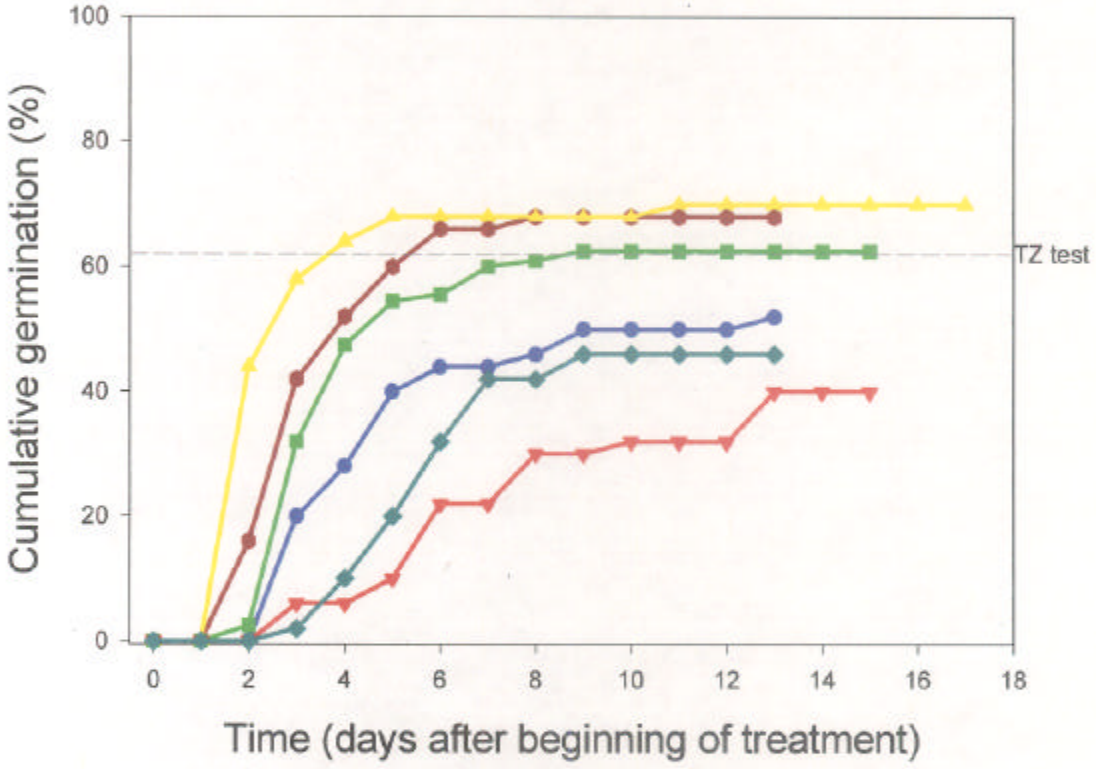


Figure 3

Effect of various temperature treatments on germination of scarified Modoc seed



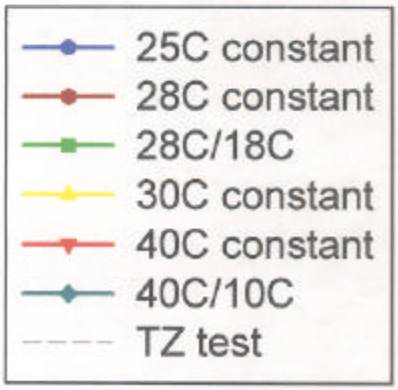


Figure 4

Effect if various temperature treatments on germination of intact 66 seed

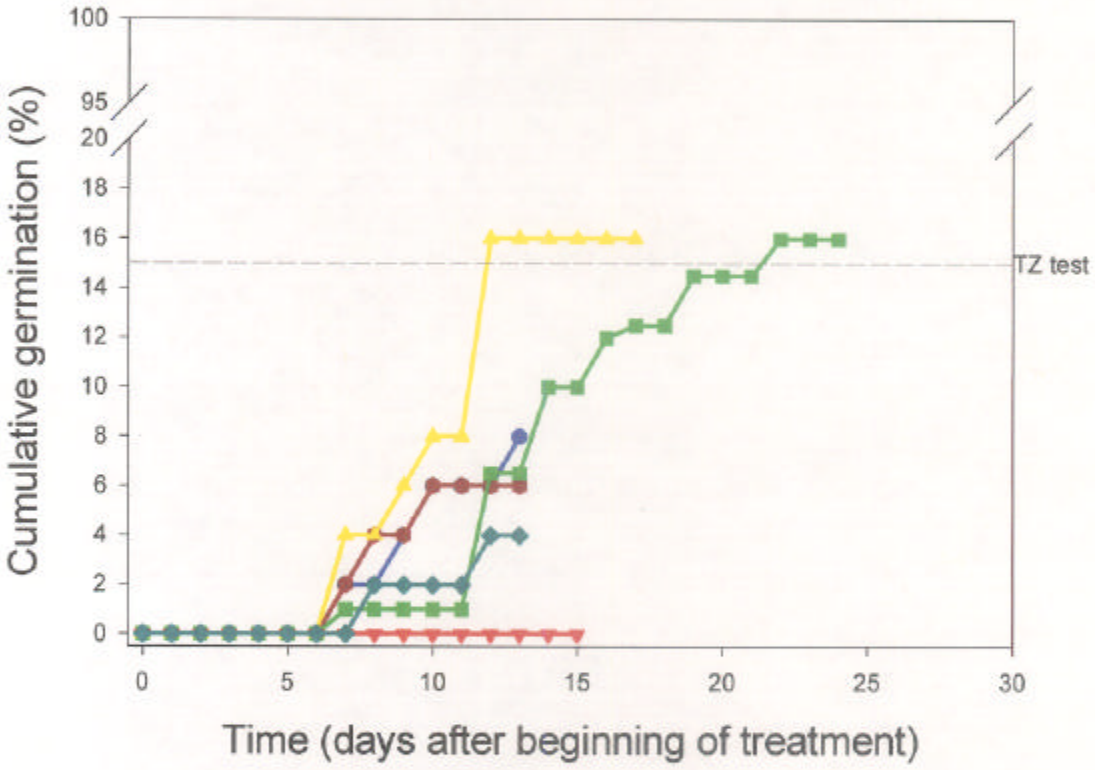
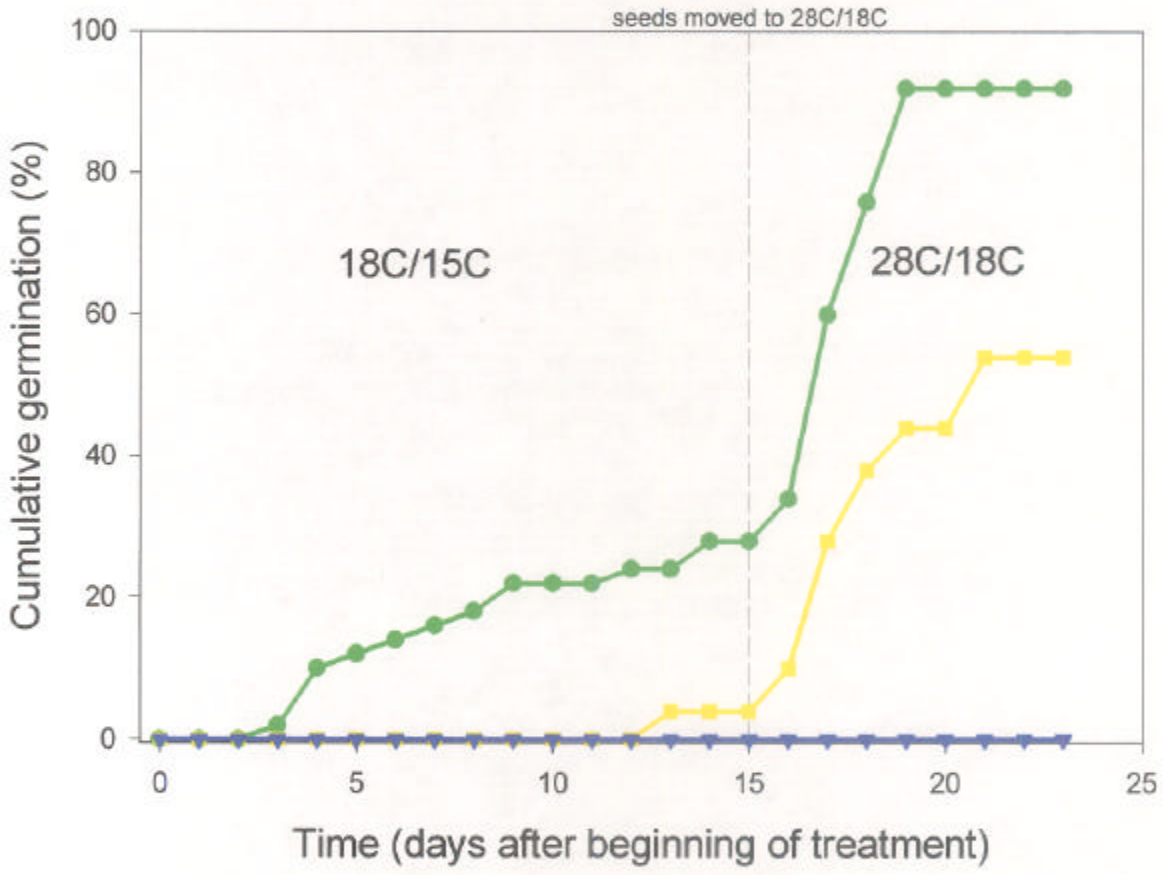


Figure 5

Effect of cool temperature on germination of scarified seeds from three seed lots

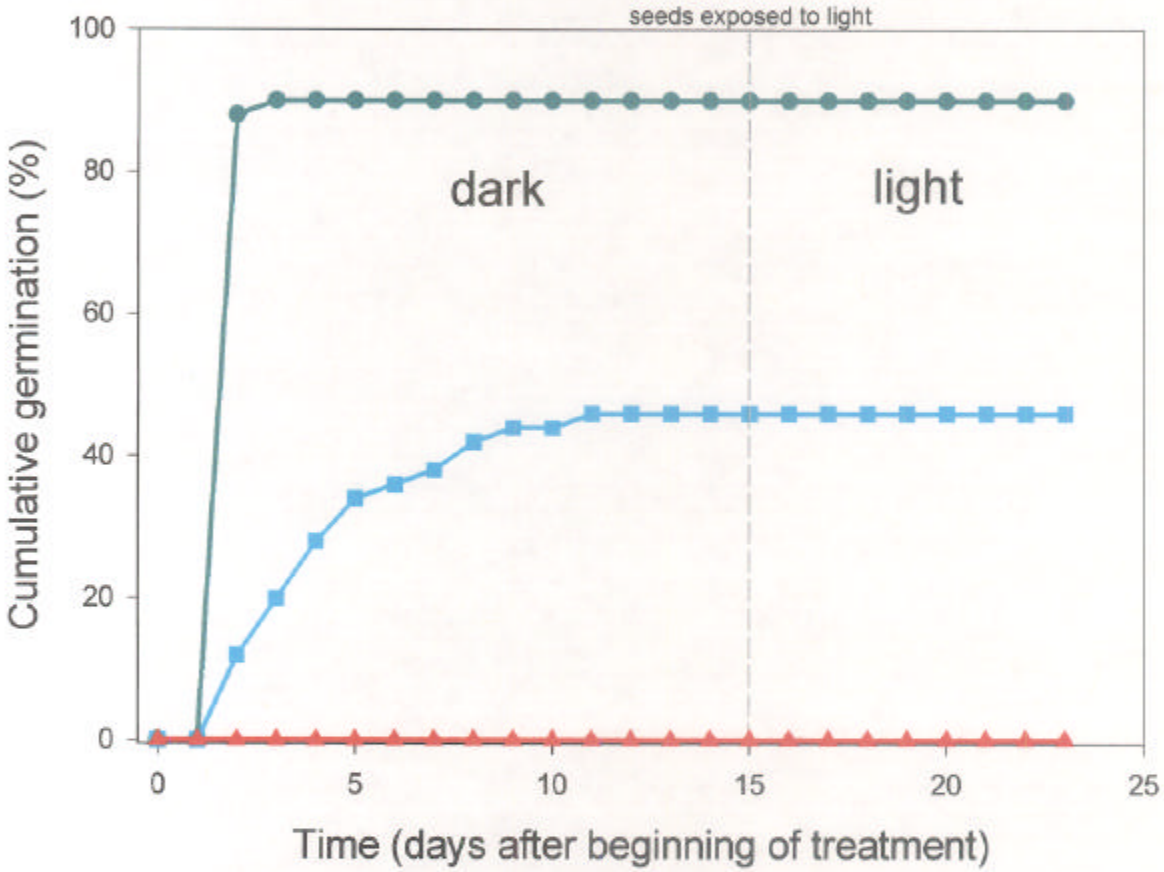


Granite seed lot
Modoc seed lot
66 seed lot
moved to 28C/18C

Granite TZ test = 92
Modoc TZ test = 62
66 TZ test = 15

Figure 6

Effect of light on germination of scarified seeds from three seed lots

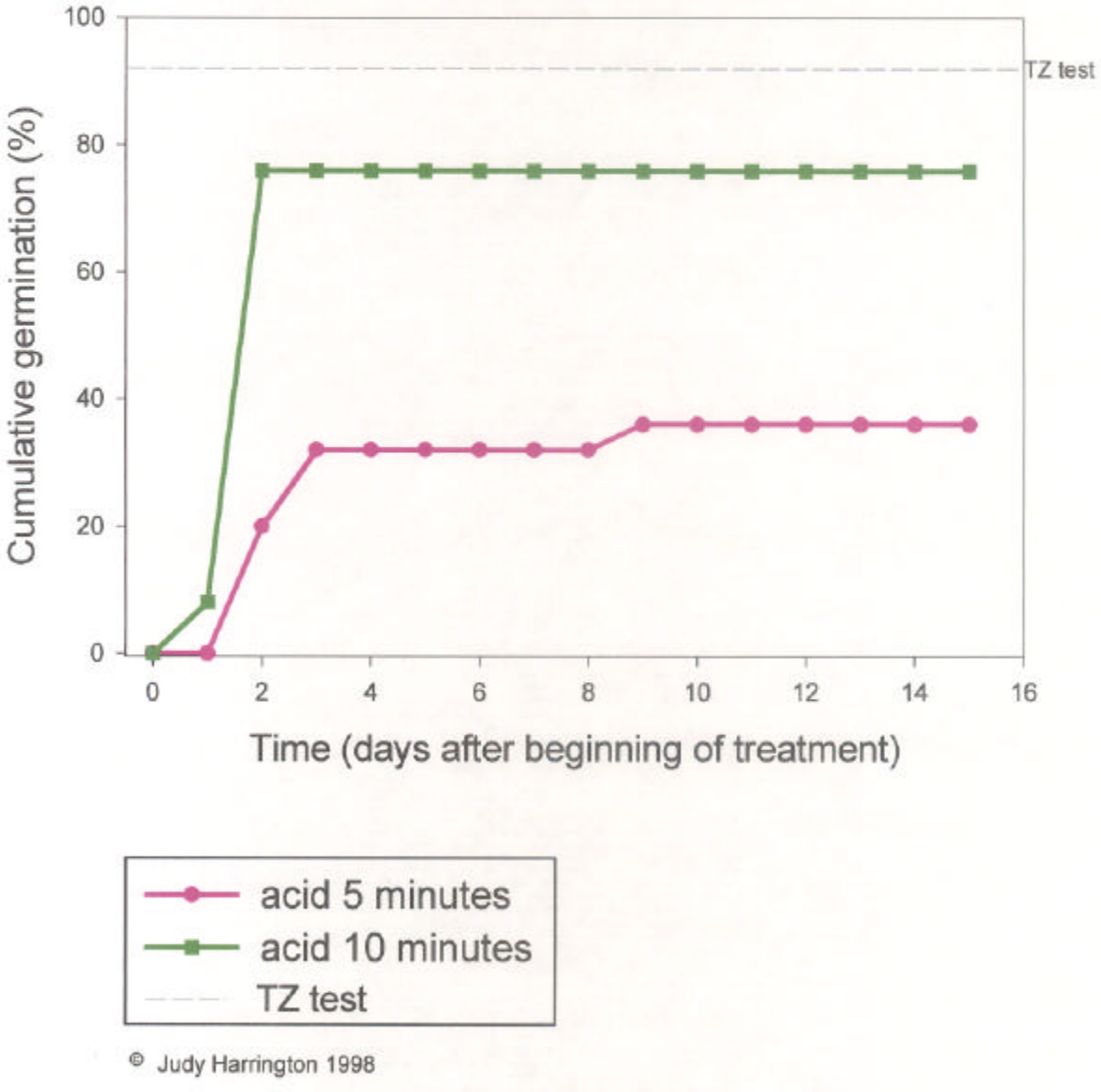


Granite seed lot
Modoc seed lot
66 seed lot
seeds exposed to light

Granite TZ test = 92
Modoc TZ test = 62
66 TZ test = 15

Figure 7

Cumulative percent germination of Granite seed treated with concentrated sulfuric acid



Progress Report

Selection of Turf Type and Seed Production in Inland Saltgrass

The proposal was written with Colorado State U. (CSU) as principal investigator, and U. of Arizona (UA) as secondary investigator (via subcontract). We still have not received any funds from CSU – we are waiting for the Sponsored Projects offices of both universities to finish a cooperative agreement. However, we have gone ahead with the project, and are on schedule. A brief summary of activities is given below.

- Fourteen saltgrass accessions from UA were increased in a greenhouse during the winter of 1997-8.
- Seven accessions from CSU were received during the spring of 1998.
- Sixty three plot container boxes were constructed out of ½" thick plywood during February-March of 1998. The purpose of the boxes is to prevent uncontrolled spread of the invasive rhizomes of the saltgrass accessions. Boxes measure 6 ft. long, 4 ft. wide, and 2 ft. deep (with no tops/bottoms).
- Planting field was leveled, and the 63 boxes buried to a depth of 2 ft, so that their tops were flush with the soil surface. This task was much more difficult than imagined, requiring two months to complete.
- Saltgrass plugs of 21 accessions were planted the first week of July into the 63 plots, comprising three replications in a randomized block design. Plots were 6 ft. x 4 ft., with 10 ft. alleyways.
- Plots are flood irrigated, fertilized, and weeded periodically. All plots are growing nicely, but are not yet filled in. They should be filled in by mid-summer of next year. In the meantime, data will be taken on establishment rates.
- The 21 saltgrass accessions are being increased in a greenhouse for upcoming salinity and drought tolerance screening. Plugs will be planted this spring in the field for drought tolerance screening, to occur during the summers of 1999-2000. The 21 accessions will also be established into greenhouse hydroponics, and screened for salinity tolerance during the summer of 1999.

Chromosome studies in *Distichlis spicata* (L.) Greene var. *spicata* and *D. spicata* (L.) Greene var. *stricta* (Torr.) Beetle

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Purpose

Variations in chromosome numbers from the basic set are common in grasses. Both aneuploidy and euploidy can affect the viability of gametes or the physiological function of plants, resulting in infertile plants, plants with reduced fertility, or plants with reduced vigor. Crosses between fertile plants of different ploidy levels may result in progeny with impaired fertility. The purpose of the cytogenetic studies of saltgrass accessions in our collection (project objective 7) is to gather basic information on ploidy levels and genomic abnormalities that may affect gamete formation and fertility and that will help prevent the selection of inappropriate parental combinations for the breeding program.

Methods

Clones from the breeding project established by Dr. Robin Cuany and additional materials from several collections were selected for study. Root tips for mitotic chromosome studies were pre-treated with colchicine to accumulate metaphase cells. Fixed and acetocarmine-stained root tips were dissected to obtain primarily meristematic tissue, digested in a cellulase-pectinase mixture, and smeared or "splashed" onto slides using standard methods modified in our laboratory to provide acceptable spreading and flattening of the very small *Distichlis* chromosomes. Preparations for observation of meiosis were primarily made from young anthers about 1.0 – 1.5mm in length that were stained in acetocarmine with iron mordant supplied in the initial fixative. Pollen mother cells were dissected and squashed. Observations were made with an Olympus BX-50 microscope with phase contrast optics.

Results

Chromosome counts have been made for 40 clonal accessions using root tip metaphase spreads. Additional mitotic divisions from several accessions have been observed in spreads made from developing tissue of young anthers. In all cases the counts obtained from this tissue agree in number with counts made from root tip cells. Coastal saltgrass accessions from California and Florida both have 40 chromosomes, the number reported by Stebbins and Love (1941) for California coastal saltgrass (figure 1A and figure 1G). Stebbins and Love (1941) also proposed a chromosome number for inland saltgrass of 2n=4x=40 and published a drawing of metaphase inland saltgrass chromosomes that is similar in appearance to our coastal saltgrass chromosome figures. Among the material examined to date, all but one clone from our inland saltgrass collection deviate in number from the reported 2n=40. The single 40 chromosome clone (AZ 11) shows a morphological deviation in two chromosomes compared to the previously published work and either of our coastal saltgrass clones (figure 1B). Given the model of 2n=4x=40 (Stebbins and Love,

1941), the majority of our accessions are aneuploids with only 38 chromosomes. There is no evidence of endomitosis in the cells examined; all unambiguous figures (considered to be unbroken and adequately flattened cells for counting) have the same chromosome number in a minimum of 12 cells to more than 50 cells examined per clone. Since the small size of the chromosomes makes karyotyping difficult with evidence from acetocarmine staining alone, it cannot be determined from these mitotic preparations if the 38-chromosome clones are nullisomic (lacking both chromosomes of a pair present in the normal genome) or double monosomics (lacking one chromosome for each of two different homologues). Observations of chromosome pairing at diakinesis in 38-chromosome clones, however, show 19 bivalents and no unpaired chromosomes (figure 2A and figure 2B). This evidence supports the hypothesis that these plants are nullisomic (2n-2=38).

Although we have found only one ambiguous reference to reports of the 38-chromosome variation in inland saltgrass (Great Plains Flora Association, 1986), it appears in our collection in material from diverse geographic origins. Accessions from California, Nevada and Idaho are 38-chromosome types, as are the majority of the Colorado collection that has been studied. We have additional samples from Utah, eastern Nebraska, and several other locations that will be examined to expand our understanding of the range and distribution of the chromosome variants as material becomes established.

The accession C-12 has 42 chromosomes (figure 1E). Forty of the chromosomes in C-12 are similar to those seen in the 2n=40 coastal saltgrass types, and the additional two chromosomes are similar in appearance to the two small, morphologically different chromosomes observed in our 40-chromosome clone AZ 11. One of the small chromosomes observed in C-12 and AZ 11 also appears in accession AZ 39 (figure 1D), which has a total of 39 chromosomes. The chromosome configurations of these three clones suggest the hypothesis that B chromosomes are present in this species. Further evidence to support the B chromosome hypothesis is seen in observations of meiosis I chromosome behavior in accession C-12. Twenty bivalents and two small, unpaired chromosomes can be seen at diakinesis (figure 2C). At this time, we interpret our observations of several C-12 figures at diakinesis to indicate that the small chromosomes do not associate as bivalents or into multivalent configurations with other chromosome pairs. Twenty bivalents and one visible unpaired chromosome aligned in an equatorial view of the metaphase plate at very early anaphase I can be seen in figure 2D. Figure 2E shows a slightly more polar view of the same stage of meiosis I in C-12 where two small, unpaired chromosomes are clearly visible. The twenty bivalents account for the regular 2n=40 chromosome number and the unpaired small chromosomes, exhibiting classic B chromosome behavior, account for the additional chromosomes seen in the mitotic metaphase preparations. Two lagging chromosomes have been observed in all late anaphase I and telophase I figures of C-12 meiosis that have been considered to be reliable for interpretation (figures 2F-H). Laggards do not appear to be connected to the spindle and seem to remain near the metaphase plate during anaphase I. Within somatic tissue bearing these small chromosomes, however, their regular appearance in all cells indicates that functional centromeres must be present. Elimination of these chromosomes or the random inclusions of one or both into daughter nuclei are possible. Progeny from a cross of the 2n-2=38 female AZ 126 and the 2n=40 + II B male C-12 are currently being grown to provide material to study transmission of the extra chromosomes.

Two accessions have been identified with 74 chromosomes. None of the chromosomes in these accessions appear to be B chromosomes. Reeder (1967) has also

reported two observations of inland saltgrass with about 72 chromosomes. These male clones, C-92 originating from the western slope of Colorado and AZ 103 from the eastern front range of Colorado, are probably the results of restitution events. These polyploids may have irregular gamete formation due to multivalent associations in meiosis that could result in severely unbalanced chromosome complements, but we have not yet made observations of meiosis in these clones. Although these clones may be poor choices for parents in the breeding program, they could be useful for the production of aneuploid lines for genetic studies. We plan to study the meiotic behavior of these clones when adequate material becomes available.

Pollen viability in *D. spicata* var. *stricta*

Purpose

Low seed set reported in some populations of inland saltgrass and the discovery of many accessions in our collection with chromosome complements deficient compared to the previously reported number raise concerns about the quality of gametes being produced. Aneuploidy often has a greater impact on pollen viability than egg viability. While the best test of gamete function is the ability to produce a zygote, estimates of pollen quality are often made from *in vitro* germination tests or examination of pollen germination directly on stigmas. The latter method is also useful for evaluating incompatibility where it exists.

Methods

Pollen germination was tested *in vitro* on a limited scale using four different liquid media (two of which were tested with four different levels of osmoticum) and two agar-solidified media. The liquid media used were sucrose-borate (Singh, 1993), calcium-borate with 10%, 20%, 30% or 40% sucrose (Dafni, 1992), Brewbaker-Kwack medium at the same four sucrose concentrations as the previous medium (Brewbaker and Kwack, 1963), and LMD medium (Leduc et al., 1990). The two agar solidified media were borate- and calcium-enhanced MS medium as described by Fernando et al. (1997) but solidified with 8% agar and an 8% agar-solidified version of the calcium-borate medium with 10% sucrose.

Pollen germination assays on stigmas were made from hand pollinations with the following genotypes:

Har-7-10 pollen on females AZ 40, AZ 31, AZ 24, AZ 126, AZ 12, AZ 76, C-11, and 96-1-1
C-1 pollen on female C-11
'A' pollen on female C-66
C-12 pollen on females AZ 126, AZ 24, AZ 138, AZ 1, C-11, and AZ 40
AZ 37 pollen on female AZ 31
AZ 46 pollen on female AZ 126
AZ 136 pollen on female AZ 126
C-8 pollen on females AZ 31 and AZ 126

Pollen germination was observed at intervals beginning 2 hours after pollination using aniline blue staining of the pollinated stigmas modified from the procedure described by Kho and Baer (1968). Callose fluorescence was visualized upon UV epi-illumination using an

Olympus Vanox microscope equipped with appropriate filters. Some samples were observed using bright field microscopy after toluidine-o staining. An acridine orange staining method using the fluorescence microscope was also tested.

Results

All attempts to germinate saltgrass pollen on artificial media were unsuccessful. Aniline blue staining is the most commonly used method to visualize pollen tube growth through stigmas and styles and it provided good results in our tests. Pollen tubes could easily be visualized growing into the stigmatic papillae and through the style (figure 3A). Pollen tube penetration into the more dense tissue at the base of the pistil around the egg was obscured in most squashes unless the pistil was cut in half before squashing (figure 3B and figure 3C). Pollen from all male genotypes was observed to have good germination and pollen tube development. No attempt was made to calculate germination percentages because of the number of pollen grains producing tubes appeared similar in all assays. Also, pollen was probably lost in differing amounts from stigmas during fixation and staining treatments depending on the maturity of the stigmas. Some pollen would adhere and germinate on immature stigmas, but the pollen tubes did not generally grow well into the styles (figure 3E). On receptive stigmas, pollen tubes extended through the base of the bifurcated styles after 2 hours. Most observations were made on material fixed 24 hours after pollination to allow time for pollen tubes to reach the eggs. We could find no evidence of impaired fertility from these assays.

Acridine orange staining did not provide sufficient differentiation of pollen tubes to be useful as a pollen germination assay (figure 3F). The technique might have some potential if it could be modified to reduce fluorescence of the stigmatic tissue. Toluidine-o staining is a simple technique that will allow observations of germinating pollen grains on the stigmas, but pollen tube growth cannot be followed far into the styles (figure 3G and figure 3H).

Summary

Both aneuploids and euploids are present in our inland saltgrass collection. The majority of our accessions have 38 chromosomes. This chromosome complement appears to have wide geographic distribution among inland saltgrass populations of the western United States. These plants are nullisomic (2n-2=38), but no phenotypic traits have been identified to distinguish these from 2n=40 types. It is possible that the 38-chromosome inland saltgrass represents an evolutionary divergence from 40-chromosome coastal saltgrass. Additional work with more collections is needed to understand the distribution of chromosome types. Gamete formation is expected to be regular in either 2n-2=38 or 2n=40 plants based on observations of chromosome pairing and separation during meiosis I. One or two B chromosomes are believed to be present in several accessions based on evidence from the behavior of the chromosomes in meiosis I as seen in accession C-12. Controlled crosses of a 38-chromosome female with a 38-chromosome male and a 38-chromosome female with the 2n=40 + II B male have both produced germinable seed. Plants from these crosses are currently being established for further study. Seeds from several additional crosses designed to test seed production potential among the 38 chromosome plants have been harvested but

not yet germinated. Seed from approximately 20 additional crosses are maturing in the greenhouse. AZ 24 x Har-7-10 seed harvested as early as 30 days after pollination had 80% germination within 48 hours with no after-ripening period. AZ 126 x C-12 seed harvested 42 days after pollination and after-ripened for 21 days had 100% germination within 48 hours. Both seed lots were germinated according to the protocol developed in our laboratory by J. E. Harrington. Pollen viability studies using pollen from eight male genotypes each assayed on stigmas of up to eight different female genotypes showed satisfactory germination of the pollen grains. Pollen tubes have been observed to grow normally through the styles of each female genotype. There is no indication of incompatibility, heteromorphic pollen types within genotypes, or male genotypes that produce pollen with low levels of fertility. Poor seed set reported for natural populations of inland saltgrass (or domestic populations in crossing blocks) is probably related to pollen availability rather than pollen quality or genetic deficiencies in most cases.

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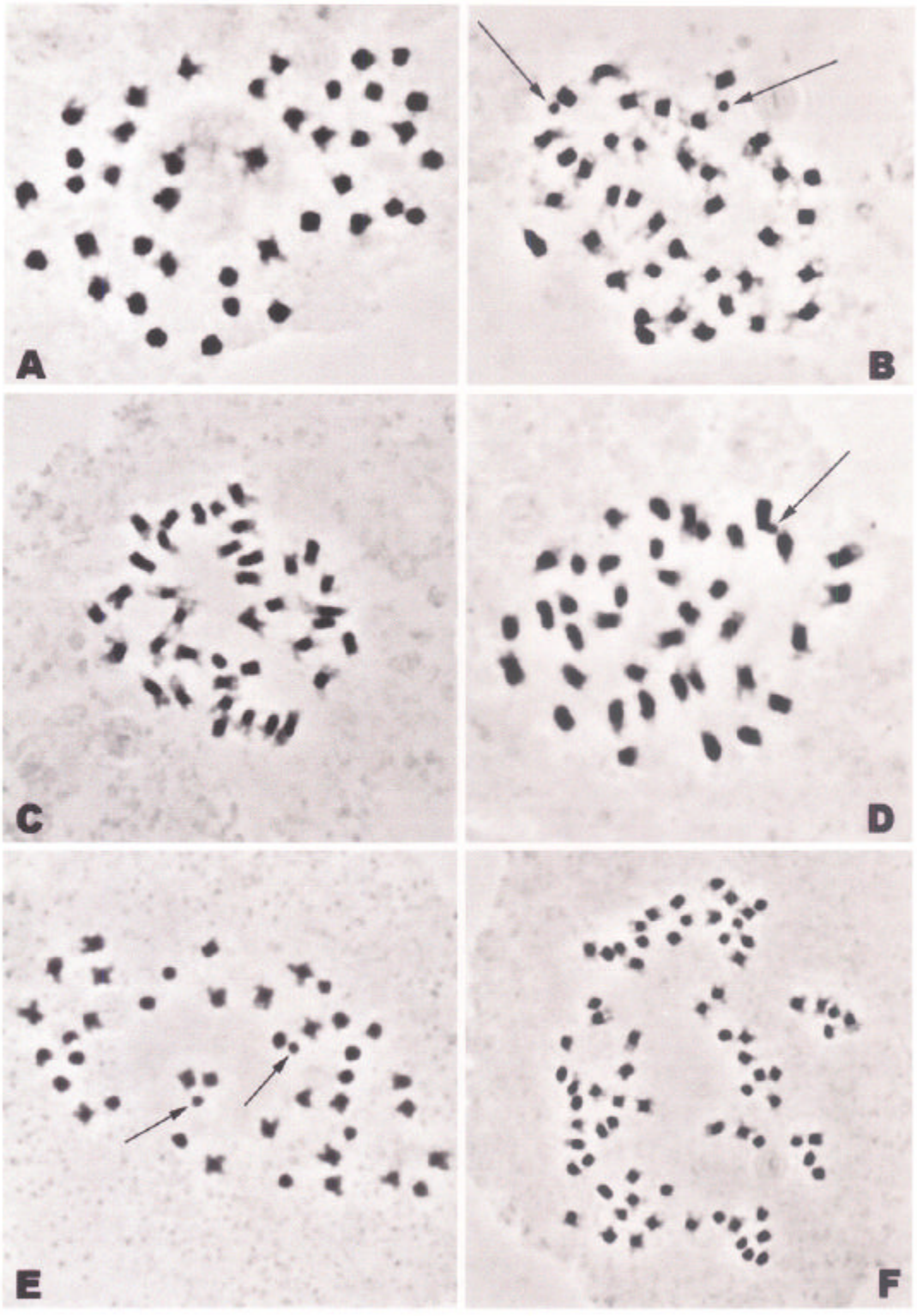

Figure 1A-F.

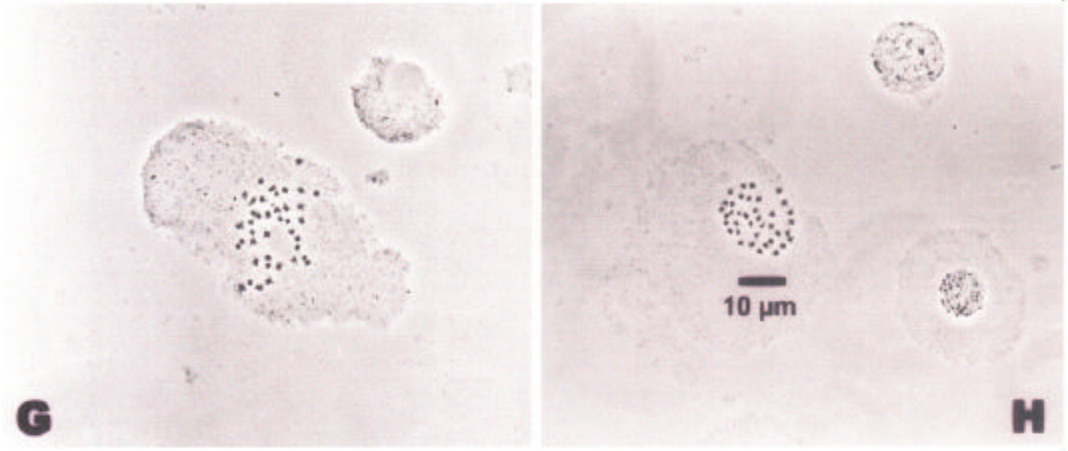

Figure 1 (continued), G-H. Root tip metaphase chromosomes in *D. spicata* v. *spicata* and *D. spicata* v. *stricta*. Fig. 1A is coastal saltgrass from San Francisco Bay, California with 2n=40 chromosomes. This represents the commonly reported chromosome number for the species. Fig. 1B is the inland saltgrass accession AZ 11 with a proposed 2n-2=38 + II B chromosome number. Arrows denote B chromosomes. Fig. 1C is accession AZ 67. Having 2n-2=38 chromosomes, AZ 67 is typical of our inland saltgrass collection. Fig. 1D is AZ 39. This accession has a proposed 2n-2=38 + I B chromosome number. Fig. 1E is a metaphase spread from C-12, an accession collected in western Colorado. This clone is our only identified accession with an A chromosome complement similar to our coastal saltgrass clones but also carries 2 probable B chromosomes (2n=40 + II B). Arrows denote the B chromosomes. Fig. 1F is AZ 103, collected from the eastern front range of Colorado near Longmont. It is one of two polyploids in our collection with 74 chromosomes. Accession C-92, collected from across the continental divide in western Colorado, also has 74 chromosomes. Fig. 1G is coastal saltgrass from Florida having 2n=40 chromosomes. Fig. 1G. *Distichlis* chromosomes are very small. Although morphological differences can be seen in the metaphase or pro-metaphase chromosomes, we cannot yet propose a karyotype. This accession is C-56 with 2n-2=38 chromosomes.

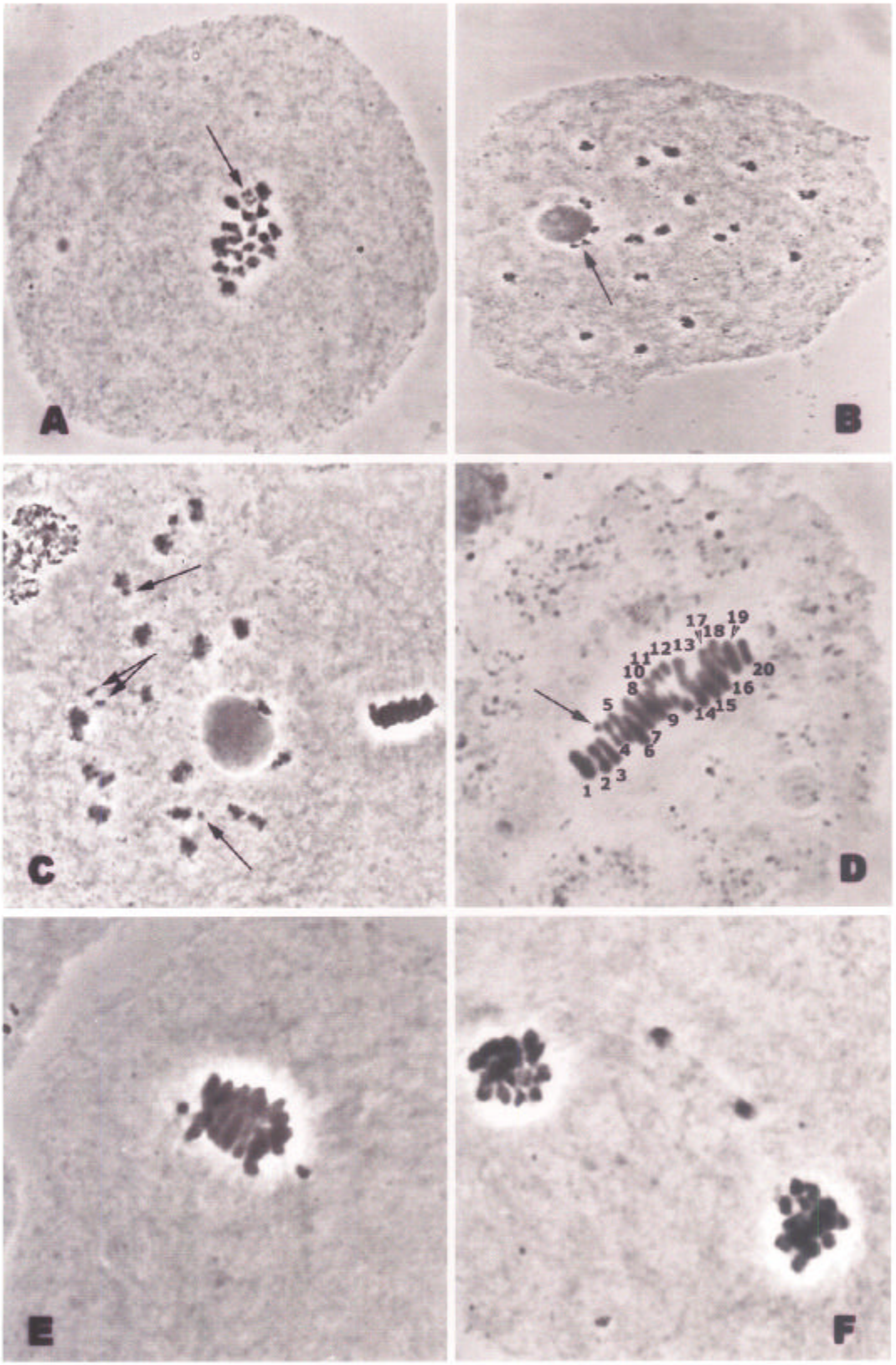

Figure 2A-F.

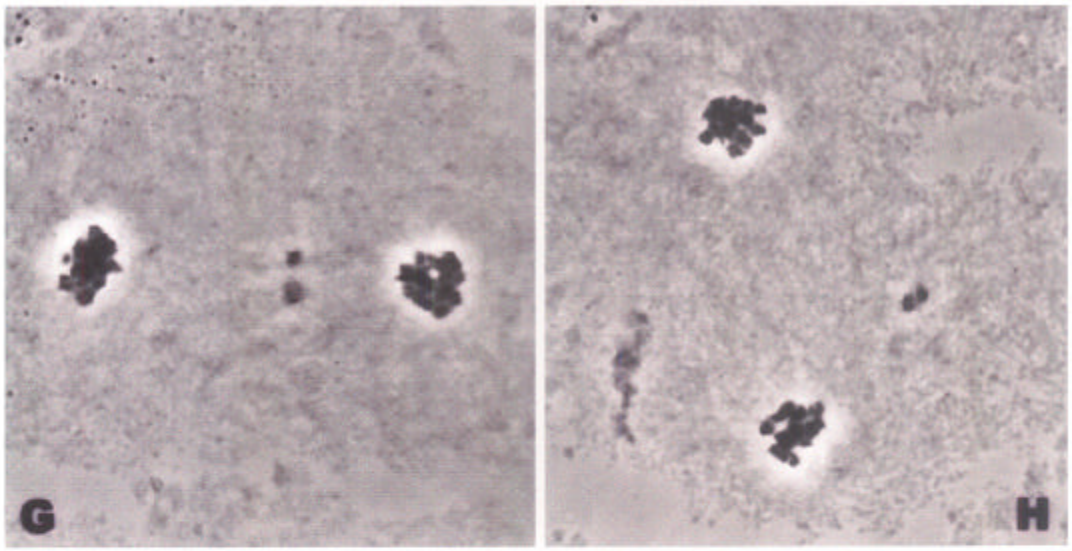

Figure 2 (continued), G-H. Meiosis I in several *D. spicata* v. *stricta* clones. Fig. 2A shows late diakinesis in an IBIS population clone with 38 somatic chromosomes. Fig. 2B is accession C-8 at diakinesis. The presence of 19 bivalents in these and other 38 chromosome types (as seen in root tip cells) suggests that the individuals are nullisomic rather than double monosomics for different homologues. The arrow in each of these figures (and the double arrow in Fig. 2C) points to a bivalent that appears to begin to desynapse early and very noticeably in diakinesis compared to the other bivalents. These chromosomes appear to proceed regularly through meiosis I. Fig. 2C shows accession C-12 at a somewhat earlier stage of diakinesis. Twenty bivalents plus two small, unpaired chromosomes are evident. Fig. 2D is an early anaphase I cell from C-12. Twenty chromosome pairs are visible. The arrow indicates a clearly visible unpaired chromosome proposed to be one of two B chromosomes in this clone. Fig. 2E is again C-12 in early anaphase I showing two unpaired chromosomes on either side of the mass of separating homologues. Figs. 2F-H are three representative views of the two lagging chromosomes present at anaphase I and telophase I in C-12 meiosis in virtually all clear divisions observed. It is not yet known at what frequency laggards are eliminated or included in one or another daughter nucleus.