Evaluation of ten strains of bromegrass prior to their use in a breeding program
by Alexander Haburchak

A THESIS Submitted to the Graduate Division in partial fulfillment the requirements for the degree of Master of Science in Agronomy
Montana State University

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The chromosome number of each of the seven strains counted was estimated to be 2n = 56.

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TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

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It was found that the ten strains used, varied slightly in the number of days between panicle emergence from the boot and anthesis. The ones that exhibited a greater time length between these two periods appeared to have a larger percent seed set under field conditions in 1948.

The photoperiod of 18 hours, which was used in this study, did not produce the large number of panicles as reported by previous workers, however a large amount of vegetative growth was obtained.

A highly significant positive correlation was found between the number of seeds per S_o panicle and the mean height of the seedlings established from the S_o seed. The mean height of the S_1 seedlings and the mean height of the S_o seedlings also were positively correlated.

The chromosome number of each of the seven strains counted was estimated to be $2n = 56$.

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INTRODUCTION

Bromegrass is an important grass crop in Montana and in many areas of the United States. There is a large demand for this grass as a pasture crop and for soil conservation. This crop spreads by rhizomes making it particularly useful in the control of erosion.

The purpose of this study was to obtain all possible data on 10 strains of bromegrass which might be of value in a breeding program. Emphasis was placed upon the self sterility and cross compatability of each of the 10 strains. This was done to determine which strains would be most suitable in developing a synthetic variety. A synthotic variety possessing all or many of the desireable qualities of the selected strains may add additional value to this grass.

A chromosome count was made to see how these strains compared with other known strains in America.

REVIEW OF LITERATURE

Smooth bromegrass, Bromus inermis Leyss, was first introduced into the United States from Europe (7). The first known seed to be imported was from Hungary in 1884. This bromegrass was first known as Hungarian brome and currently consists of a group of strains referred to as the southern strains. Bromegrass proved well adapted in the United States, so in 1896 large amounts were imported. Most of the bromegrass imported at this later date came from Russia, and led to the development in this country of the northern strains.

Newell and Keim (7) found in an experiment performed in Nebraska, that the northern strains of bromegrass are generally shorter in height and less vigorous than the southern strains. When planted in the early spring or late fall under Nebraska conditions, the seedlings of southern brome were more vigorous. Preliminary tests, made by Newell and Keim, indicated that the differences become less pronounced when grown at higher altitudes. The southern strains appeared to be more tolerant to midsummer heat and drought than the northern strains. The southern strains in Nebraska grew vigorously during the shorter day period and flowered by May 18. The northern strains were later getting started in the spring and their flowering was not completed until May 30. The southern strains grow well during the short days of spring and go into a dormant period during the hot dry weather while the northern strains continue their growth. The growth of the northern strains which continues throughout the dry period results in a smaller total amount of forage than does the discontinuous growth of southern strains. Under the conditions of their study, the designations "early" and "late" are suggested for the strains of southern and northern type, respectively.

Bromegrass is a naturally cross pollinated crop. White and McConkey (11) report that self sterility is "fairly marked" in bromegrass however, there is a wide variation between plants. Highly self fertile lines can be selected if desired. Tsiang (10) in a self fertility study of bromegrass reported that selfed lines were not as vigorous as open pollinated lines, but that there were wide variations. The selfed lines, on the average, were smaller in culm diameter and less vigorous in recovery than the open pollinated lines. Although reduction in the average forage yield of the selfed lines was significant, some selfed lines produced more than the open pollinated check.

The flowering of bromegrass is greatly influenced by the photoperiod. Evans and Wilsie (4) found in a photoperiod experiment on northern and southern strains of bromegrass in the greenhouse, that no flowering occurred in either strain with a light period of 10 hours. At a photoperiod of 15 hours the southern strains flowered while only some of the northern strains flowered. At a photoperiod of 18 hours all of the northern strains produced panicles and the southern strains at an 18 hour photoperiod produced more panicles per plant than they did at 15 hours. The average number of seeds per panicle varied from .92 for the northern strains to 1.48 for the southern strains. Gall (5) reported that longer photoperiods under some conditions gave better panicle growth in the greenhouse. In general, panicle production was considerably greater in the southern strains than in the northern strains. Panicle emergence was seven days earlier in southern strains when kept at a temperature of 75°F and a greater number of panicles were produced than at a temperature of 65°F. On cloudy days when the temperature was kept at 60°F, little or no anthesis occurred. Only 80% of the southern strains flowered when kept at a continuous temperature of 60°F.

In a bromegrass study at Illinois, Gall (5) reported that floral primordia of plants in the field were formed by early April. This indicates that a long photoperiod is not necessary for floral initiation. This observation was made in the field during an unusually cold spring so the plants were not growing normally. Due to the unfavorable environment, the floral primordia may have been later than is normally expected. It was also observed that anthesis followed about 14 days after the first inflorescence appeared. When bringing bromegrass into the greenhouse, prechilling was not found necessary for floral initiation.

Elliot and Love (3) have suggested that meiotic irregularities in clones of bromegrass introduce further limitations in obtaining desireable gene combinations in inbred or crossbred progenies.

Chromosome numbers of bromegrass have been reported as 2n = 42, 56 and 70. Nielsen found a chromosome number of 70 however, this was found only in one strain. Hill and Myers (6) found that in 193 strains examined all had 56 or approximately 56 chromosomes. Due to the length and great number of chromosomes in bromegrass it is difficult to get accurate counts. Stählin (9) reported that bromegrass had 42 chromosomes Hill and Myers (6) found no strain with a chromosome count of 42 and they believe that lines having this chromosome number are rare in the United States. They also suggest Nielsen's 2n = 70 was a 56 chromosome strain plus accessory chromosomes.

MATERIALS AND METHODS

Ten plants, representing 10 strains, of smooth bromegrass, <u>Bromus inermis</u> Leyss, were brought into the greenhouse October 28, 1948. These plants were then separated into seven to nine clones, depending upon the size of the plant. Two clones of each plant were transplanted into two gallon stone crocks. The remaining clones were transplanted into eight inch flower pots. The clones were transplanted into several pots so that upon flowering they could be moved about and crosses made in all possible combinations.

The bromegrass used in this experiment was of southern origin and desireable strains possessing the ability to grow at high altitudes had been selected at Havre, Montana. The selections were reestablished at Bozeman, Montana in 1947. The origin and desirable reasons for which these selections were made at Havre are shown in Table I.

Five panicles of S_1 (selfed) seed and five panicles of S_0 (open pollinated) seed were gathered from single plants of each strain during the summer of 1948. This seed was then germinated on blotter paper to determine the amount of viable seed produced under either self pollination or open pollination. Upon germination the seedlings were paired and planted into flats so a comparison

Table I. Original source of the material used and the reasons for which they were selected.

Original Number	Present Number	Original Source	Reason for Selecting
1-48	1-2-1	Lincoln brome known as Day strain came from 3 year old stands at Havre, June 11, 1947.	Started growth with an 80% stand in 1945 and in 1947 had a 72% stand. Had Uniform Clone.
2-48	1-2-1	"	"
3-48	2-7-1	"	"
40-48	3-23-1	B. in. 7. Havre received selection from New York. This selection was made June 16, 1947 at Havre, Montana.	Started with 77% stand in 1948 and had a 62% stand in 1947. Very good seed producer.
50-48	4-25-3	"	"
60-48	4-30-1	"	Started with 77% stand in 1948 and had a 62% stand in 1947. Good seed producer.
70-48	5-33-1	"	"
800-48	6-39-1	B. in. 9. Nebraska selection was selected at Havre, June 11, 1947.	Started with an 80% stand in 1945 and had a 82% stand in 1947. Good seed producer.
900-48	7-49-1	B. in. (Kans) Selected at Havre, June 11, 1947.	Started with a 63% stand in 1945 and had a 53% stand in 1947. Medium seed producer.
100-48	8-52-3	"	Started with a 63% stand in 1945 and had a 53% stand in 1947. Good seed producer.

of vigor between S_o and S_1 seedlings within each strain could be made. Six weeks after transplanting, the vigor was estimated by measuring the height of the S_1 and S_o seedlings.

Root tips, for chromosome counts, were obtained from the S_1 seedlings. The root tips were gathered at different times of day to determine when cell division was at its maximum. It was found that from 10:00 A. M. to 12:00 A. M. the largest number of cells could be observed dividing. Immediately after collecting the root tips, they were immersed in paradichlorobenzine for six hours, then placed into carnoy fluid (6-3-1) for 30 hours. Aceto-carmine root tip smears proved more successful than aceto-orcein smears. The chromosomes were easier to distinguish and remained stained for a longer period of time when aceto-carmine was used.

The greenhouse temperature was kept as near 75°F as possible and the relative humidity was maintained at approximately 60%. The photoperiod, from the time the clonal segments were made until the completion of the experiment, was 18 hours. The 18 hour day length was obtained by placing 200 watt bulbs at three foot intervals above the plants. The plants were sprayed weekly with "vaportone" to control insects.

Notes were taken on the date of panicle emergence from the boot. The date of anthesis was also recorded to determine whether there was any variation between strains as to the number of days between emergence from the boot and anthesis.

The crosses were made by tying the two panicles that were to be crossed together and placing a number four kraft bag over them. This was done just prior to anthesis with plants that were observed to be at the same stage of maturity. The bags were tapped three to four times daily to encourage cross pollination.

EXPERIMENTAL RESULTS

The seed from five S_1 panicles and five S_c panicles from each strain obtained from the field in 1948 was germinated. The percentage of viable seed per selfed panicle was determined for those strains where it was possible to accurately count the seeds prior to germination. The percentage of viable open pollinated seed was calculated for all strains. These results are shown in Tables II and III. Under controlled cross pollination in the greenhouse the number of viable seeds produced are shown in Table IV. This number was obtained by counting the number of seedlings 15 days after being placed in germinators. Under selfed conditions in the greenhouse no viable seed was produced.

Table II. Viable seed produced under self pollination in the field at Bozeman in 1948.

Strain	Number of seeds	Number germinated	Mean per panicle	% germinated
1-1-2	54	9	1.8	16.66
1-2-1		25	5	
2-7-1		97	19.4	
3-23-1		8	1.6	
4-25-3	195	55	11	28.20
4-30-1		14	2.8	
5-33-1	420	240	48	57.14
6-39-1		15	3.75	
7-49-1	410	274	55	66.82
8-52-3		13	2.6	

Table III. Viable seed produced per panicle under open pollination in the field at Bozeman in 1948.

Strain	Seed per Panicle 1	2	3	4	5	Mean	% Germinated
1-1-2	94	116	108	102	144	113	97.53
1-2-1	104	88	71	104	84	92	97.87
2-7-1	232	202	208	138	120	183	85.84
3-23-1	40	33	39	66	53	46	88.84
4-25-3	231	156	30	52	131	120	89.56
4-30-1	90	50	91	138	94	84	83.46
5-33-1	126	90	67	189	66	108	87.62
6-39-1	25	45	66	30	42	42	90.82
7-49-1	72	73	197	76	90	103	92.87
8-52-3	61	62	49	30	49	50	86.55
Least significant difference at the 5% point for strains =						54.2	

Table IV. Number of panicles crossed and the number of viable seeds produced under controlled cross pollination in the greenhouse.

Strain	Number of panicles crossed	Number of viable seed	Mean viable seed per panicle
1-1-2	14	9	1.6
1-2-1	0	0	0
2-7-1	4	6	0.66
3-23-1	0	0	0
4-25-3	4	15	0.26
4-30-1	4	12	0.33
5-33-1	1	3	0.33
6-39-1	3	0	0
7-49-1	6	0	0
8-52-3	3	0	0

The average height of the S_o seedlings and S_1 seedlings produced by the ten strains is presented in Table V. The seedlings resulting from the S_o seed were consistently more vigorous than the seedlings produced by the S_1 seed. There was a significant difference in seedling vigor between methods as is indicated by the high F value in Table VI.

Table V. Mean germination per strain of S_1 and S_o seeds produced under field conditions and the mean height of the S_1 and S_o seedlings grown in the greenhouse at Bozeman in 1948.

Strain	$\bar{X}$ number of seeds per panicle that germinated Selfed	Open	Vigor measured by $\bar{X}$ height of seedlings in inches at six weeks S_1	S_o	Average height of S_1 and S_o seedlings in inches
1-1-2	2	119	5.6	6.7	6.15
1-2-1	5	92	5.0	6.2	5.6
2-7-1	19	183	6.5	6.6	6.55
3-23-1	1.6	46	5.0	5.4	5.2
4-25-3	11	120	5.98	6.56	6.27
4-30-1	3	84	4.6	5.6	5.1
5-33-1	48	108	5.8	6.7	6.25
6-39-1	39	42	5.8	6.2	6.0
7-49-1	55	103	5.8	6.0	5.9
8-52-3	2.6	50	6.1	7.1	6.6
Average	18.62	94.7	5.6	6.3	5.96

Least significant difference at 5% point for Average vigor between strains = 2.92
Average vigor between methods of pollination = 1.31

Table VI. An analysis of variance of the height of S_1 seedlings and the S_o seedlings grown in the greenhouse from seed obtained in the field at Bozeman in 1948.

Variation due to:	Degrees of freedom	Sum of Squares	Mean Square	F value
Between lines	9	65.502	7.278	4.355*
Method of pollination	1	39.970	39.970	23.919**
Error	9	15.040	1.671	
Total	19	120.512		

Correlation coefficients were calculated in all possible combinations between the observations are shown in Table VII.

Table VII. Correlations coefficient of the factors presented in Table V in all combinations.

Characters Correlated	Correlation coefficient
Mean number of seeds per selfed panicle x the mean number of seeds per S_o panicle	.0999
Mean number of seeds per selfed panicle x the mean height of S_1 seedlings	.1787
Mean number of seeds per selfed panicle x the mean height of S_o seedlings	.249
Mean number of seeds per open pollinated panicle x the mean height of S_1 seedlings	.2022
Mean number of seeds per open pollinated panicle x the mean height of the S_o seedlings	.6395**
Mean height of the S_1 seedling x the mean height of the S_o seedlings	.4959*

Only two of the six correlations computed were significant. There was an association between the number of seeds per S_o panicle and the mean height of the S_o seedling as is indicated by the highly significant positive correlation of 0.6395. This indicates that the mean height of the S_0 plants and the number of seeds produced per open pollinated panicle are associated. This may indicate that strains producing vigorous seedling growth may as mature plants produce more open pollinated seed. In a comparison of Table III and Table V strains 1-1-2, 2-7-1, 4-25-3 and 5-33-1 produced a large quantity of S_0 seed and also produced the most vigorous seedlings. It appears that the most vigorous seedling grows to be the more vigorous mature plant. In strain 8-52-3, which had the highest seedling height yet produced few S_0 seeds, the number of S_0 seedlings observed was relatively small. If a larger population had been observed the estimate of seedling vigor might have been reduced. The correlation coefficient between the mean height of the S_1 seedlings and the mean height of the S_0 seedlings was significant at the 5% point. This indicates that some of the seedling vigor of specific strains is transmitted to the S_1 progeny. Although it was hoped to make crosses of the 10 strains in all possible combinations, only 19 combinations were made due to the lack of flowers. At least one panicle from each strain was selfed to get an estimate of the degree of self sterility in the greenhouse.

The number of days from emergence of the panicle from the boot until anthesis varied from 10 to 19 days. The two extremes were the result of single observations on two of the ten strains. A comparison of the number of seeds produced under field and greenhouse conditions is presented in Table VIII.

Table VIII. The number of viable seeds obtained under field and greenhouse conditions at Bozeman in 1948, and the number of days in the greenhouse between emergence from the boot and anthesis.

Strain	Mean viable seed per S_0 panicle in the field	Mean viable seed per S_1 panicle in the field	Number of days from boot until anthesis in greenhouse		Number of viable seed produced under controlled crosses in greenhouse			Number of selfed seeds produced in greenhouse	
			No. obs.	$\bar{x}$ days	No. crosses	$\bar{x}$ Fe.	$\bar{x}$ M.	No. obs.	No. Seed
1-1-2	119	2	4	14.25	11	1.3	1.3	1	0
1-2-1	92	5	4	15.5	1	0.0	0.0	1	0
2-7-1	183	19	13	16.69	4	1.5	1.75	2	0
3-23-1	46	1.6	1	10.0	1	0.0	0.0	1	0
4-25-3	120	11	6	14.16	4	3.75	2.75	1	0
4-30-1	84	3	4	12.50	4	3.0	1.25	1	0
5-33-1	108	48	1	19.0	1	3.0	0.0	1	0
6-39-1	42	39	4	12.50	3	0.0	0.0	1	0
7-49-1	103		7	15.57	6	0.0	1.5	2	0
8-52-3	50	2.6	2	13.50	3	0.0	0.0	1	0

DISCUSSION

Bromegrass is a naturally cross pollinated plant. It has been observed by other investigators that when naturally cross pollinated plants are selfed, the seedlings produced from the S_1 seed are usually less vigorous than S_o seedlings. The results from the experiment performed indicate that the S_o seedlings were more vigorous than the S_1, however the difference was significant in only two strains, 1-2-1 and 5-33-1.

In testing for self sterility of the bromegrass in the field, five panicles from each strain were placed under a bag prior to anthesis. This was done in an attempt to find those strains which were quite self sterile. Five open pollinated panicles were harvested from each strain to see how much seed they would produce under natural open pollination. It was hoped to find self sterile strains that would produce an abundance of seed when crossed with other self sterile plants. After determining the self sterility of the strains it was planned to make crosses in all possible combinations, but due to the lack of flowers only 19 combinations were made, so all the desired information was not obtained.

From the ten strains observed under field conditions, seven were quite self sterile. The panicles that were selfed in the greenhouse produced no viable seed. Strains

3-23-1, 6-39-1, and 8-52-3 produced little seed under open pollination. It can be assumed that these strains would be of little value in a synthetic variety composed of more than three strains as they would not make a proportionate contribution of their desireable genes to the total gene population. Strains 5-33-1, 6-39-1, and 7-49-1 were quite self fertile. These strains also may not be of much value in the development of a synthetic variety because a large percentage of the seed produced may be the result of selfing. A synthetic variety, when being developed, should be made up of strains that are cross compatabile and highly self sterile so that all the desireable genes occur with equal frequency thus giving rise to a superior population of plants.

The mean number of seeds per S_1 panicle and the mean number of seeds per S_o panicle were not correlated. From the data it appears that selfing of the open pollinated parental clones immediately reduced the amount of seed set, but the degree of reduction was not associated with the amount of open pollinated seed produced. The mean number of seeds per S_1 panicle and the mean height of S_1 seedlings were not positively correlated. The lack of association between these two factors suggests that plant vigor, as measured by seedling growth, is a minor factor in the production of self pollinated seed. The mean heights of the S_1 and S_0 seedlings were correlated. The mean number of seeds per selfed panicle and the mean height of the S_0 seedlings were not positively correlated. This further supports the suggestion that strain vigor is not a major factor in the amount of selfed seed produced. Apparently the sterility mechanisms mask the influence of plant vigor upon the amount of selfed seed set. The amount of open pollinated seed produced by a specific strain however, tends to be strongly influenced by plant vigor as is indicated by the highly positive correlation between the amount of open pollinated seed per panicle and the mean height of the S_0 seedling.

The growing of plants in the greenhouse often brings about a problem in temperature and light control. The optimum temperature for panicle production is believed to be 75°F for southern strains of bromegrass. The temperature was kept as near 75°F as possible, but during some parts of the day when the sun was shining the temperature rose to a higher degree.

Gall in his study of bromegrass at Illinois had very good panicle growth, but seed was not produced. The temperature may have been the cause of no seed set. It is assumed that the occassional high temperatures caused by heat from the sun may have been the reason for the poor seed set obtained in this experiment. The placing of the kraft bag over the panicles also appears to keep the panicle above room temperature. This may have contributed to the poor seed set obtained in the greenhouse. The plants in the greenhouse displayed weak panicle growth however, an abundant vegetative growth occurred.

Observations were made in the greenhouse on the number of days between panicle emergence from the boot and anthesis. It was noted that the longer the period from emergence from the boot until anthesis, the larger was the amount of seed which was produced in the field in 1948.

Chromosome counts were made in seven of the ten lines studied. Accurate counts were difficult to obtain as all the chromosomes could not be observed at a single focal plane. It was also difficult to find the somatic cells in the prophase stage of mitosis, which is the most desirable stage for making accurate chromosome counts. From the counts that were made it is believed that these strains are octaploid or 2n = 56. The basic chromosome number of bromegrass is seven and most of the strains had a mean of more than 49.0. It is believed that some chromosomes may have been missed rather than having been counted twice. If the bromegrass strains studied had been hexaploid or 2n = 42, there would probably have been some counts of less than 42. The additional chromosomes that would be needed to make a total of 56 are believed to have been partially or completely masked by the other chromosomes.

In the time allotted to this study, it was impossible to collect all the data desired. If it were carried further the crosses in all possible combinations would probably be more successful and complete if carried out in the field during the summer months. By observing the results for an additional year it could be determined whether seedling vigor would result in a more vigorous mature plant as was assumed. A more accurate chromosome count could be obtained from the pollen mother cells rather than root tips.

SUMMARY

1. The seedlings resulting from S_o seed of Bromus inermis Leyss were consistently more vigorous than the seedlings produced by S_1 seed.
2. S_o clones were significantly more vigorous than S_1 clones in strains 1-2-1 and 5-33-1.
3. There was a significant positive correlation between the mean height of the S_1 seedlings and the mean height of the S_o seedlings.
4. There was a highly significant positive correlation between the number of seeds per S_o panicle and the mean height of the S_o seedlings.
5. Temperatures higher than the optimum required by bromegrass are believed to have been the cause of poor seed set in the greenhouse.
6. The number of days between emergence of the panicle from the boot and anthesis is approximately two weeks in the greenhouse.
7. The strains of bromegrass used in this experiment are believed to have a chromosome number of 2n = 56.

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