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An Evaluation of Selfing Techniques for *Agropyron elongatum*

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AN EVALUATION OF SELFING TECHNIQUES FOR
AGROPYRON ELONGATUM

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INTRODUCTION

Tall wheatgrass *Agropyron elongatum* (Host) Beauv. has gained in importance since its introduction into the United States in 1909 from its native habitat on saline meadows and seashores of Southern Europe and Asia Minor. This very late-maturing, coarse, nonlodging, 2 1/2 to 6 foot tall bunchgrass was discarded in Utah in 1919 for being too aggressive. It is now under production in the intermountain and other regions because of its cold and drought tolerance as well as for its salt tolerance and its ability to make excellent fall and spring recovery. According to Weintraub (1953) this salt-tolerant plant gives high yields of forage on sub-irrigated alkaline soils.

Because of its ability to produce forage on wet alkaline soils, this normally cross-pollinated wheatgrass is being studied with hopes of improving forage type. Self-pollination, a method often used for plant improvement of variable practicable application for high polyploid grass species, is to be studied for tall wheatgrass. Little is known about the ability of this grass to produce desirable results under self-pollinated conditions. The purpose of the experiment designed and described in this thesis was to find information leading to the solution of the problem of low self-fertility for this species of grass, in hopes that it would contribute to salted-land forage improvement.

REVIEW OF LITERATURE

Many researchers concerned with the problem of forage grass improvement have contributed information about self-fertility in relation to forage grass breeding.

Material from which selfing bags are made was thought by Clarke (1927) and Beddows and Davis (1938) to influence the amount of self-pollinated seed set by normally cross-pollinated grass species. A recent publication of Hanson and Carnahan (1956) maintained that the type of isolator used depended upon the amount of self-pollinated seed desired and the number of inflorescences to be enclosed in the isolator. Vegetable parchment bags and sleeves are believed to be the most satisfactory isolator, though kraft paper is used by some forage research workers. Weather conditions, the species and amount of seed desired will be the determining factors between the use of sleeves or bags.

Two sheets of vegetable parchment paper 45 x 35 cm. were rolled together by Valle (1933) in such a fashion that a 3-fold isolating wall was produced without any pasting. The bag produced was called a Tammisto bag, which Valle thought was superior to Swedish or Knoll panicle bags. Swedish bags were made by gumming 2 sheets of pergamin (glassine) paper together. Knoll panicle bags were 1 thickness of vegetable parchment paper sewed together, after which the seam was waxed. Seed set of _Bromus arvensis_ was greater under the Tammisto bag, but _Festuca rubra_ and _Phleum pratense_ set less seed than when isolated under the other bags.

In the testing of seed set of Bromus inermis under various types of bagging materials, Keller (1945) found 35-pound vegetable parchment paper bags, 3 x 36 inches, superior to 43-pound parchment paper, brown kraft, and 50-pound bleached kraft paper. Bleached kraft paper was inferior only to 35-pound parchment paper. Heinrichs (1953) working with Agropyron intermedium secured 50 times the amount of self-pollinated seed in the field under vegetable parchment paper bags 3 1/2 x 12 inches than he did under glassine bags in the greenhouse. Cross-pollinating the same plants under identical conditions gave little difference in the amount of seed set. Hays and Schmidt (1943) used vegetable parchment paper bags 18 x 3 inches in selfing studies for 3 species of grass. As a controller of pollen, vegetable parchment paper was found to be superior to kraft and glassine bags by Smith (1944), though advantages were small and not consistent. Jenkin (1937) favored vegetable parchment paper bags over cotton fabrics. He concluded that even the best cotton fabrics were not entirely pollen-proof. Work of Wilsie, Ching and Hawk (1952) showed that 7 x 18 inch bags made from high grade, closely woven sheeting were as effective as vegetable parchment bags or sleeves when used as isolating materials for Bromus inermis.

Cloth bags of various types and structures as well as other selfing materials have been used with varying degrees of success by Malte (1921), Kirk (1927), Knowles and Horner (1943), and Wilsie, Ching and Hawk (1952). These investigators used 3 x 6 foot cages covered with cloth having 48 threads per square inch, or cages of similar construction. Kraft bags 4 x 10 inches were used for self-pollination of Agropyron cristatum, but proved inferior to cotton cages. Domingo

(1941) and Clark (1944) used 3 x 26 inch kraft paper bags in their work. Hays and Barker (1922) suggested covering glassine bags with manilla-paper bags as a means of protecting glassine bags against wind and rain. Malte (1921) used oiled paper isolators, but noticed reduced viability of selfed seed. Fruwirth (1916) used oil cloth protectors, while Waller and Thatcher (1917) used wax capsules. According to Hanson and Carnahan (1956), Stapledon suggested the use of small greenhouses as a substitute for bagging when undertaking the crossing of large numbers of plants.

Seed set of Dactylis glomerata when selfed under bag ranged from 1 to 17 percent. A slight increase was made over this amount when the whole plant was cage-isolated. The amount of seed set increased from 7 to 50 percent when plants were open-pollinated, as found by Wolfe and Kipps (1925). According to Smith (1944), bagging had a detrimental influence upon the amount of self-pollinated seed set. This conclusion was made from his finding that many highly self-fertile plants set less seed under the bag than they did when cross-pollinated. Myers (1942-A) thought the variation in amount of seed received to be caused by the number of inflorescences enclosed in a bag. He found that 1 to 4 panicles of Dactylis glomerata were not significantly different in the amount of selfed seed produced, while 8 panicles enclosed in a bag reduced seed set. One to several inflorescences enclosed per bag gave little difference in the amount of seed produced in Smith's work. Nilsson (1934), reporting Sylvin, claims that several inflorescences are necessary for most effective seed set. Keller's work indicates that 1 panicle of Bromus inermis yields fewer selfed seed per unit of inflorescence than 2 to 4 panicles per bag. The utilization of 2 to

7 inflorescences were reported for various grasses by Keller (1948). Five panicles of Bromus inermis were bagged by Hays and Schmid (1943) and Adams (1953), while only 4 were bagged by Murphy and Atwood (1953). Heinrichs (1953) bagged 10 spikes of Agropyron intermedium. Little evidence of the most effective number of inflorescences to place in a selfing bag can be found in the literature. This is likely due to the lack of standardization of bag size.

Beddows and Davis (1938) used cotton wool pads to protect grass culms from mechanical injury. Myers (1942-A) found that the wrapping of cotton around grass culms had no effect upon self-pollinated seed set. Hanson and Carnahan (1956) found that parchment paper was easily torn. Bags were toughened by soaking 1/3 the length from the open end, but were more easily tied around the grass culms. This soaking would make bagging less injurious to grass culms.

It appears that any adequate means used to support bags in the field could be satisfactory. Beddows and Davis (1938) used bamboo canes and tied bags and grass culms securely to them. Myers (1942-A) passed a string through an eyelet in the upper corner of the bag and tied it to a bamboo stake. Long grass culms were tied to the stake in order to prevent the wind from pulling culms from the sleeve or bag. Hanson and Carnahan (1956) mentioned that wooden stakes 1 1/2 x 1 1/2 inches could be used satisfactorily. Domingo (1941) used 4 foot, number 9 galvanized wire. He placed a 1 3/4 inch loop in the upper end of the wire to prevent the bag from collapsing against the inflorescence.

Nilsson (1934) seemed to think that low seed set was not the result of inadequate pollination if bags were large enough and not too rigidly attached to the support. Small amounts of seed set on isolated inflorescences did not appear to be caused by insects, fungi or parasites. Minor mechanical injury could be the cause of the variability in seed set among inflorescences on the same plant. According to Knowles and Horner (1943), bagging date had no effect on the amount of seed set for Agropyron cristatum. Common strains set more self-fertile seed than the Fairway strain. It was suggested by Keller (1952) and Hanson and Carnahan (1956) that the removal of leaves from culms to be bagged may increase the amount of self-pollinated seed set. Myers (1942-A) showed through his work that plants to be studied should be bagged at a similar stage of maturity because early panicles were found to set considerably more seed than later ones.

According to observations of Gregor and Sansome (1927), the ecological conditions to which grasses are subjected can exert a strong influence upon the behavior of self-sterile plants. Meteorological conditions, age of plants and origin of ecotypes affected the amount of self-pollinated seed set when selfing Bromus inermis. Papravko (1934) observed that southern ecotypes showed a greater percentage of self-fertility than northern ecotypes. Fruwirth (1916) showed that light and temperature conditions have a great influence on the development of sex organs of plants. Malte (1921) attributed the failure of plants to set viable seed in his experiments to the collection of moisture inside the selfing bags, which reduced pollen grain germination.

Through measurement of the penetration of light and air through different paper and the evaporation of water from vials in bags, Keller (1945) failed to find any characteristic which might account for the differential in seed set under isolation bag. Clark (1944) measured day time temperatures in the bag and in the shade of the bag simultaneously and found approximately 2 degrees variation. It was concluded that this was not sufficient to account for reduced amount of seed set. Pearson (1933) found that a small bag made of a dark colored, highly porous material such as muslin would keep plant tissue temperature down, while it could be raised by the use of transparent material such as cellophane or glassine paper. He concluded that ordinary manila or kraft paper would keep enclosed tissue at approximately the same temperature as fully exposed tissue.

Wilsie, Ching and Hawk (1952) concluded that low seed set under pollination bags was caused by variable environmental conditions inside these bags. Delicate balance changes among plant and ecological factors affect seed set, but the effect of the pollination bag on seed set is only minor. Gregor and Sansome (1927) were also of the opinion that bag effect upon self-pollinated seed was minor, while Keller (1944) considered high seed yields from some bags quite definite indication that the bag of itself did not interfere with seed production. Nilsson (1934) found a correlation between the amount of seed set under open-pollinated conditions and self-pollinated seed produced, for some grass species. Other species appeared to have no correlation.

The value of self-pollination in grass breeding is viewed with variable opinions by various workers. According to Hays and Schmid (1943) seeds produced under self-pollinated conditions must be sufficiently numerous to furnish the necessary material for selection. Plants produced from this seed must have sufficient vigor for convenient handling. Keller (1945) valued inbreeding as a method for producing grass hybrids or as a method to be used in genetic studies. As far as Hanson, Myers and Garber (1952) were concerned, the most feasible use of inbred lines was in the production of synthetic varieties to insure greater uniformity of important economic characters. To find the extent to which selfing or close inbreeding could be employed for improvement of partially self-sterile grasses was most important, according to Valle (1937). If reduced vigor of plants resulted, then more suitable methods of breeding should be found. Stevenson (1939) could visualize the variation in breeding methods required by the different grass species. Inbreeding, he felt, was limited as a means of grass improvement but he could see that it was the quickest means of obtaining in a relatively homozygous condition certain desirable characters of rare occurrence. If a loss of vigor occurred, then it might be restored by controlled matings.

Many thoughts have been expressed by various authors hinting at the cause of self-sterility and the reason for the production of large amounts of self-fertile seed by some plants and not by others. According to Wexelsen (1952), the retention or increase of self-fertility by selection in selfed lines may be due to the fact that selection for self-fertility automatically involves a selection for the most heterozygous individuals and that self-fertility is due to heterozygosity of sterility factors. He thought that, to transfer self-fertility of some source to other materials, was not beyond feasibility. Nielsen (1951), working with Bromus inermis, thought genetic factors governing incompatibility had a significant role in the performance of plants after inbreeding. The failures and successes received in the setting of self-pollinated seed could not be attributed to cytogenetic disturbances alone, although self-sterile plants had a high frequency of chromosome laggards throughout their meiotic cell divisions. According to Myers (1942-B), seed setting ability of grass under bag is genetically complex; any approach to homozygosity accompanying sib-mating of an autotetraploid grass may be expected to be very slow. Studies of Leffel, Kalton and Wassom (1954) showed a correlation between open-pollinated seed set and self-pollinated seed set for the year 1949, but not for 1950. They concluded that factors other than panicle size were responsible for the association found between selfed and open-pollinated seed set. The factors affecting self-fertility were thought to be constant because a strong interannual correlation for self-fertility was found. A poor correlation for cross-pollinated seed set indicated that environmental influence affected open-pollinated seed set more than self-pollinated seed set. The variation in environmental controlled effects, however, were thought to be far less important than the genetic-controlled variation for the amount of self-pollinated seed set.

According to Cheng (1946), inbreeding as a method of improvement of _Agropyron cristatum_ was of limited practical application. No correlation could be found to exist between the amount of self-pollinated seed set and the amount of open-pollinated seed set. Myers (1942-B) found an indication of such a correlation for _Dactylis glomerata_. Law and Anderson (1940), working with _Andropogon furcatus_, found a few lines that were highly self-fertile; those lines showing little loss of vigor in the S-1 generation also showed little loss of vigor in the S-2 generation. Inbreeding caused segregation into distinct entities which, when open-pollinated, exhibited a considerable increase in vigor. One difficulty was that desirable forage types were low-seed producers.

Myers (1942-B) found evidence to indicate that reduced growth vigor was not a major factor contributing to the reduction of seed set under bag. Improvement of naturally cross-pollinated species is most feasibly done by producing strains with uniform, desirable characteristics.

Many methods for interpreting results of self-fertility can be found in the literature. Smith (1944) indicated that frequent occurrence of zero seeds in 1 or more bags, where other bagged heads of the same plant had given few to fairly numerous seed, would make statistical study very difficult if not impractical. Keller (1948) felt that the mean and standard error were of little significance in expressing self-fertility data for relatively self-sterile species since they yielded skewed curves. Only slightly skewed curves can be accurately expressed by calculating the mean and its standard error. Both types of data appear to be more adequately represented and easier to interpret when presented as frequency distributions without regard to mean or the magnitude of error. This is true because the extent to which these histograms are skewed is a measure of the intensity of self-sterility in the species. Frequency distribution will probably prove most useful with species that are relatively self-sterile. A standardized number of classes on a large number of observations should be used to present data.

According to Myers (1942-B), large numbers of plants should be involved in making accurate determination of plant characteristics under study. Smith, Myers and others felt that self-fertility expressed as percent was a reliable and accurate expression of self-fertility. Keller suggested that the weight of seed per inflorescence could be used. Weight as a means of expressing self-fertility was much quicker and easier to determine than the calculation of average florets per spikelet, total calculated florets, heavy seed per inflorescence, heavy seed per 100 spikelets or other laborious methods used by Beddows and Davis (1938). Leffel, Kalton and Wassom (1954), comparing different methods used to determine self-fertility, found that the number of plump seed per panicle, number of germinable seed per panicle, number of germinable seed per 100 florets, seed weight per panicle and mean weight of unthreshed panicles were all a measure of the same attribute of interpreting self-fertility.

On the basis of 11 plants, Smith (1944) found Agropyron elongatum to set 27 percent as much self-pollinated as open-pollinated seed. He concluded that this species is intermediate in self-fertility, but reviewed foreign authors who considered it to be low in self-fertility. Keller (1948) presented a histogram showing Agropyron elongatum with a highly skewed characteristic toward self-sterility.

MATERIALS AND METHODS

Source of plant materials

Thirty individual plants of tall wheatgrass *Agropyron elongatum* (Host) Beauv., selected from an aggregate source growing on salt plots near Springville, Utah, were moved to Logan, Utah, in the fall of 1953 and held in greenhouse pots. Seed was collected from each plant prior to its removal from the field and planted in flats in the greenhouse in the spring of 1954. Parent plants and 19 progeny of each were space-planted in 3 foot rows on the Evans Farm (Forage Experimental Farm). A similar planting of 22 individual plants and 20 progeny each was made adjacent to the preceding planting during the spring of 1955.

The experimental farm soil is a clay loam of alluvial deposit left by Lake Bonneville. Being bench land soil, it is deep and well drained. Since the farm is on the east bench, it possesses a westward slope which makes it subject to full effects of the afternoon sun; but it is not well protected from prevailing southerly and northerly winds. The mean summer temperature ranges from 50 degrees to 90 degrees. The humidity is variable, but generally low except during periods of summer rain storms.

Experimental arrangement of phase 1

All plantings were well established and vigorously growing in the spring of 1956. Extreme variation could be detected among plants in color, height, spreading ability, vegetative texture and spikelet arrangement. Other than morphological characteristics, little was known about these plants.

The plantings of tall wheatgrass were divided by age of plants into

2 divisions. The older plants were used in an experiment designed to determine the extent to which the number of spikes enclosed in a selfing bag would influence the set of self-pollinated seed. Another point of interest was to ascertain to what extent selfing bags influenced seed set. To answer this last question it was assumed that the selfing bag would exert the same influence on open-pollinated seed as it would upon the set of self-pollinated seed.

In an attempt to gain the information desired, 100 two-year-old plants were selected arbitrarily in order that as many phenotypic expressions could be represented as possible. Upon each of the 100 plants selected seven, 3 x 36 inch vegetable parchment paper bags were placed. Each bag represented a treatment. Treatment number 1 was 1 spike enclosed in 1 bag. Treatment number 2 was 2 spikes enclosed in 1 bag, and so on until 6 spikes were enclosed in 1 bag. The 6 bags were placed upon the plant between June 29, 1956, and July 11, 1956, prior to anthesis, which started July 16 and lasted through July 25. On July 28, 1956, when all plants had ceased flowering, each bag was thoroughly checked for damage and a seventh bag added to each plant. Four open-pollinated spikes were enclosed by the seventh bag.

Bags were supported by 2, six-foot 2 1/2 x 1 inch redwood stakes driven into the ground next to the plant to be bagged. (Figure 1). Bags were tied as rigidly as possible around the top and as securely as possible around the center and bottom in order that wind damage to bags and culms might be prevented. Bags were alternately arranged and adjusted in order to prevent one from being on top of another or in the same position with respect to one another at all times.

Figure 1. Photograph showing 35-pound vegetable parchment paper bag arrangement on 2-year-old plants of Agropyron elongatum

Experimental arrangement of phase 2

Another phase of the experiment was conducted on the 1-year-old plants to determine the effect bag construction had upon the amount of self-pollinated seed set. Fifty plants were selected arbitrarily in order that as many different phenotypic expressions could be tested as possible. Upon each plant 4 differently constructed types of bags were placed, each containing 4 spikes. Bags were supported by number 9 galvanized wire with a 2 inch loop in the upper end to prevent the bag from collapsing against the inflorescence. Two wire supports were securely fastened to a 6 foot 2 1/2 x 1 inch redwood stake driven into the ground next to the plant being tested. (Figure 2). Each bag was securely tied to a stake.

No attempt was made to use all types of materials which have been used as isolating materials in this phase of the experiment. The 4 most commonly used isolators are as follows:

1. Thirty-five-pound vegetable parchment paper bags, 3 x 36 inches. These were the same as the bags used in phase 1 on the older plants.
2. Light weight kraft bags, 3 x 26 inches
3. Cloth bags made from high grade closely woven sheeting, 3 x 12 inches
4. Thirty-five-pound vegetable parchment sleeves, 3 x 26 inches. These sleeves were made by cutting the ends from vegetable parchment bags and stuffing the ends with cotton. Both ends were securely tied to the stake.

The 4 types of bags used were of standard construction except the cloth bag, which was locally made.

Figure 2. Photograph showing 4 types of selfing bags and their arrangement on 1-year-old plants of *Agropyron elongatum*

Bag treatment and seed analysis

Bags of both phases of the experiment were closed at the base by folding and fastening with a stapler. Grass culms were tied to the stake at the base of the bag in order to prevent movement of culms against the bag and mechanical injury, as well as to prevent the wind from pulling the culms from the bags. All the spikes placed under the bags were of approximately the same stage of maturity and size.

On August 28, 1956, four control spikes were taken from each plant tested in the field. Bags were removed with contents intact and moved into the seed laboratory, where damaged bags and contents were discarded. Damage to bags was caused largely by wind and rain.

To gain information necessary for analysis of data, number of spikelets per bag, amount of seed per bag and weight of seed per bag were carefully ascertained and recorded. Thrashing of seed was done by hand. Raw data obtained were analyzed as a randomized block design on the basis of seed per spikelet and weight of seed per spike, by standard statistical methods. Where greater numbers of plants (replications) were used as a source of data, seed weights are given on the basis of seeds per spike instead of seeds per spikelet because this method generally gave larger tabular values.

To determine significant differences among means the multiple range test suggested by Duncan (1955) was used. Symbolization and terminology used are the same as those suggested. Shortest significant ranges are given in the row headed Rp: and are computed by multiplying the standard error of the mean ($S_{\bar{x}}$) by interpolated tabular values furnished by Duncan. The row P: refers to the number of means in the interval being tested. Any 2 treatment means not compared by the same line are significantly different from each other at the .01 level.

RESULTS

Early in July prior to complete anthesis a wind and rain storm damaged many bags in the field. The loss of data to be obtained from these bags, along with the loss of a few seed weights in the seed laboratory account for the variable number of plants used when making the various analyses. Wherever the possibility of contamination of the self-pollinated seed by foreign pollen entering bags at the time of flowering existed, data from these bags were not included.

Influence of spike number upon self-pollinated seed set under selfing bags

Analysis of variance and treatment means for 6 bagging treatments are given in Table 1. Calculations given are on the basis of seeds per spikelet. No significant differences were obtained for any treatment at the .05 level, making further calculation unnecessary. Plant (replication) effect is highly significant, however, indicating that plants influence the amount of self-pollinated seed produced more than the number of spikes enclosed in a selfing bag.

On the basis of this experiment, 1 and 6 spikes enclosed in a selfing bag have little influence upon the number of self-pollinated seed produced. The mean for treatments 3 and 4 shows a slight increase over all others, but not enough to be significant. Because of the large coefficient of variation obtained in this analysis, greater differences would have to occur before significance could be gained.

Analysis of variance and treatment means calculated on the basis of the average weight of seed per spike gave the same result (Table 2).

Table 1. Analysis of variance and treatment means for the number of spikes per selfing bag and their effect on self-pollinated seed set as determined by seeds per spikelet

Source of Variation	Degrees of Freedom	Mean Square	F Value
Treatment	5	.035	.76
Plants	58	.346	7.52**
Plants x Treatment	290	.046	
Total	353		

Coef. of Var. = 102%

Treatments	1	2	3	4	5	6
Means	.197	.175	.240	.232	.200	.219

**Significant at the .01 level

Table 2. Analysis of variance and treatment means for the number of spikes per selfing bag and their effect on self-pollinated seed set as determined by average weight of seed per spike

Source of Variation	Degrees of Freedom	Mean Square	F Value
Treatment	5	.0009	.72
Plants	55	.0133	10.93**
Plants x Treatment	275	.0012	
Total	335		

Coef. of Var. = 116%

Treatments	1	2	3	4	5	6
Means	.029	.023	.029	.033	.029	.028

**Significant at the .01 level

The coefficient of variation is slightly greater but variability among means is less defined. Four spikes enclosed per bag gave highest mean seed weight, but again this difference is not significant.

Tabular values of seed weight and seed numbers upon which calculations were based proved variable and not altogether consistent. Many zero values resulted for the amount of self-pollinated seed obtained, with the majority of values being small. A few plants yielded nearly the same amount of self-pollinated seed as they did open-pollinated seed. Plants varied in seed set with respect to each other as well as not being consistent with seed relationships for spikes under the same bag.

Effect of selfing bag on seed producation

The effect of placing 35-pound vegetable parchment paper bags upon inflorescences can be seen by comparison of bagged open-pollinated seed from 4 spikes with seed from 4 spikes having no treatment. Data obtained by seed count are not significant at the .05 level for treatment (b) over treatment (a) (Table 3). The data based on seed weight per spike for comparison of the same treatments are highly significant (Table 4). Treatment (a) is 4 open-pollinated spikes from the same plant which were open-pollinated under near normal conditions. Any difference between the 2 treatments should be due to the effect of the isolation bag upon the plant.

Some difference in treatment results between Table 3 and Table 4 may be explained on the basis of fewer degrees of freedom for plants in Table 4. The most logical cause of significant difference between the 2 treatments is that open-pollinated seed that has been bagged after it has started formation weighs less than normal seed. That isolation bags

Table 3. Analysis of variance and treatment means for the bag effect on open-pollinated seed set as determined by seeds per spikelet

Source of Variation	Degrees of Freedom	Mean Square	F Value
Treatment	1	3.61	2.81
Plants	96	2.56	1.99**
Plants x Treatment	96	1.28	
Total	193		

Coef. of Var. = 11%

Treatments	(a)	(b)
Means	3.13	3.40

**Significant at the .01 level

Table 4. Analysis of variance and treatment means for the bag effect on open-pollinated seed set as determined by average weight of seed per spike

Source of Variation	Degrees of Freedom	Mean Square	F Value
Treatment	1	.076	8.54**
Plants	55	.038	4.24**
Plants x Treatment	55	.009	
Total	111		

$S_{\bar{x}}$ = .013 Coef. of Var. = 29%

Treatments	(a)	(b)
Means	.323	.375

P:	2
Rp:	.047

**Significant at the .01 level

P: Number of means in interval being tested

Rp: Shortest significant range at .01 level for a given P

influence seed weight can be seen by comparison of significant ranges for the bag-materials treatment. The large number of replications and the low coefficient of variation of 11 percent obtained for data calculated would indicate the non-significance of differences in Table 3 to be correct. Variations in the open-pollinated seed produced under bag compared to that produced under near normal conditions was great but did not show significant difference, although the mean for treatment (a) was somewhat lower than for treatment (b). Comparison of treatment (a) and (b) on a weight basis with 56 observations should be sufficiently accurate to draw inferences that would be characteristic of the species. It is recognized that replications on individual plants of both treatments would give more reliable results.

Effect of bagging material on self-pollinated seed set

The type of material used in bag construction shows a definite influence on the amount of selfed seed obtained. Table 5 gives analysis of variance and treatment means for 4 different materials, which are significant at the .01 level. Comparison of treatment means calculated on the basis of number of selfed seed per 100 spikelets indicates that 35-pound vegetable parchment paper is superior to Kraft paper (treatment (B)) or cloth (treatment (C)). No significant difference in the number of seeds was found between a 35-pound vegetable parchment bag (treatment(A)) and a 35-pound vegetable parchment sleeve (treatment (D)). No difference exists between cloth bags and 35-pound vegetable parchment bags. A significant difference does exist between cloth bags and vegetable parchment sleeves.

The differences among treatments (A), (B), (C) and (D) are very

Table 5. Analysis of variance and treatment means for types of selfing bags as determined by set of selfed seed per 100 spikelets

Source of Variation	Degrees of Freedom	Mean Square	F Value
Treatments	3	13,289.64	15.13**
Plants	34	3,970.12	4.52**
Plants x Treatment	102	878.43	
Total	139		

$S_{\bar{x}} = 5.01$ Coef. of Var. = 72%

Treatments	(B)	(C)	(A)	(D)
Means	18.42	34.21	47.67	64.21
P:	2	3	4	
Rp:	18.24	19.03	19.54	

**Significant at the .01 level

P: Number of means in interval being tested

Rp: Shortest significant range at .01 level for a given P

sharp and definite when analyses of variance and treatment means are compared on the weight basis of selfed seed per 100 spikelets (Table 6). It would appear that the bag has a greater effect upon the weight of seed than upon the number of seeds produced. A sharp, distinctive increase in seed weight was obtained in favor of 35-pound vegetable parchment sleeves (treatment (D)) over other treatments. The material and construction of bags apparently tend to influence seed set under self-pollinated conditions.

In order to determine to what extent seed numbers and seed weight were a measure of the same value, a correlation calculation was made. The weight of seed as a measure of self-fertility is a measure of the same value as the number of seeds per spikelet, as indicated by an r of 0.98 at the .01 level. This correlation would be an expected value, but as plants giving high seed yields did not always give the highest or consistent seed weight, statistical proof was necessary.

Plant effect upon seed set

In all analysis of variance Tables 1 through 6, plant effect was significant at the 1 percent level. This was true when calculated on a seed weight or a number basis. High significance existing for seed production variability of open-pollinated plants suggested that a relationship between open-pollinated and self-pollinated seed produced could exist. Correlation to indicate that the plants producing large amounts of open-pollinated seed also produced the larger amounts of self-pollinated seed, did not exist. This appeared to be true whether calculation was made on a seed number or seed weight basis. A comparison of plant 28-1(S)6, Tables 7 and 8, with 28-1(OP)6, Tables 9 and 10, set, on an average, as much self-pollinated seed as open-pollinated seed.

Table 6. Analysis of variance and treatment means for types of selfing bags as determined by weight of selfed seed per 100 spikelets

Source of Variation	Degrees of Freedom	Mean Square	F Value
Treatment	3	.515	16.13**
Plants	27	.113	3.64**
Plants x Treatment	81	.031	
Total	111		

$S_{\bar{x}} = .03$ Coef. of Var. = 68%

Treatments	(B)	(C)	(A)	(D)
Means	.72	1.53	2.06	2.98

P:	2	3	4
Rp:	.110	.114	.118

**Significant at the .01 level
P: Number of means in interval being tested
Rp: Shortest significant range at .01 level for a given P

Table 7. Significant range for plant means as determined by Duncan's multiple range test conducted on selfed seed per spike /1

	Plant Number	Mean No. of Seeds	P:	Rp:
1	28- 1(S)6	3.19	59	.40
2	14-17(S)6	1.13	38	.40
3	9- 8(S)6	1.02	37	.39
4	5-13(S)6	1.00	20	.39
5	17- 7(S)6	.72	19	.38
6	15-18(S)6	.48	14	.38
7	28- 2(S)6	.45	13	.37
8	11-15(S)6	.41	9	.37
9	20- 6(S)6	.37	8	.36
10	17- 2(S)6	.35	7	.36
11	3-15(S)6	.35	6	.35
12	14- 9(S)6	.34	5	.35
13	3- 3(S)6	.33	4	.34
14	7- 5(S)6	.33	3	.33
15	8-17(S)6	.32	2	.32
16	20- 1(S)6	.31		
17	23- 2(S)6	.31		
18	20-17(S)6	.29		
19	24-16(S)6	.28		
20	16- 9(S)6	.27	$S_{\bar{x}}$ = .09	
21	7- 6(S)6	.27		
22	14-13(S)6	.26		
23	18-15(S)6	.26		
24	4-12(S)6	.23		
25	10-15(S)6	.20		
26	16- 4(S)6	.18		
27	26-19(S)6	.17		
28	5-11(S)6	.14		
29	8-16(S)6	.12		
30	16-11(S)6	.11		
31	20-15(S)6	.08		
32	4- 6(S)6	.08		
33	6- 4(S)6	.07		
34	1- -(S)6	.06		
35	19-13(S)6	.05		
36	13-15(S)6	.04		
37	16-18(S)6	.03		
38	18- 3(S)6	.03		
39	12- 9(S)6	.02		
40	18- -(S)6	.01		
41	17-15(S)6	.00		
42	11- 8(S)6	.00		

Many plant numbers omitted for convenient table construction
/1 Refer to Table 1 for analysis of variance
P: Number of means in interval being tested
Rp: Shortest significant range at .01 level for a given P:

Table 8. Significant range for plant means as determined by Duncan's multiple range test conducted on weight of selfed seed per spike /1

	Plant Number	Mean Seed Wt. in Gms.	P:	Rp:
1	28- 1(S)6	.314	56	.065
2	5-13(S)6	.143	47	.065
3	14-17(S)6	.098	46	.064
4	15-18(S)6	.081	40	.064
5	17- 7(S)6	.074	39	.063
6	29- 3(S)6	.068	25	.063
7	28- 2(S)6	.048	24	.062
8	17- 2(S)6	.042	18	.062
9	11-15(S)6	.042	17	.061
10	14- 9(S)6	.039	14	.061
11	3- 3(S)6	.039	13	.060
12	23- 2(S)6	.038	11	.060
13	16-10(S)6	.035	10	.059
14	7- 5(S)6	.034	9	.059
15	20-17(S)6	.033	8	.058
16	8-17(S)6	.031	7	.058
17	24-16(S)6	.029	6	.057
18	20- 1(S)6	.029	5	.056
19	4-12(S)6	.028	4	.055
20	16- 4(S)6	.028	3	.053
21	16- 9(S)6	.026	2	.051
22	7- 6(S)6	.026		
23	20- 6(S)6	.025		
24	18-15(S)6	.023		
25	9-13(S)6	.023	$S_{\bar{x}}$ = .014	
26	14-13(S)6	.022		
27	10-15(S)6	.021		
28	26- 9(S)6	.020		
29	3-15(S)6	.019		
30	6-15(S)6	.014		
31	20-15(S)6	.013		
32	8-16(S)6	.012		
33	16-11(S)6	.011		
34	4- 6(S)6	.011		
35	1- -(S)6	.009		
36	6- 4(S)6	.008		
37	13-15(S)6	.005		
38	16-18(S)6	.004		
39	18- 3(S)6	.001		
40	18- -(S)6	.000		
41	17-15(S)6	.000		
42	11- 8(S)6	.000		

Many plant numbers omitted for convenient table construction
/1 Refer to Table 2 for analysis of variance
P: Number of means in interval being tested
Rp: Shortest significant range at .01 level for a given P:

Table 9. Significant range for plant means as determined by Duncan's multiple range test conducted on open-pollinated seed per spike /1

	Plant Number	Mean No. of Seeds	P:	Rp:
1	16-18(OP)6	5.69	97	3.72
2	16-11(OP)6	5.59	76	3.72
3	20-19(OP)6	5.29	75	3.71
4	18-17(OP)6	5.27	50	3.71
5	7- 6(OP)6	5.17	49	3.70
6	5-19(OP)6	4.98	48	3.70
7	8-16(OP)6	4.95	47	3.69
8	7- 5(OP)6	4.94	46	3.69
9	14-17(OP)6	4.76	45	3.68
10	7- -(OP)6	4.52	44	3.68
11	3-15(OP)6	4.45	43	3.67
12	6- 4(OP)6	4.38	42	3.67
13	28- 2(OP)6	4.35	41	3.66
14	3- 3(OP)6	4.03	38	3.66
15	5-11(OP)6	4.02	37	3.65
16	18-15(OP)6	3.99	36	3.65
17	11-15(OP)6	3.97	34	3.64
18	16- 9(OP)6	3.89	32	3.63
19	15-18(OP)6	3.64	31	3.62
20	5-13(OP)6	3.58	27	3.61
21	24-16(OP)6	3.51	25	3.60
22	26-19(OP)6	3.37	22	3.60
23	17- 2(OP)6	3.05	20	3.59
24	1- -(OP)6	3.01	19	3.58
25	28- 1(OP)6	2.99	18	3.57
26	17-15(OP)6	2.96	17	3.55
27	11- 8(OP)6	2.86	16	3.54
28	20-15(OP)6	2.82	15	3.53
29	16- 4(OP)6	2.61	14	3.51
30	17- 7(OP)6	2.59	13	3.50
31	20- 6(OP)6	2.51	12	3.49
32	14-13(OP)6	2.47	11	3.45
33	23- 2(OP)6	2.42	10	3.44
34	13-15(OP)6	2.10	9	3.41
35	18- -(OP)6	2.04	8	3.37
36	25-16(OP)6	1.62	7	3.34
37	20-17(OP)6	1.55	6	3.29
38	9-19(OP)6	1.27	5	3.25
39	19- 3(OP)6	1.14	4	3.19
40	29- 3(OP)6	.86	3	3.09
41	26- 3(OP)6	.74	2	5.54
42	11- 1(OP)6	.67	$S_{\bar{x}}$ = .80	

Many plant numbers omitted for convenient table construction

/1 Refer to Table 3 for analysis of variance

P: Number of means in interval being tested

Rp: Shortest significant range at .01 level for a given P:

Table 10. Significant range for plant means as determined by Duncan's multiple range test conducted on open-pollinated seed weight per spike /1

	Plant Number	Mean Seed Wt. in Gms.
1	8-16(OP)6	.657
2	16-18(OP)6	.570
3	28- 2(OP)6	.561
4	7- 5(OP)6	.547
5	16-11(OP)6	.541
6	13- 7(OP)6	.522
7	3-15(OP)6	.518
8	23- 5(OP)6	.516
9	15-18(OP)6	.509
10	7- 6(OP)6	.496
11	8-17(OP)6	.476
12	5-13(OP)6	.450
13	4- 6(OP)6	.438
14	26-19(OP)6	.413
15	16- 4(OP)6	.408
16	3- 3(OP)6	.406
17	14-17(OP)6	.401
18	16- 9(OP)6	.399
19	4-12(OP)6	.399
20	6-15(OP)6	.389
21	16-10(OP)6	.378
22	6- 4(OP)6	.377
23	28- 1(OP)6	.357
24	21-12(OP)6	.357
25	20-15(OP)6	.347
26	1- -(OP)6	.345
27	24-16(OP)6	.342
28	17- 2(OP)6	.277
29	11-15(OP)6	.275
30	23- 2(OP)6	.275
31	12- 9(OP)6	.261
32	18-15(OP)6	.253
33	17- 7(OP)6	.252
34	11- 8(OP)6	.226
35	18- 3(OP)6	.208
36	20- 6(OP)6	.201
37	17-15(OP)6	.200
38	13-15(OP)6	.187
39	10-15(OP)6	.161
40	18- -(OP)6	.140
41	20-17(OP)6	.114
42	29- 3(OP)6	.056

P:	Rp:
56	.311
48	.311
47	.310
45	.310
44	.309
40	.309
39	.308
37	.308
36	.307
32	.307
31	.306
29	.306
28	.305
25	.305
24	.304
22	.304
21	.303
20	.303
19	.302
18	.301
17	.300
16	.299
15	.298
14	.297
13	.296
12	.294
11	.293
10	.291
9	.289
8	.286
7	.283
6	.279
5	.275
4	.270
3	.263
2	.252

$S_{\bar{x}} = .067$

Many plant numbers omitted for convenient table construction

/1 Refer to Table 4 for analysis of variance

P: Number of means in interval being tested

Rp: Shortest significant range at .01 level for a given P:

pollinated seed. Comparison of other plants with respect to their position in the multiple range between tables would tend to show visually the lack of correlation between open-pollinated and self-pollinated seed produced.

By use of Duncan's multiple range test for plant means it is shown that the plants differed significantly in their ability to set seed. The position of the plant in the various tables whether calculated on seed numbers or seed weight assumes approximately the same position. Any difference is probably due to the inherent ability for seed size among plants and the vigorous threshing that individual seed lots received in the laboratory. By comparing significant ranges for open-pollinated seed set, Tables 9 and 10, it can be seen that no sharp differences occur among plants, while plants producing large amounts of self-pollinated seed are few and have a short range of significance in proportion to the longer ranges of significance for plants expressing greater self-sterility (Tables 7 and 8, 11 and 12).

Number of self-pollinated seed per 100 spikelets plotted against number of bags (Figure 3) shows a histogram strongly skewed toward self-sterility. The greater proportion of plants set less than 5 seed per 100 spikelets. The average selfed seed set in relation to open-pollinated seed set was 8.9 percent for 2-year-old plants. One-year-old plants set 11.6 percent as much seed as they did open-pollinated seed. Graphical presentation of these plants indicates the largest grouping occurred for 5 to 10 seeds per 100 spikelets (Figure 4) and follows in general configuration a figure presented for <u>Agropyron elongatum</u> by Keller (1948).

Table 11. Significant range for plant means as determined by Duncan's multiple range test conducted on selfed seed per 100 spikelets /1

	Plant Number	Mean No. of Seeds	P:	Rp:
1	33-20(S)6	146.6	35	67.6
2	42-11(S)6	118.4	34	67.4
3	31- 2(S)6	97.2	33	67.4
4	43-20(S)6	75.5	32	67.3
5	43- 7(S)6	66.2	31	67.0
6	42- 2(S)6	63.7	30	67.0
7	39- 6(S)6	61.5	29	67.0
8	48- 7(S)6	60.4	28	67.0
9	45- 2(S)6	49.6	27	66.9
10	38- 2(S)6	48.5	26	66.8
11	45- 7(S)6	48.5	25	66.8
12	48-13(S)6	47.9	24	66.7
13	44-10(S)6	46.1	23	66.6
14	50-20(S)6	44.6	22	66.5
15	46-17(S)6	39.2	21	66.5
16	42-18(S)6	38.5	20	66.4
17	35-13(S)6	37.5	19	66.2
18	35-11(S)6	32.3	18	65.9
19	42-15(S)6	30.6	17	65.7
20	45-12(S)6	30.1	16	65.5
21	39-19(S)6	28.2	15	65.2
22	45- 4(S)6	27.6	14	64.9
23	45- 1(S)6	27.6	13	64.7
24	46-13(S)6	24.8	12	64.4
25	41- 2(S)6	24.1	11	64.0
26	35- 6(S)6	23.0	10	63.6
27	50- -(S)6	22.2	9	63.0
28	41-11(S)6	18.8	8	62.4
29	49-17(S)6	15.8	7	61.8
30	35-15(S)6	12.5	6	60.9
31	38- 6(S)6	11.0	5	60.2
32	33-16(S)6	9.6	4	59.0
33	40- 4(S)6	6.2	3	57.2
34	49- 5(S)6	4.4	2	55.0
35	48- 3(S)6	.8		

$S_{\bar{x}} = 14.82$

/1 Refer to Table 5 for analysis of variance
P: Number of means in interval being tested
Rp: Shortest significant range at .01 level for a given P:

Table 12. Significant range for plant means as determined by Duncan's multiple range test conducted on weight of selfed seed per 100 spikelets /1

	Plant Number	Mean Seed Wt. in Gms.	P:	Rp:
1	33-20(S)6	.703	28	.400
2	31- 2(S)6	.588	27	.400
3	42-11(S)6	.575	26	.399
4	48- 7(S)6	.490	25	.399
5	39- 6(S)6	.398	24	.398
6	42- 2(S)6	.355	23	.398
7	45- 2(S)6	.318	22	.397
8	43- 7(S)6	.313	21	.397
9	38- 2(S)6	.305	20	.396
10	43-20(S)6	.283	19	.395
11	46-17(S)6	.273	18	.393
12	48-13(S)6	.265	17	.392
13	44-10(S)6	.250	16	.391
14	35-13(S)6	.233	15	.389
15	45- 1(S)6	.232	14	.388
16	45- 7(S)6	.225	13	.386
17	42-18(S)6	.218	12	.384
18	35-11(S)6	.183	11	.381
19	45-12(S)6	.168	10	.379
20	45- 4(S)6	.163	9	.376
21	39-19(S)6	.148	8	.373
22	46-13(S)6	.138	7	.369
23	35- 6(S)6	.128	6	.364
24	41-11(S)6	.120	5	.354
25	42-15(S)6	.105	4	.352
26	33-16(S)6	.053	3	.342
27	38- 6(S)6	.048	2	.328
28	35-15(S)6	.005		

$S_{\bar{x}}$ = .09

/1 Refer to Table 6 for analysis of variance
P: Number of means in interval being tested
Rp: Shortest significant range at .01 level for a given P:

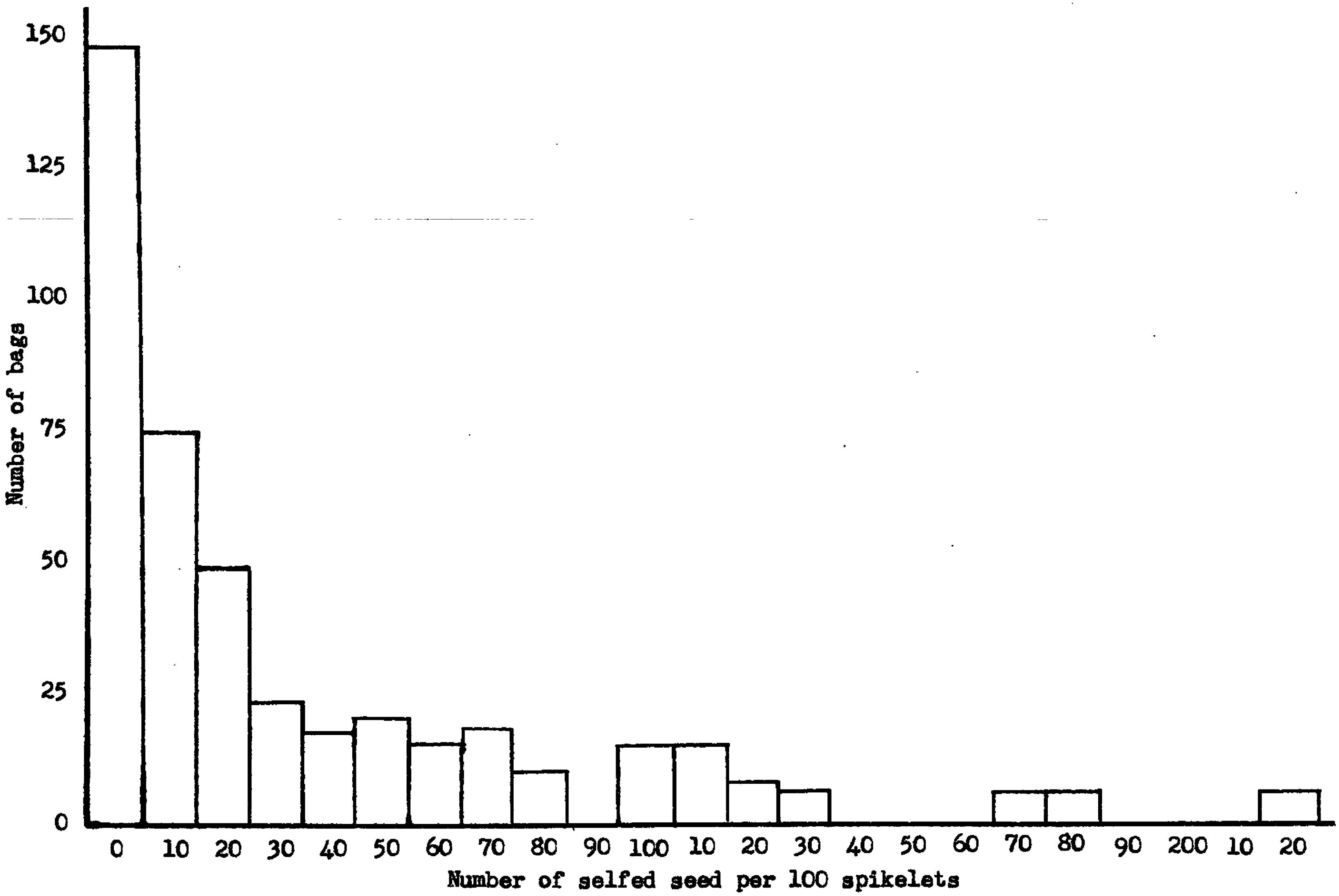

Figure 3. Histogram showing self-fertility for 2-year-old plants of *Agropyron elongatum*

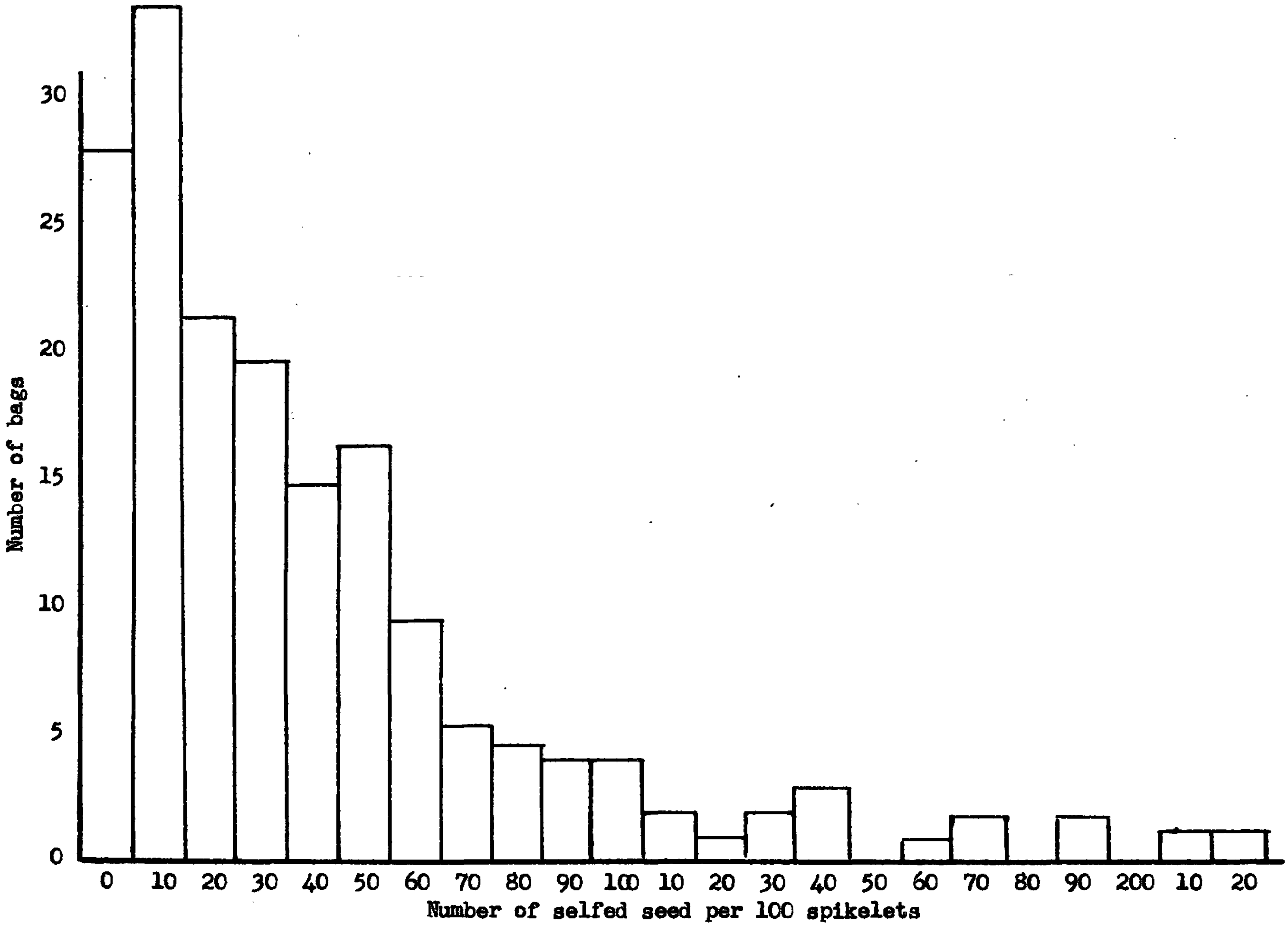

Figure 4. Histogram showing self-fertility for 1-year-old plants of <u>Agropyron elongatum</u>

DISCUSSION

Methods in field application of bags

A great amount of wind damage could be expected and was received because of the height of the selfing bags above the ground. Some bags were extended in the air above 6 feet in order to prevent damage to inflorescences, some of which elongated 11 inches after they were bagged. Because of the flexibility and height of stakes, it could be reasonably assured that sufficient movement of bags and plants occurred to insure adequate pollination.

Wind damage to bags was quite variable, depending upon the arrangement of bags upon the stakes and the type of material of which the bag was made. Cloth bags were least damaged by wind and remained in place better with the least amount of tying, however, these bags were the most difficult to place upon plants because of scabrous leaf blades. Vegetable parchment sleeves were the most easily fastened to the stake but were most vulnerable to wind. Extreme care had to be exerted to insure that sleeves were securely tied at the top, center, and bottom. Thirty-five pound vegetable parchment paper bags were most easily placed upon the plants, and remained reasonably secure with a moderate amount of tying. Kraft paper bags were equal to vegetable parchment paper bags in ease of placing upon the plants, but were inferior in efficiency.

Bags tied in groups around a stake had a tendency to work upward through wind movement, leaving inflorescences exposed (Figure 1). The exposure of inflorescences would not be hazardous provided bagged culms had been marked with wire twisters, so that they might be identified from open-pollinated culms at harvest time. The removal of culms from bags after danger of pollination of the flowers from foreign pollen is over may even increase the amount and quality of self-pollinated seed. It is most important that bags do not become removed from inflorescences before flowering has ceased. In order to prevent wind damage to selfing bags, it would seem advisable to place not more than 1 or 2 bags on a stake. Bags must be secured in such a fashion that wind movement upon the stake becomes nil. To accomplish this it is necessary for the stake to be as high as the top of the selfing bag is above the ground, and the selfing bag must be securely fastened to it. In the author's opinion, fastening of selfing bags to the stakes could be more easily accomplished if bags were constructed with ears similar to handles on many paper drinking cups. Tying pressure would be exerted around the circumference of the bag, thus alleviating the necessity for some frame to keep the bag fully inflated. Because bags would be completely anchored at 3 points along their length, less wind damage would result.

Some inaccuracy in results obtained (Tables 5 and 6) could be due to different volumetric relationships inside the different types of selfing bags used. The difference in volume was caused only by the difference in bag length; and since the inflorescences were adequately covered, it was assumed that the area of the bag along the culms had no influence on the amount of seed produced. The area within a bag may have influence upon the amount of self-pollinated seed set, though the number of spikes placed in a selfing bag (Tables 1 and 2) tended to disprove that the enclosure area per inflorescence had any effect on the set of seed under a bag.

Spike number and bag effect upon seed set

The non-significance found for treatments where a variable number of spikes are enclosed in selfing bags (Tables 1 and 2) could be caused by several factors. The first and foremost possible cause is that no difference actually occurs between 1 and 6 spikes when enclosed in a selfing bag as far as their effect on seed setting ability is concerned. This would tend to coencide with and confirm the finding of Myers (1942-A) and Heinrichs (1953). The possibility exists that because no loops were inserted to keep bags from collapsing against the inflorescences, and because several bags were tied to the same stake, volume relationships inside the bag over the large number of replications were such that any variation that might exist became counteractive. The tying of 1 bag to 1 stake would tend to delimit any possible influence on volume relationships inside the bag caused by tying more than 1 bag to a stake.

The reduced seed weight resulting when open-pollinated spikes were bagged (Table 4) compared with no difference in seed numbers (Table 3) indicates that seed weight is reduced significantly by bagging. To conclude that reduced seed weight was the only influence of a selfing bag on seed formation under self-pollinated conditions may be an erroneous assumption. Correlation of seed weight for treatment (a) with seed weight for treatment (b) resulted in an r value of 0.67 at the .01 level, indicating that seed weight reduction was not an erroneous assumption. Mean seed weight for the bagged open-pollinated plants (treatment (a)) is significantly lower than that for plants with no treatment (treatment (b)) and since the correlation value indicates that a plant's ability to produce seed under the different conditions is the same, then reduced seed weight is the only effect a selfing bag could have for comparable conditions. To prove that reduced seed weight was the only influence exerted by a selfing bag when self-pollinating plants is possible, and could be shown by comparison of seed weight of cloned material in space isolation with the same material isolated under selfing bag or sleeve. The fact that a correlation could not be obtained for the weight of selfed seed with the weight of open-pollinated seed for conditions of this experiment is only measuring the fact that plants do not have ability to produce a related amount of self-pollinated seed to open-pollinated seed, and is not measuring the lack of similarity between weight of equal seeds from each condition.

The sharp, significant distinction among treatments for different bagging materials (Table 6), comparing weight with a gradual significant distinction among treatments for the calculation on numbers (Table 5), would again indicate that weight is influenced more by selfing plants under bag than seed numbers. Since a significance among treatments calculated on a number basis occurs (Table 5), some proof is given to show that the type of material and construction of the bag have their effects on self-pollinated seed production. This may tend to disprove the assumption that seed weight is the only influence of the selfing bag upon normally cross-pollinated plants, though other effects of the selfing bag that may be exerted are only minor compared with reduced seed weight. It would appear that, if other influences are exerted by the selfing bag, reduced seed weight would intensify the result. For instance, if reduced seed numbers occur because of the selfing bag, then weight reduction of that seed would intensify the expression of significance because of reduced number and actual weight effect. This can be readily seen by comparison of treatments (a) and (b) (Tables 3 and 4) and treatments (A), (B), (C) and (D) (Tables 5 and 6). The conclusion of Wilsie, Ching, and Hawk (1952), Gregor and Sansome (1927) and Keller (1944) that the bag exerted only a minor influence on seed set may be justified. If reduced seed weight of seeds produced under bag isolation is sufficient to inhibit proper seed germination, then such a conclusion would be unjustifiable. Weight reduction of seed produced under isolation, though significant, may not be sufficient to interfere with seed germination.

Influence of plants upon seed set

The amount of open-pollinated seed set upon different plants varies quite widely. The range is significant, as shown by Tables 9 and 10, but the range is gradual. This indicates that open-pollinated seed set varies less significantly than the seed from the same plant under self-pollinated conditions (Tables 7 and 8). The range is sharp and distinct for plants producing the larger amounts of self-pollinated seed. No correlation can be found between the weight or number of selfed seed produced and that of open-pollinated seed produced; because the range of significance is sharp and distinct for plants producing the larger amounts of self-pollinated seed, some influences other than environmental seem to determine the amount of self-pollinated seed set. That some influence other than environmental affects the amount of self-pollinated seed set tends to follow the findings of Myers (1942-B) and Leffel, Kalton and Wassom (1951). The ability of a plant to produce a range of self-pollinated seed varying from zero seeds per bag to the amount of open-pollinated seed produced by plant 28-1-6 would indicate genetic control of a plant's ability to produce self-pollinated seed. The lack of correlation between seed set under open-pollinated conditions and that of self-pollinated seed produced would also indicate that the genetic factor or factors affecting seed set in the open were not the same as those preventing self-pollination.

Since plants used in this experiment varied greatly in the field in many visible characteristics, a few spikes were gathered in order to gain material to make pollen-mother-cell smears. One slide showed a distinct chromosome compliment of 70 chromosomes. Other slides showed variation in chromosome number, some appearing to be octaploid and tetraploid. Since material was gathered early and at random, no connection could be made between chromosome number for the various plants and the amount of self-pollinated seed produced. Variable chromosome numbers could account for much of the significance obtained in various analyses, as well as the wide phenotypic variation of plants in the field. Potential cytological difficulties may be related to self-sterility and therefore require further study for this species.

The variation in percent of self-pollinated seed produced between the 1 and 2-year-old plants could be caused by several factors. First the age of the plants may have some influence toward self-fertility. Gregor and Sansome (1927) thought that the age of a plant did affect self-fertility. The material or number of observations may have actually varied enough to cause the difference, though since the source for plant materials was the same, this is unlikely. The third possibility is the difference in the treatments. Omitting the loop placed in the bag on the 2-year-old plants may have reduced the set of self-pollinated seed 3.5 percent, from the 11.9 percent found for the 1-year-old plants. The highest percent of self-fertility found in this experiment is less than 1/2 that found by Smith (1944), and in configuration (Figure 4) appears closer to the findings of Keller (1948). Under conditions of this experiment, Agropyron elongatum appears to be low in self-fertility. Since a few plants were found that gave no seed and some were found that gave considerable seed under self-pollinated conditions, inbreeding as a method of strain improvement for this species would require further cytological study and breeding.

SUMMARY AND CONCLUSIONS

An experiment was designed and conducted during the summer of 1956 to determine the extent to which Agropyron elongatum could be self-pollinated. Plants were selected for their phenotypic variation. Treatments were arranged to give information about the environmental effects of selfing bags and sleeves upon the set of self-pollinated seed.

Results obtained from this experiment have shown many interesting facts. Much of the information obtained only indicates the need for further investigation in the realm of chromosome numbers and their relation to self-fertility. Cytological and genetic problems need to be solved before intensive inbreeding can be used as a means of improvement of this species.

Conclusions made on the basis of this experiment are as follows:

1. The plant material used has a greater influence on the amount of self-pollinated seed set under bag isolation than environmental influences caused by the isolation bag.

2. The number of spikes enclosed in a selfing bag do not influence the amount of self-pollinated seed set to any great extent.

3. The type of material used to construct selfing bags and the construction of the bags have their effects on the amount of self-pollinated seed obtained. The environmental influences caused by bag material and bag construction are probably far less important to the amount of self-pollinated seed set than the genetic make-up of the plants used in making the study.

4. The greatest influence exerted by the selfing bags upon the set of self-pollinated seed is the reduction of seed weight. Weight of seed is affected more by bag isolation than is the number of seeds produced. It is not known whether the seed weight reduction is detrimental to seed germination and seedling viability.

5. Thirty-five-pound vegetable parchment paper is superior to kraft paper or cloth as an isolating material for tall wheatgrass. Sleeves are superior to bags by comparison of the different bagging materials, on a seed weight basis.

6. Further studies need to be conducted before self-pollination as a means of improvement of this species can be used successfully. Cytogenetic studies will prove most valuable.

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