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CONTROLLED CROSS POLLINATION AND HOT WATER EMASCULATION OF  
CRESTED WHEATGRASS, AGROPYRON DESERTORUM (FISCH.) SHULT.

by

James Edward Hansen

A thesis submitted in partial fulfillment  
of the requirements for the degree

of

MASTER OF SCIENCE

in

Agronomy

UTAH STATE AGRICULTURAL COLLEGE  
Logan, Utah

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James E. Hansen

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## INTRODUCTION

Grasses are more important in our present agricultural economy than ever before, yet the grasslands of the world are far below their production potential. One of the most hopeful approaches to this problem is through breeding better grasses.

Controlled hybridization is a necessary element in the improvement of grasses by breeding. Consequently, emasculation and pollination techniques play vital roles in the work of grass breeders. The majority of our important forage grasses have perfect flowers, and most of these are naturally cross pollinated by the wind. The degree of self fertility varies from species to species and from plant to plant. To insure hybrid seed, breeders must resort to emasculation or the selection of self or male sterile female parents.

Emasculation has been accomplished in various ways. However, hand emasculation has been recognized as the standard method. The minuteness of the floral organs of many grasses makes hand emasculation very difficult or even impossible. Many hours of tedious work are required to produce a small number of hybrid seeds. Emasculation by hot water is another method which is used. This technique is made possible by the fact that the pollen is inactivated at a temperature slightly lower than that required to inactivate the ovary. This method is relatively rapid although not as effective as careful hand emasculation. The effectiveness of hot water emasculation was evaluated under the conditions encountered in this study.

Pollination is a universal problem in controlled hybridization. In some areas the prevalence of a species makes distance isolation laborious and impractical and the pollen control provided may not be complete.

Isolation under bags has also been used. This method has distinct advantages over distance isolation because it can be accomplished in the nursery any time prior to anthesis and furnishes excellent pollen control. However, with presently accepted pollination techniques, seed yields under bags are usually considerably lower than where the inflorescences are left unbagged. It was a prime objective of this study to evaluate several pollination treatments under bags.

An adequate quantity of pollen is required to produce good seed yields under bags. Yet, there is a lack of knowledge as to what quantity of pollen is needed. Is 1 pollinator as effective as 4? This is one of the questions that this study investigated.

Frequently, circumstances demand that controlled hybridization be made even though the clones to be hybridized are not planted adjacent to each other. Under these circumstances the culms of the male, female, or both may be cut off near the soil surface, placed in a container of water, moved to a suitable location for hybridization and bagged. Under such conditions some hybrid seed will be produced. It was an objective of this study to evaluate several solutions as media for detached culms.

## REVIEW OF LITERATURE

Hot water emasculation

Stephens and Quinby (14) in 1933 reported the successful use of hot water to emasculate sorghum. They subjected the inflorescences of genetically marked females to hot water at several temperatures for various periods of time. Their equipment was relatively crude, but they clearly demonstrated that a differential inactivation temperature existed between the pollen and the ovary. The heads that were treated just prior to anthesis at initial water temperatures of  $44^{\circ}$  C. and  $48^{\circ}$  C. for 10 minutes produced 90 percent and 50 percent seed set without the production of any selfed seed.

Domingo (4) demonstrated that hot water was an effective means of emasculating smooth brome grass, Bromus inermis. He treated 7 relatively self-fertile clones at different stages of maturity with hot water of  $37^{\circ}$  C. to  $51^{\circ}$  C. at  $1^{\circ}$  intervals for 5 minutes. The effectiveness of emasculation in his study was measured by the difference in the seed set between treated panicles that were selfed under bags and those that were unbagged. The most effective temperature appeared to be  $47^{\circ}$  C. under field conditions.

Keller (10) found that smooth brome grass panicles treated at water temperatures from  $44^{\circ}$  C. to  $49^{\circ}$  C. for 5 minutes reduced the number of open pollinated seeds by 41 percent. The same treatment reduced the number of selfed seeds under bags 7 times. He stated that the most desirable water temperature was  $47^{\circ}$  C. Tsiang (15) reported a treatment of  $47^{\circ}$  C. for 3 minutes or  $48^{\circ}$  C. for 1 minute was rather satisfactory for the emasculation of brome grass.



Clark (2) reported that a treatment of 45° C. at 6 a.m. appeared as effective for emasculation as 47° C. at noon. However, the treatments applied at noon were less injurious to the plants than those applied in the early morning or late afternoon.

Crested wheatgrass spikes were treated at water temperatures from 43° C. to 48° C. for 5 minutes by Clark (2). He stated that the thermal differential between the male and female gametophytes was best demonstrated by a treatment of 47° C. just prior to anthesis. Knowles and Horner (11) suggested a treatment of 48° C. for 1 minute to emasculate crested wheatgrass.

Fisher (6) treated 27 clones of stiffhair wheatgrass, Agropyron trichophorum, at various water temperatures ranging from 45° C. to 48° C. for 5 minutes. He also demonstrated that the pollen was inactivated at a slightly lower temperature than the ovary. However, he reported that the best temperature for the emasculation of this species was 46° C.

#### Cross pollination under bags

Several types of bags have been used to isolate grasses for controlled pollination. Smith (13) selfed many species of grasses under kraft, parchment, and glassine bags. Parchment bags were considered to be the most satisfactory for controlling pollination even though the results were not completely consistent. It was concluded that the differences in seed set due to the type of bag used were generally minor.

Keller (7) selfed many smooth brome grass plants under 27, 35, and 43 pound parchment bags, 40 pound brown kraft, and 50 pound bleached kraft paper bags. He concluded that 27 or 35 pound parchment bags

were more satisfactory as measured by the seed set than 43 pound parchment, bleached kraft, or brown kraft bags.

Bulk cross pollination under bags in grasses has been attempted in various ways with limited success. Domingo (4) pollinated smooth bromegrass plants by means of the bag transfer method to compare pollination at 4 time-intervals subsequent to anthesis. The average number of seeds per panicle decreased from 32.2 when pollinated immediately following anthesis to 3.4 when pollinated the following afternoon between 1 p.m. and 5 p.m. The average number of seeds per panicle was increased from 5.8 to 62.1 by placing the panicles of the pollen parent above rather than below the panicles of the seed parent.

Clark (2) reported that none of several pollination methods gave satisfactory seed yields when applied to smooth bromegrass, crested wheatgrass, and western wheatgrass.

In the bulk hybridization of smooth bromegrass, Keller (10) used single clones of relative high fertility as the female and male parents. The female panicles were pollinated by detached pollen-bearing panicles which were inserted in the bag just prior to general anthesis. The pollen-bearing panicles were sustained in vials of water. Inflorescences emasculated and bagged until the start of anthesis produced 59 percent as much seed as open-pollinated inflorescences. Inflorescences emasculated and crossed under bags produced 21 percent as much seed as open-pollinated inflorescences. The high seed yields from the inflorescences under some bags clearly indicated that the isolation bag does not interfere with seed production. Nilsson (12) concluded that the bag had only a minor influence on the seed set of several forage grasses.

### Detached culms in solution

Vinogradova (17) reported the following technique as the most practicable method of crossing pasture grasses. Two to 3 days before the female parent flowers, 4 to 6 stems at various stages of flowering are cut from the male parent and placed in a test tube. The detached pollinators are then introduced under the isolator of the female plant and kept there until the end of flowering. Several other grass investigators (2) (10) (6) have used detached pollinators sustained in solution in controlled hybridization.

Keller (9) reported that detached culms of Agropyron ciliare, A. cristatum, A. trachycaulum, A. semicostatum, Bromus carinatus, B. inermis, Hordeum jubatum, Festuca elatior, and Phalaris tuberosa sustained in tap water produced fair amounts of viable seed. Most lots of seeds from detached culms weighed from 40 to 83 percent of those matured on intact culms. Phalaris arundinacea and Phleum pratense failed to produce any seed when detached.

According to Decker (3) the removal of the leaves from detached culms of stiffhair wheatgrass, Agropyron trichophorum (Link) Richt., increased seed yields from 4.4 to 17.4 percent. However, the seed was somewhat lighter in weight.

Sugarcane breeders have used dilute acid solutions as media for detached stalks for crossing purposes. Verrett, et al. (16) studied many solutions in the search for a good medium for detached sugarcane stalks. A 0.05 percent solution of sulfurous acid was best suited for keeping detached stalks alive in an apparent normal condition for several weeks. Brandes and Sartoris (1) reported that sugarcane stalks detached prior to flowering and placed in a solution consisting of

equal parts of 0.01 percent sulfurous and 0.01 percent phosphoric acids produced viable seeds.

## MATERIALS AND METHODS

Controlled pollination under bags without emasculation

Sixty-four clones of crested wheatgrass, Agropyron desertorum (Fisch.) Shult., were selected for high cross fertility from a breeding nursery of approximately 21,000 spaced plants in late March of 1956. Each clone was divided into 2 segments, transplanted into paired rows next to a field of approximately 8,000 crested wheatgrass plants. This and an earlier field planting at the Evans Farm (Forage Experimental Farm, Utah Experiment Station) are shown in figure 1. Paired rows were 6 inches apart and clonal members were planted adjacent to each other within rows at 16 inch intervals. A 25 gauge galvanized piece of tin was pushed into the soil to separate plants opposite in the paired rows. These plants were fertilized with ammonium sulfate at the rate of 200 pounds of elemental nitrogen per acre. They were watered by hand as needed, and the weeds controlled to insure as near optimum conditions for growth as possible.

Twenty-four clones (12 used as male and 12 as female parents) were used to study 7 pollination treatments during the summer of 1956. A randomized block design with 12 replications was used, and each replication consisted of 7 pollination treatments imposed upon the 2 clonal members of a given clone. The clone opposite the female was used as the pollen parent in each instance. Three female and 3 male spikes of similar maturity were used in each treatment. Bagging was accomplished with 35-pound parchment bags 1 to 5 days before general anthesis. In all of the controlled crosses under bags, the male

pollinators were placed above the inflorescences of the female seed parent in a modified mutual pollination scheme.

Female inflorescences were pollinated under bags or left exposed according to the following treatments:

1. Selfed, bag shaken daily for 4 seconds at the height of anthesis, otherwise held rigid during the flowering period.
2. Modified mutual pollination; bag held rigid all during the flowering period.
3. Modified mutual pollination; bag shaken daily for 4 seconds at the height of anthesis, otherwise held rigid during the flowering period.
4. Modified mutual pollination; air current from the worker's mouth was circulated up and down in the bag daily for 4 seconds at the height of anthesis, bag held rigid during the flowering period.
5. Modified mutual pollination; bag shaken daily for 4 seconds, 30 minutes after the height of anthesis, otherwise held rigid during the flowering period.
6. Modified mutual pollination; air current from the worker's mouth was circulated up and down in the bag daily for 4 seconds, 30 minutes after the height of anthesis, bag held rigid during the flowering period.
7. Open pollination.

Each bag was supported by a number 9 galvanized wire with a  $2 \frac{3}{4}$  inch loop in the upper end to prevent the bag from collapsing and damaging the inflorescences. The bottom of each bag was folded over, paper clipped, and sealed with Scotch cellophane and masking tape. The bags were held rigid by placing a number 16 rubber band around the bag just below the loop and attaching it to a 1 inch x  $2 \frac{3}{4}$  inch x 5 foot pointed wooden stake (see figure 2) driven into the ground at the base of the plant. Air from the worker's mouth was circulated into the bags (treatments 4 and 6) by means of a piece of quarter inch rubber tubing, which was fastened to the wire with cellophane tape.

The tubing was plugged with doweling when not in use.

Spikes from the female parents were harvested when the culms began to dry one-half inch below the last spikelet, and were then placed in 1-pound kraft paper bags for storage. The number of spikelets per spike was carefully counted and recorded. Seeds from each spike were threshed out by hand. The number of seeds per spikelet was analyzed statistically.

The effect of the quantity of pollen on seed yield. Sixteen of the 64 clones of crested wheatgrass were used to study the effect of quantity of pollen on seed yield. A randomized block design replicated 12 times was used. The technique used for crossing under bags was the same as that used in treatment 3 of the pollination treatment phase of this study, except that the number of spikes bagged or exposed in each treatment was as follows:

1. Three female spikes selfed,
2. Two female spikes pollinated under a bag by 4 male spikes.
3. Four female spikes pollinated under a bag by 2 male spikes.
4. Three female spikes open pollinated.

Controlled cross pollination under bags following hot water emasculation

The 24 remaining clones were used to evaluate the effectiveness of hot water emasculation and seed yields under bags following emasculation. A randomized block design replicated 12 times was used, but in 3 of the replications the male and female failed to flower simultaneously. Consequently, the data presented are based on 9 replications. Three spikes from the male and 3 from the female plants were subjected to the following treatments:

1. Female emasculated and selfed intact.
2. Female not emasculated and selfed intact.

3. Both parents emasculated, intact, and bagged.
4. Female emasculated, both parents intact and bagged.
5. Female emasculated, male detached, and bagged.
6. Female emasculated, both parents detached and bagged.
7. Female emasculated and open pollinated.

Emascultation was accomplished in 1 day between the hours of 10:30 a.m. and 2 p.m. 1 to 5 days prior to general anthesis. Six spikes were immersed simultaneously in a wide mouth gallon thermos jug filled with water at 47° C. A piece of masonite was placed over the unoccupied portion of the mouth of the thermos jug to reduce changes in the water temperature. During the 5 minute treatment the water temperature dropped less than one-half degree centigrade. The thermos jug was placed in a trench 7 inches deep and 6 inches away from the plant being treated. This facilitated immersion of the spikes with less damage to the short crested wheatgrass culms. To reduce drying of the soil in the root zone, the trench was filled immediately following the completion of the emasculatation treatment.

Culms were detached (treatments 5 and 6) near the soil surface with a sharp razor blade, the lower leaves removed, and the culms inserted into a mason jar filled with water. To prevent heating and evaporation of the solution, the mason jars with modified lids were buried in the soil (see figure 2).

The spikes that were emasculated were allowed to dry off before being bagged. The bags were held rigid during the flowering period, except to be shaken daily for 4 seconds at the height of anthesis.

Spikes from the female were harvested, stored, and threshed in the same manner as in the controlled pollination without emasculatation phase of this study. The seeds were allowed to germinate for a period



of 14 days on porous clay plates at temperatures ranging from 67° to 77° F. Only those seeds which produced normal radicles and plumules were considered viable. The number of seeds per spikelet and the percent viable seeds were analyzed statistically.

Evaluation of several solutions as media for detached culms

Twenty-four additional clones were selected for high cross fertility late in the fall of 1955. These clones were divided into 10 segments, and each clonal piece was planted in gallon cans and placed in the greenhouse. In March of 1956, these clones were cut back and transplanted into rows adjacent to the block of 8,000 crested wheat-grass plants. The 10 clonal members were planted in equal numbers at 2 locations in the design. Management practices were the same as those described for the other planting.

Six clones of similar maturity were selected for use in the detached culm solution study. A randomized block design with 12 replications was used. Each replication consisted of 3 culms that were detached and inserted into quart jars filled with the following solutions:

1. Tap water
2. Bloomlife (a commercial product used to extend the life of cut flowers, produced by Flower Foods Incorporated, Maywood, Illinois)
3. Two-thousandth N sulfuric plus 0.002 N phosphoric acid
4. Two-ten-thousandth N sulfuric plus 0.002 N phosphoric acid
5. Two-hundred-thousandth N sulfuric plus 0.00002 N phosphoric acid.

The jars were arranged by replication near a good source of foreign pollen. Tap water was added daily to each solution until the spikes were harvested.

The length of the flowering period for the 3 spikes in each solution was recorded individually in days and the average was analyzed statistically.

Three open-pollinated intact spikes (in addition to the spikes from the 5 described treatments) were harvested, stored, threshed, and analyzed statistically in the same manner as in the controlled pollination following emasculation phase of this study.

## RESULTS

Controlled pollination under bags without emasculation

The analysis of variance and treatment means of the seeds per spikelet resulting from 7 pollination treatments are given in table 1. Duncan's (5) "new multiple range test" was used to determine significant differences among means. Symbolization and terminology follow Duncan's usage. The shortest significant ranges (or differences) are given in the row headed Rp:. These were computed by multiplying the standard error of a mean ( $S\bar{x}$ ) times tabular values furnished by Duncan. The row headed p: refers to the number of means in the interval being tested. The interval is meant to include the 2 ranked means being tested in addition to those in between. Any 2 treatment means not underlined by the same line are significantly different from each other at the .05 level.

Different clones were used as male and female parents in each replication. Replications were highly significant. This may indicate that the parents differed as to their inherent capabilities to produce seed, pollen, or both. The females that produced small quantities of seed in controlled crosses, without exception, produced small quantities of seed when exposed to open pollination. The same was true for those females that produced large amounts of seed under bags. Inherent differences among the female parents to produce seed probably accounted for most of the differences between replications.

All the other controlled cross pollination treatments resulted in significantly more seeds per spikelet than the bags which were held rigid all during the flowering period (treatment 2). Superior

Table 1. Analysis of variance and treatment means of the number of seeds per spikelet resulting from 7 pollination treatments

Source of Variation	Degrees of Freedom	Mean Square	F
Total	83		
Replications	11	4.765	10.499**
Treatments	6	11.258	24.688**
(4), (6) vs. (3), (5) <sup>1</sup>	1	4.813	10.554**
(5), (6) vs. (3), (4)	1	0.249	0.546
Replications X Treatments	66	0.456	

$\bar{Sx} = 0.195$                       Coefficient of variation = 41.9

Treatments <sup>1</sup>	(1)	(2)	(3)	(5)	(4)	(6)	(7)
Means <sup>2</sup>	0.11	0.44	1.68	1.78	<u>2.27</u>	<u>2.45</u>	<u>2.52</u>
			<u>66.77%</u>	<u>70.69%</u>	90.1	97.2	

p:	2	3	4	5	6	7
Rp:	0.55	0.58	0.60	0.61	0.62	0.63

\*\* Significant at the .01 level

p: Number of means in interval being tested

Rp: Shortest significant range at .05 level for a given p:

1. Numbers within the parenthesis refer to the following treatments:
  1. Selfed.
  2. Modified mutual pollination, bag held rigid all during the flowering period.
  3. Modified mutual pollination, bag shaken daily at the height of anthesis.
  4. Modified mutual pollination, air current from the worker's mouth was circulated up and down in the bag daily at the height of anthesis.
  5. Modified mutual pollination, bag shaken daily 30 minutes after the height of anthesis.
  6. Modified mutual pollination, air current from the worker's mouth was circulated up and down in the bag daily 30 minutes after the height of anthesis.
  7. Open pollinated.
2. Any 2 means not underlined by the same line are significantly different from each other at the .05 level.

treatments featured timely agitation of the pollen within the bags. Air circulated up and down in the bags daily during the flowering period (treatments 4 and 6) seemed to be the most effective technique for controlled cross pollination. Ninety and 97.5 percent as many seeds per spikelet resulted from these pollination treatments as from open pollination. Differences in the seed yields that resulted from the air circulation treatments and the shaking treatments could very well be due to the pollen distribution within the bags. In crested wheatgrass it appears that cross fertile parents and timely pollen distribution are the keys to good hybrid seed yields under bags. It is apparent that the isolation bags had little if any influence on seed sets in this study.

#### Effect of quantity of pollen on seed yield

Even though the maturity among the parents was quite variable, 2.03 seeds per spikelet resulted from 2 spikes pollinating 4 spikes as compared to 2.19 seeds per spikelet that resulted from 4 spikes pollinating 2 (see table 2). When the ratio of male pollinator spikes to female spikes is increased the chance for simultaneous flowering of the parents and the total quantity of foreign pollen are increased. Yet, the number of pollinators seemed to have only a minor influence on seed set.

#### Controlled pollination under bags following hot water emasculation

Emasculation with hot water at 47° C. for 5 minutes reduced the number of viable selfed seeds approximately 9 times as reported in table 3. Yet, when both parents were emasculated, left intact, and bagged (treatment 3), the seed yields were reduced approximately 3 times as much as when only the female spikes were emasculated (treatment 4). This suggests that hot water emasculation has greater power

Table 2. Analysis of variance and treatment means of the number of seeds per spikelet resulting from different numbers of male pollinator spikes in controlled pollination as compared to selfing and open pollination

Source of Variation	Degrees of Freedom	Mean Square	F	
Total	47			
Replications	11	1.529	2.478*	
Treatments	3	16.244	26.327**	
Replications X Treatments	33	0.617		
$\bar{S}\bar{X} = 0.227$		Coefficient of variation = 43.6		
Treatments <sup>1</sup>	(1)	(3)	(2)	(4)
Means <sup>2</sup>	0.13	2.03	<u>2.19</u>	<u>2.83</u>
p:	2	3	4	
Rp:	0.65	0.69	0.71	

\* Significant at the .05 level

\*\* Significant at the .01 level

p: Number of means in interval being tested

Rp: Shortest significant range at .05 level for a given p:

1. Numbers within the parenthesis refer to the following treatments:
  1. Three female spikes selfed.
  2. Two female spikes pollinated under a bag by 4 male spikes.
  3. Four female spikes pollinated under a bag by 2 male spikes.
  4. Three female spikes open pollinated.
2. Any 2 means not underlined by the same line are significantly different from each other at the .05 level.

Table 3. Analysis of variance and treatment means of the number of viable seeds per spikelet resulting from controlled pollination following hot water emasculation as compared to selfing and open pollination without emasculation

Source of Variation	Degrees of Freedom	Mean Square	F
Total	62		
Replications	8	0.829	4.044**
Treatments	6	8.142	39.717**
Replications X Treatments	48	0.205	

$\bar{Sx} = 0.151$                       Coefficient of variation = 49.8

Treatments <sup>1</sup>	(1)	(2)	(3)	(6)	(5)	(4)	(7)
Means <sup>2</sup>	0.03	0.26	0.43	0.65	0.89	1.28	<u>2.86</u>

  

p:	2	3	4	5	6	7
Rp:	0.43	0.45	0.47	0.48	0.48	0.49

\*\* Significant at the .01 level

p: Number of means in interval being tested

Rp: Shortest significant range at .05 level for a given p:

1. Numbers within the parenthesis refer to the following treatments:

1. Female emasculated and selfed intact.
2. Female not emasculated and selfed intact.
3. Both parents emasculated, intact, and bagged.
4. Female emasculated, both parents intact and bagged.
5. Female emasculated, male detached, bagged.
6. Female emasculated, both parents detached and bagged.
7. Female not emasculated and open pollinated.

2. Any 2 means not underlined by the same line are significantly different from each other at the .05 level.

to inactivate pollen being used in self fertilization than when it is used in cross fertilization.

Female spikes in treatment 4 produced 44.7 percent as many viable seeds per spikelet as the spikes that were not emasculated and open pollinated (treatment 7). If the competitive effect exerted by foreign pollen is ignored, approximately 98 percent of the seed produced in treatment 4 was hybrid as compared to 91 percent in treatment 7. The percent hybrid seed was computed by subtracting the estimated percent selfed seed from the total percentage. Similarly treated spikes from the female parents involved in the crosses were selfed (treatments 1 and 2) to obtain the estimated number of selfed seeds. The estimated number of selfed seeds divided by the total number of seeds (treatments 4 and 7) equals the estimated percent selfed seeds. In this species, if a high percent of hybrid seed is required, emasculation would be desirable.

Female spikes that were emasculated, left intact, and bagged with detached pollinators sustained in water (treatment 5), produced 0.89 of a viable seed per spikelet as compared to 1.28 in treatment 4. The difference between these treatments is almost of sufficient magnitude for significance at the .05 level. Female spikes that were emasculated, detached, and bagged with detached pollinators (treatment 6), yielded significantly fewer viable seeds than treatment 4. It appears that intact culms when applicable are superior to detached culms in controlled hybridization.

The percent viable seeds from spikes that were emasculated compared favorably with spikes that were not emasculated (see table 4). The decrease in the viability of the seeds from the female spikes in treatment 6 was probably mostly due to the seeds developing on detached



Table 4. Analysis of variance and treatment means of the percent viable seed resulting from controlled pollination following hot water emasculation as compared to selfing and open pollination without emasculation

Source of Variation	Degrees of Freedom		Mean Square	F		
Total	51					
Replications	8		511.5	5.7**		
Treatments	5		151.8	1.7		
Replications X Treatments <sup>1</sup>	38		89.7			
$\bar{Sx} = 2.7$		Coefficient of variation = 10.6				
Treatments <sup>2</sup>	(6)	(3)	(5)	(4)	(2)	(7)
Means	82	88	90	91	91	94

\*\* Significant at the .01 level

1. One degree of freedom was subtracted from the number associated with the error mean square for each of the 2 missing observations.
2. Numbers within the parenthesis refer to the following treatments:
  1. Female emasculated and selfed intact. (Omitted because too few seeds were produced to obtain reliable viability percents.)
  2. Female not emasculated and selfed intact.
  3. Both parents emasculated, intact, and bagged.
  4. Female emasculated, both parents intact and bagged.
  5. Female emasculated, male detached, bagged.
  6. Female emasculated, both parents detached and bagged.
  7. Female not emasculated and open pollinated.

culms, rather than the emasculation treatment. Data from the spikes that were selfed and emasculated (treatment 1) were not presented in table 4 because too few seeds were produced to obtain reliable viability percents.

#### Evaluation of several solutions as media for detached culms

The relative efficiency of 5 solutions as media for detached culms was measured by the length of the flowering of the culms, the number of viable seeds per spikelet, and the percent viable seeds from detached culms when exposed to open pollination.

Water, 0.00002 N sulfuric plus 0.00002 N phosphoric acid solution, and 0.0002 N sulfuric plus 0.0002 N phosphoric acid solution generally appeared to be superior media to 0.002 N sulfuric plus 0.002 N phosphoric acid solution and Bloomlife solution as media for detached culms (tables 5, 6, and 7). Detached culms in the most concentrated acid and Bloomlife solutions began to turn yellow and brown within 3 to 4 days subsequent to detaching. Apparently these solutions inhibited the normal development of the culms. For crested wheatgrass, water seemed to be as good a medium for detached culms as any of the solutions used.

The low viable seed yield reported in table 6 for the culms intact (treatment 6) may be due to the generally poor conditions for pollination that prevailed at the time of flowering. Most all the surrounding crested wheatgrass plants had completed their flowering before these plants began to flower. The detached culms (treatments 1 through 5) were placed near a good localized source of foreign pollen.

Table 5. Analysis of variance and treatment means of the number of days of flowering of detached culms in 5 solutions

Source of Variation	Degrees of Freedom	Mean Square	F		
Total	59				
Replications	11	6.31	3.41**		
Treatments	4	9.20	4.97**		
Replications X Treatments	44	1.85			
$S\bar{x} = 0.39$		Coefficient of variation = 17.1			
Treatments <sup>1</sup>	(3)	(2)	(4)	(5)	(1)
Means <sup>2</sup>	6.6	<u>7.6</u> <u>6.7</u>	<u>8.3</u>	<u>8.4</u>	<u>8.8</u>
p:	2	3	4	5	
Rp:	1.11	1.17	1.21	1.23	

\*\* Significant at the .01 level

p: Number of means in interval being tested

Rp: Shortest significant range at .05 level for a given p:

- Numbers within the parenthesis refer to the following treatments:
  - Tap water.
  - Bloomlife.
  - Two-thousandths N sulfuric plus 0.002 N phosphoric acid solution.
  - Two-ten-thousandths N sulfuric plus 0.0002 N phosphoric acid solution.
  - Two-hundred-thousandths N sulfuric plus 0.00002 N phosphoric acid solution.
- Any 2 means not underlined by the same line are significantly different from each other at the .05 level.

Table 6. Analysis of variance and treatment means of the number of viable seeds per spikelet from detached culms in 5 solutions and intact culms

Source of Variation	Degrees of Freedom		Mean Square	F		
Total	71					
Replications	11		1.126	5.716**		
Treatments	5		2.625	13.325**		
Replications X Treatments	55		0.197			
$\bar{Sx} = 0.128$		Coefficient of variation = 51.0				
Treatments <sup>1</sup>	(3)	(2)	(4)	(1)	(5)	(6)
Means <sup>2</sup>	0.08	0.76	0.86	0.98	1.09	<u>1.49</u>
p:	2	3	4	5	6	
Rp:	0.36	0.38	0.39	0.40	0.41	

\*\* Significant at the .01 level

p: Number of means in interval being tested

Rp: Shortest significant range at .05 level for a given p:

1. Numbers within the parenthesis refer to the following treatments:
  1. Tap water.
  2. Bloomlife.
  3. Two-thousandths N sulfuric plus 0.002 N phosphoric acid solution.
  4. Two-ten-thousandths N sulfuric plus 0.0002 N phosphoric acid solution.
  5. Two-hundred-thousandths N sulfuric plus 0.00002 N phosphoric acid solution.
  6. Intact culms.
2. Any 2 means not underlined by the same line are significantly different from each other at the .05 level.

Table 7. Analysis of variance and treatment means of the percent viable seeds from detached culms in 5 solutions and intact culms

Source of Variation	Degrees of Freedom	Mean Square	F			
Total	68					
Replications	11	302.2	1.5			
Treatments	5	3233.3	16.1**			
Replications X Treatments <sup>1</sup>	52	200.3				
$\bar{Sx} = 4.1$		Coefficient of variation = 20.0				
Treatments <sup>2</sup>	(3)	(2)	(4)	(1)	(5)	(6)
Means <sup>3</sup>	45	61	72	76	79	<u>93</u>
p:	2	3	4	5	6	
Rp:	11.64	12.26	12.67	12.91	13.16	

\*\* Significant at the .01 level

p: Number of means in interval being tested

Rp: Shortest significant range at .05 level for a given p:

- One degree of freedom was subtracted from the number associated with the error mean square for each of the 3 missing observations.
- Numbers within the parenthesis refer to the following treatments:
  - Tap water.
  - Bloomlife.
  - Two-thousandths N sulfuric plus 0.002 N phosphoric acid solution.
  - Two-ten-thousandths N sulfuric plus 0.0002 N phosphoric acid solution.
  - Two-hundred-thousandths N sulfuric plus 0.00002 N phosphoric acid solution.
  - Intact culms.
- Any 2 means not underlined by the same line are significantly different from each other at the .05 level.

## DISCUSSION

In the interpretation of the results some consideration should be given to the variation in maturity among parent plants. The clones were cut into 2 pieces and transplanted early in the spring and this to some extent upset their normal flowering period. Controlled crosses were made later the same year, however, data from clones involved in specific crosses that failed to flower simultaneously were not included in the analysis of variance.

Superior controlled cross pollination treatments all featured timely agitation of the pollen within the bags. Air circulated up and down in the bags daily subsequent to anthesis seemed to be the most effective technique for controlled cross pollination. Ninety and 97.5 percent as many seeds per spikelet resulted from this pollination technique as from open pollination. Spikes that were cross pollinated under bags which were held rigid throughout the flowering period, yielded only 17.5 percent as many seeds as the open pollinated. In crested wheatgrass, it appears that cross fertile parents and timely pollen distribution are the keys to good hybrid seed yields under bags. Data presented in this paper support Nilsson (12), Smith (13), and Keller (7) who found that isolation bags had little if any influence on seed set.

Atmospheric air movement is highly variable from location to location, from season to season, and from day to day. It is quite possible that on warm, calm afternoons when most of the pollen is shed in many of the cool season grasses, the agitation within undisturbed, isolation

bags is insufficient for proper pollen distribution. This may be the reason why grass breeders have reported unsatisfactory hybrid seed yields under bags when compared to distance isolation.

The number of seeds per spikelet that resulted from 2 spikes pollinating 4 spikes was 2.03 as compared to 2.19 that resulted from 4 spikes pollinating 2. The number of pollinators seemed to have only a minor influence on seed set.

Emasculation with hot water at 47° C. for 5 minutes reduced the number of viable selfed seeds approximately 9 times. Keller (10) applied a similar treatment to smooth brome grass and the number of selfed seeds was reduced about 7 times. The increase in the effectiveness of the emasculation treatment may be due to differences between the species concerned. The results of this study suggest that hot water emasculation has greater power to inactivate pollen being used in self fertilization than when it is used in cross fertilization.

The female spikes that were emasculated, left intact, and bagged with pollinators intact, yielded 44.7 percent as many viable seeds per spikelet as the spikes that were not emasculated and open pollinated. If the competitive effect exerted by foreign pollen is ignored, approximately 98 percent of the seed produced in the former treatment was hybrid as compared to 91 percent in the later treatment. In this species, if a high percent of hybrid seed is required, emasculation would be desirable.

Brome grass panicles that were emasculated and open pollinated yielded 59 percent as many seeds as panicles that were not emasculated and open pollinated (10). Assuming that the injury to the female floral organs in crested wheat grass is approximately the same as in brome grass, the efficiency of the shaking treatment in pollination

following emasculation of the female spikes compared favorably to the efficiency of the same treatment when the female spikes were not emasculated. This suggests that the total number of viable seeds per spikelet following emasculation could have been increased by using the air circulation pollination technique.

Female spikes that were emasculated, left intact, and bagged with detached pollinators sustained in water, yielded 31 percent as many viable seeds as spikes that were not emasculated and open pollinated. Keller (10) (8) reported bromegrass panicles subjected to a relatively similar treatment yielded 21 percent as many seeds as panicles that were not emasculated and open pollinated. In the study being reported the bags were shaken daily at the height of anthesis during the flowering period. The difference in the seed yields could very well have been due to the pollen distribution within the bags during the flowering period.

Female spikes that were emasculated, detached, and bagged with detached pollinators, yielded significantly fewer viable seeds than when the culms of both parents were left intact. It appears that intact culms when applicable are superior to detached culms in controlled hybridization.

Water, 0.00002 N sulfuric plus 0.00002 N phosphoric acid solution, and 0.0002 N sulfuric and 0.0002 N phosphoric acid solution generally appeared to be superior to 0.002 N sulfuric plus 0.002 N phosphoric acid solution and Bloomlife solution as media for detached culms. For crested wheatgrass, water seems to be as good a medium for detached culms as any of the solutions used. Verret, et al. (16) and Brandes and Sartoris (1) reported that dilute acid solutions were generally better media for detached sugar cane stalks than water. Although the



acid solutions used were not exactly the same, the different results are probably due to the differences in the species concerned.

## SUMMARY

Twenty-four clones of crested wheatgrass, Agropyron desortorum, of high cross fertility were used to evaluate 7 pollination treatments. Superior controlled cross pollination treatments featured timely agitation of the pollen within the bags. Air circulated up and down in the bags daily during the flowering period seemed to be the most effective technique for controlled cross pollination used. Ninety and 97.5 percent as many seeds per spikelet resulted from this pollination technique as from open pollination. Spikes that were cross pollinated under bags which were held rigid during the flowering period, yielded 17.5 percent as many seeds per spikelet as spikes that were open pollinated. In this species, it appears that cross fertile parents and timely pollen distribution are the keys to good hybrid seed yields under bags. The parchment isolation bags had little if any influence on seed sets.

Sixteen clones were used to study the effect of the quantity of pollen on seed yields. The number of seeds per spikelet that resulted from 2 spikes pollinating 4 spikes was 2.03 as compared to 2.19 that resulted from 4 spikes pollinating 2. The number of pollinators seemed to have only a minor influence on seed set.

Twenty-four clones were used to evaluate the effectiveness of hot water emasculation and the seed yields under bags following emasculation. Emasculation with hot water at 47° C. for 5 minutes reduced the number of viable selfed seeds about 9 times. Female spikes that were emasculated, left intact, and bagged with pollinators intact, produced

44.7 percent as many viable seeds as the spikes that were not emasculated and open pollinated. In this species, if a high percent of hybrid seed is required, emasculation would be desirable. It appears that intact culms when applicable are superior to detached culms in controlled hybridization.

Six clones were used to evaluate 5 solutions as media for detached culms. Water, 0.00002 N sulfuric plus 0.00002 N phosphoric acid solution, and 0.0002 N sulfuric plus 0.0002 N phosphoric acid solution generally appeared to be superior to 0.002 N sulfuric plus 0.002 N phosphoric acid solution and Bloomlife solution as media for detached culms. For this species, water seemed to be as good a media for detached culms as any of the solutions used.

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