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# Bulk Hybridization of Smooth Bromegrass (*Bromus inermis*)

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# BULK HYBRIDIZATION OF SMOOTH BROMEGRASS

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A thesis submitted in partial fulfillment of the requirements

for the degree of

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Wayne E. Domingo

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# INTRODUCTION

Large populations of controlled hybrids are essential to the most rapid progress in many phases of plant breeding programs. Plant species vary in the ease with which they may be hybridized. Hand hybridization of forage grasses is usually slow and laborious, and the minuteness of the floral parts of most of the species which have perfect flowers renders their hybridization by hand especially difficult and tedious. This difficulty limits the use that forage grass breeders are making of the significant principles of hybridization and thereby retards progress in this phase of plant breeding. Any dependable, rapid technique of hybridization which would eliminate many of the present hand operations, that is "bulk" hybridization, would make possible more rapid progress in the breeding of forage grasses.

The study herein reported was designed to estimate the feasibility of applying various methods of bulk emasculation and bulk pollination to forage grasses. In limiting the scope of the study, smooth bromegrass (Bromus inermis) was selected to receive the greatest attention because of its importance among forage grasses and the wide range of self-fertility among individual plants of the species, a characteristic which proved very helpful in the study.

## ISOLATION EQUIPMENT

Isolation was effected, in all cases, by means of 3 x 26 inch kraft bags shown in use in figure 2, page 25. Each bag was supported by a 4-foot no. 9 wire, one end of which was thrust into the ground at the base of the plant and the other end enclosed in the bag that it supported. A 1 3/4 inch loop in the upper end of the wire prevented the sides of the bag from collapsing against the inflorescences.*

Observations of the flowering processes of isolated panicles were facilitated by 1 3/4 inch square holes cut near the sealed ends of the bags and covered with a transparent plastic, Plastacele.** A good seal between the kraft and the Plastacele was obtained by soaking 1/4 inch of the border of a 2 3/4 inch square of Plastacele in acetone until a layer of partially dissolved plastic was formed (45 to 60 seconds), immediately placing the square over the 1 3/4 inch hole in the kraft bag, and pressing the edges of the plastic firmly to force the partially dissolved plastic into the pores of the kraft. The bag was ready for use as soon as the highly volatile acetone had evaporated.

Such adhesives as glue, battery wax, and a mixture of beeswax and rosin did not adhere to both the kraft and celluloid windows.

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* The bags were manufactured by the Specialty Department of the Union Bag and Paper Corporation of Chicago, Illinois. They can be obtained from most variety stores, where they are used in retailing merchandise.

** Plastacele of 0.005 inch thickness was purchased from du Pont de Nemours and Co., Arlington, New Jersey.

## EMASCULATION

### Review of Literature

Bulk emasculation has been attempted on at least 4 species of the Gramineae family by the utilization of a condition wherein the staminate organs of the perfect flowers are more susceptible to extremes of temperatures than are the pistillate organs.

Stephens and Quinby (10)* investigated the possibility of emasculating sorghum plants by subjecting the inflorescences to hot water at various temperatures for various lengths of time. Although their results were not consistent, the evidence obtained indicated that such emasculation was possible and deserved further investigation. Complete emasculation, as evidenced by the character of the endosperm of seeds produced following hand pollination of treated heads, was effected on two of the treated heads. The heads were treated for 10-minute periods just prior to normal anthesis at initial water temperatures of 44 and 48 degrees Centigrade. Neither head produced any selfed seed; the former produced 533 hybrid seeds and the latter 362, representing 90 percent and 50 percent seed-set respectively.

Jodon (5) emasculated rice by hot-water treatments at 40 to 44 degrees Centigrade and 0 to 6 degrees Centigrade for 10 minutes. Over 1000 florets were treated at temperatures varying between 40 and 44 degrees, only one of which produced a selfed seed. In other trials, from 5 percent to 73 percent of the florets treated in the same temperature range and hand pollinated produced seeds, and of

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* Figures in parentheses refer to "Literature Cited", page 32.

them the selfed seeds were "too few to be of importance".

Li et al. (6) observed no differential in the thermal death points of the staminate and pistillate organs of millet (Setaria italica). Few hybrid seeds were produced on heads which were isolated with normal flowering heads as a pollen source after being treated with hot water over a range of temperatures some of which permitted normal selfed seed to be produced and others of which destroyed both male and female organs. Since little hybrid seed was produced by this normally cross-pollinated species, even on heads which received the less severe treatments and which produced many selfed seeds, it seems possible that lack of hybrid seeds might have been due, not to lack of differential in the thermal death points of the two types of organs, but to some other factors such as failure of normal opening of the glumes.

Suneson observed widespread sterility in wheat exposed to frosts at the bloom stage. In later experiments (11) he obtained partial emasculation of wheat by subjecting plants to temperatures varying between 27 and 36 degrees Farenheit for 15 to 24 hours one to five weeks before heads emerged from the boot.

## Experimental Methods and Results

### Plant Material

Bulk emasculation of smooth bromegrass was attempted in the summer of 1938 by treating the panicles with hot water, hot air, and cold air.

Plants used were second-year clones of smooth bromegrass plants which had been selected on the basis of their relatively high self-

fertility. Treatments were made at 7 different stages of maturity, beginning when the panicles were emerging from the boot and continuing at 4-day intervals thereafter until just prior to anthesis.

## Criterion of Success

The success of emasculation was measured in all cases by selecting relatively self-fertile plants for treatment, dividing the 4 stems simultaneously receiving a given treatment into 2 groups, isolating one group in a bag and exposing the other to wind-borne pollen from adjacent plants, and comparing the seed-sets on the 2 groups of stems. Treatments which effected complete emasculation without appreciable injury to the female organs were detected by lack of seed-set on the isolated panicles accompanied by formation of seed on the panicles which received the same treatment but remained exposed to atmospheric pollen. The lack of seed set on isolated panicles indicated that as far as the formation of selfed seed was concerned the panicles were emasculated, and the formation of seed on the corresponding exposed panicles indicated that the female organs were still functional.

## Hot-Water Treatments

Hot water treatments were made for 5 minutes at one-degree intervals from 37 to 51 degrees Centigrade at each of the 7 stages of maturity by immersing the panicles in water contained in a one-gallon thermos jug.

In anticipation of stems being too short and brittle at the early stages of maturity to bend sufficiently to allow the heads to

be immersed in the thermos jug, the plants which were to be treated at the first 4 stages of maturity (4, 8, 12, and 16 days after the boot stage) were grown in nail kegs which at the time of treatment were laid on their sides thereby facilitating immersion of the panicles in the hot water. After treatment the kegs were buried in soil to a depth equal to the height of the kegs, in the center of a 900-plant, spaced, bromegrass planting to insure abundant atmospheric pollen. Except in cases of excessively short stems, most smooth bromegrass stems can be bent sufficiently near their bases to permit immersion of the panicles into water containers without breakage.

The percentages of seed-set (number of seeds produced divided by the total number of florets) obtained on the panicles which were either isolated or exposed following 5-minute, hot-water treatments of 43 to 50 degrees Centigrade are shown in table 1. The table indicates that several of the treatments prevented the formation of selfed seed yet permitted the formation of seeds on similarly treated panicles which were exposed to atmospheric pollen. More confidence is placed on the data from the last 3 stages of maturity than on those from the first 4 stages which represent the plants grown in nail kegs where existed abnormal conditions, because of accidental exposure to drought, which may have influenced seed-set. Seven of the treatments made at the last 3 stages of maturity prevented seed-set on the isolated panicles yet permitted an average of 67 seeds per panicle to be produced on similarly treated panicles exposed to atmospheric pollen. Those treatments were 45, 46, and 47 degrees Centigrade 20 days after the boot stage; 47, 48, and 49 degrees Centigrade 24 days after the boot stage; and 46 degrees Centigrade 28 days after the

Table 1. Percent seed-set on groups of 2 panicles of smooth bromegrass which were either (1) isolated or exposed to atmospheric pollen, or (2) isolated or exposed to atmospheric pollen after being treated with hot water at 43 to 50 degrees Centigrade for 5 minutes at each of 7 stages of maturity

| Days after boot stage | | 0 | | 4 | | 8 | | 12 | | 16 | | 20 | | 24 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location of panicles | | Exp'd | Bag'd | Exp'd | Bag'd | Exp'd | Bag'd | Exp'd | Bag'd | Exp'd | Bag'd | Exp'd | Bag'd | Exp'd | Bag'd |
| Temperature, degrees Centigrade | 50 | 52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 49 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 |
| | 48 | 51 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 |
| | 47 | | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 54 | 0 | 24 | 0 | | |
| | 46 | 31 | 0 | 0 | 0 | 23 | 0 | 0 | | 43 | 0 | 71 | 2 | 29 | 0 |
| | 45 | 40 | 0 | 12 | 0 | 39 | 0 | 24 | 0 | 59 | 0 | 93 | 11 | 39 | 1 |
| | 44 | 63 | 0 | 55 | 5 | 49 | 0 | 59 | 0 | | 12 | 91 | 8 | 41 | 3 |
| | 43 | 38 | 0 | 39 | 4 | 22 | 0 | 40 | 0 | 78 | 18 | 88 | 8 | 48 | 2 |
| | none | 59 | 8 | 69 | 13 | 62 | 0 | 61 | 0 | 81 | 24 | 88 | 31 | 65 | |

boot stage or just prior to normal anthesis. Since a plant of different genotype was treated at each of these 3 stages, it is possible that the inconsistency in the critical temperature was due to plant differences rather than to maturity differences.

## Hot-Air Treatments

Hot-air treatments were also made over the range of 37 to 51 degrees Centigrade at each of the 7 stages of maturity. The hot-air apparatus, figure 1, was designed to permit treatment of panicles without bending the stems and to eliminate the dead-air space which makes accurate temperature control very difficult. It consisted of a collapsible, rubber-walled air-chamber within a metal chamber containing the temperature-controlling water. The entire apparatus was lowered over the upright stems of a plant, after which the rubber-walled chamber was permitted to collapse from the weight of the water. This forced most of the air from the chamber and placed the panicles in close contact with the heat source.

The percentages of seed-set obtained on panicles which were either isolated or exposed following treatments by hot air at 43 to 50 degrees Centigrade at the 7 stages of maturity are shown in table 2. This table indicates that several of the treatments prevented the formation of selfed seed-set yet permitted the formation of hybrid seed on similarly treated panicles. It will be noted that the results are more erratic than those obtained by the hot-water treatments. No differential in the thermal death points of the staminate and pistillate organs was observed on panicles treated at stages 4 and 6; while 47, 48, 49, and 50 degree treatments effectively emasculated

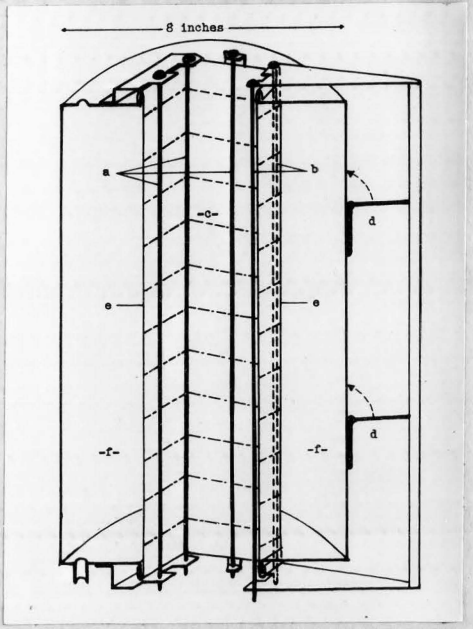


Figure 1. Cross section of collapsible, rubber-walled air-chamber within a metal chamber containing a temperature-controlling liquid. It was used to treat panicles of smooth bromegrass with hot air and cold air. Stationary wire stays (a) and movable wire stays (b) maintained the air-chamber (c) in a distended condition, as shown, while the apparatus was lowered over upright stems. Hinges (d) were then moved to the position indicated by the arrows. The movable stays then allowed the rubber walls (e) to collapse from the weight of the water (f). Much of the dead-air space was thereby eliminated and the panicles were separated from the heat source by only a thin sheet of rubber

Table 2. Percent seed-set on groups of 2 panicles of smooth bromegrass which were either (1) isolated or exposed to atmospheric pollen, or (2) isolated or exposed to atmospheric pollen after being treated with hot air at 43 to 50 degrees Centigrade for 5 minutes at each of 7 stages of maturity

| Days after boot stage | | 0 | | 4 | | 8 | | 12 | | 16 | | 20 | | 24 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location of panicles | | Exp'd | Bag'd | Exp'd | Bag'd | Exp'd | Bag'd | Exp'd | Bag'd | Exp'd | Bag'd | Exp'd | Bag'd | Exp'd | Bag'd |
| Temperature, degrees Centigrade | 50 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 7 | 0 | 48 | 8 | 2 | 0 |
| | 49 | 21 | 0 | 17 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 49 | 4 | 6 | 0 |
| | 48 | 47 | 0 | 20 | 3 | 3 | 0 | 60 | 3 | 48 | 0 | 55 | 6 | 22 | 0 |
| | 47 | 82 | 3 | 28 | 0 | 36 | 0 | 51 | 1 | 82 | 0 | 62 | 12 | 51 | 0 |
| | 46 | 82 | 7 | 21 | 0 | 42 | 0 | 67 | 3 | | | 58 | 4 | 44 | 2 |
| | 45 | 74 | 1 | 27 | 0 | 41 | 0 | 75 | 4 | 66 | 6 | 47 | 10 | 49 | 7 |
| | 44 | 81 | 5 | 16 | 7 | 45 | 2 | 76 | 14 | 78 | 9 | 62 | 7 | 60 | |
| | 43 | 78 | 7 | 41 | 4 | 48 | 3 | 83 | 12 | 81 | 12 | 58 | 21 | 46 | 18 |
| | none | 78 | 24 | 40 | 7 | 49 | 5 | 59 | 21 | 78 | 7 | 60 | 1 | 56 | 20 |

the panicles treated at stages 5 and 7.

## Cold-Air Treatments

Panicles were treated with cold air at near 0 degrees Centigrade for periods of 5, 10, and 20 minutes at each of the 7 stages of maturity. The apparatus described above was used and a mixture of ice, salt, and water in the outer portion supplied the temperature desired. None of the cold-air treatments used injured the floral organs appreciably as evidenced by the absence of appreciable decrease in the amount of seed produced on treated, selfed panicles as compared with that produced on panicles that were merely selfed.

## Conclusions

Bulk emasculation seems quite feasible by hot-water treatments and less so by either hot-air or cold-air treatments as used in these experiments. The less intimate contact between the florets and the heat source in the hot-air method, as compared with the hot-water method, may account for the more erratic results obtained thereby. Cold-air treatments for periods of time longer than 20 minutes or at temperatures other than 0 degrees may be effective, but for equal periods of time the hot-water treatments are distinctly superior to the cold-air treatments at 0 degrees.

Lower temperatures for longer periods of time would probably give similar results as was shown by the thermal death point of Bromus polyanthus when treated with hot air at 47 to 50 degrees Centigrade for periods of 5, 10, and 20 minutes. The results, presented in table 3, indicate that a 50 degree treatment for 5 minutes

Table 3. Number of seeds produced on groups of 2 panicles of Bromus polyanthus when isolated after being treated with hot air at 47, 48, 49, and 50 degrees Centigrade for periods of 2, 5, 10, and 20 minutes

| Temperature | Duration of treatment | | | |
|---|---|---|---|---|
| degrees C. | 2 minutes | 5 minutes | 10 minutes | 20 minutes |
| 50 | 117 | 0 | 3 | 0 |
| 49 | 21 | 21 | 6 | 0 |
| 48 | 68 | 19 | 4 | 0 |
| 47 | 55 | 12 | 1 | 12 |

destroys tissue to the same extent as does a 48 degree treatment for 20 minutes. Five-minute treatments appear sufficiently long for emasculation and yet allow for more uniform distribution of heat than may be possible with treatments of shorter duration.

## POLLINATION

### Review of Literature

Bulk pollen transfers have been made on several species of the Gramineae family by several different methods.

Weatherwax (12) used kraft bags to collect corn pollen and transfer it to the silks of the female parents.

Jelinek (3) and Rosenquist (9), working with wheat, and Reed (8), working with sorghum, obtained hybrids by isolating heads of the male and female parents in the same isolation bag. Rosenquist obtained an average of 10.9 hybrid seeds on 55 hand-emasculated wheat spikes. Reed obtained 8.2 percent hybrid seed on unemasculated sorghum heads by the same method.

Collected pollen of corn has been applied to the silks by means of an atomizer, a method reported by Coulter (2).

Hybrids have been produced on corn by Jenkins (4) and on wheat by Rosenquist (9) by placing the severed, pollen-bearing portion of the male parent in a vial of water inside the bag which isolated the female organs. Jenkins found that pollen was shed over a longer period of time when the water was used than when no water was used, resulting in more seeds per ear. Rosenquist produced an average of 14.3 seeds per hand-emasculated spike by this method.

Pope (7) and Reed (8), working with barley and sorghum respectively, obtained hybrids by shaking the pollen-shedding heads of the staminate parent over the heads of the pistillate parent. Pope's pollinations resulted in production of seed on 91 percent of 566 barley florets, and Reed obtained from less than 1 percent to over 18 percent hybrid seed-set on unemasculated sorghum plants.

## Experimental Methods and Results

### Plant Material

Bulk pollen transfers from male to female parents were attempted by 6 different methods, one in the summer of 1938 and 5 additional ones in the summer of 1939.

Plants used in the summer of 1938 were 3-year-old spaced plants of smooth bromegrass selected on the basis of their relatively low self-fertility, and those used in the summer of 1939 were second-year clones of individual plants from this spaced planting also selected because of their low self-fertility.

### Criterion of Success

Hybrid seed could not be identified as such because of the lack of any known genetic markers.

At least 4 panicles on each of the relatively self-sterile female parent plants were selfed. Other panicles on the same plants were pollinated by the several transfer methods with pollen from unrelated plants when many stigmas were extruded. Success of pollen transfers was measured by comparing the amount of seed produced on the selfed panicles with the amount produced on panicles of the same

plant to which foreign pollen had been transferred. The number of seeds produced on the pollinated stems in excess of the number produced on the selfed stems of the same plant was assumed to be hybrid seed and the result of the pollen transfer.

Bag Transfer

Nearly 800 stems on 30 plants were pollinated in the summer of 1938 by allowing the pollen of the male parents to dehisce into an isolation bag and transferring that bag to the previously isolated panicles of the female parents. Some of the pollinations were made at various intervals of time following anthesis, which usually occurred in late afternoon, and others were repeated on consecutive days during the week of greatest anthesis but at various times during the day. These data are presented in table 4. Over 400 selfed panicles on the 30 female parent plants indicated self-fertility sufficient to produce an average of only 2.4 seeds per stem under kraft bags.

Two hundred stems were pollinated by this method immediately after pollen had been shed, with a resultant increase in seed-set of 29.2 seeds per stem over selfed seed-set. When pollen transfers were delayed until 5:30 a.m. to 8:00 a.m. on the morning following anthesis the 147 stems thus pollinated produced an increase of 13 seeds per stem over selfed seed-set. Pollinations of 111 stems made between 8:00 a.m. and noon on the day following anthesis resulted in an average increase of 5.6 seeds per stem. Pollination of 186 stems between 1:00 p.m. and 5:00 p.m. on the day following anthesis, but prior to any pollen-shedding on that day, resulted in an increase in seed-set of 1.6 seeds per stem which is not significant at the

Table 4. Seed production on panicles of relatively self-sterile plants of smooth bromegrass following bag transfer of pollen at 4 time-intervals subsequent to anthesis and for various numbers of times during the week of greatest anthesis but at various times during the day, compared with seed production on the same plants by selfing

| Pollinations made | Number of plants used as female parents | Number of bags transferred | Total number of panicles pollinated | Avg. number of seeds per panicle | | |
|---|---|---|---|---|---|---|
| | | | | On pollinated stems | On selfed stems | Gain due to transfer |
| (a) In late afternoon immediately following anthesis | 18 | 51 | 200 | 32.2 | 3.0 | ** 29.2 |
| (b) From 5:30 a.m. to 8:00 a.m. on the morning following anthesis | 12 | 38 | 147 | 16.5 | 3.5 | ** 13.0 |
| (c) From 8:00 a.m. to noon on the morning following anthesis | 11 | 29 | 111 | 9.1 | 3.4 | * 5.6 |
| (d) From 1:00 p.m. to 5:00 p.m. on the afternoon of the day following anthesis but prior to anthesis on the same day | 19 | 49 | 186 | 3.4 | 1.8 | 1.6 |
| (e) Once on each of 3 consecutive days during the week of greatest anthesis but at various times during the day (see text) | 15 | 18 | 67 | 13.0 | 3.6 | ** 9.4 |
| (f) Once on each of 5 consecutive days during the week of greatest anthesis but at various times during the day (see text) | 11 | 20 | 74 | 18.4 | 3.5 | ** 14.9 |

* Significant at the 5-percent level.
** Significant at the 1-percent level.

5-percent level. These data indicate that the pollen of smooth bromegrass loses its viability within 24 hours after being shed under conditions existent in kraft bags and therefore stress the necessity of making such pollinations within a few hours after anthesis.

Two series of pollinations by the bag transfer method were repeated on consecutive days during the week of greatest anthesis but at various times during the day. Sixty-nine panicles were pollinated on each of 3 consecutive days, and 74 panicles were pollinated on each of 5 consecutive days. Stems pollinated on 3 consecutive days produced an increase of 9.4 seeds per stem, while those pollinated on 5 consecutive days produced an increase of 14.9 seeds per stem. All of the pollinations were made between 8:00 a.m. and noon on days following anthesis with the exception of one day on which a pollination of both the 3-day- and the 5-day-series was made. On that day pollinations were made late in the afternoon when a small amount of pollen had been shed when the 3-day-series pollination was made and more pollen had been spilled later in the afternoon when the 5-day-series pollination was made. The greater seed-set increase of the 5-day-series is possibly due more to the greater amount of pollen available for this one transfer than to the increased number of pollinations.

### Panicles of Male and Female Parents Isolated Together

Clones of 2 relatively self-sterile plants were growing sufficiently close together to permit stems from each to be enclosed in the same isolation bag. Ten such pairings were made several days

prior to anthesis with 4 stems of each parent isolated in each bag. The stems of one parent plant were consistently longer than those of the other parent, resulting in the panicles of those stems being in a position superior to that of the panicles of the other parent. Each group of 4 stems in a bag was considered a pollen source for the other 4 stems in the same bag. The panicles in the inferior position produced an increase of 62 seeds per stem over the selfed seed-set on stems of the same plant, while the panicles in the superior position produced only 5.6 hybrid seeds per stem. Table 5, method (a).

Severed Panicles of the Male Parent Placed in the Isolation Bag of the Female Parent

Eight isolation bags on the female parent contained 4 stems each. Shortly after pollen had been shed on a day of general anthesis, groups of 4 panicles were severed from the male parent and placed in the top of each bag which isolated female panicles with the resultant average increase of 7.7 seeds per stem over selfed seed-set. Table 5, method (b).

Air Current Over Severed Panicles of the Male Parent

Fourteen bags each isolated 4 stems of the female parent, and an equal number of bags each isolated 4 stems of the male parent. Shortly after pollen had been shed on a day of general anthesis each group of 4 stems of the male parent was cut below its isolation bag; the bag containing the stems was carried to the bag which isolated the female panicles; a small hole was cut in the tops of the 2 bags and the 2 holes placed together; and a current of air was blown from the worker's mouth into the bottom of the bag which isolated the

Table 5. Number of seeds produced on panicles of relatively self-sterile plants of smooth bromegrass following pollination with foreign pollen by 5 different methods, compared with the number produced on selfed panicles of the same plants

| Method of pollen transfer | Identity of female parent | No. of pollinated panicles | Avg. no. of seeds per panicle | | |
|---|---|---|---|---|---|
| | | | Pollinated panicles | Selfed panicles | Gain due to transfer |
| (a) Isolating panicles together | | | | | |
| Lower | 16-4 | 40 | 62.1 | 0.1 | ** 62.0 |
| Upper | 16-5 | 39 | 5.8 | 0.2 | * 5.6 |
| (b) Placing severed panicles of male parent in the bag isolating the panicles of the female parent | 8-30 | 32 | 8.0 | 0.3 | * 7.7 |
| (c) Passing air current over severed panicles of male parent | 8-30 | 55 | 7.9 | 0.3 | ** 7.6 |
| (d) Atomizer | 16-2 | 84 | 3.9 | 0.0 | 3.9 |
| (e) Passing air current over intact panicles of male parent | 20-4 | 79 | 3.8 | 0.2 | ** 3.6 |

* Significant at the 5-percent level.
** Significant at the 1-percent level.

male panicles over the panicles into the bag which isolated the female panicles. Sufficient viable pollen was transferred by this method to produce 7.6 hybrid seeds per stem. Table 5, method (c).

### Atomizer

A mass of pollen from the male parent was collected during anthesis and blown onto 84 panicles of the female parent by means of an atomizer, with a resultant increase of 3.9 seeds per stem over selfed seed-set, which is not significant at the 5-percent level. Table 5, method (d).

### Air Current Over Intact Stems of the Male Parent

An air current passed into the bag which isolated panicles of the male parent and out through an 8-foot rubber tube to the bag which isolated panicles of the female parent carried sufficient viable pollen to produce an average increase of 3.6 seeds per stem on 79 stems. Table 5, method (e).

### Conclusions

Although chances of cross-sterility in smooth bromegrass are not believed to be great, the obvious differences in the success of pollen transfer methods might have been diminished by the use of the same parentage for all methods. However, since these data indicate that sufficient pollen can be transferred by most of the methods to produce significant increases in seed-set over selfed seed-set, other factors such as proximity of parent plants and available labor might govern a choice of method more than the number of seeds produced.

The method of isolating panicles of the male and female parents together requires less labor than any of the others since the panicles can be isolated at any time prior to anthesis and no additional attention is required at any specific time. All of the other methods require careful observation of the flowering processes as well as definite operations at a specific time.

The method of isolating panicles of the male and female parents together and the method of passing an air current over intact stems of the male parent both require definite location of parent plants. This is not the case with the methods of bag transfer, atomizer, passing an air current over severed panicles of the male parent, and placing severed panicles of the male parent in the bag which was isolating panicles of the female parent.

The optimum time of pollen transfer is probably longer in the methods of bag transfer and passing an air current over either severed or intact stems of the male parent, where the dehisced pollen can be recovered from the paper bag, than in the method of placing the severed stems of the male parent in the bag isolating the panicles of the female parent, which necessitates transfer of pollen while the anthers are shedding viable pollen.

Contamination is possible by the method of bag transfer because of the short exposure of the panicles of the female parent to the atmosphere during the transfer of bags; however, if such transfers are not made during general anthesis but within a few hours following anthesis, it is believed that considerable seed will be produced with little contamination.

The lack of satisfactory seed increase by the atomizer method

is attributed to the fact that smooth bromegrass pollen collected in bulk at normal temperatures quickly forms large aggregates which would not be easily caught by the feathery stigmas of the female parent.

## HYBRIDIZATION UNDER GREENHOUSE CONDITIONS

An attempt was made in the greenhouse during the winter of 1939-40 to combine the 2 procedures previously studied separately by emasculating with hot water and transferring pollen to the same panicles by 3 of the methods previously studied.

Twelve-plant clones of 2 relatively self-fertile plants for female parents and a 16-plant clone for the male parent were grown in nail kegs in a greenhouse from November 18, 1939, until harvest on April 18, 1940. Effective day length was increased approximately 7 hours from January 29, 1939 through anthesis by means of Mazda lamps. An attempt was made to maintain a temperature of 60 degrees Farenheit, but periods of sunshine caused wide fluctuations above that temperature.

The 12 plants of each genotype used as female parents were divided at random into 6 groups of 2. Each group of 2 plants was assigned one of the 6 temperatures from 44 to 49 degrees Centigrade. At least 2 stems on each plant were selfed, and at least 2 were treated just prior to anthesis for 5 minutes at the assigned temperature and then selfed. A comparison of these seed-sets indicated whether emasculation was complete. Other stems on the plants were treated just prior to normal anthesis and isolated in groups of 2.

Pollen was transferred to these groups of 2 stems by 3 methods, namely: (a) isolating panicles of the male and female parents together, (b) placing severed panicles of the male parent in the isolation bag of the female parent immediately following anthesis, and (c) allowing the pollen of the male parent to dehisce into an isolation bag and shortly thereafter transferring the bag to the previously isolated panicles of the female parent.

The data, expressed in percent seed-set, obtained from the trials between 44 and 47 degrees Centigrade are presented in table 6 and the material just before harvest is shown in figure 2.

Even though the clones of the 2 female parents appeared to be vigorous, the amount of self-fertility exhibited did not compare favorably with the 24 percent and 31 percent selfed seed-set which had been obtained under field conditions. Temperatures of 44 and 45 degrees were not sufficiently severe to completely prevent the formation of selfed seed. The 47 degree treatments were too severe for even the female organs, as evidenced by the lack of seed-set on pollinated, treated panicles. One temperature, 46 degrees, resulted in complete emasculation without complete injury to the female organs. Treated panicles which were isolated from foreign pollen failed to produce a single seed whereas other panicles similarly treated and later pollinated produced seed.

The amount of seed resulting from the pollen transfers was measured by the difference between the amount of seed produced on panicles which were pollinated following heat treatment and the amount produced on panicles of the same plant which were selfed following heat treatment. Such differences are presented in table 7. When

Table 6. Percent seed-set on at least 2 panicles of 2 plants of 2 smooth bromegrass genotypes which were either (1) selfed, or (2) selfed or pollinated by one of 3 methods following treatment by hot water for 5 minutes at 44 to 47 degrees C

| Temperature degrees C. | Genotype | Plant | Percent seed-set* | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | Panicles treated and pollinated by | | |
| | | | Panicles only selfed | Panicles treated and selfed | Placing severed panicles of male parent in bag isolating panicles of female parent | Transferring isolation bags | Isolating panicles of male and female panicles together |
| 47 | 15-19 | 1 | 3.3 | 0.0 | 0.0 | 0.0 | 0.0 |
| | | 2 | 1.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 24-23 | 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | | 2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 46 | 15-19 | 1 | 0.7 | 0.0 | 0.0 | 0.0 | 1.2 |
| | | 2 | 1.7 | 0.0 | 0.0 | 4.2 | 0.0 |
| | 24-23 | 1 | 5.0 | 0.0 | 0.3 | 6.3 | 14.7 |
| | | 2 | 1.4 | 0.0 | 0.0 | 0.0 | 0.0 |
| 45 | 15-19 | 1 | 0.2 | 0.0 | 3.2 | 6.5 | 5.8 |
| | | 2 | 1.9 | 1.3 | 19.1 | 3.7 | 5.2 |
| | 24-23 | 1 | 1.3 | 1.6 | 8.0 | 7.6 | 0.0 |
| | | 2 | 9.3 | 0.0 | 13.5 | 13.5 | 24.1 |
| 44 | 15-19 | 1 | 0.4 | 1.3 | 0.8 | 2.7 | 30.5 |
| | | 2 | 0.0 | 0.0 | 7.1 | 16.3 | 1.4 |
| | 24-23 | 1 | 5.6 | 5.0 | 8.2 | 3.5 | 2.6 |
| | | 2 | 10.0 | 1.9 | 8.5 | 8.1 | 11.4 |

* The average number of florets per panicle was approximately 200.

Figure 2. Plants of smooth bromegrass on which controlled hybrids were made by emasculating by hot-water treatments and transferring pollen by three methods

Table 7. Hybrid seed-set* on panicles of 2 plants of 2 smooth bromegrass genotypes following pollination, by 3 methods, of panicles that had been either partially or completely emasculated by 5-minute, hot-water treatments at 3 temperatures

| Temperature degrees C. | Genotype | Plant | Method of pollination | | |
|---|---|---|---|---|---|
| | | | Placing severed panicles of male parent in bag isolating panicles of female parent | Transferring isolation bags | Isolating panicles of male and female parents together |
| 46 | 15-19 | 1 | 0.0 | 0.0 | 1.2 |
| | | 2 | 0.0 | 4.2 | 0.0 |
| | 24-23 | 1 | 0.3 | 6.3 | 14.7 |
| | | 2 | 0.0 | 0.0 | 0.0 |
| 45 | 15-19 | 1 | 3.2 | 6.5 | 5.8 |
| | | 2 | 17.8 | 2.4 | 3.9 |
| | 24-23 | 1 | 6.4 | 6.0 | - 1.6 |
| | | 2 | 13.5 | 13.5 | 24.1 |
| 44 | 15-19 | 1 | - 0.5 | 1.4 | 29.2 |
| | | 2 | 7.1 | 16.3 | 1.4 |
| | 24-23 | 1 | 3.2 | - 1.5 | - 2.4 |
| | | 2 | 6.6 | 6.2 | 9.5 |

* Data are percentages and represent differences between percent seed-sets on stems which were pollinated following heat treatment and the percent seed-sets on stems which were selfed following heat treatments. The average number of florets per panicle was approximately 200.

the 3 pollen-transfer methods were tested by the analysis of variance; no two of them were shown to differ significantly in their effectiveness.

## DISCUSSION

Considerable evidence is presented to show that the pistillate organs of smooth bromegrass are more susceptible to high temperatures than are the staminate organs.

The differential in thermal death points of the male and female organs when treated with hot water was 2 or 3 degrees under field conditions but apparently only 1 degree under greenhouse conditions.

The effective temperature on 2 of the genotypes on which reliable data were obtained under field conditions was 47 degrees Centigrade, while 46 degrees was effective on 2 genotypes in the greenhouse. This one-degree discrepancy might be explainable on the basis of a more hardened condition of the field plants because of the greater extremes of the diurnal temperature cycle to which they were exposed. The narrowness of the range of effective temperatures makes obvious the necessity of using accurately calibrated thermometers.

Evidence to the effect that the critical temperature may vary between species was obtained in the greenhouse during the winter of 1938-39. Inflorescences of Elymus virginicus, Elymus glaucus, Elymus canadensis, Agropyron pauciflorum, and Bromus inermis were immersed in water at 41, 44, and 47 degrees Centigrade for 5 minutes just prior to normal anthesis and then isolated. The highest temperature

at which selfed seed was produced was noted. The Elymus and Agropyron species are highly self-fertile and the Bromus plants were selected on the basis of their relatively high self-fertility. Heads of the 3 Elymus species which had been treated at 41 and 44 degrees produced small amounts of selfed seed, while those treated at 47 degrees produced none. Heads of Agropyron pauciflorum which were treated at 41 degrees produced selfed seed but those treated at 44 and 47 degrees produced none. None of the treated Bromus inermis panicles produced selfed seed.

There is a wide range in the apparent self-fertility within individual plants, as well as between plants, of smooth bromegrass as indicated by the data of selfed seed-sets in table 6. Therefore reliable estimates of the effectiveness of emasculation and the gain in seed-set due to cross pollination can be obtained only by adequate estimates of selfed seed-set on both treated and untreated panicles.

Six methods of pollen transfer were explored, and 3 of the most satisfactory were investigated in some detail. An analysis of variance indicated that the 3 methods investigated most carefully did not differ significantly in their effectiveness when the percentages in table 7 were used as raw data or when those percentages were converted to dimensions of angles by the inverse sine transformation (1).

Large-scale controlled hybridization of smooth bromegrass by a combination of hot-water emasculation and bulk pollen transfer is definitely possible because of the differential in the thermal death points of the staminate and pistillate organs and the fact that receptive stigmas are extruded from the glumes of florets treated

at temperatures sufficiently severe to destroy the viability of the pollen.

Field grown plants are more suitable material than greenhouse plants because of the apparently greater differential in the thermal death points of the 2 types of organs and the larger hybrid seed-set which might be expected.

## SUMMARY

1. Panicles of relatively self-fertile plants of smooth bromegrass (Bromus inermis) were completely emasculated by immersing them in hot water or subjecting them to hot air. More consistent results were obtained by the former method. An average of 67 hybrid seeds per stem was produced under field conditions on 14 panicles following emasculation by hot water treatments from 45 to 49 degrees Centigrade for 5 minutes just prior to normal anthesis and pollination by exposing the emasculated panicles to atmospheric pollen. One hot-water treatment under field conditions, 47 degrees Centigrade for 5 minutes, completely emasculated 2 panicles on each of 2 genotypes and sufficient stigmas remained functional to produce 130 and 84 seeds per stem on the two genotypes, representing 54 percent and 24 percent seed-set respectively.

The length of effective treatment could be decreased or increased within limits by raising or lowering the water temperature.

2. Bulk pollen transfers were made by 6 methods. Those producing significant amounts of hybrid seed were (a) allowing pollen of the male parent to dehisce into an isolation bag and transferring that

bag to the previously isolated panicles of the female parent shortly after anthesis, (b) enclosing living panicles of the male and female parents in the same isolation bag, (c) placing severed panicles of the male parent inside the bag isolating the panicles of the female parent shortly after anthesis, (d) passing an air current over the severed panicles of the male parent into the bag isolating the panicles of the female parent shortly after anthesis, and (e) passing an air current over intact stems of the male parent through an 8-foot rubber tube into the bag isolating panicles of the female parent shortly after anthesis. The one method which did not produce a significant amount of hybrid seed was that of collecting the pollen from the male parent in bulk at anthesis and applying it to the panicles of the female parent by means of an atomizer.

3. Under conditions existent in kraft isolation bags at the time of normal anthesis in the field pollen of smooth bromegrass lost much of its viability within 24 hours after being shed. When collected in bulk under atmospheric conditions it quickly forms large aggregates.

4. Controlled hybrids were produced on greenhouse plants of smooth bromegrass by a combination of hot-water emasculation followed by pollen transfer by 3 methods, namely: (a) enclosing living panicles of the male and female parents in the same isolation bag, (b) placing severed panicles of the male parent inside the bag isolating the panicles of the female parent shortly after anthesis, (c) allowing pollen of the male parent to dehisce into an isolation bag and transferring that bag to the previously isolated panicles of the female parent shortly after anthesis. The effective hot-water treatment

on 2 genotypes was 46 degrees Centigrade for 5 minutes. The amounts of seed resulting from the three methods of pollination did not prove to be significantly different.

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