SOME FACTORS INFLUENCING THE FORMATION OF F_1 SEED BETWEEN VARIETIES OF OATS

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Samuel Lineas Hager

1948

THESIS

This is to certify that the

thesis entitled

"Some Factors Influencing the Formation of F_1 Seed Between Varieties of Oats."

presented by

Samuel L. Hager

has been accepted towards fulfillment of the requirements for

MS degree in Farm Crops

E. E. Down

Major professor

Date August 28, 1948

M-795

SOME FACTORS INFLUENCING THE FORMATION OF F_1 SEED BETWEEN VARIETIES OF OATS

By

Samuel Lineas Hager

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Farm Crops

1948

THESIS

ACKNOWLEDGMENT

The writer is grateful to Dr. E. E. Down for guidance and aid in conducting this experiment and for the review of this thesis. The helpful suggestions given by Dr. S. T. Dexter are appreciated.

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INTRODUCTION

In plant breeding, it is recognized generally that the more material there is with which to work, especially during the earlier phases of the project, the greater are the chances that the desired ends will be accompolished.

When oats, a plant normally self-pollinated, are cross-pollinated artificially, a very small percentage of the florets produce grain. The difficulty with which seeds are secured following cross-pollination of oats is undesirable not only because it results in just a few seed with which to start the F_1 generation but also because it makes it impractical, if not impossible, to carry on a program of back-crossing. Back-crossing is a very useful method that plant breeders may employ to concentrate and to render in pure lines, with a substantial saving of time, desired characteristics of the varieties involved in the cross. Without back-crossing, several extra generations would be required before chance segregation and combination would unite the sought after characteristics. For a back-cross program to be very successful, many pollinations would have to be made followed by a reasonably high percentage of set.

Why oats fail to produce seeds after the flowers

have been cross-pollinated artificially is not thoroughly understood. Oat florets, emasculated and pollinated, are subjected to a process that visibly injures them. A partial drying of the outer floral parts is generally evident. The glumes can not be replaced about the pistil to give it the protection that they afforded before the flower was manipulated. Of the many things that could influence the setting of seeds after cross-pollination, air moisture was considered to be one of them. On the foregoing postulation, this experiment was based.

The purpose of this experiment was to determine whether it would be possible to increase the set of seed following cross-pollination of oats by enclosing the heads with a suitable container along with a chemical compound designed to have some influence on the moisture content of the encased air.

It is the property of any salt in a saturated solution to maintain an equilibrium vapor pressure (i. e. a constant relative humidity) with a confined atomsphere at a constant temperature (7). The vapor pressure maintained will depend upon the compound in solution and the temperature.

This experiment is to follow closely the theory and the procedures used by Dexter (2, 3) in the curing and processing of farm products to the desired moisture content by the use of suitable salts impregnated into

wooden blocks.

REVIEW OF LITERATURE

Much data are available dealing with the correlation of various phases of plant growth to air moisture. Some of the data concerns plant responses under conditions of controlled air moisture; other data show the relation of the natural atomspheric humidity to various plant responses. Work that has dealt with controlled humidity in relation to seed set, or with the correlation of seed set with humidity after cross-pollination will be cited.

Hollowell (6), working with red clover, came to the conclusion that atomspheric humidity did not affect the setting of seed following cross-pollination under greenhouse or field conditions.

Grandfield (4), working with alfalfa, found that the number of flowers setting pods increased as the relative humidity decreased from 90 to 10 percent.

Dexter (2) and Dexter and Creighton (3) describe methods to bring certain farm products to a desired moisture content by the use of wooden blocks impregnated with suitable salts. The blocks function to decrease, or increase, the relative humidity in the surrounding atomsphere.

PRELIMINARY GREENHOUSE EXPERIMENT

To obtain preliminary information on the problem as stated, work was started in the greenhouse during the winter and spring of 1947 to determine

1. what type of bag should be used to cover the pollinated heads and
2. what method should be used to apply the salts.

An attempt was made to keep the greenhouse temperatures around 60 degrees F. during the night and 75 F. during the day. Occasionally during the winter and early spring because of steam pressure failure, the temperature fell to 48-55 F. at night; and on the coldest days, the temperature would not go above 60 F. During the late spring, the temperature on the warm, sunny days would range frequently from 80 to 90 degrees F.

Several strains of oats were used to make the crosses. No attempt was made to use the same variety for the female throughout the experiment. When pollen was needed,it was secured from any variety that was shedding pollen at the time as long as this variety was not the same as the one one being pollinated. Pollination was made immediately after emasculation, but care was taken not to pollinate any floret an anther of which had showed signs of shedding pollen even after the anther had been tapped against the thumbnail.

Briefly the treatments in the greenhouse were as follows:

No. 1. Heads were covered with a 2½ by 6 inch glasine enclosing a vial filled with a saturated salt solution with a 1½ inch cotton wick extending from the vial.

No. 2. Heads were covered with a cellophane bag, 2½ by 3 by 8 inch with pleated sides, and the salts were applied by the vial and wick method as outlined above.

No. 3. Heads were covered with a cellophane bag of the type mentioned above, and the salts were applied impregnated into wooden blocks 3/4 by 3/4 by 3½ inches.

No. 4. Heads were covered with a pliofilm turkey bag, 10 by 12 by 24 inch pleated side, and the salts were applied using the wooden blocks.

No. 5. Heads were covered with the pliofilm turkey bags, and the salts applied by the vial and wick method.

No. 6. Heads were covered with the glasine bag, 5 by 3¼ by 11 inches pleated side, and the salts applied using the wooden block method.

A few heads were left in the open after they had been pollinated. In some instances water was used in the vials without any salt.

DATA FOR GREENHOUSE

The results of the treatments in the greenhouse for 1947 are as follows:

(Table on following page)

Table No. 1. Results of greenhouse experiment.

Salt	Humidity*	Florets pollinated	No. set	% set
Method 1. Glasine bag 2¼ by 6 inches with wick.				
Water	100	27	0	0
$CaSO_4$	98	71	7	9.9
Na_2SO_3	95	36	0	0
Na_2SO_4	93	68	12	17.7
$ZnSO_4$	90	82	6	7.3
KBr	84	10	1	10.1
NH_4Cl	79	17	0	0
Left in open	-	46	1	2.2
Total	-	357	21	5.9
Method 2. Cellophane bag with wick.				
Water	100	36	2	5.2
$Mg(C_2H_3O_2)_2$	65	44	1	2.3
$KC_2H_3O_2$	20	52	2	3.6
Left in open	-	42	0	0
Total	-	174	5	2.8
Method 3. Cellophane bag with blocks.				
$Mg(C_2H_3O_2)_2$	65	14	0	0
$CaCl_2$	32	20	0	0
Total	-	34	0	0

(7)* The humidity as given shows the equilibrium relative humidity that will be maintained in a closed atomsphere over a saturated solution of the given salt at a temperature of 20 degrees C. No claim is made that these humidities were maintained within the bags covering the heads.

Table No. 1. Continued

Salt	Humidity	Florets pollinated	No. set	%set
Method 4. Turkey bags with blocks.				
Na_2HPO_4	95	24	4	16.7
$NaC_2H_3O_2$	76	21	0	0
$MgC_2H_3O_2)_2$	65	24	3	12.5
NaBr	58	20	1	5.0
$CaCl_2$	32	21	9	43.0
Total	-	110	17	15.4
Method 5. Turkey bags with wicks.				
Na_2HPO_4	95	27	4	14.2
$ZnSO_4$	90	17	8	47.1
$Mg(C_2H_3O_2)$	65	9	1	11.1
$KC_2H_3O_2$	20	83	15	18.1
Total	-	136	28	20.6
Method 6. Glasine bag 5 by 3¼ by 11 inches with blocks.				
$NaC_2H_3O_2$	76	10	0	0
$Mg(C_2H_3O_2)$	65	27	7	25.9
NaBr	58	31	8	25.8
$CaCl_2$	32	10	6	60.0
Bag alone	-	15	7	46.6
Total	-	93	28	30.2

Several different kinds of salts were used in the preliminary experiment. The same ones were not used necessarily with all of the different methods of applying the salts.

The different methods are listed in the chronological order that they were applied. Method 1 was used in the colder part of the year. The last one, number 6, was used during the warmer part of the spring in May. Temperature differences in the greenhouse may have had some effect on the number of seed set following the cross-pollinations.

Moisture would collect on the inside of the pliofilm turkey bags and on the inside of the cellophane bags soon after the bags had been used to cover the pollinated heads. This water apparently had been transpired by the head. The adsorbing area seemed to be too small to take up the water as fast as it was given off by the plant. The observation, that water collected within the bags that were impervious to moisture, led to the conclusion that the plant was capable of giving off sufficient moisture through the enclosed head to bring the air within the bag to the saturation point. It seemed, therefore; that the problem would be to get rid of the excess moisture rather than to provide other means to raise the moisture content within the bags. Grandfield and Zink (5) found it necessary to introduce dry air into humidity control cabinets during the daylight hours to counterbalance the effect of the moisture transpired by the plant.

In the greenhouse experiment, the 5 by 3¼ by 11 inch glasine gave the best set after cross-pollination.

FIELD EXPERIMENT 1947

After profiting from the preliminary experiment in the greenhouse, a new study was made in the field during the summer of 1947.

The varieties selected for use in this experiment were as follows: females, two white seeded oats, Eaton and 3909 (a variety being tested at the Michigan State College Experiment Station); males, two yellow seeded oats, Benton and Clinton, and two reddish seeded oats, Bonda and Bonham. These varieties were chosen because the yellow color of the seed of Clinton and of Benton and the reddish color of Bonda and of Bonham could be used as an index of crossing when either of these varieties were crossed on to one of the white seeded varieties.

On May 6th, seed of the foregoing varieties were space planted in separate rows three feet apart. The seeds of Eaton and 3909 were planted at approximately 8 inches apart in the rows. The seeds of the varieties used to supply the pollen were sown about 4 inches apart. The planting was made in such a manner in order to facilitate working around the plants later in the season. Also, it was designed this way so the plants would tiller much more than normal to give more heads over a longer period of time than would have been usual following a drilling of the seeds in rows.

As stated earlier, the object of this experiment was to get an increased set of cross-pollinated oat flowers. An attempt was to be made to influence favorably the moisture content of the atomsphere surrounding the cross-pollinated heads by the employment of suitable salts and by covering the heads with a suitable bag.

The salts selected for use during the summer of 1947 were Na_2SO_3, $ZnSO_4$, KBr, NH_4Cl, NaBr, $Mg(C_2H_3O_2)$, $CaCl_2$, and Na_2HPO_4. These salts were impregnated into western yellow pine blocks which were 3½ by 3/4 by 3/4 inches in size. The blocks were soaked in a saturated, boiling solution of a particular salt for twenty-four hours during the first impregnation. When the blocks were made ready for re-use, they were soaked for only three or four hours. After soaking, the blocks were dried overnight in an oven at a temperature near 150 degrees C. The blocks of the different salts, after drying, were wrapped in separate cellophane bags to protect them from adsorbing moisture from the atomsphere. The blocks remained wrapped until they were used in the field.

The type of covering used to put over the pollinated heads were glasine bags: Size No. 1 white, pleated side, 5 by 3¼ by 11 inches, weight 25 lbs. This bag is semi-transparent. It is neither water proof nor air proof. It is somewhat water and air resistant. This type of bag was used in order that some of the water transpired

by the plant could escape to the free atomsphere.

On July 7th, the oats had started to head. The variety, 3909, was a few days earlier than the Eaton; therefore the 3909 was used as the female for the first two replications of the experiment.

At the beginning of the experiment, those heads that were the fartherest out of the boot were emasculated first. The florets selected for emasculation were located near the top of the head. As the season advanced, the florets emasculated were located farther and farther down the head so that near the end of the flowering period the florets emasculated were those which were located at the basal portions of the head. As the season advanced,there was a general reduction in the number and size of the florets available for emasculation and pollination.

A pair of curved nosed forcepts that had been filed down to make a flat point were used to emasculate the oat flowers. A description of the actual mode of emasculation followed during the summer will be given. Florets that had shed pollen, or that were near to shedding pollen, were cut from the plant with a pair of scissors. Then the florets on the plant that appeared to be in such a stage of development that the anthers in them would have shed pollen in the next day or two were emasculated. The remainder of

the florets that had small immature stigmas and anthers were cut from the plant. This procedure left on the average 15 to 22 florets per head. Sometimes there were more; sometimes, less.

To emasculate, the innermost of the outer glumes was folded back with the aid of the forcepts. The axillary oat was thus exposed. Next the axillary floret was removed. Then the palea of the main floret was pulled back exposing the anthers . The three anthers were removed. Finally, the palea and the outer glume were replaced to the normal position that they had occupied. Immediately after the emasculation of all of the florets left on the head, the head was covered with a small glasine bag of the type commonly used to cover corn ear buds. The bag was folded at the bottom and fastened with a paper clip. The heads were kept covered so that the chances of stray pollen fertilizing the florets would be reduced. The emasculated heads remained covered until the time of pollination. Part of the emasculation was done in the forenoon and part, in the afternoon whichever fitted into the schedule of work for the day the better. All through the process of emasculation, care was taken to prevent the florets being emasculated from becoming accidentally pollinated.

The time allowed to elapse between emasculation and

pollination varied from one to three days. No attempt was made to systematize the length of time between emasculation and pollination with the subsequent treatments. A block of heads of the same variety were emasculated in the morning before the work of pollination for that day had begun, or in the afternoon after the pollination had been completed. These heads were pollinated during the next three days until all had been used.

To get pollen, a small handfull of florets were collected from the male; and were used to supply pollen until their glumes began to wilt. The flowers were opened, and enough ripe anthers were collected to pollinate the florets on one head. These anthere were put in a small cardboard box while being collected, and then used immediately to pollinate only one head. In this manner, an attempt was made to insure fresh anthers for use on each head. Pollen from Bonda was used to cross with the 3909; and pollen from Bonham, to pollinate Eaton. In case there was no pollen being shed by the variety required, pollen was gathered from any one of the varieties designated as male. In this experiment, it was considered that the source of pollen made no difference. It may be well to note that late in the afternoon, it became difficult to find sufficient pollen for crossing the heads. Generally by 3 to 5 o'clock in the afternoon, all of the oat flowers that were going to shed pollen that afternoon had dehisced; and the florets

had opened up scattering their pollen in the wind. Near the end of the crossing season, some difficulty was experienced collecting pollen. Then only a few flowers were shedding pollen at a given time. The anthers, too, were smaller and not as well matured as the ones used in the earlier part of the season.

In the act of pollinization, the florets to be crossed were opened individually using the process described previously for emasculation. Then a ripe anther was taken from the box, dusted over the stigma, and left in the floret. The floret was then carefully closed by putting the glumes back in place before preceeding to the next floret. As soon as a head had been pollinated completely, the head was subjected immediately to an assigned treatment.

Before proceeding, an explanation of the treatments applied to the pollinated heads will be given. There were eight salts used in the experiment. The heads were kept covered for three, four, and five days. Heads were covered for a similar period with the glasine bag alone. This made a combination of twenty-seven treatments. Three heads were left in the open. All of these gave a total of thirty individual treatments. Hereafter in this experiment, a combination of these thirty treatments will be spoken of as a replication.

Immediately after a head had been pollinated, a

western yellow pine stake, three-quarters inch square, was driven into the ground to an agreeable height beside the head. From a cross arm at the top of this stake, were suspended two salt impregnated blocks that had been tied together with a string. This gave an effective moisture adsorbing area of approximately 23 square inches. One of the glasine bags was fitted down over the stake so that it enclosed the blocks and the cross-pollinated head of oats. Care was taken to insure that the head was not touched by the blocks. Most of the salts would have been toxic to the plants if the salts had touched them. A wad of cotton was wrapped around the stem of the oat. Then the lower part of the bag was tied with a string in such a manner so as to close the bag as tight as possible around the stake and the stem without injuring the stem. In the process, the stem was drawn up close to the stake, and the passage of air into and out of the bag through the lips was cut down to a minimum. For the heads that were covered with the glasine bag alone, a similar process was followed except that the salt impregnated blocks were omitted. Both blocks used with a head were impregnated with the same salt.

Within the individual replication, the order of assigning the treatments to the thirty heads was accompolished by drawing lots as soon as the head had been pollinated.

The first one of the thirty treatments that was drawn was applied to the first head pollinated. The remainder of the treatments in the replication followed in chronological order the sequence in which they were drawn. In this manner, a replication was completed over two or more days. The various treatments were applied in the morning, in the afternoon, and at the time of day determined by the time the ones drawn before them were completed.

Each head except those left in the open was covered with a bag with the appropriate treatment within two to five minutes after pollination was completed. The bags remained on the heads for three, four, or five days. Then they were removed. The removing of the bags took place in the afternoon from five o'clock to dark depending on the time the rest of the work for the day had been completed. The heads remained uncovered from the time the bags were removed until harvest several weeks later.

Only one of the two female varieties was used throughout a single replication of the thirty treatments in the experiment. Replication 1 was completed first; replication 2, next; and the others, in numerical order.

There were four different replications completed during the summer. Part of a fifth was done. The results of each are tabulated in charts appearing later in this thesis.

The seeds that were harvested from the experiment

were planted in the greenhouse in the fall of 1947. The plants that resulted from these seeds could not be identified as hybrids because the grain color, the index or crossing relied upon for this experiment, could not be determined. Red seeded and yellow seeded oats growing in the greenhouse at the same time appeared to be white seeded oats. The seeds from this F_1 generation were planted in the field during the first of June 1948. A preliminary check on the resulting material shows that segregation for date of maturity is taking place in each of the progeny rows except for about five that have one to three plants each. These results indicate that practically all of the seeds were actually a product of cross-pollination, and they will be treated as such in the folling discussion.

The heads that had been cross-pollinated in the field in 1947 were tagged for identification so that the following information could be checked against the records:

1. the day of emasculation
2. the day of pollination , and the number of florets
3. the time of emasculation and pollination, whether morning or afternoon
4. the treatment used on the head including the salt and the length of time that the head was covered.

Explanation of table 2: Table 2 shows the percentage set of the cross-pollinated heads for the summer of 1947.

Table No. 2. Result of field experiment 1947.

Salt or otherwise	Replications														
	1 Female 3909			2 Female 3909			3 Female Eaton			4 Female Eaton			5 Female 3909		
	3 days	4 days	5 days	3 days	4 days	5 days	3 days	4 days	5 days	3 days	4 days	5 days	3 days	4 days	5 days
	% set per head			% set per head			% set per head			% set per head			% set per head		
Open	19.0	50.0	61.9	5.0	23.1	27.8	0	22.2	0	13.3	0	16.7	0	25.0	—
Glasine	5.9	21.7	4.0	0	0	57.1	42.1	4.0	0	0	17.6	46.7	—	7.1	0
Na_2SO_3	21.6	26.0	13.6	0	27.3	15.8	0	16.7	45.5	20.0	0	0	9.1	16.6	—
$ZnSO_4$	40.0	34.8	42.1	0	7.7	46.7	41.2	42.1	0	40.0	0	0	31.3	—	10.0
KBr	10.3	40.0	27.8	47.4	0	57.9	13.3	71.4	20.0	0	0	7.1	0	6.7	—
NH_4Cl	31.6	61.9	—	12.5	15.4	6.3	46.2	5.9	14.3	35.8	10.0	0	—	—	30.0
NaBr	12.5	4.3	54.2	25.0	5.3	5.9	0	18.2	10.5	0	0	0	0	7.8	0
$Mg(C_2H_3O_2)_2$	26.3	0	46.1	0	21.3	40.0	50.0	33.3	5.9	0	0	62.5	—	15.4	16.7
$CaCl_2$	0	0	30.8	0	0	0	57.9	0	20.0	0	15.4	5.9	—	11.1	20.0
Na_2HPO_4	33.3	46.7	25.8	7.1	—	6.2	0	5.6	0	—	0	0	—	42.9	9.1
Total %		27.6			15.7			18.6			11.2			13.6	

Total no. pollinated 2240 Total set 417 Total % set 18.6

Table No. 3. Shows crossing results from June 8-25 incl. 1947.

Date	Day			AM			PM		
June	No. (X)	No. set	% set	No. (X)	No. set	% set	No. (X)	No. set	% set
8	122	26	21.3	—	—	—	122	26	21.3
9	56	17	30.3	56	17	30.3	—	—	—
10	105	25	23.8	70	15	21.4	35	10	28.6
11	226	75	33.2	86	16	18.6	140	59	42.1
14	232	58	25.0	64	6	9.4	168	52	30.9
15	202	31	15.3	117	6	5.1	85	25	28.3
16	81	14	17.3	81	14	17.3	—	—	—
17	246	9	3.7	127	4	3.1	119	5	4.2
18	179	67	37.4	126	43	34.1	53	24	45.3
19	152	18	11.8	103	15	14.6	49	3	6.1
23	317	64	20.2	162	18	11.1	155	46	29.6
24	232	11	4.7	134	8	6.0	98	3	3.1
25	90	2	2.2	71	2	2.8	19	0	0
Total	2240	417	18.5	1197	164	14.2	1034	253	23.6

Table No. 4. Gives the average temperature (10) for 24 hours and for the period from 7:30 Am to 6:30 PM. Humidity averages for the same periods are given. 1947

Date June	Ave. temp. for day 12:30 AM to 12:30 PM	Ave. temp. for 7:30 AM to 6:30 PM	Ave. rel. humidity 12:30 AM to 12:30 PM	Ave. rel. humidity 7:30 AM to 6:30 PM
8	67	73	57	51
9	68	72	59	57
10	68	73	66	58
11	68	74	64	62
14	71	74	79	79
15	69	71	86	82
16	70	73	91	84
17	74	79	80	66
18	70	75	79	75
19	58	60	81	68
23	63	69	55	52
24	69	76	54	47
25	72	79	45	41

The humidities were computed from psychrometric tables (9) by using only whole degrees for the air temperature and for the dew point. No correction was made for pressure. The temperatures given are in degrees F. The weather data were secured from the East Lansing weather station of the U. S. Department of Commerce.

Table No. 5. Showing the % set on a total basis for each salt and for the glasine bag and the head left in the open per AM, PM, and per day. 1947

Treatment	% set in AM	% set in PM	% Total
Open	21.4	17.8	20.4
Glasine	12.6	16.7	14.7
Na_2SO_3	14.6	25.4	17.3
KBr	21.5	27.2	23.9
NH_4Cl	18.1	27.1	23.5
$ZnSO_4$	10.4	33.1	25.3
NaBr	5.6	19.2	11.6
$Mg(C_2H_3O_2)_2$	12.9	37.1	22.3
$CaCl_2$	5.1	27.5	12.0
Na_2HPO_4	17.3	15.4	16.3
Total	13.7	24.5	18.6

Table No. 6. Percentage set per female.

Female	Total florets crossed	No. set	% set
3909	1363	282	21.1
Eaton	877	135	15.4

Chart 1.
Ave. Temp. for day plotted against
% set for day (vertical).
1947

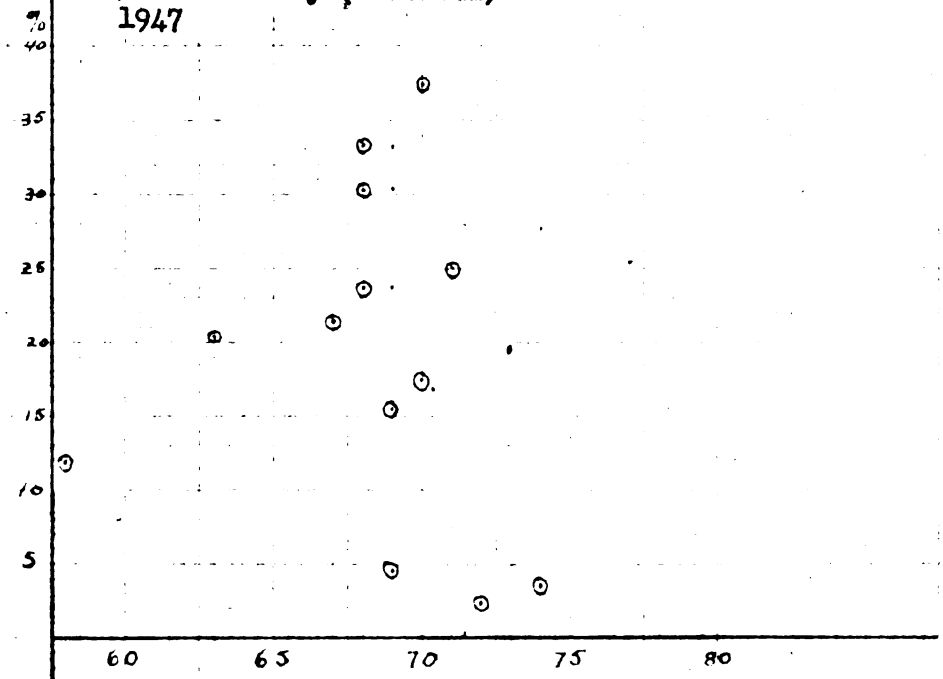


Chart 2.
Ave. temp. 7:30 AM to 6:30 PM plotted
against % set for day (vertical).
1947

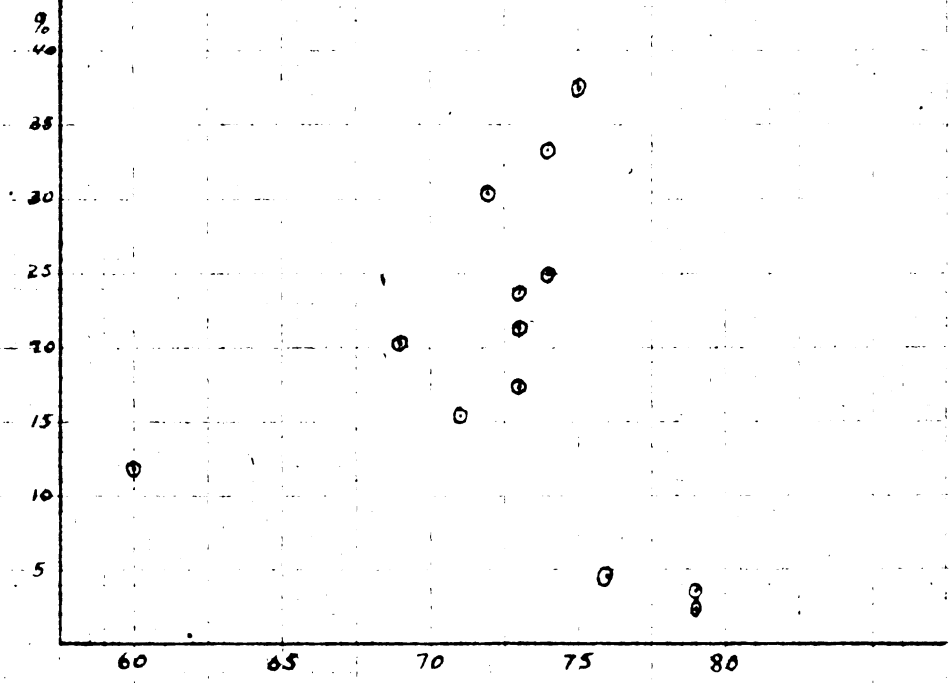


Chart 3.
Average relative humidity for day
plotted against % set for day (vertical).
1947

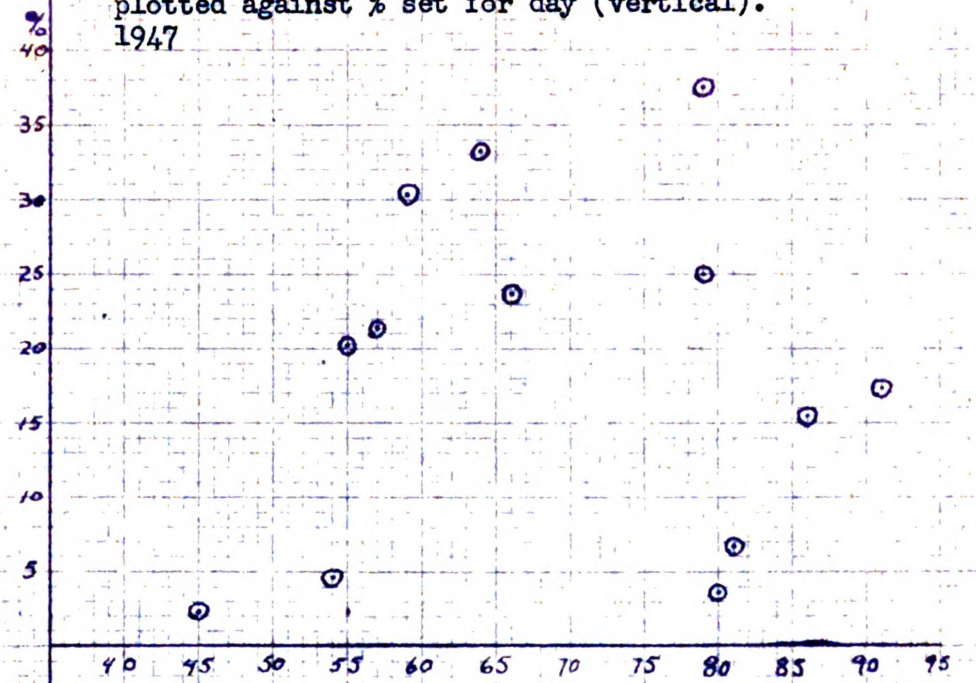
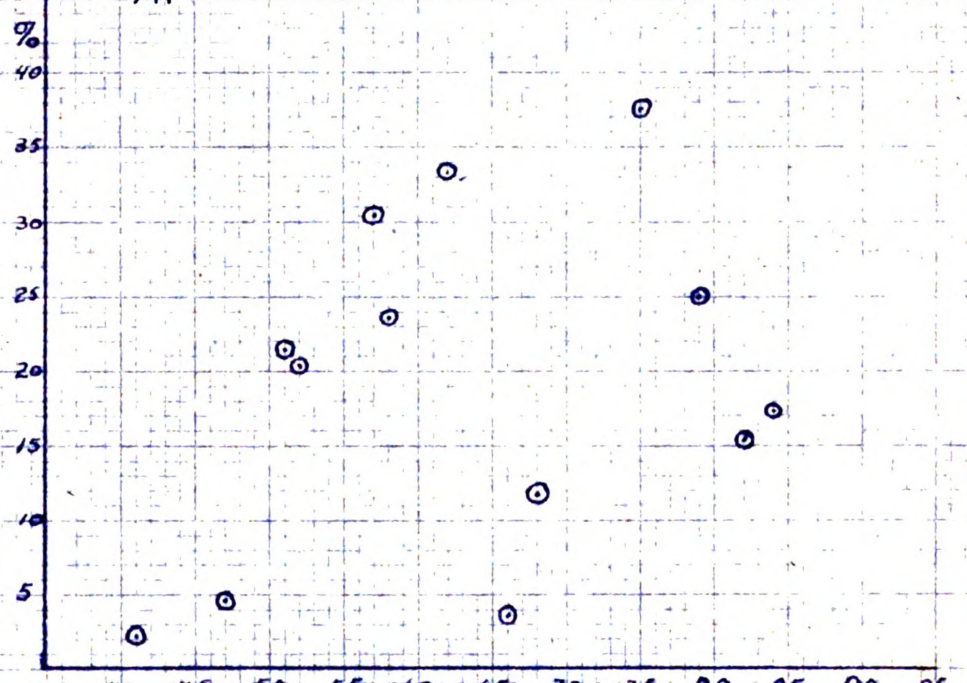


Chart 4.
Average relative humidity for 7:30 AM to 6:30 PM
plotted against % set for day (vertical).
1947



The columns headed 3, 4, and 5 days refer to the number of days that the heads were kept covered after the application of the salt. These columns have no significance conserning the row headed open. Averages always represent the total number of florets pollinated divided into the number setting seed. Replication 5 was incomplete. Blank spaces in any of the other replications represent heads that were destroyed in the field.

DISCUSSION OF RESULTS1947

An analysis of variance (1, 8) of replications 1, 2, 3, and 4, after substituting in them where data was missing corresponding percentages (if available) from replication 5, showed no significant differences between the percentage set for individual treatments, the different salts, or the different lengths of time that the heads were covered; but a significant difference was shown between replications. An analysis of variance was applied to this data after the percentage set for each treatment was divided by the average for the percentage set of the three heads left in the open of the individual replication under consideration. This analysis showed, in addition to the difference between replications, a highly significant difference between females in favor of the Eaton. This reversal was probably due to the fact that the heads of Eaton left in the open gave a lower percentage set in

comparison to the heads covered than did the 3909; for the 3909 gave the greater percentage set of the two.

The remainder of the discussion of the results will be based on the data as shown in the preceeding tables.

The total percentage set for the afternoon exceeded that of the morning by a percentage of 24.5 to 13.7 Only in the case of the heads left in the open and for the Na_2HPO_4 salt treatment did the morning set exceed that of the afternoon. In considering the total set per morning and afternoon each day, it is evident that for all the days except June 19, 24, and 25 the percentage set was greater in the afternoon (except where figures are not available for both periods).

One of the most outstanding things apparent about the results is that the amount of set following cross-pollination is very variable even under similar methods of treatment. There must be some uncontrolled factors influencing the experiment which are capable of masking the effects of the treatments used.

The treatments without salts: The heads left in the open gave a higher percentage set than the average for the whole experiment. When the glasine bag was used alone, it gave much lower results than did the heads left in the open; and it gave a lower percentage set than did any of the salts except $CaCl_2$ and NaBr.

The treatments combining the glasine bag with salts: $ZnSO_4$ gave the highest percentage set; and only $ZnSO_4$, KBr, NH_4Cl, and $Mg(C_2H_3O_2)_2$ gave better results than did the heads left in the open.

It is necessary to recognize the fact that the experiment was not set up to test the factors which will be compared with the percentage set in the following statements:

1. The percentage set in the afternoon was 24.5 as compared with a set of 18.6 for the morning.

2. There does not appear to be any direct correlation between the average temperatures for the day and the percentage set, but it seems worthy of note that the three lowest percentages of set (each less than 5%) occurred on the three hottest days (average temperature 7:30 AM to 6:30 PM). The next lowest set occurred on the coldest day of the period.

3. There seems to be no correlation between the relative humidity of the atomsphere and the percentage set.

FIELD EXPERIMENT 1948

In the winter and spring of 1948, it was impossible to continue the experiment due to the fact that the variety grown for use as the male, for some physiological reason, failed to develop plants vigorous enough to produce sufficient pollen for use; however, it was found that by using a much larger adsorbing surface the excess moisture could be prevented from collecting on the inside of the bag when the oat heads were covered with a bag of the pliofilm type. These bags are practically impervious to moisture and air.

In the summer of 1947, it had been possible to complete only four replications of thirty treatments. With such a limited amount of data, little leeway was given for statistical analysis.

The experiment for the summer of 1948 was planned so that one replication of the experiment could be done each day. In order to do this, the number of salts to be used was cut down to four. The salt treatments were to be covered by three different types of bags. The number of florets crossed per head was reduced to five. It would have been desirable to have crossed a larger number per head, but it was considered better to keep the number low in order to make possible the completion of a whole replication each day. Since the results the year before had indicated that there might be some differences resulting from forenoon

and afternoon pollinations, arrangements were made to have an equal number of pollinations subjected to the different treatments occurring in the afternoon and in the morning.

In the spring of 1948, the same varieties of oats were seeded as in the year of 1947. The varieties were space planted in rows on April 22 following the same general plan as practiced the year before.

The salts used in the summer of 1948 were $ZnSO_4$, KBr, NaBr, and $CaCl_2$. Four different methods of applying these salts were used:

1. The salts were adsorbed by wooden blocks in a manner similar to that used the previous summer.

2. The salts were adsorbed into an 18 by $2\frac{1}{2}$ inch strip of blotting paper having a surface area of approximately 90 square inches.

3. The salts were adsorbed into an 18 by 6 inch strip of blotting paper folded into a cylinder having a $4\frac{1}{2}$ inch lap giving a surface area of approximately 162 square inches.

4. The salts were impregnated into an 18 by 12 inch strip of blotting paper folded into a cylinder 12 inches high with a lap at the ends of 1 inch. This cylinder had a surface area of approximately 404 square inches. The type of blotting paper used was Arrow Blotting Paper, weight 100, color white. Two strips of the paper were stapled together one on top of the other with wire staples. Each of the strips mentioned

above were of two thicknesses of the blotting paper.

The blotters were dipped into a saturated solution of the proper salt at a temperature at or near the boiling point. As soon as the blotters were thoroughly wet with the solution (the process occurred almost immediately), they were removed. Next, the blotters were placed in an oven and dried overnight at a temperature of approximately 125 degrees C. The dry blotters were wrapped in moisture proof pliofilm bags. In these, they were taken to the field and kept until they were needed. Blotters that had been used were dried and used over again. The wooden blocks were treated in a manner similar to that used the year before.

There were three different bags used in conjunction with the four salts:

1. Glasine: 5 by 3¼ by 10 inches. The same type of bag used the year before. This bag will be referred to as the medium glasine bag.

2. Glasine: 5 by 3¼ by 19. This bag was made by glueing together at the ends, after the closed end of one of them had been cut off, two of the smaller bags. This bag will be referred to as the big, or large glasine bag.

3. Pliofilm Turkey Bag: Two types were used. Type one: Shelmar Turkey Bag 18 by 24 inch. Type two: Shellene Turkey Bag 10 by 8 by 24 inch. It had been planned to use the turkey bags for more than one replication, but the first type was not able to withstand the weather for a period of much more than three days. The supply of the

first type lasted through replications A toI . The second type of turkey bag was used for the remainder of the experiment. The turkey bag will be referred to as the pliofilm bag.

The medium glasine bag was used with the wooden blocks and with the 18 by 2½ inch blotter. The large glasine bag was used with the cylinder made from the 18 by 6 inch blotter (4½ inch overlap). The pliofilm bag was used with the cylinder made from the 18 by 12 inch blotting paper.

The use of the four different salts in combination with the four ways of applying the salts in connection with the bag types as shown above gave sixteen different treatments to be applied to pollinated heads. In addition, pollinated heads were covered with each type of bag listed with no salt, and with a smaller 1 7/8 by 6 inch glasine bag. (This bag will be referred to as the tiny glasine bag.) One head was left uncovered in the open after pollination. All of these in combination made a total of twenty-one different treatments. This total of twenty-one treatments was called a replication. One replication was completed each day. The order of doing the individual treatments was so arranged that in the end just as many of the pollinations subjected to the individual treatments occurred in the morning as in the afternoon.

The first emasculation of florets for crossing during the summer of 1948 was done on June 25th. This was almost two weeks earlier than the work was started the year before.

Again, the 3909 was the earlier of the two varieties to be used as female, and it was the only one used in the summer of 1948.

The overall plan of emasculation was the same as that followed the previous year with some variations that will be noted. Instead of selecting those heads that were the most advanced, or were the most suitable of the whole row, two heads from each of eleven consecutive clones in the space planted row were selected for emasculation. This gave a total of 22 heads for emasculation. At the beginning of the season, the more advanced heads were selected. As the season advanced, it became necessary to select heads that still had enough florets that had not shed pollen. Throughout the season, those plants which had completed blooming, and those that would be through blooming before they could have been emasculated were cut from the plant. New culms were sent up by the plants for a week or more. An attempt was made to select for emasculation those heads that would furnish florets nearest the top of the head; but as the season advanced, the florets emasculated were situated lower and lower on the plant until near the end of the period most of the florets emasculated were those located near the bottom of the head. The florets at the bottom of the head were smaller than the ones higher up. Although some of the anthers were removed during the forenoon, most of the emasculation was done in the afternoon

after the florets shedding pollen that day had opened up.

Since only five florets per head were needed for pollination, the florets selected for emasculation were more nearly at the same stage of development than the ones had been the year before. Most of them would have shed pollen the next day, or at the most, by the second day.

The actual process of emasculation was the same as that followed the year before. If any of the anthers were split as they were pulled out, or seemed ready to dehisce, the floret was discarded. The forcepts and the hands were wet with alcohol, and then allowed to dry before going on. After emasculation, the excess florets were cut off, and the head was covered immediately with a 1 7/8 by 6 inch glasine sac. This bag was slightly lighter and smaller than the one used for the same purpose the year previously.

Pollination was done in the summer of 1948 as described for 1947 with a slight variation. Since there was a need for only five anthers to pollinate each head, only a few florets from the pollen supplying plants were gathered at a time. Enough ripe anthers to pollinate one or two heads were collected. These were placed in the crease of the hand, and held there from the time of gathering until they were used. This interval was, at the most, five minutes. Usually it was less. Most of the anthers used came from the small axillary florets, for it seemed to be easier to find mature,

dehiscing ones in these florets. Generally, the pollination was done between eight o'clock in the morning and five o'clock in the afternoon.

Bonda supplied the pollen for all of the replications except for five heads done in the afternoon of replication J and for replications K, L, and M. The pollen from Bonham was used in these replications.

During the latter part of the season, the anthers used to supply the pollen for crossing were smaller, harder to find, and appeared to be in poorer physical condition than those gathered earlier from florets that had been located nearer the top of the head.

To determine the order that the twenty-one treatments would be applied, tags indicating the treatments were mixed together. Then the tags were drawn from the lot at random. The order in which the tags were drawn determined the order in which the treatments were applied to the pollinated heads. The head subjected to the first treatment was one of the two located at the south end of the eleven adjacient clones of which two heads of each had been emasculated two days previously. The remaining head of the clone was given the second treatment. The two heads of the next clone were pollinated and treated with the next two treatments drawn. For the rest of the replication for a particular day, the heads were assigned to the remaining treatments according to the occurrence of the head in the row and to the sequence in which the treatments had been drawn.

After the first day, unless an even number of afternoon and morning pollinations had been done for the individual treatments, drawings had to be made separately for the afternoon and for the forenoon in such a way to insure that certain treatments would be applied in the morning, or in the afternoon as required to bring the totals together. When it made no difference otherwise, pollinations were made in the afternoon or forenoon as the work permitted. For example, during

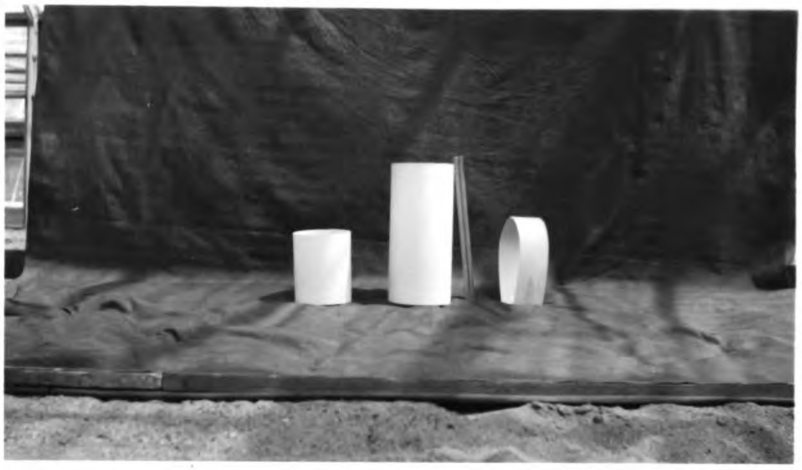


Fig. 1. Blotters used to adsorb salts.

the first day of pollination, treatments were applied in the morning according to the order drawn until the morning was over. The rest of the replication was done in the afternoon.

As soon as the heads had been pollinated, they were subjected to the treatment assigned to them. The principle of enclosing the head with the bag and the salt impregnated material was the same as that used in 1947. Slight modifications

were necessary when the blotters were used. The 18 by $2\frac{1}{2}$ inch strip of blotting paper was arched over the top of the stake and down along the inside of the bag before the bag was closed. The blotting paper cylinders were suspended in such a manner so that they were allowed to hang down around the head, more or less covering, or encircling it. A blotter with no salt adsorbed into it was used with the treatment, pliofilm bag alone, to prevent the bag from

Fig. 2. View of treatments applied in field.

collapsing. As was done the previous year, precautions were taken to keep the salt impregnated material from touching the heads.

Two days were allowed always to elapse between emasculation and pollination in the summer of 1948. Before emasculated florets were pollinated, they were examined to see if the ovary had started to grow, or to see if the stigmas had

wilted. Either of these conditions might have indicated that the floret had already been self-pollinated. If either of these conditions were present (no ovaries showed signs of growing), the floret was not used. Since these precautions were taken and time does not permit the growing of the seeds that were harvested from the cross-pollinated heads, the seeds produced will be considered as having been derived as a result of cross-pollination, and will be so considered in the results and the discussion that follow. Approximately 250 florets were left with the bag used to cover the emasculated. heads, and were never pollinated. At the time of harvest, none of these had any grains in them.

Tables and charts pertaining to the work done in the summer of 1948 will follow.

DISCUSSION OF RESULTS 1948

Again the results of the individual treatments were extremely variable. Part of this variability may be accounted for as a result of the small number of florets crossed per head.

The treatments not involving salts: The medium sized glasine bag gave the lowest set of any type bag. The pliofilm bag gave the highest actual set of the types of bags when it was used alone. The amount of seed set in the open and also in the tiny glasine bag was as large as any that was obtained by other treatments using bags alone, or larger, except in the case of the pliofilm bag.

-37-
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Table No. 7. Shows the results of each replication of the field experiment 1948. In the columns under the main heading of treatments the numbers represent the actual number of seed produced per head of which five florets had been artificially cross—pollinated. Dashes indicate no treatment.

Replication	Date June 27-30 / July 1-9	Open AM	Open PM	Tiny glasine bag AM	Tiny glasine bag PM	Medium glasine bag and blocks: Bag alone AM	Bag alone PM	$ZnSO_4$ AM	$ZnSO_4$ PM	KBr AM	KBr PM	Na Br AM	Na Br PM	Ca Cl_2 AM	Ca Cl_2 PM
A	27	1	-	-	0	0	-	0	-	5	-	0	-	0	-
C	29	-	1	-	3	-	1	-	4	-	3	-	1	-	5
D	30	2	-	2	-	3	-	1	-	4	-	5	-	1	-
E	1	-	1	0	-	-	1	-	2	0	-	1	-	-	1
F	2	-	2	-	4	-	2	-	0	-	3	-	4	-	0
G	3	1	-	0	-	0	-	1	-	0	-	0	-	0	-
H	4	-	2	-	0	-	0	-	0	0	-	-	0	-	0
I	5	2	-	0	-	0	-	0	-	-	2	0	-	0	-
J	6	-	2	-	1	0	-	2	-	2	-	0	-	-	1
K	7	0	-	2	-	-	1	-	0	-	4	-	5	1	-
L	8	-	4	-	5	-	1	-	0	-	0	-	0	1	-
M	9	0	-	0	-	0	-	0	-	-	0	-	1	-	1
Treatment total for AM AND PM		6	12	4	13	3	6	4	6	11	12	6	11	3	8
Grand total for treatment *		18		17		9		10		23		17		11	
Percentage set for grand total *		30.0		28.3		15.0		16.6		38.3		28.3		18.3	

Replication	Medium glasine bag and blotter: $ZnSO_4$ AM	$ZnSO_4$ PM	KBr AM	KBr PM	NaBr AM	NaBr PM	$CaCl_2$ AM	$CaCl_2$ PM	Big glasine bag with blotter: Bag alone AM	Bag alone PM	$ZnSO_4$ AM	$ZnSO_4$ PM	KBr AM	KBr PM	NaBr AM	NaBr PM	$CaCl_2$ AM	$CaCl_2$ PM
A	0	-	1	-	1	-	0	-	-	4	-	5	-	3	-	4	-	4
C	-	2	-	0	-	1	-	2	2	-	5	-	1	-	-	X	4	-
D	3	-	1	-	-	4	1	-	4	-	0	-	0	-	3	-	1	-
E	-	0	-	2	-	0	0	-	-	3	-	1	-	0	0	-	-	2
F	-	1	-	3	-	3	-	4	-	2	-	3	-	1	-	3	-	1
G	0	-	0	-	0	-	-	0	0	-	0	-	0	-	0	-	0	-
H	-	1	-	0	-	0	-	1	0	-	-	0	0	-	-	0	-	1
I	0	-	0	-	0	-	-	1	-	0	0	-	-	1	1	-	0	-
J	3	-	0	-	0	-	1	-	1	-	-	3	-	1	-	0	-	2
K	-	3	-	1	0	-	0	-	-	1	0	-	0	-	0	-	0	-
L	1	-	0	-	0	-	0	-	-	0	3	-	-	1	-	1	0	-
M	-	2	-	1	-	0	-	0	0	-	-	0	0	-	0	-	-	0
Treatment total for AM AND PM	7	9	2	7	1	8	2	8	7	10	8	12	1	7	4	8	5	10
Grand total for treatment *	16		9		9		10		17		20		8		12		15	
Percentage set for grand total *	26.6		15.0		15.0		16.6		28.3		33.3		13.3		21.8		25.0	

Replication	Pliofilm: Bag alone AM	Bag alone PM	$ZnSO_4$ AM	$ZnSO_4$ PM	turkey bag with blotter: KBr AM	KBr PM	NaBr AM	NaBr PM	$CaCl_2$ AM	$CaCl_2$ PM	Total no. grains set for day	% set for day out of a possible 105 florets crossed
A	-	5	-	5	-	4	-	1	-	4	47	44.8
C	2	-	-	5	-	0	4	-	-	5	51	51.0*-
D	-	5	2	-	2	-	-	3	2	-	49	46.7
E	0	-	1	-	0	-	2	-	0	-	17	16.2
F	-	3	-	2	-	4	-	3	-	5	53	50.5
G	1	-	0	-	0	-	1	-	0	-	4	3.8
H	-	0	-	1	-	0	2	-	-	0	8	7.6
I	1	-	0	-	1	-	-	1	1	-	11	10.5
J	-	4	1	-	-	0	-	3	-	2	29	27.6
K	0	-	-	4	0	-	0	-	0	-	22	30.0
L	1	-	0	-	-	3	-	4	2	-	27	25.7
M	-	1	-	2	0	-	0	-	-	4	12	11.4
Treatment total for AM AND PM	5	18	4	19	3	11	9	15	5	20	330	
Grand total for treatment *	23		23		14		24		25			
Percentage set for grand total *	38.3		38.3		23.3		40.0		41.6		26.3	"

* Total crossed for each treatment was 60 florets.

Overall figures:	Crossed	Set	% set
For summer	1255	330	26.3
For PM	625	230	36.8
For AM	630	100	15.8

X denotes head destroyed in field

*- % out of a possible 100 florets

Table No. 8. Gives the average temperatures (10) for 24 hours and for the period from 8:30 AM to 4:30 PM. Humidity averages for the same periods and the % set for the day are also given.

Date June 27-30 July 1-9	% Set for day	Ave. temp. for day 12:30 AM to 11:30 PM	Ave. temp. for 8:30 AM to 4:30 PM	Ave. rel. humidity for day 12:30 AM to 11:30 PM	Ave. rel. humidity for 8:30 AM to 4:30 PM
27	44.8	72	75	85	76
28	—	75	81	—	—
29	51.0	70	72	86	80
30	46.7	65	70	71	55
1	16.2	61	66	64	50
2	50.5	68	71	77	70
3	3.8	78	85	72	62
4	7.6	78	85	65	50
5	10.5	78	83	71	60
6	27.6	73	76	70	57
7	20.9	69	73	63	50
8	25.7	69	78	55	39
9	11.4	75	83	57	42

The humidities were computed from psychrometric tables (10) by using only whole degrees for the air temperature and for the dew point. No correction was made for pressure. The temperatures given are in degrees F. The weather data were secured from the East Lansing weather station of the U. S. Department of Commerce.

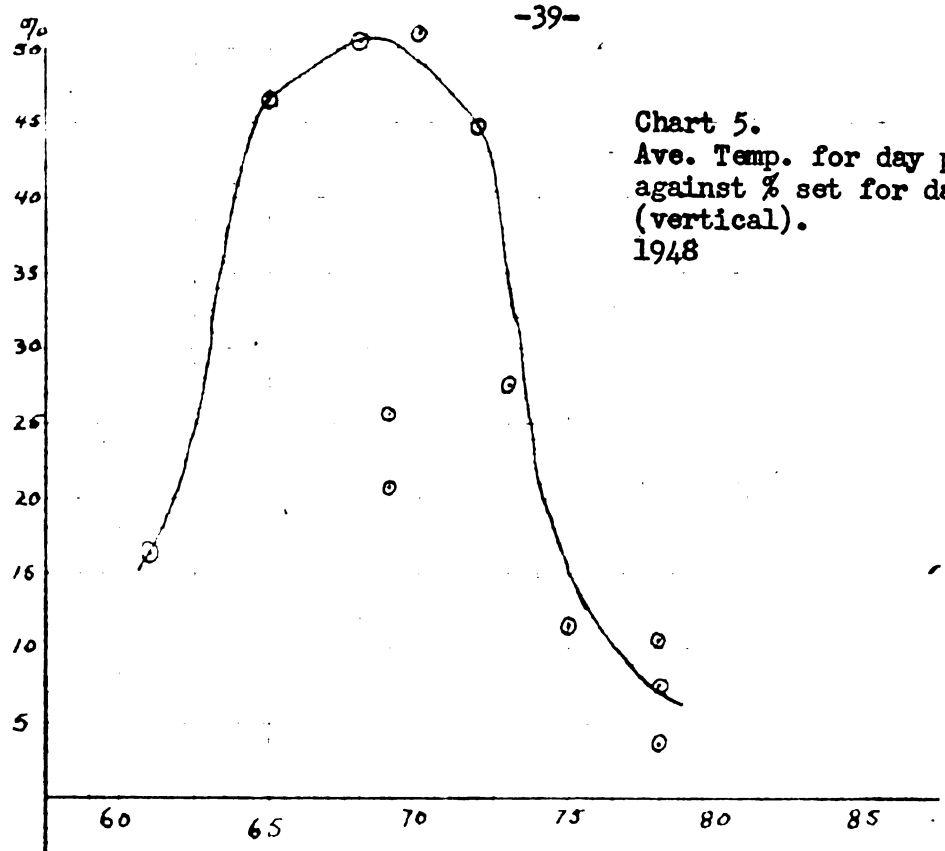

Chart 5.
Ave. Temp. for day plotted against % set for day (vertical).
1948

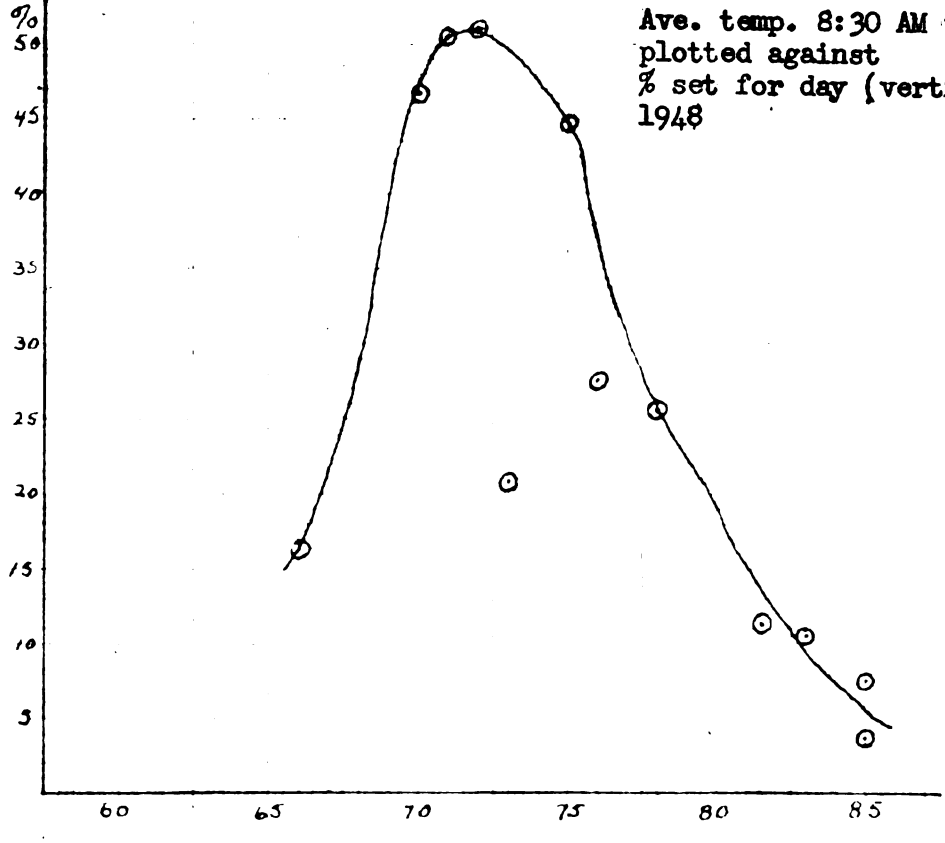

Chart 6.
Ave. temp. 8:30 AM to 4:30 PM plotted against
% set for day (vertical)
1948

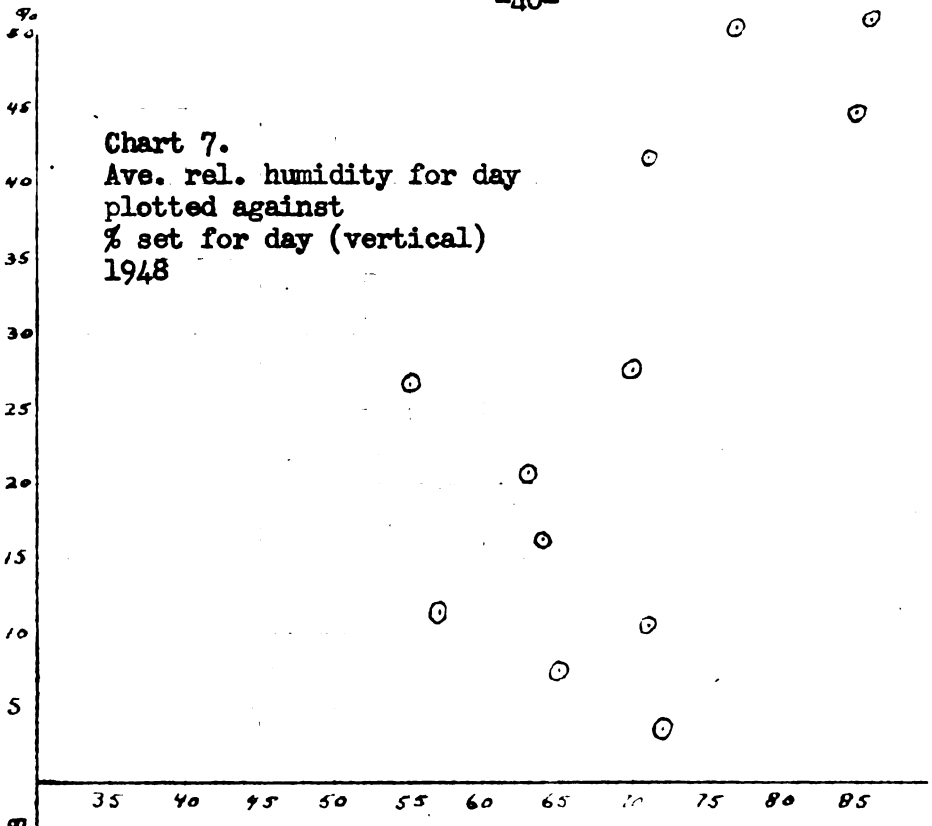

Chart 7.
Ave. rel. humidity for day plotted against % set for day (vertical) 1948

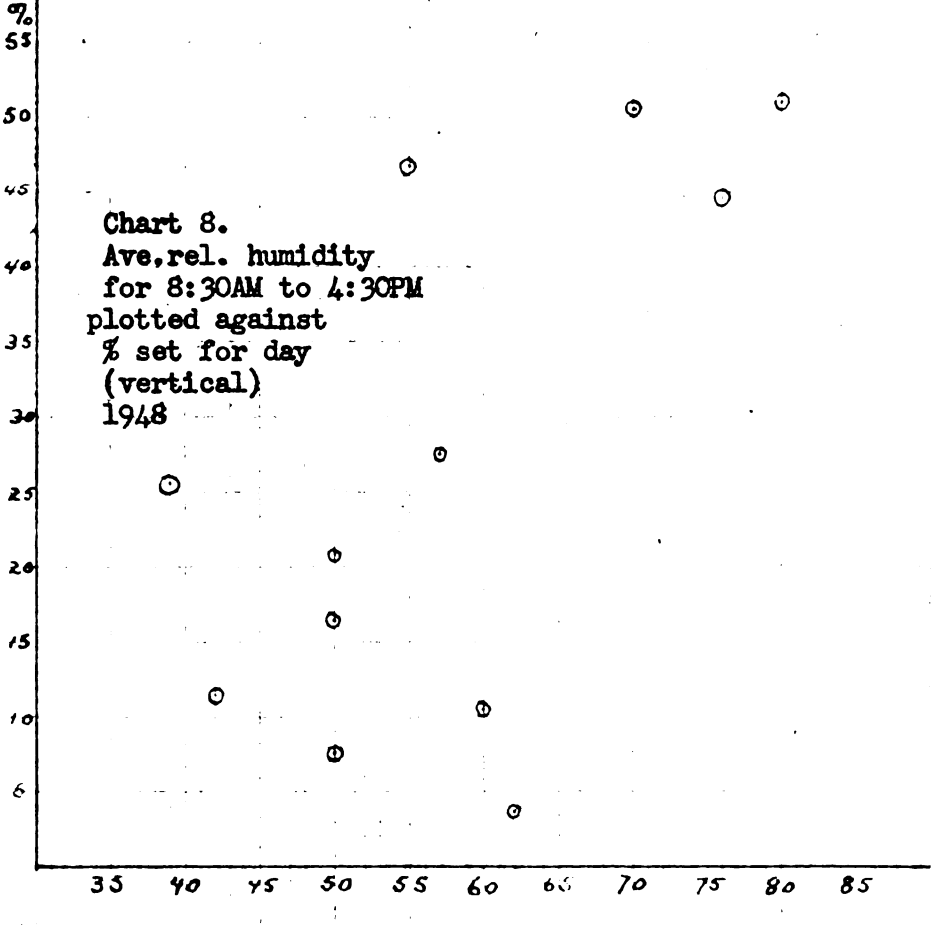

Chart 8.
Ave,rel. humidity for 8:30AM to 4:30PM plotted against % set for day (vertical) 1948

The treatments involving salts: The best results, on the average, were obtained with the pliofilm bag. The kind of salt used with the pliofilm bag did not seem to be of great importance. The set for the bag alone and for

Fig. 3. Cross-pollinated oat head covered with pliofilm bag. Note how blotter impregnated with salt is used.

each of the salts except KBr, which was more than 15% lower, were practically the same. With the exception of KBr in combination with the medium glasine bag, onon of the other bag-salt combinations gave any better results than did the heads left in the open or the tiny glasine bag.

Although the heads left in the open gave comparatively good results for cross-pollination, such a system of handling crossed heads would not be feasible because there would be no way to insure controlled pollination using this system.

The experiment for 1948 was arranged in order to allow a complete replication of the treatments each day that cross-pollination was done; consequently comparisons of the daily statistics can be more validly compared with the amount of set for the respective days.

The afternoon seems to be the more conductive to a higher set of cross-pollinated florets. In the afternoon, 230 florets set seed as against 100 that set after pollination was made in the morning.

The graph of the percentage of seed set per day against the temperature, except for two days, seems to indicate that there is some relationship between the temperature and the amount of set. The best set occurred when the average daily temperature was between 65 and 72 degrees F., or when the average temperature between 8:30 AM and 4:30 PM was 70 to 80 degrees F. On either side of these ranges, the amount of set decreased. The three lowest sets occurred on the days that were the hottest. The two days that did not give results consistent with the statements made above were at the end of the crossing season. Heads crossed late in the season, as stated before, seemed to be in poorer

physical condition than those crossed pre viously.

There seemed to be no correlation between the average humidity of the atomsphere and the amount of set.

COMPARISON OF RESULTS 1947 AND 1948

The set under the individual treatments showed great variability both years. There was a greater seed set in 1948 than in 1947, the percentages were 26.3 and 18.6 respectively.

The medium glasine bag when used alone gave about the same set, 14.7% and 15.0%, both years. The four salts used with the medium glasine bag did not give consistent results for the two years. The $ZnSO_4$ gave results 10% lower the second year than it did the year before; the KBr was over 10% better the second year.

A higher percentage of seed was set in the afternoon each year. This observation was the more pronounced the second year.

There was an indication the first year of a correlation between the daily seed set and the average temperature for the day. This tendency was more apparent the second year. It seems that there is an optimum temperature range for the seed to set after cross-pollination. Above this range, or below it, there is a reduction in the amount of set.

No correlation could be found of seed set to atomspheric humidity either year.

CONCLUSIONS

An attempt was made to increase the set of seeds following artificial cross-pollination of oats by enclosing the heads with a suitable container along with a chemical compound designed to have some effect on the moisture content of the encased air.

The conclusions are as follows:

1. A suitable bag seemed to offer the greatest possibility of increasing seed set.

2. The bag used proved to be of greater importance than the salt used.

3. The pliofilm turkey bag gave the best results. different salts used with the pliofilm bag did not seem to affect materially the seed set with this bag.

4. There were some indications that the temperature of the air materially influenced seed set.

5. There appeared to be no relation between the humidity of the air and the amount of set.

These last two observations are included even though the experiment was not designed to test the factors considered.

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