THE EFFECTS OF POLLEN MIXTURES AND OF FOREIGN POLLEN APPLIED AT DIFFERENT TIME INTERVALS ON THE SEED PHENOTYPES IN CORN

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By

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TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Corn has been a major grain crop in the United States since its discovery by the early white explorers. Since that time much improvement has been made in yield, quality, disease resistance, lodging resistance and other agronomic characters through the use of selection and other plant breeding methods. These advancements and the production of new hybrid strains have made it essential for the plant breeder to use isolated blocks in order that he may maintain pure strains. Therefore, information pertaining to the application of foreign pollen at the same time or at different time intervals to certain strains of corn would be useful to the breeder in order that he may know the effects caused by foreign pollen. This type of information would aid in determining the amount of isolation necessary and the distance between isolated blocks essential in maintaining pure strains.

The basis of such a breeding program would be dependent upon such factors as the delay in pollination, differences in pollen potency, female preference, differential rate of pollen-tube growth and other physiological factors.

The objectives of these investigations are to determine:

1. The effects of mixing pollen obtained from a Honey June plant, *Zea mays saccharata* L., and a Yellow Surcropper plant, *Zea mays indentata* L., and then applying the pollen to each plant.

2. The competitive effect of Honey June and Yellow Surcropper pollen applied at different time intervals where Honey June was used as the female parent.

REVIEW OF LITERATURE

Some of the very early investigators were familiar with the effects of foreign pollen. In a discussion of methods which insure flowers being fertilized with pollen from different plants, Darwin (2)[1] concluded that if pollen from a different species or variety is placed on the stigma of an emasculated flower, and then after a time interval of several hours, pollen from the same species is placed on the stigma, the effects of the latter are completely dominant over the pollen of the other species.

More recently, Jones (4), working with mixed pollinations in corn, has shown that the plant's own pollen has been more efficient in accomplishing fertilization than that from other varieties or individuals which may differ only in minor features. The pollen, which was less effective when in competition with the plant's own pollen, was fully able to function when not applied in mixtures.

From 23 pollen mixtures, a total of 76,620 seeds were obtained. Of these 23 mixed pollinations, 20 showed a selective action in favor of the plant's own pollen, while the other 3 exhibited opposite results. Since such a large proportion of the pollen mixtures showed a marked deviation from random assortment, it was concluded that in corn the plant's own pollen was more effective in achieving fertilization than pollen from plants of only slightly different genetic constitution (4).

A difference exists in the ability of the pollen from different types of plants to accomplish sexual fusion when acting in competition (4). Experiments with corn showed that the differences in rate of pollen-tube

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[1] Numbers in parenthesis refer to "Literature Cited", p. 16.

growth has some influence in causing this selective action to take place (3).

Evidence concerning the differences in the rate of pollen-tube growth in plants showing this selective action was obtained by separating the ears into upper and lower halves (4). The pollen tubes which grew faster furnished the gametes for fertilization of a greater number of seeds on the lower than in the upper halves. The farther they had to travel the more probable that the faster growing pollen-tubes would reach the egg first.

A similar selective action favoring the plant's own pollen has been reported in cotton. Kearney and Harrison (5) obtained evidence that selective fertilization, in favor of the like pollen, takes place in Upland, and also in Egyptian cotton.

Further investigations (6) indicated that when emasculated flowers of Pima and Upland cotton, were pollinated with approximately equal quantities of both types of pollen, a marked degree of selective fertilization was shown. The resulting populations, from such pollinations, contained a higher percentage of homozygous than of heterozygous plants.

Both types of pollen were tested in media and by observation, and, so far as could be determined, there were no differences in the viability of the two pollens that could account for selective fertilization (6).

A differential rate of growth of the tubes of the like and of the unlike pollen could not be found in the styles. This factor, which seems to be responsible for selective fertilization in Zea, and a few other plants, does not account for the situation met with in gossypium (6).

The authors (6) contend that the presence of the like pollen causes a reaction in the stigmatic tissues which causes these tissues to be less suitable for the development of the unlike pollen.

From a review of the literature concerning selective fertilization

Brieger (1) concluded that the difference between the elimination of gametes and selective fertilization lies in the fact that the elimination of gametes is fixed for a given plant, while selective fertilization appears only in certain combinations. The gametes in selective fertilization are always potentially functional and fail to function in certain combinations only. This selection is caused by an interaction between the genotype of the female diploid plant and the male gametophyte in higher plants or between the two gametes in Basidiomycetes. It may make a difference whether the factor for selective fertilization is present in the female in the homozygous or the heterozygous condition.

MATERIALS AND METHODS

This experiment was conducted in 1948 at the Oklahoma Experiment Station at Stillwater, Oklahoma. The open pollinated varieties, Honey June (white sweet) and Yellow Surcropper (yellow dent), were used.

For the paired pollinations between varieties, plantings were made on June 9, 1948, and the pollinations were made on August 4, 7, and 10. For the study on pollinations made at different time intervals, plantings were made on July 5, and the pollinations were made on August 29, 30, 31, and September 1, 2. The ear shoots were bagged before any silks appeared. The tassels were covered 14 to 16 hours before the pollinations were made, so that any foreign pollen that may have lodged on the tassels would have lost its viability. The silks were cut off evenly the day before the plants were pollinated to insure that all pollen would have an equal chance to be the fertilizing agent. Immediately after each pollination, the ear shoot was covered by a number 12 Kraft paper bag.

In order to determine the effects of pollen mixtures on the seed phenotypes obtained from a Honey June plant and a Yellow Surcropper plant, 112 paired pollinations were made. The pollen from each individual pair was placed in a paper bag and thoroughly mixed by shaking, and then applied to the plants which supplied it. Each individual ear shoot was then tagged and marked with its pair number and type of pollination made.

The ears were harvested on October 1, 1948, and the different type kernels on each ear were counted and recorded. When Honey June was used as the female parent, each seed resulting from fertilization by the Honey June pollen was indicated by a shrunken endosperm, while those fertilized by the Yellow Surcropper pollen had a plump, or starchy, endosperm (xenia). On the opposite cross, using Yellow Surcropper as the female parent, the deep yellow colored kernels indicated that these eggs were fertilized by Yellow Surcropper pollen, while those kernels showing a pale yellow color were due to fertilization by the Honey June pollen.

For the study on pollinations made at different time intervals, Honey June was used as the female parent, and pollinations were made for a 5 day period. Each day's pollinations were a duplication of the preceding day. Five Honey June plants were pollinated with Honey June and Yellow Surcropper pollen at 0.0 hour. Fifty Honey June plants were pollinated at 0.0 hour with Honey June pollen. From these 50 plants, 10 groups of 5 were pollinated with Yellow Surcropper pollen at the time intervals 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0 and 6.0 hours, respectively. In the cross where Yellow Surcropper was used as the pollen applied first, 5 Honey June plants were pollinated with Yellow Surcropper and Honey June pollen at 0.0 hour. Fifty Honey June plants were pollinated at 0.0 hour with Yellow Surcropper pollen. From these 50 plants, 10 groups of 5 were pollinated with Honey June pollen at the time intervals 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, and 6.0 hours, respectively.

A total of twenty-five pollinations for each time period were made for the cross Honey June x (Honey June + Yellow Surcropper) and also for the cross Honey June x (Yellow Surcropper + Honey June). A total of 550 plants were pollinated over the 5 day period.

The ears were harvested on October 29, 30; and the data for each day's pollinations were recorded in % of total for the pollen applied first.

The data obtained from the paired pollinations between varieties, and the pollinations at different time intervals were analyzed by the analysis of variance method.

RESULTS AND DISCUSSION

Paired Pollinations Between Varieties

Some difficulty was encountered in obtaining plant pairs between the varieties, because of a difference in the time of silking and pollen shedding. The Yellow Surcropper variety began shedding pollen on July 30, 1948; whereas, the Honey June did not start producing pollen until August 3. However, because of the large amount of variability within the varieties, the writer was able to make 112 paired pollinations over a period of 6 days. From these 112 paired pollinations, 90 pairs set seed and were harvested. A total of 80,362 kernels were obtained from the 180 ears.

The analysis of variance of the results of this study is presented in Table 1. The basic data for this analysis are given in Table 4 (Appendix). Since an F value of 1.04 obtained for the pair's comparison was not significant at the 5% level, it may be assumed that there was no significant difference between the combined ear sizes of each pair, for the 90 pairs involved.

An F value of 7.78 was obtained for the female varieties comparison. Therefore, there was a highly significant difference between the average ear sizes of the varieties, Honey June and Yellow Surcropper. The Honey June ears averaged 472 kernels and the Yellow Surcropper ears averaged 421 kernels.

The F value obtained for pollen was 0.46. This figure was not significant at the 5% level, and indicates that there was no significant difference in the average amount or potency of the 2 pollens used in the mixture.

The interaction, pairs x pollen, gave a highly significant F value of 7.26, indicating that the amount or potency of the 2 pollen varieties was not the same in all pairs.

A significant difference for the interaction of variety female x pollen was obtained and indicates that the female parent exhibited a pollen preference for 1 of the pollen types. Since fertilization of the Honey June plants with the pollen mixture gave 22,540 or 53.07% selfed kernels and 19,927 or 46.93% crossed kernels, and fertilization of the Yellow Surcropper plants gave 19,701 or 52.00% selfed kernels and 18,194 or 48.00% crossed kernels, it seems evident that the female parent preferred its own pollen instead of the foreign pollen (Table 4).

The interaction, pairs x variety female, showed no significant difference, and was included in the error term. This gave a more reliable mean square error term to use in the computations.

Table 1.--Analysis of variance of the number of selfed and crossed kernels obtained when a mixture of pollen from a Honey June and a Yellow Surcropper plant was applied to each plant.

Source of variation	D.F.	Sum of Squares	Mean Square	F value
Total	359	6,947,489	19,352	
Pairs	89	689,850	7,751	1.04
Variety Female	1	58,064	58,064	7.78**
Pollen	1	3,398	3,398	0.46
Pairs x Pollen	89	4,820,556	54,164	7.26**
Variety Female x Pollen	1	47,151	47,151	6.32*
Error	178	1,328,470	7,463	
Pairs x Variety Female	89	562,197	6,317	0.73
Pairs x Variety Female x Pollen	89	766,273	8,610	

*Significant at the 5% level.

**Significant at the 1% level.

Pollinations at Different Time Intervals

Because of the large amount of dew present on the plants each morning, pollinations could not be made at an early hour. For the 5 day series, the pollinations were started at 9:30 a.m. and completed at 3:30 p.m. From the 550 pollinations made, 433 of the ears set seed and yielded 172,443 kernels.

The analysis of variance of the data are given in Table 2. The basic data for this analysis are given in Tables 5 and 6 (Appendix).

In the analysis of variance, it was found that the differences caused by dates were significant at the 1% level, indicating that different results were obtained on different pollination dates.

There was no significant difference between the results of the crosses Honey June x (Honey June ≠ Yellow Surcropper), and Honey June x (Yellow Surcropper ≠ Honey June). The small F value obtained may be accounted for when a comparison is made between the 2 types of pollinations. When Honey June pollen was applied first 57.46% of the total kernels were produced by fertilization of the female parent by the Honey June pollen. When Yellow Surcropper pollen was applied first 58.09% of the total kernels were produced by fertilization of the female parent by the Yellow Surcropper pollen.

An F value of 23.84 was obtained for the delay in pollination, indicating highly significant differences obtained between the pollen applied at 0.0 hour and that applied at 6.0 hours.

The first order interactions, dates x varieties, dates x times and varieties x times were checked by the second order interaction, or error term, dates x varieties x times. The F value indicated no significant difference for any of the first order interactions; therefore, they were combined with the error term and a more reliable error mean square was figured and used in obtaining the F values for the main factors involved.

Table 2.—Analysis of variance of the average number of selfed kernels (Honey June) in % of total on 5 dates when Yellow Surcropper pollen was applied at various time intervals after Honey June pollen and of the average number of crossed kernels onto Honey June in % of total on 5 dates when Honey June pollen was applied at various time intervals after Yellow Surcropper pollen.

Source of Variation	D.F.	Sum of Squares	Mean Square	F Value
Total	109	64,195.36	588.95	
Dates	4	5,610.13	1,402.53	7.96**
Varieties	1	10.97	10.97	0.06
Times	10	42,011.84	4,201.18	23.84**
Error	94	16,562.42	176.20	
D x V	4	619.71	154.93	0.81
D x T	40	6,942.29	173.56	0.91
V x T	10	1,376.98	137.70	0.72
D x V x T	40	7,623.44	190.59	

**Significant at the 1% level.

The data presented in Table 3 illustrate the significant differences caused by the application of pollen at various time intervals.

The average % of total kernels produced by pollen applied first at the 0.0 hour was significantly different from that produced at 1.0 and 3.5 hours, and highly significant from that produced at 0.5, 3.0, 4.0, 5.0 and 6.0 hours.

From these results it seems evident that the pollen applied first has a significant advantage over pollen applied later, only when there is a time interval of at least 3 hours between pollen applications.

Table 3.—Average % of total kernels produced by pollen first applied to Honey June at Stillwater, Oklahoma in 1948.

Hour	Honey June applied first	Yellow Surcropper applied first	Average %
0.0	52.85	47.32	50.08
0.5	36.22	22.70	29.46**
1.0	34.51	36.25	35.38*
1.5	43.24	33.45	38.34
2.0	39.12	40.56	39.84
2.5	56.80	61.76	59.28
3.0	74.09	77.17	75.63**
3.5	63.82	67.12	65.47*
4.0	64.89	74.87	69.88**
5.0	83.51	85.14	84.32**
6.0	83.07	92.73	87.90**
Average	57.46	58.09	57.77

*Significant at the 5% level.

**Significant at the 1% level.

Significant differences of 11.79 and 15.64% were required at the 5 and 1% levels, respectively.

SUMMARY AND CONCLUSIONS

A study of the effects of paired pollinations between varieties and pollinations at various time intervals on the seed phenotypes in corn was conducted at the Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma in 1948, as an aid to breeders in determining some of the factors that contribute to the effects caused by foreign pollen. A total of 252,805 kernels were used for this study.

1. In the paired pollination study, no significant difference was obtained between the combined ear sizes of the pairs, for the 90 pairs involved.

2. A highly significant difference was obtained between the average ear sizes of the varieties Honey June and Yellow Surcropper.

3. There was no significant difference obtained in the average amount or potency of the 2 pollens used.

4. A highly significant difference was exhibited by the interaction, pairs x pollen, indicating that all pollen did not react the same in all pairs.

5. A significant difference was obtained in the interaction of variety female x pollen, indicating that the variety used as the female parent showed a preference for its own pollen.

6. The interaction, pairs x variety female, showed no significant difference and indicated that no differences were caused due to different ear sizes in different pairs.

7. In the pollinations at different time intervals, date of pollination showed a highly significant difference, indicating that different results were obtained on different pollination dates.

8. No significant difference occurred in the results of the crosses Honey June x (Honey June ≁ Yellow Surcropper), and Honey June x (Yellow Surcropper ≁ Honey June).

9. A highly significant difference was obtained in the delay in time of pollination, indicating the results obtained from pollen applied at 0.0 hours were quite different from pollen applied at 6.0 hours.

The average % of total kernels produced by pollen applied first at the 0.0 hour was significantly different from that produced at 1.0 and 3.5 hours, and highly significant from that produced at 0.5, 3.0, 4.0, 5.0 and 6.0 hours. These results indicate that the pollen applied first, to the female parent, has a significant advantage over pollen applied later, only when there is a time interval of at least 3 hours between pollen applications.

10. The interactions used in the analysis, dates x varieties, dates x times, and varieties x times gave no significant differences.

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APPENDIX

Table 4.--Number of selfed and crossed kernels obtained when a mixture of pollen from a Honey June and a Yellow Surcropper plant was applied to each plant.

Pair No.	Honey June Female		Yellow Surcropper Female	
	No. of Kernels		No. of Kernels	
	Selfed	Crossed	Selfed	Crossed
1	95	448	272	40
2	137	248	187	153
3	545	121	146	170
4	122	460	348	23
5	134	238	214	237
6	192	240	172	304
7	373	51	41	440
8	373	95	232	240
9	332	193	152	227
10	362	80	109	264
11	279	249	254	99
12	251	56	45	241
13	63	209	299	147
14	378	231	46	96
15	138	293	271	130
16	105	169	226	193
17	29	170	267	69
18	387	70	144	315
19	155	133	253	303
20	207	282	270	233
21	511	31	57	445
22	116	428	344	196
23	14	277	240	74
24	87	200	205	52
25	234	36	201	150
26	375	89	48	187
27	223	298	229	222
28	312	82	34	351
29	381	91	132	122
30	299	23	106	60
31	245	161	105	130
32	22	318	490	25
33	126	307	441	44
34	165	33	285	180
35	305	254	229	294

Table 4.—(Cont'd.)

Pair No.	Honey June Female		Yellow Surecropper Female	
	No. of Kernels		No. of Kernels	
	Selfed	Crossed	Selfed	Crossed
36	478	18	102	243
37	449	27	66	264
38	447	39	110	430
39	126	136	348	116
40	328	103	165	194
41	314	201	119	259
42	194	324	194	227
43	505	42	36	239
44	157	483	340	139
45	803	37	107	104
46	157	95	403	109
47	96	368	319	138
48	536	71	65	441
49	345	160	244	211
50	120	582	391	135
51	201	303	226	266
52	232	243	271	266
53	335	113	108	288
54	206	223	341	139
55	231	80	273	84
56	393	2	10	381
57	124	602	386	180
58	344	232	429	95
59	407	121	119	359
60	354	27	37	366
61	130	388	560	12
62	406	154	74	370
63	111	385	287	214
64	387	104	32	453
65	131	415	238	67
66	291	108	178	237
67	155	473	376	114
68	573	241	36	188
69	170	333	376	129
70	86	618	411	81

Table 4.--(Cont'd.)

Pair No.	Honey June Female		Yellow Surecropper Female	
	No. of Kernels		No. of Kernels	
	Selfed	Crossed	Selfed	Crossed
71	154	219	112	270
72	261	262	118	215
73	295	51	73	235
74	6	570	500	65
75	365	312	276	127
76	58	300	290	92
77	199	162	67	235
78	23	299	331	18
79	191	412	267	88
80	557	66	26	429
81	89	492	367	58
82	162	220	237	291
83	340	425	339	261
84	166	416	417	42
85	129	300	270	258
86	326	58	130	414
87	340	316	251	256
88	175	301	287	233
89	247	108	150	447
90	63	123	322	166
Totals	22,540	19,927	19,701	18,194

Table 5.--Average number of selfed kernels (Honey June) in % of total on 5 dates when Yellow Surcropper pollen was applied at various time intervals after Honey June Pollen.

Time Delay in Hours	Honey June x (Honey June + Yellow Surcropper) Average % Honey June Dates 1	2	3	4	5
0.0	55.51	54.41	36.86	56.59	60.90
0.5	39.45	27.74	37.63	21.88	54.42
1.0	37.61	26.15	39.63	20.80	48.34
1.5	32.78	11.65	42.98	62.41	66.36
2.0	41.52	20.22	40.14	38.61	55.10
2.5	36.53	58.07	43.70	79.57	66.12
3.0	68.15	80.05	87.04	69.40	65.81
3.5	65.35	55.38	51.23	78.24	68.90
4.0	65.50	46.10	58.94	83.96	69.97
5.0	80.34	85.03	64.38	96.67	91.13
6.0	86.54	79.36	70.82	85.81	92.81
Average	55.39	49.47	52.12	63.08	67.26

Table 6.—Average number of crossed kernels onto Honey June in % of total on 5 dates when Honey June pollen was applied at various time intervals after Yellow Surcropper pollen.

Time Delay in Hours	Honey June x (Yellow Surcropper ≠ Honey June) Average % Yellow Surcropper Dates 1	2	3	4	5
0.0	69.74	29.76	44.72	70.19	22.19
0.5	9.92	8.86	17.12	46.76	30.83
1.0	50.05	21.58	18.05	48.93	42.64
1.5	33.79	42.67	26.55	30.77	33.45
2.0	6.35	39.79	22.03	88.08	46.54
2.5	20.58	46.79	67.50	89.72	84.21
3.0	64.63	90.87	75.40	73.13	81.80
3.5	58.43	52.62	62.60	80.57	81.39
4.0	83.70	57.38	69.09	77.96	86.23
5.0	78.33	76.25	85.04	90.98	95.11
6.0	88.83	91.71	97.62	94.47	91.04
Average	51.30	50.75	53.25	71.96	63.22

Typist: Mary Wallace Spohn