THE INFLUENCE OF ENVIRONMENTAL FACTORS AND POLLINATING TECHNIQUES ON THE SUCCESS OF POTATO POLLINATION IN THE GREENHOUSE[1]

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INTRODUCTION

Each year thousands of crosses are made by potato breeders in this country. Although many of these are fruitful, crosses between some of the most desirable parental types result in very few or no viable seed. There are many causes of non-fruitfulness in the potato but it has been known for a number of years that environment plays an important role. Not only could the number of crosses producing fruit be increased but also the efficiency of the breeding program could be considerably improved if each potato breeder knew at what stages of flower maturity and at what time of day he might pollinate a flower with a reasonable chance that it would set fruit. He should also know how high the temperature could rise before further pollinating is a waste of time. Definite information concerning these and other problems connected with the effect of environment and pollinating techniques on fruit setting in potatoes is not presently available to most potato breeders.

Regarding the effect on environment on non-fruitfulness of the potato plant, Young (14) pointed out that high temperature and low moisture cause degeneration of embryo-sacs at all stages of flower development. Longley and Clark (11) found that environmental differences during two seasons had little effect on the fertility of some potato varieties but caused considerable variation in others. This variation, they believed, was due to differences in the amount of moisture during the two seasons, more meiotic disturbances occurring during the season of scanty rainfall.

Edmundson (7) found that significantly more viable pollen was produced on plants grown in the greenhouse at Greeley, Colorado, (elevation, 4800 feet) or in the field at Estes Park, Colorado, (elevation, 7500 feet) than was produced on plants of the same variety grown in the field at Greeley. No differences in amount or viability of pollen were found for plants grown at the first two locations. Low temperature and high humidity were found to be the conditions most favorable for the formation of viable pollen in Louisiana by Henderson and LeClerg (9). Clarke (2) observed that about 36 hours were required for fertilization to occur after the stigma had been pollinated. A high temperature during these critical hours, he believed, hastened macrospore degeneration and abscission.

East (6) mentions that pollen appeared to be in the best condition for use in the early morning of the second day of blooming and that it was at this time that the stigma was most receptive. Salaman (12), in describing a procedure for a potato breeding program, says that pollination should

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take place after the flowers are open. He says that flowers can be pollinated at the time of emasculation (24-48 hours before they open) but that fruit set at this time is poor. Henderson and LeClerg (9) found that flowers pollinated at the time of emasculation failed to set as much fruit as those pollinated 24 hours later. They did note pollen tubes in the styles of the buds pollinated at the time of emasculation and so assumed that the ovules were probably immature when the pollen tubes reached them.

That the position of the flower cluster may effect fruit set was shown by East (6) who found that the flower clusters produced in the center of the plant are more apt to set fruit than are those produced above or below. Amount and intensity of light are also known to influence the number of flowers and berries produced, (4), (5), and (13). Clarke and Lombard (3) report that seed-piece size may affect the number of flowers formed and, consequently, the number of fruit set.

Materials and Methods

Most of the investigations reported in this paper are based on the results of crosses made among seven potato clones—B24-58, Katahdin, Canoga, Earlaine, Houma, 9V224-3 and 9V21-11. These particular clones were selected because they seemed to be representative of the wide variation of male and female fertility found in potato clones used for breeding purposes. All of these except the male-sterile Houma produce varying amounts of viable pollen.

All crosses were made during the early spring months in the greenhouse using plants grown in soil beds or in twelve-inch clay pots. Plants were trimmed to two or three branches and kept upright by tying to stakes. The beds and benches were lighted until 10 p.m. each evening so that all plants had at least 16 hours of illumination. Lights were also used on cloudy days.

Since high temperatures are known to reduce the fertility of some potato clones the temperature at the time each cross was made was recorded. Also, during 1952 and 1953 a cylindrical recording thermograph was used to record the temperature throughout the pollinating season. The greenhouse thermostat was set at 65°F. During the night and on cool-cloudy days the temperature remained below 70°F. On sunny days, however, it often rose as high as 80° to 100°F. The amount of heat accumulating each day was calculated as the degree-hours above 70°F. The units for any one day were determined by counting the number of squares (each square being equal to one degree for one hour) below the line made by the stylus of the thermograph and above the 70° line on the thermograph chart.

Flowers were pollinated at three stages of maturity: 1. About 48 hours before the flower opened; 2. About 24 hours before the flower opened; and, 3. Just after the flower had opened. These three stages were designated as "early bud", "late bud", and "open", respectively. The approximate sizes of buds estimated to be at these three stages are shown in figure 1. In addition to the blossom clusters pollinated at these three stages, for which pollinating was done between 6 and 11 a.m., flowers of each cross were pollinated in the evening between 5 and 9 p.m. An

FIGURE 1.—The stages of maturity at which potato flowers and buds were pollinated: Open—just after the flower had opened. Late bud—approximately 24 hours before the flower opened. Early bud—approximately 48 hours before the flower opened.

attempt was made to have the flowers pollinated in the evening at the late bud stage of development, however, since most flowers open in the morning, the buds pollinated during the evening were somewhat more mature than were those listed as at the late bud stage. In 1952 and 1953 from five to eight flowers for each of the three maturity stages of 42 crosses were pollinated. Also from five to eight flowers of each cross were pollinated during the evening. One half of these pollinations, those in which Earlaine, B24-58, and Canoga were the male parents, were repeated in 1954.

In most instances the pollen was transferred directly from the anther to the stigma by means of a scalpel which was sterilized in alcohol after each cross to prevent contamination. In order to save time and obtain a more even distribution of pollen on the stigma for certain experiments in 1954, an oversize ten-penny finishing nail, its head hollowed by drilling with a 3/32 inch bit, was used as a pollinating cup. The shank of the nail acted as a handle and the cup formed by the drilling was of ideal size to fit over the stigma. A scalpel was used to scrape pollen from the anthers into the cup. The point of the nail was used to spread the corolla when buds were pollinated and the pollinating was done by dipping the stigma into the pollen-filled cup. Enough pollen to pollinate from 60 to 100 flowers could be placed in each cup.

Inflorescences were tagged in a way which permitted each flower within the inflorescences to be identified. All seedball clusters were bagged with cloth bags two or three weeks after pollination. Records were kept so that the time when each cross was made, the temperature at which each cross was made and the stage of flower maturity could be determined after seedballs had matured.

Seeds were counted in all seedballs which set in 1952, in three seedballs per cross in 1953 and in two in 1954. Whenever possible in 1953 and 1954 counts were made from the second and third berries on either side of the cluster. All other fruits in the cluster were removed as soon as it could be determined which were going to set. Only plump seeds were included in seed counts.

Most of the data were subjected to an analysis of variance. Statistically significant differences between means were determined by the Student-Neuman-Keuls Multiple Range Test (8).

Experimental Results

Stage of flower maturity at time of pollination

Prior to 1952 most flowers used in the breeding program were pollinated before they opened. During 1951 some flowers pollinated after they had opened, set fruit very rapidly. This in addition to supporting observations of East (6) and Henderson and LeClerg (9) led to the carrying out of an experiment designed to show the relationship between fruit set and age of flower at the time of pollinating. The number of fruit set and seeds per seedball from the crosses made during these three years are shown in tables 1 and 2.

An analysis of variance of the 1953 and 1954 data (the 1952 experiment was not set up so that the data could be analyzed) showed that the percentage of fruit set and the number of seeds per seedball were reduced by pollinating 48 hours before the flower opened. The percentage of fruit set did not differ significantly if the pollinating was done the day preceding, the evening preceding, or the day following flower opening, although the number of seeds per seedball were fewer when buds were pollinated 24 hours before the flower opened than they were if pollinating was delayed until after the flower had opened.

An experiment designed to test the range of flower maturity over which pollination can be expected to have a chance to succeed was set up in 1954. On April 10, 14, and 23, flower clusters with buds and flowers at various stages of development were pollinated with pollen-fertile clones used for this study. The date on which the flowers had opened or would open was estimated at the time of pollination. The results for the three pollinating dates are summarized in table 3.

Under the conditions existing in the greenhouse at Ithaca in April, 1954, the percentage of fruit set in relation to flower maturity formed a normal curve with its peak at about the time the flower opened. Crosses made on flowers at the time they opened or one day after they had opened had the best chance to set fruit but some fruit was set by flowers pollinated at maturity levels from two days before they opened until three days after they had opened.

Effect of temperature on pollination

Although the greenhouse temperature at night and on cloudy days was controlled at 65°F., it rose considerably above that point on sunny days. Since fruit set seemed to be better on some days than on others, even when the clones were crossed, it appeared possible that temperature fluctuations might be influencing fruit set.

As was mentioned previously the heat units above 70° accumulating each day were determined from thermograph charts. Since a record was kept of the date when each flower was pollinated, the percentage of successful crosses under any particular set of temperature conditions was easily determined.

The percentage of fruit set failed to show any correlation with the heat units for any one of the three 24-hour periods just before pollination or for either of the two 24-hour periods immediately following pollination. Partly because Clarke(2) had stressed the importance of temperature

TABLE 1.—*Number of pollinations and number of fruit set from crosses among seven potato clones pollinated at different stages of flower maturity and times of day during 1952, 1953, and 1954.*

Pollinated Year	From 6 to 11 a. m.						From 5 to 9 p. m.				
	Early Bud		Late Bud		Open		Late Bud		Total		
	Flowers Pollinated	Fruit Set	Flowers Pollinated	Fruit Set	Flowers Pollinated	Fruit Set	Flowers Pollinated	Fruit Set	Flowers Pollinated	Fruit Set	
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	Pct.
1952	115	6	270	114	131	71	193	97	709	288	40.6
1953	232	87	347	167	347	191	351	172	1388	617	45.1
1954	113	21	116	59	119	65	111	32	459	188	41.0
Total	460	114	733	340	597	327	655	301	2556	1093	42.8
Set Per cent	20.0		46.5		54.8		47.6				

TABLE 2.—*Seeds per seedball produced from crosses among seven potato clones pollinated at different stages of flower maturity and times of day during 1952, 1953, 1954.*

Pollinated Year	From 6 to 11 a. m.						From 5 to 9 p. m.			
	Early Bud		Late Bud		Open		Late Bud		Total	
	Fruit Counted	Seeds per Fruit	Fruit Counted	Seeds per Fruit	Fruit Counted	Seeds per Fruit	Fruit Counted	Seeds per Fruit	Fruit Counted	Seeds per Fruit
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
1952	3	55	112	108	75	117	91	120	281	114
1953	47	76	84	105	86	146	86	137	303	122
1954	13	75	30	107	30	140	20	134	93	119
Total	63		226		191		197		677	118
Average		75		107		134		129		

TABLE 3.—*Relationship of fruit set to stage of flower maturity at the time of pollination.*

Stage of Maturity	Flowers Pollinated	Fruit Set	Fruit Set
	No.	No.	Per cent
Open 3 days	42	8	19.0
Open 2 days	89	26	29.2
Open 1 day	109	47	43.1
Just opened	125	51	40.8
Late bud	104	34	32.7
Early bud	76	12	17.1
Three days before opening	23	0	0
Total	568	178	31.3

during the time immediately following pollination, the heat units accumulating .over the period from 24 hours preceding to 96 hours following pollination were compared with the percentage of fruit set. These data showed a significant negative correlation (r equalled –.88) between fruit set and degree hours for the early bud stage, but no correlation for the other stages of flower development.

Since the period of time from 24 hours preceding, to 96 hours following pollination at the early bud stage, is the same as the period of time from 72 hours preceding to 48 hours following flower opening at that stage, it seemed possible that the reduction in fruit set might be brought about by high temperature at the time of flower opening rather than at the time of pollination. Table 4 shows this relationship for the four stages of flower maturity.

TABLE 4.—*Percentage of fruit set for flowers pollinated in 1952 and 1953 in relation to the cumulative degree-hours above 70°F. for a period of time from 72 hours before to 48 hours after pollination.*

5-day Cumulative Degree-Hours	Stage or Time of Pollination				Total		
	Early Bud	Late Bud	Open	Evening	Flowers Pollinated	Fruit Set	
	Pct. Set	Pct. Set	Pct. Set	Pct. Set	No.	No.	Pct.
20-60	43	63	89	73	323	316	67
61-100	30	59	72	66	589	356	60
101-140	31	40	71	49	343	163	48
Above 140	5	44	45	43	401	123	31
Average	22	53	69	60	1656	858	52

Here the trend was consistent for all four stages. Whenever more degree-hours accumulated, the percentage of fruit set decreased. The percentage of successful pollinations was just half as great when 140 or more degree hours above 70° accumulated during the period of time from 72 hours before to 48 hours after pollination as it was when only 20 to 60 hours accumulated during that period. A series of charts set up to show the relationship between fruit set and degree-hours for each of the five 24-hour periods included in the period of time from 72 hours before

to 48 hours after flower opening disclosed that the degree-hours on any one day did not have any apreciable effect on the amount of fruit set.

The temperature recorded at the hour nearest the time when each cross was made varied from 64° to 76°F. in 1952 and 1953, but, the percentage of fruit set was about the same for crosses made at one temperature within this range as for those made at another. Also a number of crosses were made on afternoons when the thermometer registered above 90°F. Although no counts were made, it appeared that the percentage of fruit set and the number of seeds per fruit were about as good for these crosses as they were for flowers pollinated when temperatures were cooler.

TIME ELAPSE BETWEEN POLLINATION AND POLLEN GERMINATION

In 1954, the number of pollen tubes in styles of flowers pollinated at three maturity stages was determined for four crosses. For each stage of each cross, styles which had been pollinated 24, 36, 48, 60, 72 and 96 hours were flattened onto slides for the counts. The fixing, staining, and slide preparation were a modification of the methods described by Buchholz (1). Only pollen tubes which had germinated but which had not yet reached the ovules could be counted. The average number of pollen tubes in styles of four crosses for each stage at each time are presented in table 5.

TABLE 5.—*The average number of pollen tubes in eight or more styles of potato flowers pollinated at three flower maturity stages and harvested 24, 36, 48, 60, and 72-96 hours after pollination*

Stage	Hours after Pollination and Average Number of Pollen Tubes				
	24	36	48	60	72-96
	No.	No.	No.	No.	No.
Early Bud	6.2	7.2	10.0	30.5	52.0
Late Bud	40.0	48.0	69.6	142.4	105.2
Open	110.8	158.8	123.2	173.2	157.2

For flowers pollinated at the early bud stage, the number of tubes found in the style did not increase significantly until after 48 hours following pollination. For flowers pollinated at the late bud stage there was no significant increase of pollen tubes in the styles which were examined until after 48 hours following pollination, but more tubes at 60 hours than at 48 and fewer at 72-96 hours than at 60 were observed. The data available did not show significant differences in total tubes at any time from 24 to 72-96 hours after pollination for flowers pollinated after they had opened.

It is also noteworthy that there were always fewer pollen tubes in the styles of early bud crosses than there were in styles of flowers pollinated at the late bud or open stages of maturity.

DISCUSSION

Fruit set was reduced whenever buds were pollinated earlier than 24 hours before they would have opened. Also few pollen tubes were found in the style before the flower opened no matter how long it had been

pollinated. It is probable, therefore, that with the conditions under which this experiment was carried out most pollen grains germinate after the flower opens no matter at what stage of maturity it is pollinated.

Fruit set was not noticeably reduced by high temperatures on any one day, but it was reduced when high temperatures persisted for several days preceding and following flower opening. The reduction of pollen viability by persistent high temperature has received considerable attention in the past (7, 9). It is of interest that in this study the temperature effect seemed to be more closely correlated with the time of flower opening than with time of pollination. It is apparent that the pistil rather than the pollen was being affected by the high temperature. This suggests that in future studies relating to this subject it would be well to consider the effect of temperature on both the male and the female flower parts.

In addition to the experiments reported in detail in this paper, data were collected which demonstrated that the percentage of fruit set was about the same for flowers pollinated at 6, 7, 8, 9, 10, or 11 a.m. and that the position of a flower within the blossom cluster (*i.e.*, whether it was first, second, *etc.*, to open) made little difference on that flower's chance of setting fruit. Also, pollinating during the evening was as successful as pollinating during the morning hours. Apparently, the potato breeder has considerable choice as to when and how his pollinating is to be done.

Some controversy exists among breeders as to whether or not it is necessary to emasculate flowers used in a greenhouse potato breeding program. Experiments conducted in conjunction with this study showed that, where plants were not shaken during the time they were in bloom, self-pollination of unemasculated flowers was negligible. Whether or not it is necessary to emasculate and/or isolate will depend on local conditions. At Ithaca, New York, for instance, it was difficult to scrape pollen from anthers sooner than 48 hours after the flowers opened. At Aberdeen, Idaho, with the same potato clones in 1957 copious amounts of pollen could be shaken from anthers of flowers which had been open for only 24 hours. At Ithaca bees were seldom found in the greenhouse before late April. At Aberdeen large numbers entered the greenhouse through any available crack during a few sunny days in early March.

The use of the hollow nail insures that the stigma receives as much pollen as it will hold which would be of value in crosses where fruit does not set readily. The nail is of greatest value where a number of flowers are to be pollinated with the same pollen. For our purposes pollen was scraped from the anthers with a scalpel, but the pollinating process could undoubtedly be speeded by using an electric vibrator to collect pollen into a small container or by drying the anthers to aid in releasing the pollen. (10).

Summary

1. Some fruit was set by potato flowers which were pollinated at maturity stages from two days before they opened, to three days after the day on which they opened although the percentage of fruit set was highest for flowers pollinated the day on which they opened, or the day after they had opened. More seeds per fruit were developed from flowers pollinated just after they had opened than from those pollinated 24 hours

before they had opened and these latter, in turn, produced more seeds per seedball than did those pollinated 48 hours before they opened.

2. High temperature, measured in degree-hours above 70°F., for a period from 48 hours before the flower opened until 72 hours after it had opened, caused a greater reduction in fruit set than did high temperature on any one day. This heat effect seemed to be more closely associated with flower maturity than with time of pollination.

3. Most pollen grains on stigmas of clones examined in this study seemed to germinate after the time that the flower would normally open regardless of the stage of maturity at which the flower was pollinated.

4. The position of the flower within the cluster and the morning hour at which the flower was pollinated seemed to have little effect on the chances of that flowers setting fruit. The percentage of fruit set was as high for flowers pollinated in the evening as for those pollinated in the morning.

Literature Cited

1. Buchholz, J. T. 1931. The dissection, staining, and mounting of styles in the study of pollen-tube distribution. Stain Tech. 6: 13-24.
2. Clarke, A. E. 1940. Fertilization and early embryo development in the potato. Amer. Potato Jour. 17: 20-25.
3. ———— and P. M. Lombard. 1939. Relation of length of day to flower and seed production in potato varieties. Amer. Potato Jour. 16: 236-244.
4. ———— and P. M. Lombard. 1942. Flower bud formation in the potato plant as influenced by variety, size of seed-piece, and light. Amer. Potato Jour. 19: 97-105.
5. Driver, C. M. and J. G. Hawes. 1943. Photoperiodism in the potato. Imp. Bur. of Plant Breed. and Genetics. Dec. 1943: 36.
6. East, E. M. 1908. Techniques of hybridizing the potato. Amer. Soc. Hort. Sci. Proc. 5: 35-40.
7. Edmundson, W. C. 1942. Comparison of Katahdin potato pollen produced in the field and in the greenhouse. Amer. Potato Jour. 19: 12-15.
8. Federer, W. T. 1955. Experimental Design. The MacMillan Co., New York, 591 Pp.
9. Henderson, M. T. and F. L LeClerg. 1943. Studies on some factors affecting fruit setting in *S. tuberosum* in the field in Louisiana. Jour. Agr. Res. 66: 67-76.
10. King, J. R. 1955. Pollen and pollination studies on Irish Potatoes. Amer. Potato Jour. 37: 7-14.
11. Longley, A. E. and C. F. Clark. 1930. Chromosome behavior and pollen production in the potato. Jour. Agr. Res. 41: 867-888.
12. Salaman, R. M. 1926. Potato varieties. Cambridge Univ. Pres, 378 Pp.
13. Werner, H. O. 1941. Effect on berry production of varied day length during the life of two Triumph potato strains. Amer. Potato Jour. 18: 174-178.
14. Young, W. J. 1923. The formation and degeneration of germ cells in the potato. Amer. Jour. Bot. 10: 326-335.

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