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A Study of the Pollen and Pistils of Apples in Relation to the Germination of the Pollen

J. N. Martin
Iowa State College

L. E. Yocum
Iowa State College

A STUDY OF THE POLLEN AND PISTILS OF APPLES IN RELATION TO THE GERMINATION OF THE POLLEN.

J. N. MARTIN AND L. E. YOCUM.

INTRODUCTION.

The failure of a fruit tree to develop a normal amount of fruit may be due to a number of causes as stated by Kraus in volume VI of the Journal of Heredity. However, unless a plant develops fruit parthenocarpically, the development of fruit depends primarily upon how the pollen and pistil function relative to each other. As Kraus points out, fertilization by no means insures the development of a fruit, for very commonly the fruit does not develop despite the fact that normal fertilization occurred, but, on the other hand, in the absence of fertilization, fruit seldom develops among apples.

The aim of the work reported in this paper was as follows: (1) to determine the content of the pollen; (2) to investigate the germination of the pollen in solutions and on membranes with a view to discovering the requirements of the pollen for germination; (3) to determine the effects of different temperatures, of age, and of drying on the germination of the pollen; (4) to determine the structure and content of the stigma and style, whether or not secretions are present on the stigma, and the behavior of the stigma with reference to the germination of the pollen.

It was thought that such investigations might give some information concerning: (1) the effect of rainy weather during the blooming period on the setting of fruit; (2) the condition of the stigma at the time horticulturists regard it as receptive; (3) the time at which artificial pollination can be done most successfully; and (4) whether or not the bagging of flowers practiced by horticulturists in experiments involving artificial pollination has any effect upon the results of pollination due to increasing the moisture content of the air about the flowers.

It is claimed by some that rains during the blooming period prevent the pollen from functioning properly by washing away or diluting the stigmatic secretions. The glistening of the stigma at the time it is considered receptive is interpreted by some as due to the presence of a secretion. If apple flowers can be successfully pollinated at the time they are emasculated then much

time can be saved in experimental work involving artificial pollination. Keeping flowers enclosed in bags during pollinating experiments may have considerable effect upon the germination of the pollen. The air enclosed in the bag becomes moist due to the transpiration from the flowers and, if the germination of the pollen is delicately adjusted to a certain amount of moisture, increasing the atmospheric moisture about the stigma may have an effect upon the germination of the pollen.

The apples included in the investigation were Ben Davis, Gano, Wealthy, Dutchess, and Jonathan. The Winesap was included at first but it was found to have very little normal pollen and was discarded. The investigations extended over three consecutive seasons which differed considerably in the character of the weather as to rainfall and temperature during the blooming period, but the requirements for the germination of the pollen and the average percentages of germination obtained under the same experimental conditions were practically uniform for the three seasons. The flowers were collected from the same trees each season and from one tree of each variety. It was not a part of the investigation to determine whether or not different trees of the same variety differ in respect to the germination of their pollen.

SIZE AND SHAPE OF POLLEN.

Excepting the slight bulging of the germ pores, apple pollen in the varieties studied is nearly globular when turgid, and, when measured in a 5 per cent cane sugar solution, ranges in diameter from 34μ to 46μ in Ben Davis, Wealthy, and Dutchess, and 30μ to 38μ in the Jonathan and Gano.

Apple pollen loses water very rapidly when exposed to the air, and in a few minutes the wall folds as a result of shrinking, as shown in figure 163. In this condition one diameter is very much shortened and the other somewhat lengthened. Comparative measurements of pollen in this condition are not reliable because the dimensions vary with the amount of shrinking of the contents and the folding of the walls. Pollen shrinks so rapidly that under ordinary pollinating conditions, it is shrunken when it reaches the stigma. Examinations of pollinated stigmas showed them covered with pollen in the shrunken condition.

CONTENT OF THE POLLEN.

In the young bud, three or more days before the flowers opened, the pollen grains contained much starch as shown in

figure 164. The remaining tests were made on pollen from open flowers, and at this time there was no trace of starch, except in a very few apparently undeveloped grains. Occasionally slight traces of sugar were seen, when tested with phenylhydrazine

Fig. 163

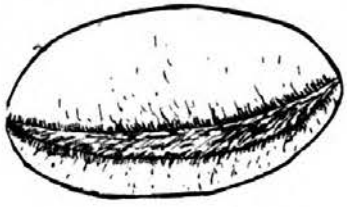
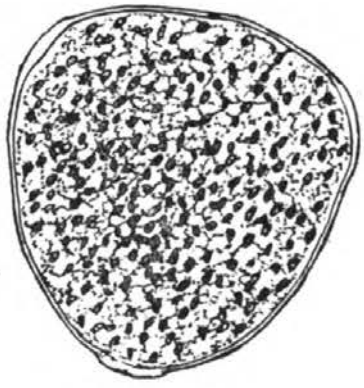
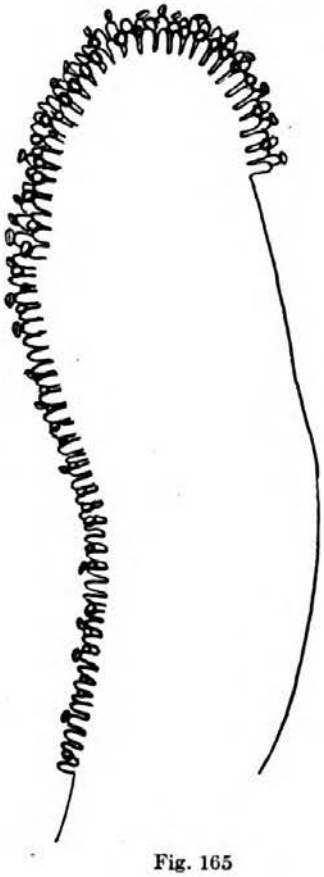


Fig. 164





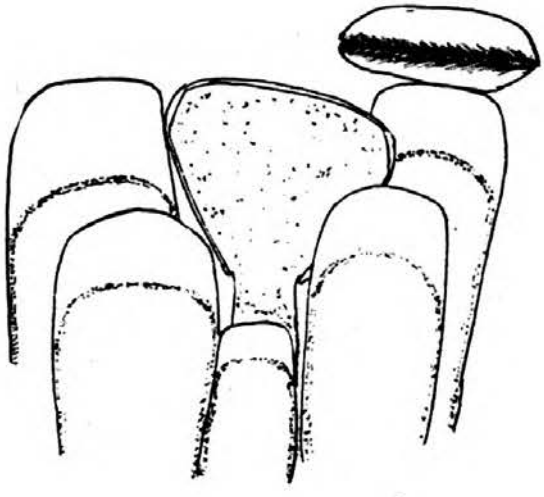


Fig. 165

Fig. 166

Fig. 163.—A pollen grain, shrunken and wall folded, a condition of apple pollen after a few minutes exposure to the air.

Fig. 164.—A pollen grain containing much starch.

Fig. 165.—A pollinated apple stigma, showing its papillate character and position of pollen with reference to the papillae.

Fig. 166.—A portion of an apple stigma, at time of pollination, showing papillae with their shrunken protoplasts, one pollen grain which has germinated and another lodged on the end of a papilla and in a shrunken condition.

hydrachloride and sodium acetate, but most of the pollen grains were apparently free from sugar at this stage. With Sudan II and alkanin no reaction for fat was obtained. With Millon's reagent, the pollen turned distinctly red. This reaction was interpreted as indicating the presence of proteins or amino acids. When treated with ruthenium red the contents became distinctly pink and were not decolored by washing. Also the walls reacted slightly to ruthenium red. The pronounced reaction of the contents with ruthenium red is likely due to amino acids. With the methylene blue the walls and sometimes the contents adjacent to the walls stained violet, indicating the presence of pectin. Both Congo red and haemotoxylin stained the walls, thus indicating the presence of cellulose. From the tests it appears that the stored food of the pollen is present in the form of starch in the young bud, but consists of proteins or amino acids, some pectic substances, and occasionally slight traces of sugar at the time of pollination. The constituents of the wall are cellulose and pectic substances.

THE WATER ABSORBING POWER OF APPLE POLLEN.

The water absorbing power of the pollen of the five varieties was found to lie in the same range of variation. Even pollen from the same anther varied much in ability to absorb water. A very small number of pollen grains in some samples became turgid in 70 per cent cane sugar solution after 72 hours. Ninety per cent remained plasmolyzed in 55 per cent cane sugar during 48 hours, while in a 35 per cent solution about 90 became turgid in less than one hour and practically all within 48 hours. The absorbing power of most of the pollen was, therefore, overcome by the osmotic force exerted by cane sugar solutions ranging from 35 to 55 per cent. Calculated according to Berkeley and Hartley[1] these solutions represent an osmotic force ranging from 45 to 105 atmospheres. Even dead pollen showed considerable absorbing power and when put in the weaker solutions dead pollen became turgid in a short time and many burst.

GERMINATION OF THE POLLEN.

In germinating the pollen in solutions, chiefly cane sugar solutions were used, although other sugars were tried and found to be good. The solutions are given in percentages and were made up by adding to the number of grams corresponding to the desired percentage of sugar enough water by weight to make 100

[1] Physical chemistry, Phillips, J. C., p. 51.

grams. About one-half c.c. of the various concentrations were spread over the bottoms of watch glasses and after the pollen, usually ranging from 50 to 200 grains in amount, was introduced, the watch glasses were sealed to prevent as far as possible changes in concentration resulting from evaporation.

The membranes used were pieces of beef's bladder, and were about 1 cm. square. After being soaked 15 or 20 minutes in distilled water and then dried between two blotters, the membranes were put in watch glasses, pollen was spread on their surfaces and the watch glasses were then sealed.

In a mass of pollen from the same flower as well as from different flowers there was such a wide variation in the requirements for germination, that no conclusions could be drawn except from the averages of a large number of tests.

In table I are given the results obtained in two germination tests to show the variations that occurred in the behavior of the pollen in the cane sugar solutions.

TABLE I.

VARIATION IN PERCENTAGES OF GERMINATION OF POLLEN IN WATER AND SUGAR SOLUTIONS AS SHOWN BY COMPARING THE RESULTS OF TWO TESTS.

Medium	Percentage of Germination in Set No. 1.	Percentage of Germination in Set No. 2.
Distilled Water	45	10
2½ per cent cane sugar	55	80
5 per cent cane sugar	50	70
10 per cent cane sugar	60	40
15 per cent cane sugar	70	40
20 per cent cane sugar	75	10
25 per cent cane sugar	30	0
30 per cent cane sugar	8	0

A temperature from 22° to 25° C. was found most favorable for germination. The pollen of the five varieties was quite uniform in requirements and average results obtained in the germination tests of any one variety are representative of those obtained for other varieties.

The investigation first had to do with determining the behavior of the pollen in distilled water and various concentrations of cane sugar solutions at a temperature of 22° to 25° C., this being the temperature found most favorable by other investigators. Table II gives the results obtained with pollen of the Wealthy.

TABLE II.

PERCENTAGES OF GERMINATION AND LENGTH OF TUBES OBTAINED IN DIFFERENT MEDIA IN 16 HOURS AT A TEMPERATURE OF 23° C.

MEDIUM	PERCENTAGE OF GERMINATION AT THE END OF 16 HRS.	APPROXIMATE LENGTH OF TUBES.
Distilled Water	25	1-2 mm
2½ per cent cane sugar	70	1-2 mm
5 per cent cane sugar	60	¾-1
10 per cent cane sugar	60	½-1
15 per cent cane sugar	50	¼-1
35 per cent cane sugar	25	$\frac{1}{6}$-¾

The average percentage of germination was highest in the 2½ per cent cane sugar solution. As the concentration increased up to 65 per cent, the per cent of germination decreased. A concentration of 65 per cent was the maximum for germination when the period allowed for germination was no longer than 16 hours. However, in some tests, as much as 2 per cent germination was obtained in 72 hours in concentrations of 70 per cent.

The fact that the pollen germinated to a considerable extent in distilled water suggested that the sugar solutions functioned in the germination of the pollen only in controlling the water supply. Comparative tests run in sugar solutions and on membranes (shown in table III) gave further evidence that this was true.

TABLE III.

A COMPARISON OF THE PERCENTAGES OF GERMINATION OBTAINED IN SUGAR SOLUTIONS WITH THOSE OBTAINED ON MEMBRANES, THE TIME PERIOD BEING 12 HOURS.

VARIETY	IN 2½ CANE SUGAR SOLUTION.	ON ANIMAL MEMBRANE
Wealthy	68	85
Gano	64	90
Jonathan	75	88
Dutchess	80	90

Table III shows that a higher percentage of germination was secured on the membranes than in the sugar solutions. Germination also took place more rapidly and the tubes grew faster and were more nearly normal in appearance on the membranes. In the sugar solutions, there was clumping of the

cytoplasm and some bursting after a few hours, while on the membranes the tubes of the same age were normal in appearance. The membranes were found suitably dried when the surface moisture was removed and this was accomplished by pressing them once between blotters.

The longest tubes obtained in any of the sugar solutions and on the membranes were about 2 millimeters. This limit in growth was attributed to the exhaustion of stored food in the pollen grain, and since no more growth was made in the sugar solution than on the membrane, it was again evident that the pollen tubes did not use the sugar as a food. Even when grown on membranes soaked in sugar solutions, thus eliminating the feature of poor aeration, the tubes were no longer than on membranes soaked in distilled water. It is evident from the behavior of the pollen in water, in the sugar solutions, and on the moist membranes that germination depends only upon a water supply. This water requirement is a little less than the amount of water absorbed when the pollen is in pure water, as is shown by the fact that the largest percentage of germination was obtained in the least time in 2½ per cent cane sugar solution and on membranes. In stronger sugar solutions the pollen, although germinating very little in 2 hours, gradually took up water until the required amount was obtained and then germinated, thus often showing a fair per cent of germination at the end of a long period. In table IV are shown the comparative rates of germination in the different per cents of sugar solution.

TABLE IV.

THE PERCENTAGES OF GERMINATION AND LENGTHS OF TUBES OBTAINED IN THE SAME SOLUTIONS WITH TIME PERIODS OF DIFFERENT LENGTHS.

Solution	Time 2 Hours		Time 6½ Hours		Time 22 Hours	
	Percentage of germination	Length of tubes	Percentage of germination	Length of tubes	Percentage of germination	Length of tubes
Water	15	1/10 mm	15	1 mm	15	1½ mm
2½ per cent cane sugar	20	1/6-1/3"	65	1½ mm	65	1½ mm
10 per cent cane sugrr	1/20	1/100-1/50	25	½ mm	60	¾-1 mm
15 per cent cane sugar	1/20	1/100-1/50	25	¼-½ mm	60	¾-1 mm
20 per cent cane sugar	1/20	1/100-1/50	15	1/50-¾mm	45	¼-1 mm
35 per cent cane sugar	0		0		30	1/10-½ mm
50 per cent cane sugar	0		0		10	1/20-¼ mm
70 per cent cane sugar	0		0		some germination a end of 72 hours.	

From table IV it is seen that the difference between the percentages of germination in the different concentrations is not so marked at the end of long periods as at the end of short periods. In the long periods there was time for many of the pollen grains in the stronger solutions to secure the required amount of water and germinate. In determining the most favorable solution for germination, it is obvious that it is essential to consider the time element.

UNIFORMITY OF GERMINATION IN THE FIVE VARIETIES.

All of the five varieties, excepting Duchess, have been reported either partly or entirely self-sterile in the work of Lewis, and Vincent (11). Self-sterility in some cases is known to be local, and these varieties may not be sterile in the orchard at Iowa State College. Nevertheless, it was thought worth while to investigate the uniformity of the water requirements of the five varieties by using membranes which had given a more even and a higher per cent of germination than the solutions. This was done by sowing the pollen of the five varieties on a piece of membrane about 2 inches long and 1 inch wide, the pollen of each variety occupying a line running the full length of the membrane. Table V gives the results obtained.

TABLE V.

PERCENTAGES OF GERMINATION AND GROWTH OF TUBES OF THE POLLEN OF THE FIVE VARIETIES UNDER SIMILAR CONDITIONS.

VARIETY	PERCENTAGE OF GERM. AT THE END OF 3 HRS.	LENGTH OF TUBES.
Duchess	90	½-1 mm.
Jonathan	92	½-1 mm.
Wealthy	85	½-1 mm.
Gano	90	½-1 mm.
Ben Davis	80	½-1 mm.

Table V shows some variation in the germination of the pollen of the different varieties, but so little that all five can be considered uniform in the water requirement for the germination of their pollen. It is, therefore, obvious that self-sterility, self-fertility and inter-fertility among these five varieties do not depend upon a difference in the water requirement for the germination of their pollen. Some pollinating experiments were carried on in the orchard to test out this assumption. For example, Jonathan, reported as entirely self-sterile by the Oregon Ag-

ricultural Experiment Station, was self-pollinated and stigmas examined after 48 hours. On 20 stigmas examined 118 pollen grains were counted and 24 of the number had germinated, the number of germinations on a stigma ranging from 2 to 6. The results show that the pollen of the Jonathan can germinate on its own stigma and if this variety is self-sterile in the orchard of the Iowa State College the cause can not be attributed to the inability of the pollen to germinate on the stigmas of the Jonathan.

THE EFFECT OF AGE AND DRYING ON THE GERMINATION OF THE POLLEN.

In determining the effect of age and drying, pollen was taken from buds about two days previous to opening, from flowers in which the anthers were dehiscing, and from flowers which had been stored in paper bags in the laboratory. The average of a number of germination tests of pollen from buds and open flowers were 75 and 87 per cent. The results show that pollen taken from flowers just previous to their opening or about the time the flowers are emasculated in pollinating experiments is about as good as pollen from anthers which are dehiscing. The pollen stored in the paper bags all died within 18 days and very little was found viable after 12 days' storage. It was also found that exposure to sugar solutions in which the pollen remained plasmolyzed soon resulted in death. More than 90 per cent were killed when exposed to a 70 per cent cane sugar solution for 72 hours.

EFFECT OF TEMPERATURE ON GERMINATION.

Table VI shows the effect of varying the temperature on germination of the pollen of the Wealthy, and the same was true for the pollen of the other varieties.

TABLE VI.

EFFECT OF TEMPERATURE ON RATE OF GERMINATION AND GROWTH OF TUBES.

Temperature 23°	Time 3 hrs.		Time 18 hrs.	
Medium	Percentage of germination	Length of tubes	Percentage of germination	Length of tubes
Water	25	1/10-1/5 mm.	30	1-2 mm.
2½ per cent sugar so.	55	1/10-1/5 mm.	80	1-2 mm.
5 per cent sugar so.	25	1/20-1/10 mm.	60	½-1 mm.
10 per cent sugar so.	15	1/20-1/10 mm.	50	¼-½ mm.
Membrane	70	1/10-1/4 mm.	95	1-2 mm.
Temperature 10°				
Water	0		35	¼-½ mm.
2½ per cent sugar so.	0		60	¼-½ mm.
5 per cent sugar so.	0		40	1/10-¼ mm.
10 per cent sugar so.	0		30	1/10-¼ mm.
Membrane	10	1/20-1/10 mm.	65	
Temperature—1.5°				
Water	0		0	
2½ per cent sugar so.	0		0	
5 per cent sugar so.	0		0	
10 per cent sugar sol.	0		0	
Membrane	0		5	1/20-1/10mm.

From table VI it is seen that low temperatures retard germination but do not prevent it, even at —1.5° C. With more time allowed the percentage of germination at —1.5° would very likely have been greater. In accord with the results of other investigators, the pollen was found to be exceedingly resistant to cold. Pollen kept frozen up solid in water and sugar solutions for three or four hours gave a normal percentage of germination when its germination capacity was tested, thus showing no bad effects from cold.

PISTIL.

THE NATURE OF THE STIGMA.

The stigma is papillate as shown in figure 165. At the opening of the flower the papillæ have reached their full develop-

ment. They have very thin cellulose walls which in some cases contain pectin and their protoplasm consists of a very thin peripheral layer enclosing a single large vacuole. The sap filling the vacuoles occasionally contained small traces of cane sugar and sometimes substances reacting to the tests for pectin. The amount of pectin in the walls varied in different papillæ on the same stigma, some giving no evidence of any, while others showed a distinct color reaction with ruthenium red and methylene blue. There was no reaction with Sudan III, or Millon's reagent. At the opening of the flower the protoplasmic layer had pulled away from the wall at the apex of some of the papillæ as shown in figure 166. This shrinking continued in the open flowers, and in a few days, ranging from three to six, when flowers were left exposed on the trees, the walls of the papillæ over the upper surface of the stigma were completely collapsed and the protoplasm was becoming brown. This death of cells moves rapidly down the style and in a period of a few days, both stigma and style are dead and withered.

It is the reflection and refraction of light from the vacuoles and from the outer surface of the plasmic membranes when pulled away from the walls that cause the glistening which by some is regarded as an indication that the stigma is receptive. At this stage the papillæ would not become turgid when water was supplied and they showed no change when immersed in strong salt or sugar solutions. Judging from the behavior of their protoplasms toward solutions, the papillæ are dead at the period of pollination. The brown color which the stigma soon takes on after the flower opens is further evidence of the early death of the papillæ.

The styles and stigmas are much more sensitive to cold than the pollen. As indicated by taking on a brown color and becoming dry and brittle very soon, the styles were killed by an exposure of an hour to a temperature of —1° C.

THE SECRETIONS AND THE GERMINATION OF THE POLLEN ON THE STIGMA

The stigma is commonly considered a secreting organ, and the notion is prevalent that stigmatic secretions have much to do with the germination of the pollen. Stigmatic secretions may have much to do with the germination of pollen in many plants, but very likely too much has been attributed to them.

During the blooming period of 1916 there were a number of warm, bright days which afforded an opportunity to study the stigmas. Warm, bright days favor insect pollination and statistics show that such days during the blooming period are most favorable for the setting of fruit. On warm, bright days the stigmas should be free from condensed atmospheric moisture and thus any liquids present could be regarded as secretions. In the study of the stigma, pistils left exposed and pistils enclosed in paper bags and cheese cloth were used.

Stigmas ready for pollination and some that were pollinated under control were brought into the laboratory and examined for secretions. On bright, warm days when the pollen was germinating well on the stigma, no liquids could be seen on the surface of the stigma with the compound microscope. When mounted in oil there was no evidence of any liquid in the space between the papillæ. The liquid was being lost from the papillæ by evaporation and not by exudation. Even on stigmas kept enclosed in paper bags no liquid was found.

Only a small percentage of the pollen on a well pollinated stigma is suitably located for germination. The favorable location is between the papillæ as shown on figure 166. Those on the tips of the papillæ were only rarely observed to absorb enough moisture to become turgid. The observations on the five varieties studied, showed that the germination of the pollen does not depend upon stigmatic secretions, which exude from the stigma but upon the ability of the pollen to draw liquids from the papillæ, and it is obvious from the nature of the pollen that water is the only liquid necessary to start germination. Pollen lodged between the papillæ was able to absorb from the papillæ the required amount of water for germination. The problem as to what effect liquids have on germination when present on the stigma was not worked out. Since apple pollen germinates poorly in water, it is reasonable to infer that pollen germinates poorly on a stigma wet with rain, and the fact that rain during the blooming period is unfavorable to the setting of fruit may be attributed in part to this. Of course such weather is usually accompanied by a low temperature, which retards the germination of the pollen, and such weather is also unfavorable to pollination by insects. A number of factors are concerned, but so far as we could determine, there is no basis for the theory that rains

wash away or dilute the stigmatic secretions and thereby prevent the setting of fruit.

It has also been stated that freezing temperatures kill the stigmas which consequently do not function properly and as a result pollination is not effective on the stigma. This point was investigated, but to a very limited extent. Clusters of flowers were exposed to freezing temperatures until the styles were killed. The flowers were then removed to a temperature of about 25° C. and their stigmas pollinated with pollen that had been at room temperature. On the stigmas of these dead styles very good germination of the pollen was obtained.

THE STYLE.

The style is grooved just below the stigma, the groove being almost a millimeter in length. In this region there are many small vascular strands which reach to the base of the stigma and within a few cells of the papillæ. The outer walls of the epidermal cells are cutinized. When stained with safranin the line separating the papillæ of the stigma and the epidermis of the style is quite distinct. Tests for sugar at the time of pollination with phenylhydrazine hydrochloride and sodium acetate gave the results shown in table VII.

TABLE VII

VARIETY	REACTION OF STYLES TO SUGAR TESTS.
Duchess	Abundance of cane sugar about 2 mm. below stigma and occupying a section of about 2 mm.
Gano	In some cane sugar about 3 mm. below stigma and occupying a section of about 2 mm.
Jonathan	No sugars found.
Ben Davis	Abundance of cane sugar about 2 mm. below stigma and occupying a region of about 2 mm.
Wealthy	Same as Gano.

In some cases in the same flower, some styles showed abundance of cane sugar while others showed none. In Gano there was much more cane sugar in the styles before the flowers opened than at pollination. The sugar when present was found abundant in the sieve-vessels.

DISCUSSION.

The investigations so far show that there are two classes of pollen grains, one requiring only water for germination, the other requiring besides water the addition of chemical substances such as acids, sugars and salts. Plants having pollen belonging to the first class have long been known, Mohl (15) having discovered in 1834 that the pollen of the *Marina* would germinate in water. As investigations go on the list of plants known to have this type of pollen is being rapidly extended. Hansgirg (5) germinated the pollen of *Phalaris brachystachya* in water. The pollen of other grasses investigated by him bursts in water. Lidfors (9, 10) germinated the pollen of some species of *Rhododendron, Azalea, Erica, Nicotiana* and *Glaucium* in distilled water. Molisch (16) germinated the pollen of *Amorpha fruticosa, Colutea arborescens* and fifteen other species of plants in saturated air. Jost (7) found that the germination of the pollen of a number of the grasses depended upon a limited water supply, and he was able to germinate the pollen of *Arrhenatherum elatius, Dactylis glomerata, Bromus mollis, Glyceria aquatica, Secale cereale, Zea Mays* on starch paste and on parchment paper soaked in water or sugar solutions and properly dried. The pollen of *Dactylis* germinated when spread on the under surface of the leaves of *Limnanthemum nymphaeoides.* The pollen of a number of the *Compositae* and *Umbelliferae* Jost germinated on parchment paper only when they were soaked in sugar solutions and properly dried. He did not determine whether or not the sugar had any other function than that of controlling the water supply. Martin (13, 14) found the germination of the pollen of *Trifolium pratense, Trifolium hybridum and Medicago sativa* to depend upon water supply which could be controlled artificially by germinating the pollen on animal membranes soaked in water and properly dried.

Tokugawa (22) found that the pollen of some of the *Compositae and Umbelliferae* which previous investigators failed in germinating, germinated in 25 to 50 per cent sugar solutions and on parchment paper. He concluded that the germination of the pollen of these plants depended only upon water supply. An examination of the stigmas of these plants showed that there was no secretion and that the pollen absorbed the required amount of water from the papillæ. Tokugawa (22) germinated the pollen of *Brassica campestris* on the under epidermis of

leaves of *Vicia faba*. He concluded after germinating the pollen of a number of plants, that, in general, germination depended upon a proper water supply.

In the germination of the pollen of the second class, in addition to water the addition of some chemical substance has been found to increase the percentage of germination. The addition of various percentages of sugar has been found helpful in germinating the pollen of many species. Rittinghaus (18) and Max Pfund (17) found that the pollen of a large number of species would germinate in cane sugar solutions, the concentrations ranging from 20 to 50 per cent. Kny (8) and Mangin (12) found that better germination was secured when gelatin was added to the sugar solution. Careful investigations of the function of those substances such as sugar, gelatin and some others which seem necessary for the germination of the pollen of some species may show that they function only in controlling water absorption. In some cases there is evidence that the pollen uses the sugar as a food. In some cases, the substance seems to act as a stimulant and only very small percentages are required. Molisch (16) found that the addition of about 0.01 per cent of calcium malate or malic acid to the sugar solution caused the pollen of *Azalea* and *Rhododendron* to germinate. In the germination of pollen of some species of *Erica* and *Menziesia,* Lidfors (9) observed that the addition of a small amount of citric acid to the sugar solution increased germination. Burck (2) was able to germinate the pollen of some species of *Mussaenda, Begonia,* and *Pavetta* in distilled water only after the addition of a portion of the stigma or a trace of levulose. Sandsten (19) found that a slightly acid medium is required for the germination of tomato pollen.

Both Strasburger (21) and Tokugawa (22) have shown that in many cases pollen will germinate on the stigmas of plants differing widely in relationship. The pollen of some Monocotyledons germinated on the stigmas of Dicotyledons, and vice versa. In ealh case the tubes penetrated the stigma ,but their growth was checked in the style and hence they did not reach the ovules.

As to the content of pollen, Van Tieghem (23) found that the pollen of many plants at the time of pollination contains much starch which disappears as germination proceeds. Sandsten (19) found starch in the pollen of a number of plants but does not state the age of the pollen or whether or not apple pollen was

included in the investigation. In apple pollen, we found abundance of starch present in the early bud stage but none at the time of pollination.

Sandsten (19) and Adams (1) have investigated the germination of apple pollen, both using sugar solutions, but neither determined the function of the sugar in the solutions or investigated the stigma in relation to the germinatiou of the pollen. Of the five varieties included in our work, Sandsten included the Duchess. We found 2½ per cent cane sugar solution most favorable for germination, which is closely in accord with their results, Sandsten using 3 per cent and Adams 5 per cent solutions. In our work the sugar was found to aid only in controlling the water supply. Better germination was secured on membranes soaked in distilled water and then dried until surface moisture was removed. Examinations of stigmas under ordinary conditions of pollination showed no liquids on the surface and that the situation of the pollen on the stigma is similar to that on the membrane.

The temperature found in our work to be the most favorable for germination was in accord with the observations of Sandsten and Adams. Schaffnit (20) and Chandler (3) exposed apple pollen to temperatures of —17° C. and —18° C. for long periods when the pollen was dry without any apparent injury. Sandsten (19) found that an exposure of apple pollen to a temperature of —1.5° for less than one hour resulted in very little injury. He exposed the pollen on dry watch glasses. Chandler (3) does not state how the pollen was exposed which he considered not dried and found not injured by a temperature of —8° C. We found that pollen could be frozen up solid in water and sugar solutions without being injured.

Sandsten found the stigmas more susceptible to cold than the pollen. They were killed when exposed a few hours to a temperature of —1.5° C. He does not state how the injury was indicated. We found that the stigma at the time of pollination is apparently dead under ordinary conditions, but that temperatures a little below freezing kill the style. We also found that pollen germinates on a stigma which has been exposed to a temperature low enough to kill the style.

The length of time which pollen can remain viable in storage depends very much upon storage conditions. Lidfors (9, 10) and Pfundt (17) have shown that pollen kept uniformly dry

remains viable much longer than pollen exposed to variations in moisture. Tokugawa (22) kept some pollen of four species of plants stored under uniformly dry conditions and some stored in a room where it was exposed to the fluctuating moisture content of the air. The pollen in constant dryness retained its vitality from 31 to 98 days while that stored in the room retained its vitality only from 9 to 24 days. Molisch (16) found the longevity in storage to range from 12 to 72 days in a number of plants which he investigated. In the grasses Jost (7) found the longevity to range from 1 to 8 days.

Sandsten (19) obtained some germination in apple pollen after a storage of six months. Adams (1) obtained germination after a storage of three months. Lewis and Vincent (11) found apple pollen to be effective after a storage of three weeks in vials plugged with cotton. Crandall (4) found that apple pollen which had been stored more than 11 days did not give satisfactory results in pollination experiments. In our work, the pollen of flowers kept in paper bags on a table in the laboratory, gave no germination after a storage of 18 days.

Observations and statistics show that the weather conditions at the time of pollination have an important influence on the setting of fruit. Hedrick (6) concluded from statistics ranging over a period of twenty-five years, that rain and the accompanying cold and wind cause the loss of more fruit than any other climatic factor. In his opinion there are several ways in which wet, cold weather interferes with the setting of fruit. One is that the stigmatic secretion, which he thinks is very much essential to the proper germination of the pollen, is either washed away or so diluted that the pollen does not germinate. His statistics show that bright, warm, dry weather at the time of blossoming is the most favorable for good crops of fruit. He adds that under these conditions there is more and better pollen produced, and that the stigmas show a greater amount of secretion. On bright, warm days, we found no evidence of any secretion on the surface of the stigmas in the five varieties of apples investigated. The pollen germinated when lodged between the papillæ in the absence of any secretion. It was found that immersing in water and low temperatures retarded the germination of the pollen. If the weather is cold and wet at the time of blossoming, both the presence of water on the stigmas and a low temperature may interfere with the proper functioning of the

pollen. On bright days the stigmas were more glistening than on cloudy days, due to the greater reflection and refraction of light by the papillæ, which to the unaided eye resembled small drops of liquid.

SUMMARY.

The pollen of the five varieties of apples studied contain proteins or amino acids, some pectin and occasionally small amounts of sugar at the time of pollination. The walls are composed of cellulose and pectin. In the early bud stage there was much starch present. The ability to absorb water varied much for different pollen grains, but most of them remained plasmolyzed in 55 per cent cane sugar solution.

The concentrations of cane sugar solutions for germination varied much for different pollen grains, ranging from pure water to 70 per cent concentration. The most favorable concentration was found to be 2½ per cent. The pollen was able to germinate in any concentration from which it could absorb the required amount of water. As the length of the germinating period increased in the higher concentrations the per cent of germination and length of tubes increased. The sugar in the solution was found to serve only in controlling the absorption of water, and better germination and tubes fully as long were obtained when the pollen was germinated on animal membrane. The conditions required for germination were found to be the same in the five varieties.

A temperature ranging from 22° C. to 25° C. was found most favorable for germination. Lowering the temperature slowed germination. Apple pollen is very resistant to cold, apparently suffering no injury from being frozen.

Pollen from flowers stored in paper bags and left on table in the office were all dead at the end of 18 days.

The stigma is papillate. The papillæ have thin cellulose walls and a thin peripheral layer of protoplasm enclosing a single large vacuole. The cell sap of the papillæ is very dilute, exerting very little osmotic pressure and at the time of pollination the papillæ covering the apex of the stigma are much shrunken and in a few days collapse and become brown. The styles of the pistils in most cases were found to contain much cane sugar, but the sugar was always found at some distance below the stigma.

No secretions were found on the stigma when conditions were most favorable for pollination. The favorable location of the pollen grains on the stigma was found to be between the papillæ. When so located they absorbed the required amount of water and germinated. The pollen on the ends of the papillæ was observed in nearly all cases to remain plasmolyzed.

DEPARTMENT OF BOTANY,
IOWA STATE COLLEGE.

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