A study of the pollen of Phoenix dactylifera with reference to its longevity and effect on the fruit,

by

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INTRODUCTION

A great many problems of vital interest to the date industry have had to be solved since the introduction of the date palm (Phoenix dactylifera) into the lower valleys of the arid Southwest. Investigations in the past have had to do with artificial ripening of fruit, offshoot propagation, packing of the fruit, and general cultural operations. Until the past two or three years very little attention has been given the quality of fruit produced or its improvement.

Date palms are monoecious and when grown in orchard formation the palms must be hand-pollinated to insure a perfect set of fruit. Date growers in the Old World have known for many years that the pollen from certain palms was inferior to that produced by others. Drummond (5) states that "when the most careful selections have been made there are seasons when some of the palms will be sterile." Brown (3) states that "many palms produce flowers which are entirely useless as fertilizing agents."

In the hope that more definite knowledge might be obtained on the reaction of pollen from different palms, these investigations were undertaken with the fol-

lowing objects in view:

1. To determine the viability of pollen from different palms;
2. To determine if pollen will remain viable from one date season to another;
3. And to determine if different varieties of pollen have an effect on the size and time of maturity of fruit.

SURVEY OF LITERATURE

Pollen Longevity

A considerable number of workers have reported observations on the life duration of date pollen. The majority of these observations indicate that pollen retains its viability one year or more. However, as far as can be found, few carefully controlled experiments have been conducted.

Knowlton (9) states that the earliest reference to pollen longevity is in regard to date pollen. Popenoe, Wilson, (13) states, "thoroughly dried pollen will retain its vitality for many years." Popenoe, Paul B., (12) reports that a pollination made in 1912 at the Mecca Experiment Station, Mecca, California, with pollen 7 years old, sent from the Tempe Date Gardens at Tempe, Arizona, was entirely successful. Tumey (18) cites Fischer, giving an account of pollen having been kept for 18 years without losing its fertility. Swingle (16) (17) reports that sometimes it happens that some of the female flowers appear in the spring before any of the male palms have blossomed. To provide a supply of pollen for such flowers, the Arabs make a practice of keeping a few bunches of male flowers from the previous year. The pollen is said to keep without deterioration for at least 2 years. Brown (3) states, "The

early male flowers which appear before those of the female palms are dried in the sun for three or four days, after which they are stored until required for use. It is said that they keep in good condition for a year if necessary. At the same time it is recognized that fresh flowers are superior to those which are dried, and the latter are only used if there is not a sufficient number of fresh male flowers when the work of fertilization is in progress." Stout (15) made 464 tests of 29 different lots of pollen, one or more years old, that had been carefully dried and stored under the best conditions employed by the date growers in the Coachella Valley. In each test hundreds of pollen grains were used. In all these tests only three germinating pollen grains were found. Stout believed these were undoubtedly stray grains of new pollen, that was being tested in the laboratory at the same time. He states that it seems conclusive that pollen one year or more old is entirely worthless in effecting fertilization and the setting of fruits.

Sandsten (14) did extensive work on the longevity of apple and plum pollen. Pollen was obtained from four different states. The samples were stored in the laboratory in a temperature ranging from 10° C. to 18° C. Once a month for six months a few pollen grains were taken from each lot and tested. Some of these data are given in Table I.

Table I.

Some of the results of Sandsten's investigations on the longevity of apple and plum pollen.

Lot		1st Germination	2nd Germination	3d Germination	4th Germination	5th Germination	6th Germination	7th Germination
1	Apple	47	43	44	38	39	38	12
	Plum	53	52	42	35	30	18	0
2	Apple	58	57	50	43	38	33	10
	Plum	54	48	38	26	21	11	0
3	Apple	42	46	40	38	39	19	5
	Plum	60	48	42	25	18	2	0
4	Apple	56	51	52	40	23	28	8
	Plum	50	47	38	20	12	0	0

Horsford (8) preserved pollen of Lilium auratum from one season to the next by wrapping it in several sheets of paraffin paper and storing it in a warm, dry place. It rapidly lost its vitality on exposure to air.

Cameraries first demonstrated sexuality in the higher plants in 1694. It was not until many years afterward that a definite idea was gained as to just how the pollen functioned in fecundation. According to Brink (2), Amici first discovered the pollen tube and later observed and described its development on the stigmas of Hibiscus and the gourd, and traced it through the tissues of the style to the ovary. Knowlton (9) gives Van Tiegham

credit as the first investigator to have germinated pollen artificially, although Von Mohl had previously germinated pollen of Morina in water. Pollens of many species of plants are not sensitive to kind or concentration of media used in artificial germination. Adams (1) found that apple and other fruit pollens germinated in sugar solution over a wide range of concentrations, and even in water alone. Stout (15) found that date pollen germinated on a 1-percent agar with 3, 5, or 10-percent of cane sugar. Winkler (20) found that a 15 and 20-percent sucrose medium gave the best results in germinating grape pollen.

EXPERIMENTAL WORK

Description of Pollen

Date pollen is produced very abundantly. It is yellow in color, has a pronounced fragrance when fresh, does not adhere when dry, and is carried by the wind. The pollen is small, lenticular in shape when dry, but when turgid in sugar solution is nearly spherical. Dry pollen averages 24.4 microns in length, and 12.8 microns in width. In a turgid state the average diameter is about 21.3 microns. Dry pollen becomes turgid in an instant after it is placed in a sugar solution. There appears to be considerable difference in the size of pollen grains produced by different varieties of date palms. It was thought that some correlation might be obtained between size of pollen and viability. However, after measuring a number of varieties, it was found that the variation of pollen grains within the variety would necessitate the counting of thousands of grains before such a correlation could be undertaken. Table II gives measurements taken of 12 varieties of pollen. Table III shows measurements of the same pollens a few minutes after being placed in a sucrose solution.

Table II.

Measurements of dry pollen
(Measured in microns)

Variety	No.	Maximum		Minimum		Average	
		Length	Width	Length	Width	Length	Width
Purdy	1	24.5	14.3	15.9	10.8	22.7	12.7
Me Mes	2	29.2	15.9	21.2	10.3	25.2	13.2
Sultan Col. B	3	27.9	13.8	23.6	10.6	25.5	12.8
Mubsali	4	26.3	13.3	18.3	10.9	23.3	12.5
Totten	5	26.6	15.9	21.2	10.6	24.2	12.8
Pump	6	26.0	14.6	18.8	10.6	23.3	12.5
Alex.	7	29.2	15.9	18.8	10.6	24.8	12.7
1-5 F	8	26.6	13.3	18.6	10.6	22.1	12.0
Stoever	9	29.2	13.8	21.8	10.9	26.7	12.9
Daker Med.	10	29.5	15.9	18.6	12.7	25.5	13.6
Daker Med. (1914)	11	29.2	15.9	21.2	13.0	25.6	14.1
Campus (1926)	12	26.6	13.3	18.6	10.6	24.4	12.2

Table III.

Measurements of turgid pollen
(Measured in microns)

Variety	No.	Maximum Diameter		Minimum Diameter		Average Diameter	
		Max.	Min.	Max.	Min.	Average Max.	Average Min.
Purdy	1	23.9	21.2	19.6	19.1	20.9	20.7
Me Mes	2	24.2	22.6	19.9	19.6	21.6	21.3
Sultan Col. B	3	21.2	21.2	19.9	19.9	22.1	21.8
Mubsali	4	21.5	21.5	18.6	18.6	20.6	20.6
Totten	5	22.8	21.5	21.2	18.6	21.2	21.1
Pump	6	21.8	21.5	19.9	19.1	20.4	20.2
Alex.	7	21.8	21.5	17.2	17.2	22.3	22.3
1-5 F	8	22.6	21.2	18.6	18.6	20.6	20.6
Stoever	9	26.6	23.1	21.0	19.1	23.4	22.7
Daker Med.	10	23.9	23.9	21.2	20.7	21.9	21.7
Daker Med. (1914)	11	23.9	21.2	18.6	18.6	20.9	20.8
Campus (1926)	12	21.2	21.2	19.9	19.9	21.1	20.4

Pollen stored for 12 years appeared to be lighter in color, less uniform in size and shape, and did not reach the same state of turgidity as did fresh pollen. However, there appeared to be no difference between fresh pollen and 1-year-old pollen.

Pollen Germination

Method of pollen germination. Two methods of germinating pollen were used. A 1-percent agar medium was prepared with 10-percent cane sugar. The method of preparing and sterilizing this medium was that used in common practice in the pathological laboratory. A tube of this medium was heated to the melting point and a portion poured into a sterilized petri dish in such a way that when it hardened a thin layer covered the bottom of the dish. Pollen was dusted over the medium and the dish placed upside down in an incubator. Stout (15) in his work placed a small drop of agar sugar solution on a glass slide, dusted pollen over the solution, and placed the slide in a moist chamber. The writer tried this method, but discarded it on account of the possibilities of mixing one variety of pollen with others being handled in the laboratory.

The method used in practically all germinations in this study was a sugar solution in the form of a hanging drop. A drop of sugar solution was placed on a cover glass and the pollen was dusted over the solution.

The cover glass was then inverted over a glass cell which was attached to an ordinary slide by means of vaseline. A thin film of vaseline was also placed on the top of the cell to seal the cover glass to it. Usually a few drops of the sugar solution were placed in the bottom of the cell as an additional precaution against evaporation.

Crushed stigmas and styles were added to the sugar solutions to determine if a higher germination could be secured. After several trials this method was discarded as no increase in the percentage of germination could be noticed. The addition of the crushed stigmas and styles made the solution so cloudy that it was impossible to make an accurate count of the pollen grains. The only noticeable effect of the addition of the stigmas and styles was an increase in the length and diameter of the pollen tube. Agar sugar solution also had the same effect on the pollen tube.

Knowlton (9) found that there were two degrees of vitality in pollens, one that is capable of producing a short tube growth but not capable of effecting fertilization, and the other producing a long tube growth which will accomplish fertilization. In these experiments no attention was paid to the length of pollen tubes in making germinations tests. Any pollen grains that had broken the exine walls and showed a pronounced protuberance were considered germinated. Bursted pollen grains showing no

protuberance were considered as not germinated. Pollen grains that did not become turgid when placed in sucrose solution were discarded.

Optimum temperature for germinating date pollen. In order to ascertain the optimum temperature for germinating date pollen, a series of tests was made with temperatures ranging from 10° C. to 50° C. The germinating medium used in these tests was a 20-percent sucrose solution in the form of a hanging drop in a sealed cell. Results of these tests are shown in Table IV.

Table IV.

Effect of temperature on the germination of date pollen.

Temperature (C.)	10°	15°	20°	25°	30°	35°	40°	50°
Germination (Percent)	0	5	32	53	75	21	0	0

A much higher percentage of germination was secured in other germination tests. Data in this experiment represent an average of several trials.

Degrees of concentration of sucrose media for germinating date pollen. Date pollen germinated well in sucrose solutions up to concentrations of 40-percent. The percentage of germination for the different concentrations varied, first one concentration giving a higher germination, then another. Four hundred or more pollen grains were counted for each concentration. The range of germina-

tion for the various concentrations is shown in Table V.

Table V.

Minimum, optimum, and maximum degrees of concentration of sucrose media for germination of date pollen.

Cane sugar (Percent)	0	5	10	15	20	25	30	40	50	60
Germination (Percent)	54	58	65	65	78	76	77	72	20	0

In germinating pollen at different concentrations it was noticed that germination was much slower at the higher concentrations.

Longevity of Date Pollen

Approximately 50 cc. of pollen were collected, thoroughly dried, placed in a tight wooden box, and the box was placed in a cabinet in the laboratory. The pollen was collected on May 12 and germination tests were made once each month until February 28. The pollen remaining in the box was taken to the Tempe Date Orchard and the first pistillate bloom that opened in the orchard was pollinated. Two other spathes that were almost ready to burst were opened and pollinated on the same palm. The method used in pollinating was the same as that often used in commercial orchards. Pollen was worked into balls of cotton and the cotton tied into the center of the pistillate flower cluster. The clusters were then covered with two thicknesses of heavy starched muslin to prevent wind-blown

pollen from coming in contact with the flowers. The covers were removed four weeks later. This method of covering the flower clusters had been checked several times the year previous and found to be reliable. The following table gives the results of this experiment.

Table VI.

Longevity of date pollen.

Date tested.	May 12	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Percent germinated.	64	75	63	42	58	30	32	--	12	8

The bunches pollinated with this pollen were examined on July 27. Each fruiting cluster appeared to have set a maximum crop.

Forbes and Simmons (7) worked with year-old pollen and obtained conflicting results. However, they concluded that since a few bunches set a normal crop of fruit the failure was due to the method of applying the pollen rather than to the lack of viability of the pollen itself.

Stout (15) made tests on several lots of pollen collected February 19, 1923. On May 31, 1923, he states that these pollens gave good germination and suggests that date pollen dies some time during the summer.

Influence of Light on Germination of Date Pollen

Sandsten (14), working on the germination of tomato pollen, found that pollen germinated more readily in bright sunshine than in cloudy weather. In order to determine what effect light would have on the germination of date pollen, the following experiment was conducted: Duplicate mounts were made in 20-percent cane sugar solution. One set of mounts was placed in a tight box and the box wrapped with two thicknesses of heavy, black paper. Another set was left on the laboratory exposed to a north light. The third set was placed in a south window exposed to direct sunshine. Results of these experiments are shown in Table VII.

Table VII.

Percentage of germination of pollen in sunshine, indirect light, and darkness.

Pollen	Total Darkness	Indirect Light	Sunshine
Mactum Seedling	81%	86%	00%

Stigma Receptivity

This experiment was undertaken to determine the length of time after the spathes opened that fertilization would take place. Spathes were bagged when they appeared, and prior to their bursting. The time of opening was then recorded. After the spathes opened, the sheaths

were cut away and the flowers clusters were divided up into several parts, each part containing five strands. Each cluster of five strands was covered with a cylinder made of two thicknesses of paraffin paper. Cotton was worked around the base of the fingers in such a way as to close the base of the cylinder. The top of the cylinder was also closed with a cotton stopper to allow ventilation and to exclude pollen that might be floating in the air. A second bag made of heavy craft paper was placed over the entire cluster as an added precaution against wind-blown pollen. Duplicate lots were pollinated each 24 hours for a period of 18 days after the spathes opened. The cylinders were removed before pollinating the blossoms. The usual field method of applying the pollen was used. The results of these experiments are shown in Table VIII.

Table VIII.

Length of time pistillate flowers remain receptive to pollen after the spathes open.

Variety	Time pollinated	Percent fertilized
Horra	Same day	100
	2nd day	100
	3d day	100
	4th day	95
	5th day	85
	6th day	75
	7th day	40
	8th day	25
	9th day	15
	10th day	10
	11th day	8
	12th day	3
	13th day	0
	14th day	0
	15th day	0
	16th day	0
	17th day	0
	18th day	0

These results show that after 6 days the percentage of fertilized dates decreased quite rapidly until the thirteenth day, after which no fertilized dates were obtained.

Effect of Pollen on Fruit

Vinson (19) was the first, so far as the writer was able to find, to note that certain pollens affected the time of maturity. Vinson, while studying the effect of notching the fruit stem on the time of maturity, noticed that one Deglet Noor palm ripened its fruit in ad-

vance of the others. This palm had been pollinated with pollen from the same male as had those palms that appeared to have matured their fruit earlier the year before.

Nixon (11) reported that certain pollens not only affected the time of maturity but also the size of fruit and seed of the Deglet Noor variety. His experiments were made under carefully controlled conditions and substantiate the observations made by Vinson.

A series of experiments was started to study the effect of pollen from different palms on the fruit of several varieties. The same method of bagging was used in these experiments as described in the study of stigma receptivity, except that a small piece of cotton was placed in the upper part of the bagged fingers. When pollen was dusted into the bag, part of it lodged in the piece of cotton and was slowly dislodged by movement of the cluster in the wind.

In collecting pollen, the spathes were cut before they opened and taken to the laboratory, where the sheaths were removed and the flower clusters placed in large paper bags. After drying for several hours, the pollen was shaken from the flowers. The pollen was then spread out in a thin layer in the bag and allowed to dry for several days, as fresh pollen will mold quickly and lose its vitality if not thoroughly dried. When dry, the

pollen was transferred to a small bellows-type insect powder gun. Pollen was blown into the bags, after forcing the spout of the gun through the cotton stopper.

Effect of pollen on moisture content of fruit. Vinson (19) in working on the chemistry of the date found that the development of a bunch of dates after pollination takes place in three principal stages. During the first few weeks of growth, development is confined almost entirely to the long main stem, which almost reaches its full size before marked development of the fruit begins. The seed next increases in size until a maximum is reached. The fruit then enters the third stage of development, marked by the accumulation of sugar with further noticeable increase in size. A date having 20-percent of dry matter may change in a short time to one of 60-percent in three ways: (1) By the addition of dry matter without loss of water, and consequently with gain in weight. (2) By loss of water and decrease in weight. (3) By increase in dry matter and loss of water without change in weight. His interpretation is that water has been replaced, to a considerable extent at least, by sugar.

According to Vinson's work it would be almost impossible to state that any slight difference in moisture content of the date was due to different pollens used, unless the fruits were of the same degree of maturity

at the time the test was made. The writer selected fruits of approximately the same degree of maturity from bunches pollinated with different pollens and placed them in an electric oven at a temperature of 40° C. They were held in the oven until the weight did not vary more than 1 gram in 24 hours. The percentage of moisture was then figured on the dry weight basis. Results of these experiments are shown in Table IX.

Table IX.

Effect of pollen on the moisture content of the Tadala date.

Pollen	Grams of fruit Fresh weight	Grams of fruit Dry weight	Percent Moisture
Ted's Seedling	237.0	136.4	73.7
Daker Med.	199.5	115.0	73.4
Pick. #2	222.3	130.6	70.2
Pick. #1	215.5	127.3	69.3
Canariensis #1	294.0	173.7	69.1
Daker Early	228.5	135.3	68.8

Effect of pollen on size of fruit and seed. In these experiments 12 different pollens were used: Unfortunately, all the data could not be obtained, as the fruit was seriously damaged by heavy rains coming just at the time the fruit started to ripen. Sufficient data were taken, however, to prove conclusively that pollen from different staminate palms had some effect upon the size of fruit and seed. Tables X, XI, and XII give the results obtained from these experiments.

Table X.

Effect of pollens on the size of fruit and seed of the Deglet Noor date. (50 fruits used in each experiment.)

Kind of Pollen	Av.weight of fruit (In grams)	Av.weight of seed (In grams)	Fruit Length (In centimeters)	Fruit Width (In centimeters)	Seed Length (In centimeters)	Seed Width (In centimeters)
Mosque	15.56	1.24	4.44	2.35	2.86	.91
Mubsali	13.05	1.04	4.20	2.19	2.66	.84
Daker Early	12.93	1.03	4.39	2.25	2.75	.87
Fard	12.13	.64	4.07	2.19	2.32	.70
Perry #1	12.01	1.03	4.20	2.19	2.63	.82
Canariensis #1	11.84	1.04	4.20	2.25	2.41	.77
Totten	11.65	.87	4.02	2.04	2.37	.77
Canariensis #2	11.06	.54	3.98	2.11	2.30	.67

Table XI.

Effect of pollens on the size of fruit and seed of the Saidy date. (50 fruits used in each experiment.)

Kind of Pollen	Av. weight of fruit	Av.weight of seed	Fruit Length	Fruit Width	Seed Length	Seed Width
Daker Early	19.43	2.23	4.32	2.65	2.88	1.23
Totten	18.70	2.139	4.26	2.63	2.86	1.18
Pick. #2	18.69	2.06	4.28	2.65	2.85	1.18
Canariensis #2	18.36	2.05	4.22	2.60	2.76	1.13
Daker Med.	18.19	2.48	4.32	2.63	2.90	1.31
Pick. #1	17.89	2.02	4.22	2.54	2.80	1.18
Ted's Seedling	16.73	2.08	4.14	2.52	2.74	1.22
Canariensis #1	15.78	1.69	4.01	2.50	2.54	1.13

Table XII.

Effect of pollens on the size of fruit and seed of the Tadala date. (50 fruits used in each experiment.)

Kind of Pollen	Av.weight of fruit	Av.weight of seed	Fruit		Seed	
			Length	Width	Length	Width
Mubsali	16.15	1.99	5.78	2.20	3.60	.98
Daker Med.	15.60	2.18	5.62	2.31	3.68	1.02
Pick. #2	14.74	2.08	5.46	2.27	3.67	1.02
Pick. #1	14.00	2.17	5.34	2.26	3.60	1.04
Daker Early	13.65	2.05	5.30	2.22	3.61	1.02
Canariensis #1	13.56	1.25	5.43	2.19	3.52	.97
Ted's Seedling	10.25	2.07	5.43	2.22	3.62	1.00

Effect of pollen on the time of maturity of fruit. The Deglet Noor variety was selected for this experiment as it undergoes pronounced changes in color before ripening. Any differences in maturity of fruit in this variety could be noted easily and quickly. All pollens were represented on the same fruiting clusters in order to counteract any variation that might occur due to position on palm or age of blossom at time of pollination. Duplicate pollinations were made on two other palms of the same variety. Results of this experiment are shown in Table XIII.

Table XIII.

Effect of pollen on the time of maturity of Deglet Noor fruit.

Pollen used	Date first fruits ripened	Percent ripe October 8
Fard	Sept. 8	84
Mosque	Sept. 18	46
Daker Early	Sept. 18	51
Mubsali	Sept. 18	46
Totten	Sept. 18	48
Perry #1	Sept. 18	62
Canariensis #1	Sept. 18	49
Canariensis #2	Sept. 23	33

DISCUSSION OF RESULTS

The results of the germination experiments with date pollen show that there are striking differences in the viability of date pollen from different palms. The writer cannot agree with Drummond (5) in his statement that when the most careful selections have been made there are seasons when some of the palms will be sterile. Certain staminate palms in the Tempe Date Experiment Station have been used to pollinate the palms used for experimental purposes for the past 15 or 20 years, and no report is on record as to their not producing viable pollen. Two other palms growing in the Station orchard have produced blossoms with very little viable pollen, most of the blossoms being sterile. Although the writer has no data to substantiate this, it seems most logical to assume that high or low viability in pollen is an inherited character rather than one caused by seasonal or cultural changes.

The results show it is possible to keep date pollen in a viable condition from one pollination season to another. The percentage of germination decreased rapidly after the seventh month. Other pollens not included in the above study did not retain their viability for that length of time. The duration of viability is leng-

hened by thoroughly drying the pollen before storing it. eath in pollen, according to Knowlton (9), is probably aused by a slow precipitation of proteins within the pro- oplasm.

These data show that the stigma of the late flower is receptive over a period of 13 days. Dorsey 4) showed that plum stigmas are receptive under orchard conditions for a maximum of 1 week, but that they begin to urn brown within 3 to 5 days. Stigmas remained receptive onger under greenhouse conditions than in the open. Ab- cission of the style does not take place until about two eeks after blossoming. The abscission layer, however, eached an advanced stage at the end of the eighth day, and t is probable that if the pollen tube had not passed this ayer before that time it would not do so.

It is doubtful whether stigmas of the late flower would remain receptive for the same length of ime under natural conditions. The bags around the flowers robably extended the period of receptivity several days.

These results indicate that pollen will erminate as readily in darkness as in the light. The ailure of the pollen to germinate in sunlight was undoubt- dly caused by high temperature. A thermometer placed near he germinating cell registered 42° C.

The time of maturity and size of the

fruit and seed of a variety is dependent to a certain extent upon the variety of pollen used to fertilize the flowers. This difference in pollens may be accounted for by the fact that all varieties were seedlings originally and, like all cross fertilized plants, are very highly heterogeneous. Just how the pollen affects the variation in time of maturity and size of seed and fruit is not known.

SUMMARY

1. Pollen stored for 12 years appeared to be lighter in color, less uniform in size and shape, and did not reach the same state of turgidity as did fresh pollen. There appeared to be no difference between fresh pollen and 1-year-old pollen.

2. The most favorable temperature for germinating pollen is from 20° to 30° C., the temperature limits being 15° to 35° C.

3. Pollen germinates in water and in any concentration of sugar solution up to and including 50-percent. The most favorable concentration is 20-percent.

4. Under favorable conditions date pollen will remain in a viable condition from one pollination season to another.

5. Indirect light and total darkness have no noticeable effect on artificial germination of pollen. Pollen placed in direct sunlight did not germinate.

6. Stigma receptivity extended over a period of 13 days when the blossoms were bagged. This is perhaps a much longer receptive period than the same stigmas have under natural conditions.

7. Pollen may affect the moisture content of the fruit, although this has not been definitely proved.

8. Pollen from different palms affected the time of maturity and the size of fruit and seed.