T H E S I S

ON

THE CAUSE OF SEEDLESSNESS OF PRUNES

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The Cause of Seedlessness of Prunes.

Different kinds of fruits show considerable variation in the extent to which their development is dependent upon an accompanying development of seeds. Some kinds are capable of maturing their fruits even though there be no growth of the fertilized ovules. Indeed in some cases it is not necessary that fertilization or even pollination take place. Such fruits are said to be parthenocarpic. There are other kinds of fruits in which the development of the fleshy tissues of the fruit is invariably accompanied by a corresponding development of the seeds. If, for any reason, seed development should be arrested in these fruits the surrounding fleshy tissues cease to grow and the miniature fruits soon drop off. There are still other kinds of fruits, intermediate between the two classes that have just been mentioned. In these pollination and fertilization must take place and the seed must start to develop or the fruit will not "set". However with them, it is not necessary for the seeds to become fully developed in order for the fruit to mature. It is only essential that seed development progress to a certain stage (the exact stage varying with species and even variety), when their abortion may occur without apparent injury to the development of the fruits enclosing them.

The Italian prune has been thought to be dependant on the continued growth of the seed for the development of the fruit as only a small percentage of the mature fruits contain undeveloped seeds. It has been claimed that the fruits, in which the partially developed seeds abort, fall at the time of the June drop or later.

It was to investigate the relation of some of these internal factors to the setting and maturing of fruit and to the June drop in this variety that this work was started.

Review of Literature.

F. A. Waugh (1), in working with a number of pollen varieties, states that 41% of the June drop had no germs. He explained the nondevelopment of the seeds with the theory that either the pollen or the female gametophyte was weak. The germ, resulting from the union of a weak and strong or two weak gametis, was not strong enough to fully develop.

Charles P. Hartley (2), while breeding tobacco, found that premature pollination gave a light set of seed. Apparently plump seeds were merely hollow shells with no embryos on the inside. He assumed that early pollination stimulated the tissues to growth but that the pollen tubes distroyed the egg.

Methods.

The work was carried on with old Italian prune trees. They were in a fair state of vigor but had received very little pruning for a few years. A late frost destroyed the fruit in the lower part of the orchard, following which, the owner did but little cultivating the remainder of the season. The trees, on which this work was carried on, were on a knoll above the frost line and so largely escaped injury from the frost.

The second year the trees were carefully pruned. Part of the tree would receive a regular pruning for trees of their age and condition. It consisted in a medium heavy thinning out of branches to let in more light. No heading back was done. Another part of the tree would receive a pruning as laid out in part of the problem which will be detailed later. Another part was left unpruned to check with the previous year's conditions.

At blossoming time the first year the trees were covered with bloom but the weather was so cold and wet that little fruit set. The second year the bloom was lighter but the weather was good.

Methods in Field.

One part of the problem dealt with the effect of thinning buds on the spurs. Each year, about one

Month before blossoming time, a number of spurs had the fruit buds thinned to one to a spur. By this term "spur" is meant the small subdivisions of a branch. On a prune tree of average growth the bearing wood grows from one-fourth of an inch to eight inches or more in length each year. The longer growths will have two to four fruit buds clustered around the base, while the shorter growths will have as many grouped around the tip. By this term "spur" we designate these small subdivisions of the branches.

On a count of large numbers of spurs it was found that by removing all but one fruit bud to a spur sixty-six and two-thirds per cent of the buds would be removed. The results of this part of the work were such the first year that during the second year it was considerably extended. Besides thinning buds on the individual spurs, the buds were thinned by pruning out whole spurs and branches.

This prunning consisted in a heavy thinning out, enough wood being removed to eliminate sixty-six and two-thirds per cent of the buds. Smaller amounts were h handled so as to thin out the buds to the amount of fifty and ninety per cent, both by thinning buds on individual spurs and pruning off spurs and branches. To check with these blossoms, a number of spurs, that had neither been

thinned or pruned, were bagged and allowed to self-pollinate.

The first year a few of the blossoms were hand pollinated and the remainder self pollinated. The second year all the blossoms of this part of the problem were allowed to self pollinate.

Emasculation of flowers.

Emasculation of the flowers to be hand pollinated was carried on one to three days before the blossoms began to open. Emasculation was performed by seizing the corolla between the nails of the thumb and second finger, at a point just below where the stamens attach to the petals. With one motion the corolla above that point, with attached stamens, could be removed. By this method three hundred and fifty to five hundred flowers could be emasculated in an hour. The flowers were then covered with paper sacks to keep out insects and pollen. A larger number must be emasculated than is wanted for pollination. In a count of twenty-five hundred emasculated flowers, fifteen per cent were destroyed by time for pollination. Of those destroyed, sixty per cent had had the stigmas broken off in the process of emasculation. The pistil and stamens, as they lay in the unopened bud are so interlocked that removing the stamens often breaks the pistil.

Pollination.

Pollen was collected a few days previous to the time for pollination by forcing out a few branches in laboratory. The anthers were collected, placed in open dishes to dry and as the anthers burst and dry pollen was exposed, the pollen was tested as to viability. Italian pollen was the variety used chiefly with Silver and Petite used as checks. Collections were also made from young trees of different ages. Later pollen was gatheredafter a heavy rain and also after a light frost. After being dried, the pollen was kept in small glass vials, closed by cotton stoppers.

Pollen was applied with a camels' hair brush. Each stigma was supplied with enough pollen to make its color noticeable to the naked eye.

To study the effect of the time of pollination, flowers were pollinated at three different stages or periods of maturity of the blossoms.

The first were hand pollinated when twenty-five per cent of the blossoms on other parts of the tree was open. The second lot was pollinated when practically all the blossoms were out, by count being ninety to ninety-five per cent. The third lot was pollinated when seventy-five per cent of the blossoms were dropping their petals and a few of the earliest stigmas were blackened.

The same experiments were carried on for two successive seasons.

Laboratory Methods.

A study of the development of the bud, with special reference to the pollen grain, was made that continued from early summer until blossoming time the following year. Buds were gathered at intervals of one week during June, July and August, or until the blossoms were well formed and distinguishable. During the fall and early winter, collections were made monthly. After the first of the year and as activity in the bud increased collections became more frequent until, at the time of the division of the pollen mother cell, material was being gathered twice daily. Collections were then continued at longer intervals until at pollination time when material was collected three times daily.

Gilson's mixture was used as a killing and fixing agent at the beginning of the season on account of its greater penetrating power. Buds were trimmed and cut away on the sides to give easier access to the interior of the buds, for the killing solution. After the buds became older the tips of the buds were cut off, exposing the cavity with the two blossoms. Later still as the blossoms became larger and more solid, they were forced out through this opening by pressing on the bud at the point of attachment of the blossoms to the bud. The blossoms were extracted readily and with no damage to them.

About the time for the mitosis of the pollen mother cell Flemming's Weaker Solution was substituted for Gilson's Solution as it gives better results when safranin and gentian violet are used in staining. It takes more time but gives a better differentiation of spindle threads and chromosomes than the first solution.

All material collected was left in the killing and fixing solution for twenty-four hours. It was then washed as long as was needful and afterwards dehydrated in successive strengths of alcohol. After Gilson's solution the strengths used were seventy-five, eighty-five, ninety-five and one hundred per cent alcohol. If Flemming's Weaker Solution was used fifteen, thirty-five, fifty, seventy-five, eighty-five, ninety-five and one hundred per cent strengths were used. They were used in the order named for a period of twenty-four hours.

After dehydration the material was put into equal parts of xylol and absolute alcohol and after twenty-four hours put into xylol alone. To this, paraffin was added gradually until the paraffin was well infiltrated through the material, which took from thirty-six to forty-eight hours. It was then placed in an electric oven at a temperature of 50°C and left for at least a week. The paraffin used had a melting point of 50° to 55°C.

Most of the material was sectioned four to seven microns in thickness, depending on conditions and

the work to be done with the sections. Whole buds collected in June and July were easily sectioned in paraffin. Those collected in August and September became increasingly difficult to section and should be imbedded and sectioned in celloidin. Blossoms and pistils section readily if infiltrated and mounted in paraffin. Sections were mounted on slides, being fastened by Mayer's fixative and then allowed to dry for twenty-four hours.

Safranin and gentian violet were used for staining most of the slides. The sections were left in safranin for twenty-four hours and then transferred to gentian violet for one to thirty minutes. The length of time varied for different materials, the younger and more rapidly growing materials took the least time to stain. If stained well the sections showed the clearest and best differentiation with the use of this combination of stains but it was easy to over stain with gentian violet which left the spindles and chromosomes in a blurred mass.

Safranin and Ehrlich's Haemotoxylin were used with considerable success, especially when the slides were studied with the aid of an electric light and blue globe. This combination of stains seemed to be easier on the eye when the sections were studied by artificial light. Chromosomes were well differentiated but the spindle threads were not so well stained. Cell walls were clear but not so heavy as with gentian violet.

Safranin was used for eighteen to twenty-four hours and Ehrlich's Haemotoxylin five to ten minutes, though there is little danger of the latter overstaining. This combination was harder to photograph than was the first combination of stains used.

Several other stains were used in different combinations but these three stains gave the best results.

The camera used was a Leitz-Wetzlar photomicrographic camera. Different lenses and different objectives of a microscope, with or without the eye piece were used to obtain the required result.

Development of the Bud.

During June and July the development of the but consisted in enlargement of all the parts present in the bud but no attempt at differentiation between fruit and leaf buds was noticeable. Except for the difference in size no distinction could be noted between buds collected the middle of June and those collected the last of July Figs 1 and 2. In buds of that period, the center or primordial tip is smoothly rounded off. The cells are in even regular rows over the crown of the tip.

About the first week in August the cells in the crown of the tip begin to divide rapidly and the rows lose their regularity as the crown is pushed up.

As time goes on the cells in the center cease to divide or divide less rapidly than those on the outer portions so that a cup shaped structure is formed. This outer portion or ridge forms the sepals from which the petals and stamen s later spring. The pistil is the last part formed but the rudiments of all parts are clearly seen by the first of September. Fig. 5.

During the next few weeks growth of all floral parts occurs. By the middle of November the anthers show differentiation of tissue in the regions of the four sporangia. Fig. 6 and 7. A cross section of an anther shows the cells arranged in more or less regular circles in the four different sporangia. The inner cell or cells, numbering one or more, is the primary sporogenous tissue and is surrounded by a single layer of endothecium cells. The sporogenous cells are plump, square to roundish in cross section, and have a large clear nucleus. The endothecium cells are a little longer tangentially than radially and do not stain quite so densely as do the sporogenous cells. The epidermal cells are one layer in thickness and approximately cubical in shape. At this time the pistil is a homegeneous mass of cells. The line of placentation shows, as does a line where the ovarian cavity later develop.

During December development consists mainly in a very gradual enlargement of the blossoms. Cross

sections of anthers show a clearer outline of the sporangia, the sporogenous tissue and endothecium being more definitely differentiated. Occasionally the endothecium is found to be divided into two layers but usually only one layer is in evidence.

By the tenth of January the endothecium was regularly divided into two layers. The ovarian cavity was slightly enlarged. Fig. 10. From this time on the growth of the bud is more rapid than during the earlier part of the winter. At no period during the dormant season was there any indication that development and growth of the blossom and its parts had suspended. Evidence, as illustrated by sections of buds during the winter, shows that growth is continuous, becoming more rapid after the first of the year and increasing more rapidly the nearer the time for blossoming approaches.

On February 8th the sporogenous tissue of the anthers sho four to sixteen cells in cross section. Fig. 11 and 12. Directly outside the sporogenous tissue is the tapetum, one layer in thickness. The tapetum is enclosed by one or two middle layers. A single layer of endothecium surrounds the whole inner part. The endothecium cells are three microns in width radially and a little longer tangentially. The middle layers are approximately the same size and can not be distinguished from the endothecium except by their position.

The tapetum and sporogenous tissue stain considerably darker than the remainder of the sporangium. The cells of the sporogenous tissue or pollen mother cells are 1.5 microns in diameter and have a large clear nucleus.

By the twenty-seventh of February the pollen mother cells are in the resting stage and are gradually becoming more spherical in shape. The tapetum is becoming slightly disorganized and shows two nuclei to each cell. The middle layers are compressed and considerably overlap each other. This is partly caused by the enlargement of the endothecum cells. The ovules are showing as two small knobs, one on each side of the placenta in the ovarian cavity and are only a few cells in cross section.

During the next twelve to fifteen days the blossoms enlarge gradually. In the anthers all energies seem bent on preparing for the division of the pollen mother cell. The tapetum is rapidly becoming disorganized to furnish food for the pollen mother cells. Each of the two nuclei in the tapetum cells has from one to four nucleoli that vary considerably in size.

Pollen mother cells are becoming spherical as the outer cells enlarge and the tapetum breaks down, leaving more room in the sporangia. The mother cell nucleus is twelve to thirteen microns in diameter and generally slightly oval rather than circular in cross

section. Around the outside is a reticulum while the center is a clear open space except for the nucleolus.

Strands of chromatin are at first around the wall of the nucleus, later extending toward the center. During synapsis the nucleolus is usually at one side of the nucleus. Fig. 29. The spireme thread is more or less coiled around the nucleolus, and shows, as a whole mass, an irregular outline. The spireme threads appear heavy and fairly uniform in thickness. No sections showed any indication of the double nature of the spireme.

After this stage of development the spireme becomes distributed through the nucleus, later appearing as chromosomes. About this time the nucleolus disappears and spindle fibers show. Fig. 31. The spindle is sharply conical towards each pole, the two poles being in the cytoplasm next to the plasma membrane and outside of the nucleus. While the cells measured twenty-two microns in diameter the spindles measured fourteen microns as an average. After a short time in this position the chromosomes can be observed to migrate toward the poles leaving a number of strands that connect the groups of chromosomes which are formed at each pole. Fig. 32. A definite nucleus is formed at each end with a light nuclear membrane around it.

Next follows the division of the two nuclei. Two spindles are formed, one at each side of the cell and in parallel places. Fig. 33. They are much smaller than the first spindles, being shorter and decidedly narrower. Of those noticed, several were bent into a slight crescent shape. The chromosomes again form at the poles and resolve themselves into the four nuclei of the tetrads or microspores. Fig. 35. Around each nucleus and enclosing a certain amount of protoplasim, cell walls are produced that complete the formation of the microspores. The microspores develop rapidly within the enlarged and thickened mother cell wall. This heavy mother cell wall later seems to dissolve and the microspores are liberated.

All of these changes occur very rapidly. Of two hundred buds collected on March eighth, practically all of them showed the pollen mother cells in the diakinetic stage or the presence spindles. Only a very few anthers were not this far advanced. On the following day the tetrad stage was reached in a majority of the mother cells while on the tenth practically all changes described, have taken place.

The second division seemed to take place most rapidly as in only a half dozen sporangia was the second mitosis observed.

After the formation of the pollen grain the endothecium cells rapidly enlarge in both directions but

expecially radially. On the eighth of March the average measurement of fifty cells was six microns radially and 8.1 microns tangentially while on the seventeenth it was 22.9 microns radially and 14.2 microns tangentially. Fig. 2 and 5. This rapid increase in size ruptures the epidermal layer until many breaks show in it. The middle layers are so compressed and distorted as to practically lose their identity. The only trace of the tapetum is a few scattered strands. Fig. 26.

Approximately at the time of the mother cell divisions, the first differentiation within the ovule occurs. On the outer side of each ovule and towards the top appears a protuberance of cells arranged in several concentric circles. Fig. 15 and 23. This forms the beginning of the nucellus. At first it stands out well by itself but as the growth proceeds the neighboring tissue or integuments rapidly surround it. By the time the female gametophyte was mature or the twenthieth of March the integuments had grown around the nucellus, leaving only the micropyle, which showed at the top of the ovule.

The Mature Pollen Grain.

Pollen grains, immediately after the anthers open, are full and plump. On exposure to air the grains shrivel up, doubling in regular folds so that viewed thru a microscope one or two creases can be noted. If we consider the pollen grain as a sphere, which it approximates, one diameter will be longer, while the other two, at right angles to the first and to each other, will be shorter than when the grain was plump. Putting them into water or weak sugar solution causes them to resume their original shape. Frequently the osmotic action that sets in when put in water creates pressure enough, in swelling the pollen grain, to burst the cell wall and force out the contents. This often happened when attempts were made to germinate pollen in weak sugar solutions. To prevent bursting denser solutions were used.

Anthers, on bursting, expose the pollen grains to the air where they dry out. Since water will burst the grains by asmotic action, may not this partly explain the cause of a poor set of fruit when a rainy period occurs during the blossoming season. The rain falling on the pollen grains, sets up an osmotic action that results in bursting of the pollen grain.

In watching the bursting of pollen grains a certain type of pollen grain was never observed to burst

and later was never seen to germinate. These were small undersized grains. When put into sugar solutions for germination tests, such a gradating of sizes was observed that no difinite line could be drawn between the sizes. But if dry pollen was mounted in absolute alcohol it retained the same shape. No filling out or rounding out of the grains occurred. There was then a clear distinction between the two kinds of pollen grains, the large plump ones, of the shape described at first, and the smaller ones. In drying out these smaller ones would be round in shape and not elongated like the larger ones. The folds in the cell walls were irregular and ran in all directions. A count of these grains was made and included in Table I in connection with germination results, to show the comparison of percentage of small grains with the percentage of germination that occurred with the different kinds of pollen.

Table I

Per cent of small grains in comparison with per cent of germination.

Kinds of Pollen.	% germination	% small grains
Silver	87%	5
Petite	71%	13.
Italian (old trees)	81%	7.9
Italian(5yr old tree)	7.3%	60.
Italian(2" " ")	50%	23.5

This table shows that the better the germination, the fewer small grains present.

The pollen having the most small grains came from young trees. It should not be inferred from this that pollen from young trees will have more small grains than pollen from old trees, for the trees from which this pollen was taken were small undersized trees that had made almost no growth the last year. The condition of the tree would undoubtedly have an influence on the pollen and if the results indicated anything, it might be that the food supply received the previous year had had some influence. The Petite pollen was collected from a young vigorously growing tree but no attempts were made to ascertain if the percent of small grains was a variety characteristic or merely an individual characteristic.

In 1915 all varieties of plum and prune pollen were germinated in a solution composed of six per cent sugar and six per cent gelatine or weaker strengths. In 1916 attempts to germinate pollen in solutions of the same strengths resulted in very little germination and considerable bursting. Repeated attempts showed that a solution composed of thirty per cent sugar and twelve per cent gelatin gave the best germination tests and prevented bursting. The results both years gave approximately the same per cent of germination for the same varieties.

In germinating in solutions of different strengths, the different varieties showed considerable

variation. Silver pollen would germinate in a solution of forty per cent sugar and twelve per cent gelatine as well as in thirty per cent sugar and twelve per cent gelatine but in twenty per cent sugar and eight per cent gelatine so much bursting would occur that the accuracy of the test was destroyed. Petite and Italian pollen gave fifty per cent less germination in forty per cent sugar and twelve per cent gelatine than in the regular solutions. Italian pollen would germinate in twenty per cent sugar and eight per cent gelatine but would have considerable bursting while Petite pollen would germinate readily with no bursting.

Three weeks after the pollen was collected it was again tested. It would not germinate very readily in the solution used at first nor wouldn't give as high tests as at first in any solutions used.

Table II.

Germination tests three weeks after the pollen was collected.

Kinds of Pollen	% germination in 30% sugar and 12% gelatin	% germination in 20% sugar and 8% gelatin	% germination in 15% sugar and 6% gelatin	% germination in 10% sugar and 4% gelatin
Silver	0	65	50	
Petite	2	13	42	50
Italian	49	43	50	51

In the weaker solutions all varieties showed more or less bursting. Italian and Petite pollen gave twelve per cent bursting in the weakest solution while Silver showed twelve per cent bursting in the fifteen per cent sugar and six per cent gelatine.

Pollen collected after a light frost tested ninety per cent in germination. That collected after a heavy rain would not test over thirty-five per cent.

In considering the results of the germination work the question arises. "If the pollen grain requires such different strengths of solution from one year to the next, are these conditions always met in nature?" In other words, does the tree always secrete stigmatic juices of density required by the contents of the pollen grain for germinating the pollen? Might not conditions arise, as a result of which, the pollen would require a dense medium for germination, while the stigmatic juices that are secreted would be of a light density in which the pollen would quickly burst? This would possibly explain why seasons have occurred when, with apparently ideal climatic conditions, the set of fruit has been very light, while the following year the set would be good.

Again, if there should be the proper balance between the stigmatic juice and the pollen of the same variety, might it not be possible that such a balance does not exist between the pollen and stigmatic juices of different varieties.

Results of Pollination.

Microscopic Study of Pistils.

In studying sections made from pistils after pollination, a great difference was noted in the behavior of the pollen on different stigmas. Collections were made from spurs under the same sacks that had been pollinated at the same time and with the same brushful of pollen. Some stigmas would show a group of pollen grains vigorously germinating and with the pollen tubes pushing down through the tissue of the style. As many as fifteen pollen tubes, from as many pollen grains, have been observed growing down through a single style. Another stigma taken under the same conditions would show no activity at all in the growth of the pollen tubes. The pollen would not even be germinated. Here is evidence that several adjacent stigmas may give a different reaction to the same uniform lot of pollen. This would indicate that there is a possibility that only a part of the total number of flowers would be fertilized if a uniform grade of pollen were furnished them. In such cases pollen would not be the limiting factor, but physiological conditions within the pistil or its stigmatic juice.

Pollination

Results in the Field.

The first year the conditions of the blossoms and spurs and the results from the same, where the buds had been thinned were such that it seemed as though factors other than thinning were concerned in the results. The second year, to check with the spurs on which the buds had been thinned on individual buds and by pruning, 1262 blossoms were sacked on parts of the trees where no thinning had been don and allowed to self pollinate as in the case of the others. As indicated by Table IV by May fourth thinning had produced no increase in the apparent set of fruit. The ones that had received no attention save sacking had as good an apparent set of fruit as the one where the buds had been thinned. It will be noticed further, that those that had been allowed to self pollinate, whether the buds were thinned or not, had given better results than those that had been hand pollinated and also better indications for a good set of fruit than the parts of the tree open to the weather. Several factors might enter in here. Emasculation and had pollination might be detrimental or in the case of the self pollinated flowers the flowers on opening would allow the pollination at the time the pistil is supposed to be most receptive. Flowers do not all open at the same time but in the case of the hand pollinated flowers

all pistils are pollinated at one time while with self pollination there is a successive pollination of opened blossoms.

In pollinating at successive periods of the maturity of the flowers no data were secured from which conclusions in any direction could be drawn. Results appeared too erratic to be relied on. One thing to be noted was the uniformly good results obtained from the use of Italian pollen in the different classes. During the two years Petite pollen gave the poorest average results of the three varieties of pollen tried. In 1916 those blossoms pollinated with Silver pollen had a greater percentage of large sized fruits at the time the count of fruit was taken.

In ascertaing the number of fruits apparently set, all fruits, that were healthy green color and were firmly attached to the spur, were included. Just how many of the smaller sized furits that start are parthenocarpic can not be told but undoubtedly there are a good many. Emasculated flowers that were sacked and not pollinated started small fruits that corresponded in size May fourth to the large numbers of small ones on the spurs where the flowers had been hand pollinated.

Summary.

The development and growth of the fruit buds continues without interruption from the time the bud is formed in the summer until blossoming time the following season. The pollen grains, excepting a few, seem completely developed and vigorous, and under proper conditions appear to be able to function normally. All female gametophytes were apparently in readiness for fertilization by the time the blossoms opened.

Pollen differs, from one season to another, in its requirements of a germinating medium. Different varieties vary in the reaction of the pollen to the different germinating solutions. Correlated in a way to the per cent of germination of a lot of pollen is the per cent of small nonfunctioning pollen grains.

Pistils on the same spur vary considerably in their ability to furnish proper donditions for the germination of the pollen grain. Some permit a good germination of pollen on their stigmas, while others show no pollen germinating at all.

Flowers that were sacked and allowed to self-pollinate gave better results than those that were emasculated and hand pollinated. Thinning of bud had no effect on the set of fruit. Pollination at different stages of maturity of blossoms seemed to have no influence on the set of fruit.

Table III.

Results of Pollination work 1915.

Time of pollination	Blossoms pollinated	fruits 4/26/15	5/21/15	6/24/15	7/19/15	Mature fruits	% mature fruits
Buds thinned 66 2/3% 25% of blossoms out.	1046	640	235	133	69	49	7.6
Pollination early	842	34	18	11	7	6	.71
Pollination 95% of blossoms out	576	31	17	12	6	3	.35
Pollination late	1213	44	0	0	0	0	0

Table IV.

Result of Pollination 1916.

Time of Pollination	Blossoms pollinated	Fruits 5/5/16	% fruits
Early Pollination 25% of blossoms open			
Italian x Italian	955	307	32.1
Italian x Petite	404	93	23
Italian x Silver	680	278	40.8
Total	2039	677	32.7
Pollination when 95% of blossoms were open			
Italian x Italian	1074	151	14
Italian x Petite	513	23	4.4
Silver	252	1	.39
Total	1839	175	9.5
Late Pollination 75% of petals dropped			
Italian x Italian	979	217	22.1
Italian x Petite	444	67	12.8
Italian x Silver	515	139	26.9
Total	1938	423	22.7
Buds thinned 66 2/3%	1282	598	46.6
Buds pruned off 66 2/3%	1029	569	55.2
Check on thinning buds	1262	663	52.5

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Explanation of Plates

Plate I.

Fig. 1. Bud is undifferentiated. The growing tip is surrounded by the layers of bracts collected June 24, 1915.

Fig. 2. Same as Fig. 1 but showing relative increase in size and lengthening of bud. Same magnification. Collected July 26, 1915.

Fig. 3. Growing tip pushing up rapidly. No differentiation of tissues within the blossom part. Collected August 17, 1915.

Fig. 4. Two blossoms. Indentation at top. Calyx developing around the outside, Collected August 31, 1915.

Plate II.

Fig. 5. Rudiments of floral organ present September 15, 1915. Showing sepal, petal, stamen and the pistil slightly.

Fig. 6 and 7. Longitudial and cross sections of blossom November 18, 1915. Shows the concentric circle of cells within the sporangia. Ovarran cavity very small.

Fig. Longitudinal section of blossom December 18, 1915. Differentiation clearer in sporangia. Ovarran cavity inlarged.

Plate III.

Figs. 9 and 10. Endothecium divided into two layers. Ovarian cavity is widening January 10, 1915.

Fig. 11 and 12. Same as Figs. 9 and 10 except for increase in size. February 8, 1915.

Plate IV.

Fig. 13. Ovules started February 27. In the anther mother cells in the resting stage, not entirely rounded and free from each other. Tapetum layer shows as a darker row of cells next to the mother cells.

Fig. 14. Cross set of blossoms on March 5, 1915. Pollen mother cell almost spherical and free from each other. Large nucleus easily discernible. Ovules quite enlarged.

Fig. 15. During mitosis March 8, 1915. Ovule showing first of differention within its tissue.

Fig. 16. Blossoms March 19, 1915. Microspores fully developed and free.

Plate V.

Fig. 17. Longitudinal section of ovary March 8, 1915. No differentiation of ovule appear.

Fig. 18. Longitudinal section of ovary March 15, 1915. Nucellus appear as a semis of concentric

cells of cell on the outside of ovule. Integuments slightly grown around it.

Fig. 19. Longitudinal section of ovary March 19, 1915. Nucellus lengthwise with the axis of the pistil. Integuments over growing the nucellus rapidly from below.

Fig. 20. Longitudinal section of ovary March 23, 1915. Nucellus entirely surrounded by integuments and female gametophyte formed.

Plate VI.

Fig. 21. Cross section of ovary March 8, 1915. No differentiation of tissue within the ovule.

Fig. 22. Cross section of ovary March 9, 1915, showing the first differentiation of tissue in the ovule.

Fig. 23. Cross section of ovary March 13, 1915, showing integuments growing around the nucellus.

Fig. 24. Ovule completed March 23, 1915.

Plate VII.

Fig. 25. Cross section of anther just before mitosis of pollen grain. The tapetum is almost entire. Middle layers are full size and endothecium longer tangentially than radially.

Fig. 26. Cross section of anther March 17. Microspores are free and endothecium has enlarged greatly tangentially. Tapetum layer almost gone.

Fig. 27. Cross section of anther wall shows condition of cells during mitosis. Several nucleoli appear in one nucleus of the tapetum cell.

Fig. 28. Pollen tube entering the tissue of the style.

Plate VIII.

Fig. 29. Pollen mother cell during synapsis nucleolus surrounded by spireme.

Fig. 30. Diakinesis of mother cell. Spireme thickened and distributed throughout the cell.

Fig. 31. Spindle stage of mitosis.

Fig. 32. Chromosomes grouped at the poles of the spindle. Spindle threads are still connecting the poles.

Fig. 33. Two daughter nuclei with the light nuclear membrane surrounding each one.

Fig. 34 Each daughter nucleus has developed a separate spindle.

Fig. 35. Three of the four nuclei of a mother cell with a nuclear membrane around each. Just before cell walls are laid down.

Fig. 36. A group of tetrad within the mother cell walls.

Fig. 1

Fig. 2

Fig. 3

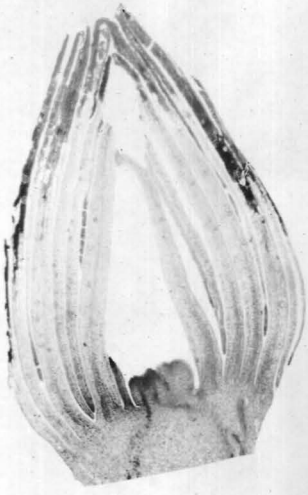
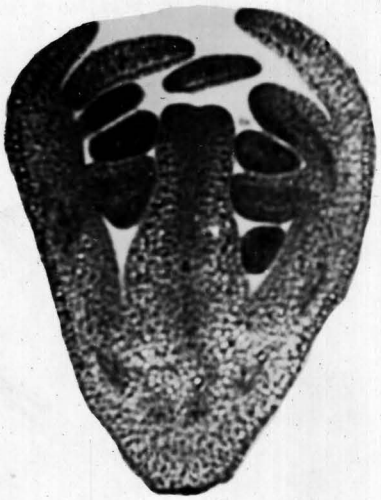


Fig. 4

Fig. 5



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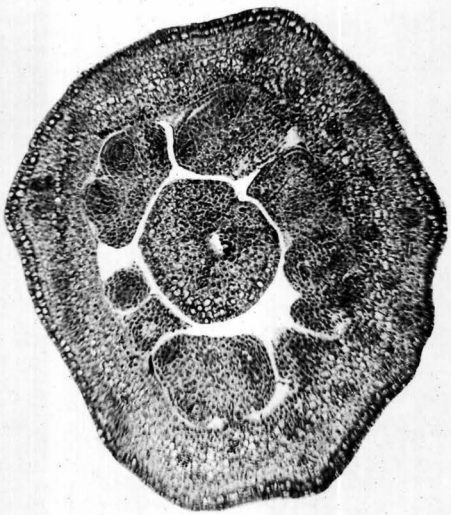

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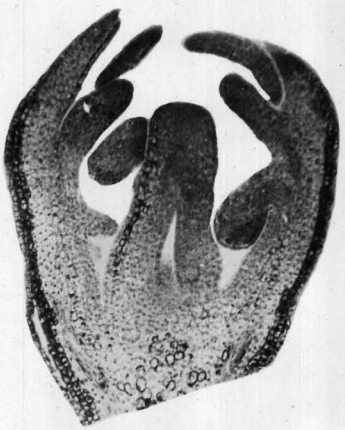

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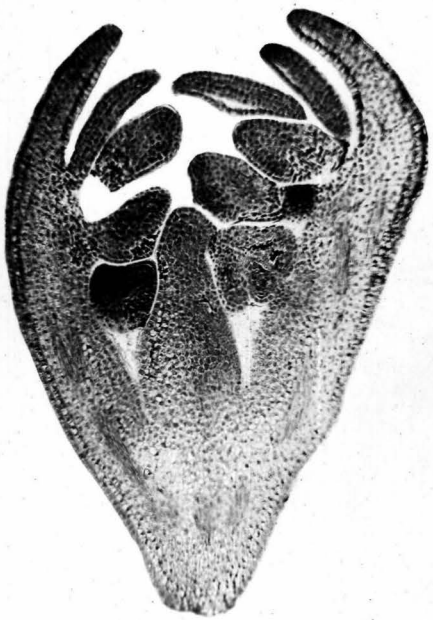

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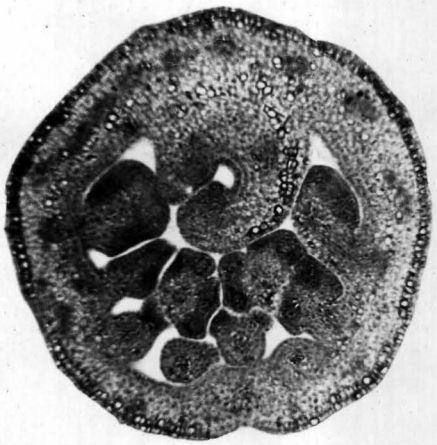

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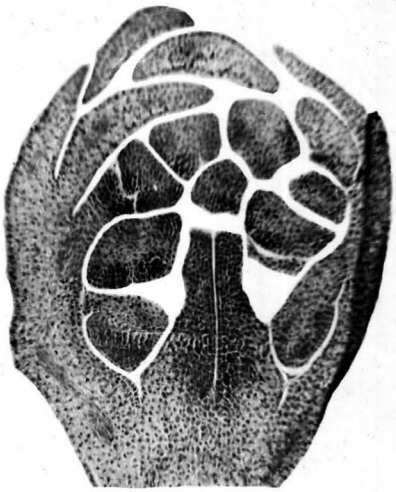
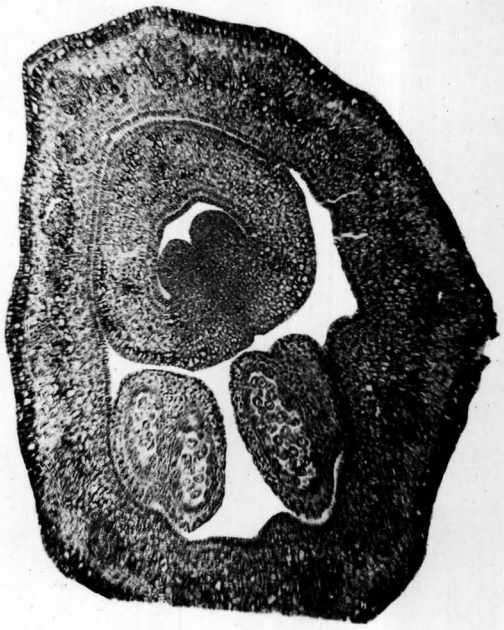


Fig. 11

Fig. 12

Fig. 13



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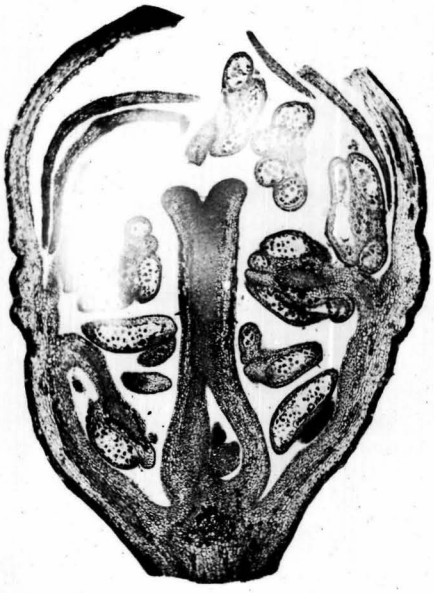
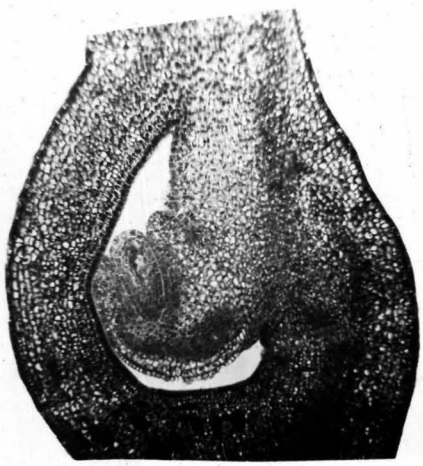


Fig. 15

Fig. 16

Fig. 17

Fig. 18



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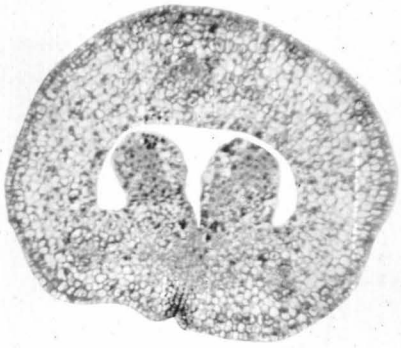

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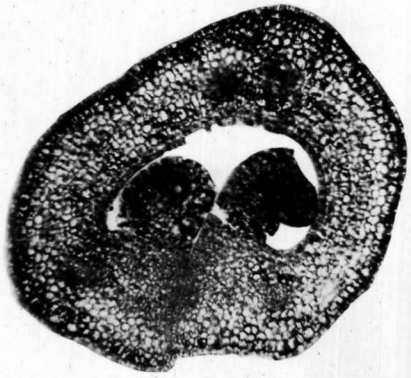

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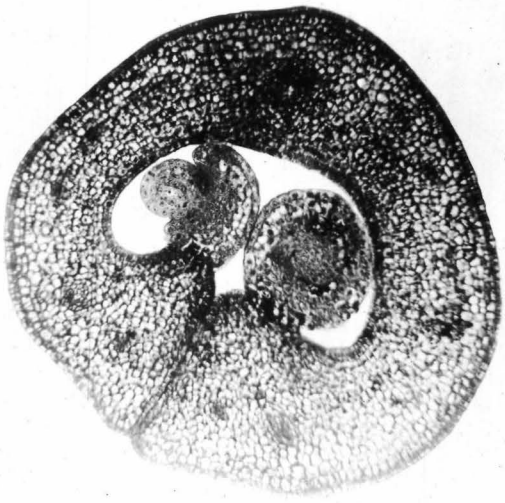

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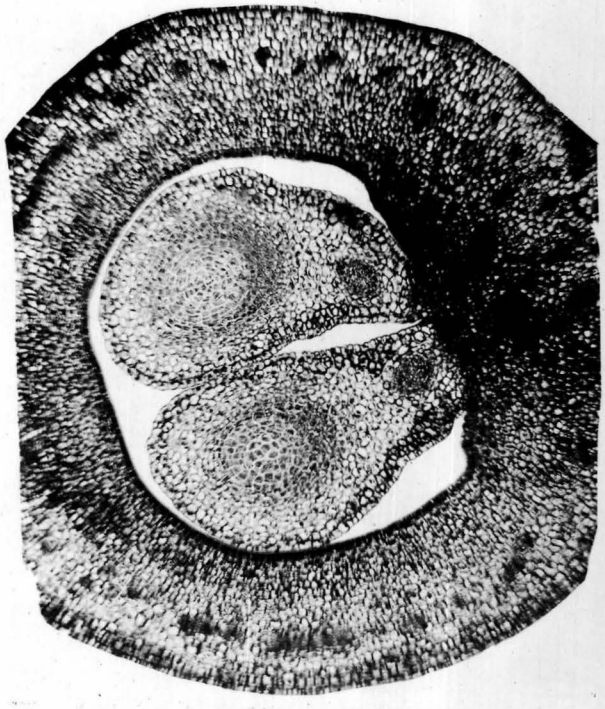

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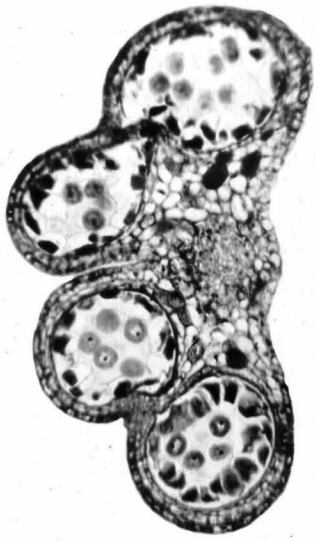

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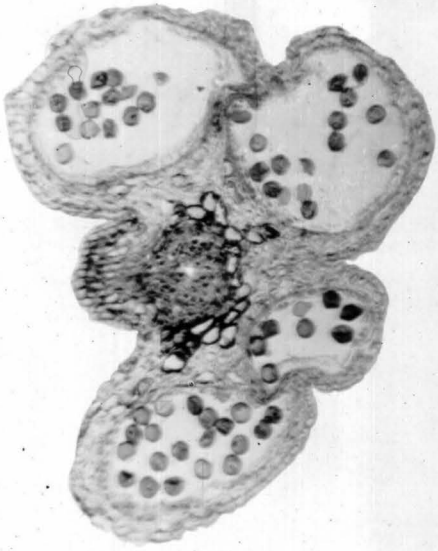
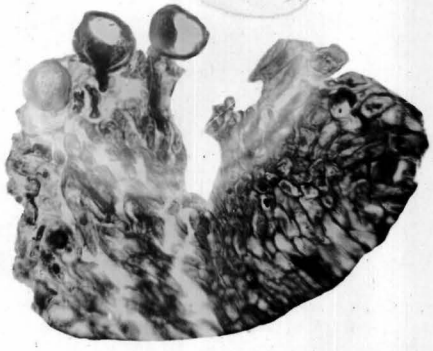


Fig. 26

Fig. 27



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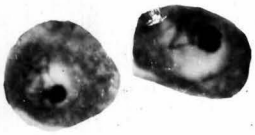

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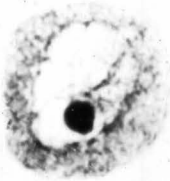

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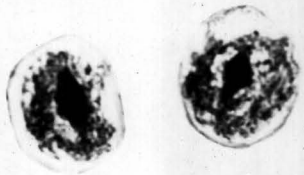

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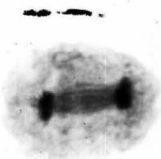

Fig. 32

Fig. 33

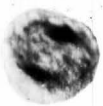

Fig. 34

Fig. 35

Fig. 36