A STUDY OF STERILITY IN THE PLUM[1]

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INTRODUCTION

In a former publication on weather in relation to fruitfulness in the plum (DORSEY 1919) it was shown that during bloom weather conditions may be such as alone to prevent the setting of fruit. It was further emphasized that, aside from the total effect of weather, certain single factors of it, acting in the extreme, might be singled out as being responsible for the failure of fruit to set. Weather conditions were shown to have their most immediate influence on such processes as dehiscence, pollination and fertilization. These conclusions are further supported by the variation in the extent of the second drop from year to year which can in general be correlated with weather conditions at bloom.

Aside from the influences affecting the functioning of the organs of reproduction which can be assigned to interference from weather or from the environment, there are others having a direct bearing upon reproduction, and hence upon fruitfulness, which appear to act within the germ plasm and are therefore inherent. An investigation of these influences forms the basis of this report.

THE STATUS OF SELF-STERILITY IN THE PLUM

Former investigators have dealt primarily with the economic phase of sterility. Tests have been made in *Prunus americana, P. Besseyi, P. hortulana, P. nigra,* and *P. triflora,* by WAUGH (1896, 1897, 1898, 1899), GOFF (1894 and 1901), HEIDEMAN (1895), WAITE (1905), and others. These show that the cultivated varieties of native species, with two exceptions, New Ulm (HEIDEMAN 1894) and Robinson (WAUGH 1898), are self-sterile. A similar condition was found in some of the Sweet Cherries by GARDNER (1913). On the other hand, the work of BACKHOUSE (1911 a, b), PETERS (1916), and SUTTON (1918), shows that in *P. domestica* only about one-half of the varieties are self-sterile.

It should be stated here that self-pollination and self-sterility are used with reference to the clone. Self-pollination in botanical usage refers to the transfer of pollen from an anther to the pistil of the same flower. In horticultural usage self-pollination has a broader sense and includes the transfer of pollen from any flower borne by a variety to any pistil of the same variety. Likewise self-sterility refers to the clone rather than to the flower or individual plant.

Since self-sterility has been found to be so prevalent, considerable interest centers around the reliability of the tests which have been made. WAUGH (1898) discusses this phase of the subject in some detail and concludes that the method used,—that of covering the blossoms with paper bags or other material,—is reliable. His conclusions agree with those of WAITE (1894) and BEACH (1898, 1899). This point was considered of sufficient importance to be checked further by different methods on account of its commercial as well as its scientific bearing. The data obtained in this test are presented in table 1. Bags were not used either on the trees grown in tubs in the greenhouse or on those tented in the orchard, so that any adverse influence the bags may have had in previous tests was eliminated in these. ALDERMAN (1917) in sterility studies in the apple, followed a similar method by covering the entire tree with muslin.

It will be noted that *P. americana, P. americana mollis, P. Besseyi, P. domestica, P. hortulana, P. nigra,* and *P. triflora* are represented in this table. The results with the trees under the tent as well as with those in the greenhouse agree with those previously reported and show that self-sterility is the outstanding feature of all the varieties included in these tests and that it is constant in expression. Considering the numbers under observation, the few exceptions found may be regarded as within the limits of experimental error, and it is even possible that some may have been self-fertilized, since, as will be shown later, pollen-tube growth takes place under these conditions. It appears safe to conclude therefore that the general condition in this genus has been correctly reported.

Since self-sterility is so general in the plum, cross-pollination, except as noted in certain varieties of *P. domestica,* is essential to fruitfulness. This makes it necessary to give careful attention to the blossoming-dates of varieties used as pollenizers. Such a classification of varieties has been compiled by a number of workers: WAUGH (1896, 1898, 1900); GOFF (1901); HEDRICK (1908), and others. In addition to planting pollenizers which bloom at the same time as the variety to be pollinated,

TABLE I

Showing the degree of self-sterility in selected varieties and species tested in the greenhouse and tented in the orchard as a check to the method previously used.

Variety	Condition of growth of trees, in self-pollination tests	No. of flowers pollinated	No. of fruits set	No. of fruits mature
Burbank	In greenhouse	Entire tree	6	0
"	" "	1 branch	0	0
Compass	" "	Entire tree	0	0
Minnesota No. 6[1]	" "	185	1	0
" " "	" "	24	0	0
" " "	" "	1 branch	—	1
Minnesota No. 10[1]	" "	1 "	0	0
" " 12[1]	" "	49	0	0
" " 21[1]	" "	1 branch	0	0
" " 35[2]	" "	84	2	0
P. Besseyi	" "	2 trees	0	0
Surprise	" "	Entire tree	0	0
Yellow Egg	" "		10	4
Minnesota No. 8[2]	Tented in orchard, 1915	Entire tree	0	0
" " 21	" " " "	" "	0	0
Sand Cherry × Apricot	" " " "	" "	0	0
Wolf[3]	" " " "	" "	0	0
Minnesota No. 9[1]	" " " 1916	" "	0	0
" " 12	" " " "	" "	0	0
" " 21	" " " "	" "	0	0
Assiniboin	" " " 1917	" "	0	0
Minnesota No. 8	" " " "	" "	0	0
" " 21	" " " "	5-yr.-old tree	0	0
Compass	" " " "	Entire tree	0	0
Etopa	" " " "	1 branch	0	0
P. americana	" " " "	4-yr.-old tree	0	0
P. Besseyi, Tree No. 1[4]	" " " "	281	1	0
" " " 2[4]	" " " "	263	2	—
" " " 3[4]	" " " "	176	8	—
" " " 5[4]	" " " "	300	1	—
Wakapa	" " " "	6-yr.-old tree	0	0
Wohonka	" " " "	6-yr.-old tree	0	0

[1] Cross between Burbank and Wolf.

[2] Cross between Abundance and Wolf.

[3] Large percentage of pistils aborted.

[4] The tents covering these trees blew off during a heavy rain on May 21st.

the effectiveness of the variety selected as a pollenizer, or the mutual "affinity" with the variety to be pollinated, must be determined. WAUGH (1899), HEIDEMAN (1895) and others have given this point some study. It is sufficient to state here that while there are differences in the effectiveness of pollenizers, inter-sterility has not been found to be extensive in the varieties of the native species, although *P. domestica* cannot be pollinated successfully with the native varieties. From the economic standpoint, therefore, the essential facts in the control of sterility in orchard plantings as a means of avoiding crop failure are already well understood.

It may be stated at this point that unfruitfulness is not considered herein from the standpoint of injury due to fungous diseases and insects.

Since the prospect of a crop, so far as the setting of fruit is concerned, may be determined by inspection as early as the five- or six-week period after the time of bloom, it will be seen that the problem of sterility, while limited in point of time, covers that period in the life cycle when delicate sex structures must not only form but must function. Consequently greatest emphasis has been given to this period in order to determine what factors are operating in this genus, not only to bring about self-sterility so extensively, but also to reduce to such an extent the number of functional pistils found in some seasons. The successive main headings will indicate clearly the phases of the problem covered.

MATERIAL AND METHODS

A representative list of varieties and hybrids has been available for this study. A part are growing in the experimental orchards at University Farm and the remainder at the Fruit-Breeding Farm six miles west of Excelsior, Minnesota. In both orchards the trees are grown under clean cultivation. Attention has been given to the species as well as to the variety. HEDRICK *et al.* (1910) have been taken as authority for the species of the different varieties except in a few of recent origin.

In the cytological investigations the usual technique has been followed. As to the killing fluids, chromo-acetic and Flemming's medium were most used, Carnoy's fluid being a poor fixative for the plum. The triple stain and Heidenhain's iron-alum-haematoxylin both proved to be excellent stains. Although the cytological phase of sterility is presented briefly, material was fixed and sectioned extensively. In all, over 2600 preparations were made in covering the different stages in anther and pistil.

POLLEN DEVELOPMENT IN RELATION TO STERILITY

Since functional pollen bears such a vital relation to fruitfulness, a careful cytological study of pollen development in the plum has been made with the object of determining the general condition in this genus.

With a knowledge of the pollen condition in species, hybrids, and varieties at hand, the relation of pollen to self-sterility can be ascertained. Moreover, normal development will serve as a basis for comparison in determining type and extent of pollen abortion.

Normal pollen development

Pollen development was studied in detail in varieties representing *P. americana, P. nigra, P. triflora, P. domestica, P. pennsylvanica, P. hortulana Mineri,* and *P. Besseyi,* but since there was so little variation from the condition common to the higher plants only a very brief description of the earlier stages will be included here. The later stages, however, especially those beyond the point of pollen degeneration, will receive more detailed treatment.

The winter stages

In Virginia, DRINKARD (1910) found that during December and January there was a slight development going on in the fruit bud of the plum. On January 10th in Abundance the pollen mother-cells were in the resting stage. As early as February 12th, there was some indication of division and by February 24th tetrads were being formed.

In Minnesota pollen development is less advanced during the winter. In Surprise on December 11th, development had progressed no farther than the archesporial-cell stage, and on March 23rd no further growth had taken place. By April 12th, the synaptic stage had been reached. Burbank and *P. nigra* on March 22nd were at the early archesporial-cell stage and it was not until April 6th that Burbank had formed pollen mother-cells which one week later were at synapsis. Material was fixed March 22nd, 1915, from varieties representing *P. triflora, P. americana mollis, P. hortulana Mineri,* and a number of hybrids between *P. triflora* and *P. americana mollis,* and in none had development advanced farther than the archesporial-cell stage. On the other hand, on January 13, 1918, *Amygdalus Davidiana* had pollen grains with two nuclei, but with scant cytoplasm.

The anther wall

During the early archesporial-cell stage of the winter months the central cells of the anther are somewhat larger than those of the outer three rows and differ from them primarily in having larger nuclei and more angular walls. The cells of the epidermal layer are small and have a staining reaction similar to the others.

At the pollen mother-cell stage the anther walls are three to four cells thick and noticeable elongation has taken place in the outer layer, while the three inner layers, particularly the innermost, are somewhat compressed. The cells of the outer layer take the orange, while the other layers, like the tapetum, have a greater affinity for the violet. The partition between the loculi of an anther is also three to four cells thick, and these cells as early as the open spireme stage are very much compressed and elongated.

In the final growth stages, marked changes take place in the cells of the anther wall. The outer wall of the epidermal cells becomes thicker, and additional elongation takes place as the anther cavity increases in size. The cells of the hypodermal layer become much broader, but show the most marked change from the earlier stages in their greater length and in the presence of conspicuous ridges in the wall. This layer is the most prominent element of the mature anther wall and is bordered on the inner side by the collapsed and very much extended walls of the inner layers. The cell layers between the loculi, which have become very much compressed at the liberation of the microspore, disappear with the tapetum,—a change which throws the pollen of both loculi together. The point of union of the partition between the loculi with the outer wall marks the place of dehiscence. In fact, at the time of the dissolution of the partition cells a part of the cells of the anther wall along the line of the suture is also dissolved.

The early pollen stages

The tissues of the anther at the pollen mother-cell stage have the characteristic differentiation and staining reaction. In cross section, the mother-cells are four to five cells deep and four or five times as long. The polyhedral walls about them are thin at first but become noticeably thicker previous to rounding up. The uninucleate tapetal cells are slightly larger than the mother-cells and stain more deeply with the blue.

The chromatin in the winter stages of the archesporial cells is coarsely granular and the relative uniformity of the deeply staining masses in number and size is striking. The number of these bodies approximates that of the double number of chromosomes.

The rather scant chromatin of the mother cells, however, in the early stages is finely granular and quite evenly distributed throughout the nucleus. Previous to the formation of the spireme thread, larger and more deeply staining masses are found. The spireme which enters synapsis is very slender, irregular in marginal outline but distinctly granular. The synaptic mass is very tight and compact, being in many cases but little larger than the nucleolus near or about which it is typically located.

At the time the synaptic mass is unraveling, a few short loops appear first. These gradually lengthen and with further loosening others are formed so that the thread is soon spread throughout the nuclear area. While the plum is not suitable material in which to study the manner of pairing of the chromosomes, well fixed preparations of the spireme at critical formative stages show a condition which strongly favors the side-by-side pairing.

During the open-spireme stage the chromatin thread increases in thickness and as diakinesis is approached becomes much looser and even more granular. Immediately following segmentation the chromosomes are very irregular in outline and in many cases the individuals of a pair lie distinctly apart. With further development, they gradually become more compact and at the end of this stage are found evenly distributed near the nuclear membrane. As the time of division is approached the chromosomes assume a homogeneous structure which completely obscures their double nature.

The heterotypic and homoeotypic division

Coincident with the appearance of the spindle fibers of the heterotypic division, the nuclear membrane becomes irregular in outline and the area of the nucleus much smaller. The multipolar spindle of the early preparatory changes forms a distinctly bipolar spindle at the metaphase. The chromosomes at the equatorial plate lie in slightly different planes in most of the preparations of this stage, and show some irregularity in the passage to the poles. The fibers of the spindle, particularly the intra-polar fibers, are very distinct. Following this division the chromosomes are drawn together at the poles in a close, compact mass. The heterotypic spindle gradually becomes less distinct as the nuclei of the dyad are formed.

After reorganization, the dyad nuclei divide simultaneously. The spindle of this division is slightly smaller and narrower at the equatorial-plate stage than is that of the first division, and the chromosomes are smaller although distinct. The spindles sometimes lie in the same plane although typically they are in planes perpendicular to each other. The chromosome number determined in the plum is presented in table 2. Judging from the number of species represented, ten chromosomes as the reduced number is quite common in this genus.

TABLE 2
Showing the number of chromosomes found in different forms of the plum.

Variety	Chromosome number
Iron Clad (*P. americana*)........................	10
Minnesota No. 12 (*P. triflora* × *P. americana mollis*)	10
Opata (*P. Besseyi* × (*P. Munsoniana* × *P. triflora*))	Near 10
P. pennsylvanica	20 2*x*
Stella (*P. americana* × *P. triflora*)................	10
Stoddard (*P. americana*)	10
Surprise (*P. hortulana Mineri*)..................	20 2*x*
Wolf (*P. americana mollis*)......................	20 2*x*
Wyant (*P. americana*)............................	0

From the above description it will be seen that pollen development proceeds through the heterotypic and homoeotypic divisions with every appearance of being normal. This condition obtains for the most part in the varieties of pure species as well as in extreme hybrid forms. Degenerative processes which become so active later do not gain expression as early as this.

The tapetum

The tapetum in the plum shows no marked variation from its usual course of disintegration. Its cells have a single large nucleus which first divides about the time of the first division in the mother-cell. The most noticeable changes which take place in the tapetum previous to the liberation of the microspores are vacuolization and further division of its nuclei. In some anthers advanced degeneration takes place at the tetrad stage, at a time when the walls are still intact. Following the liberation of the microspores, the tapetum rapidly disappears. During the period of rapid anther enlargement, while its cells are yet intact, the tapetum is often withdrawn from the anther wall. Following this stage the tapetal cells are more or less separated and undergo the most rapid disintegration. The walls about the tapetum persist much later than the mother-cell wall which disappears typically at the late tetrad stage. In the mature anther only occasional traces of tapetal cells or walls remain in the anther sap. The functioning of the tapetum and its disappearance from the anthers in which there is partial or complete pollen abortion are the same as in those bearing all normal grains. The tapetum in the plum, then, functions normally as nourishing cells and apparently has no bearing upon pollen degeneration.

The tetrad wall

Tetrad formation marks the point at which the hereditary allotment to each nucleus has been made and also the beginning of an independent existence of each microspore. The reaction of the microspore to its environment, both before and after liberation, therefore, is of particular interest from the standpoint of aborted pollen.

The stages in the formation of the wall about the rounded mother-cell in the plum are very distinct and can be easily followed. The origin of this wall in other forms has been given some attention but its relation to the wall of the mother-cell has not always been indicated. According to the evidence at hand there appears to be two distinct methods of disposing of the mother-cell wall: (a) in one case, as in the lily (ALLEN 1905) and the grape (DORSEY 1914), the original mother-cell wall disappears at the rounding-up stage; and (b) in the other, illustrated by the strawberry (VALLEAU 1918), the mother-cell wall remains intact after rounding-up has taken place. The plum belongs to the latter class and since these early stages precede the action of degenerative processes they will be presented in some detail.

The angular walls between the early mother-cells have the appearance in section of thin lines. These take the orange heavily with the triple stain and with Heidenhain's haematoxylin stain light or dark blue. At this time they are similar in thickness to the walls of the tapetum. At the time of the rounding up of the mother-cell the walls become noticeably thicker than those between the tapetal cells, but the staining reaction is similar.

The first evidence in the plum of a new wall about the rounding-up cytoplasm of the mother-cell is the separation of a thin layer from the inner surface of the old wall, first at the cell angles and subsequently farther along the sides (plate 2, 1). In some sections the new wall,—which will hereafter be referred to as the *tetrad wall* as distinguished from the persistent mother-cell wall,—appears as a line and in others as a surface, according to the angle of view. Later stages show considerable irregularity in separation. At the cell angles and narrow ends of the cell, it is generally drawn away while yet in contact with the old wall along the longer sides. From the irregularity in separation, however, it should not be inferred that there is necessarily a similar irregularity in formation, since, where partly separated or even in contact with the old wall, its outline can generally be followed distinctly around the remainder of the cell periphery. Complete separation of the tetrad wall takes place in most cases previous to the heterotypic division.

The tetrad wall appears to be a derivative of the mother-cell wall instead of the cytoplasm. This view is supported by the manner of separation as well as by the evidence from staining reaction. As noted above, in the young mother-cell the walls have the appearance in section of a thin line and later undergo slight thickening and stain heavily with the orange. Immediately after the separation of the tetrad wall, which stains a light orange, the mother-cell wall, which is again noticeably thinner than just previously, has a darker reaction to the orange. Furthermore, since the cytoplasm rounds up first, the plasma membrane is very distinct and separated in places from the tetrad wall which, at this time, is generally completely formed.

The mother-cell wall, which remains after the formation of the tetrad wall, persists in many anthers as late as the liberation of the microspores. In others it disappears soon after the heterotypic division. The rapid increase in the size of the anther cavity brings about considerable elongation in the mother-cell wall which provides ample space for the tetrad. The tapetal cell walls disappear later than those of the mother-cell so it appears that different enzymes are acting, or if a single enzyme, that dissolution is localized.

The tetrad wall, which is thin and homogeneous when first separated, does not undergo any appreciable thickening until after the heterotypic division. Subsequent to this, particularly following the organization of the tetrad nuclei but coincident with wall formation between them, the tetrad wall increases rapidly in thickness. The maximum thickness of the outer wall is found when the walls between the microspores reach the greatest thickness, i.e., in the mature tetrad.

Traces of cell plates appear between the tetrad nuclei by the deposition of material near the central point of the intra-polar spindle fibres. The staining reaction of this material is at first slightly darker than that of the thick tetrad wall and the tetrad nuclei also round up before the walls between them become thick. Further rounding of the spores is followed by a gradual entrance of the viscid tetrad-wall between them until each one is completely enveloped with the thick wall characteristic of this stage (plate 2, 2). The stages in the division of the cytoplasm between the microspore nuclei of the tetrad have been followed out in considerable detail and for the most part agree with the observations of FARR (1916, 1918). The question now enters as to what differences appear in the morphology of the tetrad as a result of the two types of mother-cell wall dissolution, i.e., a dissolution of the middle lamella at the time of tetrad-wall formation as in the grape, and dissolution subsequent to the heterotypic division, as in the plum and strawberry.

In the grape, plum and strawberry, the outer wall between a mother-cell and a tapetal cell persists after rounding-up has taken place in the cytoplasm. In this position the tetrad wall has a similar origin in each and a careful study of these stages shows a similar structure and separation. In all three forms this wall, which is very thin at first and subsequently thickens, the interpretation appears justified that it is homogeneous throughout because the stains used did not show in either that the exterior is bordered by a thin wall or membrane. Such an interpretation is in accord with the well known swelling of colloidal substances. The refractive power of the outer margin of the tetrad wall in the grape and plum is identical with the triple stain and is similar to that of the inner layer about the microspores. Furthermore, there is never a separation of an outer wall or membrane from an inner thicker portion of the tetrad wall and during dissolution there is no appreciable persistence of either the outer or the inner surface over that of the middle portion. It is possible, however, that other staining methods may show differentiation in the tetrad wall not revealed so far by the technique used. It appears then that the differences in the time of dissolution of the mother-cell wall result in no striking morphological differences in the tetrad wall. In the grape, that remnant of the mother-cell wall, whether simply the middle lamella or more, which does not enter into the formation of the tetrad wall is dissolved immediately, while in the plum and strawberry it persists for a time longer. In one case there appears to be an enzyme action which is either absent or delayed in the other. The origin of the tetrad wall, however, appears to be identical in each type.

The microspore wall

The microspore wall first appears in section as a very fine line between the plasma membrane and the thick tetrad wall (plate 2, 2). It is best seen where slight plasmolysis has occurred. Before the tetrad wall is dissolved the microspore wall becomes noticeably thicker. The sequence of events is such that the extended cross walls of the mother-cell, the tetrad walls, and the walls about the microspores can sometimes be seen in the same anther. The microspore wall is formed adjacent to the interior surface of the thick tetrad wall but outside of the plasma membrane. No evidence of it can be seen in the plum until after the tetrad wall has become thick between the microspores, but since the inner border of the tetrad wall about each nucleus at this stage stains slightly more heavily than previously the interpretation is that at least a part of the heavier staining reaction is due to the changes taking place in microspore-wall formation.

The liberated microspore

Before the microspore is set free in the anther sap its nucleus is reorganized and in the resting stage. In many anthers the microspores of a tetrad remain in their usual position in relation to each other for some time after the dissolution of the tetrad wall. Breaking down of the persistent mother-cell wall generally precedes the dissolution of the tetrad wall although both may disappear at the same time. In the plum as in Fragaria (VALLEAU 1918) there is no appreciable increase in size in the microspores before liberation. The anther sap is clear and homogeneous at the time of dissolution of the tetrad wall, and its staining reaction is not changed by the inclusion of the substance of the walls of the mother-cell and of the tetrad.

The germ pore is formed in the plum microspore immediately after liberation from the tetrad, during the early stages of thickening and growth in the microspore wall, but before the time of rapid extension. The first evidence of the suture is a bending in of the previously spherical covering forming three longitudinal grooves in the surface at places where the wall appears slightly thinner. During this stage the two distinct elements of the wall can be for the first time definitely distinguished. At the germ pore the intine is continuous and the exine in cross-section is broken or discontinuous. Further thickening takes place primarily in the exine, which in the mature pollen grain constitutes the most conspicuous part of the wall. There are three sutures and at the mid-point of each a germ pore. The germ pore is bordered by projecting, fimbriated outgrowths of the exine (plate 2, 3) which are considerably raised and are conspicuous in the mature pollen. These edges overlap, and in some varieties, as Wyant, the pore is closed by them. The germ pore is present in all forms included in this investigation, a condition which is very different from that in the grape in which it is absent in all pollen borne by reflexed stamens.

Subsequent to the formation of the germ pore the microspore wall enters a period of rapid growth both in extension and thickness. This takes place much more rapidly than the growth of stainable cytoplasm, producing that appearance characteristic of this stage in which large vacuoles are formed. The stainable cytoplasm, with the nucleus, is located mostly toward one side.

The general relation of wall, vacuoles and cytoplasm is maintained until the division of the microspore nucleus, a condition which results in the division figure being located at one side in the rather narrow crescent-shaped cytoplasmic mass. So far as observed, the division figure is perpendicular to the microspore wall, and the cell plate cuts off the typical small generative cell. Division is followed by a further increase in the size of the pollen grain in which there is a rapid growth of the stainable cytoplasm.

The mature pollen grain

The exine of the mature pollen grain is thick and its exterior is marked by prominent ridges and furrows (plate 2, 4). These are most conspicuous in *Prunus americana* and *P. nigra;* in other species, as *P. virginiana, P. pennsylvanica* and *P. Besseyi,* the surface is only slightly furrowed although distinctly rough. The sutures are prominent and extend nearly the entire length of the grain. The protrusions about the pore also vary in development in the different species, some extending only slightly over the pore and some practically covering it. In some sections the two coats in the wall can be clearly distinguished by a difference in staining, although they are very seldom separated except where cutting has been the cause.

Soon after nuclear division in the microspore there is a rapid increase in the stainable cytoplasmic content so that at maturity the conspicuous vacuoles of the earlier stages disappear. At the time that the stainable cytoplasm completely fills the space within the wall of the pollen grain the microspore nuclei reach their maximum size (plate 2, 17).

In the mature pollen grain the nuclei, particularly the generative nucleus, are characterized by their small size (plate 2, 8). This diminution in size is brought about by the withdrawal of nuclear sap, a process which first becomes evident by the irregular outline of the nuclear membrane. The staining of the nuclei when thus contracted is clear and distinct and not diffuse. There appears to be a concentration of the chromatin into larger masses as contraction progresses from the more finely granulated condition found immediately after the telophases of the division (plate 2, 9). The lightly staining network, which connects the finely granular chromatin of the earlier stages, becomes less conspicuous at maturity. This is the normal condition in forms known to produce viable pollen.

The generative cell as well as the generative nucleus decreases markedly in size as maturity is reached, as will be seen by comparing figures

5, 6, 7, 8, 9, and 10, of plate 2. In some sections the limiting membrane of the generative cell is so closely contracted about the nuclear membrane that the generative cell has the appearance of a nucleus (plate 2, 9 and 10). It may be either round or lens-shaped, and while generally located near the center of the pollen grain close to the vegetative nucleus it is in some cases found at one side adjacent to the wall. Conspicuous features of the contracted generative nucleus at pollen maturity are the dense masses of chromatin, relatively few in number, and the extremely small nucleolus (plate 2, 8). The position of the generative cell in the cytoplasm is independent of that of the germ pores. In Yellow Egg (*P. domestica*) the generative cell is larger than in the American species, and at maturity the chromatin is more finely granular. In Wyant and Iron Clad, the generative cell is usually small when first cut off.

Pollen is mature before the stigma, and owing to its maturity and the protection afforded by its thick wall and the anther wall it is more resistant to adverse weather than is the stigma. At about the time the petals are bursting the nuclei in plum pollen are entering upon the contracted stage, and because of the presence of the anther sap they remain turgid until the drying which accompanies dehiscence. When dry, instead of being spherical and turgid, they are oval in outline and have three deep folds lengthwise in the covering corresponding to the sutures. In some varieties pollen is readily removed by the wind when dry after dehiscence, and in others but little is blown away because of the adhesive action of a yellowish oily substance.

The pollen tube

Upon reaching a receptive stigma, both aborted and normal pollen grains soon become turgid and spherical. When the growth of the tube starts there is a slight bulging of the intine at the germ pore adjacent to the stigma, but the tube nucleus and generative cell still remain in their usual position. When the tube first emerges from the pore, the cytoplasm contained in it and that adjacent in the pollen grain stains more deeply than before germination. In some grains previous to germination the cytoplasm stains more heavily about the margin, near the plasma membrane. The chromatin in both nuclei at this time is finely granular and more homogeneous than before in its staining reaction. The tube is large and conspicuous when first formed and becomes noticeably more slender as it advances into the stylar tissue, and both the tube nucleus and the generative cell, as well as the larger portion of the cytoplasm, enter it by the time its length is three to four times the diameter of the pollen grain. In the micropyle, after having passed through the tissue of the style, the tube again becomes thicker. MOORE (1917) notes this difference in diameter and attributes it to food supply. It appears, however, to be due more largely to the stage of growth. Since the movement of the generative cell in the tube can be easily followed, division must take place there, although in the numerous sections of the tube at this stage no division figures have been found. In fact in sections of tubes in the micropyle (plate 2, 11) the generative cell can be found still undivided so that it is probable that ♂ gametes are formed late in the period of tube growth.

After the tube has extended as far as one-half of the length of the style its cytoplasm becomes vacuolated and the nuclei are very inconspicuous. Partitions are formed in the tube, although they are not easily found because they do not stain with Heidenhain's haematoxylin or with Flemming's triple stain. Partitions were also found by OSTERWALDER (1910) in the pollen tube of the pear and by KNIGHT (1917) in the apple, but stains were used by the latter which makes a study of this feature much easier than the stains used in this investigation. As the tube advances in the style the cytoplasm is located well toward the growing tip and the empty walls of the tube left behind can be found in the stylar tissue, in many instances still leading to the empty pollen coverings.

Aborted pollen never develops tubes. Empty coverings of grains which have developed tubes in sections of the stigma are readily distinguished from aborted grains by their slightly larger size, the broken intine or remnant of the tube, and the absence of cytoplasm. In all of the preparations of receptive stigmas many normal-appearing grains do not germinate and from many others only short tubes are formed (plate 5, L and M); yet these are under conditions where others grow normally. This condition prevails in controlled crosses as well as in cases of controlled self-pollination where all pollen is known to have been applied at the same time. In the style the tubes become fewer in number toward the base while immediately beneath the stigmatic surface there are in many cases a multitude of short tubes, which in length grade gradually into the longer ones. The great extremes in the rate of growth shown by the tubes from different grains in the same style account for the small number found at the micropyle in the later stages. Later more attention will be given to the significance of the series presented here.

From the foregoing it will be seen that normal pollen development is the typical condition in the plum. Self-sterility and cross-sterility, which are so general, are not due to degenerate pollen except in those forms where pollen abortion is complete. From plate 5, L, it will be seen that there is tube growth when the plum is self-pollinated, so it may be definitely stated that self-sterility is caused by other factors which operate subsequent to tube formation. At this point interest centers around the extent to which aborted pollen modifies the typical pollen condition in the plum.

Aborted pollen or arrested development

The types of aborted pollen

In Prunus, as in Vitis (Dorsey 1914) and Fragaria (Valleau 1918), the range in the time of pollen abortion extends from liberation from the tetrad to maturity. From selected grains taken in order of arrested development a complete series can be constructed. In fact, such a series can often be found in a single anther (plate 3, A, C, H, I). However, by far the larger number abort before division of the microspore nucleus. Since the one-nucleate grains may persist as late as the time of maturity of normal grains abortion takes the form of a delay rather than of disintegration.

A study of the late tetrad, at the time of wall dissolution, in which the microspores are still in position, shows typically an even development. Size differences between the microspores become conspicuous after further growth. In cases of early cytoplasmic abortion in grains no larger than one-half of the mature diameter, the wall undergoes partial or even nearly complete thickening. In others often of greater size, there is less thickening of the exine. The germ pore, however, is formed in all cases where development is carried beyond the normal time of its formation. In general, since there may be wall thickening and enlargement accompanying early cytoplasmic abortion, there does not appear to be an intimate interdependence between wall and cytoplasm although nearly complete wall-thickening is always found in cases in which abortion occurs at a late stage. Tischler (1908) has regarded wall formation in pollen as being more or less independent of normal cytoplasmic development. This interpretation would appear justified in view of the condition found in pollen before dehiscence, in which some aborted grains are nearly devoid of stainable cytoplasm and in others only the broken down remnants of the nucleus and cytoplasm remain. The fact however, that thickening in the wall takes place so early in growth, before degenerative processes are complete suggests that wall thickening is dependent upon cytoplasmic growth. This view is further supported by the thin walls found in those few grains in which abortion occurs before wall thickening.

Considering now the condition of the chromatin in the aborted series, those grains in which abortion occurs at the earliest stages show but little if any increase in amount in the stainable cytoplasm over that received from the mother-cell. In such cases the chromatin still persists in large masses, a condition which suggests only partial reorganization. The nuclear membrane in such cases may be either compressed and irregular in outline or very much extended. The cytoplasm has different reactions to stains, in some instances being finely granular and flocculent and in others dense and more homogeneous. In general those grains which have developed no farther than the one-nucleate stage are characterized by their small size, scant cytoplasm, large vacuole, irregular nuclear membrane, and large chromatin masses. Yet in these there are generally thick walls and normal-appearing germ pores.

Where development is carried as far as the division of the microspore nucleus, there is in most cases considerable addition to the cytoplasm, but the nuclei show a similar condition to that above described for the single nucleus in that there is a suggestion of arrested reorganization as shown by the condition of the chromatin. The cytoplasm may still show a large vacuole or the stainable cytoplasm may fill up the entire grain, in the latter case the staining reaction is lighter (plate 3, J, K). This condition in the cytoplasm indicates abortion at later stages, in which cases the nuclei may appear more normal and may even enter the contracted stage. It is probable that the latest cases of abortion cannot be detected by the appearance in stained sections but are shown by the inability of the grain to send out tubes. It has been stated in the discussion on pollen development that on the stigma some apparently normal grains do not send out tubes. It is conceivable that in these grains which do not develop tubes the end of the aborted series is to be found.

Since the development of pollen is so typically normal up to the time of liberation of the microspore, it now becomes important to determine whether the other elements of the anther show a normal growth, especially in anthers where there is a large percentage or even total pollen abortion. A careful study of anther development in a large number of forms shows that the anthers undergo the usual differentiation even when the pollen they bear is completely aborted. The exceptions to this are found where there is complete abortion early in development, in which case there is often an unusual ingrowth of the endothecium. Even in these cases the mature anther is filled with sap and the tetrad wall and tapetum completely disappear.

The general development of the different elements of the anther and especially a normal sequence of development in anthers where only a part of the pollen is aborted would appear to eliminate any influence from this source as a factor in pollen abortion. The abortion of some grains in the same substratum in which others not only develop normally but function normally indicates differences between the grains rather than localized influences from the anther.

In view of the condition in the anther, where there is approximately a normal development independent of the pollen condition, it is of interest to find the microspore wall undergoing a more or less independent development. It will be seen therefore that so far as the $2x$ tissue is concerned abnormalities which might later influence pollen development do not enter and that abortion begins with the $1x$ condition.

Earliest evidence of pollen abortion

It has been emphasized that typically abortion does not become evident until after microspore liberation. However, certain apparent exceptions to this in an extreme hybrid condition have been found. In a cross between *P. Besseyi* and *P. armenica* the stages of the heterotypic and homoeotypic division have been studied in detail and there are indications in some of the mother-cells of irregularities in chromatin distribution, which indicate that processes resulting in abortion begin earlier in some cases than liberation, which in the less radical crosses is the typical condition. In this cross 87 percent of the pollen is aborted (table 4).

The earliest indication of arrested development found is in the dyad stage (plate 2, 12) although at the heterotypic division single chromosomes sometimes lie far to one side of the plate and at the early metaphase they are even more scattered (plate 2, 13). This scattered condition of the chromosomes is suggestive, especially in view of the condition at later stages. In a number of the dyads the chromosomes of one nucleus were at late metaphase at the time of complete reorganization of the sister nucleus. In other mother-cells of this cross at the tetrad stage, the conspicuous rings or circles in the cytoplasm (plate 2, 14) which resemble small nuclei in some instances, particularly after the heterotypic division, also indicates an unbalanced condition following the reduction divisions. As many as thirteen of these rings have been counted in a single tetrad. Some of the darker-staining bodies in the cytoplasm have every appearance of being chromosomes which have not entered into nuclear reorganization. That the rings, or in some instances spheres, which appear so conspicuous in the cytoplasm (plate 2, 15, 16) are connected with chromatin distribution and are evidences of early degeneration is supported by the extreme condition found in such tetrads as that shown in figure 16, plate 2. In the tetrad illustrated here no nuclei have been formed and the rings and dark-staining bodies are conspicuous features in the cytoplasm. Two of the spherical masses may in fact be interpreted as nucleoli.

Following the heterotypic division some abnormalities are found in nuclear reorganization. The variations found at this time include the formation of as many as three nuclei (plate 2, 18) in the place of one, or rarely, the organization of one large and one small nucleus in a single microspore. Sometimes an unusually large nucleus is formed somewhat in advance of the others. Following the liberation from the tetrad wall, the unusually small microspores sometimes found (plate 2, 19) appear to complete the series of variations from the condition found in most of the other forms. While stages earlier than these—from diakinesis through to the end of the heterotypic division—have been studied, there are no outstanding conditions which would justify placing the beginning of degeneration earlier than the period of reorganization of the dyad nuclei—and degeneration at this stage is extremely rare.

Evidence of irregular chromatin distribution in the division of the mother-cells in hybrids has been reported by JUEL (1900), TISCHLER (1908), ROSENBERG (1909), LEVINE (1916) and others. This condition is of interest here primarily in that it does not result in a type of pollen abortion different from those more nearly normal cases where it has not been found. It appears, however, that the condition found at nuclear reorganization following the heterotypic division justifies the conclusion that degeneration processes may begin earlier in the plum than in Vitis or Fragaria.

The breaking up of pollen into globules

In addition to the processes resulting in the aborted-pollen series, there enters another process which dissolves pollen. This dissolution of pollen results in the production of a yellowish oily mass, which makes the pollen of some varieties sticky and therefore less easily blown away by wind. This condition is of wide occurrence in the plum, particularly where a large percentage of the pollen is aborted. All stages in the formation of this yellowish substance have been studied and it is found that the substance is made up primarily from pollen although the tapetum may also enter into its formation.

In the process of dissolution the pollen walls are first affected. Some stages in cross-section show a beaded condition of the wall, a bead or globule being formed at each ridge in the exine. In the final stages the wall may be intact, yet completely composed of small globules which gradually merge into larger ones. The small globules of the earlier stages are formed from a relatively thin wall which determines their diameter (plate 3, E). Before the exine breaks up into globules a thick homogeneous band similar in appearance to the tetrad wall is sometimes formed about the cytoplasm. Whether or not this is due to a thickening of the intine could not be determined. At the time of breaking down of the exine into globules, the cytoplasm shows a more diffused staining reaction. Later stages show some globules as large as the mature pollen grain which may further merge into even larger masses, but the final stages usually show a number of smaller globules yet separate (plate 3, D). The tapetum sometimes is affected in the same way as the pollen although it usually functions normally. This dissolution process is general in the loculus although it more commonly affects aborted pollen. It is found in some cases to affect grains with apparently normal nuclei and occurs in pure forms as well as in hybrids. This dissolution process, however, does not necessarily take place, but when it does it is found to occur most frequently near the time of maturity rather than at an earlier stage in development. There is also considerable variation in the extent of dissolution in the anthers of a single flower. Since it does not always take place even in aborted pollen, dissolution does not necessarily appear to be the final process in abortion but rather a supplementary process—undoubtedly enzymatic in nature.

Pistillody and petalody

So far pollen abortion has been discussed aside from the occurrence of any metamorphosis in the anther. There is a wide-spread tendency in the plum for stamens to change into petals on the one hand and into pistils on the other. These abnormal types, including acalycine flowers, have been noted previously by Waugh (1896, 1900) and others. In the course of metamorphism into these organs an elaborate series of intermediate forms occurs.

In pistillody (plate 4, C, D) particularly, the usual ontogeny of stamen development is upset to such an extent that the following series can be constructed showing the variation in the degree of development of different organs: (a) The nucellar tissue is developed normally in some pistilloids, and not at all in others; (b) the integuments vary from an entire enclosure of the nucellus to complete absence; (c) the position of the ovules in the pistilloids ranges from a terminal position near the anther to a basal position; (d) there are all degrees of enclosure of the ovules by the carpel wall; (e) there are all degrees of stigma and style development; (f) there are all stages in embryo-sac growth up to normal embryo-sac development; and finally (g) in anther suppression there is a great variety of odd outgrowths of stigma and anther tissue terminating the filament-like style. Some of these stigmas become receptive and in some the ovules swell. However, it is important to note that pistilloids are borne in the position of stamens, and hence on account of the abscission of the calyx tube do not persist long enough for fertilization to occur or for the style to be cut off. Even the extreme metamorphosis into an apparently normal pistil does not prevent the shedding of the calyx tube at the usual time or interfere with the usual functioning of the central or normal pistil.

Petalody like pistillody also occurs frequently, and all stages of metamorphosis of an anther into a petal are found (plate 4, A, B). The yellow anther tissue assumes various shapes and sizes as the transition to the white tissue of the petal becomes complete. Yellowish borders of anther tissue generally develop in the narrower petaloids. The tissue of the filament is very similar in external appearance to that of the petal and as with the style, the lateral wing-like appendages lead up to the broadened end which may be part anther and part petal. The most common type of anther bearing the white tissue of the petal is shown in plate 4, A, in which the end is pointed and white. There is little relation between the broadening of the petaloid and the suppression of anther tissue, since the latter may be absent on either narrow or broad petaloids. However, transformation has not gone so far in the case of the petaloids as to form an abscission layer at the base as in the petal, and all petaloids which were observed were found to be shed, like stamens, with the calyx rather than with the petals.

Sections through petaloids taken from flowers at full bloom show a variety of unusual shapes in the loculi. Some are long and narrow, others wide with irregular outlines; still others are lobed or branched, tapering in some to long points, which are devoid of any trace of reproductive tissue other than an occasional collapsed mass of cell walls. Petaloids also show great differences in the extent of pollen development; in the opposite end of a loculus bearing normal grains, pollen development may have been stopped as early as at the stage of the liberated microspore.

In pistillody and petalody three things are outstanding: (a) in the most extreme instances of metamorphosis the manner of abscission of petaloid or pistilloid is the same as that of the normal stamen, viz., with the calyx tube: (b) these metamorphic changes may result in complete abortion; and (c) they have not introduced a new type of abortion from that found where anther changes in these two directions are not taking place.

An explanation of the phenomena shown in these changes is not easy. The departure from normal growth takes place in somatic tissue first, and if growth is carried far enough, normal-appearing pollen and embryo sacs are produced. This would lead to the inference that the factors upsetting normal development are sporophytic. Something of the nature and extent of the changes which take place when petalody reaches the double-flowered commercial types is shown by its inheritance, as in stocks in which doubleness is dominant to singleness. It is probable that some of the pistilloids would actually set fruit if they were not cut off so early by the dropping of the calyx-tube. There does not appear to be any great difference, except in position, between pistilloids borne in the position of stamens and accessory pistils borne about the primary central one. In those cases in which more than one pistil has been found, pistilloids are not necessarily formed. In fact, in a peach hybrid which bears occasionally as many as five pistils and frequently three, the conspicuous feature of the flower is its doubleness. But doubleness in some of the ornamental flowering varieties of Prunus does not interfere with the growth of a normal pistil. So, while pistillody and petalody take on various strange forms in this genus, they appear to be due to forces which do not materially modify the aborted pollen types or interfere markedly with the production of a normal pistil, even though many other pistils may be produced in varying degrees.

The extent of aborted pollen

The extent of pollen abortion in selected forms has been determined partly from mounts in lactic acid and partly from stained preparations. The results are presented in tables 3 and 4.

In table 3 the individuals of two crosses between *P. triflora* and *P.*

americana mollis are classified according to the percentage of aborted pollen found. It will be noted that two varieties of *P. triflora,*—Abundance and Burbank,—have been used as the pistillate parent. In determining the extent of abortion in these hybrids an average of 425 grains was counted in each individual in the Abundance × Wolf crosses and 730 in each of Burbank × Wolf crosses. The percentages based upon such large numbers undoubtedly represent the pollen condition fairly accurately. In Burbank 32 grains were aborted in 1003 and in Wolf 52 in 226 (table 4), the condition not being determined in Abundance. It will be seen that aborted pollen is present in large quantities in the F_1 progeny of each cross, and also that there is a slightly greater range in the abortion in the Burbank crosses.

TABLE 3

The aborted pollen in the F_1 individuals of the interspecific cross, Prunus triflora (Abundance and Burbank) × P. americana mollis (Wolf).

	Number of trees	Percentage of aborted pollen in the F_1 plants																
		5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85
Abundance × Wolf..	34	2	3	4	9	4	5	2	1	2	1		1					
Burbank × Wolf....	23	4	1	3	1	2	3	3	1			2		1		1		1

From table 4 it is apparent that extreme hybrids in the genus Prunus are unable to complete the development of large proportions of pollen—a condition which also obtains in monospecific varieties and forms generally regarded as pure species. In fact, the degree of pollen abortion may lead to a question of the purity of some of the so-called pure forms included in the list. There is some variation shown in the amount of defective pollen between individual trees of a clone; this point, however, was not checked extensively.

The general pollen condition of the forms listed as pure species contrasted with that of the hybrids is shown in table 5, in which each form is classified according to the percentage of pollen aborted. In this way the greater amount of defective pollen in the hybrids is emphasized.

The pollen condition in the plum may be briefly summarized as follows: Abortion occurs for the most part between the time of liberation of the microspore from the tetrad and maturity—in other words during the gametophyte generation. The fact that anther development is normal even when there is a larger percentage of, or complete, pollen abor-

TABLE 4

The pollen condition in certain species and selected varieties of Prunus in which a number of interspecific crosses are included.

Variety	Total number	Number normal	Number aborted	Percent aborted
Aitkin (*Prunus nigra*)	214	167	47	22.0
Amygdalus nana × *P. persica*	327	51	276	84.4
Blush (*P. americana*)	216	190	26	12.0
Burbank (*P. triflora*)	1003	971	32	3.2
Cheney (*P. nigra*)	220	101	119	54.1
Compass (*P. Besseyi* × *P. hortulana Mineri*)	214	121	93	43.5
" " "	—	—	—	100.0
" " "	211	90	121	57.3
De Soto (*P. americana*)	226	209	17	7.5
Etopa (*P. Besseyi* × *P. triflora*)	200	152	48	24.0
" " "	207	182	25	12.1
Ironclad (*P. americana*)	211	181	30	14.2
" "	257	235	22	8.6
" "	232	209	23	9.9
Loring (*P. triflora* × *P. americana* ?)	—	—	—	26.0
Manitoba (*P. nigra*)	214	183	31	14.5
Ocheeda (*P. americana*)	315	295	20	6.3
" "	372	265	107	28.8
Opata (*P. Besseyi* × (*P. Munsoniana* × *P. triflora*)) (plate 3, B, F)	200	40	160	80.0
P. americana (wild)	302	237	65	21.5
" "	228	195	33	14.5
" "	214	185	29	14.0
P. angustifolia	226	185	41	18.1
P. Besseyi	211	185	26	12.3
P. Besseyi × *P. americana*	353	128	225	63.7
P. Besseyi × *P. armenica*	225	28	197	87.6
" "	209	37	172	82.3
" × *P. Simoni*	211	137	74	35.1
P. nigra	237	180	57	24.1
"	206	128	78	37.9
"	222	214	8	3.6
P. pennsylvanica	436	321	115	26.4
P. virginiana (plate 3, L)	233	198	35	15.0
"	273	253	20	7.3
Rollingstone (*P. americana*)	392	254	38	9.7
Sapa (*P. Besseyi* × *P. triflora*)	355	206	149	42.0
Surprise (*P. hortulana Mineri*)	309	204	105	34.0
" "	200	186	14	7.0
Wolf (*P. americana mollis*)	226	174	52	23.0
Wyant (*P. americana*)	248	207	41	16.5
" "	187	153	34	18.2
Yellow Egg (*P. domestica*)	510	494	16	3.1

TABLE 5

A classification of the hybrids and pure forms listed in table 4 on the basis of the percentage of aborted pollen.

	Percentage of aborted pollen																			
	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
Hybrids	1	1			2		2	1	1		1		1			2	1	1		1
Pure forms	6	5	6	4	3	1		1			1									

tion, tends to eliminate conditions in the anther as a constant factor in abortion. In extreme hybrid forms there is evidence of pollen disintegration as early as the dyad stage. The breaking down of pollen into yellowish globules appears to be a supplementary enzymatic process separate and distinct from true abortion, since it does not always occur and sometimes affects mature grains which have every appearance of being normal. A new type of aborted pollen is not introduced by pistillody and petalody. Neither have these metamorphic processes been found to affect the normal method of stamen dehiscence. Aborted pollen occurs in the plum in considerable quantities, even in many so-called pure species, but is not sufficient in these, considering self- or cross-pollination, except in a few extreme cases of complete abortion, to be of itself a prohibitive factor in the setting of fruit. However, in view of the extreme hybrid condition of many varieties, aborted pollen in them becomes of greater importance. The abortion of pollen during the haploid stage appears to point to a state or condition in the germplasm of the gametophyte as the cause of abortion.

PISTIL DEVELOPMENT IN RELATION TO STERILITY

In the section on pollen development it was shown that self-sterility and cross-sterility as well, are of the type generally referred to as incompatible and are not necessarily due to defective pollen except in extreme cases of complete abortion. It now remains to determine the factors in pistil development which enter into the general question of sterility. As in the case of the pollen, the course of normal development will be presented first.

Pistil development

Early stages

The degree of development of the pistil in late winter is shown in figure 20, plate 2. There is as yet no protrusion of the growing-point from which the ovule will develop. It will be seen that the carpel cavity is formed by the folding together of two margins which do not unite until considerable growth has taken place. The point of union forms the suture which is clearly distinguishable either at bloom or at maturity. The two ovules are borne on a parietal placenta close to but on either side of the suture. The stigmatic cells at this time appear no different from the other cells of the epidermis. The pistil, then, in the winter bud is rudimentary and its special structures are not formed until the early spring growth.

The pistil at bloom

In the treatment of weather in relation to fruitfulness (DORSEY 1919) the principal factors affecting receptiveness, namely, the stigmatic surface and the abscission of the style were discussed. These will not be dealt with further here, since, in the light of the evidence to be presented later, on the failure of so many pistils to set fruit, interest centers primarily about development in the ovule.

In the course of this investigation sections have been made of pistils at various stages of growth from a large number of different varieties and species. While some of the variations found in Prunus species have not been noted by PÉCHOUTRE (1902), his studies of pistil development in the Rosaceae in general and particularly in Prunus have been so thorough and are presented in such detail that further treatment in this connection would be largely repetition. The following discussion of the mature pistil has been carefully checked with that of PÉCHOUTRE in species available in this investigation.

At the time the flowers open, the embryo sac may contain from one to eight nuclei, although generally there are but two or four. The embryo sac increases in size but little before fertilization. The nucellus is made up of large, thin-walled cells with a "cap" of smaller, thicker-walled cells at the apex. The two integuments are concrescent at the chalaza but otherwise separate throughout their length. The outer integument is five cell layers in thickness and epidermal in origin; the inner is three cells thick and sub-epidermal in origin (PÉCHOUTRE 1902). The distal ends of the integuments, especially of the outer, make a very irregular growth and push well up into the narrow end of the carpel (plate 2, 21; and plate 5, B). This extension of the integuments forms a large canal or channel through which the growth of the pollen tube is tortuous. Tubes frequently miss the opening to this channel and grow downward in the cavity of the ovary but none has been found to enter the chalaza. At the distal end of the carpel cavity there is an irregular ingrowth of tissue—called the "obturator" by PÉCHOUTRE—which partly projects over the micopyle (plate 2, 21, a). The anatropous ovules gradually assume an upright position and at the time of fertilization are borne upright on a parietal placenta.

In external appearance pistils vary in many features. In *Prunus domestica* the style is much thicker and much more pubescent than in *P. americana,* and the characteristic green color is partly obscured by a purplish pigment in *P. Pissardi.* The suture also is deeper and more distinct in some varieties than in others. In all species studied, however, the cells of the central core in the style have much denser cytoplasm than have the cells of the surrounding tissue (plate 5, L, a), and no tube growth has been found outside toward the epidermis. The tubes pass between cells by dissolving the middle lamella, since, with the exception of a few cases in the suture for short distances below the stigma there is no canal in the style of the plum. At the first the course of the tube is very tortuous, but farther down the style it is more direct, the tube is narrower, and its stainable portion is longer. Vacuoles soon appear in the cytoplasm of the cells of the central core near the tubes; and later, before abscission all cells of the central core are more or less vacuolized. The point of abscission is much higher in some species than in others—in fact "pointed" plums are generally characterized by the persistence of a stub of the style.

The ripe seed

After fertilization there is great variation in the degree of development in the embryos in different pistils but there is a very rapid extension of the embryo sac into the canal. Coincident with embryo-sac extension there is rapid growth in the endosperm whose free nuclei form a thin jacket one cell layer thick around the inner surface of the embryo sac. Walls do not form between the nuclei of the endosperm for some time after the complete extension of the embryo sac to the chalaza. As the embryo sac enlarges, a conspicuous channel is formed in the nucellus, which is completely absorbed during the period of rapid endosperm growth. In many sections the nucellus is broken down considerably in advance of the embryo sac. After the endosperm is formed, it, like the nucellus, is largely absorbed and gives way very rapidly to the growing cotyledons. The endosperm, however, is never completely absorbed in this genus and can still be found in the ripe seed in irregular patches between the cotyledons and the seed coat, to which latter it is always joined and of which it may be considered to form a part (plate 2, 22, a). This condition led PÉCHOUTRE to suggest that the Rosaceae arose from plants with seed albumen.

The "pit" in the plum (plate 2, 22, b) is formed by the laying down of stony tissue about the inner border of the carpel. The ovary wall thickens to form the fleshy edible portion of the ripe fruit. Hardening in the stone cells does not take place until the normal size of the pit is reached. Since typically only one embryo is matured in each pit, it remains to be seen what disposal is made of the other one which is always found in the early stages.

The suppression of one ovule

During the earliest stages of floral development no differences are evident in the two growing points on the placenta. At bloom there may still be an even development of the two ovules (plate 5, A) and rarely two embryos may develop to maturity (plate 4, H) in the same "pit." Such development, however, is not the typical condition and while abortion is found at all stages between the megaspore mother-cell and maturity, in most varieties one ovule shows an arrested development previous to fertilization. After fertilization the smaller ovule is quickly surpassed by the other and in the mature seed only the brown remnants of the integuments persist (plate 5, D).

In the earliest stages of arrested growth found in the suppressed embryo, the integuments are normal in appearance and degeneration begins first in the megaspore and the cells of the nucellus immediately surrounding it. In some of the sections of the earliest stages the nucellus is a degenerate mass and the integuments are partly drawn together (plate 5, C). In these early cases of suppression the ovule turns brown and there is no further growth. In most instances, however, the embryo sac is formed. If both ovules develop normal embryo sacs, the one in which fertilization takes place first apparently gains the ascendency. Following fertilization, size differences soon become pronounced and in those in which fertilization does not take place the liquid is completely withdrawn. If fertilization takes place in both ovules, which sometimes happens, suppression takes the form of embryo abortion, in which case in the ripe seed the coats are often devoid of contents except for the partially developed embryo.

There is a great difference between varieties in the degree of development of a second ovule. The relative size of the ovule at the time of bloom was studied by outline drawings, and these show an equal development in a large number of pairs in some varieties and a large number of cases in other varieties in which one ovule is conspicuously larger than the other. The drawings show, however, that neither ovule is constantly larger than the other. Near maturity, the greatest development in the suppressed ovule was found in Stella, a cross between *P. triflora* and *P. americana*. A series in the growth of the second embryo in this variety is shown in figure I, plate 4. This is in marked contrast to Assiniboin (plate 4, J) taken at approximately the same stage of development.

Thus it will be seen that the typical condition is for one embryo to develop in each pit. In other words, approximately one-half of all ovules are suppressed, although normal development in one is all that is required for fruit formation. This condition prevails in varieties representing a single species or in extreme interspecific crosses and it occurs alike in varieties bearing normal pollen and in varieties in which most or all of the pollen is aborted.

The dropping of pistils

Under orchard conditions pistils fall at three distinct stages: (a) at or immediately after bloom, (b) from two to four weeks after bloom, and (c) later, following considerable enlargement in the pistil. For convenience in presentation these will be taken up in the order of their occurrence.

The first drop

The extent of pistil abortion has been studied by a number of investigators, and the general conditions reported are comparable to those found in Minnesota. BAILEY (1892) reports finding a wild tree of *Prunus americana* which "bears flowers without pistils." LORD (1894) found that all varieties sometimes bear flowers with aborted pistils. HEIDEMANN (1895) found 90 percent of aborted pistils in Hiawatha. GOFF (1894, 1895) determined the degree of pistil abortion in a number of varieties of *P. americana*, which in some cases, as in Moreman, amounted to as much as 74 percent. PETERS (1916) evidently does not recognize dropping from aborted pistils and ascribes the falling of flowers "a few days after the petals" to non-pollination. WAUGH (1896) found 100 percent "defective pistils" on several trees. He considers that in the aggregate they are "numerous enough to be taken into serious consideration," but that they do not exert an influence on the crop "except in uncommon cases of total defectiveness." In a later publication, WAUGH (1897) presents extensive data showing the percentage of defective pistils in a number of varieties in nine plum groups. Defective pistils have been given considerable attention in the present investigation, but the partial summary of WAUGH's data given in table 6 expresses the general condition very well. It will be seen that defective or aborted pistils are of wide occurrence and of sufficient numbers in many instances to have an influence upon the crop.

TABLE 6

A summary of the data collected by WAUGH (1897) *on the extent of pistil abortion in the plum.*

Group	Percentage of defective pistils																					No. of varieties	Average percentage for group
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100		
runus domestica	69	16	12	3	2		5		1	1												29	4.3
panese	34	7	10	7	3	2		2		1	2		1			1						22	11.2
americana	59	20	16	11	13	9	11	6	8	2	5	4	3	2			1		6		4	55	21.2
nigra	9	4	3	1	2	1	1	1	1	2												6	17.0
iner	17	7	3		2																	10	1.9
ayland	14	3	5	5	2	2	2	1	1	1					1		1					12	10.5
ildgoose	22	3	8	5	4	2		3	2			1		1	1				1		1	18	19.8
icasaw	24	11	8	4	2	2	2	2	3	2	3		1	1		2	2	1				20	10.5

Pistil abortion occurs at any stage from that of a slight growth of the flower rudiments in the early spring to the time at which the flowers have reached normal size at bloom; but for each variety the degree of growth reached before abortion is quite characteristic and varies but slightly. Flowers with the earliest-aborted pistils drop first but always come into full bloom. When abortion occurs so late that the pistil reaches normal size, but little further growth takes place and dropping may occur as late as a week after bloom. Flowers with defective pistils always drop at the pedicel base and neither the calyx tube nor style is shed by abscission because growth is not carried far enough. Pistils aborted at the earliest stages turn brown or black while the later ones to abort are distinctly yellow compared with the green of normal ones. Two features, therefore, characterize this drop: (a) the flowers bear aborted pistils, and (b) falling takes place soon after bloom. The immediate cause of the dropping of the flower appears to be pistil abortion.

Sections have been made of a large number of these defective pistils, and in all cases *both* ovules are found to be aborted (plate 5, E, F). The aborted series presented is comparable with that of the single ovule previously discussed. In the larger aborted pistils the dark color of the degenerated ovules can be seen distinctly through the carpel wall. In the earliest cases of abortion found the size of the pistil was several times that of the pistil in the dormant winter bud. It is of interest to note also that pistil and ovule abortion have no apparent influence upon pollen development.

The early abortion of pistils has been assigned to various causes. GOFF (1901) held that abortion is due "in the majority of cases to a return of cold weather" after spring growth has once started. WAUGH (1896) determined the percentage of defective pistils from nine varieties at different points between Denison, Texas, and Ottawa, Canada, which do not sustain the conjecture of GOFF because of the large numbers which he found aborted at all places.

The fact that some varieties bear fruit in abundance for a number of years, and afterward for one or more seasons suddenly produce flowers that show a complete abortion of pistils suggests a definite relation of this condition to nutrition. Observation of changes of this nature have been made by a number of investigators (LORD 1894, 1899; GOFF 1894, and others), and two striking instances have occurred at the Minnesota Station. One variety, Wickson, bore two heavy crops of crossed plums in the greenhouse, and the following year all pistils were aborted. In the second instance, Wolf under orchard conditions bore heavily in 1914, and for three consecutive seasons afterward produced less than 1 percent of normal pistils. Wickson was not subjected to killing temperatures previous to the time of abortion.

The occurrence of aborted pistils in varieties of so many different species under cultivation as well as in wild seedlings indicates that the tendency to the appearance of this condition, whatever its cause, is widespread. The occurrence of so many aborted pistils in seasons following heavy fruits suggests, as noted, a connection with nutrition. It is a matter of general observation among fruit growers that heavy fruiting is likely to be followed by a light crop, but under these conditions fruit-bud formation is usually reduced. While the relation between food supply and fruitfulness has not been definitely explained as yet on experimental grounds, the general relation shown in the orchard justifies placing some confidence in the assumption of the relation between aborted pistils and nutrition. If this assumption is correct, a small degree of abortion may be interpreted as suggesting a competition between different flowers in a bud while a total abortion indicates a much more general condition.

The earliest stages of pistil abortion have been given careful cytological study. The first appearance of disintegration is found in the ovule and consists of a breaking down in the embryo sac. The disintegration stages here closely resemble in general details those already discussed in the abortion of one ovule. In the aborted-pistils series both ovules are affected; in fact, the general suppression of one ovule may be regarded as the first step in the aborted series and needs only the suppression of the other to complete it. When this condition is taken into consideration, together with the fact that early and late abortion take place, the series is complete. This indicates a condition of instability in the pistils in this genus, which if suggestive of dioeciousness has not yet, as will be shown later, progressed so far as to show a constant morphological difference between flowers.

The second drop

The first drop is followed two weeks or so after bloom by another distinct wave of falling pistils. While there are a few intergrading forms between these two drops, certain features of the second drop separate it distinctly from the first.

Unlike the pistils of the first drop, those of the second have every external appearance of being normal. Enlargement up to a certain point takes place and in most cases the calyx tube breaks away at least in part even though there is insufficient growth in the young plum to throw it off. The style is not deciduous in the earliest pistils to fall, but, like the calyx tube, drops in those which fall later (plate 1, D). In *P. Besseyi*, however, both the style and the calyx tube persist longer than in other species (plate 1, C). Pistils which fall in the second drop, as in the first, absciss at the pedicel base while the pistil is still green, although the pedicel has become light yellow. Yet in the last pistils of the second drop to fall the abscission layer is formed at the base of the ovary (plate 5, K) and in some instances can be easily broken off at this point. Even the last pistils to fall, which are usually the larger ones, are still turgid, and while abscission generally precedes browning and shriveling, in some varieties the integuments turn brown and the sap of the nucellus is partly or completely withdrawn in many of the pistils before they fall. The browning appears first in the chalaza and gradually extends to the nucellus and integuments and sometimes even to the stone tissue.

From the foregoing it is evident that there are characteristic features which distinguish the second drop from the first.

By referring to table 7, it will be seen that at about three weeks after bloom in accounting for all the flower buds or flowers produced by a tree only four different categories are necessary. Other varieties could have been included in the table but these four illustrate something of the extremes encountered. These counts were made in the spring of 1917.

TABLE 7

A percentage classification of the total number of flowers borne by four varieties of plums, on the basis of those which were winter-killed, those which fell at the first drop, at the second drop, and those which "set."

Variety	Date of bloom	Flower buds winter-killed	First drop	Second drop	Number set
Assiniboin	May 17	0	34	55	11
Minnesota No. 18[1].........	" 21	10	50	35	5
" " 21[2].........	" 19	42	46	7	5
" " 35[1].........	" 19	15	21	54	10

[1] Abundance × Wolf. [2] Burbank × Wolf.

After deducting the number of flowers winter-killed, those which fell with aborted pistils, and those which set, a considerable number, varying from 7 to 55 percent, remains. These constitute the second drop, which in number is comparable to those of the first drop. In fact, it will be seen that in one variety the first drop may be greatest and in another the second, and that in all cases the number of pistils listed under either drop is greater than the percentage to set. Leaving out of consideration the flower buds killed during the winter and those eliminated in the first drop, as a rule there still remains a sufficient number to produce more than a crop if fertilization occurs in them. These are analyzed further in table 8 in order to determine what size differences occur between those which set and those which drop.

This analysis was begun seventeen days after bloom, when the second drop had started. The size of those dropping and those still persisting (table 8) was compiled from records made in 1917, in which the pistils in each category are put into classes according to their greatest diameter parallel with the suture. Attention is called to the relative size at the time of falling of pistils open to cross-pollination and those from which the stigma was snipped before pollination.

TABLE 8

An analysis of the pistils on trees of each variety included in table 7 beginning 17 to 24 days after bloom or about ten days after all flowers with aborted pistils had fallen. A size classification is made on the basis of those persisting and falling on the successive dates as indicated.

Variety	Assiniboin								Minnesota No. 21						Minnesota No. 35														Minnesota No. 18					
Date	June 5		June 7		June 11		June 15		June 11			June 16			June 4		June 7			June 9			June 11			June 15			June 9		June 11		June 16	
Days after bloom	20		22		26		30		24			29			17		20			22			24			28			20		22		27	
Size of pistils at different dates in mm	Falling	Persisting	Falling	Persisting	Falling	Persisting	Falling[1]	Persisting	Falling	Persisting	Falling; stigma snipped[4]	Falling[1]	Falling; stigma snipped[4]	Persisting	Falling	Persisting	Falling	Falling; stigmas snipped[4]	Persisting	Falling	Falling; stigmas snipped[4]	Persisting	Falling	Falling; stigmas snipped[4]	Persisting	Falling[1]	Falling; stigmas snipped[4]	Persisting	Falling	Persisting	Falling	Persisting	Falling[1]	Persisting
1.1- 1.5	[2]		[2]						2	2	1				14[2]		22	8		7	1								17[2]	1	2			
1.6- 2.0			2				1		11		11	1	12		105	3	44	4	1	43	6		9	7		3			44	22	46		8	
2.1- 2.5	3	1	23	1	9		13		4	2	7	26	14		10	33	4	1	21	37	9	29	34	10		9			5	37	52	8	24	
2.6- 3.0	16	40	41	38	17		44		1	6		23	9			25			32	12		41	42	12	4	13	1			25	52	10	9	
3.1- 3.5	5	24	9	46	29		32			8		5	3			2		1	9			15	8	2	16	11	6			5	9	16	10	
3.6- 4.0		14		17	11		2			3		6	1						1			9	1		14	4	5	1		1	3	12	4	
4.1- 4.5		5		6	3		2			2		1										2			7		1	7		1	1	8	5	
4.6- 5.0		5		4																		2			6			2		1		5	3	
5.1- 5.5		2		3			1							1								1			2			6				4	4	
5.6- 6.0		4		3		1		1																	5			7				6	2	1
6.1- 6.5		1		1																					1			15				13		5
6.6- 7.0						1	1							1														8				14		9
7.1- 7.5														2[3]														7				10	1	14
7.6- 8.0						1		1																				6				3		23
8.1- 8.5						1																						1[3]				3		11
8.6- 9.0						5																												9
9.1- 9.5						9																												9
9.6-10.0						11																												3
10.1-10.5						7																												3[3]
10.6-11.0						5		1																										
11.1-11.5						7		5																										
11.6-12.0						1		3																										
12.1-12.5								2[3]																										
Totals	24	96	75	119	69	49	96	13	18	23	19	62	39	4	129	63	70	14	64	99	16	99	94	31	55	40	13	60	66	93	165	112	70	87

[1] Last to fall.

[2] All pistils were measured before any of this drop had fallen; therefore these numbers show the relative proportion as well as the size of those falling and persisting on these dates. On the other dates after some pistils had fallen the total number remaining were classified according to size.

[3] These larger plums are setting.

[4] In each case in which the stigma was snipped the operation was performed early enough after bloom to make certain that pollination had not taken place.

Emphasis is placed upon the following points brought out in table 8: (a) the period of abscission of the second drop extended from 17 to 30 days after bloom; (b) beginning with the first pistils to fall, size differences between those persisting and those which fell, gradually increased with time; (c) pistils which fell within the above-mentioned time limit enlarged only up to a certain point; (d) those pistils with the stigmas snipped before pollination, enlarged before falling, to a size comparable with that of those not so treated; and (e) in each variety there was a gradual increase in the size of the pistils which fell off.

The degree of enlargement of certain pistils over others is again shown in plate 1, A and B, in which the extremes in the percentage of pistils to set, 3.1 and 63.9 percent, respectively, are shown. The distinction in size between the first and second groups of pistils to fall is appreciable, since at bloom the normal pistil is only 1.1 to 1.5 mm through the suture diameter. Only a few of this size are recorded in table 8. Plate 1, D, illustrates the difference between those falling last and those setting, in Minnesota No. 21, thirty-one days after bloom. With this analysis, showing so clearly the size distinction between those falling and those persisting, the evidence as to the cause of the falling of the second group will be presented.

The cause of the second drop

The similarity in size at the time of falling of those pistils open to pollination and those whose pollination was prevented by snipping the stigma, would alone appear to justify the conclusion that the second drop is due to non-fertilization. This point, however, was investigated further by two different methods: (a) by excluding pollen, and (b) by a cytological examination of the ovules.

In the experiments in which pollination was prevented, tents were placed over one tree each of Minnesota No. 21, Assiniboin, Wohonka, Wakapa and *Prunus americana,* during the period of bloom. On account of the total self-sterility of these varieties no emasculating was done under the tents. The entire lot of normal pistils under the tents, including branches with snipped stigmas, those self-pollinated and those not pollinated, showed an enlargement similar to those falling with the second drop under orchard conditions and fell with them. But controlled crosses under the tents set in approximately the same proportion as uncovered trees of the same variety adjacent.

As checks to the tented trees in the orchard, two trees (Manitoba and

Yellow Egg) were held under observation in the greenhouse where pollen was definitely excluded from the stigma by emasculation; on these trees the pistils which were not pollinated showed enlargement before falling, similar to those under the tents. Before concluding finally that fertilization has not taken place in pistils which fall in the second drop the state of development in the embryo sac should be determined.

The condition in the ovule has been examined in pistils definitely known not to have been pollinated. These served as a check to those examined from the second drop. In the absence of fertilization an interesting condition is created in the ovule, which shows something of the dependence upon the egg of development in other parts. The contrast will be shown between the fertilized and unfertilized condition.

The general proportions between the different parts of the pistil are maintained in the partial enlargement which takes place in the second drop. The integuments thicken and expand, a condition which is in marked contrast to that usual in the suppressed ovule. The nucellus increases in size, as was noted by BACKHOUSE (1911 a) in self-pollinated pistils which fell "generally within three weeks," but it seldom breaks down before abscission, although there may be a partial withdrawal of sap. The canal as a rule extends into the nucellus but little beyond the chalazal end of the embryo sac in the pistils which fall earliest in the second drop; later, however, there is considerable canal extension. In one pistil from which the stigma was snipped before pollination, at thirty-four days after bloom the canal through the nucellus had extended to the chalaza (plate 5, J). This ovule had grown to the largest size of any found in the absence of fertilization and was 4.4 mm long and 1.5 mm wide. PETERS (1916), working with varieties of *Prunus domestica*, states that when self-sterile varieties are self-pollinated, "the carpel swells up to the size of a culinary pea before it falls," but that if the flowers are not pollinated, they fall "a few days after the petals." Apparently he fails to make a distinction between the first and second drop. The point is that ovule development reaches only a certain size in the absence of fertilization, but the pistil in which fertilization has not occurred persists after the style and calyx tube have fallen.

Turning now to the embryo sac, the early stages after bloom where fertilization has been prevented show the normal nuclear condition. Breaking down in the nuclei of the embryo sac first appears from two to three weeks after bloom. Disintegration takes place in the antipodal nuclei first, then in the endosperm nuclei and lastly in the egg nucleus. In figures 25 and 26, plate 2, the first stages of disintegration are shown in the egg, twelve days after bloom. More advanced stages are shown in figures 23 and 24, plate 2, which are drawn from pistils collected 24 and 34 days respectively after bloom. In the last instance pollination was prevented by snipping the stigma. It will be seen that the nuclei in the embryo sac persist long enough to allow for a considerable delay in pollination. The persistence of the egg in the absence of fertilization is decidedly different from the condition in the apple, in which KNIGHT (1917) found that "at 120 hours the egg cell begins to show disintegration."

The embryo sac elongates but little if fertilization does not take place (plate 5, G, H). Even in the case noted above of exceptional ovule development there was but very slight elongation of the embryo sac. Cases of this kind suggest that canal extension is independent of the embryo-sac growth when certain sizes are reached in the ovule. Endosperm in the absence of an embryo has been found only in two cases in Yellow Egg (*P. domestica*), in which a few divisions had apparently taken place. As a single exception, an embryo four cells across was found in a pistil falling within the size limits of the second wave of dropping. These instances may be regarded as intermediate between the second wave and the third.

The condition found in the unfertilized series is in marked contrast with that found when fertilization takes place. As early as 18 days after bloom the embryo sac in which the egg has been fertilized extends the entire length of the nucellus to the chalaza, and a jacket of endosperm, usually only one cell thick, covers the entire area of the "dumb-bell-shaped" sac. With the completion of these changes in the embryo sac the embryo may be no larger than four cells across (plate 2, 27). The slow growth of the embryo in the early stages and the extremely rapid formation of endosperm have been emphasized by PÉCHOUTRE (1902).

It will be seen from the above observations that all the evidence shows that fertilization has not occurred in the pistils which fall at the second drop. This is in accord with the statement of WAUGH (1899) that "the germs of the incipient seeds are not fecundated." Pollination may have taken place, but tube growth was retarded to such an extent that fertilization was prevented probably by the abscission of the style. By referring to the article on weather in relation to fruitfulness (DORSEY 1919) it will be seen that this drop can be ascribed primarily to unfavorable weather at bloom—especially rain and low temperatures—which would also account for the differences in the extent of the second drop from year to year.

The third or "June drop"

Following the second drop there is still another—the so-called "June drop." In popular usage the term June drop applies primarily to the third drop of large plums because they are much more conspicuous, but does not include the relatively few which fall from time to time, even up to maturity. WAUGH (1899) distinguishes clearly between the second drop and the June drop. He says that "this first fall of minute fruits (which sometimes takes the whole crop) is commonly supposed to result from non-fecundation of the ovules," but that "this is not the true June drop." BACKHOUSE (1911 a) states that "it seems probable that the trouble known as the June drop of the Americans" is "to be explained as a consequence of self-pollination." According to the evidence presented previously, self-pollinated pistils drop at sizes similar to those at which unpollinated pistils drop and for the same reason. It has been shown that time and size of dropping draw a relatively sharp line between the first and second waves of dropping. Likewise these two factors separate the second drop from the third. From table 8 it will be seen that when fertilization does not take place enlargement reaches only a certain point, the maximum recorded being in the 5.6-6.0 mm class, while the mode is near 3.0 mm. Among the last of the second drop an occasional ovule is found with slight embryo development, which shows that there are connecting forms between the second and third drops as well as between the first and second. In approximately one month (table 8) the second drop is over, and those setting have so increased in size as to place them in a distinct size class from those which have fallen.

The size of plums in the third drop is shown in table 9. By comparison with table 8 it will be seen that considerable enlargement has taken place and that the size of those falling is comparable with the size of those persistent at the earlier date.

The measurements of Assiniboin and Winnipeg were made forty days after bloom and include a small number of fruits which fall without injury and a large number injured by curculio stings. These fell for the most part with the third drop and have generally been considered a part of it. Dropping occurred as late as forty to eighty-three days after bloom. In the cross Compass × Yellow Egg the entire crop fell just before maturity. So plums which fall at this drop, like those which fall at the first and second, have a characteristic size with a considerable range in the time of falling but with a pronounced mode. It should also

TABLE 9

Showing the size of plums falling at the June drop. Under the headings Assiniboin and Winnipeg the size of those falling from curculio stings is also given.

	Assiniboin			Minn. No. 6 × Manitoba[2]	Compass × Yellow Egg	Minn. No. 21 × Burbank[2]	Winnipeg		
Date	June 26			May 7	June 22	June 21	June 26		
Days after bloom	40			54	83	82	40		
Size of plums	Dropping	Dropping[1]	Persistent	Dropping	Dropping	Dropping	Dropping	Dropping[1]	Persistent
5.6- 7.5	1				1				
7.6- 9.0	2				6				
9.1-10.5	2	1			13				
10.6-12.0		1			25		1	1	
12.1-13.5		13			17	2		3	
13.6-15.0		25			15			12	
15.1-16.5	1	15			2	8		5	
16.6-18.0		7				8		3	
18.1-19.5		2	2			12			
19.6-21.0						1			1
21.1-22.5			2			1			2
22.6-24.0			1	3					
24.1-25.5				1					
25.6-27.0				2					
27.1-28.5									
28.6-30.0				1					

[1] Stung by curculio. [2] Greenhouse cross.

be noted that the method of abscission in the June drop is different from that in either of the others. The conducting tissue of the pedicel, especially at the base, has become hardened, while this condition has as yet not been reached at the base of the ovary. Consequently, instead of parting at the pedicel base as in the first and second drops, abscission takes place between the plum and pedicel and in the most advanced cases, as in the normal falling of the ripe plum, the pedicel does not drop but may persist for one or more years. A further difference is found between the second and third drop in that neither calyx tube nor style is ever present as late as the time of the third drop.

Seed development in the June drop

Sections have been made of the embryos of a large number of plums which fell at the June drop. Dissections were also made of ovules at various stages to determine the amount of growth in the embryo. The general condition found may be summarized as follows: (a) embryo development started but growth stopped at any time from the stage when the embryo was a few cells across to the time at which it had reached nearly the mature size; (b) endosperm had partly formed, but the embryo gained the ascendency to such an extent that it was often found naked in the nucellus; (c) enlargement in the seed could reach nearly the mature size when fertilization had once occurred, accompanied by only a slight growth of the embryo.

Considerable variation was found in the relative development of embryos. A characteristic of all the seeds dissected in the cross Compass × Yellow Egg (table 9 and figure E, plate 4) was the small amount of endosperm present. In fact, in some seeds none could be detected. The last stages of the seeds of this cross show a complete absorption of the sap of the nucellus and a drying and withering of the seed coats, within which the embryo appears as a lump at the pointed end. In many of the seeds of this cross neither embryo nor endosperm could be found by hand dissection, indicating an early abortion of both. The canal, however, is very prominent and it is possible that complete embryo-sac extension has taken place before abortion. An early browning of the seed coats soon follows suppression of the embryo. In this cross all plums fell before maturity but after they had reached nearly the normal size.

In a cross between Minnesota No. 21 and Burbank, embryo development differed from that described above. Many plums of nearly mature size—ranging from 15 to 20 mm through the suture diameter—were falling 82 days after bloom. Dissection of the seeds showed considerable growth in the embryo and only traces of endosperm. F, plate 4, is a series showing embryo growth, natural size. Sections were made of some of these embryos, and one of the smaller was only sixteen cells across—yet the plum had persisted for nearly three months. The external appearance of the seeds in this cross is similar to that of Stella (plate 4, G).

It may be argued that fertilization had not taken place in those cases in which an embryo sac could not be found. Even if an embryo could not be found the size of the plum was so much greater before falling than those of the second drop where fertilization is known definitely not to have taken place, that the size alone may be taken as conclusive evidence of fertilization. This position appears justifiable in view of the fact that in the experiments carried on under the tent and in the greenhouse, in which pollen was definitely excluded from the stigmas, the cases in which the size of pistils approached that of the pistils falling at the third drop were so few as to be negligible.

The great rapidity in endosperm formation and also in embryo-sac extension have been emphasized; therefore the occurrence of embryos without or with only a partial growth of endosperm was not to be expected. The above status of seed development in the Compass × Yellow Egg cross is not typical. In table 10 some of the variations found in a cross between *P. triflora* and *P. americana* are set forth. The seeds classified in this table were taken from fruit falling just before the normal plums were ripe and were typical of the plums which so often ripen earliest. From I, plate 5, it will be seen that the tissues of the seed are easily distinguished.

TABLE 10

The relative development of the embryos in plums dropping a week to ten days before maturity in a cross between P. triflora and P. americana. All were taken on the same date, August 19, 106 days after bloom and showed no evidence of external injury.

Embryo size	Seed coat	Nucellus	Endosperm	Embryo	Cotyledons
⅔	Brown	Sap absorbed	¼-developed	Present	3 lobes. 8.6 mm long
⅔	"	" "	" "	Absent	None
½	Brown and shriveled	" "	" "	Very small	1.8 mm long
½	" " "	" "	" "	Absent	None
⅔	" " "	" "	Slightly "	"	"
½	" " "	" "	¼- "	Present	5 mm long
¼	" " "	" "	" "	Absent	None
⅔	" " "	Normal	Normal	Present	12.7 mm long
¾	White and turgid	"	"	"	11.8 " "
⅔	Brown " "	"	"	"	11.7 " "
Full size	White " "	"	"	"	14.3 " "

The condition in the later stages of the development shows that there is in each case at least partial growth of the endosperm. The sap of the nucellus is withdrawn in all cases, resulting in the shriveling of the seed coat when the embryo is partially formed. When the embryo is marked "absent" the supposition is that growth stopped early so that it was too small to detect in dissection. In four seeds the endosperm appeared in the irregular patches characteristic of maturity. These plums were of nearly full size and differed from the others in that they ripened earlier. The condition of seed development near maturity in these was so similar to that found earlier at the June drop that they could, were it not for confusion, be included under that heading.

The status of development in the ovule in the third drop shows marked differences from that in the second. Firstly, a greater size is attained than is ever found in the second drop, and secondly, instead of there being disintegrating nuclei within a slightly elongated embryo sac, tissues cease growing at various stages rather than disintegrating. This latter fact alone suggests an additional stimulus absent in the second drop.

Cause of the June drop

WAUGH (1899) considered that three principal causes enter into the June drop: (a) "non-pollination," (b) "curculio-work," and (c) "the struggle for existence." It has been shown that non-pollination is the cause of the second drop and that the size element alone eliminates this factor from the third drop. After an examination of the aborted embryos in the June drop, WAUGH's conclusion that "it seems fair to conclude that pollination plays a considerable, though varying, part as a coöperating cause of the June drop," appears to be wrong. It may also be stated that although plums stung by curculio fall at this time, that curculio work is not necessarily a cause since it can be controlled and in some seasons is negligible. In addition, the third drop occurs on trees in which the small setting of fruit would not appear to create conditions of competition sufficiently intense to eliminate a large number, and it may also be very slight when there is an exceptionally heavy setting of fruit. This was the condition in one tree of *Prunus americana* in 1918, in which 67 percent of the flowers set fruit (plate 1, B). Growers generally find that the number of fruits set is not reduced to a relatively fixed maximum by the struggle for existence but that the tree matures the excess crop at the expense of size. If the struggle for existence is the primary cause, this drop would be expected to take place later, nearer the time of maturity, when greater demands are made upon the available food.

It will be seen therefore that certain considerations detract from the importance of the struggle for existence as the primary factor in this drop. However, it is not the intention to attempt to eliminate this factor entirely since recent studies (EWART 1907, 1909; MÜLLER-THURGAU 1898, 1908; HEINICKE 1917; and WHIPPLE 1917) show a specialization and adjustment in the growing parts not heretofore suspected. While the work of the authors cited has dealt primarily with the grape and apple, observations made in this connection show that influences of a similar nature, among them the fruiting habit, are at work in the plum.

Varieties of the different species vary in the production of fruit buds on the terminal one-year growth. Some of the outstanding differences are shown in *P. domestica,* in which only 22 varieties out of 158 bore fruit buds on the terminal annual growth. In *P. triflora* 19 out of 21 bore nearly a full crop. In *P. americana* 18 out of 21 varieties bore fruit buds on the terminal growths. On the other hand, *P. Besseyi* fruits primarily on the terminal twigs.

In the varieties available in this investigation there was a pronounced June drop in the plums borne on the terminal wood. In fact, on the older trees fruit seldom matured in this position. The dropping of fruit from the terminal growths can be partly accounted for on the basis of the competition from a thorn or branch which is developed between the lateral fruit buds on the terminal twigs the second season. This condition occurs over the entire outer area of the tree.

On vigorous six-year-old trees of Minnesota No. 21, an attempt was made to influence the setting of fruit on the terminal growths by removing the flowers from the remainder of the tree. On one tree all flowers were pulled off excepting on the terminal growths, and 53 fruits set on 37 shoots compared with 53 fruits on 55 shoots on an untreated check tree adjacent. The condition on the one-year shoots was not strikingly different from that on the two-year wood of the check on which 23 branches bore 33 fruits. On still another tree the fruiting thorns were cut off, leaving bloom only on the one-year terminal growths. In this case there was a smaller setting of fruit but a very luxuriant terminal growth. It is possible that the treatment noted above was made too late for complete adjustment to take place. All trees of this variety bore a light crop the year of the test,—1917. Under favorable conditions fruit matures on the terminal shoots, but the percentage to set is small considering the mass of bloom, and even the small setting noted above is far in excess of the usual condition when there is a full crop on the remainder of the tree. It is apparent that in this position competition takes place between fruit and branch as well as between different fruits.

Evidence of further adjustment in the plum is shown by the shorter terminal growths produced during the season of heavy fruit production. LOEB (1918) studied growth adjustments in Bryophyllum and found that "equal masses of sister leaves produce approximately equal masses of shoots," even when the number of shoots differs. In this form the first shoots to grow attracted "automatically the material available for shoot formation." In the plum, when there is a large setting of fruit, fruit production gains the ascendancy over vegetative growth; likewise, vegetative growth becomes uppermost in the terminal positions when the crop is limited. It appears, therefore, that within certain limits there is justification in assuming that the "struggle for existence" is a factor in the third or June drop.

Table 11 gives evidence of still another influence at work in the June drop. These data are taken from controlled crosses made on tubbed trees in the greenhouse where growth conditions were favorable. Pollination and aborted pistils may be eliminated, since all pollination was done by hand on normal pistils. The records as to the number set in each cross were made approximately four weeks after pollination when size differences in the ovary made it possible to distinguish between those in which fertilization had taken place and those in which it had not. The differences between the first and second column represent those pistils which fell because of lack of fertilization,—the second drop,—while the differences between the second and third columns show approximately the extent of the June drop.

In those crosses in table 11 on which data were taken both at the time of setting and at maturity a total of 1900 fruits which set were reduced by dropping to 726 mature fruits. In certain crosses many more fruits fell than in others; e.g., in the cross Compass × Yellow Egg, of 1327 flowers pollinated, 652 fruits set and 8 matured. The condition in the seed of these is shown in figure E, plate 4, from which it will be noted that abscission took place near maturity. Again, in the cross Minnesota No. 12 × Manitoba, of 180 fruits which set 80 matured. In contrast to this in the cross Compass × Burbank, 116 fruits set and 114 matured. The general condition shown in these results is that in many of the crosses fertilization takes place but subsequently at different stages development is stopped,—a condition which shows that in some crosses fruits cannot mature for some reason, even after fertilization has taken place. The plum is comparable, therefore, to the sweet cherry (GARDNER 1913)—namely, all varieties are self-sterile, some cross-sterile and some cross-fertile.

In summarizing the relation of the pistil to sterility, it will be seen from the evidence presented that there are three distinct periods of dropping; (a) the first drop of flowers bearing aborted pistils; (b) the second drop, including all pistils in which fertilization has not occurred;

TABLE II

Showing the relationship between the number of flowers pollinated, the number set, and the number of fruits to mature in different plum crosses.

Parentage of cross		Number of flowers pollinated	Number of fruits set	Number of fruits mature
Pistillate	Pollen			
Burbank	× Minnesota No. 1[1]	140	60	40
“	× “ “ “	208	39	—
“	× “ “ 6[1]	379	28	22
“	× “ “ “	241	46	—
“	× “ “ 9[1]	314	85	53
“	× “ “ “	274	89	—
“	× “ “ 12[1]	68	5	—
“	× Surprise	457	266	121
“	× Yellow Egg	124	43	—
Compass	× Burbank	175	116	114
“	× Yellow Egg	1327	652	8
“	× Bing	34	1	1
“	× English Morello	192	0	0
“	× S. Biggareau	169	1	1
“	× Wickson	1 tree	—	275
Etopa	× *P. Besseyi*	1 branch	—	4
Minnesota No. 1	× Minnesota No. 6	127	2	1
“ “ “	× “ “ “	9	0	—
“ “ “	× “ “ 9	237	10	5
“ “ “	× “ “ “	78	1	—
“ “ “	× “ “ 35[2]	105	1	0
“ “ “	× “ “ “	92	0	—
“ “ “	× Terry	20	6	4
“ “ “	× Yellow Egg	116	29	1
“ “ “	× “ “	54	7	—
“ “ 6	× Burbank	44	8	8
“ “ “	× “	89	18	—
“ “ “	× Manitoba	169	68	61
“ “ “	× Minnesota No. 9	238	48	39
“ “ “	× “ “ “	398	96	—
“ “ “	× “ “ 12	108	8	5
“ “ “	× “ “ “	430	43	—
“ “ “	× Surprise	291	—	96
“ “ 1	× Yellow Egg	101	44	17
“ “ 9	× Minnesota No. 6	61	3	2
“ “ “	× “ “ 12	537	0	0
“ “ “	× “ “ “	309	0	—
“ “ “	× “ “ 35	209	63	50
“ “ “	× Yellow Egg	65	13	13
“ “ 12	× Manitoba	400	180	80
“ “ “	× Minnesota No. 6	207	20	3
“ “ “	× “ “ “	341	51	—
“ “ “	× “ “ 9	300	48	20
“ “ “	× “ “ “	151	0	—
“ “ “	× “ “ 35	305	84	48

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[1] Burbank × Wolf.

[2] Abundance × Wolf.

TABLE II (continued)

Showing the relationship between the number of flowers pollinated, the number set, and the number of fruits to mature in different plum crosses.

Minnesota No. 12	× Yellow Egg	80	20	2
" " 35	× Minnesota No. 9	65	7	—
" " "	× " " 12	274	3	2
P. americana	× *P. americana*	—	—	29
P. Simoni	× Surprise	48	7	4
Surprise	× Burbank	108	24	0
"	× Double-flowering Cherry	30	0	0
Terry	× Burbank	11	0	0
Yellow Egg	× Compass	181	0	0
" "	× Manitoba	42	0	0
" "	× Minnesota No. 6	45	1	1
" "	× " " 9	508	4	—
" "	× " " 35	49	3	—
" "	× S. Biggareau	28	0	0

and (c) the third or June drop, in which embryonic development is stopped. Consequently, each drop appears to be due to a different cause, and each is distinct from the others. The size differences at the time of falling are not peculiar to the plum. Preliminary observations of the apple show essentially the same condition. An outstanding feature of the evidence presented is a stimulus to growth of the pistil which results from fertilization—a stimulus which results in distinct size differences between the fruits of the second and third drops. It appears, therefore, that, excluding the loss from fungous diseases and insect injury, there is justification for placing the pistils which fail to mature in three distinct categories.

THE GENETIC PHASE OF STERILITY IN THE PLUM

Self-sterility in the plum has been investigated from three angles: (a) the elimination of gametes in the abortion of pollen and the suppression of one ovule in each ovary; (b) the genetic relationship or "affinity" between species and varieties; and (c) a study of the pollen and pistil condition as a basis for determining the type of sterility. In the treatment of each of these divisions an attempt will be made to see in how far there is justification for placing a genetic interpretation upon the condition found to exist.

The elimination of gametes

In the strict usage of the term it is not proper to refer to the elimination of gametes in speaking of pollen or pistil abortion but for convenience of presentation this may not be misleading since potentially these would produce gametes were it not for suppression at early stages. Elimination takes place at many stages of development. In the case of pollen it was shown that, while in certain extreme hybrids there is evidence of irregularity in nuclear reorganization following the heterotypic division, for the most part pollen suppression begins at the time of microspore liberation and extends nearly to maturity. The extent of pollen suppression presented in table 4 shows that a considerable number of gametes are eliminated in this genus by means of pollen abortion.

In all plum anthers there is a considerable quantity of pollen which is never shed. Even after the pollen is shed a large number of grains are eliminated from consideration because they fail to reach the stigma. While the pollen which is lost in the process of pollination is by far the greater portion, such a loss is common to all open-pollinated plants and the element of chance deals alike with all types of pollen.

Considering now only those grains which reach the stigma, either by controlled or open pollination, sections show that while the quantity is variable, some are aborted and some are normal in appearance (plate 5, L, M). Of those which are normal in appearance some develop tubes and some do not. Aborted grains, however, never send out tubes. Again, those which germinate show great differences in the rate of tube growth, so much, indeed, that by far the larger number are eliminated so far as fertilization is concerned and are disposed of with the abscission of the style. In fact, in all the sections of pistils showing tubes in the micropyle, no cases of more than four tubes were found. It will be seen, therefore, that the number of tubes which reach the micropyle is extremely small compared with the total number of tetrads or microspores produced.

Turning now to the pistil, it has been shown that while two ovules are formed, typically one is suppressed before fertilization. This disposes of one-half the total number. In a considerable number of pistils,—those which fall at the first drop,—both ovules are suppressed. A further reduction takes place when fertilization is prevented by various causes,—this number being represented by the second drop. Consequently, as in the case of the pollen, the number of ovules which fail to function, compared with those which do function is relatively large.

Thus it will be seen that in the case of both pollen and pistil there is an extensive suppression of pollen and ovules which eliminates the gametes each would bear. The question now arises as to what causes are operating in each case to produce this condition in the germ plasm.

This phase of the subject has recently been given careful study by geneticists. They have brought forward convincing evidence which shows that there are certain factor combinations in heterozygous lines of descent which cannot undergo normal development at critical stages. The evidence upon this point has been reviewed by VALLEAU (1918) and BELLING (1918) and need not be discussed again in this connection. Suffice it to say that these researches have included both cytological studies of germ cells (TISCHLER 1908; ROSENBERG 1909; GATES 1915) and breeding tests (BRIDGES 1916; EAST 1915, and MORGAN 1914), and that the facts which have been brought out by these two methods of approach to the problem are mutually corroborative.

The suppression of one ovule in each pistil may appear at first to constitute an exception to this interpretation. And it should be emphasized that this condition does not necessarily appear to be entirely comparable genetically to that of the aborted-pollen series, since, associated with a constant suppression of one of the two ovules borne by each pistil, there may be in the same flower a complete suppression of pollen in one instance, or, in another, a normal development of all factor combinations represented in the pollen. If, however, there are certain factor combinations which cannot be brought to maturity in the 1*x* condition the suppression of one ovule would be the method of expression of this, unless certain combinations can develop in the ovule which cannot develop in the pollen. PÉCHOUTRE (1902) found that in *Prunus spinosa* two cells may start to function as embryo sacs, but that one surpasses the other in development. In the axial row of each of the two ovules in an ovary a mechanism is provided in the extra cells which do not function as embryo sacs for further elimination of genetic combinations if the cell which would normally function as the embryo sac can be replaced by another cell of the axial row. In this way factor combinations may eliminate as many as three cells of each axial row without interfering with the typical pistil development, i.e., two ovules at the start with one suppressed generally before fertilization. This mechanism would also explain why it is possible to have, say 90 percent of the pollen aborted in forms in which all of the pistils are normal. The fact that there are a considerable number of flowers with aborted pistils, representing widely the varieties and species of this genus, suggests that factor combinations may be partly responsible for aborted pistils, especially when it is considered that the suppression of one ovule takes place before fertilization.

This interpretation of the elimination of gametes is, of course, based upon the assumption of a heterozygous condition in this genus. The evidence in support of this has not been presented in this connection, but the great variability in fruit and tree characters shown by wild seedlings of *Prunus americana, P. Besseyi,* and *P. nigra* found isolated in some regions, as well as studies of a large number of F_1 seedlings of controlled interspecific crosses, show that species and varieties of Prunus are far from being homozygous. Furthermore, this condition would appear to be emphasized because of general self-sterility which would necessitate continual crossing.

The genetic relationship of varieties and species

Considerable attention has been given to the "affinity between different varieties and species by WAUGH (1898, 1899), HEIDEMAN (1895) and others. This has been judged primarily by the ease with which crosses can be made and by the effectiveness of different varieties as pollenizers with others.

In interspecific crosses varieties of *P. triflora* have been found to cross readily with such widely separated species as *P. americana* and *P. Besseyi.* Also, *P. Besseyi* can be easily crossed with *P. triflora, P. americana* and *P. armenica.* A still wider cross—Compass (*P. Besseyi* × *Mineri*) × English Morello is shown in plate 4, K. Additional interspecific crosses are listed in table 11. In fact, in the fruit-breeding work at Minnesota a large number of interspecific combinations, involving two or more species, have been made, but successful crosses between any of these species and *P. domestica* are extremely rare. It appears, therefore, from these experiences, as well as from the large number of extreme interspecific crosses which have been reported in horticultural literature in the last fifty years, that in this genus the degree of sterility between species is considerably less than in some other genera which have been studied.

Something of the condition which exists between varieties is well illustrated by Whitaker, Milton, and Sophie, all of which are open-pollinated seedlings of Wild Goose. Whitaker and Milton bloom at the same time, but are inter-sterile; both, however, are fertile with Sophie, but Sophie, used as the pistil parent, is fertile with neither (WAUGH 1901). There are instances cited also in table 11 which show complete inter-sterility. For instance, Minnesota No. 9 is completely sterile when crossed with Minnesota No. 12, but fertile with Minnesota No. 35 and Minnesota No. 6. Again, Minnesota No. 12 sets fruit when crossed with Minnesota No. 35 and No. 9, but is nearly sterile with Minnesota No. 6 and Yellow Egg.

HEIDEMAN (1894) tested further the relationship between varieties by pollinating about one hundred blossoms of Wolf with pollen from six different varieties. Those blossoms pollinated with Hiawatha (*Prunus americana*) bore fruit which "were superior in size and quality to all the rest," as contrasted with those of Early Red which set no fruit. Commenting upon experiments of this nature he states (HEIDEMAN 1895) that "the union of such crosses as possess the proper degree of affinity will prove fertile, while the union of those lacking in affinity will prove sterile." He further found that "if all of the flowers of a cluster are pollinated legitimately, they will set fruit, barring accident,"—and that many plants, especially of *P. americana,* "had the power of throwing off such ovaries as were fertilized by pollen lacking in sexual affinity."

A careful study of the data in table 11, in which are shown a large number of interspecific combinations, will show in a general way the influence of the so-called "affinity" or genetic relationship upon the setting of fruit and especially upon the third drop. While no attempt has been made in this connection to determine by means of crossing the relationship of any considerable number of varieties, something of the extremes in relationship are shown by these crosses. The question which now arises is, to what extent does the failure of certain combinations to develop enter into the falling of pistils at the second and third drops. This has been discussed in part in connection with the cause of the June drop, but it has been clearly brought out in table 11 that some combinations not only set but develop fruit more readily than others. For instance, in the cross Minnesota No. 9 × Minnesota No. 12, 537 flowers were pollinated and not a single one set fruit. This instance together with the general differences shown in many other cases between the number of flowers pollinated and those which set fruit show that there is a large mortality in all crosses.

Considering now the differences between the number of fruits which set (table 11) and of those which matured, instead of the difference between the number of flowers pollinated and of those which set fruit, it will be seen that in some of the combinations, as in the cross Compass × Yellow Egg, a large number of fruits may set and but few ripen. This condition is further emphasized when a number of different crosses are made on different branches of the same tree, in which case it is not uncommon to get a heavy set on one branch and a light set or none on another. The same differences in setting would be expected, if instead of keeping the crosses separate by branches the same combinations were mixed on different flowers on the same branch.

One interesting feature of the crosses listed in table 11 is the difference in time of dropping in certain combinations, the size of the fruit indicating definitely that fertilization has occurred. Generally the abscission of pistils in which fertilization has occurred takes place at about the six-week period; but in the cross Compass × Yellow Egg, for instance, nearly the whole crop fell about one week before maturity but not until nearly the full size had been reached.

So far the discussion has dealt with arrested development during the formation of pollen or pistil, between pollination and fertilization, and after fertilization. That this is not the complete series is shown by the fact that in the seed-bed a much higher percentage of seeds of some combinations fail to grow than of others,—even when left in place for two seasons. REEVES (1917) in the Report of the Vineland Station for 1916-1917 found out that out of a total of 19,400 seeds planted only 813 germinated,—approximately 1 in 24,—and that in some varieties none grew even though as many as 2000 seeds were planted. These results agree with those at the Minnesota Fruit-breeding Farm, where, in over 150 parent combinations including many interspecific crosses, there were great differences in the number of seeds to germinate. The plum is not unusual in the small percentage of viable seeds. EAST (1915) found that germination in Nicotiana varies from 20 to 60 percent, and in Oenothera, DAVIS (1916) found a high percentage of seeds which were not viable.

Instances such as these appear to support the hypothesis that certain factor combinations are able to develop only so far,—some failing in the zygote, some in the embryo, and some not until the time of formation of the ovule or of the pollen.

In addition to the cross relationship it has been held by some investigators that there is still another influence bearing upon the sexual status in the plum, namely, a tendency toward dioeciousness. This contention has been based upon the variation in style- and filament-length found in different trees in native species. Since dioeciousness was shown to have a direct bearing upon sterility in the grape (BOOTH 1902; BEACH 1898, 1899; DORSEY 1914) and in the strawberry (VALLEAU 1918), the condition in Prunus reported by HEIDEMAN (1895) has been investigated further.

HEIDEMAN illustrates the following flower types in the plum: (a) the dichogamous type in which the stigma passes the receptive stage before the pollen is shed, and the reverse in which the pollen is shed before the stigma is receptive; (b) heterostyled types, those in which the pistil is longer than the stamens, and the converse in which the stamens are longer; and (c) the bisexual group, which may be regarded as the extreme expression of the heterostyled types, in which stamens on one hand and pistils on the other are functionally suppressed. All of these types have been found in this investigation, but those of the third class are encountered so rarely that their significance from the standpoint of dioeciousness may be questioned.

In order to determine the degree of variation in the flowers of *Prunus americana*, a survey was made of the flower types borne by wild plants growing along roadsides and in gullies in the region bordering the Minnesota River west and south of Minneapolis. HEIDEMAN also made his observations of this species along the Minnesota River at New Ulm, sixty miles or so farther up-stream. The data obtained in this survey of over a hundred miles of road or river bank are presented in table 12.

TABLE 12

A summary of data obtained in a survey of the flower condition in 212 native trees of Prunus americana, showing the variability in pedicel-, stamen- and style-length. The data on the variation in the length of stamen and style (pistil) are presented in the table in such a way as to show the relation of the length of these structures to that of the pedicel.

	Stamen- and pistil-length	Pedicel length				
		Long 14 mm[1]	Medium 10 mm	Short 6 mm	Total	
Style longer than stamens	Style long (11.5 mm)[1]	2	18	3	23	
	" med. (9.5 mm)	9	37	12	58	95
	" short (7.5 mm)	3	5	6	14	
Stamens longer than style	Stamens long (11.5 mm)	7	14	2	23	
	" med. (9.5 mm)	4	28	8	40	73
	" short (7.5 mm)	—	8	2	10	
Pistil and stamens of equal length	Pistil and stamens long (11.5 mm)	1	2	—	3	
	" med. (9.5 mm)	5	27	1	33	44
	" short (7.5 mm)	—	4	4	8	
Total		31	143	38	212	

[1] These figures are the mid-points of the class.

This table shows that on the basis of the relative height or length of pistil and stamens the flowers of this species can be grouped into three classes: those in which the pistil is longer and projects above the stamens, those in which the stamens are longer and project about the stigma, and those in which these two structures are equal in length. It is further shown that in each category the structures in question vary from long to short. Pistil- and stamen-length apparently bear no constant relation to pedicel-length, since when the pistil or stamens are long the pedicel may vary from long to short.

Furthermore, the occurrence on occasional trees of partial pistil suppression, while producing functionally a staminate flower, is so variable as to have little significance in relation to evolutionary changes. In case of complete stamen suppression a functionally pistillate flower is likewise produced, but these cases are also rare.

The status of length relationship between stamen and pistil therefore appears to be that of independently varying structures. If dioeciousness is gaining expression in this species, as is contended by HEIDEMAN, flower structure has not as yet been sufficiently changed so that there are distinct pistillate and staminate flowers, although the stamen and pistil are of equal length in less than one-third of the flowers. Self-sterility, on the other hand, is prevalent in all flower types, and results in the same necessity of crossing as dioeciousness. Differences in length in a structure, at least up to a certain point, do not necessarily influence its functioning, but it is probable that in the case of the pistil, other things being equal, long styles would render fertilization more uncertain than short ones, when conditions at bloom were unfavorable.

It yet remains to be seen in how far the condition in the plum can be assigned to genetic causes and how far to nutrition. In this investigation the problem has been approached primarily from the genetic standpoint. It is probable that the two methods of approach are not as incompatible as may at first appear, and it is not the intention to minimize in this connection the bearing of those considerations broadly grouped under physiological influences.

Fruit development in the plum is apparently more dependent upon normal seed development than in some other fruits. In the apple there is considerable variation in seed development in different varieties, and the relation of seed development to size has been emphasized by WAITE (1894), KRAUS (1915), HEINICKE (1917) and others. In fact, fruit formation without seeds or even with only rudimentary carpels is not uncommon in the apple. Seedless grapes occur in a number of species but particularly in *V. vinifera*, and have long been a matter of comment by horticultural writers. A similar relation between seed formation and size prevails in the grape and the apple. The condition in the plum, however, in which typically a single seed is matured, and in which the stimulus to development must come from a single seed instead of from many, appears to be more sharply defined. In fact, it has been shown that development may stop at any time between fertilization and maturity. This being the case the genetic relationship would be a more decisive factor than in a fruit in which there are many seeds or in which seed development is less essential to fruitfulness.

In order to get at the question at issue, suppose for the moment that fertilization has taken place in all of the pistils on a plum tree and that all set. The question is, could all pistils develop into mature fruits? If this were possible, a single spur such as that shown at N, plate 4, would have to bear approximately three quarts of fruit (assuming 18 plums per quart), or at each node, as at L and M, plate 4, six to nine plums would have to be borne. For physical reasons alone such fruit production as this could not take place on the entire tree or even on a single spur. What factors enter, then, to reduce the number of fruits?

Among the first to suggest itself is competition for available food. But it is not clear why competition alone would be the deciding factor in view of the possible adjustment as to size which takes place when the number of persistent fruits is large. This adjustment within a variety may be so great as to make a difference on the basis of size of between 18 and 48 plums per quart. Again if only one pistil in ten sets fruit (the approximate ratio in the cases shown in table 11 is one in four to set and one in ten to mature), would there be any necessity for a third drop on the basis of competition for available food? It is conceivable that competition would be considerably reduced if only one in ten were to set fruit, but from table 11 it will be seen that even this reduction in pistils does not necessarily prevent a further loss.

A study of fruit development at various stages shows some interesting adjustments in the plum. In the terminal positions on the one-year wood, as has been noted, vegetative growth is given emphasis over fruit production. On the native species—excepting *P. Besseyi*—the greater amount of fruit is produced on two-year-old wood. In either case there are instances where the terminal positions appear to favor some buds and fruiting growths over others. As a result, there is considerable variation in the time of opening of different flowers on a tree and hence in the receptiveness of different stigmas. Consequently some pistils are not only pollinated before others but in some fertilization occurs earlier than in others. Differences in time of fertilization, other things being equal is no doubt the most important single factor,—not even excepting differences in position,—in enabling one pistil to gain the ascendency over others. The size differences in pistils are pronounced at the time of early bloom but become even more conspicuous after fertilization.

In a very detailed analysis of the factors influencing the abscission of flowers and partially developed fruits in the apple, HEINICKE (1917) shows that position, nutrition and seed development are important considerations, but these were not carefully checked by him with reference to fertilization and genetic combinations. PIETERS (1896) found that the effect of fruit-bearing upon the tissues is local, being confined in the plum and peach to "a small area in the immediate neighborhood of the fruit-stalk," but that in the apple and pear the effect is "perceptible throughout the one-year-old shoot." The local effect on the wood cylinder disappears in time. Even these adjustments, which are largely a result of differences in nutrition, do not create a condition which results in fruit setting only in certain positions, which would tend to be the case if competition were the determining factor.

Therefore, with the evidence at hand from controlled crosses in addition to that from the studies of pollen and pistil, the interpretation that normal development is determined by the factor combination either in the $1x$ or $2x$ condition appears justifiable. The influence of unfavorable factor combinations upon the June drop is especially direct in the plum, since there is but a single ovule which develops or not as the case may be. The case is different in the apple where many seeds may develop but one or more may be sufficient to furnish at least a partial stimulus to development. When there is a heavy setting of fruit resulting in increased competition for a relatively limited food supply, the uncongenial factor combinations are the first to cease development, and they are not saved either by a favorable position on the twig or by early fertilization. The difference in the time of falling at the third drop, although becoming less and less as maturity is approached, can be explained on the basis that some combinations can proceed farther in development than others. Also the greater relative number which fall at the third drop when the set is heavy would be expected, because there would be a larger number of combinations which could not develop beyond a certain point when the competition was most acute. Moreover, if this hypothesis is correct, it would appear that some combinations could be carried much farther under especially favorable conditions of nutrition than under adverse conditions. Finally, in order to check definitely the influence of position upon nutrition, careful attention would have to be given to the selection of homozygous material.

The type of sterility in the plum

The phenomena of self-sterility and cross-sterility in the plum have much in common with those reported in other forms. The outstanding features are: (a) the constancy of expression of self-sterility even in *P. domestica* in which about one-half of the varieties are self-fertile; (b) the occurrence of cross-sterility (table 11); and (c) the slow growth of pollen tubes under the condition of self- and cross-sterility.

STOUT (1916) has divided the cases of sterility reported to date into three groups: sterility from impotence, sterility from incompatibility, and sterility from embryo abortion. These are subdivided to include variations within each group. Sterility of both the second and third types appears in the plum. The type of sterility from incompatibility is comparable to that reported in Secale by JOST (1907), in Nicotiana by EAST (1915) and by EAST and PARK (1917), in Cichorium by STOUT (1918), and in the apple by KNIGHT (1917). Embryo abortion, found to be so common and to contribute so largely to the June drop, has been reported in the plum by WAUGH (1899), and also in a number of other forms, such as the apple (KRAUS 1915), and Oenothera (DAVIS 1915 a, b, 1916).

On account of the occurrence in Prunus of self- and cross-sterility, of the type characterized by slow pollen-tube growth, the results of previous studies of pollen germination are of especial interest. Attempts to germinate pollen in nutrient media have shown generally that a considerable number of grains do not send out tubes (WAUGH 1900, GOFF 1901, GARDNER 1913, VALLEAU 1918, and EAST and PARK 1917). The nutrient requirements of pollen tubes are not as yet well enough known to determine whether all grains of normal appearance can be made to germinate. While germination tests may be suggestive as to the viability of pollen, they do not serve as an index to cross- or self-relationship nor can the exact line between normal and aborted pollen be drawn as yet by this method. Judging from the general appearance of plum pollen-tubes formed in artificial media and on stigmas, there appears to be no question that true tube formation occurs in artificial media even though tube growth has been short in the media used. The conspicuous knotted and twisted terminations of apparently normal tubes found in the artificial germination tests, comparable with those illustrated by GOFF (1901) throw some doubt, however, upon the pollen-tube growth being normal beyond a certain length. The point has been emphasized by STOUT (1916) and EAST and PARK (1917) that in the most carefully controlled germination tests in nutrient media, the maximum tube growth required for fertilization has not as yet been reported.

EAST and PARK (1917), in a careful review of the studies on chemotaxis conclude that there is "certainly a probability" that pollen tubes show this phenomenon, but in their experiments in which parts of the "gynaecium" were placed in the media the results were negative although there was some evidence of increased tube growth. STOUT (1918) suggests that a "critical period in the growth of the pollen tube may result from secretions of the egg and that the different qualities of the pistil may be due to the diffusion of hormones from the gametophytes." The plum furnishes some evidence upon this point. The larger aborted pistils in the first drop become receptive even though the ovules are aborted. Pollen tubes grow in these before the flower drops. In one case, in a hand-pollinated pistil of *P. Besseyi* what appeared to be a tube was found in the carpel cavity near the aborted ovule. Tube growth has also been found in other pistils, open-pollinated, in which both ovules have aborted late before bloom. Instances like these would appear to preclude the possibility of a stimulus influencing tube growth coming from a normal egg, since in these cases development of the embryo sac had not proceeded to the formation of the egg.

The failure of so many normal-appearing grains to germinate on the same stigmas on which other grains do germinate, as is the case in the plum, raises the question as to the location of those factors which determine germination or non-germination. JOST (1907), EAST (1915), STOUT (1917), and others have emphasized the fact that the pollen tube is a $1x$ structure nourished from the $2x$ tissues of the sporophyte. This relationship is the same in self- and cross-pollination. EAST and PARK (1917) found considerable differences in tube length in Nicotiana and ascribed this condition to differences in the time of pollination rather than to differences in genetic constitution. In controlled crosses in Nicotiana (EAST 1917) 129 seeds were obtained as a result of the application of 149 pollen grains to the stigma. This is a surprisingly high proportion compared with results with the plum. A survey of the sections of a plum stigma, crossed in the greenhouse, shows that many grains were aborted and sent out no tubes, that others were normal in appearance but did not germinate, that a few sent out short tubes which at the time of fixation, 70 hours after pollination, had not extended below the stigmatic cells, and that fewer still sent out longer tubes, the longest of which extended less than one-half of the distance to the ovule.

When plum stigmas are first receptive in the greenhouse, they are often under the most favorable conditions covered with a conspicuous drop of the stigmatic fluid. When pollen is applied to such a stigma, as was the case in the above instance, all grains may be considered as entering the same substratum. Within as short a time as two to five minutes after being applied, pollen imbibes the stigmatic fluid, and even most of the aborted grains become turgid. When the amount of the stigmatic secretion is less, or when pollen is applied before receptiveness, there is greater unevenness in the matter of coming in contact with the stigmatic fluid. Such a condition would undoubtedly result in uneven germination, even if all pollen grains were viable. In the plum there appears to be another factor influencing germination and tube growth as well as time of pollination, because so many normal-appearing grains never germinate. This condition indicates that even in a fertile cross some grains are sterile, which is easily explained by the assumption that they differ in their inherited factors.

The taking up of the stigmatic fluid must certainly precede germination. In this way the protoplasmic contents of all pollen grains come in contact with the same substratum within a relatively short time; and barring selective absorption the swelling of the grains indicates that this is so. The fact that aborted grains do not send out pollen tubes shows that the growth response of the pollen comes from the nucleus instead of the cytoplasm. Up to this time, the lot of all grains would appear to be the same, and the differences noted above in germination and tube growth in the pollen of a controlled cross begin at this point. As to the style and stigma, that portion through which tubes grow would be composed of similar cells. By means of the stigmatic fluid, the gametophyte furnishes a homogeneous nutrient substance to the pollen, in the same way that the anther sap furnished a homogeneous nutrient medium to the microspores at an early stage of development. It would appear, therefore, that variations in the growth of pollen grains applied simultaneously to a receptive stigma would arise from differences in the pollen rather than in the stigma or style.

What relation, then, do self- and cross-sterility bear to the $1x$ and $2x$ conditions? STOUT (1916) points out that "a plant whose two sets of sex organs are completely incompatible is itself derived from the fusion of two cells that were compatible." This is also true of complete compatibility. This being the case, at what point in the ontogeny of a plant do the differences which bring about self-sterility arise? Cytological and genetic evidence for the most part point to a remarkable constancy in descent from cell to cell; this would be expected to continue in the stamen up to the time of the reduction divisions. The cells of the stigma and style are formed by somatic division and consequently undergo no such changes as are known to take place at the time of chromosome reduction. The ♂ gametes arise from cells in the anther which are in the same line of descent as those of the style and stigma, but under the conditions of self-pollination they bear a changed relationship to a once congenial association, and since sterility due to incompatibility must occur at this stage, and in this relationship it appears that genetically there is lacking some factor or substance in the $1x$ condition which is present in the $2x$. Whatever the basis for this changed relationship may be, it expresses itself in self- and cross-incompatibility and prevents the union of gametes which might possibly be congenial if they were permitted to come together. It appears logical, therefore, to assume that the change comes at the time of the reduction divisions, even if the change has not as yet been defined in terms of chemistry and physiology. It yet remains to be seen whether breeding experiments show an expression of self- and cross-incompatibility sufficiently definite and constant to warrant placing this character in the same category as others which have been investigated genetically.

BAUR (1911) crossed *Antirrhinum molle,* which is self-sterile, with *A. majus,* which is self-fertile, and all the F_1 plants were self-fertile. Both self-fertile and self-sterile plants appeared in the F_2 generation. COMPTON (1912) found in *Reseda odorata* that when self-sterile plants were bred *inter se* the progeny were all self-sterile; some self-fertile plants gave only self-fertile offspring when selfed, and other self-fertile plants when selfed produced a progeny in which there were approximately three plants self-sterile to one self-fertile. COMPTON held that self-fertility in this species is a simple Mendelian dominant. In working with *Reseda odorata* he obtained results from crossing and selfing experiments which, on account of constancy of expression and the ratios obtained, can be interpreted as being in accord with the hypothesis that self-sterility is a simple Mendelian dominant to self-fertility. In *Cardamine pratensis* CORRENS (1912) found a fairly well defined relationship as to sterility or fertility when two original parent plants, B and G, were pollinated with F_1 individuals. On the basis of this relationship with both parents, he grouped the 60 plants obtained from this cross into four approximately equal classes; fertile with both B and G, 16; fertile with B but sterile with G, 16; sterile with B but fertile with G, 14; and sterile with both B and G, 14. Likewise, when the F_1 plants were crossed with the parents they fell into four classes; sterile with B, 28; fertile with B, 32; sterile with G, 30; and fertile with G, 30. CORRENS advances an explanation of his results by assuming that units, representing chemical substances—"line stuffs"—segregate in germ-cell formation. Certain discrepancies in his classes have been noted by STOUT

(1916) and by East and Park (1917), which, however, do not set aside the distinct differences in cross- and self-relationship found to exist.

East and Park (1917) have presented an excellent analysis of the inheritance of self-incompatibility in four species of Nicotiana all of which are self-sterile. The data show that as to cross-relationship the plants within either a single family or more than one family, can be grouped into classes in which each member is sterile with the others but fertile with all the individuals of every other class. In the different families these intra-sterile classes varied from one to six. These crosses show not only that self-incompatibility is inherited but that reciprocal crosses are duplicated. Both self- and cross-incompatibility were found to be due to slow tube growth. The authors propose a genetic interpretation of their results in line with recent factorial analysis. On the other hand, Stout (1916, 1917, 1918) reports a different status of sterility in self- and cross-pollinations in Cichorium. His results are interpreted as showing that all grades of self- and cross-incompatibility exist in this species. In fact, self-fertility appeared in a family of red-leaved Trevios after three generations of self-sterile ancestry. The progeny of self-fertile plants do not breed true for this character. Stout holds that "the factors which determine or prohibit successful fertilization in chicory, whatever their essential nature may be, are highly variable as to degree, specificity, and transmission in heredity." It will be interesting to note from future research in which other forms this same condition obtains.

Backhouse (1912) worked with varieties of *P. domestica* and states that "there is evidence both from analogy and from the results of plum hybridization undertaken by Messrs. Laxton Bros. in the past, to show that self-sterility is a simple unit character, self-fertility being recessive, and that the heterozygote, when self-fertilized, sets a fruit here and there, as do Mallard and River's Early Prolific.

Later, Sutton (1918) reporting further on the work begun by Backhouse, says:

> "In view of the recent experiments of others two main questions arise, (1) whether self-sterility is a simple Mendelian recessive character; (2) whether the older observers were right in considering that in such cases self-steriles are fertile with the pollen of *any* other variety, or whether there are not, rather, several classes of individuals, between which there is what East has called 'cross-incompatibility.' As regards the first question there is nothing in our results which negatives the view that the property of self-sterility may be a recessive, but until a later generation can be tested, the only evidence bearing on this aspect of the

matter is the fact that the results with plums and cherries are consistent with the supposition that the plants consist of two larger classes, self-fertiles and self-steriles, with a smaller number of plants of intermediate properties. These and presumably some of the self-fertiles may be supposed to be heterozygous. The self-fertile class forms a fairly homogeneous group, and the occasional indications of partial self-fertility are probably attributable for the most part to errors."

Self-sterility tests indicate a different condition in the varieties of the American species of plum. The uniform occurrence of self-sterility indicates that this condition is dominant in the species tested. The limited data available show that besides the large group of self-sterile varieties (with only two possible exceptions) there are also cross-fertile and cross-sterile groups, as in Nicotiana and other species. So far, however, the limits of these last two groups have not been determined experimentally.

It will be seen, therefore, that while the physiology of tube growth is as yet only partly understood, much has been learned of self- and cross-incompatibility as a result of cytological and genetical studies. Inheritance studies of sterility show the behavior of this character to be quite in keeping with that of others which have been investigated genetically. There would seem to be no question concerning the segregation of a character at the time of the reduction division which produces a difference between pollen tube and stylar tissue, which, as EAST and PARK (1917) state, results in a type of sterility that is merely a physiological impediment. The type of self- and cross-sterility in the plum, therefore, is comparable with that in other forms and can be ascribed to slow tube growth.

SUMMARY

Self-sterility tests in the plum show that the varieties of the American species are self-sterile. This condition, therefore, has an important commercial bearing.

In Minnesota pollen development proceeds no farther in the fall than the archesporial-cell stage and growth in the spring begins about the first of April.

The haploid chromosome number was found to be ten in nine varieties representing seven species.

The tissues of the mature anther develop and function normally so that there appear to be no influences from this source which would contribute to pollen abortion.

The pollen mother-cell wall persists after rounding up in the mother-cell until as late as the formation of the microspores. During the period of enlargement in the anther cavity these walls stretch and thus do not interfere with anther expansion or microspore formation.

The tetrad wall in section appears as a thin layer or membrane in close contact with the mother-cell wall and separates from it first at the angles and narrow ends of the pollen mother-cell. This wall thickens subsequent to the heterotypic division and forms the thick wall characteristic of the tetrad before the liberation of the microspores.

A thin wall is formed about the microspore before liberation from the tetrad wall and the three sutures with a single germ pore in each appear as thickening in the pollen wall begins. The mature grain is characterized by thick walls, with a furrowed surface, and fimbriated margins to the germ pore. The tube nucleus and the generative cell are much contracted in mature grains and are found near the center at the time of dehiscence.

Normal pollen development is typical of the plum and, while many aborted grains are found in all varieties under investigation and in some supposedly pure species, pollen abortion is not a cause of sterility except in rare instances where suppression is complete.

The earliest evidences of suppression were found immediately following the heterotypic division in an extreme hybrid. In other varieties suppression began after miscrospore liberation from the tetrad wall and grains were found in which suppression had taken place at all stages up to maturity. The percentage of aborted pollen was higher in hybrids than in species supposed to be pure.

In many of the hybrids pollen was found to break up into yellowish oily globules. This substance accounts for the fact that pollen of some varieties is "sticky" at dehiscence and is not readily blown away by wind.

Stamens may metamorphose into either petals or pistils but in the anthers affected new types of aborted pollen are not found.

In general the status of pollen development in the forms under investigation showed that neither self- nor cross-sterility could be explained upon the basis of pollen abortion.

Pistil development in the fall showed no evidence of the growing point from which the ovule is formed, but at bloom the ovary may contain two ovules in each of which there are four to eight nuclei. Typically, either before or soon after bloom one of the ovules is suppressed but many variations were found in the degree of growth before suppression.

Pistils were found to drop in three waves which are separate and distinct in point of time and size.

The first drop takes place immediately after bloom. In all flowers which drop at this time the pistils are aborted. In some abortion occurred so early that the pistils were no longer than five millimeters, in which case the pistil at bloom was always brown. In other flowers the pistils were nearly normal in size, but in these both ovules were aborted. Flowers bearing aborted pistils generally bear normal pollen.

The second drop takes place from two to four weeks after bloom and includes all pistils in which, for any reason, fertilization has not taken place. In pistils which fall at this time the conditions in the ovules are interesting. The egg remains normal in appearance for two weeks but may persist as long as 33 days after bloom. The first nuclei to break down in the absence of fertilization are the antipodals, and these are followed by the endosperm nucleus which does not divide when fertilization is prevented. The embryo sac elongates only slightly when the egg is not fertilized but the canal, into which it lengthens normally, extends full length in the nucellus until it reaches the chalaza. The ovary reaches a size of two to five millimeters in diameter before dropping.

The third or "June drop" follows the second by an interval of about two weeks. This drop is characterized by the larger size of the plums. Fertilization has taken place but embryo development has stopped. An outstanding feature of this drop is the stimulus which results from fertilization compared with the second in which this stimulus to development is lacking.

Emphasis has been placed upon the fact that pollen development in the plum is suppressed during the period of growth when the chromosome number is reduced. Abortion can be explained by the assumption that when some factor combinations are brought together complete development cannot take place during the haploid condition of the gametophytic generation. This assumption is supported by the large percentage of aborted pollen in known hybrids.

The suppression of one of the two ovules in each ovary was found to be typical. Suppression generally took place before fertilization but sometimes afterward: when it occurred before fertilization, growth was found to stop at any stage from the megaspore mother-cell to the mature embryo-sac. When one ovule was suppressed after fertilization the one in which fertilization first took place, other things being equal, appeared to gain the ascendency.

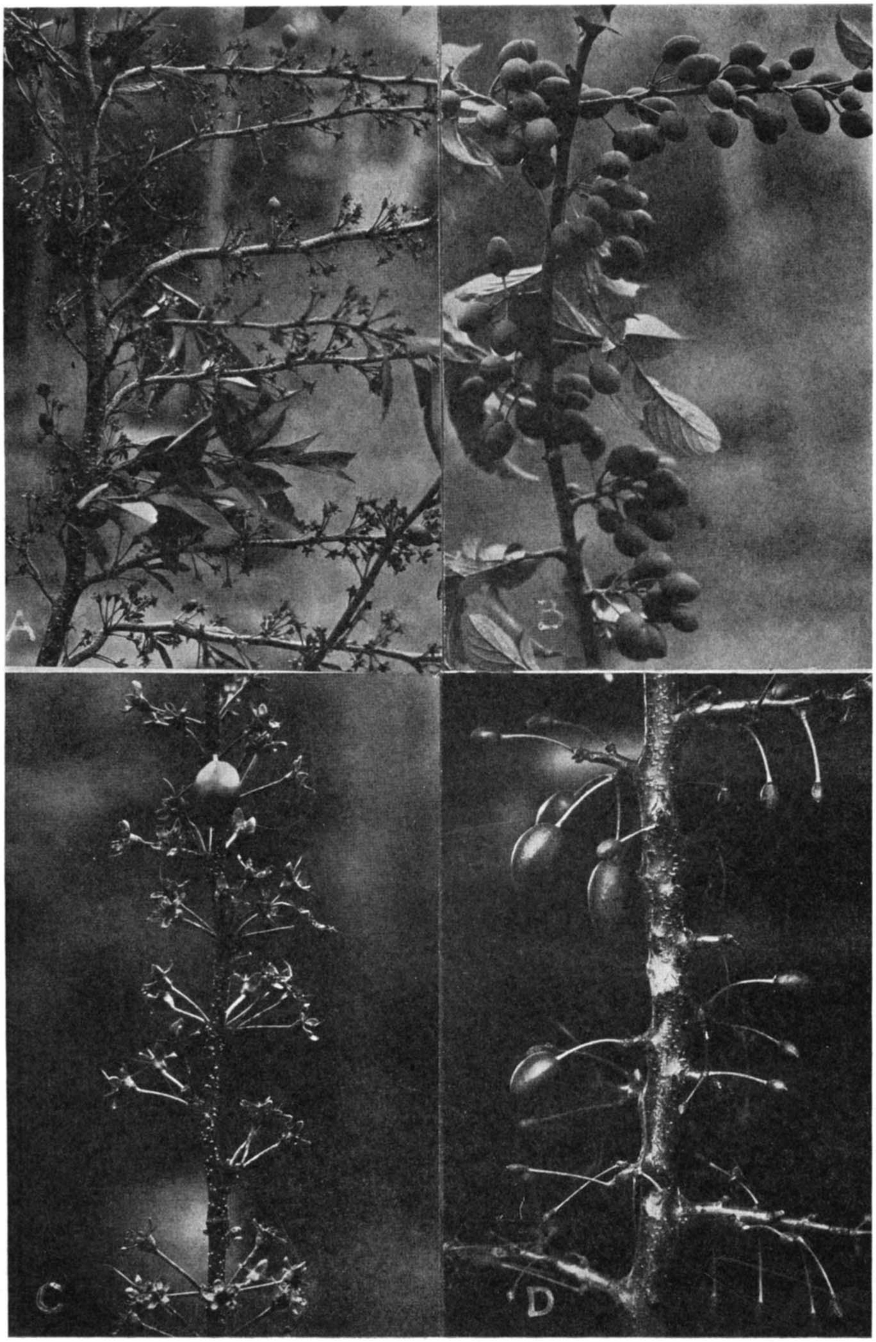
LEGEND FOR PLATE 1

A, a Sand Cherry hybrid 30 days after bloom. On the branch, a part of which is shown, there were 392 flowers. Of this number 73 had aborted pistils and only 12 set.

B, a part of a branch of *Prunus americana* 30 days after bloom, on which 138 fruits were set and only 78 pistils dropped.

C, a single twig of A (enlarged) showing the persistent calyx tubes and styles.

D, Minnesota No. 21, 31 days after bloom, showing the size differences between those setting and the pistils with unfertilized ovules which have not as yet dropped. The smaller plums illustrate the second drop. The calyx tube and style drop earlier in this variety than in that shown in C.


A
B
C
D

The reason over fifty percent of pollen may be aborted in forms in which only one-half of the ovules are suppressed can be explained on the basis that if the nucleus in the axial row of cells which would normally function as the embryo sac contains a factor combination which inhibits development its place can be taken by one of the other cells. The suppression of one-half of the ovules, however, in forms in which there is only a small percentage of aborted pollen, indicates that other than genetic causes enter into this condition in the plum.

In controlled crosses different combinations set different percentages of fruit. In some fertilization does not take place and all pistils fall in the second drop, in others fertilization occurs but a part or even all may drop. The interpretation is that different factor combinations bring this condition about through arrested development. The general relationship may be expressed as follows: All varieties under investigation were found to be self-sterile, some cross-sterile and others cross-fertile.

The type of sterility, either self- or cross-, was found to be that termed incompatibility, in which gametic fusion is prevented by slow tube growth.

Many normal-appearing grains failed to send out pollen tubes when placed on a receptive stigma under conditions similar to those in which others developed tubes. Aborted grains never send out tubes, even though they take up the stigmatic fluid.

Thus some pollen grains germinate and some do not under the conditions of the stigmatic fluid much the same as some microspores develop and some do not under the conditions of the anther sap. These differences appear to be due to something inherent in the grains rather than in their substratum, which either in the case of another sap or stigmatic fluid, may be regarded as homogeneous throughout. This condition can be explained by differences in genetic constitution.

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LEGEND FOR PLATE 2

1.—Minnesota No. 21. Showing the origin of the tetrad wall adjacent to the tapetum. In the plum the mother-cell wall persists after rounding up in the cytoplasm, but in the grape it is dissolved in this process.

2.—*P. angustifolia.* A tetrad showing the origin of the microspore wall.

3.—Minnesota No. 21. A drawing of the germ pore and suture at the one-nucleated stage of the microspore.

4.—The vegetative nucleus and generative cell in the mature pollen grain.

5.—Minnesota No. 21. The vegetative nucleus and generative cell immediately after reorganization following division in the microspore nucleus. Compare with 6, 7, 8, 9 and 10.

6, 7.—Minnesota No. 21. The generative cell of a pollen grain which did not germinate. The stigma was self-pollinated twenty-four hours before killing for sectioning.

8.—Minnesota No. 21. A contracted generative cell in mature pollen. Note the relative size of the vegetative nucleus and the generative cell.

9.—The generative cell in the final stage. In this section the generative cell has so contracted that only the nucleus is visible and the nucleolus can seldom be made out.

10.—Same as 9, another variety.

11.—A pollen tube in the micropyle showing what is probably the generative nucleus and also the cross partition in the tube.

12.—*P. Besseyi* × *P. armenica.* A dyad with only one nucleus organized. These instances are rare. The grain adjacent showed a similar condition.

13.—*P. Besseyi* × *P. armenica.* Showing the scattered condition of the chromatin at early metaphase of the heterotypic division.

14.—*P. Besseyi* × *P. armenica.* A tetrad with an extra, small nucleus and also the heavily staining bodies in the cytoplasm.

15.—*P. Besseyi* × *P. armenica.* An intermediate stage in tetrad degeneration in which extra nuclei and rings appear in addition to the deeply staining bodies.

16.—*P. Besseyi* × *P. armenica.* A final stage in tetrad nuclear degeneration in which none of the nuclei are reorganized. The remnants of spindles indicate that division has taken place. Extreme cases like this suggest a connection between the rings in the cytoplasm and chromatin. Some of the spherical bodies can be interpreted as small nucleoli.

17.—Minnesota No. 21. The nucleus of a microspore at diakinesis showing the size at this stage compared with nuclei in the mature pollen as in 4.

18.—*P. Besseyi* × *P. armenica.* A drawing of a tetrad showing three small nuclei in one lobe, where typically there should be only one.

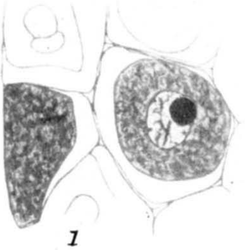
19.—*P. Besseyi* × *P. armenica.* Liberated tetrads showing the extreme contrast in size soon after liberation. The smaller one has no nuclear membrane and can be interpreted as representing an early-aborted stage. The germ pore is forming in the larger one before there is much thickening in the wall.

20.—Minnesota No. 5. The winter stage of the pistil as it appeared March 22. There is as yet no growing point in the carpel cavity from which the ovules develop.

21.—Burbank. Illustrating the general morphology of the ovule 5 days before bloom.

22.—Cross section of plum showing the embryo, cotyledons, unabsorbed nucellus (a), stone tissue (b), and suppressed ovule (c).

(Legend for plate 2 continued on page 484)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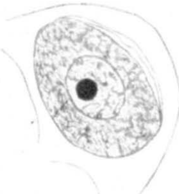
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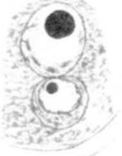
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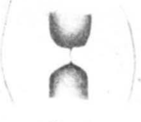
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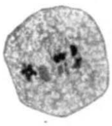
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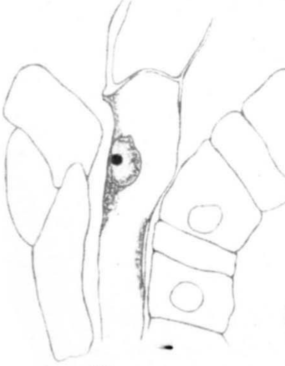
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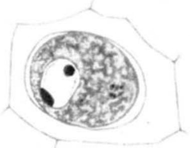
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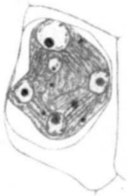
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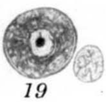
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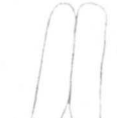
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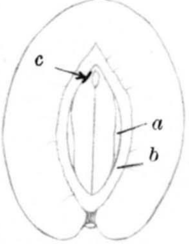

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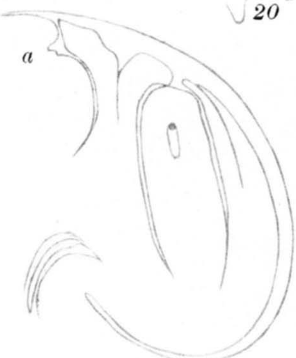

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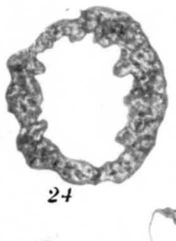
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LEGEND FOR PLATE 2 (Continued)

23.—Minnesota No. 35. An egg 24 days after bloom. There is less disintegration than in 24.

24.—Minnesota No. 35. An egg 34 days after bloom. At this stage the canal through the nucellus extends to the chalaza but there has been only a slight elongation of the embryo sac. The egg is the only cell remaining in the embryo sac.

25.—Minnesota No. 35. The appearance of the egg 12 days after bloom, the stigma being snipped before pollination. In this case there has been no elongation of the egg sac and the cytoplasm of the egg appears vacuolated. The full number of nuclei could not be found in the embryo sac.

26.—Minnesota No. 35. Same as 25 except that the embryo sac has elongated to half the distance through the nucellus and the egg shows further evidences of disintegration on account of its vacuolization and irregularity.

27.—Minnesota No. 35. An embryo 24 days after bloom in a plum which appeared to be setting normally.

LEGEND FOR PLATE 3

Photomicrographs showing the pollen condition in some extreme plum hybrids.

A.—Wohonka × Cherry (*P. triflora* × *P. americana*) × *P. cerasus.*

B.—Opata, *P. Besseyi* × (*P. triflora* × *P. Munsoniana*).

C.—Satsuma × Compass, (*P. triflora* × (*P. Besseyi* × *P. hortulana Mineri*)).

D.—*P. Besseyi* × *P. Simoni.* Note the advanced stage of the breaking down of pollen into globules.

E.—Wolf, *P. americana mollis.* An early stage of the breaking down of pollen into globules. The walls are affected first.

F.—Opata, *P. Besseyi* × (*P. triflora* × *P. Munsoniana* ?).

G.—Burbank, *P. triflora.*

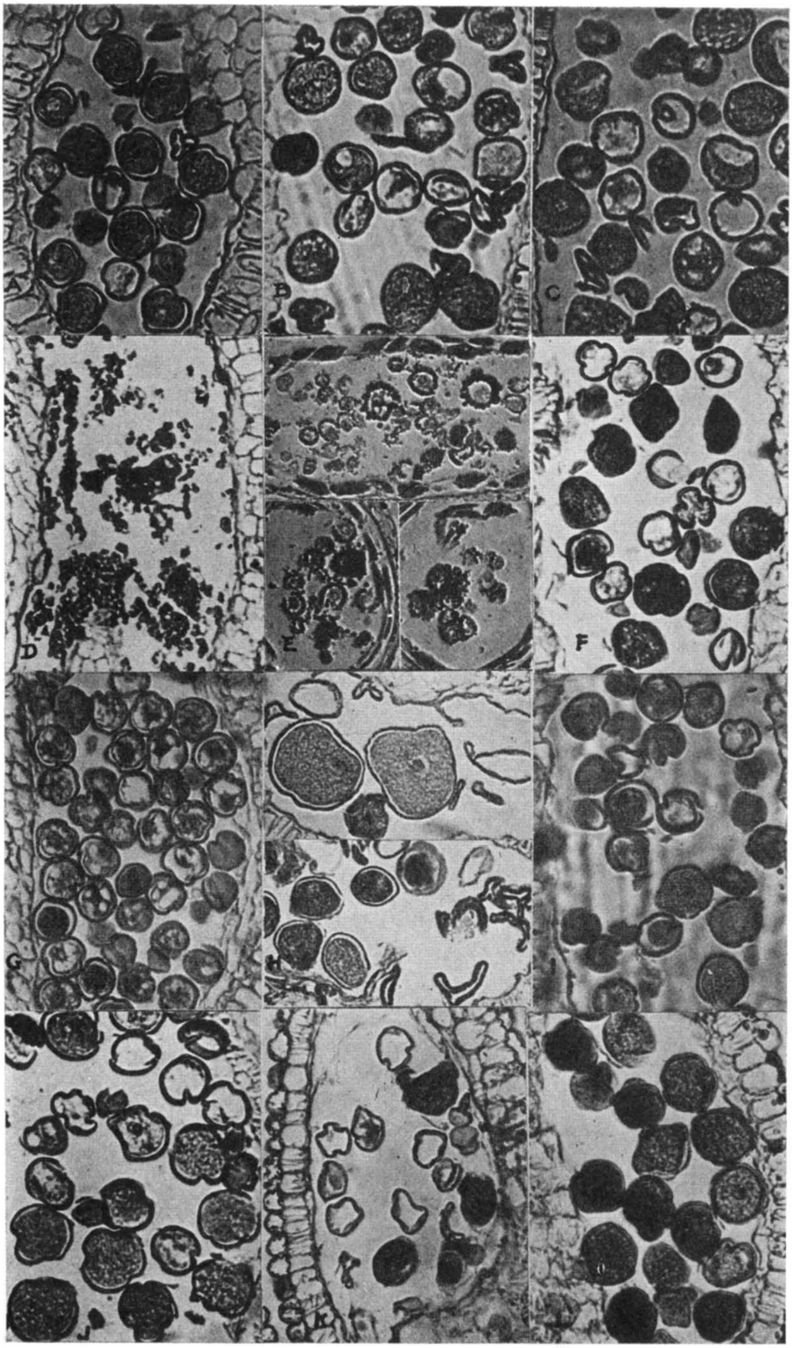
H.—*P. Pissardi.*

I.—Minnesota No. 21, (*P. triflora* × *P. americana mollis*).

J.—Minnesota No. 17, (*P. triflora* × *P. americana mollis*).

K.—Kamdesa.

L.—Chokecherry, *P. virginiana.* Pollen rarely aborted.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LEGEND FOR PLATE 4

A.—An advanced stage of petalody in a *P. triflora* cross. The normal petals have been removed.

B.—Petalody in a cross between Compass and Satsuma. The normal petals have been removed.

C.—An extreme instance of pistillody in a hybrid, parents uncertain.

D.—Pistillody in a *P. triflora* cross in which only part of the stamens are affected.

E.—Embryo abortion in a cross between Compass and Yellow Egg. These plums abscissed at the base of the plum and fell two weeks or so before maturity. The embryos in these cases aborted early in development but the plums reached nearly the normal size.

F.—A series showing the extremes in embryo growth in plums which were falling, in a cross between Minnesota No. 21 and Burbank, 82 days after pollination. These plums were 15 to 20 mm in diameter and the stone tissue was hard. In one seed of which sections were made the embryo was only 16 cells across.

G.—Ovule development in Stella (*P. americana* × *P. triflora*). The embryo can be found in only an occasional seed of this size but fertilization has taken place. The seed coats are brown on the three at the right. These plums were falling about one month after bloom.

H.—The relative size of the two ovules in a cross between Minnesota No. 21 and Burbank. At the extreme right both embryos are fertilized and are of nearly equal development. In each of the others one is being suppressed but not until an enlargement has taken place approximately equal to that in the unfertilized pistils.

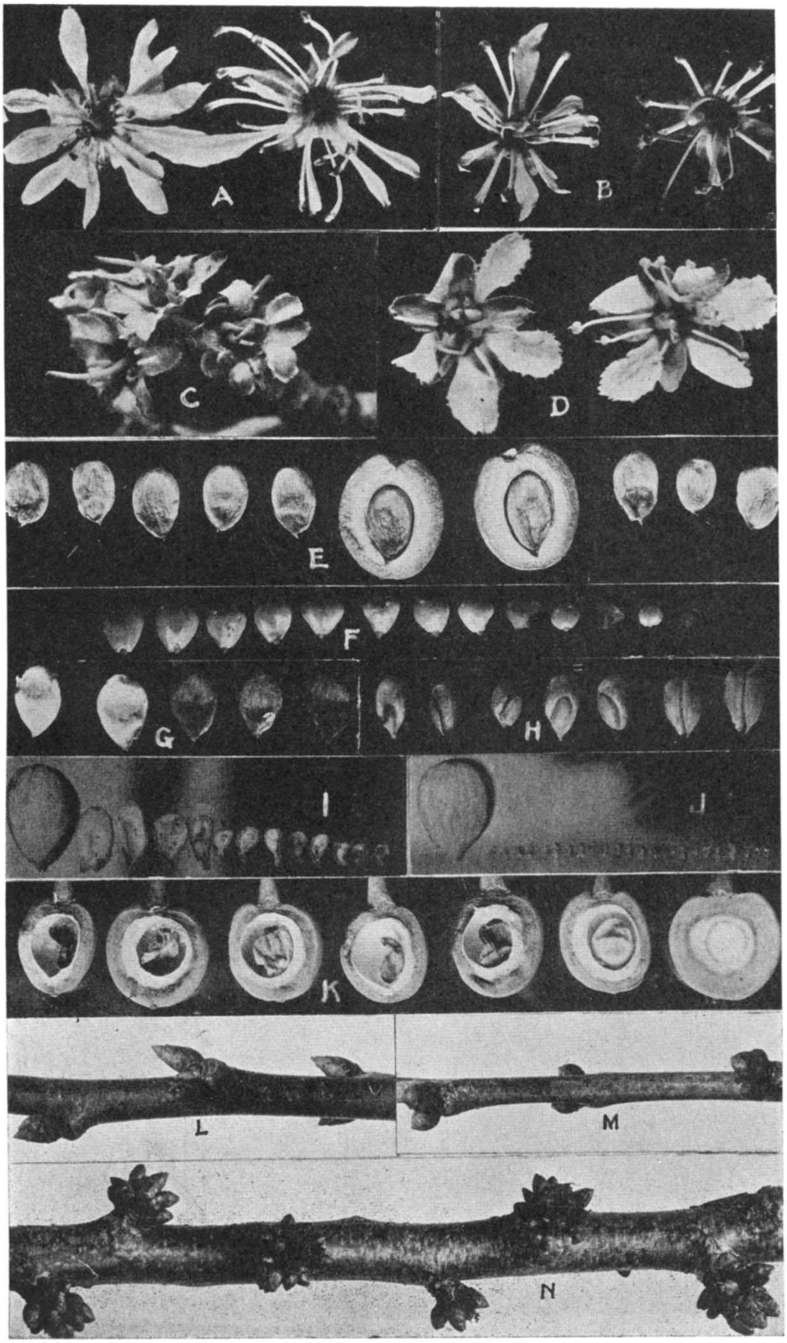
I.—The variation in the suppressed ovules in Stella compared with the normal development at the left. In this variety two seeds are often found in a pit and there is generally a partial development in the suppressed ovule.

J.—The suppressed ovules in Assiniboin (*P. nigra*). The normal seed is shown at the left. The difference between Assiniboin and Stella is largely in the time of suppression. In Assiniboin the second ovule is uniformly suppressed before bloom, so there are not normal embryo sacs to carry enlargement forward to the size of unfertilized ovules.

K.—A series in embryo development when English Morello was crossed with Compass. Those fruits with aborted seeds grew to nearly the normal size but turned yellow and fell before maturity. Fertilization had taken place.

L, M.—Fruit buds borne in one-year-old terminal twigs. These seldom set fruit in the plum. If all flowers at each node set fruit there would be 6 to 12 fruits borne at each node;—this is physically impossible on account of the weakness of the twigs if not from the standpoint of nutrition. L, Minnesota No. 21. M, Surprise.

N.—Fruiting spurs borne on the two-year wood of Burbank. There must also be a great reduction in the number of flowers borne on each spur as at each node on the one-year wood. These instances illustrate the profuse production of flowers in the plum.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LEGEND FOR PLATE 5

A.—*P. virginiana.* Showing nearly an even development of both ovules at bloom.

B.—*P. virginiana.* The suppression of one ovule before fertilization.

C.—Cheney. The suppressed ovule 11 days after bloom. This is an extreme case of degeneration in the nucellus.

D.—Minnesota No. 12 × Minnesota No. 21. The crowding of the suppressed ovule 17 days after pollination.

E, F.—Manitoba. The condition of the ovules in flowers with aborted pistils which fell soon after bloom.

G.—Minnesota No. 35. Showing the slight elongation of embryo sac 14 days after bloom when pollination was prevented by snipping the stigma.

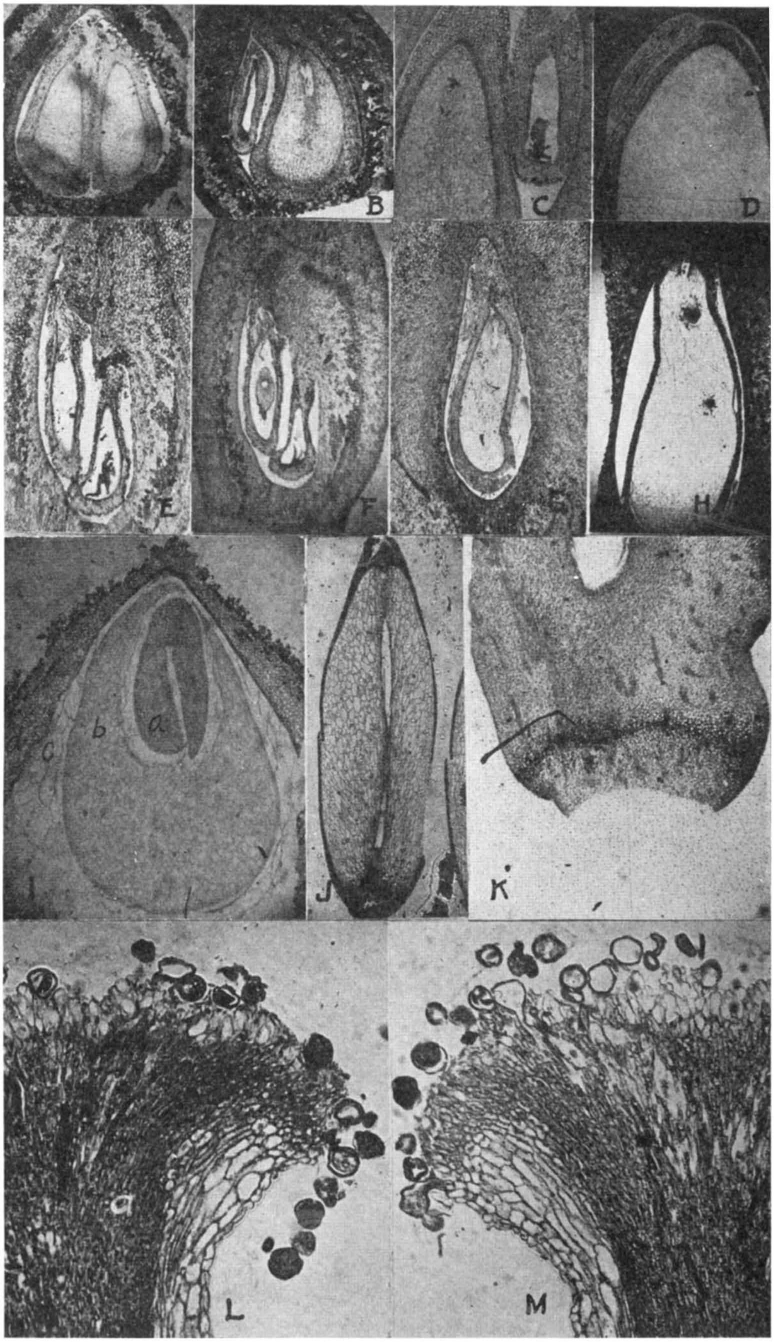
H.—Minnesota No. 35. A later stage of G, showing the extension of the embryo sac 23 days after bloom.

I.—Yellow Egg. Self-pollinated. Illustrating the embryo development two months after pollination and the three tissues in the seed, namely, a, embryo; b, endosperm; c, nucellus; and d, integument.

J.—Minnesota No. 35. The extension of the canal in a pistil 34 days after bloom in which fertilization had been prevented by snipping the stigma. Compare the slight embryo-sac extension with G and H.

K.—Assiniboin. Showing abscission layer at base of plum 12 days after bloom. Pistil from one-year terminal shoot. In this pistil fertilization has not taken place and the egg is degenerating. Plums of this type constitute the second drop.

L, M.—Minnesota No. 21. Pollen on a self-pollinated stigma 24 hours after pollination. Tree grown in greenhouse. Note the pollen-tube growth in self-pollinated stigma. Some normal-appearing grains have not germinated. Aborted grains have not sent out tubes and the empty coats of grains that have germinated are still present.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