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FRUIT-BUD AND FLOWER FORMATION IN THE SULTANINA GRAPE

A. J. WINKLER AND E. M. SHEMSETTIN

MORPHOLOGY OF THE FLOWER AND FRUIT OF THE LOQUAT

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OF THE LOQUAT[1,2]

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INTRODUCTION

THE LOQUAT (*Eriobotrya japonica*), indigenous to China, is grown more or less extensively in California, Florida, and the Gulf States. *Eriobotrya japonica* is in the family Rosaceae, subfamily Pomoideae. The Greek translation of *Eriobotrya*—"woolly inflorescence"—well depicts the extremely hairy condition of buds, flowers, fruits, and leaves. In China the loquat is called "rush orange."

Bailey[1][4] describes the tree as small and evergreen with leaves "elliptical to oblong-ovate, nearly sessile, and remotely toothed." The small, white flowers are borne in woolly panicles 4 to 7 inches long (fig. 1). Development of the panicle is acropetal. The flower panicles are terminal on the current season's growth. Growth extension occurs from terminal leaf buds on nonfruiting branches and from the distal lateral leaf bud on fruiting branches. The flowers are pentamerous, and each of the five carpels contains two ovules; ordinarily only one to eight seeds develop. Seedless varieties, though sometimes reported, are of no commercial importance. Condit[2] has described climatic adaptations, culture, and varieties of the loquat.

This paper presents the results of a study of the morphology of the loquat flower and fruit.

MATERIALS AND METHODS

Loquat buds, flowers, and fruits were collected at weekly intervals during the 1934–35 season from a tree of the Advance variety on the University Farm at Davis, California.

The extremely hairy condition of the buds made paraffin sectioning difficult. Celloidin, double infiltration as described by De Zeeuw,[3] butyl alcohol, and glycerine-butyl alcohol were therefore employed as softening agents in attempts to avoid tearing of sections. Although fairly satisfactory results were obtained with celloidin-imbedded material, the

method adopted was to kill bud samples in formalin-acetic-alcohol fixative and then expose them for several hours to 5 per cent hydrofluoric acid in 70 per cent alcohol.

Flowers and fruits were scraped practically free of hairs with a scalpel before being exposed to the killing agent. Soaking of the paraffin blocks
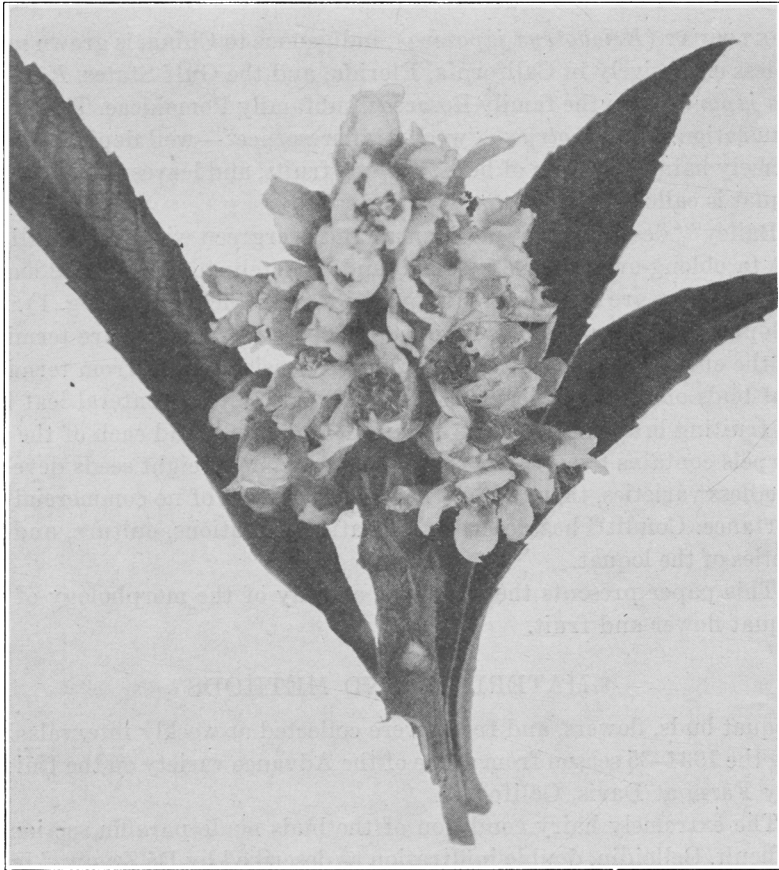

Fig. 1.—Panicle of loquat flowers at anthesis.

in water in an oven at 30° centigrade for several weeks facilitated sectioning.

Medium Flemming's killing agent and Navashin's modification of Karpechenko's were employed for flowers used in macrosporogenesis studies. The former proved more satisfactory. Delafield's haematoxylin stain was used for most of the material; gentian violet or Haidenhain's

haematoxylin for more delicate work. Although sections were usually cut 14 microns thick, flower sections used in macrosporogenesis studies were 8 microns in thickness.

FLOWER-BUD DEVELOPMENT

The period of flower-bud differentiation and of flowering is extended. Buds showing early stages of flower-bud development were collected as late as November 6. Buds were not collected early enough in 1934 to indicate at what time of year the first signs of differentiation began.
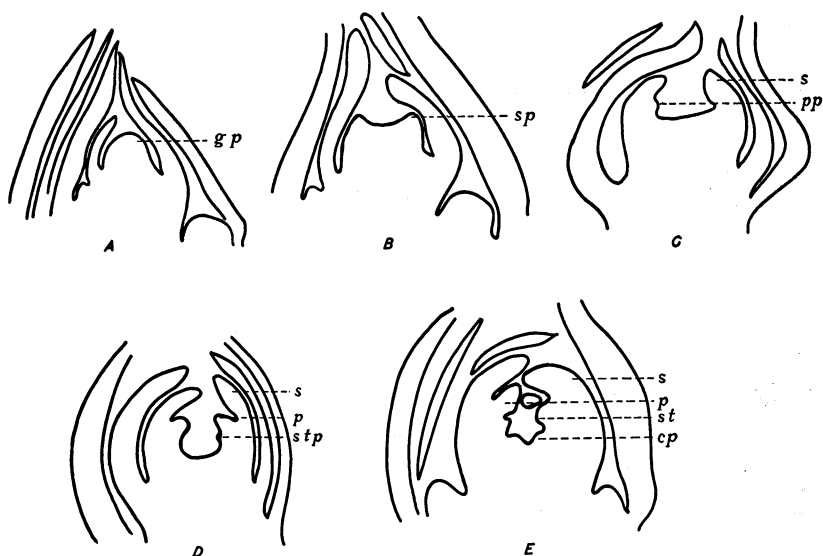

Fig. 2.—Sections showing development of typical loquat flower bud. The parts are: *gp*, growing point; *s*, sepal; *p*, petal; *st*, stamen; *sp*, sepal primordium; *pp*, petal primordium; *stp*, stamen primordium; *cp*, carpel primordium.

Buds that possessed flower primordia were tagged on October 17; these were in full bloom by December 28. The period of full bloom for the whole tree was from October 17 to February 1.

The first discernible sign of flower-bud differentiation is a distinct, blunt protuberance. As a result of a rapid multiplication of the cells at the outer edge of this protuberance, a slightly elevated ridge appears (fig. 2, *A*). Very soon cell division at five points around the circumference of this ridge results in the formation of the sepal primordia (fig. 2, *B*). The torus, meanwhile, has been undergoing rapid development, especially basal to the calyx. As a result of this toral growth the rapidly forming sepals are elevated. They develop quickly, and their apices grow towards each other so as to enclose the distal portions of the carpels. The

sepals as well as all of the exposed toral surfaces are densely covered with long epidermal hairs.

Very soon after the sepal primordia develop, those of the petals appear, arising in a whorl alternating with the sepals (fig. 2, *C*). Growth of the petals and sepals continues, and soon they cover over in "hood" fashion the cup-shaped torus (figs. 2, *D* and 2, *E*). Each petal is constricted at the point of divergence from the torus.

Shortly after the petal primordia appear, those of the stamens develop in three cycles around the torus directly inside the petals. The outer cycle of stamens develops first; then the middle, and finally the inner cycle is initiated. The innermost and middle cycles of stamen primordia number five each, while those of the outer consist of ten, there usually being twenty stamens in all. The stamen cycles are too close together to be easily differentiated from one another by the naked eye.

The five carpel primordia are formed soon after the inner cycle of stamen primordia is developed. They arise from the cup-shaped receptacle just basal to the inner cycle of stamens and some distance from the center of the torus (that is, at the apex of the pedicel). Infolding of the carpel edges takes place very early in carpel development. Carpel growth proceeds rapidly, and soon adnation of the lower portions of the carpels is so complete that one cannot discern the infolded edges, which are clearly distinguished only in the upper half of the flower. The styles are completely free. The ovules arise from the infolded carpel edges which form the placentae. The exposed surfaces of the carpels develop a very dense pubescence (plate 1).

During the development of the carpels the torus continues to grow, and thus elevates the stamens, petals, and sepals. The tip of the axis is below the carpels, and the placentae are carpel tissue only. Since the center of the torus does not undergo any growth, there is no central column of axis tissue extending up through the set of five carpels.

As the distal portions of the ovaries remain exposed and are not covered with receptacle tissue, the loquat flower is only partially epigynous. A photomicrograph of the developing floral parts is shown in plate 2.

OVULE DEVELOPMENT AND MACROSPOROGENESIS

Early in carpel development there occurs, slightly below the middle of each carpel, a growth of the placenta which develops into the ovule. Soon a second protuberance is seen—directly basal to the first—which becomes the obturator. Although the obturators are relatively large, their later growth does not keep pace with that of the ovules; at the time of full bloom the obturator is scarcely discernible to the naked eye.
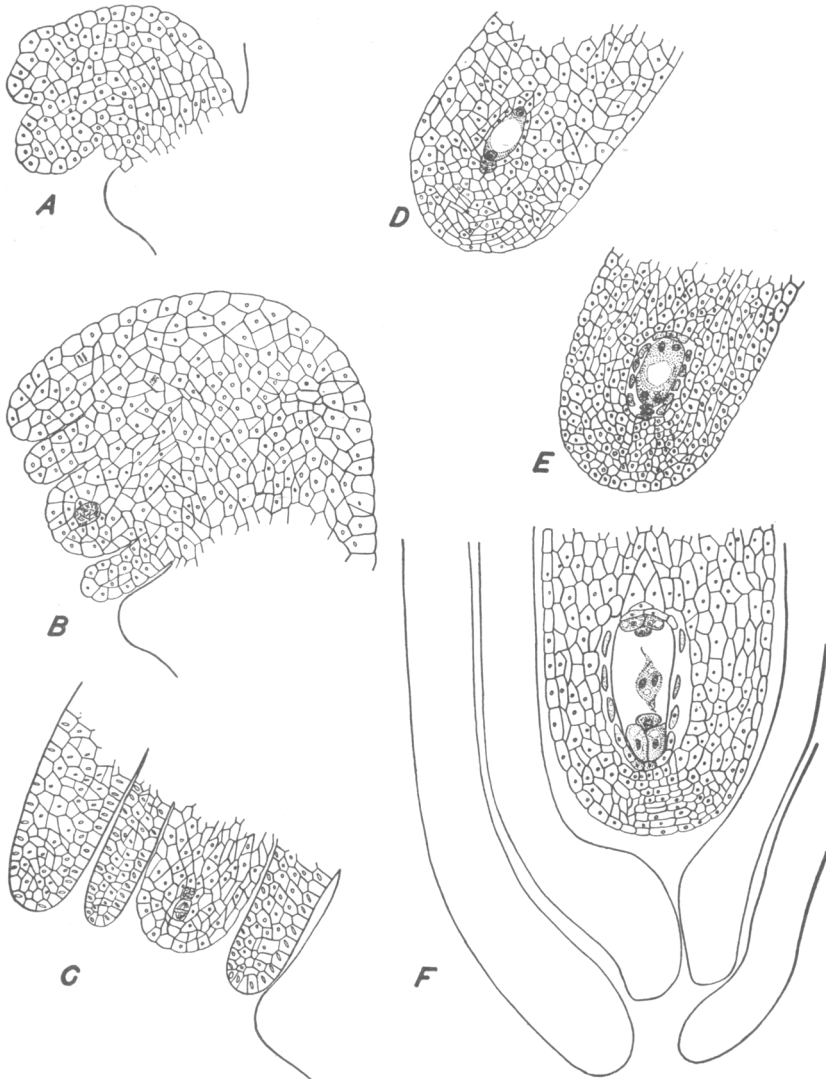
Fig. 3.—Macrosporogenesis in the loquat. *A,* Development of integuments; *B,* macrospore mother cell just before reduction division; *C,* formation of linear tetrad of macrospores; *D,* two-celled gametophyte and degenerating macrospores; *E,* four-celled gametophyte and degenerating nucellar cells, contiguous to embryo sac; *F,* mature macrogametophyte.

The two ovules in each carpel are usually side by side, one being slightly more elevated than the other. As growth of the young ovule continues, there is more cell division on one side than on the other, and soon the micropylar end points downward and outward toward the car-

pel wall, later becoming typically anatropous. At about the time when the ovule is at a right angle to the funiculus, first signs of the inner integument appear. From the epidermis of the ovule at a level just basal to a point where the macrospore mother cell will later develop arises the inner integument (fig. 3, *A*). Very soon thereafter the outer integument originates from the epidermal layer just basal to the point of origin of the inner integument. The outer integument grows somewhat faster than the inner; and, by the time the female gametophyte is fully developed, the integuments fully enclose the nucellus.

Soon after the appearance of the initials of the inner integument, the macrospore mother cell may be observed. Its origin was not noted although an archesporial cell was observed in a few instances. The macrospore mother cell usually lies two to four cells back from the micropylar end of the nucellus. Soon the macrospore mother cell undergoes heterotypic division. A chromosome plate in such a cell during heterotypic division is seen in figure 3, *B*. Thirty-two chromosomes were counted in this plate. To ascertain definitely the chromosome number, more such plates would be necessary. Moffett[5] has reported that root tips of the loquat had thirty-four chromosomes, and it is possible that two chromosomes were not visible in the plate counted. Loquat chromosomes are very small and are subject to clumping during fixation.

Heterotypic is followed immediately by homeotypic division, and a linear set of four macrospores is formed (fig. 3,*C*). The chalazal macraspore is functional, and the other three disintegrate (fig. 3, *D*). No irregularities could be seen in the formation of the eight-nucleate female gametophyte. During the development of the embryo sac there is a dissolution or breaking down of cells of the nucellus contiguous to the embryo sac. This dissolution process, which is concomitant with sac development, seems to allow for volume increase of the sac. A conspicuous feature of the nucellus when the mature female gametophyte is formed is a group of large, elongated cells at the chalazal end of the embryo sac (figs. 3, *E* and *F*).

Tetrads of microspores could be observed during the formation of the macrospore mother cell. When the linear tetrad of macrospores was formed, the microspores were still in the tetrad stage. During the formation of the two-celled macrogametophyte, pollen was observed in the anthers.

The curvature of the nucellus increases during the development of the macrogametophyte; and by the time the eight-nucleate state is attained, the ovule is completely anatropous. The micropylar end rests against the oburator.
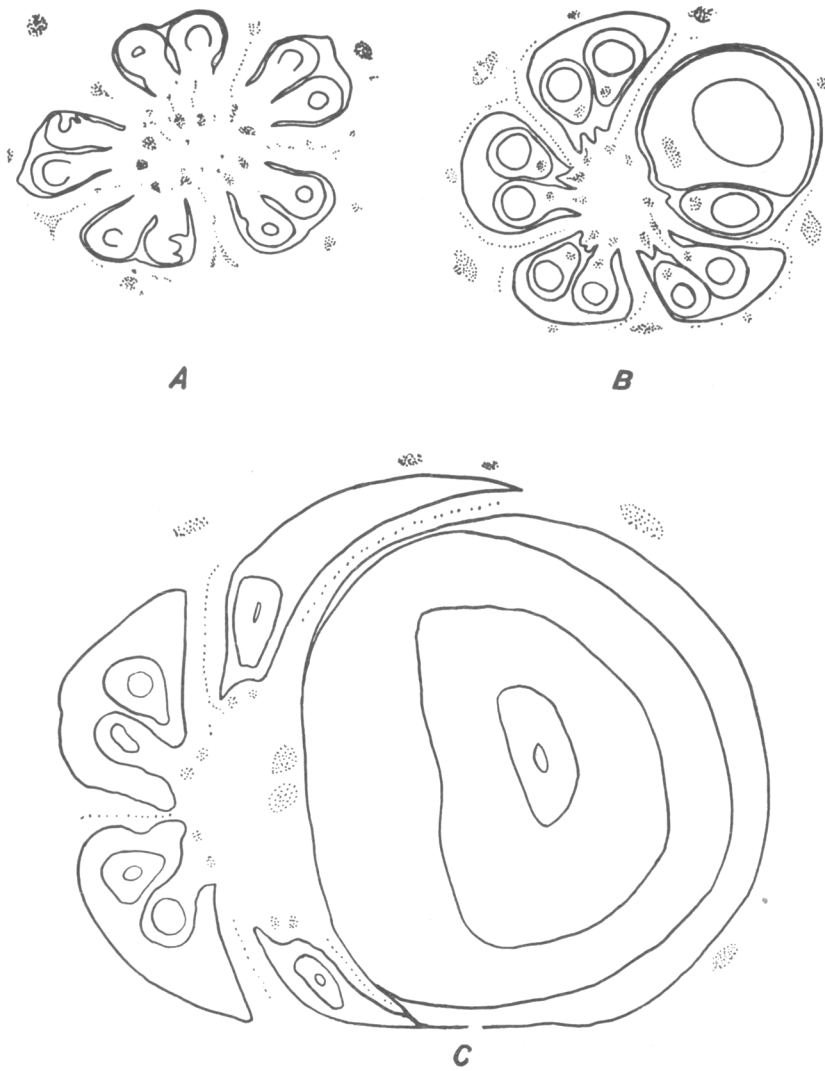
Fig. 4.—Development of seed in loquat and abortion of ovules. *A*, Ten potential seeds; *B*, initiation of abortion of nine ovules; *C*, seed and aborted ovules (note distortion of carpel walls due to growth by seed).

OVULE ABORTION

Apparently all ten ovules develop normal gametophytes. It was not discerned whether the eggs in all ten ovules are fertilized or not; however, in this variety only one or two ovules develop embryos. The cause of abortion of eight or nine ovules has not been determined. Climatic

influence on the nutritional status of the ovules may have some importance, however, since in the more southern portions of the state four or five seeds often develop in the variety Advance. In the ovules which failed to develop embryos the gametophytes remained apparently normal for several days and then degenerated. Two functional seeds seldom develop in the same carpel; but, where they do, one soon supersedes the other, and only one seed develops in the fruit. The functional seed or seeds develop very rapidly and soon distend the confines of their carpel so that the other carpels are distorted and compressed into a very small area. Degrees of abortion are illustrated in figure 4, *A*, *B*, and *C*.
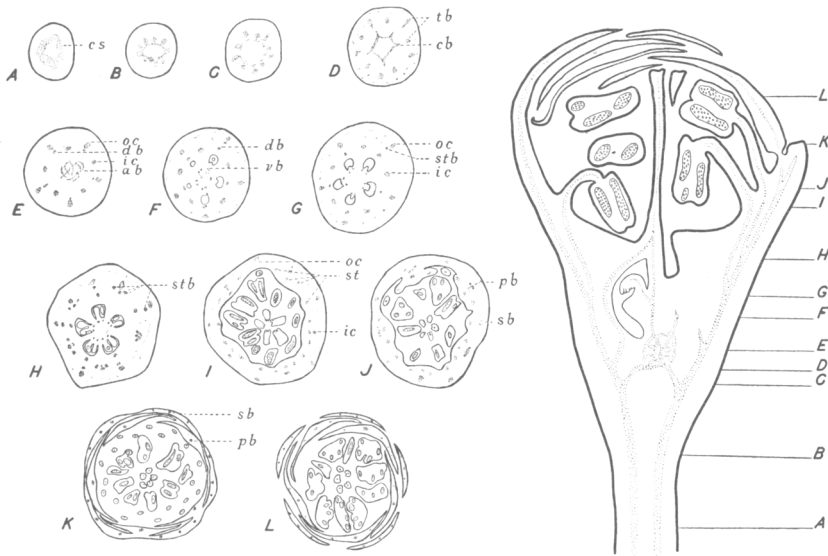

Fig. 5.—Vascular anatomy of loquat flower. Diagram on right shows levels at which depicted sections were cut. The parts are: *cs*, central stele; *cb*, central bundles; *tb*, toral bundles; *db*, dorsal bundles; *ab*, anastomosing bundles; *stb*, stamen bundles; *sb*, sepal bundles; *oc*, outer-cycle primary bundles; *ic*, inner-cycle primary bundles; *vb*, ventral bundles; *pb*, petal bundles.

VASCULAR ANATOMY OF FLOWER AND FRUIT

The vascular anatomy of a typical terminal flower is described in this report. Terminal flowers on the panicle have longer pedicels, and the method of bundle divergences is easier to trace in detail than in the pedicels of lateral flowers.

At the base of the pedicel the vascular cylinder is complete and more or less pentagonal (fig. 5,*A*). As it is traced up through the pedicel, however, it is seen to divide into ten distinct bundles (fig. 5,*B*). The exact point where this division occurs varies with different flowers, but is

usually about midway up the pedicel. The ten bundles diverge outward and are soon more or less equidistant, and the smaller bundles remaining between them diverge toward the median axis of the pedicel (fig. 5, *C*). These smaller bundles in the median portion form a star-shaped column just distal to the apex of the pedicel (fig. 5, *D*). The ten major bundles at this point form two cycles of five each, every alternate bundle being in the same cycle. At the apex of the pedicel they diverge into the toral region of the flower and follow the region between the pith and cortex.

Just basal to the carpels, the small bundles in the inner cylinder or median portion undergo extensive anastomosis and invade the central pith area to a considerable extent (fig. 5, *E*). This anastomosis continues to a level about even with the base of the carpels. Above this point the bundles become discrete. Two of these distinct small bundles extend up through each carpel and form the placental bundles of the flower. These ventral bundles furnish the vascular supply to the ovules and fuse before extending into the styles.

As has been stated, the ten primary or toral bundles follow the line of demarcation between the pith and the cortex. The bundles of the outer cycle are opposite the dorsal sutures of the carpels, and those of the inner cycle alternate with the carpels. At a level just below the bases of the carpels, five small bundles diverge from the outer cycle of toral bundles (fig. 5, *E*). All five do not branch from the primary bundles at exactly the same level. These five bundles (the dorsals) diverge toward the center of the flower, closely follow the dorsal sutures of the carpels, and then pass through the distal portions of the carpel walls and up into the styles (fig. 5, *F*). Thus there are fifteen main vascular bundles in the carpellary system—ten ventrals and five dorsals.

From each of the five primary bundles of the outer cycle arises one branch from the inner or pith side at a point on a level with the bases of the ovules (fig. 5, *G*). Although the exact point of origin of these branches varies, none branches at a point higher than at a level even with the median portion of the ovules. These five branches continue upward through the torus and terminate, one in each stamen of the inner cycle. At a point somewhat distal to the point of origin of the bundles that terminate in the inner stamen cycle, one bundle branches off from each of the inner cycle of primary toral bundles (fig. 5, *H*). These branches extend upward and terminate in the middle cycle of stamens. Slightly higher than the point of origin of the last-mentioned bundles, two more branches are given off from each of the inner cycle of primary toral bundles. These branches constitute the vascular supply of the outer cycle of stamens (fig. 5, *H*).

The petals derive their vascular supply from the inner cycle of primary bundles. After divergences for both the middle and outer cycle of stamens have been given off, each bundle of the inner cycle continues up through the cup-shaped torus (above the carpel level) and breaks into three separate bundles. The innermost of these three diverge into the petals, and the outer two extend into the sepals, as will be described later (fig. 5, K). The bundle extending into the petals forms the midrib and gives off branches to form a vascular network.

The sepals obtain their vascular supply from both the inner and the outer cycle of toral bundles. The median bundle or midrib of each sepal is the termination of one of the outer toral primary bundles. The small lateral bundles in the sepals are divergences from the inner primary bundles mentioned above. Following the divergences into the petals by one of the three bundles described in the foregoing paragraph, the second extends into a sepal to the right, and the third into the sepal to the left of the adjacent petal (fig. 5, K).

The cortex of the flower and fruit gains its vascular supply from branches from the ten primary toral bundles. These branches divide comparatively little until they reach the subepidermal region, where they branch and anastomose profusely, forming a network of fine bundles.

The vascular anatomy of the loquat resembles that of the apple as described by Kraus and Ralston[6] but differs in several respects. The anastomosis occurring in the median portion of the stele of the pedicel does not extend through so long a distance in the loquat as in the apple. Divergences for the stamen bundles occur at considerably lower levels in the loquat than in the apple.

DEVELOPMENT OF THE FRUIT

After fertilization, the loquat fruit develops very rapidly. The first indication of fruit enlargement is a thickening of the toral rim immediately above the carpel level. The whole toral region undergoes cell division and enlargement more or less uniformly throughout. The sepals grow toward the center and cover over in "hood" fashion the distal portions of the carpels. The sepal bases thicken and persist as permanent structures, whereas the petals, stamens, and styles dry up. The hood or cap enclosing the distal portions of the carpels may be excised from immature fruits, and the five carpels be exposed to view (plate 4, A). Remnants of the petals, stamens, and styles may be seen when this toral rim is cut off.

The toral rim of a number of fruits was removed on April 11, 1935, while the fruits were still on the tree. When the cut was made above the

carpels, the torus was almost completely regenerated, and the carpels were again hooded over; but whenever the cut pierced the carpel proper and cut a portion of the ovule, the fruits either died or ripened prematurely without attaining their full size.

The functional ovule develops into a fertile seed occupying the whole central region of the fruit; and the confines of the individual carpel walls which surrounded nonfunctional ovules are wholly disrupted (plate 3, *A* and *B*). The distal wall of the carpel in which the functional seed is found undergoes considerably more growth than do those of the other carpels. The result is an elevation of the distal wall of the carpel containing the functional ovule over those of the others.

By the time the fruit is mature, the carpel walls are more or less parchmentlike. The seed is extremely hard, the integuments and cotyledons being very resilient.

The skin of the mature fruit is comparatively tough, being more leathery than that of either the pear or the apple.

As has been indicated in the foregoing discussion, the edible portion of the loquat is entirely toral in nature, consisting of the pith and cortical areas. Development of the edible portion consists of a rather uniform growth of receptacle tissue throughout the fruit (plate 4, *C*). The toral cells of the mature fruit are large, thin-walled, and very juicy.

EFFECT OF LOW TEMPERATURES ON DEVELOPING LOQUAT FRUITS

Since the flowering period at Davis in the 1934–35 season was from the middle of October to February, the flowers and young fruits were subjected to comparatively low temperatures. The mean temperature of December was 48.3° F, and that of January, 45.8°; but the flowers not only were uninjured but were fertilized and developed into fruits during this period. Late in January the temperature on one night dropped to 29°. This resulted in an injury to many young fruits, thinning numerous panicles to one fruit. In nearly all cases, the terminal fruits persisted.

Many fruits injured by this cold period did not drop from the tree. In them the toral region became loosened from the carpels and could easily be "shelled" off. When the ovules of these injured fruits were not affected, these loosened areas tightened within a few weeks, and the fruits grew to normal size. The fruits that had injured ovules and yet did not drop never recovered from the injury to the torus; they grew very little in size thereafter and ripened prematurely.

SUMMARY

Floral development of the loquat (*Eriobotrya japonica*) is acropetal. The development of the floral organs is here described in detail. The loquat flower is semiepigynous and possesses ten ovules.

In the development of the macrogametophyte the macrospore mother cell undergoes heterotypic and then homeotypic division, resulting in the formation of a tetrad of macrospores. The chalazal macraspore only is functional, the remaining three disintegrating. As a result of three successive nuclear divisions, an eight-nucleate female gametophyte is formed.

Apparently normal macrogametophytes are formed in all ten ovules, but in the variety Advance only one or occasionally two seeds develop in each fruit at Davis, California.

The vascular anatomy resembles that of the apple, but the region of anastomosis in the stele of the pedicel is less extended than in the apple. Also, the point of divergence of the stamen bundles occurs at a considerably lower level in the loquat than in the apple.

The fruits have a hood or cap of toral tissue enclosing the distal portion of the ovary. This toral cap may be excised, and the distal portions of the carpels exposed to view.
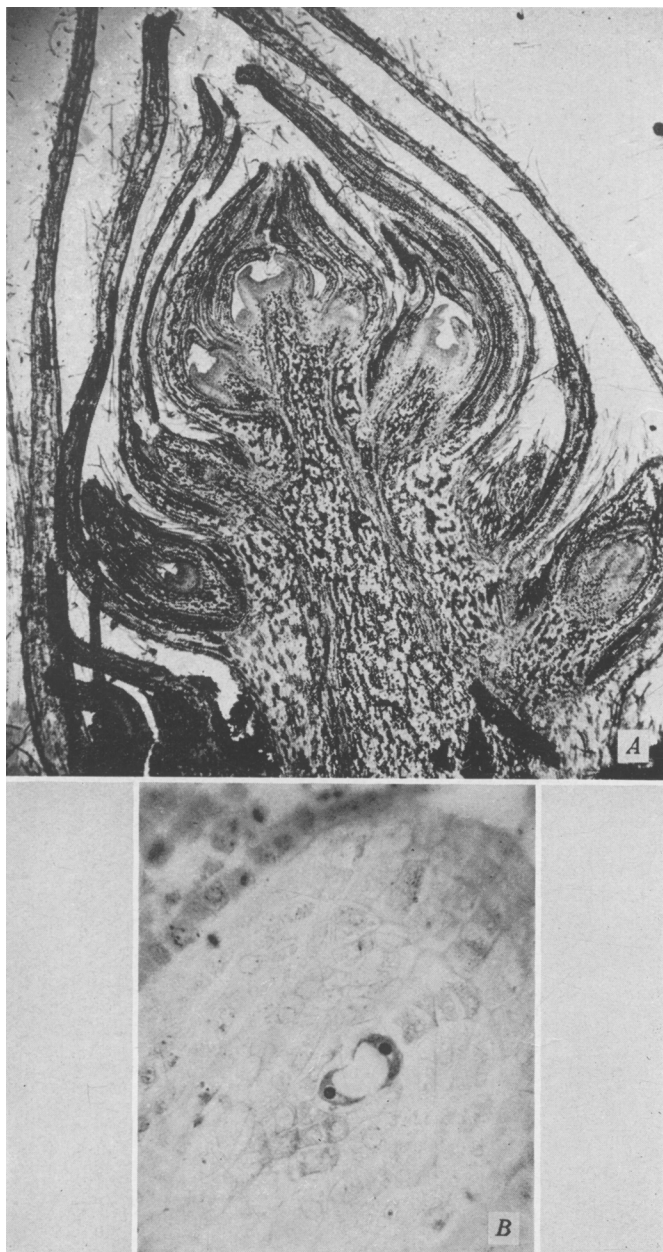
The functional seed or seeds are fertile and occupy the whole central region of the fruit. Their development results in a marked distortion of the carpel walls and a complete dislocation of the nonfunctional ovules and the carpels in which they were located.
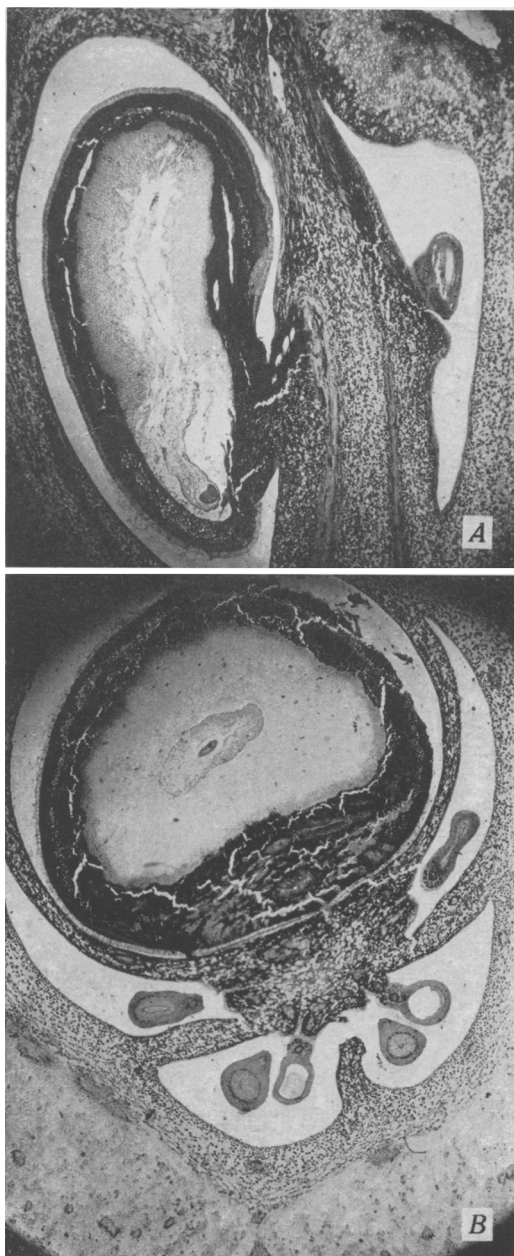
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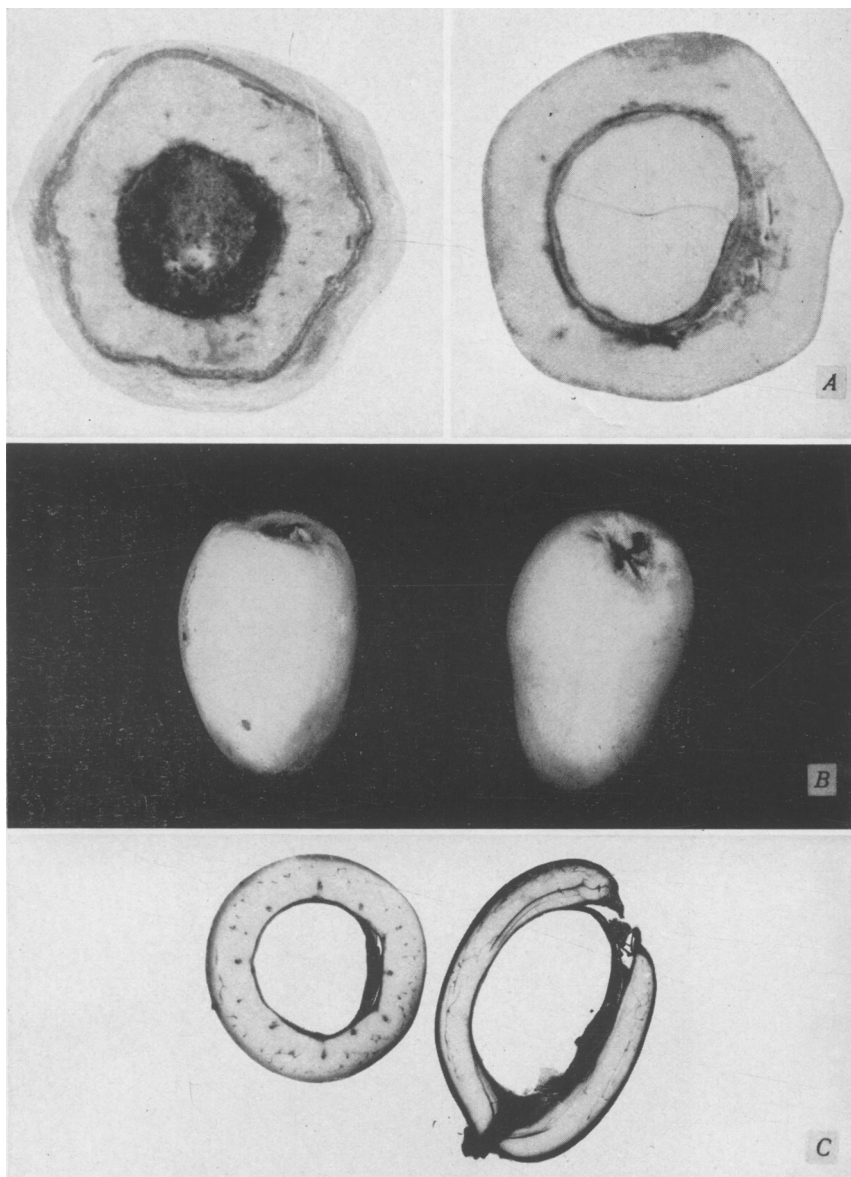   The chromosome constitution of the Pomoideae. Proc. Royal Soc. Botany 108:423–446. 1931.

Photomicrograph of very young loquat flower. Note extreme hairiness of bracts
and of the young flower.

A, Photomicrograph of developing floral parts of the loquat.
B, Photomicrograph of two-nucleate female gametophyte.

A, Photomicrograph of longitudinal section of young fruit, showing developing seed (at left) and aborting ovules (at right). *B*, Cross section of young fruit showing developing seed and aborting ovules.

A, In the fruit on the left, the toral rim has been excised to expose the carpels. The fruit on the right has been cut to show the seed and the distorted carpels. *B*, Mature loquat fruits of the Advance variety. These fruits are golden yellow. *C*, Cross and longitudinal cleared sections of loquat fruits. The seed has been removed in each case. The conspicuous and edible portion of the fruit is entirely toral in nature. The primary toral bundles in the cross section indicate the line of demarcation between pith and cortex. The compressed carpel walls may be seen in both sections.