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INTRODUCTION

INVESTIGATIONS OF THE OLIVE (*Olea europaea* L.) have been confined, for the most part, to growth habit, pollination, propagation, physiological requirements, and other horticultural aspects; few studies have been concerned with its morphology. Certain structural features of the flower and fruit have been briefly mentioned in various general sources. Ruby (1917) has surveyed varieties of *Olea europaea,* making physiological and limited morphological observations on both the mature flower and the fruit. Pirotta (1919) and Petri (1920) studied floral characteristics in relation to field conditions, whereas Weber (1928) made extensive comparative morphological investigations on flower types of the Oleaceae. More recently Andersson (1931), in his embryological studies of representative forms of the Oleaceae, has traced the development of the macrogametophyte and the early stages following fertilization in *Olea,* wherein he studied *O. chrysophylla* and *O. europaea.*

The investigations here reported are confined to *Olea europaea,* horticultural variety Mission, and are fourfold in extent, including (1) the development of the flower; (2) the general vascular relations in the flower; (3) the development of the macrogametophyte, in view of Andersson's work; and (4) the general morphological changes involved in the development of the fruit.

METHODS

Material was collected every three days from the first appearance of the inflorescence until two days before blooming, then every day for the following two weeks. The time between collections thereafter was gradually increased until maturity of the fruit.

Although several fixatives were tested, a modified Navaschin's fluid and formalin-acetic-alcohol gave the most satisfactory results with flower buds, while the latter fixative alone was used for young fruits. Young inflorescences were prepared for sectioning by the dioxan-paraffin method,

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which was replaced by the usual alcohol-xylol-paraffin series for older buds and young fruits. Buds fixed in Navaschin's fluid were vacuumed to facilitate the penetration of the fixative. Sections were cut 10 to 22 microns thick, according to the size of the material studied, and were stained in Delafield's hematoxylin or safranin and fast green. The latter schedule was modified from that suggested by Moore (1936). Both microtome and freehand sections were made of the more fleshy parts of older fruits, while the hard stone, or pit, was prepared by sawing sections 2 to 3 millimeters thick and thinning down to about 35 microns with No. 1½ to No. 2/0 sandpaper. The section was then secured to a slide with balsam (Newby and Plummer, 1936). After the balsam had hardened in an oven held at 104° F the section was ground down further with worn No. 2/0 sandpaper and was finished with crocus cloth. Staining was done with iodine green.

FLOWER DEVELOPMENT

The olive fruit has been considered similar to that of the peach, plum, cherry, and apricot. The floral development of these forms has been discussed little in the literature. Studies such as those of Goff (1899), Drinkard (1909), and Tufts and Morrow (1925) have been concerned principally with the time of occurrence of the different phases of floral-bud differentiation. The appearance and rapid differentiation and development of the olive floral axis differ from the other forms mentioned above, wherein the buds continue to develop during the season preceding bloom. In the olive, however, corresponding stages of development are not apparent until about eight weeks before the flowers blossom.

As Kraus (1913) has stated, one can best determine the true nature of any structure by studying the origin of its parts and their arrangement during their early stages. One must observe the flower bud in its earliest stages in order to understand the relations existing between the floral organs in their more mature form.

The paniculate inflorescence (fig. 1) of *Olea europaea* arises in the axil of each of the oppositely arranged leaves of a branch as a cushion of meristematic tissue that develops into a lateral axis, terminated by a flower. From this axis, in its development, arise short lateral axes, also terminated by a flower. These lateral departures themselves may form one set of lateral axes. Each of the lateral flower primordia develops in the axil of a bract. Varietal differences exist in the density and size of the inflorescence and in the size, shape, and color of the flower buds (Ruby, 1917).

Sepals.—The individual floral primordium is first evidenced by a broadening of its tip (receptacle) into a flattened conical mass. The four

sepal primordia arise as slight elevated masses at the outermost rim of the meristematic cone, which continues to broaden during the appearance of the floral parts (plate 1, *A*). Development at first is nearly vertical, but soon the upper rounded edges bend inward. The sepal tips were not observed to make contact. Soon after initiation of sepal primordial growth, meristematic activity throughout the receptacle ring underly-

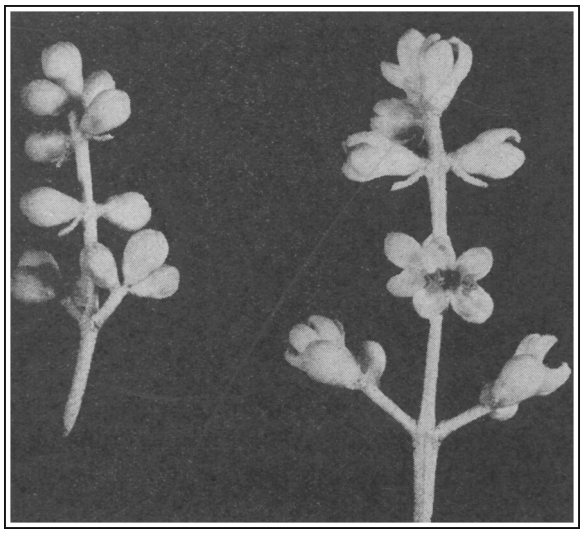

Fig. 1.—Inflorescence of *Olea europaea* var. Mission.

ing the sepal primordia elevates this ring during sepal development into a calyx tube, the four protuberances remaining as the four short distinct sepal lobes. Later a small lateral appendage arises near the base on the dorsal surface of each of the two sepals that are alternate with the bract (plate 1, *H*). Since earlier reference to such structures has not been found, the descriptive term "lateral appendage" is used here for want of a better one.

Petals.—Soon after the sepal cycle extends above the meristematic plane, an inner adjacent petal whorl of four protuberances appears, alternate with the sepals (plate 1, *B*). Apparently there is no relation between the extent of the sepal development and the appearance of the petal primordia. The petal primordia frequently arise almost simultaneously with those of the sepals. In very early stages, the petal protuberances appear as blunt elevations; later, however, meristematic growth takes place throughout the petal cycle, forming a corolla tube. The four petal primordia remain distinct upon the upper rim of the tube, developing into the four petal lobes, whose upper portions assume an almost

acute angle with the calyx, the tips approaching each other, roofing over the center of the young flower. Growth of the petal lobes, however, does not cease here: their tips bend downward, hooklike, while the contacting dorsal surfaces are frequently flattened (plate 1, *D–H*). Petal development proceeds more rapidly than sepal after the first stages; the tips of the petals extend down between the stamens in older buds (plate 1, *I*).

A distinct difference exists among varieties in the shape of the corolla lobes and in the general dimensions, depth, outward appearance, and line of the rim of the calyx (Ruby, 1917).

Stamens.—Origin of the two stamen primordia does not immediately follow that of the petals. These two protuberances arise on the ventral surface of the petal cycle and alternate with the lobes of two opposite petals. These stamen primordia arise near the base of the petal ring shortly before the four petal lobes converge toward each other; the time of origin, however, varies (plate 1, *C*). Growth is rapid both vertically and laterally. The centripetal growth of the oppositely placed stamens usually brings the ventral surfaces of the anthers into contact; their corresponding surfaces become appressed slightly against each other, while the distal regions become rounded. The lobed condition of the anthers is not evident in early stages, but develops later. In the opening of the flower, each stamen pushes backward the two petals behind it, so that the first step in its unfolding corresponds to the opening of two parts of the corolla; the petals then disjoin to form symmetrically into a cross (Ruby, 1917). The elevation of the entire petal ridge elevates the stamens at the same time, so that in later stages the stamen cycle is above that of the sepals and petals (plate 1, *F–I*).

The elevation of the sepal, petal, and stamen ridges above the meristematic receptacular surface and their almost total inclusion of this area gives the illusion that the central, undifferentiated portion is a small depression. The "depression" is, however, the tip of the floral axis and a region of checked growth (plate 1, *B*). Broadening of the receptacle accompanies further development of the floral parts, the depression appearing to widen and deepen as the surrounding areas enlarge. The now centrally inclined stamens gradually separate from each other, and their adjacent surfaces become slightly rounded. The tips of the petals penetrate to varying extents between the distal portions of the stamens.

Carpels.—The two carpel primordia appear on the innermost rim of the receptacle below the base of the stamen cycle and in the region of checked apical growth (plate 1, *D*). They are at first observed as small rounded protuberances, and their usual development is accompanied by a gradual elevation of the base of the central depressed area through the extension of the tip of the axis. The broadening of the receptacle and

the development of the stamen, petal, and sepal cycles continue with the growth of the carpellary rim (plate 1, *E–G*). Infolding of the carpel margins, with the formation of the two locules, is not evident in early stages of carpel development. Extended growth of the apical portion of the carpels forms the short style.

Thus, by the broadening and gradual central elevation of the tip of the axis, the carpellary rim is raised to a level slightly below those of the petals and sepals or, frequently, to the same level as the sepal and petal cycles (plate 1, *I*).

In studying the relation between the ovary and the other members of the flower, one must consider the several aspects of that relation from its earliest to its final state. Concerning the general subject of the origin of the carpel primordia from the wall of the depression formed by the tip of the axis (as in *Olea europaea* var. Mission), Coulter and Chamberlain (1909) state:

> In certain cases the region of the growing point belonging to the carpels ceases to develop while the rest of the growing point continues to develop *en masse*, forming a cup, or urn-like outgrowth, from the rim of which the three outer sets develop separately, forming the perigynous flower. In this case the carpels arise from what seems to be a depression in the center of the torus, but which, of course, is the region of checked growth.

The lowered position of the carpels on the receptacle of certain rosaceous forms is found by Jackson (1934) to be only apparent and is caused by the formation of the flower tube, consisting of the fused basal portions of the sepals, petals, and stamens. In *Rosa*, on the other hand, that portion of the carpels apparently below the level of the floral organs results from the invagination of the carpel-bearing part of the floral axis and from the formation of the upper part of the tube by fusion of the basal parts of the petals, sepals, and stamens.

Zonation, which is the common origin of two or more adjacent cycles, is another aspect of the perigynous condition, displayed in *Olea europaea* var. Mission especially by the nondivergence of the petal and stamen cycles. Coulter and Chamberlain (1909), viewing this condition, say:

> The tendency to zonal development, however, is carried further when a whole region arising *en masse* produces two or more cycles of floral members. In the simplest cases two cycles are thus produced, as is illustrated by the strong tendency of the petaliferous and staminiferous cycles to have a common origin in sympetalous flowers, resulting in the appearance of "stamens inserted on the tube of the corolla."

Intercalary growth of the carpels and, simultaneously, of that part of the urn-shaped torus bearing the sepals, petals, and stamens, occurring soon after formation of the floral parts and where only the carpels pro-

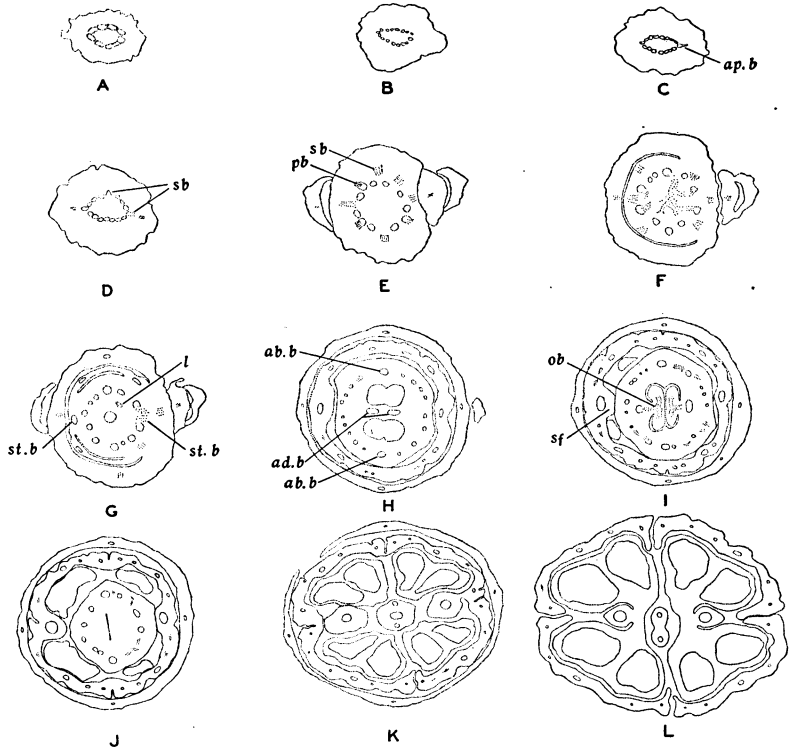

Fig. 2.—Diagrams of the floral vascular system from transverse sections, at successively higher levels. *A*, Lower petiole—showing a ring of eight bundles. *B*, *C*, *D*, Sections of the upper petiole—showing at *B*, a ring of sixteen bundles; at *C*, a diverging sepal-appendage bundle (*ap. b.*); and at *D*, diverging sepal bundles (*sb*). *E*, *F*, *G*, Sections of the receptacle. *E*, Sepal (*sb*) and petal (*pb*) diverging, leaving eight undiverged bundles to go up through the carpel walls. *F*, Divergence of branches from carpel-wall bundles in the formation of the adaxial bundles. Part of the calyx tube is separating from the receptacle, while the petal and sepal bundles are continuously departing from the vascular ring. The eight remaining carpel-wall bundles have already begun to branch (upper portion of the ring). *G*, Base of the locules, with the base of one locule evident (*l*). Both calyx and corolla are now departing from the receptacle. One stamen bundle (*st. b*) is formed and another is just forming. The mass of adaxial bundle tissue in the center of the receptacle has not yet formed into two groups. *H*, Section near the middle of the ovary; the two groups of fused adaxial bundles (*ad. b*) are now evident and the formation of the new carpel wall and petal bundles has taken place. Calyx and corolla have completely separated from the ovary. *I*, Section near the funiculus of each of the four ovules showing ovule bundles (*ob*) leaving the adaxial bundles, while there is much branching in the ring of carpel-wall bundles. One stamen filament (*sf*) with its trace is just diverging from the corolla tube. The bases of other lobes are present. Branching within the ring of carpel-wall bundles is taking place as the number of bundles is reduced. The four ovules appear to be arranged as two "pairs." The ovules of a pair, however, are actually in different locules, since the linear space separating the pairs is present only at the top of the locules. At this level and from this view, the curvature of the funiculus permits only the outer portions of the locules to be shown. In *H* these locules are present. *J*, *K*, *L*, Views, respectively, through regions of the tip of the locule, the style, and the upper portion of the stigma. In *J*, there is still branching of the ovary bundles, the two abaxials remaining large and distinct. In *K*, the two bundles of the style are clearly

duce the ovary, likewise infers perigyny. Strasburger (1921) interprets perigyny as an expansion of the end of the axis into a flat or cup-shaped receptacle (hypanthium)—an interval which separates the androecium and the gynoecium.

Evidently there are various degrees and great variation in the development of the different floral cycles, in the concavity of the receptacle, in the level of insertion of the floral organs, and in the presence and extent of the perigynous zone intercalated in the receptacle cup that separates the gynoecium from the other floral members and increases its concavity. All these are expressions of perigyny, a condition common among the higher plants, especially the Rosaceae; and, in view of the conforming relations existing among the floral parts, perigyny is apparently present in *Olea europaea* var. Mission.

VASCULAR RELATIONS IN THE FLOWER

The presence of eight large bundles is evident in the ring of vascular tissue in the upper portion of the main floral axis (fig. 2, *A*). Near the lateral floral axes, however, three of these diverge towards each of the opposite lateral axes. This divergence leaves in the main axis two large bundles that continue as such until the large gaps left by the diverging bundles are filled in again by vascular tissue. The ring of eight bundles is then restored. Distinction of the eight bundles in the pedicel is lost as the base of the flower is approached, for each bundle divides to form two; the resulting sixteen small bundles form what frequently appears as a complete vascular cylinder (fig. 2, *B*). Ruby (1917) describes the vascular ring as continuous.

The first trace to leave the vascular cylinder is that of the large bract, which leaves before the division of the eight large bundles. The gap left by this single divergence is filled in by the differentiation of additional vascular elements. Such divergence is absent, however, in the pedicel of a terminal flower, since no bract is present.

Nearer the base of the flower, two more traces from oppositely situated bundles in the ring depart to the two small sepal appendages (fig. 2, *C*). The ring gradually enlarges, becoming elliptical, while, from every fourth bundle in the ring, the four single sepal traces diverge, although not simultaneously (fig. 2, *D*).

The branching of the four single petal traces from the four bundles

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evident, while the left stamen filament has just separated from the petal cycle; the right filament is still partly united to this cycle. The large bundle of each is evident. In *L*, the sepals, shown in *K*, are absent, while the separate petal lobes show a large median vascular bundle and usually four small ones. The large stamen filament bundle in each of the two stamens and the two stylar bundles are shown.

alternates with that of the sepals and soon follows the course of the latter (fig. 2, *E*). This divergence of the eight sepal and petal bundles leaves eight undiverged bundles, each located between a sepal and a petal bundle.

As the base of the locules is approached, the eight undiverged strands of the vascular ring give off, at different levels, branches toward the center of the axis (fig. 2, *F*). These branches collectively form a central vascular cylinder, which soon divides and extends up through the placental region, each of the two branches being the fused ventral (adaxial) bundles of the adjacent carpel margins (fig. 2, *G* and *H*). Each branch contributes vascular tissue to the ovules present at the distal end of the locules. The four ovules exhibit axial placentation. A branch from the same placental bundle goes to opposite ovules in different locules (fig. 2, *I*). The ventral branch bundle then continues on to connect with the two dorsal (abaxial) bundles above the locules, and hence goes into the style and stigma.

The departure of the sepal, petal, and placental traces leaves the remaining eight bundles in the ring to continue up through the carpel walls. Two of these now become the dorsal (abaxial) bundles, while each of the two members of opposite pairs of adjacent bundles contributes to the two stamen traces (fig. 2, *G*). Frequently this divergence is evident somewhat earlier, closely following in time the branching of the ventral (adaxial) traces. Each of the two stamen bundles extends up through the corolla tube, then into the filament. They are quite distinct in the corolla tube, before divergence of the filament, by their large size and respective positions.

Although there is evidence, for the most part, of eight major bundles in the carpel walls, including the abaxials, nevertheless branching and fusion tangentially within the ring are extensive, so that the number varies at different levels from eight to as high as twenty-four (fig. 2, *G–J*). Such branching and fusion may begin soon after the departure of the adaxial bundles. Convergence and fusion with the abaxial bundles of the placental (ventral or adaxial) and other carpel wall bundles take place above the locules, and the two large vascular strands resulting continue into the style and stigma.

Each petal bundle after divergence of the corolla tube from the ovary gives off a lateral to each margin; each of these laterals, in turn, may branch, resulting in two pairs of marginal bundles. The extent of branching of the petal bundles varies, however, since shorter branches may be produced (fig. 2, *K–L*).

Sepal and bract traces seldom branch, the one strand usually sufficing for each organ (fig. 2, *H–K*).

DEVELOPMENT OF THE MACROGAMETOPHYTE

The studies of macrogametophyte development carried out here agree, in general, with those of Andersson (1931) on *Olea chrysophylla* and *O. europaea*. Development of each of the four young ovules is first evidenced by a small protuberance at the upper extremity of the placental surface, the ovules arising side by side and simultaneously in each of the two locules (plate 2, *A*). Growth continues at right angles to the placenta by periclinal and anticlinal cell divisions. The single archesporial cell is recognized by its large size, its dense cytoplasm, and its large nucleus, as the first cell beneath the epidermis of the nucellus (plate 2, *B*). In *Fraxinus, Forsythia,* and *Chioanthus,* more than one archesporial cell may be present (Andersson, 1931).

Appearance of the single integument limits the nucellus to a narrow protuberance in the center of the ovule. This protuberance consists of a single layer of large epidermal cells and usually one row of cells that separates the centrally placed archesporial cell from the epidermis. These lateral cells do not extend over the tip of the nucellus. A somewhat similar condition is also present in the Labiateae (Bushnell, 1936). The archesporial cell, preparatory to its division, enlarges both in length and width to become the embryo-sac mother cell (according to terminology of Schnarf, 1936). Then follows an elongation of the nucellus, with a slight broadening of its tip. During the origin and early growth of the integument and the lengthening of the narrow nucellus, the ovule bends; and by the time the embryo-sac mother cell arises, the nucellus and the integument are nearly at right angles to the funiculus.

Both of the meiotic divisions appear regular, the chromosomes being too small, however, for structural studies. Sax and Abbe (1932) found the haploid number of chromosomes in the Oleaceae to be twenty-three. Macrogametophyte formation differs from that present in many other genera, in that the micropylar daughter cell of the two resulting from the first division of the embryo-sac mother cell does not divide, but remains unchanged for a time, degenerating later. The nucleus of the lower (chalazal) daughter cell, on the other hand, soon divides regularly to complete the second meiotic division and, at the same time, forms the two-nucleate embryo sac (plate 2, *C*). The tetrad stage and the one-cell embryo-sac stages are not included in the development.

Decrease of nucellar tissue begins soon, so that shortly there remains only the epidermal layer of cells surrounding the embryo sac. The ovule continues to turn during its growth, and the integument to enlarge rapidly during the division of the embryo-sac mother cell. By the time the two-nucleate stage occurs, the anatropous position is reached; and

the large integument encloses the small nucellus deep within it, except for the micropylar opening.

The two-nucleate embryo sac enlarges, one nucleus going to each end, with a large vacuole forming between them. This stage is soon followed by the third nuclear division, resulting in the four-nucleate embryo sac. Apparently no cell walls are formed separating the nuclei, although conclusive evidence was not obtained. Andersson (1931) does not mention the presence of walls. An eight-nucleate embryo sac arises from the fourth nuclear division from the embryo-sac mother cell; and from this eight-nucleate sac develops the mature macrogametophyte, consisting of an egg cell, two synergids, two polar nuclei, and three antipodals (plate 4, *A*).

Variation occurs in the position of the egg cell, in the polar nuclei, and in the beaked character of the synergids. The large egg cell is usually partly below the synergids and nearer to one of them. In early stages the two polar nuclei are usually widely separated, one being in the upper half of the sac, the other in the lower half. Later they approach each other and lie in contact, most frequently just below the center of the sac. They were not found fused. In *Syringa* (Andersson, 1931) the polar nuclei do not unite until after the sperm has united with one polar nucleus. The antipodals, in the chalazal end of the sac, begin to degenerate early in embryo-sac development in *Olea europaea* var. Mission.

Development of the embryo sac of this form follows the *Scilla* type recently reviewed by Schnarf (1936). Here two macrospore nuclei take part in the development of the embryo sac, whereas only four nuclear divisions occur from the embryo-sac-mother-cell stage to the formation of the mature embryo sac.

Fertilization in the Oleaceae is hard to study because of the limited set of fruit (Andersson, 1931). In *Olea europaea* var. Mission, the fertilization of only one ovule out of the four emphasizes this difficulty still more. The act of fertilization was not seen, although abundant material was studied. Pollen tubes, however, were found in the glandlike stylar conductive tissue; in several instances, the tubes were also evident in the locule. Development of endosperm tissue in only one of the four ovules indicates that fertilization has occurred here, whereas in the remaining three ovules the embryo sacs showed no changes until signs of degeneration appeared. This degeneration does not occur until the endosperm in the fertilized embryo sac is well developed and until the ovule itself is much larger than the other three. Just what ovule is to be fertilized cannot be determined, since each develops an apparently normal mature embryo sac.

The initial stage of endosperm development was not observed; but in

other members of the Oleaceae studied (Andersson, 1931) it is cellular, as has been found in the present work for the four-celled and succeeding stages. Cell division takes place in all directions as the endosperm develops rapidly.

After fertilization, degeneration of one of the synergids is evident. A similar condition has been found in *Fraxinus* and *Syringa* (Andersson, 1931). Apparently there is no activity of the fertilized egg until the endosperm has become multicellular. At this time, however, an outgrowth develops from the egg and penetrates between the endosperm cells and the embryo-sac wall. This extension (the pro-embryo) grows down slightly beyond the narrowed micropylar region of the embryo sac, where, by successive divisions at the tip of the pro-embryo, the embryo itself is differentiated (plate 2, *D*). By this time the endosperm has developed to a great extent, the cells becoming enormous. The nucellus appears to be used up, and in its place lie plasma-rich cells, the origin of which was not determined. These cells have also been found in other members of the Oleaceae, and are thought to function as a nutrient supply for the endosperm (Andersson, 1931).

DEVELOPMENT OF THE FRUIT

Fertilization initiates changes both within the embryo sac and in the surrounding ovary tissue. These changes in the ovary accompany the development of the seed and lead to the final form and structure of the mature fruit.

The stimulation of fertilization is evidenced in all the ovary tissues, the rate of growth being greater than that during development up to this stage. This increased speed of growth is indicated especially by the thickening of the cuticle, by the increase in the size of radially elongate epidermal cells, and by the appearance of stone cells among the isodiametric parenchyma of the region enclosed by the vascular ring. These stone cells arise from the parenchyma by layered thickening of the cell wall, followed by lignification such as occurs in the formation of stone cells in pear (Crist and Batjer, 1931). In early stages they are confined mostly to the upper half of the ovary and originate at random in this region, increasing slowly at first both in size and number. They are found either singly or in clusters. Parenchyma cells, composing both the outer and inner regions of the ovary, which are separated by the vascular ring, gradually increase in size and number by radial division in the outer portion, radial and tangential divisions in the inner. The vascular tissue is confined to a ring. Cells of the inner epidermis are larger than those of the outer, though their radial elongation is less distinct.

Continued development in the ovary, after the initial changes through-

out the entire structure, is more pronounced in some tissues than in others; but, by this differentiation, distinct areas of the ovary wall now become evident. At first little change occurs in the cuticle or in the firm, compact outer epidermis, both of which form the exocarp; the same inactivity is evident in the underlying isodiametric, large nucleate, and relatively thin-walled mesocarp cells, although cell enlargement and limited cell division occur. The term *isodiametric* is used rather broadly throughout this discussion to include all stone and parenchyma cells in both the mesocarp and endocarp that even approach such a type, in contrast to the elongate type that develops later. Stomata are large and very conspicuous, with the guard cells slightly raised and with a rather large cavity present below them. Chloroplasts are seen in the mesocarp cells. Although tangential branching occurs within the vascular ring, greater growth continues in the inner region of the ovary, which, together with the vascular ring and inner epidermis, comprises the endocarp.

Cell division in both radial and tangential directions in the endocarp is very slowly accompanied by further formation of new stone cells and by the elongation and increase in size of most of the parenchyma cells. This condition may resemble that in pear, which is thought to arise from the probable shrinkage of the stone cells, leading to the radial elongation of adjacent nonlignified cells and to the formation of raylike borders around the stone-cell clusters (Crist and Batjer, 1931). The elongating parenchyma cells tend to form a network enclosing the stone cells singly or in small clusters. A region of small elongate cells, 8–10 cells wide and adjacent to the inner epidermis, extends entirely around the endocarp. This layer connects the dorsal and ventral radially directed suture bands in the endocarp, which consist of the same type of cell. The remaining small isodiametric parenchyma cells are sparsely scattered throughout the endocarp (plate 4, *B*).

Development of the single layer of large inner epidermal cells accompanies that of the remainder of the endocarp. These cells increase further in size, both tangentially and radially; and their nuclei enlarge correspondingly, becoming very conspicuous, their walls thickening to some extent.

The embryo is not large enough to be seen with the unaided eye in the oldest fruit of an inflorescence until about the fourth week after the first day of blooming. By this time the endosperm is well developed and is enclosed by the still fleshy integument.

The conductive tissue extending from the stigma to the ovary remains distinct during early fruit development, no stone cells arising, thus far, in the area.

The elongate parenchyma cells of the endocarp increase in number

and, more gradually, in size as the fruit enlarges. In dissected preserved material of the oldest fruit of an inflorescence, collected nearly 6 weeks after the first day of bloom, the three degenerated ovules are still clearly evident as minute dark-brown structures, which remain adherent to the endocarp when the young seed is removed (plate 4, *C*). The degenerate ovule that occupies the same locule as the seed is occasionally found attached to the torn placenta and can be removed with the seed. The three degenerate structures remain conspicuous in the nearly mature fruit. A peculiar ridge extending beneath the funiculus is initiated soon after the beginning of growth of the egg. It is a localized enlargement of the ovule and, in later stages, becomes very pronounced, its position being marked by a depression in the enclosing endocarp (plate 4, *C*).

The compact and slightly radially elongate outer epidermal cells of fruits about eight weeks old are overlaid with a thick cuticle. Mesocarp cells become progressively larger from the outer epidermis to the center of the region, where stone cells are occasionally found. The vascular strands in their confined ring enlarge and branch considerably in a tangential direction, but thus far penetrate the endocarp very little and the mesocarp not at all.

Stone cells in the endocarp arise continuously from the isodiametric parenchymatous tissue and are found singly or in clusters isolated by the network of elongated parenchyma cells, which now compose most of the parenchyma tissue of the endocarp. The isodiametric parenchyma cells are present, for the most part, immediately adjacent to the vascular ring. The endocarp, at this stage, is hardening because of the increase in the number of stone cells but can be cut, with difficulty, by a knife.

The inner epidermis continues to enlarge slowly by cell enlargement and by cell division. The embryo in the eight-weeks-old fruit is still a minute structure to the unaided eye (plate 4, *D*).

In the succeeding two weeks, the principal changes in internal structure are evidenced in the further hardening of the endocarp relative to continued increase in the number of stone cells, together with increase in wall thickness of each individual cell. The elongate parenchyma cells now show thickening of the walls as they gradually develop into stone cells. The cotyledons in the seed can be detected without a lens, while the cell walls of the integument thicken, and the cell contents shrivel. Occasionally a fruit contains two developing seeds.

During the ensuing weeks the cuticle continues to thicken slightly, and the outer tangential wall of the upper epidermis to thicken and to become convex. In the inner half of the mesocarp the now large parenchyma cells tend to become slightly elongate and more compact, which reduces both the size and extent of the intercellular spaces. The cell walls,

however, remain relatively thin; their nuclei large and conspicuous. This slight elongation is less evident in the outer portion of the mesocarp.

Differentiation of the vascular tissue is continued by further tangential branching within the ring and also now by shallow penetration into the endocarp. This limited radial extension of the vascular strands results in the formation of depressions that sculpture the surface of the endocarp. A substance whose nature was not definitely revealed by microchemical tests was present in the shriveled protoplast and branched pits of the stone cells. It may, however, be tannin. Many individual cells or small groups of cells scattered throughout the endocarp are also distinguished by such a blackening of these regions. The large and small isodiametric stone cells in the endocarp itself are enclosed in a network of the thick-walled elongate cells. The cell walls of the small elongate cells adjacent to the inner epidermis and the cells in the radial-dorsal and ventral suture bands also thicken. The endocarp, exclusive of the inner epidermis, therefore soon consists of lignified, thick-walled cells, making the structure now very hard (plate 3, *B–D*). All these cells are now considered as stone cells, regardless of their time of origin. There is little change in the inner epidermis.

The large embryo is ensheathed by a rather thick endosperm, while the integument has become thinner by shrinkage of the cells. Radial decrease of the compressed, but tough or membranous integument has left the large and ramifying vascular strands as large ridges, which are conspicuous as light-colored branching bands enveloping the seed (plate 4, *E*).

Further growth continues slowly in all regions of the fruit. As maturity is reached, the final expressions of development are found in the formation of a firm exocarp, the "skin," resulting from the thick cuticle, and in the single layer of dome-shaped outer epidermal cells. Ruby (1917) believes that a varietal difference may exist in the shape of the epidermal cells, but does not consider his evidence sufficient to prove that such is positively the case. He notes on the surface of the olive projections which are lighter in color than the rest of the exocarp and which appear before the ripening of the fruit of such varieties as Pegale, Verdale, and Des Vaux. These projections, he states, correspond to a subepidermal mass of lignified oval cells, separated from the normal adjacent mesocarp cells by several layers of compressed cells; they are generally detached, at least partially, from the overlying mesocarp. Such structures are present also in the variety Mission. Radial enlargement accounts for final increase in size of the mesocarp, the cells retaining their general shape although a slight elongation takes place. This elongation is most evident in the lower portion of the mesocarp, where the cells also become more

compact (plate 3, *A*). Stone cells may be present, but sparingly. Ruby represents the mesocarp of an unnamed variety as consisting of large but very compact parenchyma cells with no intercellular spaces. He finds also that the cells of the mature mesocarp situated immediately beneath the epidermis are smaller, with a slightly thicker wall than the underlying elements forming the mass of the mesocarp. In the variety Mission, the walls of the smaller subepidermal cells seem no thicker than other mesocarp cells. The latter cells, at this stage, constitute the "pulp" and are now filled with oil. The vascular strands still furrow only the surface of the endocarp, while the final stages of development in the endocarp center upon the filling, with the tanninlike substance, of the shrunken cell contents and branched wall canals of the cells, except the dorsal and ventral suture bands and the layer of elongate stone cells connecting them (plate 3, *B–D*).

The thin seed coat of the mature seed, in which the tapering, flat, leaflike cotyledons and short radicle and plumule are enclosed by starch-filled endosperm, consists of compressed thickened cells, indistinct in structure. The vascular strands form great ridges or bulges in its continuity.

The structural features of the olive fruit seem partly in agreement with those distinguishing the drupe. Various interpretations of this type of fruit agree that it possesses two distinct pericarp layers—"an outer fleshy and an inner stony layer" (James and Clapham, 1935), or, according to Strasburger (1921), a pericarp "differentiated into a succulent exocarp and a hard endocarp." Others, however, carry the distinction still further by adding that a drupe is derived from a single carpel, that it is usually one-seeded, and that the flower from which this fruit develops is perigynous. The determination, therefore, centers principally upon two factors: the number and position of the carpels involved and the structure of the developed ovary wall.

The olive, considered ontogenetically, does not conform to the drupe type as defined by Strasburger and others, since it (the olive) has originally two carpels, each containing two mature normal ovules capable of fertilization and development. The fact, however, that in the olive fruit only one carpel in its entirety is actually involved and only one seed is present agrees with such characters in the drupe. Likewise, the fruit consists entirely of carpel tissue, the wall of the ovary having both fleshy and dry portions. On such a basis, therefore, it may be considered a drupe, since fruit classification cannot be confined to hard-and-fast rules and since all fruits cannot be segregated into definite categories.

SUMMARY

These investigations of *Olea europaea,* variety Mission, were concerned with (1) the development of the flower, (2) the general vascular relations in the flower, (3) the development of the macrogametophyte, and (4) the general morphological changes involved in the development of the fruit.

Differentiation in the floral axes of the paniculate inflorescence is in acropetal succession and is characterized by the presence of zonation between the petal and stamen cycles, together with the formation of a cup-shaped receptacle through retardation of growth of the tip of the axis. The carpels, therefore, apparently arise from a depression in the center of the axis. These characters are considered as expressions of perigyny.

Eight vascular bundles are present in the pedicel. A trace diverges to the bract, but the gap is soon filled by vascular tissue. Near the base of the receptacle, each of the eight bundles divides; and the resulting sixteen small bundles frequently appear to form a complete vascular cylinder. Four of the sixteen bundles contribute to the four sepals and the two sepal appendages. Four other bundles, alternating with the sepal traces, supply the four petals. The eight remaining strands of the original sixteen contribute to the formation of the two ventral (adaxial), the two dorsal (abaxial), and the two stamen bundles, then continue as ovary wall bundles.

Development of the eight-nucleate macrogametophyte follows the *Scilla* type, wherein two macrospore nuclei take part in the development of the embryo sac, while only four nuclear division stages occur from the embryo-sac mother cell to the formation of the mature embryo sac.

The general development of the ovary was traced from fertilization to maturity. In the mature fruit the "stone" (endocarp) consists of both elongate and isodiametric stone cells, while the mesocarp remains parenchymatous. The exocarp includes only the parenchymatous outer epidermis and a thick layer of cutin.

The fruit of *Olea europaea,* variety Mission, is regarded as a drupe, since usually but one carpel and one ovule are actively involved in the development of the ovary, and since the fruit consists entirely of carpel tissue, the wall of the ovary having both fleshy and dry portions.

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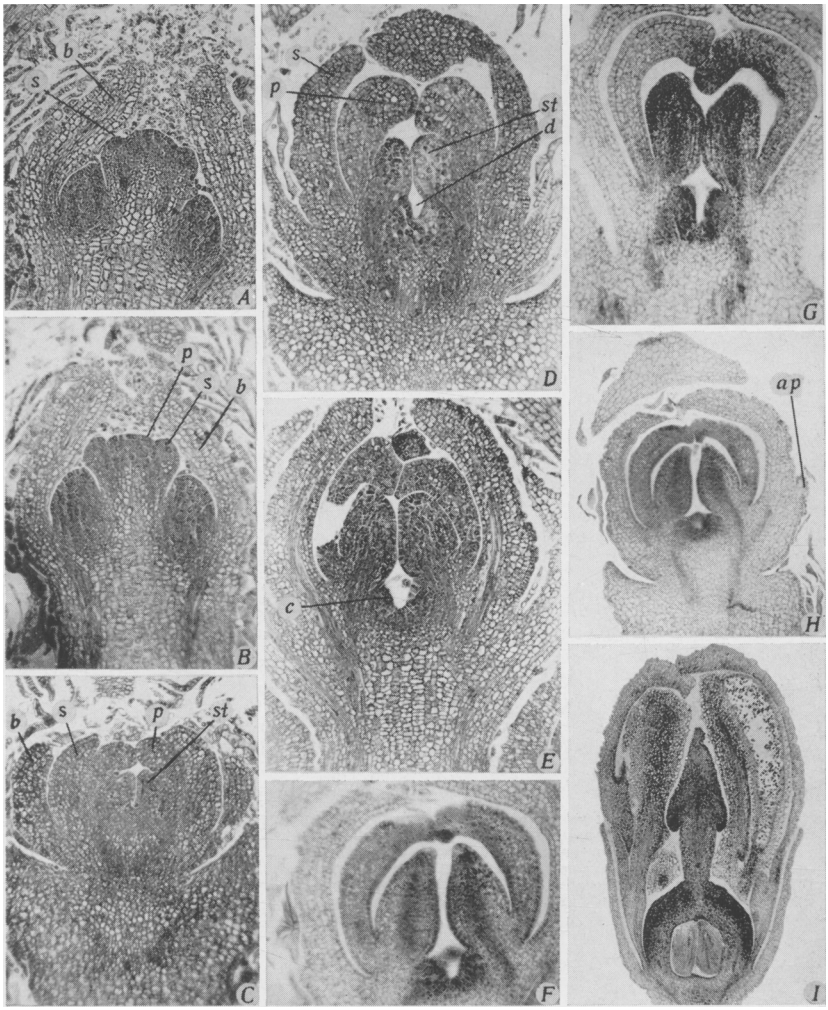

Plate 1.—Development of floral parts in the young flower bud: *A–C*, Appearance of sepal, petal, and stamen primordia respectively. *D*, Depression of the tip of the axis. *E–G*, Successive stages in early carpel development. *H*, One of the two sepal appendages in a young bud. *I*, Floral bud one day before blossoming, showing zonation of the petal and stamen cycles, the position of the ovary in respect to the stamen, petal, and sepal cycles, and the incurving of the tip of a petal. Details are: *s*, sepal primordium or sepal; *p*, petal primordium or petal; *st*, stamen primordium or stamen; *c*, carpel primordium; *b*, bract; *d*, depression of the tip of the axis; *ap*, sepal appendage. For further explanations see the text. (*A–F*, × 71; *G*, × 60; *H*, × 47; *I*, × 16.)

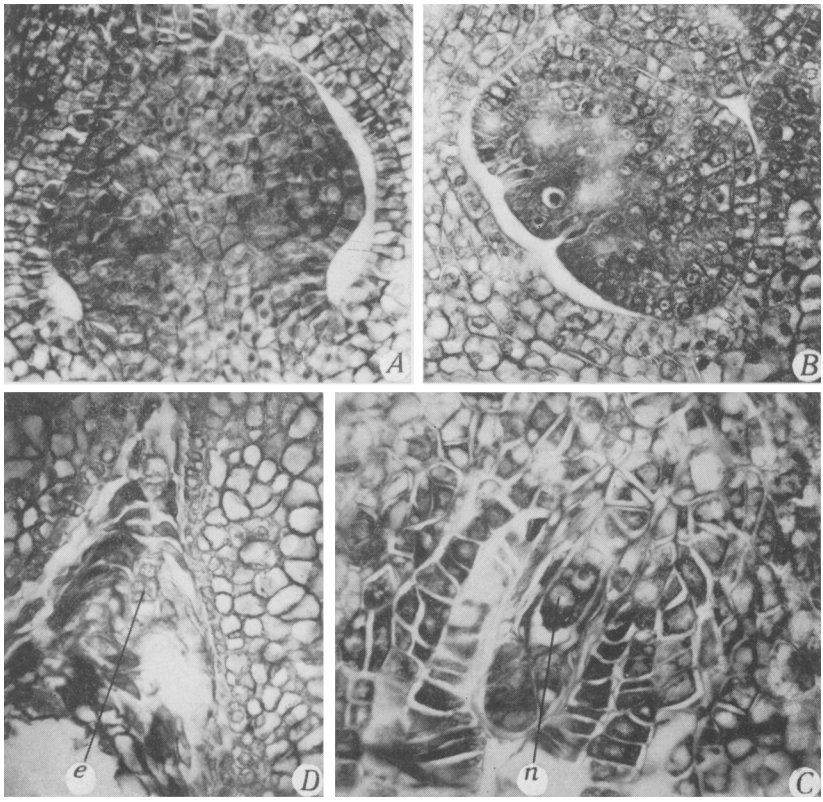

Plate 2.—*A*, Two very young ovules—each in a different locule. *B*, Archesporial cell in the small nucellus, surrounded by the single large integument. The archesporial cell is separated laterally from the nucellar epidermis by one cell layer. *C*, Two-nucleate embryo sac; one of the two nuclei is shown at *n*, the other at the right of the one indicated. Cells surrounding the nucellus are of the integument. *D*, Section through young fruit, showing very early embryo development, at the tip of the tubular pro-embryo. Endosperm surrounds the young embryo. (All × 441.)

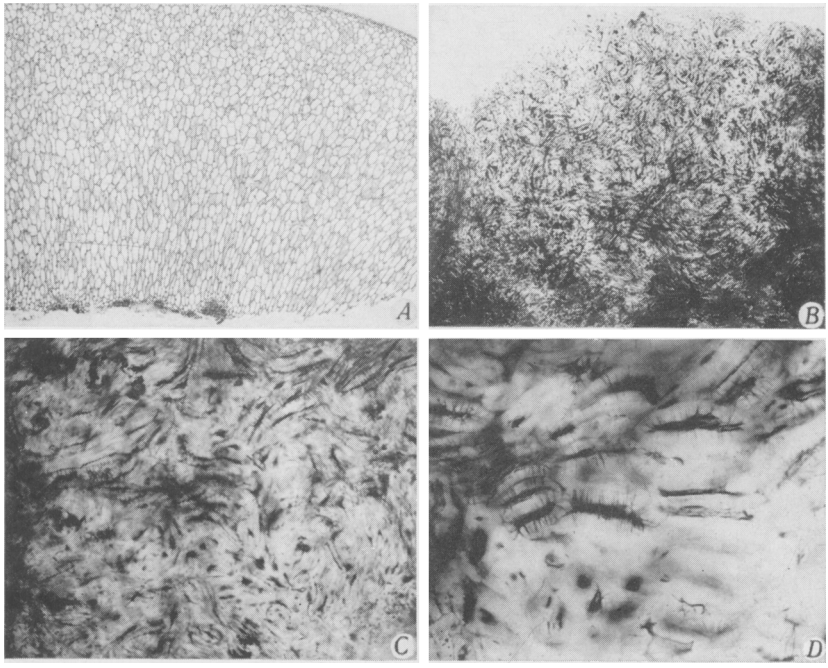

Plate 3.—*A*, Transverse section through the mature mesocarp, showing also a small section of the exocarp (at upper right) and of the vascular ring (lower margin). The cells of the inner mesocarp region are more elongate than those of the outer. *B*, *C*, *D*, Stone cells constituting the mature endocarp; the elongate cells form a network enclosing the isodiametric cells. The black tanninlike substance is present within the shriveled protoplast and branched pits of the stone cells. (*A*, × 32; *B*, × 32; *C*, × 80; *D*, × 358.)

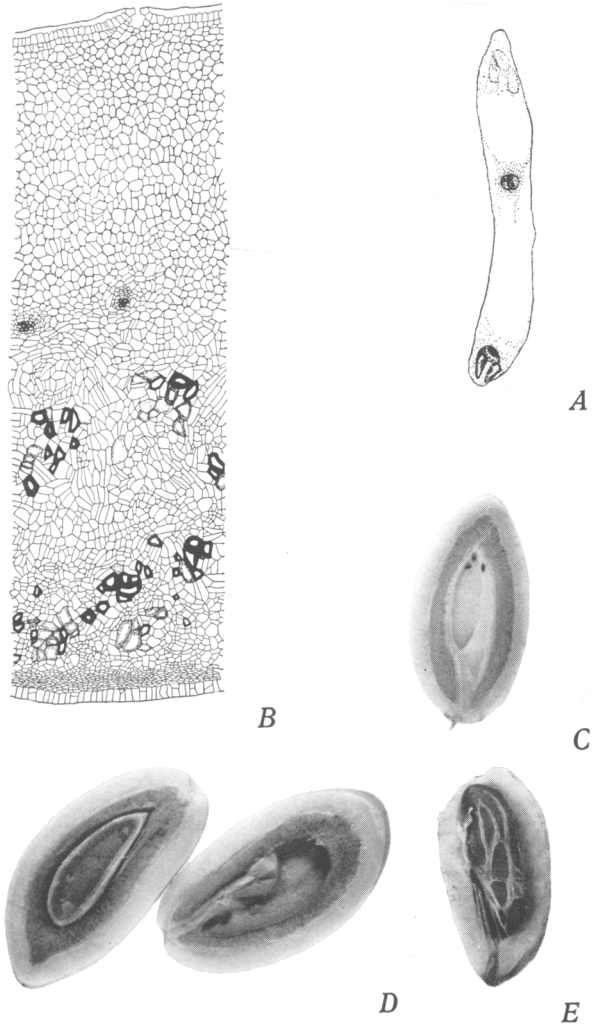

Plate 4.—*A*, Mature eight-nucleate embryo sac—consisting of two synergids, an egg cell, two polar nuclei, and three antipodals, which are shown in a stage of degeneration. *B*, Diagram of a section through a four-weeks-old fruit, showing the thick cuticle, the parenchymatous mesocarp cells, and the early development of stone cells and elongate parenchyma in the endocarp. Two vascular bundles are evident in the outermost region of the endocarp. Note the layer of small cells adjacent to the large inner epidermis. *C*, Longitudinal view of a fruit five and one-half weeks old, with the maturing ovule removed and showing three degenerate ovules and the ridge formed beneath the funiculus in which the middle and right degenerate ovules are seen to lie. The exocarp is the peripheral layer of tissue; the mesocarp, the adjacent lighter layer; and the endocarp, the dark granular region of the fruit. *D*, Eight-weeks-old fruits, the left specimen showing a maturing ovule with the small embryo at the lower and broader (micropylar) end. The right specimen shows a second ovule that began growth; the normally maturing ovule is removed. *E*, Nearly mature "pit" (endocarp) enclosing normally one seed. The integumental vascular tissue envelops the seed as light-colored bands. (*A*, × 120; *B–E*, enlarged.)