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ULTRASTRUCTURAL STUDY OF THE DEVELOPMENT OF OIL CELLS IN THE MESOCARP OF AVOCADO FRUIT

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The development of idioblastic oil cells in the mesocarp of avocado fruit was studied with the electron microscope. Observations concentrated on the formation of the complex cell wall and on the process of oil accumulation. The cell wall of the mature oil cells has three distinct layers: an external primary wall, a suberin lamella, and an interior tertiary wall. No significant oil accumulation was observed until after the suberin layer was deposited and tertiary wall formation had begun. Formation of an extensive network of smooth tubular endoplasmic reticulum was observed concomitant with the initial accumulation of oil in the cytoplasm. In the latter stages of tertiary wall formation, the primary site of oil accumulation shifted from the cytoplasm to the vacuoles. By the time the deposition of the tertiary wall was complete, most of the cell volume was occupied by a massive oil droplet; and the cytoplasm, which was devoid of membranes, was displaced to the cell periphery.

Introduction

Oil cells are idioblastic secretory structures that differentiate in the ground parenchyma of many taxonomically diverse angiosperm species but occur most commonly in species of the woody Ranales complex (WEST 1969).

In the mesocarp tissue of avocado (*Persea americana* Mill.) fruit, 2% of the mature fruit volume is occupied by oil cells (CUMMINGS and SCHROEDER 1942; SCOTT, BYSTROM, and BOWLER 1963), which differ from surrounding parenchyma cells in two major ways. First, the wall of the oil cell is a complex structure consisting of an inner cellulosic tertiary layer, a middle suberized layer, and an outer cellulosic primary layer (PLATT-ALOIA 1980). The walls of the parenchyma cells lack this distinct layering. Second, the oil in mature oil cells is localized in a single large vacuole or "oil sac" (CUMMINGS and SCHROEDER 1942; SCOTT et al. 1963). In the parenchyma cells, the lipids occur as many individual cytoplasmic lipid bodies (PLATT-ALOIA and THOMSON 1981). Also, the lipid in oil cells stains differently with osmium tetroxide than that of the parenchyma cells (PLATT-ALOIA 1980). These differences in the location and the staining properties of the lipids in the two cell types suggest that they may be compositionally different (CUMMINGS and SCHROEDER 1942). This suggestion is supported by the finding that the parenchyma cell lipids are primarily triacylglycerides (MAZLIAK 1970), whereas those of the oil cells are generally considered to be lower terpenoid "essential" oils (SCOTT et al. 1963; HEGNAUER 1966).

The intracellular location of oil synthesis in oil

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cells is uncertain. The observation of large lipoidal inclusions in the plastids of some developing oil cells has led to the conclusion that these organelles may be involved in oil synthesis (AMELUNXEN and ARBEITER 1967; AMELUNXEN and GRONAU 1969; HEINRICH 1969, 1970). Similar observations in other terpene-producing cells support this conclusion (HEINRICH, SCHULTZE, and WEGENER 1980; BOSABALIDIS and TSEKOS 1982a, 1982b). However, ultrastructural studies of terpene-producing trichomes and oil glands in *Citrus* have reported extensive networks of smooth endoplasmic reticulum (ER), which may be involved in oil synthesis (AMELUNXEN 1965; THOMSON, PLATT-ALOIA, and ENDRESS 1976) or in transport of oil from the plastids to the oil cavity (BOSABALIDIS and TSEKOS 1982b).

The present study was undertaken to follow the development of oil cells in avocado fruit to observe the ultrastructural changes associated with (1) the production of oil and (2) the formation of the complex cell wall.

Material and methods

Ovularies ranging in size from less than 1 mm to 2-3 mm long were dissected from flowers of avocado (*Persea americana*) and fixed either whole or after being cut in half. Samples were also taken from the mesocarp of young developing fruit (1 cm long).

The tissue was fixed either in 2.5% glutaraldehyde in 50 mM cacodylate buffer, pH 7.0, or in KARNOVSKY'S (1965) paraformaldehyde-glutaraldehyde fixative made with 0.1 M phosphate buffer, pH 7.2. All samples were postfixed overnight in 1% osmium tetroxide in the appropriate buffer, dehydrated through an acetone series, and embedded in SPURR'S (1969) epoxy resin.

Thin sections were stained with aqueous uranyl

acetate followed by REYNOLDS's (1963) lead citrate and were examined with a Philips EM400 electron microscope.

Results

The smallest ovularies prepared for study (1–3 mm long) contained oil cells in stages of development ranging from those with walls that were not fully suberized to those with a complete suberin lamella and a developing tertiary wall. This variability indicated that ovulary size is an inadequate developmental index for the oil cells of avocado fruit. Therefore, our ultrastructural observations will be grouped into the following categories: cells with a developing or complete suberin lamella only, cells with a developing tertiary wall, and cells with a complete three-layered wall.

CELLS WITH A DEVELOPING OR COMPLETE SUBERIN LAMELLA

The cell walls of the least developed oil cells observed were slightly more electron opaque than those of adjacent parenchyma cells (figs. 1, 2). Close examination revealed small individual plaques of suberin deposited on the inner surface of the wall (fig. 1, curved arrows). The cytoplasm of these cells contained numerous small plastids with many plastoglobuli, very few small starch grains, and electron-opaque primary lamellae (fig. 2, inset). Rough ER was abundant (fig. 2, inset), and a small amount of smooth ER was also present. The oil cell plasmalemma possessed numerous shallow invaginations which were not observed in the parenchyma cells (fig. 1, arrowheads). At this early stage of development, the oil cells contained a few small lipid droplets in the cytoplasm as well as several small and a few large vacuoles (fig. 2). The plasmodesmata connecting the two cell types were unique in that they penetrated a wall protrusion that was present only on the oil cell side (not shown).

With further development, the suberin layer in the oil cell wall thickened to 50 nm and was composed of several layers (fig. 3, curved arrows). The plasmalemma still appeared convoluted in outline, and no obvious change in cell oil content was observed.

CELLS WITH A DEVELOPING TERTIARY WALL

An accumulation of flocculent material between the oil cell plasmalemma and the suberin lamella was the first visible sign of tertiary wall formation (figs. 4–6). During early tertiary wall development, the rough ER occurred predominantly in parallel arrays near the cell periphery (fig. 4), and the amount of tubular, smooth ER increased (figs. 4, 5). The other cytoplasmic components appeared to remain the same as described.

The tertiary wall, in the latter stages of its for-

mation, had a coarse granular appearance and was more electron opaque than either the suberin or the primary wall layers. The plasmodesmata were apparently not completely occluded at this stage (fig. 6); although the wall protrusions with which they were associated were enveloped by both the suberin and tertiary wall layers, the plasmodesmata still appeared to penetrate at least the suberin layer (fig. 6). Rough ER appeared to vesiculate and decrease in amount, while there was a large increase in smooth tubular ER (figs. 5, 7 [inset]). At this stage of development, large quantities of oil were observed as droplets in the cytoplasm and vacuoles (figs. 5, 7). The oil droplets did not appear to be associated with any particular subcellular organelle but were located in the cytoplasm close to aggregates of electron-opaque particulate material and ribosomes (fig. 5, inset). Neither the plastids nor the ER contained a substance similar to the oil in either texture or staining characteristics (figs. 5, 7).

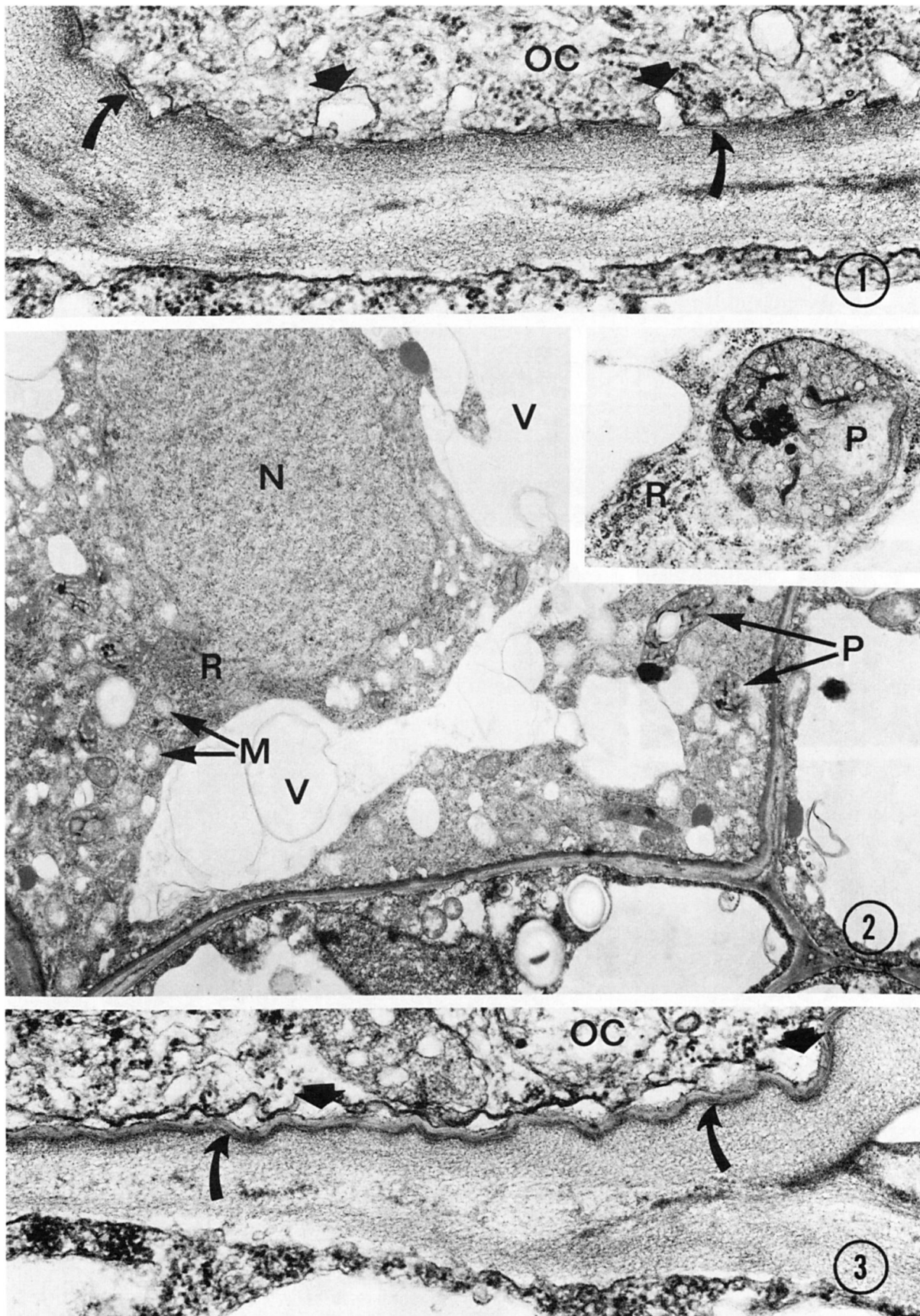
CELLS WITH A COMPLETE THREE-LAYERED WALL

The fully developed tertiary wall had a compact fibrillar to granular appearance similar to that of the primary wall but was coarser and slightly more electron opaque (fig. 8). Serial sections of plasmodesmata showed them to be completely occluded by both the suberin layer and tertiary wall (not shown). Once the oil cell wall was complete and oil was abundant, there was a rapid degradation of all cytoplasmic components. Organelles became ill-defined, and membranes appeared to break down (fig. 9). In the fully mature oil cell, the cytoplasm existed as a narrow, irregular electron-opaque band around the periphery of the cell (not shown).

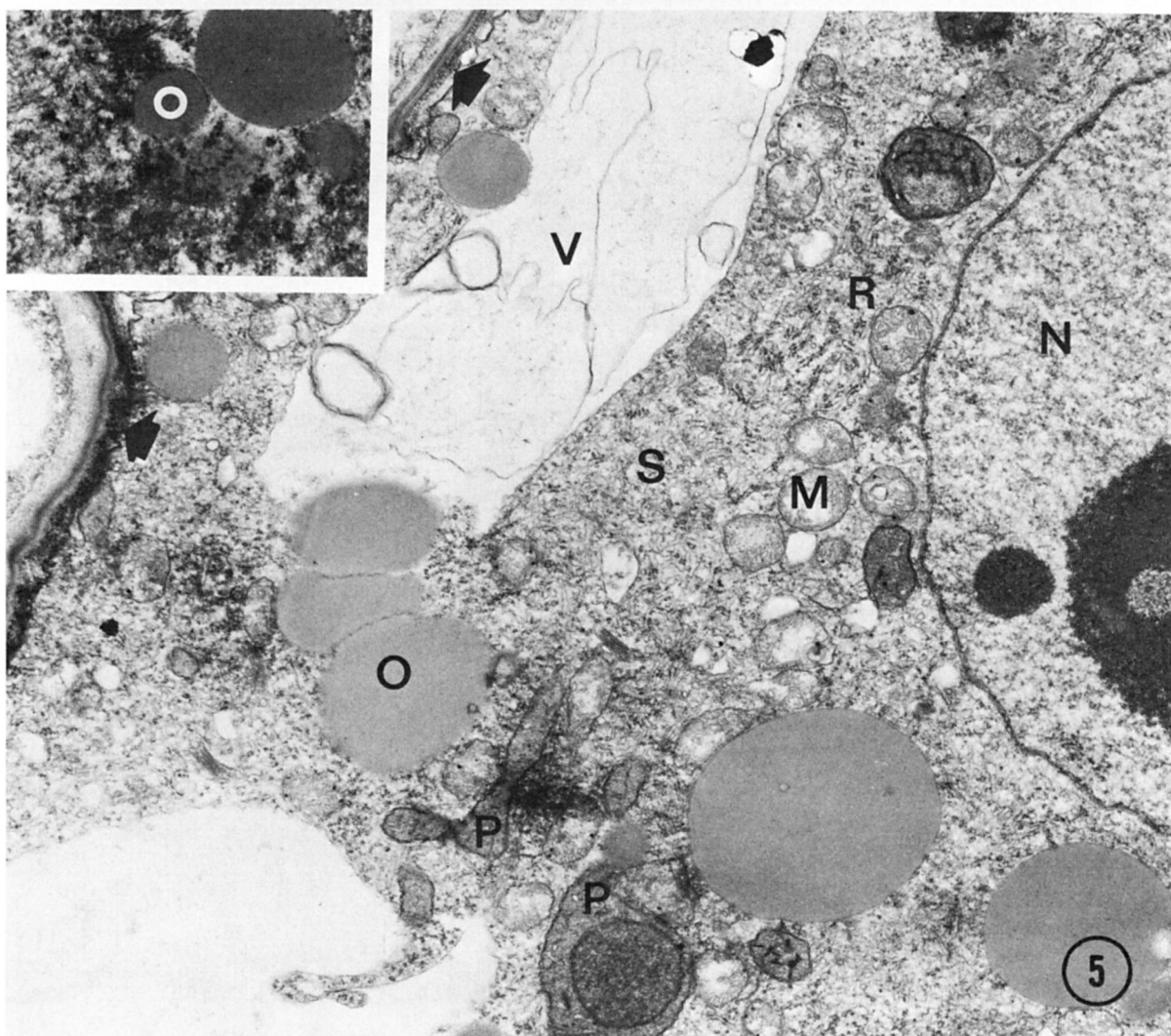
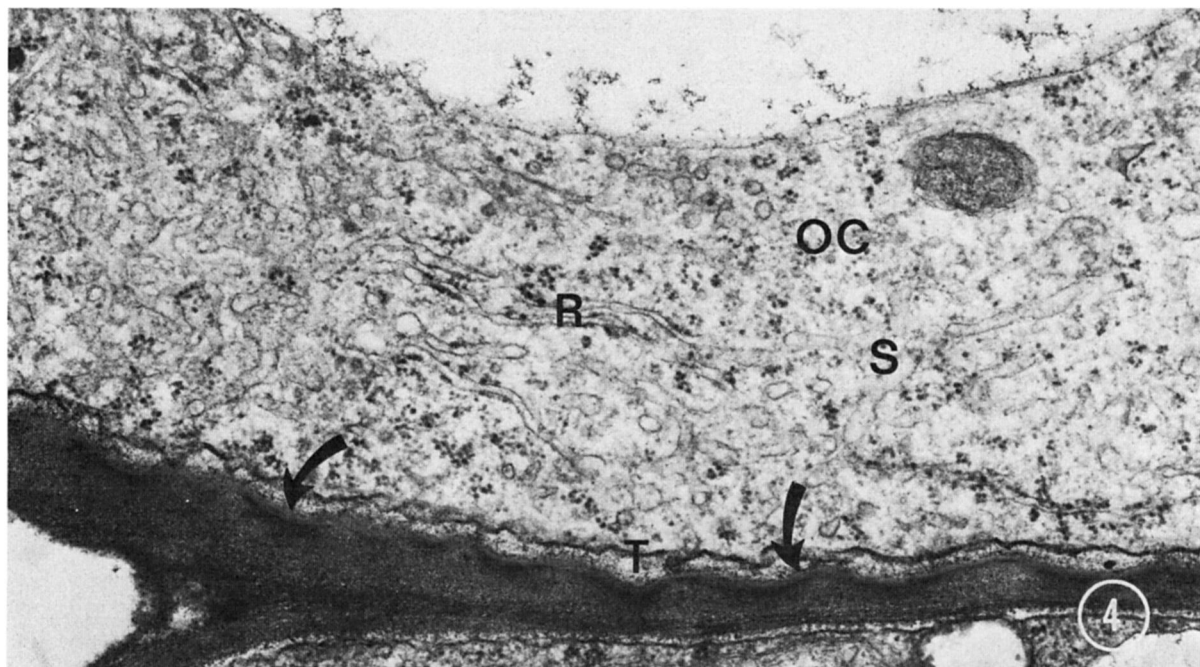
Discussion

The oil cells of avocado fruit mesocarp are similar in ultrastructure to the oil cells of *Laurus* leaves (MARON and FAHN 1979). Oil cell differentiation is recognizable by an increased electron density of the primary cell wall, accompanied by the initiation of suberin deposition. In addition, plasmodesmata in the wall of these cells are associated with a unique bulge in the primary wall of the oil cell. The cytoplasm of the oil cell initials is less vacuolate than that of surrounding parenchyma cells, and the plastids appear to contain less starch. These features have also been associated with early stages of oil cell development in other studies (OROSS 1977; MARON and FAHN 1979).

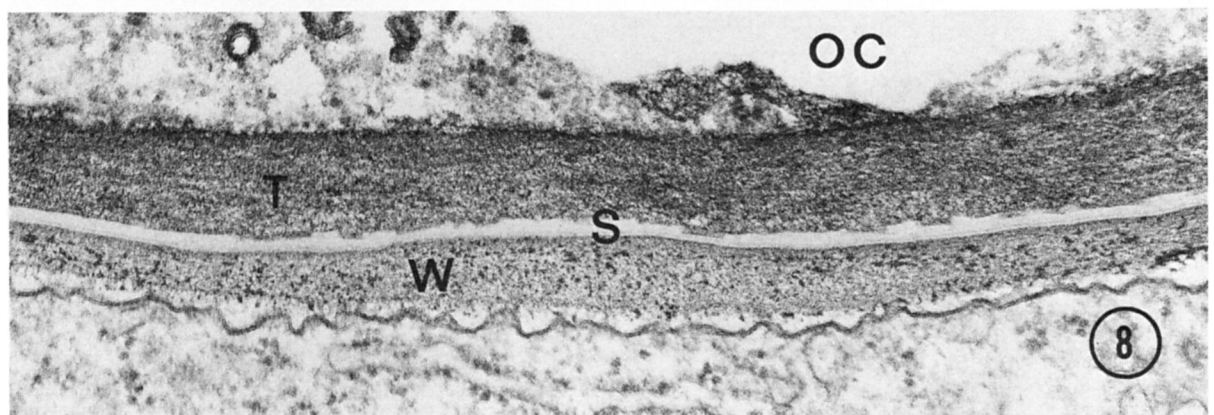
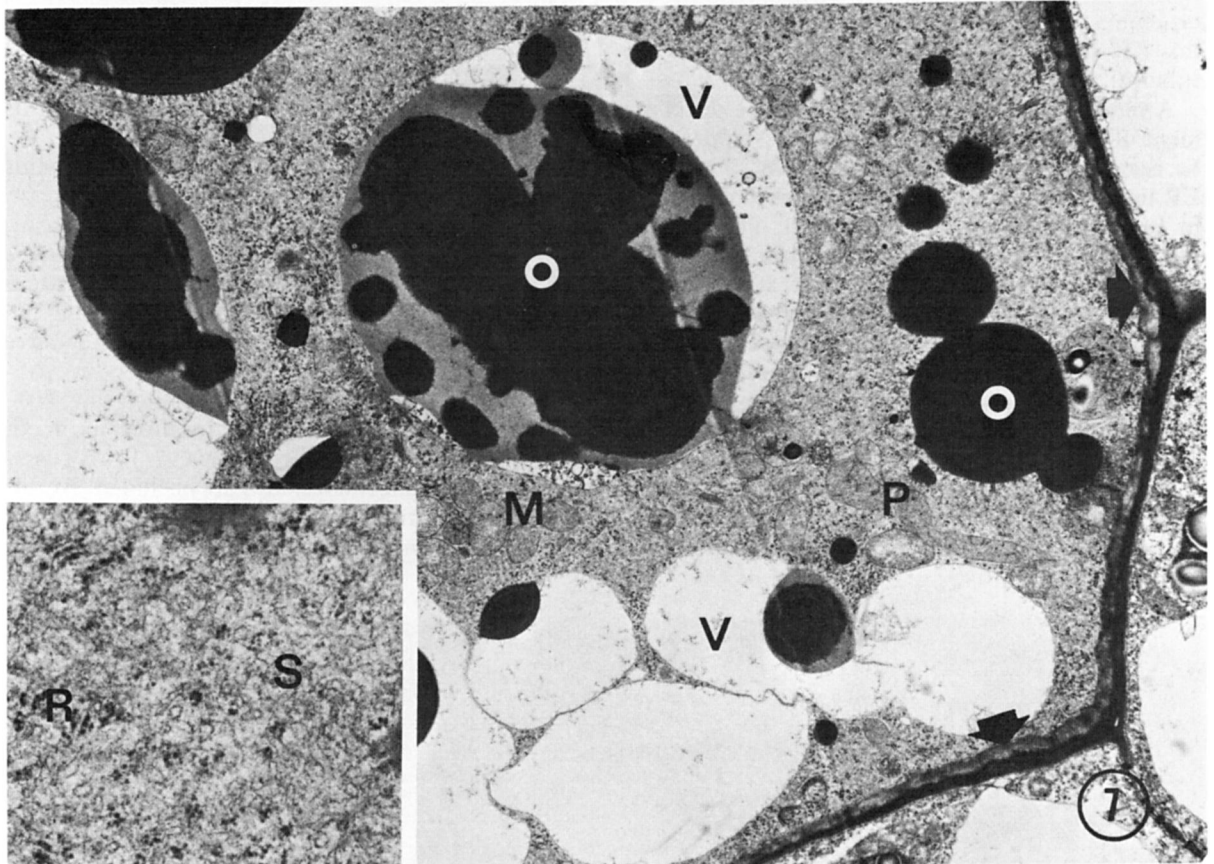
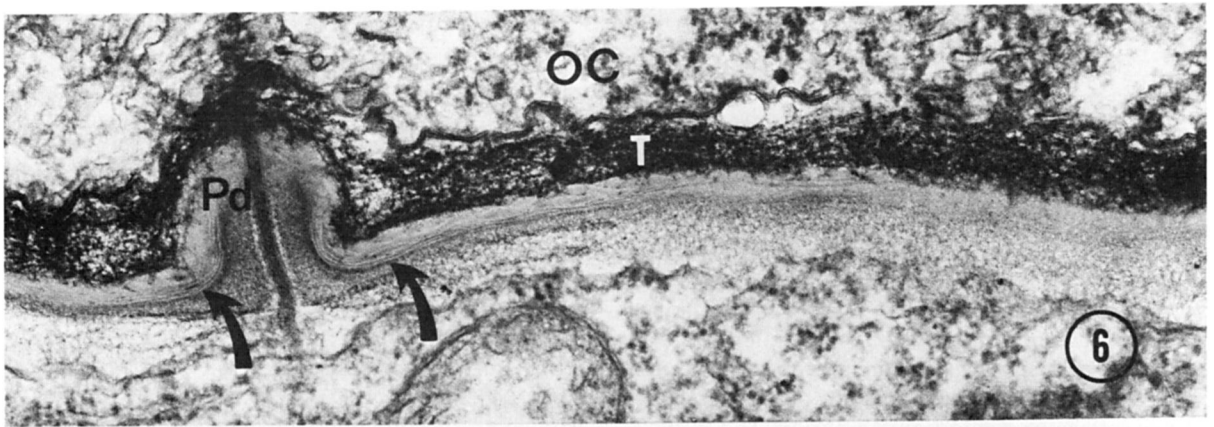
A suberized layer in oil cell walls has been reported in several species (LEHMANN 1925; LEE-MANN 1928; SCOTT et al. 1963; AMELUNXEN and GRONAU 1969; OROSS 1977; MARON and FAHN 1979). However, suberin has not always been detected (LEHMANN 1925; KASAPLIGIL 1951; TUCKER



FIGS. 1-3.—Electron micrographs of developing oil cells, which are undergoing deposition of a suberin layer. These cells were in small (1-3 mm long) ovaries. Fig. 1, Cell wall of an oil cell (OC) at an early stage of development. The wall shows a higher electron density on the side toward the oil cell, and individual plaques of suberin (curved arrows) are apparent between the wall and the plasmalemma, which is highly irregular (arrowheads). $\times 62,000$. Fig. 2, A developing oil cell from the parenchyma of a 2-mm ovary. The dense cytoplasm is filled with mitochondria (M), small undifferentiated plastids (P, see inset), and rough ER (R, and inset). The wall shows greater electron density on the side toward the oil cell. N = nucleus, V = vacuole. $\times 8,200$; inset, $\times 27,400$. Fig. 3, Later stage of oil cell wall differentiation. Characteristic lamellations (curved arrows) are visible in the well-developed suberin layer. A few fibrils of the incipient tertiary wall (arrowheads) are seen between the suberin and plasmalemma. OC = oil cell. $\times 52,000$.



FIGS. 4, 5.—Electron micrographs of developing oil cells showing deposition of the tertiary cell wall. Fig. 4, The suberin layer (curved arrows) is complete, and tertiary wall (*T*) is beginning to be deposited. Both rough (*R*) and smooth (*S*) tubular ER are present. $\times 36,000$. Fig. 5, Oil cell in which the tertiary wall (arrows) is partially deposited. Oil (*O*) is beginning to accumulate in the cytoplasm which contains abundant smooth tubular ER (*S*) and some rough ER (*R*). Mitochondria (*M*) and plastids (*P*) are similar to earlier stages. *N* = nucleus, *V* = vacuole. $\times 13,400$. Inset, Aggregates of ribosomes and electron-opaque particulate material associated with small oil droplets in the cytoplasm of a developing oil cell; $\times 34,000$.



FIGS. 6-8.—Electron micrographs of oil cells that have a nearly complete or complete three-layered wall. Fig. 6, Oil cell wall in which the tertiary wall (*T*) is at an intermediate stage of development with an irregular substructure. Suberin lamellations (curved arrows) are visible, especially near the plasmodesma (*Pd*) which has an unusual structure, typical of oil cells, and appears to traverse the suberin layer. *OC* = oil cell. $\times 62,000$. Fig. 7, Oil cell with nearly complete tertiary wall (arrowheads). The oil (*O*), which is not uniformly stained, is in both the cytoplasm and vacuoles. The cytoplasm is filled with smooth tubular (*S*) ER and apparently vesiculate rough (*R*) ER (see inset). Mitochondria (*M*) and plastids (*P*) appear unchanged. *V* = vacuole. $\times 7,200$; inset, $\times 31,000$. Fig. 8, Fully developed oil cell wall with a well-defined suberin layer (*S*) and an organized tertiary wall (*T*). *W* = primary wall, *OC* = oil cell. $\times 65,200$.

1976). The structural pattern of suberization of the oil cell wall is similar to that described in root exodermal cells (OLESEN 1978) and cork cells (WATTENDORFF 1974). In all cases, the first visible sign of suberization is the appearance of individual plaques of electron-opaque material on the inner surface of the primary cell wall. These plaques represent the first lamellae of the incipient suberin layer. In addition, accumulations of abundant rough and some smooth ER are present near the cell periphery close to shallow invaginations of the plasmalemma. WATTENDORFF (1974) hypothesized that this ER is somehow involved in the deposition of suberin.

A tertiary wall develops following the development of the suberin layer. This process appears to be associated with a reorganization of the rough ER into parallel arrays, as has also been described in *Laurus* (MARON and FAHN 1979). The tertiary wall has been reported to be cellulosic in composition (SCOTT et al. 1963); however, it appears to be more granular and usually is more electron opaque than the primary wall of the same cell.

The plasmodesmata of oil cells appear to maintain cytoplasmic continuity with adjoining cells until after suberization is complete and tertiary wall formation is well advanced. This is similar to the situation in oil cells of *Laurus* (MARON and FAHN 1979). Because the oil cell is completely surrounded by suberin, this occlusion of the plasmodesmata

marks a complete isolation of the oil cells, both apoplastically and symplastically, from the rest of the tissues.

The first noticeable increase in oil content of the oil cells occurs after the initiation of the tertiary wall and concomitantly with a substantial increase in tubular smooth ER within the cell. Similar associations of tubular smooth ER with oil or lipid synthesis have been reported in *Citrus* (THOMSON et al. 1976) and *Inula* (WERKER and FAHN 1981). However, plastids have also been suggested as the primary source of essential oils in other secretory cells (AMELUNXEN and GRONAU 1969; HEINRICH 1969, 1970; BOSABALIDIS and TSEKOS 1982a). In the present study, although plastids in the oil cells contained rather large plastoglobuli, a direct relationship between oil accumulation and plastids was not observed.

Oil accumulation is seen first in the cytoplasm, and as SMITH (1974) reported in developing *Crambe* seeds, the oil bodies are often associated with areas of electron-dense particulate material. As oil cell development continues, droplets appear to move into the vacuoles, which increase in size, and apparently coalesce to form large oil-filled vacuoles. By the time the oil content is reaching its maximum, formation of the tertiary wall is nearly complete, and the plasmodesmata are occluded. At this time the structural integrity of the organelles decreases, membranes become less distinct, and the

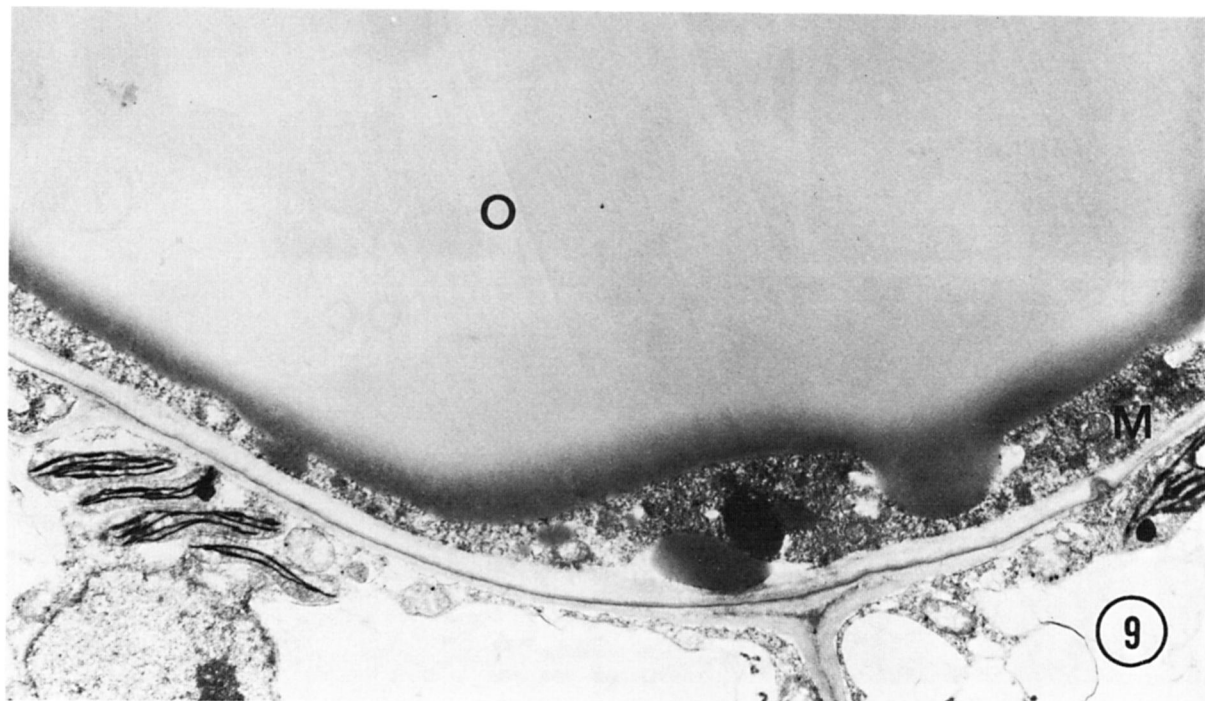


FIG. 9.—An oil cell in a later stage of development that is almost completely filled with oil (O). The cytoplasm is confined to the cell periphery and is apparently degrading; however, some mitochondria (M) are still apparent. $\times 8,300$.

cytoplasm increases in electron density. The mature oil cell is almost completely filled with oil, and all that remains of the protoplast is an electron-dense band around the periphery of the cell.

The oils produced by, and stored in, the oil cell may be toxic, ultimately leading to the death of the cell and degradation of the cytoplasm (AMELUNXEN and ARBEITER 1967; AMELUNXEN and GRONAU 1969). If so, the specialized wall surround-

ing the idioblast and including the suberin layer possibly isolates the oil cell and its contents from neighboring parenchyma cells.

Acknowledgments

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