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IDIOBLAST OIL CELLS OF AVOCADO: DISTRIBUTION, ISOLATION, ULTRASTRUCTURE, HISTOCHEMISTRY, AND BIOCHEMISTRY

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Procedures for the isolation of highly enriched preparations of specialized oil cells of avocado (*Persea americana* Mill.) fruit and clearing of plant tissues were applied to an examination of the idioblast oil cells of avocado. These cells are present in all organs examined (leaf, fruit, root, and seed cotyledon) and are dispersed throughout the tissues. Histochemical tests indicate the presence in the oil cells of alkaloids and sesquiterpene hydroperoxides and, possibly, other terpenes. Freeze-fracture electron microscopy of oil cells of the fruit (both in situ and in vitro) indicates that some of the components of the oil contained in these cells undergo a phase transition to a crystalline state at 4 C, while the storage lipid in the mesophyll cells, primarily triacylglycerols, does not. Thin-layer chromatography (TLC) of a lipid extract of the isolated oil cell preparation shows at least six different components with a polarity less than phospholipids but greater than tri- or diacylglycerols. The TLC-detectable composition of the lipid in oil cells remained unchanged during fruit maturation and ripening.

Introduction

Idioblast oil cells have been reported to occur in the leaves and other organs of dicotyledonous plant species in numerous families (Postek and Tucker 1983; Baas and Gregory 1985). The structure and ultrastructure of mature and developing oil cells of several species have been described (Murty 1960; Armstrong 1964; Tucker 1976; Platt-Aloia et al. 1983; Postek and Tucker 1983; Mariani et al. 1989; Bakker and Gerritsen 1990). The composition of the oil of these cells, however, has been surmised only as consisting of oils or lipid, based on staining with Sudan dyes (Armstrong 1964; Tucker 1976; Maron and Fahn 1979) or as "terpenes, fats, and flavonoid aglycones" (Baas and Gregory 1985). Mariani et al. (1989) included histochemistry in their study of oil cells of *Liriodendron tulipifera* and found evidence for sesquiterpene lactones. The primary reason for this lack of biochemical information on the oil is probably that these solitary oil cells make up only a small percentage of the total volume of the tissue. Any quantitative or even qualitative biochemical analysis would require that the cells be isolated in reasonably pure preparation and in fairly large quantities.

Avocado (*Persea americana* Mill.) is a species that contains oil cells in the leaves, seed, roots (Armstrong 1964), and fruits (Platt-Aloia et al. 1983; Platt-Aloia and Thomson 1992). The mesocarp of the avocado fruit has a fairly uniform cellular composition, consisting primarily of large (40–60 μm diam.) parenchyma cells (Platt-Aloia and Thomson 1981) that contain numerous droplets of lipid, primarily triacylglycerol (Biale and Young 1971). The idioblast oil cells are scattered throughout the mesocarp and compose approximately 2% of the tissue volume (Cummings and Schroeder 1942). The oil in these specialized cells occurs as a single large drop filling the cell, rather than the smaller individual drops that occur in the parenchyma cells (Cummings and Schroeder 1942). It also stains with a different density than the triacylglycerols of the parenchyma cells (Platt-Aloia et al. 1983), has a different appearance in freeze-fracture replicas compared with the triacylglycerols (Platt-Aloia and Thomson 1989, 1992), and, therefore, is thought to have a different composition.

The oil cells of avocado fruit, as those described for other species (Maron and Fahn 1979; Postek and Tucker 1983; Mariani et al. 1989; Bakker and Gerritsen 1990), are surrounded by a specialized cell wall, composed of the primary cellulosic wall, a secondary suberin layer, and a tertiary wall (Platt-Aloia et al. 1983). During ripening of avocados, the activities of the wall hydrolytic enzymes, cellulase and polygalacturonase, increase dramatically (Awad and Young 1979). As a result, the primary walls of the parenchyma cells are degraded (Platt-Aloia et al. 1980), and fruit softening occurs. The suberized wall of the idioblast oil cells, however, is immune to the activity of these enzymes and remains intact during ripening (personal observation).

Because of these observations, we felt that it may be possible to isolate the oil cells from a soft, ripe avocado, thus providing an opportunity to perform further analysis of their contents. This article describes the methodology for isolating a highly enriched preparation of a single cell type, the idioblast oil cells of avocado fruit. This is first done using mature ripe fruit; then isolation of oil cells from mature unripe fruit and immature fruit was accomplished with the use of commercial enzymes. In addition to thin-section and freeze-

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fracture electron microscopy, oil composition was investigated with thin-layer chromatography (TLC) for lipids and histochemical tests for terpenes and alkaloids.

Additionally, a method is described for rapid detection and determination of the distribution of idioblast oil cells in the fruit, leaves, seeds, and roots.

Material and methods

Avocados (*Persea americana* vars. Hass and Fuerte) were harvested from trees grown at the South Coast Field station of the University of California or from trees in field plots on the Riverside campus.

Tissue clearing for detection of oil cells

A modification of a tissue clearing technique (Bevege 1968) was developed to determine the presence and distribution of idioblast oil cells in the various tissues of avocado. Whole leaves and roots and thin slices of fruit mesocarp and of seed cotyledon were submerged in 5% NaOH in a beaker and placed in a microwave oven (Sharp carousel model, 650 W). The samples were irradiated for 1–3 min at 50% power. The length of time depended on the rigidity of the tissue and on tannin content; soft tissues such as roots and young leaves require less time than older leaves or seed cotyledons. The tissue was then washed with distilled water, placed in household bleach adjusted to pH 7.5 with 1N HCl, and left standing until it cleared, usually 3–5 min. When this cleared tissue was examined in the light microscope, the oil cells remained dark, in contrast to the surrounding translucent tissues. Vascular tissue could also be visualized.

Isolation of oil cells

The idioblast oil cells were isolated from soft, ripe fruit that were past the climacteric peak. Three to 5 g of mesocarp tissue was cut with a razor blade into small (1–2 cm^3) pieces and placed in a Ten-Brock homogenizer with 10–20 mL distilled water. The tissue was homogenized until it was completely fluid (10–15 strokes), and additional water was added as necessary. The homogenate was filtered (washing with excess water) through a 200-μm nylon mesh to remove vascular strands and unhomogenized groups of cells. The filtrate was saved and filtered through a 48-μm nylon mesh. This second filtrate contained small cell debris and the triacylglycerols. The residue remaining on the 48-μm mesh was thoroughly washed with water and saved. This residue was transferred to conical centrifuge tubes and centrifuged at 80 g for 2–3 min. The pellet was washed several times with distilled water to remove remaining triacylglycerols and some of the cell walls. The pellet contained the isolated oil cells and some larger cell debris, primarily cell walls and small pieces of vascular strands.

Oil cells were also isolated from immature fruit and from mature unripe fruit. Approximately 5 g of mesocarp from freshly harvested fruit, both mature and immature, was removed with a #3 cork borer and cut into 1–2-mm slices. These were placed in 10 mL of digestion medium containing 1.5% Cellulysin from *Trichoderma viride* (CalBiochem), 0.1% pectolyase Y-23 (Karlan), and 0.25% macerozyme (CalBiochem) in 10 mM MES buffer, 100 mM Sorbitol, 1 mM $CaCl_2$, 1 mM DTT, and 0.2% BSA, pH 5.5 (Balsamo and Uribe 1988). The tissue was incubated for 2–3 h on a stir plate with continuous agitation and then treated as above for ripe tissue, i.e., homogenized with a Ten-Brock, filtered, and centrifuged. When the cells from ripe fruit contained large amounts of cell wall debris, the final milieu of oil cells and cell walls was incubated in the above enzyme mixture for about 1 h and then refiltered through the 48-μm nylon, saving the residue.

Light microscopy

Photomicrographs of isolated oil cells and of cleared tissues were taken with a Zeiss micro-

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Figs. 1–4 Fig. 1*A*, Light micrograph of an avocado fruit tissue slice that was cleared by microwave irradiation. Most of the contents of the parenchyma cells have been removed, resulting in translucent cells. The idioblast oil cells (arrows), however, have retained their contents and are visible as dark spots. × 60. Fig. 1*B*, Low-magnification electron micrograph of a thin section of an avocado fruit. The lipid (*L*) in the parenchyma cells occurs as several large droplets in the cytoplasm, while the idioblast oil cell (*OC*) contains a single mass of oil that stains with a different density than the parenchyma lipid. × 640. Fig. 2*A*, Light micrograph of a slice of cleared avocado seed cotyledon. The opaque idioblast oil cells (arrows) stand out in contrast to the transparent parenchyma cells. × 60. Fig. 2*B*, Low-magnification electron micrograph of a thin section of avocado cotyledon. The parenchyma cells contain large amounts of starch (*S*) and electron-dense deposits in the vacuoles (*V*). The idioblast oil cell (*OC*) is almost filled with oil. × 760. Fig. 3*A*, Light micrograph of a portion of an avocado leaf that has been cleared as described in the text. The vascular tissue (*Vt*) and the oil cells (arrows) are easily visualized. × 50. Fig. 3*B*, Low-magnification electron micrograph of a portion of an avocado leaf cross section. The large idioblast oil cell (*OC*) is filled with an almost electron-transparent oil. A thin layer of electron-dense cytoplasm is around the cell periphery. × 1,300. Fig. 4*A*, Light micrograph of a longitudinal view of a cleared avocado root that was cut in half lengthwise. The oil cells appear as dark spots scattered throughout the cortex, and the vascular tissue (*Vt*) is also visible. × 50. Fig. 4*B*, Low-magnification electron micrograph of a longitudinal section of an avocado root. The oil cell contains irregular deposits of oil (*O*) and of a granular/fibrillar substance (*F*). × 600.

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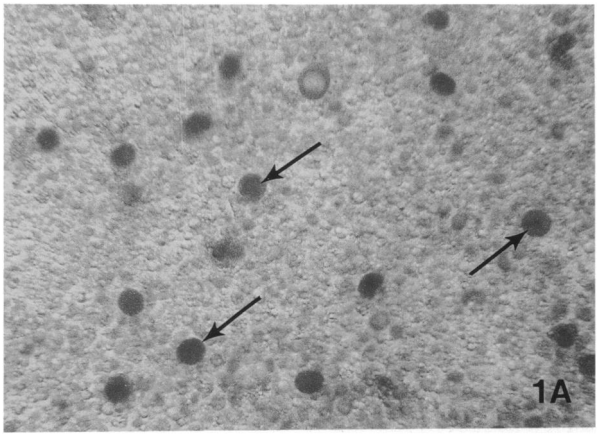

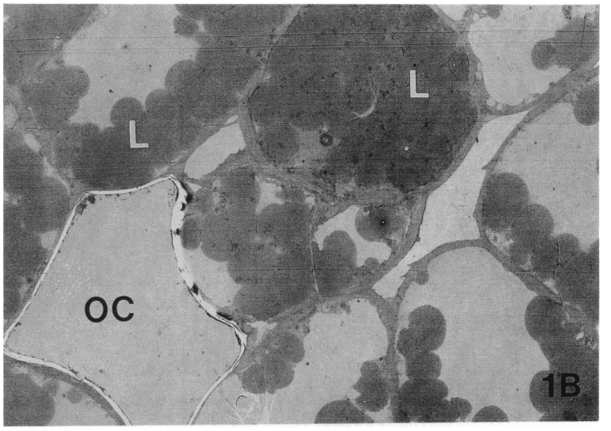

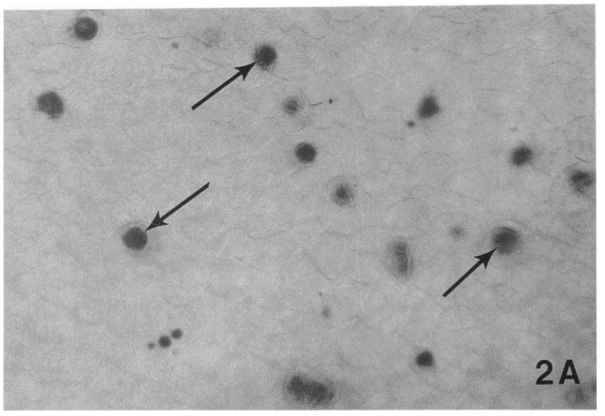

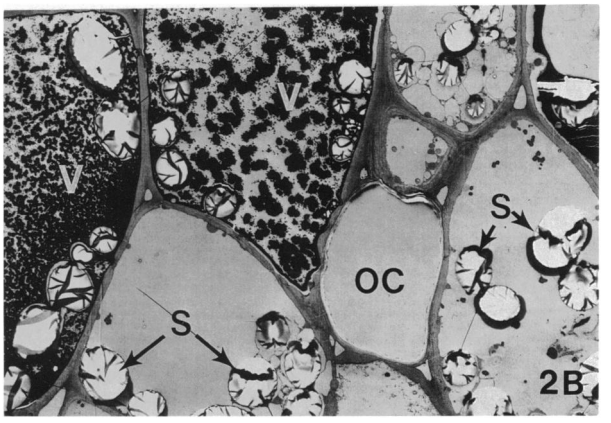

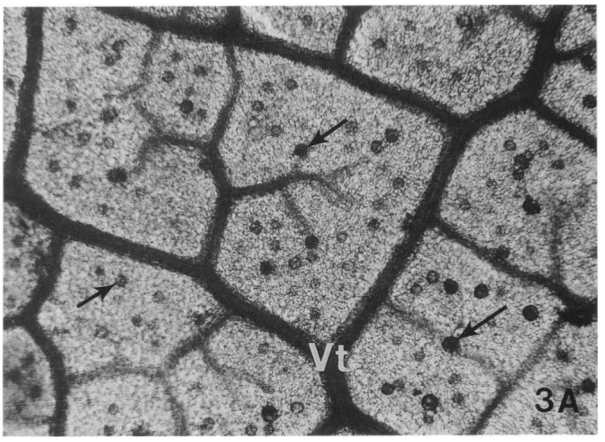

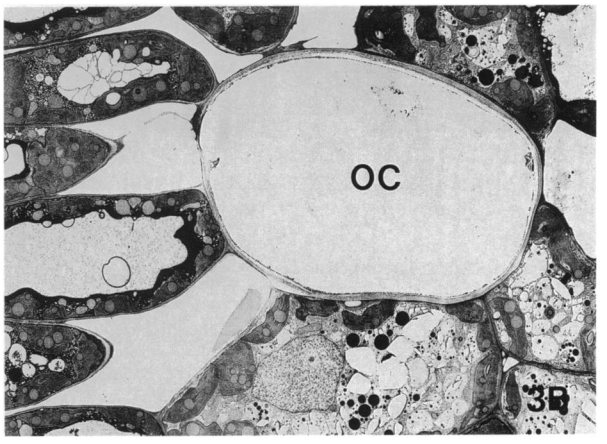

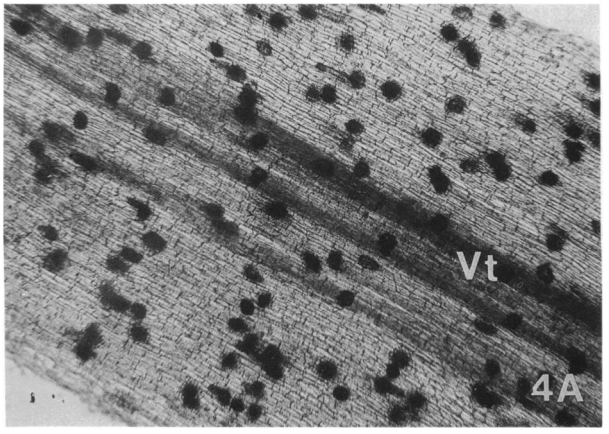

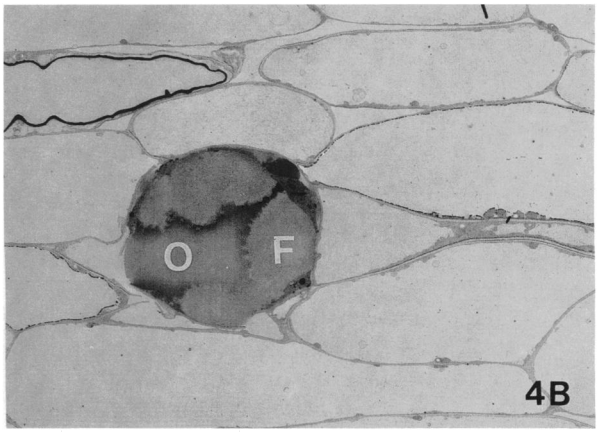

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scope equipped with a Nikon Optiphot and Kodak Tmax film. Dittmar and Wagner reagents were used as indicators for alkaloids (Furr and Mahlberg 1981). These stains were prepared according to Furr and Mahlberg (1981) as follows: Dittmar reagent—1 g potassium iodide, 1 g sodium nitrite, 30 mL hydrochloric acid, 30 mL distilled water; Wagner reagent—1.27 g iodine, 2.0 g potassium iodide, 100 mL distilled water. Terpenoids were stained with the Nadi reagent (0.5 mL 1% w/v α-naphthol in 40% ethanol, 0.5 mL 1% dimethyl phenylenediamine-HCl, 49 mL 50 mM potassium phosphate buffer, pH 7.2) (David and Carde 1964) and by the use of concentrated sulfuric acid (Kelsey and Shafizadeh 1980; Cappelletti et al. 1986; Mariani et al. 1989). The indicators were applied directly to a suspension of isolated oil cells on a microscope slide and observed in the light microscope. Photographs were taken using Kodak Kodachrome slide film, ASA 64.

Thin-section electron microscopy

Samples of fruit for thin-section electron microscopy were removed with a #3 cork borer. These plugs were placed in glutaraldehyde-formaldehyde fixative (Karnovsky 1965), sliced into 1–2-mm slices, and cut into smaller pieces in the fixative. Leaf slices, roots, and seed cotyledon slices were cut in the same fixative. All samples were fixed at room temperature for 2–4 h, rinsed in buffer, postfixed in two changes of 1% OsO_4 (1–2 h, then overnight), dehydrated in acetone, and embedded in Spurr's resin (Spurr 1969). Large (>1 mm^2) thin sections for low-magnification electron micrographs were handled according to the method of Galey and Nilsson (1966), except that freeze-fracture grids (Pelco, Redding, Calif.) were used instead of one-hole grids.

Freeze-fracture electron microscopy

Samples to be examined using freeze fracture were frozen in liquid propane, and replicas were prepared in a Balzers BAF301 freeze-fracture apparatus according to the methods of Moor and Mühlethaler (1963) as modified by Platt-Aloia and Thomson (1982). All electron microscopy was performed on a Philips EM 400 electron microscope at 80 kV.

TLC of oil cells

Lipids of the isolated oil cells were extracted by a modification of the technique of Bligh and Dyer (1959). Extraction was with chloroform : methanol 2:1 (v/v), followed by centrifugation. The lower phase was washed twice with distilled water and then concentrated under N_2 gas. Thin-layer chromatography was performed on silica plates (Silica Gel 60A from Whatman), using a solvent system of benzene : ether : acetic acid (80:20:1, v/v/v), and treated with iodine vapors.

Results and discussion

Microwave irradiation speeds up the preparation of tissue clearing from several days to less than half an hour (figs. 1*A*–4*A*). The vascular tissue and the oil cells can be easily distinguished, and the distribution of the oil cells in each organ can be determined. In all samples thus far examined, the distribution of oil cells appears to be dispersed. This technique will also prove useful in determining the presence, distribution, and abundance of oil cells in tissues of other plant species.

Electron microscopy confirms the presence of oil cells in all tissues, verifying the validity of the clearing procedure. Oil cells from the fruit (fig. 1*B*), seed cotyledon (fig. 2*B*), and leaf (fig. 3*B*) all have essentially the same ultrastructural organization of both the wall structure and the oil contents. The contents of root oil cells (figs. 4*B*, 5), however, do not form one large oil drop, and the cytoplasm does not always appear deteriorated as it does in oil cells of the other tissues (fig. 5). Additionally, the contents of the root oil cells frequently stain more densely with OsO_4 than the contents of the oil cells of the other tissues and sometimes have a significant amount of some component other than oil (figs. 4*B*, 5). These differences are probably significant in terms of both function and composition and are being investigated further. Another possibility is that fully mature oil cells were not observed in the roots prepared for this study.

The different staining density of the oil in an oil cell from a mature unripe avocado fruit compared with the storage lipid of the parenchyma cells is evident (figs. 1*B*, 6). The cell wall of the idioblast is composed of an outer primary wall, a secondary suberin layer, and an inner tertiary wall (fig. 6). This organization is also observed in the oil cells of the leaf, root, and cotyledon of avocado, has been described in oil cells of other species (Maron and Fahn 1979; Postek and Tucker 1983; Mariani et al. 1989), and may be considered as a characteristic of this cell type. The cytoplasm of the mature oil cells, as described previously for avocado (Platt-Aloia et al. 1983) and for other species (Fahn 1979; Maron and Fahn 1979; Postek and Tucker 1983), is apparently degenerated. However, this could result from inadequate preservation because of poor penetration of fixative through the suberized cell wall. Oil cells isolated from mature ripe avocado fruit (fig. 7*A*) have a similar appearance to those isolated from unripe immature fruit (fig. 7*B*) and from unripe mature fruit (fig. 7*C*).

The use of various stains and reagents for identification of alkaloids and terpenes has been re-

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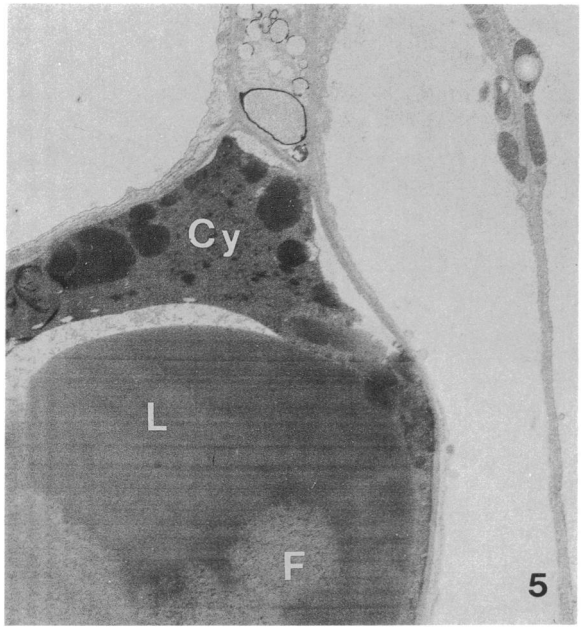

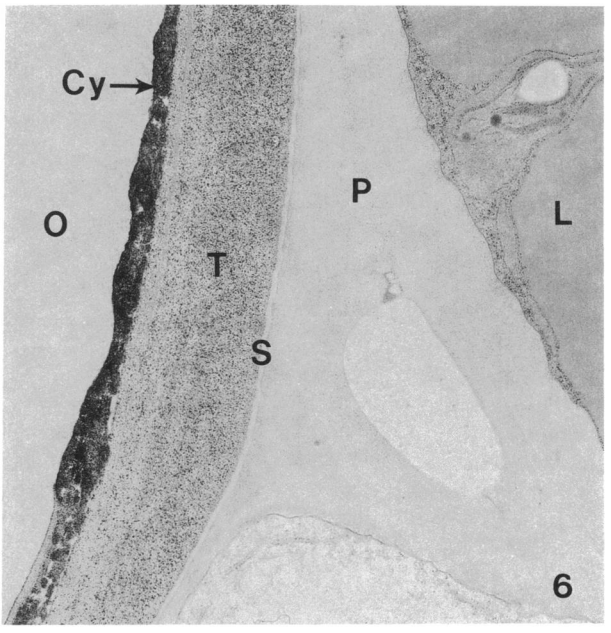

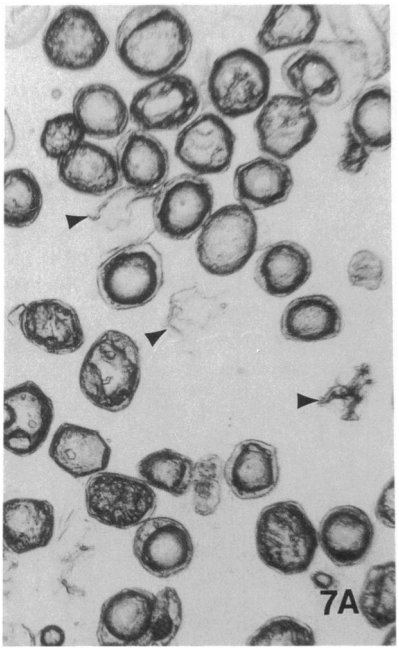

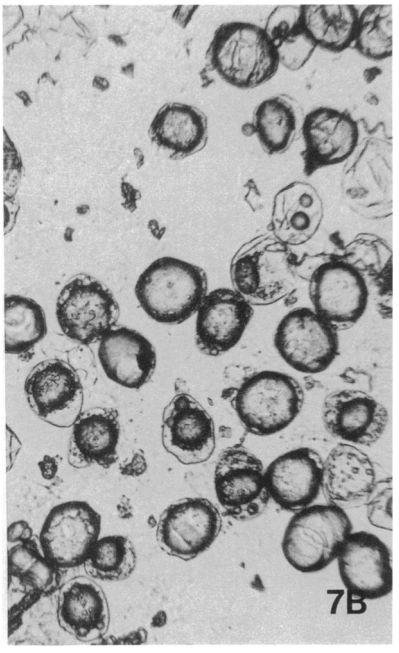

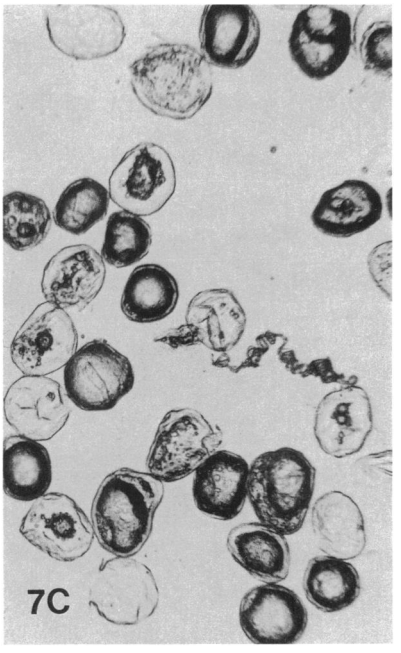

Figs. 5–7 Fig. 5, Electron micrograph of a portion of an oil cell in the root of an avocado. Although electron dense, the cytoplasm (*Cy*) is not completely degenerated. The contents are partially lipid (*L*) but contain a considerable portion that is fibrillar (*F*), indicating a component other than lipid. × 2,700. Fig. 6, Electron micrograph of a section through a portion of the complex cell wall of an oil cell from an avocado fruit. *P* = primary cellulosic wall; *S* = suberized layer; *T* = tertiary wall. The electron-dense cytoplasm (*Cy*) occurs as a thin layer around the cell periphery, and the oil (*O*) has less electron density than the lipid (*L*) in the adjacent parenchyma cell. × 12,100. Fig. 7*A*, Light micrograph of the residue of a homogenate of a mature ripe avocado after filtration through a 48-μm nylon mesh. This preparation is composed of a highly enriched population of idioblast oil cells and some large cell debris (arrowheads). × 110. Fig. 7*B*, Light micrograph of isolated oil cells from an immature avocado fruit. × 110. Fig. 7*C*, Light micrograph of isolated oil cells from a mature unripe avocado fruit. × 110.

ported for secretory trichomes of *Artemesia* sp. (Kelsey and Shafizadeh 1980; Cappelletti et al. 1986; Ascensao and Pais 1987), *Cannabis sativa* (Furr and Mahlberg 1981), *Humulus lupulus* (Oliveira and Pais 1988), *Teucrium scorodonia* (Antunes and Sevinate-Pinto 1991), and *Geranium robertianum* (Pedro et al. 1990). A histochemical examination of oil cells in *Liriodendron tulipifera* (Mariani et al. 1989) has also been described. These studies have reported the presence of a variety of lipid classes, including essential oils, sterols, resins, and terpenes as well as alkaloids and phenols.

We have applied several of these tests to iso-

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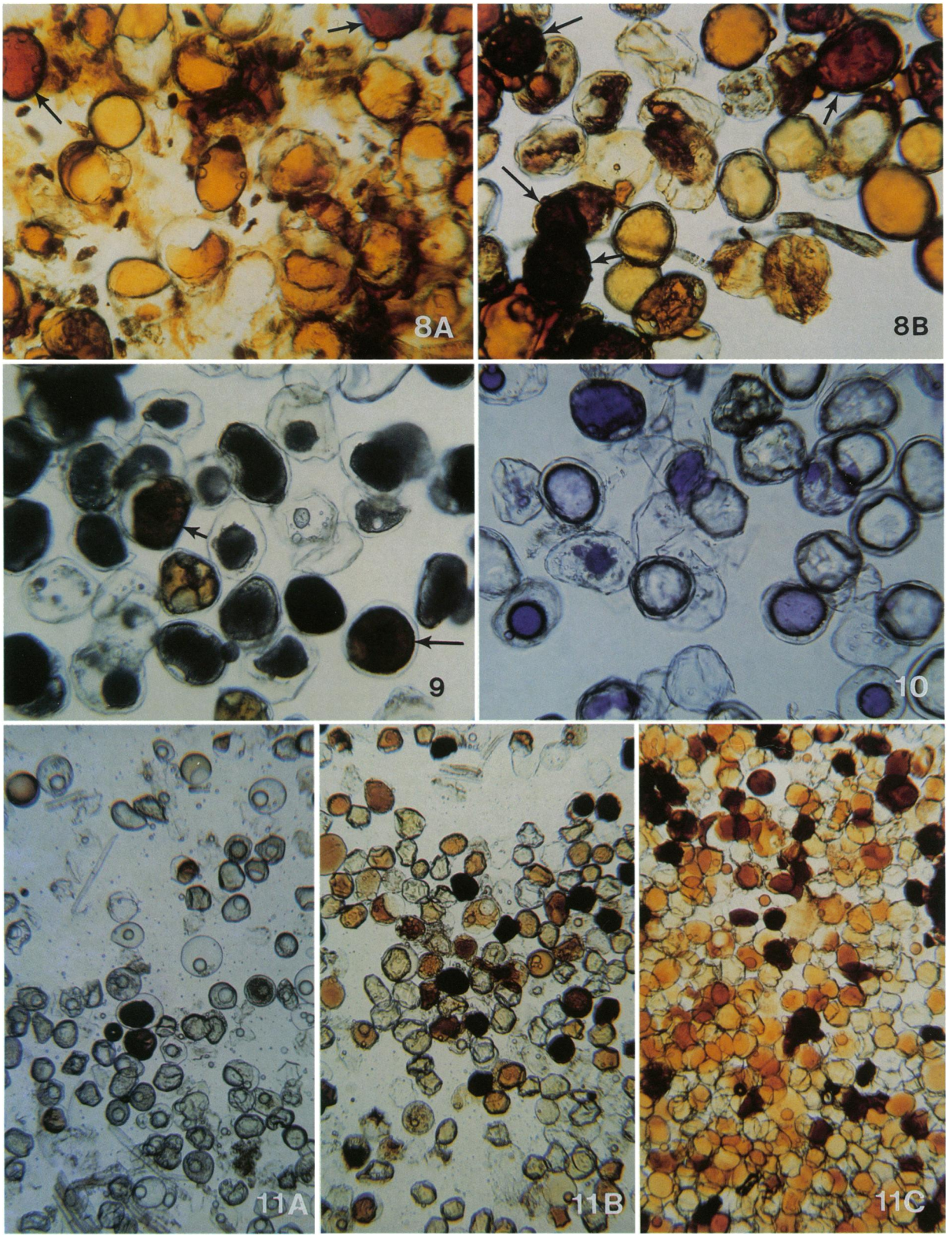

Figs. 8–11 Fig. 8*A*, Oil cells isolated from an immature avocado fruit and stained for alkaloids with Wagners reagent. Only a few of the cells (arrows) show the positive red-brown reaction color. × 170. Fig. 8*B*, Oil cells isolated from a mature avocado fruit and stained with Wagners reagent. Several of the cells show a positive red-brown reaction for alkaloids (arrows). × 170. Fig. 9, Oil cells isolated from a mature unripe avocado fruit and stained with Dittmar reagent. Some of the cells (arrows) show a positive red-brown reaction product for alkaloids. × 170. Fig. 10, Oil cells isolated from a mature unripe avocado fruit and stained with the Nadi reagent. Several cells show a violet reaction product, indicative of the presence of terpenes. × 170.

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lated oil cells from avocado fruit at three developmental stages: immature, mature unripe, and mature ripe. The tests for alkaloids using the Wagner reagent (figs. 8*A, B*) and Dittmar reagent (fig. 9) give positive, although incomplete, reactions. Some cells stain very deep red-brown, indicating the presence of alkaloids (Furr and Mahlberg 1981; Ascensao and Pais 1987), and the whole range from light yellow to red-brown occurred with both stains. This result may indicate either variations in the amount or types of alkaloids present in different cells or differences in the penetration of the stain or may result from leakage of contents from some cells during isolation. Results from the Wagner reagent indicate that more cells from the mature fruit react with a darker staining reaction than cells from the immature fruit (figs. 8*A, B*). There is, however, no variation observed in the staining pattern with the Dittmar reagent of cells isolated from immature, mature unripe, or mature ripe fruit (fig. 9).

Two different stains were employed for the detection of terpenoids. The Nadi reagent has been used as an indicator of various components based on the color of the reaction product (David and Carde 1964; Ascensao and Pais 1987, 1988; Pedro et al. 1990; Antunes and Sevinate-Pinto 1991), with pink indicating resiniferous acids; blue, essential oils; and violet, a mixture of both of these compounds. The isolated oil cells of both immature and mature avocado fruit show a violet reaction product in some but not all of the cells (fig. 10). Again, the incomplete reaction may result from variation in either the composition of the cells or penetration of the reagent.

The second reagent used for terpenoid detection is concentrated sulfuric acid. Sesquiterpene hydroperoxides have been shown to give a red color when reacted with this compound (Kelsey and Shafizadeh 1980; Cappelletti et al. 1986; Mariani et al. 1989). When isolated oil cells from avocado fruit are treated with H_2SO_4, not only did some cells show a definite color reaction but there is a noticeable difference in the reaction of cells isolated from immature, mature unripe, and mature ripe fruit (figs. 11*A, B, C*). Almost no cells from the immature fruit show a color change when treated with H_2SO_4, while those from the mature fruit show considerable reaction color, with the ripe fruit cells showing more staining than those from the unripe fruit. Variations in the staining intensity between individual cells result most likely from a real difference in the composition, since poor penetration of the H_2SO_4 is unlikely and because there is an increase, with development, in the number of cells showing a reaction.

Although these histochemical tests are not definitive, the results indicate that both alkaloids and terpenoids, including sesquiterpene hydroperoxides, in particular, may be present in these oil cells of avocado fruit. The results of the H_2SO_4 treatment further indicate a change in the composition of the "oil" with maturation, and possibly with ripening.

Freeze-fracture electron microscopy of whole avocado tissue produces replicas that contain fractures through both the triacylglycerol storage lipid (fig. 12), as well as through the oil of the oil cells (fig. 13). The pattern of fracture of each of these lipids is different compared with the other. Freeze-fracture of storage oils and triacylglycerols from other tissues (Moeller and Thomson 1979) have been shown to have an appearance similar to the pattern seen here for the avocado triacylglycerols. When avocado fruit tissue is held at 4 C for several days before preparation for freeze fracture, the fracture pattern of the triacylglycerols remains unchanged. However, the oil of the idioblast oil cells undergoes a transition, and replicas of oil cells from low-temperature-treated fruit contain crystalline patterns (fig. 14). The oil has formed ridges with a regular repeat distance of 40 nm (Thomson and Platt-Aloia 1990). When pellets of isolated oil cells of avocado fruit are are stored at 4 C for 3–4 d before preparation for freeze-fracture electron microscopy, the oil also undergoes a similar transition (fig. 15). These patterns are reminiscent of replicas of some phospholipids held below their fluid or liquid crystalline to gel phase transition temperature (Borovyagin and Sabelnikov 1989). Although the lipids in the oil cells are not phospholipids, they apparently do exhibit temperature-induced polymorphism.

Previous studies on the lipids of avocado report the major component to be triacylglycerols (TAG), with diacylglycerols, free fatty acids, and phospholipids as minor but measurable constituents (Biale and Young 1971). Many of these studies also include about 2% of the lipid fraction as containing "other" or nonsaponifiable lipids (Biale and Young 1971). Because of the staining differences of the idioblast oil cell lipid compared with the storage oil in the parenchyma cells, it

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Fig. 11*A*, Oil cells isolated from an immature avocado fruit and stained with concentrated sulfuric acid for the detection of sesquiterpene hydroperoxides. Only a very few of the cells show a positive reaction. × 70. Fig. 11*B*, Oil cells isolated from a mature unripe avocado fruit and treated with sulfuric acid. A greater percentage of the cells show a positive reaction than was seen in the immature fruit (fig. 11*A*). × 70. Fig. 11*C*, Oil cells isolated from a mature ripe avocado fruit and treated with sulfuric acid. Almost all of the cells show some reaction product color, and many have stained with an intense coloration. × 70.

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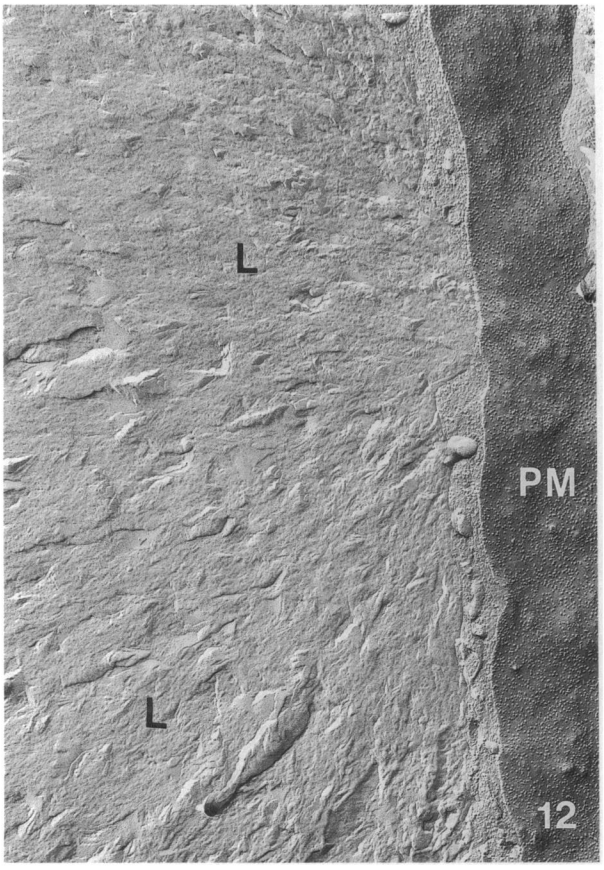

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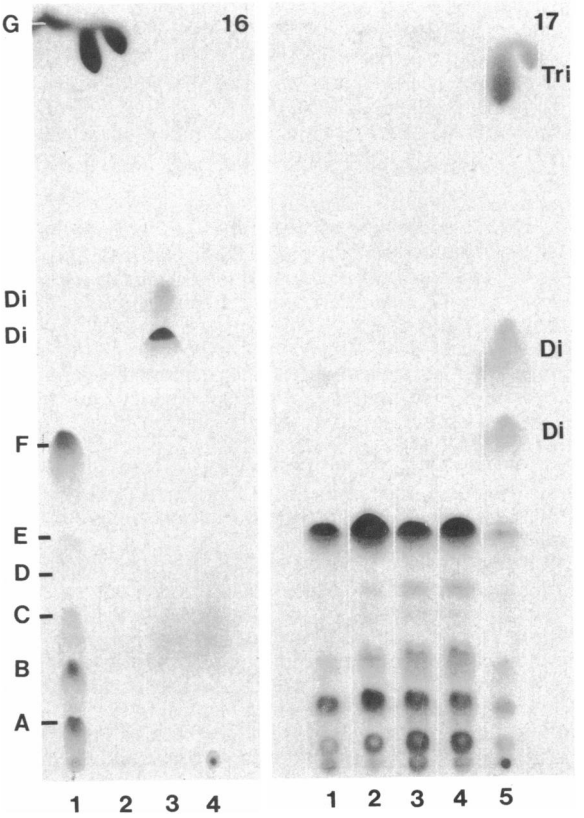
was hypothesized that the lipid contained in these cells could be an important contributing factor to the "other" category described in earlier studies. In order to test this hypothesis, lipid extracts of whole avocado homogenate, of isolated oil cells, and of standards corresponding to the major lipid categories reported for avocados, namely, phospholipid, diacylglycerol, and TAG, were compared using thin-layer chromatography. Solvent systems that allowed optimum separation and identification of TAG did not achieve adequate separation of the oil cell components (not shown). Therefore, the system was modified so that the TAG moved with the solvent front. This achieved separation of several different components present in the oil cell extract (fig. 16) and confirmed that the lipid contained in the oil cells is indeed not TAG, diacylglycerol, or phospholipid but a combination of several lipids that have a polarity between phospholipids and diacylglycerols.

Because of the differences in the staining reactions obtained with the histochemical studies, we extracted oil from isolated oil cells of immature, mature unripe, and mature ripe fruit (fig. 17). Chromatograms of total extracts of immature and mature unripe fruit (fig. 17, lanes 1 and 5) indicate an increase in diacylglycerols (*Di*) and triacylglycerols (*Tri*) with maturation of avocado fruit. Comparison of chromatograms of oil cell extracts from immature, mature unripe, and mature ripe fruit (fig. 17, lanes 2, 3, and 4) indicate that there is no significant change in the composition of oil cells during maturation and ripening detectable by thin-layer chromatography as applied in this study.

Earlier studies on the ultrastructure of oil cell development in avocado fruit (Platt-Aloia et al. 1983) showed that oil cells reach a mature structural stage in very small fruit (<1 cm long). The immature fruit used in the present study were well past this stage of maturity, and the oil cells from these fruit were therefore structurally mature and, based on the chromatography, also mature in composition. The apparent chemical differences as detected by the histochemical tests may result from a component not identifiable by TLC.

The overlying question concerning idioblast oil cells is their function, if any. This requires isolating the oil cells and determining the composition of the oil.



Figs. 16, 17 Fig. 16, Thin-layer chromatogram of avocado lipid extracts and standards. Lane 1 = oil cells isolated from a mature avocado fruit, corresponding to fig. 7*A*; 7 different spots are visible. Lane 2 = triacylglycerol fraction. This component stays with the front, using this solvent system. Lane 3 = diacylglycerol standard, 1,2 diolein. Two spots are visible (*Di*), with a retention between the oil cell components and the triacylglycerols. Lane 4 = phospholipid standard, phosphatidylcholine, which stays at the origin. Fig. 17, Thin-layer chromatogram of extracts of avocado fruit at different stages of maturity and ripening. Lane 1 = extract of total lipid fraction from an immature avocado fruit. Lane 2 = oil cells isolated from an immature avocado. Lane 3 = oil cells isolated from a mature unripe avocado. Lane 4 = oil cells isolated from a mature ripe avocado. Lane 5 = extract of the total lipid fraction from a mature unripe avocado fruit. The appearance of diacylglycerols (*Di*) and triacylglycerols (*Tri*) corresponds to the increase in lipid content of avocado fruit during maturation.

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Figs. 12–15 Fig. 12, Electron micrograph of a freeze-fracture replica of the storage lipid (*L*) in a parenchyma cell of a mature avocado fruit. The fracture pattern is distinctive and typical of freeze-fracture patterns of triacylglycerols. *PM* = *P* face of plasmalemma. × 27,500. Fig. 13, Electron micrograph of a replica of the contents of a freeze-fractured idioblast oil cell in a mature avocado fruit at room temperature. The fracture pattern is distinctive and different from that obtained with the storage lipid shown in fig. 12. × 16,500. Fig. 14, Freeze-fracture replica of the oil in an idioblast oil cell of an avocado that was stored at 4 C. Some component of the oil has undergone a transition to a crystallized form. × 52,500. Fig. 15, Replica of the oil in an isolated oil cell that was stored at 4 C and frozen from that temperature. The oil has crystallized. × 52,500.

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