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DEVELOPMENTAL ANATOMY OF THE AVOCADO STIGMA PAPILLA CELLS AND THEIR SECRETION

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The development of the avocado stigma was observed by light and electron microscopy from ca. 3 wk prior to anthesis to flower opening. The stigma papilla cells grew from 50 to 250 μm long. At the earliest stages the cells had many features of the mature cells, including extensive smooth endoplasmic reticulum and dictyosomes. Plastid clusters appeared at 3 days and wall thickenings containing lipid at 2 days prior to anthesis. Some secretion containing carbohydrate and protein was present at all stages, but secretion of most of the carbohydrate and all of the lipid occurred during the 2 days prior to anthesis. The lipid bodies appeared to become surrounded by carbohydrate, as stained by the thiosemicarbazide-silver proteinate method, prior to passing through the wall thickenings by eccrine secretion. This method also stained the secretion, cell wall, dictyosomes, and plastids. Serial sectioning showed that most of the plastid clusters were arranged with the large starch-containing heads on the periphery and the long tails pointing toward the center. Many plastids were large and complex, but the clusters were composed mainly of discrete organelles. We suggest that the plastids have a role in the lipid secretion of the papilla cells, which passes through the wall, along with some carbohydrate, by eccrine secretion via specialized areas of thickened cell wall.

Introduction

Stigma ultrastructure has been studied in a number of species, particularly in relation to pollen recognition, hydration, germination, and tube growth (KNOX 1983). The angiosperm stigma is generally considered to be a glandular structure whose secretion is important in the pollen-stigma interaction. The avocado stigma has an extracellular secretion that contains carbohydrate, lipid, and protein (SEDGLEY and BUTTROSE 1978). The papilla cells have many characteristics of secretory cells, including extensive smooth endoplasmic reticulum (SER), plastids with little internal structure, and dictyosomes. The ultrastructure is somewhat unusual in that the plastids are arranged in clusters and the cell walls have small thickenings that often contain lipid (SEDGLEY 1979). In this study, the structure of the developing avocado stigma was observed, with special reference to the possible function of these features and to the production of the stigma secretion.

Material and methods

Grafted avocado (*Persea americana* Mill. 'Fuerte') plants that had initiated floral buds were grown in a controlled-environment cabinet with temperatures of 25/20 C, day/night, a 12-h photoperiod, and a photon flux density of 500 μE m^{-2} s^{-1} (400–700 nm), or in a glasshouse under similar conditions. Avocado flowers are produced in inflorescences. Under these conditions, the period between the emergence of the individual flower buds from the inflorescence bud and anthesis was ca. 3 wk. During this period the flower buds increased from 1.5 to 6 mm long. The age of a bud was estimated from longitudinal measurements of buds that were allowed to open. Using this method, we could distinguish sampling periods of 1, 2, 3, 4–6, 7–9, and > 9 days prior to anthesis. Stigma tissue was sampled at each stage and at anthesis.

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ELECTRON MICROSCOPY

Tissue was fixed in 3% glutaraldehyde in 0.025 M phosphate buffer, pH 7, for 18 h, postfixed in 1% osmium tetroxide in the same buffer, dehydrated in an ethanol series, through propylene oxide, and embedded in Araldite. Silver sections mounted on grids were stained with uranyl acetate and lead citrate. In addition, serial sections were cut through the plastid clusters at anthesis at each of 0.9–1.9 nm (gold-purple sections) and 2.4–3.2 nm (green-yellow sections) (GALEY and NILSSON 1966; WELLS 1974) and stained as above.

ELECTRON MICROSCOPE CYTOCHEMISTRY

Tissue was processed as above with the omission of osmication. Silver sections were collected on gold grids and stained with the thiosemicarbazide-silver proteinate method for carbohydrates (THIERY 1967; LEWIS and KNIGHT 1977). Control sections received no periodic acid.

LIGHT MICROSCROPY AND HISTOCHEMISTRY

Glutaraldehyde-fixed tissue was embedded in glycol methacrylate (FEDER and O'BRIEN 1968). Tissue embedded in both Araldite and glycol meth-

acrylate was sectioned at 2 μm and stained with periodic acid–Schiff's reagent (FEDER and O'BRIEN 1968), Coomassie brilliant blue (FISHER 1968), ruthenium red (LUFT 1971), toluidine blue O (TRUMP, SMUCKLER, and BENDITT 1961; FEDER and O'BRIEN 1968), or Sudan black B (BRONNER 1975).

Results

At the earliest stages sampled, the papilla cells had a maximum length of 50 μm compared with 250 μm at maturity. The young stigma papilla cells had many of the ultrastructural features of the mature cells, including extensive areas of ER, small vacuoles, and plastids with starch (fig. 1). By 1 day prior to anthesis (figs. 2, 5), the ultrastructure was indistinguishable from that at maturity. The papilla cells showed marked polarity at anthesis, with most of the cytoplasm, organelles, and small vacuoles at the base of each cell and a large distal vacuole. The young cells did not have this polarity as the large distal vacuole did not commence development until 7–9 days prior to anthesis. The plastid clusters first appeared at 3 days and the wall thickenings containing lipid at 2 days before flower opening.

Some extracellular secretion was present at all stages and stained with periodic acid–Schiff's treatment and toluidine blue O, indicating the presence of polysaccharides. There was no staining with ruthenium red, indicating the absence of pectins. Coomassie brilliant blue gave a positive reaction at all stages, particularly to the outer surface of the secretion. Staining with Sudan black B showed that, at the earliest stages of papilla cell development, there was lipid inside but not outside the cells. Lipid first appeared in the secretion at 2 days prior to anthesis and was abundant by 1 day before flowering (fig. 3). Maximum secretion of both the lipid and carbohydrate components of the secretion occurred during the 2 days prior to anthesis.

Lipid droplets were present in the cytoplasm at all stages. They had no surrounding membrane and were observed in close association with all of the major organelles, including dictyosomes, ER, plastids, mitochondria, and microbodies (fig. 4). Lipid bodies were observed in thickenings of the cell wall during the 2 days prior to anthesis and at flower opening (fig. 5). As in the cytoplasm, no membrane was observed around the lipid in the cell wall or in the secretion.

The thiosemicarbazide-silver proteinate treatment produced a fine precipitate in the outer surface, in the body of the secretion, and in the cell wall at all stages (fig. 6). The only organelles specifically stained by this method were the dictyosomes (fig. 7) and the plastids. The lipid droplets in the cell wall thickenings did not stain but appeared to become surrounded by precipitate before entering the cell wall (fig. 6). There was no specific precipitate produced in the control sections without periodic acid.

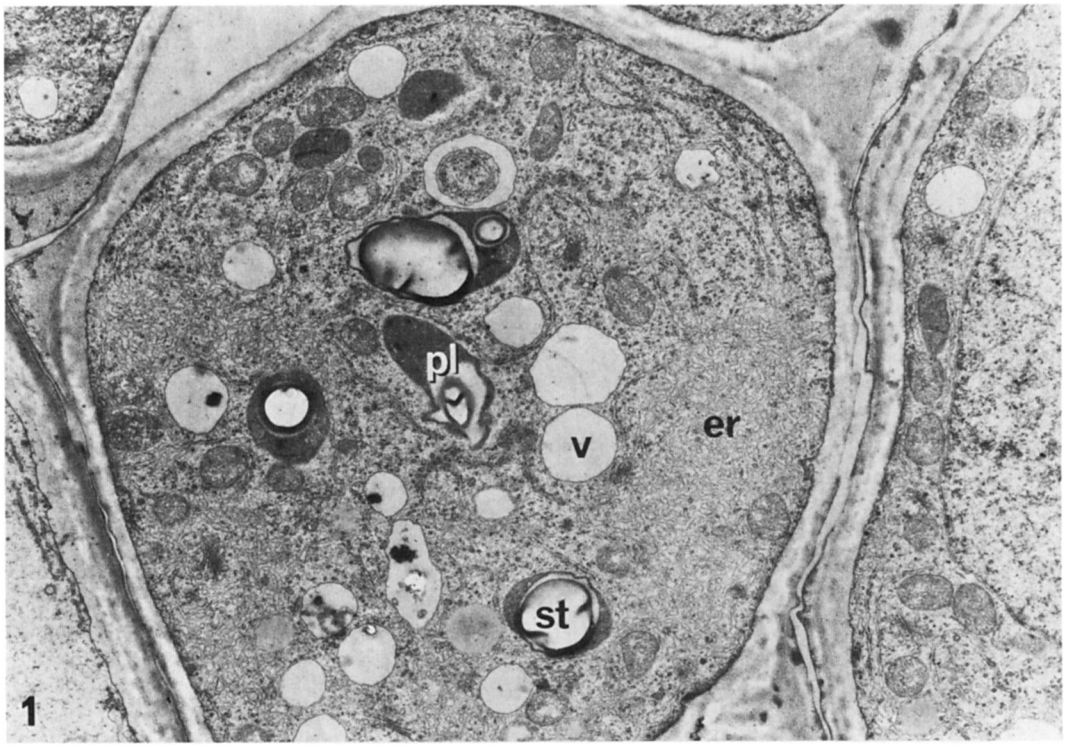
Most of the papilla cells had more than one plastid cluster at anthesis (fig. 3). Serial green-yellow sections of the clusters showed that most of the plastids were arranged with the large starch-containing heads at the periphery of the cluster and long tails pointing toward the center (fig. 8). Some of the plastids were very large and complex structures with multiple heads and tails (fig. 9), but serial gold-purple sections confirmed that most of the plastids were discrete organelles with a single head and tail (figs. 10–12). Not all the plastids of the cell were grouped into clusters, although some apparently distant plastids were associated with a cluster via their long tails (fig. 9). Many organelles were present in the plastid clusters, but none was particularly associated with the plastids.

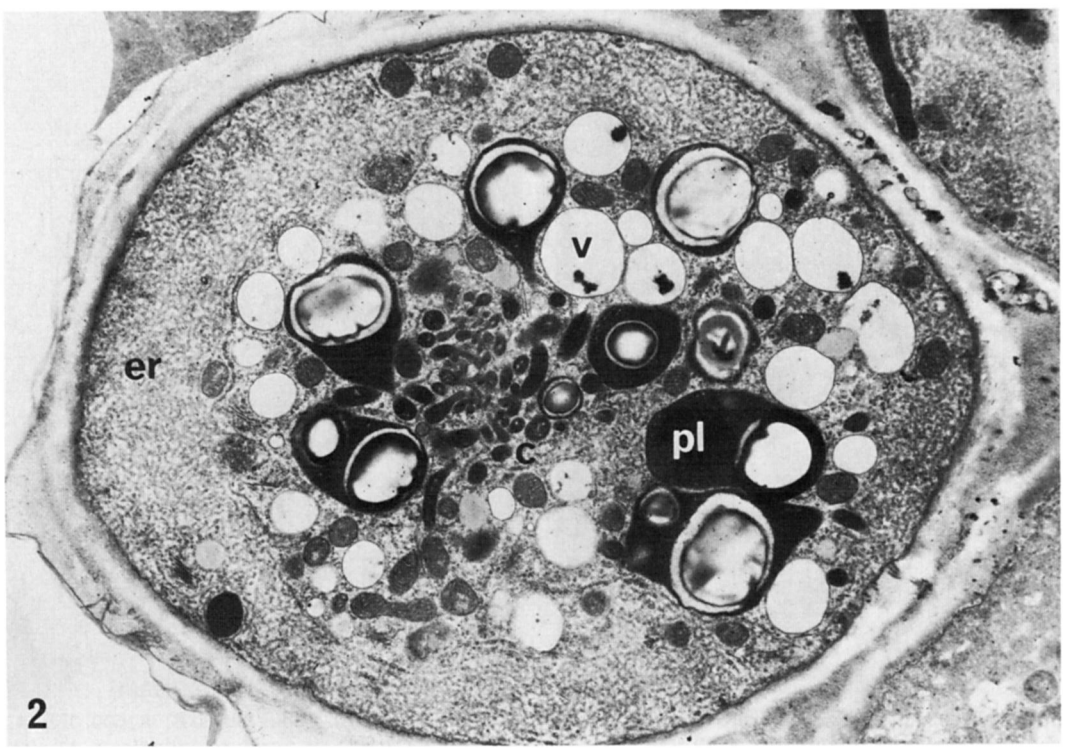
Discussion

Production of extracellular secretion by the avocado stigma occurred largely during the 2 days prior to anthesis, which coincided with the grouping of the plastids into clusters and the appearance of lipid in the cell wall and secretion. Plastids with little internal structure are common in cells that secrete lipophilic substances (SCHNEPF 1974), and plastid clusters of discrete organelles with complex three-dimensional structure have also been described in the glandular hairs of *Hygrophila difformis* (ROHR, DEXHEIMER, and KIEFFER 1980) and in the stigma papilla cells of *Gladiolus* (AMEELE 1982). Plastids from avocado mesocarp tissue are capable of synthesizing fatty acids (WEAIRE and KEKWICK 1975), and the accumulation of secretory lipid in or near the plastids of glandular cells has been reported (JOEL and FAHN 1980; ROHR et al. 1980; PACINI and CASADORO 1981). The avocado stigma plastid clusters may be involved in production of the secreted lipid.

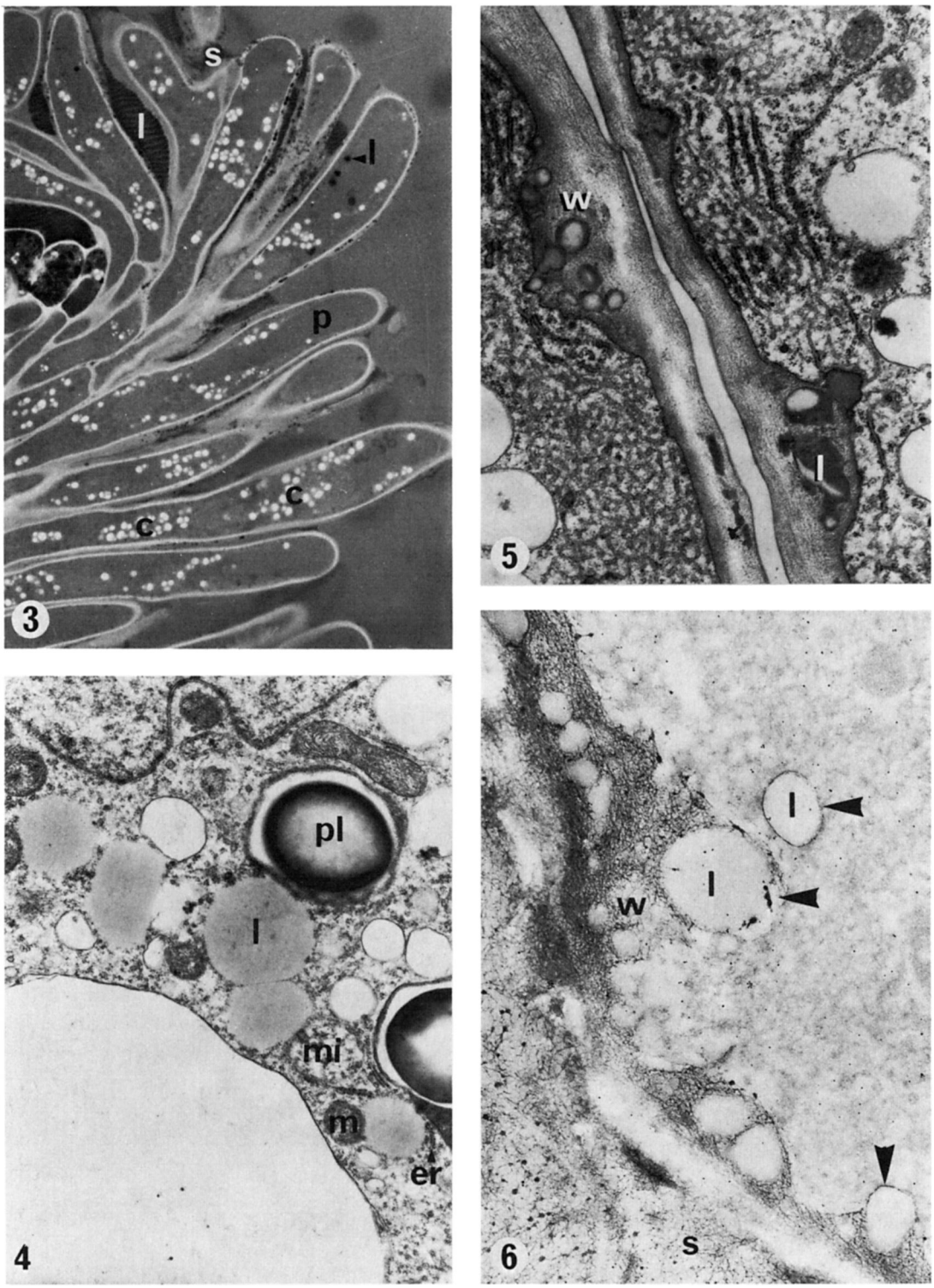
The lipid droplets did not have a surrounding membrane and were similar to those described in ripening avocado mesocarp tissue (PLATT-ALOIA and THOMSON 1981). Because of the lack of a membrane, granulocrine secretion involving membrane fusion was not possible in the avocado stigma, and the lipid appeared to pass through the wall by the eccrine mechanism (SCHNEPF 1974). Eccrine secretion of lipid was suggested for the *Forsythia intermedia* stigma (DUMAS 1977) where the lipid bodies also lacked a bounding membrane (DUMAS 1974).

The close associations between the lipid bodies and cell organelles suggest that the bodies may have been modified on their passage through the cytoplasm. One such modification appeared to be the accumulation of carbohydrate around the lipid body before it reached the cell wall. The dictyosome was

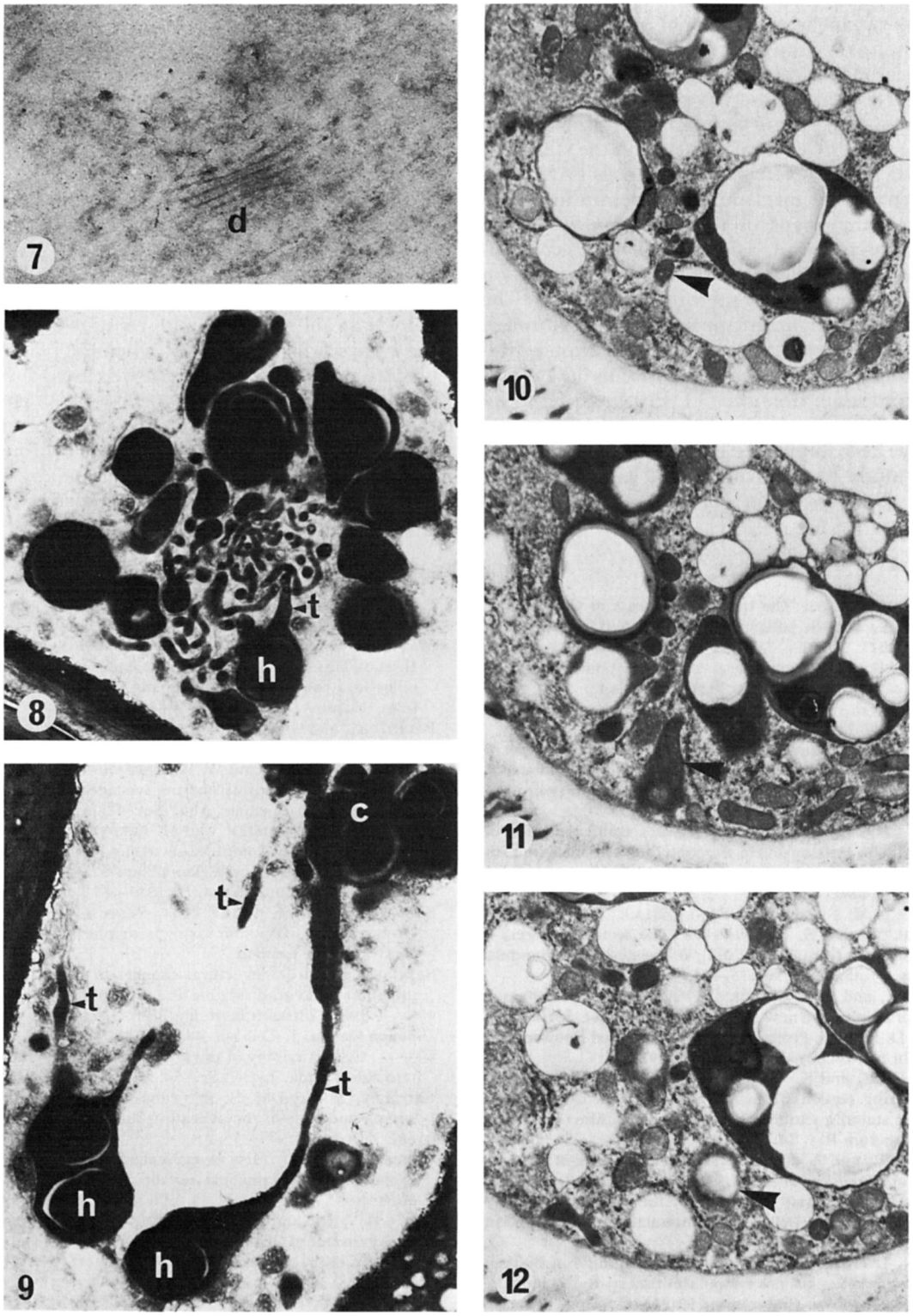




FIGS. 1, 2.—Fig. 1, Cross section of the basal portion of an avocado stigma papilla cell at 7–9 days prior to anthesis. Fig. 2, Cross section of the basal portion of an avocado stigma papilla cell at 1 day prior to anthesis. *c* = plastid cluster, *er* = endoplasmic reticulum, *pl* = plastid, *st* = starch, *v* = vacuole. Both × 7,400.



FIGS. 3–6.—Fig. 3, Stigma papilla cells at 1 day prior to anthesis stained with Sudan black B; × 600. Fig. 4, Stigma papilla cell at 2 days prior to anthesis; × 10,500. Fig. 5, Stigma papilla cell at 1 day prior to anthesis; × 13,400. Fig. 6, Stigma papilla cell at anthesis stained by the thiosemicarbazide-silver proteinate method; × 21,000. *c* = plastid cluster, *er* = endoplasmic reticulum, *l* = lipid, *m* = mitochrondrion, *mi* = microbody, *p* = papilla cell, *pl* = plastid, *s* = secretion, *w* = wall thickening; arrowheads show lipid surrounded by carbohydrate.



FIGS. 7–12.—Fig. 7, Stigma papilla cell at 2 days prior to anthesis stained by the thiosemicarbazide-silver proteinate method; × 36,800. Figs. 8, 9, Green-yellow sections of papilla cells at anthesis; both × 7,600. Figs. 10–12, Gold-purple sections of avocado stigma papilla cell at anthesis from a series of 47 sections through a plastid cluster; × 9,600. *c* = plastid cluster; *d* = dictyosome; *h* = head of plastid; *t* = tail of plastid; arrowheads show one completely sectioned plastid.

the only organelle to show polysaccharide accumulation apart from the plastids, which store insoluble carbohydrate. Carbohydrate may have been transferred from the dictyosomes to the lipid bodies. Close associations between lipid droplets and dictyosome vesicles were observed in the stigma of *Lycopersicum peruvianum* (DUMAS et al. 1978). This may also be the mechanism whereby the carbohydrate component of the secretion is passed through the cell wall, as no carbohydrate-containing secretory vesicles were observed in association with the cell wall at any stage of development. The lipid and carbohydrate appeared to pass through the cell wall at the thickened areas. Simple wall thickenings have also been described in the stigmas of *Ornithogalum caudatum* (TILTON and HORNER 1980) and *Citrullus lanatus* (SEDGLEY 1981, 1982) and have been implicated in secretion in both cases.

This study has shown that the extracellular secretion of the avocado stigma consists of carbohydrate and lipid bounded by a layer containing carbohydrate and protein. Proteins or glycoproteins were implicated in pollen-stigma recognition (MATTSON et al. 1974; CLARKE and KNOX 1978), and components of the secretion may be used by the growing pollen tube (LOEWUS and LABARCA 1973). Some carbohydrate and protein are present in the secretion up to 3 wk prior to anthesis. We have shown that extensive SER is present throughout development, and this may have a major role in secretion as suggested in other species (KRISTEN 1977; TILTON and HORNER 1980). Most of the carbohydrate and all of the lipid secretion occur during the 2 days prior to anthesis, when the plastid clusters are present and many plastids have developed into large and complex organelles. We suggest that the plastids have a role in the lipid secretion of avocado papilla cells, which passes through the wall, along with some carbohydrate, by eccrine secretion via specialized areas of thickened cell wall.

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