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Ultrastructure of Extrafloral Nectaries in *Aphelandra* spp. (Acanthaceae)

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Light and electron microscopy was employed to study the morphology and development of the bracteal nectaries of several species of *Aphelandra* (Acanthaceae). Special emphasis was given to the sub-secretory cells that show striking differences in wall structure and cytoplasmic detail when compared to the cells of the adjacent secretory layer. The appearance and location of these cells in the nectary are considered in the over-all functioning of this gland.

INDEX DESCRIPTORS: Ultrastructure; extrafloral nectaries; *Aphelandra;* Acanthaceae.

Extrafloral nectaries of the "Schuppennektarien" type (Zimmermann 1932) have been reported for a number of species (Maheshwari 1954; Inamdar 1969; Elias and Prance 1978). As this name implies, such nectaries are scale-like and slightly elevated above the surface. Although most common on leaves, they also have been observed on petioles, bracts, and fruits. The genus *Aphelandra* (Acanthaceae) contains a large number of species with bracteal nectaries of the scale type (Mattei 1909; Zimmerman 1932; Durkee 1972 and personal observations).

All of the observations on the structure of such nectaries have been based on light microscopy and show that they are typically composed of a layer of columnar secretory cells with dense cytoplasm and small vacuoles. Below this is one or more highly vacuolate sub-secretory cells which may stain so lightly as to appear empty. These sub-secretory cells have conspicuously thickened lateral walls. The nectaries are not vascularized.

The appearance of the sub-secretory layer with its distinctive cell walls led to the suggestion by some workers (Maheshwari 1954; Maheshwari and Chakrabarty 1966) that these cells had a regulatory role. Their radial walls were believed to be lignified and therefore impervious. Under conditions of high humidity, water taken up by the plant would be channeled through the cytoplasm of these cells so that they could act selectively on this fluid, retaining essential components while allowing metabolic by-products to pass through for final elimination by the secretory cells. The glands were thus postulated to serve as elaborate hydathodes, with the nectar sugar as an osmoticum, driving the movement of water with dissolved substances towards the nectary. Earlier, Radtke (1926) had disproved the idea that nectar could act in this fashion and today it is commonly accepted that the increased nectar secretion observed in conditions of high humidity is a reflection of the hygroscopic nature of the secreted sugar. Nectaries are glands specialized for the secretion of sugar, although the mode of secretion and its significance remain controversial (O'Dowd and Catchpole 1983; Durkee 1983). In *Aphelandra* and other plants with "Schuppennektarien" or the morphologically similar "Flachnektarien" (Zimmerman 1932), however, the function of the sub-secretory cells remains problematic. Because no ultrastructural studies have been done on these nectaries, it was felt that such a study, coupled with information on the quality and pattern of secretion, would be a logical first step in understanding the role of these unusual cells.

The purpose of this investigation therefore, was to examine the extrafloral nectaries of several species of *Aphelandra* at the ultrastructural level, comparing the secretory cells to those of other kinds of nectaries, with particular attention to the sub-secretory cells and the nature of the secretion.
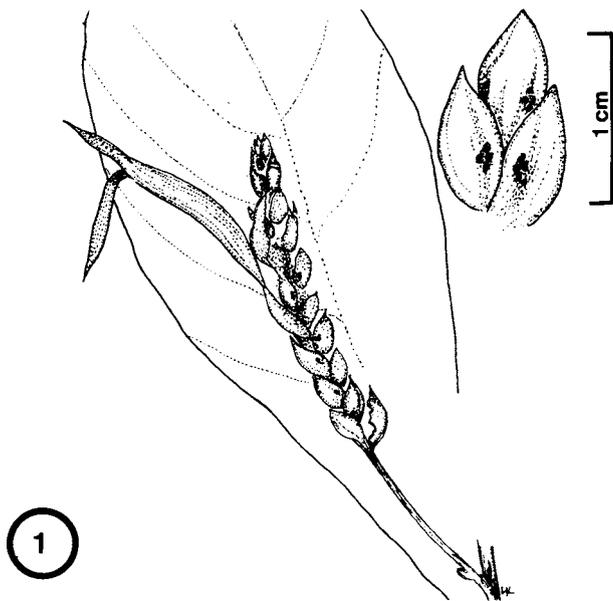

Fig. 1 *Aphelandra golfodulcensis.* Inflorescence and detail of bracts with nectaries.

Fig. 2. *A. scabra.* Scanning electron micrograph of a nectary cluster on the abaxial surface of a bract. X 100. Fig. 3. *A. golfodulcensis.* Initiation of a nectary. The outer cell (small arrow) will give rise to the secretory layer while the inner cell (large arrow) will produce the sub-secretory layer. The cells (starred) will remain in continuity with the adjacent bract epidermal cells. X 125. Fig. 4. Light microscope view of a mature nectary showing the secretory layer (S), the sub-secretory layer (Ss), the cell layer continuous with the bract epidermis (E), mesophyll (M), fibers (F) and cuticle (arrow). Transverse sections through strands of phloem and xylem are indicated (arrows). X 125. Fig. 5 *A. sinclairiana.* Secretory cells prior to secretion. Dictyosomes (G) present. X 20,000. Fig. 6, 7. *A. scabra.* Fig. 6. Laminated appearance (arrows) of the plasma membrane of adjacent active secretory cells. X 28,200. Fig. 7. Plasma membrane evaginations along the wall of adjacent active secretory cells (arrows). X 49,500.

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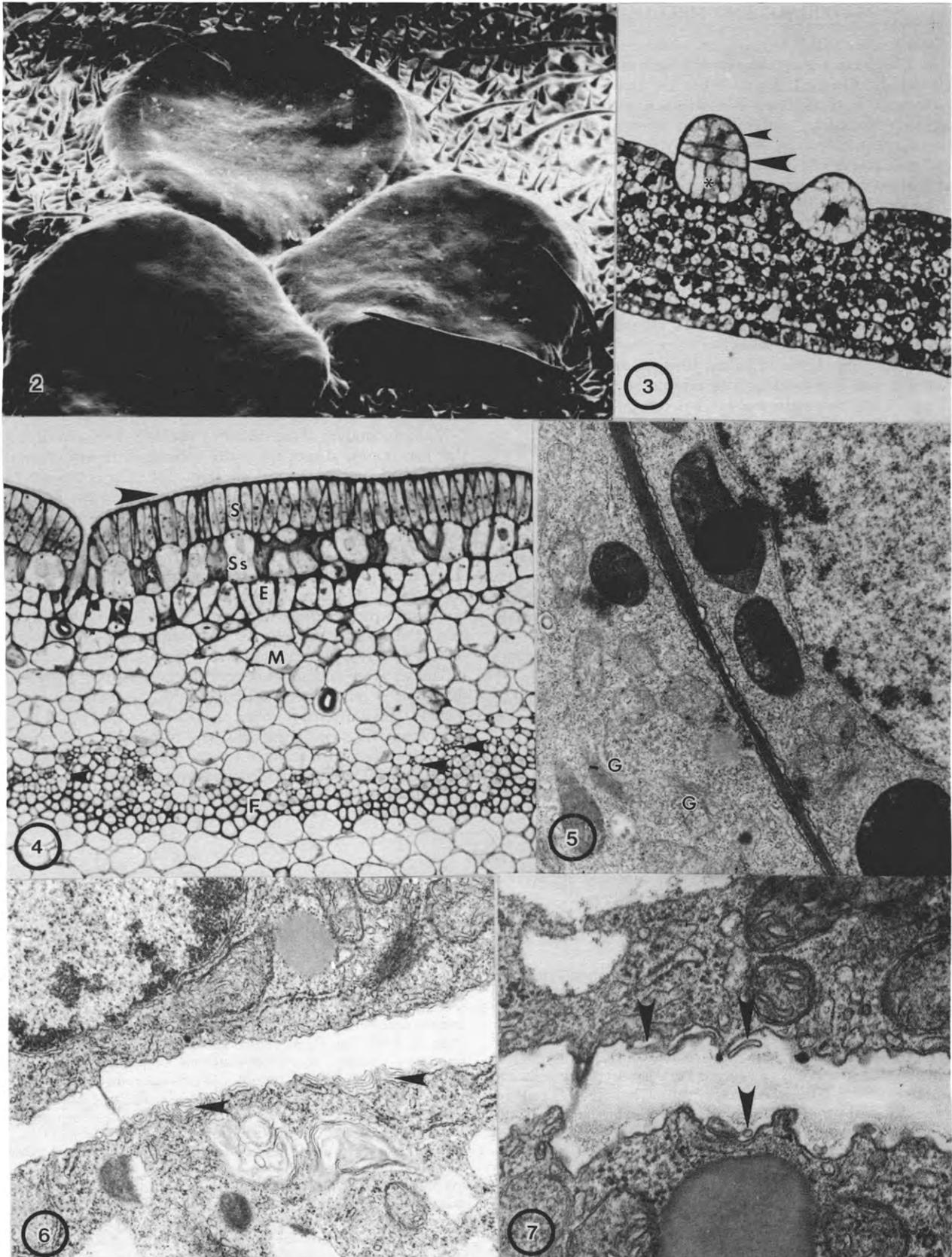
MATERIALS AND METHODS

Aphelandra scabra (Vahl) Sm., *A. golfodulcensis* McDade, and *A. sinclairiana* Nees were grown under natural light in the greenhouse at Grinnell College, Grinnell, Iowa. When the flowering spikes appeared, the bracts were harvested. Nectaries at four maturation stages were selected. Proceeding basipetally, they were: 1) differentiating, non-secreting nectaries at the apex; 2) fully developed but not yet secreting nectaries about 1 cm below the spike apex; 3) fully developed, secreting nectaries at midpoint or just below on the spike; 4) older, no longer secreting nectaries, located at the base of the spike. These were excised and fixed in 2-3% glutaraldehyde in 0.1M phosphate buffer, pH 7.2, for two hours R.T., post-fixed in 2% OsO_4 in the same buffer for 2hr in the cold, dehydrated in a graded acetone series, and embedded in Spurr's resin (Spurr 1969).

Silver-gold sections were cut, stained in 2% aqueous uranyl acetate and lead citrate, and then examined with a Hitachi H-300 electron microscope at an accelerating voltage of 75hv. For light microscopy, 1-2 μm sections were cut on a DuPont JB-4 microtome and stained with toluidine blue. Free-hand sections were also prepared and stained with Sudan IV and phloroglucinol-HCl to test for cutin and lignin respectively.

The nectar was tested for sugars and amino acids according to methods described earlier (Durkee et al. 1981).

RESULTS

Morphology and Development of the Nectary

The nectaries of the three species studied were similar in development and anatomy at maturity and the following observations apply to all. In these species two clusters of nectaries occur on the abaxial surface of each bract in the inflorescence, one cluster on each side of the midrib at or slightly below midpoint (Fig. 1, 2). Individual nectaries arise from a single protoderm cell that first enlarges and then undergoes a series of anticlinal and periclinal divisions (Fig. 3) culminating in a structure composed of secretory and sub-secretory cells resting on a layer of cells that is continuous with the epidermis of the bract (Fig. 4, 13). This ontogenetic sequence has been described for *A. lingua-bovis* (as *A. sinclairiana*, Durkee 1972) and is typical for these scale-like nectaries that are actually modified trichomes (Elias 1983).

Fairly early in nectary development, wall thickening is initiated at a point midway along the radial walls of the sub-secretory cells. An increase in vacuole size accompanies this change. At maturity, the walls reach their maximum thickness and are a conspicuous feature of the nectary (Fig. 4). The walls stain positively for cutin.

Ultrastructure of the Nectary

Secretory cells: A developing secretory cell has a dense cytoplasm with scattered cisternae of rough endoplasmic reticulum (ER), many mitochondria, a few small vacuoles, and plastids with poorly developed internal membranes, lacking starch but frequently containing one to several electron dense deposits. A few Golgi profiles can be observed (Fig. 5). Plasmodesmata occur between secretory cells and between these cells and the sub-secretory cells. The secretory cells are tightly packed with no intercellular spaces evident.

The ultrastructure of these cells does not change markedly with maturity and the onset of secretion except that the vacuoles are considerably larger than in younger secretory cells. The most noticeable difference can be seen in the plasmalemma. Outpocketings, invaginations, and layering of this membrane occur frequently along the periphery of the cell (Fig. 6, 7). Although vesicles and cisternae of ER and Golgi are in proximity to the plasmalemma, there is no suggestion of a fusion of these components.

The nectary cuticular layer (Fig. 4) contains a network of channels continuous with the wall (Fig. 8). It is bounded externally by the cutin and occasional deposits of amorphous material, probably waxes. In many sections through secreting nectaries, the cuticle shows separation from the walls.

Sub-secretory cells: As the nectary matures, these cells become easily distinguished from the secretory cells as their cytoplasm is more electron translucent and contains scattered cisternae of smooth ER (Fig. 9). Mitochondria and vacuoles are abundant, but dictyosomes are rarely seen. Plastids similar to those in the secretory cells are also found here (Fig. 12).

Of particular interest is the presence of globules of homogeneous, slightly electon-dense material that can be found along the radial walls or in the paramural space (Fig. 10). Each globule is clearly enclosed by a membrane when it is in the cytoplasm proximal to the wall, but this membrane appears to lose its integrity when the globule is observed in the paramural area. The impression gained from the static views offered by the photomicrographs is of an integration of the globules into the wall material with accompanying loss of the enclosing membrane. Since these walls test positively for cutin, the globules may represent early deposition of this material. Their origin is not clear.

With maturation of the nectary, these cells develop a large vacuole that may occupy almost the entire volume of the cell. Raphides are frequently found in the vacuole (Fig. 12). The cytoplasm contains scattered ER cisternae, mitochondria, and plastids, but lacks the density characteristic of the cytoplasm of younger cells. In older secreting nectaries, the vacuolar membranes appear to lose definition (Fig. 11). The radial walls have reached their maximum thickness and are extensively cutinized. The globules described above are not seen. No intercellular spaces are present in this layer. Plasmodesmata are confined to the periclinal walls between these cells and the secretory cells. The contrast between the secretory cells and the sub-secretory cells of an active nectary can be seen in Fig. 11.

The secretory and sub-secretory layers described above rest on a third layer of cells that is continuous with the epidermis of the bract (Fig. 13) and is derived from the original protoderm cell that gave rise to a nectary (Fig. 3). These cells tend to resemble the sub-secretory cells rather than adjacent non-nectary epidermal cells (Fig. 12). The bract mesophyll is located below this layer. The vascular tissue is about 5-6 cell diameters from the sub-secretory layer. Adjacent to each vascular bundle is a well-developed mass of fibers (Fig. 4).

Fig. 8. *A. sinclairiana.* Cutinization of the external walls of the secretory cells. Shown are the cutin (arrow), the cuticular layer (CL), and the extensions of the dark-staining wall material into the cuticular layer. X 2000. Fig. 9. *A. golfodulcensis.* The sub-secretory cells of an immature nectary. Scattered cisternae of smooth endoplasmic reticulum (ser), and small vacuoles (V) compared to secretory cells are characteristic. X 30,000. Fig. 10. *A. sinclairiana.* Cutinization of the radial walls of the sub-secretory cells. Globules of a homogeneous material (G) are associated with the wall. X 20,000. Fig. 11. *A. scabra.* Shown are a portion of the secretory layer (S), the sub-secretory layer (Ss), and the cutinized, thickened wall (W) of an older active nectary at the base of a spike. The tonoplast lacks definition (arrows). Plasmodesmata (P) are present in the walls between the secretory and the sub-secretory cells. X 6800. Fig. 12. *A. sinclairiana.* The sub-secretory cells (Ss) and the adjacent layer (E) that is continuous with the bract epidermis show considerable similarity at the ultrastructural level. The walls of cell layer (E) are quite electron dense (see also Fig. 4). Holes in the section represent areas where raphides were present. X 10,000.
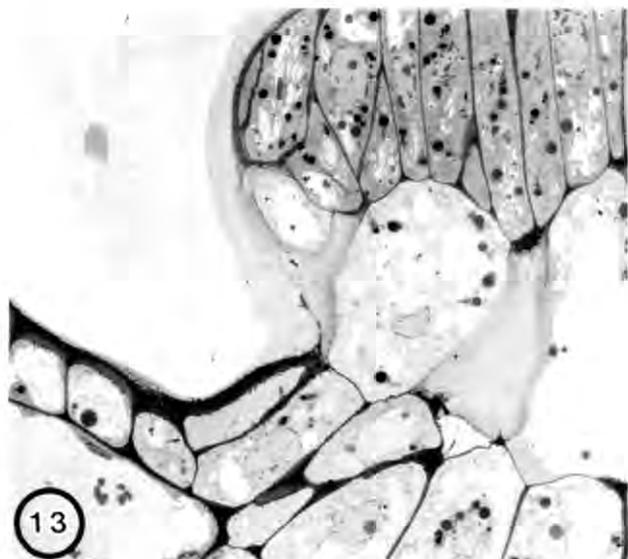
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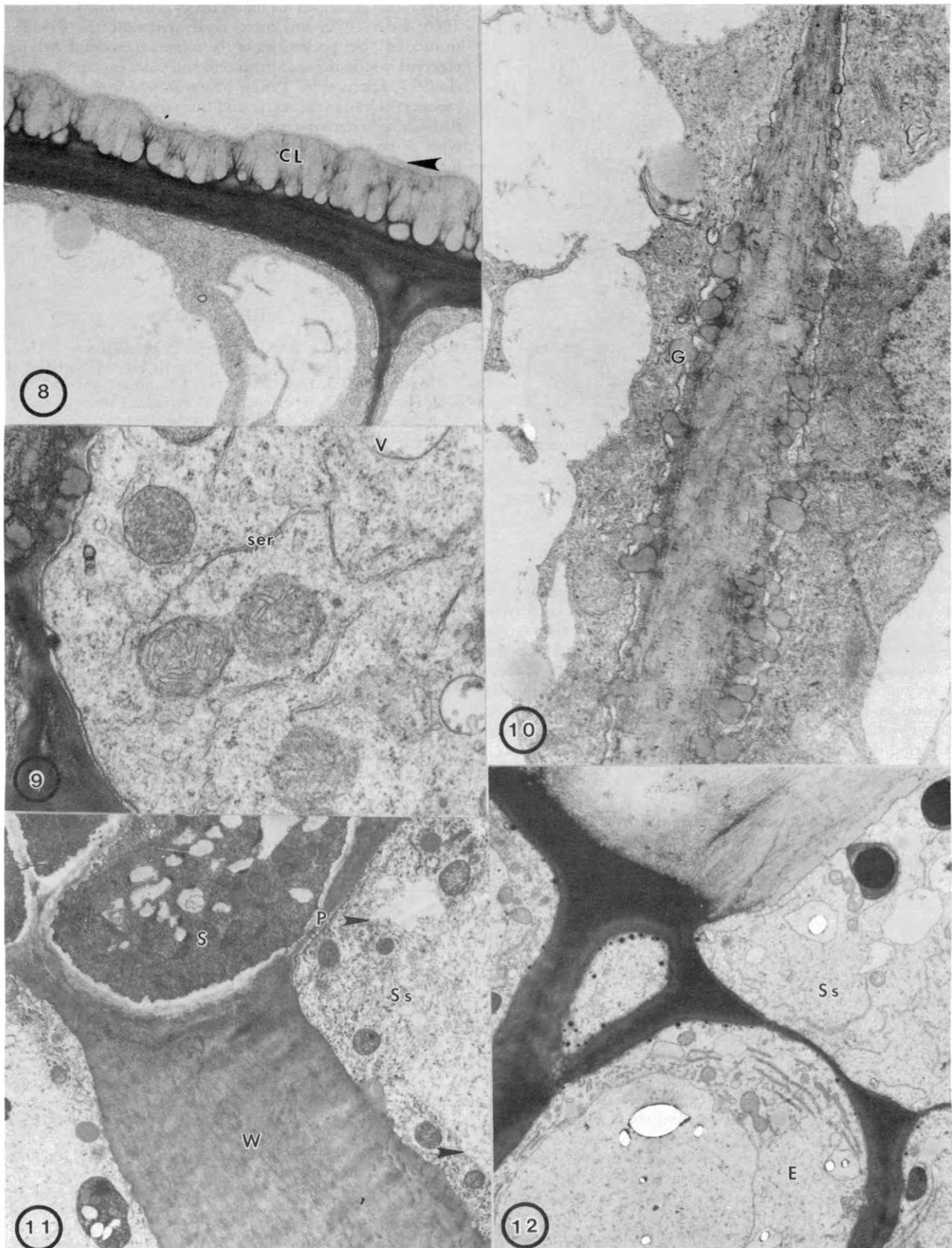
Fig. 13. *A. golfodulcensis*. Section through a portion of the nectary and adjacent bract epidermis. X 9300.

Nectar Analysis

Fresh extrafloral and floral nectar of *A. scabra* was selected for testing. The extrafloral nectar contained both fructose and sucrose with only traces of glucose. Several samples showed that the average sugar concentration in sucrose equivalents was 39% and that the amino acid concentration was approximately 12.5 mM. Both of these figures are higher than those reported in field studies of *A. scabra* (as *A. deppeana*) by Deuth (1977). The floral nectar contained fructose and sucrose with no traces of glucose. The average sugar concentration was 36% and the amino acid concentration was about 0.78 mM.

DISCUSSION

In nectaries without a direct vascular supply, it has been assumed that sucrose produced in nearby mesophyll or possibly derived from a distant phloem strand is the source of the "pre-nectar" (Wergin et al 1975). The bract nectar of *Aphelandra* contains sugar concentrations that are higher than is commonly reported from phloem sap (Zimmermann and Ziegler 1975) and glucose and fructose as well as sucrose. In addition, the amino acid concentration far exceeds that typically found in nectar (Baker and Baker 1983) and in phloem exudate (Ziegler 1975). Therefore, some modification of the pre-nectar is occurring prior to its final release as nectar.

The point at which such modification is occurring cannot be established with certainty from ultrastructural studies alone. However, the architecture of the nectary points to the possibility of the subsecretory cells functioning in this process. The heavy cutinization of the radial walls, the restriction of plasmodesmata to anticlinal walls, and the position of these cells between the secretory layer and the bract mesophyll may effectively prevent any lateral movement of materials, resulting in their being channeled through the subsecretory layer and thence to the secretory cells. More sophisticated methods are needed to determine the role played by these unusual cells.

The extrafloral nectaries of *Aphelandra* are different from other nectary types in additional ways. For example, the configurations of the secretory cell plasma membranes are unlike the "undulating

membranes" described for the secretory cells of many nectaries (Eyme 1966; Fahn 1979) and more nearly resemble the so-called "plasmatubules", out-pocketings of the plasma membrane that have been observed in scutellar epidermal cells and phloem parenchyma (Evert et al. 1977; Harris et al. 1982), tissues in which rapid, short-distance transport of solutes occurs. In addition, the internal cuticle deposition that has been demonstrated in these nectaries is not found in other types of nectaries and deserves further study, especially since little information about this phenomenon exists in the literature.

It is hoped that these findings will suggest profitable new avenues for investigating nectar secretion and other processes in nectaries of this type.

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