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# FLORAL NECTARY STRUCTURE AND NECTAR COMPOSITION IN *ECCREMOCARPUS SCABER* (BIGNONIACEAE), A HUMMINGBIRD-POLLINATED PLANT OF CENTRAL CHILE[1]

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Surface features, anatomy, and ultrastructure of the floral nectary of *Eccremocarpus scaber* (Bignoniaceae), pollinated predominantly by the largest-known hummingbird (*Patagona gigas gigas*), were studied together with nectar sugar content and secretion rate. The annular disk nectary comprises epidermis, secretory and ground parenchyma with intercellular spaces, and branched vascular bundles terminating in the secretory parenchyma where only phloem is found. Amyloplasts and vacuoles increase in size throughout development, the latter becoming sites of organelle degradation. Transferlike cells in nectary phloem and P-proteinlike fibrillar material in phloem parenchyma were observed. Flowers produced around 32 μl of nectar (mostly after anthesis) with 11 mg of sugar composed of fructose, glucose, sucrose, and maltose in a ratio of 0.34:0.32:0.17:0.17. Morphological studies as well as the presence of maltose and glucose in nectar suggest storage of the originally phloem-derived sugars as starch with its subsequent hydrolysis. The low sucrose/hexose ratio (0.25) and high nectary secretion force (nectar per flower biomass) observed places *E. scaber* close to large-bodied bat-pollinated plants. A hypothesis based on nectar origin and nectar secretion is advanced to explain pollinator-correlated variation in sucrose/hexose ratio.

Ecologists focusing on the plant/pollinator interaction have detected correlations between nectar composition and quantity, and pollinator type (Baker and Baker, 1980, 1983; Cruden, Hermann, and Peterson, 1983). However, the extent to which such correlations are reflected at the level of nectary structure is unknown. Floral nectaries vary enormously with respect to position, anatomy, and secretion mechanisms (Elias and Gelband, 1976; Fahn, 1979a, b; Subramanian and Inamdar, 1985, 1989). Available ultrastructural studies (Fahn and Rachmilevitz, 1970, 1975; Rachmilevitz and Fahn, 1973; Fahn and Benouaiche, 1979; Durkee, Gaal, and Reisner, 1981; Davis, Peterson, and Shuel, 1988), moreover, reveal a high degree of diversity in endoplasmic reticulum development, quantity of mitochondria and chloroplasts, dictyosome activity, and amount of starch present (Durkee, Gaal, and Reisner, 1981). To date the emphasis in studies of nectar has been strongly morphological or physiological. To explore the relationship between floral nectary structure, nectar composition, and pollinator type more studies are required of plant species for which there is good pollinator knowledge.

*Eccremocarpus scaber* R. et P. (Bignoniaceae) is a twining suffrutice of the matorral vegetation and sclerophyllous forests of central and southern-central Chile (Uslar, 1982). The tubular red-orange flowers, borne in subopposite pairs, are disposed in erect racemes bearing nine to 13 flowers. Each flower contains a large annular nectary located at the base of the superior ovary.

The principal pollinators of *E. scaber* are hummingbirds (Uslar, 1982). Two-thirds of the hummingbird visits to *E. scaber* are made by *Patagona gigas gigas* Vieillot, with an additional one-third made by *Sephanoides galeritus* Mol. (Belmonte, 1988). The species is also visited by the bumblebee *Bombus dahlbomi* Guér. (an ineffective pollinator) and very sporadically by the nectar-seeking butterfly *Tatochila mercedis* (Eschscholtz) (Belmonte, 1988).

*Patagona gigas gigas* is the largest known hummingbird (Grant and Grant, 1968). Weighing about three times that of an average-sized hummingbird, it consumes 12,000 calories during the daylight hours (Kruger, Prinzinger, and Schuchmann, 1982), approximately twice that of a typical species. *Patagona gigas gigas* is by no means restricted to *E. scaber*. It has also been reported to visit species of *Puya, Lobelia, Tropaeolum, Fuchsia, Lapageria, Tristerix,* and *Sophora* throughout its distribution along the Andes from Ecuador to Aysén, Chile (Ortiz-Crespo, 1974).

Given the high energy requirement of *Patagona gigas gigas, Eccremocarpus scaber* provides ideal material for exploring the relationship between nectary structure, nectar composition, and pollinator type. In this paper we study the surface features, anatomy, and ultrastructure of the floral nectary of *Eccremocarpus scaber*. The sugar constituents of *E. scaber* nectar, the rate of nectar secretion, and the total amounts of nectar secreted per flower are also determined.

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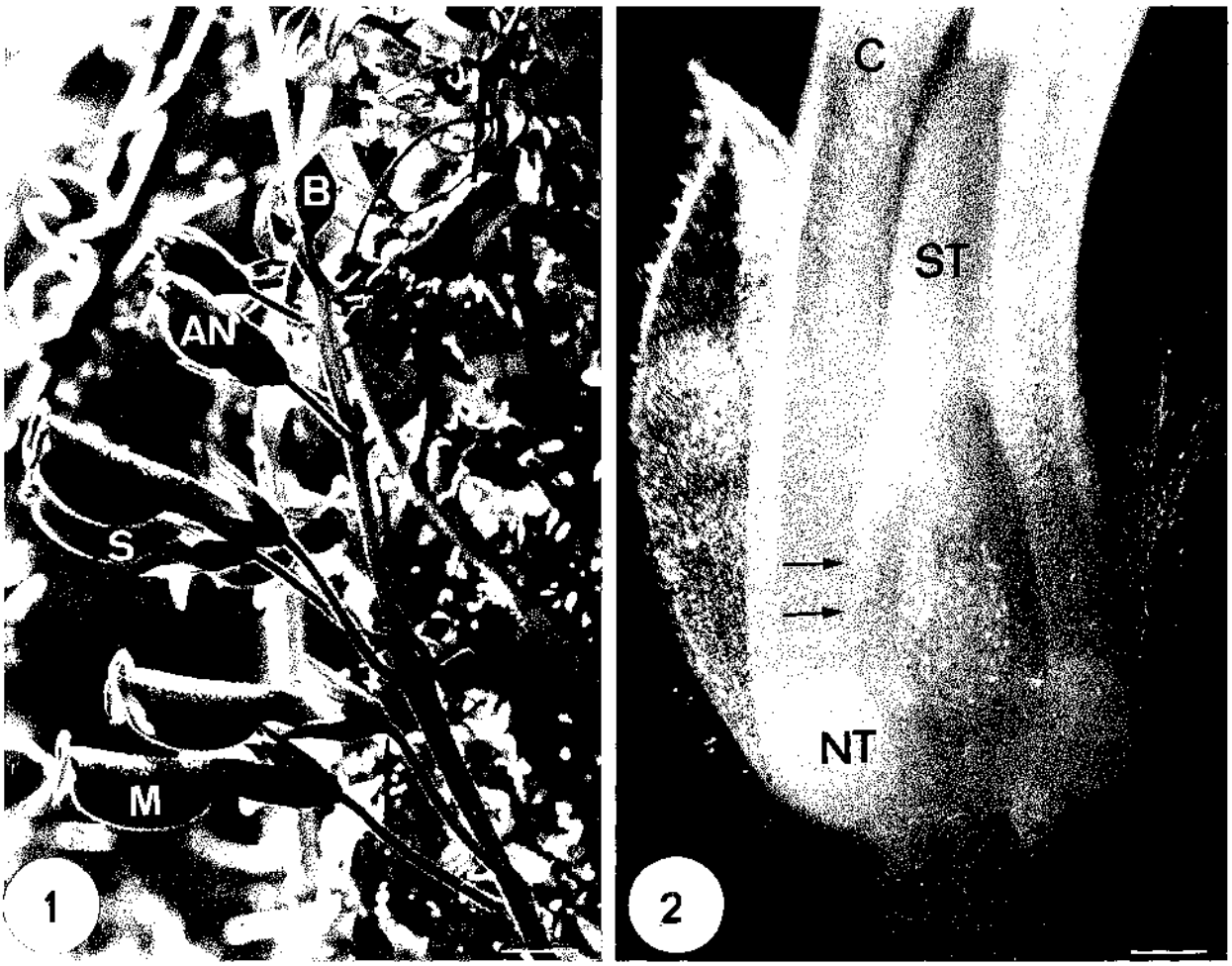



Figs. 1, 2. Floral morphology of *Eccremocarpus scaber*. **1.** Inflorescence, flower bud (B) (Stage I); early anthesis (AN) (Stage II); flower at stage of active secretion (S) (Stage III); mature flower (M) (Stage IV). Bar = 0.83 cm. **2.** Longitudinal section of *E. scaber* flower. Nectary (NT), style (ST), corolla (C), ovary (arrows). Bar = 0.05 cm.

## MATERIALS AND METHODS

***Plant material***—Flowers of *Eccremocarpus scaber* were collected in the wild, in the Santuario de la Naturaleza Yerba Loca (Región Metropolitana, 33°S, 1,789 m altitude), Chile.

Five developmental stages were defined, taking into account corolla length and nectary diameter. Flowers of *E. scaber* last 6 days from stage I to V: *Stage I:* preanthesis; flower buds with immature corollas 17–19 mm long; nectaries 0.8–2.2 mm diameter. *Stage II:* early anthesis flowers; corollas 20–22 mm long and nectaries 2.3–3.2 mm diameter; nectar is evident. *Stage III:* open flowers in which the border of the corolla tube is still erect; corollas 23–25 mm long and nectaries 3.3–4.0 mm diameter; nectar is abundant. *Stage IV:* flowers with the border of the corolla rolled back in the mature position; corollas 26–28 mm long and nectaries 4.0–4.9 mm diameter; nectar is abundant. *Stage V:* flowers showing clear signs of senescence; corollas 29 mm long or more; nectar may or may not be present. Twenty samples were processed for each developmental stage described.

***Scanning electron microscopy***—For studies of surface features, excised whole nectaries of Stage III flowers were fixed for 30–60 min in 1% osmium tetroxide, following several 5-min changes of distilled water and dehydration in a graded acetone series. Tissues were subjected to critical-point drying with liquid $CO_2$ as a transitional fluid and coated with gold (300A° thick). The material (ten samples) was examined in a Siemens scanning electron microscope.

***Light microscopy***—Three developmental stages were studied: Stages I, III, and IV. Excised whole nectaries were fixed in FAA (ethyl alcohol-glacial acetic acid-formaldehyde-water, 50:5:10:35, v/v) and aspirated for 24 hr. Fixed nectaries were dehydrated in a graded alcohol series, terminating with 100% tertiary butyl alcohol at 60 C, and embedded in paraplast. Sections 7–20 μm thick were stained with safranin-fast green or bromophenol blue. Cell volume was calculated for 100–200 cells of each nectary tissue. Cell measurements were derived from ten samples

for each of the three developmental stages (Dengler, Dengler, and Hattersley, 1986).

***Transmission electron microscopy***—Pieces of nectaries about 1 $mm^3$ from developmental Stages I, III, and IV were fixed in 2% glutaraldehyde in 0.2 M phosphate buffer pH 7 for 2 hr at room temperature with postfixation in 1% osmium tetroxide in the same buffer for 90 min. Material was then dehydrated in a graded acetone series, infiltrated, and embedded with Spurr's resin.

Thick sections about 1 nm thick were stained with 1% methylene blue or with 1% iodine ($KI-I_2$) in 30% ethanol (to stain starch) and examined under a phase-contrast microscope. Silver sections were stained by immersion in 4% uranyl acetate and 2% lead citrate and observed with a Philips M-300 electron microscope.

Nuclei, vacuoles, amyloplasts, mitochondria, and cell walls of the nectary tissues were measured directly and the vacuole/cytoplasm ratio calculated for 50 cells in the electron microscope. Cell measurements were taken from ten samples from each developmental stage.

***Nectar composition and accumulation patterns***—Nectar of Stage II–IV flowers was studied for its sugar components. Total sugar, sugar concentration, and water content were determined for Stage I–V flowers. Sugar components were identified by thin layer chromatography (TLC). The relative proportions of the sugar components were determined using the anthrone method (Dische, 1962). A bulk sample of fresh nectar was extracted from 200 flowers from Stage II–IV in the field with micropipettes and placed on ice. The nectar was immediately transported to the laboratory and diluted 20 times, spotting 1 $\mu$l of the solution (mirror-image system) onto microcrystalline cellulose thin layer plate squares (10 × 10 cm) 100 $\mu$m thick (Merck Chemical Co. Santiago, Chile) alongside an equimolar application of the following standard sugars: fructose, glucose, sucrose, and maltose. The chromatograms were run consecutively five times in ethyl acetate : acetic acid : formic acid : distilled water (18:3:1:4, v/v), air drying the plate between each run in order to ensure accurate separation of the sugars in organic solvents. One of the mirror images on the plate was developed for reducing sugars (glucose and fructose) using KOH 0.5 N and 0.5 ml of a standard solution of silver nitrate in 100 ml acetone. The silver staining was fixed with 10% sodium sulfate solution. Nonreducing sugars (sucrose and fructose) were detected with resorcinol and hydrochloric acid (1:5), maintaining the plate at 60–70 C for a few minutes waiting for the spots to appear. The Rf values of the detected sugars were compared with those of the standards.

The corresponding sugar areas on the nondeveloped side of the plate were scraped away and supplemented with 1 ml of distilled water, heated at 70 C for 15 min in a water bath, and centrifuged (microcentrifuge, 10,000 × g) for 10 min to remove cellulose fragments. Two ml of fresh anthrone solution (Dische, 1962) was added to the supernatant. Absorbance was read at 620 nm (Spectronic 20, Bausch and Lomb, Rochester, NY) and converted to $\mu$g of sugar by means of comparison with calibration curves constructed for the four sugar standards.

In order to determine total sugar accumulated during flower development, previously marked unopened buds of different sizes found on a total of 57 inflorescences were covered with fine netting (holes of 1 × 2 mm) to exclude pollinator visits to the flowers as they opened. Several days later the open flowers and remaining preanthesis buds were assigned to one of the five developmental stages described earlier. Nectar accumulated in each flower (or unopen bud) was measured with calibrated micropipettes and the sugar concentration (weight/weight) determined with a pocket refractometer (Bausch and Lomb). Flowers were sampled at random over a continuous 24-hr period to ensure that each developmental stage was subjected to similar microenvironmental conditions. The refractometer weight/weight sugar values were converted to sugar per volume according to Cruden and Hermann-Parker (1983). Nectar water content was estimated in the laboratory from the amount of water added to the known amount of sugar in the nectar to obtain the measured flower nectar volume.

## RESULTS

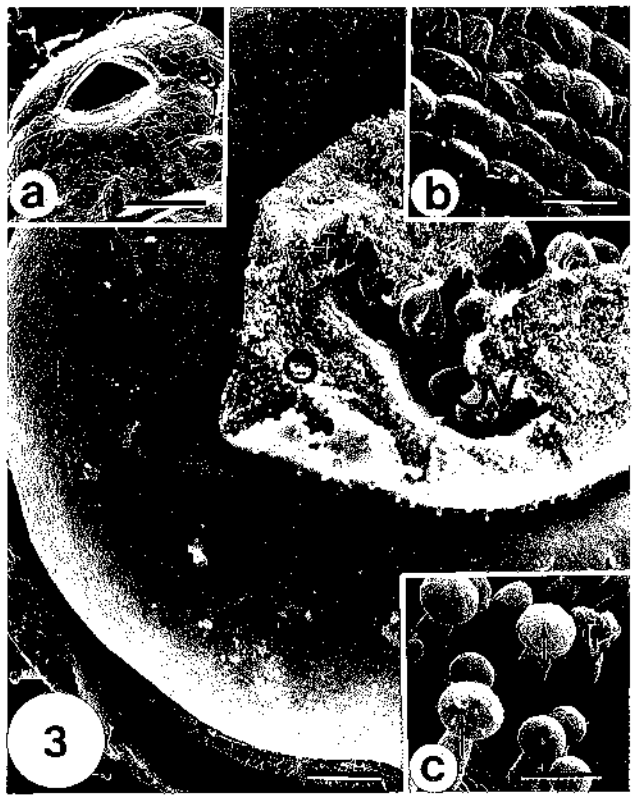
***Surface features***—Flowers in different stages of development are shown in Fig. 1. The annular nectary surrounds the ovary (Fig. 2). It originates from the thickening of the basal part of the ovary and the receptacle tissues and undergoes color change from yellow to red with age.

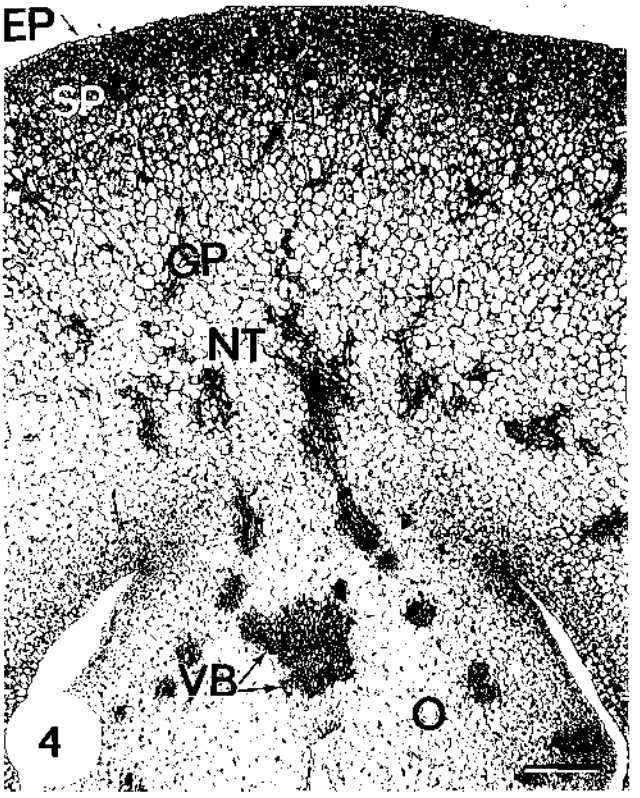
The nectary surface is smooth (Figs. 3, 3b), in contrast with the ovary which has trichomes with large heads and short stalks (Fig. 3c). Prominent stomata are located on the epidermis of the nectary facing the corolla tube (Fig. 3a). The stomatal complex is formed by two guard cells (Fig. 3a), without subsidiary cells.

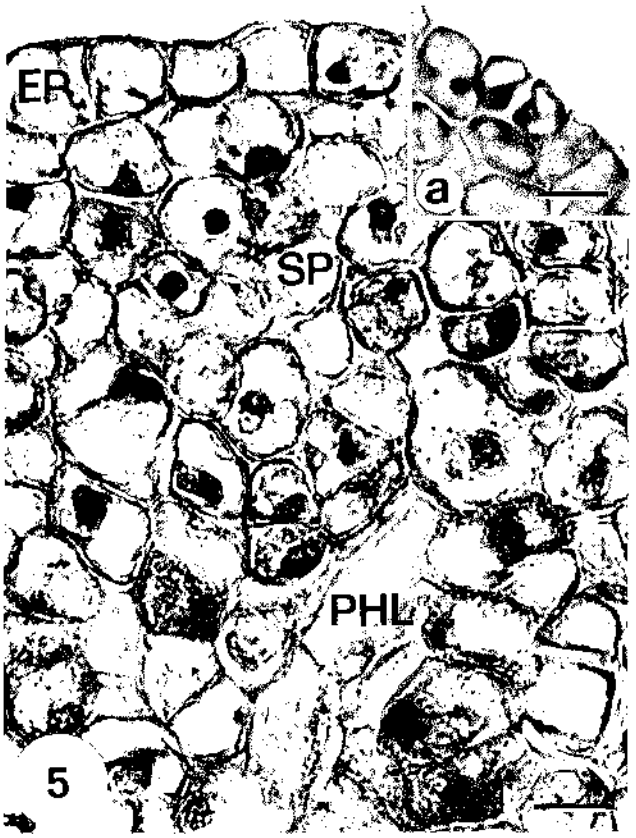
***Anatomy and ultrastructure***—Very early in development (Stage I) three zones are clearly demarcated in the nectary (Fig. 4). Following Durkee, Gaal, and Reisner (1981), these are designated epidermis (EP), secretory parenchyma (SP), and ground parenchyma (GP). The secretory and ground parenchyma are traversed by a profuse vascular system (Figs. 4–6).

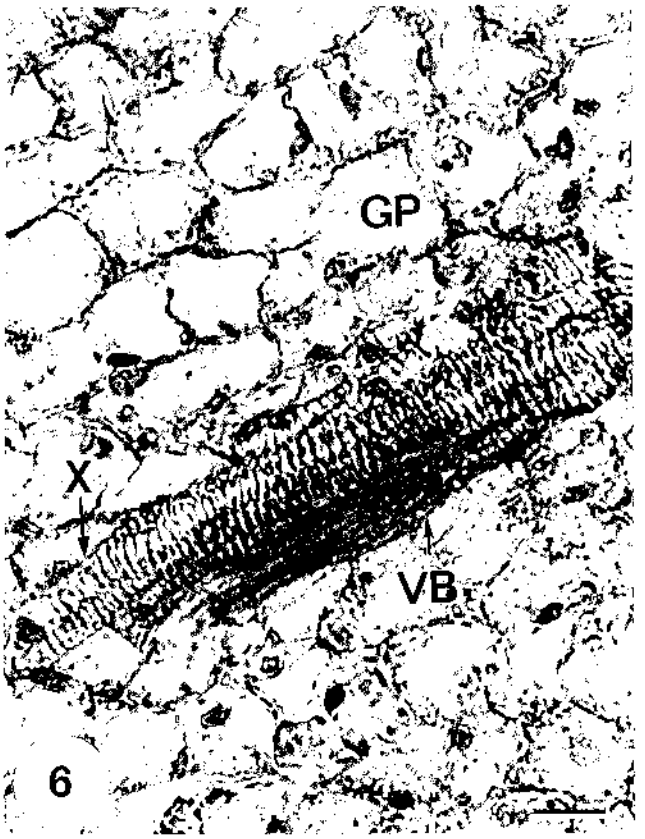
***Epidermis***—The epidermal cells are highly vacuolate and bear an external cuticle. Intercellular spaces are lacking except at the stomates (Figs. 5, 7, 8). At Stage I (Tables 1, 2) cells measure 12.6 $\mu$m average diameter and have a large vacuole occupying around 50% of the cytoplasmic volume (Fig. 7). The vacuoles contain sharply outlined electron-dense bodies (DB), 1.4 $\mu$m average diameter (Fig. 7), similar to the condensed tannins described by Ledbetter and Porter (1970). Rough endoplasmic reticulum and numerous mitochondria are present (Fig. 7a).

As development proceeds (Stages III and IV), conspicuous changes are seen in the epidermal cells. The external walls become thinner and the cytoplasm denser (Fig. 8; Table 2). Numerous vesicles, characteristic of some secretory cells, appear (Heinrich, 1975) (Fig. 8a). These vesicles are also seen in the secretory parenchyma cells, adjacent to the epidermis. Mitochondria and amyloplasts increase in size (Table 2). By Stage IV the vacuole occupies most of the cell volume and the nucleus is reduced in size (Tables 1, 2).

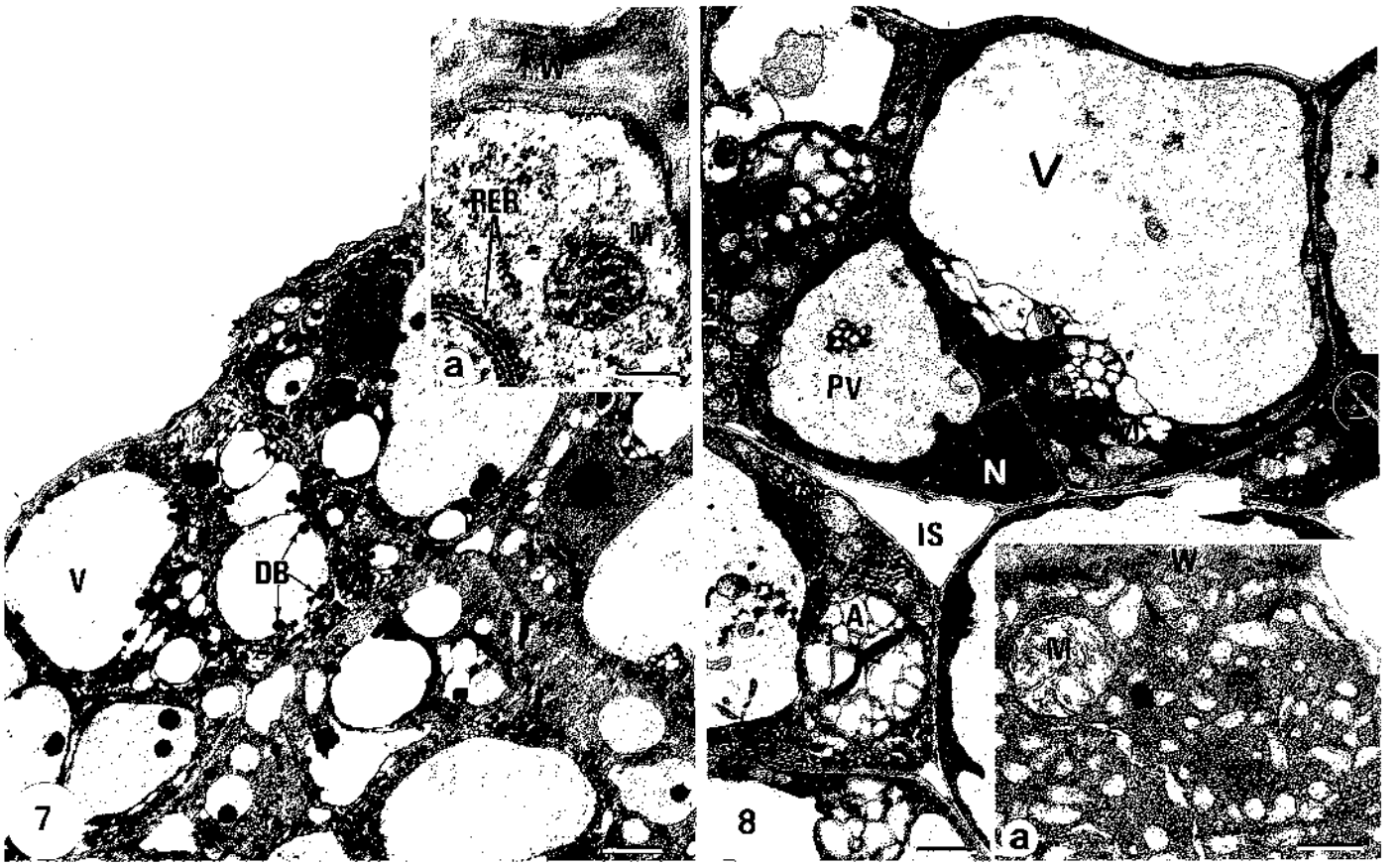



Figs. 3–6. Surface features and anatomy of *Eccremocarpus scaber* nectary at developmental Stage IV. **3.** Scanning electron micrograph (SEM) of nectary surface. Annular nectary in situ (NT); ovary (O) in transverse section showing ovules (OV). Bar = 250 μm. a. Inset showing SEM of nectary surface with a stoma on outer epidermis. Bar = 6 μm. b. Inset showing SEM of nectary surface lacking stomata on inner epidermis. Bar = 16 μm. c. Inset showing SEM of ovary surface with trichomes (arrows). Bar = 25 μm. **4.** Transverse section of nectary at late secretion stage.




Figs. 7, 8. Transmission electron micrograph (TEM) of *Eccremocarpus scaber* nectary epidermis at developmental Stages I and IV. **7.** Epidermal cells at developmental Stage I (preanthesis). Vacuole (V), dense bodies (DB, arrows). Bar = 5 μm. a. Inset. Details of the outer cell wall (W) showing a smooth cuticle with a continuous wax deposit (arrow). Rough endoplasmic reticulum (RER), mitochondrion (M). Bar = 0.2 μm. **8.** Ultrastructure of the epidermis and secretory parenchyma of nectary at stage of active secretion (Stage IV). Amyloplast (A), vacuole (V), phagocytic vacuole with membranes and organelle sequestered (PV), intercellular space (IS), epidermal cell nucleus (N), mitochondrion (M). Bar = 3.3 μm. a. Inset shows details of the epidermal cell cytoplasm. Arrowheads indicate vesicles probably fusing with the plasmalemma. Mitochondrion (M), cell wall (W). Bar = 0.6 μm.

***Secretory parenchyma***—The secretory parenchyma of the nectary is seven to ten cell layers thick at maturity and consists of highly vacuolate isodiametric cells staining strongly with safranin and fast green (Figs. 4, 5). These cells are slightly smaller than the epidermal cells until Stage IV at which time they exceed the epidermal cells in volume by a factor of 2 (Table 1). In general, secretory parenchyma cells undergo a twofold increase in volume during their development. Intercellular spaces (IS) (Figs. 9, 10), which increase in size and frequency toward the epidermis, and plasmodesmata (Fig. 10a) are recurrent features. The vacuole increases from close to 40% (Stage I) to 80% (Stage IV) of the cytoplasmic volume (Tables 1, 2). Especially conspicuous in the cytoplasm, from Stage I onward, are large complex amyloplasts with several starch crystallization points that greatly exceed those in the epidermis (Figs. 10, 11; Table 2). Also abundant in the secretory cells are mitochondria with conspicuous cristae; rough endoplasmic reticulum and dictyosomes are also present (Figs. 9, 10, 10a). From Stage III onward these organelles and membranes were observed within the vacuole along with partially degraded amyloplasts (Fig. 11). These degraded amyloplasts gave positive starch reaction when thick plastic sections were stained with KI-$I_2$ reagent. By Stage IV the amyloplasts are found sequestered in the vacuole where they have undergone severe degradation (Fig. 11a).

***Ground parenchyma***—The ground parenchyma comprises 25–33 cell layers of isodiametric, highly vacuolated

←

Monostratified epidermis (EP, arrow), secretory parenchyma (SP), ground parenchyma (GP). Numerous vascular bundles (VB, arrows) are seen in the nectary (NT) and ovary (O). Bar = 135 μm. **5.** Detail of nectary in transverse section at late stage of secretion. Monostratified epidermis (EP) and secretory parenchyma (SP) with phloem (PHL). Bar = 18.4 μm. a. Inset showing transverse section of stomatal complex on outer epidermis. Bar = 20 μm. **6.** Vascular bundle (VB, arrow) showing xylem elements (X, arrow) with annular thickenings in nectary ground parenchyma (GP). Bar = 11.7 μm.

TABLE 1. Cell diameter (μm), cell volume (μm³), and vacuole/cytoplasm ratio (%) for nectary tissues at different developmental stages.[a]

| Nectary tissue | Diameter $\bar{x}$ ± SE (range) | Volume $\bar{x}$ ± SE (range) | Vac/cyt ratio $\bar{x}$ ± SE (range) |
|---|---|---|---|
| Epidermis ($N$ = 100) | | | |
| Stage I | 12.6 ± 0.5 (6.7–28.2) | 2,152 ± 162 (38–6,320) | 49.2 ± 1.9 (35–65) |
| Stage III | 16.3 ± 0.6 (7.3–27.1) | 4,245 ± 159 (132–11,951) | 79.7 ± 5.6 (55–85) |
| Stage IV | 13.0 ± 1.1 (4.2–29.9) | 1,947 ± 119 (54–5,632) | 88.3 ± 2.5 (70–90) |
| Secretory parenchyma ($N$ = 200) | | | |
| Stage I | 12.4 ± 0.4 (3.5–25.8) | 1,971 ± 109 (45–3,212) | 38.7 ± 5.2 (10–56) |
| Stage III | 15.6 ± 0.43 (5.3–31.3) | 3,120 ± 113 (371–11,845) | 58.0 ± 4.1 (25–95) |
| Stage IV | 16.5 ± 0.4 (2.5–28.2) | 4,093 ± 202 (197–11,489) | 81.2 ± 2.8 (60–99) |
| Ground parenchyma ($N$ = 200) | | | |
| Stage I | 16.4 ± 0.4 (10.7–22.1) | 4,196 ± 226 (220–10,857) | 55.6 ± 2.0 (45–90) |
| Stage III | 20.2 ± 0.8 (5.9–29.4) | 8,486 ± 441 (448–22,991) | 70.1 ± 2.1 (62–95) |
| Stage IV | 28.4 ± 1.0 (10.9–44.7) | 24,992 ± 203 (980–85,490) | 92.6 ± 2.0 (85–90) |

[a] $N$ = cell number.

cells that stain poorly with safranin and fast green (Fig. 4). Cell size exceeds that in the epidermis and secretory parenchyma (Table 1). The cell walls are very thin and contain numerous plasmodesmata (Table 2; Fig. 12). Intercellular spaces are smaller compared with those of the secretory parenchyma. Larger spaces, however, occur in the area adjacent to the secretory parenchyma. The vacuole of the parenchyma cells was crossed by cytoplasmic strands more often than that of the secretory parenchyma

TABLE 2. Organelle size (μm) ($N$ = 50) in nectary tissues of *Eccremocarpus scaber* at different developmental stages.

| Nectary tissue | Developmental stage I $\bar{x}$ ± SE | III $\bar{x}$ ± SE | IV $\bar{x}$ ± SE |
|---|---|---|---|
| Epidermis | | | |
| Vacuole | 6.20 ± 1.73 | 13.00 ± 1.23 | 11.50 ± 0.35 |
| Nucleus | 7.32 ± 0.40 | 4.58 ± 0.31 | — |
| Amyloplast | 2.84 ± 0.34 | 3.77 ± 0.33 | — |
| Mitochondria | 0.53 ± 0.05 | 0.97 ± 0.04 | — |
| Outer wall | 0.36 ± 0.04 | 0.26 ± 0.02 | 0.15 ± 0.02 |
| Lateral (side) wall | 0.14 ± 0.01 | 0.27 ± 0.04 | 0.18 ± 0.01 |
| Inner wall | 0.23 ± 0.01 | 0.18 ± 0.01 | 0.18 ± 0.01 |
| Cuticle | 0.04 ± 0.02 | 0.08 ± 0.01 | 0.04 ± 0.01 |
| Secretory parenchyma | | | |
| Vacuole | 4.80 ± 0.52 | 9.00 ± 1.20 | 13.40 ± 0.56 |
| Nucleus | 6.81 ± 0.37 | 6.88 ± 0.65 | — |
| Amyloplast | 4.22 ± 0.53 | 7.92 ± 1.08 | — |
| Mitochondria | 0.86 ± 0.04 | 1.55 ± 0.12 | — |
| Wall | 0.31 ± 0.02 | 0.27 ± 0.02 | 0.25 ± 0.02 |
| Ground parenchyma | | | |
| Vacuole | 9.20 ± 1.10 | 14.10 ± 1.36 | 26.30 ± 4.04 |
| Nucleus | 7.47 ± 0.10 | 5.60 ± 0.27 | — |
| Amyloplast | 3.72 ± 0.08 | 5.43 ± 0.29 | — |
| Mitochondria | 0.63 ± 0.09 | 1.01 ± 0.04 | — |
| Wall | 0.17 ± 0.04 | 0.21 ± 0.02 | 0.22 ± 0.01 |

TABLE 3. Sugar components of the flower nectar at the stage of active secretion, Stage III. Each figure corresponds to the average of 250 determinations.[a]

| Sugars | mg/μl $\bar{x}$ ± SE | Range | Sugar % |
|---|---|---|---|
| Fructose | 0.35 ± 0.01 | 0.22–0.51 | 34 |
| Glucose | 0.33 ± 0.01 | 0.15–0.53 | 32 |
| Sucrose | 0.17 ± 0.01 | 0.11–0.22 | 17 |
| Maltose | 0.17 ± 0.01 | 0.09–0.27 | 17 |

D/M = 0.5; S/(F + G) = 0.25.

[a] D/M is the disaccharides/monosaccharides ratio. S/F + G is the sucrose/fructose + glucose ratio.

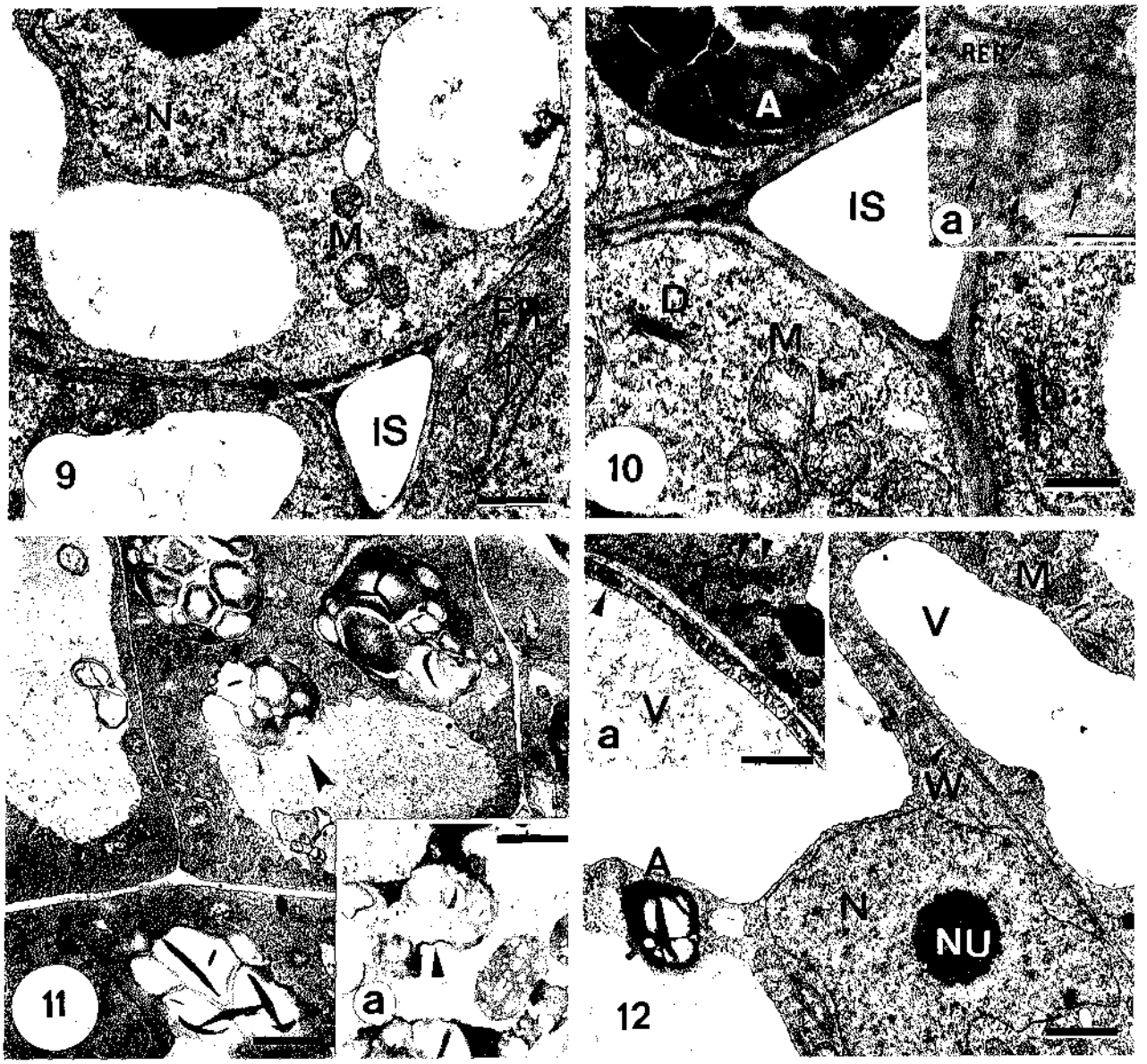
cells (Fig. 12). Smaller amyloplasts are seen in comparison with the secretory parenchyma (Table 2; Fig. 12).

The ground parenchyma cells undergo an even greater increase in size during nectary development than their secretory parenchyma counterparts (Table 1). An initially larger vacuole (Fig. 12) continues to increase in size until Stage III and IV (Fig. 12a), at which time it occupies around 90% of the cytoplasm (Tables 1, 2). At Stage III dense granular material may be seen in its interior (Fig. 12a). At this point the cytoplasm containing electron-dense mitochondria is reduced to a very thin layer along the cell wall (Fig. 12a). Autophagic characteristics of the vacuole as those seen in the secretory parenchyma are also present in the ground parenchyma cells. All organelles at Stage IV show senile degradation.

***Vascular tissues***—The nectary vascular tissues are derived from a ring of vascular bundles located at the base of the ovary (Fig. 4). Branches of the ovarian bundles enter the nectary where they divide several times, finally reaching the secretory parenchyma close to the epidermis (Figs. 4, 5). The vascular system is well developed in Stage I flowers. In the ground parenchyma the bundles contain tracheary elements with annular thickenings and phloem elements (Fig. 6). Bundles reaching the inner secretory parenchyma are less branched and contain fewer vascular elements. In the outer secretory parenchyma, only phloem elements persist (Fig. 5). Sieve elements and companion cells are seen in Figs. 13, 14. Some of the parenchyma cells next to the sieve element have very compact cytoplasm with numerous mitochondria and wall ingrowths typical of transfer cells (Fig. 14) (Gunning and Pate, 1969); the vacuoles of these cells are sinuous. Other parenchyma cells adjacent to the vascular bundles are particularly rich in mitochondria, dictyosomes, and endoplasmic reticulum (Fig. 15) and contain electron-dense fibrillar material with filaments similar in appearance and thickness (18 nm thick) to sieve tube P-protein filaments (Kollmann, Dörr, and Kleinig, 1970; Cronshaw, 1974) (Figs. 15, 16).

***Nectar sugars***—The four standard sugars—fructose, glucose, sucrose, and maltose—were found in *Eccremocarpus scaber* nectar (Table 3) in a ratio of 0.34:0.32:0.17:0.17, respectively. The disaccharide/ monosaccharide ratio is 0.5, while the sucrose/hexose ratio is 0.25.

***Nectar production and accumulation pattern***—Nectar production was highly variable among flowers (Fig. 17A). Surprisingly, flowers with zero nectar production were

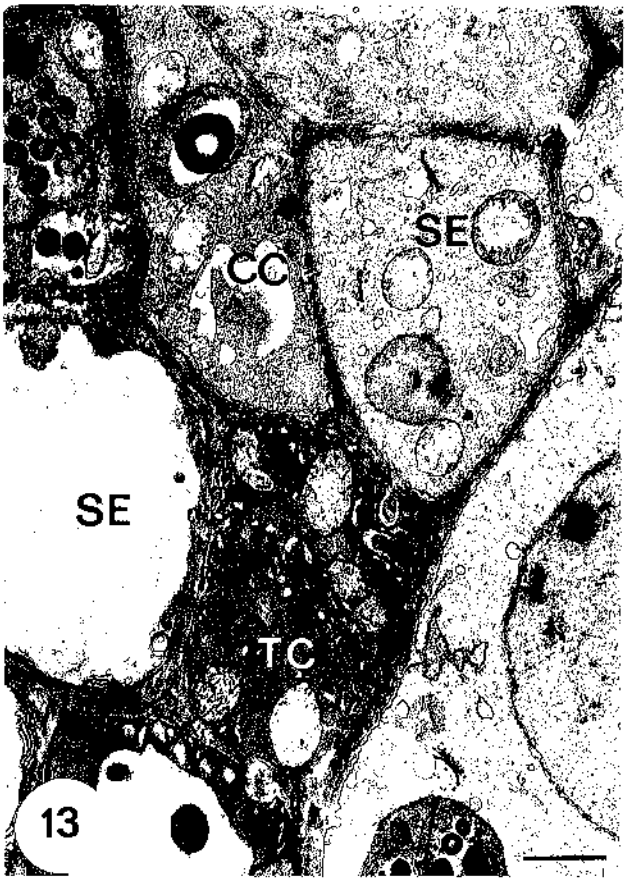



Figs. 9–12. TEM of secretory and ground parenchyma of *Eccremocarpus scaber* nectary during different developmental stages. **9.** Ultrastructure of a typical secretory parenchyma cell at developmental Stage I (preanthesis). Nucleus (N), endoplasmic reticulum (ER), mitochondria (M), intercellular space (IS). Bar = 1.5 μm. **10.** Details of secretory parenchyma cell cytoplasm at developmental Stage I. Amyloplast (A) with several starch crystallization points, dictyosome (D), mitochondria (M), intercellular space (IS). Bar = 0.8 μm. a. Plasmodesmata (arrows) between cells at developmental Stage I; rough endoplasmic reticulum (RER). Bar = 200 nm. **11.** Ultrastructure of secretory parenchyma cells of nectary at an advanced developmental stage (Stage IV) with amyloplast beginning degradation at the edge contacting the vacuole (arrowhead). Bar = 2.3 μm. a. Amyloplast sequestered in the vacuole in a more advanced stage of degradation. Part of it is falling apart (arrowhead). Bar = 2 μm. **12.** Ultrastructure of a typical nectary ground parenchyma cell at developmental Stage I. Cell wall (W, arrowhead), vacuole (V), nucleus (N), nucleolus (NU), amyloplast (A), mitochondria (M). Bar = 1.3 μm. a. Inset shows ground parenchyma cell at secretion Stage IV. Vacuole (V) and cytoplasm containing electron-dense mitochondria (arrowheads). Bar = 6 μm.

registered even in the most advanced developmental stage. Zero nectar production was not the result of pollinator extraction because pollinators were excluded at the bud stage. Variation in nectar secretion per flower was particularly high in Stage V flowers (Fig. 17A). The variation is partially due to variation in the initial timing of nectar secretion; 2.3% of the flowers initiated secretion in preanthesis; however, by Stage III, 98% of the flowers were engaged in secretion.

Because flowers occur in subopposite pairs, we hypothesized that some of the variation could be due to the proximal flower competing more successfully than the distal one for xylem and phloem content. However, evidence for such positional effects was not obtained (ts = 1.10; $P = 0.2$; Wilcoxon Signed-Rank Test comparing subopposite flower pairs for Stage II and IV flowers).

Secretion takes places in 4 d, from the day before anthesis to stage IV and is especially rapid in the 3 d between Stage II and Stage IV ($\bar{X} = 32$ μl per flower). It decreases notably at Stage IV (Fig. 17A). The nectar accumulated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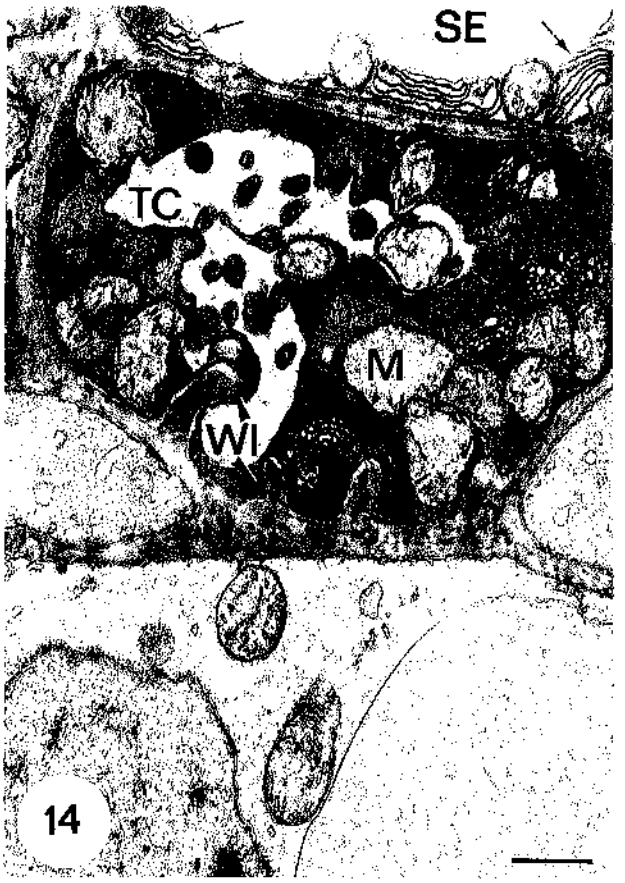
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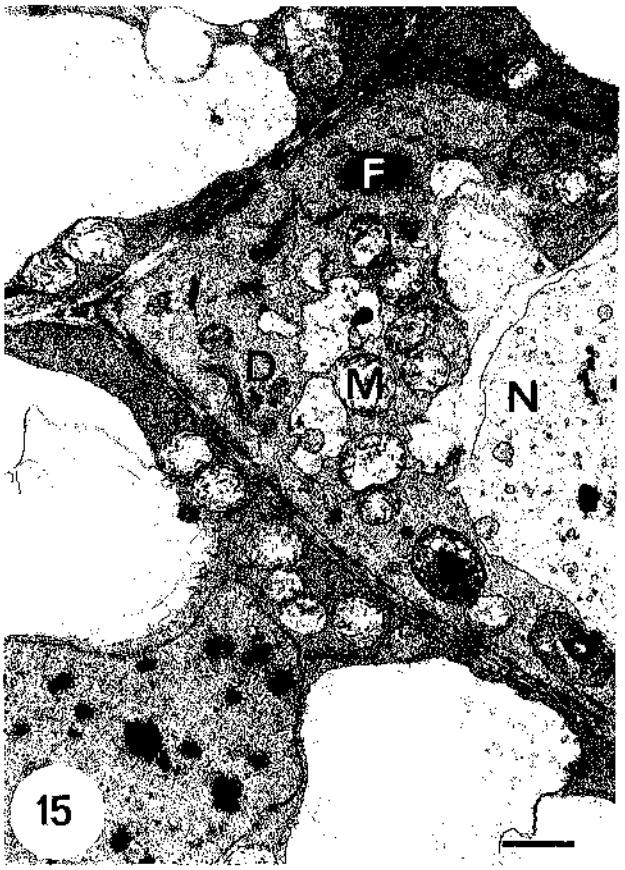
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by each flower contained the equivalent of an average of around 26 μl of water (Fig. 17A). At maturity, individual flowers contained an average of around 11 mg of sugar (Fig. 17B). Half of the sugar secreted was already available in Stage III flowers. As with volume, sugar concentration varied enormously between flowers at any one developmental stage (Fig. 17B). However, there was no significant trend in concentration with developmental stage (Wilcoxon 2-Sample Test). At Stage IV as nectar secretion ceased, concentration averaged 32%.

## DISCUSSION

Conceivably, the high energy requirement of *Patagona gigas gigas,* the principal pollinator of *Eccremocarpus scaber,* might be reflected in the secretory capacity, internal structure, and functioning of the *E. scaber* nectary. Several features of the nectary revealed in this study are consistent with this hypothesis. The *Eccremocarpus scaber* nectary produces twice the amount of sugar secreted during the lifetime of *Combretum farinosum* flowers pollinated by more typical-sized hummingbirds (Schemske, 1980). *Campsis radicans,* another hummingbird-pollinated species of the Bignoniaceae, accumulated three times the amount of sugar produced by a single *E. scaber* flower. However, the flowers of the last-mentioned species are three times as large as those of *E. scaber.*

A more appropriate measure of secretory capacity for evaluating selective effects is the amount of nectar secreted per unit of total floral biomass (here called "secretion force"). Opler (1983) compared "maximum nectar" (largest amount of nectar found in a single flower) against floral biomass for many plant species and showed that what we call "secretion force," is lowest in flowers pollinated by smaller vectors such as lepidopterans and hymenopterans and highest for species pollinated by bats, nocturnal moths, large bees, and hummingbirds. Flowers of *E. scaber* weigh 0.17 g and produce 32 μl of nectar over their lifetimes. The "secretion force" of *E. scaber* flowers is around 50% higher than that of more typical hummingbird-pollinated species and very similar to that of bat-pollinated species (Opler, 1983).

Although there are other studies on nectaries in Bignoniaceae, these only describe the external anatomy, general histology, starch accumulation, and degradation (Elias and Gelband, 1976; Subramanian and Inamdar, 1985, 1986, 1989). Very little is reported about the sugar composition (Subramanian and Inamdar, 1989), and nothing at all about the accumulation pattern of the nectar.

Ultrastructurally the *E. scaber* nectary finds its closest parallel in *Passiflora* spp. pollinated by hummingbirds and bees. In both *E. scaber* and *Passiflora* spp. nectaries there are many anatomical features in common; i.e., the internal and external anatomy, similar type of amyloplasts, large vacuoles, epidermis with stomates, fibrillar deposits in the phloem parenchyma cells, etc. (Durkee, Gaal, and Reisner, 1981; Durkee, 1983).

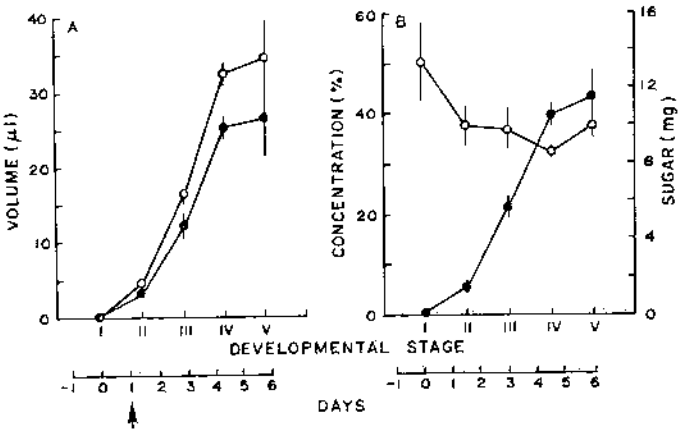


Fig. 17. Nectar volume and water volume (A), sugar concentration and amount of sugar (B) secreted throughout the life of a flower of *Eccremocarpus scaber.* Vertical lines are standard error. A. Nectar volume (empty circles), water volume in nectar (filled circles). B. Sugar concentration (empty circles), amount of sugar (filled circles). Arrow indicates when nectar secretion begins.

However, there are some notable differences between the two nectaries. Whereas nectary development ceases immediately after anthesis in *Passiflora,* nectaries of *E. scaber* undergo a fivefold postanthesis increase in diameter. In *Passiflora,* starch degradation is completed at the time of anthesis and occurs within the amyloplast envelope which remains intact. In *E. scaber,* total degradation of the large amyloplasts only becomes evident in the advanced floral stages. That secretion has begun well before this stage suggests that the nectary of *E. scaber* is structured for an unusually long period of secretion. Finally, the engulfment of amyloplasts by the vacuole and subsequent starch hydrolysis in the vacuole is quite distinct and as far as we can tell, unique, among floral nectaries (Durkee, Gaal, and Reisner, 1981).

The suggestion that the stomatal pores may be the secretory opening is supported by the external wall morphology of the epidermal cell covered by an external cuticle. No apertures or channels for nectar secretion are seen in the epidermal cell wall at any stage of development, and the cells maintain their integrity even at Stage V of development, which is the senescent stage. However, vesicles, probably originating in the endoplasmic reticulum and fusing with the plasma membrane, could be seen in the epidermis and adjacent secretory parenchyma cells during the secretion stages. These vesicles are similar to those seen in Aloë nectaries (Heinrich, 1975; Fahn, 1979a) and suggest that the epidermis and adjacent parenchyma

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Figs. 13–16. TEM of vascular bundles of *Eccremocarpus scaber* nectary secretory parenchyma at developmental Stage IV (secretory stage). **13.** Sieve cell (SE), transfer cell (TC), companion cell (CC). Bar = 1.0 μm. **14.** Ultrastructure of a transfer cell (TC). Wall ingrowths (WI, arrowheads) and mitochondria (M). Sieve element reticulum (arrows). Bar = 1.4 μm. **15.** Ultrastructure of a vascular bundle parenchyma cell showing a fibrillar structure (F), mitochondria (M), dictyosomes (D), and part of the nucleus (N). Bar = 1.8 μm. **16.** Details of the fibrillar structure (F). Bar = 0.18 μm.

cells could be involved in secretion toward the exterior or toward the intercellular spaces. Exudation of nectar via stomata occurs in floral nectaries of many plants, i.e., *Colchicum, Citrus, Bupleurum, Tropaeolum* (Fahn, 1979b). In nectaries exuding nectar through stomata, the intercellular spaces are in general well developed in the secretory parenchyma (Fahn, 1979a), as is the case of *E. scaber* nectary.

Amyloplasts in nectaries potentially serve not only as a source of nectar sugars, but also of energy through starch hydrolysis (Durkee, Gaal, and Reisner, 1981). Storage of nectar precursors as inert starch undoubtedly provides the most efficient means of accumulating nectar constituents in species whose pollinators demand rapid unloading of large quantities of nectar when the flower opens (Durkee, Gaal, and Reisner, 1981). However, the nectar of many plant species is derived directly from phloem exudate (Fahn and Rachmilevitz, 1975; Fahn, 1979a; Meyberg and Kristen, 1981). Such direct unloading in plant species under selection for large nectar volumes would presuppose the existence of significant energy availability. The possibility that amyloplasts also play a subsidiary role in nectar secretion in heavy nectar secretors rather than a direct one cannot be discarded. Because the nectar of *E. scaber* contains much glucose and maltose (direct hydrolysis products of starch) and fructose (readily derived from sucrose through invertase action), it is probably safe to assume that appreciable amounts of *E. scaber* nectar are produced via a starch intermediary. The products of starch hydrolysis perhaps join phloem sucrose in the intercellular spaces. In the *E. scaber* nectary, starch hydrolysis might be very efficient within the vacuole given the expected greater level of enzymatic activity there (Matile, 1976; Van der Wilden, Herman, and Chrispeels, 1980; Marty, Branton, and Leigh, 1980). Also, the second important component of nectar—water—is abundant in the vacuole.

Many of the changes that take place during the development of the *E. scaber* nectary could be explained by the physiological events that occur in the vascular elements that bring water and sugars to the nectary. In the *E. scaber* nectary, as in the nectaries of *Passiflora* (Durkee, Gaal, and Reisner, 1981; Durkee, 1983) or *Lonicera* (Fahn and Rachmilevitz, 1970, 1975), the xylem and sieve elements reach the ground parenchyma. However, in the secretory parenchyma the vascular bundles have only phloem elements. Since no xylem elements are found in the secretory parenchyma, water and sugars must reach the secretory parenchyma via the sieve tubes during the onset of nectary development. This would explain the presence of transfer and phloem parenchyma cells, these last having deposits of dense fibrillar material. Similar deposits have been reported for the nectaries of *Passiflora,* although the deposits lack a surrounding membrane (Durkee, Gaal, and Reisner, 1981; Durkee, 1983). In *E. scaber* nectary, the filaments forming the fibrillar deposits fit the average thickness reported for P-protein filaments (Kollmann, Dörr, and Kleinig, 1970; Stone and Cronshaw, 1973). However, further investigation is required to identify these fibrillar materials as P-protein, the physiological role of which is under discussion (Cronshaw, 1974; Raven, Evert, and Eichhorn, 1986).

Baker and Baker (1983) have shown that hexose sugars (glucose and fructose) predominate in plants pollinated by passerine birds and bats, while hummingbird-, bee-, and moth-pollinated species have sucrose-rich nectars. No totally convincing explanation for these trends has been forthcoming. The sucrose/hexose ratio of 0.25 for *E. scaber* is low for hummingbird-pollinated plants. Indeed, *E. scaber* nectar tends to resemble that of plant species pollinated by larger organisms such as bats. Such convergence in nectar composition for a species pollinated by the world's largest hummingbird and for plant species pollinated by high energy-demanding vectors might shed considerable light on the trends illustrated by Baker and Baker (1983). Given that the primary breakdown products of starch are glucose and maltose, plant species relying heavily on starch as an intermediary for nectar production may be expected a priori to have small amounts of sucrose in their nectar. The decrease in the sucrose/hexose ratio from small to large-bodied pollinators thus might reflect a general evolutionary shift away from nectar production via direct phloem unloading to nectar production dependent on starch accumulation rather than the "esoteric" taste preferences of pollinator groups as suggested by Hainsworth and Wolf (1976). Careful studies of the nectary structure of closely related plant species pollinated by organisms with widely different energy requirements would be useful for evaluating this hypothesis.

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