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Anatomy, ultrastructure and secretion of *Hibiscus pernambucensis* Arruda (Malvaceae) extrafloral nectary[1]

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ABSTRACT – (Anatomy, ultrastructure and secretion of *Hibiscus pernambucensis* Arruda (Malvaceae) extrafloral nectary). This paper reports on the extrafloral nectary (EFN) of *Hibiscus pernambucensis*, a native shrub species occurring in mangrove and restinga along Brazil's coastline. EFNs occur as furrows with a protuberant border on the abaxial surface veins of the leaf blade. Each nectary consists of numerous secretory multicellular trichomes, epidermal cells in palisade-like arrangements and non-vascularized parenchyma tissue. Nectar secretion is prolonged, since secretion starts in very young leaves and remains up to completely expanded leaves. Reduced sugars, lipids, and proteins were histochemically detected in all the nectary cells; phenolic substances were detected in the vacuoles of the epidermal palisade cells and in some secretory trichome cells. The secretory cells that constitute the body of trichomes have large nuclei, dense cytoplasm with numerous mitochondria, dictyosomes, scattered lipid droplets and plastids with different inclusions: protein, lipid droplets or starch grains; vacuoles with different sizes have membranous material, phenolic and lipophilic substances. The palisade cells show thick periclinal walls, reduced cytoplasm with voluminous lipid drops and developed vacuoles. The nectary parenchyma cells contain abundant plasmodesmata and cytoplasm with scattered lipid droplets, mitochondria, plastids with starch grains and endoplasmic reticulum. Mucilage idioblasts are common in the inner nectary parenchyma. Protoderm and ground meristem participate in the formation of EFN. Our data indicate that all nectary regions are involved in nectar production and secretion, constituting a functional unit. Longevity of the extrafloral nectaries is likely associated with the presence of mucilage idioblasts, which increases the capacity of the nectary parenchyma to store water.

Key words - anatomy, extrafloral nectary, *Hibiscus pernambucensis*, histochemistry, ultrastructure

RESUMO – (Anatomia, ultra-estrutura e secreção do nectário extrafloral de *Hibiscus pernambucensis* Arruda (Malvaceae)). Este trabalho descreve o nectário extrafloral (NEF) de *Hibiscus pernambucensis*, uma espécie nativa encontrada ao longo do litoral brasileiro, vegetando áreas de manguezal e restinga. NEFs ocorrem como sulcos profundos com bordo saliente sobre as nervuras na face abaxial da lâmina foliar. Cada nectário consiste de numerosos tricomas secretores multicelulares, células epidérmicas dispostas em paliçada e parênquima não vascularizado. Açúcares redutores, lipídeos e proteínas foram histoquimicamente detectados em todas as células do nectário. Compostos fenólicos ocorrem nos vacúolos das células epidérmicas e na porção bisseriada dos tricomas secretores. As células que formam o corpo dos tricomas secretores apresentam núcleo volumoso, citoplasma denso com muitas mitocôndrias, dictiossomos, gotas lipídicas esparsas e plastídios com diferentes tipos de inclusões: proteínas, gotas de óleo ou grãos de amido; os vacúolos possuem diferentes tamanhos e podem apresentar material membranoso, substâncias lipofílicas e fenólicas. As células em paliçada mostram paredes periclinais espessas, citoplasma reduzido com gotas lipídicas conspícuas e vacuoma desenvolvido As células do parênquima caracterizam-se por apresentar muitos plasmodesmos e citoplasma abundante com gotas lipídicas dispersas, mitocôndrias, plastídios repletos de grãos de amido e retículo endoplasmático. Idioblastos de mucilagem são comuns na região mais interna do parênquima nectarífero. A protoderme e o meristema fundamental participam na formação do nectário extrafloral. Os dados mostram que todas as regiões do nectário estão envolvidas na produção e secreção do néctar, constituindo assim, uma unidade funcional. A presença de idioblastos de mucilagem no parênquima nectarífero aumenta a capacidade de retenção de água neste tecido e, provavelmente, colabora na longevidade do nectário.

Palavras-chave - anatomia, *Hibiscus pernambucensis*, histoquímica, nectários extraflorais, ultra-estrutura

Introduction

Extrafloral nectaries (EFNs) are plant-secretory glands most commonly linked to defensive mutualisms (Doak *et al.* 2007). From the anatomical point of view EFNs vary widely in ontogeny, morphology, and structure. Sometimes the morphological characteristics of nectaries, as for example the volume of nectariferous tissue seem to be correlated with the quantity of the

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nectar secreted but not its quality (see Nepi 2007). The diversity in nectary shape and location is taxonomically valuable in addition to their ecological role in plant-insect interactions (Bentley 1977, Bentley & Elias 1983, Koptur 1992, Rudgers & Gardener 2004, Wäckers & Bonifay 2004, Díaz-Castelazo *et al.* 2005; Doak *et al.* 2007).

In Malvaceae, EFNs occur in the form of furrows, chambers or depressions coated with multicellular secretory trichomes (Sawidis *et al.* 1987a, b, 1989, Sawidis 1991, 1998, Vogel 2000, Rocha *et al.* 2002). Despite the representativeness of Malvaceae in tropical ecosystems, the secretory structure and activity of EFNs have been investigated in only a few members of this family, involving some species of the *Abutilon* (Findlay & Mercer 1971a, b, Reed *et al.* 1971, Gunning & Hughes 1976), *Gossypium* (Tyler 1908, Reed 1917, Wergin *et al.* 1975, Eleftheriou & Hall 1983) and *Hibiscus*, especially *H. rosa-sinensis* L. (Santos 1959, Sawidis *et al.* 1987a, b, 1989, Sawidis 1991, 1998). Indeed, for some time, the greater part of anatomical and ultrastructural studies on Malvaceae EFNs reports on the secretory trichomes or subglandular tissue, as separate components of the nectary.

Hibiscus pernambucensis Arruda, known popularly as *guaxima-do-mangue, algodão-do-brejo and embira-do-mangue*, is a native shrub commonly occurring along Brazil's coastline in mangroves and restinga (coastal sandy plains vegetation) (Pio Corrêa 1984, Lorenzi 1992). This species has an economic potential as a source of textile and cellulose fibers for the manufacture of paper, as well as of mucilage with the same medicinal applications as those of other mallows, in addition to its lightweight wood utilized in the fabrication of small objects, toys and boxes for packaging (Pio Corrêa 1984). It is an ornamental species and indicated for planting in degraded areas (Lorenzi 1992). Moreover, *H. pernambucensis* plays an important role in the regeneration of mangroves, providing favorable conditions for the reestablishment of species typical of this habitat (Araújo & Maciel 1979).

The purpose of this work was to study the extrafloral nectary of *H. pernambucensis*, especially regarding the anatomy, ultrastructure, ontogeny and histochemistry, and to relate these to secretion mechanisms.

Material and methods

Hibiscus pernambucensis plants occurring in natural communities along the *restinga* of Sahy Beach in the municipality of Mangaratiba, state of Rio de Janeiro, were selected for observation and periodic collection of samples during 2003-2004. Plant voucher specimens were deposited in BOTU Herbarium of the Departamento de Botânica, Instituto de Biociências, Universidade Estadual Paulista (UNESP) in Botucatu municipality, São Paulo state, Brazil. The insects on the EFNs were collected and identified on the Departamento de Entomologia of the Universidade Federal Rural do Rio de Janeiro (UFRRJ).

Anatomical studies – To characterize the anatomical structure and analyze the development of the extrafloral nectaries, samples of vegetative apices and basal region of leaf blade in different development phases were fixed in FAA (formaldehyde, acetic acid, ethanol 70%, 1:1:18v/v) (Johansen 1940), dehydrated in an ethyl series, embedded in Leica® plastic resin according to the manufacturer's recommendations, and sliced in a rotating microtome. The sections (6-8 μm) were stained with 0.05% toluidine blue in acetate buffer, pH 4.3 (O'Brien *et al.* 1964) and mounted between slides and coverslips using synthetic resin (Gerlach 1969).

Histochemical tests – Sections of nectaries in the secretory stage, occurring in freshly collected completely expanded leaves, were obtained with a Ranvier microtome and treated with Sudan IV to detect lipids (Johansen 1940); with 10% ferric chloride to detect phenolic compounds (Johansen 1940); 0.02% ruthenium red to detect pectic substances (Jensen 1962); Fehling reagent for reducing sugars (Purvis *et al.* 1964); aniline blue black for proteins (Fisher 1968); and Lugol reagent for starch grains (Johansen 1940). In all these tests, a control was conducted simultaneously following the authors recommendations. The sections were mounted with glycerin between slides and coverslips.

Scanning electron microscopy (SEM) – Samples were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer solution at pH 7.3, for 12-18 h at 4 °C, dehydrated in an ethyl alcohol series, critical point dried with CO_2 as the transition liquid (Robards 1978), mounted with a double adhesive tape on stubs, and sputter coated with gold. The samples were observed at 20 kV using a Philips SEM 515.

Transmission electron microscopy (TEM) – Samples of nectaries in the secretory stage, occurring in completely expanded leaves, were fixed as described above, post-fixed in 1% osmium tetroxide in the same buffer for 2 h, dehydrated in acetonic series and embedded in Araldite resin. Ultrathin sections were contrasted with uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963), and observed at 60 kV using a TEM Philips, CM 100.

Results

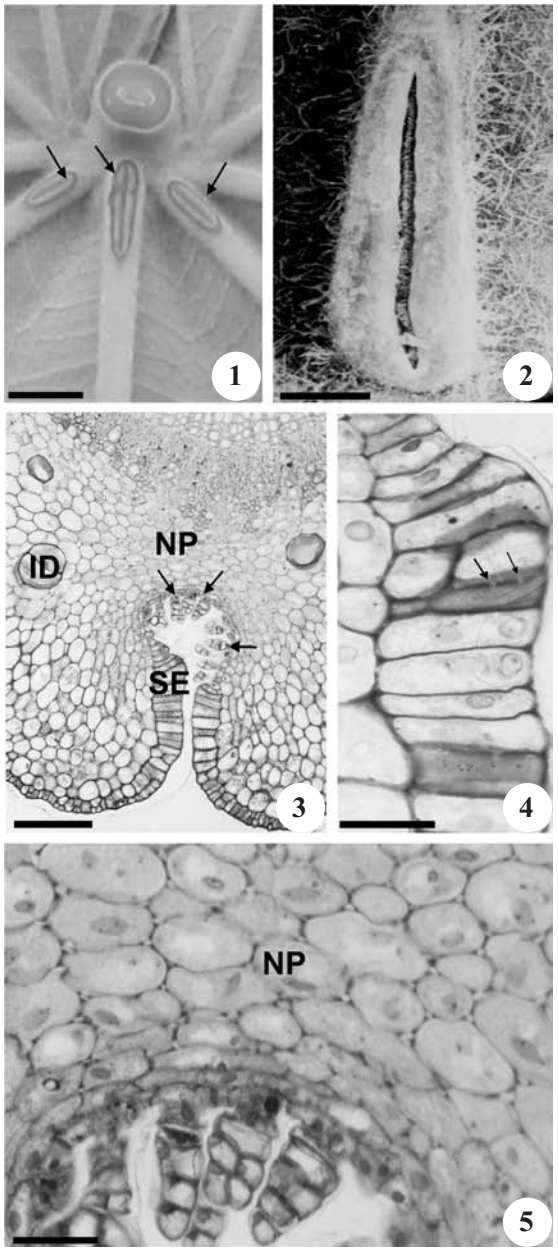
Distribution, morphology and secretion of EFNs – The extrafloral nectaries (EFNs) of *Hibiscus pernambucensis* occur on the abaxial surface of the leaf blade located

on the basal portion of the median vein, but they can also occur on the lateral veins (figure 1). Within each individual leaf, nectaries have a similar morphology, but the one located on the median vein is more developed. They consist of a furrow with raised glabrous-surfaced edges (figure 2) which widens toward the inside of the vein, forming a chamber containing numerous secretory trichomes (figure 3). Nonglandular trichomes cover the abaxial surface of the leaf blade except in the region of the nectary (figure 2).

Nectaries at the secretory stage present a tumescent border and are visible on very young leaves with *c.* 0.5-1 cm in length. The secretion is abundant and has a hyaline aspect, filling the inside of the chamber and sometimes spilling over the edges. The EFNs are constantly foraged by various insects, among which ants of the genera *Camponotus* and *Solenopsis* and bees of the genus *Trigona* are the most common. On senescent leaves, the nectary is inactive and insects are absent.

Anatomy and ontogeny of EFNs – Anatomically, the nectary consists of three regions (figure 3): 1) edge of the furrow, a single-layered epidermis constituted of column-shaped cells covered by a thick cuticle; 2) multicellular trichomes lodged at the bottom of the furrow or nectariferous chamber; 3) a large region formed by parenchyma, non-vascularized tissue. The tall epidermal cells of the nectary edge present conspicuous pores in the anticlinal walls (figure 4). The nectary parenchyma is differentiated in two zones: one, located beneath epidermis layer, constituted by tangentially flattened cells compactly arranged (figures 3, 5), and an inner region, constituted by several layers of larger parenchyma cells and characterized by the presence of large idioblasts with mucilage.

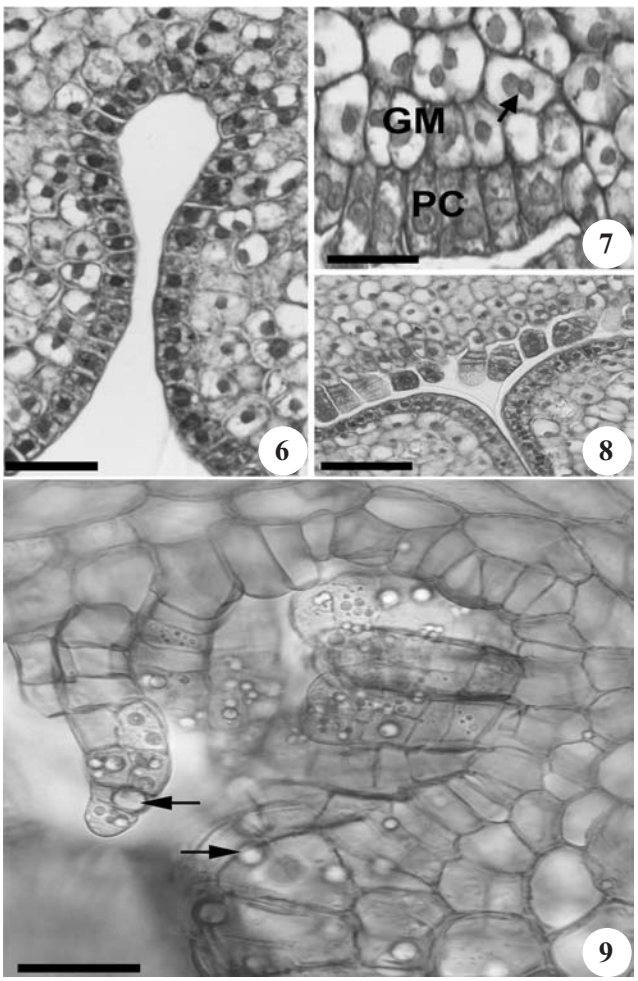
The nectary located on the median vein initiates as a furrow covered by protoderm, constituted by homogeneous size cells with a voluminous nucleus, dense cytoplasm and little developed vacuome (figure 6). The protodermal cells lining the bottom of the furrow show a marked elongation allied to an increase of the nucleus volume, which in this phase characteristically occupies a central position in the cell (figure 7). These cells divide in a periclinal direction, each one originating a basal cell and an apical cell. The basal cell remains inserted in the epidermis, constituting the basal cell of the trichome. The apical cell divides periclinally, and its derivatives divide successively in the transversal plane forming a series of up to 10 cells; after this series is formed, the cells of the intermediary portion divide anticlinally, originating a biseriate portion, while the



Figures 1-5. Extrafloral nectary (EFN) of *Hibiscus pernambucensis*. Frontal view (1-2) and cross leaf sections (3-5). 1. Part of a leaf (petiole removed) showing the location of the EFNs (arrows) on the median and lateral veins. 2. Scanning electron micrograph of the nectary showing the glabrous-surfaced edge surrounded by non-glandular trichomes, and the furrow full with a flocculent material, probably nectar. 3. Light micrograph through the nectary showing the edge composed of palisade-like epidermis (SE), nectariferous chamber with secretory trichomes (arrows) and the parenchyma tissue (NP). Note mucilage idioblasts (ID). 4. Detail showing pores (arrows) in the anticlinal walls of the tall epidermal cells. 5. Part of a nectary showing multicellular secretory trichomes at the bottom of the furrow and parenchyma tissue (NP). Bar = 6 mm (1); 100 μm (2); 40 μm (3); 25 μm (4, 5).

stalk and the cell of the apical region remains uniseriate. Secretory trichomes in different stages of development occur side by side in the same nectary (figures 8, 9). Cells of the ground meristem divide at different planes (figure 7), originating the nectary parenchyma.

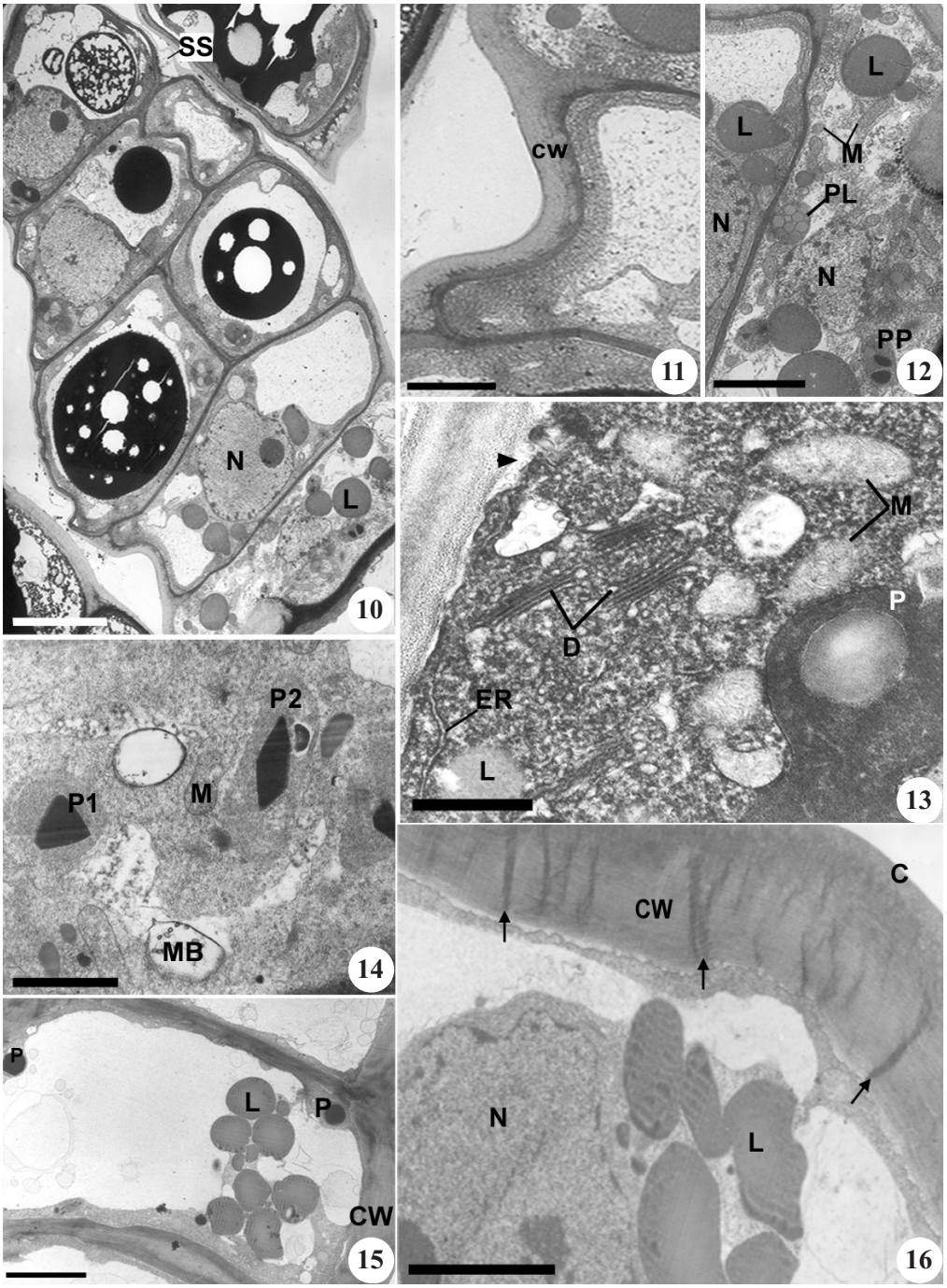
Ultrastructure of EFNs – The ultrastructure of the EFNs is showed in the figures 10-20. TEM analysis showed electron-dense bands (probably pectic substances), extending from the internal surface of the outer periclinal cell wall toward the cuticle, but not penetrating it (figure 16). Each epidermal cell tipically contains a voluminous nucleous (figure 4) and a large vacuole traversed by cytoplasmic strands (figure 16). Voluminous oil droplets (figures 15, 16) occur in the cytoplasmic strands. Occasionaly, very small plastids with osmiophilic inclusions (figure 15) are seen in the outer cytoplasm.



Figures 6-9. Light micrographs showing some EFN development stages. 6. Future nectary furrow covered with protodermal cells. 7. Protodermal precursors (PC) of the secretory trichomes in furrow region and ground meristem (GM) cells in division (arrow). 8. Part of the EFN bottom showing secretory trichomes in different stages of development. 9. Mature nectary treated with Sudan IV, showing conspicuous oil droplets (arrows) in the apical regions of the secretory trichomes and inside palisade-like epidermal cells. Bar = 35 µm (6); 25 µm (7, 9); 100 µm (8).

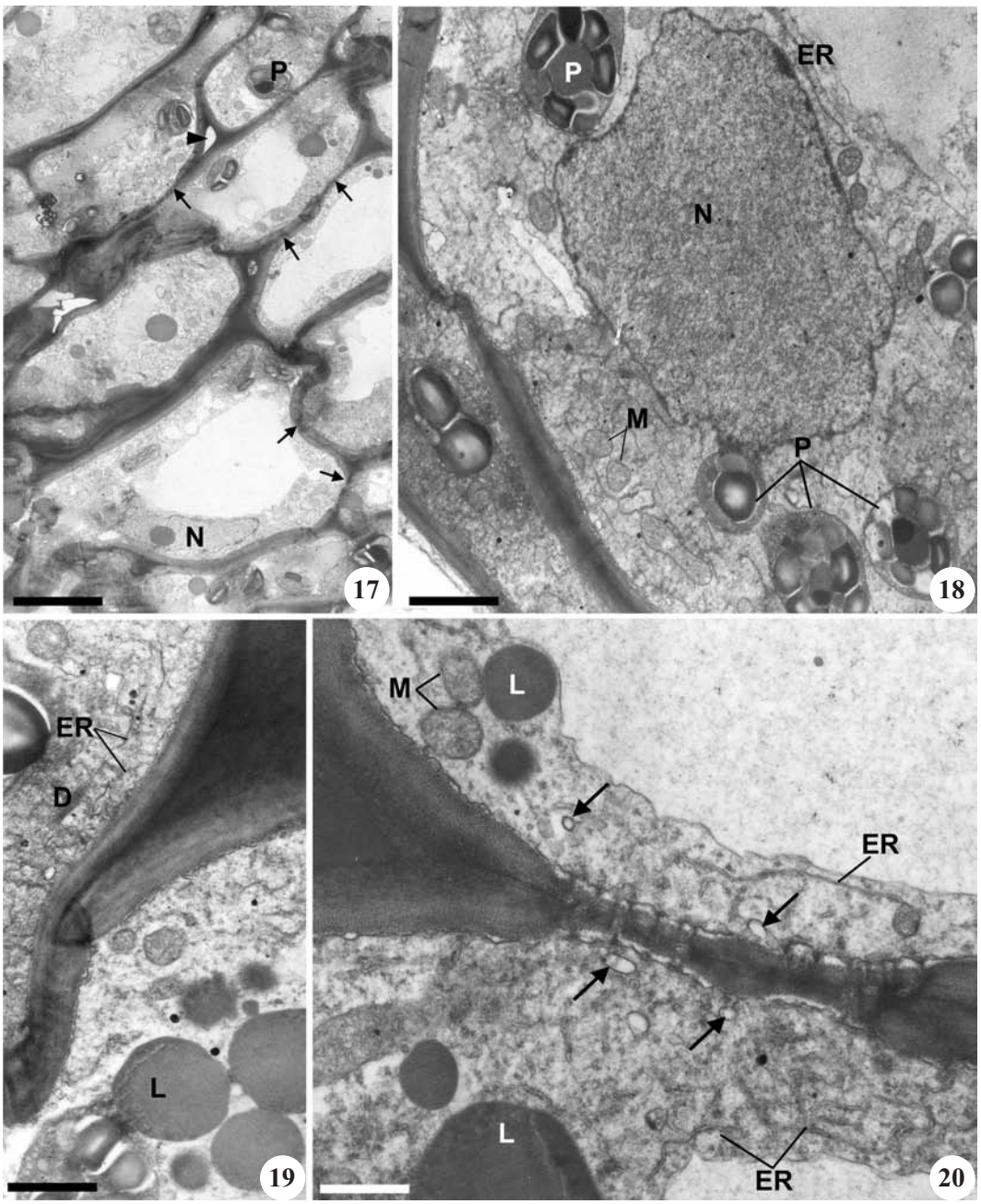
The secretory trichomes are clavate (figures 5, 10) and constituted by a basal cell, a short stalk which has two or three tabular cells, and a body constituted by a biseriate portion with two to six strata of voluminous square cells and a conical apical cell. In the stalk cells the protoplast remais compressed between the tangential cell walls (figures 10, 11); however, small spaces between the plasmalemma and cell wall (figures 10, 13 – arrowhead) are observed at several points in the body and apical cells. The stalk cells have thick lateral walls (figures 10, 11), which are electron-opaque due to the impregnation of lipids, while the transversal cells are thinner, strongly electron-dense (figures 10-12) and traversed by plasmodesmata. A thin cuticle remains attached on the tangential walls of the stalk cells (figures 10, 11), but cuticle covering the body and apical cells forms swellings origining small subcuticular spaces (figure 10). A spherical or more irregular nucleous, mitochondria with prominent cristae, plastids with oil droplets or cuneiform protein inclusions, voluminous oil droplets and various small vacuoles are common in the stalk cell located near the basal cell (figure 12). The stalk cell located in the vicinity of the body cells shows distinct characteristics, as prominent nucleus and two large vacuoles (figure 10). The cells constituing the body of the secretory trichomes are characterized by voluminous nucleus and a dense-staining cytoplasm with abundant ribosomes, many dictyosomes with adjacent vesicles, mitochondria with well developed cristae and plastids with different inclusions (figures 13, 14). Prominent plastids contained electron-dense matrix, very few thylakoids and a single starch grain (figure 13), and plastids with cuneiform proteins, with or without starch grains (figure 14) are commonly observed. Rough endoplasmic reticulum (figure 13) and multilamellar body (figure 14) were occasionally present. The vacuoles contain a flocculent material or a strongly electron-dense content (figure 10), probably phenolic substances as detected in histological sections.

Parenchyma cells located below epidermal layer tipically have irregularly thickened walls with very small spaces between them, conspicuous nucleus with variable size and shape, abundant cytoplasm and a large vacuole or many small vacuoles (figure 17) filled with a flocculent material. Mitochondria, plastids with abundant starch grains and smooth endoplasmic reticulum are



Figures 10-16. Transmission electron micrographs through the secretory trichomes (10-14) and tall epidermal cells (15-16) of *Hibiscus pernambucensis* extrafloral nectary. 10. Secretory trichome showing stalk cells, a biseriate portion and a conical apical cell. Note prominent nucleous (N), oil droplets (L) and small subcuticular space (SS). 11. Part of a stalk cell, showing a thick lateral wall (CW) impregnated with lipidic substance. 12. Part of two stalk cells showing the nucleus (N), mitochondria (M), plastids with oil droplets (PL) or protein inclusions (PP) and voluminous oil droplets (L) in the cytoplasm. 13. Detail of a cell of the biseriate portion of the trichome, showing numerous dictyosomes (D) and adjacent vesicles, mitochondria (M), oil droplets (L), plastid (P) with starch grain and endoplasmic reticulum (ER), and periplasmic space (arrowhead). 14. Detail of a cell of the biseriate portion of the trichome, showing plastid (P1) with cuneiform protein inclusion, plastid (P2) with protein inclusion and starch grain, mitochondria (M) and multivesicular body (MB). 15. Part of an epidermal cell showing conspicuous oil droplets (L), small plastids (P) and outer periclinal wall (CW). 16. Detail showing electron-dense bands (arrows) passing through the outer periclinal wall (CW). Note the homogeneous cuticle (C), nucleus (N) with lobular contour and conspicuous oil droplets (L). Bar = 6 µm (10); 5 µm (11,12); 0,6 µm (13); 1,5 µm (14); 4,5 µm (15); 2 µm (16).

the most abundant organelles (figure18). Dictyosomes are very scarse in these cells (figure 19). Oil droplets of variable size are common in the cytoplasm (figures 19, 20). Electron-translucent vesicles of variable sizes are seen in the outer cytoplasm and associated with the plasmallema, which is sinuous in the pit fields regions (figure 20). Smooth endoplasmic reticulum is very abundant at the vicinities of pit fields (figure 20). Plasmodesmata connect all parenchyma cells (figures 17, 20).



Figures 17-20. Transmission electron micrographs through the nectary parenchyma of *Hibiscus pernambucensis*. 17. Parenchyma cells tangentially flattened located below epidermis layer showing peripheral nucleus (N), amyloplasts (P), pit fields (arrows) and small intercellular space (arrowhead). 18. Detail of a parenchyma cell showing nucleus (N) with sinuous contour, amyloplasts (P), mitochondria (M) and endoplasmic reticulum (ER). 19. Part of two parenchyma cells showing voluminous oil droplets (L), dictyosome (D) and endoplasmic reticulum (ER). 20. Detail showing one pit field with numerous plasmodesmata, abundance of endoplasmic reticulum (ER), translucent vesicles (arrows) in the outer cytoplasm and associated to the plasma membrane, oil droplets (L) and mitochondria (M). Bar = 8 µm (17); 2 µm (18); 1,5 µm (19); 1 µm (20).

Histochemical tests – The active nectaries on completely expanded leaves reacted positively to reducing sugars, lipids, pectic substances, proteins and phenolic compounds (table 1). Reducing sugars were detected in all the cells of the secretory trichomes and parenchyma tissue. Proteins were detected in the cells of the glandular trichomes, nectary parenchyma and in the palisade-like epidermal cells. Oil droplets were detected in the palisade-like epidermal cells, secretory trichomes and parenchyma tissue. Lipidic substances were also detected in the lateral walls of the stalk cells of the secretory trichomes. Pectic substances were more abundant in the outer periclinal wall of the palisade-like cells. Phenolic compounds were detected in the palisade cells and in the biseriate portion of the secretory trichomes, but were absent in the parenchyma cells.

Discussion

The extrafloral nectaries (EFNs) on the leaves of *Hibiscus pernambucensis* are "hollow-type nectaries", according to Zimmermann's classification (1932). These nectaries are characterized by the presence of numerous multicellular, linear secretory trichomes lodged at the bottom of the furrow and represent a marked characteristic of the order Malvales (Arbo 1972, 1973, Wergin *et al.* 1975, Kronestedt *et al.* 1986, Sawidis *et al.* 1987a, b).

Our observations showed that the protoderm and ground meristem participate in the formation of EFNs in *H. pernambucensis*, with an ontogenetic pattern similar to that documented for different species of Malvales (Arbo 1972, Sawidis *et al.* 1987a).

In *H. pernambucensis*, the extrafloral nectaries begin to secrete nectar from very young to totally expanded leaves, with the secretion end coinciding with the beginning of senescence. This observation differs from most of the reports (Paiva & Machado 2006), which describe active extrafloral nectaries only on young leaves, and inactive nectaries on completely expanded leaves. The constant presence of *Camponotus* ants visiting the extrafloral nectaries of young and totally expanded leaves of *H. pernambucensis* plants was also reported by Cogni & Freitas (2002), who found that these ants are aggressive and attack termites. Arbo (1972) considered that the extrafloral nectaries of *Byttneria*, a genus of Sterculiaceae, which, together with Malvaceae, comprise the order Malvales, constitute a source of food that attracts ants. It is remarkable that the EFNs of *H. pernambucensis* produce abundant lipids and, as described for other species (Roth 1968, Durkee *et al.* 1981, Machado 1999), these substances may be part of the exudate acting as a food source, especially for ants.

In active nectaries, lipid droplets, reduced sugars, proteins bodies and phenolic compounds were detected by histochemistry. The EFNs of *H. pernambucensis* are devoid of their own vascularization and the reducing sugars and water components of the nectar possibly are provided by the phloem sap of the vein into which the EFN is inserted, as already described for *Gossypium* species (Wergin *et al.* 1975) and for *Hibiscus tiliaceus* L. (Rocha *et al.* 2002). Alternatively, in some species, nectar sugars are produced in the own nectary cells by photosynthesis, whereupon starch grains remains in the plastids for a short period only (Paiva & Machado 2008). Phenolic compounds in the EFNs of *H. pernambucensis* may be associated with protection against biotic (herbivores and microbes) and abiotic (protection against UV-B radiation) stresses, which are functions related to the chemical structure of their components (Hutzler *et al.* 1998). Proteins bodies in mature EFNs have been

Table1. Histochemistry of the active extrafloral nectaries at the mature leaves of *Hibiscus pernambucensis*. (– = negative; + = slightly positive; ++ = strongly positive)

Staining procedure	Target compounds	Colour observed	Reactivity		
			Epidermal cells	Secretory trichomes	Nectary parenchyma
Sudan IV	Total lipids	Orange to red	++	+	++
Ferric trichloride	Phenolic compounds	Black blue or black green	+	+	–
Ruthenium red	Mucilage/pectin	Red to pink	++	+	+
Fehling's solution	Sugars (glucose and fructose)	Pink to brilliant red	–	+	+
Aniline blue black	Proteins	Blue	+	++	+
Lugol	Starch grains	Dark blue to brownish	–	+	++

associated with storage of retained nitrogen, and their participation in nectar production is uncertain (Nepi 2007).

The secretory trichomes of *H. pernambucensis* have common characteristics to nectaries of different species, such as plastids with prominent starch grains, abundant dictyosomes, numerous mitochondria and endoplasmic reticulum (Fahn 1979; Durkee *et al.* 1981; Durkee 1983; Eleftheriou & Hall 1983; Robards & Stark 1988; Sawidis *et al.* 1989; Sawidis 1991; Figueiredo & Pais 1992; Pais & Figueiredo 1994; Jian *et al.* 1997; Sawidis 1998; Fahn & Shimony 2001; Nepi *et al.* 1996; Paiva & Machado 2006, 2008). Plastids, especially amyloplasts, play an important role in the production of nectar, with the starch totally or partially hydrolyzed during the manifestations of secretory activity. Numerous mitochondria in the nectariferous cells are possibly related to the process of starch grains hydrolysis, since these organelles are essential to the metabolic processes involving the consumption of energy. Endoplasmic reticulum is one of the principal components of nectariferous cells and plays a major role in the translocation of sugars, as well as being the site for lipid synthesis; dictyosomes are related to production of hydrophilic components of secretions (Fahn 1979, 2000; Nepi 2007).

The cytological characteristics of the *H. pernambucensis* nectary parenchyma including many mitochondria, extensive endoplasmic reticulum, the presence of vesicles and numerous plasmodesmata suggest the participation of this region of the nectary in the process of unloading of sugars originated from the phloem, as well as in the cell-to-cell transport of the nectar precursors. This mechanism of transport favors the enrichment of the pre-nectar, which is transported via plasmodesmata to the secretory trichomes (Wergin *et al.* 1975, Gunning & Hughes 1976, Durkee 1983, Eleftheriou & Hall 1983, Kronestedt *et al.* 1986, Sawidis *et al.* 1987a, b, 1989, Fahn 2000, Sawidis 1991, 1998). The considerable development of the endoplasmic reticulum in the parenchyma cells of *H. pernambucensis* is matching with the EFNs of *Hibiscus rosa-sinensis* (Sawidis *et al.* 1989, Sawidis 1991) and, according to these authors, this is the principal cellular component involved in the transport of the pre-nectar.

As described in other species of Malvaceae (Sawidis *et al.* 1987a, Sawidis 1991), in *H. pernambucensis*, after entering the trichomes, pre-nectar flows from cell to cell via plasmodesmata reaching the apical cell; the apoplastic route is impeded by a strong cutinization of the lateral walls of the trichome stalk cells. In addition, presence of a very elaborate system of membranes as plasmodesmata, endoplasmic reticulum, dictyosomes and vesicles are evidences of the symplastic route for nectar transportation. Final modification of the nectar occurs within the trichome cells and, in *H. pernambucensis*, well-developed dictyosomes and endoplasmic reticulum are involved in this process. Presence of vesicles in the outer cytoplasm and images suggesting vesicle fusions with the plasmallema are evidence of granulocrine secretion, and seem to be the cause of the sinuosity observed in this membrane (Machado *et al.* 2006). In *H. pernambucensis* it is likely that the nectar is then secreted into the small periplasmic spaces which are commonly observed in the trichome body and apical cells. After, having crossed the outer tangential wall the nectar accumulates beneath the cuticle. Absence of cuticle rupture may indicate that the cuticle is permeable to nectar, as has pointed for a number of other species (Stpiczynska *et al.* 2005).

It is likely that the release of the secretion to the surface of *H. pernambucensis* extrafloral nectary occurs also through microchannels; these are seen as wide electron-dense bands of a pectic nature in the outer periclinal wall of the palisade-like epidermal cells. Microchannels emerging as fibrillar outgrowths of the outer epidermal cell wall were reported in *Abutilon* sp. by Kronestedt *et al.* (1986). In *H. pernambucensis*, the wall pectin bands can act as hydrophilic filler, increasing the porosity of the wall to macromolecules (Taiz & Zeiger 1998), thus constituting an apoplastic exit route of the nectar to the nectary surface.

The presence of idioblasts with mucilage in the inner parenchyma region of *H. pernambucensis* nectary, due to its hydrophilic properties, helps maintain the relatively water potential of nectary tissues (Sawidis 1998). As a consequence, increases the capacity of the nectaries to store water and this in turn improves its longevity. A remarkable feature of the extrafloral nectary of *H. pernambucensis* is that the nectar secretion period is prolonged, since secretion starts in very young leaves and remains up to completely expanded leaves. Similar data were reported on floral nectaries of Orchidaceae species (Stpiczynska *et al.* 2005).

In summary, the extrafloral nectary of *H. pernambucensis* has a number of common characteristics to other Malvaceae species. This study indicates that epidermal cells that constitute the border of the nectary furrow are involved in nectar production and secretion, besides the secretory trichomes and the subglandular parenchyma tissue. Although relevant field data are not available, the prolonged nectar secretion suggests that extrafloral nectaries in this species constitute an

efficient protection for the aerial biomass by maintaining a permanent colony of aggressive ants. Nevertheless, a long-term study is necessary to evaluate the role of the EFNs in the *H. pernambucensis*-ant interactions and the factors that influence and determine this nectar secretion pattern.

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