The LINNEAN SOCIETY of London BOTANICAL Journal of the Linnean Society

Botanical Journal of the Linnean Society, 2015, **179**, 533–544. With figures

Stages of development of the floral secretory disk in *Tapirira guianensis* Aubl. (Anacardiaceae), a dioecious species

ELISABETH E. A. DANTAS TÖLKE[1]*, LEONARDO GALETTO[2], SÍLVIA RODRIGUES MACHADO[3], ANA PAULA STECCHAHN LACCHIA[4] and SANDRA MARIA CARMELLO-GUERREIRO[1]

[1]*Instituto de Biologia, Universidade Estadual de Campinas – UNICAMP, Departamento de Botânica, CEP 13083-079, Campinas, São Paulo, Brasil*
[2]*Instituto Multidisciplinario de Biologia Vegetal (CONICET-UNC) y FCEFyN, Universidad Nacional de Córdoba, Vélez Sarsfield 1611, CC 495, 5000 Córdoba, Argentina*
[3]*Instituto de Biociências, Universidade Estadual Paulista Júlio de Mesquita Filho – UNESP, Campus de Botucatu, CEP 18618-000, Botucatu, São Paulo, Brasil*
[4]*Departamento de Biologia, Universidade Estadual da Paraíba – UEPB, Campus I, CEP 8109-790, Campina Grande, Paraíba, Brasil*

Received 1 March 2015; revised 28 July 2015; accepted for publication 11 August 2015

The goal of this study was to analyse possible structural and ultrastructural differences between the secretory disk of male and functionally female flowers of *Tapirira guianensis* (Anacardiaceae) at different developmental stages. Studies were carried out using light, scanning and transmission electron microscopy. Biochemical tests were employed to determine the proportion of sugars in the nectar of the floral morphotypes: they were found to be similar, both predominantly composed of sucrose. In addition to sugars, lipids and phenolic substances were identified in anthetic flowers; thus, the secretory disk is a mixed secretion gland, also called a *sensu lato* nectary. During anthesis, granulocrine and eccrine secretory mechanisms occur in both floral morphotypes. After anthesis and fertilization of the functionally female flower, only the lipophilic and phenolic secretion continues until the early stages of fruit development. An intrastaminal secretory disk that produces both nectar and lipids is reported for the first time in Anacardiaceae. © 2015 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2015, **179**, 533–544.

ADDITIONAL KEYWORDS: floral nectary – intrastaminal disk – lipids – mixed secretion – nectar – ultrastructure.

INTRODUCTION

Tapirira guianensis Aubl. (Anacardiaceae) is a dioecious tropical tree widely distributed throughout Brazil (Silva-Luz & Pirani, 2011). There are three floral morphotypes present in this species: structurally and functionally male flowers; functionally female flowers with non-fertile stamens; and, occasionally, hermaphroditic flowers on otherwise female plants (Lenza & Oliveira, 2005). Thus, *T. guianensis* is cryptically dioecious. In Anacardiaceae, cryptic dioecy is common among species in several genera, including *Harpephyllum* Bernhardi ex Kraus, *Pleiogynium* Engl., *Rhus* L., *Pistacia* L., *Schinus* L., *Rhodosphaera* Engl., *Pentaspadon* Hook.f., *Campnosperma* Thwaites (Wannan & Quinn, 1991).

All floral morphotypes have an intrastaminal disk that produces nectar as a reward during anthesis. In male and hermaphroditic flowers, pollinators are in addition rewarded with pollen (Lenza & Oliveira, 2005).

*Corresponding author. E-mail: elisabeth.tolke@gmail.com

An intrastaminal disk is common in Anacardiaceae (Cronquist, 1981; Wannan & Quinn, 1991; Gallant, Kemp & Lacroix, 1998) and also occurs in many other families of Sapindales (Bachelier & Endress, 2009). Its nectariferous function plays an important role in reproduction as nectar is one of the main rewards offered to the pollinators (Baker & Baker, 1973; Nicolson, 2007). Sugars and amino acids are the most significant components of nectar, but other substances may be present in smaller quantities, such as proteins, antioxidants, alkaloids, phenolic substances, vitamins and organic acids (Baker & Baker, 1973; Vesprini, Nepi & Pacini, 1999; Galetto & Bernardello, 2005). More rarely such disks can also produce lipid substances and have then been named *sensu lato* nectaries (Durkee, Baird & Cohen, 1984; Machado *et al*., 2008).

Preliminary histochemical studies have shown that the floral disk of *T. guianensis* also produces both nectar and lipids and is thus a *sensu lato* nectary (Lacchia, 2006). This gland continues to secrete lipids during and after the formation of the fruit (Lacchia, 2006), which had not been reported for Anacardiaceae earlier.

The aim of the present study was, therefore, to investigate the structure and ultrastructure of the *sensu lato* nectary of *T. guianensis*, at anthesis and during fruit formation in order to understand whether the structure of the disk differs between the morphotypes, whether the sugar composition in the nectar varies between the two floral morphotypes, what classes of substances are being produced and stored in addition to the nectar, which organelles are involved at the different phases of the secretion process and what mechanisms are involved in the release of the secretion.

MATERIAL AND METHODS

Male and functionally female flower buds and anthetic flowers as well as fruits at various developmental stages of *T. guianensis* were collected in three areas of the state of São Paulo, Brazil: the Itirapina experimental station (22°13′S; 47°51′W), the Mogi Guaçú experimental station (22°10′S; 47°07′W) and a cerrado (Brazilian savannah) fragment in the District of Sousas, Campinas (22°51′S; 46°57′W). Collections were made from March to December in 2011 and from January to February in 2012. Vouchers are deposited in the UEC herbarium (UEC 182229).

Analysis of the secretion produced by the secretory disk

The secretion produced by the disk at anthesis was tested for glucose with reagent strips (Uri-test 11) to confirm the production and release of nectar. For the biochemical analysis of the nectar, the secretion of each floral morphotype was collected using glass capillary tubes and stored in Whatman n. 1 paper filter at low temperature (Galetto & Bernardello, 2005). The composition of sugars was analysed for 50 flowers from three male and three female trees in a population belonging to the fragment of the District of Sousas. After storage in filter paper, the samples were dissolved in distilled water. For quantitative analysis of the sugars, reagents kits for glucose, fructose and sucrose (Sigma) were used following the methodologies proposed by Bergmeyer & Bernt (1974), Kunsst, Draeger & Ziegenhorn (1984) and Southgate (1976). The absorbance reading was determined in a spectrophotometer at a wavelength of 340 nm. The calculation of the rate of sugar was used to classify the species according to Baker & Baker (1982). The results were expressed as mean ± standard deviation. For comparison of the arithmetic means, Bioestat 5.3 software (Ayres *et al*., 2007) was used to analyse the variance (ANOVA) in accordance with the t-test at a significance level of 5% ($P \leq 0.05$).

Histological analysis

For anatomical studies, floral buds, anthetic flowers and fruits at various developmental stages were fixed in FAA (formaldehyde, acetic acid, 50% ethanol; 1:1:18 v/v) for 24 h (Johansen, 1940). The material was then dehydrated in a butyl alcohol series and embedded in Paraplast (Johansen, 1940). Transverse and longitudinal sections 10 μm thick were obtained using a Microm HM340E rotary microtome and stained with Astra Blue and Safranin (Gerlach, 1984). All slides were mounted in Entellan synthetic resin, and the images were captured with an Olympus DP71 digital camera coupled to an Olympus BX51 microscope.

For the histochemical tests, the material was fixed in FAA (for hydrophilic substances) for 24 h and in FNT (phosphate buffer solution, formalin; 9:1 v/v) (for lipophilic and phenolic substances) for 48 h. The treatments performed and their chromatic positive reactions can be found in Table 1. The results were recorded through images captured by an Olympus DP71 digital camera couple to an Olympus BX51 microscope.

Scanning electron microscopy

Flowers fixed in FAA were dehydrated in an ethanol dilution series, critical point dried, and sputter coated with gold. Observations were carried out using a Jeol JSM 5800 LV scanning electron microscope (SEM) at 10 kV equipped with a digital camera.

Transmission electron microscopy

For transmission electron microscopy (TEM), samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3 for 24 h at 5 °C. They were post-fixed in 1% osmium tetroxide in the same buffer for 1 h at 25 °C, dehydrated in an acetone dilution series and embedded in araldite (Machado & Rodrigues, 2004). Ultrathin sections were obtained with a Diatome diamond knife and stained with 1% methylene blue and contrasted with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963). The material was observed with a Tecnai Spirit TEM (FEI) at 60 KV.

RESULTS

Disk structure

The gluco-strip tests for the presence of glucose tested positive in droplets secreted from the disk of anthetic fresh flowers from both morphotypes. It can therefore be confirmed that the intrastaminal disk functions as a nectary. Sugars were, conversely, absent in the droplets secreted from the disks of the fruits.

The disk is located between the filament bases and the ovary base in both flower morphotypes (Fig. 1A, B). Its structure is similar throughout the flowering phase and has ten lobes, which alternate with the fertile or sterile filaments (Fig. 2A). Its epidermis is uniseriate, relatively smooth and secretory and is covered with a cuticle (Fig. 2B, C). The stomata are densely distributed across the surface and are slightly sunken below the level of their neighbouring cells (Fig. 2D, E).

The region just below the epidermis was considered as nectary parenchyma and the vascularized region as sub-nectary parenchyma (*sensu* Nepi, 2007) (Fig. 2B). Both regions are characterized by sparsely distributed druses with polyhedral shape (Fig. 2B). After fertilization, there are no significant structural changes in the gland in relation to the previous stages.

Sugar composition and additional substances produced

Detailed sugar analysis showed that the composition of nectar was similar among all individuals tested, including male and functionally female flowers (Table 2). The three main sugars (fructose, glucose

Table 1. Histochemical tests used in the characterization of the substances

Test	Substance detected	Positive chromatic reaction
Sudan Black B (Pearse, 1980)	Total lipids	Blue to black
Nile Blue Sulphate (Cain, 1947)	Acidic and neutral lipids	Light blue for acidic lipids and pink to purple for neutral lipids
Lugol's iodine (Berlyn & Miksche, 1976)	Starch	Purple to black
Iron(III) chloride (Johansen, 1940)	Phenolic compounds	Brown and black
Wagner reagent (Furr & Mahlberg, 1981)	Alkaloids	Red
Schiff reagent (PAS) (McManus, 1948)	Total polysaccharides	Pink
Ruthenium Red (Johansen, 1940)	Pectins	Intense pink
Tannic acid and iron (III) chloride (Pizzolato & Lillie, 1973)	Mucilage	Black
Copper acetate/rubeanic acid (Ganter & Jollés, 1969, 1970)	Fatty acids	Dark green

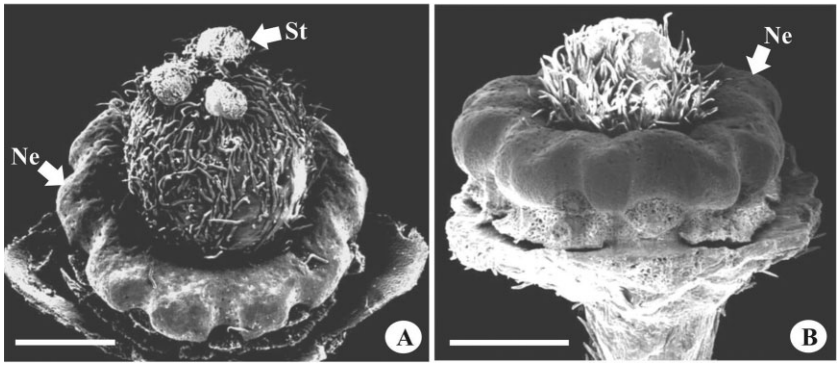

Figure 1. Electron micrographs of flowers of *Tapirira guianensis* Aubl. (Anacardiaceae). A, Functionally female flower. B, Male flower. Ne, nectary; St, stigma. Scale bars: 250 μm (A), 300 μm (B).

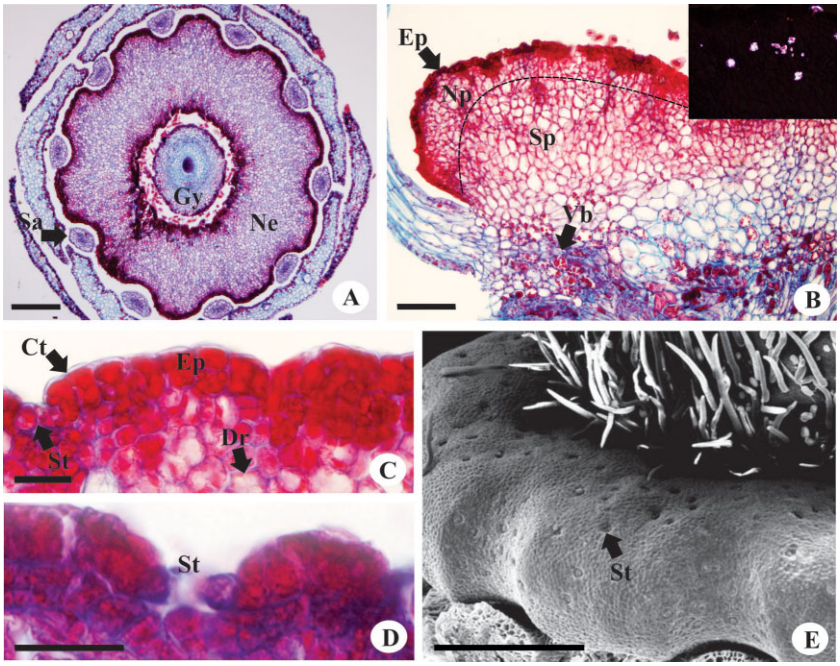

Figure 2. Structure of the secretory disk of *Tapirira guianensis* Aubl. (Anacardiaceae). A, Cross-section showing the nectary around the gynoecium, which contains ten lobes alternating with the stamens. B, General appearance of the nectary in longitudinal section showing the epidermis, nectary parenchyma, sub-nectary parenchyma and vascular bundles. In the upper right corner there is a higher magnification of the druses using polarized light. C, Longitudinal section of the nectary showing detail of the epidermis coated with cuticle; stomata and druses can also be observed. D, Detail of the stomata in longitudinal section showing its position relative to the neighbouring cells. E, Electron micrograph of the nectary evidencing the widely distributed stomata. Ne, nectary; Gy, gynoecium; Sa, stamens; Ep, epidermis; nectary parenchyma; Sp, sun-nectary parenchyma; Vb, vascular bundles; Ct, cuticle; St, stomata; Dr, druses. Scale bars: 10 μm (C, D), 50 μm (B), 100 μm (A, E).

and sucrose) were detected in all samples and their proportion was fairly homogeneous among the individuals sampled. The few changes that occurred were not significantly different ($P > 0.05$).

Table 2. Sugar composition of the nectar

Variables	Male flowers ($n = 3$)	Female flowers ($n = 3$)	Paired T-test
Sucrose %	48.34% ± 0.18	57.47% ± 0.05	0.563
Fructose %	30.01% ± 0.09	29.40% ± 0.02	0.903
Glucose %	21.56% ± 0.08	13.10% ± 0.06	0.449
r = S/G + F	1.03 ± 0.70	1.33 ± 0.35	0.644
rh = G/F	0.71 ± 0.08	1.03 ± 0.54	0.354

The composition of the nectar is analysed showing individual sugars (sucrose, fructose and glucose) and rates of sugars (r) and hexoses (rh) between male and functionally female flowers of three plants. The tabulated data are expressed as mean ± standard deviation. The variables were not significantly different between plants with male and female flowers ($P > 0.05$). Three female and three male individuals were sampled (50 flowers per sample).

Male and functionally female flowers had sucrose-dominant nectar ($r > 1.0$), and the relationship of hexoses (rh) demonstrates that fructose is the predominant sugar, in functionally female flowers (rh = 1.03) and in male flowers (rh = 0.71). The variables evaluated did not differ significantly between the two floral morphotypes ($P > 0.05$) (Table 2).

Histochemical tests revealed the presence of starch grains in the protoplast of both nectary and sub-nectary parenchyma cells before anthesis (Fig. 3A), but they were absent during anthesis and in later phases (Fig. 3B). Lipids were present in the secretory epidermis of the nectary in flower buds and flowers in anthesis in both morphotypes. This was also the case for the nectary parenchyma (Fig. 3C, D). Phenolic compounds (Fig. 3E) and fatty acids (Fig. 3F) were also found in the same structures.

During the development of the fruit, both the epidermis and subepidermis of the disk still reacted positively for lipids, phenolic compounds and fatty acids. The histochemical tests are summarized in Table 3.

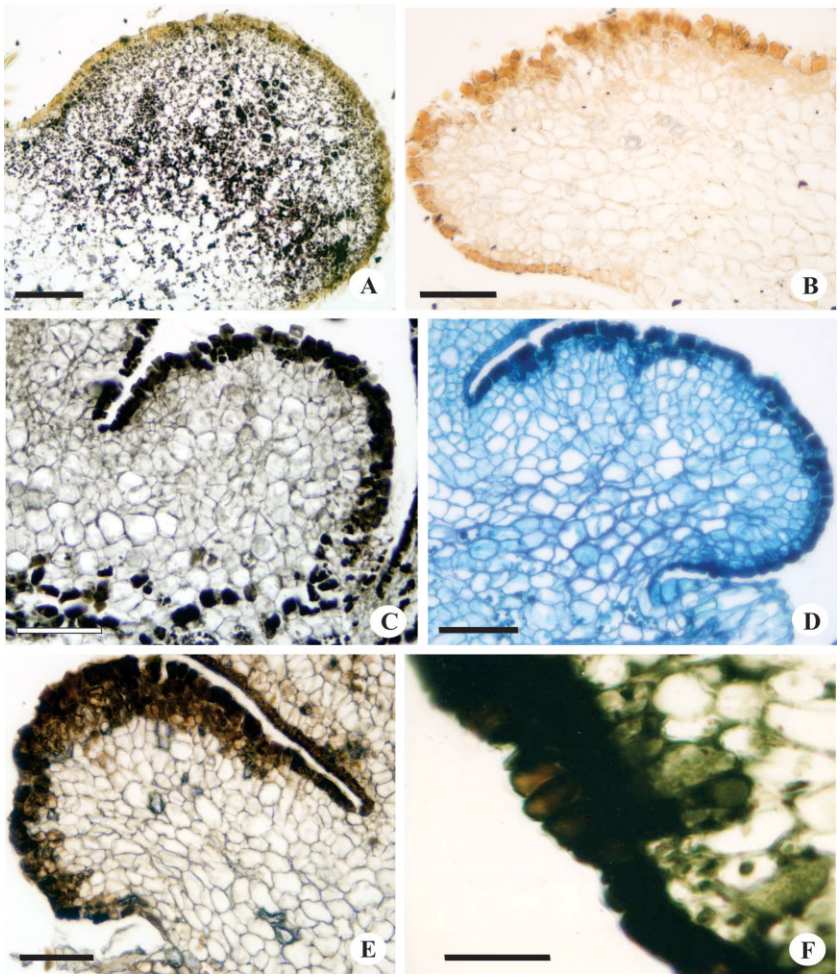

Figure 3. Histochemical tests in longitudinal sections. A, Positive reaction to Lugol's iodine for starch grains in the flower buds. B, Negative reaction to Lugol's iodine for starch grains in the anthesis. C, Positive reaction to Sudan Black B for lipids in flower buds. D, Positive reaction to Nile Blue sulphate for acidic and neutral lipids in fleshy gland of young fruit. E, Positive reaction to ferric chloride for phenolic compounds in the anthesis. F, Positive reaction to copper acetate/rubeanic acid for fatty acids in the flower buds. Scale bars: 40 μm (C, E), 50 μm (A, B, D, F).

Table 3. Histochemical tests on the secretory disk in different phases of development

Histochemical tests	Phase I	Phase II	Phase III
Sudan Black B	ep., np. (+)	ep., np. (+) (Fig. 3C)	ep., sub. (+)
Nile Blue sulphate	ep., np. (+)	ep., np. (+) (Fig. 3D)	ep., sub. (+)
Copper acetate/rubeanic acid	ep., np. (+) (Fig. 3F)	ep., np. (+)	ep., sub. (+)
Iron (III) chloride	ep., np. (+)	ep., np. (+) (Fig. 3E)	ep., sub. (+)
Lugol's iodine	ep., np., sn. (+) (Fig. 3A)	(–) (Fig. 3B)	(–)
Wagner reagent	(–)	(–)	(–)
Schiff reagent	ep., np., sn. (+)	ep., np. (+)	ep., sub. (+)
Ruthenium Red	c.w. (+)	c.w. (+)	c.w. (+)
Tannic acid/iron(III) chloride	(–)	(–)	(–)

(+) positive, (–) negative. (ep.: epidermis; np.: nectary parenchyma; sn: sub-nectary parenchyma; sub.: subepidermal cells; c.w.: cell wall).

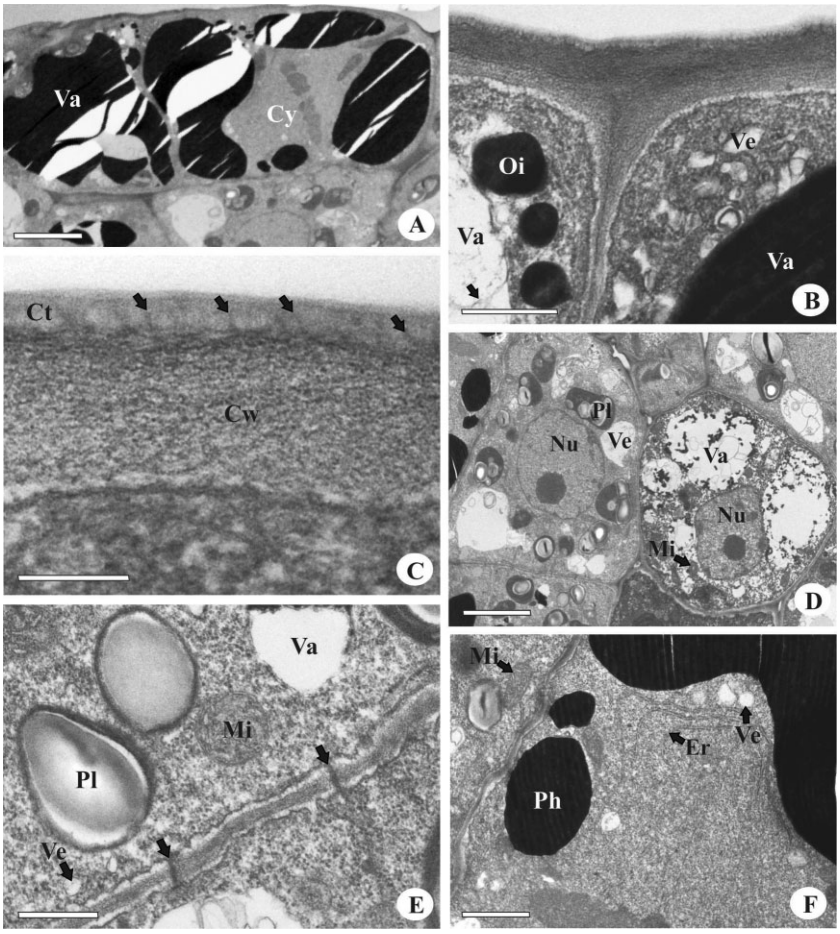

Figure 4. Ultrastructural aspects of the secretory disk of *Tapirira guianensis* Aubl. in phase I. A, Epidermal cells with prominent vacuole with strong electron-dense content and dense cytoplasm. B, Cells of the epidermis with vesicles near the plasma membrane (arrow = fibrillar material). C, Detail of the cuticle highlighting microchannels (arrows = microchannels). D, General aspect of the nectary parenchyma. E, Detail of the nectary parenchyma showing plasmodesmata (arrows), mitochondria, plastids containing starch grains and vacuole. F, Detail of the nectary parenchyma cells showing the rough endoplasmic reticulum associated with small vesicles, mitochondria, vacuole of phenolic content. Va, vacuole; Ve, vesicle; Cy, cytoplasm; Oi, oil droplets; Ct, cuticle; Cw, cell wall; Nu, nucleus; Pl, plastid; Mi, mitochondria; Er, endoplasmic reticulum; Ph, cavuole of phenolic content. Scale bars: 1 μm (A), 2 μm (B–F).

Organelles involved in the different phases of the secretion process and the mechanism involved in secretion release

Since male flowers and non-fertilized female flowers abscise after anthesis, the function of the disk was only observed in functionally female flowers. The results obtained were grouped into three distinct phases: phase I – secretory disk in flower buds; phase II – secretory disk in anthetic flowers; and phase III – persistent secretory disk in young fruits.

Phase I

In this phase the cells of the epidermis show large vacuoles completely filled with phenolic substances; the other organelles are located peripherally (Fig. 4A). Some cells have small vesicles near the membrane, many of them containing fibrillar material (Fig. 4B). The cytoplasm is dense due to the large amount of dispersed ribosomes. Their abundance hinders the visualization of organelles (Fig. 4B). The cuticle presents microchannels at various points (Fig. 4C).

The nectary parenchyma cells have small vacuoles that partially contain electron-dense material, indicating the presence of lipophilic and phenolic substances. In addition, the cells have a large nucleus with evident nucleolus, dense cytoplasm containing ribosomes, mitochondria, under-developed dictyosome and segments of rough endoplasmic reticulum

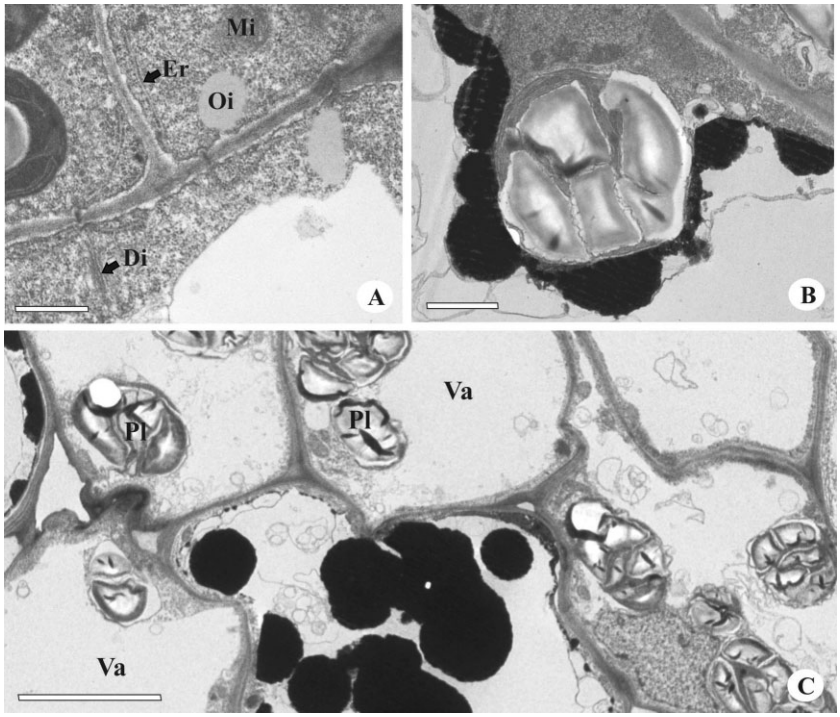

Figure 5. Ultrastructural aspects of the secretory disk of *Tapirira guianensis* Aubl. in phase I. A, Nectary parenchyma highlighting the oil droplets near the cell membrane, dictyosome, endoplasmic reticulum and mitochondria. B, Sub-nectary parenchyma with plastid containing starch grains; osmiophilic inclusions dispersed in the vacuole; organelles in parietal position. C, General aspect of the sub-nectary parenchyma. Oi, oil droplets; Di, dictyosome; Er, endoplasmic reticulum; Mi, mitochondria; Pl, plastid; Va, vacuole. Scale bars: 2 μm (A–C).

together with small vesicles (Fig. 4D, F). The plastids store starch grains (Fig. 4E). Parenchyma cells are connected by plasmodesmata (Fig. 4E). In some cells, lipid droplets near the plasma membrane are present (Fig. 5A).

The sub-nectary parenchyma cells store a large amount of starch in their plastids (Fig. 5B). A large vacuole fills every cell, some with phenolic inclusions, pushing the organelles to a parietal position (Fig. 5C). Fibrillar material is displayed inside the vacuoles (Fig. 5C). Some intercellular spaces are present (Fig. 5C).

Phase II

The epidermis does not show significant changes (Fig. 6A, B), but the endoplasmic reticulum and mitochondria are more easily observed (Fig. 6B). The nectary parenchyma exhibits a pattern similar to the previous phase, but some cells are in intense secretory process (Fig. 6C) and others in the final stage of secretion, with fibrillar material in the vacuoles (Fig. 6D). The plastids do not contain starch as in the previous phase; instead, they are full of lipophilic material (plastoglobules) (Fig. 6E).

In the sub-nectary parenchyma, only a small number of plastids contain starch; they are of smaller size than in previous phase (Fig. 6F). This feature is recognized as the final phase of hydrolysis of the starch grains. The cells in this region have intercellular spaces with organelles located in parietal position and a single vacuole occupying almost the entire cell; abundant electron-dense droplets can be observed mainly in the periphery of the vacuoles; moreover, oil droplets are present (Fig. 6F).

Phase III

In this phase, the vacuoles occupy the entire volume of the epidermal cells and contain electron-dense material (Fig. 7A). The subepidermal cells contain numerous lipid droplets located close to the plasma membrane, as well as a few vesicles (Fig. 7B). Some plastids contain lipid droplets (Fig. 7C).

In the central parenchyma cells, there is scarce osmiophilic material and plastids are completely absent (Fig. 7D). Later stages of fruit development showed similar patterns but are not shown here.

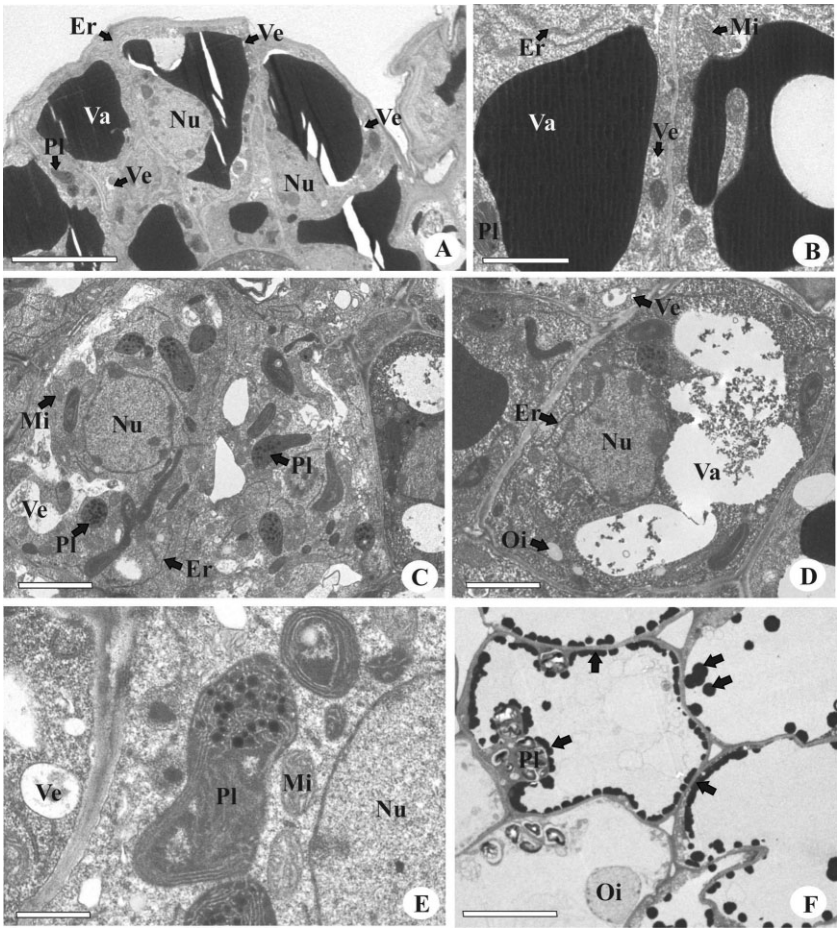

Figure 6. Ultrastructural aspects of the secretory disk of *Tapirira guianensis* Aubl. in phase II. A, Cells of the epidermis with prominent vacuole, large nucleus in peripheral position, plastids containing lipophilic droplets and small vesicles near the plasma membrane. B, Detail of the cells of the epidermis. C, General aspect of the nectary parenchyma. D, Nectary parenchyma cell with vacuole occupying most of the cell. E, Detail of the plastid in the nectary parenchyma containing oil droplets; the cells still display vesicles near the plasma membrane, mitochondria and nucleus. F, General aspect of the sub-nectary tissue with an abundance of electron-dense droplets (arrows) and oil droplets; some plastids can still be observed. Va, vacuole; Nu, nucleus; Pl, plastids; Ve, vesicle; Er, rough endoplasmic reticulum; Mi, mitochondria; Oi, oil droplet. Scale bars: 2 μm (A–F).

DISCUSSION

Our histochemical and ultrastructural tests indicate that the intrastaminal secretory disk of male and functionally female flowers of *T. guianensis* has a dual function: at anthesis it secrets nectar and lipids (see also Durkee *et al*., 1984; Machado *et al*., 2008), and in fruiting stage lipids only. To date, *T. guianensis* is the only known representative of Anacardiaceae to show this phenomenon.

Floral nectaries that persist during fruit formation are sometimes called post-floral nectaries (Schmid, 1988); however, in the case of *T. guianensis*, the secretory disk does not continue to secrete nectar after fertilization but lipid and phenolic substances and would be better named post-floral secretory gland.

DISK STRUCTURE

According to our results, there are no structural differences in the disk between the two floral morphotypes. In other families alterations in form and position of the disk between male and female are common, e.g. in *Cucurbita pepo* L. (Nepi & Pacini, 1993) and *Croton lachnostachyus* (synonym. *C. sarcopetalus* Müll.Arg.; Freitas *et al*., 2001).

The secretory disk of *T. guianensis* shows typical characteristics of nectary tissues, among them small thin-walled cells, relatively large nuclei, small vacuoles and dense cytoplasm (Fahn, 1979; Durkee, 1983; Nepi, Ciampolini & Pacini, 1996; Galetto & Bernardello, 2005; Nepi, 2007). Modified stomata are present across the entire epidermis, which indicates

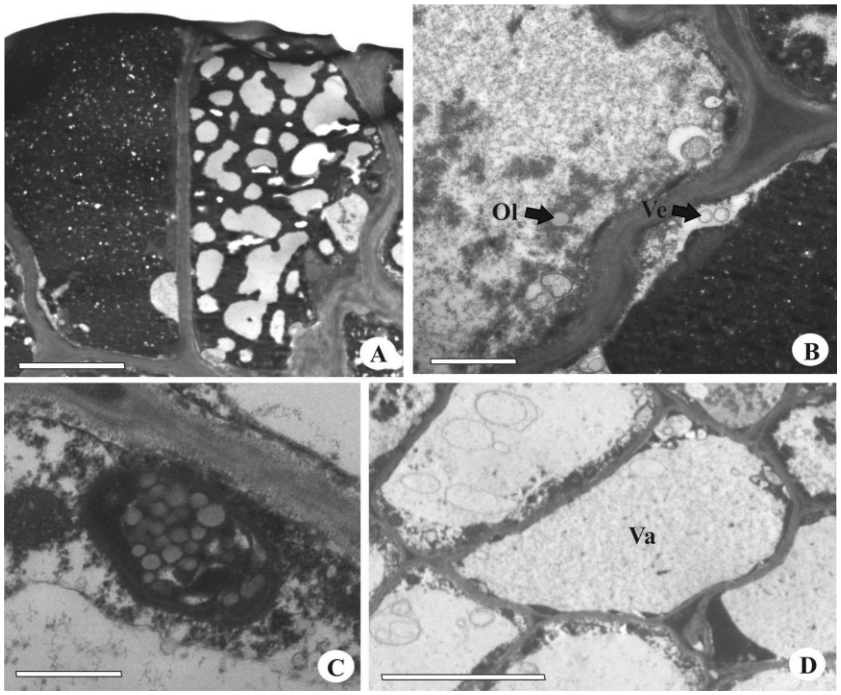

Figure 7. Ultrastructural aspects of the secretory disk of *Tapirira guianensis* Aubl. in young fruit. A, Epidermal cells. B, Detail of a subepidermal cell showing lipid droplets and vesicles. C, Plastid containing oil droplets in subepidermal cell. D, Parenchyma cells showing large vacuole that occupies almost the entire cell volume. Oi, oil droplets; Ve, vesicle; Va, vacuole. Scale bars: 2 μm (A–D).

the site of nectar secretion (Gaffal, Heimler & El-Gammal, 1998; Wist & Davis, 2006).

The presence of druses in the nectariferous tissue is reported for the first time in Anacardiaceae. They may function as a physical and chemical barrier protecting the nectariferous tissue against foraging insects and other organisms (Korth *et al*., 2006; Horner *et al*., 2007). These crystals can also act to eliminate excess cytosolic calcium and assist symplast transport of several substances (Paiva & Machado, 2005).

Sugar composition and additional substances produced

The nectar of *T. guianensis* is sucrose-dominant ($r > 1.0$) in both floral morphotypes and does not vary between the two floral morphotypes. The ratio of sugars present in the nectar in Anacardiaceae has been studied only in two other species. In *Anacardium occidentale* L. the predominance of hexoses (fructose and glucose) was verified with no significant difference between the chemical composition of the nectar among the floral morphotypes (Wunnachit, Jenner & Sedgley, 1992) and is, thus, similar to *T. guianensis*. In *Schinus terebinthifolius* Raddi, however, sucrose was predominant, although there was no indication of the floral morphotype studied (Van Handel, Haeger & Hansen, 1972).

In bud tissue, we observed the abundant presence of starch grains; however, they were not present in later stages. This disappearance most probably indicates that the starch was used as a resource in the formation of the nectar sugars or that it was involved in providing energy to the secretory process (Fahn & Shimony, 2001; Stpiczyńska *et al*., 2005). Fahn & Shimony (2001) stated that the pre-nectar (sucrose) originates in the phloem and is mainly stored in the plastids in the form of starch grains, which are hydrolyzed into fructose and glucose for the secretory phase of the nectar.

The secretory epidermis of the disk stores lipids and phenolic substances. The phenols are also observed with TEM as strong electron-dense inclusions inside vacuoles. The secretion of oils by flowers it is not widespread (Neff & Simpson, 2005) and the occurrence of a disk secreting both nectar and lipids is reported here for the first time for Anacardiaceae. Lipids and nectar often provide a particular flavour and odour that can be essential for the maintenance of certain groups of pollinators (Southwick, 1990; Galetto & Bernardello, 2004); lipids are twice as energy-rich as

the nectar and may also be a component of volatile oils, serving as an odour appeal to attract pollinators (Neff & Simpson, 2005). However, the phenolic compounds present may rather have the function of repellents to herbivores (Galetto & Bernardello, 2005; Nicolson, 2007) as they are stored in vacuoles.

Organelles involved in the different phases of the secretion process and the mechanism involved in secretion release

The ultrastructural features observed here in phases I and II are similar to those reported for nectaries of other angiosperms and are indicative of high metabolic activity (Fahn, 1979; Durkee, 1983; Nepi *et al*., 1996; Stpiczyńska *et al*., 2005; Paiva & Machado, 2006). The ultrastructural changes observed in the flower buds and at anthesis were similar to those in other families (Figueiredo & Pais, 1992; Zer & Fahn, 1992; Fahn & Shimony, 2001; Stpiczyńska *et al*., 2005; Horner *et al*., 2007; Paiva & Machado, 2008), which suggests that these alterations are involved in the conversion of the sugars to nectar.

Cells with predominant occurrence of dictyosomes are related to the production of hydrophilic substances (Durkee, 1983); such cells were observed in floral buds. On the other hand, cells with a predominant occurrence of endoplasmic reticulum are related to the synthesis of lipophilic substances, but they may also be involved in the translocation and/or temporary concentration of sugars (Durkee, 1983; Figueiredo & Pais, 1992; Paiva & Machado, 2008). The rough endoplasmic reticulum is abundant both in phase I and II, this production is more accentuated beginning in phase II.

Oil droplets in the vacuoles and in the plastids or dispersed in the cytoplasm, more evident in phases II and III than in phase I, are ultrastructural evidence for the production of lipids. This process is quite similar to the plastids involved in the synthesis of terpenoids in lipophilic glands (Gleizes *et al*., 1980; Figueiredo & Pais, 1992; Turner *et al*., 1999; Stpiczyńska *et al*., 2005; Machado, Gregório & Guimarães, 2006; Possobom, Guimarães & Machado, 2010), but are unusual in nectary tissues (Rocha & Machado, 2009).

Melo, Borba & Paiva (2010), studying species of Orchidaceae, found that the structural and ultrastructural characteristics of floral nectaries and osmophores are quite similar, except that in the latter there is a predominance of endoplasmic reticulum and a reduced number of dictyosomes. This observation is a feature compatible with phase II of the secretory disk of *T. guianensis*. The ability to secrete both nectar and lipids is also reported for the extrafloral nectaries of *Diplopterys pubipetala* (A.Juss.) W.R.Anderson & C.Davis (Possobom *et al*., 2010) and *Hiptage sericea* Hook.f. (Subramanian, Arumugasamy & Inamdar, 1990), both Malpighiaceae.

Although the secretory disk has stomata, we cannot eliminate the possibility of some nectar being exuded through the cuticle, as channels were present in the cuticle. Such cuticle channels normally allow secretion as they may increase porosity and facilitate the passage of macromolecules (Wist & Davis, 2006; Rocha & Machado, 2009; Melo *et al*., 2010; Stpiczyńska, Davies & Kaminska, 2011).

The secretory vesicles near the plasma membrane, observed mostly in phases I and II, indicate a granulocrine release of nectar (Durkee, 1983; Fahn, 2000). For lipophilic substances, however, there is evidence of eccrine release, as they are commonly present as oil droplets close to the plasma membrane. The oil droplets can fuse with the membrane and thereby cross the membrane. It has been shown that one structure may perform both ways of secretion (Zer & Fahn, 1992; Razem & Davis, 1999).

ACKNOWLEDGMENTS

We thank CNPQ (National Council for Scientific and Technological Development) for the master's scholarship granted during the first few months of the development of this work and FAPESP (São Paulo Research Foundation) for the master's scholarship and technical reserve granted (Process 2011/02293-0) for the Research Aid Proc. 08/55434 and thematic project Proc. 00/12469. This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and SECyT (UNC) which provided grants to L. Galetto and CNPQ grant to S.R. Machado.

REFERENCES

Ayres M, Ayres-Júnior M, Ayres DL, Santos AA. 2007. *BIOESTAT – aplicações estatísticas nas áreas das Ciências Bio-Médicas*. Belém: Mamirauá.

Bachelier JB, Endress PK. 2009. Comparative floral morphology and anatomy of Anacardiaceae and Burseraceae (Sapindales), with a special focus on gynoecium structure and evolution. *Botanical Journal of the Linnean Society* **159:** 499–571.

Baker HG, Baker I. 1973. Studies of nectar-constitution and pollinator-plant coevolution. In: Gilbert LE, Raven PH, eds. *Coevolution of animals and plants*. Austin: University of Texas Press, 100–140.

Baker HG, Baker I. 1982. Some chemical constituents of floral nectars of *Erythrina* in relation to pollinators and systematic. *Allertonia* **3:** 25–37.

Bergmeyer HU, Bernt E. 1974. *Methods of enzymatic analysis*, 2nd edn. New York: Academic Press, 1177–1179.

Berlyn GP, Miksche JP. 1976. *Botanical microtechnique and cytochemistry*. Ames: Iowa State University Press.

Cain AJ. 1947. The use of Nile Blue in the examination of lipids. *Quarterly Journal of Microscopical Science* **88:** 383–392.

Cronquist A. 1981. *An integrated system of classification of flowering plants*. New York: Columbia University Press.

Durkee LT. 1983. The ultrastructure of floral and extrafloral nectaries. In: Bentley B, Elias T, eds. *The biology of nectaries*. New York: Columbia University Press, 1–29.

Durkee LT, Baird CW, Cohen PF. 1984. Light and electron microscopy of the resin glands of *Passiflora foetida* (Passifloraceae). *American Journal of Botany* **71:** 596–602.

Fahn A. 1979. *Secretory tissue in plants*. London: Academic Press.

Fahn A. 2000. Structure and function of secretory cells. *Advances in Botanical Research* **31:** 37–75.

Fahn A, Shimony C. 2001. Nectary structure and ultrastructure of unisexual flowers of *Ecballium elaterium* (L.) A. Rich. (Cucurbitaceae) and their presumptive pollinators. *Annals of Botany* **87:** 27–33.

Figueiredo ACS, Pais MS. 1992. Ultrastructural aspects of the nectary spur of *Limodorum abortivum* (L) Sw. (Orchidaceae). *Annals of Botany* **70:** 325–331.

Freitas L, Bernardello G, Galetto L, Paoli AAS. 2001. Nectaries and reproductive biology of *Croton sarcopetalus* (Euphorbiaceae). *Botanical Journal of the Linnean Society* **136:** 267–277.

Furr M, Mahlberg PG. 1981. Histochemical analyses of laticifers and glandular trichomes in *Cannabis sativa*. *Journal of Natural Products* **44:** 153–159.

Gaffal KP, Heimler W, El-Gammal S. 1998. The floral nectary of *Digitalis purpurea* L. Structure and nectar secretion. *Annals of Botany* **81:** 251–262.

Galetto L, Bernardello G. 2004. Floral nectaries, nectar production dynamics and chemical composition in six *Ipomoea* species (Convolvulaceae) in relation to pollinators. *Annals of Botany* **94:** 269–280.

Galetto L, Bernardello G. 2005. Nectar. In: Dafni A, Kevan PG, Husbana BC, eds. *Practical pollination biology*. Cambridge: Enviroquest, 261–313.

Gallant JB, Kemp JR, Lacroix CR. 1998. Floral development of dioecious staghorn sumac, *Rhus hirta* (Anacardiaceae). *International Journal of Plant Sciences* **159:** 539–549.

Ganter P, Jollés G. 1969. *Histologie normale et pathologique*, Vol. *1*. Paris: Gautier, Villars.

Ganter P, Jollés G. 1970. *Histologie normale et pathologique*, Vol. *2*. Paris: Gautier, Villars.

Gerlach D. 1984. *Botanische Mikrotechnik: eine einführung*, 3rd edn. Stuttgart: Georg Thieme.

Gleizes M, Carde JP, Pauly G, Bernard-Dagan C. 1980. *In vivo* formation of sesquiterpene hydrocarbons in the endoplasmic reticulum of pine. *Plant Science Letters* **20:** 79–90.

Horner HT, Healy LA, Ren G, Fritz D, Klyne A, Seames C, Thornburg RW. 2007. Amyloplast to chromoplast conversion in developing ornamental tobacco floral nectaries provides sugar for nectar and antioxidants for protection. *American Journal of Botany* **94:** 12–24.

Johansen DA. 1940. *Plant microtechnique*. New York: McGraw-Hill.

Korth KL, Doege SJ, Park S, Goggin FL, Wang Q, Gomez SK, Liu G, Jia L, Nakata PA. 2006. *Medicago truncatula* mutants demonstrate the role of plant calcium oxalate crystals as an effective defense against chewing insects. *Plant Physiology* **141:** 188–195.

Kunsst A, Draeger B, Ziegenhorn J. 1984. *Methods of enzymatic analyses*, Vol. *2*, 3rd edn. New York: Academic Press, 163–172.

Lacchia APS. 2006. Estruturas secretoras em órgãos vegetativos e reprodutivos de espécies de Anacardiaceae: anatomia, histoquímica e ultra-estrutura. PhD Thesis, Campinas: Universidade Estadual de Campinas.

Lenza E, Oliveira PE. 2005. Biologia reprodutiva de *Tapirira guianensis* Aubl. (Anacardiaceae), uma espécie dioica em mata de galeria do Triângulo Mineiro, Brasil. *Revista Brasileira de Botânica* **28:** 179–190.

Machado SR, Gregório EA, Guimarães E. 2006. Ovary peltate trichomes of *Zeyheria montana* (Bignoniaceae): developmental, ultrastructure and secretion in relation to function. *Annals of Botany* **97:** 357–369.

Machado SR, Morellato LPC, Sajo MG, Oliveira PS. 2008. Morphological patterns of extrafloral nectaries in woody plant of the Brazilian cerrado. *Plant Biology* **10:** 660–673.

Machado SR, Rodrigues TM. 2004. Anatomia e ultraestrutura do pulvino primário de *Pterodon pubescens* Benth. (Fabaceae – Faboideae). *Revista Brasileira de Botânica* **27:** 135–147.

McManus JFA. 1948. Histological and histochemical uses of periodic acid. *Stain Technology* **23:** 99–108.

Melo MC, Borba EL, Paiva EAS. 2010. Morphological and histological characterization of the osmophores and nectarines of four species of *Acianthera* (Orchidaceae: Pleurothallidinae). *Plant Systematics and Evolution* **286:** 141–151.

Neff JL, Simpson BB. 2005. Other rewards: oils, resins and gums. In: Dafni A, Kevan PG, Husbana BC, eds. *Practical pollination biology*. Cambridge: Enviroquest, 314–328.

Nepi M. 2007. Nectary structure and ultrastructure. In: Nicolson SW, Nepi M, Pacini E, eds. *Nectaries and nectar*. Dordrecht: Springer, 129–166.

Nepi M, Ciampolini F, Pacini E. 1996. Development and ultrastructure of *Cucurbita pepo* nectaries of male flowers. *Annals of Botany* **78:** 95–104.

Nepi M, Pacini E. 1993. Pollination, pollen viability and pistil receptivity in *Cucurbita pepo*. *Annals of Botany* **72:** 526–536.

Nicolson SW. 2007. Nectar consumers. In: Nicolson SW, Nepi M, Pacini E, eds. *Nectaries and nectar*. Dordrecht: Springer, 289–342.

Paiva EAS, Machado SR. 2005. Role of intermediary cells in *Peltodon radicans* (Lamiaceae) in the transfer of calcium and formation of calcium oxalate crystals. *Brazilian Archives of Biology and Technology* **48:** 147–153.

Paiva EAS, Machado SR. 2006. Ontogênese, anatomia e ultra-estrutura dos nectários extraflorais de *Hymenaea stigonocarpa* Mart. ex Hayne (Fabaceae – Caesalpinioideae). *Acta Botanica Brasilica* **20:** 471–482.

Paiva EAS, Machado SR. 2008. The floral nectary of *Hymenaea stigonocarpa* (Fabaceae, Caesalpinoideae): structural aspects during floral development. *Annals of Botany* **101:** 125–133.

Pearse AGE. 1980. *Histochemistry theoretical and applied preparative and optical technology*, 4th edn. Edinburgh: Churchill Livingston.

Pizzolato TD, Lillie RD. 1973. Mayer's tannic acid-ferric chloride stain for mucins. *The Journal of Histochemistry and Cytochemistry* **21:** 56–64.

Possobom CCF, Guimarães E, Machado SR. 2010. Leaf gland act as nectaries in *Diplopteris pubipetala* (Malpighiaceae). *Plant Biology* **12:** 863–870.

Razem FA, Davis AR. 1999. Anatomical and ultrastructural changes of the floral nectar of *Pisum sativum* L. during flower development. *Protoplasma* **206:** 57–72.

Reynolds ES. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *The Journal of Cell Biology* **17:** 208–212.

Rocha JF, Machado SR. 2009. Anatomy, ultrastructure and secretion of *Hibiscus pernambucensis* Arruda (Malvaceae) extrafloral nectar. *Revista Brasileira de Botânica* **32:** 489–498.

Schmid R. 1988. Reproductive versus extra reproductive nectaries – historical perspective and terminological recommendations. *The Botanical Review* **54:** 179–232.

Silva-Luz CL, Pirani JR. 2011. *Anacardiaceae*. In: Lista de espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Available at: http://floradobrasil.jbrj.gov.br/2011/FB000044 Access in: 29/04/2012.

Southgate DAT. 1976. *Determination of food carbohydrates*. London: Applied Science Publishers.

Southwick EE. 1990. Floral nectar. *American Bee Journal* **130:** 517–519.

Stpiczyńska M, Davies KL, Kaminska M. 2011. Comparative anatomy of the nectar spur in selected species of Aeridinae (Orchidaceae). *Annals of Botany* **107:** 327–345.

Stpiczyńska M, Milanesi C, Faleri C, Cresti M. 2005. Ultrastructure of the nectary spur of *Platanthera chlorantha* (Custer) Rchb. (Orchidaceae) during successive stages of nectar secretion. *Acta Biologica Cracoviensia Series Botanica* **47:** 111–119.

Subramanian RB, Arumugasamy K, Inamdar JA. 1990. Studies in the secretory glands of *Hiptage sericea* (Malpighiaceae). *Nordic Journal of Botany* **10:** 57–62.

Turner G, Gershenzon J, Nielson EE, Froehlich JE, Croteau R. 1999. Limonene synthase, the enzyme responsible for monoterpene biosynthesis in peppermint, is localized to leucoplasts of oil gland secretory cells. *Plant Physiology* **120:** 879–886.

Van Handel E, Haeger JS, Hansen CW. 1972. The sugars of some Florida nectars. *American Journal of Botany* **59:** 1030–1032.

Vesprini JL, Nepi M, Pacini E. 1999. Nectary structure, nectar secretion patterns and nectar composition in two *Helleborus* species. *Plant Biology* **1:** 560–568.

Wannan BS, Quinn CJ. 1991. Floral structure and evolution in the Anacardiaceae. *Botanical Journal of the Linnean Society* **107:** 349–385.

Watson ML. 1958. Staining of tissue sections for electron microscopy with heavy metals. *Journal of Biophysical and Biochemical Cytology* **4:** 475–478.

Wist TJ, Davis AR. 2006. Floral nectar production and nectar anatomy and ultrastructure of *Echinacea purpurea* (Asteraceae). *Annals of Botany* **97:** 177–193.

Wunnachit W, Jenner CF, Sedgley M. 1992. Floral and extrafloral nectar production in *Anacardium occidentale* L. (Anacardiaceae): an andromonoecious species. *International Journal of Plant Sciences* **153:** 413–420.

Zer H, Fahn A. 1992. Floral nectaries of *Rosmarinus officinalis* L. Structure, ultrastructure and nectar secretion. *Annals of Botany* **70:** 391–397.