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Ontogenetic, anatomical and histochemical study of the extrafloral nectaries of *Sapium biglandulosum* (Euphorbiaceae)

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Abstract. The present paper aims to confirm the nature of secretory structures found on the petiole and leaf margins of *Sapium biglandulosum* Müell. Arg. The anatomy, ontogenesis and histochemistry were studied by light microscopy, whereas mono- and disaccharides in the exudates were detected by high performance liquid chromatography. The exudate from the petiole had a total sugar concentration of 32.5% (w/v), of which 38.1% was fructose, 43.7% glucose and 18.2% sucrose. The petiolar gland started its development from a group of meristematic cells that underwent asynchronous divisions. At the end of the ontogenesis, a well structured vascularised gland made up of a palisade secretory epidermis, secretory parenchyma and a secretory pore was observed. Leaf-margin glands showed a similar anatomy. Histochemical tests revealed the presence of proteins, pectins, carbohydrates, tannins and anthocyanins. On the basis of our results, there is compelling evidence that the studied glands are in fact extrafloral nectaries.

Introduction

The genus *Sapium* (Euphorbiaceae) is characterised by its arboreal size, latescent branch and fruit, and leaves that are generally glandulous (Müller 1895), that is, a pair of bulbous glands at the blade–petiole junction (Correll and Johnston 1979). Glands are distinct groups of highly specialised cells that work together to discharge substances to the exterior or into special intercellular cavities. They are composed of secretory cells and multiple types of auxiliary cells (Schnepf 1974; Fahn 1979). *S. biglandulosum* is a latescent species, with secretory glands on both the petiole and the leaf margins (Müller 1895).

These types of glands in Euphorbiaceae are frequently recognised as extrafloral nectaries (Metcalfe and Chalk 1950) that have also been reported for other species of the genus *Sapium* (Rogers *et al*. 2003; So 2004). However, these reports were based only on the topography. These glands are well developed structures and have different shapes. They are found on the petiole, either associated to stipules or substituting them, on the leaf blade between the teeth, and on floral parts (Metcalfe and Chalk 1950; Schnepf 1974; Dave and Patel 1975; Fahn 1979; Fiala and Maschwitz 1991; Freitas *et al*. 2001; Linsenmair *et al*. 2001).

Glands morphologically similar to extrafloral nectaries have been reported as extrafloral nectaries when they were actually resin glands (Curtis and Lersten 1978; Durkee *et al*. 1984). To avoid such a mistake, an anatomical description and an analysis of the contents present in the secretion of secretory glands are necessary to confirm the kind of glands present on a particular plant.

Nectaries are specialised glands that exude nectar, a sweet solution composed primarily of glucose, fructose and sucrose. They may be found on both vegetative and reproductive organs (Fahn 1979; Bentley and Elias 1983; Roshchina and Roshchina 1993; Nicolson and Thornburg 2007). Extrafloral nectaries usually function as a defensive strategy against herbivores (Elias 1972, 1980; Curtis and Lersten 1978; Freitas and Oliveira 1996; McDade and Turner 1997; Oliveira 1997; Madureira and Sobrinho 2002; Almeida and Figueiredo 2003; Paiva and Machado 2006).

Therefore, the present paper will study the ontogenesis and structure of the secreting tissues and the chemical nature of the exudates to clarify the nature of the glands present on the petiole and leaf margins of *S. biglandulosum*, and to contribute to the understanding of the defensive strategies of this species.

Materials and methods

Twigs of *S. biglandulosum* were collected at Sítio Bom Sucesso, Viçosa Municipality, and Parque Estadual Serra do Brigadeiro, Araponga Municipality, in the state of Minas Gerais, Brazil. Eight twigs were collected at Sítio Bom Sucesso in May 2004 and September 2008. In November 2005 and January 2007, seven twigs were collected at Parque Estadual Serra do Brigadeiro.

The first node considered was the first leaf primordium, which comes right after the shoot apex. The shoot apex usually contained 8–10 leaf primordia (Fig. 1*A*).

The samples were fixed in formalin–acetic acid–alcohol (FAA_{50}) for 24 h, then stored in 70% ethanol (Johansen 1940).

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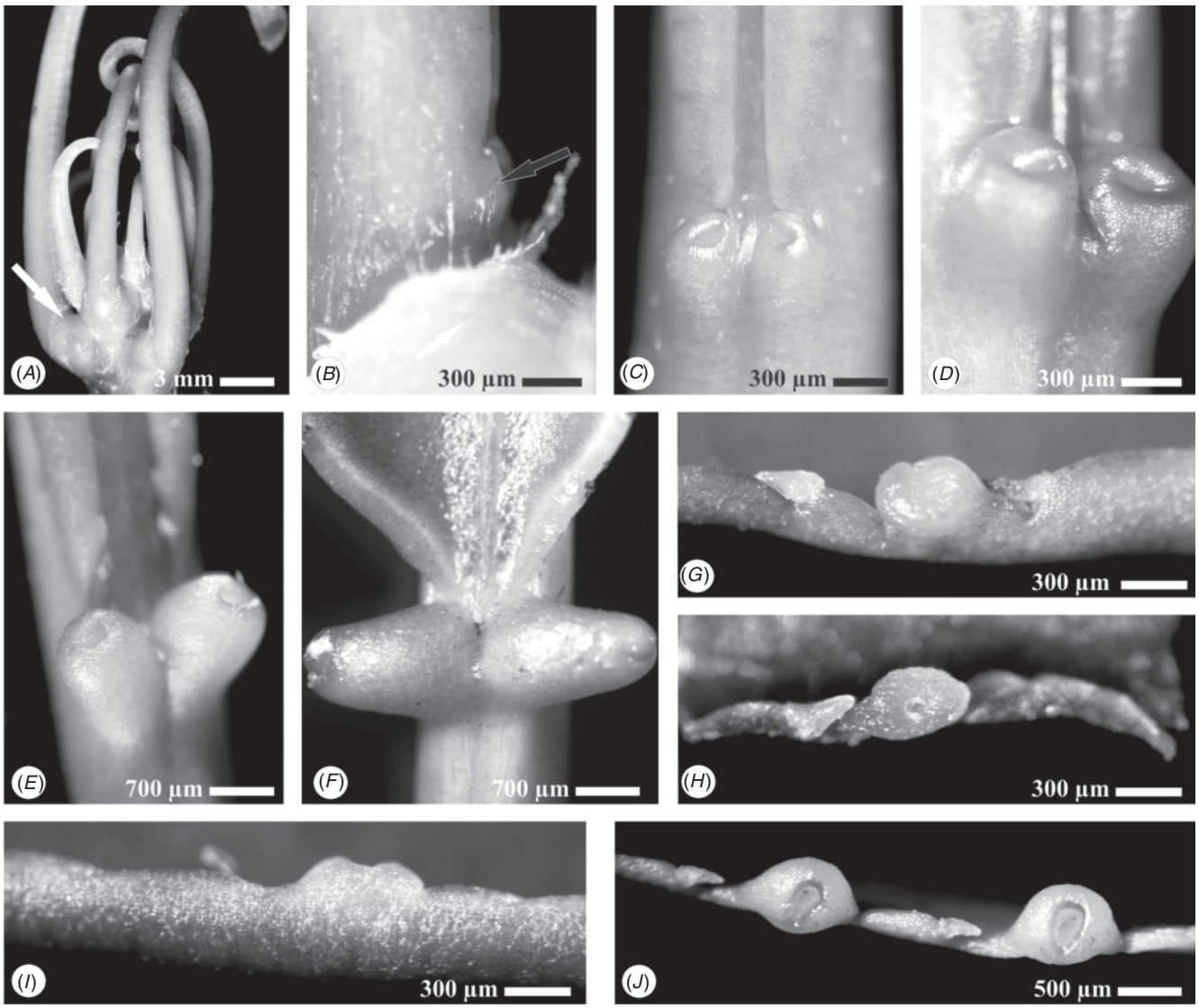

Fig. 1. Developmental stages of the glands of *Sapium biglandulosum* seen under the stereo microscope. (*A*) Shoot apex formed by nine leaf primordia. Note that one of the two stipules (white arrow) found on the leaf primordia covers the meristematic area where the gland originates. (*B–F*) Pair of petiolar glands during the development. (*B*) The first node. Note the gland primordium (black arrow). (*C*) The second node. (*D*) The third node. (*E*) The sixth node. (*F*) The ninth node. (*G–J*) Development of the leaf-margin glands. (*G*) The eleventh node. (*H*) The twelfth node. (*I*, *J*) The thirteenth node: (*I*) side view and (*J*) frontal view.

These samples were dehydrated through an ethyl alcohol–xylene series and then embedded in paraffin plus 8% wax (Roeser 1972). The blocks were sectioned with a rotary microtome (Spencer 820, American Optical Corporation, Buffalo, NY, US), producing cross- and longitudinal serial sections 7–9 µm thick. For the ontogenetic characterisation, sections were stained with safranin and astra blue (Roeser 1972) and mounted in resin (Permount, Fisher Scientific, Bridgewater, NJ, US). For the structural characterisation of mature glands, fixed samples were embedded in methacrylate (Historesin, Leica Instruments, Heidelberg, Germany) and sectioned as described above. Sections were stained with toluidine blue (O'Brien and McCully 1981) and mounted in resin.

The samples used in the histochemical tests for detection of phenolic compounds and fats were fixed in a solution of ferrous sulfate in formalin (Johansen 1940) and in buffered neutral formalin (Clark 1981), respectively. Control sections were performed simultaneously. For detection of carbohydrates, proteins and pectins, some of the fixed samples were embedded in Histosec® (Merck). Sections varying from 7 to 9 µm were cut with a rotary microtome (Leica 2155, Leica, Deerfield, IL, US). For carbohydrate detection, slides were stained with periodic acid Schiff (PAS) (Maia 1979); for total proteins, with xylidine pounceau (O'Brien and McCully 1981); and for pectins, ruthenium red (Johansen 1940). All slides were mounted in resin (Permount, Fisher).

Freehand sections of fresh samples made on an LPC table microtome (Rolemberg and Bhering Comércio e Importação LTDA, Belo Horizonte, Brazil) were used in the following histochemical tests: sudan IV (70% (v/v) ethanol) for detection

of lipid compounds (Pearse 1980); Nadi reagent for essential oils and oleoresins (David and Carde 1964); autofluorescence under UV (O'Brien and McCully 1981) and potassium dichromate (Gabe 1968) for phenolic compounds; vanillin–hydrochloric acid for tannins (Mace and Howell 1974); phloroglucinol for lignins (Johansen 1940); Dittmar and Wagner reagents for alkaloids (Furr and Mahlberg 1981); and lugol for starch (Johansen 1940).

Observations and photographic documentation were performed with a light microscope (Model AX70TRF, Olympus Optical, Tokyo, Japan) equipped with a U-Photo system and an epifluorescence HBO 50W mercury vapour lamp and a filter block A (exciter filter BP 340–380, dichroic mirror 450, barrier filter LP-430).

Images of the leaves showing gland development were taken with a SZX7 Olympus stereo microscope equipped with an EVOLT E-300 Olympus digital camera (Olympus Optical).

Branches kept in a bucket with tap water were kept in the laboratory for 2 days. Samples of exudates from 15 randomly chosen secreting petiolar glands were collected directly with capillary tubes on both days. Because of the small amount of secretion, it was not possible to collect exudates from leaf-margin glands. The secretion was then transferred to an Eppendorf tube and stored in a freezer. The analysis was carried out in the Laboratório de Análises e Síntese de Agroquímicos at the Universidade Federal de Viçosa, Minas Gerais State, Brazil. The exudate (45.4 mg) was diluted with 5 mL of ultra-pure water and analysed by high performance liquid chromatography (HPLC). It was used in a chromatographer (Type LC-6AD, Shimadzu, Osaka, Japan) equipped with a refractive index detector (RID), a data collecting system (Type Class 5000, Shimadzu, Osaka, Japan), a Techspherene column NH2 (250 mm × 4.6 mm internal diameter (id); 5 µm) at 40°C, and a Techspherene pre-column NH_2 (20 mm × 4.6 mm id; 5 µm). Deionised water–acetonitrile (80 : 20, v/v) at a column flow of 0.5 mL min^{-1} was used as the mobile phase. Aliquots of 20 µL of fructose, glucose and sucrose solutions at 0.0, 0.1, 0.6, 1.0 and 2.0 mg mL^{-1} concentrations were used to achieve a calibration curve. Three 20-µL samples of the diluted exudate were used.

Results

Gland ontogenesis

In the very beginning of gland development, it was observed that the area that would later form the glands was protected by one of the two stipules found on the leaf primordium (Fig. 1*A*, *B*). The stipules dropped off and were not found on leaf primordia placed after the first node. Each gland of the petiole originated from a group of meristematic cells. As the cells started to divide, the gland primordia appeared as two cushion-like structures (Fig. 1*B*) on the apex of the petiole. These structures increased in size, and a deepening at the central area was observed (Fig. 1*C*). As gland development went on, the structures continued to emerge (Fig. 1*D*, *E*). Apart from moving to the opposite sides of the petiole (Fig. 1*F*), the gland primordia from the fourth node up to their mature stage – the ninth node – did not show any drastic changes in their morphology. The final result is a pair of opposite glands (Fig. 1*F*), each one bearing a slit-like secretory opening.

For the glands on the leaf margin, we observed that a round protuberance would form a gland, whereas a pointy protuberance would form a tooth (Fig. 1*G*). Similarly to the petiolar glands, a deepening at the centre of the round protuberance appeared. As glandular development went on, this deepening transformed into a concavity (Fig. 1*G*, *H*). By the end of the gland ontogenesis, round glands bearing a red ring at the concavity opening (Fig. 1*J*) had formed. The concavity openings of these glands were aligned with the leaf margin (Fig. 1*I*).

During the first stage of development of petiolar glands, it was observed that the cells from the ground meristem slightly increased their volume, which precipitated the formation of a convex protrusion (Fig. 2*A*). Protodermal cells began dividing by means of anticlinal divisions, greatly increasing the superficial area. At that stage, the protodermal cells were cubical, with dense staining cytoplasm and large nuclei with thin walls, but were not larger than the cells from the ground meristem. (Fig. 2*A*).

The divisions occurred at a lower rate at the centre of the gland primordium than at its margin. This peculiar way of cellular proliferation resulted in a deepening of the central area of the gland primordium (Fig. 2*B*). The cellular proliferation in the ground meristem started later than in the protoderm. These meristematic cells were small, polyhedral and contained conspicuous nuclei. Anticlinal, periclinal and oblique (Fig. 2*C*, *D*) divisions occurred asynchronously.

The protodermal cells of the sunken area slowly took the typical columnar shape of secretory cells. Thus, they could be clearly distinguished from the cells of the ground meristem. Although the differentiation began in the protoderm, the proliferation process continued (Fig. 2*E*).

During this stage, the ground-meristem cells became a little bigger and continued to divide (Fig. 2*F*). Procambial cells were observed among the ground-meristem cells near the protoderm (Fig. 2*G*).

In the stages that followed, cellular expansion and differentiation were intensified while cell divisions continued. (Fig. 2*H*, *I*). This process culminated in the formation of an enlarged structure with a deep central cavity (Fig. 2*J*). Protodermal cells became higher and more columnar-shaped (Fig. 3*A*), characterising a secretory epidermis; however, the gland was not functional by that time. The cells at the cavity aperture were similar to the non-secretory epidermal cells (Fig. 3*B*). The ground meristem was differentiated into a secretory parenchyma (Fig. 3*A*).

A single procambial vascular bundle from the vascular system of the petiole branched towards the gland (Figs 2*J*, 3*A*). Such venation was later observed surrounding and entering the secretory parenchyma.

Cell divisions were not observed in leaves measuring more than 50 mm in length. A quick cellular expansion was observed as the leaves expanded.

Finally, a fully developed vascularised secretory gland made up of a secretory epidermis and secretory parenchyma was observed.

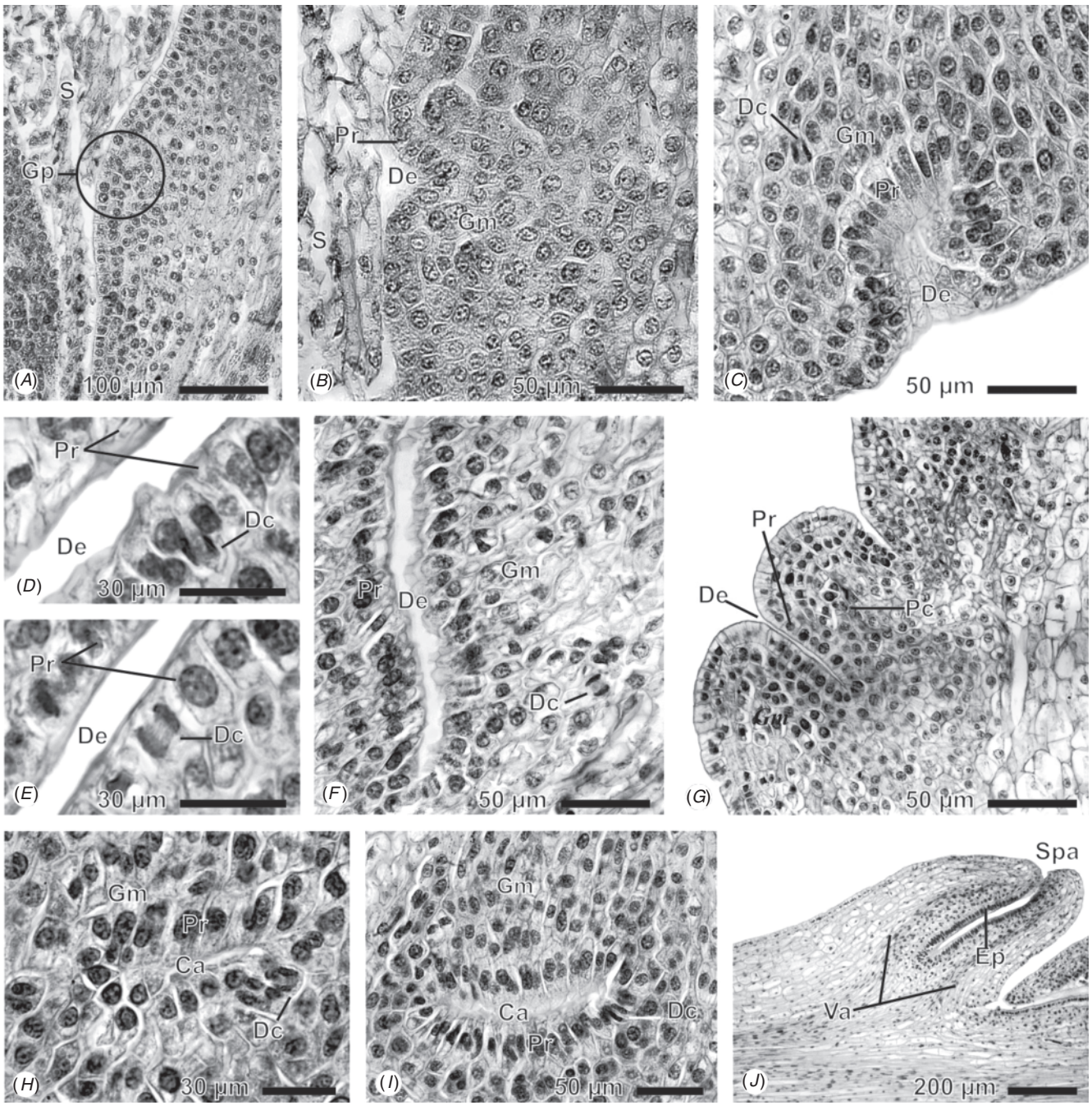

Fig. 2. Developmental stages of the petiolar glands of *Sapium biglandulosum* seen under light microscopy. (*A–C*, *G*, *J*) Longitudinal sections. (*D–F*, *H*, *I*) Cross-sections. (*A*) Gland primordium of a shoot protected by the stipule. (*B*) Gland primordium with the deepened central area. (*C*) The second node leaf primordium. (*D*, *E*) The third node. (*F*, *G*) The fourth node showing the very beginning of the gland differentiation process. (*H*, *I*) The fourth node. Note the cavity formed in the central area. (*J*) The fifth node. Note the ramification of the petiolar vascular bundle. Ca, cavity; Dc, dividing cell; De, depression; Ep, epidermis; Gm, ground meristem; Gp, gland primordium; Pc, procambium; Pr, protoderm; S, stipule; Spa, secretory pore aperture; and Va, vascularisation.

Gland characterisation

The glands of the petiole contained a single-layered secretory epidermis lacking stomata, with adjoining columnar-shape cells making up a palisade and a very thin cuticle (Fig. 3*C*). The nuclei of these cells were large and located at the bottom of the cells (Fig. 3*C*). The cytoplasm was more densely stained than the secretory parenchyma cells (Fig. 3*A*).

The closer the epidermal cells were to the cavity aperture the less columnar-shape they had, until the epidermal cells abruptly became cubical, similar to the other non-secretory epidermal cells (Fig. 3*B*).

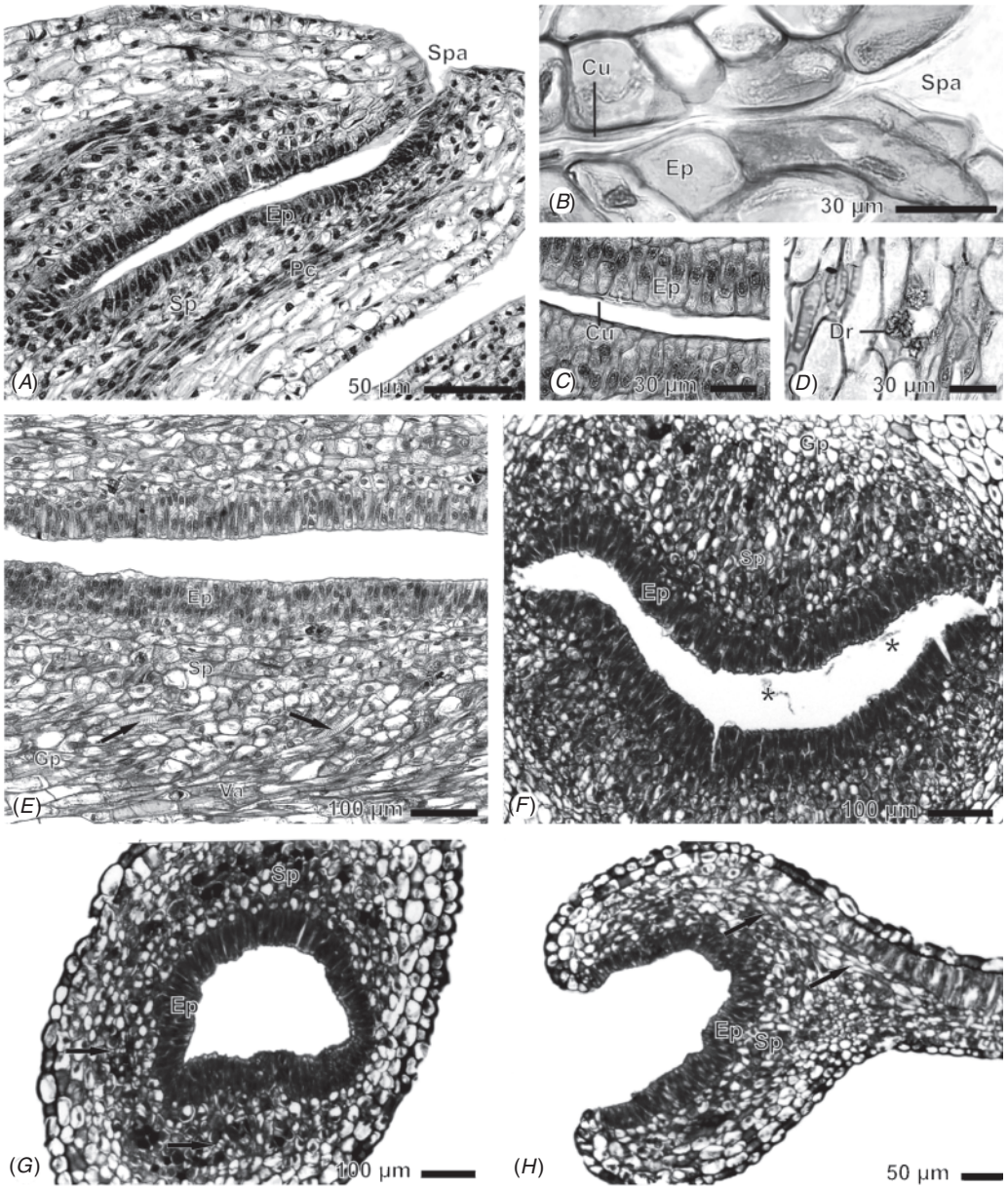

Fig. 3. Mature glands of *Sapium biglandulosum* as seen under light microscopy. (*A–F*) Petiolar glands in (*A–E*) longitudinal sections and (*F*) cross-section. (*G*, *H*) Mature secretory leaf-margin glands in (*G*) cross-section and (*H*) longitudinal sections. (*A*) Maximisation of the Fig. 2*J*. (*B–D*) The ninth node. (*E*, *F*) Mature secretory petiolar glands. Note the vascularisation surrounding and entering the gland. Xylem (black arrows). Secretion (asterisk). Ep, epidermal cells; Gp, ground parenchyma; Sp, secretory parenchyma; Pc, procambium; Cu, cuticle; Dr, druses; Spa, secretory pore aperture (Spa); and Va, vascularisation.

The secretory parenchyma was made up of three to nine layers of cells (Fig. 3*E*, *F*). These cells were smaller than those belonging to the ground parenchyma. Scattered druses were found in the ground parenchyma (Fig. 3*D*). The walls of the secretory parenchyma cells were thin. These cells contained large nuclei and dark-staining cytoplasm (Fig. 3*E*, *F*).

A vascular bundle branched from the petiolar vascular system into the gland. The mature extrafloral glands were vascularised by xylem and phloem, with phloem more abundant than xylem. The vascular bundle surrounded the secretory parenchyma and entered among its cells (Fig. 3*E*).

The glands on the leaf margins were in structure similar to those on the petiole (Fig. 3*G*, *H*). The major difference between the two types of glands was that the petiolar glands had a deep cavity with an aperture (Fig. 3*A*), whereas the leaf-margin glands had a concavity with a round aperture (Fig. 3*H*). Such a difference influenced the manner of the exudate-elimination pattern. In the petiolar glands, exudate accumulated for a short period and then dripped off, whereas in the leaf-margin glands exudate was kept within the concavity.

The histochemical tests detected carbohydrates (Fig. 4*A*, *B*), pectins (Fig. 4*C*, *D*) and proteins (Fig. 4*E*, *F*) at the secretory

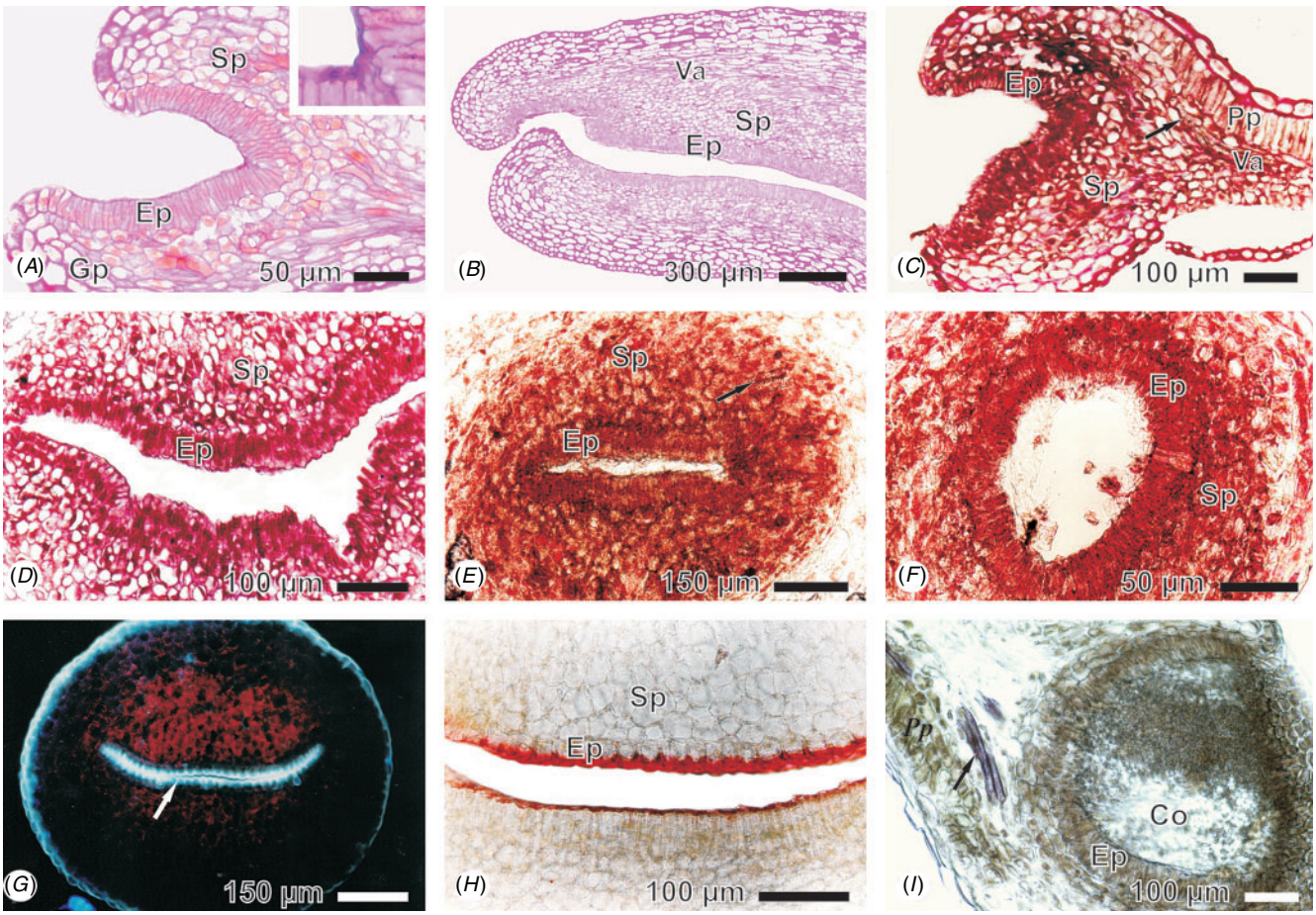

Fig. 4. (*A*–*F*, *H*, *I*) Histochemical tests and (*G*) UV-light autofluorescence of the (*A*, *C*, *F*, *I*) leaf margin and (*B*, *D*, *E*, *G*, *H*) petiolar glands. (*A*, *B*) PAS reaction. The maximisation on the upper right side in *B* shows the sugar grains formed outside the secretory epidermis. (*C*, *D*) Ruthenium red. (*E*, *F*) Xylidine pounceau. (*G*–*I*) Phenolic compounds. (*H*) Vanillin–hydrochloric acid. (*I*) Phloroglucinol. Ep, epidermal cells; Co, concavity; Gp, ground parenchyma; Sp, secretory parenchyma; pp, palisade parenchyma; and Va, vascularisation bundle. Black arrow indicates xylem. White arrow indicates epidermal cells with phenolic compounds.

epidermis and secretory parenchyma of both types of glands. The UV light detected phenolic compounds only at the outer cell walls of the secretory epidermal cells for both the petiolar (Fig. 4*G*) and the leaf-margin glands. However, the phenolic compounds in the petiolar glands were identified as tannins as they reacted positively with vanillin–hydrochloric acid (Fig. 4*H*), whereas the phenolic compounds in the leaf-margin glands were identified as anthocyanin. Anthocyanin formed a round ring at the borders of the leaf-margin glands (Fig. 1*J*). UV light also showed the xylem that reacted positively to the test for lignins (Fig. 4*I*). The other histochemical tests returned negative results.

Exudate and exudate secretion

The total concentration of sugars in the secretion was 32.5% (mg mL^{-1}). Of this, 38.1% was fructose, 43.7% glucose and 18.2% sucrose. The disaccharide–monosaccharide ratio was 0.22 (sucrose/(fructose + glucose)).

Exudate secretion in *S. biglandulosum* occurred before the complete leaf expansion, starting while the leaf margins were still coiled. The exudate from the petiolar glands was secreted and flowed to the outer surface from where it later dropped off, whereas the exudate produced by the leaf-margin glands was accumulated into the concavity.

During the collections of *S. biglandulosum*, insects such as ants, flies and bugs were observed collecting exudate in the early morning and late afternoon. Glands were observed secreting even when they had been partially wounded.

Discussion

The structural features of the secreting tissues, the position and the detection of glucose, fructose and sucrose in the exudates allow us to characterise these glands as extrafloral nectaries.

The pattern of development observed in the nectaries of *S. biglandulosum* was similar to what has been reported for extrafloral nectaries of other plant families (Arbo 1972; Dave and Patel 1975; Roshchina and Roshchina 1993; Silva and Machado 1999; Leitão *et al*. 2002; Paiva and Machado 2006; Thadeo *et al*. 2008). The asynchronous cellular divisions during the nectary ontogenesis are an ordinary process, as described for *Pedilanthus tithymaloides*, Euphorbiaceae (Dave and Patel 1975), and *Triumfetta semitriloba*, Tiliaceae (Leitão *et al*.

2002). Arbo (1972) described such asynchrony for the ontogenesis of the nectaries of *Byttneria*. However, such asynchronous divisions caused a deepening of the nectary itself instead of its emergence, as shown for *S. biglandulosum*.

The extrafloral nectaries of *S. biglandulosum* can be classified as structural nectaries, according to Fahn (1979). These structures originate from a small group of meristematic cells in the petiole, which are covered by two stipules. It is believed that the stipules act on the protection of the structure being formed, because meristematic cells are fragile.

Because the epidermis is secretory, has a thin cuticle and no stomata, it is believed that the secretion is released through the cuticle as reported for the extrafloral nectaries of *Croton sarcopetalus*, Euphorbiaceae (Freitas *et al*. 2001), where the secretion is exudated by channels. In species belonging to other botanical families (Elias *et al*. 1975; Bentley and Elias 1983; Roshchina and Roshchina 1993; McDade and Turner 1997), cuticular pores have been reported as a way to exudate the nectar. In *S. biglandulosum*, nectar may or may not accumulate. For the smaller marginal nectaries, the nectar was found to accumulate in the concavity. This may be an advantage because it reduces the evaporative loss of nectar, thus increasing the volume of available nectar (Keeler and Kaul 1979; Leitão *et al*. 2005). In contrast, the petiole nectaries are larger, with nectar accumulating for a short period and then dropping off.

Nectaries are closely related to the vascular system, often having their own vascular bundle and sometimes an underlying nectary parenchyma (Bentley and Elias 1983; Contreras and Lersten 1984; Roshchina and Roshchina 1993; Leitão *et al*. 2005; Paiva and Machado 2006; Nepi 2007). This pattern of organisation was observed in *S. biglandulosum*. The continuous flow of organic matter from the phloem is necessary for nectar production in *S. biglandulosum* because chloroplasts are not found in the nectaries.

Nectar was classified as hexose-rich according to Baker and Baker (1983). Such a sugar profile may be related to visitors (Baker and Baker 1983; Nicolson 2007). Freitas *et al*. (2001) also found the sugars in the extrafloral nectar of *Croton sarcopetalus* (Euphorbiaceae) to be primarily monosaccharides, with a predominance of glucose over fructose. Crop load increases with increasing sucrose concentration; however, when sucrose concentration is higher than 43%, it diminishes because of nectar viscosity (Nicolson 2007). Experimental data demonstrated that nectar-feeding ants normally forage for sucrose-poor nectar because high sucrose concentrations increase viscosity and ants are not able to carry such heavy loads (Nicolson 2007). Heil *et al*. (2005) reported that for the extrafloral nectaries of myrmecophytic *Acacia* species, mutualistic ants prefer extrafloral nectars that have glucose and fructose in their profile, while nonsymbiotic ants prefer extrafloral nectars that have sucrose. Once extrafloral nectar of *S. biglandulosum* has sucrose, that attracts patrolling ants that may play a defensive role; such ants seem to be generalists; that is, they randomly look for extrafloral nectars available instead of having a close relationship with *S. biglandulosum*.

Although *S. biglandulosum* has two large nectaries on the petiole, smaller nectaries are also found on the leaf margin, thus increasing the area patrolled by ants and allowing for protection of the whole leaf. Elias and Gelband (1976) considered the presence of many small nectaries at a particular site as a form of specialisation. In support of their conclusion, Elias and Gelband (1976) stated that malfunction or damage, from either internal or external factors, in one or two of these many small nectaries will not eliminate that site as an ant-attracting location. This nectary-distribution pattern may be considered an ecological advancement compared with a single large nectary (McDade and Turner 1997; Leitão *et al*. 2005).

The premature secretion of nectar in *S. biglandulosum* is important for attracting ants which then protect the buds, because during development the buds are delicate structures and subject to injuries. Additionally, ants visit the leaf blade, thus protecting the photosynthetic sites. The ant–extrafloral nectaries mutualistic interactions can reduce the damage caused by herbivores to the host plant (Elias 1972, 1980; Elias *et al*. 1975; Keeler 1977; Curtis and Lersten 1978; Anderson and Symon 1985; McDade and Turner 1997; Oliveira 1997; Koptur *et al*. 1998; Madureira and Sobrinho 2002; Almeida and Figueiredo 2003; Paiva and Machado 2006). Anderson and Symon (1985) demonstrated that ant activity was higher during the younger leaf stages, which matches the period of higher activity of the extrafloral nectaries of *Solanum.* Anderson and Symon (1985) also noted the protection afforded by insects during the early leaf stages.

The detection of polysaccharides by PAS and ruthenium red in the secretory cells of the nectaries confirmed the chemical results found for disaccharides and monosaccharides. Similar reports are found in studies of other Euphorbiaceae species and other botanical families (Rocha *et al*. 2002; Leitão *et al*. 2005; Thadeo *et al*. 2008). The detection of protein by xylidine pounceau indicates that proteins may be found in the nectar. These results showed that the nectar of the *S. biglandulosum* is nutritionally rich. The presence of proteins, sugars, polysaccharides and pectin is common among several kinds of nectary glands (Bentley and Elias 1983; Roshchina and Roshchina 1993; Leitão *et al*. 2002; Nicolson and Thornburg 2007).

The characteristics of the secretory epidermis and nectariferous tissue observed in the nectary of *S. biglandulosum* match the description of structural extrafloral nectaries in other species as reported by several authors (Dave and Patel 1975; Elias and Gelband 1976; Fahn 1979; Metcalfe and Chalk 1979; Bentley and Elias 1983; Anderson and Symon 1985; Gaffal *et al*. 1998; Freitas *et al*. 2001; Paiva *et al*. 2001; Leitão *et al*. 2002; Rocha *et al*. 2002; Thadeo *et al*. 2008).

As observed by other authors studying different nectaries, druses are scattered among the ground parenchyma (Arbo 1972; Elias *et al*. 1975; Metcalfe and Chalk 1979; Bentley and Elias 1983; Thadeo *et al*. 2008) and may be related to calcium immobilisation in the nectary region where active sugar transport is presumably occurring (Vezza *et al*. 2006). Because sucrose transport in plants involves ATPase activity (Giaquinta 1979) and Ca^{2+} inhibits plasma membrane ATPase (Leonard and Hodges 1980), this immobilisation may be a way to guarantee the continuous transport of sucrose.

Tannins, alkaloids and phenolic compounds are known to be important substances acting on the anti-herbivore system of the plants (Croteau *et al*. 2000; Dyer *et al*. 2001; Almeida and Figueiredo 2003; Nicolson and Thornburg 2007). However,

because the secreting cells are hidden within the secretory pores of this species, producing these chemicals to help with nectary protection could be a waste of energy. Because producing nectar is already a high-cost investment for the plant, the lack of these compounds could be considered an adaptation to save energy. Moreover, as tannins were found at low concentrations and ants were observed on the nectaries, a shift in which the protective chemicals are substituted by the patrolling ants could be possible. Such a hypothesis concurs with what Elias (1980) had earlier hypothesised while studying *Leonardoxa africana*.

Conclusion

On the basis of the ontogenetic process of the secretory structures of *Sapium biglandulosum*, anatomical description, histochemical tests, and disaccharide and monosaccharide analysis of the exudate, it can be concluded that the studied glands are in fact extrafloral nectaries. Although the presence of ants on the meristematic areas may be important to the plant's survival, a specific study on the role of extrafloral nectaries in *S. biglandulosum* is required for further conclusions.

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