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OIL GLANDS IN *PTERODON PUBESCENS* BENTH. (LEGUMINOSAE-PAPILIONOIDEAE): DISTRIBUTION, STRUCTURE, AND SECRETION MECHANISMS

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Pterodon pubescens Benth., a legume of the Brazilian Cerrado, produces oil that is employed in folk and conventional medicine. In this study, we investigated the distribution, structure, and secretion mechanisms of the secretory cavities in the mature embryo, seedlings, and vegetative organs of adult plants of *P. pubescens*, using light and electron microscopy as well as cytochemical methods (ZIO). Secretory cavities occur in the epicotyl and eophylls of seedlings and in the primary stem and leaves of adult plants, and they are absent in the embryo. These cavities are constituted by a wide lumen and a uniseriate epithelium of secretory cells. A parenchyma sheath surrounds each gland. Oils and terpenes were histochemically detected in the epithelium and in the lumen of the cavities. Ultrastructurally, epithelial cells showed proliferation of endoplasmic reticulum, abundant mitochondria, polyribosomes, polymorphic plastids, and lipid droplets. Multivesicular bodies and paramural bodies were also common in these cells. The secretion was released from the protoplast of the epithelial cells toward the lumen by eccrine, granulocrine, and holocrine mechanisms. The features of sheath cells evidenced by ZIO, such as the presence of Golgi bodies and smooth endoplasmic reticulum, indicate that these cells have secretory activity and participate in producing secretions.

Keywords: anatomy, cell ultrastructure, Leguminosae, secretory cavity.

Introduction

Secretory cavities and canals are common in many members of Leguminosae and can occur in vegetative (Lersten and Curtis 1986; Turner 1986; Teixeira et al. 2000; Teixeira and Gabrielli 2000; Marcati et al. 2001; Paiva and Machado 2007; Rodrigues and Machado 2009; Teixeira and Rocha 2009; Rodrigues et al. 2011*a*, 2011*b*; Milani et al. 2012) or reproductive organs (Paiva et al. 2008; Teixeira and Rocha 2009). These structures in legumes have taxonomic (Turner 1986; Lersten and Curtis 1996; Teixeira et al. 2000; Teixeira and Gabrielli 2000, 2006), ecological (Langenheim et al. 1982; Arrhenius and Langenheim 1983; Langenheim 2003), and economic (Coelho et al. 2001; Langenheim 2003; Plowden 2003) aspects. Some of the substances produced by the cavities or canals, mainly the terpenes, aid in plant resistance to microbial attack and protection against predators (Langenheim et al. 1982; Arrhenius and Langenheim 1983). The active substances synthesized by these secretory structures have been exploited by cosmetic, pharmaceutical, and other industries (Langenheim 2003).

Pterodon (Papilionoideae) is a genus of woody plants that are widely distributed in central and eastern Brazil (Lorenzi 1998). *Pterodon pubescens* Benth., popularly known as sucupira-branca, is native to the Brazilian Cerrado (Lorenzi 1998) and is important in folk and conventional medicine in this country. The oil from sucupira-branca, obtained from samara fruits, is used to treat rheumatism (Correa 1984), arthritis (Coelho et al. 2001), and schistosomiasis (Katz et al. 1993) and shows anti-inflammatory (Nunan et al. 1982) and analgesic (Duarte et al. 1996) properties.

Despite the potential value of the oil produced by *P. pubescens*, little is known about the structural and functional features of their oil-producing glands. Rodrigues et al. (2011*b*) noted that secretory cavities occur also in the stem of *P. pubescens*, where their lumen is initiated by schizogenesis and epithelial cells undergo programmed cell death in their late developmental stages. According to Rodrigues et al. (2011*a*), dead epithelial cells are replaced by new ones originating from the parenchyma sheath, characterizing an open pattern of development. However, information on the structure and function of these glands in different stages of vegetative development as well as in different organs of *P. pubescens* is lacking. Furthermore, the cellular features involved in the synthesis, accumulation, and release of the secretion have not been described.

In this study, we investigated the distribution, anatomy and ultrastructure of secretory cavities in different developmental stages and organs in this economically valuable species, *P. pubescens*, in order to elucidate the occurrence, morphology, and functioning of these glands during the secretory cycle.

Material and Methods

Samples of lateral roots, stems (at developmental stages of both primary and secondary growth) and leaves were collected from five adult individuals of *Pterodon pubescens* Benth. living in an area of Cerrado vegetation located in the

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municipality of Botucatu (22°55′S, 48°30′W), in São Paulo State, Brazil, during the growing season (September through February). Vouchers were deposited in the herbarium Irina Delanova Gemtchújnicov (BOTU) under number 25305.

Mature embryos were obtained from 30 seeds extracted from 30 fruits, using scissors. To obtain seedlings, 150 seeds were disinfected in 70% ethanol for 1 min and in 2% sodium hypochlorite for 20 min. Then the seeds were washed in distilled water and placed in acrylic boxes lined with moist filter paper. The boxes with seeds were maintained at 25°C and a 12-h photoperiod under white fluorescent light. Samples were collected from 15-d-old seedlings. Here, the term seedling refers to the developmental stage from the emergence of the primary root to the expansion of the first eophyll pair (Oliveira 2001).

For LM, samples (stem, leaves and lateral roots from adult plants; primary root, hypocotyl, epicotyl, cotyledons, and eophylls of seedlings and mature embryos) were fixed in FAA 50 (Johansen 1940), dehydrated in an ethanol series, and embedded in methacrylate resin (Leica Embedding Kit). Longitudinal and cross sections (5 μm thick) were stained with 0.05% toluidine blue, pH 4.3, in 0.1 M phosphate buffer (O'Brien et al. 1964). All the specimens were examined and documented with an optical microscope (BX 40, Olympus) equipped with a digital camera. Sudan IV (Johansen 1940) was employed to detect total lipids, and Nadi's reagent (David and Carde 1964) for terpenes.

For SEM, samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, postfixed in 0.5% osmium tetroxide in the same buffer, and washed in distilled water; critical-point drying was performed using liquid carbon dioxide. The samples were coated with 10 nm gold (Robards 1978) and observed on a FEI QUANTA SEM.

Samples for conventional TEM were initially fixed in 2.5% glutaraldehyde solution in 0.1 M phosphate buffer at pH of 7.3 for 24 h at 5°C, postfixed with 1% osmium tetroxide in the same buffer for 1 h at 25°C, dehydrated through an acetone series, and embedded in Araldite resin (Machado and Rodrigues 2004). Ultrathin sections were obtained with a Diatome diamond knife and poststained with uranyl acetate and lead citrate (Reynolds 1963).

To improve the staining of certain populations of organelles in the secretory and neighboring cells, samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, for 12 h and incubated in ZIO solution (zinc, iodine, TRIS-aminomethane buffer, and osmium tetroxide) at 10°C for 24 h (Machado and Gregório 2001). Then the samples were processed according to the conventional technique. The material was analyzed using a Philips EM 301 TEM.

Results

Secretory cavities were observed in the different organs on the vegetative axis of seedlings and adult plants of *Pterodon pubescens* (table 1). Secretory cavities were absent in the embryo. In seedlings, secretory cavities were present in the cortical region of the epicotyl and in the internerval areas and margin of the eophylls (fig. 1*A*, 1*B*). In adult plants, secretory cavities occurred in the cortex of the stem, primary, and secondary pulvinus, petiole, and rachis and in the internerval areas and margin of the leaf blade (fig. 1*C*–1*H*). In the margin of leaflets, the secretory cavities occupied the entire leaf width normally occupied by mesophyll (fig. 1*H*).

Structurally, in all the samples analyzed, the mature secretory cavities were constituted by a uniseriate secretory epithelium and a wide lumen (fig. 1*I*), where the released secretion accumulates. In both cross (fig. 1*A*–1*C*, 1*E*–1*J*) and longitudinal sections (fig. 1*D*), the secretory cavities showed a spherical or slightly elongated lumen in all the organs investigated. However, the epithelial cells showed different degrees of swelling and variable shape. Most of the epithelial cells were

Table 1

Occurrence and Distribution of the Secretory Cavities in Different Developmental Stages of *Pterodon pubescens*

Developmental stage, organ	Presence (+)/absence (−) of secretory cavities	Region of occurrence of secretory cavities in the organ
Embryo:		
Hypocotyl-radicle axis	−	...
Cotyledons	−	...
Seedling:		
Epicotyl	+	Cortex
Hypocotyl	−	...
Eophyll blade	+	Internerval areas and margin
Primary root	−	...
Adult plant:		
Stem (primary growth)	+	Cortex
Stem (secondary growth)	−	...
Primary pulvinus	+	Cortex
Secondary pulvinus	+	Cortex
Petiole	+	Cortex
Rachis	+	Cortex
Leaf blade	+	Internerval areas and margin
Root (secondary growth)	−	...

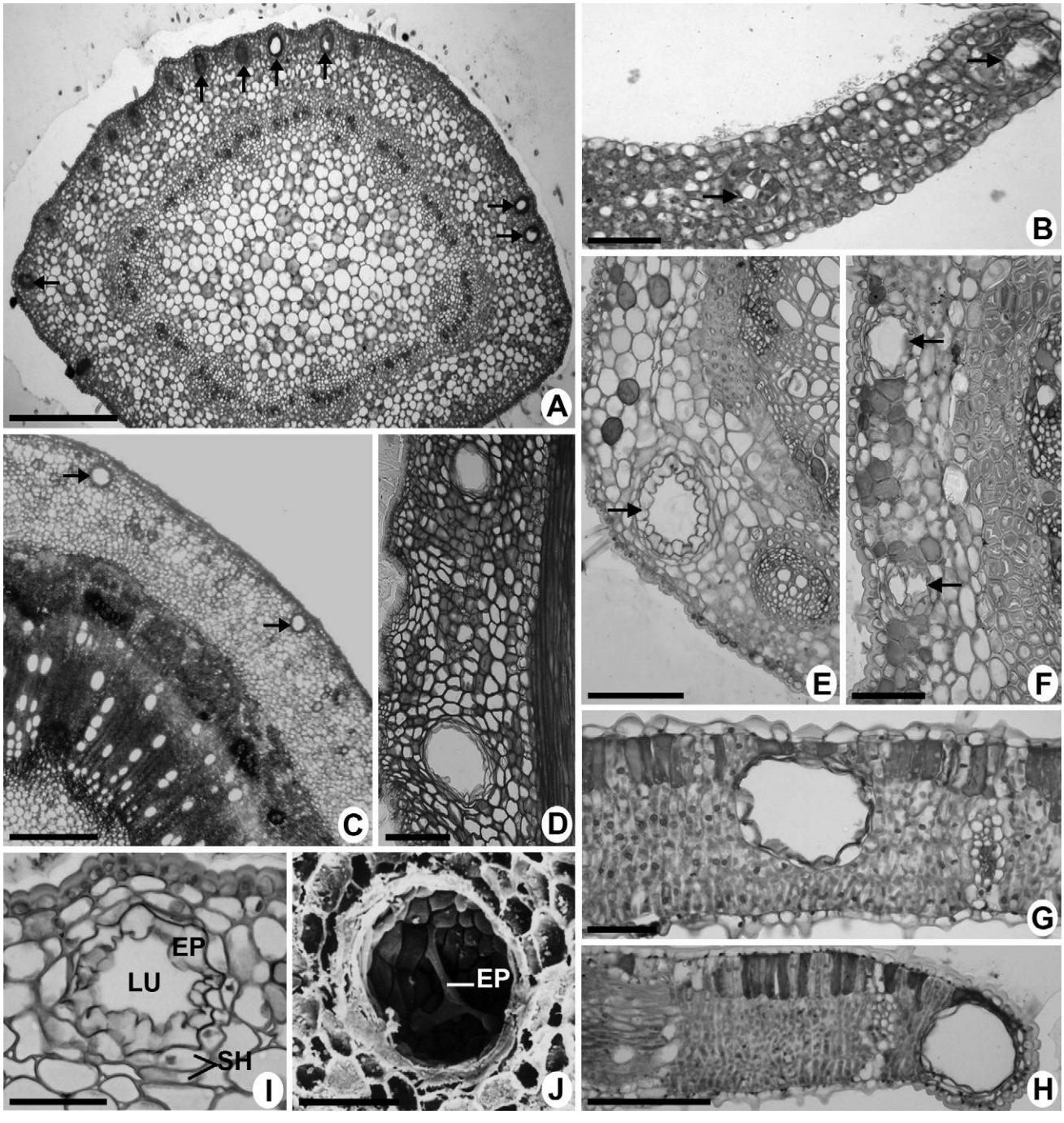

Fig. 1 Secretory cavities in different organs of *Pterodon pubescens*. *A–I*, Light microscopy. *A*, Epicotyl in cross section, showing secretory cavities (arrows) in the cortical region. *B*, Eophyll in cross section, showing secretory cavities (arrows) in the internerval areas and margin of the blade. *C*, Cross section of stem showing cavities (arrows) in the cortex. *D*, Longitudinal section of primary pulvinus showing rounded cavities. *E*, Oil cavity (arrow) in the cortex of petiole in cross section. *F*, Cavities (arrows) in the cortex of rachis in cross section. *G*, *H*, Cross sections of metaphyll, showing oil cavity in internerval area (*G*) and margin of the leaf blade (*H*). *I*, Detail of oil cavity in cross section, constituted by uniseriate epithelium (EP) and lumen (LU). Note the parenchyma sheath (SH) around the cavity. *J*, Scanning electron micrograph showing elongated epithelial cell (EP) crossing the lumen. Scale bars: *A*, *C*, 500 μm; *B*, *F*, *H*, 100 μm; *D*, *E*, 150 μm; *G*, *I*, *J*, 50 μm.

papilliform or pyramidal (fig. 1*I*); some epithelial cells were more elongated and protruded into the lumen, showing a finger-like shape (fig. 1*J*). An evident sheath (fig. 1*I*) constituted by one or more layers of tangentially elongated parenchyma cells with dense cytoplasm and voluminous nuclei surrounded the cavities in all the organs. Total lipids (fig. 2*A*) and terpenes (fig. 2*B*) were histochemically detected in the sheath cells, epithelial cells, and the lumen of the secretory cavities.

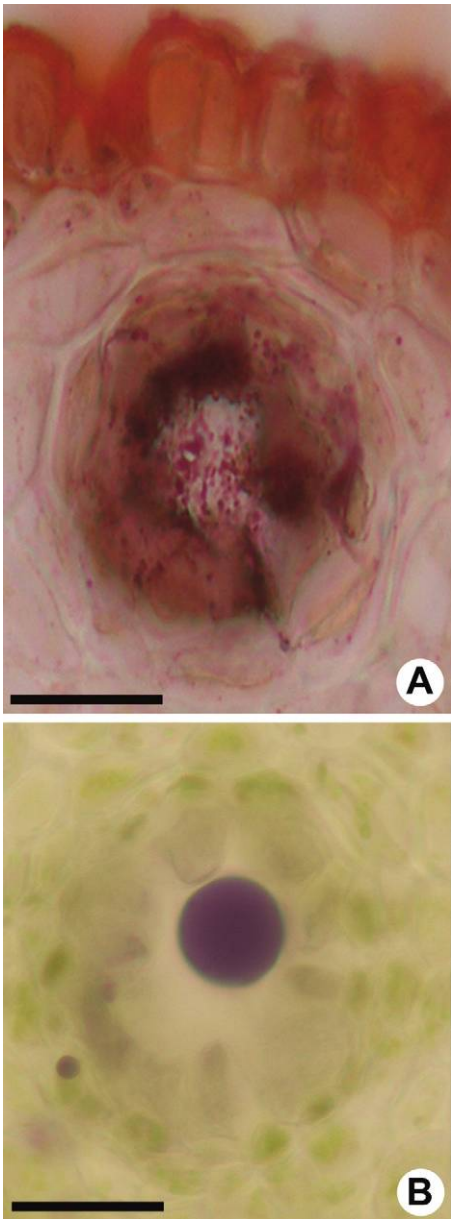

Fig. 2 Histochemistry of the secretory cavities in *Pterodon pubescens* stem. *A*, Sudan IV. *B*, Nadi's reagent. Scale bars, 40 μm.

Observed by TEM, the epithelial cells of mature secretory cavities were characterized by very thin primary walls, plasma membrane with irregular contours (fig. 3*A*, 3*C*), voluminous nucleus with evident nucleolus, and abundant electron-dense cytoplasm; the vacuoles were small (fig. 3*A*). Plasmodesmata were abundant in the anticlinal (fig. 3*A*) and in the inner periclinal cell walls adjacent to the parenchyma sheath. Polyribosomes, mitochondria with developed cristae, polymorphic plastids, and proliferated smooth endoplasmic reticulum (fig. 3*B*, 3*C*) as well as hyperactive dictyosomes (fig. 4*A*) were observed in the cytoplasm. The plastids were devoid of grana, and their matrix was granular with small oil droplets and osmiophilic inclusions (fig. 3*B*). Conspicuous oil droplets were scattered in the cytoplasm (fig. 3*B*, 3*C*) and next to (fig. 3*E*) or inside (fig. 3*F*) vacuoles. Oil droplets were also present in the peripheral cytoplasm, juxtaposed to the plasma membrane (fig. 3*D*, 3*E*). The images suggested that the oil droplets can cross the cell wall and reach the lumen of the secretory cavities (fig. 3*G*) or traverse the anticlinal cell walls between adjacent epithelial cells. Vesicles were also observed in the peripheral cytoplasm and sometimes fused to the plasma membrane of the epithelial cells in secretory cavities (fig. 4*A*). Multivesicular bodies were common in the peripheral cytoplasm of the epithelial cells (fig. 4*B*). In the vacuoles, membranous material was observed along with myelin figures (fig. 4*C*). The larger periplasmic spaces observed in some epithelial cells frequently contained paramural bodies (fig. 4*D*).

The periclinal cell wall tangential to the lumen appeared loose and underwent progressive peeling, releasing parietal material to the lumen of the secretory cavity (figs. 3*C*, 4*C*). Flocculant material and osmiophilic clusters accumulated in the lumen (figs. 3*C*, 4*C*).

In the same secretory cavity, cells with different degrees of electron density were seen side by side (fig. 4*E*). Some epithelial cells showed a highly electron-dense cytoplasm, impeding observation of the organelles (fig. 4*F*). However, the proliferated endoplasmic reticulum and mitochondria were more evident (fig. 4*F*, 4*G*). In these dark cells, the vacuoles were larger and were filled with flocculant material and electron-dense inclusions (fig. 4*F*). Oil droplets were abundant in the peripheral cytoplasm (fig. 4*G*). The periplasmic space of these cells was more developed and contained accumulated oil bodies. The middle lamella between the dark cells and adjacent active epithelial ones became swollen and sometimes dissolved (fig. 4*G*). In a later developmental stage, the dark cells ruptured and released their contents to the lumen of the secretory cavity (fig. 4*H*).

The parenchyma sheath cells were flattened and showed thicker primary walls, less dense cytoplasm, and an inconspicuous nucleus (fig. 4*I*). Plasmodesmata were common in all of their walls, connecting them to the epithelial cells and subjacent layers of ground parenchyma (fig. 4*I*). Typical chloroplasts, sparse endoplasmic reticulum, polyribosomes, and abundant dictyosomes occurred in the cytoplasm of the sheath cells (fig. 4*I*). The vacuole was more developed than in the epithelial cells (fig. 4*I*).

The ZIO method confirmed a greater abundance of dictyosomes and small vesicles in the cytoplasm of the parenchyma sheath cells, in comparison to the epithelial cells (fig. 5*A*). In the epithelial cells, the ZIO method stained tubular portions of smooth endoplasmic reticulum (fig. 5*B*), and numerous small vesicles appeared scattered in the cytoplasm (fig. 5*C*). The reaction products were seen as electron-dense deposits in the lumen of the smooth endoplasmic reticulum and of the dictyosome cisternae. Vesicles attached to the extremity of the dictyosome cisternae (fig. 5*D*) and inside multivesicular bodies (fig. 5*E*) also showed reaction products. Some dictyosomes were fully impregnated, and in others only some cisternae were stained (fig. 5*D*). Reaction products were also observed in the vacuoles (fig. 5*F*).

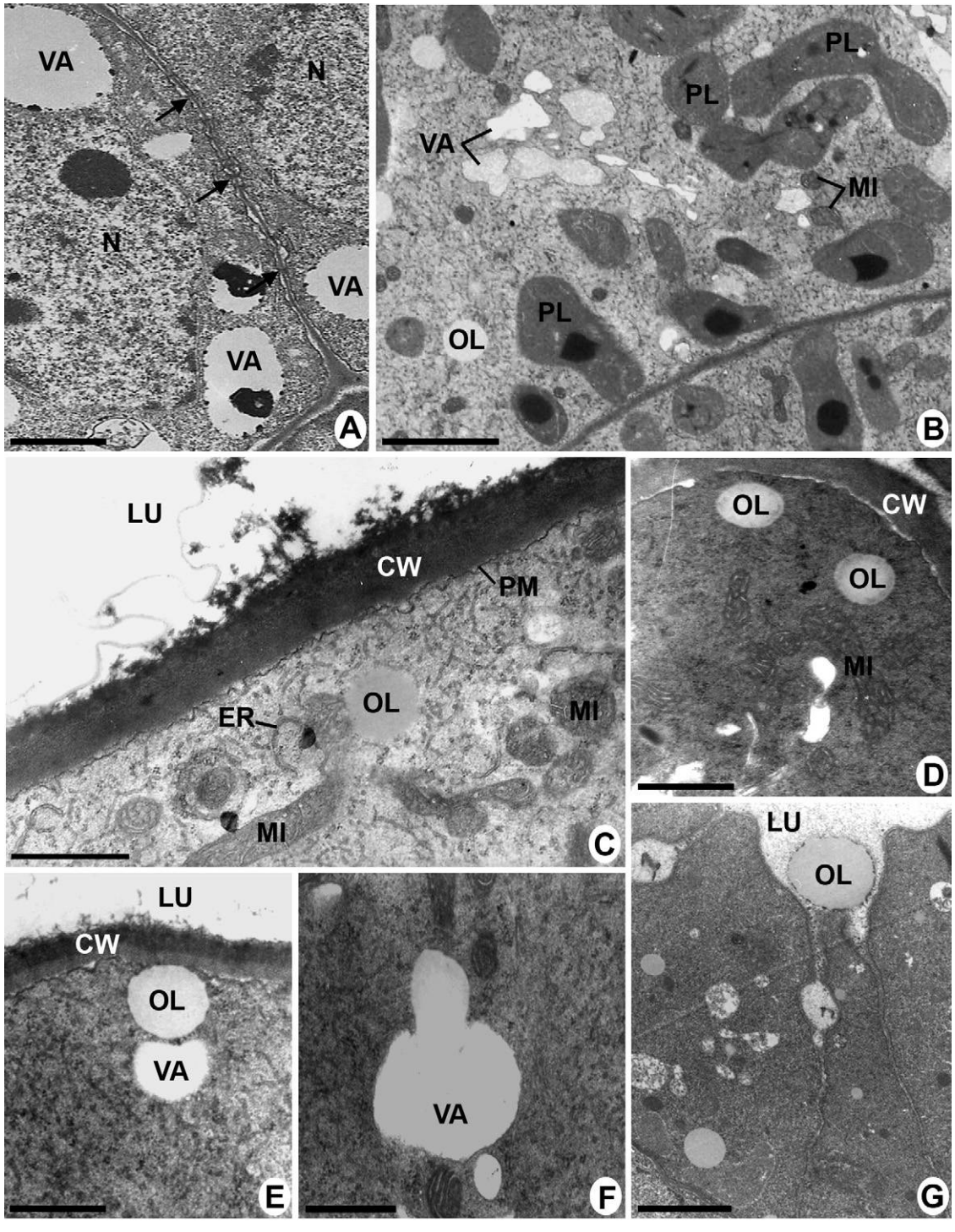

Fig. 3 Electron micrographs (conventional TEM) of epithelial cells of secretory cavities of *Pterodon pubescens* pulvinus. *A*, Neighboring epithelial cells, both showing thin walls with plasmodesmata (arrows), conspicuous nucleus, dense cytoplasm, and small vacuoles (VAs). *B*, Detail of cytoplasm showing polyribosomes, mitochondria (MI), endoplasmic reticulum, and oil (OL) droplets. The abundant polymorphic plastids (PL) contain electron-dense bodies and are devoid of grana. The VAs are small. *C*, Peripheral region of epithelial cell showing polyribosomes, MI, endoplasmic reticulum (ER), and OL in the cytoplasm. Note the sinuous contour of the plasma membrane (PM) and the loose feature of the cell wall (CW) releasing parietal material to the lumen (LU) of the cavity. *D*, Note the developed cristae of the MI. *E*, OL in the peripheral cytoplasm juxtaposed to the plasma membrane. *F*, OL inside a vacuole. *G*, OL in the LU of the cavity. Scale bars: *A*, *B*, 1.0 μm; *C*, *D*, 0.6 μm; *E*, *F*, 0.4 μm; *G*, 1.2 μm.

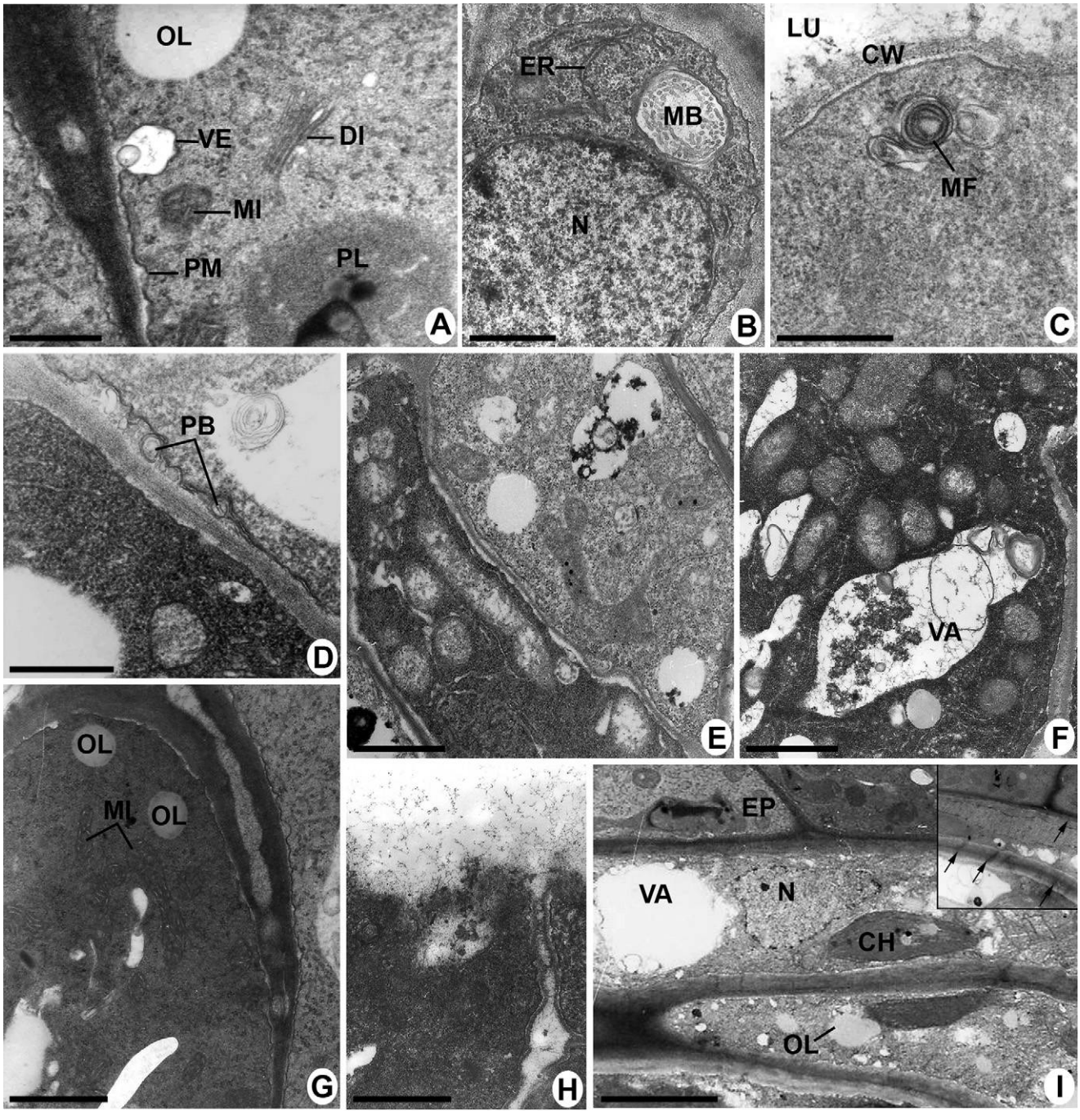

Fig. 4 Electron micrographs (conventional TEM) of secretory cavities of *Pterodon pubescens* pulvinus. *A*, Epithelial cell, showing a vesicle (VE) merging with the plasma membrane (PM). Note polyribosomes, mitochondria (MI), dictyosome (DI), plastid (PL), and oil droplet (OL) in the cytoplasm. *B*, Multivesicular body (MB) in the peripheral cytoplasm of epithelial cell. N, nucleus; ER, endoplasmic reticulum. *C*, Myelin figure (MF) in vacuole of epithelial cell. CW, cell wall; LU, lumen. *D*, Paramural bodies (PB) in the periplasmic spaces of epithelial cell. *E*, Cells with different degrees of electron density, occurring side by side in the same epithelium. *F*, Epithelial cell with dark cytoplasm and vacuole (VA) with flocculant material and electron-dense inclusions. *G*, Dark epithelial cell showing proliferated MI and OLs in the peripheral cytoplasm. Note the swelling and dissolution of middle lamellae between the dark cell and the adjacent active one. *H*, Disruption of a dark epithelial cell. *I*, Parenchyma sheath cells containing chloroplasts (CH) and OLs. The inset shows part of a wall region with plasmodesmata (arrows). N, nucleus; VA, vacuole; EP, epithelial cells. Scale bars: *A–D*, *G*, *H*, 0.4 μm; *E*, 1.0 μm; *F*, 0.6 μm; *I*, 1.8 μm.

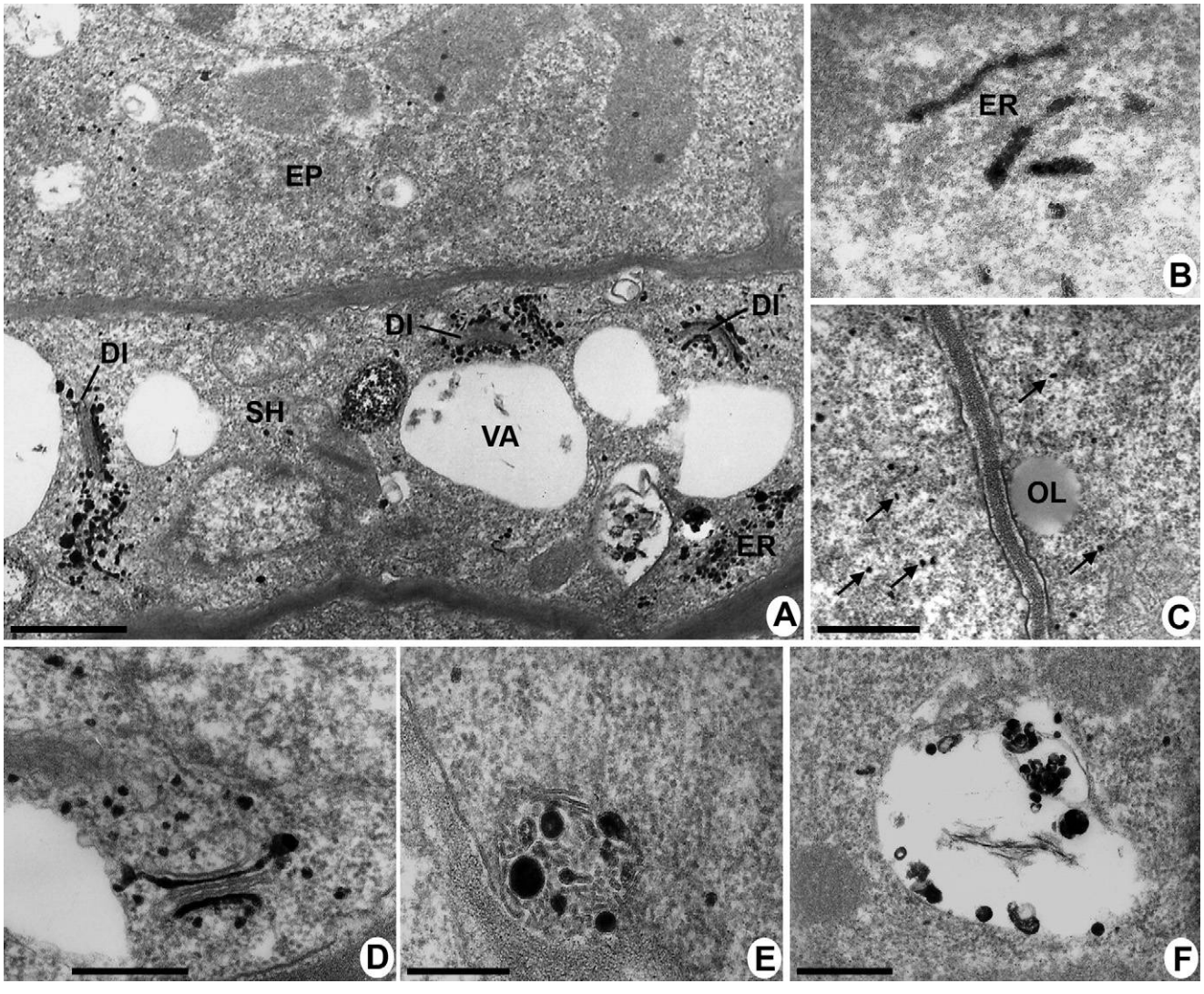

Fig. 5 Electron micrographs (TEM) of secretory cavities of *Pterodon pubescens* pulvinus processed according to the ZIO technique. *A*, Observe the different marking of organelles in epithelial (EP) and parenchyma sheath (SH) cells. DI, dictyosomes; ER, endoplasmic reticulum; VA, vacuole. *B*, Detail of an epithelial cell, showing ER impregnated with the reaction product. *C*, Mall vesicles (arrows) in epithelial cells. OL, oil droplet. *D*, Detail of sheath cell, showing deposits of reaction products in the external cisternae of the dictyosome, and in the vesicles attached to these cisternae or released to the cytoplasm. *E*, Vesicles inside a multivesicular body in a parenchyma sheath cell. *F*, Products of ZIO reaction in vacuole of parenchyma sheath cell. Scale bars: *A*, 0.8 μm; *B*, 0.1 μm; *C*, 0.3 μm; *D–F*, 0.2 μm.

Discussion

Secretory cavities are present in all aerial vegetative organs of adult individuals of *Pterodon pubescens*, as reported in other legume species (Turner 1986; Teixeira et al. 2000; Teixeira and Gabrielli 2000; Marcati et al. 2001; Paiva and Machado 2007; Paiva et al. 2008). This study demonstrated that secretory cavities were absent in the mature embryo and were first observed in the eophylls of *P. pubescens* seedlings. Terpenes detected in the secretory cavities of *P. pubescens* with the use of Nadi's reagent have been associated with resistance to microbial attack and protection against herbivores (Harbone 1993; Taiz and Zeiger 1998). This species is epigeous-phanerocotyledonar, and the presence of secretory cavities helps to protect the exposed young and vulnerable parts. The protective function of the secretory cavities begins in the seedling stage, a critical period of plant development, as reported by Rodrigues et al. (2011*b*) for *Copaifera langsdorffii* seedlings. As mentioned above, secretory cavities are lacking in the mature embryo. On the other hand, an abundance of oil ducts in the pericarp of the indehiscent fruit was described by Paiva et al. (2008) in *Pterodon emarginatus* (syn. *P. pubescens*), and this is a protective factor that probably compensates for the absence of secretory cavities in the mature embryo.

The variable shape of the epithelial cells in secretory cavities of *P. pubescens* can be related to the different stages of the secretory process. The occurrence of elongated finger-like epithelial cells is a remarkable feature and characterizes the trabecular cavities described in some species of Papilionoideae (Turner 1986). These cells have been suggested to have

taxonomic importance (Solereder 1908; Turner 1986; Teixeira et al. 2000; Teixeira and Rocha 2009).

The ultrastructural features observed in the epithelial cells of *P. pubescens*, such as the proliferation of smooth endoplasmic reticulum and polymorphic plastids, are common characteristics described for oil cells in other species (Paiva and Machado 2007; Teixeira and Rocha 2009; Rodrigues et al. 2011*b*). Proliferation of smooth endoplasmic reticulum and plastids without grana are associated with lipid synthesis (Fahn 1979; 2000) and have been described for many glands secreting lipophilic substances, mainly monoterpenes (Cheniclet and Carde 1985; Monteiro et al. 1999; Turner et al. 1999; Machado et al. 2006; Paiva et al. 2008). Lipophilic droplets were observed in the cytoplasm of the epithelial cells and in the lumen of the secretory cavities in *P. pubescens*, by means of TEM. This is consistent with the positive results of the histochemical tests.

The occurrence of myelin figures in vacuoles is another remarkable feature of the epithelial cells in *P. pubescens* and is probably associated with the incorporation of lipophilic material or its precursors into the hydrophilic contents of the vacuole (Raatikainen et al. 1992). The presence of dictyosomes and the abundance of polyribosomes in the epithelial cells of *P. pubescens* is probably associated with the synthesis and elimination of enzymes (Hall et al. 1984; Carmello et al. 1995; Machado and Carmello-Guerreiro 2001; Paiva and Machado 2007). These enzymes may be involved in processes of cell wall degradation and also in the peeling of the epithelial cell wall facing the lumen of the cavities.

With respect to the mechanisms of secretion release from the protoplast to the lumen cavity, our data suggest the occurrence of eccrine, granulocrine, and holocrine processes. The presence of oil droplets juxtaposed to the plasma membrane of the epithelial cells and in the lumen of the secretory cavities suggests an eccrine secretion process. In this process, the material passes over the porous cell walls (via loosely arranged cellulose microfibrils) and accumulates on the cell surface (Evert 2006). In addition, the abundance of vesicles filled with dense contents in the peripheral cytoplasm and juxtaposed to the plasma membrane suggests a granulocrine secretion process (Fahn 1979). Some images, such as multivesicular bodies and paramural bodies in the epithelial cells, are evidence of granulocrine secretion (Nair et al. 1983; Venkaiah 1992; Carmello et al. 1995). Finally, the disruption of the dark epithelial cells followed by the release of their contents into the lumen cavity characterizes a holocrine secretion process (Fahn 1979), which in this species is associated with programmed cell death, as reported by Rodrigues et al. (2011*b*). To our knowledge, the involvement of holocrine as well as eccrine and granulocrine processes in the same secretory system is not common.

The features of sheath cells evidenced by ZIO, such as the presence of Golgi bodies and smooth endoplasmic reticulum, indicate that these cells have secretory activity and participate in producing secretions. In addition, these cells have meristematic potential and can replace the senescent epithelial cells during the secretory cycle of the oil glands (Rodrigues et al. 2011*b*). This fact, added to the observation of epithelial cells at different stages of the secretion process, provides evidence that these glands continue actively secreting for a long time.

Acknowledgments

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