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Ultrastructure of secretory and senescence phase in colleters of *Bathysa gymnocarpa* and *B. stipulata* (Rubiaceae)[1]

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ABSTRACT – (Ultrastructure of secretory and senescence phase in colleters of *Bathysa gymnocarpa* and *B. stipulata* (Rubiaceae)). Colleters are secretory structures formed by a parenchymatic axis with vascular bundles, bound by a layer of secretory palisade-like epidermis. Some studies regarding the structure of colleters have focused on secretory cells structure, but not distinguished the secretory and senescent phases. Generally, in mucilage-secreting cells such as colleters, the endoplasmic reticulum and Golgi apparatus are involved in secretion production and transport. In these study, colleters structure of *Bathysa gymnocarpa* K. Schum. and *B. stipulata* (Vell.) C. Presl. (Rubiaceae) were determined in two phases: a secretory phase and a senescence one. Samples were collected and processed by usual light and electron microscopy techniques. Studied colleters are constituted by an epidermal palisade layer and a central axis formed by parenchymatic cells with rare vascular traces. During the secretory phase, epidermal cells presented a dense cytoplasm, small vacuoles, enhanced rough and smooth endoplasmic reticulum, and a Golgi apparatus close to large vesicles. During the senescence phase epidermal cells presented a disorganized membrane system. No intact organelles or vesicles were observed. The outer cell wall exhibited similar layers to that observed during the secretory phase. The senescent phase is easily defined by the morphology of the colleters, but not well defined at subcellular level. Our research suggests that programmed cell death starts on secretory phase. However, more evidences are needed to evaluate the phenomena.

Key words - light and electron microscopy, plant cell ultrastructure, programmed cell dead, secretory structure

RESUMO – (Ultraestrutura da fase secretora e da senescente dos coléteres de *Bathysa gymnocarpa* e *B. stipulata* (Rubiaceae)). Coléteres são estruturas secretoras formadas por um eixo parenquimático que inclui feixes vasculares, circundado por uma camada de células epidérmicas secretoras em paliçada. Em estudos sobre a estrutura dos coléteres tem sido observada a ultraestrutura das células secretoras, mas não discriminam as fases secretora e senescente. Geralmente, em células secretores de mucilagem como os coléteres, o retículo endoplasmático e o complexo de Golgi estão envolvidos na produção e no transporte da secreção. Neste estudo, foram determinadas duas fases baseadas na estrutura dos coléteres de *Bathysa gymnocarpa* K. Schum. and *B. stipulata* (Vell.) C. Presl. (Rubiaceae): a fase secretora e a fase senescente. Amostras foram coletadas e processadas utilizando técnicas usuais de microscopia óptica e eletrônica. Os coléteres estudados são constituídos por uma camada epidérmica em paliçada e um eixo central parenquimático com traços vasculares raros. Durante a fase secretora, as células epidérmicas se apresentam com o citoplasma denso, pequenos vacúolos, retículo endoplasmático liso e rugoso evidente e complexo de Golgi próximo a grandes vesículas. Durante a fase senescente, as células epidérmicas apresentaram o sistema endomembranar desorganizado. Nenhuma organela intacta ou vesícula foi observada. A parede celular mais externa exibiu camadas similares às observadas durante a fase secretora. A fase senescente é facilmente definida pela morfologia do coléter, mas não é bem definida em nível subcelular nas células secretoras. Nossa investigação sugere que a morte celular programada se inicia na fase secretora. Contudo, mais evidências são necessárias para avaliar esse fenômeno.

Palavras-chave - estrutura secretora, microscopia óptica e eletrônica, morte celular programada, ultraestrutura celular de planta

Introduction

Secretory structures can be present in different plant regions and vary in morphology. They form a polyphyletic group; divergence in the evolution may have originated distinct mechanism of synthesis and externalization of exudates (Dickinson 2000). These variations can be identified by anatomy and ultrastructure of secretory colleter cells. This secretory structure can be present at stipules, calyx, leaves, and stem apex.

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Rubiaceae colleters are secretory structures associated to the stipule. They can occur at adaxial surface or at margins; and it is possible to notice at calyx, close to trichomes (Majumdar & Pal 1958, Horner & Lersten 1968, Lersten 1974a, b, Miller *et al.* 1983, Robbrecht 1988, Thomas 1991, Da Cunha & Vieira 1997, Klein *et al.* 2004, Miguel *et al.* 2006, 2009, Vitarelli & Santos 2009). Rubiaceae colleters show little morphological variation comparing to literature, however, there is a great diversity of length, form and development of a basal constriction, and central axis diameter (Robbrecht 1988). The presence or absence of central axis vascularization was discussed by Thomas (1991).

The term colleter originates from the Greek "colla", meaning glue, and refers to the production of a glue-like secretion (Thomas 1991). This structure has been described under different names that include trichomes (Horner & Lersten 1968), squamellae (Ramayya & Bahadur 1968) and stipular glands (Van Hove & Kagoyre 1974). The most used term for this structure is colleter. Lersten (1974a, b) characterized some types of colleters in Rubiaceae species, and denominated the commonest as standard type. One significant feature of the colleters is the protodermal and fundamental origin (Thomas 1991). Klein *et al.* (2004) reported that the colleters of some *Simira* (Rubiaceae) species are formed as emergentia from the stipule, originating from protodermis and fundamental meristem. Miguel *et al.* (2006) visualized the same structure to *Bathysa nicholsonii* K. Schum. colleters.

The anatomical structure of colleters can be described as a parenchymatic cells axis surrounded by one layer of secretory palisade-like epidermis (Thomas 1991, Da Cunha & Vieira 1997). Occasionally, the middle axis presents vascular bundles (Thomas 1991, Appezzato-da-Gloria & Estelita 2000, Klein *et al.* 2004). In various Rubiaceae genera, the colleters ultrastructure was described recently (Klein *et al*. 2004, Miguel *et al.* 2006, 2009, Barreiro & Machado 2007). Several studies reported colleter structure with emphasis on secretory cells, especially in secretion production phase. The secretory stage of the colleters occurs during the leaf expansion, after which these structures turn brown and senesce (Paiva 2009). Epidermal cell of the colleters presents a dense cytoplasm, enhanced endoplasmic reticulum, Golgi apparatus, mitochondria and nuclei. Endoplasmic reticulum presence close to the plasma membrane is remarkable in Rubiaceae (Horner & Lersten 1968, Dexheimer & Guenin 1981, Miller *et al.* 1983, Klein *et al.* 2004, Miguel *et al.* 2006). Generally, in mucilage-secreting cells, such as those found in Rubiaceae colleters, the endoplasmic reticulum and Golgi apparatus are involved in secretion production and transport (Fahn 1988), the latter being mediated by vesicle traffic (Fahn 1988).

The outer cell walls of the colleters represent the limit between the environment and the plant (Brett & Waldron 1990) and, as such represent the last barrier to prevent secretion to the environment. In *Simira* and *Bathysa* species, the outer cell wall presents three layers: a polysaccharide portion, a cuticular membrane, and a cuticle proper. The secretion, in these species, could be externalized without cuticle rupture, passing through all cell wall layers (Klein *et al*. 2004, Miguel *et al*. 2006).

In general, characteristics change rapidly in secretory structures, such as ultrastructure making their understanding more difficult (Dickinson 2000). Some of these changes can be related to senescence controlled by programmed cell death (Doorn & Woltering 2004), a process that is an integral part of plant development and defense (Doorn & Woltering 2005). In *Allamanda cathartica* L., senescent colleters were found to begin with epidermal cell walls lignification, followed by the central axis (Thomas & Dave 1989). However, this anatomical study is the only one available about senescence of colleters and a gap in our knowledge remains with regard to cell conditions under the senescence phase in colleters.

The Atlantic Rain Forest is a high moisture environment, facilitating the development of many microorganisms in various plant interactions, including pathogeny. So, the study of colleters and their secretion can provide a better understanding of de role of secretion in defense mechanisms. The studied species are representative of Atlantic Rain Forest and according to the International Union for Conservation of Nature and Natural Resources (IUCN), *Bathysa stipulata* (Vell.) J. Presl. (*quina-da-serra*) and *B. gymnocarpa* K. Schum. (*guapeba-branca*) are vulnerable and need to be protected (Germano-Filho 1999). The aim of this study was to characterize the anatomy and ultrastructure of colleters from *Bathysa gymnocarpa* and *B. stipulata*, in order to distinguish secretory and senescence phases.

Material and methods

Plant material – *Bathysa gymnocarpa* and *B. stipulata* shoot apices, with completely expanded stipules, were collected from many specimens at Reserva Biológica de Tinguá (22°28'-22°39' S, 22°35'-43°34' W), in the cities of Duque de Caxias and Nova Iguaçu, in Rio de Janeiro State, Brazil. These cities are located between 220 and 1.600 m above sea level, the annual average temperature is 21.6 °C and pluviosity level is 2.268 mm (SOS Mata Atlântica 2002).

The plants were found in vegetation recognized as Atlantic Rain Forest. For visualization under different microscopy techniques it was necessary to separate stipule and apex using tweezers and blades. By convention, the nodes found at shoot apex were numerated according to separated stipule pairs: the pair closest to apical meristem was considered the first node, the next, second node and so on. Colleters were named senescent on the third or old node.

Scanning electron microscopy – Samples at different stages were fixed for two hours in a solution of 2.5% glutaraldehyde and 4.0% formaldehyde in 0.05 M cacodylate buffer, pH 7.2 (Klein *et al.* 2004). Subsequently, the samples were rinsed in the same buffer and post-fixed for one hour at room temperature with 1.0% osmium tetroxide in 0.05 M cacodylate buffer, pH 7.2 (Klein *et al.* 2004). The post-fixed samples were dehydrated in an ascending acetone series (1 hour each step). Afterwards, the samples were submitted to the critical-point-drying method using CO_2. Dried samples were placed in stubs, sputtered with 20 nm gold, and then observed with a digital scanning electron microscope (DSEM 962 Zeiss).

Light microscopy – Stipule fragments were fixed, post-fixed and dehydrated as described for scanning electron microscopy. Subsequently, the material was embedded in epoxi resin (Polybed). Thin sections (1.0 μm) were stained with 1% toluidine blue (Klein *et al.* 2004). The glass slides were sealed with Entellan® (Merck) and examined with an Axioplan Zeiss microscope.

Transmission electron microscopy – The stipule fragments were fixed, post-fixed, dehydrated, and embedded as described above. Ultrathin sections (80 nm) were collected in copper grids (300 mesh) and stained with 1.0% alcoholic uranyl acetate followed by 5.0% aqueous lead citrate. Three other methods were used to elucidate the cytochemical aspects of colleter cells: 1) the preservation and contrast of lipids was enhanced using imidazole-buffered osmium tetroxide (Angermüller & Fahimi 1982); 2) 1% ruthenium red was used to detect pectins and acid polysaccharides (Luft 1971) of the colleter palisade cells; 3) Periodic acid-thiocarbohydrazide (THC)-silver proteinate (PATAg) was used to detect polysaccharides containing 1,2-glycol groups (Thiéry 1967). Ultrathin sections exposed to all these techniques were observed at 80 kV using a transmission electron microscope (EM 900 Zeiss).

Results

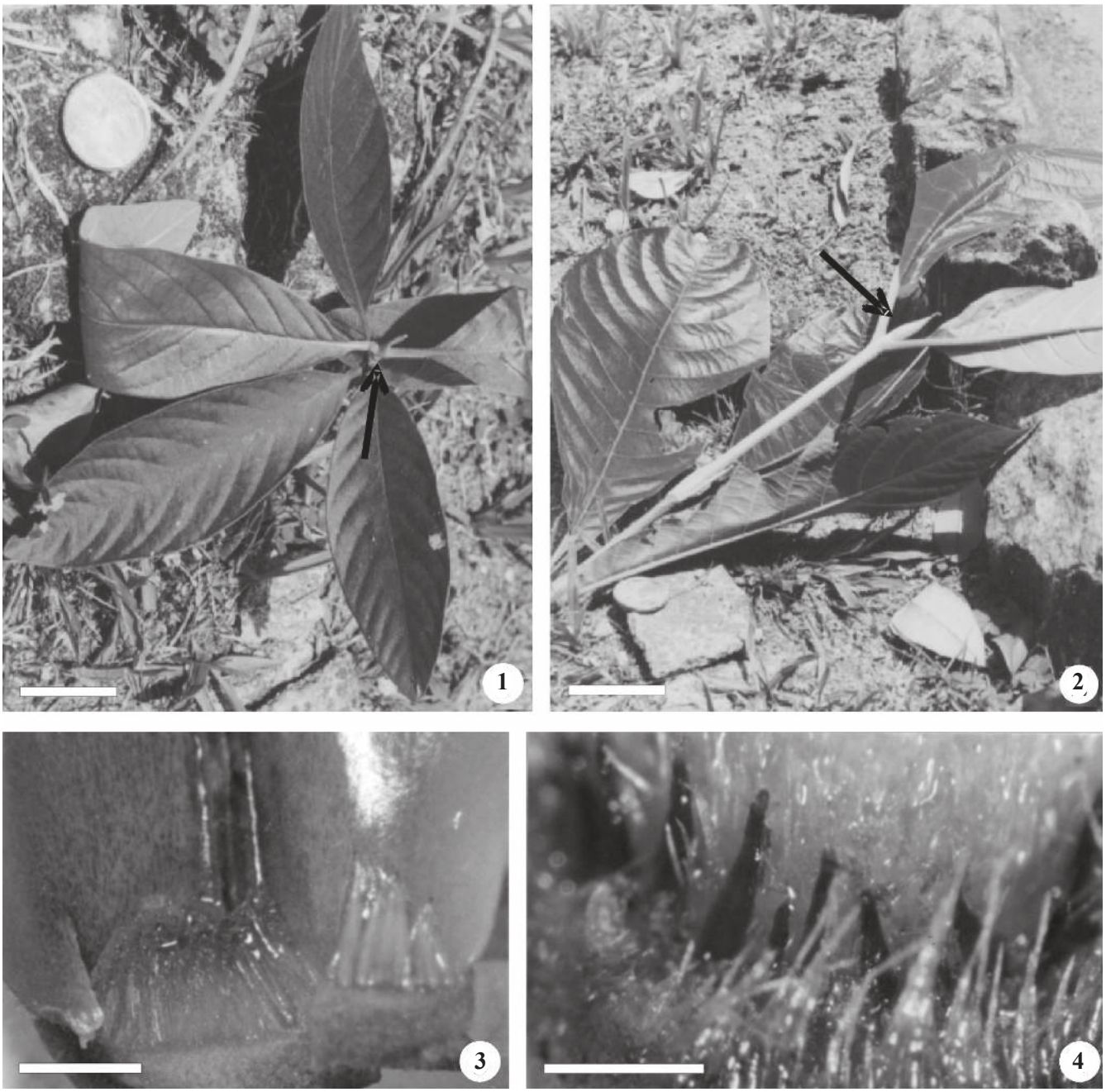
Bathysa stipulata and *B. gymnocarpa* have persistent stipules (figures 1 and 2) in the shoot apex. The colleters are located on stipule adaxial surface and are about 1 mm in height. These secretory structures are distributed in lines (figures 3 and 4). *Bathysa gymnocarpa* has a greater amount of colleters than *B. stipulata*. The secretion of the colleters is translucent and covers the interior of the shoot apex. Even after the exposure of samples to all the procedures described, it was possible to observe secretion over the stipule adaxial surface (figure 5).

During this study, in order to better understand the colleters structure, two phases were distinguished: a secretory phase and a senescence one. In the secretory phase (figure 5), standard colleters denominated in this study like lachrymiform type presented yellow color in fresh material (data not shown). In the senescence phase (figure 6), the colleters present brown color in fresh material (data not shown), and a rough surface (figure 5-8). Colleters from both species fall easily, during the last phase.

Colleters from the studied species are constituted of an epidermal palisade layer and a central axis formed by non secretory parenchyma cells (figures 9-11) with rare vascular traces (figure 9). A constriction at the colleter base was seen (figure 10). This structure is non-secretory and easily distinguished at the secretory phase.

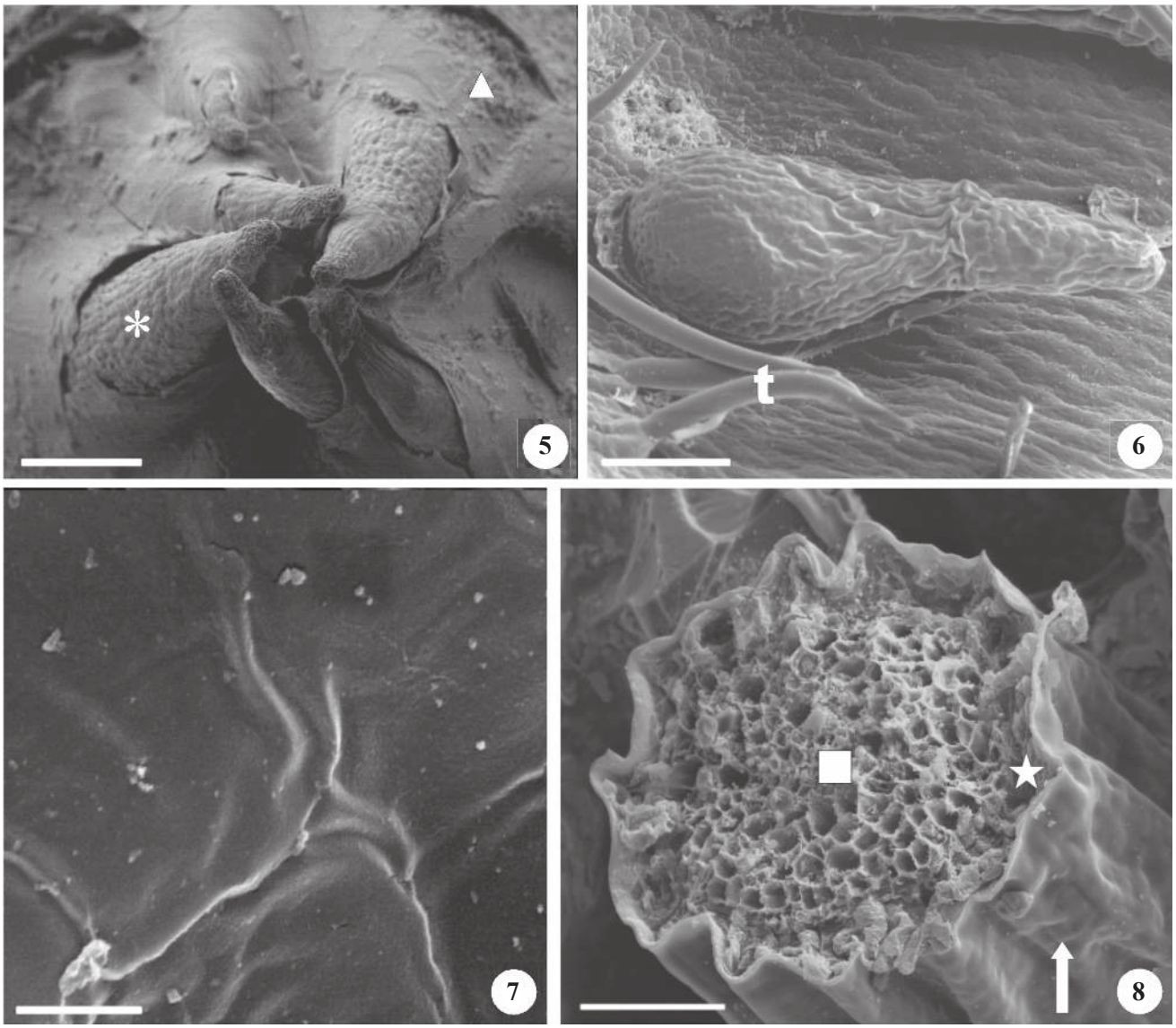
During the secretory phase, the palisade epidermal cells presented a dense cytoplasm in transversal section (figure 12). All epidermal secretory cells presented undefined contours, which provided a difficult visualization of the secretory tissues (figure 12). Secretory cells of both *B. gymnocarpa* and *B. stipulata* presented dense cytoplasm, vacuoles, enhanced rough and smooth endoplasmic reticulum (data not show) distributed along the cytoplasm, hypertrophy of Golgi apparatus (figure 13) secreting small vesicles that fuse with large vesicles (figures 13 and 14), mitochondria, and evident nuclei. In *B. stipulata,* lipid vesicles were observed in the cytoplasm close to the cell wall (figure 14). These vesicles appeared to be fusing with the membrane (figure 14). In *B. gymnocarpa,* electrondense vesicles were observed all over the cytoplasm, including the region close to the plasma membrane. These vesicles seem to dock, fuse and unload their content in a space between the cell wall and the plasma membrane (figures 15-18).

The outer cell wall of the *Bathysa* colleters during the secretory phase exhibited a polysaccharide portion, a cuticular membrane and a cuticle proper (figures 19 and 20). The cuticular membrane presented arborescent and reticulated strata (figures 19 and 20). No cuticle rupture was observed. Both the polysaccharide portion and the cuticular layer reacted with PATAg (figure 21), for acid polysaccharides, and with ruthenium red, for pectin (data not shown). The lipid portion of the cuticular membrane, which includes the cuticle proper and the matrix of the cuticular layer, reacted with osmium/imidazole (figure 22).



Figures 1-4. Leaves and shoot apex. 1. *Bathysa stipulata* leaves and shoot apex (arrow). 2. *Bathysa gymnocarpa* leaves and shoot apex (arrow). 3. Colleters distributed in lines at stipule adaxial surface base in shoot apex of *Bathysa stipulata*. 4. *Bathysa gymnocarpa* stipule base adaxial surface showing colleters organization. Bar = 2.5 cm (1, 2), 0.15 cm (3), 0.3 cm (4).

The epidermal secretory cells of studied species observed in the senescence phase were anatomically disorganized (figures 23 and 24) and presenting irregular outlines. These cells presented a collapsed aspect, making their individualization difficult (figures 23 and 24). The cellular disorganization started in the epidermal cells of the colleter tip (figure 24) and progressively reached colleter base. The epidermal cells presented a dramatic change between secretory (figure 9) and senescence phases, when compared to vascular and parenchymatic cells. In these tissues there were only slight changes between the secretory and senescence phases (figure 23). Most of the parenchymatic cells did not exhibit a collapsed aspect, although cell walls outline were not as regular as during the secretory phase. In addition, in some parenchyma cells a highly-stained material was accumulated (figure 23).



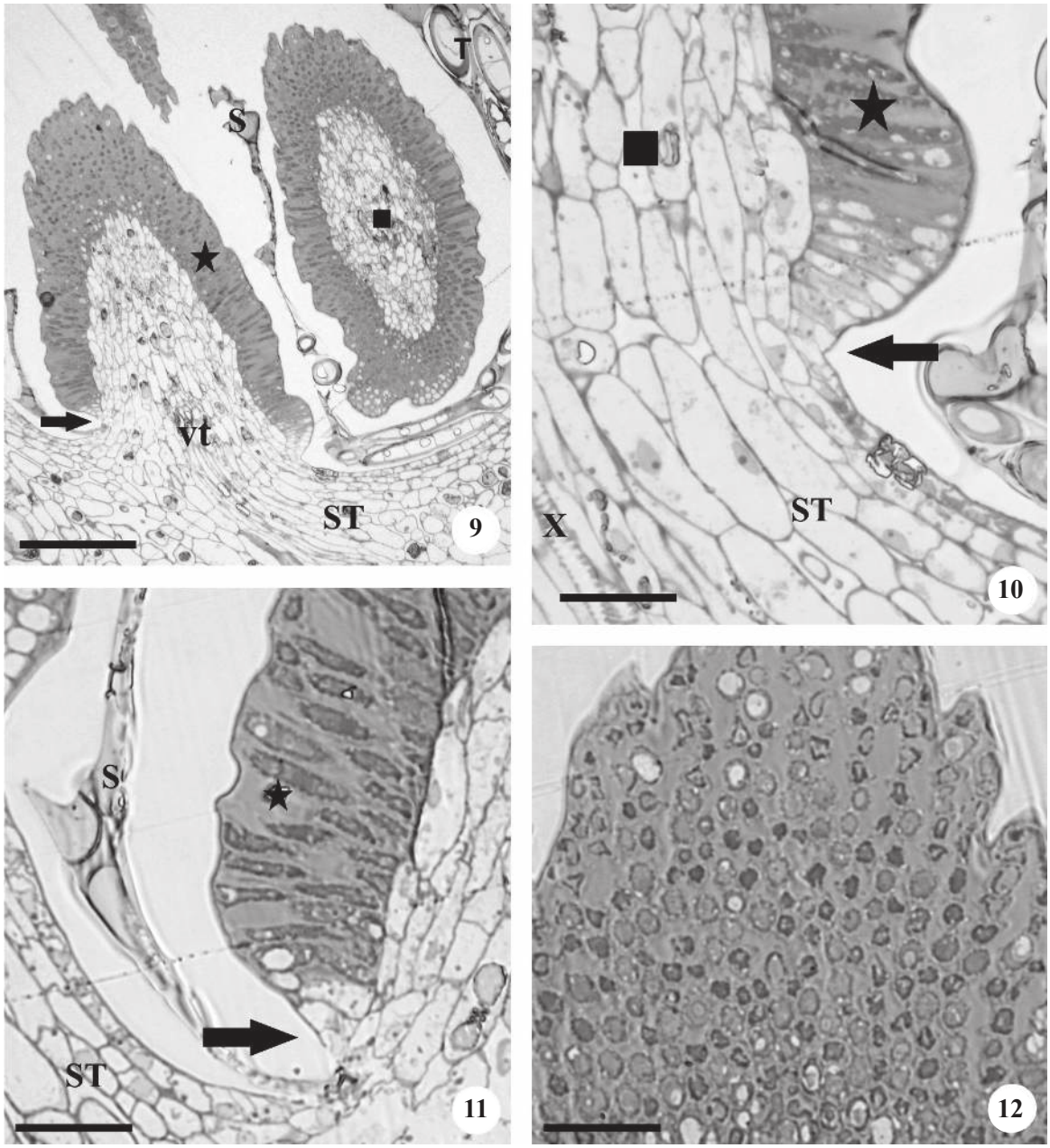
Figures 5-8. Scanning electron microscopy of *Bathysa* colleters. 5, 8. *Bathysa stipulata*. 6, 7. *Bathysa gymnocarpa*. 5. Colleters (asterisk) covered by secretion (triangle). 6. Colleter inserted in the stipule base, close to trichomes (t). 7. Colleter rough surface detail 8.Senescent colleter transverse section showing the rough surface (arrow) and disorganized epidermal cells (star) and central axis (square). Bar = 200 µm (5, 7), 2 µm (6), 100 µm (8).

During the senescence phase, epidermal secretory cells of the *B. stipulata* and *B. gymnocarpa* colleters presented a disorganized membrane system. No intact organelles or vesicles were observed (figure 25). The outer cell wall exhibited a cuticular membrane that seems to disrupt network (figures 19 and 20).

Discussion

Based on morphological data, the stipular colleters of *Bathysa stipulata* and *B. gymnocarpa* were described in secretory and senescence phases. The studied colleters are standard type and were classified as lachrymiform type, similar to the one described by González (1998). The main features that characterize this type of colleter and distinguished them from other types are their palisade-like secretory epidermal cells (Lersten 1974b) and the base diameter that is larger than the tip diameter, forming a tear-shaped structure (González 1998). In the literature, the standard type does not depict colleter morphology, thus the classification of this structure is still confused and requires additional studies.

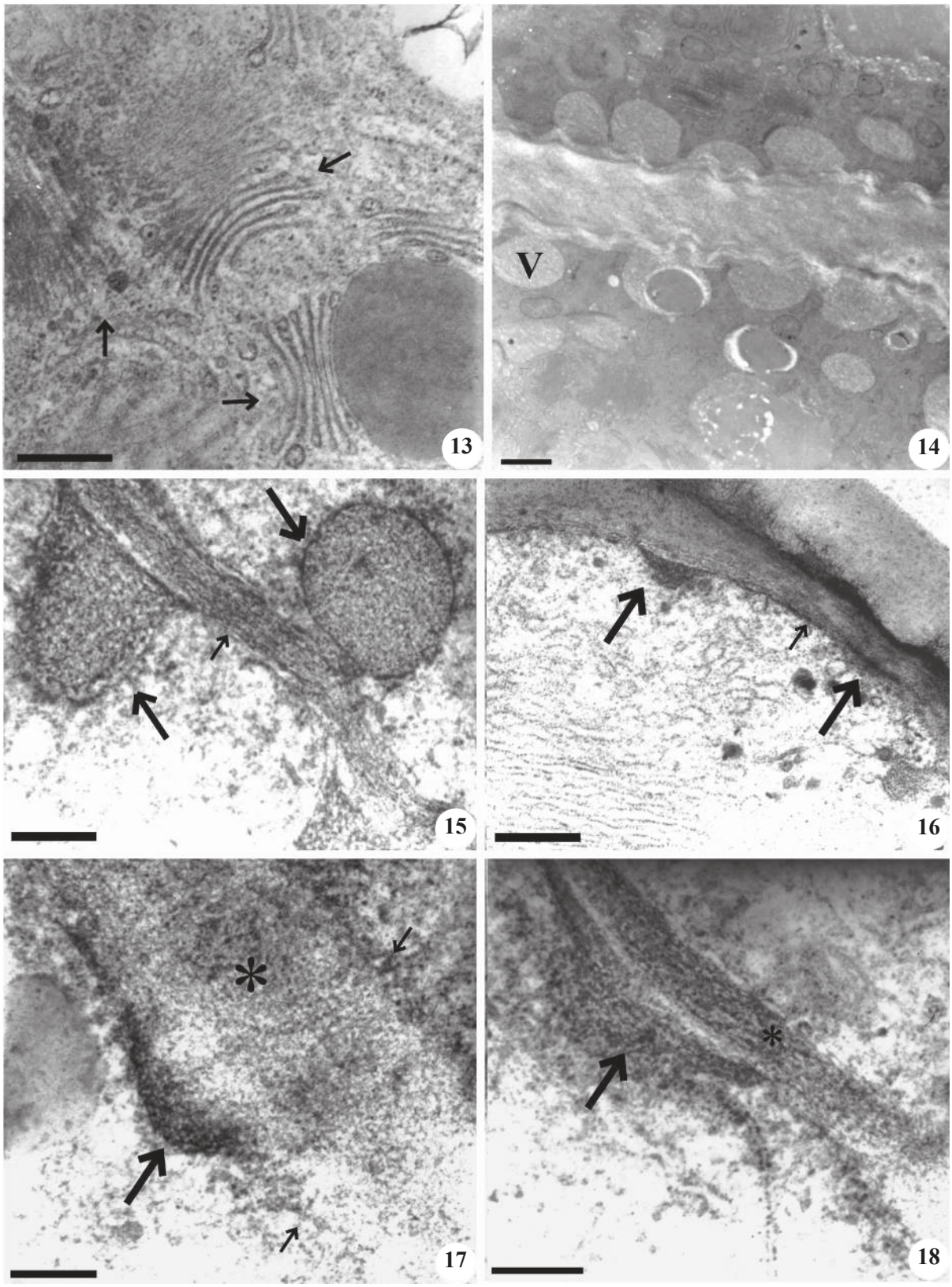
These results showed that the general aspects of anatomy in colleters do not differ much in Rubiaceae



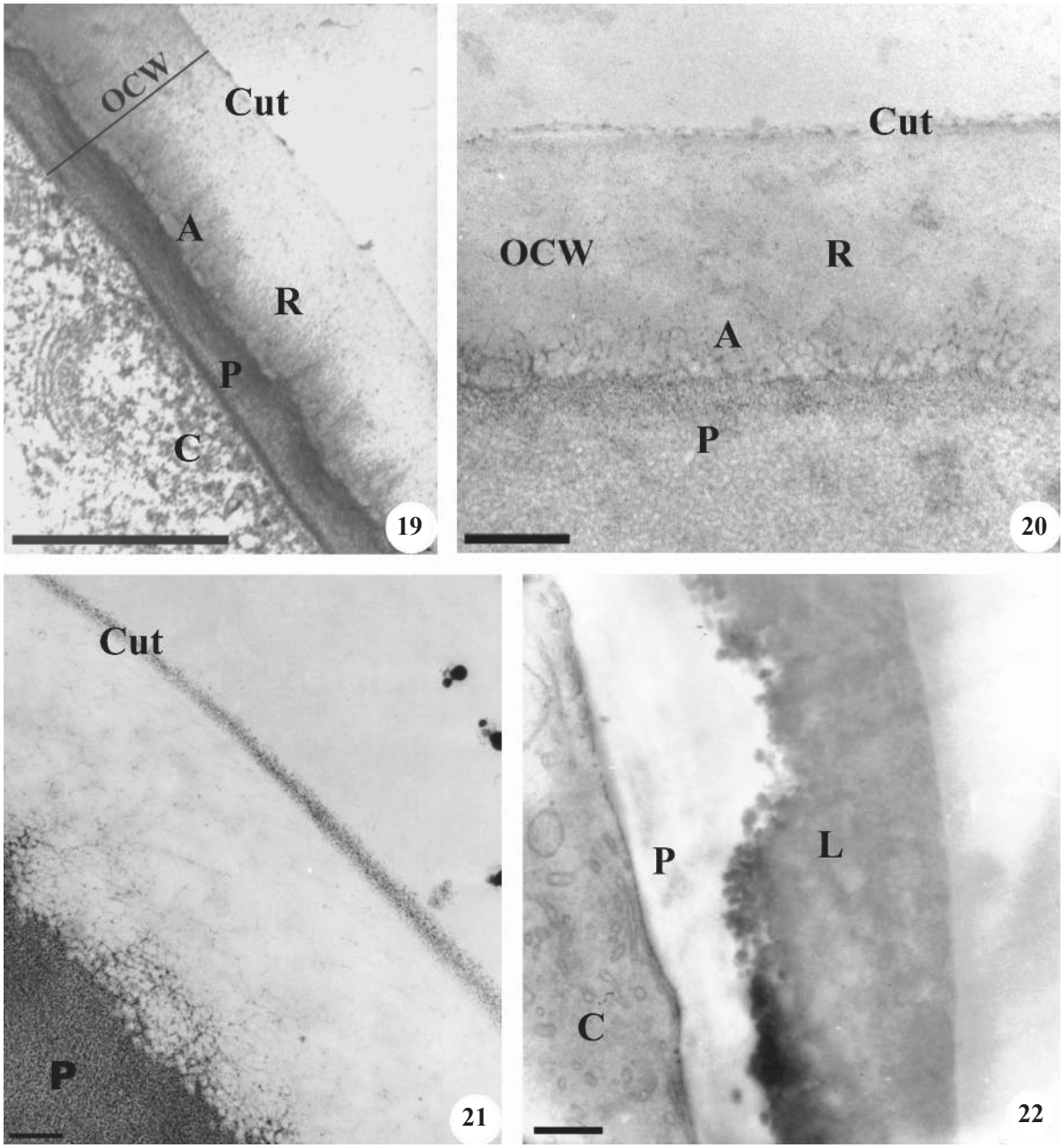
Figures 9-12. Light microscopy of *Bathysa* colleters. 9, 10. *Bathysa stipulata*. 11, 12. *B. gymnocarpa*. 9, 10, 11. Colleter longitudinal section showing the base constriction (arrows), the secretory epidermis (star) and central axis (square). Note secretion (S) outside the cells. 12. Detail of the palisade secretory tissue in longitudinal section. (ST = stipule; T = trichome; vt = vascular trace; X = xylem). Bar = 50 μm (9), 25 μm (10, 11, 12).

genera, as seen for a few species of *Pavetta*, *Neorosea*, *Tricalysia* (Lersten 1974b), *Simira* (Klein *et al.* 2004), and in previously studied *B. nicholsonii* K. Schum. (Miguel *et al.* 2006). Most anatomical features are also similar to colleters from other families, *i.e.*, Apocynaceae (Appezzato-da-Glória & Estelita 2000) and Turneraceae (González 1998). *Bathysa gymnocarpa* and *B. stipulata* presented rare vascular bundle in the middle axis, as seen in most species (Thomas 1991, Appezzato-da-Glória & Estelita 2000, Klein *et al.* 2004). But the vascular traces seem not to be involved with secretion production, as observed by Appezzato-da-Glória & Estelita (2000).

Ramayya & Bahadur (1968) suggested a protection role for colleter, which was mainly attributed to the secretion produced. According to Ramayya & Bahadur (1968), Williams *et al*. (1982), and Mueller (1985), the



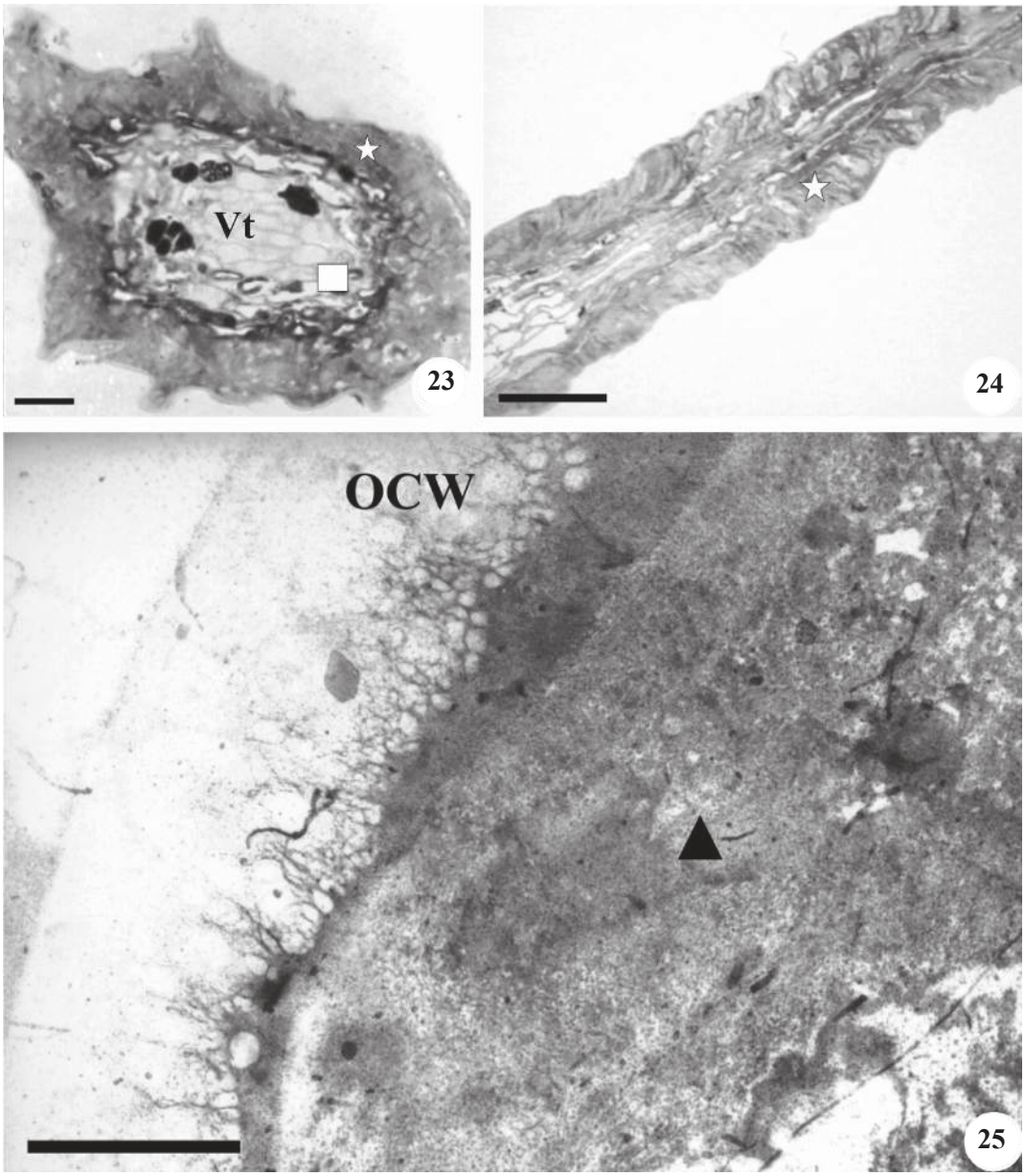
Figures 13-18. Transmission electron microscopy of *Bathysa* colleters secretory cells. 13, 15-18. *Bathysa stipulata*. 14. *B. gymnocarpa*. 13. Dense cytoplasm with enhanced Golgi apparatus (arrows). 14. Cell wall and lipid vesicles (V) appear to be fusing to membrane. 15-18. Vesicle fusion with plasma membrane sequence. Vesicles (large arrows), cell wall (asterisk), plasma membrane (small arrows). Bar = 0.5 µm (13, 14), 0.3 µm (15, 17, 18), 0.8 µm (16).



Figures 19-22. Transmission electron microscopy of *Bathysa* colleters outer cell wall. 19, 20, 21. *Bathysa stipulata*. 22. *B. gymnocarpa*. 19, 20. Outer cell wall layers. 21. Outer cell wall treated according to Thiéry method. Note polysaccharide portion. 22. Outer cell wall treated with osmium/imidazole. Note lipid portion (L). (A = arborescent layer; Cut = cuticle proper; L = lipid portion; OCW = outer cell wall; P = polysaccharide portion; R = reticulated layer;). Bar = 0.1 µm (19, 21), 0.4 µm (20, 22).

exudates can protect the apical meristem preserving it from several damages, such as desiccation and pathogens. *Bathysa nicholsonii* secretion has been tested *in vitro* for anti-fungal activity and presented inhibiting properties against fungal growth (Miguel *et al*. 2006). The secretion of the *Bathysa* colleters is translucent and covers the interior of the shoot apex, suggesting a physical mechanism for the protection of meristematic tissues.

Mucilage secretory cells are characterized by the presence of a dense cytoplasm, nuclei, mitochondria, enhanced endoplasmic reticulum, and Golgi apparatus (Rachmilevitz & Fahn 1972, Fahn 1979). Besides these general features, colleters secretory cells from *Bathysa nicholsonii* present an enhanced endoplasmic reticulum that is particularly close to the cell membrane (Miguel *et al.* 2006). The ultrastructure



Figures 23-25. Senescent colleters of *Bathysa*. 23. Transverse section of senescent colleters of *B. gymnocarpa* under light microscopy showing disorganized epidermis (star) and central axis (square). 24. Longitudinal section of senescent colleters of *B. stipulata* under light microscopy showing disorganized epidermis (star) and central axis. 25. General observation of disorganized cytoplasm (triangle) in *B. stipulata* colleters secretory cells. OCW = outer cell wall, Vt = Vascular trace. Bar = 50 μm (23, 24), 0.12 μm (25).

of the colleters secretory cells of *B. stipulata* and *B. gymnocarpa* presented general features of mucilage secretory cells. These cells were also similar to *B. nicholsonii* secretory cells (Miguel *et al.* 2006), presenting dense cytoplasm, vacuoles, and enhanced rough and smooth endoplasmic reticulum, hypertrophy of Golgi apparatus, mitochondria and evident nuclei. Furthermore, *B. gymnocarpa* also presented a peculiar feature, *i.e.* the presence of electrondense vesicles all over the cytoplasm, in contrast to *B. stipulata*, where lipid vesicles were noted mainly close to the cell wall, and to *B. nicholsonii*, in which none of those aspects were seen (Miguel *et al.* 2006), suggesting different secretory routes for these species.

Dexheimer & Guenin (1981) reported that the Golgi apparatus and endoplasmic reticulum have a role in mucilage production of *Psychotria bacteriophyla* Val. colleters whereas Werker & Kislev (1978) suggested

that mitochondria may can have a role in mucilage production in species of *Sorghum* (Poaceae). The secretion in *B. gymnocarpa* was probably produced in the Golgi apparatus, since a great amount of these organelles were found close to large vesicles, in contrast to *B. stipulata* that exhibited apparently less Golgi stacks. These results indicated that colleters secretory cells of *Bathysa stipulata* and *B. gymnocarpa* transport the secretion mainly by vesicles. This has been previously observed in secretory structures such as nectaries, secretory trichomes, and mucilage secretory cells (Fahn & Shimony 1996).

The paths taken by vesicular traffic have been intensively researched in animal cells but are less well characterized in plant cells (Hawes *et al*. 1999). However, Fahn (1988) proposed models for the passage of secretion to the cell membrane; one of these models is similar to the complete vesicle fusion process observed in *B. gymnocarpa*. Another model proposed by Fahn (1988) is the fusion of the endoplasmic reticulum to the cell membrane for secretion unloading, as also observed in *B. nicholsonii* colleters (Miguel *et al*. 2006). Fricke *et al.* (2000) reported that the unloading of the vesicle, in plant cells, is achieved by a pore formation composed by the partial fusion of the vesicle and the cell membrane. After the unloading, the vesicle becomes flat and recycles back to the endoplasmic reticulum. Modifications in the secretory pathway may be related to secretion composition (Machado 2005).

The senescence is a normal process development controlled by plant genetic program. There are several senescence types in plants, from organs to specialized cells. The colleters after performing their secretory function enter into senescence. Secretory structures, possibly due to their short life, rapidly change aspects such as anatomy and ultrastructure, making their understanding more difficult (Dickinson 2000). Epidermal cells are disorganized, making individualization difficult. The membrane system is also disorganized. Membrane integrity and cellular compartmentalization are maintained until senescence, suggesting that there is little or no leakage of cellular contents (Pennell & Lamb 1997).

Thomas & Dave (1989) showed that senescence in colleters of *Allamanda cathartica* L. was an organized process that involves, first, the epidermal secretory cell lignification, completely loosing tissue identity. Subsequently, the central axis cells were involved in part of this process acquiring the same features. In the senescence phase of *Bathysa gymnocarpa* and *B. stipulata* colleters, the epidermal secretory cells were anatomically disorganized, presenting a collapsed aspect, making their difficult individualization. The cellular disorganization began in the epidermal cells of the colleter tip and progressively reached colleter base. During secretion production and externalization, the ultrastructure of secretory cell was modified according to its life cycle and/or product. These alterations can lead the cell to programmed cell death (PCD) (Gaffal *et al.* 2007). Some studies about structural variation have shown that some organelles and cell wall changed significantly during this process (Machado *et al*. 1995, Klein *et al.* 2004, Miguel *et al.* 2006, Gunawardena *et al.* 2007).

Thomas & Dave (1989) observed the colleters lignification during senescence, however, cell wall structure did not suffer lignification, as in the presently studied species. In secretory structure studies, there is still little knowledge relating cell wall structure and function, especially for secretion passage through outer cell wall. In this way, the knowledge of cell wall structure can be useful to infer its function and physiological aspects. The present study showed that the outer cell wall of the secretory phase of the colleter developed a reticulated network of polysaccharides in its cuticular layer. In contrast, the senescence phase the cell wall seems to disrupt this network. Although many studies report that secretions are released via cuticular rupture (Horner & Lersten 1968, Thomas & Dave 1989), in the cuticle it was not observed to rupture in colleters of *Bathysa* studied species. This observation suggests an involvement of the outer cell wall in the secretion process already discussed in Klein *et al.* (2004) and Miguel *et al.* (2006).

Programmed cell death (PCD) is defined as a sequence of events that can provide controlled and organized cell destruction (Lockshin & Zakeri 2004). This process is important for plant development, pathogen response (McCabe & Leaver 2000), and senescence (Doorn & Woltering 2005). Ultrastructural aspects of senescence secretory cells were similar to classic programmed cell death signals, and Doorn (2005) suggested that senescence may be motivated by PCD. However, there is no literature available relating PCD to ultrastructural changes and senescence in secretory structures.

A secretory phase, the studied colleters present cells with hypertrophic endoplasmic reticulum and Golgi apparatus suggesting activity of synthesis. However, some organelles present membrane disorganization. Senescent colleters are shriveled with secretion at the surface. The epidermic cells are disorganized, with collapsed aspect and internal structures difficult to

individualize. The senescent phase is easily defined by cell structure, but not well defined at secretory cells level. Our investigation suggests that programmed cell death starts in the secretory phase. However, more evidence are needed to evaluate the phenomena.

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