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Ontogenesis, histochemistry and seasonal and luminous environmental characterization of secretory cavities in leaves of *Myrcia splendens* (Myrtaceae)

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Abstract

Secretory cavities are structures that secrete compounds that protects plants against herbivory and pathogenic microorganisms. These cavities have been reported in many genera. However, there are few studies on secretory cavity ontogeny in the genus *Myrcia* (Myrtaceae) as well as the effects of luminosity and seasonality on such secretory cavities. Therefore, the objective of this is to provide new information regarding the ontogenesis, structure, histochemistry and effects of seasonality in *M. splendens*. We collected and analyzed leaves from ten *M. splendens* specimens exposed to sun and shade during both wet and dry seasons. Samples were subjected to standard anatomical techniques for light microscopy. *Myrcia splendens* has schizo-lysigenous ontogenesis with exudates composed of lipids, essential oils, oil-resins and alkaloids. The largest secretory cavities were found in leaves exposed to sun during the dry season. The presence of lipophilic compounds may be an important strategy to plant protection against herbivores. The seasonal variations observed in the leaf secretory cavities demonstrate the anatomical plasticity of such species to light and water availability. As total area of leaf cavities in *M. splendens* reflects the seasonal variation, this should be taken into account when aiming to produce crops meant to essential oils/alkaloids extractions.

Keywords: anatomy; histochemical test; secretory structures; environment.

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Introduction

Plant secretory cavities are widespread internal structures that are present in the tissues of many plant organs such as leaves (Curtis and Lersten 1990; Naidoo et al. 2009; Zhou et al. 2014; Fernandes et al. 2017), stems (Fahn 1988; Palermo et al. 2017), roots (Cury and Appezzato-da-Glória 2009), flowers (Bosabalidis and Tsekos 1982; Chen and Wu 2010), fruits (Bennici and Tani 2004; Liang et al. 2006) and seeds (Rodrigues et al. 2011). The structure consist of an isodiametric lumen (Fahn 1988) surrounded by a layer of epithelium that produces the secretion (Lersten and Curtis 1989).

The lumen of secretory cavities may be filled with mucilage (Ameele 1980; Setia 1984; Fortuna-Perez et al. 2012), oils (Bosabalidis and Tsekos 1982; Curtis and Lersten 1990, 1994; Luna et al. 2013) or a mixture of both (Luna et al. 2019). The secretion in the lumen performs important functions for plants, offering protections against herbivory and pathogenic microorganisms (Fahn 2002).

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Ecological interpretations regarding the possible effects of seasonality (rainy *versus* dry season as well as lightness) on leaves usually take into consideration the cavity contents such as the essential oil yields (Defaveri et al. 2011; Silva et al. 2012) rather than modification in the structure of leaf secretory cavities such as observed by Donato and Morretes (2007).

Secretory cavities are common in Myrtaceae and occur frequently in genera such as *Eucalyptus* L'Hér. (Goodger et al. 2010), *Eugenia* L. (Lemos et al. 2018), *Melaleuca* L. (List et al. 1995), *Myrcia* DC. (Cardoso et al. 2009), *Myrtus* L. (Ciccarelli et al. 2008), *Psidium* L. (Cardoso et al. 2009), and *Syzygium* P.Browne ex Gaertn. (Al-Edany and Al-Saadi 2012).

Myrcia splendens (Sw.) DC. is a perennial species with edible fruits (Lucena et al. 2011). This species is a traditional medicinal plant (Scio et al., 2012) with essential oils

produced by the leaves (Nakamura et al. 2010). *Myrcia splendens* is present from Central to South America in several biomes (McVaugh 1968; Zappi et al. 2015). Specimens growing in the sandy coastal plains of northeastern Brazil, face seasonal variations common of that semiarid region, where the wet season occurs from December to May and the dry season from June to November (Moro et al. 2015). Therefore, species such as *M. splendens* growing in such environments are good models to study the influences of seasonal variations on secretory cavities.

Besides, there are few studies on secretory cavities in *Myrcia* (Cardoso et al. 2009; Gomes et al. 2009; Donato and Morretes 2009; Jorge et al. 2018), especially regarding the ontogeny of such cavities (Ciccarelli et al. 2008). As for *M. splendens*, there are no reports in the literature elucidating the ontogeny of the secretory cavities nor the chemical nature of the secretion. Thus, this paper provides offers new information regarding the ontogenesis, structure, histochemistry and effects of seasonal variations on the leaf secretory cavities of *M. splendens*.

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Material and Methods

Tissue collection

Shoot apices, immature and mature leaves were collected from *M. splendens* specimens growing at the Jardim Botânico de São Gonçalo do Amarante (3°34'07.0"S, 38°53'12.8"W; São Gonçalo do Amarante, Ceará, Brazil). Voucher material was deposited at the Herbário Prisco Bezerra (EAC), acronyms according to Thiers (2019), of the Universiade Federal do Ceará, under the registration number EAC62243 (Sampaio, VS - 170, 26/X/2017). Samples were collected in March 2018 (wet season) and October 2018 (dry season). The region around the garden is semi-arid and most of the annual

precipitation falls between January and May (Castro et al. 2012). In 2018, total annual precipitation was ~1,830 mm (Supplementary Table S1). Minimum precipitation occurred from September to November (~5 mm each) and maximum from January to May (~282 mm) (Supplementary Table S1). Average annual temperature is ~27° C (Supplementary Table S1). Weather data was taken from the nearest weather station, Estação Agrometeriológica at the Universidade Federal do Ceará, ~60 km from Jardim Botânico de São Gonçalo do Amarante.

Light microscopy study

For the ontogenetic study tissues were fixed in a solution of 4% paraformaldehyde and 1% glutaraldehyde in 0.2 M phosphate buffer at pH 7.2 (Karnovsky 1965). The plant material was dehydrated in increasing ethyl alcohol series up to 95% ethanol and embedded in methacrylate resin according to the recommendations from the manufacturer (Leica, Heidelberg, Germany). Cross and longitudinal sections 3-5 µm thick were made in an automatic rotary microtome (Leica RM 2065, Leica Instruments GmbH, Nussloch, Germany) with glass blades. Sections were stained with a solution composed of 1% Astra blue and 0.05% safranin for 12 h. Permanent slides were dried at 37 ºC and mounted with glass varnish.

For the qualitative detection of the chemical nature of cavity exudates, histochemical tests were applied on sections of both developing and fully expanded leaves. Cross and longitudinal sections of the middle third of leaves of embedded material and as well as cross and longitudinal freehand sections of fresh leaves were used. Sudan black B and Sudan IV were used for detection of total lipids (Pearse 1980), NADI reagent for essential oils, oil-resins or a mixture of both (David and Carde 1964), Nile blue sulphate for neutral lipids (Cain 1947), sulfuric acid for sesquiterpene lactones (Geissman

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and Griffin 1971), Wagner's reagent for alkaloids (Furr and Mahlberg 1981), Ruthenium red for pectins/mucilage (Johansen 1940), periodic acid Schiff for total polysaccharides (O'Brien and McCully 1981), xylidine Ponceau for total proteins (O'Brien and McCully 1981) and fixation with 10% ferrous sulphate for phenolic compounds (Johansen 1940).

For measuring the secretory cavities surface area, fully expanded leaf samples were cleared with 10% sodium hydroxide and 20% hypochlorite solutions (Johansen 1940), stained with a mixture of 0.05% safranin and 0.05% fuchsin in 50% ethanol (1:1 by vol) and mounted with glycerin gelatin. Secretory cavities measurements are described below. Observations and photographs were taken using a digital camera Olympus UC 30 (Hamburg, Germany) equipped with light microscope Olympus BX 41TF (Tokyo, Japan).

Seasonal and light variation influence on secretory cavities

Quantitative analysis of the seasonal variation on secretory cavities was performed by comparing the secretory cavities mean area of fully expanded leaf collected during the wet and dry seasons of plants exposed to the same amount of daily sun. Sun leaves were not shaded during anytime of the day while shade leaves were shaded during the whole day. For each treatment (sun and shade *versus* wet and dry), five leaves were used. The middle third of cleared leaves was stained with safranin/fuchsin for measuring the secretory cavities area by using the Image J program (Abràmoff et al. 2004).

After obtaining the data, a completely randomized experimental design was used, with factorial 2 (wet and dry) x 2 (sun and shade) and 15 replications. The results were submitted to the normality test, followed by variance analysis observing the significance by the F test and if statistically significant, the Tukey test was performed at the 5% probability level, using Estimating Statistics (ESTAT)

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Results

Development and structure of secretory cavities

In *M. splendens,* the cavities originate from protodermal cells in leaf primordia. Cells participating in the formation of the secretory cavity in the species may be easily distinguished from the surrounding cells after staining. They are usually larger and present densely stained cytoplasm and large nuclei with conspicuous nucleoli (fig. 1*A*). The mother cell undergoes a single periclinal division originating an initial cell (fig. 1*B*). The innermost initial cell subsequently divides anticlinally generating a second initial cell (fig. 1*C*). After that, these two innermost initial cells undergo continuous divisions in different planes giving rise to a rounded cluster of dividing cells (fig. 1*D*, *E*). The cluster of cells is composed of a single bulky central cell with densely stained cytoplasm cells and a large nucleus which is surrounded by a sheath of cells (fig. 1*E*). The central cell divides in several planes originating a group of large central cells (fig. 1*F*, *G*) while the sheath cells divide mostly anticlinally giving rise to a group of dorsiventrally flat tabular cells which will originate the cavity epithellium (fig. 1*F*, *G*). During the differentiation process of the rounded cluster of dividing cells, the remaining outermost cell (mother cell) divides anticlinally and both cells will originate the cap cells. Cap cells are similar to the other ordinary epidermal cells (fig. 1*H*).

The lumen formation of the secretory cavity begins when the central cells centrifugally move away from each other (schizogeny) (fig. 1*G*, *H*) until a central lumen emerges (fig. 1*I*). As a result, the surrounding cells simultaneously become even flatter (fig. 1*H*, *I*). At the end of development, the central cells undergo autolysis (lysigeny) and the remnants of these autolyzed cells can be observed afterwards (fig. 1*J*). In the fully

developed cavity, the lumen is isodiametric and surrounded by a flat double layered epithelium (fig. 1*K*).

The rising of secretory cavities in *M. splendens* is schizo-lysigenous and asynchronous. Cavities at different stages of development were observed at the same time (fig. 1*K*). Secretory cavities began to develop early in the primordial leaves and persist throughout leaf life span. The secretory cavities in *M. splendens* are spherical or hemispheric and are not seen as translucent dots on the leaf blade when exposed to light (fig. 2*A*). However, the presence of such structures is detected on cleared leaves (fig. 2*B, C*). They are distributed irregularly throughout the whole leaf. In the mesophyll they are predominantly found on the adaxial surface near the epidermis and may be surrounded by the palisade and/or spongy parenchyma. Cap cells can be identified on paradermic sections or cleared leaves by their resemblance to jigsaw puzzle pieces (fig. 2*D*).

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Histochemistry

In developing cavities from young leaves during the schizo-lysigenous process, histochemical tests were positive only for total proteins (fig. 3*A*) and polysaccharides (fig. 3*B*) (Table 1), as indicated by the orange color when stained with xylidine Ponceau and magenta/purple when stained with periodic acid Schiff. Total proteins were found in the central cells while polysaccharides in both central cells and lumen.

In fully developed cavities present in fully developed leaves, there is only synthesis and accumulation of total lipids (fig. 3*C*, *D*), black color when stained with Sudan black or orange/red when stained with Sudan IV; essential oils and oil-resins (fig. 3*E*), as indicated by the violet color which is a mixture of blue (essential oils) and red (oil-resins) in the NADI reaction; acid lipids (fig. 3*I*), intense blue color when stained with Nile blue sulphate; and alkaloids (fig. 3*F*) dark brown/black of the Wagner's reagent

(Table 1). Total proteins and polysaccharides were found in the epithellium cells only while total lipids, essential oils and oil-resins, acid lipids and alkaloids in both epithellium cells and lumen.

Tests for proteins (fig. 3*G*), polysaccharides (fig. 3*H*), neutral lipids (fig. 3*I*), sesquiterpene lactones (fig. 3*J*), pectins/mucilage (fig. 3*K*) and phenolic compounds (fig. 3*L*) were negative for both epithellium cells and lumen of mature cavities (Table 1). All negative tests showed no staining of the secretion. A blank, unstained leaf section is given for comparisons (fig. 3*M*). Histochemical tests for identifying the chemical nature of the cell walls of the cap cells turned positive results only for total lipids as indicated by the orange color when stained with Sudan IV (fig. 3*N*) and black color when stained with Sudan black (fig. 3*N*, *O*). However, both reactions were not very strong.

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Seasonal variation influence on size of secretory cavities

The leaf secretory cavities present in the *M. splendens* showed differences regarding seasonality (Table 2). Regarding the lightness effect, cavities exposed to sun are larger than the ones exposed to shade, despite of being collected during the wet or dry season.

When the cavities exposed to sun are analyzed regarding water availably, the larger cavities were found during the dry season while for cavities exposed to shade there was no significance variance. The statistical significance was 1%.

Discussion

Development and structure of secretory cavities

Secretory cavities in *M. splendens* originate from protoderm and begin with periclinal division of a protodermal cell. Only the innermost cell participates in the

development of the secretory cavity by a series of successional divisions. Protodermal origin is commonly reported in the literature for other plant families such as Asteraceae (Curtis and Lersten 1986; Milan et al. 2006), Leguminosae (Turner 1986; Paiva and Machado 2007), Polygonaceae (Curtis and Lersten 1994), Rutaceae (Bennici and Tani 2004; Liang et al. 2006; Zhou et al. 2014), Velloziaceae (Naidoo et al. 2009) as well as Myrtaceae (Ciccarelli et al. 2003; Kalachanis and Psaras 2005; Retamales et al. 2014). A similar manner for the differentiation of cavities was previously reported for other taxa such as *Citrus* L. (Bosabalidis and Tsekos 1982) and *Myrtus* (Kalachanis and Psaras 2005). However, in some species an anticlinal or more cell divisions preceding periclinal divisions was also reported for other species (Curtis and Lersten 1994; Ciccarelli et al. 2003). Although Bosabalidis and Tsekos (1982) and Ciccarelli et al. (2003) have worked with the same species *Myrtus communis* L., the authors found a different pattern for the first cell division. Kalachanis and Psaras (2005) reports that the first division is periclinal in the same species while Ciccarelli et al. (2003) reports an anticlinal division. It is possible that the two studies examined different subspecies or varieties of *M. communis*. However, neither Kalachanis and Psaras (2005) nor Ciccarelli et al. (2003) specify whether they worked with any variety or subspecies.

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Lumen formation in *M. splendens* is complex and occurs via a process described as schizo-lysigeny (Fahn 1979, 1987). During cavity development two sets of cells are formed: one that makes up the outer layers (epithellium) of the cavity and the other that forms a central globous group of cells. When the cavity is full of tightly grouped large cells, the removal of the central cells begins by separation of the cell walls. This first part of the lumen formation is analgous to the process of schizogeny and has been documented in numerous taxa (Bosabalidis and Tsekos 1982; Turner 1986; Ciccarelli et al. 2003; Liang et al. 2006; Zhou et al. 2014). Development of the cavity continues as the central

cells undergo autolysis, at which point the cells disintegrate in a centrifugal direction (Liang et al. 2006) with visible cell debris present in the lumen (Ciccarelli et al. 2003, 2008). This second stage for lumen formation is known as lysigeny (Fahn 1979). At the end of development, the lumen of the mature secretory cavity in *M. splendens* is bounded by a double-layer of compact epithelial cells that generate the secretions.

In Myrtaceae all three ways for cavity development are found: lysigeny alone (Carr and Carr 1970), schizogeny alone (Johnson 1980; Retamales et al. 2014) and schizo-lysigeny (List et al. 1995; Ladd et al. 1999; Ciccarelli et al. 2003; Kalachanis and Psaras 2005; Ciccarelli et al. 2008). Lumen formation may even vary within a species. For instance, in *M. communis*, Kalachanis and Psaras (2005) found that the development of cavities are schizogenous while Ciccarelli et al. (2003) and Ciccarelli et al. (2008) observed schizo-lysigeny. A possible explanation for such divergence is raised by Turner et al. (1998) who consider lysigeny to be an artifact of fixation. In *M. splendens*, there is asynchronous development of the cavities. The asynchrony observed is not exclusive of Myrtaceae as it was also observed in *Casearia sylvestris* Sw. (Fernandes et al. 2017). The topography and shape of fully developed secretory cavities in *M. splendens* are similar to observations for other Myrtaceae (Ciccarelli et al. 2003; Cardoso et al. 2009; Arruda and Victório 2011; Lemos et al. 2018) and are an important taxonomic character for the family (Kawasaki 1989; Coutinho et al. 2015; Santana et al. 2017). However, secretory cavities in *M. splendens* are only observed in cleared leaves or in leaf sections.

Histochemistry

The chemical composition of the exudates from secretory cavities found in leaves of *M. splendens* is heterogeneous and changes as the leaves mature. In the developing cavities present in immature leaves, the histochemical tests indicated the presence of total

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proteins and polysaccharides in the final stage of schizogeny and during lysigeny, respectively. The presence of total proteins during the schizogeny indicates an intense cellular activity, probably related to the presence of proteolytic enzymes which will be later responsible for the lysigeny during the programmed cell death (Beers et al. 2000; Chen and Wu 2010; Zhou et al. 2014). Consequently, the presence of total polysaccharides during the lysigeny is mostly related to cell walls remnants of degraded cells. Polysaccharides are known to be components of the energy reserve in seeds (Buckeridge 2010; Sechet et al. 2018). Therefore, as polysaccharides are not find in secretions of mature cavities in *M. splendens*, it is possible that this class of compounds from degraded cells are metabolized/resorbed by the surrounding cells.

In the developed cavities only lipophilic compounds were observed in the secretion, that is, total lipids, essential oils/oil-resins and alkaloids. The presence of these metabolites were are common in several plant families (Fahn 1979; Bottega et al. 2004; Cury and Appezzato-da-Glória 2009; Fernandes et al. 2017). In Myrtaceae the presence of lipophilic compounds was found in leaf secretory cavities of *Campomanesia adamantium* (Cambess.) O. Berg (Kuster and Vale 2016), *Eugenia copacabanensis* (Arruda and Victório 2011), *Eugenia neonitida* Sobral*, Eugenia rotundifolia* Casar. (Defaveri et al. 2011), *Myrciaria glomerata* O. Berg (Pacheco-Silva and Donato 2016), *Myrtus communis* (Ciccarelli et al. 2003), *Plinia cauliflora* (DC.) Kausel (Souza-Moreira et al. 2010) and *Ugni molinae* Turcz. (Retamales et al. 2014). In *Myrcia* species, essential oils were also found (Limberger et al. 2004).

Lipophilic compounds secreted by plants are often to protection against herbivores (Fahn 1979, 2002; Spiteller 2008; War et al. 2012; Mithöfer and Boland 2012). Essential oils/oil-resins were also verified in *Eucalyptus* spp. (Heskes et al. 2012*b*, 2012*a*), *E. copacabanensis* (Arruda and Victório 2011) and *Melaleuca armillaris* (Sol Ex Gateau)

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Sm (Chabir et al. 2011). Tests involving essental oils produced by Myrtaceae species showed biological activity against both microbial and herbivores (Chaieb et al. 2007; Regnault-Roger 2013).

The presence of alkaloids was reported in other Myrtaceae species (Porter et al. 2000; Cascaes et al. 2015; Kuster and Vale 2016; Umah et al. 2017). Alkaloids in leaves are related with toxicity and bitter tasting for animals (Harborne 2014), thus protecting mainly against herbivores (Vieira et al. 2001). It is not common for plants to produce large amounts of both lipids and alkaloids in the same organ as compounds may play the same role in antiherbivore strategies (Vieira et al. 2001). Although Cascaes et al. (2015) did not report the presence of alkaloids in *M. splendens*, our histochemical findings detected such compounds in the lumen of the leaf secretory cavities which agrees with Scio et al. (2012) who reported such secondary metabolite in leaf extracts of *M. splendens*. There are two possible explanations for the differences regarding the presence/absence of alkaloids in *M. splendens*: we are dealing with a different variety of *M. splendens* and in fact there are six varieties recognized for this species (IPNI 2020); this could be the result of seasonal variations in the secretion as reported for other species (Elgorashi et al. 2002; Lien 2002; Ncube et al. 2015; Šebrlová et al. 2015).

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Environmental analysis

Secretory cavities of *M. splendens* leaves exposed to sun were always larger than cavities exposed to shade, regardless the season. In studies of secretory cavities in *Eugenia brasiliensis* Lam., larger cavities were observed in plants from sandy coastal plains (*restinga*) where plants grew under direct exposition to sun, in comparison to plants from forest, where plants grew under the canopy (Donato and Morretes 2007). Similar results were obtained from studies of *Eugenia luschnathiana* (O.Berg) Klotzsch ex

B.D.Jacks (Lemos et al. 2018). This could be due to the presence of alkaloids in the secretory cavities. Leaves exposed to higher light intensities are consequently exposed to the harmful effects of UV light (Buchanan et al. 2015) while alkaloids absorb UV-B light and serve putatively to protect the plant from harmful radiation (Binder et al. 2009). Plants that are unable to avoid exposure to enhanced levels of UV-B radiation are at risk (Hollósy 2002). Therefore, the presence of larger cavities in leaves exposed to sun could promote a better way to face the harmful effects of UV light.

Despite total area of leaf cavities of *M. splendens* not showing differences regarding water availability for leaves exposed to shady environments, leaves exposed to sunny environments presented larger cavities in the dry season. Consequently, increased essential oils/oil-resins contents yield is expected. Leaves exposed to sunny environments may present higher total oil yield or higher yield of particular oils (Cruz et al. 2014; Feijó et al. 2014; Souza et al. 2018). In different genotypes of *Lippia gracilis* Phil. (Verbenaceae) for instance, essential oil yield of leaves varied between 1.25% and 1.92% in the rainy season and 1.42% and 2.70% in the dry season (Cruz et al. 2014). However, the under harsh conditions of water deficit, essential oil yield may change as observed for *Cuminum cyminum* L. (Bettaieb et al. 2011). In moderate water deficit (50% of field capacity), the essential oil yield in *C. cyminum* increased. However, in severe water deficit (25% of field capacity) decreased (Bettaieb et al. 2011). Therefore, as *M. splendens* is a traditional medicinal plant (Scio et al. 2012), such variations in total area of leaf cavities should be taken into consideration when trying to find a better way to cultivate such species for extraction of essential oils.

Conclusion

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On the basis of ontogeny this study demonstrates that the secretory cavities in *M. splendens* leaves have a protodermal origin and undergo schizo-lysigenous ontogenesis. The presence of lipophilic compounds (lipids and alkaloids) within the lumen may be an important strategy to plant protection against herbivores. The seasonal variations observed in the leaf secretory cavities demonstrate the plasticity of such species to abiotic environment factors, representing an important anatomical response to light and water availability. As total area of leaf cavities in *M. splendens* reflects the seasonal variation, increasing the total area of leaf cavities of leaves exposed to sun, this should be taken into account for better yields in the extraction of essential oils in this species.

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Figure 1. Ontogenesis of secretory cavities of *Myrcia splendens* as observed in light microscopy. *A*, Protodermal mother cell (arrowhead). Note the densely stained cytoplasm, large nucleus and conspicuous nucleolus. *B*, First periclinal division of the mother cell originating an initial cell (arrow). *C*, Anticlinal division of the initial cell forming a second initial cell (doublearrow). *D*, Cell divisions in different planes giving rise to a rounded cluster of dividing cells. The remaining mother cell divides anticlinally originating the cap cells (asterisk). E, Note the presence of a single bulky central cell (Cc) surrounded by a sheath of cells (Sc). *F* – G, The central cell divides in several planes originating a group of large central cells while the sheath cells divide giving rise to a group of dorsiventrally flat tabular cells. *H*, I - Note the beginning of the lumen formation (Lu) while the sheath cells become flatter by schisogeny and intensely stained epithelium. *J*, Cells autolysis indicating lisogeny. Note the cell debris (Cd). *K*, Mature secretory cavity after schisologenesis process. The mature cavity has one or two epithelium (Ep) layers cells and large lumen. Note the asynchrony of the cavity development as it is possible to observe a developing cavity next to a fully developed one. Cc, central cell; Cd, cell debris; Ep, epithelium; Lu, lumen; Sc, sheath of cells; arrow, initial cell; arrowhead, protodermal mother cell; asterisk, cap cells; doublearrow, second initial cell. Scale bars: 20 µm.

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Figure 2. Morphoantomical characteristics of fully expanded leaves of *Myrcia splendens*. *A*, Fresh leaf. Translucent dots are not observed on the leaf blade. *B*, Cleared leaf. Note the secretory cavities (arrows) througout the leaf blade. *C*, Detail of a secretory cavity. Note the oil drop within. D, Cap cells as observed in front of view as observed in leaf epidermal dissociation. Scale bars: A = 500 µm; B = 200 µm; C–D = 50 µm.

Figure 3. Histochemical tests in developing (A-B) and fully developed (C-O) secretory cavities of *Myrcia splendens* as observed in leaf cross sections under light microscopy. *A*, Presence of total proteins (xylidine Ponceau) in the lumen of developing cavities towards the end of schisogeny. *B*, Positive reaction for total polysaccharides (periodic acid Schiff) in cell debris at the end of lisogeny. *C*, Detection of total lipids (Sudan black B). *D*, Presence of total lipids (Sudan IV). *E*, Positive reaction for essential oils and oil-resins (NADI reagent). *F*, Positive reaction for alkaloids (Wagner reagent). *G*, Absence of total proteins (xylidine Ponceau) in the secretion. *H*, Absence of total polysaccharides (periodic acid Schiff) in the secretion but presence in the epithellium. *I*, Absence of neutral lipids and presence of acid lipids (Nile blue) in the secretion. *J*, Negative reactions for sesquiterpene lactones (sulfuric acid). *K*, No presence of pectins/mucilage (Ruthenium red). *L*, Absence of phenolic compounds (ferrous sulphate). *M*, Blank, untreated section. *N* - O, Detection of total lipids (Sudan IV and, Sudan Black B, respectively) in the cell wall of the cap cells. Note that only the walls which reasemble jigsaw puzzle pieces were stained. Scale bars = 20 µm.

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Table 1. Histochemical tests performed in *Myrcia splendens* secretory cavities.

Histochemical test	Compounds	Color yielded for the test	Developing cavity			Fully developed cavity	
			Epithellium	Central cells	Lumen	Epithellium	Lumen
Xylidine Ponceau	proteins	orange	-	+	-	+	-
Periodic acid Schiff	polysaccharides	magenta/purple	-	+	+	+	-
Ruthenium red	pectins	no staining	-	-	-	-	-
Sudan black B	lipids	black	-	-	-	+	+
Sudan IV	lipids	orange/red	-	-	-	+	+
NADI reagent	essential oils and oil-resins	violet	-	-	-	+	+
Wagner's reagent	alkaloids	dark brown/black	-	-	-	+	+
Nile blue sulphate	neutral lipids	red	-	-	-	-	-
Nile blue sulphate	acid ipids	blue	-	-	-	+	+
Sulfuric acid	sesquiterpen lactones	no staining	-	-	-	-	-
Ferrous sulphate	phenolic compounds	no staining	-	-	-	-	-

(+) refers to the presence of a compound while (-) absence.

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Table 2. Seasonal variation of total area (μm^2) of leaf secretory cavities in *Myrcia splendens* during the wet and dry seasons under sun and shade.

Season	Lightness	
	Sun	Shade
Wet	4.182,6366Ab	3.710,9344Ba
Dry	5.482,1144Aa	3.718,4881Ba

Means followed by the same uppercase letter in the row and the same lowercase letter in the column do not differ statistically from each other by the Tukey test at the 5% probability level.

Draft

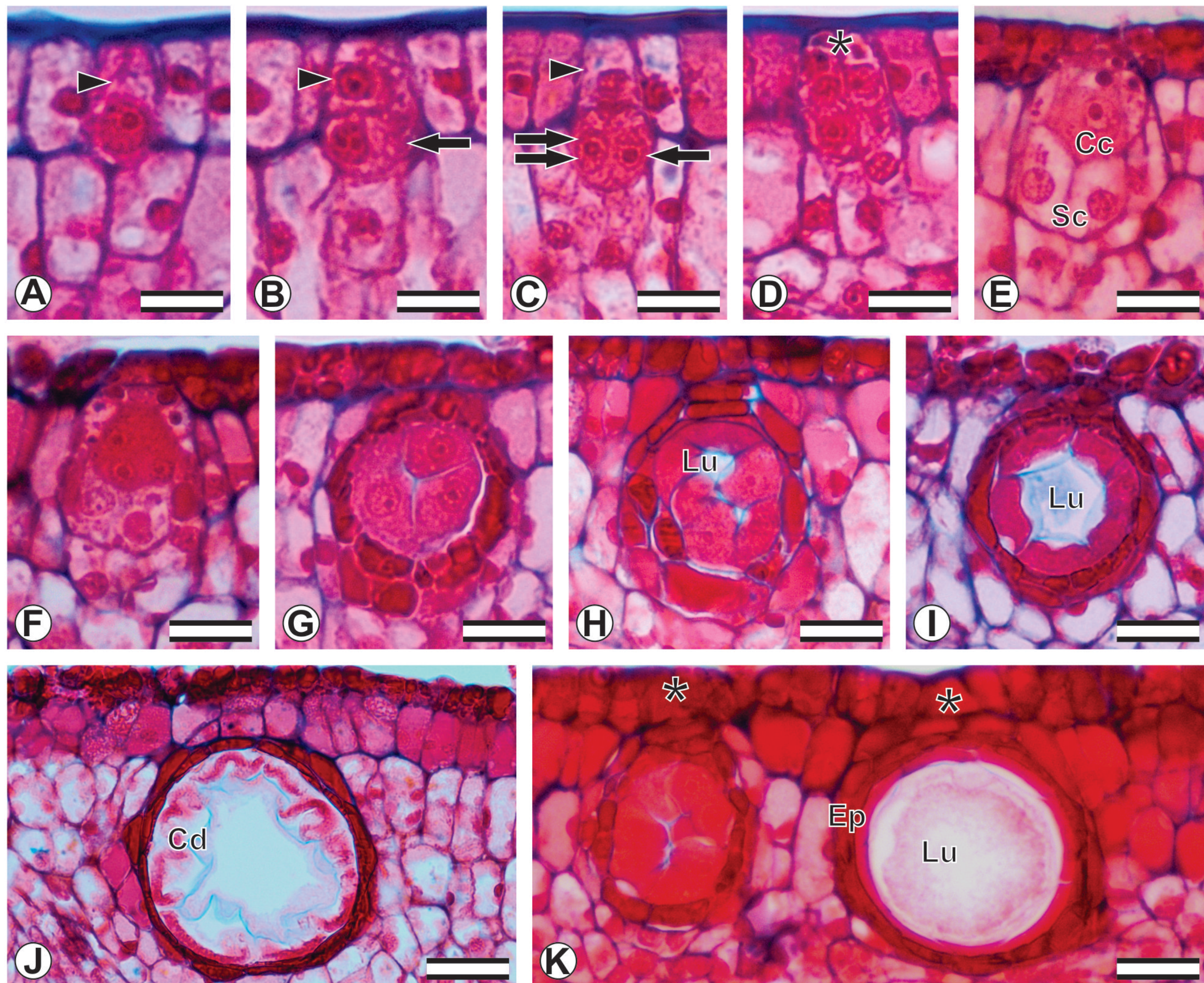

Figure 1. Ontogenesis of secretory cavities of *Myrcia splendens* as observed in light microscopy. *A*, Protodermal mother cell (arrowhead). Note the densely stained cytoplasm, large nucleus and conspicuous nucleolus. *B*, First periclinal division of the mother cell originating an initial cell (arrow). *C*, Anticlinal division of the initial cell forming a second initial cell (doublearrow). *D*, Cell divisions in different planes giving rise to a rounded cluster of dividing cells. The remaining mother cell divides anticlinally originating the cap cells (asterisk). E, Note the presence of a single bulky central cell (Cc) surrounded by a sheath of cells (Sc). *F* – G, The central cell divides in several planes originating a group of large central cells while the sheath cells divide giving rise to a group of dorsiventrally flat tabular cells. *H*, I - Note the beginning of the lumen formation (Lu) while the sheath cells become flatter by schisogeny and intensely stained epithelium. *J*, Cells autolysis indicating lisogeny. Note the cell debris (Cd). *K*, Mature secretory cavity after schisologenesis process. The mature cavity has one or two epithelium (Ep) layers cells and large lumen. Note the asynchrony of the cavity development as it is possible to observe a developing cavity next to a fully developed one. Cc, central cell; Cd, cell debris; Ep, epithelium; Lu, lumen; Sc, sheath of cells; arrow, initial cell; arrowhead, protodermal mother cell; asterisk, cap cells; doublearrow, second initial cell. Scale bars: 20 µm.

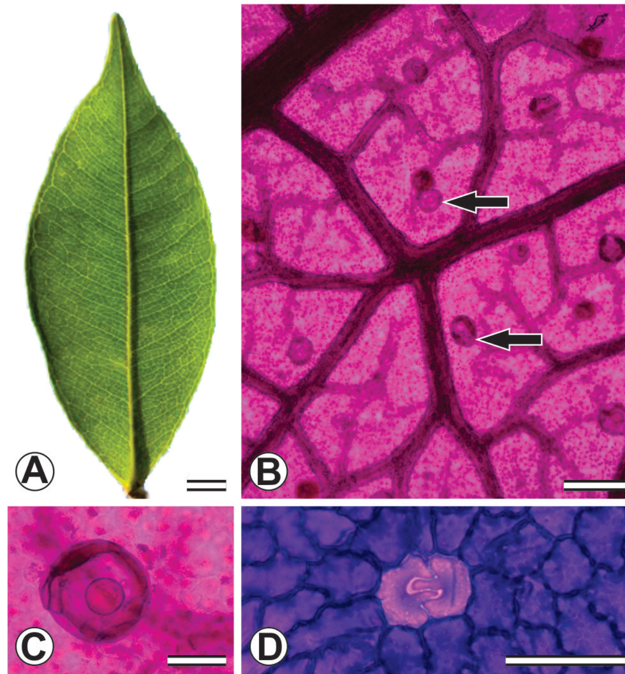

Figure 2. Morphoantomical characteristics of fully expanded leaves of *Myrcia splendens*. *A*, Fresh leaf. Translucent dots are not observed on the leaf blade. *B*, Cleared leaf. Note the secretory cavities (arrows) througout the leaf blade. *C*, Detail of a secretory cavity. Note the oil drop within. D, Cap cells as observed in front of view as observed in leaf epidermal dissociation. Scale bars: A = 500 µm; B = 200 µm; C–D = 50 µm.

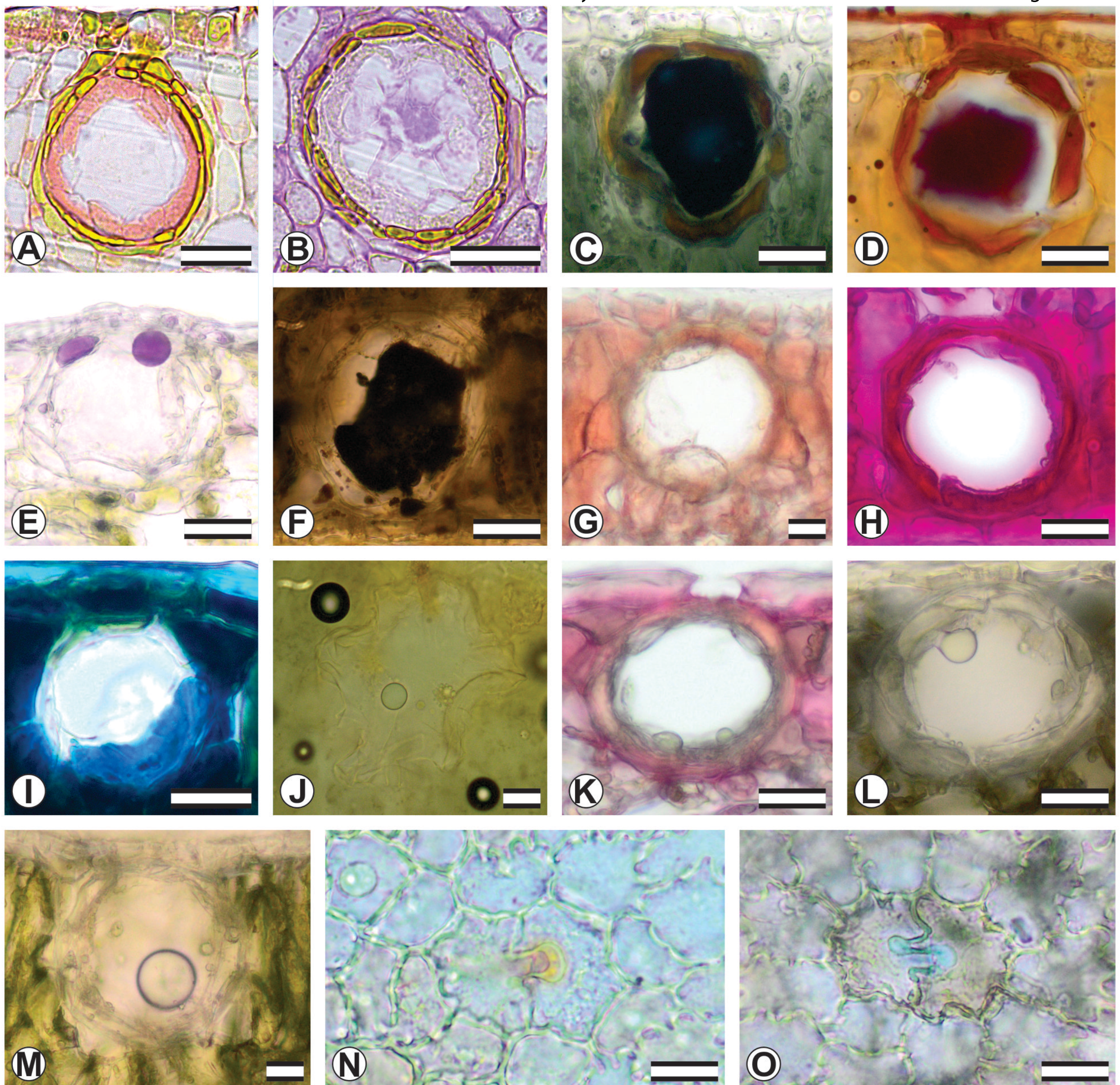

Figure 3. Histochemical tests in developing (A-B) and fully developed (C-O) secretory cavities of *Myrcia splendens* as observed in leaf cross sections under light microscopy. *A*, Presence of total proteins (xylidine Ponceau) in the lumen of developing cavities towards the end of schisogeny. *B*, Positive reaction for total polysaccharides (periodic acid Schiff) in cell debris at the end of lisogeny. *C*, Detection of total lipids (Sudan black B). *D*, Presence of total lipids (Sudan IV). *E*, Positive reaction for essential oils and oil-resins (NADI reagent). *F*, Positive reaction for alkaloids (Wagner reagent). *G*, Absence of total proteins (xylidine Ponceau) in the secretion. *H*, Absence of total polysaccharides (periodic acid Schiff) in the secretion but presence in the epithellium. *I*, Absence of neutral lipids and presence of acid lipids (Nile blue) in the secretion. *J*, Negative reactions for sesquiterpene lactones (sulfuric acid). *K*, No presence of pectins/mucilage (Ruthenium red). *L*, Absence of phenolic compounds (ferrous sulphate). *M*, Blank, untreated section. *N* - O, Detection of total lipids (Sudan IV and, Sudan Black B, respectively) in the cell wall of the cap cells. Note that only the walls which reasemble jigsaw puzzle pieces were stained. Scale bars = 20 µm.