Protoplasma (2024) 261:245–256
https://doi.org/10.1007/s00709-023-01892-0

ORIGINAL ARTICLE

Leaf secretory structures in *Rosa lucieae* (Rosaceae): two times of secretion—two ecological functions?

Valdnéa Casagrande Dalvi[1] · **Maycon de Sousa Silva**[1] · **Alex Batista Moreira Rios**[1] · **Ítalo Antônio Cotta Coutinho**[2]

Received: 28 February 2023 / Accepted: 25 August 2023 / Published online: 12 September 2023

Abstract

Secretory trichomes and colleters are two of the secretory structures whose exudates may cover the body of the plant. Such secretions comprise resins or mucilages which are associated with an array of ecological roles. In Rosaceae, secretory trichomes have been reported for the leaves while colleters associated with leaf teeth. Our study aimed to identify the secretory structures of *Rosa lucieae* and understand the ecological role played by these glands as interpreted by morphoanatomical and histochemical studies. Samples from developing and fully mature leaves were collected, fixed, and processed according to usual techniques for light and scanning electron microscopy. In *R. lucieae*, colleters are restricted to the leaf and stipular margins and are associated with the teeth. They present a parenchymatous axis surrounded by a secretory palisade epidermis and usually fall off after the secretory activity is finished. Different from colleters, secretory trichomes are persistent. They present a multicellular secretory head and stalk. They are found at the base of the leaflet, petiolule, rachis, and petiole and occasionally on the stipular and leaf margins. The colleters predominantly secrete mucilages while the secretory trichomes secrete lipids and terpenes, both via cuticle rupture. The secretory activity of colleters is predominant in the leaf primordia, holding leaflets together and protecting meristems and leaves from desiccation, while the secretory trichomes maintain their secretory activity at different stages of leaf development, protecting different regions of the leaf against pathogens and herbivores.

Keywords Colleter · Leaf teeth · Rosaceae · Sticky secretion · Glandular trichome · Secretory trichome

Introduction

Plant secretions are most of the time complex compounds as they may be composed of a great diversity of both secondary and primary metabolites and are responsible for playing an array of ecological roles (Fahn 1979; Roshchina and Roshchina 1993; Prado and Demarco 2018). Sticky secretions are widely spread in plants and may include resins, mucilage, or even a mixture of both compounds (Paiva 2009; Meira et al. 2014; Dáttilo et al. 2015; Macêdo et al. 2016; Sadala-Castilho et al. 2016; Demarco 2017; Sánchez-Sánchez and Morquecho-Contreras 2017).

Plant resins are mostly composed of volatile and non-volatile high-molecular terpenoids (i.e., in a broad sense as to include terpenes) as well as flavonoids and lipids (Dell and McComb 1979; Roshchina and Roshchina 1993). Their terpene composition differs from that of essential oils as essential oils contain low-molecular mass terpenoids with monoterpenoid being the most representative molecule (up to 90%) (Roshchina and Roshchina 1993; Bakkali et al. 2008; Markus Lange and Turner 2013; Herman et al. 2019). Resins serve a variety of functions in plants, including herbivore deterrence, pollinator attraction, and preventing the invasion of insects, fungi, and bacteria into the leaf (Dell and McComb 1979; Roshchina and Roshchina 1993; Lerdau et al. 1994; Paré and Tumlinson 1999; Langenheim 2003; Sánchez-Sánchez and Morquecho-Contreras 2017).

Handling Editor: Hanns-Heinz Kassemeyer

✉ Valdnéa Casagrande Dalvi
valdnea.dalvi@ifgoiano.edu.br

[1] Laboratório de Anatomia Vegetal, Instituto Federal de Educação, Ciência e Tecnologia Goiano (IF Goiano, Campus Rio Verde), Rodovia Sul Goiana, Km 01, Zona Rural, Rio Verde, Goiás 75901-970, Brazil

[2] Departamento de Biologia, Centro de Ciências, Universidade Federal Do Ceará, Campus Do Pici, Bloco 906, Av. Mister Hull S/N, 60.440-900, Fortaleza, Brazil

Mucilages are complexes of water-soluble acid and/or neutral polysaccharide polymers of high molecular weight (Fahn 1988). They have a wide distribution in plants, forming colloidal solutions (hydrocolloids) that in contact with water become viscous (Patten et al. 2010; Calle et al. 2021). They may be involved in plant protection against excessive sunlight, in anti-herbivore strategies, in the capture of insects by carnivorous plants, and many others (Fahn 1979; Baas and Gregory 1985; Roshchina and Roshchina 1993; Paiva 2009; Patten et al. 2010; Krimmel and Pearse 2013; Tresmondi et al. 2017; Caperta et al. 2020; Calle et al. 2021).

Both resins and mucilages may become sticky and in plants and apart from ducts and cavities, two secretory structures are commonly related to the production of such substances: glandular trichomes and colleters (Dell and McComb 1979; Fahn 1979; Wagner 1991; Roshchina and Roshchina 1993; Langenheim 2003; Paiva and Martins 2011; Chin et al. 2013; Meira et al. 2014; Coutinho et al. 2015; Costa et al. 2020). Most types of glandular trichomes found all over the plant body are an important source of lipophilic substances such as lipids, waxes, essential oils, and resins (Maffei et al. 1989; Wagner 1991; Tozin and Rodrigues 2019; Muravnik 2020). These lipophilic secreting trichomes are often involved in the protection of plants against herbivores and pathogens (Fahn 1979; Roshchina and Roshchina 1993).

On the other hand, colleters are secretory structures that produce a sticky secretion mainly composed of mucilages or a mixture of mucilages and terpenoids (Fahn 1979, 1990; Thomas 1991; Klein et al. 2004; Miguel et al. 2006; Dalvi et al. 2014). Colleters act as lubricating and protecting shoots and buds against dehydration (Mayer et al. 2013).

Although predominant on the basal adaxial side of stipules, leaf blades, bracts, calyx, and corolla (Thomas 1991; Mayer et al. 2011; Coutinho et al. 2015; Macêdo et al. 2016), recent studies have highlighted the presence of colleters associated with leaf teeth in different groups of plants (Gonzalez and Tarragó 2009; Paiva 2012a; Chin et al. 2013; Mercadante-Simões and Paiva 2013; Vitarelli et al. 2015; Fernandes et al. 2016; Meira et al. 2020; Rios et al. 2020; Silva et al. 2022).

Both secretory trichomes (Hashidoko et al. 2001; Faghir et al. 2011; Adumitresei and Gostin 2016; Chwil and Kostryco 2020) and colleters (Chin et al. 2013; Kumachova et al. 2021; Silva et al. 2022) have been found on leaves of Rosaceae Juss. For the genus *Rosa* L., although the anatomical studies have focused on secretory trichomes found in floral parts (Caissard et al. 2006; Sulborska and Weryszko-Chmielewska 2014; Wang et al. 2019), trichomes on vegetative parts have also been described such as those curiously found on the prickles (Zhou et al. 2021) or the ones found on the leaves and stems (Wang et al. 2021).

Thus, given the different functions attributed to sticky secretions and the occurrence of glandular trichomes and colleters in Rosaceae species, our study aimed to use *Rosa lucieae* Franch. & Rochebr. ex Crép as a model to understand the ecological role played by the leaf glands found in *Rosa* as interpreted by the morphoanatomical characterization of such glands as well as the histochemical nature of their secretion in accordance with the life leaf span.

Material and methods

Plant material

A population of *R. lucieae* being cultivated on the campus of the Instituto Federal de Educação, Ciência e Tecnologia Goiano, campus Rio Verde (State of Goiás, Brazil), Brazil (17°48′15″S, 50°54′24″W; 750 m asl), was used for collection of plant material. Flowering branches were collected, dried, and deposited in the collection of the herbarium at the Instituto Federal Goiano, campus Rio Verde, Goiás, Brazil (IFRV 1256).

Samples of leaf primordia and expanded leaves (Fig. 1) were fixed in FAA (formalin:acetic acid:70% ethanol, 1:1:18 by volume) for 48 h, and later dehydrated and stored in 70% ethanol (Johansen 1940). The sampling included the leaflet margins, petiolule, rachis, stipules (adnate to the petiole), and petiole. Photographs were made on Bel Photonix stereomicroscope (WF10X, China).

Light microscopy and histochemistry

For evaluating the presence and distribution of the glands on the leaf, samples from the material stored in 70% ethanol were cleared with 10% commercial sodium hydroxide and 20% sodium hypochlorite, stained with 0.1% basic fuchsin in 50% ethanol (Foster 1949, modified), dehydrated, and mounted with synthetic resin (Permount, Fisher Scientific, NJ, USA).

For the anatomical characterization, samples from the material stored in 70% ethanol were either dehydrated in an ethyl series for embedding in glycol methacrylate resin (Leica Instruments, Heidelberg, Germany) following the manufacturers recommendation. Alternatively, part of the material was dehydrated in a tert-butyl series for paraffin embedding (Johansen 1940) (i.e., paraffin added of dimethyl sulfoxide; Histosec Merck, Darmstadt, Germany). Cross, transversal, and paradermal sections at 5–7 µm thick from material embedded either in paraffin or resin were obtained using the rotary microtome (Model 1508R, Logen Scientific, China).

The sections from the resin-embedded material were stained with toluidine blue at pH 4.7 (O'Brien et al. 1964) and mounted with synthetic resin (Permount, Fisher Scientific, NJ, USA). The sections from the paraffin-embedded

Fig. 1 Morphological characterization of the leaves and colleters of *Rosa lucieae* as observed under the stereomicroscope (**a**, **e**, **f**, **h**, **i**) and in the field (**b**–**d**, **g**). **a** Translucent glands on the stipule margins (black circles). **b** Young leaflets with reddish margins. **c** Leaflets with sealed margins due to colleter exudates. **d** Expanding leaflet with colleters associated with the teeth (white circles). **e** Secretion (exudates) droplet (se) produced by colleters. **f** Note the sticky aspect of the secretion (se). **g** Expanded leaflets. **h** Detail of the colleter exudates on an expanded leaflet. **i** Blackish color of colleters (black circles) on fully expanded leaflets

material were subjected to histochemical tests including Xylidine Ponceau for proteins (O'Brien and McCully 1981) and Sudan III for structural lipids (Pearse 1980). The slides were mounted with synthetic resin (Permount, Fisher Scientific, NJ, USA) or water. The histochemical tests for total lipids with Sudan III (Pearse 1980) or with neutral red as a fluorophore (Kirk 1970) and for essential oils or oil-resins with NADI (David and Carde 1964) were performed in hand-sectioned fresh material. Tests with ruthenium red for pectins (Johansen 1940) and periodic acid-Schiff (PAS) for the detection of total polysaccharides (McManus 1948) were carried out in both fresh and embedded materials. Observations were made on a light microscope (Leica DM500, Heerbrugg, Switzerland) while photographs on an Olympus photomicroscope (BX61, Tokyo, Japan) equipped with an image capture system (DP-73 camera).

Scanning electron microscopy

For the micromorphological study, fragments of the leaflet margins, petiolule, rachis, and stipules stored in 70% ethanol were dehydrated in an increasing ethanol series up to 100% ethanol, and dried at critical point with CO_2 (Bozzola and Russel 1992) (Autosamdri®, 815, Series A, Tousimis

Research Corporation, Rockville, MD 20852, USA). The samples were mounted on stubs using double-sided tape and coated with gold (25 nm) in a Denton Vacuum (Desk V, Denton Vacuum LLC, Moorestown, NJ, USA). Observations and photographs were performed using a scanning electron microscope (Jeol, JSM – 6610, Tokyo, Japan) equipped with an energy-dispersive X-ray spectroscopy (EDS Thermo scientific NSS Spectral Imaging, Tokyo, Japan).

Results

Two distinct secretory structures were found on the leaves of *R. lucieae*: colleters and secretory trichomes, which differed on the distribution and timing of the secretory activity.

Colleters Colleters were found at the margins of the stipules and leaflets (Fig. 1). The color of the gland and texture of the timing of secretion varied in accordance with the leaf development (Fig. 1). In the leaf primordia, colleters were translucent, as observed on the stipule margins (Fig. 1a), but soon acquired a reddish color (Fig. 1b), as red as the leaflet margins (Fig. 1c–f). At this stage, colleters produced transparent sticky exudates (Fig. 1e) which kept their margins sealed and held the leaflets closed (Fig. 1b, c). Exudates in fully expanded leaves (mature leaves) become solid (Fig. 1g, h) if found at all on the colleters, while the colleters themselves turned brownish or blackish (Fig. 1i).

Colleters were found on the leaflet basal portion (Fig. 2a), between the teeth, and in the margins associated to the teeth (Fig. 2b, c). On young leaves, when teeth were not fully developed, colleters formed a continuous gland-teeth structure, being distinguished only by a constriction placed at the end of the teeth and beginning of the colleter (Fig. 2b). As the teeth developed and become bigger and longer, the constriction turned more evident, marking the end of the teeth, and beginning of the gland (Fig. 2c, d).

Throughout the teeth, a higher number of stomata were observed (Fig. 2e), making the epidermis on the teeth quite different from the remaining leaflet blade. Cuticle rupture (Fig. 2e, f) was found as a way to release the secretion to the outer side of the colleters. Older colleters withered, and therefore, their surface becomes wrinkled (Fig. 2g–i). At this stage, bacteria were found on the colleter exudates (Fig. 2j).

The secretory portion of the colleters was composed of a parenchymatous central axis surrounded by a uniseriate palisade (Fig. 3a, b) covered by a thick cuticle (Fig. 3c). Calcium oxalate crystals, druses, were found on the parenchymatous central axis (Fig. 3d). Colleter secretion (still within the colleter) and exudates (on the outer side) were composed of total polysaccharides (Fig. 3d) and pectins (Fig. 3e), as shown by the PAS test and ruthenium red, respectively. Other compounds such as total lipids (Fig. 3f), terpenes (Fig. 3g), and proteins (Fig. 3h) were found in the secretion and exudates. The chemical nature of the secretion for all colleters, that is, on the teeth tip, between the teeth, or at the base of the leaflet, was the same. On older fully expanded leaves, accumulation of phenolic compounds on the central parenchymatous axis and basal area of colleters was observed, marking the abscission area (Fig. 3i).

Secretory trichomes Secretory trichomes were found at different areas on the leaves (Figs. 4, 5, and 6). They are unusual between the leaflet teeth (Fig. 4a) and at the leaflet basal area (Fig. 5a) but were commonly found on the petiolule (Figs. 4b and 5b–d), rachis (Fig. 4c), stipules (Figs. 4d and 5e), and petiole (Fig. 4e) and on the abaxial side of the leaflet and midvein. Secretory trichomes exhibited the same pattern of color as observed for the colleters, that is, translucent when young, becoming later reddish and then turning red (Fig. 4a). However, strikingly different from the colleters, they kept red in fully expanded leaves. Sticky exudates were produced by the secretory trichome (Fig. 4d–f).

The secretory trichomes presented a round to oval secretory head and a stalk (Figs. 5 and 6). The head multicellular (Fig. 6a, b) is covered by a cuticle and the stalk is multicellular (Fig. 6a, b). Trichomes were persistent on leaves and were developed early, so they were fully formed even in the leaflet primordia, and, different from colleters, kept their secretory activity even in adult leaves (Fig. 5e).

Total lipids (Fig. 6b) and terpenes (Fig. 6c) were found in the exudates while total polysaccharides (Fig. 6d, e), pectins (Fig. 6g), and proteins (Fig. 6h) were absent. It is interesting to notice that such compounds were found within the secretory head but not in the exudates (Fig. 6d, e, g, h). Cuticle rupture was observed at the very apex of the secretory head, in its central area (Fig. 6e, f).

Discussion

Gland classification and systematic implications

Based on the morphoanatomical analyses and histochemical study of the secretion along with its characteristics throughout the leaf development, the secretory structures present at the leaflet teeth apex in *Rosa lucieae* are characterized as colleters. In *R. lucieae*, colleters are responsible for secreting small translucent secretion drops which could be easily mistaken for water drops such as those observed in the guttation process.

Reports of hydathodes or just the guttation process are common for all three subfamilies of Rosaceae (Belin-DePoux 1969; Lersten and Curtis 1982; Curtis and Lersten 1986; Donnelly and Skelton 1989; Appezzato-da-Glória and

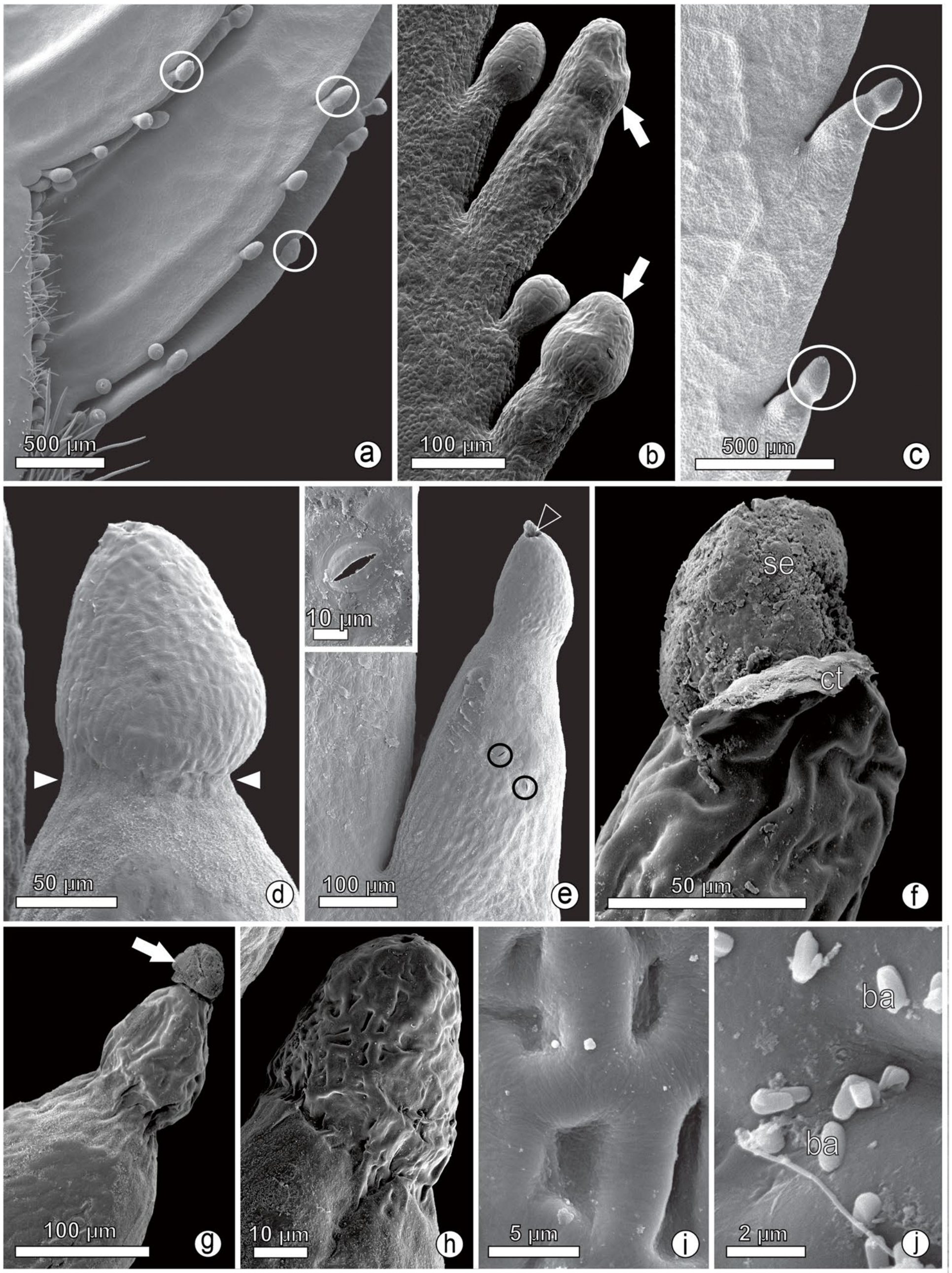
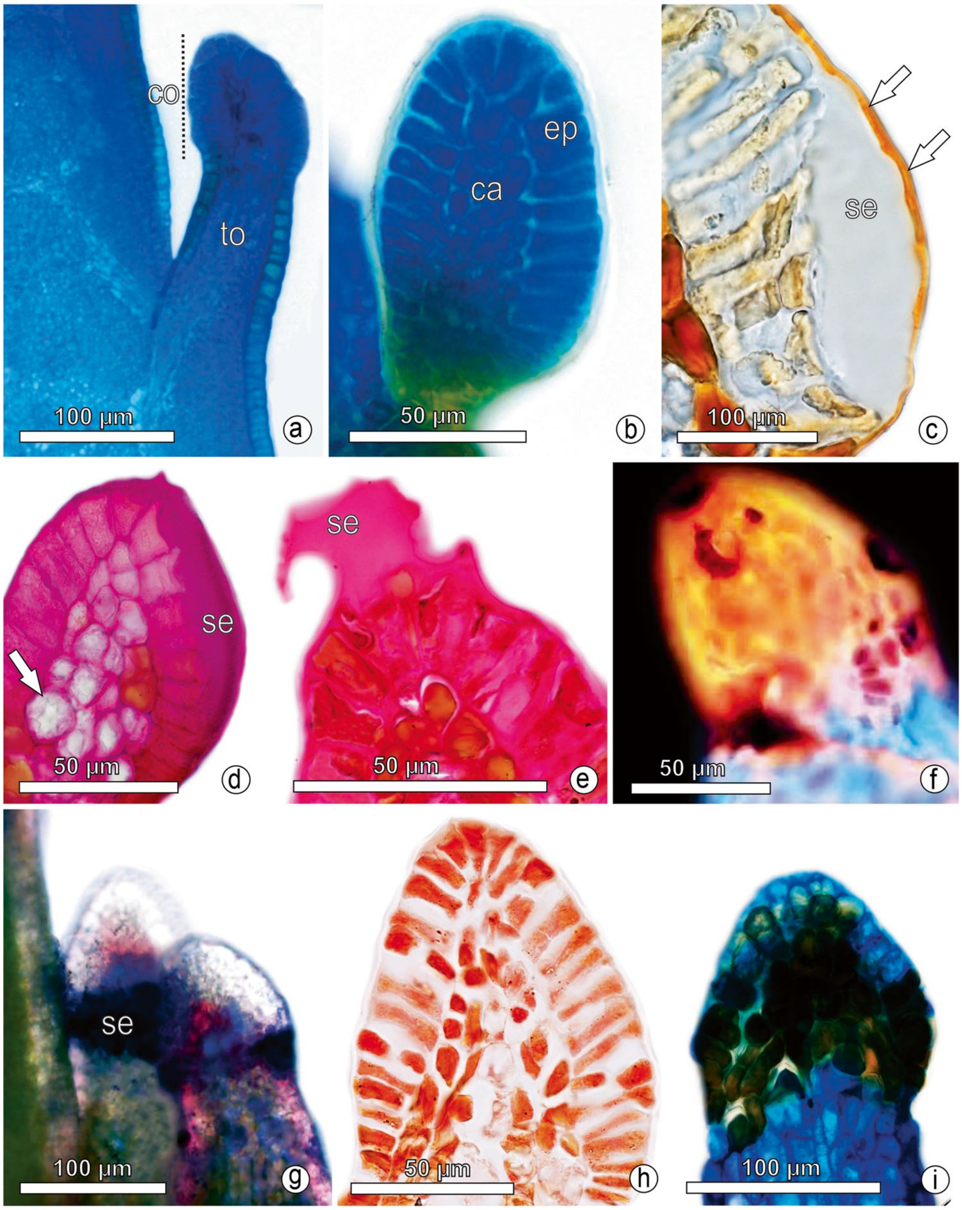


Fig. 2 Micromorphology of the leaf colleter in *Rosa lucieae*. **a** General view of the colleters found on the leaflet basal portion (white circles). **b** Detail of a young leaflet margin showing colleters associated with the very tip of the leaf teeth (white arrows). Note the gland-tooth continuous formed by the association of colleters to leaf teeth. **c** Leaf margin of an expanded leaflet with colleters at the tip of the teeth (white circles). **d** Note the constriction (white arrowhead) between the leaf tooth and colleter. **e** Stomata (black circles) along the leaf tooth and cuticular rupture at the colleter apex (black arrowhead). **f** Cuticular rupture (ct) showing the secretion release (se) to the outer side. **g** Withered colleter with exudates (white arrow) on fully expanded leaflet. **h**, **i** Detail of the wrinkled surface of a colleter. **j** Bacteria (ba) on the surface of a colleter found on fully expanded leaflet

Stalder-Miranda 1991). However, this is the first record of colleters, associated with the leaf teeth for the subfamily Rosoideae. An easy way to differentiate the colleter exudates from guttation drops is the fact that colleter exudates in *R. lucieae* are sticky while guttation drops are not. Colleters on the leaf teeth were indeed reported for *Prunus* spp. (Chin et al. 2013) and *Rhaphiolepis loquata* (formerly *Eriobotrya japonica*) (Silva et al. 2022), genera belonging to the subfamily Amygdaloideae. Colleters in Rosoideae were reported on the stem and midrib for a newly described species, *Rubus alutaceus* B. Moreno, Casierra & Albesiano (Moreno-Medina et al. 2020).

The rosoid teeth present in the Rosaceae family are characterized by the widening of vascularization towards its apex, and as showed in our results, stomata are commonly placed on teeth. Therefore, bearing in mind that the colleter associated with the teeth in *Rosa* sp. may fall off in the adult leaves, as per abscission zone observed in our study, the presence of a vascularized leaf tooth bearing stomata could easily be mistaken for a hydathode. In the past decade, several studies have reevaluated the presence or classification of glands associated with the leaf tooth glands in several plant families and proved that different types of glands may be associated with the leaf teeth (Chin et al. 2013; Fernandes

Fig. 3 Morphoanatomical and histochemical characterization of the colleters found in *Rosa lucieae*. **a** Detail of a leaflet margin showing colleter (co) associated with of the tooth (to). **b** Standard type colleter with a palisade secretory epidermis (ep) and parenchymatous central axis (ca) stained with Toluidine blue. **c** Detail of the thick cuticle (white arrow) stained with Sudan III. Note the secretion (se) accumulated under the cuticle. **d** PAS staining of colleter showing the presence of total polysaccharides (se) as shown by the magenta color. Note the oxalate crystals (druses) in the parenchymatous central axis (white arrow). **e** Ruthenium red test indicating the presence of pectins (red-dark pink staining) in the secretion (se). **f** Neutral red under fluorescence indicating the presence of total lipids—yellowish fluorescence. **g** NADI reagent showing the presence of terpenes (blue color). **h** Total proteins as indicated by red color as a result of the Xylidine Ponceau test. **i** Dark brown color showing the presence of phenolic compounds at the colleter abscission zone



et al. 2016; Meira et al. 2020; Rios et al. 2020; Gonçalves et al. 2020).

In *Casearia* Jacq. (Salicaceae), for instance, hydathodes associated with theoid teeth have been recorded (Thadeo et al. 2014), but upon a reexamination combining the ontogenetic process of the glands as well as histochemical results, authors concluded that such glands were in fact colleters (Fernandes et al. 2016). The same was true for *Prunus* L. as the gland associated with the leaf teeth had been previously called extrafloral nectaries or resin glands when in fact they were colleters (Chin et al. 2013).

Not only the presence of colleter is an important taxonomic trait but also their position on the plant body (Thomas 1991; Silva et al. 2012, 2017; Coutinho et al. 2015; Vitarelli et al. 2015). Although there are few reports of colleters in Rosaceae, colleters were found to be associated with leaf teeth only in two subfamilies: in Rosaceae, as shown in our study for *Rosa*, and in Amygdaloideae for *Prunus* (Chin et al. 2013), *Mespilus*, *Amelanchier*, *Aronia*, *Crataegus* (Kumachova et al. 2021), and *Rhaphiolepis loquata* B.B.Liu & J.Wen (Silva et al. 2022). Kumachova et al. (2021) have also found colleters on the stipules of the genera mentioned above, similarly to our results for *Rosa*. Therefore, a broader study is needed to verify the taxonomical importance of such structures for the Rosoideae and Amygdaloideae families.

Apart from colleters, secretory trichomes were also found in *R. lucieae*. Although several studies have reported the presence of secretory trichomes all over the plant body in representatives of the subfamily Rosoideae, an anatomical and histochemical study of such kind of trichomes has

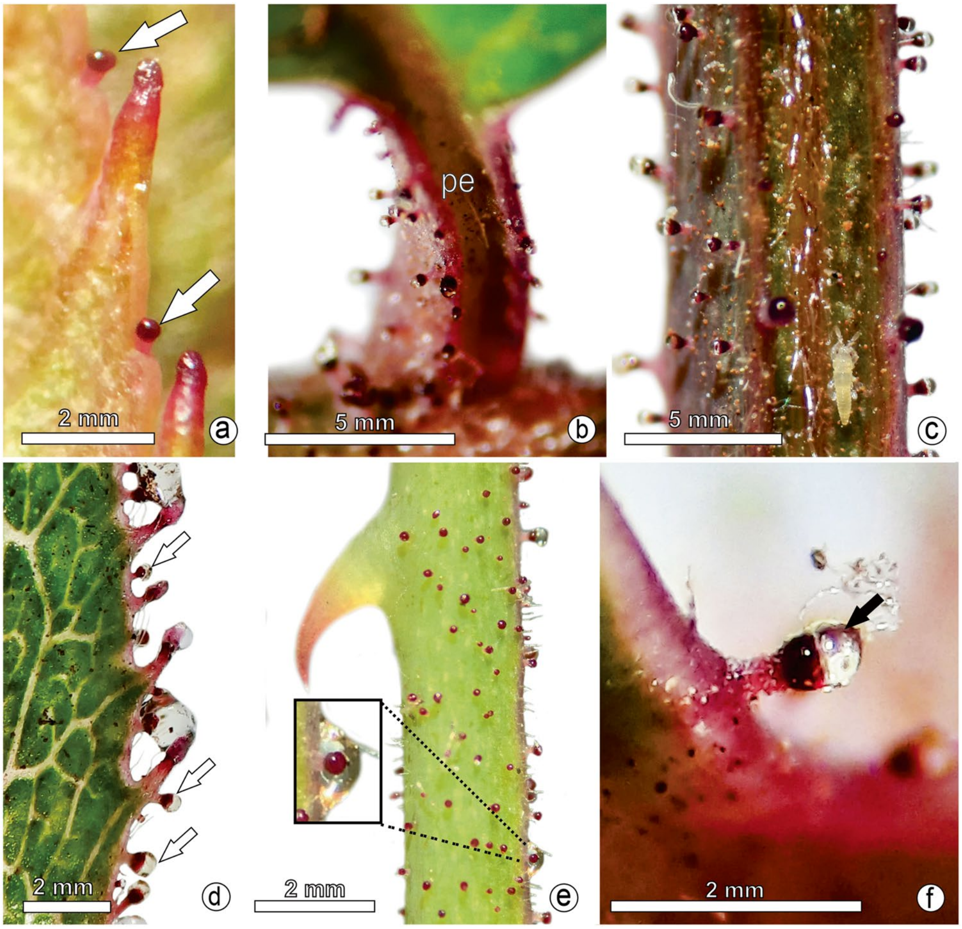


Fig. 4 Morphology of the secretory trichomes found at different leaf areas in *Rosa lucieae* as observed under the stereomicroscope. **a** Secretory trichomes (white arrow) between the leaf teeth in a young leaflet. Note the reddish color at the secretory head. **b** and **c** Secretory trichomes on the petiolule (pe) and rachis, respectively. **d** Secretory trichomes with secretion (white arrows) on the stipule margins. **e** Natural red color of secretory trichomes on the petiole. **f** Detail of translucent secretion (black arrow)

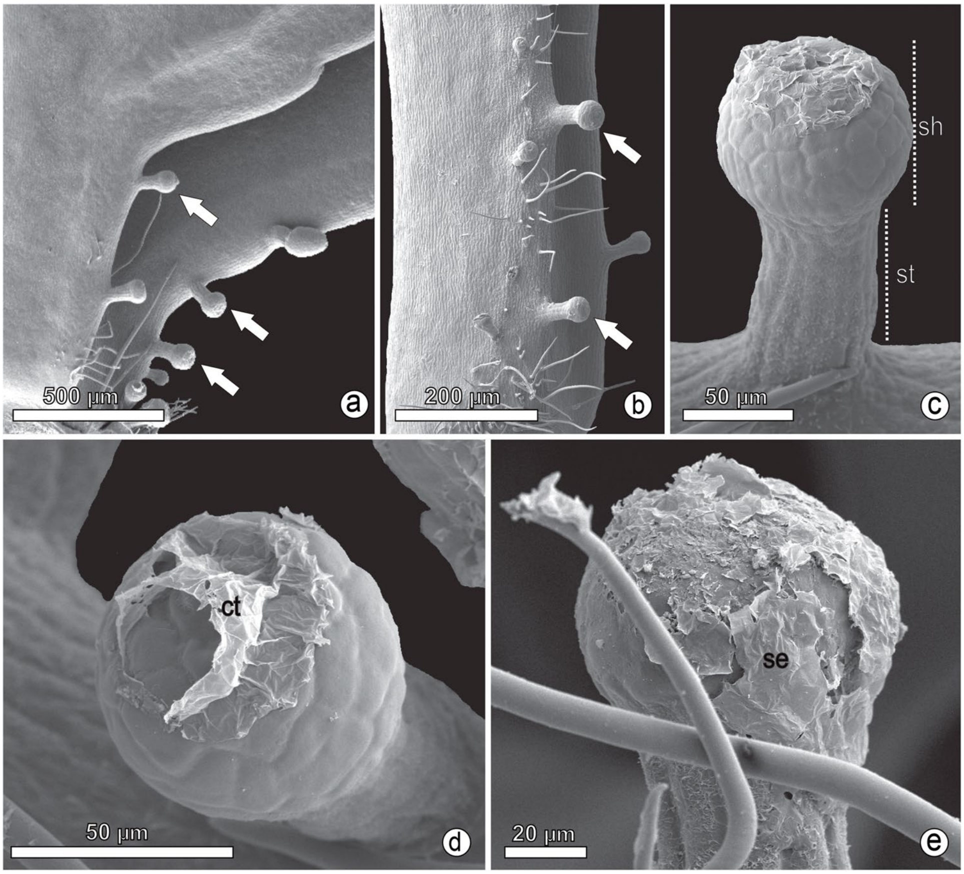
been reported only for hybrids of the genus *Rosa* (Caissard et al. 2006), for *Rosa rugosa* Thunb. (Sulborska and Weryszko-Chmielewska 2014) and *Rubus idaeus* L. (Chwil and Kostryco 2020). Once again, this fact advocates for a broader study of trichomes in the Rosaceae family.

The topography, that is, the position on the plant body, combined with the anatomical and histochemical studies as well as the timing of secretion is important for the correct classification of the glands as pointed out by previous authors (Mayer et al. 2013; Vitarelli et al. 2015; Meira et al. 2020). In *R. lucieae*, for example, colleters are morphoanatomically similar to the secretory trichomes as they both present a round to oval multicellular secretory head composed of a parenchymatous central axis covered with a palisade secretory epidermis and a multicellular stalk. However, both glands are placed at different parts on the plant body. The timing of the secretion and the composition of the exudates also differ. Colleters are exclusive to the leaflet margins and stipules being secretory active only in leaf primordia. On the other hand, secretory trichomes occur on the leaflet base and along the midvein on the abaxial side of the leaflet, and on the petiolule and along the rachis, petiole, and stipules. Secretory trichomes start secreting when leaves are very young, still developing, and keep secreting in fully expanded leaves.

Gland secretion and ecological role

Regardless of being associated with the leaflet teeth, our results show that the colleters in *R. lucieae* are placed on the leaf margin. Colleters are common secretory structures on marginal teeth of several plant families (Gonzalez and Tarragó 2009; Paiva 2012b; Chin et al. 2013; Feio et al. 2016; Fernandes et al. 2016; Meira et al. 2020; Rios et al. 2020) but considering the existing plant diversity that bears leaf teeth, their descriptions in these projections (i.e., leaf teeth) are still scarce. The position of such colleters in *R. lucieae* seems to have evolved in a strategic way as secretion from colleter may aid leaflets to maintain their margins sealed. This would in turn avoid the exposition of a soft, young, and fragile developing organ such as leaves to high levels of solar radiation. This sealing strategy has been suggested by other authors (Mayer et al. 2013; Costa et al. 2020; Meira et al. 2020). Mayer et al. (2013) suggested that the colleter exudates in coffee flowers played a role in keeping the petals united, acting as an adhesive. Therefore, flower buds would

Fig. 5 Micromorphology of the secretory trichomes in *Rosa lucieae*. **a** Secretory trichomes (white arrows) at the leaflet base. **b–d** Secretory trichomes on the petiole. **b** General view. **c** Detail of the trichome stalk (st) and the secretory head (sh). **d** Detail of a cuticle rupture (ct). **e** Detail of the secretion (se)



be sealed preventing the exposition of the developing flowers to low air humidity, hence preventing dehydration. As the colleters in *R. lucieae* are fully developed when leaves are still developing, the secretion produced by such secretory structures acts as lubricating vegetative and reproductive meristems and organs in early stages of development, reducing excessive water loss and protecting organs from dehydration as pointed out by other authors (Fahn 1979; Thomas 1991; Mayer et al. 2013; Ribeiro et al. 2017; Costa et al. 2020).

In *R. lucieae*, colleter exudates form through cuticle rupture. The accumulation of the secretion under the cuticular space may force the bursting of the cuticle as a result of the pressure generated by the substances accumulated in the periplasmic space (Paiva 2016). The dynamic distension, bursting, and final detachment of the cuticle were observed in our study. Besides, secretory pores are not observed in the cuticle, as suggested by other authors as another way for releasing the secretion to the outer side (Paiva 2016; Miguel et al. 2017). Cuticle rupture also occurs in the secretory trichomes of *R. lucieae*.

Bacteria were found on the colleter exudates of *R. lucieae*. In leaf nodulating species of Rubiaceae, it has been suggested that the secretion product of the colleters must sustain the symbionts, that is, the bacteria found living within the secretion (Lersten 1974a, b, 1975; Klein et al. 2004). Bacteria, resident in colleter exudates, would invade stomatal pores and proliferate, establishing the leaf nodules (Lersten 1975; Miller et al. 1984).

The histochemical tests show the presence of heterogenous secretion for the colleter exudates, that is, hydrophilic and lipophilic compounds in *R. lucieae*. Such compounds are widely spread among plants bearing colleters (Mercadante-Simões and Paiva 2013; Coutinho et al. 2015; Tresmondi et al. 2015; Fernandes et al. 2016; Silva et al. 2017; Meira et al. 2020; Teixeira et al. 2021, 2022; Dourado et al. 2022).

The histochemical results obtained in our study are compatible with the hypothesis for the ecological function performed by the leaf colleters. As previously suggested in the literature for reproductive (Mayer et al. 2013; Coutinho et al. 2015) and vegetative (Meira et al. 2020) organs, colleter secretion seals the leaflet margins, reducing the area of exposure to the sun, avoiding excessive water loss, thus preventing these organs from becoming dehydrated.

Tresmondi et al. (2017) showed that colleters producing hydrophilic-rich exudates tend to be more common in forest species (shadier, cooler, and moister microclimates) while lipophilic-rich secretions in savanna species (drier and hotter microclimates) (Tresmondi et al. 2017). Although cosmopolitan to sub-cosmopolitan, the rose family distribution

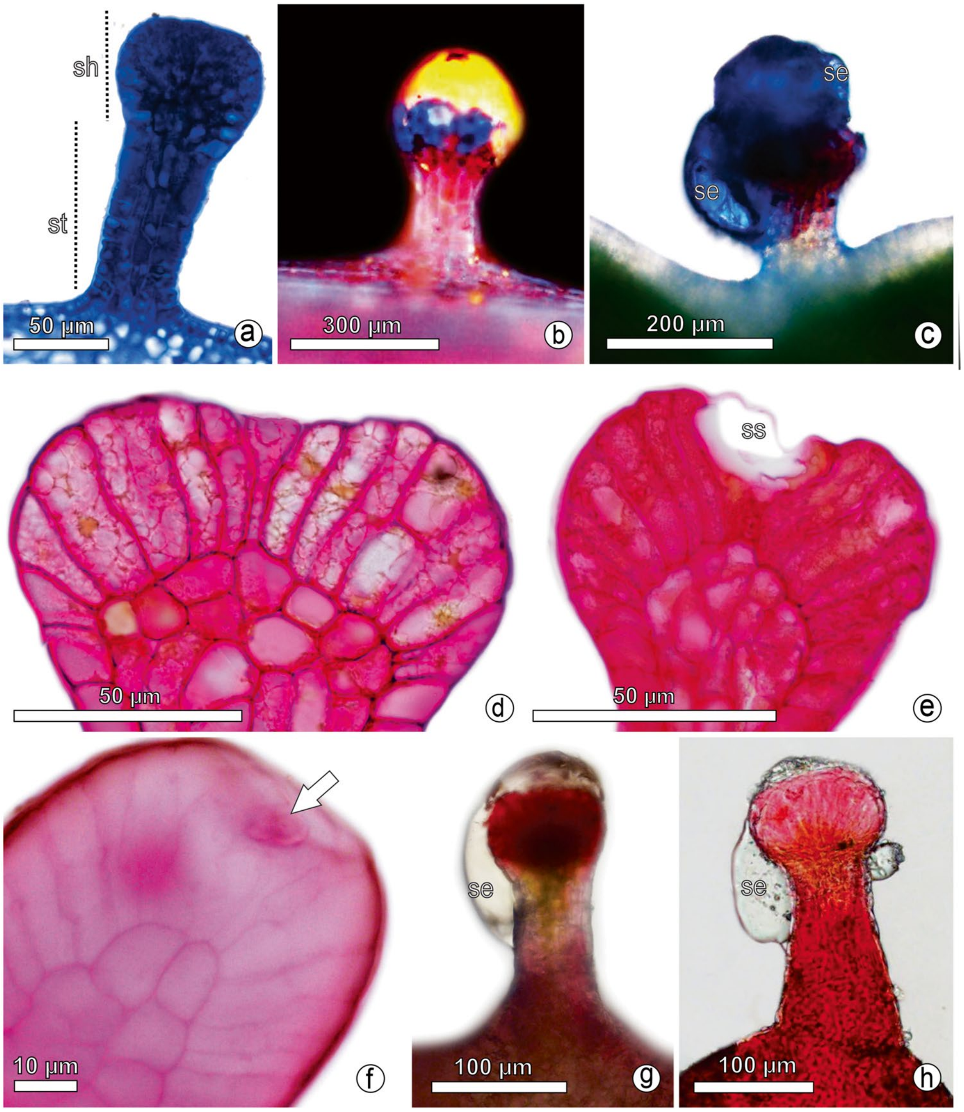


Fig. 6 Morphoanatomical and histochemical characterization of the secretory trichomes in *Rosa lucieae*. **a** Secretor trichome stained with Toluidine blue showing the stalk (st) and secretory head (sh). **b** Yellowish fluorescence of neutral red showing the presence of total lipids in the secretion. **c** Blue staining as the result of the NADI reagent showing the presence of terpenes. **d** Total polysaccharides found at the secretory head of trichomes as shown by PAS reaction. **e** Absence of total polysaccharides in the secretion accumulated in the subcuticular space (ss). **f** Cleared secretory trichome showing the cuticle rupture (white arrow). **g** Absence of pectins in the secretion (se) on the outer side of trichome. Note that the exudates do not stain red with Ruthenium red. **h** Absence of total protein in the secretion as it does not stain with Xylidine Ponceau

is particularly diverse in the Northern hemisphere (Hummer and Janick 2009). *Rosa* is one of the genera that extend southward into regions with Mediterranean climate or even into tropical latitudes (often montane) (Kalkman 2004). If taken as a model species for the genus *Rosa*, *R. lucieae* shows that having colleters with a mixture of hydrophilic and lipophilic compounds may have aided the genus *Rosa* towards such a successful distribution, that is, towards different environmental conditions (i.e., temperate or Mediterranean climates), as buds would be protected against dehydration regardless of the type of environment where species occurs.

The presence of lipids and terpenes in the secretion of the secretory trichomes of *R. lucieae* is in agreement with the histochemical results found for hybrids of *Rosa* (Caissard et al. 2006) and *R. idaeus* (Chwil and Kostryco 2020). Lipids and terpenes on leaves are the main chemical compounds associated with chemical defense against pathogens and herbivores (Langenheim 2003; Combrinck et al. 2007). Their sticky lipophilic exudates are associated with their main function, protection against herbivory as insect may get stuck in the secretion (Patten et al. 2010; Krimmel and Pearse 2013). Therefore, the presence of secretory trichomes spread throughout the leaves, the chemical nature of their exudates, and their constant secretory activity even in fully developed leaves support the ecological function of such trichomes in *R. lucieae*, indicating their involvement in anti-herbivore strategies.

Conclusion

The two leaf secretory structures identified in *Rosa lucieae*, colleters and secretory trichomes, differ in their timing of secretory activity throughout the leaf lifespan, the chemical nature of the exudates, and their position on the plant body. The premature secretory activity of the colleters and the mucilaginous composition of the sticky secretion were important features to recognize the protective function of the developing leaflets against solar radiation. The prolonged secretion time of the glandular trichomes and the lipophilic composition of the secretion corroborated for a defense function against herbivores and pathogens in the leaves.

Author contribution The research project was designed by Valdnéa Casagrande Dalvi. The samples were collected by Valdnéa Casagrande Dalvi and Maycon de Sousa Silva; light microscopy and histochemical analyses were performed by Maycon de Sousa Silva; scanning microscopy was performed by Valdnéa Casagrande Dalvi. The manuscript was written by Valdnéa Casagrande Dalvi, Maycon de Sousa Silva, Alex Batista Moreira Rios, and Ítalo Antônio Cotta Coutinho.

Funding This study was supported by the Ministério da Ciência e Tecnologia/Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Brasília, Brazil; Grant 406824/2016–9 to Valdnéa Casagrande Dalvi).

Data availability All data generated or analyzed during this study are included in this published article.

Code availability Not applicable.

Declarations

Ethics approval Not applicable

Consent to participate Not applicable

Consent for publication Not applicable

Conflict of interest The authors declare no competing interests.

References

Adumitresei L, Gostin I (2016) Morphological and micromorphological investigations regarding the leaves of several *Rosa* L. species. J Plant Dev 23:127–138

Appezzato-da-Glória B, Stalder-Miranda S (1991) Anatomia foliar e do pedúnculo floral de plantas de morangueiro (*Fragaria* x *ananassa*) "Sequoia" Tratados com fitoreguladores. An Da Esc Super Agric Luiz Queiroz 48:127–154

Baas P, Gregory M (1985) A survey of oil cells in the dicotyledons with comments on their replacement by and joint occurrence with mucilage cells. Isr J Bot 34:167–186. https://doi.org/10.1080/0021213X.1985.10677020

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils - a review. Food Chem Toxicol 46:446–475. https://doi.org/10.1016/j.fct.2007.09.106

Belin-DePoux M (1969) Contribution à l'étude des hydathodes. I. Remarques sur le type "à épithème" chez les dicotyledones. Rev Générale Bot 76:631–657

Bozzola JJ, Russel LD (1992) Electron microscopy. Jones and Bartlett Publishers, Boston, Massachusetts, USA

Caissard J-C, Bergougnoux V, Martin M et al (2006) Chemical and histochemical analysis of 'Quatre Saisons Blanc Mousseux', a moss rose of the *Rosa* × *damascena* group. Ann Bot 97:231–238. https://doi.org/10.1093/aob/mcj034

Calle J, Gasparre N, Benavent-Gil Y, Rosell CM (2021) Aroids as underexplored tubers with potential health benefits. Adv Food Nutr Res 97:319–359. https://doi.org/10.1016/bs.afnr.2021.02.018

Caperta AD, Róis AS, Teixeira G et al (2020) Secretory structures in plants: lessons from the Plumbaginaceae on their origin, evolution and roles in stress tolerance. Plant Cell Environ 43:2912–2931. https://doi.org/10.1111/pce.13825

Chin S, Lutz S, Wen J, Potter D (2013) The bitter and the sweet: inference of homology and evolution of leaf glands in *Prunus* (Rosaceae) through anatomy, micromorphology, and ancestral–character state reconstruction. Int J Plant Sci 174:27–46. https://doi.org/10.1086/668219

Chwil M, Kostryco M (2020) Histochemical assays of secretory trichomes and the structure and content of mineral nutrients in *Rubus idaeus* L. leaves. Protoplasma 257:119–139. https://doi.org/10.1007/s00709-019-01426-7

Combrinck S, Du Plooy GW, McCrindle RI, Botha BM (2007) Morphology and histochemistry of the glandular trichomes of *Lippia scaberrima* (Verbenaceae). Ann Bot 99:1111–1119. https://doi.org/10.1093/aob/mcm064

Costa ISC, Lucena EMP, Bonilla OH et al (2020) Seasonal variation in colleter exudates in *Myrcia splendens* (Myrtaceae). Aust J Bot 68:403. https://doi.org/10.1071/BT20020

Coutinho ÍAC, Francino DMT, Meira RMSA (2015) New records of colleters in *Chamaecrista* (Leguminosae, Caesalpinioideae s. l.): structural diversity, secretion, functional role, and taxonomic importance. Int J Plant Sci 176:72–85. https://doi.org/10.1086/679016

Curtis JD, Lersten NR (1986) Hydathode anatomy in *Potentilla palustris* (Rosaceae). Nord J Bot 6:793–796. https://doi.org/10.1111/j.1756-1051.1986.tb00482.x

Dalvi VC, Cardinelli LS, Meira RMSA, Azevedo AA (2014) Foliar colleters in *Macrocarpaea obtusifolia* (Gentianaceae): anatomy, ontogeny, and secretion. Botany 92:59–67

Dáttilo W, Aguirre A, Flores-Flores RV et al (2015) Secretory activity of extrafloral nectaries shaping multitrophic ant-plant-herbivore interactions in an arid environment. J Arid Environ 114:104–109. https://doi.org/10.1016/j.jaridenv.2014.12.001

David R, Carde JP (1964) Coloration differentielle des inclusions lipidique et terpeniques des pseudophylles du Pin maritime au moyen du reactif Nadi. Comptes Rendus I'academie Des Sci Paris 258:1338–1340

de S Silva M, Coutinho ÍAC, Dalvi VC (2022) Anatomical and histochemical characterization of glands associated with the leaf teeth in Rhaphiolepis loquata B.B.Liu & J.Wen (Rosaceae Juss.). Flora 293:152110. https://doi.org/10.1016/j.flora.2022.152110

Dell B, McComb AJ (1979) Plant resins - their formation, secretion and possible functions. In: Advances in botanical research, pp 277–316. https://doi.org/10.1016/S0065-2296(08)60332-8

Demarco D (2017) Histochemical analysis of plant secretory structures. In: Methods in molecular biology, pp 313–330. https://doi.org/10.1007/978-1-4939-6788-9_24

Donnelly D, Skelton F (1989) Comparison of hydathode structure in micropropagated plantlets and greenhouse-grown "Queen Elizabeth" rose plants. J Am Soc Hortic Sci 114:841–846

Dourado DM, Rocha DI, Kuster VC, Fernandes VF, Delgado MN, Francini DMT, Dalvi VC (2022) Structural similarity versus secretion composition in colleters of congeneric species of *Prepusa* (Gentianaceae). Flora 294:152120. https://doi.org/10.1016/j.flora.2022.152120

Faghir MB, Attar F, Ertter B (2011) Foliar anatomy of the genus Potentilla L. (Rosaceae) in Iran and its taxonomic implication. Iran J Sci Technol Trans A Sci 35:243–256. https://doi.org/10.22099/IJSTS.2011.2149

Fahn A (1979) Secretory tissues in plants. Academic Press, London

Fahn A (1988) Secretory tissues in vascular plants. New Phytol 108:229–257. https://doi.org/10.1111/j.1469-8137.1988.tb04159.x

Fahn A (1990) Plant anatomy, Fourth. Pergamon Press, Oxford

Feio AC, Riina R, Meira RMSA (2016) Secretory structures in leaves and flowers of two dragon's blood *Croton* (Euphorbiaceae): new evidence and interpretations. Int J Plant Sci 177:511–522. https://doi.org/10.1086/685705

Fernandes VF, Thadeo M, Dalvi VC et al (2016) Colleters in *Casearia* (Salicaceae): a new interpretation for the theoid teeth. Bot J Linn Soc 181:682–691. https://doi.org/10.1111/boj.12432

Foster AS (1949) Practical plant anatomy, 2nd edn. D. van Nostrand Company Inc., Princeton, NY, USA

Gonçalves JR, Rios ABM, Dalvi VC (2020) Unravelling the structure of cucurbitoid teeth in the Cucurbitaceae. Plant Syst Evol 306:65. https://doi.org/10.1007/s00606-020-01694-4

Gonzalez AM, Tarragó JR (2009) Anatomical structure and secretion compounds of colleters in nine *Ilex* species (Aquifoliaceae) from southern South America. Bot J Linn Soc 160:197–210

Hashidoko Y, Endoh K, Kudo T, Tahara S (2001) Capability of wild *Rosa rugosa* and its varieties and hybrids to produce sesquiterpene components in leaf glandular trichomes. Biosci Biotechnol Biochem 65:2037–2043. https://doi.org/10.1271/bbb.65.2037

Herman RA, Ayepa E, Shittu S et al (2019) Essential oils and their applications - a mini review. Adv Nutr Food Sci 4. https://doi.org/10.33140/ANFS.04.04.08

Hummer KE, Janick J (2009) Rosaceae: taxonomy, economic importance, genomics. In: Folta KM, Gardiner SE (eds) Genetics and genomics of rosaceae. Springer New York, New York, NY, pp 1–17

Johansen DA (1940) Plant microtechnique. McGraw-Hill Book, New York

Kalkman C (2004) Rosaceae. Flowering plants dicotyledons. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 343–386

Kirk PW (1970) Neutral red as a lipid fluorochrome. Stain Technol 45:1–4

Klein DS, Gomes VM, Silva-Neto SJ, Cunha M (2004) The structure of colleters in several species of *Simira* (Rubiaceae). Ann Bot 94:733–740. https://doi.org/10.1093/aob/mch198

Krimmel BA, Pearse IS (2013) Sticky plant traps insects to enhance indirect defence. Ecol Lett 16:219–224. https://doi.org/10.1111/ele.12032

Kumachova T, Babosha A, Ryabchenko A et al (2021) Leaf epidermis in Rosaceae: diversity of the cuticular folding and microstructure. Proc Natl Acad Sci India Sect B - Biol Sci 91:455–470. https://doi.org/10.1007/s40011-021-01244-z

Langenheim JH (2003) Plant resins: chemistry, evolution, ecology and ethnobotany. Timber Press., Portland, Cambridge

Lerdau M, Litvak M, Monson R (1994) Plant chemical defense: monoterpenes and the growth-differentiation balance hypothesis. Trends Ecol Evol 9:58–61. https://doi.org/10.1016/0169-5347(94)90269-0

Lersten NR (1974a) Colleter morphology in *Pavetta*, *Neorosea* and *Tricalysia* (Rubiaceae) and its relationship to the bacterial leaf nodule symbiosis. Bot J Linn Soc 69:125–136. https://doi.org/10.1111/j.1095-8339.1974.tb01620.x

Lersten NR (1974b) Morphology and distribution of colleters and crystals in relation to the taxonomy and bacterial leaf nodule symbiosis of *Psychotria* (Rubiaceae). Am J Bot 61:973–981

Lersten N (1975) Colleter types in Rubiaceae, especially in relation to the bacterial leaf nodule symbiosis. Bot J Linn Soc 71:311–319. https://doi.org/10.1111/j.1095-8339.1975.tb01207.x

Lersten NR, Curtis JD (1982) Hydathodes in Physocarpus (Rosaceae: Spiraeoideae). Can J Bot 60:850–855. https://doi.org/10.1139/b82-109

Macêdo TP, Cortez PA, Costa LCB (2016) First record of colleters in *Zanthoxylum* Linn. species (Rutaceae Juss., Sapindales): structural, functional and taxonomic considerations. Flora 224:66–74. https://doi.org/10.1016/j.flora.2016.07.007

Maffei M, Chialva F, Sacco T (1989) Glandular trichomes and essential oils in developing peppermint leaves. New Phytol 111:707–716. https://doi.org/10.1111/j.1469-8137.1989.tb02366.x

Markus Lange B, Turner GW (2013) Terpenoid biosynthesis in trichomes-current status and future opportunities. Plant Biotechnol J 11:2–22. https://doi.org/10.1111/j.1467-7652.2012.00737.x

Mayer JLS, Cardoso-Gustavson P, Appezzato-da-Glória B (2011) Colleters in monocots: new record for Orchidaceae. Flora 206:185–190. https://doi.org/10.1016/j.flora.2010.09.003

Mayer JLS, Carmello-Guerreiro SM, Mazzafera P (2013) A functional role for the colleters of coffee flowers. AoB Plants 5:1–13. https://doi.org/10.1093/aobpla/plt029

McManus JFA (1948) Histological and histochemical uses of periodic acid. Stain Technol 23:99–108. https://doi.org/10.3109/10520294809106232

Meira RMSA, Francino DMT, Ascensão L (2014) Oleoresin trichomes of *Chamaecrista dentata* (Leguminosae): structure, function, and secretory products. Int J Plant Sci 175:336–345. https://doi.org/10.1086/673538

Meira RMSA, Miranda JDB, Coutinho ÍAC (2020) Anatomical reevaluation and novelties on the leaf marginal tooth glands in *Sapium glandulosum* (L.) Morong. (Euphorbiaceae): the importance of distinguishing colleters from nectaries. In: Demarco D (ed) Plant ontogeny: studies, analyses and evolutionary implications. Nova Science Publishers, Inc., New York, USA, pp 63–82

Mercadante-Simões MO, Paiva EAS (2013) Leaf colleters in *Tontelea micrantha* (Celastraceae, Salacioideae): ecological, morphological and structural aspects. C R Biol 336:400–406. https://doi.org/10.1016/j.crvi.2013.06.007

Miguel EC, Gomes VM, Oliveira MA, Cunha M (2006) Colleters in *Bathysa nicholsonii* K. Schum. (Rubiaceae): ultrastructure, secretion protein composition, and antifungal activity. Plant Biol 8:715–722. https://doi.org/10.1055/s-2006-924174

Miguel E de C, Pireda S, Barros CF et al (2017) Outer cell wall structure and the secretion mechanism of colleters of *Bathysa nicholsonii* K. Schum. (Rubiaceae). Acta Bot Brasilica 1–9. https://doi.org/10.1590/0102-33062016abb0420

Miller IM, Scott A, Gardner IC (1984) The occurrence of calyx nodules in *Psychotria* spp. (Rubiaceae). Protoplasma 121:199–208. https://doi.org/10.1007/BF01282313

Moreno-Medina BL, Casierra-Posada F, Albesiano S (2020) *Rubus alutaceus* (Rosaceae), a new species for Colombia with agronomic potential. Rev Bras Frutic 42:1–12. https://doi.org/10.1590/0100-29452020542

Muravnik LE (2020) The structural peculiarities of the leaf glandular trichomes: a review. In: Reference series in phytochemistry, pp 1–35

O'Brien TP, McCully ME (1981) The study of plant structure: principles and selected methods. Termarcarphi Ptey. Ltd., Melbourne, Australia

O'Brien TP, Feder N, McCully ME (1964) Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma 59:368–373. https://doi.org/10.1007/BF01248568

Paiva ÉAS (2009) Occurrence, structure and functional aspects of the colleters of *Copaifera langsdorffii* Desf. (Fabaceae, Caesalpinioideae). C R Biol 332:1078–1084

Paiva EAS (2012a) Colleters in *Cariniana estrellensis* (Lecythidaceae): structure, secretion and evidences for young leaf protection. J Torrey Bot Soc 139:1–8

Paiva EAS (2012b) Anatomy, ultrastructure, and secretory activity of the floral nectaries in *Swietenia macrophylla* (Meliaceae). Am J Bot 99:1910–1917. https://doi.org/10.3732/ajb.1200122

Paiva EAS (2016) How do secretory products cross the plant cell wall to be released? A new hypothesis involving cyclic mechanical actions of the protoplast. Ann Bot 117:533–540. https://doi.org/10.1093/aob/mcw012

Paiva EAS, Martins LC (2011) Calycinal trichomes in *Ipomoea cairica* (Convolvulaceae): ontogenesis, structure and functional aspects. Aust J Bot 59:91. https://doi.org/10.1071/BT10194

Paré PW, Tumlinson JH (1999) Plant volatiles as a defense against insect herbivores. Plant Physiol 121:325–331

Patten AM, Vassão DG, Wolcott MP et al (2010) Trees: a remarkable biochemical bounty. In: Comprehensive natural products II. Elsevier, pp 1173–1296. https://doi.org/10.1016/B978-008045382-8.00083-6

Pearse AGE (1980) Histochemistry theoretical and applied, 4th edn. Churchill Livingston, Edinburgh

Prado E, Demarco D (2018) Laticifers and secretory ducts: similarities and differences. In: Ecosystem services and global ecology. IntechOpen. https://doi.org/10.5772/intechopen.75705

Ribeiro JC, Ferreira MJP, Demarco D (2017) Colleters in Asclepiadoideae (Apocynaceae): protection of meristems against desiccation and new functions assigned. Int J Plant Sci 178:465–477. https://doi.org/10.1086/692295

Rios ABM, Menino GCO, Dalvi VC (2020) Leaf teeth in eudicots: what can anatomy elucidate? Bot J Linn Soc 193:504–522. https://doi.org/10.1093/botlinnean/boaa028

Roshchina VV, Roshchina VD (1993) The excretory function of higher plants. Springer-Verlag, Berlin, Germany

Sadala-castilho R, Machado SR, Lima HA (2016) Oil-resin glands in Velloziaceae flowers : structure, ontogenesis and secretion. Plant Syst Evol 302:585–599. https://doi.org/10.1007/s00606-016-1287-5

Sánchez-Sánchez H, Morquecho-Contreras A (2017) Chemical plant defense against herbivores. In: Shields VDC (ed) herbivores. InTech, pp 3–28

Silva CJ, Barbosa LCA, Marques AE, Baracat-Pereira MC, Pinheiro AL, Meira RMSA (2012) Anatomical characterisation of the foliar colletersin Myrtoideae (Myrtaceae). Aust J Bot 60:707–717. https://doi.org/10.1071/BT12149

Silva M dos S, Coutinho ÍAC, Araújo MN, Meira RMSA (2017) Colleters in *Chamaecrista* (L.) Moench sect. *Chamaecrista* and sect. *Caliciopsis* (Leguminosae-Caesalpinioideae): anatomy and taxonomic implications. Acta Bot Brasilica 31:382–391https://doi.org/10.1590/0102-33062016abb0339

Sulborska A, Weryszko-Chmielewska E (2014) Characteristics of the secretory structures in the flowers of *Rosa rugosa* Thunb. Acta Agrobot 67:13–24. https://doi.org/10.5586/aa.2014.056

Teixeira RS, Rocha RI, Dalvi VC (2021) Leaf colleters in Clusia burchellii Engl.: structural and ultrastructural features of a little-known gland in Clusiaceae. Flora 280:151834. https://doi.org/10.1016/j.flora.2021.151834

Teixeira RS, Rocha RI, Gonçalves JR, Dalvi VC (2022) Development, structure, and secretion of leaf colleters in Clusia criuva Cambess. subsp. criuva (Clusiaceae). Acta Bot Brasilica 36:e2021abb0103. https://doi.org/10.1590/0102-33062021abb0103

Thadeo M, Azevedo AA, Meira RMSA (2014) Foliar anatomy of neotropical Salicaceae: potentially useful characters for taxonomy. Plant Syst Evol 300:2073–2089. https://doi.org/10.1007/s00606-014-1037-5

Thomas V (1991) Structural, functional and phylogenetic aspects of the colleter. Ann Bot 68:287–305. https://doi.org/10.1093/oxfordjournals.aob.a088256

Tozin LR dos S, Rodrigues TM (2019) Glandular trichomes in the tree-basil (*Ocimum gratissimum* L., Lamiaceae): morphological features with emphasis on the cytoskeleton. Flora 259:151459. https://doi.org/10.1016/j.flora.2019.151459

Tresmondi F, Nogueira A, Guimarães E, Machado SR (2015) Morphology, secretion composition, and ecological aspects of stipular colleters in Rubiaceae species from tropical forest and savanna. Sci Nat 102:73. https://doi.org/10.1007/s00114-015-1320-5

Tresmondi F, Canaveze Y, Guimarães E, Machado SR (2017) Colleters in Rubiaceae from forest and savanna: the link between secretion and environment. Sci Nat 104. https://doi.org/10.1007/s00114-017-1444-x

Vitarelli NC, Riina R, Caruzo MBR et al (2015) Foliar secretory structures in Crotoneae (Euphorbiaceae): diversity, anatomy, and evolutionary significance. Am J Bot 102:833–847. https://doi.org/10.3732/ajb.1500017

Wagner GJ (1991) Secreting glandular trichomes: more than just hairs. Plant Physiol 96:675–679

Wang D-J, Zeng J-W, Ma W-T et al (2019) Morphological and structural characters of trichomes on various organs of *Rosa roxburghii*. Hort Science 54:45–51. https://doi.org/10.21273/HORTSCI13485-18

Wang D-J, Lu M, Ludlow RA, Zeng J-W, Ma W-T, An H-M (2021) Comparative ultrastructure of trichomes on various organs of Rosa roxburghii. Microsc Res Tech 84:1–9. https://doi.org/10.1002/jemt.23765

Zhou N, Simonneau F, Thouroude T et al (2021) Morphological studies of rose prickles provide new insights. Hortic Res 8:221. https://doi.org/10.1038/s41438-021-00689-7