Abstract
Colleters are secretory structures that produce a sticky substance, consisting of a mixture of mucilage, proteins, terpenes, pectic substances and even alkaloids, which lubricates and protects the shoot apical meristem. Several colleter types have been described and have taxonomic value in many botanical families. In Myrtaceae, the colleters description is recent and presents three new morphological types (conic, euryform and petaloid) that differ those already described for other eudicots. In this work, we report the colleters morphological types in six species of three genera belonging to the Myrteae tribe of Myrtoideae from the Brazilian Cerrado. The samples were fixed for light and scanning electron microscopy. Histochemical tests were carried out on the fresh and methacrylate-embedded material. The conic and euryform colleters from Myrtoideae species of the Cerrado did not differ either morphologically nor as to the secretion nature from those described for Myrtoideae species from others biomes, which may indicate their potential use for taxonomic purposes. Considering the hypothesis that the multiple fleshy-fruit lineages have evolved independently in Myrteae tribe, our results indicate the relevance of additional studies in order to recognize the pattern of distribution of colleters in Myrtaceae.

Key words: histochemistry, mucilage, Myrtoideae, secretory structure, vegetative meristems.

Introduction
The plant’s vegetative shoot apex is essential to ensure their growth. They are composed of meristematic tissues (Fahn 1979). These tissues are thin and susceptible to desiccation due to environmental factors such as wind and solar radiation, which are more intense in the Cerrado (Brazilian Savanna), where the dry period is prolonged and the plants resume their growth after winter dormancy (Sano et al. 2008). In this context, the colleters are an important secretory structure that protects the shoot apex.
The term colleter comes from the Greek *colla* and refers to the secretion produced by the colleters, which occur in the vegetative shoot apex of many plant species and in young foliar organs, or on the adaxial face of stipules, petioles, bracts, calyx and corolla (Thomas 1991). The colleter’s sticky secretion is a mixture of mucilage, proteins, terpenes, pectic substances and even alkaloids, which lubricates and protects the shoot apical meristem (Fahn 1979; Mayer et al. 2013).

Colleters are widely distributed on young vegetative and reproductive organs in plants and have been reported in ferns (Oliveira et al. 2017), monocots (Leitão & Cortelazzo 2008; Mayer et al. 2011; Cardoso-Gustavson et al. 2014) and in sixty-five eudicots families (Thomas 1991; Muravnik et al. 2014) such as Apocynaceae, Gentianaceae, Rubiaceae, (Thomas 1991; Renobales et al. 2001; APG 2009; Lopes-Mattos et al. 2015; Tresmondi et al. 2015). Anacardiaceae (Lacchia et al. 2016), Aquifoliaceae (González & Tarragó 2009), Euphorbiaceae (Machado et al. 2015; Vitarelli et al. 2015), Fabaceae (Barros & Teixeira 2016), Lecythidaceae (Paiva 2012), Moraceae (Machado et al. 2013), Rutaceae (Macêdo et al. 2016), Rhizophoraceae (Sheue et al. 2012) and Myrtaceae (Silva et al. 2012).

In Myrtaceae, the colleters present three morphological types that differ those already described for other eudicots: (1) the petaloid type, with an axis that is flattened dorsiventrally, being much wider than thick and short; (2) the conic type has an axis, observed in the cross-section as a circular or ellipsoid form, with a decreasing diameter towards the apex and (3) the euryform type that is flattened dorsiventrally, but has an axis that is much wider than thick and longer than wide (Silva et al. 2012).

Myrtaceae is the largest family of Myrtales and there are about 140 genera and more than 3,000 species (Johnson & Briggs 1984; Wilson et al. 2001; Moura & Franzener 2015). In Brazil, Myrtaceae is one of the ten richest angiosperm families and is also considered one of the ten main Cerrado families in the number of genera (14) and species (211) (Mendonça et al. 1998).

Myrtaceae is divided into two subfamilies: Myrtoideae (with 15 tribes) and Psiloxyloideae (with two tribes: Psiloxyleae and Heteropyxideae) (Wilson et al. 2005; Biffin et al. 2010). In Brazil, there are representatives of the current subfamily Myrtoideae.

Due to their morphological diversity, the colleters has been successfully used in taxonomic studies involving several botanical families (Woodson & Moore 1938; Thomas 1991; Rio et al. 2002, 2005; Rio & Kinoshita 2005; Simões et al. 2006; Silva et al. 2012). In addition, they have ecological importance because of their function of protection against dissection and pathogen attack (Lersten 1974; Mangalan et al. 1990; Miguel et al. 2009; Mayer et al. 2011; Rocha et al. 2011).

Considering that the colleters in Myrtoideae may help clarify the phylogenetic relationships of the Myrtaceae family (Silva et al. 2012) and the scarcity of descriptive studies about colleter of the Cerrado species (Mercadante-Simões & Paiva 2013), the purpose of this study was to analyze the occurrence and morphological types of the colleters in six species belonging to three genera of Myrteae tribe belonging to Myrtoideae subfamily. These species were chosen because of their wide geographical distribution in Cerrado.

**Materials and Methods**

**Plant material**

Shoot meristems were collected from six species of three genera belonging to tribe Myrteae of Myrtoideae (sensu Wilson et al. 2005). Voucher material was deposited in the Herbarium (VIC) of the Universidade Federal de Viçosa (UFV) (Tab. 1).

**Light microscopy**

The collected material was selected under a stereoscopic microscope (Zeiss Stemi 2000-C Carl Zeiss, Germany), fixed in FAA (formalin: acetic acid: 70% ethanol (1:1:18 by volume) and stored in 70% ethanol (Johansen 1940). Samples were dehydrated in ethanol series and embedded in methacrylate (Historesin, Leica Instruments, Heidelberg, Germany). Cross and longitudinal sections (4–6 µm) were made using an automatic microtome (Zeiss - RM55) and stained with toluidine blue (O’Brien & McCully 1981), pH 4.0, for anatomical characterization. The slides were mounted in synthetic resin (Permount, Fisher Scientific, NJ, USA). The colleters classification was performed according to Silva et al. (2012).

**Histochemical analysis**

For histochemical tests on fresh material, branch sections with colleters were selected using a stereoscopic microscope and tested
with periodic acid-Schiff reagent (PAS) to detect general polysaccharides (McManus 1948), tannic acid-ferric chloride to detect mucilage (Pizzolato & Lillie 1973), ruthenium red for acid mucilage (Gregory & Baas 1989), Sudan IV to identify the lipophilic compounds (Pearse 1985), Wagner reagent for alkaloids (Furr & Mahlberg 1981) and ferric chloride for phenolic compounds (Johansen 1940). In the Methacrylate-embedded material were applied the same tests and the Xylidine Ponceau (XP) for proteins (O’Brien & McCully 1981). The controls were performed simultaneously, following recommendations in the literature. Material analysis and photographic documentation were performed using a light microscope (Primo Star, Carl Zeiss, Germany) equipped with a digital camera (model Axiocam ERC MRc 5s) and software Axiovision documentation (Carl Zeiss, Germany).

**Scanning electron microscopy**

The samples (leaf primordia and leaf buds bearing colleters) were fixed in a solution of 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate-buffered saline, pH 7.3 (Karnovsky 1965), dehydrated in ethanol series and critical point-dried with CO₂ in a Bal-Tec 020 CPD dryer (Bal-Tec, Balzers, Liechtenstein). Samples were mounted on stubs and coated with gold by using an FDU 010 sputter coater (Bal-Tec). Examinations and photographs were carried out using a Leo 1430VP scanning electron microscope (Zeiss, Cambridge, UK) at the Microscopy and Microanalysis Center, UFV.

**Results**

Colleters were observed in the vegetative apices in all six species studied. Conic colleters were observed in *Myrcia* and *Psidium* genera and euryform colleters occurred in *Eugenia* genus (Figs. 1-3). Petaloid colleters were not found.

In all of the studied species, in the fresh and methacrylate-embedded material, the histochemical analysis revealed only the presence of hydrophilic compounds. The hydrophilic fraction of secretion stained with PAS (Figs. 1b,e; 2b,e,g), tannic acid-ferric chloride (Fig. 1f,h-i) and ruthenium red (Figs. 1c; 2m) indicating the presence of an acidic mucilage. No presence of lipophilic compounds, alkaloids and phenolic compounds was detected.

Colleters observed herein were translucent during the secretory phase turning to brown as they senesce (Fig. 1g).

In *E. dysenterica* the euryform colleters were higher than wide and some of them presented bifurcation in the apex (Fig. 3a). In longitudinal section, it was observed that they have a narrow base (Fig. 2a). Conic colleters in *P. cattleyanum* occurred in a row between the leaf primordia surrounding the buds (Fig. 2k-l). They presented tapered apex and increases diameter towards the base. In *P. grandifolium*, *P. guineense* and *M. tomentosa* the conic colleters were found between several trichomes (Figs. 2g,i; 3b-c,g). The colleters in *P. grandifolium* presented a broad base (Fig. 2c).

In all species, the colleters were non-vascularized and showed simple and homogeneous cellular compositions, with no secretory epithelium.

### Table 1 – List of plant species studied.

<table>
<thead>
<tr>
<th>Species</th>
<th>Popular name</th>
<th>Georeferencing</th>
<th>Collection location</th>
<th>Voucher (VIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eugenia dysenterica</em> DC.</td>
<td>Cagaita, cagaiteira</td>
<td>19°28’30”S, 44°11’50”W</td>
<td>1</td>
<td>48.717</td>
</tr>
<tr>
<td><em>Myrcia tomentosa</em> (Aubl.) DC.</td>
<td>Goiaba brava</td>
<td>21°15’50”S, 44°41’10”W</td>
<td>2</td>
<td>48.718</td>
</tr>
<tr>
<td><em>Psidium cattleyanum</em> Sabine</td>
<td>Araçá-amarelo</td>
<td>21°16’26”S, 44°40’48”W</td>
<td>2</td>
<td>48.714</td>
</tr>
<tr>
<td><em>Psidium grandifolium</em> Mart. ex DC.</td>
<td>Araçá catuba</td>
<td>21°15’16”S, 44°41’22”W</td>
<td>2</td>
<td>48.722</td>
</tr>
<tr>
<td><em>Psidium guineense</em> Sw.</td>
<td>Araçá-do-campo</td>
<td>21°15’28”S, 44°41’14”W</td>
<td>2</td>
<td>48.713</td>
</tr>
<tr>
<td><em>Psidium laruoetteanum</em> Cambess.</td>
<td>Araçá-cascudo</td>
<td>19°28’32”S, 44°11’56”W</td>
<td>1</td>
<td>48.716</td>
</tr>
</tbody>
</table>

1 Campus Sete Lagoas of UFSJ; 2 Rural property in Itutinga, MG.
In longitudinal section, the parenchymatic cells were elongated, with cytoplasm similar to epidermal cells. They presented thin walls, dense cytoplasm and nucleus in medial or basal position.

The scanning electron microscopy revealed the secretion accumulation in the apical and medial region of the colleters (Fig. 3a,d-f). It was not detected pores or cracks in the cuticle, either by light or scanning electron microscopy which indicates the mechanisms of secretion release.

**Discussion**

Euryform colleters observed herein in *E. dysenterica* was reported in *E. brasiliensis* Lam., *E. floribunda* (H. West ex Willd.) O. Berg, *E. involucrata* DC. and *E. uniflora* L. (Silva et al. 2012). However, conic colleters were also reported in *Eugenia* (Silva et al. 2012), which confirms that more than one colleter type may occur in the same genus (Silva et al. 2012).

Although the conic colleters have been reported in *Myrcia* and *Psidium* genera (Silva et al. 2012), there is no record of this broad-based structure as it was observed in *P. guineense* and *P. grandifolium*.

Euryform colleters seem to be common in the *Eugenia* genus as well as conic colleters in *Myrcia* and *Psidium* genera, both of them from...
Figure 2 – a-n. Anatomical structure of collers of Myrtoideae species in longitudinal sections (a,c,d-e,g,i,k-l,n) and cross-sections (b,f,h,j,l,m) – a-b. euryform colletter Eugenia dysenterica; c-g. conic collers in Psidium grandifolium; h-i. conic collers in P. guineense; j. conic collers in Myrcia tomentosa; k-l. conic collers in P. cattleyanum; m-n. conic collers in P. larrouiteanum. (a,c-d,f,h-l,n. toluidine-blue staining; b,e,g. positive periodic acid-Schiff’s reaction, indicating polysaccharides in the secretion [arrowhead]; m. positive ruthenium red reaction). (*) = broad base; scale bars: a,h = 80 μm; b = 20 μm; c,f-g,i,k = 200 μm; d-e,j,m = 40 μm; l,n = 100 μm).
the Myrteae Tribe, although they are not restricted to these tribes. The colleters morphological type in the Myrtoideae tribes seems to be variable and according to Silva et al. (2012) may support the hypothesis proposed by Biffin et al. (2010) that the multiple fleshy-fruit lineages have evolved independently in Myrteae Tribe. Conic colleters have been reported in 19 species belonging to three tribes (Leptospermeae, Melaleucae and Myrteae), the petaloid colleter in 10 species from three Tribes (Lophostemoneae, Melaleucae and Sizygeae), the euryform colleter in 12 species from five tribes (Leptospermae, Melaleucae, Myrteae Syncarpieae and Syzygieae) and in the Eucalypteae tribe was reported the nonoccurrence of colleter (Silva et al. 2012).

The absence of petaloid colleters observed herein was also reported for Syzygium jambos and Callistemon viminalis (Silva et al. 2012) and seems to be related to the morphological organization of the structures that surrounds and protects the apical meristems. When the apical meristems are completely covered by imbricated cataphylls, it was observed petaloid colleter occurrence between them. But in the absence of this arrangement, the conic and euryform colleters occurred in the axils of the foliar primordia or young leaves that cover the buds on young branches (Silva et al. 2012), as noted in the species in this work.

The non-vascularisation in the Myrtoideae colleters has already been described (Silva et al. 2012). The transport of secretory precursors may occur by the symplastic pathway, through the plasmodesmata as discussed by Souza (2014). The same process was reported in species of the families Apocynaceae (Appezzato-da-Glória & Estelita 2000; Souza 2014), Caryocaraceae (Paiva & Machado 2006) and Rubiaceae (Vitarelli & Santos 2009).

The mucilaginous nature of colleter secretion in Myrtoideae, confirmed by histochemical tests, is known in the literature for this secretory structure (Lersten & Horner 1968; Lersten 1974; Fahn 1979; Vitarelli & Santos 2009; Silva et al. 2012). Its ecological function is to lubricate and protect buds in the initial phase of the development, especially against desiccation (Fahn 1979; Foster 1942; Paiva & Machado 2006; Barreiro & Machado 2007;}

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**Figure 3** – a-g. Colleters in vegetative apices of Myrtoideae species viewed under a scanning electron microscope – a. euryform colleter in Eugenia dysenterica with the bifurcate apex; b-d. conic colleters in Psidium guineense; e-f. conic colleters in P. cattleyanum; g. conic colleters in P. grandifolium. (arrowheads and * = secretion accumulation; scale bars: a-b = 200 μm; c-d,g = 400 μm; e = 100 μm; f = 20 μm).
Sheue et al. 2012; Mayer et al. 2013; Coutinho et al. 2016). This function is essential for Cerrado species, which are exposed to excessive solar radiation and constant winds and can be understood as a factor that contributes to adaptation to this biome.

The non-occurrence of proteins in the secretions exuded by the colleters seems to be common for species of the subfamily Myrtoideae and has been previously reported (Silva et al. 2012). The presence of proteins in the colletter secretions was observed in families such as Apocynaceae (Leite 2012), Rubiaceae (Castro et al. 2006), Caryocaraceae (Paiva & Machado 2006) e Malvaceae (Rocha et al. 2011), and are understood as a defense mechanism against pathogens and herbivores (Klein et al. 2004; Demarco 2005; Leite 2012).

The mechanisms of secretion liberation seem to be by cuticle permeability as suggested by Silva et al. (2012), since the secretion accumulates in the apex and medial region of the colletter, however without evidence of pores or cracks for its release.

The conic and euryform colleters from Myrtoideae species of the Cerrado did not differ either morphologically nor as to the secretion nature from those described by Silva et al. (2012) for Myrtoideae species from others biomes, which may indicate their potential use for taxonomic purposes. Considering the hypothesis that the multiple fleshy-fruit lineages have evolved independently in Myrteae tribe (Biffin et al. 2010), our results indicate the relevance of additional studies in order to recognize the pattern of distribution of colleters in Myrtaceae.

References


