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Occurrence, structure and functional aspects of the colleters of *Copaifera langsdorffii* Desf. (Fabaceae, Caesalpinioideae)

Elder Antônio Sousa Paiva

Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, 31270-901 – Belo Horizonte, MG, Brazil

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Abstract

Reports concerning colleters in Fabaceae have been scarce, mainly in the Caesalpinioideae subfamily. The present work reports the occurrence, structure, and functional aspects of the colleters of *Copaifera langsdorffii*. Shoot apices and developing leaves were fixed and processed for examination by light and electron microscopy. Secretion samples were studied to determine their chemical nature and physical properties. The colleters are clavate and occur on the adaxial face of the stipules, petiole and rachis. The secretory stage of the colleters occurs during the leaf expansion, after which these structures turn brown and senesce. The secretion is composed of highly hygroscopic acidic polysaccharides and lipids. The colleters are composed of cells with thin walls, large nuclei, and dense cytoplasm with dictyosomes, mitochondria, plastids and the endoplasmic reticulum. Analyses of the secretion, placement, and functional aspects of the colleters present in *C. langsdorffii* indicate that these structures help protect young leaves from desiccation. ***To cite this article: E.A.S. Paiva, C. R. Biologies 332 (2009).***

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1. Introduction

Colleters are difficult to precisely describe as they show significant structural diversity and can consist of trichomes or projections or even more complex structures in some cases. Some authors do not consider colleters as trichomes (see [1]) and argue that these structures are developed from primordia consisting of both protoderm and elements from ground meristem. On the other hand, colleters that develop exclusively from protoderm are described [2,3] and appear to be widespread. So, the definition of colleters as multicellular appendices or trichomes which produce a sticky secretion, as per Dickison [4], appears to be adequate. These structures differentiate early and their function is to protect the shoot meristem and leaf primordia [1,5,6]. Thus, colleters help protect plants from potentially damaging abiotic agents such as low relative humidity or high levels of solar radiation, that are capable of dehydrating young leaves and other undifferentiated plant tissue.

Colleters are known to occur in about 60 angiosperm families [1], although these structures have been misidentified in many studies as simple trichomes. Recent studies have paid more attention to colleters and they have been identified in new families, including the recent report of these structures in the Orchidaceae [7] and

E-mail address: epaiva@icb.ufmg.br.

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Aquifoliaceae [8]. The identification of a given structure as a colleter is not always straight-forward, and may demand detailed anatomical and histochemical studies, which reinforces the hypothesis that the search for these secretory appendages has been incomplete and inconsistent.

Reports of the occurrence of colleters in Fabaceae have been scarce and, until the study by Paiva and Machado [3], they seemed to be restricted to the Faboideae and Mimosoideae subfamilies (see [1]). Another recent report of colleters in Caesalpinioideae was presented by De Paula and Oliveira [9] whose added details about the unusual occurrence of these structures in *Chamaecrista* embryos. Due to the taxonomic importance of colleters (see [10]) it would be informative to widen the search for these structures in the Fabaceae, as a way to constitute a basis to future phylogenetic analyses.

The species of *Copaifera* are trees native of Tropical America and Occidental Africa. In Brazil *C. langsdorffii* is important in folk medicine and is widely distributed (see [11]). According to these authors the medicinal properties of the terpene rich oleoresin are determinant in economic uses of this species. This study presents the first report of colleters in the genus *Copaifera*, and describes their origin, distribution, ultrastructure and secretion properties.

2. Material and methods

Samples of shoot apices were collected from adult plants of *C. langsdorffii* Desf. growing on the Pampulha campus of the Universidade Federal de Minas Gerais (UFMG), Minas Gerais State, Brazil. The buds and young leaves were examined using a stereomicroscope to determine the presence and distribution pattern of the colleters.

Plant samples were fixed for light microscopy in Karnovsky solution [12], dehydrated in an ethanol series [13], subjected to pre-infiltration, and subsequently embedded in synthetic resin (Leica Embedding Kit) using standard techniques. Longitudinal and transversal sections (5 μm-thick) were stained with toluidine blue [14], and the following histochemical tests were performed: 10% aqueous ferric chloride to locate phenolic substances [13]; Sudan IV to detect lipids [13]; and ruthenium red solution to detect pectic substances [13]. These two latter tests were employed in secretion samples too. Morphometric measurements of the colleters were performed using a micrometric eyepiece mounted on an Olympus BHZ light microscope.

Secretion samples were collected and submitted to cycles of dehydration (60 °C for 12 hours) and rehydration by immersion in droplets of distilled water.

Portions of the petioles and rachises of young leaves starting their expansion phase were examined by transmission electron microscopy (TEM). For this, samples were fixed in Karnovsky solution [12] for 24 hours, post-fixed in 1% osmium tetroxide (0.1 M pH 7.2 phosphate buffer) for 2 hours, dehydrated in an acetone series, and then embedded in Araldite [15]. Ultra-thin sections were stained with uranyl acetate and lead citrate [15] and examined using a Philips CM 100 TEM at 60 kV.

For scanning electron microscopy (SEM), samples of leaf portions with colleters were fixed as above, and dehydrated using an increasing ethyl alcohol series, and subsequently dried to their critical point using CO_2. The samples were gold-coated according to Robards [16] and examined using a Quanta 200 (Fei Company) SEM at 20 kV; images were captured digitally.

3. Results

3.1. Morphology, distribution, and secretory activity

Copaifera langsdorffii is deciduous, producing new leaves in the spring. The buds are protected by scales that cover a succession of young leaves in different stages of development. Each leaf shows a pair of deciduous stipules that abscise before complete leaf expansion.

The colleters of *C. langsdorffii* studied were long, sessile, clavate and slightly sinuous (Fig. 1A). They occurred on the adaxial face of the stipules and were restricted to the insertion lines of the stipules on the stem, being absent from other stipular portions. Colleters likewise occur among simple non-secretory trichomes on the petiole and rachis; being concentrated at the insertion region of the leaflets along the rachis. Colleters vary from 400 to 800 μm in length and from 100 to 150 μm in diameter.

The fresh secretion produced by these colleters was viscous and hyaline. The secretion was found on the colleters and accumulated in volumes greater than the size of the colleters themselves (Figs. 1B and 1C). After remaining on the plant for some time the secretion tended to dehydrate and crystallize. The secretion was highly hygroscopic and rapidly returned to a viscous state when exposed to conditions of high relative humidity. Laboratory experiments with secretion samples have demonstrated that they can tolerate numerous drying and rehydration cycles without any apparent modification of their physical and chemical properties.

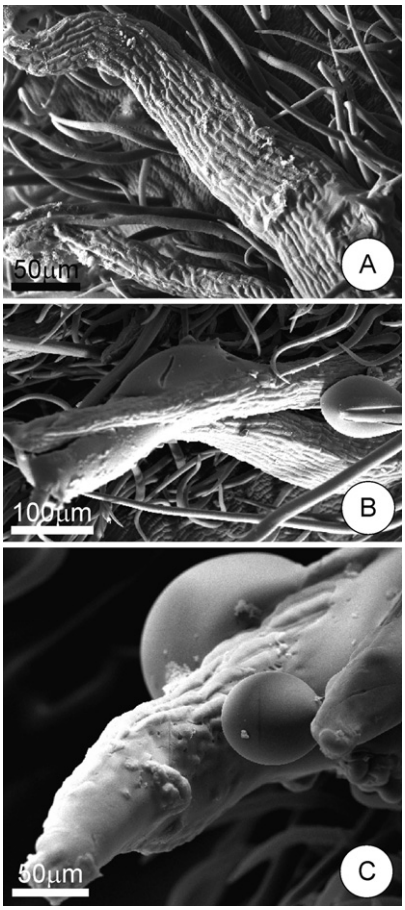

Fig. 1. General view of the colleters on leaf rachis of *Copaifera langsdorffii* by SEM. (A) Colleter body between non-secretory trichomes. Note the epithelial cell aspect which denotes a thin cuticle. (B–C) Colleter surface showing a secretion releasing; in (C) the globular drop of secretion was recently released, just before that they turn spread over the leaf surface.

The colleters of *C. langsdorffii* were observed to initiate their secretory phase during the initial stages of leaf formation while the new leaves were still protected by the stipules; their secretory phase lasts for the entire period of leaf expansion.

The colleters were observed to gradually turn brown near the end of the leaf expansion phase, and this change in color appears to be related to their senescence and death. Colleters were rare on completely expanded leaves and were not observed on mature leaves.

In addition to colleters, the aerial portions of *C. langsdorffii* have other secretory structures, including resin secretory cavities in the mesophyll of the stipules and the leaflets as well as in the cortical and medullar regions of the petioles and rachises (Fig. 2A). Extrafloral nectaries occur along the edges of the stipules (Fig. 2A) and at the base of the leaflet blade.

3.2. Ontogenesis and structural aspects

Colleters are formed from protoderm cells without the participation of any internal tissues (Figs. 2B and 2C). The differentiation of the colleters starts with a small group of protodermal initials that were identifiable by their dense cytoplasm and large nuclei. The surrounding differentiating epidermal cells, by contrast, showed well-developed vacuoles and the initial stages of accumulation of phenolic substances. The protoderm initials expand in an anticlinal direction and then pass through successive periclinal divisions and, finally, in different planes giving rise to the fully-formed colleter.

The cells of the superficial layer of the colleters showed to be slightly axially elongated (Figs. 1A, 2E) and exhibited thin cuticle at the outer periclinal walls (Figs. 1C, 2D and 2E). The protoplasm of the colleter cells showed dense cytoplasm and a large nucleus (Fig. 2D); older colleters, near the end of their secretory phase, showed more developed vacuoles which accumulated some phenolic substances (Fig. 2E). The cells in these aging structures were more densely packed, with reduced free spaces between them.

The cells at the base of the colleter were similar to the other colleter cells, but were very distinct from the subjacent highly vacuolated parenchymatic cells that occupied the cortical region of the petiole (or rachis) and stem.

All cells of the colleter body stained with ruthenium red indicating the presence of pectic compounds in the cytosol and intercellular spaces. There were occasionally small quantities of phenolic compounds in the vacuole of secretory cells, in old colleters. Lipids were detected in secretion samples, and at the cytoplasm of the secretory cells as small droplets.

The cuticle on the surfaces of the colleter showed neither visible pores nor ruptures (Fig. 2D). The recently liberated secretion was very fluid, and was secreted at non-specific points on the colleter surface.

3.3. Ultrastructural aspects

Both the core and surface cells of the colleter appear very similar, having dense cytoplasm and large nuclei, with conspicuous nucleoli (Fig. 3A). Among the notable features in the protoplasm of these cells are their highly developed endomembrane systems and their large numbers of active dictyosomes. From these latter organelles sprout innumerable vesicles that can be seen scattered throughout the ground cytoplasm, and they apparently move towards the peripheral region of the cell and fuse with the plasma membrane, or the vacuoles, to lib-

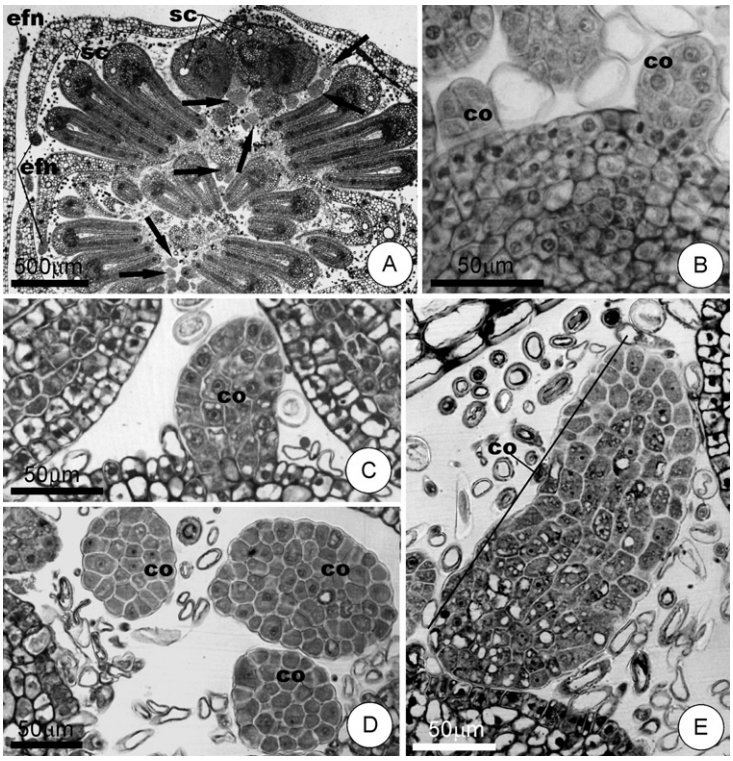

Fig. 2. Ontogenesis and structural aspects of the colleters of *Copaifera langsdorffii*. (A) General view of vegetative shoot apex in transverse section. Note the colleter presence (arrows), extrafloral nectaries at stipule margin and secretory cavities. (B–C) The initial stages of colleter formation, note the absence of cell divisions in subjacent tissue; in (C), young colleter showing cells with dense cytoplasm and evident nuclei. Note highly vacuolated cells in subjacent tissue. (D) Colleter transverse section showing superficial and core cells very similar in shape and cytoplasm density. (E) Mature colleter in longitudinal section showing some vacuolated cells and beginning of phenolic substances storage (co – colleter; efn – extrafloral nectaries; sc – secretory cavities).

erate secretion products. Well-developed periplasmatic spaces appear between the surface cells of the colleter and apparently accumulate the substances released by the dictyosome vesicles (Figs. 3B and 3C).

Secretory products were observed to accumulate in the periplasmatic spaces (Fig. 3C), and in the intercellular spaces of the colleters (Figs. 3D and 3E), which enlarges due to secretion accumulated (Fig. 3E).

The endoplasmic reticulum of the colleter cells is quite well-developed and is associated with ribosomes and plastids (Figs. 3F and 3G); the amount of ribosomes increases during the final stages of the secretory phase. The plastids showed poorly developed internal membrane systems and oil droplets were seen inside them and sometimes dispersed within the stroma (Figs. 3D and 3G). The mitochondria were globose, randomly distributed, and have well-developed cristae (Figs. 3D and 3G).

The number of vacuoles in the secretory cells increased as secretory activity progresses. These organelles were small and exhibited phenolic compounds that seemed to increase in amount inside them until colleters senescence.

4. Discussion

4.1. Structural and functional aspects

As far I know this is the first relate of colleter occurrence in this species. Other secretory structures, as the extrafloral nectaries, were reported by Leonard [17] to the leaflets. Reports of the occurrence of colleters among the Fabaceae have been rare [3], especially in the Caesalpinioideae. Colleters may have significant taxonomic importance, and Robbrecht [10] has pointed out that it would be very interesting to widen the search for these structures in order to aid future phylogenetic analyses.

The occurrence of colleters exclusively on the adaxial face of plant organs is a common characteristic (see [1,2]). The adaxial faces of the young leaves of *C. langsdorffii* are directly exposed to solar radiation

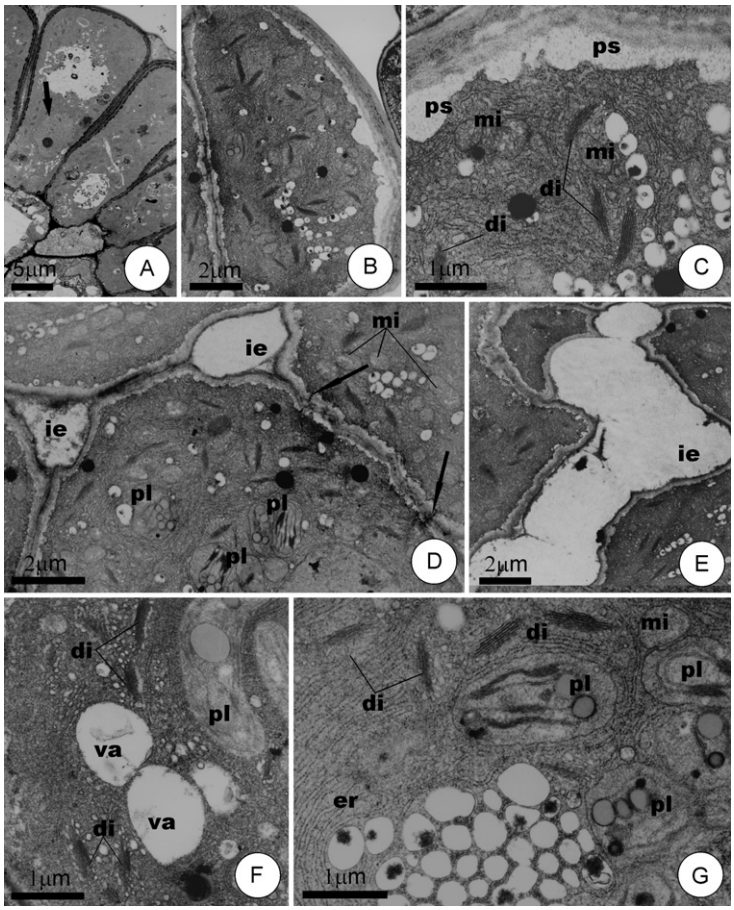

Fig. 3. Ultrastructural aspects of secretory cells of the colleters of *Copaifera langsdorffii*. (A) General view of colleter showing cytoplasm dense and conspicuous nucleus (arrow). (B–C) Secretory cell showing dictyosomes; note a conspicuous periplasmatic space. (D) Cells at beginning of secretory stage, showing plastids, dictyosomes and plasmodesmata (arrows). (E) Cells at secretory stage, note the well-developed intercellular space, which accumulate secretion products. (F–G) Detail showing cytoplasm at the secretory stage; note the active dictyosome with associated vesicles and plastids with oil droplets (di – dictyosome; er – endoplasmic reticulum; ie – intercellular space; mi – mitochondria; pl – plastid; ps – periplasmatic space; va – vacuole).

and, consequently, to desiccation – which supports the presumed protective role of colleter secretion.

The fact that *C. langsdorffii* colleters have exclusive protodermal origin allows them to be characterized as trichomes, *sensu* Solereder [18]. Colleters of exclusively protodermal origin seem to be rare; the most common are those where there are both protodermal and ground meristem originated [1]. Colleters of protodermal origin were also observed by Paiva and Machado [3] in *Hymenaea stigonocarpa* (Fabaceae Caesalpinioideae) a species that belongs to a Detarieae *sensu strictu* clade like *C. langsdorffii*, being both included in a resin producing Detarieae [19].

The observed homogeneity of the cells composing the colleters of *C. langsdorffii* is probably the result of their common ontogenetic origin. Strictly epidermal colleters were also observed to be structurally homogeneous in *Hymenaea stigonocarpa* [3].

Observations concerning the functional duration of the colleters of *C. langsdorffii* reinforce the hypothesis that these structures offer protection to the young leaves, since they are present only during the phase in which the developing leaves are especially susceptible to dehydration. The colleters of *C. langsdorffii* are deciduous, and necrosis of these structures after leaf expansion is likewise quite common in other species [1,8], especially colleters that occur on durable plant organs or portions of them such as petioles or rachises, as observed in the present study.

The presence of hygroscopic polysaccharides on the leaf cuticle, as observed with the young leaves *C. langsdorffii*, can improve water retention. Leaf cuticles normally absorb up to about 18% of their dry weight

in water vapor from near-saturated air [20,21], but if the polysaccharides associated with the cuticular membranes are chemically removed the water-absorbing capacity of the leaf drops dramatically [21]. Water uptake through the cuticle is mainly driven by the relative humidity [22].

Observations on the rehydration capacity of colleter secretion confirm its hygroscopic nature, and reinforces the hypothesis that colleters help protect young plant organs from dehydration [1]. The presence of this hydrophilic material on the leaf surface reduces water loss to the external environment and apparently helps to maintain humidity levels adequate for leaf development. Additionally, the resistance of this secretion to successive cycles of drying and hydration allows this material to maintain sufficient humidity long enough to develop other forms of protection against desiccation, as a thick cuticle layer, for example.

For developing organs that are not photosynthetically self-sufficient, xylem transport is not efficient and considerable amounts of water can be delivered by the phloem [23]. In this way, a protection to insure water maintenance in young leaves is interesting, once phloem water flow seems to be dependent on carbohydrate needs and not necessarily water demand as a consequence of transpiration flow.

The role of colleter secretion on plant protection is not restricted to the abiotic factors (see [8]). In this sense, the effect of oils in colleter secretion, as occur in *C. langsdorffii*, demands more detailed studies about their chemical nature. Miguel et al. [24] describe the presence of protein in the secretion of *Bathysa nicholsonii* (Rubiaceae) colleters and reports its antifungal properties.

Data from the literature do not establish a direct relationship between the types of tissues that constitute the colleters and the secretion volumes that they produce. In a similar way there does not appear to be any relationship between colleter size and volume of the secretion products.

4.2. Ultrastructural aspects

The secretory cells of the colleters of *C. langsdorffii* show plastids with oil droplets, which demonstrate associations of these organelles with oil synthesis. Oils have been reported among the secretion products of colleters during one or more stages of the development in *Allamanda* [25], *Gardenia* [26], *Mandevilla* [27], and *Simira* [28]. Although oils have been reported in the secretory cells of colleters and in secretion samples, it must be reinforced that this secretion is predominantly hydrophilic and is composed of pectic substances that are characteristic of the colleter secretion for many plant species (see [1]).

The accumulation of the secretion products inside colleters appears to promote a pressure that permits secretion flux to cross a cuticular barrier, probably by ruptures. Thus, the secretion can be released without energy requirements even against a gradient of concentration.

The ultrastructural characteristics of *C. langsdorffii* colleter cells are similar to those described for secretory cells of colleters in general (see [1,3,24]), especially in relation to the highly developed Golgi apparatus.

The large number of dictyosomes in colleter secretory phase is evidence of polysaccharide secretion [29] and the vesicles sprouting from these organelles, possibly loaded with secretion products, indicate that secretion in *C. langsdorffii* is granulocrine (see [30]) this pathway usually found in cells of higher plants secreting non-cellulosic polysaccharides [31]. According to Meyberg [32] the synthesis of the secretory polysaccharides probably begins in the Golgi cisternae, once the necessary enzymes are known to occur in plant dictyosomes [31]. The relatively large number of mitochondria in secretory cells should be attributed to their intense metabolic activity [33] and this fact is reported to colleters [34] as well to other plant secretory structures [35].

4.3. Conclusion

The data presented here, especially that concerning the brief functional period of the colleters in *C. langsdorffii* and the chemical nature of the secretion, allows us to infer that these structures help protect young leaves from desiccation. It is interesting to note that *C. langsdorffii* is native to tropical regions in which forest formations often show leaf falls during the transition period between the dry season and the rainy season (see [36]). During this period there is usually a water deficit in the soil, temperatures are elevated and the relative humidity is low, so that newly forming leaves require extra protection from desiccation.

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