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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.