Distribution and structural aspects of extrafloral nectaries in leaves of *Pyrostegia venusta* (Bignoniaceae)

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**ABSTRACT.** *Pyrostegia venusta* (the orange trumpet or commonly called *cipó-de-São-João* in Brazil), a medicinal plant that grows with other plants, has an ecological importance due to the presence of nectaries on the leaves. The aim of this work was to study structural and histochemical aspects and the distribution of extrafloral nectaries (ENFs) in *P. venusta* leaves. Young leaves were collected, fixed and processed by usual techniques, and studied under light microscopy and scanning electron microscopy. Analyses showed that the extrafloral nectaries are dispersed throughout the leaf, with concentrations mainly in the basal third section. Nectaries lie in epidermal depressions and can be found in abaxial and adaxial leaf blades. Their morphology may be characterized macroscopically by achlorophyllous halos and microscopically as structures consisting of a short pedicel, oval multicellular head, covered by a thin cuticular layer with a secretory pole. Reducing sugars, neutral polysaccharides, proteins, starch and phenolic compounds were detected in extrafloral nectaries.

**Keywords:** ENFs, morphology, secretory structure.

**Introduction**

Nectaries may be classified, by their position, as floral (present in the floral parts) and extrafloral (present in the non-reproductive organs) (MARAZZI et al., 2013). According to Paiva (2011), the term “extrafloral” takes on a broader meaning and comprises any nectary that is not directly involved in the process of pollination, but rather in the protection of plants against herbivores (NASCIMENTO; DEL-CLARO, 2010).

Extrafloral nectaries (ENFs) are specialized glands or tissues that secrete nectar and are found on the vegetative portions of many plant species (DELGADO et al., 2011; MACHADO et al., 2008; PAIVA; MACHADO, 2006). Secretion may be composed of sugars, amino acids, proteins and other compounds (HEIL, 2011). These nectar-secreting glands show external morphological diversity, distinct anatomical structures and different ways of releasing nectar. They occur on virtually all aboveground plant organs such as leaf blade, petiole, young stems, stipules, and on reproductive structures such as buds, calyx, inflorescence axis, flower peduncles and fruits. ENFs are well distributed among angiosperms and very common in the flora of the Brazilian savanna (MACHADO et al., 2008) where they seem to mediate interactions between insects and plants linked to defensive mutualisms (GONZALEZ, 2011). ENFs in...
Bignoniaceae are conservative characters of a unified value, useful in generic delimitation (SEIBERT, 1948). Machado et al. (2008) describe morphologically the Bignoniaceae structures as flattened dishes that may be shifted upward or downward in relation to the level of leaf blade and peltate glandular trichomes.

*Pyrostegia venusta* (Ker Gawl.) Miers. (Bignoniaceae) (also known as *Pyrostegia ignea* and *Bignonia venusta*) is a liana creeper that easily adapts to hygrophyte and mesophyte environments. It is found at the edge of woods and fields, near the seaside and roadside, with a wide distribution throughout most of southern and southeastern Brazil. *P. venusta* leaves exhibit opposite phyllotaxy and are composed of two or three leaflets. In fact, it is an important component of the Brazilian savannah landscape (DUARTE; JURGENSEN, 2007; POOL, 2008). Even though considered a weed, it is not uncommon to see *P. venusta* as an ornamental plant, as it multiplies rapidly and may be used for adorning walls and pergolas. Its leaves and stems are employed in folk medicine as a tonic and to treat diarrhea, vitiligo, coughs and common infection-related diseases of the respiratory system (MOSTAFA et al., 2013).

The presence of EFNs in *Pyrostegia venusta* leaves was initially reported by Seibert (1948) as "glandular fields". The species has been recently listed in the World List of Angiosperms with extrafloral nectaries, updated by Keeler in 2010 (GONZALEZ, 2011). EFNs are structurally diverse and anatomically consist of a modified epidermis, with or without trichomes, and specialized parenchyma. Moreover, the tissue which constitutes the nectary is called nectariferous tissue (NEPI, 2007). According to Marazzi et al. (2013), in anatomically specialized EFNs, at least three different kinds of tissues may be recognized: the epidermis, the nectary parenchyma and the subnectary parenchyma. EFNs may be vascularized or non-vascularized structures. The most frequent vascularization consists of phloem or phloem and xylem (NEPI, 2007).

Currently, many studies are concerned with understanding the evolutionary significance of EFNs and their role in mutualist relationships involving insect-plant interactions neglecting their morphology, which is extremely important for taxonomic studies. Although much information on EFNs in different species of Bignoniaceae exists, the data related to the occurrence and morphology of these structures in *P. venusta* are limited and practically non-existent. In current paper, the distribution and the structural and histochemical aspects of EFNs are studied to contribute towards the morphological characterization of these structures in *P. venusta* leaves.

**Material and methods**

**Plant material**

Young leaves of *Pyrostegia venusta* (Ker Gawl.) Miers (Bignoniaceae) located in the apical region of branches were collected in the Brazilian savanna or cerrado, in Divinópolis, Minas Gerais State, Brazil, at 20º10'45.9"S and 44º55'07.2"W. The voucher specimen was identified by Andréia Fonseca Silva of the Herbarium PAMG (PAMG 56307) of Agricultural Research Company of Minas Gerais.

**Density of EFNs**

Nectaries were observed immediately after collection with fresh and whole material. EFN density (EFNs cm⁻²) was estimated by fifteen fully expanded leaves (thirty leaflets) of each specimen under analysis (five individuals), with the determination of the limbus area and the number of nectarines, as proposed by Paiva and Machado (2006).

Leaves were analyzed under an Olympus SZ51 zoom stereo microscope to observe and count the nectaries, evaluate leaf area and determine nectary density. Data were analyzed by ANOVA tests and their means compared by Tukey’s test at 5% significance. Results were given as mean ± standard deviation.

**Light and scanning electron microscopy**

For structural analysis, the leaves were fixed and stored directly in 70% alcohol (JOHANSEN, 1940). Hand-cut transverse and longitudinal sections of the fixed material were thus obtained. The series underwent the staining procedure with iodine green and Congo red. Sections were examined with a Zeiss Primo Star light microscope and documented by AxioVision.

Samples were SEM-observed (LEO Evo 40 XVP) by immersion in fixative (modified Karnovsky, 2.5% glutaraldehyde, 2.0% paraformaldehyde, 0.05 M cacodylate buffer, pH 7.2) for 24h, and prepared according to protocol by Bossola and Russell (1999).

**Histochemical analyses of EFNs**

The following histochemical methods were used on the fresh material: ferric chloride for phenolic compounds; zinc chloride iodine for lignin and
starch (JENSEN, 1962); Sudan III (SASS, 1951) and IV (GERLACH, 1984) for lipids in general; Lugol for the identification of starch (JOHANSEN, 1940); bromophenol blue for proteins (MAZIA et al., 1953); Schiff reagent (PAS) for neutral polysaccharides; Fehling’s reagent for reducing sugars (SASS, 1951).

Results and discussion

EFNs of P. venusta were characterized macroscopically by achlorophyllous halos with approximately 0.80 ± 0.11 mm in diameter, randomly distributed throughout the leaf blade (Figures 1a and b). Density was 5.27 ± 1.74 EFNs cm⁻² and the occurrence of these structures in the basal third section of the leaflet was 6.39 ± 2.10, while this measurement in the middle and apical third sections was 4.30 ± 1.93 and 3.53 ± 1.11, respectively (Figure 1c). EFNs were microscopically identified in the adaxial and abaxial surfaces as small epidermal depressions (Figure 1d). P. venusta’s EFNs could be morphologically characterized as peltate glandular trichomes with short pedicel and multicellular ovoid heads, covered by a thin layer of cuticle and provided with a secretory pole (Figure 1d, e and f). The information obtained by scanning electron microscopy corroborated the report made under light microscopy (Figures 1g and h).

The presence of EFNs on leaves in the species studied may be the result of evolutionary adaptation and may be explained by the fact that P. venusta is a liana vine, which facilitates access of herbivores and other predators to the plant bodies, because it requires brackets (walls and other plants) for their growth that allow access to EFNs. As suggested by Chamberlain and Holland (2009), staining some EFNs may provide a visual aid for revealing the location of these structures to some animals, which cannot be eliminated, such as in the case of EFNs in P. venusta.

The randomization, the insertion of EFNs in epidermal depressions and their presence in the adaxial and abaxial surfaces may significantly reduce exudate loss by evaporation, as suggested by Paiva and Machado (2006). The above increases their availability of secretions and facilitates the patrol of limbus leaflets by insects, which contributes to the reduction of herbivore pests (HEIL; KOST, 2006; KOST; HEIL, 2005; PAIVA; MACHADO, 2006). The random distribution of EFNs and their preferential accumulation at the basal regions of the leaflet have been reported in Hymenaea stigonocarpa Mart. ex-Hayne (Fabaceae) (PAIVA; MACHADO, 2006).

Morphologically, the nectaries found in P. venusta corroborate the description by Seibert (1948) and Machado et al. (2008) for the Bignoniacae family. Gonzalez (2011) recently reported the presence of these structures in different species of the family Bignoniacae, including the species studied in current research. Duarte and Jurgensen (2007) refer to EFNs of P. venusta only as glandular trichomes, which are not related to the interactions between insects and plants. However, glandular trichomes and EFNs are distinct secretory structures (EVERT, 2013). The glandular trichomes are epidermal appendix originating in protoderm cells, whereas cells subjacent to tissues such as parenchyma or even vascular tissues also participate in the ontogeny of EFNs (ASCENSÃO, 2007; EVERT, 2013).

According Ascensão (2007), it is highly difficult to distinguish between these two types of structures, often referred to generically as glandular trichomes, without ontogenetic studies. Other structures involved in plant interactions, such as colleters, food bodies and similar structures are also considered trichomes in some species (PAIVA; MACHADO, 2006). Denardi et al. (2012) reports that the glandular trichomes in Connarus suberosus function as food bodies because of their presence on the exposed surfaces of developing organs and the chemical nature of their reserve products.

EFNs in P. venusta are similar to flattened dishes with a secretory pole and a more elevated cupule sunk on the leaf surface (Figure 1d, g and h). These nectaries are named scale-like nectaries (MACHADO et al., 2008). The secretory pole has an upper secretory region, formed by one layer of cells covered by a thin cuticle and by an underlying region with one layer of isodiametric epidermal cells connected to the leaf parenchyma (Figure 1d). The nectary parenchyma’s cells are not anatomically distinguished from other cells of the parenchyma and no vascularization was reported in these structures.

In the histochemical characterization of EFNs, a positive reaction of ferric chloride (Figure 2b), zinc chloride iodine (Figure 2c), bromophenol blue (Figure 2d), Periodic Acid Schiff (Figure 2e) and Fehling (Figure 2f) revealed the presence of phenolic compounds, starch, proteins, neutral polysaccharides and reducing sugars, respectively. Tests conducted with lugol, Sudan III and Sudan IV did not indicate the presence of starch and lipids.
According to Pimentel et al. (2011), the detection of different chemicals in the cells of nectaries does not imply their presence in the secretion, although nectaries still have ecologically potential functions. The presence and availability of different metabolite groups in EFNs may serve as attractive and nutritional supplements for different insects, such as hymenopterans (ants and others) and homopterans, a well-discussed fact in the literature (NOGUEIRA et al., 2012). The nectaries attract ants that protect the leaves against herbivores (CHAMBERLAIN; HOLLAND, 2009). In return for extrafloral nectar, ants are the bodyguards and patrol young leaf surfaces, removing or deterring unwanted pests (BIXENMANN et al., 2011; ROSUMEK et al., 2009). Phenolic compounds identified in nectaries cells of the species under analysis may provide protection against herbivores, microorganisms, and excess ultraviolet radiation. They may also protect the protoplast maintaining cellular integrity when subjected to water stress (PAIVA; MACHADO, 2008). Although the presence of plastids with starch
grains in nectariferous tissues have been extensively reported in the literature (ROCHA; MACHADO, 2009), these compounds in the EFNs of *P. venusta* were detected only in the reaction with zinc chloride iodine. Starch accumulated in the plastids may be the source of some components of nectar which is total or partially degraded during manifestations of secretory activity (HEIL, 2011). It is likely that sugars detected in *P. venusta*'s nectaries originate from degradation starch grain present in plastids, as has been reported by Pimentel et al. (2011) on the secretion structures of *Pavonia alnifolia* (Malvaceae). Detected proteins and polysaccharides suggest that such compounds might be found in exudates, which is an evidence of the nectar's complexity (COUTINHO et al., 2012). These substances in EFNs have also been described by other authors (COUTINHO et al., 2010; ROCHA et al., 2009).

**Conclusion**

The present study is an initial investigation on the distribution and morphology of EFNs in *P. venusta*. Since the nectaries presented close vascularization, further anatomical studies must be developed to carefully examine and describe the relationships between these nectaries and the leaves vascular systems.

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**References**


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