Secretory spaces in species of the clade Dipterygeae (Leguminosae, Papilionoideae)

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ABSTRACT

Dipteryx, Pterodon and Taralea are legume genera known for secreting oils, produced in secretory canals and cavities, with medicinal properties. We analyzed the distribution, morphology and histochemistry of these glands in leaves and stems of Dipteryx alata, Pterodon pubescens and Taralea oppositifolia, three Neotropical species, using standard techniques for anatomy and histochemistry. Secretory spaces, i.e. secretory cavities and canals, exhibited a wide lumen and a single layer of epithelium. Digitiform epithelial cells, forming trabeculae protruding into the lumen, were seen in all three species. Secretory cavities with a rounded or oval lumen and secretory canals with an elongated lumen in longitudinal sections were found only in T. oppositifolia. In D. alata and P. pubescens, only secretory cavities were found. In P. pubescens, secretory cavities occurred in the leaf blade margin. In T. oppositifolia, secretory spaces were much more numerous than in the other two species. Terpenes, total lipids, phenolic compounds, alkaloids and polysaccharides were detected in the secretory spaces of the three species. The abundance of secretory spaces, the presence of canals in T. oppositifolia and the position of cavities in P. pubescens are features with potential diagnostic value for their respective genera.

Keywords: anatomy, Fabaceae, gland, secretory canals, secretory cavities

Introduction

Secretory cavities and canals are common in vegetative (Lersten & Curtis 1986; Turner 1986; Teixeira et al. 2000; Teixeira & Gabrielli 2000; Marcati et al. 2001; Paiva & Machado 2007; Rodrigues & Machado 2009; Teixeira & Rocha 2009; Rodrigues et al. 2011a; b; Milan & al. 2012; Rodrigues & Machado 2012) and reproductive (Paiva et al. 2008; Teixeira & Rocha 2009; Leite et al. 2014) organs of species of Leguminosae. They produce and accumulate compounds involved with mechanisms of defense against herbivores and pathogens, and therefore are responsible for ecological interactions (Fahn 1979; Langenheim 2003).

The bioactive substances produced by secretory cavities and canals of many legume species possess economic value and have been exploited by the cosmetic and pharmaceutical industries and have been used in, among other things, varnishes (Langenheim 2003). In addition, the occurrence of secretory cavities and/or canals in vegetative and/or reproductive organs may be taxonomically important, and help to identify genera and species of Leguminosae (Turner 1986; Lersten & Curtis 1996; Teixeira et al. 2000; Teixeira & Gabrielli 2000; Leite et al. 2014).

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The early diverging papilionoid clade Dipterygeae is comprised of about 25 species in four genera, Dipteryx, Pterodon, Taralea and Monopteryx (Cardoso et al. 2012; 2013). Secretory spaces have already been documented in some members of Dipteryx, Pterodon and Taralea (see Rodrigues & Machado 2004; Paiva et al. 2008; Rodrigues & Machado 2012; Leite et al. 2014), but are absent in Monopteryx (FH Palermo unpubl. res.).

Species of Pterodon occur in drier areas of central Brazil and eastern Bolivia. Members of Dipteryx & Taralea are found in more-humid areas in the Amazon; however, some species of Dipteryx are also found in central Brazil (Barham 2005). Dipteryx alata, popularly named “baru” or “cumbaru”, possesses intensely green-colored glabrous leaves with a winged petiole and a blade with 6 to 12 leaflets (Lorenzi 2002). It is exploited for food and medicinal purposes and the wood is used in domestic and naval construction (Almeida et al. 1998). Dipteryx alata exhibits rapid growth and a low requirement for nutrient supplementation (Sano et al. 2004), and so it has been useful in the restoration of degraded areas. Taralea oppositifolia is known as “cumaruda-praia”; its leaves are pseudoparipinnate, with four to eight leaflets and a terminal appendix containing a winged rachis (Francisco 2010). The oil produced by this species is odorless and widely used for several industrial purposes (Barham 2005), and its wood is used for making furniture and in civil construction (Sousa et al. 2007). Pterodon pubescens is popularly named “sucupira-branca” in the Brazilian Cerrado. Its paripinnate leaves are comprised of 20 to 36 leaflets (Lorenzi 2002) and its pale wood is resistant to xylophagous insects and microorganisms (Rizzini 1971). The oil extracted from its fruits and seeds has analgesic potential (Coelho et al. 2005) and can inhibit penetration of the skin by the cercaria that cause schistosomiasis (Mors et al. 1964).

Recent studies showed that D. alata, P. pubescens and T. oppositifolia have secretory canals and cavities in their flowers (Leite et al. 2014). However, data of the presence of secretory structures in the vegetative axis of these species are scarce and refer only to P. pubescens (Rodrigues & Machado 2012). In this study, we analyzed the morphology and histochemistry of the secretory spaces in leaves and young stems of D. alata, P. pubescens, and T. oppositifolia. Potential diagnostic characters for the genera and the clade Dipterygeae are also discussed.

Materials and methods

Plant Material

We sampled adult individuals of Dipteryx alata Vogel. growing on the campus of the University of São Paulo (USP), in the city of Ribeirão Preto, São Paulo State, Brazil, where the climate is tropical with dry winters, and the coldest month with a mean temperature higher than 18 °C. Adult individuals of Pterodon pubescens Benth. were collected from a remnant of Cerrado in the city of Botucatu, São Paulo State, where the climate is subtropical with dry winters, and a mean temperature in the coldest month lower than 18 °C, and rainy summers with a mean temperature of 22 °C. Adult individuals of Taralea oppositifolia Aubl. were collected from Amazon Forest in the city of Roraima, Roraima State and the city of Belém, Pará State, Brazil, where the climate is humid tropical, without a dry season, with mean temperatures in all months higher than 18°C, and total rainfall in the driest month above 60 mm. For each species, we collected samples of stems at 1.5 cm and 4 cm below the shoot apex, and mature leaves located at the third node of branches of four individuals. Samples of the median region of petioles, rachis and leaf blade were extracted from the leaves.

Vouchers were deposited in the herbarium “Irina Delanova de Gemchujnicov” (BOTU) of the Universidade Estadual Paulista, Botucatu; the herbarium of the Instituto Nacional de Pesquisas da Amazônia (INPA), and the herbarium of São Paulo State University (SPFR), Ribeirão Preto, with the following register numbers: BOTU 29969 (P. pubescens), INPA 259958 (T. oppositifolia), IAN 186796 (T. oppositifolia) and SPFR 12 557 (D. alata).

Light microscopy

Samples were fixed in FAA (formaline: acetic acid: 50 % ethanol, 1:1:18 by volume) (Johansen 1940). Part of the fixed material was dehydrated in an ethanol series, embedded in methacrylate resin (Leica Historesin®, Heidelberg, Germany), and serially sectioned using a rotary microtome (Leica® RM2145, Nussloch, Germany). Cross and longitudinal sections (6 µm thick) were dyed with 0.05 % toluidine blue, pH 4.7 (O’Brien et al. 1964). Permanent slides were mounted with Entellan (Merck®, Darmstadt, Germany). Another lot of the fixed material was sectioned using a Ranvier microtome; the 12 µm-thick sections were cleared with 20 % sodium hypochlorite, rinsed in 1 % acetic acid, and stained with Safrablau (Bukatsch 1972); semi-permanent slides were mounted with glycerin jelly.

The slides were viewed under an Olympus BX41 microscope (Hamburg, Germany) with the relevant observations being documented using an Olympus Camedia C-7070 digital camera (Hamburg, Germany).

Secretory spaces in the organs were quantified in transverse sections obtained from ten samples of stems and ten samples of leaves excised from four individuals using the software Olympus CellB. The data were submitted to ANOVA followed by a Tukey test, at the 5 % probability level, using the software BioEstat 5.0.

Importantly, we considered secretory canals to be those structures in which the length of the lumen was at least twice.
as long as the diameter in longitudinal sections (modified from Fahn 1979).

**Histochemical tests**

Sections of fresh material were obtained using a Ranvier microtome (Naugra®, Haryana, India) and treated with Sudan Black B for detection of total lipids (Pearse 1980); Nadi reagent for terpenes (David & Carde 1964); 10 % ferric chloride for phenolic compounds (Johansen 1940); 0.02 % Ruthenium red for pectin substances (Johansen 1940); periodic acid/Schiff reagent (PAS) for complex polysaccharides (Feder & O’brien 1968); Wagner reagent for alkaloids (Furr & Mahlberg 1981); and bromophenol blue for total proteins (Mazia et al. 1953).

**Results**

Secretry spaces occurred in the cortex of the stem (Fig. 1A-C), petiole (Fig. 1D, F), rachis (Fig. 1E) and midrib (Fig. 1G-I). Wide secretory spaces occupied the entire area of the leaf margin in *P. pubescens* (Fig. 2A). In *T. oppositifolia*, the secretory spaces in the stem were much more abundant than in the other species (Tab. 1). In stems with incipient secondary growth, secretory spaces were not produced by the cambium or phellogens in any species.

In cross sections, the secretory spaces had a rounded lumen in all the organs (Figs. 1A-I, 2A-C). In longitudinal sections of stem and leaf portions of *P. pubescens* (Fig. 2D) and *D. alata* (Fig. 2E), the secretory spaces maintained the rounded or oval shape of the lumen, characterizing typical secretory cavities; on the other hand, the longitudinal sections of the stem and leaves of *T. oppositifolia* exhibited secretory spaces with an elongated lumen (Fig. 2F), featuring secretory canals. The distribution of the different morphotypes of secretory spaces in the stems and leaves of the three species is presented in Tab. 2.

In all organs, the secretory spaces had a uniseriate epithelium consisting of secretery cells of various shapes. The epithelial cells were voluminous and ranged from papilliform to rectangular (Figs. 1F, 2B-C); digitiform epithelial cells protruded into the lumen, forming trabeculae (Fig. 2G-I) in all three species.

A sheath comprised of one to two layers of tangentially elongated parenchyma cells surrounded the secretory spaces in the leaves and stem of all three species (Figs. 1F, 2B-C, 2H). Periclinal divisions were observed in the parenchyma sheath (Fig. 1F).

Histochemical tests detected the presence of essential oils (Fig. 3A), total lipids (Fig. 3B), polysaccharides (Fig. 3C, D), phenolic compounds (Fig. 3E) and alkaloids (Fig. 3F-G) in the epithelial cells and/or lumen of the secretory spaces of the stems and leaves of all three species.

**Discussion**

We observed secretory spaces characterized by a rounded lumen in transverse and longitudinal section in *D. alata* and *P. pubescens*; such features are consistent with typical secretory cavities according to Fahn (1979) and Evert (2006). In contrast, *T. oppositifolia* possessed, in addition to typical secretory cavities, secretory spaces with elongated lumen in longitudinal sections, which represent secretory canals (Fahn 1979; Evert 2006). This is the first report of the occurrence of secretory canals in the vegetative organs of a member of the Dipterygeae clade; the presence of these glands in floral part, was also described for *T. oppositifolia* by Leite et al. (2014).

Secretory spaces with digitiform elongated epithelial cells intersecting the lumen and forming trabeculae, previously observed in *P. pubescens* by Rodrigues & Machado (2012), were found in all three species studied here. The trabeculae in the lumen of these secretory spaces probably play a functional role as a framework to support secretion inside the wide lumen of these glands, since secretion products accumulate within the interstices. Trabeculate cavities seem to be a rare condition in Leguminosae, having been previously described only in species of Amorpheae, Psoraleae (Turner 1986) and Millettieae (Teixeira & Rocha 2009). These traditional tribes, although also belong to Papilionoideae, are not closely related to Dipterygeae, indicating that trabeculate secretory spaces have evolved independently in several lineages of the family.

The presence of a parenchyma sheath with meristematic potential around the secretory spaces appears to be a common feature among legume species (Rodrigues et al. 2011a; Rodrigues & Machado 2012). This sheath plays an important role in the maintenance of secretory activity and in the replacement of degenerated cells in the epithelium of secretory spaces (Rodrigues et al. 2011a). After degeneration, the epithelial cells can be replaced by other cells originating from the parenchyma sheath that surrounds the secretory spaces in legumes (Rodrigues et al. 2011a; Rodrigues & Machado 2012).

Our histochemical tests showed the presence of hydrophilic and lipophilic substances in secretory spaces in leaves and stems of all the species, indicating the mixed nature of the secretion, according to Fahn (1979) and Evert (2006). Alkaloids, phenolic compounds, total lipids and terpenes can function to provide protection against herbivores and pathogens (Harbone 1993; Langenheim 2003) while polysaccharides can help to maintain water potential (Sawidis 1998). The production of these compounds of different chemical categories in secretory spaces seems to be a feature shared by species of Dipterygeae, in a manner similar to the resinous species of Detarieae (Langenheim 2003; Rodrigues et al. 2011b).

The remarkable abundance of secretory spaces in the stem cortex of *T. oppositifolia* deserves special attention.
Figure 1. Secretory spaces (SS) in Pterodon pubescens (A, D, G), Dipteryx alata (B, E, H) and Taralea oppositifolia (C, F, I). Cross section of stem (A, B, C), petiole (D, F), rachis (E) and midrib (G, H, I). In F, note the wide lumen (LU) and the uniseriate epithelium (EP). The arrowhead in F and insert indicates dividing cells in the parenchyma sheath (SH). Scale bars = 150 µm (B, C, D, E, G, I), 100 µm (A, F, H).
Figure 2. Secretory spaces in *Pterodon pubescens* (A, C, D, H), *Dipteryx alata* (B, E, I) and *Taralea oppositifolia* (F, G). A, Cross section of leaf blade showing a secretory cavity consisting of uniseriate epithelium (EP) and a wide lumen (LU) in the leaf margin. B-C, Cross sections detailing secretory cavities in a stem with a wide lumen (LU), uniseriate epithelium (EP) consisting of cells with variable shapes, and parenchyma sheath (SH). D, E, Longitudinal sections of stem showing secretory cavities (CV). F, Longitudinal section of stem showing secretory canal (CN). G-I, Details of secretory spaces showing trabeculae (arrows) formed by the protrusion of epithelial cells into the lumen. Scale bars = 150 µm (D, E, F), 100 µm (A, G), 50 µm (B, C, H, I).

Table 1. Analysis of variance of the number of secretory spaces in transverse sections of stem and leaf portions in *Taralea oppositifolia*, *Dipteryx alata* and *Pterodon pubescens*. Capital letters indicate comparison between columns, and lowercase letters indicate comparison between lines (Tukey test *P* < 0.05).

<table>
<thead>
<tr>
<th>Organ/leaf portion</th>
<th><em>T. oppositifolia</em></th>
<th><em>D. alata</em></th>
<th><em>P. pubescens</em></th>
<th>P</th>
<th>F(2,27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem</td>
<td>29 Aa</td>
<td>3.4 Bb</td>
<td>3.2 Ba</td>
<td>&lt;0.0001</td>
<td>3715.4</td>
</tr>
<tr>
<td>Petiole</td>
<td>6.2 Ab</td>
<td>4.3 Ba</td>
<td>3.5 Ca</td>
<td>&lt;0.0001</td>
<td>42.5</td>
</tr>
<tr>
<td>Rachis</td>
<td>5.2 Ac</td>
<td>3.4 Bb</td>
<td>3.8 Ba</td>
<td>&lt;0.0001</td>
<td>19.2</td>
</tr>
<tr>
<td>Midrib</td>
<td>3.5 Ad</td>
<td>2.7 Bb</td>
<td>1.3 Cc</td>
<td>&lt;0.0001</td>
<td>31.3</td>
</tr>
<tr>
<td>Mesophyll</td>
<td>2.5 Be</td>
<td>3.4 Ab</td>
<td>2.3 Bb</td>
<td>0.002</td>
<td>8.4</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>0.0002</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F(4,45)</td>
<td>2298.3</td>
<td>7.5</td>
<td>16.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Secretory spaces in species of the clade Dipterygeae (Leguminosae, Papilionoideae)

The presence of a large number of secretory spaces in this species indicates the production of great amount of biologically active substances. This feature may be related to the environment where this species occurs in Central and South America, including its wide distribution in the Amazon Forest (Francisco 2010), where the hot humid climate favors the occurrence of herbivores and pathogens. The high abundance of canals and cavities that secrete substances with deterrent and antimicrobial properties (Langenheim 2003) may be fundamental for the development of these plants. Our findings showed that the very high number of secretory spaces in stems is a reliable character for distinguishing *T. oppositifolia* from *P. pubescens* and *D. alata*.

The presence of secretory spaces is a condition shared by with some other members of the ADA clade (Angylocalyceae, Dipterygeae, Amburaneae, see Cardoso *et al.* 2012; 2013). In addition to the Dipterygeae clade (present study, Rodrigues & Machado 2004; 2012; Paiva *et al.* 2008; Leite *et al.* 2014), secretory spaces were reported in the leaves of *Myrocarpus*, *Myrospermum* and *Myroxylon* (Sartori & Tozzi 2002), which are part of the Amburaneae clade (Cardoso *et al.* 2012; 2013). Surprisingly, however, secretory spaces were not observed in leaves of *Monopteryx auacu* (FH Palermo unpubl. res.), a lineage of the Dipterygeae clade.

On one hand, the presence of secretory spaces can group species into some subclades of the ADA clade; on the other hand, the morphology and distribution of secretory spaces are highly variable, and can be employed as diagnostic characters at the specific and supraspecific levels, especially for the clade Dipterygeae (present study; Leite *et al.* 2014). In this clade, the presence of secretory spaces in vegetative and reproductive organs is a well-known characteristic (Rodrigues & Machado 2004; 2012; Leite *et al.* 2014), which

Table 2. Distribution of secretory cavities and canals in young stem and leaf portions of *Dipteryx alata*, *Pterodon pubescens* and *Taralea oppositifolia*. Symbols: CV: secretory cavity; CN: secretory canals; –: absence of secretory spaces.

<table>
<thead>
<tr>
<th>Organ/region</th>
<th>Species</th>
<th>D. alata</th>
<th>T. oppositifolia</th>
<th>P. pubescens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem cortex</td>
<td>CV</td>
<td>CV; CN</td>
<td>CV</td>
<td></td>
</tr>
<tr>
<td>Petiole cortex</td>
<td>CV</td>
<td>CV; CN</td>
<td>CV</td>
<td></td>
</tr>
<tr>
<td>Rachis cortex</td>
<td>CV</td>
<td>CV; CN</td>
<td>CV</td>
<td></td>
</tr>
<tr>
<td>Midrib cortex</td>
<td>CV</td>
<td>CV; CN</td>
<td>CV</td>
<td></td>
</tr>
<tr>
<td>Leaf mesophyll</td>
<td>CV</td>
<td>CV</td>
<td>CV</td>
<td></td>
</tr>
<tr>
<td>Leaf margin</td>
<td>–</td>
<td>–</td>
<td>CV</td>
<td></td>
</tr>
</tbody>
</table>

appears in the early developmental stages of individual plants (Rodrigues & Machado 2012).

In conclusion, the secretory spaces we found in the three species of Dipteryggeae studied here are morphologically diverse and abundant. Our results show that the presence of secretory spaces with trabeculate epithelial cells, that produce mixed secretions in leaves and young stems, is a synapomorphy of Dipteryggeae. *Dipteryx alata* and *P. pubescens* are more anatomically similar to each other than to *T. oppositifolia*, confirming floral developmental data (Leite et al. 2014) and reflecting the topology of the phylogenetic tree of the tribe, which places them as sister genera (see Cardoso et al. 2012; 2013). However, the occurrence of secretory cavities in the leaf-blade margin of *P. pubescens* should be noted as a distinctive feature in comparison to the other species studied here. Further studies employing a larger number of species from these genera, as well as taxa distributed more widely throughout the ADA clade, would be interesting and generate robust data for understanding the phylogenetic relationships within the group. It is interesting that although these species colonize environments with contrasting characteristics and possess structural peculiarities in their secretory systems, they produce, as we found, secretions comprised of the same classes of chemical compounds, which suggests that these compounds may be of value to the success of these species under a range of environmental factors.

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**References**


Evert RE. 2006. Esau’s plant anatomy; meristems, cells, and tissues of the plant body - their structure, function, and development. New Jersey, John Wiley & Sons.


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Teixeira SP, Rocha JF. 2009. Oil glands in the Neotropical genus Dahlstedtia (Leguminosae-Papilionoideae, Millettiaeae). Revista Brasileira de Botânica 32: 57-64.