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## REFERÊNCIA

PAIVA, Élder Antônio Sousa e *et al.* Occurrence and structure of extrafloral nectararies in *Pterodon pubescens* Benth. and *Pterodon polygalaeflorus* Benth. **Pesquisa Agropecuária Brasileira**, Brasília, v. 36, n. 2, p. 219-224, fev. 2001. DOI: <https://doi.org/10.1590/S0100-204X2001000200002>. Disponível em: [http://www.scielo.br/scielo.php?script=sci\\_arttext&pid=S0100-204X2001000200002&lng=en&nrm=iso](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-204X2001000200002&lng=en&nrm=iso). Acesso em: 04 dez. 2020.

## Occurrence and structure of extrafloral nectaries in *Pterodon pubescens* Benth. and *Pterodon polygalaeiflorus* Benth.<sup>(1)</sup>

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**Abstract** – Extrafloral nectaries (EFNs) are structurally variable and widely spread among the angiosperms. The occurrence of EFNs in leaves of *Pterodon polygalaeiflorus* Benth. and *Pterodon pubescens* Benth. (Fabaceae: Papilionoideae) were detected in adult specimens, at the time of production of new buds and flowers. The goals of the present study are to register the occurrence of the EFNs in *P. pubescens* and *P. polygalaeiflorus*, and provide comparative data on the anatomical structures. The EFNs occur in the rachis and are located under the insertion of each petiolule. Each nectary consists of a small elevation whose apical portion is deeply invaginated, resulting in a depression (secretory pole), a common characteristic of both species. Unicellular, non-glandular trichomes occur along the rachis, being less numerous in *P. polygalaeiflorus* while in *P. pubescens* they cover the EFNs. The secretory tissue consists of parenchyma cells with dense cytoplasm compactly arranged. The nectar reaches the surface of the EFNs by rupturing the thin cuticle which covers the secretory pole, since both species lack stomata or any other interruption at the epidermis. The basic difference between the two species, in relation to the EFNs, is the density of the pubescence, which is always greater in *P. pubescens*. Structural and dimensional modifications may be observed, even between basal and apical nectaries in the same rachis, so it does not constitute a taxonomical tool.

**Index terms:** nectaries, plant anatomy, plant secretions, nectar, plant protection, herbivores, plant animal relations.

### Ocorrência e estrutura de nectários extraflorais em *Pterodon pubescens* Benth. e em *Pterodon polygalaeiflorus* Benth.

**Resumo** – Nectários extraflorais (NEFs) são estruturalmente variáveis e de ampla ocorrência entre as angiospermas. A ocorrência dos NEFs nas folhas de *Pterodon polygalaeiflorus* Benth. e *Pterodon pubescens* Benth. (Fabaceae: Papilionoideae) foi detectada em espécimes adultas, durante a produção de novas gemas e flores. Os objetivos deste estudo foram registrar a ocorrência de NEFs em *P. pubescens* e *P. polygalaeiflorus*, e fornecer dados comparativos sobre a estrutura anatômica destas estruturas. Os NEFs ocorrem na raque e estão localizados sob a inserção de cada peciólulo. Cada nectário consiste de uma pequena elevação cuja porção apical é fortemente invaginada, resultando em uma depressão (o pólo secretor), característica comum a ambas as espécies. Tricomas tectores unicelulares ocorrem ao longo da raque, sendo menos numerosos em *P. polygalaeiflorus*, enquanto em *P. pubescens* eles cobrem todo o NEF. O tecido secretor consiste de células parenquimáticas com citoplasma denso. O néctar alcança a superfície dos NEFs pela ruptura da fina cutícula que cobre o pólo secretor, uma vez que ambas as espécies não apresentam estômatos ou qualquer outra interrupção da epiderme neste local. A diferença básica entre as duas espécies, em relação aos NEFs, é a densidade da pubescência, que é sempre maior em *P. pubescens*. Modificações estruturais e de dimensões podem ser observadas até mesmo entre os nectários basais e apicais de uma mesma raque, e portanto tais modificações não apresentam valor taxonômico.

**Termos para indexação:** nectários, anatomia vegetal, secreção vegetal, néctar, proteção das plantas, herbívoros, relação planta-animal.

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## Introduction

*Pterodon pubescens* Benth. and *Pterodon polygalaeflorus* Benth. are common species from Central Brazil vegetation. Their distribution is disjunctive, and mixed populations were not observed, which may indicate some soil characteristic as a limiting factor.

Recently, some researches have tried to establish distinctive characteristics between the two species, which were taxonomically situated as synonyms for *P. emarginatus* (Lewis, 1987).

Extrafloral nectaries are extremely variable in structure and may occur all over the shoots (Elias, 1983; Oliveira & Leitão Filho, 1987). The EFNs differ from hydroids, resin glands and other secretory structures by their sugar watery secretion, where amino acids are common (Bentley, 1977). The great structural variability and wide occurrence of the EFNs are evidenced by several authors (Keeler, 1980; Oliveira & Leitão Filho, 1987; Morellato & Oliveira, 1991; Oliveira & Oliveira-Filho, 1991; Schupp & Feener, 1991; Fiala & Linsenmair, 1995).

The EFNs function as defensive traits against herbivores and their efficiency vary spatially and temporarily (Pickett & Clark, 1979; Keeler, 1980; Heads & Lawton, 1985; Barton, 1986).

Mutualistic relationship involving EFNs and insects have been studied. Ants feed on the nectar, which is rich in carbohydrates and amino acids, and protect the plants from other insects (Pickett & Clark, 1979). The flora of the "Cerrados" is rich in plant species with EFNs and many ant species have been found associated to the EFNs (Oliveira & Brandão, 1991; Oliveira et al., 1995). *Qualea grandiflora* and *Q. multiflora* (Vochysiaceae) are typical "Cerrado" species and the ants that visit their EFNs actually act as antiherbivores agents (Costa et al., 1992; Del-Claro et al., 1996).

Beyond the antiherbivore action, some researches have demonstrated that the insects which visit the EFNs are responsible for an increase in seed production by significantly reducing the predation of the reproductive structures (Inouye & Taylor, 1979; Keeler, 1981).

The occurrence, morphology, density and disposition of the EFNs in plants may have taxonomical

value (Metcalf & Chalk, 1979). Among the Fabaceae from "Cerrado", the EFNs are more frequent in the Mimosoideae (Oliveira & Leitão Filho, 1987). According to Elias (1983), the EFNs are also common in the Caesalpinioideae, but not frequent in the Papilionoideae. Oliveira & Oliveira-Filho (1991) studied the distribution of the EFNs in woody plants of the "Cerrado", including areas of occurrence of *P. pubescens* and *P. polygalaeflorus*, but these species were not included in the lists of species with EFNs.

The goals of the present study were to register the occurrence of the EFNs in *P. pubescens* and *P. polygalaeflorus*, and provide comparative data on the anatomical structures.

## Material and Methods

Leaves of *P. pubescens* and *P. polygalaeflorus* were collected during the end of the dry season, in five specimens of each species which were producing new flowers and buds, in Brasília, DF, Brazil. Nectar production was observed, allowing the detection and localization of the nectaries. The sugar watery secretion of the EFNs was tested with Fehling reagent (Kraus & Arduin, 1997).

The leaves collected were completely expanded and free from injuries, and were fixed in FAA<sub>50</sub> Johansen (1940). In the laboratory, they were submitted to the usual processes for studies of the anatomical organization of extrafloral nectaries.

Some of the leaves were dissected in the stereomicroscope. Rachis portions, including the EFNs, were dehydrated in butanolic series (Strasburger, 1924; Johansen, 1940; Sass, 1951) and embedded in Paraplast. Transverse and longitudinal sections, 12 µm thick, were done in a rotative microtome – Reichert Jung. Staining procedures were done in safranin and astrablue (Johansen, 1940) and/or astrablue and basic fuchsin (Kraus & Arduin, 1997). Slides were mounted in Entellan.

## Results and Discussion

Extrafloral nectaries occur in the rachis, under the insertion of each petiolule (Figures 1 and 2). They consist of a small elevation which apical portion is deeply invaginated, forming a depression, where the secretory pole is located (Figures 3 and 4). These characteristics are common to *P. pubescens* and *P. polygalaeflorus*.

Unicellular, non-glandular trichomes occur along the rachis. These trichomes are more numerous in

*P. pubescens* and scarcely distributed in *P. polygalaeflorus*. Because of these characteristics the EFNs are difficult to visualize in *P. pubescens*, where they are covered under a dense layer of trichomes. *P. polygalaeflorus* exhibit trichomes on the margins of the EFNs, which protect the aperture. Nevertheless, the number of these structures is variable and they can be lacking, especially, in the superior portion of the rachis.

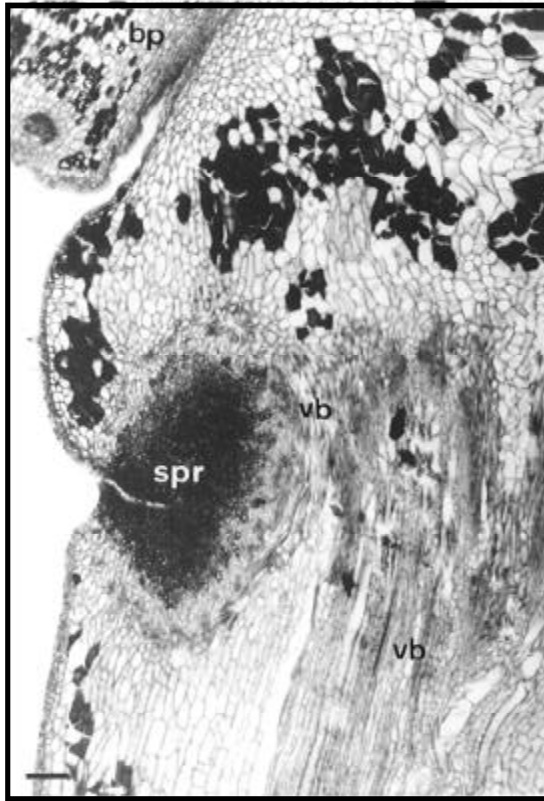
In the basal portion of the leaf, the EFNs are slightly larger than those on the apical portion, in both species. This difference is also reflected in nectar production, which can be registered through field observations.

The EFNs in both species present compactly arranged parenchymatous cells with dense cytoplasm and large nucleus, which constitute the secretory tis-

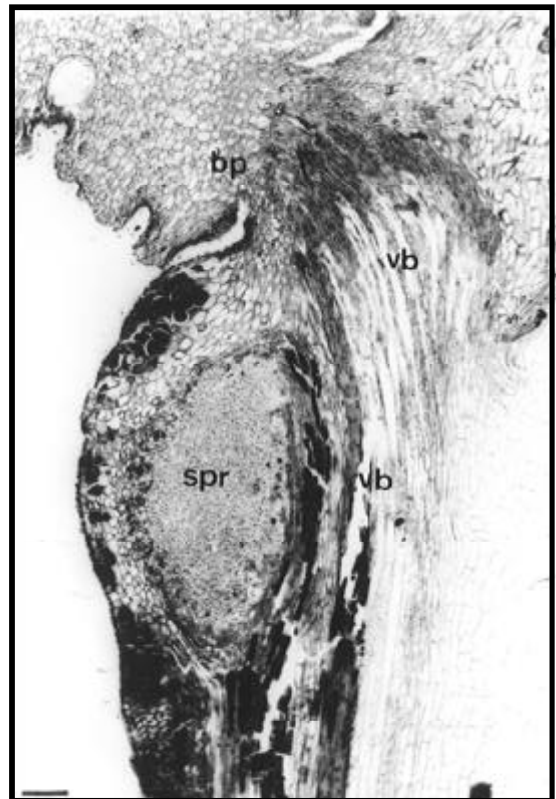
sue (Figures 5 and 6). Sclereids are observed in the margins of the depression, around the secretory pole.

These cells confer mechanical protection to the parenchyma, therefore, they are not always present, especially in *P. pubescens*. According to Elias (1983), these sclereids are related to protection against sucking insects. In the EFNs studied, this mechanical barrier may not act as a protection against insects, once it does not involve the secretory tissue completely, and is concentrated mainly in the margin of the secretory pole.

Crystals of calcium oxalate may be present in the vicinity of the parenchyma, associated to the phloem. Even though these crystals are more abundant in *P. polygalaeflorus* specimens, this difference may be consequence of the differential availability of calcium in the soil, since there is a positive correlation between calcium supply and the amount of crystals of



**Figure 1.** Longitudinal section of the rachis of *P. polygalaeflorus* through the EFN (bar = 100  $\mu$ m; bp: base of petiolule; spr: secretory parenchyma; vb: vascular bundle).



**Figure 2.** Longitudinal section of the rachis of *P. pubescens* through the EFN (bar = 100  $\mu$ m; bp: base of petiolule; spr: secretory parenchyma; vb: vascular bundle).

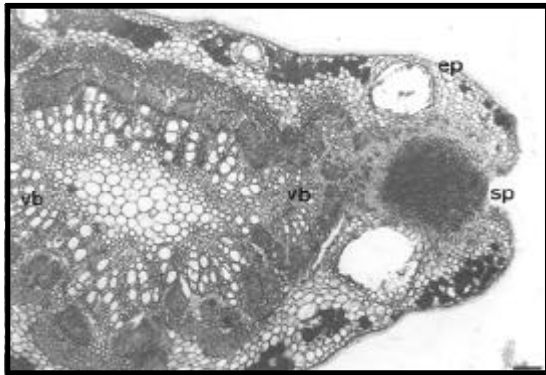
calcium oxalate in plant tissues (Zindler-Frank, 1995).

The secretory pole, in both species, is covered by simple epidermis, lacking trichomes and stomata (Figures 5 and 6). The cuticle is thin, when compared to other parts of the leaf. In some transverse sections, cuticular fragments are observed detaching from the epidermis, which suggests that the secretion is accumulated between the cuticle and the epidermis. Therefore, to reach the surface of the EFN, it causes the rupture of the cuticle.

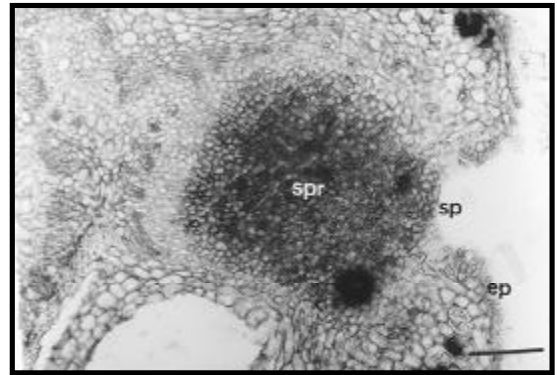
The nourishment of the nectaries is provided by the main bundles disposed in a circle, at the center of the rachis, and by two other vascular bundles, lo-

cated laterally to the rachis in *P. pubescens* as well as in *P. polygalaeiflorus*. The vascular system of the rachis is similar in both species; the lateral bundles are present all along the rachis, being involved in the vascularization of the leaflets.

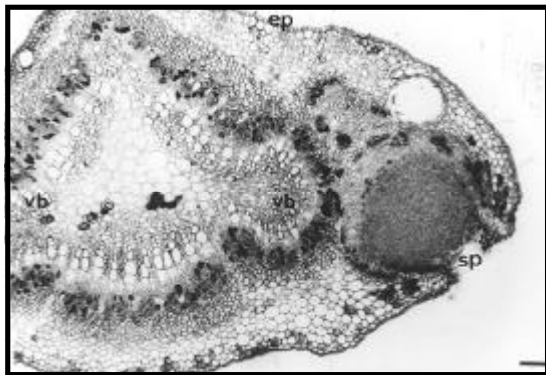
In the vicinity of the nectary, pericyclic fibers do not occur and the phloem cells are directed towards the secretory parenchyma; xylem cells do not follow this pattern. According to Fahn (1979), the nectaries of the Fabaceae may lack vascular tissue or may be supplied only by phloem, which could be seen in both of the species studied. In addition, the vascular type may vary among the genera in the same family or even among specimens of the same genera.



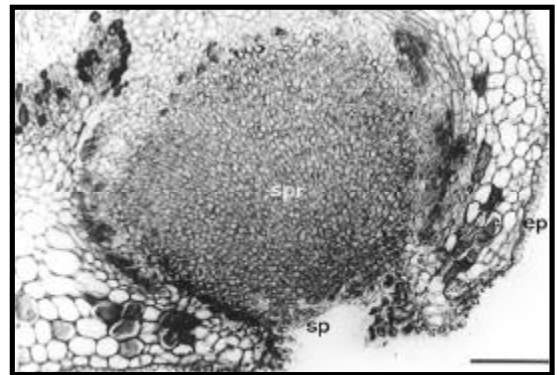
**Figure 3.** Transverse section of the rachis of *P. polygalaeiflorus* through the EFN (bar = 100  $\mu$ m; ep: epidermis; sp: secretory pole; vb: vascular bundle).



**Figure 5.** Transverse section of the rachis of *P. polygalaeiflorus* through the EFN (bar = 100  $\mu$ m; ep: epidermis; sp: secretory pole; spr: secretory parenchyma).



**Figure 4.** Transverse section of the rachis of *P. pubescens* through the EFN (bar = 100  $\mu$ m; ep: epidermis; sp: secretory pole; vb: vascular bundle).



**Figure 6.** Transverse section of the rachis of *P. pubescens* through the EFN (bar = 100  $\mu$ m; ep: epidermis; sp: secretory pole; spr: secretory parenchyma).

The vascular pattern and the anatomical structure of the EFNs in *P. pubescens* and *P. polygalaeiflorus* is similar. The differences encountered are related to the dimensions and density of trichomes near the secretory pole and may be observed within each species and even within the nectaries in the same leaf. Therefore, in *P. pubescens*, the density of the trichomes in the rachis is considerably higher than that in *P. polygalaeiflorus*, covering completely the EFN. This characteristic constitutes a relevant taxonomical tool in the distinction of these two species.

Oliveira & Leitão-Filho (1987) include *P. pubescens* among the species with no EFNs, which lead to the conclusion that the activity of the EFNs in this species, as well as in *P. polygalaeiflorus* may be concentrated in a short period of time, during the production and expansion of new leaves. This fact should limit the distinction of the EFNs in field observations. The register of EFNs in *P. pubescens* and *P. polygalaeiflorus* should represent the first step to include them as potential species for ecological and interactional studies in the “Cerrados”.

### Conclusions

1. This is the first register of the occurrence of the EFNs in *P. pubescens* and *P. polygalaeiflorus*.
2. Difficulties in the identification of the EFNs, in the field, may be due to the fact that the production of nectar is practically restricted to the period of leaf expansion and differentiation.
3. The restricted period of the production of nectar represents an efficient protection against herbivores during the critical period of susceptibility of the leaves.
4. *P. pubescens* has a higher number of trichomes, not only over the EFNs, but all over the primary plant body.
5. The anatomical characteristics of the EFNs are not confident for the taxonomical identification of the two studied species.

### Acknowledgements

To Dr. Evelyn S.A.M. Frazier for the English review and valuable criticisms on the manuscripts.

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