Anatomy of extrafloral nectaries in Fabaceae from
dry-seasonal forest in Brazil

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Extrafloral nectaries (EFNs) are found in many species of Fabaceae. The aim of this work is to describe the internal morphology of the EFNs from species of Fabaceae found in areas of dry-seasonal forest in north-eastern Brazil. All species of Fabaceae with EFNs found were collected and samples were submitted to conventional techniques for anatomical and scanning electronic microscopy analysis. EFNs were found in 35 species, of which 32 were examined anatomically. All types have epidermal cells, secretory tissues and vascular bundles in the EFNs. Sclerenchymatous cells were found between the secretory tissues and the vascular tissues, with a few exceptions. The function of these cells is not clear; however, a role in the transportation of the sap in the nectary or with the support of the secretory tissue is possible. The nectar is released through glandular trichomes, secretory pores or even by breaking the epidermal cells and cuticle. The internal patterns found in the EFNs from different species and genera can provide important information for taxonomic and evolutionary studies in the family. © 2010 The Linnean Society of London, Botanical Journal of the Linnean Society, 2010, 163, 87–98.

ADDITIONAL KEYWORDS: caatinga – legumes – secretory structures.

INTRODUCTION

Extrafloral nectaries (EFNs) are secretory structures that release nectar, which is a source of nutrients for some organisms. Besides sugar, the nectar contains lipids that are a secondary resource used to attract ants, which generally defend the plants against herbivores, birds, wasps and other animals (Koptur, Rico-Gray & Palacios-Rios, 1998; Junqueira, Diehl & Diehl-Fleig, 2001; Heil & McKey, 2003; Katayama & Suzuki, 2005; Bronstein, Alarcón & Geber, 2006; Melo et al., 2009).

These structures have been shown to occur in more than 100 angiosperm families and are most abundant and diverse in Fabaceae (Metcalfe & Chalk, 1979; Bentley & Elias, 1983). According to Melo (2008) and Keeler (2010), 3991 species of angiosperms with EFNs have been reported and 1049 of these species, in 98 genera, are members of Fabaceae.

The distribution of EFNs in Fabaceae has already been investigated for some ecosystems in Brazil, including Amazonia (12 species), the Atlantic Forest (39 species) and cerrado (ten species) (Elias, 1983; Oliveira & Leitão-Filho, 1987; Lewis & Owen, 1989; Morellato & Oliveira, 1991; Mendonça-Filho, 1996; Machado et al., 2008). However, there is only one unpublished study, by Melo (2008), of EFNs found on plants in caatinga, a seasonally dry ecosystem in Brazil.

Caatinga is a diverse ecosystem with adverse climate conditions, including a dry season that lasts up to 8 months each year (Prado, 2003) or sometimes longer. The most diverse family in the caatinga are Fabaceae, represented in this ecosystem by almost 300 species, of which approximately 81 are endemic (Queiroz, 2002; Córdula, Queiroz & Alves, 2008; Queiroz, 2008).
The presence of EFNs in numerous plant groups has led to a range of studies of these structures, focusing on anatomy (Fahn, 2000; Dickison, 2001; Paiva & Machado, 2006), ecology (Del-Claro, 1995; Koptur et al., 1998) and evolution (Pascal, Motte-Florac & McKey, 2000; Marazzi et al., 2006). Morphologically, EFNs can vary in location (rachis, petiole, pedicel), in form (elevated-concave, elevated–convex, embedded) and in their anatomical structure (e.g. some are formed only of epidermis and others are formed of epidermis, secretory parenchyma and vascular bundles) (Fahn, 1979a, 2000; Elias, 1983; Machado et al., 2008; Melo, 2008).

The objective of this work was to describe and differentiate anatomically the EFNs in the different subfamilies of Fabaceae that occur in caatinga. This was performed because of the known diversity of EFNs in Fabaceae, the known diversity of this family in the caatinga and the possibility of there being morphological variation in the EFNs found on the plants in this ecosystem.

**MATERIAL AND METHODS**

The collections were made in the municipality of Mirandiba, a semi-arid region of north-eastern Brazil, c. 500 km inland from the coast of the state of Pernambuco (8°7’13″S, 38°43′46″W). The area has an average annual temperature of 27 °C and the dry season can last up to 11 months, without rain (Alves et al., 1998). A dense, spinose, shrubby–arborescent vegetation covers most of the study area and, in this region, Fabaceae are the most abundant and diverse family of plants with EFNs (Parahyba et al., 1998; Beltrão et al., 2005; Melo, 2008; Córdula, Queiroz & Alves, 2009).

Within the study area, 35 species of Fabaceae with EFNs were recorded, of which 32 were analysed anatomically. Bauhinia cheilantha (Bong.) D.Dietr. (Caesalpinioideae) has calyciform and elevated EFNs, but they are vestigial and therefore this species was not included in this study. The EFNs of Crotalaria incana L and Rhynchosia minima (L.) DC. (Papilionoideae) have been previously described (Bhattacharya & Maheshwari, 1970; Díaz-Castelazo et al., 2005) so the EFN descriptions of these species were only revised.

The specimens were collected between October 2006 and March 2008, during both the dry and rainy seasons. All the EFNs were collected when they were fully developed and in their secretory phase. Samples were collected from three to five individuals per population, from the following ten populations: Fazenda Areia Malhada (8°08′30″S, 38°43′10″W), Fazenda Salinas (8°13′54″S, 38°32′12″W), Limoeiro (8°12′S, 38°32′W), Serrotinho (8°07′17.6″S, 38°43′21.2″W), Serra do Tigre (8°03′35″S, 38°43′07″W), Sítio Chacau (8°08′01″S, 38°39′22″W), Riacho das Pedras (8°12′07″S, 38°35′48″W), Baixio Grande (8°14′18.6″S, 38°43′19.6″W), Serra das Umburanas (8°12′S, 38°32′W) and Vertentes (8°16′8.9″S, 38°40′55.4″W). Approximately 20 EFNs were collected from each individual. The samples were fixed in formaldehyde–acetic acid–alcohol (FAA)50 and preserved in 70% ethanol for the anatomical study (Kraus & Arduin, 1997) and 2.5% glutaraldehyde in 0.1 M phosphate buffer for the scanning electron microscopy (SEM) analyses (Haddad et al., 1998). Vouchers from the study are archived at the Universidade Federal de Pernambuco Herbarium (Table 1).

Ten to 20 EFNs were sectioned for each species. The samples were embedded in methacrylate resin (Gerrits, 1964), sectioned with a rotary microtome and stained with toluidine blue (O’Brien, Feder & McCully, 1964). Free-hand sections, followed by depigmentation in 30, 50 and/or 70% sodium hypochlorite, were also made and stained in safranin blue. The samples were exposed to Fehling’s reagent in order to confirm their role as a nectary (Machado, Gregório & Guimarães, 2006; Paiva & Machado, 2006). Images were taken using an Olympus BX41 and Zeiss Standard 25 photomicroscope. The EFNs were separated into two categories: substitutive and non-substitutive. Substitutive nectaries are common structures, such as stipules, floral pedicels and trichomes that have evolved to function like non-substitutive EFNs. Non-substitutive EFNs are glandular structures that have developed specifically to be extrafloral nectaries (Vogel, 1997; Díaz-Castelazo et al., 2005).

**RESULTS**

In general, the EFNs studied have epidermal tissue, a secretory zone composed of parenchyma cells with large nuclei and dense cytoplasm and a polygonal cell zone that is lignified with pit fields and vascular bundles, which are composed of phloem (predominantly compound) and xylem. Druses (oxalate crystals) and prismatic crystals are present in the secretory parenchyma and in the lignified cells (Fig. 1). Secretory cells occur throughout the secretory zone and stipe (where present), along the outer region of the EFN.

Caesalpinioideae (a paraphyletic assemblage) are represented in the study area (Table 1) by 15 species, some with non-substitutive and some with substitutive nectaries. The EFNs of Chamaecrista have an epidermis of one or two cell layers (papillose in the secretory phase), which sometimes have a thick external pericinal wall and thin cuticle. Trichomes are absent in these EFNs. The secretory zone is comprised of six to eight layers of cells with intercellular...
spaces. Adjacent to these, there is a zone of two or three layers of cells with lignified walls (Fig. 1B), which delimits the secretory zone and the vascular bundles (Fig. 1A). Secretion occurs when nectar accumulates in the central zone of the nectary and breaks through the cuticle (Fig. 1C).

The EFNs of *Hymenaea courbaril* L. have a thin cuticle and an epidermis comprised of one or two layers of large cells with thick walls. The secretory zone has approximately eight layers of cells with large nuclei and dense cytoplasm. The cells become larger the closer they are to the epidermis. Below this, there are two or three layers of polygonal, lignified cells, which delimit the bundles and the secretory zone. The secretory region is totally surrounded by vascular bundles and secretion occurs when both the cuticle, in the central zone of the nectary, and the leaf tissue surround the EFN fragments (Fig. 1D–F).

Table 1. Species of Fabaceae with EFNs found in an area of dry-seasonal forest in Mirandiba (PE), north-eastern Brazil

<table>
<thead>
<tr>
<th>Species</th>
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<tr>
<td>Caesalpinioideae</td>
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<tr>
<td><em>Bauhinia cheilantha</em> (Bong.) D.Dietr.</td>
<td>EC et al. 252</td>
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<tr>
<td><em>Chamaecrista calycioides</em> Greene.</td>
<td>EC et al. 298</td>
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<tr>
<td><em>Chamaecrista ducheanea</em> (Bezerra &amp; Fernandes) H.S.Irwin &amp; Barneby</td>
<td>EC et al. 233</td>
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<tr>
<td><em>Chamaecrista pilosa</em> Greene var. <em>luxurians</em> (Benth.) H.S.Irwin &amp; Barneby</td>
<td>EC et al. 55</td>
</tr>
<tr>
<td><em>Chamaecrista repens</em> (Vogel) H.S.Irwin &amp; Barneby</td>
<td>KP 479</td>
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<td><em>Hymenaea courbaril</em> L.</td>
<td>EC et al. 345</td>
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<tr>
<td><em>Libidibia ferrea</em> (Mart.) L.P. Queiroz var. <em>ferrea</em></td>
<td>EC et al. 244</td>
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<tr>
<td><em>Poincianella bracteosa</em> (Tul.) L.P. Queiroz</td>
<td>EC et al. 277</td>
</tr>
<tr>
<td><em>Poincianella gardneriana</em> (Benth.) L.P. Queiroz</td>
<td>EC et al. 253</td>
</tr>
<tr>
<td><em>Senna macranthera</em> (DC. ex Collad.) H.S.Irwin &amp; Barneby var. <em>pudibunda</em> (Mart. ex Benth.)</td>
<td>EC et al. 305</td>
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<tr>
<td>H.S.Irwin &amp; Barneby.</td>
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<tr>
<td><em>Senna obtusifolia</em> (L.) H.S.Irwin &amp; Barneby</td>
<td>YM et al. 149</td>
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<td><em>Senna occidentalis</em> (L.) Link</td>
<td>EC et al. 296</td>
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<tr>
<td><em>Senna splendida</em> (Vogel) H.S.Irwin &amp; Barneby var. <em>gloriosa</em> H.S.Irwin &amp; Barneby</td>
<td>EC et al. 271</td>
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<tr>
<td><em>Senna trachypus</em> (Benth.) H.S.Irwin &amp; Barneby</td>
<td>EC et al. 273</td>
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<td><em>Senna uniflora</em> (P.Miller) H.S.Irwin &amp; Barneby</td>
<td>YM et al. 149</td>
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<td>Mimosoideae</td>
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<tr>
<td><em>Anadenanthera colubrina</em> (Vell.) Brenan</td>
<td>EC et al. 206</td>
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<tr>
<td><em>Chloroleucon dumosum</em> (Benth.) G.P.Lewis</td>
<td>KP et al. 249</td>
</tr>
<tr>
<td><em>Chloroleucon foliolosum</em> (Benth.) G.P.Lewis</td>
<td>EC et al. 210</td>
</tr>
<tr>
<td><em>Desmanthus pernambucanus</em> Thell.</td>
<td>YM et al. 280</td>
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<tr>
<td><em>Enterolobium contortisiliquum</em> (Vell.) Morong.</td>
<td>EC et al. 178</td>
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<td><em>Inga vera</em> Wild.</td>
<td>EC et al. 340</td>
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<td><em>Neptunia plena</em> Benth.</td>
<td>YM et al. 161</td>
</tr>
<tr>
<td><em>Parapiptadenia zehntneri</em> (Harms) M.P.Lima &amp; H.C.Lima</td>
<td>EC et al. 203</td>
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<tr>
<td><em>Parapiptadenia aff. zehntneri</em> (Harms) M.P.Lima &amp; H.C.Lima</td>
<td>EC et al. 356</td>
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<tr>
<td><em>Piptadenia stipulacea</em> Ducke</td>
<td>YM et al. 183</td>
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<td><em>Piptadenia viridiflora</em> Benth.</td>
<td>EC et al. 330</td>
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<tr>
<td><em>Pithecellobium diversifolium</em> Benth.</td>
<td>EC et al. 208</td>
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<tr>
<td><em>Pityrocarpa moniliformis</em> (Benth.) Luckow &amp; R.W.Jobson</td>
<td>EC et al. 223</td>
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<tr>
<td><em>Prosopis juliflora</em> DC.</td>
<td>YM et al. 273</td>
</tr>
<tr>
<td><em>Senegalia piauhiensis</em> (Benth.) A.Bocage &amp; L.P. Queiroz.</td>
<td>EC et al. 212</td>
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<tr>
<td><em>Senegalia polyphylla</em> (DC.) Britton &amp; Rose in Britton &amp; Killip</td>
<td>EC et al. 355</td>
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<tr>
<td><em>Senegalia riparia</em> (Kunth.) Britton &amp; Rose in Britton &amp; Killip</td>
<td>EC et al. 190</td>
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<td>Papilionoideae</td>
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<td><em>Crotalaria incana</em> L.*</td>
<td>EC et al. 54</td>
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<tr>
<td><em>Erythrina velutina</em> Willd.</td>
<td>YM et al. 279</td>
</tr>
<tr>
<td><em>Rhyncchosia minima</em> (L.) DC.*</td>
<td>EC et al. 36</td>
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*Previously studied (Bhattacharyya & Maheshwari, 1970; Díaz-Castelazo et al., 2005).
The vouchers are deposited in Universidade Federal de Pernambuco Herbarium. Voucher: EC, Elisabeth Córdula; KP, Katarina Pinheiro; YM, Yanna Melo.
In *Senna* spp., the EFNs are formed (from a longitudinal perspective) by a layer of epidermal cells, which are quadrangular or rectangular and in a palisade formation (Fig. 1G, H). The secretory zone has four to six cell layers and one to three layers of polygonal, lignified cells. The vascular bundles are located in the central part of the EFN and travel through the stipe to the secretory region (Fig. 1I). In *S. uniflora* (P.Miller) H.S.Irwin & Barneby, however, the lignified cells are absent and the cells from the secretory zone are larger than in the other species studied (Fig. 1J). In *Senna* spp., the nectar is released through stomata, pores and glandular trichomes, where the exudate accumulates until the cell walls and cuticle break (Fig. 1K, L). No EFNs of species of Caesalpinioideae were active during the dry season.

Substitutive EFNs were also observed in *Libidibia ferrea* (Mart.) L.P. Queiroz var. *ferrea*, *Poincianella bracteosa* (Tul.) L.P. Queiroz and *P. gardneriana* (Benth.) L.P. Queiroz. In *L. ferrea* var. *ferrea*, the EFNs are made of a layer of epidermal cells and 10 layers of parenchyma cells with dense cytoplasm. The bundles are derived from the floral pedicel. The
nectar is secreted from the epidermis, which is near the bundles (Fig. 3A). Non-glandular trichomes sometimes exist in the nectariferous region (Fig. 3B). Poincenella breacteosa and P. gardneriana have EFN-like glandular trichomes, which are multicellular and associated with the cavities that also secrete nectar (Fig. 3C). Histochemical analyses of the content tested positive for sugars in both structures. The nectar exudes when the cells of the trichomes break (Fig. 3D, E).

Mimosoideae are represented in the study area by 17 species with non-substitutive EFNs (Table 1). Although there are some differences in the shape of the EFNs of these species, the structural organization is almost the same (Fig. 2A–G). Some variation was found in the number of cell layers and position of the vascular bundles. The secretory zones are in the central part of the nectary (Fig. 2A). The epidermal cells are papillose during the secretory phase, usually have trichomes and sometimes have thick cell walls and a thin cuticle (Fig. 2B, C). The secretory tissue (secretory zone) has parenchyma cells of various sizes, which have dense cytoplasm and large nuclei (Fig. 2B). Below the secretory zone, there is a zone of lignified cells with scarce cytoplasm (Fig. 2A); except for Senegalia piuahui (Benth.) A.Bocage & L.P. Queiroz (Fig. 2D). Below this zone there are vascular bundles, which can be independent and exclusively irrigate the nectary (Fig. 2E) or irrigate the nectary and the surrounding tissues (Fig. 2F). In Desmanthus pernambucanu Thell. and Parapiptadenia aff. zehntneri (Harms) M.P.Lima & H.C.Lima, a zone with three or four layers of parenchyma cells between the lignified cells and the bundles was found (Fig. 2G). Exudation occurs when the cuticle and the epidermal cells break (Fig. 2G–I). For species of Mimosoideae, only the EFNs of Neptunia plena Benth. were active during the dry season.

Papilionoideae are represented in the study area by three species with non-substitutive and substitutive EFNs, but only Erythrina velutina Willd. is presented here because the other species were previously described (Bhattacharyya & Maheshwari, 1970; Díaz-Castelazo et al., 2005). The EFNs found in E. velutina are substitutive and identical, despite their occurrence in distinct locations on the foliar rachis and floral pedicel. They are comprised of a uniseriate epidermis, parenchyma cells, starch storage cavities and vascular bundles randomly arranged in the parenchyma (Fig. 3F). Exudation occurs through glandular trichomes, which are grouped in the EFN secretory region (Fig. 3G–I). For the species of Papilionoideae, only the EFNs of E. velutina were active during the dry season.

DISCUSSION

The present study describes the EFNs of species of Fabaceae that occur in caatinga. Of the 82 species of Fabaceae cited by Córdula et al. (2008) in the study area, 35 (43%) have EFNs. Caesalpinoideae are represented by 15 species (18%), Mimosoideae by 17 species (21%) and Papilionoideae by three species (4%).

The number of species of Fabaceae known to have EFNs in the study area is higher than that found in some other environments, as mentioned in other studies (Oliveira & Leitão-Filho, 1987; Morellato & Oliveira, 1991; Machado et al., 2008). Although caatinga is an ecosystem characterized by high water stress (Prado, 2003), the data gathered in this study suggest that it is a favourable environment for plants
with EFNs because of the considerable number of the species that have these structures in this ecosystem.

The anatomical arrangement observed in the EFNs studied is common in secretory structures, especially those that produce and secrete nectar (Esau, 1960; Fahn, 1979a; b; Dickison, 2001; Castro & Machado, 2006). According to Fahn (1979a, 2000), the anatomical arrangement of the EFNs that have an epidermis, secretory zone and vascular tissue is also common. This is supported by the characteristics observed in this study and those observed for species (including those in Fabaceae) in other ecosystems (Elias, Rozich & Newcombe, 1975; Elias & Gelband, 1977; Paiva et al., 2001; Machado et al., 2008).

Besides the characteristics mentioned above, a group of cells with lignified walls and pits is sometimes present between the secretory and vascular tissue, but the function of these cells is still not clear. The cells are located near the bundles and it is possible that they are involved in the transport of nectar precursors to the nectariferous tissue. Lignified cells that were similar in appearance and location have also been found in other taxa of Fabaceae (Françino et al., 2006). These cells probably function like an endoderm, as observed by Paiva & Machado (2006), who cited a similar structure in *Hymenaea stigonocarpa* Mart. ex Hayne (Caesalpinioidae), which they called an endoderm. According to these authors, the endoderm prevents the secretory zone from breaking and appears to participate in the transfer of substances to the secretory tissue, because it is in contact with the vascular bundles.

Sclerenchyma cells (fibres) can store starch, which provide nutrients to the adjacent parenchyma cells (Scatena & Dias, 2006), and the compounds involved in the synthesis of starch, mainly glucose, are one of the main components of nectar (Vieira, Gazzinelli & Mares-Guia, 1999; Castro & Machado, 2006). However, among the species studied, starch was absent in the lignified cells, which may support the hypothesis that the cells mentioned above are involved with the transport of nectar precursors to the secretory tissue.

Francino et al. (2006), while studying *Chamaecrista triechopoda* (Benth.) Britton & Rose in Britton & Killip, found EFNs with morphological and anatomical aspects similar to those of the *Chamaecrista* spp. analysed here. The authors cited the occurrence of cells with thick walls and scarce cytoplasm, which might refer to the lignified cells observed in this study.

The embedded EFNs noted in *Hymenaea courbaril* have already been observed in other species of Fabaceae, such as *Hymenaea stigonocarpa* and *Leonardoxa africana* (Baill.) Aubrév., with similar anatomical structures (Elias, 1980; Paiva & Machado, 2006). Embedded EFNs have also been found in species of Acanthaceae, Bignoniaceae and Verbenaceae, and the nectariferous region of this type of nectary is often formed only by the epidermis (Maheshwari, 1966; Elias & Gelband, 1976; McDade & Turner, 1997).

The anatomical study of *Cassia* L. and *Senna* of India, including *S. occidentalis* (= *Cassia occidentalis* L.) studied by Bhattacharyya & Maheshwari (1970), corroborates with what is presented here.

Even although the EFNs vary morphologically in Mimosoideae, structural similarity has been observed, even among species of different genera (Lima, Garcia & Sartori, 2007; Morim & Barroso, 2007). The same anatomical arrangement was found in other species, such as *Anadenanthera falcata* (Benth.) Speg. and *Inga feuillei* DC. (= *Feuillea feuillei* DC.) Kuntze) (Pascal et al., 2000; Machado et al., 2008).

Besides the non-substitutive EFNs, which occur in most species, some species have substitutive extrafloral nectaries. In *Libidibia ferrea* var. *ferrea*, the EFN is a modified floral pedicle, which possibly explains why this type of nectary has an elaborate group of cells compared with others. According to Díaz-Castelazo et al. (2005), substitutive nectaries can...
Figure 3. Substitutive extrafloral nectaries (EFNs) in species of Caesalpinioideae and Papilionoideae (Fabaceae) from caatinga. A–B, Caesalpinia ferrea var. ferrea. A, longitudinal section (LS) of the EFN showing the nectariferous epidermis near the bundles and the parenchyma zone. B, scanning electron microscopy (SEM) image highlighting the trichomes in the external region of the EFN and the pedicel. C–E, Caesalpinia bracteosa. C, cross section of the leaf showing the nectariferous trichome and associated cavity. D, SEM showing in detail the stipitate nectariferous trichome. E, SEM of the surface of an EFN showing in detail the cells that have broken to secrete nectar (white arrow). F–I, Erythrina velutina. F, LS highlighting anatomical organization of an EFN with stalk cavities. G, glandular trichomes in the secreting region. H, SEM of an EFN highlighting the secretory pore made of trichomes. I, SEM highlighting the glandular trichomes in the secreting pore. Ca, stalk cells; Cv, associated cavities; Ep, epidermis; Fv, vascular bundle; PP, parenchyma; Tg, glandular trichome; Tr, trichome. Scale bars, 50 μm (G and I); 100 μm (A and C); 150 μm (F); 200 μm (E); 300 μm (D); 500 μm (B and H).

Lersten & Brubaker (1987) reported stipel and stipule EFNs for other Erythrina spp. and the internal organization of these structures is identical to those of E. velutina. Similar EFNs and trichomes have been observed in many angiosperm families, including Fabaceae (Metcalfe & Chalk, 1979; Wunna-chit, Jenner & Sedgley, 1992; Pascal et al., 2000; Machado et al., 2008). According to Fahn (1979a, b), the glandular trichomes located in the EFNs help with the exudation mechanism.

Papilllose cells, the cuticle, stomata, pores and trichomes associated with nectaries make it easier for nectar to exude, which can occur when the cuticle and epidermal cells break (Fahn, 1979a, b; Mauseth, 1988; Castro & Machado, 2006). The cuticle and cells break and nectar is released because of the accumulation of nectar under the cuticle and inside the cells (Sticzynska, Davies & Gregg, 2003; Machado et al., 2006).

Cruden, Hermann & Peterson (1983) argued that the viscosity of nectar, when secreted, can change during the dry season because of the reduction in humidity. In the study area, many plants lose their leaves and consequently their EFNs during the dry season (Prado, 2003). However, in E. velutina (Papilionoideae) and N. plena (Mimosoideae), the EFNs remain active during this period and the leaves remain on the branches. In addition, E. velutina has EFNs on the floral pedicel that remain active during its reproductive phase, which also occurs during the dry season. So, it is possible that the activity of these EFNs is related to the protection of the reproductive organs or even to aid in the attraction of pollinators (Schemske, 1980; Beckmann & Stucky, 1981; Castro & Machado, 2006).

The availability of water is probably one factor that influences the production of nectar. Erythrina velutina is a tree that grows close to aquatic environments, where it is guaranteed water because of its deep root system (Gonçalves, 1982; Santos & Carles-son, 1998; Larcher, 2004), and N. plena is an aquatic.

In the majority of species studied, the EFNs were only active during the rainy season, when most
species are simultaneously fertile. Therefore, it is likely that weather conditions influence nectar secretion. The periodicity in the activity of the nectaries must be one of the main factors that has influenced the distribution of EFNs in plants from caatinga, especially in the case of the effective defence mechanism developed by species of Fabaceae (Polhill, Raven & Stirton, 1981; Pascal et al., 2000; Paiva et al., 2001).

In angiosperms, especially Fabaceae, EFNs seem to have developed to help ants, which defend the plants (Schemske, 1980; Polhill et al., 1981; Charão, 2005). However, it is now known that plants with EFNs also have relationships with birds, bees and other animals, suggesting that there is also an evolutionary relationship related to pollination (Castro & Machado, 2006). Although EFNs seem to have independent origins (McKey, 1989), a high level of morphological similarity occurs among nectaries belonging to groups that are phylogenetically related and among those that are not related (Pascal et al., 2000; Machado et al., 2008). The same is true with substitutive nectaries (Vogel, 1997; Lopes, Vogel & Machado, 2002; Diaz-Castelazo et al., 2005).

In Papilionoideae, the reports of EFNs are for modified structures (Polhill et al., 1981; Lersten & Brubaker, 1987; Diaz-Castelazo et al., 2005; Lewis et al., 2005). It can be inferred that, in Fabaceae, the substitutive EFNs might indicate a recent evolutionary acquisition. However, the assertion related to the independent origin of the EFNs makes it hard to interpret these structures (McKey, 1989; Pascal et al., 2000).

The present study confirmed anatomical patterns among most of the analysed species of Fabaceae, providing important data for understanding the systematics of the family. Even although some authors have emphasized the independent evolution of extrafloral nectaries in different plant groups, including Fabaceae (McKey, 1989), from an anatomical point of view the EFNs studied have similar structural patterns in related groups and especially in species of the same genus. However, more studies about the anatomy of EFNs are needed to clarify this hypothesis.

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