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Secretory structures of *Ipomoea asarifolia*: anatomy and histochemistry

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Abstract: *Ipomoea asarifolia* (Desr.) Roem. & Schult., Convolvulaceae, is a weed that infests agricultural areas and is toxic to cattle. In spite of its toxicity, the leaves of this plant are used in traditional remedies in the state of Bahia, Brazil. The present work describes the leaf anatomy of *I. asarifolia* and characterizes the exudates of its secretory structures. The leaves have a unistratified epidermis composed of ordinary cells with straight to slightly sinuous anticlinal walls and thin cuticles. Paracytic stomata are found on both surfaces of the leaves at the same level as the ordinary epidermal cells. Trichomes producing polysaccharide secretions occur on the petiole and leaf blade and are considered colleters. The mesophyll is dorsiventral and the vascular bundle of the central vein is bicollateral. Two opposed nectaries occur on the petiole near the leaf blade. Each nectary is composed of a small canal with internal ramifications and numerous secretory trichomes. The laticiferous glands are articulated, not anastomosed, and are composed of large diameter cells with thin cell walls. The secretions of the laticiferous glands are lipidic.

Article

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Introduction

The family Convolvulaceae has a worldwide distribution and comprises about fifty genera and 2000 species. Most genera are weeds or creeping vines, with white latex, simple alternate leaves, and showy flowers with infundibuliform corollas (Souza & Lorenzi, 2005).

The genus *Ipomoea* is the largest in the family, comprising about 700 species that grow in tropical and temperate areas of the world. In the semi-arid region of northeastern Brazil this genus is represented by seven species, mostly weeds that of which are weeds that are common in cultivated areas (Meira, 2008).

One of these species is *Ipomoea asarifolia* (Desr.) Roem. & Schult., popularly known as *salsa-brava* or *batatarana*. This species prefers to grow in sandy soil and is found with several agricultural crops, along the margins of ponds and on ocean beaches. Despite its ornamental value and importance for dune fixation, *I. asarifolia* is sometimes a perennial weed and toxic to livestock. Its toxicity has been demonstrated experimentally (Döbereiner et al., 1960) and under natural conditions in cattle, sheep and goats (Méndez & Riet-Correa, 2008; Riet-Correa et al. 2003). However, despite this, its leaves are used to relieve insect bites and its roots are used as purgative by traditional peoples from the state of Bahia.

The goal of this work was to describe the leaf anatomy and chemical nature of the exudates from the leaves and secreting tissues of *I. asarifolia.*

Materials and Methods

Plant material

The material was collected in pastures in the municipality of Cruz das Almas, in the state of Bahia, Brazil. The samples were deposited in the herbarium at the Federal University of Bahia Recôncavo (HERB), Bahia: Brazil, Bahia: Cruz das Almas, 28/11/09, F. M. Martins (HERB 2182); Brazil, Bahia: Cruz das Almas, 28/11/09, F. M. Martins (HERB 2180); Brazil, Bahia: Cruz das Almas, 20/11/10 F. M. Martins (HERB 2177); Brazil, Bahia: Cruz das Almas, 20/11/10 F. M. Martins (HERB 2179). Developed leaves were fixed in 50% FAA (37% formaldehyde, glacial acetic acid, 50% ethanol) for 24 h (Johansen, 1940) and ferrous sulfate in formalin (37% formaldehyde, ferrous sulfate heptahydrate III, distilled water) for 48 h (Johansen, 1940). The material was then stored in 70% ethanol.

The samples were dehydrated through a tertiary butyl series and then embedded in paraffin for the histological studies (Histosec; Merck; Johansen, 1940). The blocks were sectioned with a rotary microtome (Model RM2245, Leica Microsystems), producing serial cross- and longitudinal sections 10-15 µm thick. For

structural characterization, sections were stained with safranin and astra blue (Gerlarch, 1969) and mounted in synthetic resin (Permount; Fisher).

In parallel, histochemical tests were performed with sections taken from fresh samples using a cryomicrotome (Model CM1850; Leica Microsystems). The exudate was studied using histochemical methods, which are listed in Table 1. Control tests for lipophilic substances were performed with an extraction solution composed of methanol:chloroform:water:HCl (66:33:4:1 v/v; High, 1984).

Table 1. Histochemical tests performed to identify the main metabolites secreted by colleters, nectaries and latex from the leaves of *Ipomoea asarifolia.*

	Metabolite	Reagent
Lipids	Total lipids	Sudan black B (Pearse, 1985)
	Acid and neutral lipids	Nile blue (Cain, 1947)
Terpenoids	Essential oils and oleoresins	Nadi reagent (David & Carde, 1964)
	Sesquiterpene lactones	Sulfuric acid (Geissmen & Griffin, 1971)
	Resin	Cupric acetate (Johansen, 1940)
Phenolic compounds	Phenolic general	Potassium dichromate (Gabe, 1968) Ferric chloride (Johansen, 1940)
	Tannin	Vanillin hydrochloric (Mace & Howell, 1974)
Alkaloids	Lignin	Phloroglucinol (Johansen, 1940) Draggendorf reagent (Furr & Mahlberg, 1981) Dittmar reagent (Furr & Mahlberg, 1981)
Polysaccharides	Total polysaccharides	Ellram reagent (Furr & Mahlberg, 1981)
		PAS (McManus, 1948)
	Starch	Lugol (Johansen, 1940)
	Acid mucopolysaccharides	Alcian blue (Pearse, 1985)
	Mucilage	Pizzolato & Lillie Reaction (Pizzolato, 1977)
Proteins	Total proteins	Coomassie brilliant blue (Fisher, 1968)
		Xilidine Ponceau (O´Brien & McCully, 1981)
		Mercury bromophenol (Mazia et al., 1953)
Rubber		Aniline blue black (Fisher, 1968) Oil red (Pearse, 1985)

Observations and image documentation were performed with a light microscope (Model BX51; Olympus Optical) equipped with an Olympus A330 digital camera. The scales of the figures were obtained by projecting a slide micrometer photographed/scanned under the same conditions.

Results

In cross section, the outline of the petiole is concave on the adaxial side and convex on the abaxial side (Figure 1A). The epidermis is uniseriate with isodiametric cells, a thin cuticle and stomata on both surfaces (Figure 1A). On the adaxial epidermis of the petiole there are projections that look similar to extrafloral nectaries; however, close observation of the anatomy of these structures reveals that they consist only of parenchymatous tissue (Figure 1A) and are devoid of stomata and vascular tissue. In the proximal region of the leaf lamina there are two opposing nectaries. These occur as crypts on the surface, with a system of internal channels and an epidermis covered by trichomes (Figure 1B-E). The presence of polysaccharides was demonstrated by the PAS and the Pizzolato & Lillie reactions (Figure 1D-1F).

Trichomes were observed on the epidermis, consisting of a single cell at the base and up to five secreting cells (Figure 2A). The secretions present in the interior and on the exterior of the trichomes gave a positive reaction to the PAS stain, potassium dichromate and ferric chloride (Figure 2B, C, D). These tests indicated the presence of polysaccharides and phenolic compounds, respectively, in the secretion (Table 2).

The chlorenchyma consists of up to three cell layers and the angular collenchyma of 5 layers. The epidermis of the samples was significantly damaged, with the loss of epidermal and subepidermal tissue; however, the formation of scar tissue originated from the collenchyma was observed, as well as the formation of cells with meristematic activity and cell walls containing suberin (Figure 3B). In the chlorenchyma and ground parenchyma, articulated non-anastomosing laticifers occur that consist of large-diameter cells with thin walls (Figure 3A, C). There is no dissolution of the walls of the laticifers, which makes their articulated nature very clear (Figure 3D). When the petiole is injured, latex exudes from the damaged region, which has a fresh milky appearance and can be observed within the laticifer when dyed with safranin (Figure 3C). The secretion from the laticifer is lipid in nature, as shown by a positive reaction to tests with Sudan black B and Nile blue (Table 2) (Figure 3E, F, G). The results also highlight the presence of rubber in the laticifer secretion (Figure 3H). The vascular system of the petiole is composed of bicollateral bundles, comprising up to five units of varying size (Figure 1A, B).

Table 2. Histochemical characterization of colleters, nectaries and laticifers from the leaves of *Ipomoea asarifolia.*

Metabolite	Reagent	colleters	nectaries	laticifers
Lipids	Sudan black B	-	-	+
	Sudan scarlet	-	-	+
	Nile blue	-	-	+
Terpenoids	Nadi reagent	-	-	-
	Sulphuric acid	-	-	-
	Cupric acetate	-	-	-
Phenolic compounds	Potassium dichromate	+	-	-
	Ferric chloride	+	-	-
	Vanillin hydrochloric	-	-	-
Alkaloids	Phloroglucinol	-	-	-
	Wagner reagent	-	-	-
	Dittmar reagent	-	-	-
	Ellram reagent	-	-	-
Polysaccharides	PAS	+	+	-
	Lugol	-	-	-
	Alcian blue	-	-	-
	Pizzolato & Lillie Reaction	+	+	-
Proteins	Coomassie brilliant blue	-	-	-
	Xilidine Ponceau	-	-	-
	Mercury bromophenol	-	-	-
	Aniline blue black	-	-	-
Rubber	Oil red	-	-	+

The leaf blade has a thin cuticle and epidermis (Figure 4A, B). In frontal view the ordinary cells of both sides have anticlinal walls that are straight or slightly wavy in cross section. Some of the epidermal cells are quite small and some have convex outer periclinal walls (Figure 4C, D). The stomata are of the paracytic type, located on both sides and at the same level as the other epidermal cells. The trichomes present on the leaf blade are identical to those found in the epidermis of the petiole.

The mesophyll is dorsiventral with palisade parenchyma composed of 2-4 layers of elongated cells that make up half of the leaf mesophyll and there are many extracellular spaces (Figure 4A, E) in this region. The spongy parenchyma occupies the lower half of the mesophyll and is composed of up to five layers of cells that vary widely in shape. Large extracellular spaces can be seen in the spongy parenchyma (Figure 4E), as well as idioblasts containing druses.

In the midrib, the cortical region is composed of four layers of chlorenchyma, with an irregular distribution and cells of varying diameter (Figure 4F), which represents the only supporting tissue present in the leaf. The parenchyma cells vary in diameter and in the parenchyma around the phloem it is possible to observe large-caliber laticifers and idioblasts with druses. The vascular bundles of the midrib are bicollateral; however, this region does not contain subdivisions like the petiole, and forms a single arc of xylem and phloem (Figure 4F).

Discussion

The anatomical study of the leaf of *Ipomoea asarifolia* (Desr.) Roem. & Schult. showed that the histological organization reflects the predominant features of the Convolvulaceae, as cited by Metcalfe & Chalk (1950). The thin, smooth cuticle of *I. asarifolia* differs from that described by Monqueiro et al. (2004), who reported the occurrence of a rough cuticle in *I. grandeifolia*, and Procopio et al. (2003), who described the presence of a thick cuticle in *I. cairica*. Moreover, the thin cuticle of *I. asarifolia* is something of an anomaly considering the environment in which the plant is found; it would be expected that a plant that grows in full sun, in an environment with high temperatures, would possess a thick cuticle to prevent excessive transpiration. It is possible that the thinness of the cuticle is compensated by the presence of an underground water storage organ that has not been histologically described. The epidermal cells of *I. asarifolia* with predominantly straight anticlinal walls are in agreement with those described by Arruda et al. (2009) for *Ipomoea imperiati* and *I. pes-caprae*. *Ipomoea grandifolia* (Monqueiro et al., 2004) and *I. carica* (Procopio et al., 2003) showed markedly wavy anticlinal walls. According to Fahn & Cutler (1992), the epidermis of plants that grow in a xeric environment is composed of small cells with straight anticlinal cell walls; the authors believe that this feature may be an adaptation to drought conditions. Furthermore, according to Iljin (1957) there is an inverse correlation between cell volume and the ability of the plant to survive drought. Moreover, Cutler et al. (1977) state that the reduction in cell size appears to be one of the main responses to water stress. The presence of cells with convex outer periclinal walls in the epidermis agrees with that described by Metcalfe & Chlak (1950) for the Convolvulaceae; however, this characteristic is common in plants that live in a low-light environments, where papillose cells act as convex lenses that focus rays of light into the mesophyll for photosynthesis (Uphof, 1962).

The trichomes present on the adaxial surface of the leaf have a restricted distribution in taxa of the Convolvulaceae, occurring only in some species of *Ipomoea* (Metcalfe & Chalk 1950). In work carried out

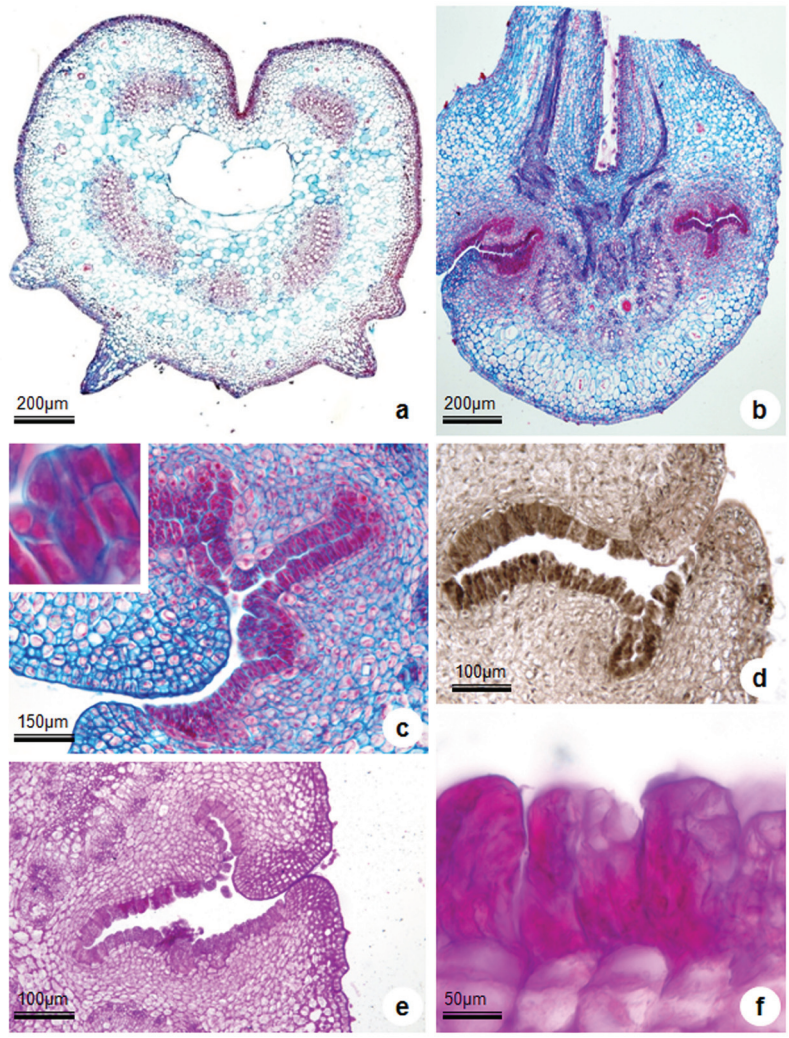

Figure 1. Transversal section the petiole of *Ipomoea asarifolia*: structure and histochemistry. A. Petiole of mature leaf. B. Petiole of immature leaf, region near the leaf blade. C. Petiolar nectary and detail of nectary trichomes. D. Positive reaction to Lillie & Pizzolato test showing the presence of mucilage secretion. E. Total polysaccharides identified by the PAS reaction. F. Detail of trichomes with drainage inside and outside of cells that were secreting.

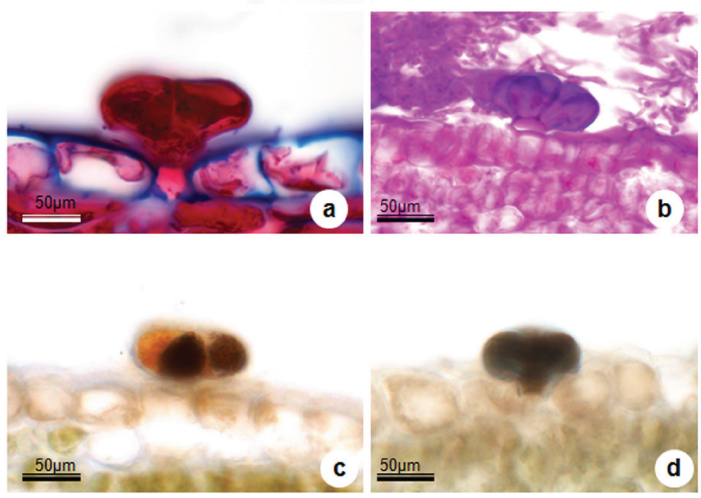

Figure 2. Colleters on the petiole of *Ipomoea asarifolia*: structure and histochemistry. A. Colleters on the petiole strongly stained by safranin. B. Polysaccharides identified by the PAS reaction. C. Positive reaction to potassium dichromate test. D. Phenolic compounds inside the cell, which were revealed with ferric chloride.

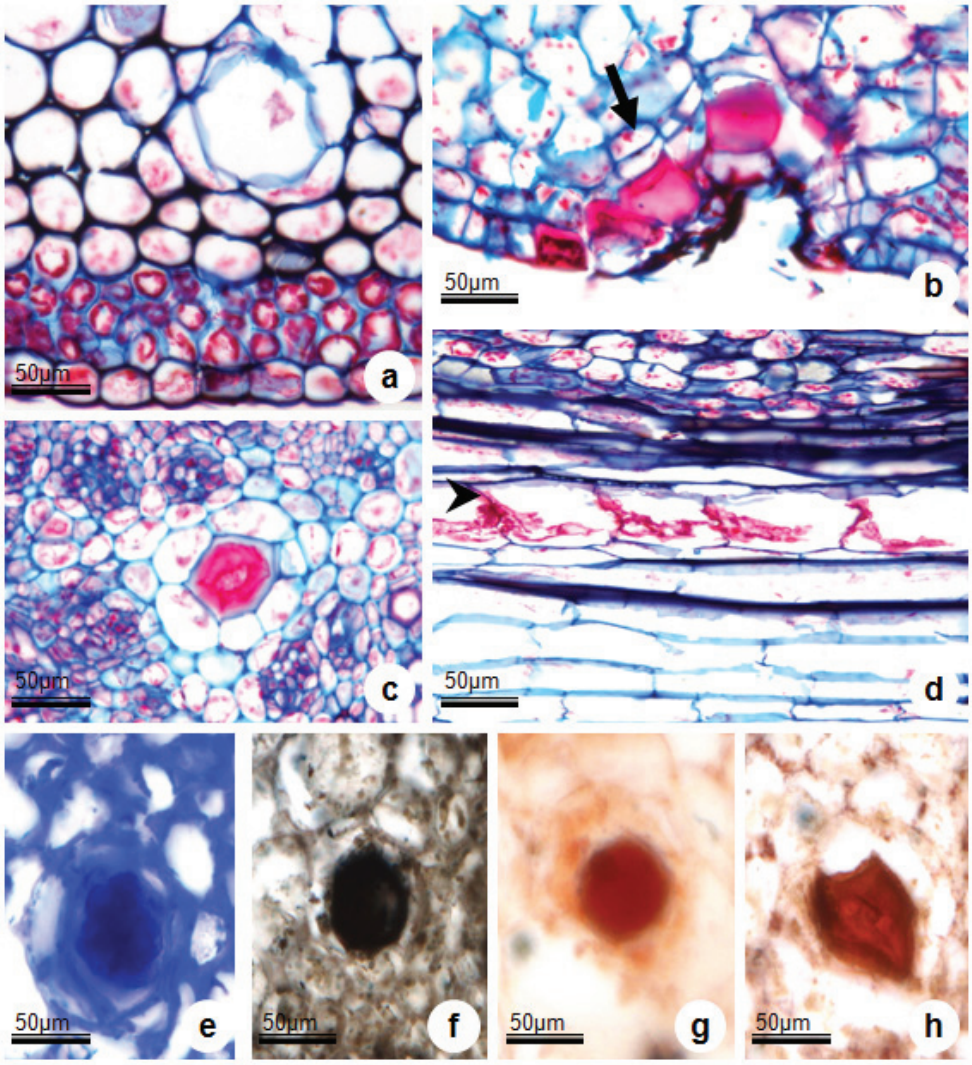

Figure 3. Transversal section the petiole of *Ipomoea asarifolia*: structure and histochemistry. A. Cortex showing chlorenchyma and collenchyma laticifer. B. Formation of scar tissue cells with meristematic activity (arrow) and cells with suberized wall. C. Details of laticifer discharge. D. Laticifer (longitudinal view) shown in the undissolved walls (arrowhead). E. Nile blue. F. Sudan Black B. G. Sudan Scarlet. H. Identification of rubber in the secretion of the laticifer.

by Monqueiro et al. (2004) and Procopio et al. (2003), trichomes were not observed in the *Ipomoea* species studied. However, Arruda et al. (2009) found trichomes in *I. imperiati* and *I. pes-caprae* as described in this study. The secretion from the trichomes is composed of hydrophilic polysaccharides and indicates that the trichomes are colleters. According to Fahn (1979), colleters may be present on vegetative or reproductive organs, releasing a viscous fluid (mucilaginous or resinous) that protects and lubricates meristems early in development. The structural similarity of colleters with other types of secretory structures led researchers to confuse them with extrafloral nectaries and resin glands (Arekal & Ramakrishna, 1980; Mohan & Inamdar, 1986; Subramanian et al., 1989; Thomas, 1991). For this reason, histochemical tests are crucial to detect the mucilaginous secretion and to confirm that structures are colleters. Keeler & Kaul (1979), in a paper on nectaries of *Ipomoea*, described the function of the nectary trichomes present in this genus, although they did not perform tests to confirm the presence of a secretion.

Crypt nectaries are described for only six angiosperm families, including the Convolvulaceae (Keeler & Kaul, 1979), and they are considered the most complex type of nectary in the family. The occurrence of a nectary in a crypt may be related to protecting the trichomes from direct contact with pathogens.

The stomata are distributed on both sides of the leaf as previously described by Metcalfe & Chalk (1950) for the Convolvulaceae, and as reported for *Ipomoea*

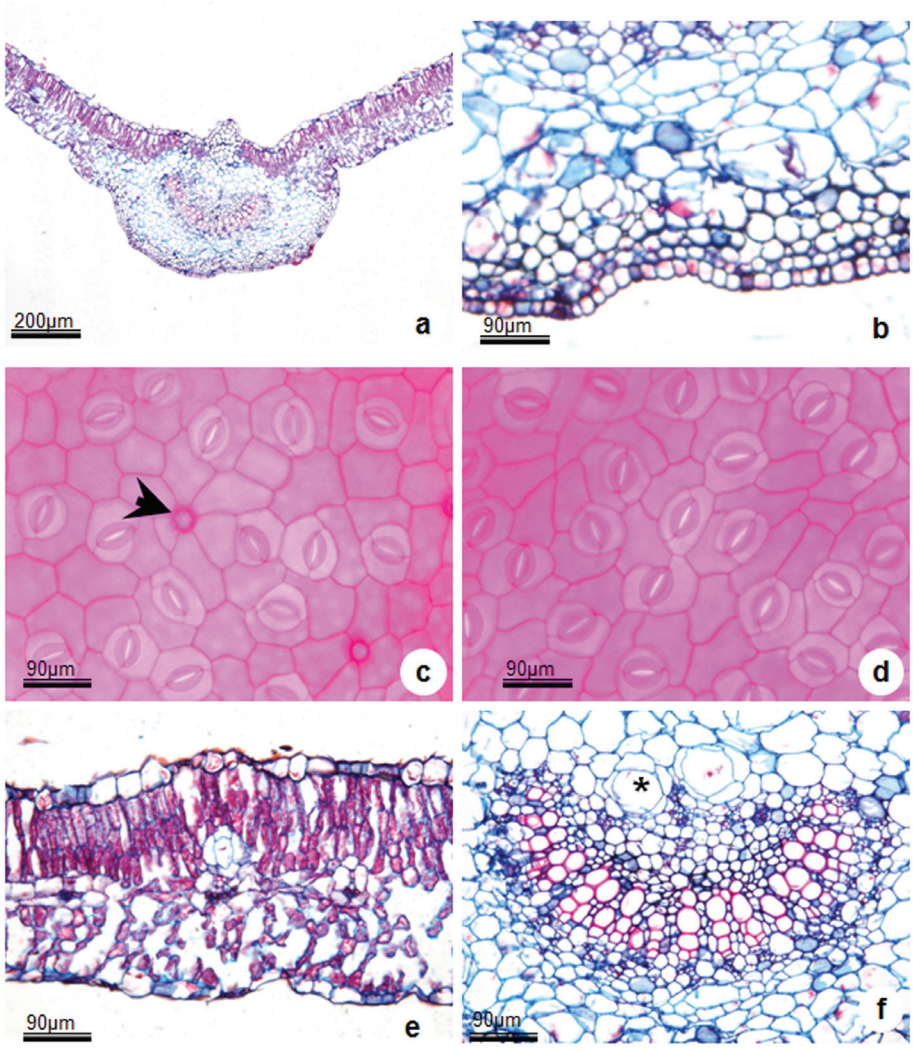

Figure 4. Transverse sections of leaf of *Ipomoea asarifolia.* A. Overview of the leaf. B. Detail of the abaxial epiderms. C. Front view of adaxial secretory trichome (arrow). D. Front view of the abaxial surface. E. Dorsiventral mesophyll with spongy parenchyma showing large intercellular spaces. F. Bicolateral vascular bundle of midrib, accompanied by laticifers. Bar: A: 200 µm; B-F: 90 µm.

grandifolia (Monquero et al., 2004) and *I. carica* (Procopio et al., 2003). In regards to the stomatal typology of *Ipomoea,* the information is conflicting; Solereder (1908) describes paracytic stomata, Metcalfe & Chalk (1979) report anomocytic and paracytic stomata, and Procopius et al. (2003) and Monqueiro et al. (2004) report anomocytic stomata. Arruda et al. (2009) did not mention the type of stomata in their description of *I. imperiati* and *I. pes-caprae*. A recent study by Guterres et al. (2008) confirmed the presence of stomata in *I. aquatica* and anisocytic paracytic stomata in *I. squamosa*. According to Dickison (2000), several taxonomic groups can be characterized by the type of stomata; however, some taxa possess a combination of two or more types. Dorsiventral mesophyll in *I. asarifolia* is consistent with that described by Metcalfe & Chalk (1950) for the Convolvulaceae.

Laticifers are present in several families of angiosperms, including the Convolvulaceae (Metcalfe & Chalk, 1983). Many of these families are not taxonomically related, which suggests that the ability to produce latex appeared more than once during the evolution of these groups (Fahn, 1979). Although laticifers are universally present in the Convolvulaceae (Solereder, 1908, Metcalfe & Chalk, 1950), few studies have described their presence in *Ipomoea* (Pickard, 2008). They occur on the midrib of the leaf (where they are associated with the phloem), the cortex and pith of the stem and the rhizome (Solereder 1908, Metcalfe & Chalk 1950; Condon & Fineran, 1989). Non-

anastomosing articulated laticifers were also described in *I. alba* (Pickard, 2008) and *Calystegia silvatica* (Condon & Fineran, 1989). In *I. asarifolia*, there is no dissolution of the cell wall ends of the laticifers, and it is possible to observe the cells that were articulated in these structures. In addition, the laticifers of this species showed thin cell walls, which are consistent with Mahlberg (1993), who stated that the walls of laticifers can be as thin as the walls of parenchyma cells or very thick. The cell walls of the laticifer system are always primary and isolate the latex so it does not leak into adjacent tissues (Fahn, 1990).

Many functions have been attributed to latex. Due to its distribution throughout the plant body it has been compared to the blood tissue of animals. Being close to the phloem, it was believed to perform the function of assisting in the translocation of assimilates. Later, it was suggested that it might be responsible for maintaining nutritional reserves of the plant. However, it was later proven that the material present inside the laticifer was not mobilized in unfavorable conditions. The current view is that the laticifer is a secretory structure that produces several substances that are not reused in the plant's primary metabolism. Probably the laticifers use latex to close wounds and protect the plant against attacks from herbivores and microorganisms (Fahn, 1979). For these reasons the presence of latex may confer an advantage over other competing species that lack laticifers. The lipid nature of the latex secretion can be considered toxic and may inhibit the proliferation of microorganisms, and lipids also have the ability to clot, which seals wounds and provides a physical defense, because the jaws of insects can get stuck to the plant itself (Dussourd, 1990).

The presence of crystals in the parenchyma of *I. asarifolia* is consistent with that described by Metcalfe & Chalk (1950) for several members of the Convolvulaceae, including the genus *Ipomoea*. Being a creeping plant, with stoloniferous stems, the crystals may be a defense against attacks by ruminant animals. According to Barbosa et al. (2005), in the dry season there is a predominance of *I. asarifolia*, and it is one of the only forages available. The crystals are useful as structural supports, provide protection from herbivores and maintain ionic balance by storing calcium and oxalate in idioblasts (Larrosa & Duarte, 2005). The vascular organization of the petiole and midrib of the leaf blade with the formation of bicollateral vascular bundles was demonstrated by Solereder (1908) and Metcalfe & Chalk (1950) for several genera of the family, including *Ipomoea*. Besides the occurrence of bicollateral bundles, the authors also mention that the distribution of the arc forms an arc pattern in Convolvulaceae. Bicollateral vascular bundles were observed by Arruda et al. (2009) in *I. imperiati* and *I. pes-caprae*.

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