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ARTICLE

The influence of parasitoidism on the anatomical and histochemical profiles of the host leaves in a galling Lepidoptera - *Bauhinia ungulata* system

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ABSTRACT: (The influence of parasitoidism on the anatomical and histochemical profiles of the host leaves in a galling Lepidoptera - *Bauhinia ungulata* system). Galls induced by insects develop through a complex series of plant cell responses. *Bauhinia ungulata* L. (Fabaceae: Caesalpinioideae) has leaf-folding galls, which were studied by means of anatomical and histochemical approaches. These approaches compared the non-galled leaves, non-parasitoidized galls, and parasitoidized galls by addressing two questions: (1) what structural and histochemical changes are caused by the gall inducer in the leaf tissues of the host plant? And (2) is the parasitoid capable of affecting the gall structure? The non-galled leaf is amphistomatic, with anomocytic stomata, and multicellular non-glandular and navicular glandular trichomes. The mesophyll is dorsiventral, and the vascular system is surrounded by a lignified bundle sheath. In the gall, the outer epidermis is derived from the abaxial leaf epidermis. The outer cortex of the gall originates from the chlorophyllian parenchyma, and is homogeneous, with polyhedral cells and few intercellular spaces. The vascularization net is enhanced, and the secondary veins are disorganized. The nutritive tissue derives from the cells of the adaxial epidermis and adjacent parenchyma, which accumulate lipids, and is partly consumed by the chewing larvae. The cells of the outer cortex and those around the vascular bundles accumulate secondary metabolites, functioning as protective layers against natural enemies. The parasitoidized galls are similar to the non-parasitoidized ones, except for the sites of intact hyperplasic nutritive tissue with hypertrophied cells, which are visible macroscopically. This indicates the maintenance of the stimuli from the Lepidoptera larvae feeding on the host-plant cells, which continue to enlarge, but will no longer be used for larvae nutrition.
Key words: Leaf-folding gall, nutritive tissue, parasitoids.

RESUMO: (Influência do parasitoidismo nos perfis anatômico e histoquímico das folhas em um sistema Lepidoptera galhador – *Bauhinia ungulata*) Galhas induzidas por insetos se desenvolvem por meio de uma série complexa de respostas celulares vegetais. *Bauhinia ungulata* L. (Fabaceae: Caesalpinioideae) apresenta galhas de dobramento foliar, aqui estudadas por meio de técnicas de anatomia e histoquímica, com o objetivo de comparar folhas não galhadas e galhas com e sem parasitoides, e responder as seguintes questões: (1) quais são as alterações estruturais e histoquímicas causadas pelo indutor nos tecidos da planta hospedeira? (2) O parasitoide é capaz de afetar a estrutura da galha? A folha não galhada é anfistomática, com estômatos anomocíticos, tricomas multicelulares não glandulares e glandulares naviculares. O mesofilo é dorsiventral, e o sistema vascular está envolvido por bainha lignificada. Na galha, a epiderme externa é derivada da epiderme abaxial; o córtex se origina do parênquima clorofiliano e é homogêneo, com células poliédricas e poucos espaços intercelulares. A vascularização é mais intensa e as nervuras secundárias se mostram desorganizadas. O tecido nutritivo, derivado de células da epiderme adaxial e do parênquima adjacente, apresenta células com conteúdo lipídico o qual é consumido em determinados pontos pela larva do indutor. O córtex externo e células ao redor do feixe vascular acumulam metabólitos secundários, funcionando como camadas protetoras contra inimigos naturais. As galhas com parasitoides são similares às sem parasitoides, exceto por apresentarem locais com tecido nutritivo intacto, hiperplásico, com células hipertrofiadas, os quais formam pontos visíveis macroscopicamente. Isso indica a manutenção do estímulo desencadeado pela larva nas células vegetais, que continuam sua expansão. Porém não mais servirão a sua função prévia de nutrição da larva.
Palavras chave: Galhas de dobramento foliar, tecido nutritivo, parasitoides.

INTRODUCTION

Galls induced by insects result from a complex series of plant cell responses (Mani 1992). Espírito-Santo & Fernandes (2007) estimated that approximately 133,000 species of gall-inducing insects exist, most of them highly specific to their host plant species or even to their host organ (Mani 1992, Hardy & Cook 2010).

The formation of galls produces structural changes in tissues that originally had specialized functions and become adapted to provide nutrition, protect against natural enemies, and maintain a favorable microenvironment for the gall-inducing organisms (Mani 1964, Price *et al.* 1987, Dreger-Jauffret & Shorthouse 1992, Rohfristsch 1992, Stone & Schonrögge 2003, Oliveira & Isaias 2010a). These organisms feed on plant tissues or fluids, with the exception of fungus-feeding gall midges (Stone & Schönrogge 2003), which induce the so called Ambrosia galls (Arduin & Kraus 2001). Thus, the process of cecidogenesis is a reaction to this feeding beha-

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vior (Mani 1992). According to Rohfritsch (1992), the feeding habit and the position of the larvae in the gall chamber are the pressures that generate the various forms observed in insect galls. Stone & Schönrogge (2003) argued that natural enemies are the main selective pressure responsible for the variety of gall shapes. Gall development involves a differentiation of plant tissue caused by mechanical and chemical stimuli provided by the insect, such as chewing the plant tissue, fluids injected during oviposition, salivary secretions, excretions, and hormone production (Hori 1992). Thus, the gall-inducing organism stimulates changes in the developmental pattern of the host leaf (Rohfritsch 1992), accompanied by biochemical modifications, which are mainly apparent in the nutritive tissue (Mani 1964, Bronner 1992) as well as in the outer cortex, where the accumulated substances can perform defensive functions (Hartley 1998, 1999).

In addition to the modifications that result directly from the interaction of the host plants with the gall inducers, a guild of organisms can modify gall features to a greater or lesser extent. These organisms include the inquilines, predators, ectoparasitoids, endoparasitoids, scavenging species, fungi, consumers of these fungi, and microorganisms (Wiebes-Rijks & Shorthouse 1992). The term "inquiline" refers to the phytophagous species, while "parasitoid" refers to entomophagous species (Wiebes-Rijks & Shorthouse 1992, László & Tóthmérész 2006). The inquilines, which are not able to induce the formation of their own galls and must use galls induced by other organisms, may cause tissue modifications, inducing cellular hypertrophy, and consequently changes in gall size (Wiebes-Rijks & Shorthouse 1992, László & Tóthmérész 2006). The influence of parasitoids on the structure of galls, however, is not usually mentioned. These include ectoparasitoids, which feed externally, and endoparasitoids, which develop inside the body of the gall inducer (Wiebes-Rijks & Shorthouse 1992).

The leaf folding gall induced by an unidentified species of Lepidoptera on *Bauhinia ungulata* L. (Fabaceae: Caesalpinioideae) has two trophic levels: the Lepidoptera and its endoparasitoids, with anatomical features that depend on the presence of one or both organisms. Here, we describe the morphology of the non-galled leaves of *Bauhinia ungulata*, and of the leaf folding galls, by addressing two questions: (1) what structural and chemical changes are caused by the gall-inducing Lepidoptera on its host leaves? And (2) is the parasitoid capable of affecting the gall structure?

MATERIAL AND METHODS

Study site and sampling

The samples of non-galled leaves and galls of *Bauhinia ungulata* were collected in September 2010 in the Parque Estadual do Pau Furado, covering the municipalities of Uberlândia and Araguari, Minas Gerais, southeastern Brazil, an area within the Cerrado domain (Brazilian savanna). The material was fixed in FAA_{50} (37% formaldehyde, glacial acetic acid and 50% ethanol, 1:1:18, v/v), and stored in 70% ethanol (Johansen 1940).

Gall separation according to the gall inducer and associated guild

Galls in different developmental stages were dissected under a stereomicroscope and separated according to the gall inhabitants (larvae of the gall-inducing Lepidoptera, parasitoids, or both). The macroscopic features and insects were photographed.

Anatomical analysis

For anatomical observations, permanent slides of histological sections were prepared by dehydration in an *n*-butyl series, embedding in Paraplast® (Kraus & Arduin 1997), sectioning (12 μm) in a rotation microtome (Leica® 2035 BIOCUT), deparaffinization, and staining in safranin-astra blue 9:1 (v/v) (Bukatsch 1972, modified to 0.5%). The slides were mounted on colorless Acrilex® varnish (Paiva *et al.* 2006), examined under a light microscope (LM), and the conspicuous aspects were photographed with a Canon PowerShot A650 digital camera.

Histochemical analyses

For histochemical analyses, part of the samples were embedded in polyethylene glycol (PEG) 6000 (Kraus & Arduin 1997), sectioned in a rotation microtome (20 μm), and treated with Lugol's reagent for detection of starch, 2% ferric chloride for phenols, acidified phloroglucinol for lignins, Jeffrey's reagent for alkaloids (Yoder & Mahlberg 1976), Coomassie blue for proteins (Dunn 1933), Fehling's reagent for reducing sugars (Sass 1951), Sudan Black B for lipids (Jensen 1962), and α-naphthol-*p*-phenylenediamine (NADI) for terpenoids and essential oils (David & Carde 1964). All slides were observed by means of a light microscope (LM).

Scanning Electron Microscopy (SEM)

Non-galled leaves and galls fixed in FAA_{50} were dehydrated in an ethanol series (Johansen 1940), critical-point dried, mounted on stubs, and covered with 15 nm of gold (Balzers SCD 050) (O'Brien & McCully 1981). The specimens were observed in a scanning electron microscope at 20 kV (Leo Evo® 40).

RESULTS

General aspects

Bauhinia ungulata shows leaf-folding galls induced by a Lepidoptera (Fig. 1A-D). A typical nutritive tissue differentiates in response to the feeding activity of the

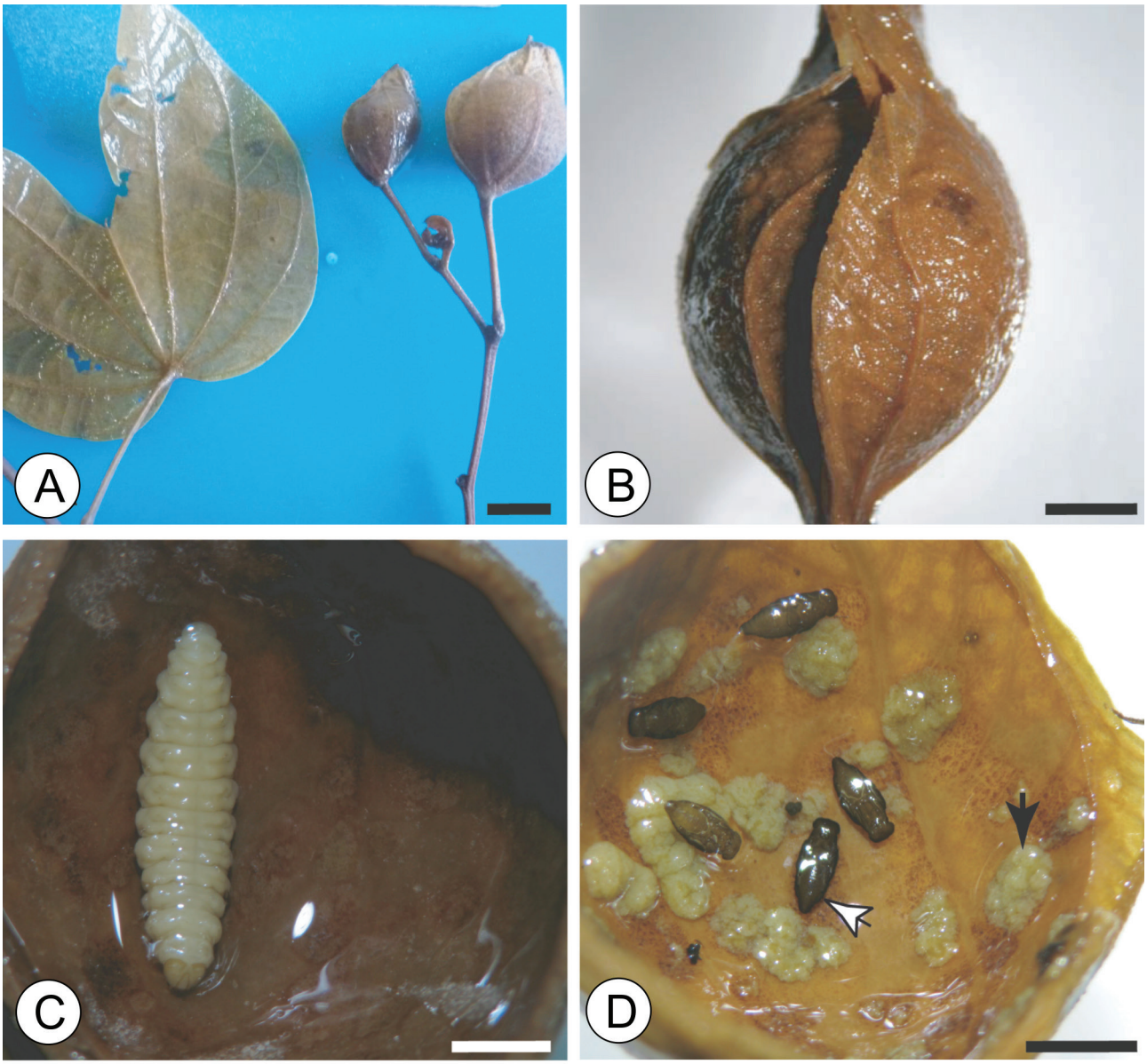

Figure 1. Morphological aspects of the host leaves and leaf folding galls on *Bauhinia ungulata* (Fabaceae). **A.** Non-galled leaf and leaf folding gall. **B.** Gall in detail. **C.** Larva of the galling Lepidoptera inside the chamber. **D.** Parasitoids (white arrow) and sites of the nutritive tissue (black arrow). Bars: 10 mm (A); 2.5 mm (B-D). Colors altered by fixation.

gall-inducing larvae (Fig. 1C). The galls with parasitoidized larvae contain a large amount of intact nutritive tissue, which can be observed macroscopically (Fig. 1D).

Anatomical features

The midrib and secondary veins of the non-galled leaves of *B. ungulata* have collateral bundles surrounded by sclerenchymatic sheath (Fig. 2A). The mesophyll is dorsiventral, with 1-2 layers of palisade parenchyma and 2-3 layers of spongy parenchyma (Fig. 2B). The epidermis is papillose (Fig. 2C-E), uniseriate, and amphistomatic with anomocytic stomata (Fig. 2F). On the abaxial epidermal surface, multicellular simple non-glandular trichomes and navicular glandular trichomes (Fig. 2B-D) with lipid accumulation are present. A thin cuticle covers the leaf on both surfaces. Epicuticular wax platelets are present (Fig. 2F).

During gall formation, the host leaves are completely transformed into galls through the processes of tissue hyperplasia and cell hypertrophy. The vascular bundles retain the collateral arrangement (Fig. 3A). The wall of the leaf folding galls can be divided into five tissue zones. The outer epidermis of the gall derives from the abaxial leaf epidermis, which is papillose. The outer cortex has polyhedral cells and few intercellular spaces, and is separated from the inner gall by the vascular layer. The inner gall, which derives from the adaxial parenchyma and the adaxial epidermis of the host leaf lamina, has smaller, compact cells (Fig. 3A-C). The nutritive tissue is discernible within the inner gall by the periclinal divisions of its cells. The cells of the nutritive

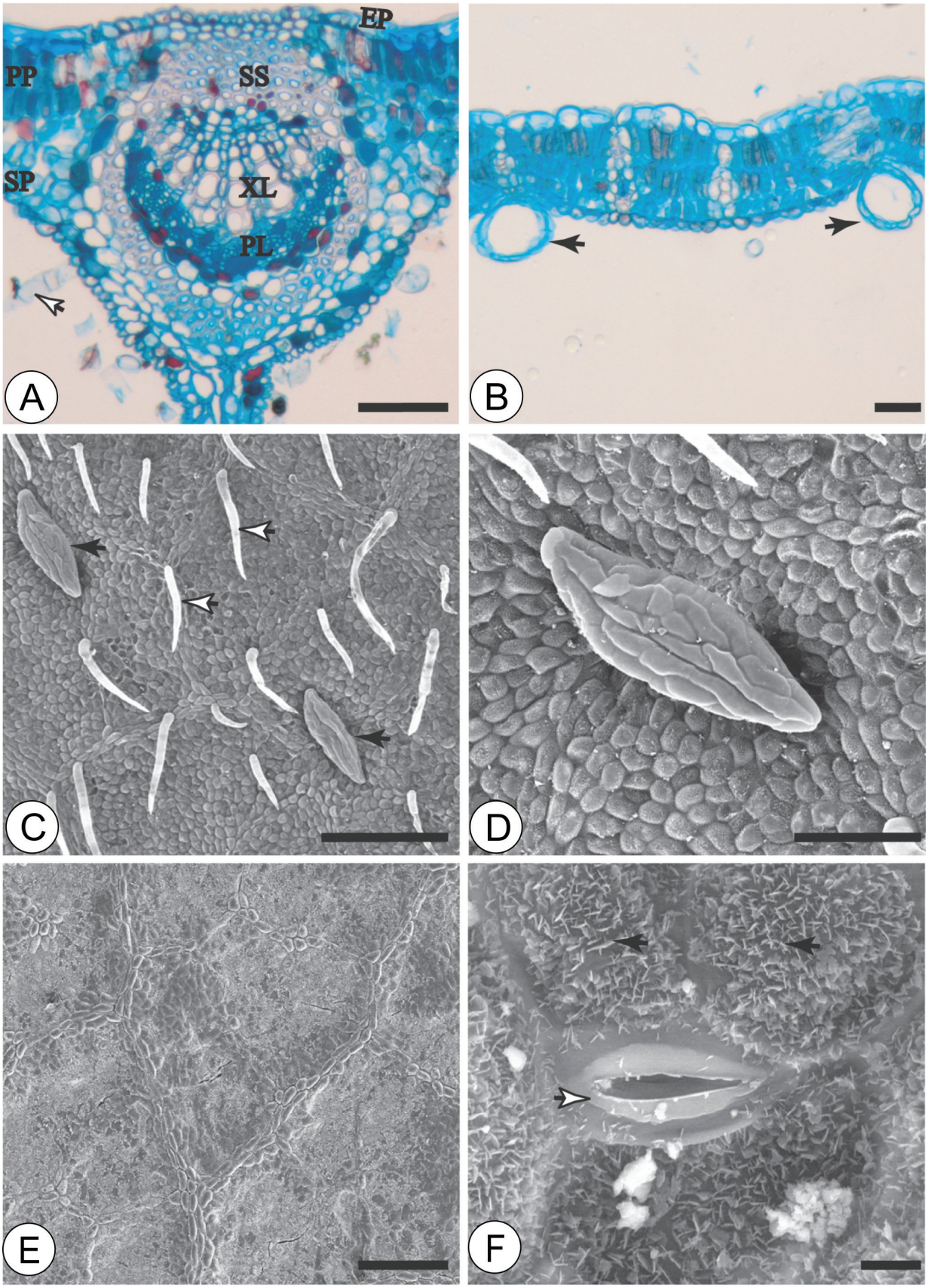

Figure 2. Structural aspects of the non-galled leaf of *Bauhinia ungulata* (Fabaceae) in transverse sections (A-B), and in scanning electron microscopy (C-F). **A-B.** Mesophyll. **A.** Simple non-glandular trichome (arrow). **B.** Navicular trichomes (arrows). **C-D, F.** Abaxial epidermal surface. **C.** Simple non-glandular trichomes (white); navicular trichomes (black arrow). **D.** Navicular trichome. **E.** Adaxial epidermis. **F.** Anomocytic stomata (white); epicuticular wax (black arrows). Abbreviations: EP, epidermal cells; PL, phloem; PP, palisade parenchyma; SP, spongy parenchyma; XL, xylem; SS, sclerenchymatic sheath. Bars: 80 µm (A-E); 5 µm (F).

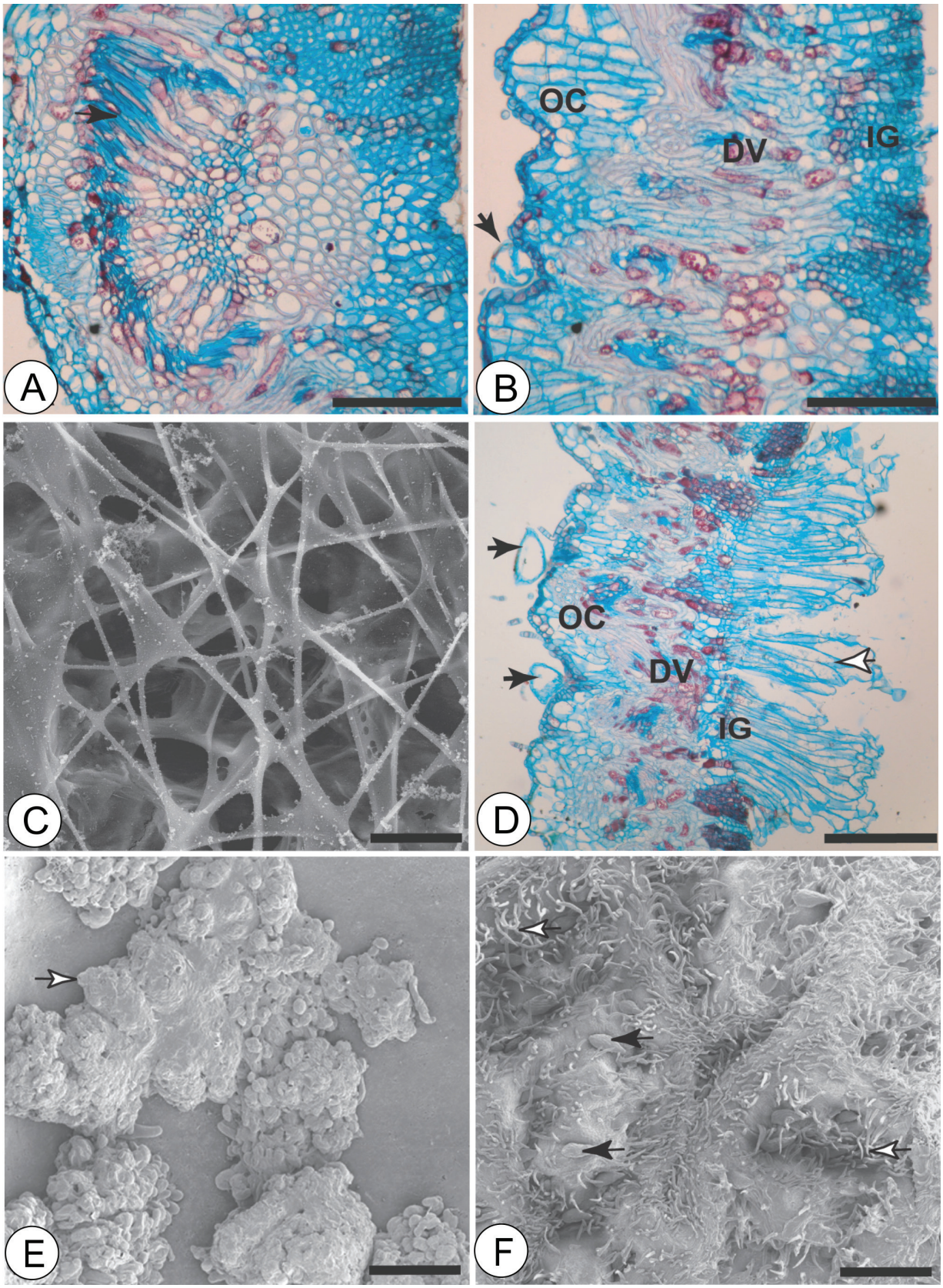

Figure 3. Structural aspects of the galls on *Bauhinia ungulata* (Fabaceae) in transverse sections (A, B, D), and surfaces observed in scanning electron microscopy (C, E, F). **A.** Hypertrophied secondary vein and redirection of the phloem (arrow). **B.** Gall without proliferated nutritive tissue; navicular trichome (arrow). **C.** Surface of the larval chamber, with destroyed nutritive cell walls. **D.** Navicular trichome (black arrows) and inner gall (IG) with hypertrophied nutritive cells (white arrow). **E.** Surface of the larval chamber, with proliferated nutritive tissue in parasitoidized galls. **F.** Simple non-glandular (white arrow) and navicular trichomes (black arrow) on abaxial surface. Abbreviations: DV, disorganized vascularization; IG, inner gall; OC, outer cortex. Bars: 150 µm (A-B, D-F); 50 µm (C).

tissue are chewed by the larvae, and are no longer observed in mature galls (Fig. 3B and 3C). The parasitoidized galls contain sites at which the nutritive tissue proliferates intensely and hypertrophies (Fig. 3D and 3E). Neoformations of vascular tissues are present, and the secondary veins are highly disorganized (Fig. 3C). The structure of the simple non-glandular trichomes and the glandular navicular trichomes remains unaltered (Fig. 3F), and the secondary veins are hypertrophied (Fig. 3A-C).

Histochemistry

Lipids and terpenoids were detected in the cuticle and as droplets in the glandular trichomes, and in the mesophyll of the non-galled leaves of *B. ungulata.* Polyphenols, alkaloids and reducing sugars were observed in the vascular bundles and mesophyll. Starch and proteins were detected in the mesophyll, and lignified walls in the cells of the vascular bundle sheath and xylem.

The histochemical profiles of non-parasitoidized and parasitoidized galls were similar to each other. In these structures, terpenoids and lipids were detected in the navicular glandular trichomes and in the nutritive tissue. Polyphenols, reducing sugars, and alkaloids were detected in the inner gall, except in the nutritive cells, and in the vascular bundles; starch was detected only in the nutritive tissue (Table 1).

Table 1. Histochemical profile of leaves and leaf-fold galls of *Bauhinia ungulata* (Fabaceae).

	Tissues	Leaf	Leaf folding gall
Lipids	Trichomes	++	++
	Mesophyll	+	
	Outer cortex		-
	Nutritive tissue		++
Terpenoids	Trichomes	++	++
	Mesophyll	+	
	Outer Cortex		-
	Inner Gall		-
	Nutritive tissue		++
Polyphenols	Mesophyll	+	
	Vascular bundles	+	+
	Outer cortex		-
	Inner gall		+
	Nutritive tissue		-
Alkaloids	Mesophyll	+	
	Vascular bundles	+	+
	Outer cortex		-
	Inner gall		+
	Nutritive tissue		-
Reducing sugars	Mesophyll	+	
	Vascular bundles	+	+
	Outer cortex		-
	Inner gall		+
	Nutritive tissue		-
Starch	Mesophyll	+	
	Outer cortex		-
	Inner gall		+
	Nutritive tissue		-
Protein	Mesophyll	+	
	Outer Cortex		-
	Inner Gall		-
	Nutritive tissue		-

+, positive reaction; -, negative reaction

DISCUSSION

The anatomical features of *Bauhinia ungulata* are similar to those of other species of the genus *Bauhinia*. Dorsiventral mesophyll with interspersed collateral vascular bundles surrounded by a sclerenchyma sheath is a generic feature (Miyake *et al.* 1986, Bicalho *et al.* 2005, Duarte *et al.* 2007, Lusa & Bona 2009). Leaf papillae are observed in several genera of the tribe Caesalpinieae (Solereder 1908), including *Bauhinia* (Duarte & Debur 2003, Duarte *et al.* 2007), as well as thin cuticle, epicuticular wax, and anomocytic stomata (Duarte & Debur 2003, Duarte *et al*. 2007, Lusa & Bona 2009). Glandular trichomes were described in *B. forficata* (Miyake *et al*. 1986) and *B. holophylla* (Bicalho *et al*. 2005), and navicular glandular trichomes were described in *B. forficata* and *B. variegata* (Lusa & Bona 2009).

The non-galled leaves and the leaf folding galls of *B. ungulata* differed in both morphological and histochemical features. The leaf lamina folds along the midrib, originating a simple structure, the leaf folding gall, which Price (1992) considered the first type of true galls to arise during the evolution of this kind of insect-plant interaction. This gall morphotype has been described for 0.4% of the galls in the Neotropics (Isaias *et al.* 2013). As a consequence of the leaf folding, the margins come together and form a single ample chamber where the gall-inducing larva develops. The host leaf tissues undergo hyperplasia and cell hypertrophy (Rohfrisch 1992), and generate the tissue zonation of the gall. The origin of the nutritive tissue, the adaxial leaf-tissue layers, epidermal and parenchymatic, was determined by the position of the protoxylem archs towards the larval chamber. The vascular position does not seem to be easily altered during the development of simple galls, as also observed in the *Aspidosperma australe-Pseudophacopteron* sp. system (Oliveira & Isaias 2010b).

The feeding habit of the gall inducer may cause the distinct patterns of differentiation of gall tissues, especially the nutritive tissue that surrounds the gall larval chamber (Rohfritsch 1992). This special tissue, a vacuolated parenchyma stimulated by the feeding of the larvae, is common in galls induced by chewing insects (Mani 1964). The nutritive tissue can accumulate lipids, which are the main compounds for the feeding and survival of the larvae in Lepidoptera-induced galls (Mani 1964), and the presence of lipids corroborates the nutritional hypothesis (Price *et al.* 1986, Stone & Schönrogge 2003). Therefore, lepidopteran gall tissues have a high energy content compared to the adjacent non-galled areas (Florentine *et al.* 2005).

The larvae of the inducing Lepidoptera chew on the epidermal cells, and stimulate either the epidermis or

adjacent cells to divide continuously, replacing the recently eaten nutritive cells (Florentine *et al*. 2005, Oliveira *et al.* 2010, Formiga *et al.* 2011, Raman 2011). In the galls of *B. ungulata*, this process of division is similar to that of the vascular cambium, phellogen, or secondary thickening meristem in monocots, and occurs via cell redifferentiation (*sensu* Lev-Yadun 2003); i.e., the meristematic capability is reassumed by the epidermis or parenchyma, which produce rows of aligned cells.

In parasitoidized galls, a larger amount of intact nutritive tissue, arranged at macroscopically visible sites, may develop. This cell proliferation is only possible after the death of the galling larva and the halting of tissue consumption. Even though inquilines can induce the formation of nutritive tissues (Noort *et al.* 2007), in the Lepidoptera - *B. ungulata* system, the sites of proliferated nutritive tissue must have been stimulated previously, and the cells were not consumed by the voraciously feeding larvae. After the death and consumption of the host larvae, some polyphagous parasitoids can feed on the gall tissue, in some cases simply in order to excavate the escape channel (Wiebes-Rijks & Shorthouse 1992). In the Lepidoptera - *B. ungulata* system, the site of proliferated nutritive cells in parasitoidized galls remains intact, which indicates that the associated endoparasitoid is a monophagous predator species.

Secondary metabolites such as alkaloids, terpenoids, polyphenols, and flavonoids have been described for some species of *Bauhinia* (Salatino *et al*. 1999, Maia-Neto *et al.* 2008, Cechinel-Filho 2009), and were detected by histochemical methods in leaf cells of *B. ungulata* (Maia-Neto *et al*. 2008). Secondary compounds, such as alkaloids or polyphenols, which were not detected in the nutritive tissue, proved to be deleterious to herbivores (Abrahamson *et al*. 1991, Isaias *et al*. 2000, Cuevas-Reyes *et al.* 2004, Detoni *et al.* 2010), and their accumulation must be blocked in order to prevent intoxication of the larvae. However, their presence in the cortical layers may constitute a chemical defense against natural enemies (Yang *et al.* 2003), which should favor the parasitoids but not the gall inducer, whose natural enemy is capable of entering the system. The concentration of these secondary compounds around the gall vascular bundles may prevent the destruction of the vascular system by the Lepidoptera. On the other hand, this protection does not seem to inhibit the attack of certain natural enemies, and according to Carmona *et al.* (2011), "most secondary metabolites are perhaps relics of past co-evolutionary interactions", a hypothesis supported by the normal occurrence of parasitoidism on the galling Lepidoptera.

In the gall-inducing Lepidoptera - *B. ungulata* system, the cell hypertrophy, tissue hyperplasia, and the accumulation of reserve substances in the nutritive tissue suggest that the development of this new organ, the gall, supplies the nutritional needs of the insect. Moreover, the attack of parasitoids kills the galling Lepidoptera, resulting in intact nutritive cells lining the larval chamber. This system seems to represent a better adaptation to provide nutrition for the gall inducer, than to form a defense against its natural enemies. Also, the stimuli from the feeding Lepidoptera on host-plant cells are maintained, and these cells continue to divide just after the death of the Lepidoptera, even though they will no longer fulfill their original function.

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REFERENCES

ABRAHAMSON, W.G., McCREA, K.D., WHITWELL, A.J. & VERNIERI, L.A. 1991. The role of phenolic compounds in goldenrod ball gall resistance and formation. *Biochemical Systematics and Ecology, 19*: 615-622.

ARDUIN, M. & KRAUS, J.E. 2001. Anatomia de galhas de ambrosia em folhas de *Baccharis concinna* e *Baccharis dracunculifolia* (Asteraceae). *Revista Brasileira de Botânica, 24*(1): 63-72.

BICALHO, G.O.D., CARDOSO, M.G., SILVA, V.F., MUNIZ, F.R., CASTRO, E.M. & GAVILANES, M.L. 2005. Estudo morfológico das folhas de *Bauhinia holophylla* Steud. *Caderno de Pesquisa, Série Biologia*, Santa Cruz do Sul, *17*(1): 13-19.

BRONNER, R. 1992. The role of nutritive cells in the nutrition of cynipids and cecidomyiids. In SHORTHOUSE, J.D. & ROHFRITSCH, O. (Eds.) *Biology of Insect-Induced Galls*. New York: Oxford University Press. p. 118-140.

BUKATSCH, F. 1972. Bermerkungen zur Doppelfärbung Astrablau-Safranin. *Mikrokosmos*, *61*: 255.

CARMONA, D., LAJEUNESSE, M.J. & JOHNSON, M.T.J. 2011. Plant traits that predict resistance to herbivores. *Functional Ecology, 25*(2): 358-367.

CECHINEL-FILHO, V. 2009. Chemical Composition and Biological Potential of Plants from the Genus *Bauhinia*. *Phytotherapy Research, 23*: 1347-1354.

CUEVAS-REYES, P., QUESADA, M., HANSON, P., DIRZO, R. & OYAMA, K. 2004. Diversity of gall-inducing insects in a Mexican tropical dry forest: the importance of plant species richness, life forms, host plant age and plant density. *Journal of Ecology, 92*: 707-716.

DAVID, R. & CARDE, J.P. 1964. Coloration différentielle des inclusions lipidiques et terpéniques des pseudophylles du Pin maritime au moyen du réactif Nadi. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences, 258*: 1338-1340.

DETONI, M.L., VASCONCELOS, E.G., MAIA, S.C.I.O, E., AGUIAR, J.A.K., ISAIAS, R.M.S. & SOARES, G.L.G. 2010. Differential biochemical responses of *Calliandra brevipes* (Fabaceae, Mimosoidae) to galling behaviour by *Tanaostigmodes ringueleti* and *T. mecanga* (Hymenoptera, Tanaostigmatidae). *Australian Journal of Botany, 58*: 280-285.

DREGER-JAUFRET, F. & SHORTHOUSE, J.D. 1992. Diversity of gall-inducing insects and their galls. In: SHORTHOUSE, J.D. & ROHFRITSCH, O. (Eds.) *Biology of Insect-Induced Galls*. New York: Oxford University Press. p. 8-33.

DUARTE, M.R. & DEBUR, M.C. 2003. Caracteres morfo-anatômicos da folha e do caule de *Bauhinia microstachya* (Raddi) J. F. Macbr. (Fabaceae). *Revista Brasileira de Farmacognosia, 13*(1): 7-15.

DUARTE, M.R., SILVA, A.G., COSTA, R.E. & FARIA, L.T. 2007. *Bauhinia variegata*: Diagnose morfoanatômica e análise comparativa en-

tre exemplares de regiões climáticas distintas. *Latin American Journal of Pharmacy, 26*(6): 837-45.

DUNN, M.J. 1933. *Gel Electrophoresis: Proteins*. Oxford: Bios Scientific Publishers.

ESPÍRITO-SANTO, M.M. & FERNANDES, G.W. 2007. How many species of gall-inducing insects are there on Earth, and where are they? *Annals of the Entomological Society of America, 100*: 95-99.

FLORENTINE, S.K., RAMAN, A. & DHILEEPAN, K. 2005. Effects of gall induction by *Epiblema strenuana* on gas exchange, nutrients, and energetics in *Parthenium hysterophorus*. *BioControl, 50*: 787-801.

FORMIGA, A.T., SOARES, G.L.G. & ISAIAS, R.M.S. 2011. Responses of the host plant tissues to gall induction in *Aspidosperma spruceanum* Müell. Arg. (Apocynaceae). *American Journal of Plant Sciences, 2:* 823-834.

HARDY, N.B. & L.G. COOK. 2010. Gall-induction in insects: evolutionary dead-end or speciation driver? *BMC Evolutionary Biology, 10*: 257.

HARTLEY, S.E. 1998. The chemical composition of plant galls: are levels of nutrients and secondary compounds controlled by the gall former? *Oecologia, 113*: 492-501.

HARTLEY, S.E. 1999. Are gall insects large rhizobia? *Oikos, 84*: 333-342.

HORI, K. 1992. Insect secretions and their effect on plant growth, with special reference to hemipterans. In: SHORTHOUSE, J.D. & ROHFRITSCH, O. (Eds.), *Biology of Insect-Induced Galls*. New York: Oxford University Press. p. 157-170.

ISAIAS, R.M.S., CARNEIRO, R.G.S., OLIVEIRA, D.C. & SANTOS, J.C. 2013. Illustrated and annotated checklist of Brazilian gall morphotypes. *Neotropical Entomology*, *42*(3): 230-239.

ISAIAS, R.M.S., SOARES, G.L.G., CHRISTIANO, J.C.S. & GONÇALVES, S.J.M.R. 2000. Análise comparativa entre as defesas mecânicas e químicas de *Aspidosperma australe* Müell. Arg. e *Aspidosperma cylindrocarpon* Müell. Arg. (Apocynaceae) contra herbivoria. *Floresta e Ambiente, 7*: 19-30.

JENSEN, W.A. 1962. *Botanical Histochemistry: Principles and Practice*. San Francisco: W. H. Freeman and Company. 408 p.

JOHANSEN, D.A. 1940. *Plant Microtechnique*. New York: McGraw-Hill Books. 523 p.

KRAUS, J.E. & ARDUIN, M. 1997. *Manual Básico de Métodos em Morfologia Vegetal.* Rio de Janeiro: Editora da Universidade Federal Rural do Rio de Janeiro, Seropédica. 198 p.

LÁSZLÓ, Z. & TÓTHMÉRÉSZ, B. 2006. Inquiline effects on a multilocular gall community. *Acta Zoologica Academiae Scientiarum Hungaricae, 52*(4): 373-383.

LEV-YADUN, S. 2003. Stem cell in plants are differentiated too. *Current Topics in Plant Biology, 4*: 93-100.

LUSA, M.G. & BONA, C. 2009. Análise morfoanatômica comparativa da folha de *Bauhinia forficata* Link e *B. variegata* Linn. (Leguminosae, Caesalpinioideae). *Acta Botanica Brasilica, 23*(1): 196-211.

MAIA-NETO, M., ANDRADE NETO, M., BRAZ FILHO, R., LIMA, M.A.S. & SILVEIRA, E.R. 2008. Flavonoids and alkaloids from leaves of *Bauhinia ungulata* L. *Biochemical Systematics and Ecology, 36*: 227-229.

MANI, M.S. 1964. *Ecology of Plant Galls*. The Hague: Dr. W. Junk Publishers. 434 p.

MANI, M.S. 1992. Introduction to Cecidology. In: SHORTHOUSE, J.D. & ROHFRITSCH, O. (Eds.), *Biology of Insect-Induced Galls*. New York: Oxford University Press. p. 3-7.

MIYAKE, E.T., AKISUE, G. &. AKISUE, M.K. 1986. Caracterização farmacognóstica da pata-de-vaca *Bauhinia forficata* Link. Revista Brasileira de Farmacognosia., *1*: 58-68.

NOORT, S.V., GRAHAM, N.S., WHITEHEAD, V.B., & NIEVES-ALDREY, J.L. 2007. Biology of *Rhoophilus loewi* (Hymenoptera: Cynipoidea: Cynipidae), with implications for the evolution of inquilinism in gall wasps. *Biological Journal of the Linnean Society, 90*: 153-172.

O'BRIEN, T.P., McCULLY, M.E. 1981. *The Study of Plant Structure: Principles and Selected Methods.* Melbourne: Termacarphi Pty Ltd.

OLIVEIRA, D.C. & ISAIAS, R.M.S. 2010a. Redifferentiation of leaflet tissues during midrib gall development in *Copaifera langsdorffii* (Fabaceae). *South African Journal of Botany, 76*: 239-248.

OLIVEIRA, D.C. & ISAIAS, R.M.S. 2010b. Cytological and histochemical gradients induced by a sucking insect in galls of *Aspidosperma australe* Arg. Muell (Apocynaceae). *Plant Science, 178*: 350-358.

OLIVEIRA, D.C., MAGALHÃES, T.A., CARNEIRO, R.G.S., ALVIM, M.N. & ISAIAS, R.M.S. 2010. Do Cecidomyiidae galls of *Aspidosperma spruceanum* (Apocynaceae) fit the pre-established cytological and histochemical patterns? *Protoplasma, 242*: 81-93.

PAIVA, J.G.A., FANK-DE-CARVALHO, S.M., MAGALHÃES, M.P., & GRACIANO-RIBEIRO, D. 2006. Verniz vitral incolor 500®: uma alternativa de meio de montagem economicamente viável. *Acta Botanica Brasilica, 20*(2): 257-264.

PRICE, P.W. 1992. Evolution and ecology of gall-inducing sawflies. In: SHORTHOUSE, J.D. & ROHFRITSCH, O. (Eds.) *Biology of Insect-Induced Galls*. New York: Oxford University Press. p. 208-224.

PRICE, P.W., FERNANDES, G.W. & WARING, G.L. 1987. Adaptive nature of insect galls. *Environmental Entomology, 16*:15-24.

PRICE, P.W., WARING, G.L. & FERNANDES, G.W. 1986. Hypotheses on the adaptive nature of galls. *Proceedings of the Entomological Society of Washington, 88*(2): 361-363.

RAMAN, A. 2011. Morphogenesis of insect-induced plant galls: facts and questions. *Flora, 206*: 517-533.

ROHFRITSCH, O. 1992. Patterns in gall development. In: SHORTHOUSE, J.D. & ROHFRITSCH, O. (Eds.), *Biology of Insect-Induced Galls*. New York: Oxford University Press. p. 60-86.

SALATINO, A., BLATT, C.T.T., SANTOS, D.Y.A.C. & VAZ, A.M.S.F. 1999. Foliar flavonoids of nine species of *Bauhinia*. *Revista Brasileira de Botânica, 22*: 17-2.

SASS, J.E. 1951. *Botanical Microtechnique*. 2nd ed. Ames: Iowa State College Press. 391 p.

SOLEREDER, H. 1908. *Systematic Anatomy of the Dicotyledons: A Handbook for Laboratories of Pure and applied Botany*. v. 2. Oxford: Clarendon Press. 1182 p.

STONE, G.N. & SCHÖNROGGE, K. 2003. The adaptive significance of insect gall morphology. *Trends in Ecology & Evolution, 18*(10): 512-522.

WIEBES-RIJKS, A.A. & SHORTHOUSE, J.D. 1992. Ecological Relationships of Insects Inhabiting Cynipid Galls. In: SHORTHOUSE, J.D. & ROHFRITSCH, O. (Eds.) *Biology of Insect-Induced Galls*. New York: Oxford University Press. p. 238-257.

YANG, C.M., YANG, M.M., HUANG, M.Y., HSU, J.M. & JANE, W.N. 2003. Herbivorous insect causes deficiency of pigment-protein complexes in an oval-pointed cecidomyiid gall of *Machilus thunbergii* leaves. *Botanical Bulletin of Academia Sinica, 44*: 314-321.

YODER, L.R. & MAHLBERG, P.G. 1976. Reactions of alkaloid and histochemical indicators in laticifers and specialized parenchyma cells of *Catharanthus roseus* (Apocynaceae). *American Journal of Botany, 63*: 1167-1173.