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# Structural and histochemical profile of *Lopesia* sp. Rübsaamen 1908 pinnula galls on *Mimosa tenuiflora* (Willd.) Poir. in a Caatinga environment

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ABSTRACT - (Structural and histochemical profile of *Lopesia* sp. Rübsaamen 1908 pinnula galls on *Mimosa tenuiflora* (Willd.) Poir. in a Caatinga environment). Gall-inducing insects can change the anatomical pattern of host plant tissues by inducing peculiar gall morphotypes. In this study, the structural changes observed in *Lopesia* galls on *Mimosa tenuiflora* resemble those found in other Cecidomyiidae, with two tissue compartments. Nevertheless, the parenchyma layers of the inner compartment, between the mechanical zone and the nutritive tissue, are peculiar. Gall development does not impair the synthesis of any compounds detected by histochemical tests on non-galled tissues of *M. tenuiflora*. Lignin, polyphenols, alkaloids and terpenoids were detected in the outer compartment, suggesting their involvement in chemical defence of galls. Proteins, reducing sugars and lipids were detected both in outer and inner compartments, whereas nutritive tissue is rich in reducing sugar. This profile is linked with the nutrition of the gall-inducing insect. The Caatinga environment does not seem to constrain the development of galls, but the thick periclinal cell wall and homogeneous parenchyma may contribute to the control of humidity and light radiation, thus favouring the survival of the gall-inducing insect.
Keywords: Cecidomyiidae, gall anatomy, leaf gall, plant insect interaction

RESUMO - (Perfil estrutural e histoquímico de galhas foliares de *Lopesia* sp. Rübsaamen 1908 em *Mimosa tenuiflora* (Willd.) Poir. em ambiente de Caatinga). Insetos galhadores podem alterar o padrão anatômico dos tecidos das suas plantas hospedeiras, induzindo morfotipos peculiares de galhas. Neste estudo, as modificações estruturais observadas nas galhas de *Lopesia* sp. em *Mimosa tenuiflora* assemelham-se àquelas evidenciadas em outras galhas de Cecidomyiidae, com dois compartimentos teciduais. No entanto, as camadas de parênquima no compartimento interno entre a zona mecânica e o tecido nutritivo são peculiares. O desenvolvimento da galha não bloqueia a síntese de quaisquer compostos detectados nos tecidos não galhados de *M. tenuiflora*, por meio de testes histoquímicos. Ligninas, polifenóis, alcaloides e terpenoides foram detectados no compartimento externo, sugerindo seu envolvimento na defesa química das galhas. Proteínas, açúcares redutores e lipídios foram detectados tanto no compartimento externo quanto no interno, e o tecido nutritivo é rico em açúcares redutores. Esse perfil está ligado à nutrição do galhador. O ambiente da Caatinga parece não impor restrições ao desenvolvimento da galha, mas, a parede celular periclinal espessa e o parênquima homogêneo parecem contribuir para o controle da umidade e da radiação, favorecendo à sobrevivência do inseto galhador.
Palavras-chave: anatomia de galha, Cecidomyiidae, galha foliar, interação inseto-planta

## Introduction

All plant groups may be attacked by mites, nematodes, bacteria, fungi, viruses, lichens, and mostly by insects, resulting in the development of galls (Mani 1964, Rohfritsch 1992). Galls are the result of abnormal growth of plant tissues due to an increase in the cell hypertrophy and cell division induced by the feeding stimuli of the galling insects (Raman 2007). These structural changes and the physiology of the host plant cells and tissues are redirected toward a new organ, the gall (Harper *et al.* 2004, Raman 2007).

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Among the most common family of insects capable of inducing galls, the one most representative is the Cecidomyiidae (Diptera) (Gagné & Jaschhof 2017).

An important aspect of the Cecidomyiidae is that they induce profound modifications in their host plant organs, both at the cell and tissue levels (Arduin & Kraus 1995, Moura *et al*. 2009, Oliveira & Isaias 2010, Isaias *et al*. 2011). After the establishment of the galling insect, the growth of gall tissues is associated with changes in the accumulation of carbohydrates, proteins, lipids, as well as secondary metabolites, such as phenols and alkaloids (Arya *et al.* 1975, Oliveira *et al.* 2011*,* Amorim *et al.* 2017, Bragança *et al.* 2017). Accordingly, the histolocalization of these compounds is related to the nutrition of the galling insect, as well as to the defense against natural enemies and unfavorable environmental factors (Stone & Schönrogge 2003). Consequently, the metabolites may be compartmentalized in gall tissues (Bragança *et al.* 2017).

A common species of Leguminosae-Caesalpinioideae, *Mimosa tenuiflora* (Willd.) Poir. is infested by a galling *Lopesia* Rübsaamen 1908 (Cecidomyiidae) in the Caatinga vegetation in the Northeast region, Brazil (Maia *et al.* 2010, Santos *et al.* 2011, Carvalho-Fernandes *et al.* 2012). *Mimosa tenuiflora* occurs in Brazil, Colombia, El Salvador, Honduras, Mexico, and Venezuela. In Brazil, it has been recorded in all states of the Northeast region, extending to the State of Minas Gerais, and is one of the best-studied species of Fabaceae. Its anatomy, ecology, chemical constituents, biological activity, and usages have been addressed in more than 30 scientific publications (*cf*. Santos-Silva *et al.* 2015), reflecting its large economic and ecological importance.

The adaptive success of *M. tenuiflora* in Caatinga environment may be related to its anatomical traits. Among the adaptive traits commonly related to xeric environments, a reduction in volume-surface ratio, thick wax, cuticle and periclinal cell walls, dense palisade parenchyma and trichome covering, and abundant water storage tissues (Fahn & Cutler 1992) can be expected in both host leaves and galls. Phenolics and calcium oxalate crystals may also occur (Fahn & Cutler 1992, Fahmy 1997, Burrows 2001, Rotondi *et al*. 2003). This work analyzes the *M. tenuiflora*-*Lopesia* sp. system as a model of study to map traits that may favor plant survival over the abiotic peculiarities of the Caatinga, and that can be overexpressed during gall development.

Herein, we address a new approach for this plant species, focusing on its structural and histochemical profiles developed under the influence of the associated galling insect, *Lopesia* sp. Moreover, we discuss structural and histochemical profiles of *Lopesia* galls under the influence of abiotic and biotic stresses, such as high temperatures and natural enemies, in the Caatinga environment. It is assumed that the gall-inducing insect stimuli, together with the environmental stress, should drive the morphogenesis of the gall toward anatomical and chemical traits that could induce positive responses to the adaptive value of both *M. tenuiflora* and the galling *Lopesia* sp. The main question is: can the characteristics of Caatinga vegetation impact both the structural and histochemical profile of the galls induced by *Lopesia* sp. on *M. tenuiflora*?

## Materials and methods

Sampling and Fixation - Non-galled pinnulae and galls were randomly sampled from individuals of *M. tenuiflora* (n = 10) located at Lagoa Rasa Ranch (13°95'S e 42°47'W), Cachoeirinha Farm, Caetité municipality, Bahia State, Brazil. The voucher specimen is deposited at HUNEB herbarium under the registration number 24.975.

Anatomical analysis - Samples (n = 5) of non-galled pinnula, mature, and senescent galls were fixed in FAA (37% formaldehyde, glacial acetic acid, and 50 % ethanol, 1:1:18, v/v) for 48 hours, dehydrated in an ethanol series, embedded in Paraplast® (Kraus & Arduin, 1997), and cross-sectioned (12 µm) in a Reichert Jung® rotary microtome. The histological sections were stained with 0.5% safranin and 0.5% astra blue, 9:1, v/v (Kraus & Arduin 1997).

Histochemical analysis - Handmade sections (n = 5) of fresh non-galled pinnulas, mature and senescent galls were used for histochemical analyses. For detection of proteins, the sections were immersed in 0.1% bromophenol blue in a saturated solution of magnesium chloride in ethanol during 15 minutes, and later washed in acetic acid and water (Backer 1958). For reducing sugars, the sections were immersed in Fehling's reagent (solution A- 7.9% copper sulphate and solution B- 34.6% potassium sodium tartrate and 1% sodium hydroxide, followed by heating) for 15 minutes (Sass 1951). The presence of lipids and starch was verified with saturated solution of Sudan red B in 70% ethanol for 20 minutes (Brundett

*et al.* 1991), and with Lugol's reagent for 5 minutes (Johansen 1940). For detection of lignins, acidified phloroglucinol (solution A- 2% phloroglucin and solution B-25% hydrochloric acid) was applied for 5 minutes (Johansen 1940). For detection of polyphenols, 1% ferric chloride was used for 5 minutes (Johansen 1940). The accumulation of alkaloids was verified with Jeffrey's reagent (10% nitric acid and 10% chromic acid), for 15 minutes (Johansen 1940), and terpenes were detected with 1% α-naphthol and 1% dimethyl-*p*-phenylenediamine (NADI reagent) in 0.01 M phosphate buffer, pH 7.2, for 30 minutes (David & Carde 1964). The sections were mounted in Kaiser's jelly glycerin (Kraus & Arduin 1997). All reactions were followed by control tests according to the authors, compared to blank sections, and photographed on a light microscope (Leica ICC50 HP).

## Results

General aspects of host plant and gall morphology - *M. tenuiflora* is a tree (figure 1), approximately 8 m high. Its leaves are compound twice-pinnate with 4-10 pairs of leaflets, and 17-25 pairs of pinnula per leaflet (figure 2). The leaflets are papery, oblong with entire margins, oblique base and rounded apex, glabrous to pilosulous, with minute sessile glands especially on the abaxial surface (Santos-Silva *et al.* 2015). *Lopesia* sp. galls on *M. tenuiflora* are unilocular, covered with sparse non-glandular trichomes. The galls are bivalve-shaped, non-fused along the margins, and turn from green to brown along development (figure 3).

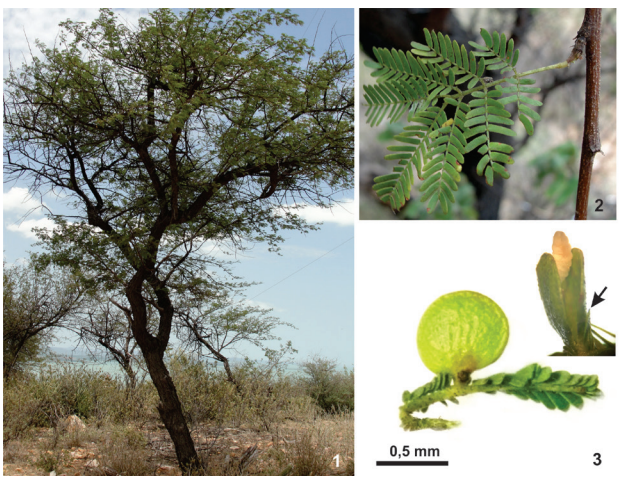


Figure 1-3. *Mimosa tenuiflora* (Leguminosae - Caesalpinioideae) non-galled pinnula and galls of *Lopesia* sp. (Cecidomyiidae - Diptera). 1. Plant habit 2. Leaf. 3. Leaflet with bivalve-shaped gall, evidencing the pinnula at the base of valves (arrow).

Anatomical and histochemical profiles of the non-galled pinnula - The epidermis is uniseriate, with irregular shaped cells, thick-walled and covered by thin cuticle both on adaxial and abaxial surface. Pinnula lamina is dorsiventral, with a 3-layered palisade parenchyma and a 3-4 layered spongy parenchyma (figure 4). The unicellular non-glandular trichomes (figure 5) vary in size, and are straight, with sharp apex. Multicellular glandular trichomes occur (figure 6) in adaxial and abaxial surfaces. The vascular system has collateral arrangement involved predominantly by a parenchymatic sheath with isolated pericyclic fibers (figure 7).

The non-galled pinnula has positive reactions for all the analyzed compounds, except starch (tables 1-2). Proteins occur in palisade and spongy parenchyma. Reducing sugars occur in epidermis and spongy parenchyma, and lipids were detected in epidermis, non-glandular trichomes, palisade and spongy parenchyma. Lignin is detected in the walls of xylem cells and pericyclic fibers. Polyphenols occur in cells of epidermis and palisade parenchyma (figure 8); alkaloids in epidermis and terpenoids in the vascular bundles (figure 9), and in the basal cells of the unicellular non-glandular trichomes.

Anatomical and histochemical profiles of mature galls - The outer tissue compartment is formed by the epidermis, parenchyma, vascular bundles and sclerenchyma. The epidermis is uniseriate, with thick-walled elongated cells, covered by thin cuticle. Glandular and non-glandular trichomes occur (figure 10). The parenchyma has 3-4 cell layers and the sclerechyma 4-5 cell layers. The neoformed vascular bundles are collateral and immersed in the sclerechymatic layer. The inner compartment is formed by 4-5 layers of parenchymatic cells, which limits the nutritive tissue, with periclinally elongated cells (figure 11).

The histochemical profile of mature galls reveals positive results for all tested substances, except for starch (tables 1-2). Proteins occur in parenchyma cells of the outer and inner compartments, in sclerenchyma (figure 12), and in nutritive cells. Reducing sugars are detected in the ordinary epidermal cells, non-glandular trichomes, parenchymatic cells of the outer compartment (figure 13), and nutritive tissues. Lipids are detected in the epidermis, non-glandular trichomes, and parenchymatic cells of outer and inner compartments (figure 14-15), and nutritive tissue. Lignins are detected in the cell walls of the

sclereids (figure 16). Polyphenols occur in epidermis, parenchymatic cells of the outer compartment and cytoplasm of the sclerenchyma cells (figure 17). Alkaloids occur in cells of epidermis and parenchymatic cells of the outer compartment (figure 18). Terpenoids occur in non-glandular trichomes and in cytoplasm of the sclereids (figures 19-21).

Anatomical and histochemical profiles of senescent galls - The dermal and ground system is suberized in the outer tissue compartment. The vascular system does not alter toward senescent gall. The inner compartment and nutritive tissue has necrotic cells. For senescent galls, the histochemical tests were positive for suberin in necrotic nutritive cells and lignins in cell walls of the sclereids (tables 1-2; figures 22-23).

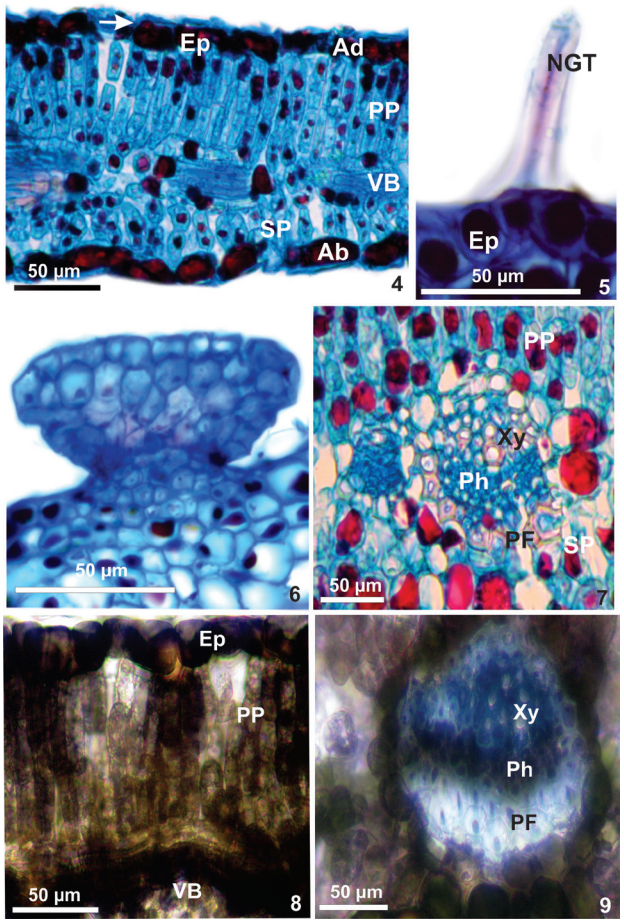


Figure 4-9. Transverse sections of the non-galled pinnulae of *Mimosa tenuiflora* (Leguminosae - Caesalpinioideae). 4-7. Anatomy. 4. Pinnula evidencing uniseriate epidermis covered by a thin cuticle (arrow), palisade and spongy parenchyma, and vascular bundles. 5. Non-glandular trichomes. 6. Multicellular glandular trichomes. 7. Vascular bundles evidencing xylem and phloem involved by pericyclic fibers. 8-9. Histochemistry. 8. Polyphenols detected on the epidermis and in the palisade parenchyma. 9. Terpenoids in the vascular bundles. Ad (adaxial), Ab (Abaxial), Ep (epidermis), Ph (phloem), PF (pericyclic fibers), NGT (non-glandular trichomes), PP (palisade parenchyma), SP (spongy parenchyma), VB (vascular bundles) Xy (xylem).

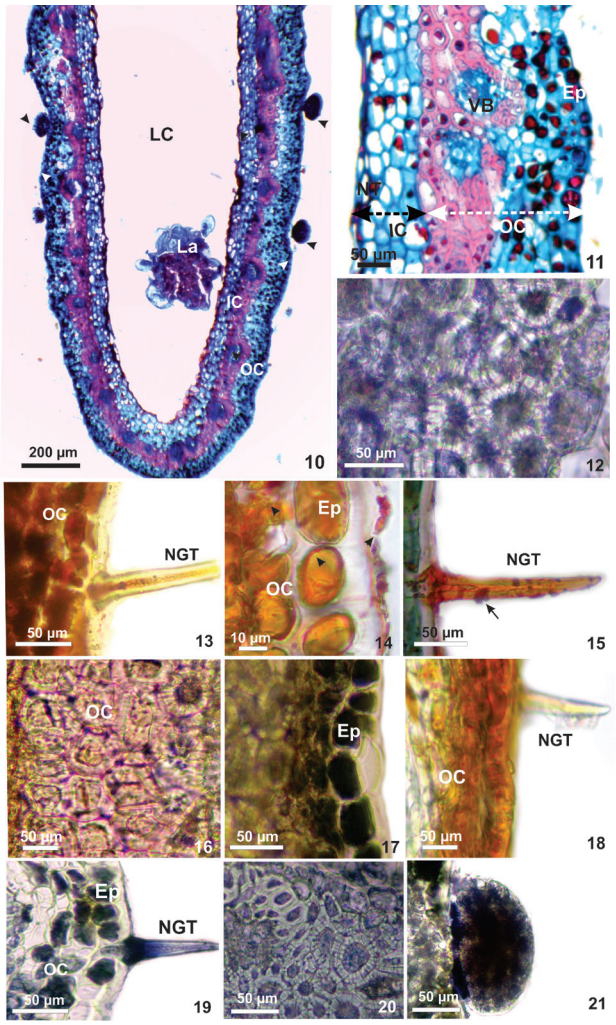


Figure 10-21. *Lopesia* sp. mature galls (Cecidomyiidae - Diptera) on pinnulae of *Mimosa tenuiflora* (Leguminosae - Caesalpinioideae). 10-11. Gall anatomy.10. Longitudinal section evidencing larval chamber and larva, cuticle (white arrow) and glandular trichomes (black arrow). 11-21. Transverse sections. 11. Outer and inner compartments. 12-21. Gall histochemistry. 12. Proteins detected in sclerenchyma cells. 13. Reducing sugars detected on the epidermis and in the parenchyma cells of the outer compartment. 14-15. Lipids evidencing the cuticle (black arrow) on epidermal ordinary cells and non-glandular trichomas. 16. Lignin detected on cell walls of the sclerenchyma (white arrow). 17. Polyphenols detected on the epidermis and in the cytoplasm of the sclereids. 18. Alkaloids detected on the epidermis and in the parenchyma cells of the outer compartment. 19-21. Terpenoids detected in non-glandular trichomes, cytoplasm of the sclereids, and glandular trichomes. Ep (epidermis), IC (inner compartment), LC (larval chamber), La (larva), NT (nutritive tissue), NGT (non-glandular trichomes), OC (outer compartment), VB (vascular bundles).

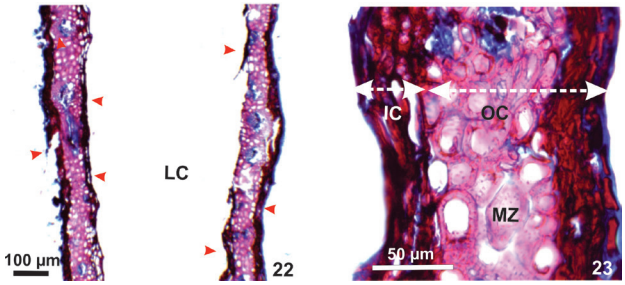


Figure 22-23. Transverse sections of *Lopesia* sp. senescent galls (Cecidomyiidae - Diptera) on the pinnulae of *Mimosa tenuiflora* (Leguminosae - Caesalpinioideae). 22. Gall evidencing necrotic cells of the epidermis and nutritive tissue (red arrow). 23. Detail of the gall evidencing accumulation of suberin in the outer compartment parenchyma, higher lignification in mechanical zone, inner compartment and nutritive tissue with necrotic cells. Ep (epidermis), IC (inner compartment), LC (larval chamber), MZ (mechanical zone), NGT (non-glandular trichomes), OC (outer compartment).

## Discussion

The anatomical alterations from non-galled pinnulae toward galls - The structural alterations induced by *Lopesia* sp. on the pinnulae of *M. tenuiflora* are quite similar to those induced by other species of Cecidomyiidae in the neotropics (Arduin & Kraus 1995, Moura *et al.* 2009, Oliveira & Isaias 2009, Oliveira *et al.* 2010). Cecidomyiidae galls commonly have parenchyma homogenization and the formation of sclerenchyma layers adjacent to the nutrient tissue (Rohfritsch 1992). Accordingly, gall features seem to be independent of the host plant potentialities. Nevertheless, we can consider that in *M. tenuiflora*-*Lopesia* sp. system, the redifferentiation of an inner parenchymatic layer between the sclereids and the nutritive tissue is peculiar. Concerning the dermal

Table 1. Histochemical tests in non-galled pinnula, mature and senescent galls of *Lopesia* sp. (Diptera - Cecidomyiidae) on *Mimosa tenuiflora* (Leguminosae - Caesalpinioideae).

| Reagents | Substances | Non-galled pinnula | Mature galls | Senescent galls | Color result |
|---|---|---|---|---|---|
| Bromophenol blue | Proteins | + | + | - | Dark Blue |
| Ferric chloride | Polyphenols | + | + | - | Bluish Black or dark green |
| Fehling | Reducing sugars | + | + | - | Bright Red |
| Phloroglucinol | Lignins | + | + | + | Rose |
| Jeffrey | Alkaloids | + | + | - | Red brown |
| NADI | Terpenoids | + | + | - | Blue |
| Sudan red | Lipids | + | + | + | Red |
| Lugol | Starch | - | - | - | Brown |

Results: (+) positive reaction; (-) negative reaction.

system, there is apparently no alteration in the structure of ordinary epidermal cells and trichomes, an indicative that *Lopesia* sp. stimuli is constrained by the morphogenetical pattern of *M. tenuiflora*. Anyhow, the galling stimuli promote the differentiation of two laminar appendages on the base of two pinnulae. These hypertrophied appendages develop the valve-like leaves peculiar of this bivalve-shaped gall.

The thin cuticle covering the gall outer epidermis may be compensated by the thick cell walls. Due to the cuticle slight thickness on *M. tenuiflora* - *Lopesia* sp. system, its primary function, *i.e.*, protection against abiotic factors, may be performed by the thick periclinal cell walls, which can control the excess of water loss due to the high temperatures of Caatinga (Gal *et al.* 2015). Together with the thick cell walls, the glandular and non-glandular trichomes observed both in host pinnula and galls may enhance the protection against unfavorable environmental features (Stone & Schonrogge 2003, Moura *et al.* 2009, Oliveira & Isaias 2010). Also, gall tissues and trichomes are terpenoid-rich, which may add in chemical defense against natural enemies (Souza-Silva *et al.* 2017).

The neoformation of vascular bundles is a peculiarity of the galls of *Lopesia* sp. on *M. tenuiflora.* These neoformed vascular bundles are connected to the vascular system of the rachis and may transfer nutrients to the nutritive cells. Consequently, they are crucial both to the galling herbivore nutrition, and to the maintenance of gall structure (Dias *et al.* 2013). The collateral bundles are common for Cecidomyiidae galls (Arduin & Kraus 1995, Oliveira & Isaias 2009,

Table 2. Histolocalization of metabolites in the non-galled pinnulae of *Mimosa tenuiflora* (Leguminosae - Caesalpinioideae) and galls of *Lopesia* sp. (Diptera - Cecidomyiidae).

| Histolocalization | Protein | Lipids | Reducing sugars | Polyphenols | Terpenoids | Alkaloids | Lignin |
|---|---|---|---|---|---|---|---|
| Non-galled tissues | | | | | | | |
| Epidermis | - | + | + | + | - | + | - |
| Non-glandular trichomes | - | + | - | - | + | - | - |
| Palisade parenchyma | + | + | + | + | - | - | - |
| Spongy parenchyma | + | + | + | - | - | - | - |
| Vascular bundle | - | - | - | - | + | - | + |
| Pericyclic fibers | - | - | - | - | - | - | + |
| Mature galls | | | | | | | |
| Outer compartment | | | | | | | |
| Epidermis | - | + | + | + | - | + | - |
| Non-glandular trichomes | - | + | + | - | + | - | - |
| parenchyma | + | + | + | + | - | + | - |
| Sclerenchyma | + | - | - | + | + | - | + |
| Inner compartment | | | | | | | |
| parenchyma | + | + | + | - | - | - | - |
| Nutritive tissue | + | + | + | - | - | - | - |
| Senescent galls | | | | | | | |
| Outer compartment | | | | | | | |
| Epidermis | - | + | + | - | - | + | - |
| Non-glandular trichomes | - | + | - | - | - | - | - |
| Parenchyma | - | + | - | - | - | - | - |
| Sclerenchyma | + | - | - | - | - | - | + |
| Inner compartment | | | | | | | |
| Parenchyma | - | + | - | - | - | - | - |
| Nutritive tissue | - | - | - | - | - | - | - |

Results: (+) positive reaction; (-) negative reaction.

2010, Fleury *et al.* 2015), and have been previously reported for the galls on *Copaifera langsdorffii* Desf. (Oliveira & Isaias 2009, 2010) and *Aspidosperma spruceanum* Benth. *ex* Müell. Arg. (Formiga *et al.* 2011) in Neotropical region.

Due to gall stimuli, the ground system reassumes its potential meristematic capacity for cell division and hypertrophy (Moura *et al.* 2009, Oliveira & Isaias 2010). Cell divisions occur in several and distinct planes at gall site, which result in the increased number of cell layers. Hyperplasia and cell hypertrophy are common phenomena in gall development, and have been already reported for several host plant-galling herbivore systems (Moura *et al*. 2008, Moura *et al.* 2009, Fleury *et al.* 2015). The ground system of the galls on *M. tenuiflora* is altered from the pinnula palisade and spongy parenchyma toward the homogenous parenchyma, similar to the galls induced by Cecidomyiidae on *C. langsdorffii* (Oliveira *et al.* 2010) and *Lantana camara* L. (Moura *et al.* 2008). On the galls of *Lopesia* sp. on *M. tenuiflora*, the homogeneous parenchyma of the outer and inner compartments may help avoiding desiccation, as large cells, with diminutive intercellular spaces may efficiently accumulate water (Kraus 2009). Accordingly, gall tissue compartments in *Lopesia* sp. galls may help tolerating the hydric stress of the Caatinga.

Cecidomyiidae galls commonly have hypertrophied parenchymatic cells, and the development of a mechanical layer around the nutritive tissue (Rohfritsch 1992). Lignin accumulation may be stimulated by biotic stresses, such as the attack of pathogens, or abiotic stresses, such as water deficit (Lee *et al.* 2007). The over accumulation of lignin on *Lopesia* sp. galls proved the deviation of secondary metabolites, commonly produced by the host plant toward neo-formed tissue layers, due to the biotic stress imposed by the larvae (Oliveira *et al.* 2017). Moreover, lignification can also confer protection against natural enemies on *Lopesia* sp. galls (Mani 1964, Stone & Schönrogge 2003).

As is true for most Cecidomyiidae galls, *Lopesia* sp. altered the innermost portions of the ground system toward its nutritive demands. The nutritive cells are consumed by the larvae, and quickly differentiate and divide continuously. The nutritive tissue has small cells when compared to the other gall tissues, similarly to the leaf galls induced by *Pisphondylia brasiliensis* Couri and Maia, 1992 (Cecidomyiidae) on *Guapira opposita* (Vell.) Reitz. (Nyctaginaceae) (Fleury *et al.* 2015) and by *Lopesia* sp. on *Lonchocarpus cultratus* (Vell.) Azevedo-Tozzi & H.C.Lima (Leguminosae-Papilionoideae) (Suzuki *et al.* 2015). In *Lopesia* sp. galls, nutritive cells accumulate lipids and proteins, with poor accumulation of defensive substances, as reported for other galls (Hartley 1998).

The cells of the outer and inner parenchyma of *Lopesia* sp. galls on *M. tenuiflora* get through a process of suberization after the emergence of the galling insect, which may protect the galling site from the invasion of pathogens (Isaias & Oliveira 2012). The suberization repeats a morphogenetical pattern present in other sites of the host plant, which is activated in gall final phase. Such expression of a plant potentiality in an uncommon site is not exclusive of Cecidomyiidae galls, as it has been described in galls induced by *Aceria lantanae* Cook (Acari) on *Lantana camara* L. (Moura *et al.* 2009) and by *Callophya duvauae* Scott (Psylloidea) on *Schinus polygamus* (Cav.) Cabrera (Dias *et al.* 2013).

The histochemical profile in gall tissue compartments - The activity of *Lopesia* sp. does not prevent or induce the neo-synthesis of any chemical compounds histochemically detected in the non-galled pinnula of *M. tenuiflora*. However, metabolites detection in specific gall sites can be a result of the manipulation of the chemical composition of the host plant by the galling insect (Nyman & Julkunen-Tiito 2000).

Lipids accumulate both in non-galled tissues and galls outer and inner tissue compartment, which indicates the maintenance of a host plant capability. Lipids are high energetic molecules (Buchanan *et al*. 2000), which can be converted into structural and metabolic components, as proposed for *Lonchocarpus muehlbergianus* Hassl. (Leguminosae-Papilionoideae) due to the activity of *Euphalerus ostreoides* Crawf. (Oliveira *et al.* 2006). Such accumulation is important for the maintenance and development of *Lopesia* sp. galls.

Proteins and reducing sugars accumulate in the parenchymatic layers of the outer and inner compartment in mature galls, which indicates that these metabolites are not only related to galling nutrition. Both proteins and reducing sugars can be translocated from the outer toward the inner tissue compartment, conferring resources for the development of the structure (Schrönrogge *et al.* 2000, Raman 2007), and reallocation to the nutritive cells (Oliveira *et al*. 2010, 2011) of *Lopesia* sp. galls on *M. tenuiflora*.

The accumulation of polyphenols in gall cells is usual for some Cecidomyiidae galls (Formiga *et al.* 2009, Nyman & Julkunen-Tiitto 2000, Bedetti *et al.* 2014, 2017), and has been related to chemical defense, possibly by inhibiting oviposition and feeding of natural enemies (Oliveira *et al.* 2006, Moura *et al.* 2008). The enhancement of phenolics accumulation may also be a response to abiotic factors, such as insolation and low pluviosity (Formiga *et al.* 2009). As the stress of high insolation and low pluviosity is characteristic of the Caatinga environment, phenolics accumulation may be important for adjusting the microenvironment of the gall both for the host plant and for *Lopesia* sp. galls. The accumulation of polyphenols, terpenes and alkaloids, restricted to the outer tissue compartment in galls, may enhance the potential chemical protection (Nyman & Julkunen-Tiitto 2000; Amorim *et al*. 2017, Silva *et al*. 2017) in the galls of *Lopesia* sp. on *M. tenuiflora*.

## Conclusion

The reorganization and compartmentalization of the tissues in gall developmental site resemble mostly those already described for Cecidomyiidae galls, but the parenchyma layers of the inner compartment between the mechanical zone and the nutritive tissue is a peculiarity of *Lopesia* sp. galls on *M. tenuiflora*. The histochemical profile of the gall is similar to that of its

host plant, and the secondary metabolites are restricted to the outer compartment. Therefore, we assume that the polyphenol accumulation may help gall establishment, by avoiding the excess of light irradiation and low pluviosity. Even though the Caatinga environment does not seem to impose a special constraint for *Lopesia* sp. gall development, the thick periclinal cell walls and the homogeneous parenchyma may contribute to the control of humidity and light radiation, protecting the galling insect against the dry environment.

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