Redifferentiation of leaflet tissues during midrib gall development in *Copaifera langsdorffii* (Fabaceae)

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Abstract

The tree *Copaifera langsdorffii* is a superhost for galling herbivores. This plant species has great morphogenetic potential, and responds differently to the stimuli of more than 20 gall-inducing insects. Among these, an undescribed species of Cecidomyiidae induces a midrib gall in which a radial cecidogenetic field is generated and the leaflet tissues redifferentiate. Our objectives were to assess the amplitude of this cecidogenetic field, in which the leaflet tissues were influenced by the feeding action of the cecidomyiid; how the final gall shape was generated; and if tissue redifferentiation conferred any adaptive value on the galling herbivore. Leaflet morphogenesis followed the pattern described in the literature for simple leaves, resulting in a mesophytic arrangement. Tissue redifferentiation due to gall formation revealed that in a midrib gall, abaxial epidermal cells divided to enlarge the gall; spongy parenchyma cells originated the storage tissue, secretory structures, and vascular bundles; palisade parenchyma cells became homogeneous; and adaxial epidermis originated the nutritive tissue. Cell elongation, a necessary step towards cell redifferentiation, is triggered by an increase in water transport to the gall site and vacuole pressure due to neoformed xylem bundles. The generation of the final shape of the midrib gall involved repetitive histological steps in response to the amplitude of the cecidogenetic field. The largest impact of the cecidomyiid feeding action occurred in gall tissues redifferentiated from protoderm and adaxial ground meristem, which provided advantages to the gall maker of the *C. langsdorffii* midrib gall in terms of nutritional value, microenvironment, and protection against natural enemies.

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1. Introduction

Insect galls are products of intimate relationships between the inducers and their host plants; indeed, the capacity for gall formation is one of the most efficient strategies of plant usage by herbivores (Roskam, 1992). Galls originate from abnormal cell development in host–plant organs regulated by the action of a gall-inducing agent, which obtains, directly or indirectly, shelter, food, and protection against natural enemies and abiotic factors (Price et al., 1986; Rohfritsch and Anthony, 1992). This development is closely dependent on chemical stimuli and the continuous feeding activity of the gall maker (Mani, 1964; Meyer and Maresquelle, 1983; Bronner, 1992).

Gall makers alter the normal morphogenetic patterns of their host plant (Mani, 1964; Meyer and Maresquelle, 1983; Rohfritsch, 1992), and are responsible for the formation, development, and maintenance of its gall. Therefore, each specific agent defines its gall morphotype (Mani, 1964, 1992; Dreger-Laffret and Short-house, 1992; Nyman, 2000; Cook and Gullan, 2008).

Gall ontogenesis comprises a complex series of interactions between plant cells and the gall-inducing organisms (Rohfritsch, 1992). This complexity involves several cytogenetic and morphogenetic events that are frequently simultaneous or combined, which lead to gall-formation processes (Mani, 1964). A superhost for galling herbivores such as *Copaifera langsdorffii* constitutes an ideal model system to study gall developmental patterns. The alterations of its tissues during the establishment of each gall inducer and the formation of each morphotype may shed light on the intimate relationships between the organisms involved. Also, it must be inferred that...
the generation of one or more cecidogenetic fields redirects auxin flux to the gall site, and as a consequence, the fates of the host–plant cells are altered. In this context, following the differentiation and redifferentiation of these cells may reveal the origins and pathways of the peculiar shapes and variability of C. langs dorffii gall morphotypes. This is particularly relevant to the discussion of hypotheses regarding nutritional, microenviron mental, and enemy effects (Stone and Schönrogge, 2003), as well as a way of demonstrating the adaptation to gall-inducing agents, based on a functional design.

C. langs dorffii shows great morphogenetic potential to respond differently to several gall-inducing stimuli (Oliveira et al., 2008). Among its gall morphotypes, one of the most frequent over the course of a year is a leaf-midrib swelling induced by an undescribed species of Diptera, family Cecidomyiidae, in either young or mature leaflets (Oliveira and Isaias, 2009). Our objective was to describe the morphogenetic steps from the emergence of leaf primordia until the maturation of the leaflets, passing through gall induction in mature leaflets, and ending with gall senescence, in order to answer the following questions: (i) what is the pattern of non-galled leaflet lamina differentiation?, (ii) What is the direction of the cecidogenetic field and its role in the determination of the final gall shape?, (iii) Which leaflet tissues are more impacted by the Cecidomyiidae feeding action?, and (iv) Do alterations in leaflet tissue have any adaptive value for the galling herbivore?

2. Material and methods

Leaves and galls (Fig. 1a, b) in successive developmental stages were collected from July 2006 to October 2007, from 15 specimens of a natural population of C. langs dorffii Desf. (Fabaceae) trees, located in an area of canga at Retiro das Pedras (20°05′35″S, 43°59′01″W), Serra da Calçada, Municipality of Brumadinho, Minas Gerais, Brazil. Canga is a subdivision of the “Cerrado”, the Brazilian savanna, with a predominance of herbaceous vegetation growing in very nutrient-poor, rocky soils (Silva et al., 1996). During this one-year collection period, two periods of leaf sprouting and two cycles of leaf maturation were followed. Leaf development was analyzed from the emergence of leaf primordia (1 mm long) until final expansion (3–4 cm long), when the first marked leaf reaches the seventh node (45–60 days). Gall development was analyzed from the induction phase, which occurred in leaflets from the fifth to the seventh node, until gall senescence (~90 days).

For ontogenetic studies, entire shoots (n = 15) were collected. Entire leaves from the first and second nodes and mid-leaflets from the third to the seventh node were detached and numbered. Galls in four developmental stages were sorted by size (n ≥ 10, for each developmental stage) and collected. The induction phase was considered as tissue swelling (≤0.5 mm), the growth and developmental phases as the initiation of abaxial leaflet folding (≤3 mm), the maturation phase as the completion of gall enlargement (4 to 5 mm), and the senescent phase (≥5 mm) as the absence of the gall-inducing insect in the larval chamber. The samples were fixed in FAA (Johansen, 1940), dehydrated in an n-butyl series, and embedded in Paraplast® (Kraus and Arduin, 1997). Entire leaves, leaflets, and galls were serially cross-sectioned (8–12 μm), and stained with astra blue and safranin (8:2, v/v) (Bukatsch, 1972, modified to 0.5%). Fresh leaflet fragments (1 cm²) were heated to 60 °C in Jeffrey’s mixture (Johansen, 1940) until epidermis detachment. Epidermal peels were stained in 0.5% safranin (Johansen, 1940).

Cell areas were measured in five cross-sections per leaf or leaflet lamina, and per gall in each developmental stage, at mid-organ position. The measurements of the non-galled (n = 40) and galled tissue areas (n = 40) were made from drawings done with the aid of a drawing tube mounted on an Olympus light microscope, and analyzed with the Quantikov Image Analyzer software (Pinto, 1996).

Numerical data were submitted to an ANOVA, followed by Tukey’s test (p < 0.05%) using JUMP 5.0 software (Sas Institute Inc. 2002, the statistical discovery software).

3. Results

3.1. General features

Leaves of C. langs dorffii are compound and paripinnate, with lateral leaflets evaginating from a central axis. At maturity, the leaflets are glabrous and elliptical, with entire margins, an acute apex, and an obtuse base (Fig. 1a) where the nectaries are located. Gall induction promotes leaf lamina arching (Fig. 1a, b), forming a pocket with a central elongated larval chamber (Fig. 1c, d) permanently opened to the adaxial surface (Fig. 1e). The gall maturation phase is characterized by an increase in size (Fig. 1f), and during the senescent phase, the cells adjacent to the gall opening and the external epidermis lignify.

3.2. Leaf morphogenesis

At the first node, leaf primordia are protected by cataphylls and numerous trichomes. The cataphylls have simple epidermis, 2–3 parenchymatous cell layers with secretory structures interspersed, and poorly developed vascular bundles. In cross-section, leaf primordia have isodiametric protodermal cells, and a 4-layered ground meristem; cells of both tissues have dense cytoplasm and conspicuous nuclei and nucleoli (Fig. 2a). The midrib region protrudes from the abaxial surface, and the procambial cells occupy a central position, and have dense cytoplasm (Fig. 2a). At the second node, the leaflet lamina is more expanded by the increment in submarginal cell divisions, the vascular tissues of the midrib develop, and secretory structures differentiate (Fig. 2b). The cataphylls begin to degenerate and the leaves emerge. Secretory structures and colleters are observed at the base of the petiolules. The ground meristem is 4-layered, with 1 adaxial, 2 middle, and 1 abaxial layer. Procambial strands may be distinguished in the middle layers as tiny cells within the wider ground meristem cells (Fig. 2e). Also, the initial cells of the secretory cavity epithelium elongate, divide, and separate in a schizogenous process (Fig. 2f–g).

At the third and fourth nodes, the epidermal cells enlarge approximately 18%. The ground meristem adaxial layer cells divide anticlinally and elongate, forming the adaxial layer of the palisade parenchyma (Fig. 2g). A second palisade layer...
originates from the adjacent middle layer. In the mesophyll central portion, secretory structures differentiate. Second-order veins begin to differentiate in the middle layers of the ground meristem (Fig. 2g). At the fifth node, epidermal cells are almost isodiametric in cross-section, with inconspicuous nuclei. Both layers of the palisade parenchyma cells continue to elongate. The midrib vein is almost completely differentiated with collateral arrangement, and minor veins are still undifferentiated. At the sixth node, the mesophyll is completely differentiated, and the cells adjacent to the phloem in the midrib vein begin to lignify (Fig. 2h). In the abaxial ground meristem and in its adjacent middle layer, intercellular spaces develop, forming the spongy parenchyma (Fig. 2h). Nectaries are well developed, and probably enter the secretory phase (Fig. 2i). At the seventh node, leaf maturation is complete. In mature leaflets, the lamina is dorsiventral, amphistomatic, with non-glandular trichomes restricted to the leaflet margin. The palisade and spongy parenchyma are 2-layered. When leaflets develop in full sunlight, the mesophyll is homogeneous. Secretory structures and vascular bundles are completely differentiated. In the midrib region, the vascular tissue is surrounded by 1–2 layers of lignified cells (Fig. 2j).

### 3.3. Gall morphogenesis

The induction phase begins with larvae hatching in the midrib region next to the leaflet apex (Fig. 3a). The growth and developmental phases are characterized by cell divisions and elongation causing lamina hypertrophy and arching (Fig. 3b). From maturation through senescent phase, tissue hyperplasia

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**Fig. 1.** General features of leaves and midrib galls of *Copaefera langsdorffii*. (a) Non-galled (left) and galled leaves (right); (b) detail of leaflets with midrib galls; (c–f) diagrams of midrib galls in cross-section (arrows). (c) Induction phase causes leaf lamina arching; (d–e) growth and developmental phases. Tissue development forms a pocket towards the abaxial leaflet surface; (f) maturation phase. Larval chamber is partially obliterated due to parenchyma hyperplasia. lc = larval chamber, lt = lignified tissue, p = gall parenchyma, ss = secretory structures, vs = vascular system.
and cell lignification occur. Also, the number of secretory structures increases (Fig. 3c).

At the induction phase, epidermal, palisade, and spongy parenchyma cells hypertrophy (Fig. 3d). The neoformation of secretory structures from the palisade cells originated from the adaxial middle layer is evidenced in a schizogenous process similar to that observed in the leaflet lamina (Fig. 3d, e). Vascular bundles in the midrib region do not alter.

The growth and developmental phases (Fig. 3b) are characterized by an increase in cell division. Epidermal cells
hypertrophy and divide anticlinally, and the cuticle thickens. The ground system is multi-layered, due to cell divisions in random planes, and homogeneous with hypertrophied cells. A two-layered nutritive tissue originates from the adaxial epidermis and adaxial layer of the palisade parenchyma. The second layer of palisade and the two layers of spongy parenchyma form a storage tissue. Neoformation of secretory structures is observed over the entire gall mesophyll, probably

Fig. 3. Morphogenesis of *Copaifera langsdorffii* midrib gall. Cross-sections. (a) Induction phase; larvae at the adaxial surface of the midrib region. (b) Growth and developmental phases; larvae inside the larval chamber formed by cell hypertrophy and lamina arching. (c) Senescent phase; the insect has left the gall chamber and the gall is wide open. (d–e) Anticlinal elongation of adaxial epidermal cells, hypertrophy of palisade parenchyma, and neoformation of secretory structures (dotted circle). (f) Maturation phase, detail of gall tissues with lignification of the subepidermal layer (white arrow) and asymmetrical thickening constriction of anticlinal cell walls at abaxial epidermis (black arrows), cells of storage tissue are vacuolated. (g) Detail of gall tissue, nutritive tissue with small cells surrounding the larval chamber. (h) Gradient of cell growth and elongation in gall tissues, the size and shape of the circles inside the squares indicate the relationship between cell expansion and the direction of elongation; the thickness of the arrows indicates the main axis of cell elongation. la = larva, lc = larval chamber, NT = nutritive tissue, ST = storage tissue.
due to cell redifferentiation within the storage tissue. The vascular bundles in the midrib vein are not altered, but neoformed vascular bundles are interspersed with the gall parenchyma. The gall chamber is already formed, and shelters 3–6 gall-inducing larvae.

In the maturation phase, the gall structure is larger due to cell hypertrophy and tissue hyperplasia. The abaxial epidermal cells have anticlinal walls with poorly lignified asymmetrical thickenings (Fig. 3f). The two cell layers adjacent to the abaxial epidermis (Fig. 3f) and the cells close to the ostiole lignify, forming two mechanical zones. The cells of the storage tissue are hypertrophied and more vacuolated than the ones adjacent to the adaxial epidermis (Fig. 3f) which surrounds the larval chamber, a 3–4-layered nutritive tissue differentiates by periclinal divisions of the outer palisade parenchyma (Fig. 3g). At the gall site, the midrib region remains unaltered.

In the senescent phase, gall tissues reach the maximum point of the reactive process (Fig. 3c). The ostiole is open wide and bounded by lignified cells, the gall-inducing larvae have left the gall chamber, and the nutritive tissue remains unaltered. The gall has a distinct zonation of tissues: a 3–4-layered nutritive tissue around the larval chamber, a 13–15-layered storage tissue, and an external 2–3-layered mechanical tissue. The gall structure is covered externally by simple epidermis.

The patterns of cell growth and elongation are altered during gall development and are responsible for the formation of gall size and shape. The epidermal cells elongate anticlinally, whereas the cells of the storage tissue that were originated from the spongy parenchyma hypertrophy and expand in all directions. The 3–4 layers of nutritive tissue cells are smaller and homogeneous, due to cell divisions and little expansion (Fig. 3h).

The midrib gall induced by the Cecidomyiidae is a product of alterations in the leaflet morphogenetic pattern; nevertheless, the zonation observed in non-galled tissue is maintained during gall differentiation, with a clear relationship between the origin and fate of the cells. Also, due to this relationship, specific tissue functions are established in the new morphological design (Fig. 4).

3.4. Histometric analyses

Histometric analyses of leaflets revealed that either the abaxial or adaxial epidermis have the same rhythm of cell growth and anticlinal divisions. The mean cell areas of the abaxial epidermis, in cross-section, did not differ significantly from those of the adaxial epidermis during leaflet development (Fig. 5a, d). However, adaxial epidermis near the larvae originated hypertrophied nutritive cells, which attained their maximum size (336.81 µm²) during the growth and developmental phases. In the maturation and senescent phases, the cell areas of nutritive tissue decreased, 262.86 and 251.76 µm², respectively, because of cell division (Fig. 5a). From non-galled leaflet maturation to gall senescence, the mean cell area of the abaxial epidermis did not alter significantly, 121.43 µm² at the sixth node of non-galled tissue, and 124.2 µm² for the senescent gall (Fig. 5d).

The elongation of palisade parenchyma cells is responsible for the increase in thickness during non-galled leaflet differentiation, and at the fifth node, when gall induction takes place, these cells have totally expanded (260.82 µm²) (Fig. 5b). The subepidermal layer of the nutritive tissue originates from hypertrophy and hyperplasia of palisade parenchyma, and reached its largest areas during the growth and developmental phases (336.81 µm²), with a decrease in size in the maturation and senescent phases (262.86 and 251.76 µm², respectively) (Fig. 5a).

The storage tissue originates from the spongy parenchyma cells, which divide both anticlinally and periclinally. Cell hypertrophy contributes to gall enlargement, reaching the largest areas in the senescent phase (2098.32 µm²) (Fig. 5c).

![Fig. 4. Diagrammatic representation of tissue differentiation and redifferentiation from leaflet morphogenesis to cecidomyiid gall formation in *Copaifera langsdorffii*, showing the origins of gall tissues.](image)
4. Discussion

The midrib gall of *C. langsdorffii* develops along the midrib vein, because of the feeding action of a species of Cecidomyiidae. In general, cecidomyiid galls exhibit a high degree of tissue specialization (Mani, 1964; Dreger-Jauffret and Shorthouse, 1992; Harris et al., 2003), and are capable of significantly altering events of their host organ morphogenesis.

The events of *C. langsdorffii* leaflet morphogenesis followed the patterns described in the literature for simple leaves (Foster, 1936; Cutter, 1986; Fahn, 1990; Villani and Demason, 1999), resulting in a mesophytic structure, even though the *C. langsdorffii* individuals were growing in ferruginous canga. It seems that the leaves of this species do not respond to abiotic stresses as they do to galling herbivore activity. Midrib gall formation results in cell types with specific functions, after changes in cell identities, a process referred to in this article as redifferentiation, sensu Lev-Yadun (2003), which results in galls with constancy in form and cell layers. This structure also fits the modern definition by Raman (2007) of galls induced by insects, because they are symmetrical structures.

The dermal system of non-galled leaflets is one-layered from the first to the seventh node, where the leaflet is completely differentiated. The adaxial epidermis originates exclusively from the adaxial protoderm, and the abaxial epidermis from the abaxial protoderm, a common pattern (Foster, 1936; Cutter, 1986; Fahn, 1990). In midrib galls, the adaxial epidermis of leaflets is continuous with the covering cells of the larval chamber, forming the outermost layer of nutritive tissue, which originates primarily from cell hypertrophy and anticlinal divisions. On the opposite side, the gall dermal system continuous with the abaxial leaflet epidermis has thick cell walls covered by thick cuticle. These anatomical features commonly stabilize temperature and humidity in leaves (Fahn, 1990; Woodman and Fernandes, 1991; Gutschick, 1999; Press, 1999), and therefore should provide a favorable microenvironment for the gall-maker establishment and

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**Fig. 5.** Histometric analyses of leaflet and gall development. (a) Cellular area of leaflet adaxial epidermis and gall nutritive tissue. (b) Cellular area of leaflet palisade parenchyma and gall nutritive tissue. (c) Cellular area of leaflet spongy parenchyma and gall storage tissue. (d) Cellular area of leaflet abaxial epidermis and gall external epidermis. Same letters indicate that the values did not differ significantly from each other at the 5% probability level, by Tukey’s test.
gall development as stated previously by Pike et al. (2002) and Álvarez et al. (2009). In other gall systems, these structural features in galls have commonly been related to the microenvironment hypothesis (Price et al., 1986, 1987; Stone and Schönrogge, 2003), which has been frequently invoked but nevertheless rarely tested experimentally (Miller III et al., 2009).

The ground and vascular systems of *C. langsdorffii* originate from the four layers of ground meristem, whose stratified arrangement allows the origins of the palisade and spongy parenchymas, secretary structures, and procambial strands to be distinguished. Due to the feeding activity of the gall maker, the formerly differentiated leaflet tissues of *C. langsdorffii* reassemble the capacity for cell division, generating a significant impact, especially in the ground-system cells. The palisade parenchyma cells hypertrophy during the gall induction phase, and reassemble their meristematic capacity during the growth and developmental, and the maturation phases. At this time, the cells of its adaxial layer redifferentiate into the second layer of nutritive tissue. The spongy parenchyma also hypertrophies and divides either anticlinally or periclinaly, forming a storage tissue outwards from the nutritive layers.

As is true for plant organs in general (Steeves and Sussex, 1989; Obroucheva, 2008), the direction of cell elongation plays a key role in the determination of gall morphology, which was demonstrated by distinct sites of cell hypertrophy and tissue hyperplasia during the development of a few galls in the Neotropics (Souza et al., 2000; Arduin et al., 2005; Oliveira et al., 2006; Moura et al., 2008), as well as in the temperate zone (Meyer and Maresquelle, 1983; Bronner, 1992; Rohfritsch, 1992; Álvarez et al., 2009). In the midrib gall of *C. langsdorffii*, the change in shape from elongated palisade parenchyma to spherical cells in storage tissue is an important step for gall development. This alteration is a common pattern in gall development (Rohfritsch, 1992), and was previously described in galls induced by *Aceria lantanae* in *Lantana camara* (Moura et al., 2009) in the Neotropics. This alteration in cell shape may be an indication that cytoskeletal microtubules are regulating cell hypertrophy, but redifferentiation and tissue hyperplasia coincide in time during gall formation. Nevertheless, a definite cecidogenetic field radial to the position of the cecidomyiid larvae deep in the chamber is generated.

Prior to the definition of the final gall morphology, parenchyma cells store water and hypertrophy. During the growth and developmental phases, nutritive and phenolic substances also accumulate. In galls, the activation or inhibition of growth is probably influenced by water accumulation, and an increase in the biosynthesis of phenols, which inhibit the action of IAA oxidases, and so expand the action of the auxins responsible for cell hypertrophy, as discussed by Hori (1992) for some hemipteran gall systems.

During the maturation phase of the *C. langsdorffii* midrib gall, parenchyma cells of the outermost layers of the storage tissue gradually redifferentiate into seleroids. Cell lignification with the formation of a mechanical zone is a common process in galls induced by both Cecidomyiidae and Cynipidae (Rohfritsch, 1992) and has already been reported for other gall systems (Kraus et al., 1996; Isaias et al., 2000; Kraus et al., 2002; Gonçalves et al., 2005; Oliveira et al., 2006; Álvarez et al., 2009). This mechanical zone confers protection against natural enemies (Vance et al., 1980; Rohfritsch, 1992; Stone and Schönrogge, 2003; Hanley et al., 2007), and is related to hormone activity, particularly decreases in auxins (Dorchim and Aloni, 2002), which should coincide with the end of gall maturation.

Secretary structures, differentiated from the ground meristem in *C. langsdorffii*, are globular and distributed within mesophyll cells. In galls, some cells from the storage tissue redifferentiate into new initial cells of secretary structures. These structures are also of schizogogenous origin and vary in shape due to the cecidogenetic field, which was previously recorded by Álvarez et al. (2009) in galls induced by *Forda formicaria* in *Pistacia terebinthus*, and was supposed to be a striking feature in gall development. Some other secretary structures could be observed in *C. langsdorffii*, extrafloral nectaries (EFNs), and colleters. EFNs can be observed only in young leaflets of *C. langsdorffii*, and the colleters occur at the base of the petiole. Even though there are no ultrastructural and histochemical studies to confirm the secretary nature of these structures in *C. langsdorffii*, the colleters may be involved in the process of topographical or chemical signaling for gall ing herbivores. The set of secretary structures of *C. langsdorffii* can take part in host organ location by the gall maker, and should therefore influence its establishment, but studies on this topic are few.

In *C. langsdorffii*, procambial strands differentiate in the middle layers of the ground meristem, similarly to *Nicotiana tabacum*, *Pelargonium zonale*, *Alternanthera philoxeroides*, *Erythroxylum rural*, *E. nanum* and *E. tortuosum* (Foster, 1936; Cutter, 1986; Fahn, 1990). First, the vascular system differentiates in the midrib from the second node on. The differentiation of smaller veins begins, and will be completed only at the seventh node. In midrib galls, new vascular bundles redifferentiate from storage tissue cells, and are connected to the pre-existing veins, which according to McCrea et al. (1985), Bronner (1992), Rohfritsch (1992), and Larson and Whitham (1997) increase nutritional resources for the maintenance of the gall maker and formation of the nutritive tissue.

Cell lignification, just below the gall abaxial epidermis and at the ostiole, characterizes the senescent phase in midrib galls, and indicates that by this time the gall growth is complete. In senescent galls, neither larvae nor pupae are observed, possibly because the larvae leave the gall to pupate in the soil, a common behavior in Cécidomyiidae (Gagné, 1994).

As a natural microlaboratory for developmental studies, *C. langsdorffii* has been the object of different analyses...
(Fernandes and Price, 1992; Almeida et al., 2006; Oliveira et al., 2008; Oliveira and Isaías, 2009). The midrib galls represent the first object of ontogenetic study in this superhost model system, and reveal crucial developmental steps in gall systems. Leaflet folding occurs because the ceccidogenetic field generates more conspicuous cell responses at the opposite site of gall induction, corroborating previous observations that next to the feeding site, tissues are less reactive (Weis et al., 1988; Rohfritsch, 1992). Even though tissue zonation is maintained until maturation, the fates of all leaflet tissues are altered during gall development. Cells nearest the larval chamber differentiate into a nutritive tissue, where the orientation of cell division is similar to that of the original protodermal layer. This cell layer maintains its physiological youth, being capable of entering continuous mitotic cycles, which are crucial for nutritive tissue metabolism. Lignification in peripheral tissue layers represents the end of cell cycles. Storage cells hypertrophy, and tissue hyperplasia thickens the gall wall. These two morphological strategies provide protection against natural enemies, which according to Stone and Schönhöfge (2003) represent the main selective pressure for gall diversity.

The histometric analyses related to the morphogenesis from healthy leaf until gall senescence in the C. langsdorffii-Cecidomyiidae system are important to comprehend histological patterns involved in the final gall morphotype. Current analysis supports the close relationship between storage tissue development and the morphology of the midrib gall. It also represents an effort to understand the morphogenetic responses leading to gall diversity in a superhost plant of galling herbivores. The comparison of this model system of gall development with other gall morphotypes in C. langsdorffii will aid in the comprehension of the anatomical responses that generate the diversity of gall morphotypes within the same host–plant potential.

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