



BUILDING TWO HOUSES ON A SINGLE HOST PLANT: GALLING INSECT SYNCHRONIZES ITS LIFE CYCLE WITH PLANT PHENOLOGY

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Abstract: Galls are specific interactions between specialist herbivores and their host plants. They are considered neofomed plant organs developed from cellular hypertrophy, tissue hyperplasia and cellular redifferentiation of the host tissues. Among several organisms capable of inducing galls, insects induce them with high morphological complexity. The induction and development of galls depend on the availability of responsive sites in the host plant that react to the chemical and/or mechanical stimuli of galling insects. The synchronization between the timing of availability of these responsive sites and the galling insect life cycle is essential for the establishment of the interaction. Galling insects are subject to several chemical, physiological and phenological changes in their host plant. Thus, changes in the host cycle may alter the insect's life cycle, distribution and abundance. This study focused on the morphological aspects and phenological relationship of the *Matayba guianensis* Aubl. (Sapindaceae) – *Bystracoccus mataybae* Hodgson, Isaias & Oliveira (Eriococcidae) system, carried out in a semi-deciduous forest located at the Estação Ecológica do Panga (EEP), Uberlândia, MG, Brazil. We monitored the host plant phenology monthly from April 2015 to April 2016, and galls were sampled throughout the year to determine the stage of development of the galling insect. During leaf flushing, galling insects were collected every two days. The second-instar nymph induced leaf galls during leaf sprouting (peaks in September and October). The growth, development and maturation of the gall and of the galling insect occur concomitant to leaf maturation (peaks from February to May). Before the first leaves fall (August), the first-instar nymph moves from the senescent leaflet gall to branches and induces a stem gall, where it remains during part of the dry season until the next leaf flush. The synchrony between the life cycle of the galling insect and the host plant phenology maintains the univoltine cycle.

Keywords: insect galls; insect–plant interaction; leaf galls; phenological synchrony; stem galls.

INTRODUCTION

The capacity to induce galls is an efficient herbivory strategy (Roskam 1992), and these structures are the product of one of the most intimate relationships between specialist herbivores and their host plants (Stone & Schönrogge 2003). Galls

develop from abnormal growth on host plant species triggered by inducing organisms that look for shelter, food and protection against their natural enemies (Price *et al.* 1986, Rohfritsch & Anthony 1992). Gall development depends on continuous chemical stimuli originating from the feeding activity of the galling herbivore (Bartlett &

Connor 2014), which acts as a biotic modulator of plant morphogenesis (Mani 1964, Meyer & Maresquelle 1983, Rohfritsch 1992, Oliveira & Isaias 2010, Oliveira *et al.* 2016). Galling herbivores, such as insects, cause alterations that may greatly influence the host plant life cycle and produce an overlap of reactions that establish the dynamic of the interaction (Price & Hunter 2005). The galling organisms have dependent life cycles, either partially or totally, on host plant tissues.

This very specific interaction between insects and plants can be affected by climatic conditions which may influence the host plant phenology (Mani 1964, Floate *et al.* 1996, Yukawa 2000, Oliveira *et al.* 2013). Seasonal changes on environmental conditions modulate plant features, such as morphology, physiology, chemistry and phenology (Floate *et al.* 1996), which may determine the establishment and life cycle of the galling insect (Yukawa 2000, Castro *et al.* 2012, Magalhães *et al.* 2015 Oliveira *et al.* 2016). In the Neotropical Region, the most important factor that determines the vegetative phenophases, especially the timing of leaf sprouting, is water availability (Wright & Van Schaik 1994, Myers *et al.* 1998, Lemos-Filho & Mendonça-Filho 2000, Oliveira & Gibbs 2000, Bencke & Morellato 2002, Pedroni *et al.* 2002, Huberty & Denno 2004, Lenza & Klink 2006, Oliveira *et al.* 2013). Once galling insect species search for reactive plant sites to induce their galls, they need to synchronize their capacity to induce galls with the availability of reactive tissues that occur rather during leaf sprouting (Weis *et al.* 1988). However, some galling insects can induce galls on mature leaves, as occurs in *Copaifera langsdorffii* (Fabales, Caesalpinoideae) (Oliveira & Isaias 2009).

Considering of Eriococcidae-induced galls, the sex of the galling insect and the length of its life cycle are determinant factors for gall structure and establishment (Magalhães *et al.* 2015). In the Eriococcidae (Gullan *et al.* 2005), male-induced galls can usually be distinguished from female-induced ones by their external shape (Gullan *et al.* 2005), as observed in the *Pseudotectococcus rollinae* (Hemiptera, Coccoidea, Eriococcidae) – *Rollinia laurifolia* (Magnoliales, Annonaceae) system (Gonçalves *et al.* 2005). However, in galls induced by *Eriogallococcus isaias* (Hemiptera, Coccoidea, Eriococcidae) on leaves of *Pseudo-*

bombax grandiflorum (Malvales, Malvaceae), sexual dimorphism was not detected in the external morphology (Magalhães *et al.* 2015). The length of the galling insect life cycle is also important for gall shape and depends on synchronization with host phenology.

In the Neotropical Eriococcidae, the male galling insects have five instars (four nymphs and one adult) and leave the mature gall only to fertilize a female. The females are neotenic, with three instars (two nymphal and one adult), are sessile and never leave the gall structure (Gullan *et al.* 2005). The first nymphs (crawlers) leave the gall and overwinter in the bark, where they molt to the second instar, and move to young leaves to begin a new gall cycle (Magalhães *et al.* 2015). For instance, in the gall induced by *P. rollinae* on *R. laurifolia*, the first instar moves to the stem, induces a new rudimentary gall morphotype, and overwinters until the next period of leaf sprouting (Gonçalves *et al.* 2009). Thus, it is clear that the study of gall morphology, host plant phenology, galling insect life cycle, and population dynamics are essential for understanding the natural history of the specific interactions between Eriococcidae-induced galls and Neotropical host plants.

Our study focused on the phenological relationship between *Matayba guianensis* (Sapindales, Sapindaceae) and *Bystracoccus mataybae* (Hemiptera, Coccoidea, Eriococcidae) to evaluate the synchrony between galling insects and host plants and describe different gall morphologies induced by the same galling insect. Based on Hodgson *et al.* (2013), we assumed here that the galling insect has a univoltine life cycle synchronized with vegetative plant phenology and that the crawlers overwinter on the stem in the dry season. We also assumed that when leaf sprouting begins, there will be responsive reactive tissues and the second-instar nymph will move to the leaves in order to induce a new gall cycle.

MATERIAL AND METHODS

Study area

This study was carried out in a semi-deciduous forest at the Estação Ecológica do Panga (EEP) in the south of the Uberlândia municipality (19°10'S and 48°23'W), State of Minas Gerais, Brazil. This

reserve area is over than 800 m high, covers an area of 410 ha and presents all the Cerrado main physiognomies (Schiavini & Araújo 1989). The region is characterized by an Aw tropical climate, according to Alvarez (2013), presenting a dry winter and a rainy summer; the average annual rainfall is 1,482 mm, while the average monthly temperature is 22.8°C. The rainfall distribution is seasonal, with a rainy season from October until April and a dry season from May until September (Rosa *et al.* 1991).

Morphological studies

Samples of non-galled leaflets (N = 5) and stems of *M. guianensis*, leaflet galls (N = 5) and stem galls (N = 5) induced by *B. mataybae* were fixed in 37% formaldehyde, acetic acid, 50% ethanol (FAA, 1:1:18), dehydrated in *n*-buthyl series, and embedded in Paraplast® (Kraus & Arduin 1997). The samples were sectioned in a rotary microtome (12 µm), and stained in astra blue-safranin, 8:2 (Bukatsch 1972, modified to 0.5%). Observations and photomicrographs were obtained in a Leica DM500® coupled with an ICC50® HD camera. For scanning electron microscopy, samples fixed in FAA were dehydrated in an ethanol series, dried in CO₂ critical point, glued to the stubs, covered with gold (35 nm) (O'Brien & McCully 1981), and observed in a scanning electron microscope (SEM) (Leo Evo 40XVP).

Host phenology and galling censuring

In order of appearance in the population, 30 individuals of *Matayba guianensis* were selected and the plant phenology monitored once a month, from April 2015 until April 2016. The vegetative phenophases, leaf sprouting, mature leaves, senescent leaves and leaf falling, were monitored according to the phenological method proposed by Fournier (1974). This method is semi-quantitative, ranking five categories of phenophase (0 = absence of phenophase; 1 = 1–25% of intensity of the phenophase; 2 = 26–50%; 3 = 51–75%; and 4 = 76–100%). The presence and absence of the different phenophases were evaluated for each plant and the activity index was measured. The activity index method estimates the proportion of individuals in the population with phenological synchrony at a specific time (Bencke & Morellato 2002). Leaf flushing was considered as

spanning from the beginning of the first leaves until their complete expansion (Pedroni *et al.* 2002), and leaf falling was estimated from the percentage of canopy covering.

During host plant phenophase transitions, galls were sampled and then evaluated in the laboratory. The identification of the different gall developmental stages was related to *B. mataybae*'s phase, as described in Hodgson *et al.* (2013). The first instar nymph overwinters in a stem gall, its body is oval, reddish, about 0.25 mm long and 0.18 mm wide with well-developed legs. The second instar nymph (on young galls) is similar to the adult female (growth and development gall), but smaller (length about 0.5 mm and width about 0.46 mm), and the body is round and red. The adult female (mature gall) is 5–6 mm wide, red, and its body is flask-shaped with a heavily sclerotized dorsal plate. According to Hodgson *et al.* (2013), *B. mataybae* is a parthenogenetic species, which possibly explains why no male individuals were found.

The galls were dissected monthly (N = 50 per month) and the insects were removed to determine their stage of development. During the period of leaf sprouting (July–October), other 36 individuals of *M. guianensis* were monitored, and the galls were sampled at two-day intervals (N = 2400). The galling insect developmental stage (first instar, second instar, adult female and adult female with eggs and/or crawlers) were determined according to Hodgson *et al.* (2013) and counted in the laboratory.

Data analysis

The overlap between mean angles of the different plant phenophases and gall development was tested by the Watson-Wheeler test using the Circular R package (Agostinelli & Lund 2013) in R software (R Core Team 2014). We performed this test because the circular variables of plant phenophases and gall developmental stages did not fulfil the uniformity and von Misses distribution premises (Zar 2010). Although all *p*-values demonstrated that the mean angles were different (*p* < 0.05), we had to observe the overlapping and not only their mean angles. If the mean intensity (I) and mean activity (A) of the two phenomena compared in each test differed, this was evidence that even if they had an overlap, they were considered events with a maximum peak of

different occurrence. To estimate the overlap between the insect developmental phases and the plant phenophase activities and intensities, we used the Pianka Index (1973).

The data of the insect population dynamics sampled during the leaf-flushing period (July–October 2015) with a two-day interval were organized in histograms of 11-day intervals. Each data was based on the mean percentage of the samples collected in the respective time interval.

RESULTS

Gall morphology in the Matayba guianensis–Bystracoccus mataybae system

The second instar nymph of *B. mataybae* induces galls on green and red leaflets of *M. guianensis* (Sapindaceae) (Figure 1a–c). The leaflet galls are light green at maturity, and glabrous and intralaminar with an opening toward the adaxial leaflet surface (Figure 1d). These leaflet galls are approximately 5–6 mm wide (Figure 1d) and shelter mature females with eggs (Figure 1d) the crawlers (first instar nymphs) hatch from the eggs (Figure 1e–f) and leave mature galls. The adult females die inside the gall (Figure 1e). The crawlers move to the host twigs, where they induce stem pit galls for overwintering during the dry season (Figure 1g–h). Each pit stem gall contains one individual and has approximately 2 mm in diameter (Figure 1h). Sometimes, when in high abundance, the pit galls can fuse.

The first alteration induced by *B. mataybae* on the young host leaflets of *M. guianensis* (Figure 2a) is the hyperplasia of the epidermis and the adjacent mesophyll parenchyma to develop a primary nymphal chamber (Figure 2b). The female galling insect induces a second nymphal chamber, below the first (Figure 2c) and thus, gets pregnant. At maturity, the adult females' bodies of *B. mataybae* with crawlers in the expanded abdomen occupy the whole space of the secondary chamber. At leaflets senescence, the crawlers leave the galls and move towards stem branches to induce a new gall structure in the same host plant (Figure 2d). Stem galls have increasing activity of the phellogen (Figure 2d), with over-differentiation of the phelloderm instead of the suber.

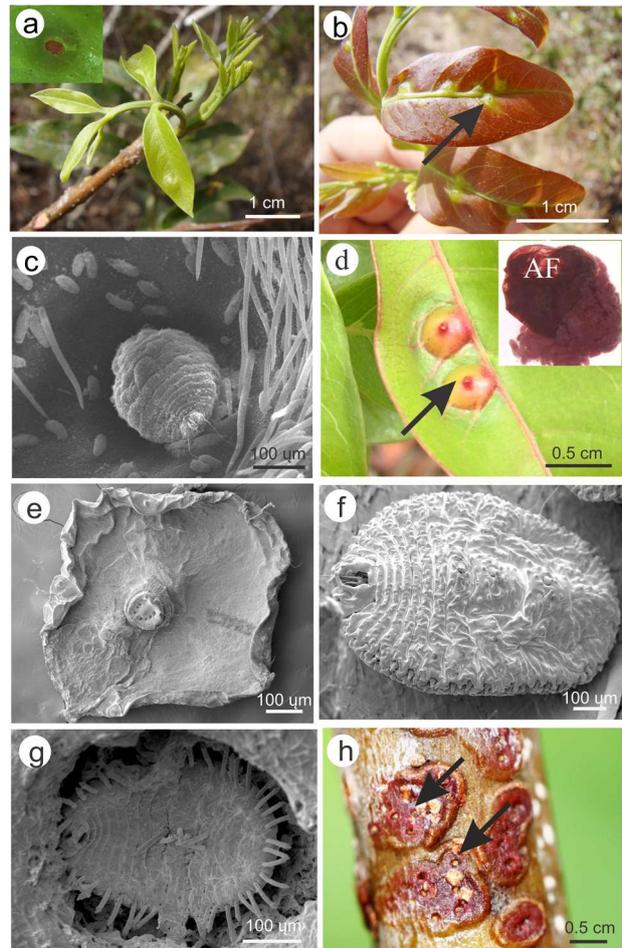


Figure 1. (a) Young leaflets of *Matayba guianensis* with galls induced by second instar nymphs of *Bystracoccus mataybae* (detail). (b) Young gall induced by a second instar nymph on the adaxial surface of red leaflets (arrow). (c) Second instar nymph on the young leaflet surface. (d) Mature leaf with galls protruding to the adaxial surface, gall opening (arrow) and detail of the adult female with many crawlers (first instar nymphs – (f) crawler detail). (e) Senescent adult female without crawlers and sclerotized dorsal plate. (f) First instar nymph (crawler). (g) First instar nymph in a stem gall. (h) Morphological aspects of a stem gall on *M. guianensis* (arrows indicate the insects inside the stem galls).

Host plant phenology

The host plant, *M. guianensis*, is semi-deciduous and has its vegetative phenophases spread along the year (1-year cycle). Leaf flushing occurs from June until December with maximum activity and intensity in September (A = 83.8%, I = 39.2%) and October (A = 82%, I = 39.5%), where the vector shows the mean concentration (Figure 3a). In December, all individuals of *M. guianensis* have fully expanded leaves (Figure 3b). Although mature leaves have the vector direction pointing

Table 1. Statistical values of Watson-Wheeler tests for mean angle overlap between vegetative phenophases and insect developmental phases. Abbreviated names indicate: Leaf flushing (LF), mature leaves (ML), senescent leaves (SL) and leaf absence (LA). All tests have freedom degree equal 2 and p-values ≤ 0.001 says there is no mean angle overlap.

Instars	LF	ML	Activity		Intensity			
			SL	LA	LF	ML	SL	LA
I1	101.50	90.07	38.39	62.35	85.19	107.80	40.95	30.24
I2	63.86	171.75	150.50	127.39	32.88	178.16	146.39	118.29
A1	188.14	359.99	525.12	339.15	96.24	316.39	356.61	194.44
A2	396.30	77.18	32.72	141.78	205.74	51.36	13.55	81.40

between March and April, the distribution of mature leaves occurs over the year. At the end of February, the number of leaves entering senescence increases ($A = 31.5\%$; $I = 22\%$) with a peak in June (Figure 3c) and thereafter until August, leaf falling increases monthly with the vector pointing between July and August (Figure 3d).

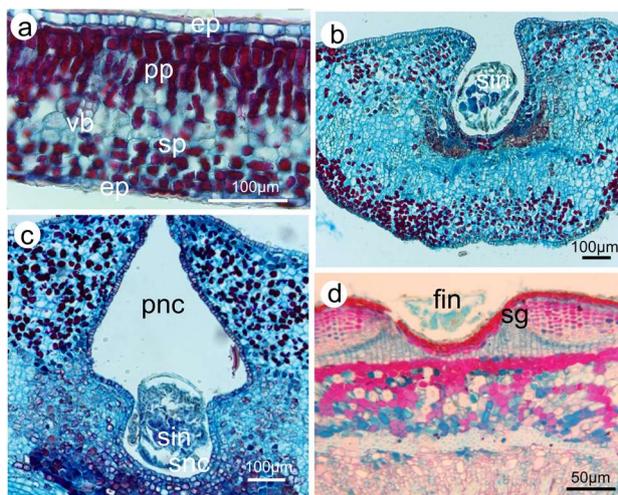


Figure 2. (a) Non-galled tissue of leaflet of *Matayba guianensis* showing unstratified epidermis, 2-3 layers of palisade parenchyma and 5-7 layers of spongy parenchyma. (b) Gall in growth and development phase; increase of ground and epidermal tissues around the insect. (c) Beginning of formation of the secondary nymphal chamber. (d) Anatomy of stem galls induced by the first instar. ep = epidermis; pp = palisade parenchyma; sp = spongy parenchyma; vb = vascular bundles; sin = second nymphal instar; pnc = primary nymphal chamber; snc = secondary nymphal chamber; fin = first nymphal instar; sg = stem gall

Galling insect life cycle

From July to August, there are peaks of leaf falling, and the host canopy decreases significantly (Figure 3c-d). During this period, there is an increase in the number of first instar nymphs

inducing galls on stems of *M. guianensis* (Figure 4). The first nymphs in stem galls are found in February, which corresponds to the end of the rainy season. These first instar nymphs move from senescent leaves toward stem branches before leaf falling. Leaf flushing begins at the end of June, with a peak in September, thus the first leaflet galls are induced by second instar nymphs and are visible.

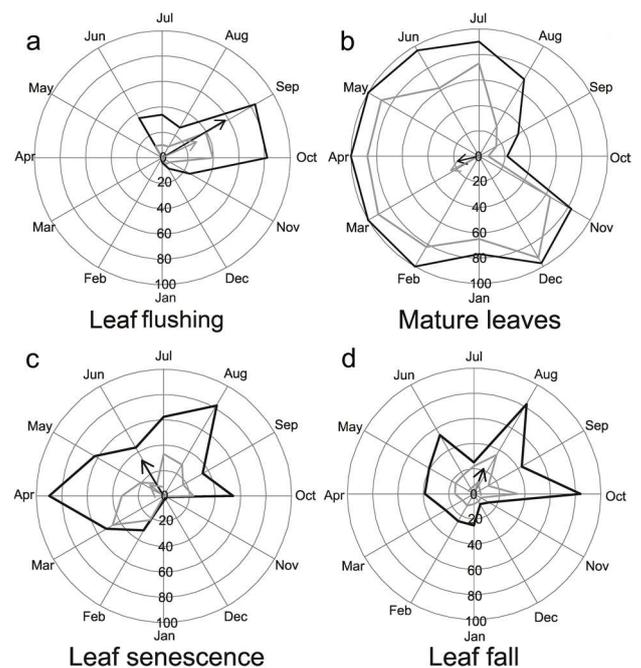


Figure 3. Representations of *Matayba guianensis* vegetative phenology, from April 2015 to April 2016, according to two methods of data analyses: index of activity (black) and percentage of intensity (grey). (a) Leaf flushing; (b) expanded leaves; (c) senescent leaves and (d) leaf falling.

Gall growth and development phases occur from August (3.2%) until November (96.3%). Also in November, the first galls in the maturation

phase are observed (3.7%), increasing significantly by February (71.8%) and reaching their maximum occurrence (85%) in April (Figure 4). The mean number of crawlers per pregnant female is 12.38 (range: 1 to 272).

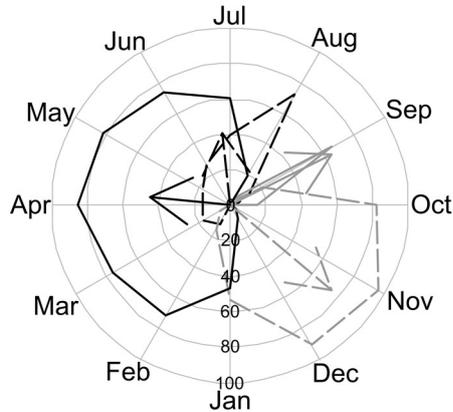


Figure 4. Representation of the different developmental phases of *Brystacoccus mataybae*, from April 2015 until April 2016. Each pattern corresponds to a phase: black-dashed = first instar nymphs (stem gall); grey = second instar nymphs (young leaflet gall); grey-dashed = adult females (growth and developmental gall); black = adult females with eggs and/or crawlers (mature gall).

The highest values of Pianka's index for the first instar nymph are reached during leaf senescence activity (0.83) and leaf fall intensity (0.82). Since the second instar nymph is responsible for inducing galls on young leaflets, the overlap for second instar nymphs and leaf sprouting activity (0.77) and intensity (0.81) are the highest. There is no difference between the adult female overlap for the different plant phenophase activities or intensities, but the presence of adult females with eggs and/or crawlers is higher in the presence of mature leaves (activity overlap = 0.89; intensity overlap = 0.89), decreasing with leaf age.

Although there are overlaps among phenophases and gall developmental phases, the mean angle are not superposed. Leaf induction begins and is extended during leaf flushing. Gall growth, development and maturation occur concomitant to mature leaves, and stem gall induction occurs in February, when the first leaves begin to fall.

Regarding the population dynamics of insects, from July 13th to 24th 2015, the occurrence of mature insects (60.7%) overlaps those of first instar nymphs at stem galls (39.3%). Between August 10th and 21st, the first signs of leaf gall

induction appear (6.3%) and the insects are already turning into female adults (0.7%). Thus, until the 12th week after the induction, growth and developmental phases are increasing, and the percentage of stem galls (first instar nymphs) are decreasing significantly. From the 13th to the 16th week, the induction of leaf galls (second instar nymphs) declines, and the growth and development phases are increasing largely (Figure 5). Adult insects with eggs and/or crawlers do not emerge until January 2016.

DISCUSSION

The Matayba guianensis–*Bystracoccus mataybae* system

The galling insect *B. mataybae* associated to *M. guianensis* has three instars: the first instar nymph (crawler), the second instar nymph and the adult female. The first instar nymph, presumably female, is oval shaped, reddish and approximately 0.25 mm long. It moves from senescent leaflet galls to twigs, where it induces pit galls. The second instar nymph induces leaflet galls with two nymphal chambers; its body is red, round and similar to the adult female, being flask-shaped with a heavily sclerotized dorsal plate. There are only females on this host plant-galling system and consequently, no sexual dimorphism could be detected (Hodgson *et al.* 2013), although dimorphism is very common for the Ericoccidae gall system (Gullan *et al.* 2005, Gonçalves *et al.* 2009, Magalhães *et al.* 2015). Although all p-values demonstrated that the mean angles were different, some overlaps may have occurred due to different periods of phenophases. Thus, especially because the vegetative phenophase is the first and the galling-insect can react to this modification, the mean concentration of each phenophase and gall developmental stages was not be superposed. In the process, *B. mataybae* has a univoltine life cycle, which is phenologically synchronized with its host plant phenophases, this galling insect build two different gall structures on the same host plant: stem galls, induced by the first instar nymphs; and leaflet galls, induced by the second instar nymphs.

Both the stem and the leaflet galls on *M. guianensis* are permanently open and start to differentiate by hyperplasia of the parenchyma around the body of *B. mataybae* nymphs. The

adult females do not leave their galls, and the ostiole of the leaflet galls facilitates the emergence of the crawlers. The stem galls induced by the first instar nymphs are structurally simple, i.e., an increase in phellogen activity around the insects' body results in a large phellogen production, but not of phellem. The new phellogen activity could protect the *B. mataybae* nymphs, probably hindering damage inflicted by high temperatures and low humidity, as proposed to the stem galls induced by first instar nymphs of *P. rollinae* on *R. laurifolia* (Gonçalves *et al.* 2009).

On young leaflets, the feeding activity of the second instar nymphs induces hyperplasia and cell hypertrophy around the gall induction site, a common feature for other host plant-galling herbivore systems in the Neotropics (Oliveira *et al.* 2006, Oliveira & Isaias 2010, Isaias *et al.* 2011, Magalhães *et al.* 2015, Oliveira *et al.* 2016). Anticlinal divisions of the epidermis and periclinal divisions of parenchyma cells adjacent to the induction site form the primary larval chamber, adaxially located, as illustrated by Gonçalves *et al.* (2005), Hodgson *et al.* (2013), and Magalhães *et al.* (2015). Regardless the gall inducer taxa, most galls have just one chamber (Moura *et al.* 2008, Oliveira & Isaias 2009, Isaias *et al.* 2011, Vecchi *et al.* 2013), while the galls of *B. mataybae* on the leaflets of *M. guianensis* have two chambers, herein nominated upper and lower nymphal chambers (Hodgson *et al.* 2013). This feature seems to be diagnostic for Eriococcidae galls and serves as the entrance of the males for copulation, and as accommodation for the crawlers until the time of their emergence (Gullan *et al.* 2005).

Host plant phenology and galling insect life cycle

The leaf sprouting peak between September and October in the population of *M. guianensis* opens a window of opportunity for gall induction and establishment. Weis *et al.* (1988) and Rohfritsch (1992) have proposed the important relation between the responsive host tissues and gall induction. Host plant tissues must react to insect feeding and chemical or mechanical stimuli for gall formation (Weis *et al.* 1988), a process more efficient in young tissues (Rohfritsch 1992). Such relationship has been confirmed for the *R. laurifolia* (Annonaceae)–*P. rollinae* (Eriococcidae) system (Gonçalves *et al.* 2009). In the *M.*

guianensis–*B. mataybae* system, the galling insect induces galls only in young leaflets, after leaf sprouting. The gall induction concomitant with the time of leaf sprouting have also been reported in the *Aspidosperma austral* (Gentianales, Apocynaceae) – *Pseudophacopteron* sp. (Hemiptera, Phacopteronidae) system (Campos *et al.* 2010) and in the *C. langsdorffii*–multiple Cecidomyiidae system (Oliveira *et al.* 2010). For species that strictly oviposit on young leaves, food resources are available for a limited period of the host plant life cycle (Oliveira *et al.* 2016). This is the case in the *M. guianensis* – *B. mataybae* system, whose synchrony between the gall inducer life cycle and plant phenology is vital and maintains the single annual generation. Despite this, *B. mataybae* triggered gall formation on the mature stem tissue. The induction of leaf galls on *M. guianensis* is similar to that of *P. rollinae* on *R. laurifolia* (Gonçalves *et al.* 2005) and *E. isaias* on *P. grandiflorum*, other Neotropical Eriococcidae in which the second instar nymphs induce leaf galls (Magalhães *et al.* 2015). These three galling

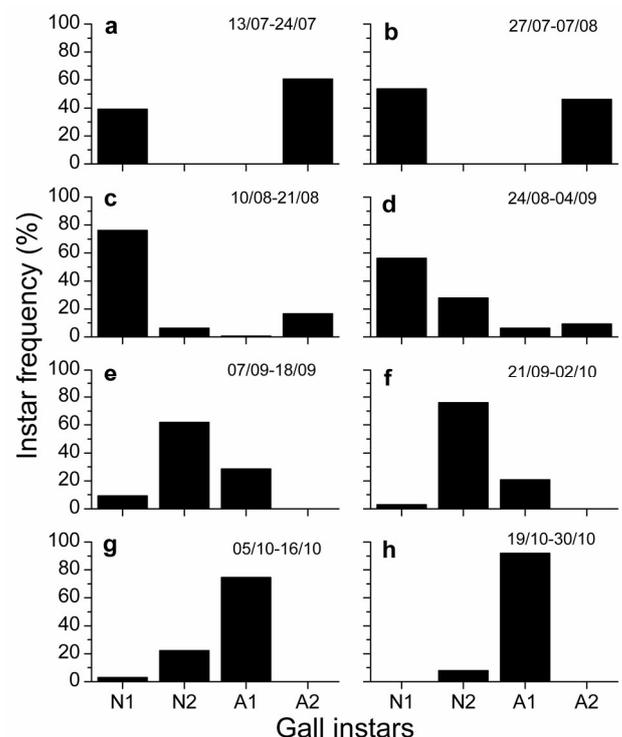


Figure 5. Galling insect developmental phases during the leaf-flushing period (July 13th to October 13th 2015). N1 = first instar nymphs; N2 = second instar nymphs; A1 = adult females; and A2 = adult females with eggs and/or crawlers.

herbivores are univoltine, and gall induction is synchronous with leaf sprouting (Gonçalves *et al.* 2005, Magalhães *et al.* 2015). The synchrony between the host plants and the galling insect is particularly important for determining the univoltine life cycle (Weis *et al.* 1988, Gonçalves *et al.* 2009, Campos *et al.* 2010), to provide resources and water for gall development, and for the insect's diet, corroborating the nutritional hypothesis (Weis *et al.* 1988, Bronner 1992). However, in species such as *Aspidosperma spruceanum*, gall induction occurs either in young or in mature leaves (Campos *et al.* 2010). This was previously recorded for the Neotropical galls induced by *Gynaikothrips ficorum* on *Ficus microcarpa* (Souza *et al.* 2000), and for unidentified species of galling herbivores on *Baccharis concina*, *B. dracunculifolia* (Arduin & Kraus 2001) and on *Copaifera langsdorfii* (Oliveira & Isaias 2009). This behavior generates high specificity to host plants in situations where responsive tissues are not available; the galling herbivores use alternative inducing sites in mature tissues, as observed by Oliveira & Isaias (2009). The galling insect, *B. mataybae*, induces leaflet galls only on young leaves of *M. guianensis*.

The galling insects need an efficient strategy to survive during periods when the host plant loses its leaves. When the crawlers of *B. mataybae* disperse, the host plant has no young leaflets and, consequently, no leaf galls can be induced. In this system, the stem galls as a second gall morphotype on the host plants, represent an efficient strategy for the survivorship of the first instar nymphs, which remain sheltered during the deciduous phase of the host plant. This strategy is peculiar to the two already reported Eriococcidae Neotropical systems. While rudimentary lenticel-like galls are induced by *P. rollinae* on the stems of *R. laurifolia* (Gonçalves *et al.* 2005), *E. isaias* crawlers remain in stem bark of *P. grandiflorum* during the dry season but do not induce stem galls (Magalhães *et al.* 2015), suggesting a similar strategy to survive during dry periods but less specialized. The first instar nymphs of *B. mataybae* induce stem galls during the dry season, different from other Neotropical galls induced by Eriococcidae. In the case of the present study, the pregnant female remains in the galls for a long time, probably as a consequence of the survival strategy of this insect,

which is to induce stem galls in the absence of leaves for overwintering, which is an efficient strategy to protect its progeny. That is, adult females with eggs and/or crawlers are abundant in the presence of mature leaves, and when these get older and begin to fall, the crawlers leave the gall structure and induce stem galls for overwintering.

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