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Host plant phenology and hypergaller-induced endogalls shape the gall life cycle and structure in Myrcia ovata Cambess

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Abstract

The availability of reactive sites for gall induction is tightly linked to specific phenophases of the host plant and may determine the interactions with associated fauna. Among this fauna, inquilines can induce endogalls within a pre-existing gall (primary gall), altering gall structure and developmental dynamics. Herein, we investigated the *Myrcia ovata* host phenology and the life cycles of primary galls induced by *Myrciamyia maricaensis* and endogalls induced by hypergaller inquiline. Also, we describe the anatomical profile of both gall and endogall. We observed that leaf sprouting in *M. ovata* occurred year-round with pronounced fluctuations, creating recurrent windows for gall induction on apical and lateral stem buds. Primary galls were smaller and thin walled and occurred year-round with temporal fluctuations, with mature stages peaking at the onset of the rainy season followed by a decline. Endogalls were more robust and persisted across a broader temporal range, extending through later periods of the rainy season. Endogall formation also involves tissue hyperplasia, increased vascularization and formation of a novel nutritive tissue on the parenchyma of primary galls. Several endogall can develop in the primary gall effectively hijacking host resources and compressing the primary gall chamber. Phenological decoupling between *M. ovata* and *M. maricaensis* may lead to multivoltine gall cycles, whereas inquiline hypergallers hijack the primary gall, restructuring gall tissues and modifying the developmental dynamics of this multitrophic system.

Introduction

Gall-inducing insects manipulate host plant tissues to form specialized structures known as galls, which provide protection and nutrition for their offspring (Oliveira et al. 2016; Ferreira et al. 2019; Harris and Pitzschke 2020). These interactions often exhibit a high degree of specificity between gall-inducing insects and their host plants (Price et al. 1987; Oliveira and Isaias 2025). Beyond protecting their inhabitants from environmental stress and natural enemies, galls also represent valuable model systems for exploring ecological dynamics in plant–insect interactions (Michell and Nyman 2021; Rezende et al. 2021; Gonçalves et al. 2025). Thus, understanding these interactions requires considering the environmental and phenological host contexts in which gall induction occurs, particularly seasonality and the availability of reactive sites for gall formation (Oliveira et al. 2013; Pfeffer et al. 2018; Costa et al. 2022; Marquesine et al. 2024; Nobrega et al. 2025).

The availability of meristematic tissues, which serve as reactive sites for gall induction, is tightly linked to specific phenophases of the host plant (Weis et al. 1988; Carneiro et al. 2013; Ferreira et al. 2022; Isaias et al. 2025). On these interactions gall-inducing insects need to synchronize their reproductive cycles with these phenophases of the host plant to optimize gall induction and larval development (Oliveira et al. 2013; Pfeffer et al. 2018; Costa et al. 2022; Nobrega et al. 2025). However, abiotic factors such as temperature, precipitation, and photoperiod can influence host plant phenology, potentially disrupting the synchrony between gall inducers and their hosts (Rezende et al. 2019) and triggering cascading effects on gall-inducing insect fitness and the dynamics of their associated trophic guilds (Raether 2021; Lombardero et al. 2022; Arriola et al. 2024). Nevertheless, biotic pressures also act in

concert with abiotic factors, shaping not only the timing of these interactions but also their outcomes in terms of gall structure and community composition (Rezende et al. 2021; Guiguet et al. 2023; Gonçalves et al. 2025).

The gall-inducing cycle throughout the year reflects an adaptive response not only to abiotic environmental factors, which directly influence host plant physiology and phenology but also to biotic interactions involving the associated gall fauna (Rezende et al. 2021; Baine et al. 2024; Kechrid et al. 2025). Biotic interactions with natural enemies, such as inquilines, play pivotal roles in shaping gall populations, development, and structure (Rezende et al. 2019, 2021, 2023). Inquilines may regulate gall-inducing populations by exerting competitive pressure for resources, ultimately leading to the mortality of gall-inducing insects (Baine et al. 2024; Gonçalves et al. 2025; Kechrid et al. 2025). Although most inquilines exploit gall tissues without inducing major changes, some can also induce structural modifications within existing gall tissues (herein called the primary gall). These inquilines, categorized as hypergallers, modify existing galled tissues, forming endogalls by directly interacting with plant tissues and triggering the development of a whole new gall apparatus within the primary gall induced by the galling insect (Hawkins and Goeden 1984; Fenwick 2013; Baine et al. 2023). This process alters the internal structure and resource allocation of the primary gall, often creating a more complex microenvironment that forms new nutritive, reserve and vascular tissues (Fenwick 2013; Baine et al. 2023). The arrival of the hypergaller inquiline can disrupt the phenological synchrony between the host plant and the primary gall inducer, extending developmental times, increasing mortality rates, and influencing interactions with natural enemies such as parasitoids (Guay et al. 2016; Kechrid et al. 2025).

Given these dynamics, systems involving hypergallers and endogalls provide a powerful framework for investigating how structural complexity and biotic pressure influence gall development across trophic levels. *Myrcia ovata* Cambess. (Myrtaceae) is a key host plant species in the Restinga ecosystem (Maia 2001). This species is attacked by the gall-inducing insect *Myrciamyia maricaensis* (Diptera: Cecidomyiidae), which induces fusiform galls on apical and lateral stem buds. These galls may subsequently be modified by inquiline species, particularly a species of Tetrastichinae eulophid (Hymenoptera: Eulophidae) capable of inducing endogalls within primary gall tissues (Ferraz and Monteiro 2003; Maia 2021). Chalcidoid microhymenopterans, particularly eulophids, are commonly associated with insect galls as parasitoids or phytophagous inquilines (Maia and Silva 2021; Soares et al. 2021; Mete et al. 2025), although some few species are reported as true inquilines, in which their larvae are sedentary, modify gall tissues or produce new tissues (endogalls), and their presence results in death of the inducer (Maia 2001; Maia 2021; Ferraz and Monteiro 2003).

These complex interactions with *M. ovata* take place in the unique environmental context of the Restinga ecosystem, which provides a distinctive ecological setting for studying insect–plant interactions. Restinga ecosystems are coastal formations of the Atlantic Forest biome and are characterized by nutrient-poor soils, high solar radiation, and relatively stable year-round precipitation (Cerqueira 2000; Scarano et al. 2002). These ecosystems support unique flora that includes endemic species as well as taxa adapted from adjacent forests (Cerqueira 2000; Scarano et al. 2002; Pereira et al. 2004;). This plant

composition sustains a remarkably diverse and specialized fauna of gall-inducing insects, making Restingas an important host spot of diverse galls in Brazil (Maia et al. 2001; Arriola et al. 2016; Silva et al. 2017; Maia et al. 2024).

The combination of environmental predictability and biotic specificity creates ideal conditions for the emergence of complex trophic networks, including intricate interactions between *Myrciamyia maricaensis* (Diptera: Cecidomyiidae) galls on the stem buds of *Myrcia ovata* (Myrtaceae) and endogalls induced by a Tetrastichinae eulophid (Hymenoptera: Eulophidae: Tetrastichinae) hypergaller inquiline, as reported in inventories and ecological studies of gall-inducing insects (Maia 2001; Maia 2024; Ferraz and Monteiro 2003). In this study, we investigated how host plant phenology is related to the development of primary galls induced by *Myrciamyia maricaensis* and its eulophid hypergaller-induced endogalls on *Myrcia ovata*, as well as how endogall formation affects primary gall structure. Specifically, we tested the following hypotheses: i) gall induction is constrained by the temporal availability of meristematic tissues, reflecting limitations imposed by the availability of responsive host tissues; (ii) hypergaller colonization occurs predominantly during the early stages of *M. maricaensis* gall development, when tissues are actively growing; and (iii) modifications by the eulophid hypergaller involve tissue neoformation and hyperplasia of primary gall tissues, resulting in endogall formation.

Material and methods

Biological system and study area

Myrcia ovata Cambess. (Myrtaceae) is a shrub considered endemic to the Atlantic Forest, with records of occurrence in the northern, northeastern, southern and southeastern regions of Brazil, especially in the states of São Paulo, Rio de Janeiro, Espírito Santo, Bahia, Pernambuco and Sergipe (Santos et al. 2024; Species link 2025). *Myrcia ovata* is a host plant for the galling insect *Myrciamyia maricaensis* (Maia 1995) (Diptera: Cecidomyiidae), which induces fusiform apical and lateral stem bud galls, varying from light green to dark green and purple. Inserted into this multitrophic system, a Tetrastichinae eulophid (Hymenoptera: Eulophidae: Tetrastichinae) inquiline induces endogalls on *M. maricaensis* galls modifying the fusiform shape to globoid (Maia 2001; Maia 2024).

Phenological analysis of *M. ovata* was performed in a population located in Restinga da Barra de Maricá (RBM) (22°57'39"S 42°50'36"W and 22°57'38"S 42°36'36"W), in the municipality of Maricá, Metropolitan Region of Rio de Janeiro, Brazil, with authorization granted by the Instituto Estadual do Ambiente (INEA) (N^{O} 057/2022). The ecosystem known as Restinga is coastal sandy plains originating from Quaternary sediments and features flora derived from the Atlantic Forest. Key environmental markers include sandy soils with low water retention, reduced air humidity, and the strong influence of maritime winds (Scarano 2002). Although the climatic seasons in the RBM are not clearly defined, the months of July and August are typically characterized by a water deficit (Rodarte et al., 2022). A total of 17 *M. ovata* individuals distributed across an area of approximately 5 km within the same continuous Restinga ecosystem were

monitored. The area was chosen as a single study site because of the lack of significant phytophysiognomic differences.

Meteorological data for the study location were collected from the automatic station (A667) Saquarema, Sampaio Correia, Rio de Janeiro (22°52'12.0"S, 42°36'36.0"W) and sourced from the database of INMET – Instituto Nacional de Meteorologia do Brasil (2024). The data were selected because of the consistent availability of monthly records throughout the phenological monitoring period. According to Alvares et al. (2013), the climate in Maricá is classified as “Aw” (humid tropical) under the Köppen system, characterized by a hot and rainy summer and a dry winter. Climatological analysis over the last six years (2018–2023) revealed a wide range of thermal amplitudes, with the average maximum temperature ranging from 24–30 °C and the average minimum temperature ranging from 17–23 °C (Fig. 1). The rainy season starts in October and lasts until late March, after which the dry season spans from April until September. These seasonal precipitation patterns typically show the highest average rates occurring during the summer, whereas the lowest rates are observed in late fall and winter (Fig. 1 and Fig. 3A). The area is strongly influenced by persistent desiccating northeast trade winds and maintains a high average evaporation rate (124.5 ± 27.6 mm) throughout most of the year (Louro and Santiago 1984; Mantovani and Iglesias 2008).

M. ovata vegetative and reproductive phenology

Monthly visits were made to the 17 selected individuals of *M. ovata* for 12 months (November 2022 to October 2023). Vegetative phenophases (leaf flushing, mature leaves, senescent leaves, and fallen leaves) (Figure 2A-B) and reproductive phenophases (flower buds, flower anthesis, immature fruits, and mature fruits) (Figure 2C-D) were evaluated. The phenophase intensity index was evaluated according to the methods of Fournier (1974). The Fournier method semiquantitatively evaluates the intensity of each component, which in the present study was modified to four semiquantitative categories, in which 0 = absence of phenophase; 1 = 1–33% canopy cover; 2 = 34–66%; and 3 = 67–100% (Rodarte et al. 2022). The fournier intensity indices (IF) in each month were expressed via the following formula: (IF =: Σ Fournier scores/(3 × N)) × 100), where Σ Fournier is the sum of the IF of all individuals, 3 is the maximum value of the semiquantitative categories, and N is the total number of plants analyzed (Fournier 1974, adapted; Rodarte et al. 2022).

The life cycles of galls and endogalls

The different developmental stages of primary galls and endogalls in *M. ovata* were monitored monthly in conjunction with the results of a phenological study of the host plants, observing the whole host individual and accounting for every gall seen on each of the 17 host plants used in this study. For this purpose, the stages of the developmental cycle were described morphologically and differentiated according to characteristics such as size, shape, color and presence of signs of senescence or inducer exit (Isaias et al. 2013, Arduin et al. 1994) and categorized into the following phases: (1) growth and development; (2) maturation; and (3) senescence, according to Shorthouse and Rohfritsch (1992) and Arduin and Kraus (1995).

The gall and endogall morphotypes were defined according to the morphotype standardization proposed by Isaias et al. (2013) and were then categorized by gall type, size and color. Fusiform galls were induced by *M. maricaensis* and were categorized as follows: light green, up to 0.8 cm and 0.5 cm width - young galls (YGs); green and dark green, with 1.0–1.5 cm height and up to 0.5 cm width - mature galls (MGs); yellow, beige and brown, with 1.0–1.5 cm height and up to 0.5 cm width, with visible ostiole - senescent galls (SGs) (Fig. 2 E-G). The globoid morphotype was also induced by *M. maricaensis,* but its morphology changed because of the formation of numerous endogalls within the parenchymatic tissues of the primary gall induced by a Tetrastichinae eulophid. These were categorized as follows: light green and red, up to 1 cm in height and 0.8 cm in width, hypergaller inquiline young endogalls (YEs); light, dark green and light brown, 1.5–3.0 cm in height and 1.0–2.0 cm in width, hypergaller inquiline mature endogalls (MEs); and brown, 1.5–3.0 cm in height and 1.0–2.0 cm in width, multiple exit holes, and hypergaller inquiline senescent endogalls (SEs) (Fig. 2 H–J).

The gall phases were also evaluated semiquantitatively according to the methods of Fournier (1974). Additionally, a total of 20 galls and 20 endogalls from each morphotype were evaluated under a stereomicroscope to record the developmental stages of the associated insects to support the definition of both gall stages of maturation.

Histological profiles

For anatomic analysis, mature galls (without and with young and mature endogalls) were collected (n = 10 per category), fixed in FAA_{50} (formaldehyde, acetic acid, 50% ethanol, 1:1:18 v/v) for 48 hours and then transferred to an ethanol series for dehydration (Kraus and Arduin 1997). Afterward, the samples were embedded in 2-hydroxyethylmethacrylate (Historesin, Leica® Instruments, Heidelber, Germany) and cut into 5-µm-thick sections with a rotary microtome (YD-315 model, China). The sections were then stained with 1% toluidine blue, pH 4.0 (O'Brien et al. 1965), and mounted with Entellan®.

Statistical analysis

To understand how the intensity indices of the Fournier (IF) index of vegetative and reproductive phenophases and the different phases of gall induction change, circular graphics were constructed. Statistical comparisons among the circular patterns of different development cycles of galls (YG, MG and SG) and endogalls (YE, ME, and SE), precipitation and the phenophases of *M. ovata* (leaf flushing, mature leaves, senescent leaves, and leaf fall) were performed to test whether host-plant phenophase, gall, and endogall cycles overlap, using the Whatson–Wheeler test with the Cramér–von Mises distribution in R software (R Core Team®) via the Circular, Plotrix and Vegan packages (Oksanen et al. 2007; Agostinelli and Lund 2023).

Results

Host vegetative phenology

Leaf flushing (Fig. 3B) in *M. ovata* occurred in all months throughout the year, with the lowest IF in July and August. The highest peaks of this phenophase occurred in September (IF = 70.6%), January (IF = 66.7%), and June (IF = 64.7%), with the average vector set between October and November. The Watson–Wheeler test revealed significant differences in the circular distribution of precipitation and the occurrence of leaf flushing ($W = 72.778$, $df = 2$, $p < 0.001$), indicating that the timing of young leaf occurrence significantly differed from that of the precipitation periods.

Mature leaves (Fig. 3B) occurred throughout the year, with fluctuations in their intensity. From November (IF = 64.7%) to February (IF = 71.1%), we recorded the lowest rates of mature leaf occurrence, followed by a peak from March (IF = 94.1%) to April (IF = 91.1%). A slight decrease occurred in May (IF = 75.6%), followed by a consistent period from June (IF = 64.7%) to August (IF = 82.4%). We also observed another slight decrease in September (IF = 66.7%), with a peak in October (IF = 86.3%). The average vector occurred in October. The Watson–Wheeler test revealed significant differences in the circular distribution of precipitation and the occurrence of mature leaves ($W = 207.08$, $df = 2$, $p < 0.001$), suggesting that the timing of mature leaf occurrence is significantly different from that of the precipitation patterns.

Senescent leaves (Fig. 3C) occurred throughout the year, with four peaks occurring in January (IF = 46.7%), May (IF = 53.5%), August (IF = 51.0%), and October (IF = 52.9%), with the average vector pointed out between October and November. We detected the lowest points of leaf senescence in March (IF = 37.3%), June (IF = 39.2%), and September (IF = 37.3%). The Watson–Wheeler test revealed significant differences between the circular distributions of leaf senescence and precipitation ($W = 77.656$, $df = 2$, $p < 0.001$), suggesting that leaf senescence timing does not coincide with precipitation patterns. Leaf fall (Fig. 3B) showed a distinct seasonal pattern, with peaks in February (IF = 40.0%), June (IF = 39.2%), and October (IF = 37.2%). The lowest percentages of leaf fall occurred in December (IF = 29.4%), January (IF = 31.1%), March (IF = 33.3%), and July (IF = 33.3%) to September (IF = 33.3%). The Watson–Wheeler test for homogeneity of angles between the circular data of leaf fall and precipitation revealed significant differences in their distributions ($W = 73.408$, $df = 2$, $p < 0.001$). This suggests that the distributions of precipitation and leaf dropping do not overlap, indicating that there is no overlap between the two processes.

Host reproductive phenology

The appearance of floral buds (Fig. 3D) in *M. ovata* presented two distinct peaks throughout the year. The first floral buds began emerging in December (IF = 3.9%) and January (IF = 4.4%), with the initial peak occurring in February (IF = 40.0%) and reaching its highest intensity in March (IF = 45.1%), followed by a decline in April (IF = 13.3%). A second peak emerged in May (IF = 26.7%) and continued into June (IF = 35.3%) between March and April. The average vector of occurrence is pointed out between April and May. From July to November, no floral buds were recorded (IF = 0). These peaks occurred at the transition from the rainy season to the middle of the dry season. Notably, the distributions of precipitation and floral buds did not overlap, as confirmed by the Watson–Wheeler test for homogeneity

of angles (W = 192.15, df = 2, p < 0.001). Floral anthesis (Fig. 3D) exhibited a pattern similar to that of floral buds. The first peak of this phenophase occurred in March (IF = 20.0%), followed by a decrease in April (IF = 11.1%) and a subsequent peak in May (IF = 44.4%), with the highest rate occurring in June (IF = 49.0%). The average vector indicates activity between March and April. There was no overlap between the distributions of precipitation and floral anthesis (W = 224.7, df = 2, p < 0.001).

The immature fruit phenophase (Fig. 3E) occurred from February to August and lasted 7 months throughout the year. We recorded the highest peaks in March (IF = 43,1%) and July (IF = 58.8%), and a slight decrease occurred in May (IF = 31.1%), with the average vector pointing between March and April. There was no similarity between precipitation and the available immature fruits (W = 179.34, df = 2, p < 0.001). The phenophase of mature fruits (Fig. 3E) was similar to that of immature fruits from February to August. The first peak began in February (IF = 24.4%), reaching a peak in March (IF = 43.1%), with a slight decrease in May (IF = 31.1%). The second peak started in June (IF = 49.0%) and reached its highest rate in July (58.8%). The differences between the distributions of the precipitation periods and the occurrence of mature fruits were statistically significant (W = 307.38, df = 2, p < 0.001). Therefore, there is no similarity in the circular distribution between precipitation-treated and mature fruits, indicating that these distributions do not overlap.

Life cycle of M. maricaensis galls

Young *M. maricaensis* galls (YGs) on *M. ovata* buds were present throughout the year, with fluctuations in their occurrence (Fig. 4A). A peak was observed in November (IF = 17.6%), followed by a decrease in December (IF = 5.9%) and January (IF = 8.9%). Another increase occurred in February (IF = 13.3%) and March (IF = 13.7%), with the average peak identified between January and February. A slight decrease was recorded in April (IF = 8.9%), followed by a minor peak in July (IF = 9.8%) before stabilizing from August (IF = 3.9%) to October (IF = 3.9%). Statistically significant differences were detected between YG and precipitation (W = 14.294, df = 2, p < 0.001) and between YG and leaf flushing (W = 12.717, df = 2, p < 0.001), indicating that these distributions do not overlap.

Mature *M. maricaensis* galls (MGs) occurred predominantly during the rainy season (Fig. 4A). The occurrence rate began to increase in November (IF = 13.7%) and peaked in December (IF = 27.5%), with the highest occurrence occurring between these two months. A slight decline was observed in January (IF = 22.2%), after which no occurrences were recorded from March to April. The occurrence rate slightly increased in May (IF = 2.2%), with small peaks in June (IF = 7.8%) and July (IF = 7.8%). Afterward, the occurrence rate stabilized between August (IF = 2.0%) and October (IF = 2.0%). The distribution of MGs did not overlap with that of leaf flushing (W = 37.308, df = 2, p < 0.001), but it did overlap with that of precipitation (W = 2.5227, df = 2, p = 0.2833). These findings indicate significant differences between the MG and leaf flushing but no significant differences between the MG and precipitation.

Senescent *M. maricaensis* galls (SGs) presented a single strong peak in occurrence, followed by a decline throughout the year (Fig. 4A). Their occurrence began increasing in April (IF = 11.1%), reaching the highest peak in May (IF = 40.0%), with the average peak spanning these two months and persisting

into June (IF = 29.4%). A decline started in July (IF = 15.7%), followed by consistently low occurrence rates from August (IF = 5.9%) to January (IF = 6.7%), with a slight increase in February (IF = 13.3%) before decreasing again in March (IF = 5.9%). Statistical analyses revealed significant differences between the distributions of leaf flushing and SG ($W = 50.458$, $df = 2$, $p < 0.001$) and between precipitation and MG ($W = 110.68$, $df = 2$, $p < 0.001$). These results indicate that SG occurrence does not overlap with leaf flushing or precipitation patterns.

Development cycle of endogalls induced by hypergaller inquiline

Young endogalls (YEs) were observed during both the rainy and dry seasons, with one peak occurring each (Fig. 4B). The most prominent peak occurred in November (IF = 23.5%), after which the occurrence slightly decreased and stabilized between December (IF = 11.8%) and February (IF = 13.3%), with the average vector pointing between December and January. A sharp decline was observed from March (IF = 3.4%) to April (IF = 4.4%). The second peak appeared in May (IF = 20.0%), followed by a significant decrease in June (IF = 2.0%) and stabilization from July (IF = 7.8%) to August (IF = 7.8%). No occurrences were recorded from September to October. Statistical analysis revealed significant differences between leaf flushing and YEs ($W = 11.788$, $df = 2$, $p = 0.002$), indicating that their occurrence does not overlap. YEs did not significantly differ from precipitation ($W = 0.20464$, $df = 2$, $p = 0.9027$), suggesting that these variables overlap.

Mature endogalls (MEs) presented two peaks throughout the year, with two larger peaks and two decreases, and the average vector pointed between December and January (Fig. 4B). The first peak occurred in November (IF = 29.4%), whereas the largest peak was observed in May (IF = 44.4%). In the remaining months, the number of MEs was lower but still present. The lowest occurrence rates were recorded in August, September, and October (IF = 3.9%). Statistical analysis revealed significant differences between leaf flushing and MEs ($W = 19.565$, $df = 2$, $p < 0.001$), suggesting that their distributions do not overlap. However, when the MEs were compared with precipitation, no significant differences were found ($W = 0.51477$, $df = 2$, $p = 0.7731$), indicating that the occurrence of both variables overlaps.

Senescent endogalls (SEs) were found throughout the year, with greater rates during the rainy season (Fig. 4B). The first peak started in November (IF = 39.2%) and lasted until January (IF = 40.0%), followed by a slight decrease in February (IF = 24.4%), stabilization in March (IF = 27.5%) and a decrease in April (IF = 8.9%), and the lowest rate was reached in May (IF = 2.2%). The second peak started in June (IF = 25.5%) and started decreasing in July (IF = 17.6%), with a strong decrease in August (IF = 3.9%). Afterward, it started increasing again in September (IF = 11.8%) and October (IF = 11.8%). There were significant differences between SE and leaf flushing ($W = 35.06$, $df = 2$, $p < 0{,}001$), indicating that there was no overlap. However, when precipitation and SE were compared, they did not significantly differ ($W = 2.8424$, $df = 2$, $p < 0.2414$).

Histological analysis

The *Myrciamyiia maricaensis* galls of *Myrcia ovata* not altered by hypergallers form a single larval chamber with only one inducer (Fig. 5A). The gall is attached to the stem in the basal region, and a gall wall occurs in the median portion surrounding the gall inducer (Fig. 5A). A thin covering occurs in the apical region (Fig. 5B), where the cells are smaller than those in surrounding tissues and contain prismatic crystals and vacuolar metabolites (Fig. 5B). An outer epidermis surrounds all the galls and presents a single layer without trichomes and with small cells (Fig. 5C). The gall cortex in the median region is differentiated into outer and inner compartments (Fig. 5C). The outer cortex consists of 15 to 30 layers of polyhedral parenchymatic cells, which are visibly larger than those in the inner cortex (Fig. 5C). Subepidermal layers of the outer cortex are photosynthetic. Secretory cavities are predominantly located below the epidermis in the outer cortex of the gall (Fig. 5B, C). The inner cortex, also referred to as the nutritive tissue, comprises 8 to 15 layers of parenchymatic cells, characterized by a conspicuous presence of polyphenols stained green by toluidine blue (Fig. 5C). A uniseriate epidermis is also present internally and remains in contact with the larval chamber (Fig. 5C). Vascular bundles originate from the bottom of the gall and extend longitudinally between the outer and inner cortex (Fig. 5A, B, C). The ostiole is closed and formed by a uniseriate epidermis and small parenchymatic cells, with no distinction between the inner and outer cortex in this region (Fig. 5D).

Galls of *Myrcia ovata* containing endogalls exhibit modifications to their original structure, including a noticeable increase in the number of parenchymatic cells and increased vascularization (Fig. 6A, B, C). The larval chamber of the gall inducer *M. maricaensis* remains singular, although it is reduced in size (Fig. 6A, B, C). The endogalls are multiple, with one small chamber per hypergaller, and occur exclusively within the cortex, with a loss of distinction between the outer and inner cortices (Fig. 6A-F). The cortex adjacent to the larval chamber shows a loss of vacuolar metabolite staining, with a cecidogenous gradient (Fig. 6B-C, E). The epidermis of *M. ovata* galls remains similar to that of galls without endogalls, maintaining the frequent presence of secretory cavities below it (Fig. 6D). The initial formation of endogalls induces significant hyperplasia, as observed in the surrounding cells (Fig. 6E), with increased cell division, particularly in cells in direct contact with the endogall inducer (Fig. 6E, arrow). At maturity, the endogall contains a parenchymatic cortex, with a nutritive role, surrounded by lignified cells (Fig. 6F), and features a single chamber with each single inducer (Fig. 6G). Vascularization may extend near the endogalls (Fig. 6G).

Discussion

Our results indicate a clear decoupling between the host vegetative phenophase and the life cycle of its primary galls induced by *Myrciamyia maricaensis*. The constant availability of sites for gall induction, such as buds and leaf flushing, occurs throughout the year, providing recurrent sites for gall induction, reinforcing the hypothesis that the constant availability of reactive tissues favors gall establishment and a multivoltine gall-inducing life cycle (Weis et al. 1988; Oliveira et al. 2016). The multivoltine pattern observed for *M. maricaensis* reflects its ability to exploit several windows of tissue availability rather than following a single annual cycle, as seen in other galling systems (Fagundes et al. 2018, Costa et al. 2021). Although peaks of gall induction temporally overlap with periods of higher rainfall, our statistical

analyses indicate that precipitation does not act as a direct driver of host phenology or gall induction. However, rainfall overlapped with the presence of mature galls, likely because host growth and water status may favor gall development rather than induction per se, with higher gall abundance in rainy seasons (Araújo and Santos 2009, Costa and Araujo 2019, Santos-Silva et al. 2022, Melo et al. 2023). In galls induced by dipterans, for example, the mature stage is commonly associated with maximal gall size and the main trophic activities of gall-inducing larvae (Bronner 1992). Our results are quite different from those reported for *Eugenia copacabanensis* (Myrtaceae), where the vegetative activity of some important host plants, associated with the availability of sites for gall induction, likely reflects the indirect influence of rainfall on Restinga ecosystems (Nobrega et al. 2025).

Although precipitation did not directly influence *Myrcia ovata* phenophases or the gall-inducing life cycle, as evaluated from gall development stages, it may impact gall-inducing behavior. Adult Cecidomyiidae have extremely short lifespans (1–3 days), and their emergence, mating and oviposition are concentrated within narrow temporal windows (Skuhravý et al. 1996; Hidayat et al. 2020; Yukawa and Kim 2021). Consequently, factors that restrict adult flight during this period may strongly limit effective dispersal. Given their millimetric size and low body mass, rainfall can mechanically interfere with flight and reduce aerial activity, as documented for small migratory insects in general (Wainwright et al. 2022). Consequently, rainy periods may further constrain the already limited dispersal window of Cecidomyiidae, potentially influencing the dispersal and spatial patterns of gall establishment. Thus, multivoltinism can give the gall-inducing an important adaptive strategy.

Despite the lack of influence of precipitation on both host plant phenophases and most gall development stages, we found an important association between endogall induction and development with precipitation. We expected that endogalls would be induced only in young primary galls, which are also associated with increased precipitation and constitute the main reactive phase of primary gall development. Another important finding of this study is the strong anatomical distinction between primary galls induced by *M. maricaensis* and the endogalls formed within them by inquiline hypergallers. Our observations reveal that primary mature galls are thinner-walled, smaller, and decline after an early peak during the rainy season, whereas mature primary galls with endogalls are more robust and maintain structural integrity during this period. This suggests a developmental shift in gall identity once hypergaller larvae are established.

Asynchrony x multivoltinism: the relationship between Myrcia ovata and Myrciamyia maricaensis

The continuous presence of young and mature leaves throughout the year in *M. ovata*, with multiple peaks in leaf flushing, indicates that this species provides repeated windows of meristematic tissue availability rather than a single seasonal flush. This pattern allows *M. maricaensis* to exploit several temporal opportunities for gall induction, supporting a multivoltine life cycle (Oliveira et al. 2016). The constant availability of meristematic tissues increases the chance of phenological asynchrony or decoupling and, in some cases, reduces larval success (Yukawa 2000; Tooker and Helms 2014, Fagundes et al. 2018). This strategy reflects an adaptive response to a relatively predictable resource

base, allowing *M. maricaensis* to maintain reproductive success in a heterogeneous environment where leaf sprouting occurs across several months rather than being confined to a single wet-season flush.

In contrast to vegetative phenology, the reproductive phenophases of *M. ovata* flowering and fruiting exhibit two well-defined peaks, and none of the gall developmental stages overlap temporally with these phenophases, indicating that the association is not statistically significant. By not coinciding with reproductive peaks, galls may experience reduced competition with flowers and fruits, as both structures function as strong sinks for photoassimilates and nutrients, and phenological mismatches are known to modulate interactions between galling insects and host reproduction in other systems (Dorchin et al. 2006, De Carvalho-Sposito et al. 2022, Fernandes et al. 2022, Jansen-González et al. 2024). Data also show that during part of this reproductive period, *M. maricaensis* primary galls are in advanced developmental phases, such as senescence, which commonly corresponds to the pupal stage in Diptera: Cecidomyiidae (Rohfritsch 1992). However, in general, a high abundance of galls on host trees may reduce investment in reproductive structures, as observed in plants from Cerrado vegetation (Martini et al., 2021). This phenomenon occurs because gall development depends on the expression of genes associated with reproductive organ development, and galls can be interpreted as ectopic reproductive structures (Martini et al., 2021; Schultz et al., 2019).

Climate change and potential impacts on host plants and gall system

Projected shifts in rainfall distribution and temperature in coastal Restinga may play a pivotal role in adjusting the synchrony between host plants and gall-inducing insects (Aguirrebengoa et al. 2020). In the Restinga da Barra de Maricá, precipitation is seasonal, with the highest average rates during summer and lower values in late fall and winter. Despite this seasonality, there is continuous production of reactive sites for gall induction, resulting in a weak temporal adjustment between *M. ovata* phenology and the life cycle of *M. maricaensis*, which allows the occurrence of multivoltine patterns of gall development. However, shifts in the onset or duration of rainy or dry seasons may alter leaf flushing and the availability of sites for gall induction, potentially concentrating these events within shorter time windows or shifting their timing. Such changes may delay or advance gall induction periods (Rezende et al. 2019; Nobrega et al. 2025). Under these conditions, not only the primary gall life cycle but also its interactions with associated organisms, especially endogalls, may change.

As demonstrated by Yukawa (2000), small temporal mismatches between host phenology and gall-inducing organisms can significantly reduce galling success and population persistence. In *M. maricaensis* galls, such mismatches can reduce the number of cycles per year and ultimately decrease endogall success. Consequently, climate-induced asynchronies may lead to cascading effects across trophic interactions, including inquiline populations associated with these galls. Additionally, projections indicate that coastal Restinga vegetation faces significant risks under climate change scenarios, including reductions in woody plant diversity and shifts in the phenological timing of key host species (Inaque et al. 2021, Lourenço et al. 2021). These changes may alter the onset of leaf flushing and

reproductive events, disrupting the synchronization between gall inducers and their hosts and potentially acting as a selective filter that favors the persistence of highly specialized interactions capable of tracking narrower and less predictable phenological windows. Although Restinga areas are considered seasonal, the phenology of plant species such as *Myrcia* spp. is not strongly correlated with precipitation (De Medeiros et al. 2007). The plant species phenophases in these areas are not influenced by rainfall (Alves et al. 2021), but other abiotic factors, such as soil moisture, wind exposure, salinity, and substrate dynamics, may play a more prominent role in shaping the vegetative and reproductive cycles and plasticity of Restinga species (Londe et al. 2021, Lourenço et al. 2021), consequently modulating the temporal patterns of gall formation.

The presence of young and mature endogall peaks throughout the rainy season suggests that hypergaller may be particularly vulnerable to environmental stress or phenological mismatch with the primary gall. The sites for endogall induction, that is, mature galls, peak in November when endogalls are induced and develop until May, which is the rainy season. In this scenario, changes in precipitation regimes can directly impact the induction, establishment and abundance of endogalls.

Developmental connection between the primary gall and the endogall

The developmental stages of primary galls and endogalls on *M. ovata* reveal slightly different life cycle strategies between the primary gall-inducer *M. maricaensis* and its associated eulophid hypergaller. While primary bud galls were not synchronous with host plant phenophases or precipitation, endogalls were temporally associated with the presence of mature primary galls and overlapped with precipitation. These patterns suggest that hypergallers succeed by hijacking and remodeling mature gall tissues, the main trophic gall stage (Bronner 1992). The temporal coupling between these two guilds of gallers emphasizes the role of interspecific interactions and seasonal filtering in determining gall persistence and development across plant–insect tropic nets once inquiline hypergallers demand the presence of a gall to induce its endogalls (Fenwick 2013; Guay et al. 2016; Baine et al. 2023).

Previous work on the same galling system in the Restinga of Maricá reported a different seasonal pattern. Ferraz and Monteiro (2003) reported that the population of primary galls and modified galls containing hypergallers broadly fluctuated throughout the year, with both types occurring mainly during the dry season. In contrast, our results show that endogalls become more frequent during periods when mature primary galls decline. This difference suggests that the temporal relationship between primary gall inducers and hypergallers may change after many years of interaction in the system of an established population, resulting in a temporal shift in which gall structures become progressively dominated by hypergallers.

The formation of endogalls within the primary galls induced by *M. maricaensis* indicates that gall tissues retain sufficient developmental plasticity to support secondary gall induction by hypergaller inquilines, establishing a hierarchical dependency among the host plant, primary galler and hypergaller. The hypergaller, which acts as an inquiline of primary galls, exploits the nutritive resources of the primary gall

and induces a secondary gall structure, an endogall (Fenwick 2013; Gobbo et al. 2020; Baine et al. 2023). The onset of endogall development occurs when the primary gall is still in its maturing phase, suggesting that the hypergaller synchronizes its oviposition with the maturing development stage of *M. maricaensis*. During this phase, hypergaller larvae trigger new rounds of hypertrophy and tissue redifferentiation, redirecting plant resources toward their own nutritive cells (Ferreira et al. 2019; Tooker and Helms 2014).

At the young endogall stage, hyperplasia predominates in the host cells surrounding the larval chamber, resulting in the formation of dense nutritive tissue, similar to that of galls induced by hymenopteran (Rohfritsch 1992, Isaias et al. 2018). As the endogall matures, distinct tissue layers differentiate, including new parenchymatic tissues, similar to those of the nutritive tissue (Fenwick 2013; Gobbo et al. 2020). These changes suggest that endogall alters the metabolic flow and structural integrity of the primary gall, potentially shortening the lifespan of the galling insect. Consequently, the presence of hypergallers alters the population dynamics of *M. maricaensis*, increasing mortality and decreasing reproductive success (Ferraz and Monteiro 2003; Baine et al. 2023).

The increase in endogall formation during the rainy season coincides with the decrease in mature primary galls. Anatomical observations revealed that endogall formation involves larval inquilines inducing novel gall chambers within the parenchyma of primary galls, resulting in the enlargement and thickening of the gall structure. This development comes at a cost to the primary gall inducer, as endogall growth compresses the primary gall larval chamber, likely leading to mortality (Ferraz and Monteiro 2003, Fenwick 2013). Therefore, the poor persistence of mature primary galls during the rainy season may be due not only to abiotic filtering but also to the structural and physiological hijacking imposed by hypergaller larvae; thus, the sequestration of gall resources by endogalls may be a key driver of primary mature gall decline under rainy conditions, reinforcing the role of inquiline antagonism in shaping gall phenology and persistence.

Similar structural consequences of endogall formation have been described for this galling system (Ferraz and Monteiro 2003). The development of multiple endogalls within *Myrciamyia maricaensis* galls drastically alters the internal gall architecture, resulting in the production of numerous secondary chambers that progressively compress and eventually close the original larval chamber. As a consequence, mortality of the cecidomyiid inducer becomes extremely high, indicating that hypergallers may act as important mortality factors in this system (Ferraz and Monteiro 2003). Our anatomical observations support this mechanism, as the proliferation of parenchymatic tissues and the formation of multiple endogall chambers reduce the available space for the primary larva by altering the structural organization of the gall cortex.

Because primary galls are thin walled and composed of parenchymatic layers, they tend to degrade quickly when they reach senescence and therefore do not persist long attached to the host plant. In contrast, inquiline hypergallers in this system act as ecosystem engineers, inducing abnormal tissue proliferation and the formation of multiple secondary larval chambers within the primary gall. This

remodeling enlarges the gall structure and creates several internal endogalls. Once endogalls reach senescence, the reinforced structure allows them to remain attached to *M. ovata* stems, where they may function as shelters for a variety of successor arthropod species. Rezende et al. (2023) demonstrated a similar pattern in galls induced by *Palaeomystella oligophaga* on *Macairea radula*, where persistent bud galls serve as shelter for other arthropods after senescence. Such structural persistence likely enhances local arthropod diversity and promotes trophic continuity, reinforcing the ecological importance of hypergallers as agents of habitat formation in gall-associated communities.

Anatomical differentiation between primary and endogall structures

The primary galls induced by *M. maricaensis* display typical Cecidomyiidae-induced features: continuous nutritive layers, as an inner tissue that surrounds the larval chamber, and an outer tissue formed specifically by chlorophyll layers under the epidermis, associated with vascular bundles that establish the continuity of vascularization from the host organ (Ferreira et al. 2019; Costa et al. 2022; Costa 2025). This morphofunctional compartmentalization of the gall cortex reveals the high structural complexity of *M. maricaensis* galls, with tissues functionally specialized in terms of their nutritive and photosynthetic roles. In the star-shaped leaf gall of *Myrciaria floribunda* (Myrtaceae), the cecidomyiid also has a complex structure, with lipid-rich nutritive tissue surrounding the larval chamber enclosed by a sclerenchymatous ring and bordered by vascular bundles (Castilho et al. 2025).

As in primary galls, endogall-inducing organisms also invest in the formation of two specialized tissues, including the induction of a new nutritive tissue surrounding themselves, a feature commonly reported in other endogall systems (Fenwick 2013; Baine et al. 2023). The hyperplasic pattern is typical of hymenopteran galls (Rohfritsch 1992, Isaias et al. 2018), reflecting the chewing behavior of the galling organism and the distinctive pattern in comparison to a cecidomyiid-induced gall. In a novel and complex reconfiguration, the endogall inducers promote the neoformation of lignified tissue that envelops the entire endogall, which may provide structural support and mechanical protection against natural enemies (Ronquist and Liljeblad, 2001). The alteration of nutritive and sclerenchyma patterns confirms that endogalls induced by hymenopterans have morphogenetic changes similar to those of hymenopteran primary galls (Bronner 1992, Rohfritsch 1992, Isaias et al. 2018). This shift reflects the reactivation of plant morphogenetic programs mediated by biochemical effectors produced by the hypergaller (Costa 2025; Oliveira et al. 2016; Gätjens-Boniche 2019). In this context, the production of a tissue that is structurally and functionally absent in the primary gall highlights the high capacity of the hypergaller to reconfigure preexisting tissues, even under constraints imposed by the host plant (Isaias et al. 2014; Formiga et al. 2013), and the stimulation of the main galling inducer. Multiple larval chambers with a single endogaller are formed in the primary gall, within which an endogall inducer is established, embedded in nutritive tissue and protected by sclerenchymatous tissue. Such modifications also resemble the structural rearrangements described for *Periclistus* inquilines in cynipid galls, in which larvae induce new nutritive and sclerenchymatic layers within preexisting gall structures (Fenwick 2013; Gobbo et al. 2020).

The vascular system of the primary galls is well developed, originating at the base of the galls and forming a continuous layer between the outer and inner cortex. This vascular configuration is further reorganized following endogall induction, and connections with endogalls are typically established. Consequently, the vascular system initially established in primary galls (Isaias et al. 2024, Takeda et al. 2024) continues to support the metabolic demands of endogalls, underscoring their dependence on preexisting host-derived structures. In this context, endogall-inducing organisms exhibit a specialized life strategy, exploiting the structural complexity of primary galls while operating at lower energetic costs. However, the formation of endogalls disrupts the morphophysiological stability of the gall microhabitat, altering nutrient fluxes, mechanical properties, and, potentially, the chemical defense profile of gall tissues. Owing to multiple inductions within a single primary gall, hypergallers transform the original structure into a heterogeneous system composed of several larval niches. Ferraz and Monteiro (2003) reported that such modifications may allow additional phytophagous and parasitoid species to exploit galls, thereby increasing the diversity of associated organisms.

Complex gall systems can host not only gall-inducing organisms and their natural enemies but also a wide variety of associated life forms, with galls functioning as products of ecosystem engineering (Rezende et al. 2023). In this context, hypergallers may also act as ecosystem engineers within the gall system, reshaping both the structural organization of the gall and the ecological interactions occurring within it.

Conclusion

Despite the mismatch in occurrence, the multivoltine strategy exhibited by *Myrciamyia maricaensis* reflects an adaptation to the phenology of *Myrcia ovata*, in which meristematic tissues remain available throughout the year and are not constrained by seasonal factors such as precipitation. The gall inducer exploits multiple temporal windows of bud availability, producing successive induction peaks during wet and dry seasons, which ensures favorable sites for gall formation and larval development. However, the establishment of a hypergaller species capable of inducing endogalls within *M. maricaensis* galls adds new layers of ecological and structural complexity to this system. Hypergaller activity alters tissue organization, resource partitioning, and developmental timing, ultimately disrupting the phenological synchrony between the host and the primary gall inducer. Together, these findings highlight the dynamic interplay between biotic and abiotic drivers in shaping gall systems and emphasize the vulnerability of such finely balanced interactions to environmental change.

Declarations

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Author contributions

PHPG and DCO contributed to experimental design, data collection, data analysis, and manuscript preparation. LPN, RRMC, VCK and BGF performed experimental sampling and evaluation, spectrometric analyses, and data collection. DCO and PHPG wrote the first draft of the manuscript. All authors reviewed and approved the final version of the manuscript, and DCO. supervised all stages of the study.

Competing interests

The authors declare no competing interests.

Data Availability

All the data generated or analyzed during this study are included in this published article. The data used can be requested on demand.

References

1. Agostinelli C, Lund U (2023) R package ‘circular’: circular statistics, version 0.5-0. CRAN. https://cran.r-project.org/package=circular
2. Aguirrebengoa M, Menéndez R, Müller C, González-Megías A (2020) Altered rainfall patterns reduce plant fitness and disrupt interactions between below- and aboveground insect herbivores. Ecosphere 11: e03127
3. Alvares CA, Stape JL, Sentelhas PC, Gonçalves JDM, Sparovek G (2013) Köppen’s climate classification map for Brazil. Meteorol Z 22:711–728
4. Alves IDJ, Paz JRLD, Moreira ALDC, Pigozzo CM (2021) Fenologia reprodutiva e vegetativa de três espécies ocorrentes em uma área de restinga urbana em Salvador, Bahia, Brasil. Hoehnea 48:e722020
5. Araújo WS, Santos BB (2009) Efeitos da sazonalidade e do tamanho da planta hospedeira na abundância de galhas de Cecidomyiidae (Diptera) em *Piper arboreum* (Piperaceae). Rev Bras Entomol 52: 300-303
6. Arduin M, Kraus JE, Montenegro G (1994) Morphology and phenology of leaf galls of Piptadenia gonoacantha (Fabales, Mimosaceae). Rev Bras Entomol 38:79–89
7. Arduin M, Kraus JE (1995) Anatomia e ontogenia de galhas foliares de Piptadenia gonoacantha (Fabales, Mimosaceae). Bol Bot Univ São Paulo:109–130

8. Arriola ÍA, Costa EC, de Oliveira DC, Isaias RMS (2024) Soil–plant–gall relationships: from gall development to ecological patterns. Biol Rev 99:1948–1975
9. Baine Q, Casares EE, Carabotta E, Martinson VG, Martinson EO (2023) Galls on galls: a hypergall-inducing midge and its parasitoid community. Ecology 104:e3976
10. Baine Q, Hughes DW, Casares EE, Martinson EO, Martinson VG (2024) External insect gall morphology influences the functional guilds of natural enemy communities. Proc R Soc B 291:20242424
11. Bronner R (1992) The role of nutritive cells in the nutrition of cynipids and cecidomyiids. In: Shorthouse JD, Rohfritsch O (eds) Biology of insect-induced galls. Oxford University Press, New York, pp 118–140
12. Carneiro RG, Burckhardt D, Isaias RM (2013) Biology and systematics of gall-inducing triozids (Hemiptera: Psylloidea) associated with *Psidium* spp. (Myrtaceae). Zootaxa 3620:129–146
13. Castilho LG, Campos NV, Matos SD, Konno TUP, Souza MAA, Vieira ACM (2025) Anatomical, histochemical, and micromorphological aspects of leaf gall in *Myrciaria floribunda* H. West ex Willd. O. Berg (Myrtaceae). Flora 326:152721
14. Cerqueira R (2000) Biogeografia das restingas. In: Esteves FA, Lacerda LD (eds) Ecologia de restingas e lagoas costeiras. Núcleo de Pesquisas Ecológicas de Macaé, Rio de Janeiro, pp 65–76
15. Costa K, Araújo W (2019) Distribution of gall-inducing arthropods in areas of deciduous seasonal forest of Parque da Sapucaia (Montes Claros, MG, Brazil): effects of anthropization, vegetation structure and seasonality. Pap Avulsos Zool 59:e20195931
16. Costa E, Martini V, Souza-Silva A, Lemos-Filho J, Oliveira D, Isaias R (2021) How galling herbivores share a single superhost plant during their phenological cycle: the case of Mimosa gemmulata Barneby (Fabaceae). Trop Ecol 63:61–74
17. Costa EC, Oliveira DC, Isaias RM (2022) Parasitoid impairment on the galling Lopesia sp. activity reflects on the cytological and histochemical profiles of the globoid bivalve-shaped gall on Mimosa gemmulata. Protoplasma 259:1585–1597
18. Costa EC (2025) The structural development and functional traits of Diptera-induced galls. In: Plant galls: structure and functions. Springer Nature Switzerland, Cham, pp 349–380
19. De Carvalho-Sposito S, Urso-Guimarães M, da Silva F (2022) Temporal resource partitioning and stochastic colonization explain the co-occurrence of gall-inducing insects in the super-host plant *Copaifera langsdorffii* Desf. (Fabaceae). Austral Ecol 47: 1340-1349
20. De Medeiros DPW, Lopes AV, Zickel CS (2007) Phenology of woody species in tropical coastal vegetation, northeastern Brazil. Flora 202:513–520
21. Dorchin N, Cramer M, Hoffmann J (2006) Photosynthesis and sink activity of wasp-induced galls in *Acacia pycnantha*. Ecology 87:1781–1791
22. Fagundes M, Xavier R, Faria M, Lopes L, Cuevas-Reyes P, Reis-Junior R (2018) Plant phenological asynchrony and community structure of gall-inducing insects associated with a tropical tree species. Ecol Evol 8:10687–10697

23. Fernandes G, Maia R, Arantes-Garcia L, Pereira E, Oki Y, França M, Costa L, Barbosa M (2022) Deep capillary impact of a psyllid gall on its host ecophysiology, architecture and performance. Trees 36:1193–1206
24. Fenwick B (2013) Comparative morphogenesis of cynipid leaf galls induced by Diplolepis polita and Diplolepis nebulosa (Hymenoptera: Cynipidae) and modification by inquilines of the genus Periclistus (Hymenoptera: Cynipidae). Doctoral dissertation, Laurentian University of Sudbury
25. Ferraz FFF, Monteiro RF (2003) Complex interactions involving a gall midge Myrciamyia maricaensis Maia (Diptera, Cecidomyiidae), phytophagous modifiers and parasitoids. Rev Bras Zool 20:433–437
26. Ferreira BG, Álvarez R, Bragança GP, Alvarenga DR, Pérez-Hidalgo N, Isaias RM (2019) Feeding and other gall facets: patterns and determinants in gall structure. Bot Rev 85:78–106
27. Ferreira BG, Moreira GR, Carneiro RG, Isaias RM (2022) Complex meristematic activity induced by *Eucecidoses minutanus* on *Schinus engleri* turns shoots into galls. Am J Bot 109:209–225
28. Formiga AT, Oliveira DC, Ferreira BG, Magalhães TA, Castro AC, Fernandes GW, Isaias RMS (2013) The role of pectic composition of cell walls in the determination of the new shape-functional design in galls of Baccharis reticularia (Asteraceae). Protoplasma 250:899–908
29. Fournier LA (1974) Un método cuantitativo para la medición de características fenológicas en árboles. Turrialba 24:422–423
30. Gätjens-Boniche O (2019) The mechanism of plant gall induction by insects: revealing clues, facts, and consequences in a cross-kingdom complex interaction. Rev Biol Trop 67:1359–1382
31. Gobbo E, et al (2020) From inquilines to gall inducers: genomic signature of a lifestyle transition in Synergus gall wasps. Genome Biol Evol 12:424–438
32. Gonçalves PHP, Costa EC, Ferreira BG, de Oliveira DC (2025) Natural enemies' influence on the population, structure, and function of insect-induced galls. In: Plant galls: structure and functions. Springer Nature Switzerland, Cham, pp 443–462
33. Guay JF, Bulot D, Béland JM, Cloutier C (2016) Gall-maker Paradiplosis tumifex (Diptera: Cecidomyiidae) and its inquiline Dasineura balsamicola (Diptera: Cecidomyiidae): an update on epidemic episodes and seasonal ecology in Québec, Canada. Can Entomol 148:452–465
34. Guiguet A, McCartney NB, Gilbert KJ, Tooker JF, Deans AR, Ali JG, Hines HM (2023) Extreme acidity in a cynipid gall: a potential new defensive strategy against natural enemies. Biol Lett 19:20220513
35. Harris MO, Pitzschke A (2020) Plants make galls to accommodate foreigners: some are friends, most are foes. New Phytol 225:1852–1872
36. Hawkins BA, Goeden RD (1984) Organization of a parasitoid community associated with a complex of galls on Atriplex spp. in southern California. Ecol Entomol 9:271–292
37. Hidayat P, Guntoro DA, Takasu K, Overholt W (2020) Biology and rearing of the cogongrass gall midge, *Orseolia javanica* Kieffer & Docters van Leeuwen-Reijnvaan (Diptera: Cecidomyiidae). bioRxiv 10.1101/2020.02.27.966499

38. Inaque GM, Zwiener VP, Marques MCM (2021) Climate change threatens the woody plant taxonomic and functional diversities of the Restinga vegetation in Brazil. Perspect Ecol Conserv 19:53–60
39. Instituto Nacional de Meteorologia (2024) Dados meteorológicos da estação automática A667 – Saquarema, Sampaio Corrêa, RJ [dataset]. INMET. https://bdmep.inmet.gov.br/
40. Isaias RMS, Carneiro RGS, Oliveira DC, Santos JC (2013) Illustrated and annotated checklist of Brazilian gall morphotypes. Neotrop. Entomol. 42: 230-239
41. Isaias RMS, Oliveira DC, Carneiro RGS, Kraus JE (2014) Developmental anatomy of galls in the Neotropics: arthropods stimuli versus host plant constraints. In: Neotropical insect galls. Springer Netherlands, Dordrecht, pp 15–34
42. Isaias RMS, Ferreira BG, Alvarenga DR, Barbosa LR, Salminen JP, Steinbauer MJ (2018) Functional compartmentalization of nutrients and phenolics in the tissues of galls induced by Leptocybe invasa (Hymenoptera: Eulophidae) on Eucalyptus camaldulensis (Myrtaceae). Aust Entomol 57:238–246
43. Isaias RMS, Kraus JE, Costa EC, Carneiro RGS (2024) The anatomy of neotropical galls and the untold lessons about the morphogenetical potentialities of plants. Rodriguésia 75:e01542023
44. Isaias RMS, Arriola ÍA, Ferreira BG (2025) Meristematic features influence on gall development. In: Plant galls: structure and functions. Springer Nature, Cham, pp 1–17
45. Jansen-González S, Teixeira S, Pereira R (2024) Conflicting dynamics of galling and pollination: *Arastichus gallicola* (Hymenoptera, Eulophidae), a specialized parasitic galler in pistillate flowers of *Thaumatophyllum bipinnatifidum* (Araceae). Plants 13: 3520.
46. Kechrid R, Adjami Y, Kermiche K, Ouakid ML (2025) How natural enemies shape gall wasp diversity in oak cynipid communities. Afr J Ecol 63:e70037
47. Kraus JE, Arduin M (1997) Manual básico de métodos em morfologia vegetal. Editora da Universidade Federal Rural do Rio de Janeiro, Seropédica
48. Lombardero MJ, Ayres MP, Álvarez-Álvarez P, Castedo-Dorado F (2022) Defensive patterns of chestnut genotypes (Castanea spp.) against the gall wasp, *Dryocosmus kuriphilus*. Front For Glob Change 5:1046606
49. Londe V, Pereira JA, de Sousa HC (2021) Phenological study of congeneric Myrcia species and Clethra scabra in wetland and drained habitats in a Montane Forest. J For Res 32:1419–1427
50. Lourenço J, Newman E, Ventura J, Milanez C, Thomaz L, Wandekoken D, Enquist B (2021) Soil-associated drivers of plant traits and functional composition in Atlantic Forest coastal tree communities. Ecosphere 12: e03629
51. Louro LR, Santiago LJM (1984) A região da Barra de Maricá, RJ, e a importância de sua preservação. Atas Soc Bot Bras 2:109–118
52. Maia VC (2001) The gall midges (Diptera, Cecidomyiidae) from three restingas of Rio de Janeiro State, Brazil. Rev Bras Zool 18:583–629
53. Maia VC (2021) Review of the inquilinous fauna associated with insect galls in Brazilian restingas. Braz J Biol 82:e235395

54. Maia VC (2024) Gall-inducing insects from the Maricá Environmental Protection Area (RJ, Southeastern Brazil). Rev Bras Entomol 68:e20230086
55. Maia V, Silva B (2021) Insect galls of the Brazilian Cerrado: associated fauna. Biota Neotrop. 21: e20211202
56. Mantovani A, Iglesias RR (2008) Factors limiting seed germination of terrestrial bromeliads in the sandy coastal plains (restinga) of Maricá, Rio de Janeiro, Brazil. Rodriguésia 59:135–150
57. Marquesine RR, Gonçalves PHP, Nobrega LP, Mantuano D, Ferreira BG (2024) How does the life cycle of Clinodiplosis profusa (Cecidomyiidae) adjust to phenological variations of the host plant Eugenia uniflora (Myrtaceae) in sun and shade? Sci Nat 111:44
58. Martini VC, Raymundo D, Prado-Júnior J, Oliveira DC (2021) Botton-up and top-dwon forces in plant gall relationships: testing the hypothesis of resource concentration, associated resistance, and host fitness reduction. Ecol. Entomol. 46: 1072-1081.
59. Melo GAB, Santos-Silva J (2023) Occurrence and characterization of insect galls in two areas of tropical dry forest (caatinga) in São Francisco River Natural Monument, Brazil. Entomol Beginners 4:e058
60. Mete Ö, Tataroğlu M, Katılmış Y (2025) New records for the association between eulophid parasitoids (Hymenoptera: Chalcidoidea: Eulophidae) and gall wasps (Hymenoptera: Cynipoidea: Cynipidae and Diplolepididae). Turk J For. 26: 20-24.
61. Michell CT, Nyman T (2021) Microbiomes of willow-galling sawflies: effects of host plant, gall type, and phylogeny on community structure and function. Genome 64:615–626
62. Nobrega LP, Marquesine RR, Gonçalves PHP, Maia VC, Oliveira DC, Ferreira BG (2025) Synchrony strategies of six gall inducers that share a superhost, *Eugenia copacabanensis* (Myrtaceae). Plant Biol. 28: 225-239.
63. O'Brien TP, McCully ME (1987) The study of plant structure principles and selected methods. Termarcar phi Pty. Ltd., Melbourn.
64. Oksanen J, Kindt R, Legendre P, O'Hara B, Stevens MHH, Oksanen MJ, Suggests MASS (2007) The vegan package. Community ecology package 10:631–637
65. Oliveira DC, Mendonça Jr MS, Moreira ASFP, Lemos-Filho JP, Isaias RMS (2013) Water stress and phenological synchronism between *Copaifera langsdorffii* (Fabaceae) and multiple galling insects: formation of seasonal patterns. J Plant Interact 8:225–233
66. Oliveira DC, Isaias RMS, Fernandes GW, Ferreira BG, Carneiro RGS, Fuzaro L (2016) Manipulation of host plant cells and tissues by gall-inducing insects and adaptive strategies used by different feeding guilds. J Insect Physiol 84:103–113
67. Oliveira DC, Isaias RMS (2025) Plant galls: structure and function. Springer 465p.
68. Pereira MCA, Cordeiro SZ, Araujo DSDD (2004) Estrutura do estrato herbáceo na formação aberta de Clusia do Parque Nacional da Restinga de Jurubatiba, RJ, Brasil. Acta Bot Bras 18:677–687

69. Pfeffer L, Rezende UC, Barônio GJ, de Oliveira DC (2018) Building two houses on a single host plant: galling insect synchronizes its life cycle with plant phenology. Oecol Aust 22.
70. Price PW, Fernandes GW, Waring GL (1987) Adaptive nature of insect galls. Environ Entomol 16:15–24
71. Raether C (2021) Vigor, phenology, and genotype: how plant traits explain variation in herbivory on *Quercus lobata*. Doctoral dissertation, California State University, Chico
72. Rezende UC, Cardoso JCF, Kuster VC, Gonçalves LA, Oliveira DC (2019) How the activity of natural enemies changes the structure and metabolism of the nutritive tissue in galls? Evidence from the Paleomystella oligophaga (Lepidoptera)–Macairea radula (Melastomataceae) system. Protoplasma 256:669–677
73. Rezende UC, Cardoso JCF, Hanson P, Oliveira DC (2021) Gall traits and galling insect survival in a multienemy context. Rev Biol Trop 69:291–301
74. Rezende UC, Cardoso JCF, Gonçalves PHP, de Oliveira DC (2023) The multitrophic system configuration on galls of *Macairea radula* (Melastomataceae) induced by *Paleomystella oligophaga* (Lepidoptera) depends on abiotic events. Int J Trop Insect Sci 43:2095–2104
75. Rohfritsch O (1992) Patterns in gall development. In: Shorthouse JD, Rohfritsch O (eds) Biology of insect-induced galls. Oxford University Press, New York, pp 60–86
76. Rodarte AT, Benevides CR, Moreira MM, Greco AV, Leão LCS, de Oliveira P, de Lima HA (2022) Flowering phenology in a restinga community: seven years of study. In: Aspects of Brazilian floristic diversity: from botany to traditional communities. Springer International Publishing, Cham, pp 3–45
77. Ronquist F, Liljeblad J (2001) Evolution of the gall wasp-host plant association. Evolution 55:2503–2522
78. Santos ATBD, Moitinho RR, Lima VP, Calado DC (2024) From leaves to inflorescences: gall induction of Iatrophobia brasiliensis Rübsaamen, 1915 on inflorescences of Manihot caerulescens Pohl (Euphorbiaceae) during the dry season. Rev Bras Entomol 67:e20230055
79. Santos-Silva J, Santos G, Santos J (2022) Soils and seasonality influence the richness of gall-inducing insects and their host plants in a tropical dry forest. J Arid Environ. 196: 104651
80. Scarano FR (2002) Structure, function and floristic relationships of plant communities in stressful habitats marginal to the Brazilian Atlantic rainforest. Ann Bot 90:517–524
81. Schultz JC, Edger PP, Body MJA, Appel HM (2019) A galling insect activates plant reproductive programs during gall development. Scientific reports **9**: 1833.
82. Shorthouse JD, Rohfritsch O (eds) (1992) Biology of insect-induced galls. Oxford University Press, New York
83. Silva MMD, Boeger MRT, Melo JCFD, Santos BFSA (2017) Antiherbivory defense mechanisms along an environmental gradient in restinga. Acta Bot Bras 31:583–596
84. Skuhravý V, Skuhrava M, Brewer J (1996) Some survival adaptations of gall-inducing midges (Dipt., Cecidomyiidae). J Appl Entomol 120: 237-239

85. Soares E, de Oliveira A, Lima V, Calado D (2021) Insect galls associated with *Copaifera sabulicola* J.A.S. Costa & L.P. Queiroz (Fabaceae): characterization and new records. Rev Bras Entomol. 65: 4
86. Takeda S, Yoza M, Ueda S, Takeuchi S, Maeno A, Sakamoto T, Kimura S (2024) Exploring the diversity of galls on Artemisia indica induced by *Rhopalomyia* species through morphological and transcriptome analyses. Plant Direct 8: e619
87. Tooker J, Helms A (2014) Phytohormone dynamics associated with gall insects, and their potential role in the evolution of the gall-inducing habit. J Chem Ecol 40:742–753
88. Weis A, Walton R, Crego CL (1988) Reactive plant tissue sites and the population biology of gall makers. Ann. Re. Entomol. 33: 467-486.
89. Wainwright CE, Volponi SN, Stepanian PM, Reynolds DR, Richter DH (2022) Using cloud radar to investigate the effect of rainfall on migratory insect flight. Methods Ecol Evol 13:2506–2518
90. Yukawa J (2000) Synchronization of galls with host plant phenology. Popul Ecol 42:105–113
91. Yukawa J, Kim W (2021) A new and a described species of Cystiphora (Diptera: Cecidomyiidae) from Japan, with reference to geographically diversified intraspecific populations of C. taraxaci and its host range expansion from native Japanese to alien and hybrid species of Taraxacum (Asteraceae). Entomol Sci 24:241–257

Figures

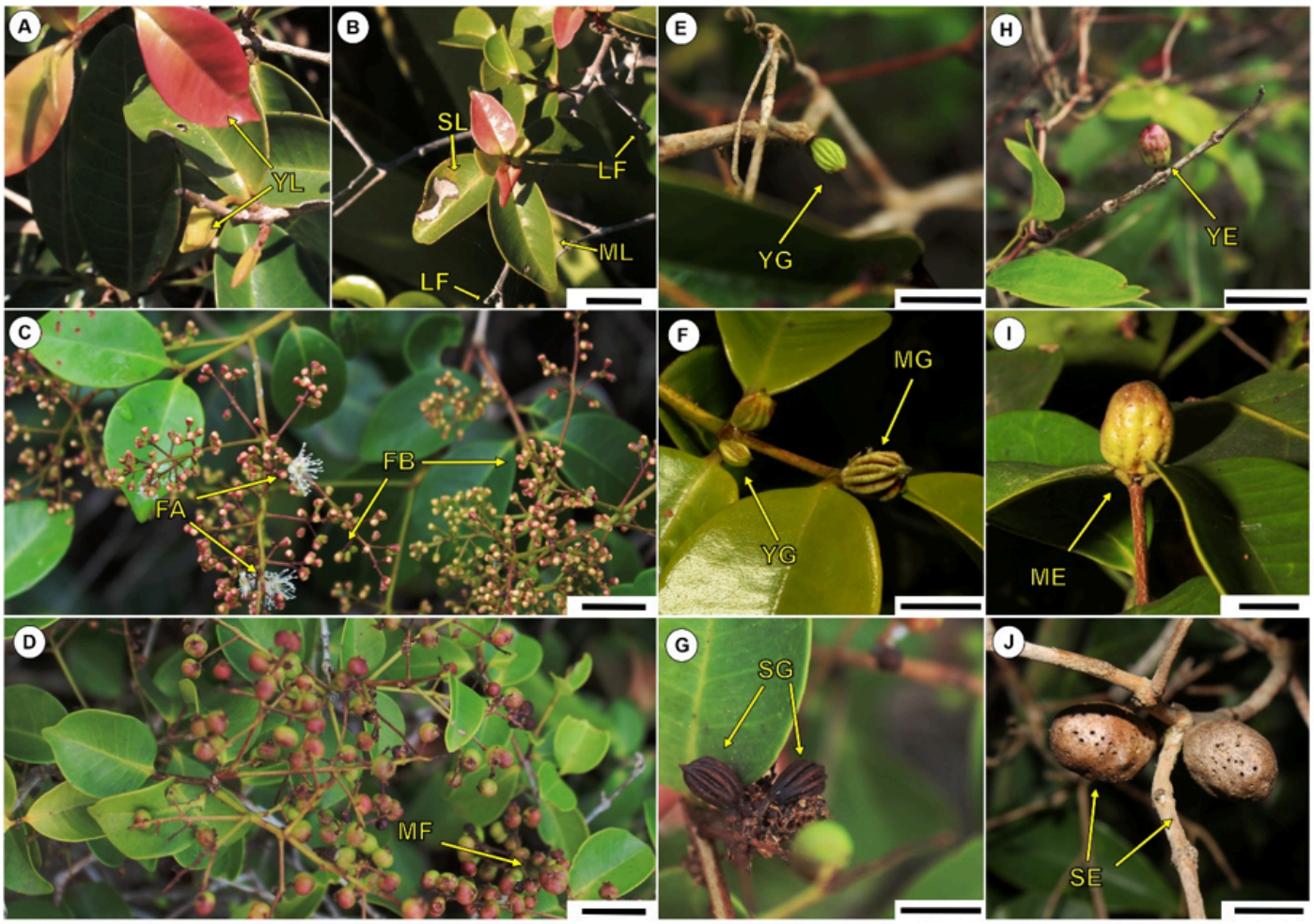


Figure 1

Myrcia ovata vegetative phenophases, *Myrciamyia maricaensis* galls and hypergaller inquiline endogalls. (A-B) Young and mature leaves – scale bar: 1cm. (C) Floral buds and floral anthesis – scale bar: 1.3cm. (D) Mature fruits – scale bar: 2cm. (E) Young *M. maricaensis*Gall – scale bar: 1cm. (F) Mature *M. maricaensis* Gall – scale bar: 1cm. (G) Senescent *M. maricaensis* galls; scale bar: 1cm. (H) Eulophid Young Endogall – scale bar: 1.5cm. (I)Eulophid Mature Endogall – scale bar: 1cm. (J) Eulophid Senescent Endogall – scale bar: 1cm.

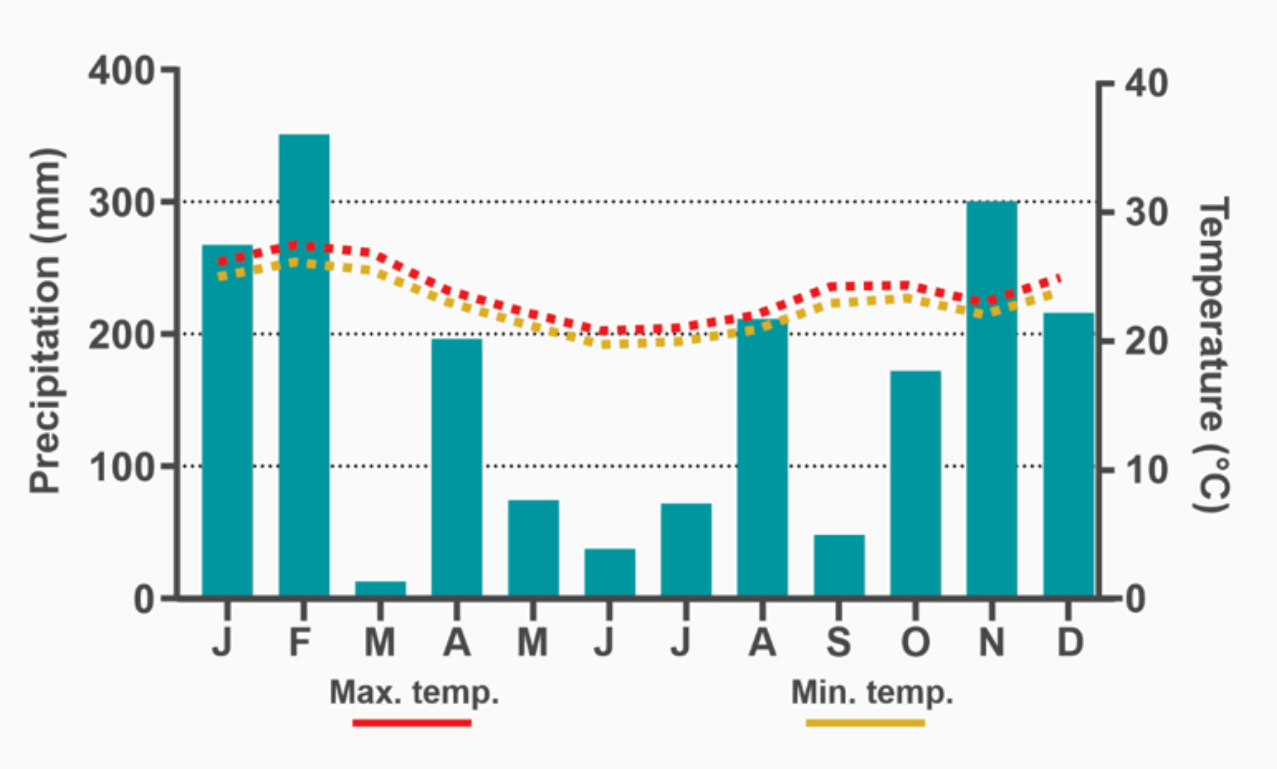


Figure 2

Climogram of monthly climatic data for the study area from 2018-2023. The red line indicates the average maximum temperature, and the green line indicates the average minimum temperature (°C). The black bars indicate the total monthly precipitation (mm). Data were obtained from INMET (2018–2023) via an automatic station (A667) in Saquarema, Sampaio Correa, Rio de Janeiro, Brazil.

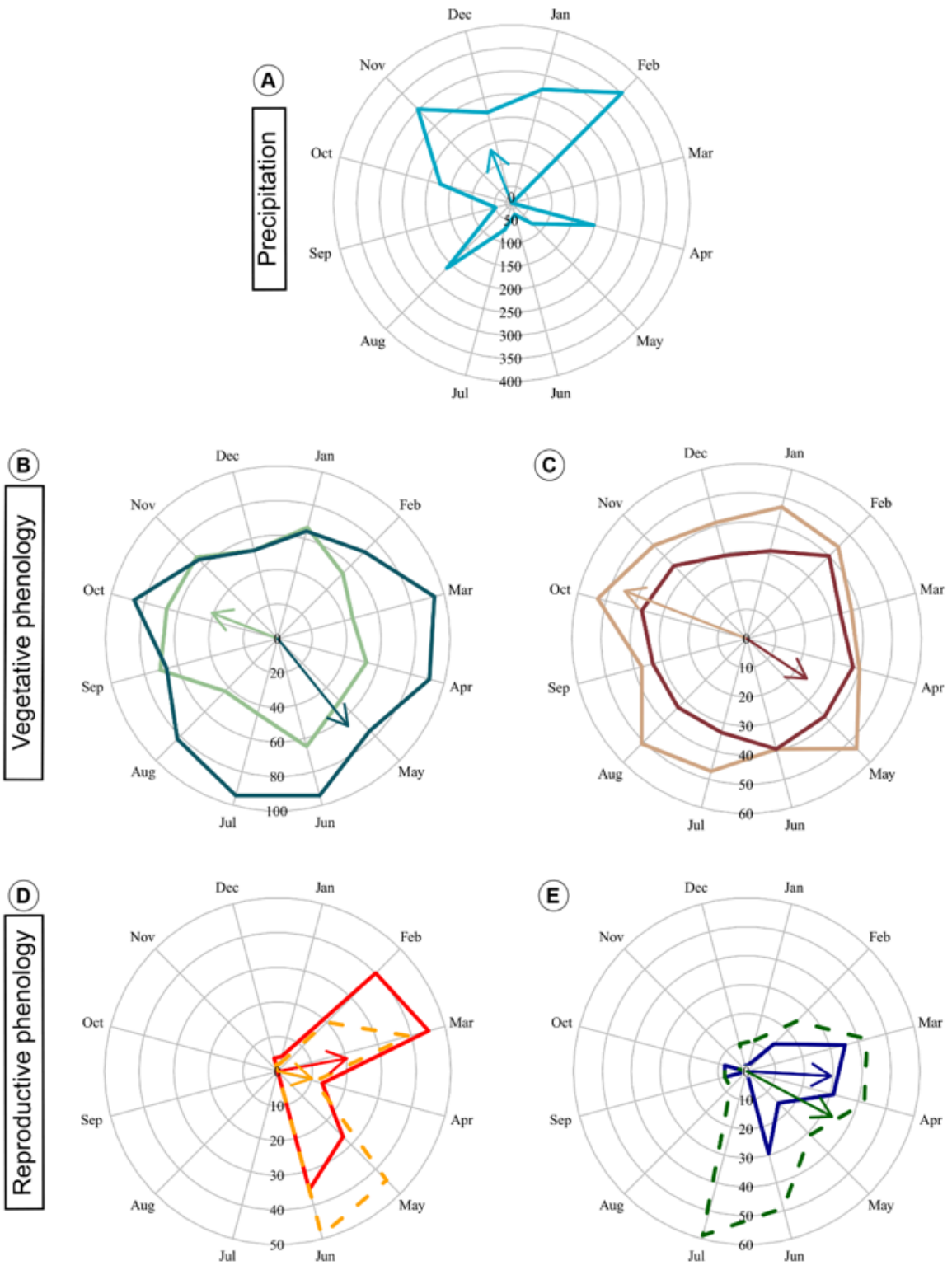


Figure 3

Circular analysis of phenological features from November 2022 to October 2023. (A) Circular analysis of precipitation rates. The solid light blue line indicates precipitation during the 1-year phenological study. The values indicate the monthly precipitation in millimeters. (B–C) Circular analysis of intensity indices of vegetative phenophases: young and mature leaves, leaf senescence and leaf fall of *Myrcia ovata*. The solid light green line represents young leaves; the solid dark green line represents mature leaves; the

solid brown line represents leaf fall; and the solid beige line represents leaf senescence. The values are percentages of the intensity of each phenophase. (C-D) Circular analysis of intensity indices of reproductive phenophases: floral bud, floral anthesis, immature fruit and mature fruit of *Myrcia ovata*. The solid red line represents floral buds; the dotted orange line represents floral anthesis; the solid marine blue line represents immature fruits; and the dotted dark green line represents mature fruits. The values are percentages of the intensity of each phenophase.

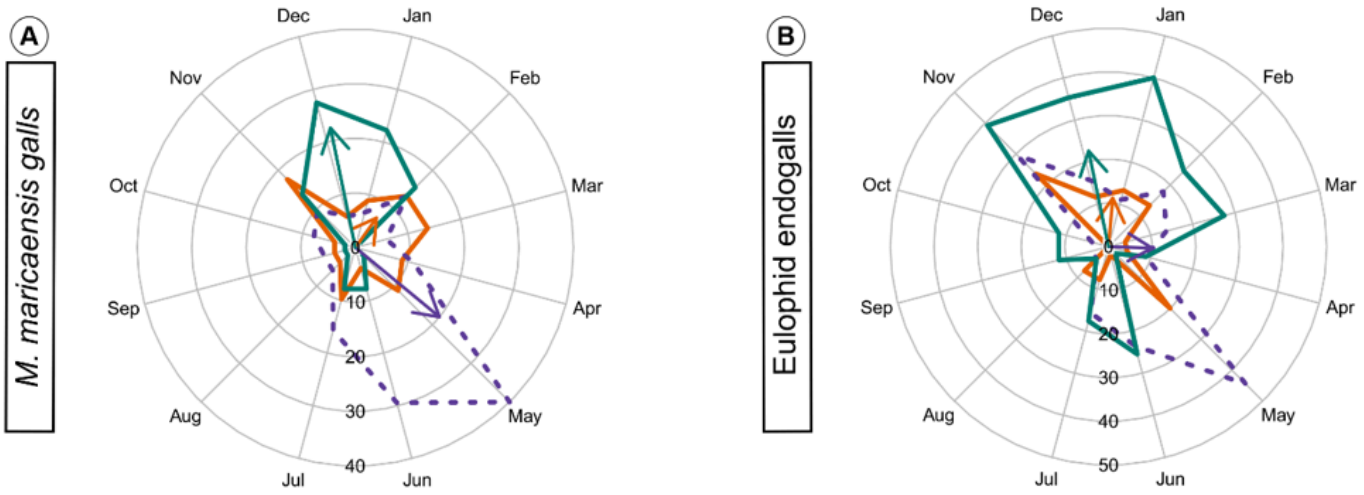


Figure 4

Circular analysis of the life cycle intensity indices of *M. maricaensis* galls and inquiline endogalls on *M. ovata* from November 2022 to October 2023. (A) Year-round galls induced by *M. maricaensis*. (B) Year-round eulophid endogalls. The solid orange line represents the young stage; the solid green line represents the mature stage; and the dotted purple line represents the senescent stage. Arrows indicate the subsequent medium vector of occurrence. The values are percentages of the intensity of each phenophase.

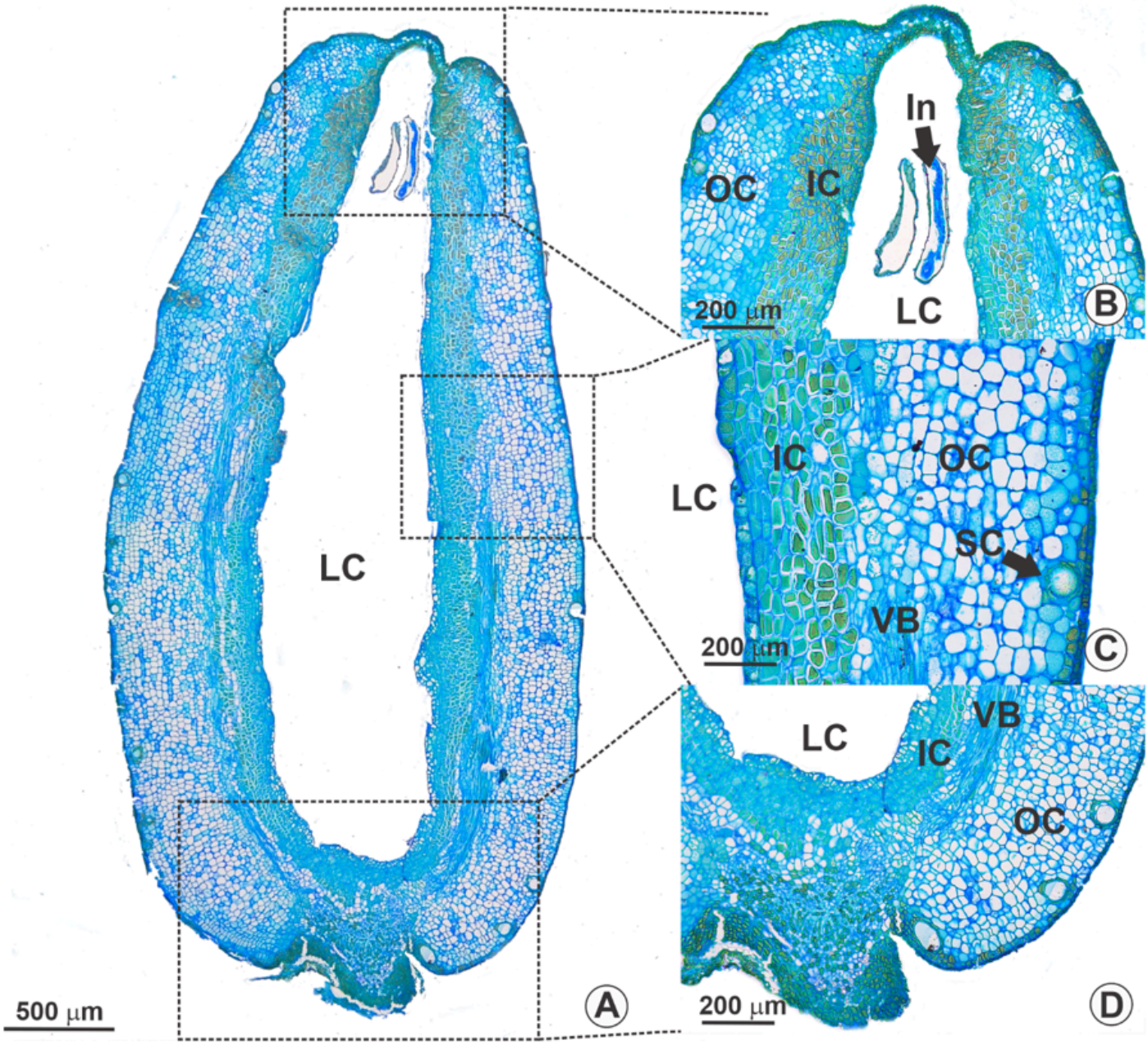


Figure 5

Histology of mature galls of *Myrcia ovata* (Myrtaceae) induced by *Myrciamyiia maricaensis*(Diptera: Cecidomyiidae) without the endogall, longitudinal sections. A – Overview of the gall, showing a single larval chamber; B – Apical portion of the gall, well vascularized; C – Gall cortex, composed of outer and inner cortices; D – basal portion of the gall, attached to the host plant stem. *Abbreviations:*IC- Inner cortex; In – Inducer; LC- Larvar chamber; OC- Outer cortex; SC- Secretory cavity; VB- Vascular bundle.

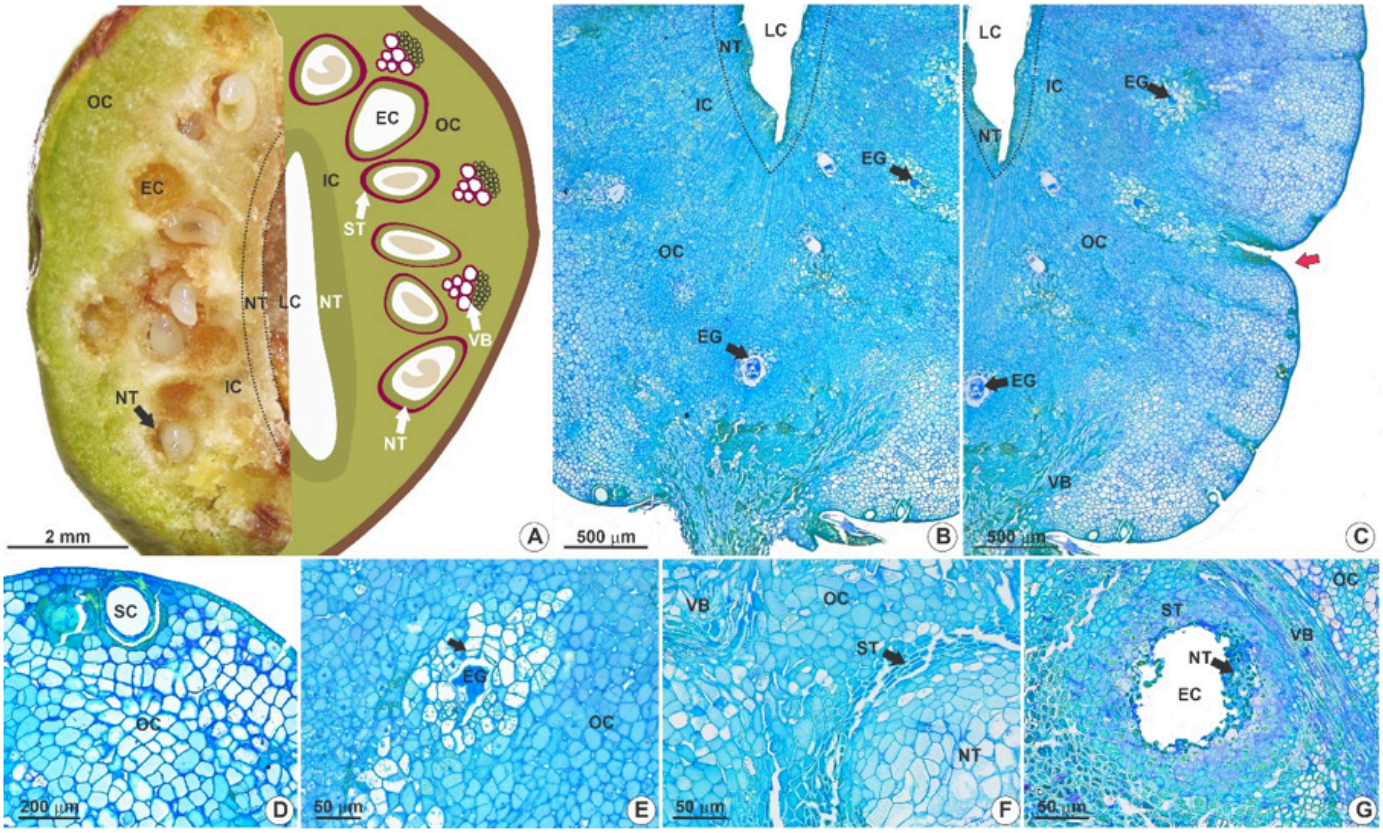


Figure 6

Histology of Eulophid endogalls induced on *M. maricaensis* gall cortex. A – Scheme overview of the endogalls within the gall cortex; B – Gall cortex with multiple endogalls present on the basal portion. C – Invaginations (red arrow) in the lateral portion of the gall; D – Uniseriate epidermis and secretory cavities; E – Close-up of an endogall surrounded by recent cell divisions (arrow); F – Vascularization near the endogall; G – Endogall with the presence of neoformed tissues. *Abbreviations:* OC – Outer Cortex; IC – Inner Cortex; LC – Larval Chamber; EC – Endogall chamber; EG – Endogall; SC – Secretory cavity; VB – Vascular bundle; NT – Nutritive Tissue; ST – Sclerenchymatic Tissue.