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Short communication

Rainfall increases gall morphological metrics in the Brazilian Cerrado

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ABSTRACT

The shape of the galls can be adjusted by factors such as natural enemies and climatic conditions to which the galls are subjected. Studies that evaluate the climatic regime as a possible driver of the morphology of galls are scarce and do not consider Neotropical systems. To fill this knowledge gap, this study evaluated the influence of seasonality on the morphology of Cecidomyiidae galls induced on *Myrcia neoobscura* (Myrtaceae) growing in the Cerrado of Bahia. A total of 270 galls were sampled during the dry and rainy seasons. The average monthly precipitation was obtained. The galls were refrigerated, weighed, and measured within 72 h of collection. The average weight of the galls ranged from 0.106 g to 0.780 g; while, the volume ranged from 35.07 mm^3 to 726.70 mm^3 and the surface area from 20.03 mm^2 to 719.57 mm^2. The average weight, volume, and surface area of galls were approximately 50% higher during the rainy season than during the dry season. These three variables were also positively related to average precipitation. These results support the hypothesis that seasonality may contribute to the observed variation in the final shape of galls on a local scale.

Keywords: Cecidomyiidae, leaf gall, insect-plant interaction, Myrtaceae, Semiarid

Galls are examples of extended phenotypes of gall-inducing insects, composed of host plant tissues whose development is completely controlled by the genes of their inducers (Stone & Schönrogge 2003). Because of the intimate contact with plant cells, the gall-inducing insect complex changes the structure and physiology of its host plants, inducing morphological modification in the host plant tissue (Miller III & Raman 2019). Insect galls vary enormously in complexity, and generally possess tissue types that are absent in host plants without galls (Raman 2011; Isaias *et al.* 2013). The formation of galls has a high adaptive value for inductors because of the nutritional and microenvironmental improvements and protection conferred against their natural enemies (Miller III & Raman 2019).

Many insects, approximately 13,000 species from different orders (Hemiptera, Thysanoptera, Coleoptera, Hymenoptera, Lepidoptera, and Diptera) induce galls. These structures represent microhabitats that support relatively closed communities of specialized inhabitants within which insects develop. Galls are distinguished from other insect-generated shelters (*e.g.*, rolled leaves) by their involvement in the differentiation and growth of plant tissues (Shorthouse & Rohfritsch 1992; Cornelissen *et al.* 2016).

The shape of the galls can be adjusted by different abiotic factors, such as natural enemies, the physical and climatic

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conditions of the habitat to which the galls are subjected (Woods *et al.* 2021), or by aspects of their physiology and demography (Shorthouse & Rohfritsch 1992; Williams 1994; Stern 1995; Ferreira *et al.* 2019). Although the importance of these factors has been demonstrated, explaining the variation in gall shape remains a major challenge imposed by the large number of galling insect-host plant systems and little empirical support for the influence of these factors on gall shape (Stone & Schönrogge 2003). For example, the only study that evaluated the effect of climatic regime as a possible driver of gall morphology was conducted in Spain (Gil-Tapetado *et al.* 2020).

In this study, we tested the hypothesis that seasonality, including temperature and precipitation, may contribute to the observed variation in the shape of galls based on their weight, volume, and surface area, for the following reasons: the drastic seasonal climatic changes that occur in the Cerrado affect the interactions between galling insect and host plants, consequently resulting in variations in gall morphology (Gehring *et al.* 2020; Gil-Tapetado *et al.* 2020). For example, in the dry season in the Cerrado, the hydrothermal stress that the host plant undergoes results in more stressed tissues, limiting the ability of gall-inducing insects to shape the galls, resulting in galls with smaller sizes and volumes.

The population of *Myrcia neoobscura* E.Lucas & C.E.Wilson (Myrtaceae), studied during 2022 and 2023, hosts Cecidomyiidae-induced galls and is found in a cerrado area of the Chapada Diamantina National Park, in the municipality of Lençóis, Bahia, Brazil (Fig. 1A-B). The individuals studied were found along a trail (12º34'17" S 41º23'24" W) at 371m altitude. The characteristic climate of the region is mesothermal, of the Cwb type, with maximum rainfall occurring in the summer (November, December, and January) (Alvares *et al.* 2013). Between August and November, there is a markedly dry season. The average annual precipitation is above 1,000 mm, and the average annual temperature ranges from 22 °C to 25 °C, with average annual lows of approximately 15 °C (Funch *et al.* 2002).

Myrcia neoobscura is a subshrubby species (Fig. 1C) endemic to Brazil, where it grows in the Northeast (Bahia), Southeast (Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo), and South (Paraná, Santa Catarina) regions (Santos *et al.* 2023). Its leaves are simple, lanceolate, glabrous, and emerge in pairs from the apical meristem. It is a perennial plant that loses few leaves throughout the year (Bencke & Morellato 2002). The months with a greater intensity of leaf fall were concentrated during the rainy season. In the same season, sprouting of new leaves, blooming, and fruiting occur at the end of the season. On their leaves, conical, green or yellowish, unilocular galls clustered on the abaxial leaf surface (Figs. 1D-F, 2) and were induced by an unidentified species of Cecidomyiidae (Diptera). Their larval chambers are relatively narrow and house a single inducer. To our knowledge, this is the first report of gall occurrence in *M. neoobscura*.

To evaluate the influence of seasonality on gall shape, 270 leaf galls induced on three adult *M. neoobscura* individuals ranging in height from 1.20-1.30 m were randomly collected from five to seven leaves of each plant during the dry period of 2022 (April, May, and June) (n=15 galls per individual; n=45 galls per month/total 135) and the rainy periods of 2022 and 2023 (November, December, and January) (n=15 galls per individual; n=45 galls per month/total 135). The average temperature (°C) and precipitation (mm) for each month were obtained from the climatic station of Lençóis municipality (INMET 2023). Only galls without fungi, parasitoids, or damage caused by herbivorous insects were considered in this study. Moreover, only fresh, completely formed and grown greenish galls were collected to minimize the effect of growth stage differences. Galls (n=45 galls per month/total 270) and leaves collected (n=10 leaves per month/total 60) were fully formed and grown to minimize the effect of developmental stage on gall size. The collected samples were transported in sealed, properly labeled plastic bags under refrigerated conditions. In the laboratory, galls were characterized morphologically, measured, and weighed. Some of the samples were dissected to describe their internal structure and obtain immature insects, whereas others were dissected to obtain adult insects and associated fauna. The pots were inspected daily to check for hatching of adult insects.

To identify gall-inducing insects, the immature forms were collected, conditioned in microcentrifuge tubes, and fixed in 70% ethanol. The samples were sent to the Diptera Laboratory at the Universidade Federal do Rio de Janeiro, Museu Nacional, Brazil.

To obtain the individual mass of the galls (fresh weight), each gall was detached from the leaf and weighed using an electronic scale for no more than 72 h under refrigerated conditions (Gil-Tapetado *et al.* 2020). To estimate the volume and surface area of each gall, the following equations were used.

$$Volume = \frac{4}{3} x \pi x r1 x r2 x r3$$

$$Surface\ area = 4\pi \sqrt[p]{\frac{r1^p r2^p + r1^p r3^p + r2^p r3^p}{3}}$$

Where r1, r2 and r3 are the radii along the three planes of the gall (length and two measurements of right angle for width) measured using a caliper (Alca, Pie de Rey 150 mm) and ∏= 1.6075 (Cooper & Rieske 2010; Gil-Tapetado *et al.* 2020).

Linear mixed models (LMM; lmer function) were used to compare the effects of seasonality (dry *vs.* rainy) on the weight, volume, and surface area of Cecidomyiidae-induced galls in *M. neoobscura*. These three variables were square root transformed (sqrt) to assume normality of the analyses. For each model, seasonality was considered a fixed explanatory

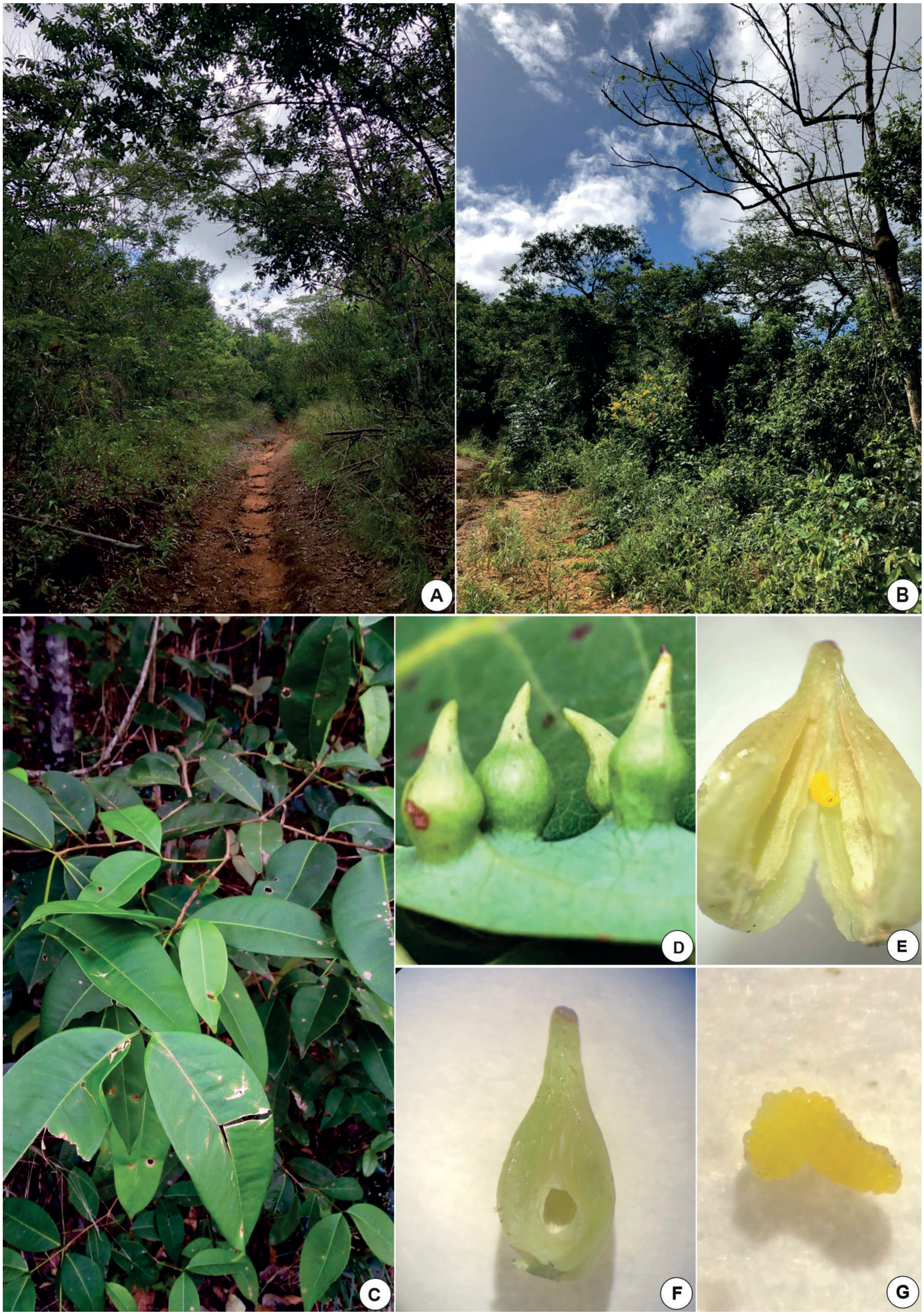

Figure 1. Cecidomyiidae galls induced on *Myrcia neoobscura* E. Lucas & C. E. Wilson (Myrtaceae) in Cerrado areas of the Parque Nacional da Chapada Diamantina, Lençóis, Bahia, Brazil. **A-B.** Areas sampled during rainy (A) and dry (B) seasons. **C.** Detail of branches. **D.** Conical galls. **E.** Detail of the gall with the inducing insects inside. **F.** Detail of the single larval chamber. **G.** Young larva of Cecidomyiidae. Photos: Gabriela Bomfim.

variable, whereas plants and replicates were included as random factors. To investigate the relationship between average monthly precipitation (fixed explanatory variable) and the weight (sqrt), volume, and surface area (sqrt) of the galls (response variables), LMM was used with plants and replications as random factors. The average temperature was not evaluated in this study because it correlated with precipitation during the sampled months (r_s = 0.71).

All analyses were performed in the R statistical program (R Core Team 2020). The packages, lme4 (Bates *et al.* 2015) and lmerTest (Kuznetsova *et al.* 2017), MuMIn (Barton & Barton 2015), and DHARMa (Hartig 2021) were used to perform the mixed models, significance (*p*-value), coefficient of determination (R^2), and diagnostic assumptions of the models.

No species other than Cecidomyiidae were present on the sampled plants. In the dry period, their average weight ranged from 0.106 g to 0.408 g, volume from 35.07 mm^3 to 427.40 mm^3 and surface area from 20.03 mm^2 to 277.40 mm^2. While, in the rainy season the weight ranged from 0.265 g to 0.780 g, the volume from 27.40 mm^3 to 726.7 mm^3 and the surface area from 60.91 mm^2 to 719.57 mm^2. The analyses indicated that the weight, volume, and surface area of the galls varied between the seasons (Figs. 2B-C, 3A-C), demonstrating that seasonality had an important effect on the weight and morphometry of Cecidomyiidae galls induced in *M. neoobscura*. The weight of the galls was, on average, almost 50% higher during the rainy season compared to the dry season (t_{264} = 5.59, p = 0.005; mean ± standard deviation: rainy: 0.68 ± 0.08 g; dry: 0.46 ± 0.07 g) (Fig. 3A). Similarly, significantly higher values of 50% were observed during the rainy season for their volume (t_{264} = 5.23, p = 0.006; rainy: 17.29 ± 4.19 mm^3; dry: 10.46 ± 2.68 mm^3) and surface area (t_{264} = 4.21, p = 0.014; rainy: 16.23 ± 3.56 mm^2; dry: 11.02 ± 2.11 mm^2) (Fig. 3B-C). Moreover, the results indicated that precipitation positively increased weight (t_{264} = 3.56, R^2 = 0.52, p = 0.024), volume (t_{264} = 5.17, R^2 = 0.45, p = 0.006) and area (t_{264} = 5.31, R^2 = 0.46, p = 0.006) during gall development (Fig. 3D-F).

There is a wide diversity of gall shapes in the Neotropics, ranging from pits or open folds to structures that completely surround insects (Isaias *et al.* 2013). The morphology of the gall is an important predictor of gall-inducing insect survival and is the result of environmental adaptation of gall-inducing insects (Price *et al.* 1987; Stone & Schönrogge 2003). This hypothesis is widely accepted as a selective advantage for galls (Cornell 1983; Ferreira *et al.* 2019; Miller III & Raman 2019). Other factors may affect the morphological characteristics of galls, such as differences in the age and population density of the host plants, years of settlement of the galls in the area (Bonsignore & Bernardo 2018; Gil-Tapetado *et al.* 2020), and the number of eggs laid by females on the host organ (Panzavolta *et al.* 2012; 2013; Bernardo *et al.* 2013; Gil-Tapetado *et al.* 2020), stage of gall development, age of the host organ at the time of oviposition (Oliveira & Isaias 2009), and sex of the gall inducer (Gonçalves *et al.* 2009). In this study, we demonstrated the effect of climate on gall morphology, where Cecidomyiidae leaf galls induced on *M. neoobscura* individuals growing in Cerrado environments responded to seasonality, with up to a 50% increase in weight, volume, and surface area during the rainy season. These increments were also positively associated with precipitation. Dry and hot environments with high temperatures, such as those in the Cerrado, promote special adaptations in insects, including galling (Fernandes *et al.* 1995; Gonçalves-Alvim & Fernandes 2001). The combination of low humidity and high temperature acts with potential hydrothermal stress in the dry season and is probably one of the physiological factors affecting host plant vigor, and consequently, the

Figure 2. Detail of the leaf of *Myrcia neoobscura* E. Lucas & C. E. Wilson (Myrtaceae), showing of Cecidomyiidae galls induced on abaxial surface. **A.** leaf. **B.** Gall sampled during rainy (**B**) and dry (**C**) seasons. Scale bar = 2mm. Photos: Gabriela Bomfim.

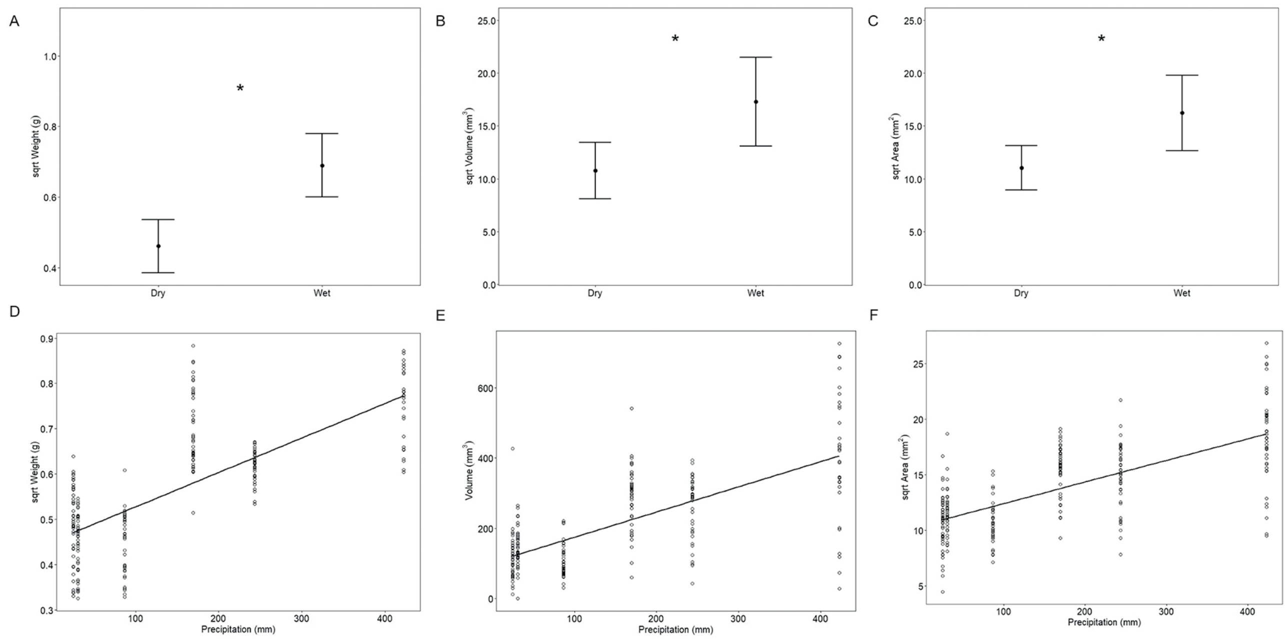

Figure 3. Statistical analyses of seasonality of Cecidomyiidae galls induced in *Myrcia neoobscura* E. Lucas & C.E. Wilson (Myrtaceae). **A-C:** Mean ± Standard Deviation indicating the variation in (A) weight (g), (B) volume (mm^3) and (C) area (mm^2) in relation to the wet season (November, December and January) and the dry season (April, May and June). The legend '*' indicates $p < 0.05$. **D-F:** Significant and positive relationship ($R^2 > 0.45$, $p < 0.01$) between precipitation (mm) and (D) weight, (E) volume and (F) area of galls.

morphology of galls (Askew 1961; Weis 1982a; b; Weis & Abrahamson 1985; 1986).

Periods of higher humidity provide better environmental conditions for the development of the host plant, allowing for better development and modification of the morphological characteristics of the Cecidomyiidae gall, as observed in this study. According to Araújo and Santos (2009), as the arrival of rainfall favors the sprouting of new leaves and branches in the Cerrado. Under these conditions, resource availability is higher, favoring the colonization of gall-inducing insects and an increase in the abundance of galls. Therefore, under these conditions, gall size is not limited by climate, given the favorable conditions for gall growth and development, but may result from an adaptation of the gall-inducing insect to wetter environments, adding evidence supporting the adaptive significance of galls as advocated by the microenvironment hypothesis (Price *et al.* 1987; Stone *et al.* 2002), although other factors may also affect gall morphology, such as differences in plant age, evaluation period, and population density of the inducer (Gil-Tapetado *et al.* 2020).

Seasonality is directly related to insect abundance (Kishimoto-Yamada & Itioka 2015). Seasonal changes directly influence host plant development, and thus alter the quantity and quality of nutrients offered to galling insects. The only study that evaluated the climatic regime as a possible driver of gall morphology induced by *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae), on chestnut trees (*Castanea* spp.), was conducted in Spain (Gil-Tapetado *et al.* 2020). The authors observed the effect of climate on *D. kuriphilus* growth and found that the mass and volume of galls followed a pattern that could be associated with a climatic line. In particular, the gall from the south of the country was larger than the other samples and occurred under more favorable conditions, with high rainfall and rare drought, which corroborates our results. One of the factors that may justify such pattern is that seasonal changes also influence host plant development and consequently affect the quantity and quality of nutrients offered to gall-inducing insects (Araújo & Santos 2009). Further studies, encompassing other gall-inducing insect-host plant systems, are crucial to investigate the influence of these factors on the morphological characteristics of galls and to determine the patterns of responses of gall-inducing insects to these variations at different scales.

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