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Leaf asymmetry and the presence of insect galls on three plant species in a phytophysiognomy of Atlantic Forest

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ABSTRACT

The relationship among three plant species, their respective gallers and leaf asymmetry were evaluated in restinga habitat of the Environmental Protected Area of Maricá, RJ, Brazil. Fieldwork was done in February 2017, when 20 leaves, ten galled and ten ungalled, were sampled from each of ten haphazardly-chosen individuals of each plant species. Fluctuating asymmetry was not found in the present study, but all species showed antisymmetry (AS). *Clusia lanceolata* Cambess. and *Maytenus obtusifolia* Mart. also exhibited directional asymmetry (DA). AS and DA are predicted in populations under high levels of stress. The galled leaves of *C. lanceolata* and *Eugenia astringens* Cambess. exhibited higher leaf asymmetry than did ungalled leaves, showing that galls increase leaf asymmetry or that galling insects select more asymmetric leaves. No differences in asymmetry were found between galled and ungalled leaves of *M. obtusifolia*, which may indicate that this plant tolerates and minimizes the presence of galling insects, showing a high capacity for homeostasis in face of biotic stress. The abundance of galling insects was significantly higher in *E. astringens* than in *M. obtusifolia* and *C. lanceolata*, possibly because the leaves of *M. obtusifolia* exhibit greater sclerophilia and *C. lanceolata* possesses latex, characteristics that make herbivory difficult.

Keywords: antisymmetry; directional asymmetry; fluctuating asymmetry; galling insects; Restinga.

Assimetria foliar e a presença de galhas de insetos em três espécies de plantas em uma fitofisionomia da Mata Atlântica

RESUMO

A relação entre três espécies de plantas, suas respectivos galhadores e assimetria foliar foi avaliada no habitat de restinga da Área Protegida Ambiental de Maricá, RJ, Brasil. O trabalho de campo foi realizado em fevereiro de 2017, quando 20 folhas, dez galhadas e dez não galhadas, foram amostradas de cada um dos dez indivíduos escolhidos ao acaso para cada espécie de planta. A assimetria flutuante não foi encontrada no presente estudo, mas todas as espécies apresentaram antissimetria (AS). *Clusia lanceolata* Cambess. e *Maytenus obtusifolia* Mart. também exibiram assimetria direcional (DA). AS e DA são previstos em populações sob altos níveis de estresse. Folhas galhadas de *C. lanceolat*a e *Eugenia astringens* Cambess. exibiram maior assimetria do que folhas não galhadas, mostrando que as galhas aumentam a assimetria das folhas ou que os insetos galhadores selecionam mais folhas assimétricas. Não foram encontradas diferenças na assimetria entre folhas galhadas e não galhadas de *M. obtusifolia*, o que pode indicar que esta espécie tolera e minimiza a presença de insetos galhadores, mostrando alta capacidade de homeostasia diante de um estresse biótico. A abundância de insetos galhadores foi significativamente maior em *E. astringens* do que em *M. obtusifolia* e *C. lanceolata*, possivelmente porque as folhas de *M. obtusifolia* apresentam maior esclerofilia e *C. lanceolata* possui látex, características que dificultam a herbivoria.

Palavras-chave: antissimetria, assimetria direcional, assimetria flutuante, insetos galhadores, Restinga.

Introduction

Galls can affect leaf development and cause asymmetry, which can be evaluated by differences in leaf morphometry between galled and ungalled leaves (SANTOS et al., 2003). A variety of types of asymmetry are known, including fluctuating asymmetry (FA), antisymmetry (AS) and directional asymmetry (DA). Among these, FA is the most common estimate of development instability (GRAHAM et al., 2010; SANTOS et al., 2003), since it measures the imperfect growth of supposed bilateral structures. Large deviations are reflected in a bimodal distribution of leaf side area (i.e. a population can have leaves of both increased left and increased right sides), while DA is present when one leaf side is invariably greater than the other (GRAHAM et al., 1998). According to Cornelissen and Stiling (2005), asymmetric leaves tend to have greater nutritional quality and fewer defensive compounds compared to symmetric leaves (CORNELISSEN; STILING, 2011). These traits can be positively related to the presence of galls, since galling insects are known for their ability to manipulate plant metabolism in their favor (STONE; SCHÖNROGGE, 2003).

Although the several inventories of insect galls have been performed in the Neotropical Region, mainly in Brazil, including areas of Cerrado (such as MAIA; FERNANDES, 2004) and Atlantic Forest (MAIA et al., 2014; MAIA; MASCARENHAS, 2017), and less frequently in Caatinga (CARVALHO-FERNANDES et al., 2012), the Pantanal (URSO-GUIMARÃES et al., 2017) and the Amazon (ALMADA; FERNANDES, 2011; MAIA, 2011). Studies about the relationship between the presence of galls and leaf asymmetry are very scarce for Brazil. To date, only four publications are known, three developed in areas of Cerrado in the state of Minas Gerais, Southeast of Brazil (ALVES-SILVA, 2013; SANTOS et al., 2013; SANTOS et al., 2017) and one in an area of Atlantic Forest in the state of Pernambuco, Northeast of Brazil (SILVA et al., 2015). The first study investigated this relationship between *Ditylenchus* Filipjev (Nematoda) galls and *Miconia fallax* DC. (Myrtaceae), the second and the third ones studies investigated *Schizomyia macropillata* Maia, 2005 (Cecidomyiidae: Diptera) galls and *Bauhinia brevipes* Vogel. (Fabaceae), while the fourth one analysed the relationships of the galls of an undescribed species of Cecidomyiidae and *Ouratea polygyna* Engl. (Ochnaceae).

In the present study, the relationship among three native Brazilian endemic plants, *Clusia lanceolata* Cambess. (Clusiaceae), *Eugenia astringens* Cambess. (Myrtaceae) and *Maytenus obtusifolia* Mart. (Celastraceae); their respective gallers, *Clusiamyia nitida* Maia, 1996, *Dasineura globosa* Maia, 1995 and *Mayteniella distincta* Maia, 2001; and leaf asymmetry were evaluated for an area of restinga in the state of Rio de Janeiro. These plants and their respective galls were chosen because they occurred in high frequency and were abundant (MAIA, 2001). All three galling species belong to the family Cecidomyiidae (Diptera) and induce conspicuous leaf galls.

Materials and Methods

Study area

Fieldwork was done in February 2017 at the Environmental

Protected Area of Maricá (22°52'-22°54'S and 42°48'-42°54'W), located in the municipality of Maricá, state of Rio de Janeiro, Southeast of Brazil (Figure 1). The area encompasses about 800 hectares (SILVA, 2011) of restinga vegetation comprising 379 plant species (SILVA; OLIVEIRA, 1989) and about 100 galling insect species (MAIA, 2001).

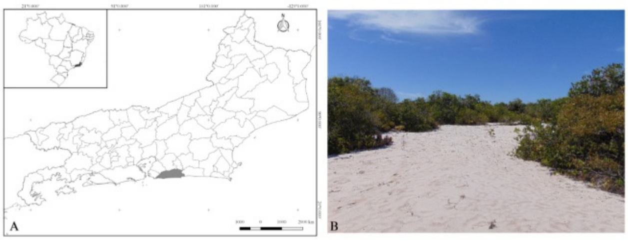

Figure 1. (A) Municipality of Maricá, state of Rio de Janeiro, Southeast Brazil. (B) Location of study area: Environmental Protected Area of Maricá. / **Figura 1.** (A) Município de Maricá, estado do Rio de Janeiro, Sudeste do Brasil. (B) Localização da área de estudo: Área Protegida Ambiental de Maricá.

Plant species

Clusia lanceolata Cambess., *Eugenia astringens* Cambess. and *Maytenus obtusifolia* Mart. (Fig. 2) are evergreen shrubs or small trees, with the first two occurring only in Atlantic Forest, while the third also occurs in Cerrado and Amazonian forest (FLORA DO BRASIL, 2020). *Clusia lanceolata* (Figure 2A) is very ornamental, especially when in flower, and so is often used in landscaping (LORENZI, 2009). It hosts two gall morphotypes, both on leaves, one induced by a lepidopteran, which occurs in low abundance and is rare, and the other induced by a gall midge, *Clusiamyia nitida* (Maia, 1996) (Diptera, Cecidomyiidae) (MAIA, 2013) (Figure 2B), which is very abundant, common and occurs throughout the entire year.

Eugenia astringens (Figure 2C) is also an ornamental species and possesses hypoglycemic properties (SILVA; PEIXOTO, 2009). It hosts seven gall morphotypes, five on leaves, one on buds and one on stems, the most common of which is induced on leaves by *Dasineura globosa* (Maia, 1995) (Cecidomyiidae) (Figure 2D).

Maytenus obtusifolia (Figure 2E) possesses anti-inflammatory properties and anti-ulcer effects. Additionally, its wood is used to make handles of tools and as firewood (http://museunacional.ufrj.br/hortobotanico/restinga/maytenusobtusifolia.html). This plant species hosts two gall morphotypes, one on fruit induced by *Bruggmanniella maytenuse* (MAIA; COURI, 1992) and the other on leaves induced by *Mayteniella distincta* (MAIA, 2013) (Figure 2F); the former is very rare while the latter is abundant and common (MAIA, 2001). Both galling species are gall midges (Cecidomyiidae).

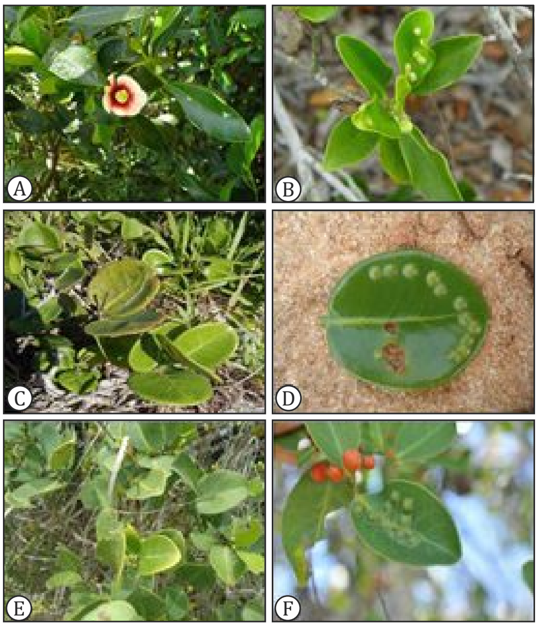

Figure 2. Plant and galling insect species. (A) *Clusia lanceolata* Cambess., (B) *Clusiamyia nítida* Maia, 1996, (C) *Eugenia astringens* Cambess., (D) *Dasineura globosa* Maia, 1995, (E) *Maytenus obtusifolia* Mart., (F) *Mayteniella distincta* Maia, 2001. / **Figura 2.** Plantas e espécies de insetos galhadores. (A) *Clusia lanceolata* Cambess., (B) *Clusiamyia nítida* Maia, 1996, (C) *Eugenia astringens* Cambess., (D) *Dasineura globosa* Maia, 1995, (E) *Maytenus obtusifolia* Mart., (F) *Mayteniella distincta* Maia, 2001.

Study design

Twenty leaves, ten galled and ten ungalled, were sampled from ten haphazardly-chosen individuals of each plant species. The widths of each lateral-side of the leaves were measured with a 0.5mm caliper. Differences between the left and right sides of the leaves were evaluated using a paired Students' *t*-test for each plant species. Based on the value of the right side (Rs) minus that of the left side (Ls), the occurrence of AS or FA was determined, as proposed by Palmer and Strobeck (1986). Differences significantly greater than zero indicate that one side is consistently wider than the other, representing directional asymmetry in the population. A mean difference between both sides of zero (Rs-Ls=0), indicates fluctuating asymmetry, which describes random and generally small departures from symmetry.

To verify AS, we checked the differences between the left and right sides of the leaves and then created a histogram. When data do not represent a normal distribution or a mesocurtic distribution, there is anti-symmetry in the population.

Statistical analysis

An important consideration in studies evaluating FA is the assessment of measurement error (PALMER, 1996). We estimated measurement error by re-measuring 10% (n = 10) of the leaves sampled per species and correlating the two measurements using an Index of Repeatability (FALCONER, 1981).

The data were log-transformed in order to seek normality, but figures show untransformed values for the sake of clarity. Comparison of leaf asymmetry and abundance of galls among species were performed using analyses of variance (ANOVA). A paired Student's *t*-test was used to compare leaf asymmetry of galled and ungalled leaves. The mean value of leaf asymmetry per plant was used in the statistical tests. All statistical analyses were performed using STATISTICA 12.

Results

The Index of Repeatability showed that the measurements of the three species were performed with sufficient precision, eliminating possible errors during leaf morphometry (*C. laceolata*: $IR = 0.945$, $p < 0.05$; *E. astringens*: $IR = 0.985$, $p < 0.05$; *M. obtusifolia*: $IR = 0.997$, $p < 0.05$). Fluctuating asymmetry was not found in the present study, while all species exhibited AS (*C. lanceolata*: $w = 0.939$, $p < 0.001$; *E. astringens*: $w = 0.967$, $p = 0.01$; *M. obtusifolia*: $w = 0.942$, $p < 0.001$). The species *C. lanceolata* (t= -3.24; p = 0.002) and *M. obtusifolia* (t = -2.47; p = 0.015) also exhibited DA, with the left side of the leaves being larger than the right.

There were no significant differences in leaf asymmetry between the three species. However, *E. astringens* possessed values slightly above the other species ($F_{2,27} = 1.426$ $p = 0.258$; Figure 3).

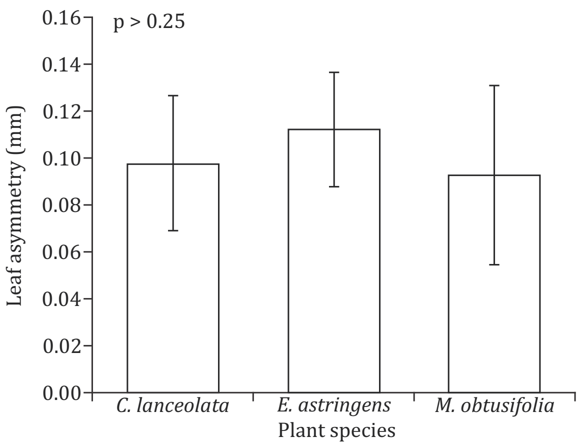

Figure 3. Comparison (mean and standard deviation) of leaf asymmetry among the plant species studied. Differences were not statistically significant (p > 0.25). / **Figura 3.** Comparação (média e desvio padrão) da assimetria foliar entre as espécies de plantas estudadas. As diferenças não foram estatisticamente significativas (p > 0,25).

Galled leaves were significantly more asymmetric in *C. lanceolata* (t= -2.967; p= 0.01) and *E. astringens* (t = -3.402; p = 0.007), while *M. obtusifolia* did not exhibit a significant difference in leaf asymmetry between galled and ungalled leaves (t= -1.95; p= 0.08) (Figure 4). The abundance of galls was significantly greater in *E. astringens* (n = 481), followed by *M. obtusifolia* (n = 311) and *C. lanceolata* (n= 150) ($F_{2,27}$ = 9.462 p < 0.001).

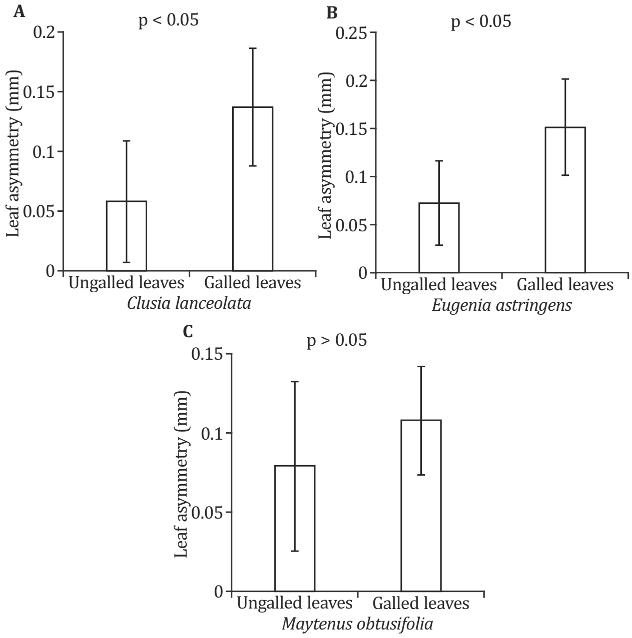

Figure 4. Comparison (mean and standard deviation) of leaf asymmetry between ungalled and galled leaves. (A) *Clusia lanceolata* Cambess., (B) *Eugenia astringens* Cambess., (C) *Maytenus obtusifolia* Mart. / **Figura 4.** Comparação (média e desvio padrão) da assimetria foliar entre folhas não galhadas e galhadas. (A) *Clusia lanceolata* Cambess., (B) *Eugenia astringens* Cambess., (C) *Maytenus obtusifolia* Mart.

Discussion

Most studies on AS have been performed using animals. In plants, the relationship between stress and AS remains poorly investigated (PRATT; MCLAIN, 2002; WILL; LIEBHERR, 2015). AS may arise from “transitions in symmetry breaking”, which occur when organisms are subjected to strong stress, resulting in non-linear developmental processes (GRAHAM et al., 1993; GRAHAM et al., 1998; LENS; DONGEN, 2000). According to Sakai and Shimamoto (1965), different types of symmetry may manifest themselves in the same plant species. In the present study, *C. lanceolata* and *M. obtusifolia* exhibited both AS and AD while *E. astringens* exhibited only AS. This finding may indicate that, in some cases, different types of leaf asymmetry may depend on spatial scales and different stressors (VENÂNCIO et al., 2016).

Restingas (or sandy coastal plains) are habitats characterized by limiting environmental conditions for the development of plants (LANE et al., 2008). Our results corroborate other studies where plants under stress exhibited AS and DA (SILVA et al., 2015; VENÂNCIO et al., 2016). AS and DA are predicted to occur in populations that are under high stress levels. Lens and Dongen (2000) showed that FA was common in habitats with low levels of disturbance, unlike DA, which prevailed in populations with high levels of disturbance. As expected, leaf asymmetry did not differ significantly among the species studied, possibly because the response of these plants to the restinga is manifest in a similar way.

The galled leaves of *C. lanceolata* and *E. astringens* exhibited higher levels of leaf asymmetry compared to leaves without galls, similar to what was found by Silva et al. (2015) for insect galls of *Ouratea polygyna* Engl (Ochnaceae) and Alves-Silva (2012) for nematode galls of *Miconia fallax* (Melastomataceae). In these two species (*C. lanceolata* and *E. astringens*) the results may indicate that the galls increased leaf asymmetry. In a review, Møller (1996) showed that in 63% of the studies, insect galls were positively related to developmental instability of their host. Santos et al. (2013) also found a positive relationship between *Schizomyia microcapillata* Maia (Cecidomyiidae: Diptera) and fluctuating asymmetry in leaves of *Bauhinia brevipes* Vogel. (Fabaceae), and Zvereva et al. (1997) demonstrated that herbivorous beetles increased levels of FA in willow trees.

However, Cornelissen and Stiling (2005) found no causal relationship between herbivorous insect attack and FA for two oak species, but observed that herbivores selected more asymmetric leaves due to higher nitrogen levels. Therefore, we can not rule out the possibility of female galling insects selecting previously asymmetric leaves for oviposition (SANTOS et al., 2013).

Maytenus obtusifolia did not exhibit significant differences between galled and ungalled leaves. One possible explanation concerns the plastic potential of the species. Phenotypic plasticity is the ability of an organism to change its physiology or morphology in response to an unstable and heterogeneous environment as a way of maintaining vigor (DEBAT; DAVID, 2001). Lomônaco and Germanos (2001) points out that biotic factors are also involved in the mechanisms underlying phenotypic plasticity. In this sense, the morphological changes in *M. obtusifolia* caused by the galls may have been buffered to maintain the vigor of the species (ALVES-SILVA, 2012).

In a study involving *Miconia prasina* (Sw.) DC (Melastomataceae), the authors verified that the presence of galls of *Ditylenchus* did not affect the vigor of the plant and agreed that *M. prasina* could tolerate the impact of the nematoid by compensation (SANTOS et al., 2009). The difference between galled and ungalled leaves in relation to leaf asymmetry indicates that *M. obtusifolia* can tolerate and minimize the presence of galling insects, revealing a high capacity for homeostasis in the face of biotic stress.

The abundance of galls was significantly higher in *E. astringens*, followed by *M. obtusifolia* and *C. lanceolata*. One possible explanation would be differences in the degree of sclerophilia of these species: *M. obtusifolia* possesses leaves with greater sclerophilia than *E. astringens*, a characteristic that hinders the endophytic oviposition of gallers or, in the case of exophytic oviposition, the perforation of the leaf epidermis by galling larvae. Additionally, *C. lanceolata* has an extremely thick and abundant latex, which is another characteristic that confers protection against herbivory.

Conclusions

All species presented antisymmetry, *C. lanceolata* and *M. obtusifolia* also presented directional asymmetry. In the present study the hypothesis that leaves with gall has a greater asymmetry was corroborated by the species *C. lanceolata* and *E. astringens*. *M. obtusifolia* and *C. lanceolata* for having defenses against herbivory hindered the action of the galling insect on the induction of gall.

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