Acta Botanica Brasilica 29(2): 274-277. 2015.
doi: 10.1590/0102-33062014abb3727

Short Communication

Relationship between gall-midge parasitism, plant vigor, and developmental instability in *Ouratea polygyna* Engl (Ochnaceae) in a patch of a Brazilian Atlantic Forest

Maíra Honorato de Moura Silva[1], Jarcilene Silva de Almeida-Cortez[2], Estevão Alves Silva[3] and Jean Carlos Santos[1*]

Received: August 28, 2014 Accepted: November 6, 2014

ABSTRACT

We tested the preference prediction of plant vigor hypothesis by examining the relationship between gall occurrence and leaf size. We also examined the effect of galls on leaf asymmetry, which is a measure of developmental instability. Gall occurrence did not increase with leaf size, thereby providing no support to the preference prediction. Galled leaves were significantly more asymmetric than ungalled leaves. Moreover, leaf asymmetry increased with both gall occurrence and gall size, indicating that galls boosted the stress levels in *Ouratea polygyna*.

Keywords: biotic disturbance, Cecidomyiidae, leaf asymmetry, preference prediction, stress

As stated by the preference prediction, gall abundance in leaves and/or shoots should be significantly greater on large plant modules than on small ones (Fritz *et al*. 2000), and galls tend to follow this prediction because the fitness of the inducer is expected to increase with plant module size (Price 1991). For instance, in *Bauhinia brevipes* Vog. (Fabaceae), longer shoots are rare; however, the abundance of gall-midges was recorded as 10-fold higher on the largest shoots (Santos *et al*. 2008). Once established in leaves, galls can alter leaf development (Souza *et al*. 2000) and cause asymmetries and distortions (Santos *et al*. 2013). Studies focusing on the influence of galls on leaf bilateral symmetry show unclear relationships between leaf asymmetry and galls (see Telhado *et al*. 2010; Alves-Silva 2012). Differences in leaf morphometry between galled and ungalled leaves may indicate that besides causing changes in leaf development (Santos *et al*. 2013), galls also affect leaf symmetry, which may compromise plant fitness (Venâncio *et al.* 2014). In this study, we investigated (1) the relationship between gall-midge abundance and leaf size in *Ouratea polygyna* Engl. (Ochnaceae) at the period of leaf expansion and (2) the relationship between leaf morphometry and both gall abundance and size. We also compared the abundance of galled and ungalled leaves per plant. We hypothesized that (i) galls are more abundant in larger leaves, (ii) galled leaves are more asymmetric than ungalled leaves, and (iii) gall size is positively related to leaf asymmetry. In *O. polygyna*, gall midge oviposition takes place in young, filiform, rolled leaves when there is still no pattern of bilateral symmetry. Therefore, we were able to examine whether mature unfolded leaves presented asymmetry associated with the presence, abundance and size of galls.

The fieldwork was performed throughout November of 2007 at the Charles Darwin Ecological Refuge, a 60-ha patch of Atlantic Forest located in Igarassu, Pernambuco, Brazil (7°48.915'S, 34°57.274'W; for details about the study site see Costa *et al.* 2009). A still undescribed species of gall midge (Diptera: Cecidomyiidae) induces spheroid, hairy, one-chambered galls of reddish coloration, which generally occur in clusters throughout the leaf surface of *O. polygyna*. This tree is <3 m in height and is evenly distributed throughout the study area. Young leaves have a filiform or cylindrical aspect, which is common in *Ouratea* species; mature leaves exhibit completely unfolded blades, which are lanceolate, greenish, and may reach up to 15 cm in length and 7 cm in width; leaves are petiolated, and their margins are smooth (Santos & Ramalho 1996).

[1] Programa de Pós-Graduação em Ciências Biológicas, Universidade Federal de Pernambuco, 50670-901, Recife, PE, Brazil
[2] Departamento de Botânica, Universidade Federal de Pernambuco, 50670-901, Recife-PE, Brazil
[3] Instituto de Biologia, Universidade Federal de Uberlândia, 38408-100, Uberlândia, MG, Brazil
* Corresponding author: jcsantos@inbio.ufu.br

We haphazardly tagged 28 *O. polygyna* individuals, and 50 leaves were sampled from each tree (n = 1,400 leaves). After a preliminary observation, 87 leaves were discarded because of large distortions or area loss, which may otherwise bias subsequent analyses (following Alves-Silva & Del-Claro 2013). Therefore, the sample included 1,313 leaves with variable numbers of galls, including some ungalled leaves. The abundance of galled and ungalled leaves in *O. polygyna* was compared using a paired Student's *t*-test. Gall size (mm) was measured in each leaf with a caliper. In this study, leaf size was considered a measure of "plant vigor" and used to test the preference prediction (following Gonçalves-Alvim *et al.* 1999; Cornelissen & Fernandes 2001). To examine whether galls predominantly occurred in larger or wider leaves, the length and width of all leaves were measured with a digital caliper (0.01 mm accuracy). We then ranked leaves according to their size (length and width) to compare the abundance of galls in each leaf class interval. Leaf length and width classes spanned intervals of 9 mm (such as 20–29 mm and 30–39 mm, see Fig. 1A–F). The abundance of galls in each leaf size class was examined with Kruskal–Wallis tests, and the frequency of leaves in each size interval was examined with goodness of fit chi-squared tests. To determine whether galls induced asymmetry in leaves, both the right and left sides (*Rs* and *Ls*) of all leaves collected from *O. polygyna* (n = 1,313) were measured (mm). The midrib was used as a mark to separate both leaf sides. Bilateral structures (e.g., leaves) can display three different types of asymmetry, which can be distinguished by exploratory analyses using the values of *Rs* minus *Ls* (Palmer & Strobeck 1986). "Antisymmetry" occurs when data do not fit the assumptions of a normal distribution. In "directional asymmetry" (DA), the mean difference between sides ($Rs - Ls$) is significantly greater than zero, indicating that one side of the bilateral character is consistently longer than the other. "Fluctuating asymmetry" describes random and generally small departures from symmetry with a mean value of zero ($Rs - Ls = 0$). Our data met the assumptions of DA, as the mean *Rs* minus *Ls* value was significantly different from zero (mean = 0.431 mm; $t = 11.264$; $p < 0.0001$). DA was neither related to leaf length nor width ($p > 0.05$ in both cases); thus it could be examined without ambiguity in the subsequent analyses. A paired Student's *t*-test was used to compare DA of galled and ungalled leaves, and a linear regression was performed to test the relationship between DA (intercept) and mean gall size. In both tests, the mean value of DA per plant was used.

Gall midges were observed in 57.7% (n = 759) of leaves sampled from *O. polygyna* ($t = 2.135$; $p < 0.05$). Leaves with galls supported, on average, 17.6 ± 20.39 (mean ± SD) galls (range 1–155 galls per leaf; n = 13,356 galls in the 759 galled leaves). Mean gall size was 2.75 ± 0.56 mm (n = 3,774 galls). Leaves were 111.18±19.41 (mean ± SD) mm in length and 42.28 ± 8.22 mm in width (n = 1,313 leaves). Most leaves sampled from *O. polygyna* were of an intermediate size, measuring from 110–119 mm in length ($\chi^2 = 26.017$; $p < 0.05$) and 40–49 mm in width ($\chi^2 = 60.114$; $p < 0.05$; Fig. 1A-B), and the abundance of galls was also highest within these intermediate leaf classes (leaf length $\chi^2 = 897.03$; $p < 0.0001$; leaf width $\chi^2 = 9309.162$; $p < 0.0001$; Fig. 1C-D). When we compared the mean abundance of galls in each leaf size class, no difference was observed, indicating that gall-midges occurred in the plant irrespective of leaf length ($H = 2.440$; $p > 0.05$) and leaf width ($H = 3.823$; $p > 0.05$; Fig. 1E-F). Galled leaves were significantly more asymmetrical than ungalled leaves (23% difference; $t = 3.870$; $p < 0.001$; Fig. 1G). Furthermore, DA exhibited a positive though marginally significant relationship with mean gall size ($F = 3.7678$, $r^2 = 0.1357$; $p = 0.064$; intercept = 0.310, slope = 0.358; Fig. 1H).

Although *O. polygyna* was severely attacked by gall midges, the hypothesis of galls preferentially occurring on larger leaves was rejected because gall incidence was related to leaf availability, rather than leaf size. One expected consequence of the presence of galls in *O. polygyna* was a difference in leaf symmetry between galled and ungalled leaves; we observed a markedly significant difference in leaf asymmetry (DA), according to the presence and size of galls. Our results are surprising, given that similar studies found that plants under disturbance should display fluctuating asymmetry, not DA (see Santos *et al.* 2013). Nonetheless, DA is presumed to occur in populations that experience high levels of stress; Lens & Dongen (2000) showed that FA was common under low levels of habitat disturbance, but DA prevailed under elevated perturbation levels. The consideration of how galls stimulate or elicit changes in leaf shape is a cornerstone of gall–host interactions (Cornelissen & Stiling 2005; Telhado *et al.* 2010), since changes in leaf shape because of parasitism are usually associated with low fitness of the host (Fay *et al.* 1996). Furthermore, since galls are leaf parasites, their presence and abundance are assumed to decrease plant vigor and fitness (Sacchi *et al.* 1988; Hartley 1998). Leaf asymmetry is widely recognized to reflect the inability of a population to buffer developmental perturbations (Zvereva *et al.* 1997). The present study is one of the few to demonstrate that galls are related to increased leaf asymmetry in the host plan. Nonetheless, we also take into account that gall midge females may have chosen to oviposit on previously stressed undeveloped leaves (see Cornelissen & Stiling 2005). Thus, developing galls boosted the levels of asymmetry in mature leaves, further increasing leaf stress (see Møller 1995). In conclusion, we reject the preference prediction of plant vigor hypothesis, because gall-midge occurrence was not related to leaf size in *O. polygyna*; however, gall abundance and size increased leaf asymmetry, measured via DA. Future studies should aim to understand the underlying physiological mechanisms of the massive occurrence of gall midges in this plant as well as the long-term effects of this interaction and its fitness consequences on *O. polygyna*.

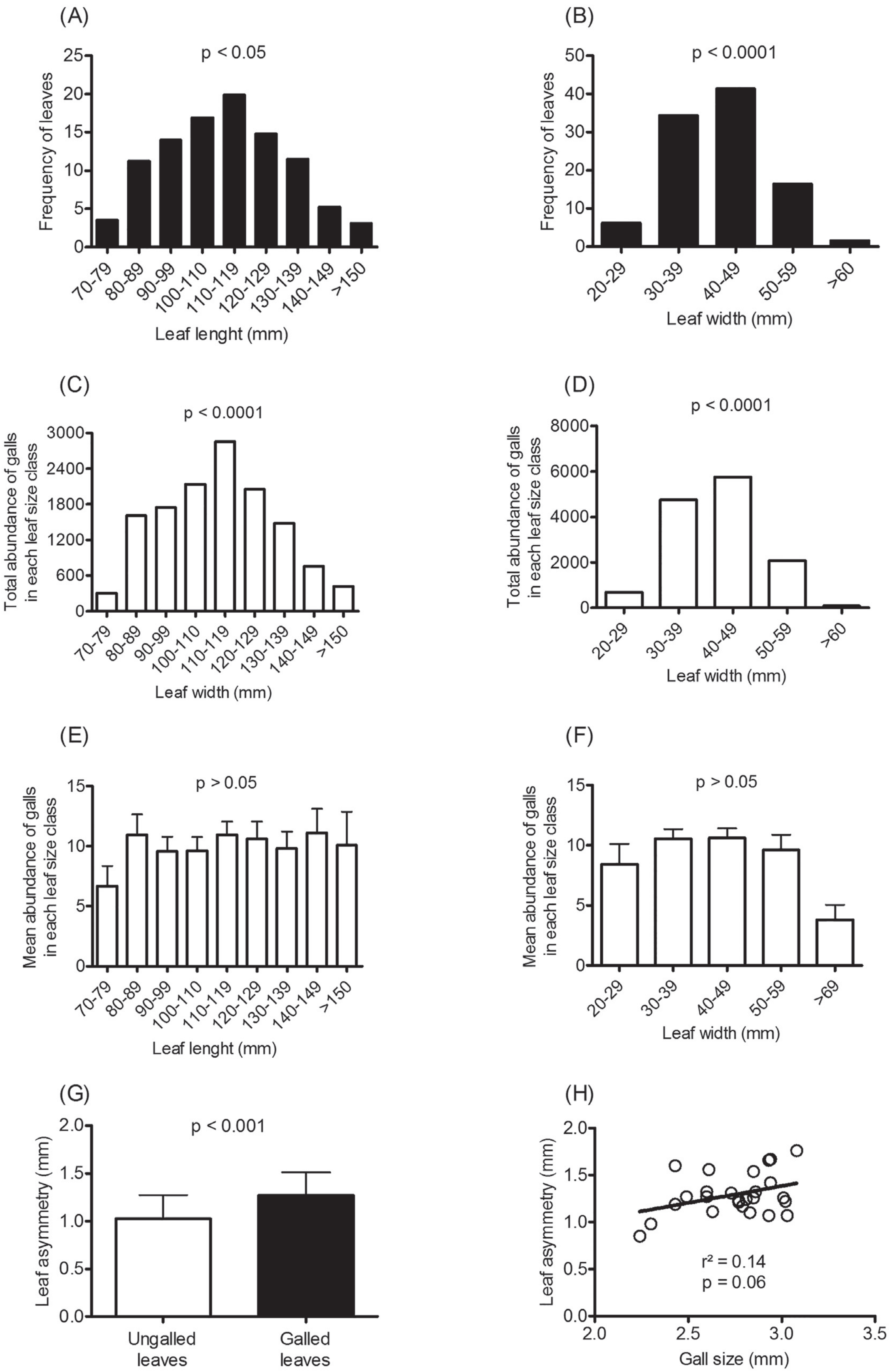

Figure 1. Frequency of leaves in length (A) and width (B) intervals; abundance of galls according to leaf length (C) and width (D); mean and standard deviation of galls according to leaf length (E) and width (F); (G) comparison (mean and standard deviation) of the directional asymmetry (DA) levels between galled and ungalled leaves; (H) positive relationship between DA and gall size (both in mm).

Acknowledgements

The authors thank Sheila P. Carvalho-Fernandes for field assistance; CAPES, FAPEMIG, FACEPE, and CNPq provided financial support.

References

Alves-Silva E. 2012. The influence of *Ditylenchus* (Nematoda) galls and shade on the fluctuating asymmetry of *Miconia fallax* (Melastomataceae). Ecología Austral 22: 53-61.

Alves-Silva E, Del-Claro K. 2013. Effect of post-fire resprouting on leaf fluctuating asymmetry, extrafloral nectar quality, and ant–plant–herbivore interactions. Naturwissenschaften 100: 525-532.

Cornelissen TG, Fernandes GW. 2001. Patterns of attack by herbivores on the tropical shrub *Bauhinia brevipes* (Leguminosae): vigor or chance? European Journal of Entomology 98: 37-40.

Cornelissen T, Stiling P. 2005. Perfect is best: low leaf fluctuating asymmetry reduces herbivory by leaf miners. Oecologia 142: 46-56.

Costa CMQ, Silva FAB, Farias AI, Moura RC. 2009. Diversidade de Scarabaeinae (Coleoptera, Scarabaeidae) coletados com armadilha de interceptação de vôo no Refúgio Ecológico Charles Darwin, Igarassu-PE, Brasil. Revista Brasileira de Entomologia 53: 88-94.

Fay PA, Hartnett DC, Knapp AK. 1996. Plant tolerance of gall-insect attack and gall-insect performance. Ecology 77: 521-534.

Fritz RS, Crabb BA, Hochwender CG. 2000. Preference and performance of a gall-inducing sawfly: a test of the plant vigor hypothesis. Oikos 89: 555-563.

Gonçalves-Alvim SJ, Faria ML, Fernandes GW. 1999. Relationships between four Neotropical species of galling insects and shoot vigor. Anais da Sociedade Entomológica do Brasil 28: 147-155.

Hartley SE. 1998. The chemical composition of plant galls: are levels of nutrients and secondary compounds controlled by the gall-former? Oecologia 113: 492-501.

Lens L, Dongen S. 2000. Fluctuating and directional asymmetry in natural bird populations exposed to different levels of habitat disturbance, as revealed by mixture analysis. Ecology Letters 3: 516-522.

Møller AP. 1995. Leaf-mining insects and fluctuating asymmetry in elm *Ulmus glabra* leaves. Journal of Animal Ecology 64: 697-707.

Palmer AR, Strobeck C. 1986. Fluctuating asymmetry: measurement, analysis, patterns. Annual review of Ecology and Systematics 17: 391-421.

Price PW. 1991. The plant vigor hypothesis and herbivore attack. Oikos 62: 244-251.

Sacchi CF, Price PW, Craig TP, Itami JK. 1988. Impact of shoot galler attack on sexual reproduction in the arroyo willow. Ecology 69: 2021-2030.

Santos E, Ramalho R. S. 1996. Caracterização dendrológica e potencialidades paisagísticas de *Ouratea polygyna* Engl. (Ochnaceae) e *Melanopxylon brauna* Schott (Caesalpinaceae) na arborização urbana. Árvore 20: 535-543.

Santos JC, Silveira FAO, Fernandes GW. 2008. Long term oviposition preference and larval performance of *Schizomyia macrocapillata* (Diptera: Cecidomyiidae) on larger shoots of its host plant *Bauhinia brevipes* (Fabaceae). Evolutionary Ecology 22: 123-137.

Santos JC, Alves-Silva E, Cornelissen TG, Fernandes GW. 2013. The effect of fluctuating asymmetry and leaf nutrients on gall abundance and survivorship. Basic and Applied Ecology 14: 489-495.

Souza SCPM, Kraus JE, Isaias RMS, Neves LJ. 2000. Anatomical and ultrastructural aspects of leaf galls in *Ficus microcarpa* L. F. (Moraceae) induced by *Gynaikothrips ficorum* Marchal (Thysanoptera). Acta Botanica Brasilica 14: 57-69.

Telhado C, Esteves D, Cornelissen T, Fernandes GW, Carneiro MAA. 2010. Insect herbivores of *Coccoloba cereifera* do not select asymmetric plants. Environmental Entomology 39: 849-855.

Venâncio HS, Alves-Silva E, Santos JC. 2014. Environmental and biotic factors affecting the fluctuating asymmetry and flower set in *Miconia fallax* (Melastomataceae). Tropical Ecology (in press). DOI unavailable.

Zvereva EL, Kozlov MV, Niemelä P, Haukioja E. 1997. Delayed induced resistance and increase in leaf fluctuating asymmetry as responses of *Salix borealis* to insect herbivory. Oecologia 109: 368-373.