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Herbivory-induced stress: Leaf developmental instability is caused by herbivore damage in early stages of leaf development

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ABSTRACT

Herbivory is a major source of plant stress and its effects can be severe, decreasing plant fitness, or subtle, affecting the development of leaves by influencing the normal pattern of growth and expansion of leaf blades. Fluctuating asymmetry (FA) analysis is recognized as a measure of plant stress, and can be used to evaluate subtle effects of herbivory on the imperfect growth of bilaterally symmetrical traits, such as leaves. One general issue is that authors usually consider FA as an indicator of stress, which can attract herbivores (plant stress hypothesis), and studies showing that herbivores themselves affect leaf symmetry (herbivory-induced stress hypothesis) are scarce, with mixed results. Here, we investigated the relationship between herbivory by thrips and leaf FA in *Banisteriopsis malifolia* and *Heteropterys escallonifolia* (Malpighiaceae). *Pseudophilothrips obscuricornis* is a free-living, non-pest, sucking species that feeds mainly on leaf buds. We hypothesized that herbivory by thrips in the early stages of leaf development would provoke increased FA levels in mature leaves. The results showed that thrips herbivory rate was low, affecting barely more than 1% of the leaf blade. Nonetheless, thrips-attacked leaves of *B. malifolia* and *H. escallonifolia* presented increases of 15 and 27% in leaf asymmetry, respectively, compared to uninjured leaves, corroborating the herbivory-induced stress hypothesis. Since herbivory by thrips in leaf buds was related to significant increases in the stress of mature leaves, we assume that under these circumstances, FA can be used as a biomarker for plant stress following herbivory damage. To be useful as a biomarker of stress, FA in plants must be investigated with caution, taking into account the natural history of the herbivore species and timing of leaf damage.

1. Introduction

Plants are distributed along a gradient of stressful conditions (Cuevas-Reyes et al., 2011a) and may suffer from physiological tensions that might eventually cause small deviations from perfect symmetry in otherwise bilaterally symmetrical traits (Cornelissen and Stiling, 2010; Klingenberg, 2003). One common measure of developmental instability is fluctuating asymmetry (FA) analysis, a component of character size variation widely used to estimate a population's response in relation to stressful conditions (Palmer, 1996; Palmer and Strobeck, 1986). For instance, FA in plants is associated with a wide range of environmental variables, such as pollution (Zvereva et al., 1997a), soil salinity (Cornelissen and Stiling, 2010), shade conditions (Puerta-Piñero et al., 2008) and climate (Cowart and Graham, 1999), among others. In these circumstances, leaves present increased levels of difference in their bilateral symmetry (Santos et al., 2013), which tends to become more severe as the stressor becomes stronger (Cuevas-Reyes et al., 2011b).

In spite of the extensive literature concerning FA and environmental stressors (reviewed by Møller and Shykoff, 1999), few studies have addressed the relationship between FA and insect herbivory (see Cornelissen and Stiling, 2005; Santos et al., 2013; Telhado et al., 2010). These studies, however, show alternatively strong, weak or no association at all between FA and insect herbivory (Alves-Silva, 2012; Costa et al., 2013; Telhado et al., 2010). This is because the FA–herbivory literature (studies examining the relationship between FA and herbivory) is still relatively controversial and two lines of research can be recognized. The first considers FA as a surrogate for herbivore attack, as stressed plants have more free nitrogen and less defensive or secondary defense compounds (Cornelissen and Stiling, 2005). Such an approach follows the concepts of the *plant stress hypothesis* (discussed in Møller, 1995; see also Mattson and Haack, 1987; White, 1984). Conversely, other studies demonstrate that herbivores themselves might act as plant stressors, affecting the bilateral pattern of growth in leaves

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and thus inducing FA (Santos et al., 2013; Zvereva et al., 1997b). This approach, still scarcely studied, will be hereafter named as the *herbivory-induced stress hypothesis* for the sake of clarity. The hypothesis states that herbivore damage, either in young or mature leaves, will affect the expected pattern of growth in bilaterally symmetrical characters eventually giving rise to increased FA levels. The purpose of this work was to investigate whether or not insect herbivores cause leaf FA.

Thrips (Insecta: Thysanoptera), a group of ubiquitous, sap-sucking small insects (Mound and Marullo, 1996) were chosen as our study herbivores. Aggregations of *Pseudophilothrips obscuricornis* Priesner, 1921 (Tubulifera: Phlaeothripidae) (hereafter *Pseudophilothrips*) are commonly found on Neotropical Malpighiaceae (Alves-Silva and Del-Claro, 2014). Both adults and larvae feed on leaf buds (rudimentary and undeveloped foliage) and young leaves, causing mature leaves to show distortions and numerous brown/dark necrosis marks. In general, herbivory by thrips influences the nutrient balance as well as the photosynthetic rate of their host plants, ultimately causing a loss in overall plant vigor and fitness (Cuda et al., 2008; De Borbón and Cardello, 2006; Mound and Zapater, 2003).

The system *Pseudophilothrips* – Malpighiaceae is ideal as a model to examine whether herbivory by thrips causes leaf FA. To advance knowledge of FA–herbivory systems, study designs should be able to examine unambiguously whether herbivores induce FA or rather search for asymmetric leaves to feed on (Díaz et al., 2004). In our study system, thrips preferentially feed on leaf buds, thus FA on mature leaves would be evidence that earlier herbivory by thrips increased the developmental instability of leaves, which would be evidence for the *herbivory-induced stress hypothesis*.

Throughout the leaf flush season of the two study plants (see below), we examined the occurrence and feeding behavior of thrips on leaf buds. Further sampling and analyses of mature leaves aimed to evaluate the FA levels in uninjured and damaged leaves, i.e. the ones that were attacked by thrips during the leaf bud stage. We hypothesized that the latter would display higher FA. Should this hypothesis be corroborated, it might give support for the *herbivory-induced stress hypothesis*. We also examined the relationship between thrips abundance and leaf herbivory rates, and we predicted a positive relationship. To conclude, we compared the abundance of adults and larvae of *Pseudophilothrips* and we examined their occurrence according to leaf developmental stage. We hypothesized that thrips, especially larvae (the stage where thrips feed voraciously on plants), would be found feeding predominantly on leaf buds and will contribute to FA of mature leaves.

2. Material and methods

2.1. Study system

Fieldwork was conducted in a Brazilian savanna area (230 ha; 18°59′ S–48°18′ W) in Uberlândia city, Brazil, from January to November 2011, and in March 2015 (see Alves-Silva and Del-Claro, 2013; Vilela et al., 2014 for details on the study area). The study plants, *Banisteriopsis malifolia* (Nees and Martius) B. Gates and *Heteropterys escallonifolia* A. Juss. are shrubs (<2 m high) with many branches. Leaves from both species have small trichomes distributed along the leaf blade on both sides, and a pair of extrafloral nectaries occurs at the base near the petiole at each side. *B. malifolia* leaf budding begins at the onset of the rainy season (October) and lasts until early April. Leaves are smooth-margined, ovate, greenish and can reach up to 15 cm in length and 10 cm in width; the apex is acute and the base is rounded (Alves-Silva and Del-Claro, 2014). *H. escallonifolia* phenology is also markedly seasonal. This plant produces leaves from late August to January, during the rainy season. Its leaves are oblong, dark green and can reach up to 10 cm in length and 5 cm in width. The leaf apex is obtuse and the base is rounded (Urbanetz et al., 2013).

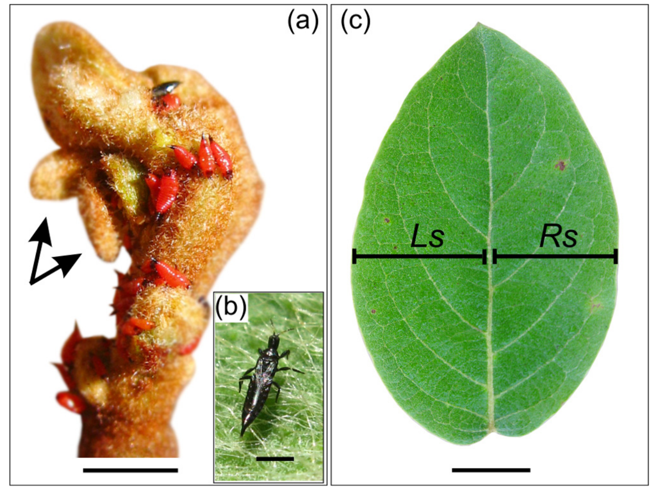

Fig. 1. (a) Larval stages of *Pseudophilothrips obscuricornis* feeding on a leaf bud (rudimentary foliage – arrows). (b) An adult individual of *P. obscuricornis*. (c) *Banisteriopsis malifolia* leaf, indicating how fluctuating asymmetry measurements were performed. *Rs* – right side; *Ls* – left side. Scale bars: a, c – 20 mm; b – 10 mm.

Thrips (Thysanoptera) are mostly known for their pest status in many economically important crops (Kirk and Terry, 2003), but in fact, only about 2% of the ~5000–6000 species are considered pests, and belong to the suborder Terebrantia (Cavalleri et al., 2010; Monteiro et al., 2001; Morse and Hoddle, 2006; Mound and Marullo, 1996; Mound and Morris, 2007). Species within the suborder Tubulifera (like *Pseudophilothrips*) are far less studied and are not regarded as pests (Mound, 2002). Nonetheless, some species, like *Liothrips* are voracious herbivores, being under consideration for biological control of invasive plant species (Cuda et al., 2008).

Pseudophilothrips is the sole herbivorous insect that feeds on meristems, shoots, short-shoot leaves and leaf buds of *B. malifolia* and *H. escallonifolia* (Fig. 1a, b). Both adults (~2 mm in length) and larvae (~1 mm in length) are found in aggregations, but larvae occur predominantly on meristems, shoots and leaf buds, whereas adults are more mobile and are found especially on young leaves. Adults are black and winged, whereas larvae are wingless and reddish. Females lay eggs on lateral meristems, shoots and leaf buds.

2.2. Sampling

B. malifolia ($n = 30$) and *H. escallonifolia* ($n = 48$) were randomly chosen within the Brazilian savanna area. All individual plants selected for this study belonged to the same population and were spread evenly in an area of ~6 ha, and therefore, were prone to similar biotic and abiotic stresses (following Telhado et al., 2010). Shrubs were not shaded by the canopy of large trees and received direct sunlight throughout the whole day. Plants were tagged prior to the leaf budding season, (*B. malifolia* in December and *H. escallonifolia* in August) and from each shrub, we selected the most apical stem for FA and herbivory analyses (following Alves-Silva and Del-Claro, 2013), because variation in leaves from different parts of plants might influence FA levels (Sibio and Rossi, 2012).

Pseudophilothrips individuals colonized *B. malifolia* and *H. escallonifolia* in January and September, respectively, at the first sign of leaf budding. From the onset of leaf flush until leaf sampling, all other herbivores that might feed on leaves and affect the study were removed from the study-tagged plants and were placed on distant non-studied plants of the same species. Fortunately, all of

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