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Patterns of growth, development and herbivory of *Palicourea rigida* are affected more by sun/shade conditions than by Cerrado phytophysiognomy

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ABSTRACT

Plant development is influenced by several abiotic factors, which in turn influence morphological traits and life history. We investigated whether leaf area, herbivory, toughness, fluctuating asymmetry, structural complexity and the number of inflorescences of *Palicourea rigida* are influenced by sun/shade conditions or by Cerrado phytophysiognomy (typical cerrado or rupestrian field). We expected to find greater structural complexity, leaf toughness and more inflorescences in sun plants; shaded plants were expected to exhibit a greater degree of fluctuating asymmetry (an index of plant stress), reduced leaf toughness and greater herbivory. As for phytophysiognomies, we expected to find higher levels of leaf toughness and reduced structural complexity in plants from the rupestrian field. We sampled plants in the sun and shade from both phytophysiognomies. Leaf area, toughness, herbivory and fluctuating asymmetry, were influenced more by sun/shade conditions than phytophysiognomy; leaf toughness was the only variable to show greater values in conditions of sun. Our results indicate that exposure to sunlight is not a requirement for increased plant development, but plants in shade are experiencing stress, as shown by increased fluctuating asymmetry; increased leaf area, a strategy to compensate for lower light exposure for plants and higher herbivory, which depicts lower toughness.

Keywords: Brazilian savanna, environmental stress, fluctuating asymmetry, leaf area loss, Rubiaceae, rupestrian grassland

Introduction

Plants are sessile organisms able to acclimate their ontogeny to different environmental conditions (Rozendaal *et al*. 2006; Jan *et al*. 2013). An efficient way of acclimation exhibited by plants occurs through the modification of leaves in order to optimise light capture (Bongers & Popma 1988; Raven *et al*. 2005). Individuals in a population of plants distributed in shaded and illuminated areas present distinct morphological and functional characteristics. In areas where light is not a limiting factor, plants show a higher photosynthetic rate and tendency to produce thicker leaves, raising the amount of nitrogen available to the photosynthetic machinery (Björkman 1981; Gulmon & Chu 1981). However, in some cases, the excess of light may represent a stress for plants, causing them to cool their leaves by increasing evapotranspiration (Givnish 1984). In contrast, plants in shaded areas may enhance the efficiency of light capture

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by increasing leaf area (Evans & Poorter 2001; Valladares *et al*. 2007). This increase enables plants to maintain their photosynthetic rate as do plants in sunnier areas. Nonetheless, this is followed by a reduction of leaf toughness (Björkman 1981; Sims & Pearcy 1989), which is a measure of mechanic resistance and an important factor of plant defence against herbivory (Lucas *et al*. 2000; Korndörfer & Del-Claro 2006; Read & Stokes 2006; Peeters *et al*. 2007). For early successional plant species, shade also may be classified as a stressing environmental condition, because these plants are naturally adapted to and depend on sunlight exposure for their development (Venâncio *et al*. 2016a).

Sunlight exposure is an important factor responsible for the growth and development of plants and may influence several traits and even fitness (Lomônaco & Reis 2007; Pascarella *et al*. 2007; Venâncio *et al*. 2016b). For instance, differences in structural complexity (e.g., number of leaves, height, canopy, trunk diameter) are common in plants growing in different habitats (Farnsworth & Ellison 1996), and this could ultimately influence plant performance and attractiveness to herbivores (Lawton 1983; Alves-Silva & Del-Claro 2014).

Environmental stress, including light deficiency and intense herbivory, can induce other biological responses in plants (Velasque & Del-Claro 2016), for instance, regarding developmental instability (Puerta-Piñero *et al*. 2008; Alves-Silva & Del-Claro 2016). Developmental instability occurs in plants as a response to a given stressing factor, such as pollution, radiation, nutrient deficiency, light/shade conditions, herbivory and parasitism, among others (Kozlov *et al*. 1996; Møller & Shykoff 1999; Cuevas-Reyes *et al*. 2011; Alves-Silva 2012; Santos *et al*. 2013). Plants display developmental instability by increasing the difference between leaf sides, and this feature can be statistically assessed with fluctuating asymmetry (FA) analysis. FA is based on the deviations from perfect symmetry in bilaterally symmetrical leaves (Cowart & Graham 1999; Cornelissen & Stiling 2005). Under non-stressed conditions, leaf sides should exhibit no difference between the right and left sides. However, stressed environments tend to increase leaf side differences, causing increased FA (Martel *et al*. 1999). Usually, high FA values are evidence of high stress (reviewed by Møller & Shykoff 1999). In the case of plants with widespread occurrence, it has been shown that sun/shade conditions may provoke developmental instability (Puerta-Pinero *et al*. 2008; Alves-Silva 2012; Miljković 2012; Alves-Silva & Del-Claro 2016) and decrease plant fitness (Venâncio *et al*. 2016b). Furthermore, abiotic factors allied to FA measures are fundamental in population biology studies, as they allow us to understand adaptations and coadaptations in population ecology (Graham *et al*. 2010).

In this study, we aimed to investigate whether leaf area, herbivory, toughness, FA, structural complexity intensity and the number of inflorescences are influenced by phytophysiognomy – typical cerrado or rupestrian field – and/or microhabitat conditions – shade or sun. We studied individuals of *Palicourea rigida*, which is a common and attractive plant found in many Cerrado phytophysiognomies (Ratter *et al*. 2003; Ribeiro & Walter 2008). Plants were sampled in natural sun/shade conditions in two different Cerrado phytophysiognomies, including a plateau with typical cerrado vegetation and a rupestrian field with rocky soils. These phytophysiognomies present distinct soil characteristics; the rocky soil of rupestrian field, for instance, is rich in aluminum (Benites *et al*. 2003; Negreiros *et al*. 2008). Aluminum is well-known by its toxic effects in many plant species, mostly by its capacity of soil acidification; this metal is also related to leaf toughness (Foy *et al*. 1978; Ma *et al*. 2001). Considering the distinct abiotic conditions provided by both microhabitat and Cerrado phytophysiognomies, we hypothesised that plant parameters are affected by both factors.

As for our predictions, we expected to find increased levels of leaf area and FA in plants in the shade compared to plants in the sun. FA is a measure of plant stress; thus, shaded plants were expected to show increased FA and leaf area to intercept more light. We expected higher leaf toughness in sunny plants, owing to increased plant performance in the sun (Björkman 1981; Gulmon & Chu 1981; Sims & Pearcy 1989) and lower values of herbivory (Lucas *et al*. 2000; Korndörfer & Del-Claro 2006; Read & Stokes 2006; Peeters *et al*. 2007). Considering the structural complexity, we assumed that plants in sunny sites would develop better than their shaded counterparts (Gulmon & Chu 1981); above-ground plant parts would show increased performance, including phenology intensity, and hence, a high structural complexity. Regarding the potential toxicity of aluminium rich soils, we expected to find higher levels of leaf toughness and reduced structural complexity in plants from the rupestrian field.

Materials and methods

Study Area

We conducted this study at the Caldas Novas State Park (Parque Estadual da Serra de Caldas Novas, PESCAN, 17°47’13.0”S - 48°40’12.0”W). The park has an elliptical shape with approximately 123 km² of area. Its top has a large plateau, with slopes at its sides that form natural walls and the ridge of the foot is surrounded by farms and urban allotment. The park vegetation is predominantly *sensu stricto* cerrado, but cerradão, campo sujo, campo rupestre (rupestrian grassland) and seasonal semi-deciduous forests are also very frequent phytophysiognomies (Oliveira-Filho & Ratter 2002; PESCAN 2015).

Two vegetation types were studied in this work. The first was a plateau with a *sensu stricto* cerrado phytophysiognomy, about 990 m in altitude, presenting mainly a red-yellow oxisol. The second site was a rupestrian field that occurs at the lower part of the ridge, toward the top. It is characterised by flat and rocky soils, with low water retention capacity that are rich in aluminium, magnesium and calcium (Benites *et al*. 2003).

Leaf area and herbivory

We sampled a total of 32 individual plants, distributed equally between the plateau and the rupestrian field (*n* = 16 plants at each site). Half of the plants at each site (*n* = 8 plants) were exposed directly to sunlight, and the other half were shaded plants. The criteria used to establish plants as in the sun or shade was their occurrence in the vicinity of large canopied trees. Plants in the sun were far from large trees and received both direct and lateral sunlight all day long, whereas shaded plants were under the canopy of trees or rocks and received only weak lateral sunlight (adapted from Venâncio *et al*. 2016b).

We collected two mature, completely expanded leaves (approximately 10 % of total leaves per plant) from all individual plants at both sites (plateau and rupestrian field). In the laboratory, we took pictures of those leaves and used Image J software (Wayne Rasband version 1.5b) to quantify the leaf area, the amount of leaf area loss (herbivory) caused by chewing herbivores and the asymmetry (described below).

Leaf toughness

In order to evaluate leaf toughness, we performed a second survey at each phytophysiognomy, sampling two leaves from a new set of individual plants, accounting for 60 individual plants (*n* = 30 per site). Half of the leaves were collected from sunny plants and the other half from shaded ones. In the laboratory, we measured leaf toughness using a penetrometer with a punch able to pierce a leaf blade positioned over a flat surface (Aranwela *et al*. 1999). We standardised the tests by hitting the same spot of leaves, at the centre of a leaf blade, between the main leaf vein and its edge. We calibrated the instrument with each new measure and used Newton as our standard unit.

Fluctuating asymmetry

We measured all collected leaves of *Palicourea rigida* Kunth (first survey only) on both sides (*Rs* – right side; *Ls* – left side) from the leaf midrib, which was taken as a reference, to the leaf edges at the middle part of *P. rigida* leaves, which is usually the largest part of the leaf blade (Santos *et al*. 2013; Alves-Silva & Del-Claro 2016). We measured all leaves from digital images using the Image J software, which was calibrated to 0.01 mm accuracy (adapted from Cornelissen & Stiling 2005). Prior to this, we placed leaves individually under transparent glass, which flattened the leaf blade (adapted from Ivanov *et al*. 2015). Near the leaves, we positioned a ruler to act as a reference and to permit calibration of the Image J software. We also measured leaf length in order to determine whether leaf asymmetry was related to leaf size (Telhado *et al*. 2017).

FA is the pervasive asymmetry type in leaves; however, in some cases, leaves might show either directional asymmetry (DA) or antisymmetry (AS), two other categories of symmetry (Graham *et al*. 2010). In DA, one side of the character is always greater than the other, so *Rs* > *Ls* or *Ls* > *Rs*, and a histogram shows skewed distribution of data of *Rs minus Ls* measurements. AS depicts a scenario where the population presents individuals with both *Rs* > *Ls* and *Ls* > *Rs* – that is, a bimodal distribution of *Rs minus Ls* measurements in a histogram. In contrast, in a population showing FA, the variation between leaf sides is random, small and normally distributed. The main difference between these asymmetries is that FA is caused and boosted by environmental (e.g., pollution – Kozlov *et al*. 1996) and/or biotic (herbivory – Møller 1995) conditions, while DA and AS have a genetic basis (Graham *et al*. 2010).

Structural complexity and inflorescences

In the field, we measured several parameters indicative of plant structural complexity, such as the stem diameter (in millimetres) at 10 cm from the soil; plant height; and canopy length and width, all in centimetres (following Lawton 1983; Costa *et al*. 2010). We obtained these measures from the same 32 individuals found at the plateau and in the rupestrian field (first sampling). We counted plant structures made by eye examination, and performed appropriate measures using a digital calliper and measuring tape. We then submitted these variables to a principal component analysis (PCA), a multivariate test that summarises all variables and provides an index of plant structural complexity (following Alves-Silva & Del-Claro 2014). This index formed by PCA retains as much variability as possible from the original variables and is commonly used to group morphometric data in order to create a composite single index (Jolliffe 2002). We also noted the number of open flowers and inflorescences, as they could show whether plant phenology intensity changed according to microhabitat and/or phytophysiognomy.

Statistical analyses

In order to check whether our measurements of leaf asymmetry were accurate to permit the use of subsequent statistical tests without incurring errors (i.e., measurement error – Yezerinac *et al*. 1992; Cornelissen & Stiling 2005), we once again measured the *Rs* and *Ls* subset of the 32 leaves. We used two-way analysis of variance (ANOVA) to test whether variation between leaf sides was larger than measurement error (Alves-Silva & Del-Claro 2016). We employed *Rs* and *Ls* measurements (millimetres) as dependent variables, and leaf sides and individuals were regarded as factors (Cuevas-Reyes *et al*. 2011). In addition, we used the index of repeatability of Falconer, which is based on the variance within and between leaves, sides and individuals, to examine the reliability of our original and repeated measurements (Cornelissen & Stiling 2005). To conclude, we performed a correlation test between original and re-measured *Rs* and *Ls* measurements in order to examine how comparable and related the variables were (Hódar 2002).

In order to rule out DA, we performed a Student's *t* test with the mean equal to zero using the *Rs minus Ls* measurements. A significant result indicates the presence of DA in *P. rigida* leaves. To investigate AS, we subjected the *Rs minus Ls* measurements to a normality test together with the visual examination in a histogram, which might reveal bimodality, which is indicative of AS. Once these tests were not statistically significant, FA could be considered the type of asymmetry found in *P. rigida* leaves. As the last exploratory test, we investigated the relationships between FA and leaf length in order to see whether FA could be assessed without ambiguity in subsequent tests (Telhado *et al*. 2017).

We used two-way ANOVA tests to verify the effects of phytophysiognomy (rupestrian field and plateau, including its potential interaction effects) and microhabitat (sun and shade) on leaf area, leaf herbivory, leaf toughness, FA and structural complexity of *P. rigida*. We conducted these tests to check which variable influenced plant parameters the most and whether a given parameter was affected both by phytophysiognomy and microhabitat. We examined the relationship between leaf FA and herbivory with an analysis of covariance (ANCOVA), where microhabitat (sun/shade) was employed as factor and herbivory was regarded as the covariate. The relationship among inflorescences (intercept – dependent variable), herbivory, FA and plant structural complexity (slopes) we examined using a multiple regression. The number of flowers and inflorescences per plant were positively related, so we used the number of inflorescences as a measure of plant fitness, as this variable had less variance than the number of flowers and could better fit the model. We performed further regression tests examining the relationship between inflorescences and plant complexity in each microhabitat (sun/shade). All statistical tests we conducted using *log + 1* transformed data, as a standardisation. We performed statistical tests in *R* statistical software, version 3.2.3. Quantitative data are shown as mean ± standard deviation whenever appropriate.

Results

Leaf asymmetry

We conducted the measures of leaf morphometry with enough precision to rule out measurement errors, as depicted by the significant side*individual effect (F_{31} = 84.8478; $P < 0.0001$). In addition, the index of repeatability of Falconer was 98 %, and the correlation between the first and second measurements yielded values greater than 0.99 for both *Rs* and *Ls*. Therefore, we consider our measurements were accurate and appropriate to be used in subsequent analyses. Both DA and AS were not present in the leaves of *P. rigida* (t_{63} = 0.1409; $P > 0.05$, Lilliefors normality test $P > 0.05$). Thus, we assumed *P. rigida* leaves to show purely FA as the type of asymmetry. The relationship between leaf asymmetry (i.e., *Rs minus Ls*) and leaf length was not statistically significant ($F_{1,62}$ = 0.1511; R^2 = 0.0024; $P > 0.05$); therefore, the use of an uncorrected absolute FA index per plant (FA = $[(\Sigma|(Rs - Ls)| / n)$ is appropriate.

Plant parameters according to microhabitats and phytophysiognomies

No plant parameter, except for leaf toughness, was statistically affected by the Cerrado phytophysiognomies (Tab. 1, Fig. 1A). On the other hand, microhabitat affected leaf toughness, FA, leaf area and herbivory (Tab. 1, Fig. 1B-D). Leaf area was influenced by the interaction effect between phytophysiognomies and microhabitat and was the only parameter to show such interaction effects (Tab. 1). FA was, on average, higher at shaded sites, and this pattern was found for leaf area and numbers of flowers and inflorescences. Herbivory was, in general, higher in shaded plants but varied according to the phytophysiognomy (Tab. 1, Fig. 1D); in fact, herbivory in rupestrian shaded plants was 9.7-fold greater compared to sunny plants in the same phytophysiognomy. Neither the number of inflorescences per plant nor plant structural complexity were affected by phytophysiognomies and/or sun/shade (Tab. 1, Fig. 1E-F). Leaf FA was not related to herbivory but rather to microhabitat only (Tab. 2).

Plant structural complexity

Palicourea rigida structural complexity varied in sunny and shaded plants. For instance, stem diameter and height were 13 % and 12 % higher in sunny plants, respectively, but the median of structural complexity (Fig. 1F) was lower for sunny plants, presumably because plant canopy varied according to microhabitat, being either higher or lower in sun and shade in each Cerrado phytophysiognomy (Fig. 2).

The number of inflorescences per plant was positively related to plant structural complexity only (Tab. 3), and separate regressions showed that this interaction was also significant for both microhabitats (sun: $F_{1,14}$ = 10.586, R^2 = 0.4306, slope = 0.1373, $P < 0.01$; shade: $F_{1,14}$ = 6.6726, R^2 = 0.3228, slope = 0.1647, $P < 0.05$) (Fig. 3).

Discussion

Palicourea rigida traits were affected mostly by sun/shade conditions than Cerrado phytophysiognomies. Shaded plants presented increased FA levels, larger leaf

Table 1. Two-way ANOVA tests were conducted to examine the influence of phytophysiognomy (typical cerrado – plateau and rupestrian field) and microhabitat (sun and shade) on several plant parameters of *Palicourea rigida*. Interaction effects between phytophysiognomy and microhabitat were also investigated. Bold values indicate statistical significant differences. The symbols *, ** and *** mean $P < 0.05$, $P < 0.001$ and $P < 0.0001$, respectively. 'n.s.' means non-significant. D.f. – degrees of freedom. *Log+1* transformed data were used in the analyses.

Variables	D.f.	Mean Sq.	F-value
Leaf toughness			
Phytophysiognomy	1	0.3397	**79.5048** ***
Microhabitat	1	0.0488	**11.4199** *
Interaction effects	1	0.0007	0.1754 $^{\text{n.s}}$
Residuals	55	0.0042	
Fluctuating asymmetry			
Phytophysiognomy	1	0.0545	0.6823 $^{\text{n.s.}}$
Microhabitat	1	0.6831	**8.5470** *
Interaction effects	1	0.0248	0.3109 $^{\text{n.s.}}$
Residuals	28	0.0799	
Leaf area			
Phytophysiognomy	1	0.0143	0.9173 $^{\text{n.s.}}$
Microhabitat	1	0.5186	**33.1766** ***
Interaction effects	1	0.1344	**8.6001** *
Residuals	28	0.0156	
Leaf herbivory			
Phytophysiognomy	1	0.0278	0.1333 $^{\text{n.s.}}$
Microhabitat	1	0.9142	**4.3759** *
Interaction effects	1	0.2968	0.2433 $^{\text{n.s.}}$
Residuals	28	0.2089	
Inflorescences			
Phytophysiognomy	1	0.0040	0.0579 $^{\text{n.s.}}$
Microhabitat	1	0.0067	0.0968 $^{\text{n.s.}}$
Interaction effects	1	0.0046	0.0669 $^{\text{n.s.}}$
Residuals	28	0.0695	
Structural complexity			
Phytophysiognomy	1	0.3743	0.3468 $^{\text{n.s.}}$
Microhabitat	1	0.0274	0.0254 $^{\text{n.s.}}$
Interaction effects	1	0.3789	0.3511 $^{\text{n.s.}}$
Residuals	28	1.0792	

area and higher herbivory. The number of inflorescences and structural complexity were affected neither by sun/shade conditions nor phytophysiognomy; in fact, only leaf toughness was affected by the phytophysiognomies.

Regarding the parameters measured, we also observed an interaction effect of microhabitat*phytophysiognomy and leaf area. This interaction is probably due to the topography and different levels of canopy cover affecting *P. rigida* in each phytophysiognomy. The rupestrian field has many slopes and sustains both covered and open areas, with the presence of sparse taller trees (Eiten 1987; Alves & Kolbek 2010). In contrast, the plateau has a flatter terrain with vegetation distributed in a more continuous way (Silva *et al*. 2002). These differences reflect unequal amounts of light over the region, and consequently, the exposure of leaves to sunlight.

Shaded plants presented high levels of FA, as expected. Shrubs of *P. rigida* are found mainly in open and disturbed areas and on the edges throughout the Cerrado vegetation, suggesting that its performance, including germination, may be enhanced in sun-exposed environments (Vieira *et al*. 1996; Felfili *et al*. 2000). In this context, plants occurring in the shade might experience increased stress, which, in our study, was reflected by the elevated FA levels in shaded plants. FA is commonly used as a biomarker of population stress, as it more often than not positively relates to elevated levels of stress, such as pollution, soil conditions, herbivory and parasitism, among others (Kozlov *et al*. 1996; Møller & Shykoff 1999; Cuevas-Reyes *et al*. 2011). Microhabitat conditions are also among the detrimental factors that influence population distribution and individual performance (Raz *et al*. 2011), and sun/shade conditions already have been shown to influence to a large extent FA levels (Puerta-Piñero *et al*. 2008; Alves-Silva & Del-Claro 2013; Venâncio *et al*. 2016a), indicating that plants do respond to sunlight conditions by showing stress, which can be statistically evaluated with FA. In some cases, FA is negatively related to fitness, so this biomarker can be

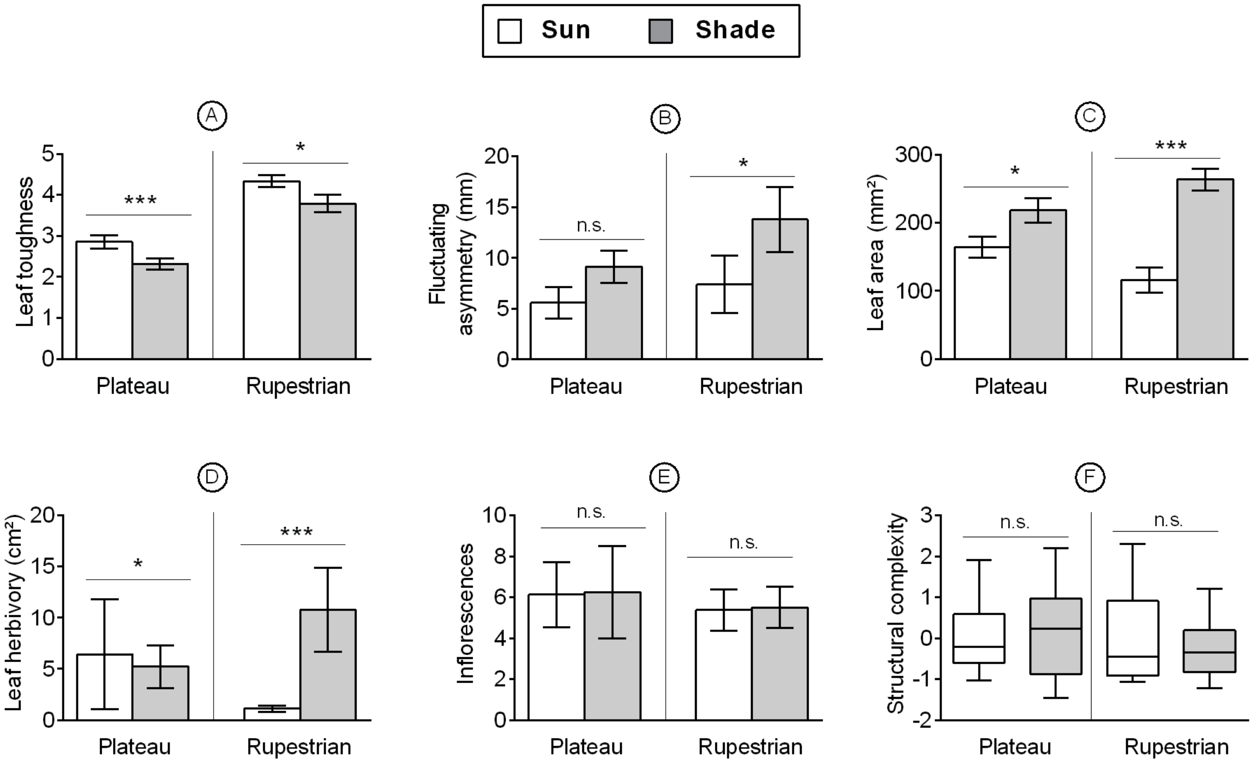

Figure 1. Comparison (mean and standard error) of several parameters of *Palicourea rigida* (a – leaf toughness, b – fluctuating asymmetry, c – leaf area, d – leaf herbivory, e – inflorescences per plant, f – structural complexity) according to Cerrado phytophysiognomies (typical cerrado – plateau and rupestrian field) and microhabitat (sun and shade). Data from structural complexity is unitless and is shown in boxplot to be more visually attractive. Figures show untransformed data. The symbols * and *** mean $P < 0.05$ and $P < 0.0001$, respectively. 'n.s.' means non-significant.

Table 2. Multiple regression results for the relationship among inflorescences (intercept – dependent variable), plant structural complexity, herbivory and FA and (slopes). The symbol *** means $P < 0.0001$. 'n.s.' means non-significant. D.f. – degrees of freedom. *Log+1* transformed data were used in the analyses.

Regression coefficients			
Variables	**Estimate**	**Std. Error**	***t*-value**
Intercept – inflorescences	0.5606	0.1472	3.8086 ***
Plant struc. complexity	0.1804	0.0467	3.8626 ***
Leaf herbivory (%)	-0.1793	0.0974	-1.8408 $^{\text{n.s.}}$
Leaf fluct. asymmetry (mm)	0.2156	0.1506	1.4316 $^{\text{n.s.}}$
Analysis of variance	**D.f.**	**F-value**	**Multiple R^2**
Regression	1.28	6.8727	0.4240

used to predict and anticipate organismal investment in reproduction (Møller 1997; Venâncio *et al*. 2016b).

We found no significant relationship between FA and fitness (number of inflorescences) in *P. rigida*, revealing that other factors aside from FA are affecting plant flowering intensity. In fact, plant structural complexity was the only variable that potentially influenced plant flowering. The relationship between plant structure and number of inflorescences was positive in both microhabitats. This is related to the ability of taller plants to intercept more light and allocate photosynthetic resources to fitness (Falster & Westoby 2003). According to our data, some architecture parameters of *P. rigida*, such as stem diameter and height, were higher in sunny plants, indicating that plants grew better in sunny locations, as expected.

Shaded plants had larger leaf areas, as this is a strategy to enhance the efficiency of light capture (Bongers & Popma 1988; Evans & Poorter 2001; Valladares *et al*. 2007). Both light insufficiency and intense herbivory are characteristics of environmental stress (Puerta-Piñero *et al*. 2008), and, in our study, herbivory in general was also higher in shaded plants, presumably due to the low toughness of shaded

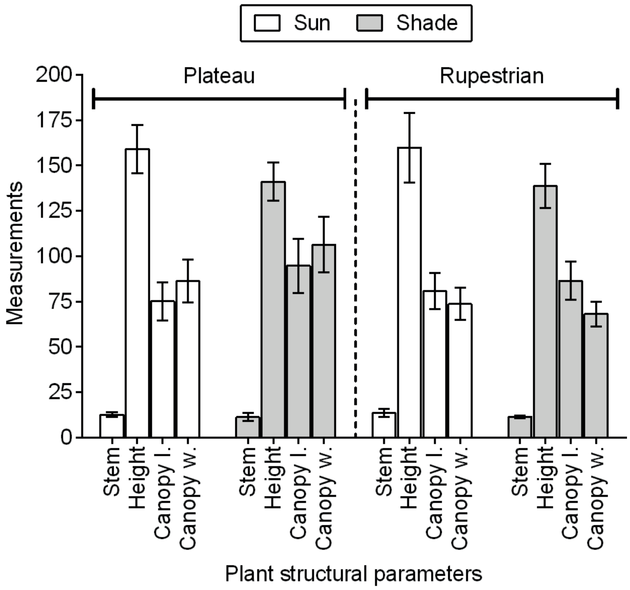

Figure 2. Structural parameters measured in *Palicourea rigida* according to microhabitat (sun/shade) and phytophysiognomy (typical cerrado – plateau and rupestrian field). Stem diameter is shown in mm, the other variables are in cm; "Canopy l." and "Canopy w." mean canopy length and width, respectively.

leaves. Louda & Rodman (1996) found that shaded plants had lower leaf toughness and thickness, and Rodrigues *et al*. (2010) demonstrated that increases in these variables are detrimental to insect herbivory.

However, sunny plants from the plateau presented thicker leaves and were more attacked by herbivores than the thin shaded plants from the same phytophysiognomy. We believe this result cannot be explained only by toughness and/or by soil characteristics, as we did not observe a clear pattern for herbivory. Leaf toughness might not be enough to reduce herbivory in *P. rigida*, therefore, we believe that the unequal distribution of plants observed at rupestrian field (Eiten 1987; Alves & Kolbek 2010) influenced our results; shaded plants were often found under the canopy of large trees at more humid spots, which favours the occurrence of arthropods, while sunny plants were found mostly isolated, at wide open areas. The homogeneous plant occurrence at the plateau might have allowed a better distribution of herbivores, favouring their access to *P. rigida* plants. However, we do not know why herbivory was higher at sunny plants from plateau.

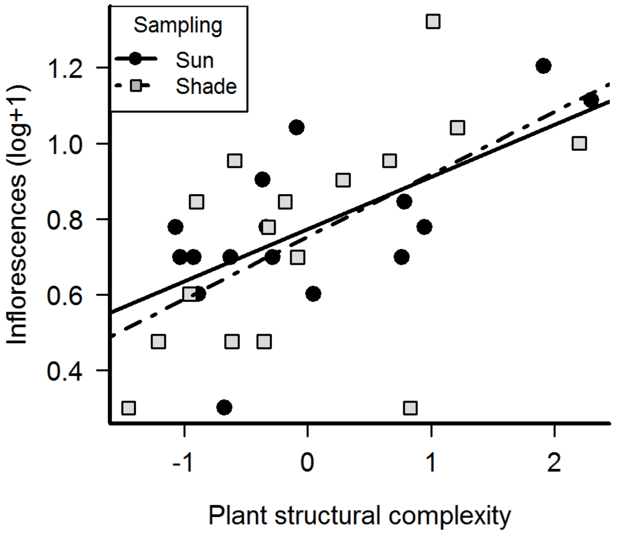

Figure 3. Relationship between the number of inflorescences and the structural complexity of *Palicourea rigida* in two distinct microhabitats.

Plants from the rupestrian field presented higher toughness, and this can be attributed to soil characteristics. Its rocky soil is rich in aluminium, calcium and magnesium (Benites *et al*. 2003; Negreiros *et al*. 2008). Due to the soil acidification, aluminium is toxic for many plant species, which reduces their vegetative parts and increases leaf toughness (Foy *et al*. 1978; Ma *et al*. 2001). In general, *P. rigida* is one of the most aluminium-accumulating plants in the Cerrado, and such plants usually have the leatheriest leaves (Haridasan 1982), thus supporting the assumption that leaf toughness was higher in the rupestrian field due to edaphic conditions. In addition, sun-adapted plants also tend to have more sclerophyllous leaves as an adaptation to high irradiance levels (Givnish 1988).

Our study showed that *P. rigida* presents phenotypic differences related to distinct levels of sunlight, which ultimately affected leaf morphometry, growth and consistency (toughness, area, herbivory and FA). Phenotypic differences associated with distinct small-scale variations might be detrimental to subpopulations but are necessary to maintain genetic diversity (Bell *et al*. 1991). The high levels of FA in shaded plants are evidence of elevated stress, which, together with the large leaf area of shaded plants, shows how the life history of *P. rigida* adjusts to stressful environments.

Table 3. Relationship between fluctuating asymmetry and herbivory in sunny and shaded *Palicourea rigida*.

Variables	D.F.	Mean Sq.	F-value	P-value
Herbivory	1	0.0001	0.0016	0.9684
Microhabitat	1	0.7234	9.8686	0.0039
Interaction effects	1	0.2245	3.0631	0.0910
Residuals	28	0.0733		

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