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Effect of post-fire resprouting on leaf fluctuating asymmetry, extrafloral nectar quality, and ant–plant–herbivore interactions

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Abstract Fires in the Cerrado savanna are a severe form of disturbance, but some species are capable of resprouting afterwards. It is unknown, however, how and whether post-fire resprouting represents a stressful condition to plants and how their rapid re-growth influences both the production of biochemical compounds, and interactions with mutualistic ants. In this study, we examined the influence of post-fire resprouting on biotic interactions (ant–plant–herbivore relationships) and on plant stress. The study was performed on two groups of the extrafloral nectaried shrub Banisteriopsis campestris (Malpighiaceae); one group was recovering from fire while the other acted as control. With respect to biotic interactions, we examined whether resprouting influenced extrafloral nectar concentration (milligrams per microliter), the abundance of the ant Camponotus crassus and leaf herbivory rates. Plant stress was assessed via fluctuating asymmetry (FA) analysis, which refers to deviations from perfect symmetry in bilaterally symmetrical traits (e.g., leaves) and indicates whether species are under stress. Results revealed that FA, sugar concentration, and ant abundance were 51.7 %, 35.7 % and 21.7 % higher in resprouting plants. Furthermore, C. crassus was significantly associated with low herbivory rates, but only in resprouting plants. This study showed that post-fire resprouting induced high levels of plant stress and influenced extrafloral nectar quality and ant-herbivore relationships in B. campestris. Therefore, despite being a stressful condition to the plant, post-fire resprouting individuals had concentrated extrafloral nectar and sustained more ants, thus strengthening the outcomes of ant–plant mutualism.

Keywords Cerrado · Camponotus crassus · Developmental instability · Mutualism · Extrafloral nectaries · Herbivory

Abbreviations

FA · Fluctuating asymmetry
mg/μL · Milligrams per microliter
Rw · Right width
Lw · Left width

Introduction

Fire is a major source of deforestation in tropical biomes as it can destroy large areas in a short period of time, killing plants and the associated fauna, changing landscapes and soil properties and releasing tons of carbon to the atmosphere, thereby contributing to global warming (Kauffman et al. 1994; Gade 1996; Matos et al. 2002; Van der Werf et al. 2010). On an evolutionary scale, many plants have been positively selected for their ability to survive different types of fire regimes and resprout afterwards (Pausas and Keeley 2009; Simon et al.
2009; Pyke et al. 2010). Nonetheless, fire can be a stressful condition and might impose structural and physiological tensions which might alter plant performance and development (Schmidt et al. 2005; Vivian and Cary 2012 and references). Such stress levels experienced by plants can be assessed via fluctuating asymmetry (FA) analysis.

FA in plants refers to small and random departures from bilateral symmetry in otherwise bilaterally symmetrical leaves (Cornelissen and Stiling 2005) and can estimate a population’s inability to buffer its growth against assorted types of perturbations (Parsons 1992). As a general rule, higher FA levels indicate higher stress from environmental, biotic, and genetic conditions (Wilsey et al. 1998; Cowart and Graham 1999; Cuevas-Reyes et al. 2011). Disturbances affect not only the growth patterns of bilateral organs but also negatively influence plant development and fitness (Diaz et al. 2004). In the case of resprouting plants, the rapid growth of aboveground parts might also attract herbivores, which benefit from the tender tissues of leaves and flowers, often inflicting severe damage to the plant (Prada et al. 1995; Vieira et al. 1996). Nonetheless, fire can also affect the production of extrafloral nectar and thus, plants might sustain a richer and more diverse ant-guard fauna (Alves-Silva 2011; see also Koptur et al. 2010).

Ant–plant mutualisms mediated by the presence of extrafloral nectaries are commonplace in the neotropics, particularly in the Cerrado (Oliveira and Freitas 2004), where plants provide sugar solutions and ants act as herbivore deterrents (Guimarães et al. 2006). Since the Cerrado is susceptible to frequent fires (Hoffmann and Moreira 2002), it is relevant to understand to what extent resprouting following fire affects the quality of extrafloral nectar, as sugar quality might strengthen or weaken ant–plant mutualism stability (Taylor 1977; Josens et al. 1998). Since the protective behavior of ants on plants is highly dependent on their density on hosts, changes in resource quality are presumed to influence the outcomes of ant–plant interactions (Blüthgen and Fiedler 2004; Riedel et al. 2012).

In this context, the extrafloral nectaried shrub Banisteriopsis campestris Juss., was ideal as a model with which to study the influence of fire on extrafloral sugar quality, FA levels and ant–plant–herbivore interactions. In 2010, part of the area where the plant grows was burned to the ground. Resprouting leaves had functional extrafloral nectaries and soft tissues, thus attracting the patrolling ant Camponotus crassus Mayr (Formicinae) and herbivores, respectively. Plants at an adjacent site were unaffected by the fire. Thus, in this work we were able to investigate the effect of the fire on (1) plant stress measured as FA and (2) biotic interactions (sugar quality and ant–herbivore interactions). Four main questions were addressed: Does resprouting influence (a) FA levels? and (b) the quality of extrafloral nectar?; (c) Do changes in extrafloral nectar quality affect the abundance of ant-guards?; and (d) is there an association between ant abundance and herbivory rates?

Materials and methods

Study site

Fieldwork was conducted in a sensu strictu cerrado area (18°59' S–48°18' W; 890 m above sea level) in Uberlândia city, Brazil, from July to November 2010. The Cerrado covers about 400 ha, of which 230 ha are characterized by sensu strictu cerrado, a type of vegetation dominated by herbaceous, shrubs, and trees ranging between 2 and 4 m tall, and a fair amount of grasses. Malpighiaceae, Bignoniaceae, and Myrtaceae are the most abundant and diverse shrubs at the area, while Caryocaraceae, Ochnaceae, and Vochysiaceae are the dominant trees. Soils are hydromorphic and acid with a significant amount of aluminum and potassium (Cardoso and Lomônaco 2003). The average rainfall in the region in 2010 was 1,248.1 mm, of which 94 % was concentrated during the wet season (October to April). Mean monthly temperature ranged from 25.3 °C in February to 20.2 °C in June, with an annual mean of 23.4 °C (Laboratory of Climatology, UFU, Brazil).

Study organisms

B. campestris is a shrub, rarely exceeding 1.5 m in height. Mature leaves can reach up to 7 cm in length and 5 cm in width. Small, non-glandular trichomes are present on both leaf surfaces, and leaves also bear a pair of extrafloral nectaries at the base (Fig. 1), near the petiole (Machado et al. 2008). B. campestris phenology is markedly seasonal, with flowering occurring from December to February and fruiting (samaras) peaking in January. Leaf flush occurs from September to December. During the dry season (May–September), plants have only a few leaves (<10), all of which are in an advanced state of senescence, with no active extrafloral nectaries. B. campestris is patrolled by C. crassus, one of the most common ground-nesting ant species in the study area. These ants feed on extrafloral nectar and are known for their aggressive behavior towards herbivores in general (Oliveira and Freitas 2004). Individuals forage on the whole plant and dislocate rapidly from plant structures (e.g., branches, leaves, stems). The attacks towards insect herbivores involve several bites (Oliveira et al. 1987).

Sampling

Two groups of B. campestris were used in the study. The resprouting group contained 29 individuals distributed evenly within a site recovering from fire (≈2.5 ha). About 3 months after the fire, in late September, B. campestris developed new shoots and leaves with active extrafloral nectaries. The control (unburned) B. campestris group contained a further 29 individuals in an adjacent area (≈3 ha) which was not affected by the fire. All plants at
both sites were <1.5 m in height and had approximately the same phenological status (i.e., young leaves with functional extrafloral nectaries). In addition, all plants belonged to the same population and were located in an area of 6–7 ha, and therefore were prone to similar biotic and abiotic stresses. Plants were tagged in November, and sugar measurements and FA were examined on the ten most apical leaves from each individual plant \( n = 290 \) leaves from each group; total \( n = 580 \) leaves). Within this period, leaves showed no signs of herbivory. Extrafloral nectar was measured with a pocket refractometer and each leaf was measured only once. Sugar quantity (milligrams) was calculated as “\[ y = 0.00226 + (0.00937x) + (0.0000585x^2) \]”, where “\( y \)” indicates the sugar content in milligrams and “\( x \)” is the brix (percent) value displayed on the refractometer (sensu Dafni et al. 2005). Studies suggest that nectar concentration can be altered by biotic factors, such as herbivory and nectar consumption by insects (Heil et al. 2000; Ness 2003). Therefore, \( B. \) campestris leaves were bagged in nets to prevent the visits of insects to extrafloral nectaries. Leaves were bagged in the evening and nectar measurements were performed the morning after (0700–0800 hours), as this is the period in which \( B. \) campestris releases extrafloral nectar and drops of nectar are commonly observed on extrafloral nectaries. The short period of bagging also prevented the nectar from accumulating, which otherwise might influence sugar concentration (see O’Brien et al. 1996). Extrafloral nectar production was asynchronous among leaves within individual plants. Therefore, nectar measurements were conducted over 2 weeks to obtain as much data as possible. The number of tagged leaves that did not release/produce nectar during the period of the study (inactive extrafloral nectaries) was counted for each individual plant.

\( B. \) campestris supports several herbivores from different guilds, such as gallers, miners, sap suckers, and chewers (personal observation). In this study, it was more appropriate to consider herbivory as a whole, regardless of insect herbivore guild. In the field, we noticed that leaves might be attacked by more than one insect guild simultaneously, but leaf damage was small, barely affecting 5 % of the leaf blade. For instance, sap suckers usually fed on small portions of leaves, and the resulting necrosis rarely occupied >1 % of the leaf area. Thus, in this study, the herbivory rates for \( B. \) campestris were not measured according to leaf damage classes (see García-Guzmán and Dirzo 2001). The use of photographs to measure leaf herbivory was also discarded, since it would involve a degree of disturbance (to place leaves for a certain period on a flat surface with an area scale), which might influence ant behavior. \( C. \) crassus individuals move very rapidly and tend to climb on anything that touches the plant, including the researcher’s body, equipment, and clothes. Therefore, the \( B. \) campestris herbivory rate was estimated via the percentage of damaged leaves per plant, by counting the number of damaged leaves and dividing it by the total number of leaves. Galls, mining, necrotic spots, and leaf area loss were all considered as evidence of herbivory. Herbivory was then ranked on a scale from 0 to 4, where 0 = no herbivory; 1 = 1–25 % damaged leaves (low herbivory); 2 = 26–50 % (moderate herbivory); 3 = 51–75 % (high herbivory); and 4 = more than 76 % of damaged leaves (extreme herbivory). All \( B. \) campestris individuals were patrolled by \( C. \) crassus. Ant counting per individual plant was performed three times, within each 7-day period, to assess the temporal variation in ant abundance.

Fluctuating asymmetry assessment

To assess \( B. \) campestris leaf FA, widths of all leaves \( n = 580 \) were measured on both the right (\( R_w \)) and left sides (\( L_w \)), from the leaf edge to the midrib, at the central point of the leaf, which corresponded to its widest part. This procedure was performed at the time of extrafloral nectar assessment, when leaves showed no signs of herbivory. FA measurements were carried out in the field to the nearest 0.01 mm using a caliper. To test the accuracy of the measurements, a subsample of 50 leaves was re-measured (on the same day within a 5–10-min interval from the first measurements) and compared with the original \( R_w \) and \( L_w \) measurements. The reproducibility of measurements is...
mandatory in FA studies and indicates whether leaf sides were measured with sufficient precision to discard measurement errors. A two-factor analysis of variance (ANOVA) was used to determine whether the between-sides variation was significantly larger than measurement error. Among the 580 leaves collected from *B. campestris*, 24 showed large variations in Rw minus Lw values (outliers) due to unknown biological/physical causes and were discarded in subsequent FA analyses to avoid biasing the results. The significance of the interaction (individual–leaf side) was greater than that expected by measurement error (*F*$_{1,49}=65.4403$; *p*<0.0001), indicating that leaves were measured with sufficient accuracy (see Cuevas-Reyes et al. 2011).

According to Palmer and Strobeck (1986), it is necessary to discriminate FA from the other kinds of asymmetry, such as directional asymmetry and antisymmetry. FA indicates random and small departures from symmetry in leaves with a mean value of zero. Directional asymmetry depicts a scenario in which leaves have one side consistently larger than the other and mean differences of Rw minus Lw are always greater or less than zero. Antisymmetry reflects a lack of symmetry, but in no specific direction, so significant differences on the Rw minus Lw distribution from the normal curve (bimodal distribution) are apparent and significant. The main ecological significance between the tree types of asymmetry is that FA is caused by environmental and biotic factors, and can be measured and analyzed, permitting conclusions about how some variables affect the development of organisms (see Cornelissen and Stiling 2005). On the other hand, directional asymmetry and antisymmetry and not well understood, and the literature is still scarce since researches argument that both may have genetic basis, restraining their use in ecological studies which aim to determine how exterior causes affect the species development (see Palmer and Strobeck 1986; Graham et al. 1993; Rowe et al. 1997; Van Dooren et al. 2010). In this context, to assure that our data fitted purely FA and not other types of asymmetry we conducted the following tests. Directional asymmetry was checked by showing that the mean Rw minus Lw values did not differ from zero (one sample Student’s *t* test). To check for antisymmetry, Rw minus Lw values were checked for normality (Møller 1995). There was no difference in the mean Rw minus Lw measurements, consequently directional asymmetry was discarded (*t*$_{555}=0.6612$; *p*>0.05). Rw minus Lw measurements also did not depart from the normal curve (*p*>0.05), thus antisymmetry was rejected. Therefore, the Rw minus Lw measurements were considered to reflect FA. Absolute FA was calculated as the mean difference between the right and left sides [i.e., FA=(Σ(Rw−Lw))/n] (Palmer and Strobeck 1986)]. FA dependence on leaf size was rejected (n=100 leaves, *R*²=0.0089; *F* = 0.8768; *p*>0.05) indicating that FA can be assessed unambiguously in subsequent tests (Alves-Silva 2012).

**Statistical analyses**

Quantitative data are presented as the mean±SD. In those cases where data did not satisfy the assumptions of normal distribution (*p*<0.05) and variance homoscedasticity, and where transformations were unable to achieve data normality, we used non-parametric statistical tests. The Mann–Whitney *U* test was used to compare the FA levels between resprouting and control *B. campestris* individuals (question a). Student’s *t* tests were used to compare the differences in sugar concentration (milligrams per microliter) between *B. campestris* groups (question b); and the abundance of leaves with active extrafloral nectaries in each group. Pearson correlation tests were performed to verify the relationship between FA and extrafloral sugar concentration in each *B. campestris* group. The abundance of *C. crassus* did not vary during the study (resprouting plants: *F*$_{2,84}=0.4703$; *p*>0.05; control plants: *F*$_{2,84}=0.1433$; *p*>0.05). Therefore, the number of ants from the first observation was used in subsequent statistical analyses for the sake of clarity. The difference in *C. crassus* abundance between *B. campestris* groups was compared using a Student’s *t* test. The relationship between *C. crassus* abundance and extrafloral nectar sugar concentration was examined via Pearson correlation tests in both *B. campestris* groups (question c). The differences in herbivory levels according to the number of *C. crassus* individuals were compared using ANOVA (followed by the Tukey post hoc test, resprouting plants) and Kruskal–Wallis tests (control plants) (question d).

**Results**

FA levels were very distinct between the *B. campestris* groups; leaves from resprouting individuals were on average 51.7 % more asymmetric than leaves from control plants (*U*$_{29,29}=147.5$; *p*<0.0001) (Fig. 2a). In resprouting *B. campestris* individuals, extrafloral nectar was measured in 209 of the 278 leaves examined (7.21±1.08 leaves per plant), accounting for 75.2 % of leaves. In control *B. campestris*, 197 out of 278 (70.9 %) leaves were observed to produce nectar (6.79±1.11 leaves per plant). No difference was found for the number of leaves with functional extrafloral nectaries between the two *B. campestris* groups (*t*$_{56}=1.4350$; *p*>0.05). Sugar concentration, however, was markedly different between resprouting and control *B. campestris* (Fig. 2b). Sugar concentration ranged from 0.04 to 0.48 mg/μL and 0.05 to 0.35 mg/μL in resprouting and control *B. campestris*, respectively (*t*$_{56}=4.1051$; *p*<0.0001).

Sugar concentration was positively related to leaf FA in resprouting plants (*r*$_2$=0.3739; *p*=0.0456) and marginally significant in control *B. campestris* individuals (*r*$_2$=0.3559; *p*=0.0580). The abundance of *C. crassus* ranged from two to 12 individuals in resprouting *B. campestris* (6.24±2.32,
and three to eight individuals in the control group (4.97±1.43, n=144) (t_{56}=2.5192; p<0.05). The abundance of *C. crassus* was positively related to sugar concentration in both *B. campestris* groups (resprouting: r_{27}=0.52539; p<0.001; control r_{27}=0.4236; p<0.05) (Fig. 3).

### Discussion

**FA and environmental stress due to fire**

Results showed that FA in *B. campestris* was influenced by habitat disturbance, as individuals recovering from the fire showed elevated FA levels. Positive relationships between habitat disturbance and FA are commonplace and

As regards herbivory, 13 of the 29 *B. campestris* individuals of the resprouting group presented low herbivory levels, moderate herbivory was observed in six individuals and high herbivory in ten (n=29 *B. campestris* individuals analyzed). In control plants (n=29 *B. campestris* individuals analyzed), the scenario was different, low herbivory levels were noticed in four individuals, moderate herbivory in 14, and high herbivory in 11 plants. No individual at the resprouting or control area had extreme herbivory (>76 % of damaged leaves per individual). The abundance of *C. crassus* was associated with low herbivory levels in resprouting *B. campestris* individuals (F_{2,26}=11.4703; p<0.001). In contrast, ant abundance was not related to herbivory levels in control *B. campestris* plants (H_{2}=3.2276; p=0.1991) (Fig. 4).
environmental factors are a major source of stress to plants (Kozlov et al. 1996; Puerta-Piñero et al. 2008; Cornelissen and Stiling 2011). To the best of our knowledge, only one study has assessed the effect of burning on FA (Freeman et al. 2005) and showed significant results. In the Cerrado savanna, fire is the foremost form of disturbance, but it still remains widely unknown whether and how it affects the patterns of growth of bilaterally symmetrical traits. In our study, B. campestris was shown to be under severe stress as leaf FA levels in resprouting plants were 51.7 % higher in comparison to those of control plants. In our study system, several environmental factors might cause FA in B. campestris, the more important being differences in sunlight and wind exposure (burned area). Both sunlight (and the lack thereof) and wind exposure are major stress factors for plants (Møller 1995; Puerta-Piñero et al. 2008). For example, Miconia fallax DC. (Melastomataceae) is highly susceptible to differences in sunlight and individuals growing in shaded conditions have increased FA levels (Alves-Silva 2011). The opposite might occur in B. campestris individuals, as in the field, this plant grows shaded by the canopy of large trees. Thus, open areas and direct sunlight exposure comprise departures from the normal environmental conditions for B. campestris and might cause severe stress (high FA) in individuals. The intense exposure of resprouting B. campestris individuals to direct sunlight might explain not only the increased FA levels, but also the higher number of leaves with active extrafloral nectaries. As shown by Radhika et al. (2010), extrafloral nectary function might be light-dependent, since light elicits changes in plant physiology, affecting the production of nectar. Additionally, high temperatures might also influence extrafloral sugar quantity and quality (Wyatt et al. 1992; Petanidou and Smets 1996).

Sugar quality and ant–plant–herbivore interactions

Sugar concentration and C. crassus abundance were 35.7 % and 21.7 % higher in post-fire resprouting B. campestris, respectively, in comparison to unburned plants. Furthermore, the high abundance of ants in resprouting B. campestris was reflected in reduced herbivory rates. Ants are indeed attracted to high-quality sugar sources and their abundance on plants is negatively related to herbivory (Heil and McKey 2003; see also Josens et al. 1998; Kost and Heil 2005). As shown in other studies, FA can directly influence plant metabolism and the production of chemical compounds (Lempa et al. 2000; Cornelissen and Stiling 2005), and in this study, FA was positively related to extrafloral sugar concentration. Our results, however, are correlative and it appears unlikely that the genes responsible for the growth of paired organs might influence mechanisms of nectar physiology. Thus, we also conclude that other unmeasured variables might have played a role in the differences in extrafloral nectar quality in B. campestris. In general, resprouting after fire involves trade-offs, random effects, and differential allocation of resources in order to maximize plant growth, performance, the development of aboveground structures, and the restitution of biomass (Bellingham and Sparrow 2000; Nzunda and Lawes 2011; Vivian and Cary 2012). The rapid resprouting of B. campestris at the disturbed site, together with increased plant metabolism to recover its aboveground parts might be the reason why extrafloral sugar was more concentrated, and similar results were found for other Banisteriopsis species in the Cerrado vegetation (Alves-Silva 2011). Moreover, in the case of B. campestris, the rapid resprouting of aboveground parts might attract herbivores (see plant vigor hypothesis; Vieira et al. 1996). Thus, the higher sugar concentration in resprouting B. campestris individuals might be beneficial in sustaining more ant-guards to protect the plant.

In conclusion, this study has shown that fire influences plant metabolism (extrafloral nectar), increases the stress rates (FA), and affects trophic interactions (abundance of predatory ants and reduced herbivory) in B. campestris. FA studies in the neotropics are still scarce (Telhado et al. 2010), but are increasing rapidly in number and providing new tools for the understanding of several ecological features responsible for the performance of organisms, both animals and plants, in distinct habitats (Alves-Silva 2012; Costa et al. 2012). Innovative approaches that consider major disturbances, trophic chains, and FA might provide important advances for the knowledge of insect–plant interactions, especially in the Cerrado savanna, where extrafloral nectariied shrubs are common and fire is frequent.

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