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Plant Stress in Determining Attack Rates of Herbivorous Chewing Insects

O Estresse das Plantas na Determinação das Taxas de Ataque de Insetos Herbívoros Mastigadores

Luana Dos Reis Fiuza[1]
Hosana Rosa da Silva[2]
Karen Luiza Rodrigues Duarte[3]
Ronaldo Reis Junior[4]
Walter Santos de Araújo[5]
Paulo Henrique Costa Corgosinho[6]
Pablo Cuevas-Reyes[7]
Magno Augusto Zazá Borges[8]
Maurício Lopes de Faria[9]

ABSTRACT

Objective: this study examines the relationship between fluctuating asymmetry (FA) and herbivory in *Qualea parviflora* and *Eugenia dysenterica* across five Cerrado areas, testing the Plant Stress Hypothesis (PSH) and the Environmental Harshness Hypothesis (EHH). **Methods:** we evaluated how stressful conditions affect plants, herbivory rates, and the richness and abundance of herbivorous insects, considering variation in species composition between

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[1] Mestre em Biodiversidade e Uso dos Recursos Naturais pela Universidade Estadual de Montes Claros (UNIMONTES), Montes Claros MG - Brasil. luanna.fiuza22@gmail.com. https://orcid.org/0009-0002-2777-5255

[2] Mestre em Biodiversidade e Uso dos Recursos Naturais. Universidade Estadual de Montes Claros (UNIMONTES). Montes Claros MG - Brasil. hosanarosa.bio@gmail.com. https://orcid.org/0009-0000-6887-8950

[3] Mestre em Biodiversidade e Uso dos Recursos Naturais pela Universidade Estadual de Montes Claros (UNIMONTES), Montes Claros MG - Brasil. karenlrduarte@gmail.com. https://orcid.org/0000-0001-8610-3577

[4] Doutor em Entomologia pela Universidade de São Paulo - USP. Docente do Programa de Pós-Graduação em Biodiversidade e Uso dos Recursos Naturais. Departamento de Biologia Geral, Universidade Estadual de Montes Claros (UNIMONTES). Montes Claros - MG -Brasil. chrysopa@gmail.com. https://orcid.org/0000-0002-8646-1451

[5] Doutor em Ecologia e Evolução pela Universidade Federal de Goiás – UFG. Docente do Programa de Pós-Graduação em Biodiversidade e Uso dos Recursos Naturais. Departamento de Biologia Geral, Universidade Estadual de Montes Claros (UNIMONTES). Montes Claros - MG -Brasil. walterbioaraujo@gmail.com. https://orcid.org/0000-0003-0157-6151

[6] Doutor em Biologia de Água Doce e Pesca Interior pelo Instituto Nacional de Pesquisas da Amazônia - INPA. Docente do Programa de Pós-Graduação em Biodiversidade e Uso dos Recursos Naturais. Departamento de Biologia Geral, Universidade Estadual de Montes Claros (UNIMONTES). Montes Claros - MG -Brasil. pcorgo@gmail.com. https://orcid.org/0000-0002-2851-6557

[7] Doutor em Ciências pela Universidad Autonoma do Mexico - UNAN. Docente da Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Departamento de Ecología y Recursos Naturales. Morelia – Michoacán – Mexico. pcragalla@gmail.com. https://orcid.org/0000-0002-5897-919X

[8] Doutor em Ciência Animal. Universidade Federal de Minas Gerais - UFMG. Docente do Programa de Pós-graduação em Biodiversidade e Uso dos Recursos Naturais. Departamento de Biologia Geral, Universidade Estadual de Montes Claros (UNIMONTES). Montes Claros - MG -Brasil. magno.borges@unimontes.br. https://orcid.org/0000-0001-5126-1794

[9] Doutor em Conservação e Manejo da Vida Silvestre pela Universidade Federal de Minas Gerais - UFMG. Docente do Programa de Pós-graduação em Biodiversidade e Uso dos Recursos Naturais. Departamento de Biologia Geral, Universidade Estadual de Montes Claros (UNIMONTES). Montes Claros - MG -Brasil. fariaml@gmail.com. https://orcid.org/0000-0003-4976-1874

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phytophysiognomies. **Results:** the results showed significant differences in structural complexity: rocky field < cerrado *sensu stricto* < cerradão. FA varied with complexity for *Q. parviflora*, but not for *E. dysenterica*. Leaf area removed differed by species but did not significantly correlate with complexity. For *Q. parviflora*, leaf area removed decreased with FA, while for *E. dysenterica*, it increased with FA and complexity. **Conclusion:** contrary to predictions, herbivore richness and abundance were greater in the most stressed phytophysiognomy, with variation in species composition mainly due to species replacement.

Keywords: Asymmetry; Cerrado; Herbivores; Phytophysiognomies; Stress.

RESUMO

Objetivo: este estudo examina a relação entre assimetria flutuante (AF) e herbivoria em *Qualea parviflora* e *Eugenia dysenterica* em cinco áreas de Cerrado, testando a Hipótese de Estresse da Planta (HEP) e a Hipótese de Adversidade Ambiental (HAA). **Métodos:** avaliamos como as condições estressantes afetam as plantas, as taxas de herbivoria e a riqueza e abundância de insetos herbívoros, considerando a variação na composição de espécies entre fitofisionomias. **Resultados:** os resultados mostraram diferenças significativas na complexidade estrutural: campo rupestre < cerrado *sensu stricto* < cerradão. A AF variou com complexidade para *Q. parviflora*, mas não para *E. dysenterica*. A área foliar removida diferiu por espécie, mas não se correlacionou significativamente com a complexidade. Para *Q. parviflora*, a área foliar removida diminuiu com a AF, enquanto para *E. dysenterica* aumentou com a AF e a complexidade estrutural da vegetação. **Conclusão:** contrariamente às previsões, a riqueza e abundância de herbívoros foram maiores na fitofisionomia mais estressada, com variação na composição de espécies principalmente devido à substituição de espécies.

Palavras-chave: Assimetria; Cerrado; Herbívoros; Fitofisionomias; Estresse.

INTRODUCTION

The Plant Stress Hypothesis (PSH)[1,2] predicts that plants subjected to hygrothermal and/or nutritional stress become more susceptible to herbivore attack than less physiologically stressed plants. This pattern can be explained by stress inducing an increase in the nutritional quality of plant tissues due to the degradation of nitrogen-rich enzymes[3]. This enzymatic degradation reduces protein synthesis, leading to an increase in the concentration of free amino acids and other soluble nitrogenous constituents in plant tissues[4-2]. Furthermore, there may be a reduction in the concentration of defensive compounds or an alteration in the ratio of nutrients to chemical defenses, as plants invest energy and resources primarily in maintaining homeostasis[1,2,3]. Thus, influenced by the combination of higher nutritional quality and lower defense, plants under stress become more vulnerable to herbivory (Castagneyrol *et al.* 2018[5]; Price 1991[6]).

Currently, one of the most widely used methods to infer plant physiological stress is Fluctuating Asymmetry (FA)[7]. FA represents small random deviations in the symmetry pattern of bilateral characters of a specific morphological trait and can be used to measure changes in ontogenetic development in plants and animals[8]. Under stressful conditions, plants may exhibit developmental instability, increasing the difference between the sides of fully expanded leaves. This difference can be numerically assessed with FA analysis[9]. Some authors relate FA to susceptibility to herbivore attack, which can be explained by the higher nutritional quality of asymmetric leaves compared to symmetric leaves [10,11].

In addition to considering the nutritional quality of resources, free-living herbivorous insects face physiological constraints related to habitat characteristics where they feed. Therefore, in the most stressful environments—those that are hotter and drier, with constant desiccation threats—herbivore attack rates and distribution are limited[12].

The physiological tolerances to abiotic conditions play a crucial role in explaining the spatial and temporal distributions of some insect species[13]. Consequently, Fernandes and Price[14] proposed the Environmental Harshness Hypothesis (EHH), which predicts that endophagous herbivorous insects tend to feed on plants in stressful environments, while free-living herbivores prefer plants in less stressful environments[15,16]. Furthermore, due to environmental stress conditions, plants develop mechanisms that increase sclerophylly, thereby reducing attractiveness to free-living herbivores[14,17]. Thus, it is evident that the Plant Stress Hypothesis (PSH) and the Environmental Harshness Hypothesis (EHH) operate on distinct scales; the effects of environmental harshness can influence plant physiological stress by altering expected patterns.

This study tested the Plant Physiological Stress Hypothesis (PSH)[1] predicting that plants subjected to hygrothermal and nutritional stress will experience higher herbivore attack rates compared to physiologically unstressed plants, and the Environmental Harshness Hypothesis (EHH)[14] which predicts that more stressful environments disadvantage free-living herbivores. Additionally, we investigate the interaction between these hypotheses by assessing how stressful conditions influence individual plants and how these conflicting effects determine herbivore insects attack rates, as well as the richness and abundance of free-living herbivorous insects among different phytophysiognomies of the Cerrado. Consequently, less stressful and structurally more complex phytophysiognomies are expected to exhibit higher richness and abundance of free-living herbivores, thereby resulting in higher herbivore attack rates.

METHODS

Study Area

The study was conducted in five areas, four of which are located in the municipality of Jequitaí, and one in the municipality of Francisco Dumont (Fig. 1). These areas constitute the study stations for fauna monitoring related to the construction of the Jequitaí dam, conducted by the Laboratory of Ecology and Biological Control of Insects at Unimontes, in collaboration with the “Rio Jequitaí” consortium. The municipalities are located in the northern region of the State of Minas Gerais, Brazil, characterized by a megathermal rainy climate classified as Aw, corresponding to a humid tropical savanna climate with a dry winter[18]. The average annual temperature is 23ºC, with precipitation totaling 1000 mm/year, concentrated mainly from November to January, while the period from May to October is characterized by dry conditions[19].

Each area was selected to represent a gradient of environmental stress. Thus, the Água Fria points (campo rupestre) represent the structurally least complex areas, extending to the Água Branca points (cerradão), which are the most complex. The cerrado areas (Estrada Velha and Fazenda Gentios) represent intermediate complexity, while the Cataratas points represent a transitional environment between cerrado *sensu stricto* and dry forest (Fig. 1)

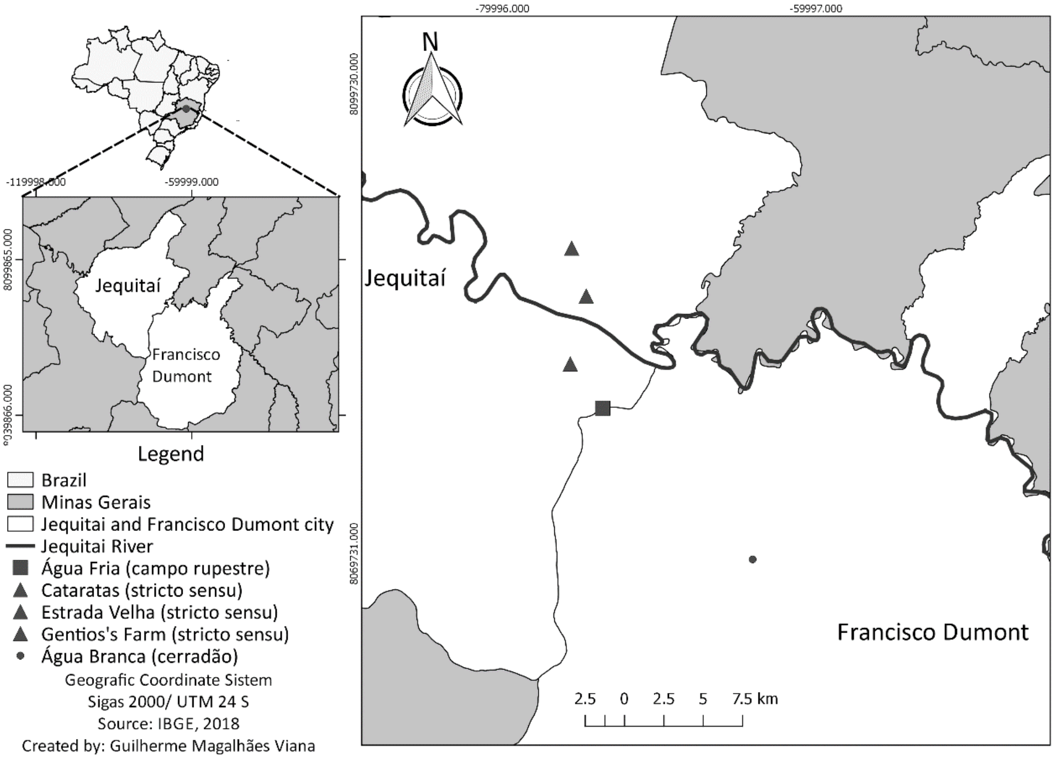

Figure 1- Location of the five sampling areas in the municipalities of Jequitaí (Água Fria, Cataratas, Estrada Velha, Fazenda Gentios) and Francisco Dumont (Água Branca) in Minas Gerais, Brazil.

Data collection

In each study area, a 100 x 100 m² plot was demarcated. Within each plot, five 10 x 10 m² subplots were designated: four at each corner and one centrally located, totaling 25 subplots for sampling. All trees with a Diameter at Breast Height (DBH) of 10 cm or more were identified and tagged with numbered metal plates. Subsequently, the DBH of each tree was measured, and its height was estimated visually. Finally, air temperature and relative humidity were recorded in each subplot using a thermohygrometer.

To evaluate the impact of Fluctuating Asymmetry (indicative of physiological stress) on herbivory rates in plants, we used the two most abundant tree species in the study subplots: *Qualea parviflora* Mart. - Vochysiaceae and *Eugenia dysenterica* Mart. DC - Myrtaceae. Data collection took place in December 2019, during the rainy season.

In each subplot of the study areas, all individuals of the two selected plant species were sampled to assess fluctuating asymmetry. From each individual, three randomly selected branches from around the canopy were sampled, and 20 fully expanded leaves were chosen from these branches. Leaves damaged by herbivory were excluded from the analysis. Next, the leaves of each individual were photographed and digitized for measuring fluctuating asymmetry using ImageJ software. On each leaf, measurements of the width of the right (RW) and left (LW) sides were taken, using the midpoint of the central vein as a reference to the leaf edges. Fluctuating asymmetry of each leaf was calculated as the mean difference between the widths of the right and left sides of the leaf (RW - LW), divided by the sum of the right and left sides of the leaf [(RW - LW) / (RW + LW) / 2], to mitigate the influence of leaf size[10, 20,21].

To quantify the percentage of leaf area consumed by herbivores, an additional 20 randomly selected leaves from around the tree canopy of the same individuals were sampled. These leaves were photographed and digitized, and total leaf area as well as the percentage of leaf area removed were calculated using ImageJ software.

In May 2022, to sample the herbivore community associated with plants, 30 samples were collected per plant using the beating technique. Due to time constraints and to ensure the feasibility of the study, 25 tree individuals were randomly selected, considering specific criteria of similar height and circumference within the Cataratas, Estrada Velha, and Água Fria plots, totaling 75 samples. This approach ensured a representative sampling of the herbivore community.

Herbivorous insects and all fallen foliage were collected from the canopy and transported to the Laboratory of Ecology and Biological Control of Insects at Unimontes for identification at the family level and determination of morphospecies.

Data Analysis

Vegetation Structural Complexity Index and Hypothesis Testing

For the analysis of vegetation structural complexity in each area, we utilized the following variables: plant richness and abundance, mean basal area, mean temperature, and humidity. These variables underwent Principal Component Analysis (PCA) using PAST software[23], to correlate all phytosociological and environmental variables and summarize them into two principal axes. Following the analysis, qualitative terms were grouped using contrast analysis to assess significant differences among the areas. Consequently, we employed PCA scores as an index of structural complexity in sub-plots, serving as an explanatory variable in tests of the Plant Stress Hypothesis (PSH) and Environmental Harshness Hypothesis (EHH). The analyses were performed using R software[24].

To test whether plant physiological stress differs between areas, generalized linear models (GLMs) were constructed, using Fluctuating Asymmetry as the response variable and the Vegetation Structural Complexity Index as the explanatory variable. Additionally, we examined the correlation between the leaf area removed by free-living insects (response variable) and Fluctuating Asymmetry (explanatory variable) to evaluate whether plant physiological stress increases susceptibility to herbivory, as predicted by White's 1969 hypothesis. The statistical analyses were conducted separately for the two species studied, given that they are different species and may respond differently to the environment. All analyses assumed a normal error distribution and were performed using R software[24].

Environmental harshness was assessed using the Structural Complexity Index of vegetation in the study areas, which ranged from the simplest to the most complex forms, representing a stress gradient for the free-living herbivore. For this, a generalized linear model (GLM) was constructed with removed leaf area as the response variable and the Structural Complexity Index as the explanatory variable. The statistical analysis was performed assuming a normal error distribution using R software[24].

Diversity, Abundance, and Beta Diversity of Herbivores (Dβ)

Insect abundance at each site was determined by counting the total number of individual insects collected. These data were subjected to analysis of variance (ANOVA), and a generalized linear model (GLM) with a normal distribution was used to compare mean insect abundance across the sites. Insect diversity across the three sampled sites was assessed using the Shannon index (H'). Both analyses were performed using R software. Herbivore diversity was further decomposed into local and regional components, and Beta diversity (Dβ) was analyzed using the Tidyverse, Reshape2, and Betapart packages within R software[24].

RESULTS

In the arboreal vegetation, 67 plant species distributed across 24 families were sampled. The Fabaceae family was the most representative, comprising approximately 24.6% of the species. The most abundant plant species were characteristic of the Cerrado biome, including *Plathymenia reticulata*, *Acosmium dasycarpum*, *Astronium fraxinifolium*, *Hymenaea stigonocarpa*, *Qualea parviflora*, *Q. multiflora*, and *Eugenia dysenterica*, which were frequently encountered in the study subplots. Total species richness ranged from a minimum of 12 species in the campo rupestre (CR) area to a maximum of 31 species in the cerradão (CD) area. Mean density varied from a minimum of 2.9 individuals per subplot to a maximum of 15.5 individuals in the Fazenda Gentios area. *Qualea parviflora* was the most abundant species in three out of the five study areas.

Principal Component Analysis (PCA) indicates that subplots in the campo rupestre and cerradões are structurally distinct from cerrado *sensu stricto* areas (Fig. 2). The first PCA component captured the majority of data variance (77.58%), primarily composed of variables describing vegetation structure (basal area, richness, abundance, and average height). The second component, explaining 20.17% of data variance, primarily relates to subplot conditions (temperature and humidity). Therefore, we interpret PC1 scores as an index of vegetation structural complexity to visualize the complexity gradient (Fig. 2).

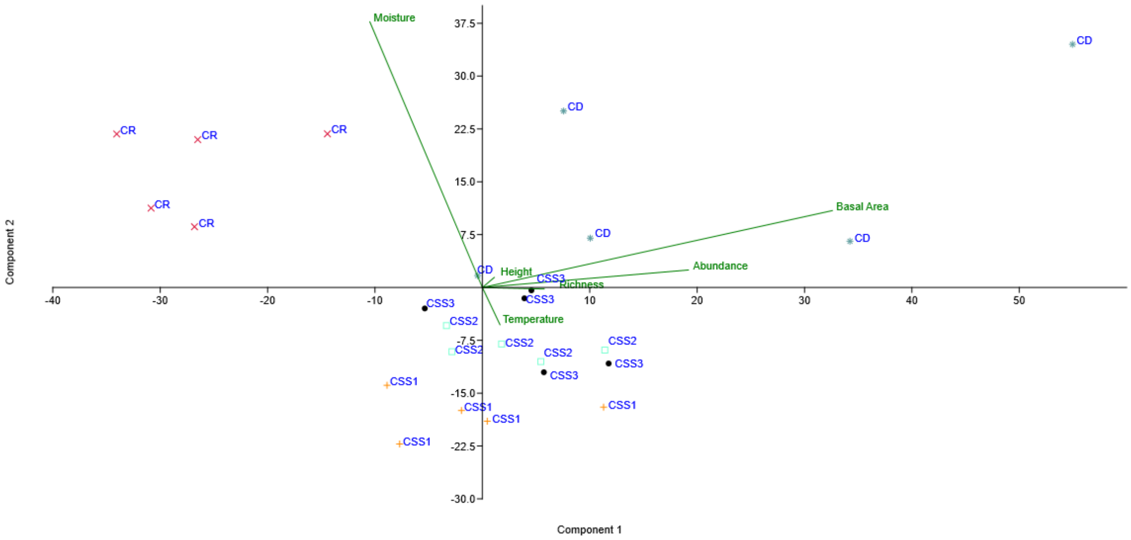

Figure 2 - Principal Component Analysis (PCA) representation of environmental and phytosociological variables across five study areas, with four located in the municipality of Jequitaí and one in Francisco Dumont, MG. The areas include: CR - Campo Rupestre, CD - Cerradão, and CSS - Cerrado *sensu stricto* 1, 2, 3.

A total of 53 individuals of *Qualea parviflora* (Vochysiaceae) and 49 individuals of *Eugenia dysenterica* (Myrtaceae) were sampled across the study areas. For Q. *parviflora*, 2120 leaves were collected, with 1060 leaves used for calculating Fluctuating Asymmetry and the remaining 1060 leaves used for calculating removed leaf area. For *E. dysenterica*, 1960 leaves were collected, with 980 leaves used for calculating Fluctuating Asymmetry and the other 980 leaves used for calculating removed leaf area.

The average leaf fluctuation asymmetry per plant varied negatively with increasing vegetation Structural Complexity Index (F = 5.0372, P < 0.05) for *Q. parviflora* (Fig. 3a). In *E. dysenterica*, on the other hand, fluctuating asymmetry did not vary significantly with increasing vegetation structural complexity (P = 0.582) (Fig.3b).

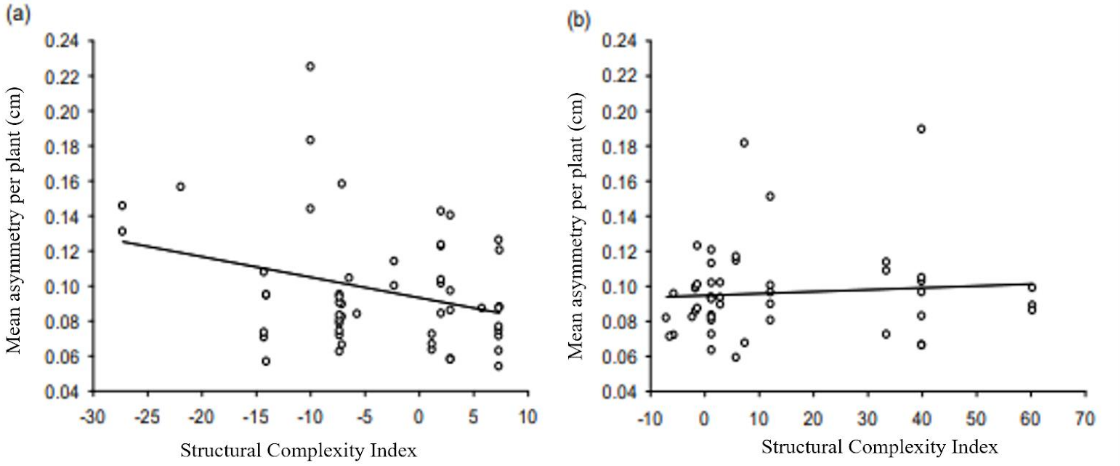

Figure 3- Structural Complexity Index influencing leaf Fluctuating Asymmetry. (a) *Qualea parviflora* and (b) *Eugenia dysenterica*.

Leaf area removed did not vary significantly with increasing complexity index in *Q. parviflora* (F = 0.2267, P = 0.636). Similarly, leaf area removed in *E. dysenterica* individuals did not vary with changes in the complexity index (F = 0.0139, P = 0.906). In contrast, leaf area removed decreased significantly with increasing fluctuating asymmetry in *Q. parviflora* (F = 4.379, P < 0.001) (Fig. 4a). On the other hand, in *E. dysenterica*, leaf area removed increased significantly with increasing fluctuating asymmetry (F = 16.231, P < 0.001) and with the interaction between complexity index and fluctuating asymmetry (F = 11.378, P < 0.001) (Fig. 4b)

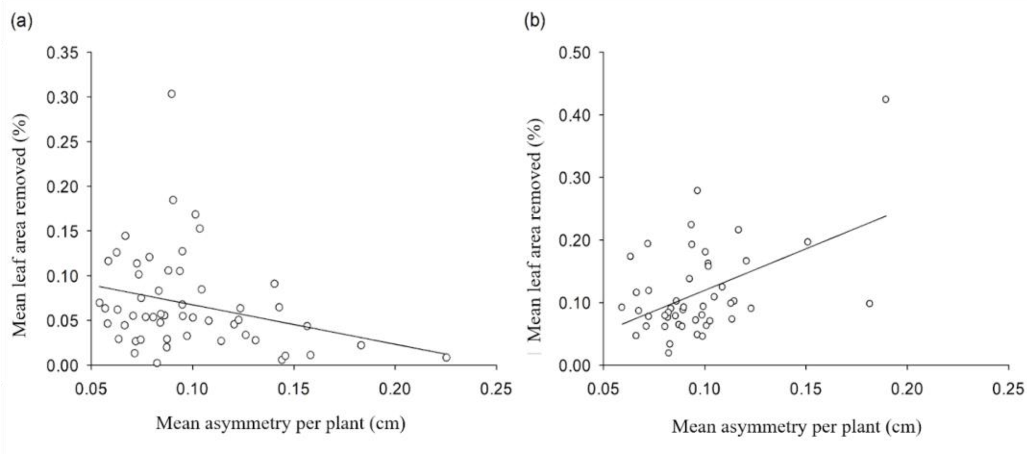

Figure 4 - Average percentage of leaf area removed varying as a function of leaf fluctuating asymmetry in the two studied species, (a) *Qualea parviflora* and (b) *Eugenia dysenterica.*

During the study, 361 insects were sampled, of which 158 were identified as herbivores using a taxonomic key. The three most abundant orders were Hemiptera, with 86 herbivores (54.09%), followed by Coleoptera, with 39 herbivores (24.53%), and Orthoptera, with 18 herbivores (11.32%). The average abundance was highest in the Campo Rupestre (mean of 1.94 individuals), followed by the Cerrado *sensu stricto* (1.31) and the transition between Mata Seca and Cerrado (0.89), although the difference was not significant between the locations (F=0.6339, P=0.5372). The other orders were less frequent, contributing lower abundance to the set of identified herbivores. The total herbivore richness was 38 morphospecies, with the Shannon Index (H’) indicating a diversity of H’=2.64 in the Cerrado *sensu stricto*, H’=1.97 in the Mata Seca/Cerrado transition, and H’=2.06 in the Campo Rupestre. However, richness did not differ between the studied areas (F=2.1681, P=0.1171).

Regarding Beta diversity (β), a presence-absence matrix was constructed using Sorensen dissimilarity as a metric and subsequently partitioned into its turnover and nestedness components, following Baselga's method[27]. Separate matrices were created for each component across the study phytophysiognomies, resulting in the following Beta diversity values: 0.852439 for turnover, 0.03373219 for nestedness, and 0.8861712 for total Beta diversity. Notably, the Sorensen index revealed a Beta diversity (β) of 0.75, indicating significant differences in community composition among study areas or plots (Fig. 5).

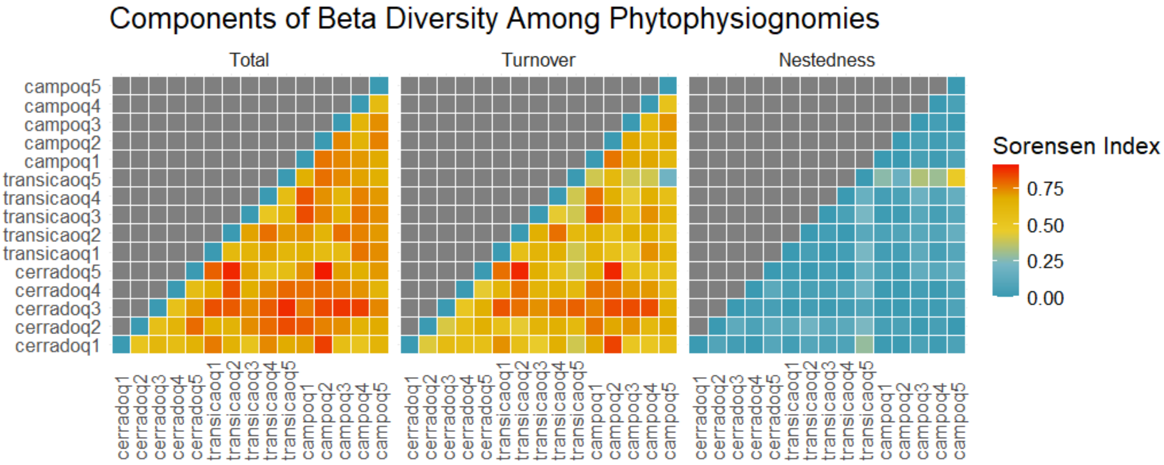

Figure 5 – Components of Beta Diversity (Dβ) determined by Sorensen dissimilarity. The distribution of morphospecies among plots highlights a turnover relationship, with the second matrix showing the majority of variation attributed to turnover, while nestedness (third matrix) contributes less to the observed variation. Areas colored more intensely in red indicate higher variation in species composition.

DISCUSSION

The Cerrado domain is characterized by phytophysiognomies with distinct structural characteristics and species compositions[28]. In this study, vegetation structure varied in terms of species richness, abundance, and Structural Complexity Index. The sampled areas formed a gradient of structural habitats, with the cerradão areas at Água Branca site exhibiting the highest complexity indices. The cerrado *sensu stricto* areas at Estrada Velha, Cataratas, and Fazenda Gentios showed intermediate complexity indices and did not vary significantly among themselves.

Differences in species tolerances influencing their distribution are well-documented in the literature[29,30]. In our study, *Qualea parviflora* and *Eugenia dysenterica* were the dominant species across the sampled sites, occurring prominently in most areas. The species *Q. parviflora* is found across the campo rupestre to cerrado *sensu stricto* formations, while *E. dysenterica* is distributed from cerrado *sensu stricto* areas to cerradão formations. This distribution pattern suggests that *Q. parviflora* tends to occupy more open, well-lit, and dry areas. Indeed, the family Vochysiaceae is typical of open areas, primarily occurring in transition zones between cerrados and campo rupestres. Studies on *Vochysia tucanorum* suggest that individuals growing under shaded conditions experience greater stress, as indicated by alterations in leaf water potential and stomatal conductance, compared to those growing under full sun conditions[32]. On the other hand, Myrtaceae species like *E. dysenterica* appear to be more sensitive to desiccation, which leads them to prefer forest formations with structurally more complex areas[33,34]. Thus, comparing these two species enabled us to assess the impact of structural complexity across the gradient represented by the study areas.

Fluctuating asymmetry, taken as a measure of plant physiological stress, responded idiosyncratically to variations in the structural complexity index in the two studied species. In *Q. parviflora*, asymmetry decreased with increasing habitat complexity. However, in *E. dysenterica*, asymmetry showed no correlation with increasing complexity. Habitats with greater complexity tend to mitigate microclimatic harshness by increasing shading, air circulation, and enhancing local humidity[35,36]. Consequently, greater microclimatic stability reduces disturbances in plant development, resulting in fewer asymmetric leaves[37,38]. Clearly, different plant species exhibit varying tolerances to stress induced by temperature and humidity. Thus, conditions stressful to one species may not be so for another[39, 40,41].

In this study, *E. dysenterica* was significantly more attacked than *Q. parviflora*. The characteristics of the sites where plants survive are determining factors in attack rates[42]. At each site, historical factors interact with ecological factors in determining attack rates. Thus, observed attack rates are influenced by physical and chemical defenses[43,44,45], intrinsic species characteristics[46], nutritional quality[10,37], plant richness and abundance, as well as plant-to-plant interactions [47,48].

Herbivory, measured by the extent of leaf area removed, reflects a cumulative result of successive attacks often carried out by different herbivore species, each varying in susceptibility to environmental stress. However, contrary to expectations, structural complexity did not influence attack rates at the study sites. Indeed, the impact of environmental harshness has been documented as a primary factor in determining attack rates and the diversity of gall-inducing insects on their host plants[14,49,50]. Free-living herbivores, on the other hand, do not appear to respond in the same way. Oliveira *et al.*[51] working in some of the same sites in northern Minas Gerais, demonstrated that networks composed of gall-forming insects exhibited greater specialization, whereas networks composed of free-living herbivorous insects showed greater modularity. Thus, while structural complexity may influence the species richness of chewers (which cause leaf damage), the observed attack rates on leaves may be driven by one or a few more abundant species[49].

In *Q. parviflora*, plants with higher asymmetry were less attacked. Despite fluctuating asymmetry varying negatively with structural complexity, neither habitat complexity nor the interaction between complexity and asymmetry were related to leaf area removed. Zvereva e Kozlov[52] similarly observed in their experiments that *Melasoma lapponica* beetles avoided plants with high fluctuating asymmetry. Thus, intrinsic characteristics of *Q. parviflora* associated with its environment are presumed to account for these differences[53]. The success of herbivores on moderately stressed plants appears to be related to increased nutritional quality of plant tissues, resulting from reduced protein synthesis and increased circulating nitrogen concentration, associated with decreased synthesis of secondary defense compounds[54,55]. However, the PSH was proposed for plants experiencing intermediate physiological stress[1]; therefore, very severe deficits often lead plants into senescence and death[56].

Contrary to our expectations, our results regarding the abundance of herbivorous insects in the study areas did not support the environmental harshness hypothesis. We anticipated that more stressful environments, characterized by lower resource availability and being less suitable habitats for insects, could lead to a reduction in herbivore populations. However, upon analyzing the study areas, we observed that the campo rupestre, which is the most stressful phytophysiognomy, exhibited the highest insect abundance compared to less stressful and more complex phytophysiognomies. Indeed, Monteiro *et al.*[57] reported similar results when studying different altitudes in mountainous environments in Minas Gerais. When analyzing the abundance of free-living herbivores at three different altitudes, each characterized by distinct vegetation structures, they observed a higher abundance of insects at the intermediate altitude. Here, where campo rupestre predominates, insect abundance was greater compared to the lower altitude dominated by cerrado. Both cases may have occurred due the unique structural characteristics of each phytophysiognomy and the specific relationships that certain insects have with their host plants[58], resulting in an overlap of insects from the same species[59].

Similarly, insect richness was predominantly concentrated in the most stressed phytophysiognomy. This result contradicts our initial expectations related to environmental harshness, as we anticipated lower diversity of free-living herbivores in campo rupestre. However, despite these results opposing the environmental harshness hypothesis, they are not isolated findings. Silva *et al.*[60], in studies conducted in dry tropical forests, recorded higher richness and abundance in environments with early successional states, where disturbance and regenerating vegetation were predominant. Similarly, Barbosa *et al.*[61] reported high richness of leaf chewers in a comparable study, indicating stress levels in the vegetation and environment. These findings suggest that, in these contexts, the presence of stress in vegetation and the environment may promote the diversity and abundance of insects, contrary to traditional expectations[62]. Moreover, these results lead us to speculate that this particular trend may be related to the low tree density in this area compared to other studied phytophysiognomies, thereby creating a high insect density effect on the few available trees for colonization.

Beta diversity primarily reflected turnover and, to a lesser extent, nestedness among phytophysiognomies, indicating that communities within each phytophysiognomy are not subsets of the richest one, but rather exhibit different species compositions[25]. The turnover component refers to the exchange of species among the different sampled locations, while nestedness represents the directional loss or gain of species among these locations[26]. Differences in vegetation structure were responsible for changes in the composition of herbivorous insects between campo rupestre and cerrado *sensu stricto* at different altitudes in a mountain range in Minas Gerais[57], indicating that variations in vegetation structure contribute to species turnover among phytophysiognomies. This highlights the predominant effect of heterogeneity among phytophysiognomies on ecological niches, influencing the variation of arthropods collected in the region[63]. The same pattern was identified among gall-inducing herbivores in this area, where variations in floristic composition along the altitudinal gradient directly influenced the diversity and distribution of these species[64]. In this sense, the campo rupestre fields are composed of a variety of intertwined vegetation on unique soils, each exhibiting its own distinct physical and chemical characteristics[65], which significantly influence the observed beta diversity. In contrast, in closed environments, vegetation density, soil moisture, and the availability of decomposing organic matter may be the primary factors determining the presence and diversity of insects[66]. These environmental variations result in resource partitioning and ecological niche differentiation among insect communities, facilitating the coexistence of distinct communities in each type of phytophysiognomy[67,68].

CONCLUSION

In conclusion, our findings highlight the intricate plant-herbivore relationships under physiological and environmental stress, emphasizing the variability in fluctuating leaf asymmetry in relation to habitat structural complexity. We observed a unique relationship between fluctuating leaf asymmetry and environmental complexity across different species, indicating the crucial role of this measure of stress in understanding plant-herbivore interactions in different phytophysiognomies of the Cerrado.

Furthermore, our analysis of beta diversity revealed significant variation in herbivorous insect communities among different phytophysiognomies of the Cerrado, highlighting the role of environmental complexity in shaping these communities. These results underscore the complexity of insect-plant interactions in the Cerrado and emphasize the necessity of adopting a broader approach that considers temporal and spatial contexts in understanding insect beta diversity across environmental stress gradients. Such a comprehensive understanding is essential for preserving Cerrado biodiversity and ensuring the sustainability of associated ecosystems.

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