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Padrões de herbivoria e efeitos na sobrevivência de plantas lenhosas jovens em uma Savana Neotropical

Herbivory patterns and effects on survival of woody juvenile plants in Neotropical savanna communities

PAOLA PISETTA RAUPP

2021

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Dissertação apresentada à Universidade Federal de Uberlândia, como parte das exigências para obtenção do título de Mestre em Ecologia e Conservação de Recursos Naturais.

Orientador: Prof. Dr. Alan Nilo da Costa

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“o que não somos, nós outras, é essa multidão vibrátil, mais ingênua, pronta a extasiar-se com os efeitos de luz das metáforas eloquentes sobre os quadros de filha, esposa e mãe numa resignação com as belezas que se mostram e as fealdades que se escondem...o que não aprovamos...é tudo isso que decorre da desproporção de direitos e dos desencontros dos deveres."

Trecho do discurso de Edith Gama proferido na inauguração da 2 Convenção Nacional Feminista, com homenagem a Bertha Lutz - 1934.

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RESUMO GERAL

As folhas frequentemente apresentam danos causados por insetos herbívoros nos ecossistemas terrestres. No primeiro ano de desenvolvimento das plantas, a herbivoria é considerada o principal estressor biótico responsável pela redução na sua sobrevivência. Nesse sentido, os herbívoros podem modificar a abundância e distribuição das espécies vegetais e, consequentemente, moldar a estrutura de comunidades. Entender o funcionamento e o papel da herbivoria durante os primeiros estágios de vida das plantas representa a linha de base para desenvolver estratégias de conservação, o que é, particularmente, importante para a savana brasileira, considerada a savana tropical mais rica em biodiversidade do mundo. No primeiro capítulo desse estudo, nós caracterizamos e comparamos a herbivoria foliar de acordo com a frequência e nível de dano em plantas jovens lenhosas, distinguindo-se os danos entre seis guildas de insetos (minadores, galhadores, sugadores, raspadores, mastigadores e cortadores) em formações vegetais de savana aberta e fechada. Nós encontramos que a frequência de folhas atacadas por herbívoros variou de acordo com a formação vegetal e guilda de herbívoro, sendo maior para cortadores em savana fechada e para raspadores e mastigadores em savana aberta. A comunidade de plantas na savana fechada teve um nível de herbivoria foliar 1.4 vezes maior que na savana aberta, principalmente devido a guilda dos cortadores. Essa guilda foi responsável pela maior redução de área foliar (~ 95%), incluindo a remoção da parte aérea das mudas através de um corte ao nível do hipocótilo feito pelas formigas-cortadeiras-de-folha, registro até então nunca contabilizado. No segundo capítulo, nós avaliamos a sobrevivência e a capacidade de rebrota de 1520 mudas de 10 espécies arbóreas com diferentes tamanhos de sementes após simular quatro níveis de herbivoria experimental de insetos mastigadores (0%, 50%, 100% e 100% mais hipocótilo) em formação vegetal de savana aberta e fechada. Nós encontramos que a sobrevivência de mudas variou de acordo com a espécie, formação vegetal e intensidade de herbivoria. Desfolhas até 50% não afetaram a sobrevivência das mudas em nenhuma formação vegetal. Alternativamente, altos níveis de herbivoria (100% e 100% mais hipocótilo) reduziram a sobrevivência das mudas. As mudas em formação vegetal aberta tiveram menor sobrevivência para todos os níveis de herbivoria. Além disso, as características intrínsecas das espécies, como o tamanho da semente e a capacidade de rebrota das mudas, foram positivamente relacionadas com o aumento na sobrevivência das mudas. No geral, os resultados encontrados nos dois estudos ilustram o papel dos insetos herbívoros em moldar as comunidades de plantas em diferentes formações vegetais. Mais que isso, fornecem informações importantes para o desenvolvimento de planos de restauração e conservação da savana brasileira, principalmente para ambientes com alta incidência de herbívoros, como formigas-cortadeiras-de-folha.

Palavras-chave: Cerrado; dano foliar; estabelecimento de plantas; formigas-cortadeiras; guilda de insetos; herbivoria; mudas.

ABSTRACT

Leaves often show damage caused by insect herbivores in terrestrial ecosystems. During the first year of plants' development, herbivory is considered the main biotic stressor responsible for reducing their survival. In this sense, herbivores can modify the abundance and distribution of plant species and, consequently, shape the structure of terrestrial communities. Understanding the functioning and role of herbivory during the early stages of plants life is the baseline to develop conservation strategies, which is particularly important for the Brazilian savanna, considered the richest tropical savanna in biodiversity of the world. In the first chapter of this thesis, we characterized and compared herbivory according to the frequency and leaf damage levels of woody saplings accounted for six insect guilds (mining, galling, sucking, rasping, chewing, and cutting) in two savanna formations (open and closed). We found that the frequency of herbivory on leaves varied according to vegetation formation and herbivore guild, being higher for cutting in closed savanna and for rasping and chewing in open savanna. Leaves of woody saplings in closed savanna showed a 1.4 times higher leaf herbivory level than open savanna, mainly due to cutting guild. This guild exhibited the highest level of leaf damage (~ 95%), including the removal of the shoot-system of plants through a cut in the hypocotyl made by leaf-cutter ants, which was never counted before. In our second chapter, we evaluated the survival and regrowth capacity of 1520 seedlings of 10 tree species with different seed sizes after simulating four levels of experimental herbivory of leaf-chewing insects (0%, 50%, 100%, and 100% plus hypocotyl) in open and closed savanna formation. We found that the survival of seedlings varied according to species, environment, and herbivory intensity. Defoliation up to 50% did not affect seedling survival in any savanna formation. Alternatively, high levels of herbivory (100% and 100% more hypocotyl) reduced seedling survival. Seedlings in open savanna had lower survival for all herbivory levels. In addition, intrinsic traits of plants such as seed size and growth capacity were positively related to successful plant establishment. Overall, our results found in both studies reinforce the role of herbivores insects in shaping plant communities. Finally, they provide important information for the development of restoration and conservation strategies for the Brazilian savanna, especially in places with the dominance of leaf-cutter ants.

Keywords: Cerrado; herbivory; insect feeding guild; leaf-cutter ants; leaf damage; plant establishment; saplings.

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INTRODUÇÃO GERAL

A herbivoria vem aumentando ao longo dos anos (Meineke et al., 2019). Nos ecossistemas terrestres é praticamente impossível encontrar plantas que não exibam danos causados por insetos herbívoros (Kozlov and Zvereva, 2017). Globalmente é estimado que eles consumam entre 5% e 10% do tecido foliar das plantas (Brezzi et al., 2017; Galmán et al., 2018; Mendes et al., 2021). Estudos demonstraram que tais danos causados por insetos são capazes de impactar a dinâmica de populações de plantas (Myers and Sarfraz, 2017), afetando a estrutura e o funcionamento de comunidades (Costa et al., 2017) e ecossistemas (Hunter, 2001). Entretanto, a maioria dos estudos que estima herbivoria e os seus impactos, dedica-se somente às plantas adultas, tendo relativamente pouca atenção dada aos primeiros estágios de desenvolvimento das plantas, que é representado por plântulas e mudas (Barton and Hanley, 2013).

Apesar dos diversos estressores ambientais que podem dificultar a sobrevivência das mudas, como a competição, a seca e os patógenos, a herbivoria é considerada o principal fator responsável pela sua mortalidade (Moles and Westoby, 2004). Sabemos que as mudanças morfológicas e fisiológicas que ocorrem ao longo do desenvolvimento das plantas podem influenciar sua performance no ambiente (Massad, 2013). Nesse sentido, as mudas são consideradas particularmente vulneráveis ao ataque de herbívoros devido seu menor tamanho e baixa reserva de nutrientes e defesas químicas comparada com plantas adultas (Hanley et al., 2004). Devido a esses fatores, os mesmos níveis de herbivoria impostos em plantas adultas frequentemente geram consequências mais drásticas quando impostos em mudas, afetando negativamente seu crescimento, sua função foliar e até sua sobrevivência (Blundell and Peart, 2001; Massad, 2013). Dessa forma, os danos causados em mudas podem causar a modificação na composição das plantas que comporão a vegetação (Hanley and Sykes, 2009), por isso, as mudas desempenham uma função crucial na dinâmica e estrutura de comunidades (Barton and Hanley, 2013).

As plantas geralmente possuem suas folhas atacadas por insetos, os quais podem pertencer a diferentes guildas alimentares (Kozlov and Zvereva, 2017). Essas guildas reúnem grupos de espécies de herbívoros que principalmente se assemelham quanto ao seu tipo de alimentação. Nesse sentido, cada guilda de insetos herbívoros pode ocasionar diferentes tipos de danos e impactar diferentemente as plantas dependendo da frequência e nível de dano foliar (Brezzi et al., 2017). Guildas de sugadores, minadores e galhadores apresentam em sua maioria insetos especializados, alimentando-se de uma ou poucas espécies de plantas. Geralmente, eles apresentam um nível baixo de dano foliar nas comunidades de plantas e, por isso, suas estimativas de herbivoria são pouco reportadas na literatura (Kozlov and Zvereva, 2017; Oliveira et al., 2020). Entretanto, apesar dessa baixa frequência de ataque e nível de dano, indica-se que eles podem afetar o crescimento e a reprodução de plantas hospedeiras (Zvereva et al., 2012, 2010). Todavia, os herbívoros mastigadores (ou desfolhadores) são mais estudados e reportados na literatura (Kozlov and Zvereva, 2017). Eles normalmente se alimentam de forma generalista (Oliveira et al., 2020) e possuem uma alta contribuição na remoção da biomassa de plantas em ecossistemas terrestres (Crawley, 1989). Através da remoção dos tecidos vegetais, os herbívoros mastigadores podem impactar a sobrevivência e, consequentemente, o estabelecimento das plantas dependendo da quantidade foliar consumida (Barton and Shiels, 2020). Reporta-se que desfolhas de até 50% parecem não afetar a sobrevivência das mudas ao nível da comunidade, enquanto desfolhas completas causam sua diminuição (Barton and Shiels, 2020; Hinman and Fridley, 2020). Dessa forma, os impactos da herbivoria nos primeiros estágios de vida parece variar de acordo com o tipo de herbívoro, a frequência de ataque e a intensidade do dano.

Fatores intrínsecos e extrínsecos às plantas e às suas espécies podem modular os efeitos da herbivoria no estabelecimento das mudas (Schuldt et al., 2012). Particularmente, o tamanho da semente das espécies é considerado um atributo funcional que auxilia a predizer a performance das mudas (Suárez-Vidal et al., 2017). Relata-se que, em resposta a herbivoria, espécies de mudas com sementes maiores possuam maior sobrevivência em comparação às espécies que possuem sementes menores devido à sua maior quantidade de reserva nutritiva disponível para a produção de raízes e caules maiores (Moles and Westoby, 2004). Ainda, a capacidade das mudas de rebrotar suas folhas é considerada um atributo de tolerância frente à herbivoria (Mundim et al., 2012). Nesse sentido, mudas que conseguem rebrotar após remoção foliar completa reduzem os impactos negativos causados pela perda fotossintética, o que é essencial durante o período de seu desenvolvimento (Kraft et al., 2015).

As condições ambientais são fatores extrínsecos às plantas e que podem influenciar as respostas de sobrevivência das mudas (Salazar et al., 2012). Os nutrientes no solo, a serrapilheira, o microclima e a luminosidade são algumas das condições abióticas que podem mudar ao longo dos gradientes ambientais e modificar as respostas de tolerância das mudas à herbivoria (Hoffmann et al., 2004). Especificamente, a luminosidade é amplamente conhecida por impactar a capacidade de rebrota e, consequentemente, a sobrevivência das mudas após eventos de herbivoria (Norghauer et al., 2008). Por exemplo, em ambientes florestais, onde a luz é o agente limitante para o desenvolvimento vegetal, a presença de clareiras parece aumentar a sobrevivência de mudas danificadas (Norghauer et al., 2008). Em contrapartida, em ambientes com presença de luz constante, o estresse abiótico promovido pelo aumento da temperatura e da escassez hídrica podem ocasionar uma maior mortalidade das mudas (Salazar et al., 2012).

A savana brasileira (bioma Cerrado) é composta por uma vasta riqueza vegetal e heterogeneidade de habitats (Ribeiro and Walter, 2008), o que a qualifica como um excelente ambiente para o estudo sobre as interações herbívoro-planta em diferentes condições ambientais. Devido à sua riqueza e à elevada porcentagem de perda da sua cobertura vegetal nativa, ela é classificada como um *hotspot* de biodiversidade (Myers et al., 2000). Suas paisagens exibem contrastantes formações vegetais que incluem uma diversa e abundante comunidade de insetos herbívoros (Marquis et al., 2001). Dentre eles, as formigas-cortadeiras-de-folhas que, em sua maioria, pertencem ao gênero *Atta*, são consideradas os herbívoros dominantes em termos de consumo de biomassa foliar (Costa and Vieira-Neto, 2016; Wirth et al., 2003). Elas são herbívoros generalistas que podem consumir desde quantidades parciais das folhas até quase completamente as mudas, cortando seu hipocótilo alguns centímetros acima do solo (Wirth et al., 2003; observações pessoais). Dada a atuação das formigas-cortadeiras na remoção foliar, elas também são consideradas importantes filtros ecológicos por modificarem a composição e a abundância das espécies de plantas arbóreas (Costa et al., 2017; Garcia et al., 2020). Entretanto, sua frequência de ocorrência e o efeito dos seus níveis de dano na sobrevivência de mudas ao nível da comunidade permanecem inexplorados.

Vários estudos reportam estimativas e efeitos da herbivoria na sobrevivência das plantas aliado a fatores intrínsecos e extrínsecos das espécies. Entretanto, permanece pouco explorado, como a associação das condições ambientais e a herbivoria interagem influenciando os danos foliares e a sobrevivência das mudas nas comunidades. Menos ainda, como os fatores intrínsecos das espécies vegetais impactam nessa sobrevivência (e.g., tamanho de semente e capacidade de rebrota). Conhecer a magnitude dos danos causados por insetos herbívoros e seus efeitos na sobrevivência de plantas jovens em contrastantes formações savânicas representa uma oportunidade para melhor compreender os fatores que determinam a estrutura das comunidades. Nesse sentido, esse estudo foi desenvolvido em formações savânicas fechada e aberta em uma savana Neotropical. No primeiro capítulo foi avaliado os padrões de herbivoria foliar em comunidades de mudas, investigando a frequência e o nível de dano e as principais guildas de insetos responsáveis por tais danos. No segundo capítulo foi avaliada a sobrevivência de mudas de 10 espécies lenhosas após simular quatro níveis de herbivoria de insetos mastigadores (i.e., controle, 50%, 100% e 100% mais hipocótilo), considerando os diferentes tamanhos de semente e a capacidade de rebrota dessas mudas após o dano. As informações contidas nos dois capítulos a seguir providenciam discussões sobre como determinados fatores abióticos e bióticos afetam a dinâmica da vegetação nos ecossistemas savânicos neotropicais. Por fim, também são fornecidas implicações importantes relacionadas à ecologia, conservação e restauração de paisagens nas regiões tropicais.

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CAPÍTULO 1.

Intensity and frequency of insect guilds herbivory on saplings across two tropical savanna formations

Capítulo formatado conforme as normas da revista Acta Oecologica

ABSTRACT

Plants frequently exhibit some level of herbivory imposed by insect herbivores. However, there are scarce herbivory estimates on the first stages of plant life, especially at the community level across non-forest environments. Concurrently, there are no studies that investigated to what extent the damages caused by different insect feeding guilds in saplings of Neotropical savannas. Accessing such information represents the initial step to better understand the role of herbivore insect food-webs in tropical savannas. Here, we aimed to characterize and compare herbivory according to the frequency and level of foliar damage distinguishing six insect-feeding guilds (i.e., mining, galling, sucking, rasping, chewing, and cutting) across two savanna formations (i.e., closed and open savanna). We measured visible damage in marked leaves of 22 juvenile woody species to determine the frequency and level of accumulated leaf herbivory in closed and open savanna formation. Our results demonstrated that the frequency of leaves attacked by herbivores into communities varied according to the plant formation and herbivore guild, being higher for cutting guild in closed, and rasping and chewing guilds in open savanna. Also, we showed that the proportion of leaf loss varied widely across individuals, ranging from 0.01% to 100%, but mostly reaching 10%. The environment altered the amount of leaf tissue removed by herbivores, which was 1.40 times higher in closed than open savanna. Cutting insects (mainly leaf-cutting ants) were the guild that most reduced vegetal tissues (~95%), removing even the shoot system of approx. 27% of the plants, drastically increasing the herbivory levels into communities. Our results highlight the role of leaf-cutting ants in drastically removing vegetal tissues in closed savanna, where their nests are most present in the study area. Based on that, we indicate that herbivores distribution can modify the spatial pattern of herbivory in saplings of Brazilian savanna.

Keywords: herbivory, insect feeding guilds, leaf-cutting ants, leaf damage, plant-insect interaction, saplings, savanna vegetation.

INTRODUCTION

Plants frequently exhibit some level of leaf damage by herbivory (Barton and Hanley, 2013; Kozlov and Zvereva, 2017), which is a major determining of variation in growth and survivorship in the first stages of plant life (Benitez-Malvido et al., 1999; Moles and Westoby, 2004). Despite this importance, most studies have estimated herbivory in adult plants (e.g., Kozlov et al., 2015; Mendes et al., 2021), while few efforts have been concentrated in the juvenile stages (Barton and Hanley, 2013). Saplings present smaller sizes and lower nutrients reserves than adult individuals (Hanley et al., 2004). These characteristics make them more vulnerable to herbivore attacks (Moles and Westoby, 2004; Vadigi and Ward, 2014), which can impact the abundance and distribution of plants depending on the damage intensity (Maron and Crone, 2006). Thus, studies of herbivory in saplings are needed to clarify the impact of herbivores on the structure and composition of plant communities.

Plants are often attacked by numerous types of insect herbivores (Kozlov and Zvereva, 2017). Herbivore guilds differ in feeding mode, overall abundance, and mean specialization, and frequently have a distinct impact on the frequency and amount of foliar damage (e.g., Eichhorn et al., 2007). The estimation of these damages can indicate the overall background losses of a plant to different insects, helping to understand the insect-vegetation dynamic (Kozlov and Zvereva, 2017). To our knowledge, few studies investigated the pattern of leaf damage in sapling communities (see, De la Cruz and Dirzo, 1987; Martini and Goodale, 2020; Souza et al., 2013; Stiegel and Mantilla-Contreras, 2018). Moreover, these few studies were all carried out in forested landscapes. Therefore, it is unknown whether different insect feeding guilds can show a similar frequency and level of damage in sapling communities across non-forest environments.

Herbivory levels are known to vary along environmental gradients (Eichhorn et al., 2007, 2006; Howlett and Davidson, 2001). However, remain uncertain how environmental conditions can boost or weak herbivory levels at the community level since some studies have shown contradictory results. For example, plants growing in the sun have shown greater damage compared to those growing in shady habitats (Salgado-Luarte and Gianoli, 2010). In divergence, it has also been shown that saplings under high light irradiance display less herbivory than under canopy closure (e.g., Goodale et al., 2014). Finally, it was also reported that light incidence has no overall effect on herbivory (Eichhorn et al., 2007, 2006). Nevertheless, herbivory patterns can change among environments as a result of differences in the quality, quantity, and spatial distribution of plant species (e.g., V. C. Martini et al., 2021; Salgado-Luarte and Gianoli, 2010). Such variations can hinder predictions about the damage frequency and intensity of different feeding insect guilds on saplings throughout vegetations types.

Heterogeneous environments represent excellent study models to assess the impact of abiotic variations on herbivory patterns of juvenile woody plants. The Neotropical savanna (also called Brazilian Cerrado) is a biodiversity hotspot with various habitats that differ in environmental conditions and vegetation structure (Myers et al., 2000; Ribeiro and Walter, 2008). No other tropical savanna in the world has such species, shapes, and functional richness (Myers et al., 2000). These ecosystems have a diverse and abundant community of insect herbivores (Marquis et al., 2001), which include leaf-cutting ants of the *Atta* genus (Costa and Vieira-Neto, 2016). These ants are considered dominant invertebrate herbivores in the consumption of plant biomass (Costa et al., 2017, 2008; Meyer et al., 2011; Wirth et al., 2003). However, to our knowledge, no study has distinguished their cutting foliar damages and contrasted them with different insect guilds to understand their role in herbivory patterns and community structure in savanna formations.

Patterns of leaf herbivory in non-forest environments are rarely recorded (Martini and Goodale, 2020), especially in saplings of different vegetation types. This information is particularly important to understand the Cerrado dynamics, a biodiversity hotspot that has been intensively devastated in the last decades (Myers et al., 2000; Strassburg et al., 2017). By estimating the herbivory in communities of woody saplings over time, distinguishing six insect-feeding guilds across two vegetation formations (open and closed savanna), we aimed to make a general characterization of saplings herbivory in an area of Neotropical savanna by testing if the (ii) frequency and (iii) level of leaf damage vary according to the savanna formations and insect-feeding guilds. Our main hypotheses were that there would be a significant difference in the frequency and level of leaf damage among the (H1) savanna formations and (H2) insect-feeding guilds, (H3) with the greatest impacts from leaf-cutting ants.

MATERIAL AND METHODS

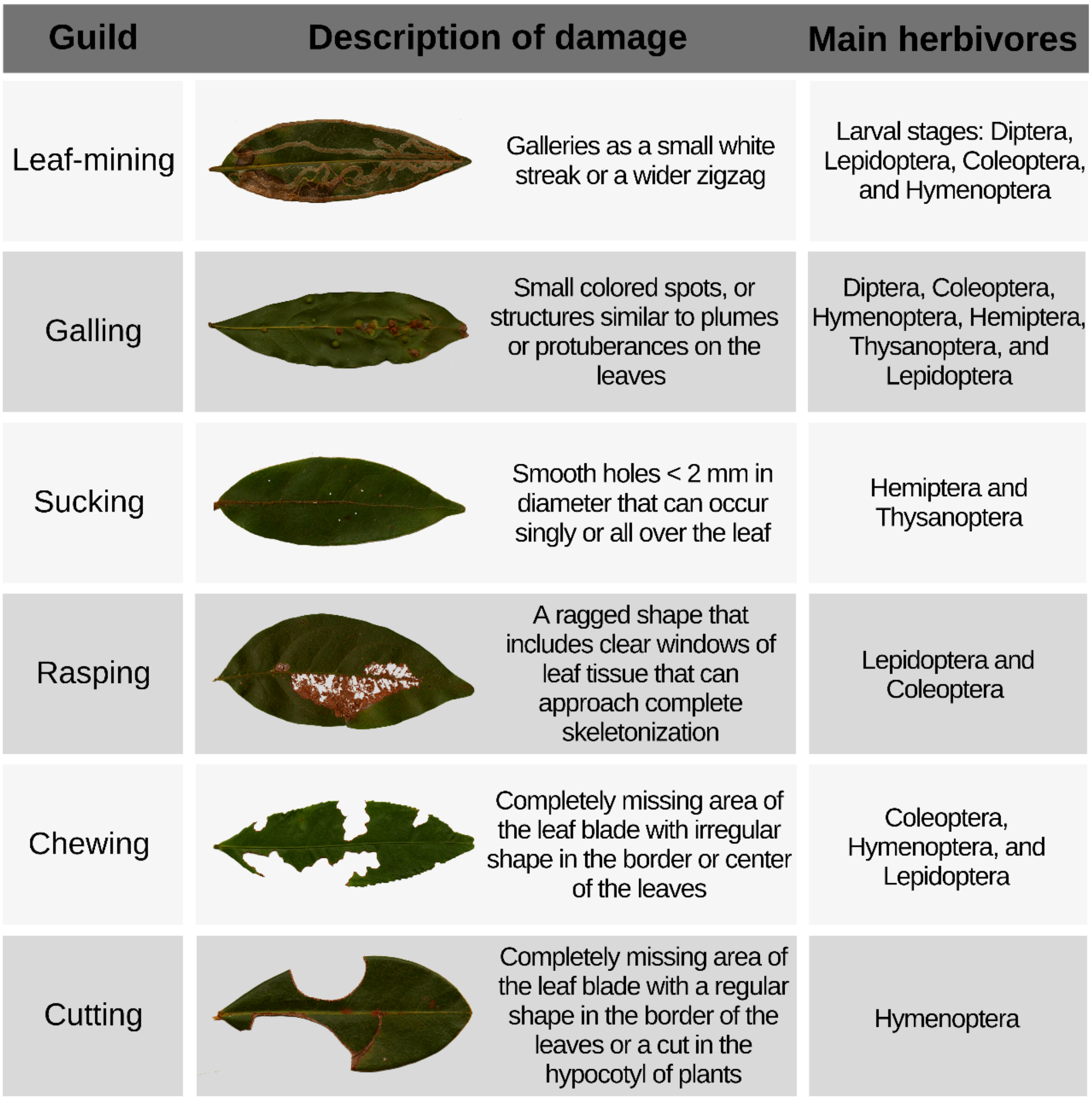
Study area

We carried out this study at Panga Ecological Station (hereafter: PES; 19°10’ S and 48°23’ W), a 405-ha reserve owned by the Federal University of Uberlândia (UFU), located 30 km south of Uberlândia, Brazil. The climate of the region according to the Köppen-Geiger classification is AW, presenting two well-defined seasons: a rainy season from October to April and a dry season from May to September (Alvares et al., 2013). The annual average temperature and accumulated precipitation are approx. 23 °C and 1650 mm, respectively (Cardoso et al., 2009). The PES vegetation is composed of several plant formations typical of Brazilian Cerrado (Gonçalves et al., 2021). Our fieldwork was conducted in two formations based on their contrasting vegetation cover: *cerrado denso* (hereafter: closed savanna) and *cerrado ralo* (hereafter: open savanna; Ribeiro & Walter 2008). The former has a dense woody layer with a canopy cover of around 60% and has practically no grass in understory vegetation, while the latter is characterized by a dense herbaceous layer with sparse shrubs and small trees with a canopy cover of less than 20% (Ribeiro and Walter, 2008).

Experimental design

We delimited 10 plots of 4 m^2 in each savanna formation at least 30 m apart. In each plot, we tagged saplings of shrubs and trees less than 30 cm height above the ground. We identified them to species or genus, randomly selecting up to three individuals per species in each plot. Overall, we selected between nine and 14 individuals per species according to their natural abundance and presence in each savanna formation. Then, we used a permanent pen to mark two or three leaves from top to bottom per individual, depending on the availability of leaves. The marked leaf method does not damage the leaves or prevent herbivores to attack, and it enables perform temporal monitoring of herbivory (Lowman, 1984). From March to August 2020 (ranging from wet and dry seasons in the region), we monthly photographed each marked leaf in the field. These photos were analyzed in the ImageJ software version 1.48 (Rasband, 2018) to measure the total leaf area and the total damaged/consumed area (sensu Calixto et al., 2015). We evaluated 814 leaves from 276 woody saplings of 22 species across 20 plots to determine the leaf damage accumulated over six months (Table S1).

We measured and distinguished leaf injuries in six diagnosable damage types: mining, galling, sucking, rasping, chewing, and cutting (Fig. 1). The last type was a new category created to include leaf-cutter ants (*Atta* and *Acromymex* genera) and leaf-cutter bees (*Megachile* spp.). We separate these insects from the chewing guild because they do not consume directly the leaves and they display damage patterns easily diagnostically (Fig. 1). Leaf-cutter ants collect plant tissues to serve as a substrate for the growth of a mutualistic fungus that is the main food source for the colony (De Fine Licht and Boomsma, 2010), while leaf-cutter bees use leaf tissues to construct nests (Eigenbrode et al., 1999). Pathogen necroses were not counted. During the field surveys, juvenile trees with absent marked leaves and no herbivory evidence were considered as a result of the natural process of leaf turnover or senescence (Dent, 2004). These leaves losses were not attributed to herbivores, but their accumulated damage level was assumed as the same recorded in the previous monitoring. All the herbivory measurements were conducted by a single person to avoid inconsistencies.



Guild	Description of damage	Main herbivores
Leaf-mining	Galleries as a small white streak or a wider zigzag	Larval stages: Diptera, Lepidoptera, Coleoptera, and Hymenoptera
Galling	Small colored spots, or structures similar to plumes or protuberances on the leaves	Diptera, Coleoptera, Hymenoptera, Hemiptera, Thysanoptera, and Lepidoptera
Sucking	Smooth holes < 2 mm in diameter that can occur singly or all over the leaf	Hemiptera and Thysanoptera
Rasping	A ragged shape that includes clear windows of leaf tissue that can approach complete skeletonization	Lepidoptera and Coleoptera
Chewing	Completely missing area of the leaf blade with irregular shape in the border or center of the leaves	Coleoptera, Hymenoptera, and Lepidoptera
Cutting	Completely missing area of the leaf blade with a regular shape in the border of the leaves or a cut in the hypocotyl of plants	Hymenoptera

Figure 1. Herbivore feeding guild responsible for the damage on leaves. Classification adapted from De la Cruz e Dirzo (1987), Andrade *et al*., (2020), and the identification key for Dyer *et al*. (2010).

Statistical analyses

To investigate whether the frequency of accumulated herbivory in leaves at the community level is different according to the environment and insect feeding guild, we first transformed the data to presence-absence and fitted a GLMM (generalized linear mixed-effects model). We used this data as the response variable with binomial (logit link) distribution, setting the interaction term between environment and insect-feeding guild as a fixed effect, and sampling plot identity and leaf identity nested on plant individual nested on species as random effects.

To investigate whether the level of accumulated herbivory in leaves differs according to the environment and insect feeding guild, we used the data of herbivory occurrence and fitted another GLMM having the level of herbivory as a response variable and the same categories of fixed and random effects described above. We used the beta distribution with a logit link, which is appropriate to model proportions (Stroup, 2012). The beta family allows values greater than 0 and less than 1. The smallest proportional value we had was 0.0001 and the highest was 1 (i.e., total leaves removed). Then, we redistributed data setting 0.0001 as minimum and 0.9999 as maximum using the R-package *scales* version 1.1.1 (Wickham and Seidel, 2020). This procedure keeps the relative distances between data observations and changes their scale.

The GLMMs were fitted using the R-package *glmmTMB* version 1.0.2.1 (Brooks et al., 2017). We checked the fit of models using the QQ plot of residuals and the plot of residuals *vs.* predicted values by simulating the residuals 1000 times in the R-package *DHARMa* version 0.3.3.0 (Hartig, 2020). Models' significance was assessed through type II Wald chi-square tests using the R-package *car* version 3.0.10 (Fox and Weisberg, 2019). Post-hoc tests were conducted through the comparison of the estimated marginal means adjusting the p-values using Tukey's method at the 0.05 level of significance in the R-package *emmeans* version 1.5.3 (Lenth, 2020). Having a significant result for an interaction term, we performed posthoc analysis with the environment and insect-feeding guild as filters. We back-transformed the estimated marginal means (EMMs) and standard errors for plotting using the R-package *RVAideMemoire* version 0.9.78 (Hervé, 2020). These same EMMs were used to quantitatively describe model results. We also plotted raw data using dots and added jittering (i.e., random noise on points distribution) to prevent overplotting and improve visualization. All analyses were carried out in R software version 4.0.3 (R Core Team, 2020).

RESULTS

Frequency of herbivory

From 814 leaves of woody saplings monitored, 69.5% suffered herbivory damage (566 leaves of 238 plants), while the remaining 30.5% were undamaged (248 leaves of 38 plants). We observed that 228 leaves (40.0%) were attacked by more than one herbivore guild. Also, we found a significant interaction of environment and herbivore guild on the frequency of leaf herbivory ($\chi^2 = 71.21$, df = 5, $p < 0.001$, Fig. 2). Cutting damage was the most frequent in closed savanna (0.31) while rasping and chewing were the most frequent in open savanna (both with 0.35). Galling also was more frequent in the open (0.05) than in the closed savanna (0.01). Mining (0.01) and sucking (0.21) had no differences between environments. We also found no effects related to the environment (EMM= 0.10; $\chi^2 = 2.72$, df = 1, $p = 0.099$), but a significant difference related to insect-feeding guilds ($\chi^2 = 257.75$, df = 5, $p < 0.001$).

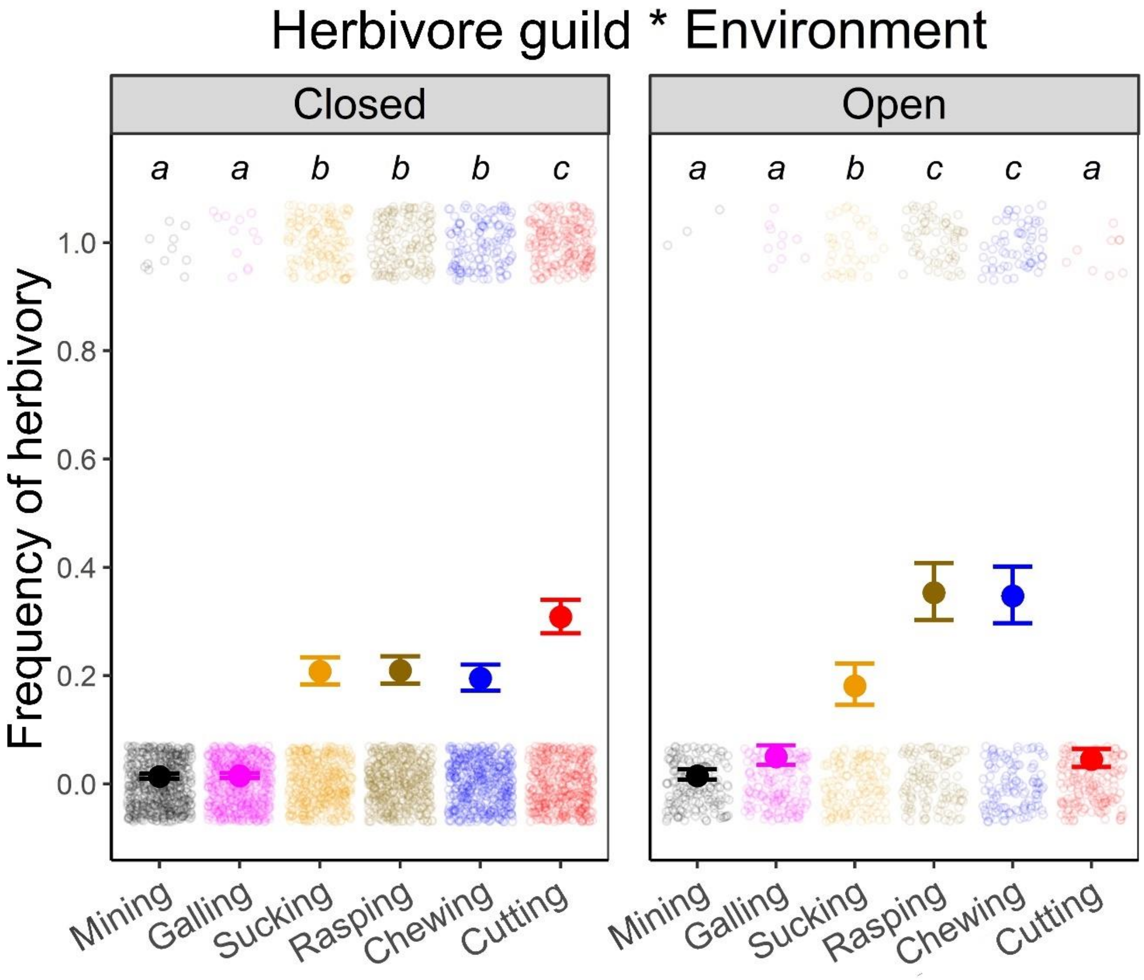


Figure 2. Differences in the frequency of leaf herbivory according to herbivore guild (A) and the interaction term filtered by environment (B). Points and line segments show back-transformed marginal (model adjusted) means and standard errors, respectively. Dots show raw data using jitter values (herbivory guild*environment: height= 0.07 width= 0.40). Distinct letters indicate significant differences ($p \leq 0.05$).

Level of herbivory

The total leaf herbivory in the sapling communities was 30.3% ± 27.6 (mean ± SD). The herbivory level per leaf ranged from 0.01% to 100% but was generally between 0.001 and 10.0% (contemplated in 50.0% of the values). The interaction term between environment and insect-feeding guilds was not significant for leaf herbivory level (χ^2 = 8.47, df = 5, p = 0.132). However, we found a significant effect of environment on the level of leaf area loss (χ^2 = 5.33, df = 1, p = 0.021, Fig. 3A). The EMM of leaf herbivory level in closed savanna (0.21) was 1.40 times higher than in open savanna (0.15).

Moreover, we found an effect of herbivore guild ($\chi^2 = 2053.83$, df = 5, $p < 0.001$, Fig. 3B). Cutting had the highest EMM of herbivory level (0.95), removing even the shoot system of 74 plants, followed by chewing (0.12), mining (0.11), rasping (0.09), galling (0.08), and sucking (0.05). Also, we observed that the presence of cutting guild increased the leaf herbivory by species, resulting in a 6.9 and 1.9 times higher raw mean of leaf damage for the community in closed and open savanna, respectively (Fig. S1).

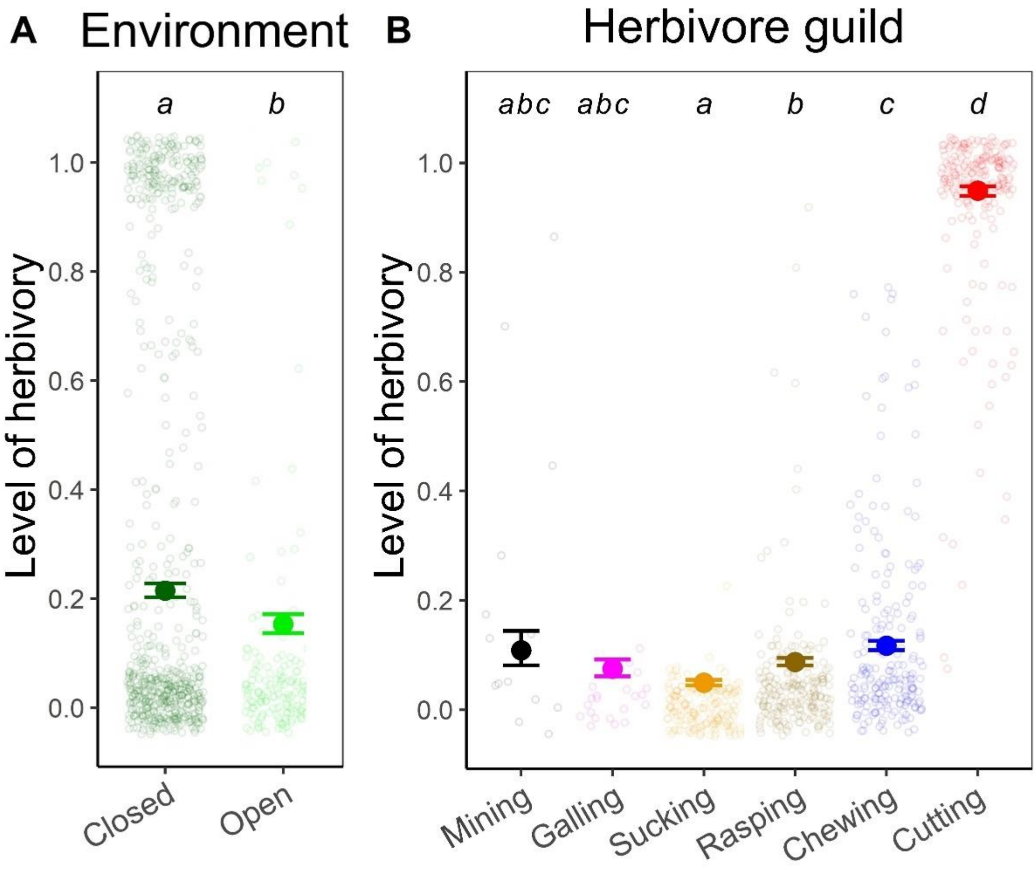


Figure 3. The level of leaf herbivory according to the environment (A) and herbivore guild (B). Points and line segments show back-transformed marginal (model adjusted) means and standard errors, respectively. Dots show raw data using jitter values (environment: height= 0.05 width= 0.34, and herbivory guild: height= 0.05 width= 0.40). Distinct letters indicate significant differences ($p \leq 0.05$).

DISCUSSION

By contrasting insect-herbivore guilds responsible for the damages on the juvenile plants, we identified their relative importance in terms of frequency and level of damage in two savanna formations. We demonstrated that the frequency of leaf damage was different according to the savanna formation (H1) and herbivore guild (H2), being cutting the most frequent in closed, and rasping and chewing the most frequent in open savanna. In addition, the level of herbivory was higher in closed than open savanna, which was mainly driven by leaf-cutter ants that frequently removed the whole plant foliage (H3). We highlight that the presence of cutting insects may alter the outcomes of herbivory level for the sapling communities in Neotropical savannas. Overall, these findings provide important insights into the potential impact of different herbivore insects on the plant community of contrasting savanna formations.

The total leaf herbivory in sapling communities (approx. 30.3%) of the studied savanna area was at least 2.2 times greater than has been reported for juvenile plant communities of tropical forests (9.2%, Benítez-Malvido and Lemus-albor, 2005; 10.5%, De la Cruz and Dirzo, 1987), subtropical forests (14.0%, F. Martini et al., 2021), and temperate forests (2.7%, Murphy et al., 2016). Moreover, comparing our results with available estimates for mature plants in other Neotropical savannas areas (4.6 to 9.3%; Fowler and Duarte, 1991; Marquis et al., 2001), we observed that woody saplings are under higher herbivory pressure. This result is consistent with studies showing that seedlings and saplings are more vulnerable to herbivores (Hanley et al., 2004). All these comparisons highlight the importance of insect herbivores on the vegetation dynamic of savannas (Costa et al., 2008).

We observed a high likelihood of foliar attack since most leaves (69.5%) had traces of herbivory. This result indicates an intense activity of herbivores insects in the savanna community, such as recorded in forests with *Croton lachnostachyus* (Euphorbiaceae) plants showing 80.8% of the investigated leaves damaged (Rossetti et al., 2014). The frequency of herbivory varied according to the environment and herbivore feeding guild. The greatest change was observed with cutting guild that was the most frequent damage in closed savanna, being far less representative in open savanna. Although leaf-cutter bees occur in the study area (Costa A.N., personal observations), we only registered damages by leaf-cutter ants within the cutting guild. Populations of leaf-cutter ants are generalists and tend to show an aggregated distribution across Cerrado landscapes, regardless of vegetation type and species identity (Costa and Vieira-Neto, 2016). The greatest distribution of these ants may indicate a higher frequency of leaves being attacked (Meyer et al., 2011). In contrast, chewing and rasping insects were important in both environments, but they had an increased frequency of damage in open savanna. Both herbivore guilds are mostly generalist species, feeding in a wide range of plants (Brezzi et al., 2017), which can explain their higher leaf damage frequency contrasted to most guilds. As well as for leaf-cutter ants, the abundance and distribution of these insect-feeding guilds can explain the difference in the level of herbivory between environments. More than explains part of the results observed, this indicates that herbivores distribution can modify the spatial pattern of herbivory among savanna formations.

Sucking and mining damages were similar between environments while galling damages were higher in open savanna. All of them are mostly specialist insects in the use of food resources (Brezzi et al., 2017; Novotny et al., 2010; Oliveira et al., 2020). Specialized insects feed on a single or few phylogenetically close plants, which can result in a less attack frequency in leaves at the community level compared with more generalist insects such as in the chewing guild (Oliveira et al., 2020). However, plant communities with one host plant species dominating can be more prone to herbivory pressure by specialist insects than communities with less host dominance and more non-host neighbors (V. C. Martini et al., 2021). Thus, the increase in the herbivory pressure of galling insects in open savanna can be a result of a higher abundance of host plant species.

We found a leaf herbivory level 1.4 times higher in closed than open savanna communities. Closed savannas present a higher litter accumulation and milder microclimatic conditions compared to open savannas (Salazar et al., 2012). Studies have shown that the high presence of litter and canopy closure may increase herbivory levels on juvenile plants as a feasible result of the higher abundance of insects sheltering in the litter (Benitez-Malvido et al., 1999; García-Guzmán and Benítez-Malvido, 2003; Goodale et al., 2014). Also, juvenile trees in subtropical forests are subject to greater insect feeding under favorable microclimates (F. Martini et al., 2021). In contrast, the direct and constant light incidence in open savannas might naturally result in juvenile plants with sun-adapted leaves that are often smaller, tougher, and thicker compared to shade-adapted leaves, which may improve plant resistance reducing the intensity of leaf herbivory (e.g., Coley, 1983; Salgado-Luarte and Gianoli, 2010). However, although the palatability of leaves or the environmental conditions might diverge among environments making them more or less prone to herbivores feeding (Salgado-Luarte and Gianoli, 2010), here we suggested that the differences found between savanna formations were boosted by cutting insects. Excluding their herbivory level values from the total raw mean per savanna formation, the herbivory between environments became similar (~ 5.20%, Fig. S1). It highlights the influence of cutting ants distribution as a driver of high herbivory levels in saplings. More studies are suggested to determine the recurrence of greater frequency and level of herbivory in closed savanna made by cutting insects across Cerrado landscapes.

Herbivore feeding guilds are known to cause different levels of damage on leaves at the community level (see also, Brezzi et al., 2017). Cutting guild caused approx. 4.8 times greater leaf loss compared to the rest of the insect guilds together, which supports the findings showing some of these insects (i.e., leaf-cutter ants) as the dominant herbivores of Neotropical savannas (Costa et al., 2008; Costa and Vieira-Neto, 2016). A study that measured leaf-cutter ants within the chewing guild found that they can remove the same amount of foliar tissue to other insects combined (i.e., sucking, rasping, mining, and unknown damages, Andrade et al., 2020). Otherwise, mining and galling caused similar leaf damage to sucking, rasping, and chewing, which were different from each other in order from least to most consumer guild. Overall, differences in herbivory levels are frequently based on the movement capability of insects from chewing, sucking, and rasping guilds to exploit many leaves on various plants (Stiegel and Mantilla-Contreras, 2018). In contrast, as insects from mining and galling guilds show a limited capability to move, their distribution might be restricted to one or few plant species (Stiegel and Mantilla-Contreras, 2018). Here, although all these feeding guilds caused similar minor damages contrasted with cutting, they have been shown to result in a long-term pronounced reduction of approx. -45% in plant vertical growth (Zvereva et al., 2012). A deficit in growth might reduce the ability of juvenile stages of plants to compete and obtain resources in the environment.

In this study, approx. 27% (n= 74) of the monitored woody saplings had their shoot systems removed by leaf-cutter ants, which can indicate a lower probability of survival compared with little leaf-damaged individuals. Studies have shown that herbivory until 50% does not affect the survival of seedlings trees while 100% of defoliation significantly reduces their survival (Barton and Shiels, 2020; Hinman and Fridley, 2020). In this sense, leaf-cutter ants may act as an ecological filter regulating the establishment of plants and the vegetation structure (Costa et al., 2017). Thus, a high density of their nests can represent a problem for restoration and mitigation plans (see also, Garcia et al., 2020). In contrast, the lower levels of herbivory reported here by non-cutting guilds are unlikely to cause mortality (Zvereva et al., 2012). However, they can increase minor detrimental effects over years on the growth and reproduction of plants (Kozlov and Zvereva, 2017), which can also impact plant communities. Moreover, it is important to consider that the herbivory levels on saplings can be even higher since we did not use metrics that overestimate damages (Getman-Pickering et al., 2020), and the continuous plant exposition to herbivore pressure may increase the amount of tissue damaged (Filip et al., 1995; Raupp P. P, personal observations.).

CONCLUSION

This study represents an initial step to better understand the patterns and role of background and foreground herbivory in woody saplings of tropical savannas. Overall, we found a higher herbivory pressure in the saplings of the Brazilian Cerrado compared with studies developed in forest environments. Specifically, we demonstrated four key results regarding herbivory in our study area: (1) the frequency of herbivory on leaves of juvenile woody plants was variable according to the vegetation type and insect feeding guild, being higher for cutting in closed and rasping and chewing in open savanna. This was a likely result of the abundance of these herbivores in these environments; (2) closed savanna had higher herbivory intensity than open savanna as a possible result of the higher frequency of cutting damages in this environment; (3) cutting guild exhibited the highest damage levels, including the removal of the shoot-system of plants, which was never counted before. They drastically increased the herbivory levels of the community, potentially acting as a demographic filter to most of the species in tropical savanna plant communities, and; (4) the herbivory level by mining, galling, sucking, rasping, and chewing guilds was low compared with cutting, but according to studies could still play a major role in shaping the community of juvenile woody plants. Finally, we increased the ecological knowledge of herbivory in sapling communities for tropical savannas, helping to understand the role of different groups of herbivores and abiotic conditions in determining vegetation structure in non-forest environments.

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SUPPORTING INFORMATION

Table S1. Species-level information for woody saplings studied in closed and open savanna formation. The number of plants and leaves monitored and the frequency and level of accumulated herbivory for all guilds (raw mean values ± standard deviation) per species and community that we found are indicated.

Family, Species	**Number**				**Mean of herbivory (%)**			
	Plants		**Leaves**		**Frequency**		**Proportion**	
	Closed	**Open**	**Closed**	**Open**	**Closed**	**Open**	**Closed**	**Open**
Anacardiaceae								
Tapirira guianensis Aubl	10	0	27	0	56.67 ± 50.40	—	52.09 ± 50.61	–
Annonaceae								
Cardiopetalum calophyllum Schltdl.	11	0	33	0	39.39 ± 49.62	—	1.03 ± 1.29	–
Caryocaraceae								
Caryocar brasiliense Camb.	9	9	27	27	92.59 ± 26.69	92.59 ± 26.69	18.06 ± 32.22	9.56 ± 12.16
Chrysobalanaceae								
Hirtella gracilipes (Hook.f.)	10	0	30	0	43.33 ± 50.40	—	5.70 ± 9.42	–
Euphorbiaceae								
Maprounea guianensis Aubl.	11	0	33	0	30.30 ± 46.67	—	18.99 ± 40.09	–
Fabaceae								
Copaifera langsdorffii Desf.	11	0	33	0	48.48 ± 50.75	—	20.41 ± 39.42	–
Vatairea macrocarpa (Benth.) Ducke	13	0	39	0	61.54 ± 49.29	—	36.47 ± 44.75	–
Leguminosae								
Bauhinia rufa (Bong.) Steud.	0	11	0	32	—	87.87 ± 33.14	–	14.94 ± 29.76
Malpighiaceae								
Byrsonima sp.	0	10	0	30	—	43.33 ± 50.40	–	1.67 ± 3.45
Melastomataceae								

Family, Species	**Number**				**Mean of herbivory (%)**			
	Plants		**Leaves**		**Frequency**		**Proportion**	
	Closed	**Open**	**Closed**	**Open**	**Closed**	**Open**	**Closed**	**Open**
Miconia albicans (Sw) Triana	11	0	32	0	84.85 ± 36.41	—	14.86 ± 28.48	–
Myristicaceae								
Virola sebifera Aubl.	12	0	33	0	55.56 ± 50.40	—	56.01 ± 47.97	–
Myrtaceae								
Eugenia involucrata DC.	10	10	30	30	30.00 ± 46.61	93.33 ± 25.37	40.24 ± 51.43	7.37 ± 4.61
Myrcia splendens (Sw.) DC.	11	0	33	0	30.30 ± 46.67	—	1.79 ± 2.34	–
Nyctaginaceae								
Guapira graciliflora (Mart. ex Schmidt) Lundell.	10	0	30	0	16.67 ± 37.90	—	56.72 ± 49.74	–
Guapira noxia (Netto) Lundell	10	0	29	0	63.33 ± 49.01	—	80.58 ±40.95	–
Polygalaceae								
Bredemeyera sp.	13	0	37	0	56.41 ± 50.23	—	70.04 ± 46.78	–
Proteaceae								
Roupala montana Aubl.	10	10	30	30	63.33 ± 49.01	40.00 ± 49.83	20.05 ± 31.33	12.80 ± 30.96
Rubiaceae								
Cordiera myrciifolia (K. Schum.) C. H. Perss. & Delprete	13	0	38	0	56.41 ± 50.23	—	9.52 ± 27.31	–
Salicaceae								
Casearia sylvestris Swartz	10	11	30	33	50.00 ± 50.85	60.60 ± 49.62	10.46 ± 31.46	11.51 ± 29.66
Sapindaceae								
Matayba guianensis Aubl.	10	0	29	0	60.00 ± 49.83	—	91.75 ± 26.09	–
Siparuna guianensis Aubl.	10	0	30	0	36.67 ± 49.01	—	80.25 ± 41.64	–
Vochysiaceae								
Qualea multiflora Mart.	10	0	29	0	66.67 ± 47.95	—	44.70 ± 48.16	–
Community-level	**215**	**61**	**632**	**182**	**55.92 ± 49.68**	**52.09 ± 49.94**	**35.11 ± 28.64**	**9.76 ± 4.70**

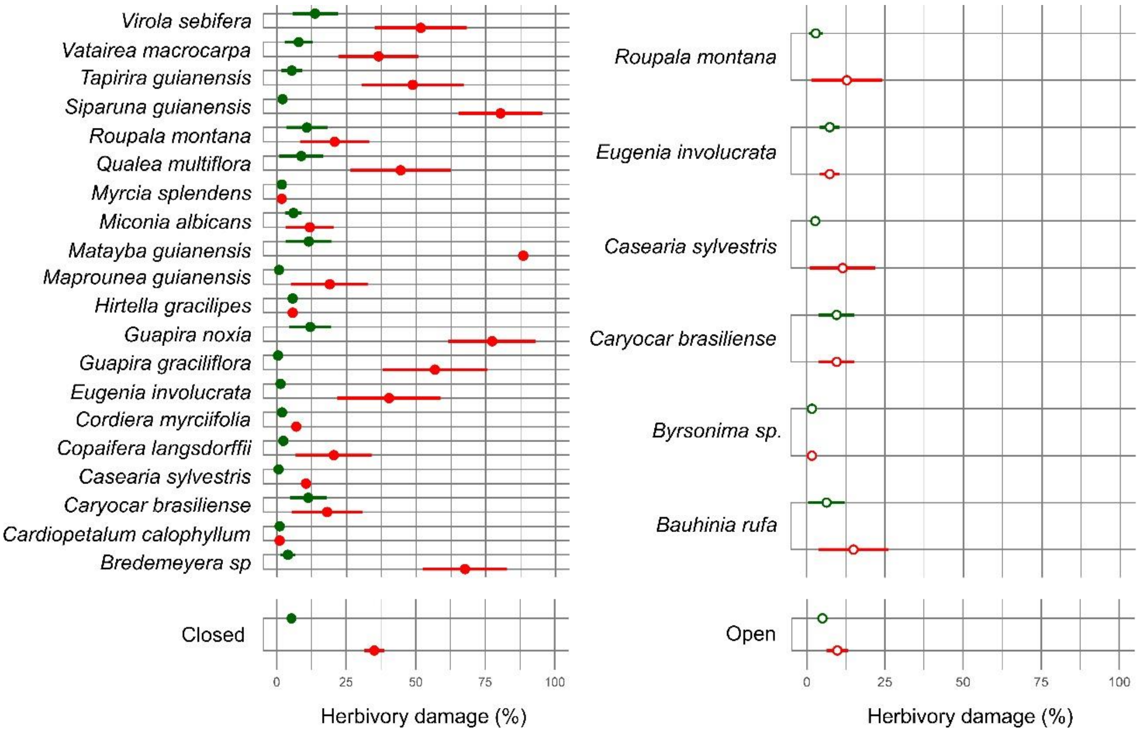


Figure S1. Mean herbivore damage and standard deviation (raw data) on saplings of 22 species studied in open and closed savanna formations at Panga Ecological Station, Uberlândia, Brazil. Each species is considering two groups of herbivory damage: mining, galling, sucking, rasping, and chewing feed guilds in green, and all these feed guilds plus cutting guild in red. Filled points represent closed savanna and hollowed points, open savanna.

CAPÍTULO 2.

Herbivory intensity, vegetation formation and plant species traits drive seedlings survival in the Neotropical savanna

Capítulo formatado conforme as normas da revista Journal of Applied Ecology

ABSTRACT

1. Tropical regions exhibit high herbivore pressure which represents a driver of biodiversity patterns across ecosystems by determining the establishment of seedlings. However, it is still unknown how intrinsic and extrinsic factors drive the susceptibility of seedlings in contrasting vegetations. This is a baseline to develop mitigation and conservation strategies, especially in plant communities from a Neotropical savanna biodiversity hotspot, the Brazilian Cerrado, which is the largest and most threatened tropical savanna in the world.

2. Here, we assessed the re-growth capacity and survival of 1520 seedlings of 10 woody species with different seed sizes after simulating four levels of experimental leaf-chewing insect herbivory (0%, 50%, 100%, and 100% plus hypocotyl) in closed and open savanna formations in a Neotropical savanna.

3. The survival of tree seedlings varied according to species, savanna formation, and herbivory intensity. Defoliation until 50% did not affect the survival of seedlings in either environment. Alternatively, high levels of herbivory (100% and 100% plus hypocotyl) reduced seedling survival up to 47%. For all herbivory levels, seedlings under open savanna had lower survival. Moreover, plant traits such as seed size and re-growth capacity were generally positively related to the success of the seedling establishment.

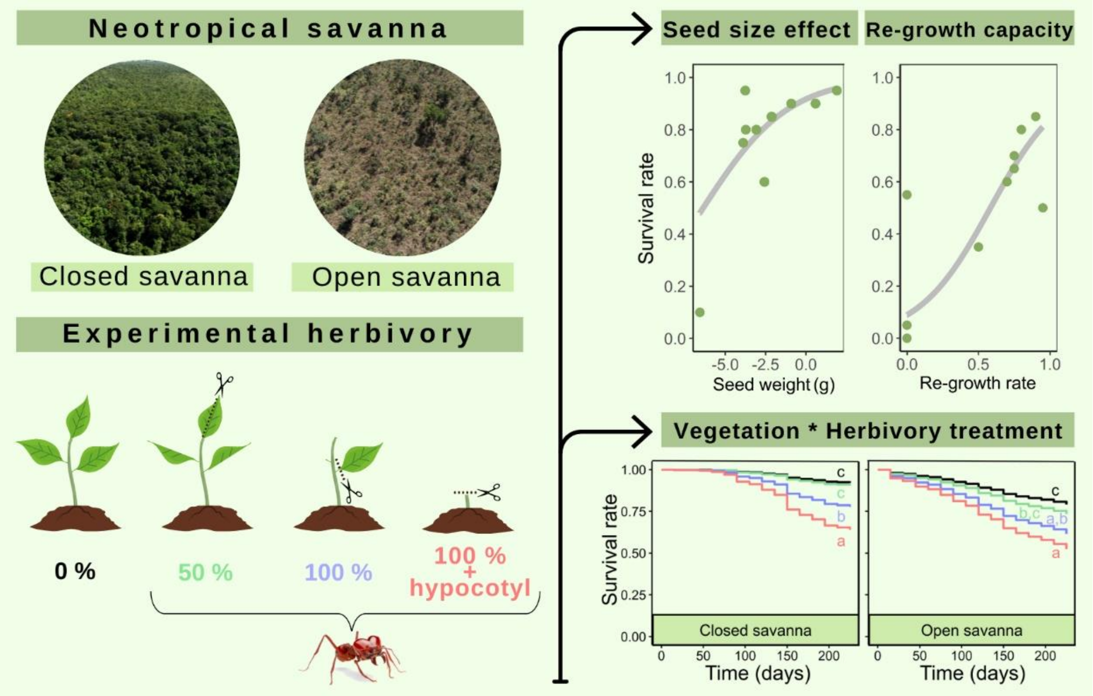
4. *Synthesis and applications.* Our study highlights the role of plant traits and environmental factors such as vegetation and herbivory intensity primarily affecting the survival of native seedlings in communities of a Neotropical savanna hotspot. Our findings bring implications for the process of natural succession and ecological restoration by providing species susceptibility traits and mortality rates of seedlings in different vegetations of a tropical savanna. We point out that to increase the successful establishment of plant species in regions dominated by leaf-cutter ants and/or other chewing herbivores, important actions include (i) invest a greater number of less tolerant species to herbivory based on the studied functional traits to compensate

for their higher mortality, and (ii) use an adequate number of seedlings considering the mortality rate illustrated for each vegetation type.

Keywords

Ecological restoration, herbivory, leaf-cutter ants, savanna vegetation, seedling establishment, seed size, re-growth capacity, tree seedlings survival.

Graphical abstract



INTRODUCTION

Herbivory is an important and widespread biotic stressor to plants (Denno, 1983; Karban & Myers, 1989). Its effects may be especially harmful during the seedlings stage when the tolerance to damage is generally weaker than in older ontogenetic stages due to plant smaller size, low reserve, and high rates of resource allocation on growth (Boege & Marquis, 2005; Hanley et al., 2004; Massad, 2013). It is estimated that the mortality caused by herbivores may reach 30–40% of seedlings (Latorre et al., 2013; Lindquist & Carroll, 2004), indicating herbivore feeding as a major biotic stressor responsible for seedlings death (Moles & Westoby, 2004). Despite the high herbivory-induced mortality in seedlings, their probability of survival might be directly associated with the damage level. Studies have shown that defoliation up to approximately 50% does not appear to affect the persistence of seedlings (Barton & Shiels, 2020; Hinman & Fridley, 2020), while severe defoliation between 90 to 100% seems to cause a significant reduction in growth, leaf function, and ultimately in survival (Blundell & Peart, 2001; Hinman & Fridley, 2020).

Because plant survival is related to specific life-history traits (Hanley, 1998), the tolerance of seedlings to damage tends to vary substantially among species (e.g., Barton & Shiels, 2020; Meiners et al., 2000). It is hypothesized that larger-seeded species would be more tolerant to damage and have higher survival capacity compared with smaller-seeded species due to their amount of nutritive reserve available to produce large roots and stems (Ganade & Westoby, 1999; Maron et al., 2019). Studies have supported this hypothesis evidencing faster and higher mortality rates of seedlings from smaller-seeded species after natural or simulated herbivory (Armstrong & Westoby, 1993; Bonfil, 1998; Costa et al., 2017). Besides, seedlings from larger-seeded species generally present higher re-growth capacity after total above-ground herbivory (Edwards & Gadek, 2002; Green & Juniper, 2004). This capacity gives a selective advantage over those seedlings that do not re-growth by replacing the photosynthetic tissue lost

(Green & Juniper, 2004; Kraft et al., 2015). Although mortality due to herbivory appears to vary according to damage intensity and physical traits of species such as seed size (Costa et al., 2017), it remains unclear whether these responses are widespread across plant communities and vegetation formations since most studies are not conducted under natural settings and at the community-level (Blundell & Peart, 2001; Hinman & Fridley, 2020).

Multiple environmental stressors influence the tolerance of plants attacked by herbivores (Atkinson & Urwin, 2012; Wise & Abrahamson, 2005). Along an environmental gradient, several abiotic factors can impact the survival of seedlings such as soil nutrients and microclimate (Hoffmann et al., 2004; Salazar et al., 2012). Furthermore, light availability often drives herbivory outcomes by affecting the survival, growth investment, and re-growth capacity in seedlings (Blundell & Peart, 2001; Hinman & Fridley, 2020; Norghauer et al., 2008). In forest formations, where light is one of the most limiting resources of plant growth (Chazdon et al., 1996), the presence of canopy gaps can increase the photosynthetic capacity of the seedlings, and therefore their chances of survival after herbivory (Norghauer et al., 2008). On the other hand, open formations have continuous light incidence that can negatively impact the survival of seedlings by increasing stressful environmental conditions such as soil water deficits (López-Sánchez et al., 2019; Salazar et al., 2012).

Neotropical savannas are highly heterogeneous ecosystems, have well-characterized fluctuations in climatic variables, and show a substantial difference in soil parameters and light incidence through canopy cover (Mistry & Beradi, 2014), which combined represent an advantageous system to test how the variation of abiotic and biotic factors can influence the seedling establishments. The dominant herbivores in numerical terms and biomass consumption in Neotropical savannas are chewing insects, such as the leaf-cutter ants of the genus *Atta* that can play a role impacting ecosystem dynamics similar to that of large mammalian herbivores from other savanna ecosystems (Costa et al., 2008; Costa & Vieira-Neto, 2016; Mundim et al., 2012). These ants present defoliation patterns ranging from partial to almost complete removal of the shoot-system seedlings (i.e., clipping the hypocotyl), which represent a common behavior of leaf-cutter ants (Costa, Vasconcelos, et al., 2018; Vasconcelos & Cherrett, 1997). To our knowledge, no study demonstrated the direct effect of this severe damage on the survival and re-growth capacity of seedlings across plant formations of Neotropical savannas and also considering the influence of the seed size species.

The Neotropical savanna (also called Brazilian Cerrado) is a biodiversity hotspot that is considered the largest and most threatened tropical savanna in the world (Silva & Bates, 2002). It is suggested that the current suppression of their native vegetation can drive to the extinction of approx. 480 endemic plant species by 2050 (Strassburg et al., 2017). Hence, there is a need for urgent measures to avoid the collapse of this hotspot (Hofmann et al., 2021), which points out restoration as a key solution (Strassburg et al., 2017). In this sense, there is a baseline demand to evaluate factors that can decrease the field establishment of early-stage native plants in this region. It remains unknown information on the susceptibility traits of tree seedlings to the simultaneous effects of herbivory and the vegetation formations. As herbivores may restrict ecosystem recovery (Tanentzap et al., 2009), these questions have special relevance helping to understand the patterns of tree distribution in the Neotropical savanna and develop successful ecological restoration practices in their different vegetations.

In this study, we assessed seedling survival responses between four levels of simulated leaf-chewing insect herbivory and two vegetation formations (i.e., open *vs*. closed savanna) considering a seed size gradient and re-growth capacity of 10 native woody species of the Brazilian Cerrado. Specifically, we predicted that (1) species of seedlings would have different survival rates according to savanna formation and herbivory intensity due to differences in plant species traits, leading to high and low survival patterns. (2) High levels of herbivory would negatively impact the survival of seedlings in both vegetations. (3) Seedling survival would be more negatively affected at any herbivory level under open than closed savanna since the former has greater canopy gaps and a warmer and drier microclimate, which are abiotic stressors for seedlings. (4) Seedling survival would be influenced by intrinsic traits of species, with a lower survival to smaller-seeded compared with larger-seeded species. We also expected that (5) lower re-growth capacity (i.e., resprout) after high levels of herbivory should equate to lower survival. Overall, our study builds toward a better understanding of how biotic stressors, abiotic conditions, and species intrinsic traits can influence the survival of initial plant stages in Neotropical savanna communities, which is a baseline for future conservation and restoration studies of this biodiversity hotspot.

MATERIAL AND METHODS

Study site and species

This study was carried out at the Panga Ecological Reserve (hereafter, PER; 19°10’ S and 48°23’ W; ~800 masl; Fig. S1A). This area is a 404-ha reserve owned by the Federal University of Uberlândia (UFU), located 30 km south of the city of Uberlândia, Minas Gerais, Brazil. The climate of the region according to the Köppen-Geiger classification is AW, characterized by two defined seasons: a rainy summer (October to April) and dry winter (May to September; Alvares et al., 2013). The average annual temperature and precipitation are 23 °C and 1650 mm, respectively (Cardoso et al., 2009). The PER presents high environmental heterogeneity, being composed of several vegetation types found at the Brazilian savanna (Gonçalves et al., 2021). The experiment was conducted in two savanna formations, known as open and closed savanna, which mainly differ in terms of canopy cover and wood plant density. The closed savanna is a woodland with a dense wood layer of shrubs and trees (range: 3-7 m in height) that form a homogeneous canopy cover of 85.7 ± 8.4% (mean ± SD) without grasses. The open savanna is a typical savanna formation characterized by a dense and continuous grass layer with sparse shrubs and small trees (range 2–3 m in height), and a canopy cover of 6.0 ± 6.0% (Ribeiro & Walter, 2008).

To produce seedlings for our simulated herbivory experiment, we selected ten native species of trees from six different families: *Myracrodruon urundeuva* Allemão (Anacardiaceae); *Jacaranda cuspidifolia* Mart. (Bignoniaceae); *Enterolobium gummiferum* (Mart.) JF Macbr*, Hymenaea martiana* Hayne, *Senegalia polyphylla* (DC.) Britton & Rose, and *Tachigali rubiginosa* Mart. ex Tul. (Fabaceae); *Guapira graciliflora* (Mart. ex Schmidt) Lundell (Nyctaginaceae); *Magonia pubescens* St. Hil. (Sapindaceae); *Solanum lycocarpum* A. St.-Hil, and *Solanum paniculatum* L. (Solanaceae) (Fig. 1). These species are naturally found in closed and open savanna formations and have variations in different traits including seed size (ranging from 0.001 to 6.800 g; mean ± SD: 0.93 ± 2.13 g; *sensu* Consolaro et al., 2019), seedling height (root plus stem range: approx. 10 to 35 cm), and leaves morphology (e.g., simple and compound leaves; Fig.1).

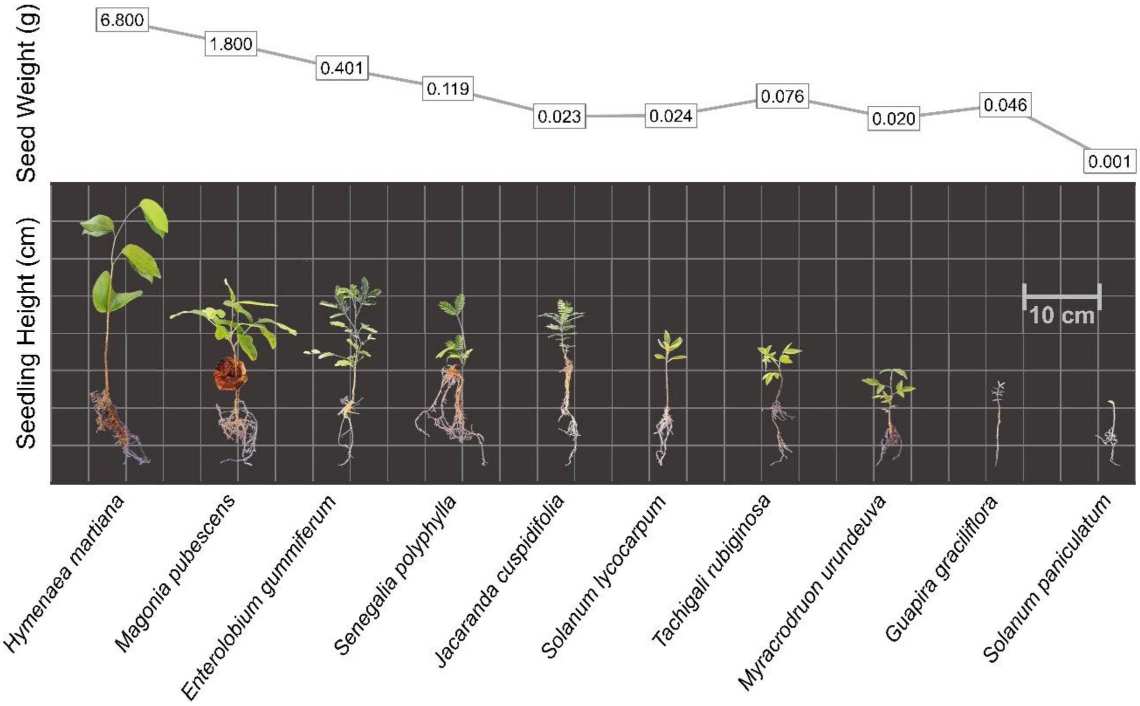


Figure 1. Seedlings before field transplant (i.e., 3 months of age) and the average seed weight of the 10 tree species (from six families) were used in the experiments of simulated herbivory. Seed weight was retrieved from Consolaro et al. (Consolaro et al., 2019).

Experimental Design

To investigate how different levels of herbivory and vegetation formations impact the survival of savanna trees during their initial stages of development, we performed an experiment simulating herbivory with seedlings of the selected species. We produced the seedlings used in this study from seeds collected in the PER and other remnants located in the region. Seeds were germinated in plastic bags filled with savanna soil and cultivated in a greenhouse for around three months (October to December of 2019) at PER. In January of 2020, we transplanted the seedlings to 40 plots of 1.5 m^2, which were equally distributed between open and closed savanna areas (n = 20 per environment), placing a minimum distance of 30 m between plots (Fig. S1A). One species (i.e., *S. paniculatum*) was transplanted to ten plots per environment. Each plot received four seedlings per species. In this sense, we used 760 seedlings per savanna formation (36 or 40 seedlings per plot). The density of seedlings used in experimental plots (approx. 27 seedlings m^{-2}) was comparable to the average density that naturally occurs in the study site (38.8 ± 17.3 plants m^{-2}, Costa, Bruna, & Vasconcelos, 2018). Other plants present in plots were removed to standardize the local conditions. After the seedlings transplant, all plots were covered with rectangular cages (1.5 x 1.0 x 0.5 m; Fig. S1B-C) to avoid access by herbivores and other interferences on herbivory treatments. We used transparent fabrics which caused minimal interference in luminosity and ventilation to plants (Fig. S1B-C).

In February 2020, we replaced those seedlings which were dead, probably due to the manipulation and transplant stress with additional seedlings kept in the greenhouse. Then, to simulate leaf-chewing insect damages, using a scissor, we applied four different treatment levels of herbivory intensity: I) 0% of damage, representing undamaged control seedlings; II) 50% of damage, clipping vertically half of all leaf blades without injuring the midrib; III) 100% of damage, clipping all leaves by cutting their petioles; and IV) 100% of damage plus the hypocotyl removal, removing the whole plant foliage by clipping the hypocotyl under the first leaf emerged (cotyledons). This last damage simulates specific high-level herbivory commonly caused to seedlings by *Atta* leaf-cutter ants in the study area (Costa, Vasconcelos, et al., 2018). Seedlings were randomly assigned to their location within plots and among damage treatments using a randomization matrix through the *sample* function in R software. We monitored the seedlings fortnightly from March to October 2020 (totaling 15 monitoring periods). In each evaluation, we visually classified seedlings as belonging to one of the following plant statuses: dead, alive, or regrowth after 100% of damage (considered only treatments III and IV).

Statistical analyses

We used cluster analysis to assess how each species responds to the different levels of herbivory for each savanna formation and identify a potential group of species more and less tolerant to herbivory according to seedling survival rate (prediction 1). We used the Euclidean distance coefficient and Ward's method, which identifies the strongest clustering structure. The response variable was the proportion of seedlings surviving at the end of the experiment. Since the height of the cut on the dendrogram controls the number of groups obtained, we used the Average Silhouette method in the R-package *factoextra* version 1.0.7 (Kassambara & Mundt, 2020) to indicate the optimal number of groups. After having the formation of two final clusters based on survival (i.e., higher and lower survival groups), we tested the significance between these groups using an analysis of similarity (ANOSIM) with Euclidean distance and 999 permutations through R-package *vegan* version 2.5-6 (Oksanen et al., 2019).

To investigate how herbivory intensity (0%, 50%, 100%, and 100% plus hypocotyl) and savanna formations (open and closed) influence the survival rates of seedlings over time (predictions 2 and 3, respectively), we fitted a mixed-effects Cox proportional-hazards model using the R-package coxme version 2.2-16 (Therneau, 2020). We specified savanna formations (two levels), treatments (four levels), and the interaction term between these two factors as fixed effects. Plot number and species identity were set as random effects. We performed post-hoc comparisons using Tukey’s adjusted contrasts in the R-package *emmeans* version 1.5.0 (Lenth, 2020) considering the environments and treatments as factors.

We investigated whether the survival of seedlings was determined by species seed size. For that, we used the average seed weight per species as a proxy to seed size (log-transformed; prediction 4) by using the number of seedlings that survived at the end relative to the number of those that died as our dependent variable. These two variables were combined through the *cbind* function in R software. Then, we modeled this proportional data by conducting Generalized Linear Models (GLMs) with a binomial distribution and logit link function (Crawley, 2012; Zuur et al., 2009). We conducted separate models for each treatment within each environment as it allows us to investigate whether the same pattern applies to all the possible conditions. Due to our lower sample size and presence of some outliers, we fitted GLMs adjusting the Mallows or Huber type robust estimators (method Mqle in *glmrob* function) (Cantoni & Ronchetti, 2001) using the R-package *robustbase* version 0.93-7 (Maechler et al., 2021). This procedure allows us to avoid the distorting influence of these observations and keep model reliability.

We also investigated whether the rate of previous regrowth in the plant species was further related to their respective seedling survival (i.e., the death/alive outcome; prediction 5) by using the same robust GLM routine described previously. We only selected data related to treatments III and IV, where leaves were completely removed and re-growth could potentially occur.

We tested the significance of robust GLMs using the robust quasi-deviance test (Cantoni & Ronchetti, 2001). The model’s fit was assessed visually by inspecting the distribution of residual *vs.* fitted values and the histogram of residuals. To investigate the amount of variance explained by those significant models, we calculated the Tjur’s R^2 (i.e., coefficient of discrimination for GLMs with binary outcomes; Tjur, 2009) in the R-package *performance* version 0.4.8. (Lüdecke et al., 2020). Using the R-package *ggeffects* version 0.16.0 (Lüdecke, 2018), we extracted the estimated marginal effects for plotting. All analyses were carried out in R statistical environment version 4.0.2 (R Core Team, 2020).

RESULTS

A general framework of seedling survival across species

The cluster analysis (Cophenetic correlation = 77%) revealed two clusters of species in each savanna formation that were significantly different according to the survival rates of seedlings in each treatment (ANOSIM, R = 0.879, p = 0.001; Fig. 2). The first cluster was characterized by species in the environment with higher survival for most treatments (survival rate mean ± SD = 0.834 ± 0.170; Fig. 2). The second cluster was characterized by species in the environment with lower survival (survival rate mean ± SD = 0.350 ± 0.248; Fig. 2). Three of the ten species studied (i.e., *G. graciliflora*, *S. lycocarpum*, and *T. rubiginosa*) were in both clusters, depending on the savanna formation where they were inserted.

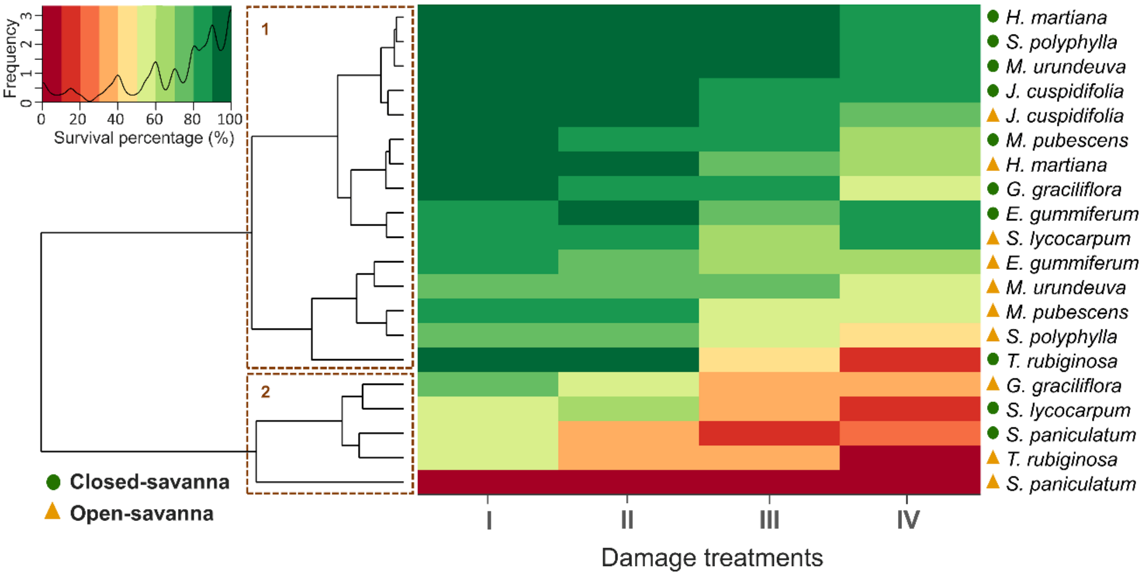


Figure 2. Species clustering according to seedling survival after different levels of herbivory (I: 0%, II: 50%, III: 100%, and IV: 100% plus hypocotyl) in two different savanna formations (open savanna: yellow triangle; closed savanna: green dots). The different colors in the cluster represent the range survival percentage as indicated in the histogram. The dendrogram shows

similarities in survival between species per environment, in which similar survival rates are combined into branches that are themselves fused at a less height.

Seedling survival according to herbivory level and savanna formation

We found a significant effect of the environment on seedling survival (χ^2 = 334.33; df = 1; p < 0.001; Fig. 3A), with those plants occurring in a closed savanna having a higher survival rate (81.18% of final survival) than those occurring in the open savanna (66.49%). Herbivory treatments had also a significant effect on seedling survival (χ^2 = 122.44; df = 3; p < 0.001; Fig. 3B). Treatments I and II had a final survival rate of 85.79% and 81.89% (respectively) and their curves were not different from each other. Treatment III had significantly lower survival rates (69.47%) when compared to treatments I and II, and higher when compared to treatment IV, which had the smallest survival rates (58.22%). Finally, the interaction between environment and treatment significantly influenced seedling survival rate over time (χ^2 = 9.17; df = 3; p = 0.027; Fig. 3C). We found differences among groups within each environment. In the closed savanna, the higher survival rates were those of treatments I (92.11%) and II (90.53%), which were not different from each other. The survival curve of treatment III was significantly different from treatment I and II, with 77.89% of survival. Treatment IV had the lowest survival rates in the closed savanna (64.21%), significantly different from all other treatments in this closed formation. In the open savanna, treatments I (79.47%) and II (73.30%) had the highest survival rates, not different from each other. Treatment III (61.05%) had smaller survival rates, being significantly different from treatment I but not from treatment II. Finally, although treatment IV had the smallest survival rates (52.33%), it was not significantly different from treatment III. We found that seedlings in the closed environment had higher survival rates than the open in all treatment levels (Fig. 3C).

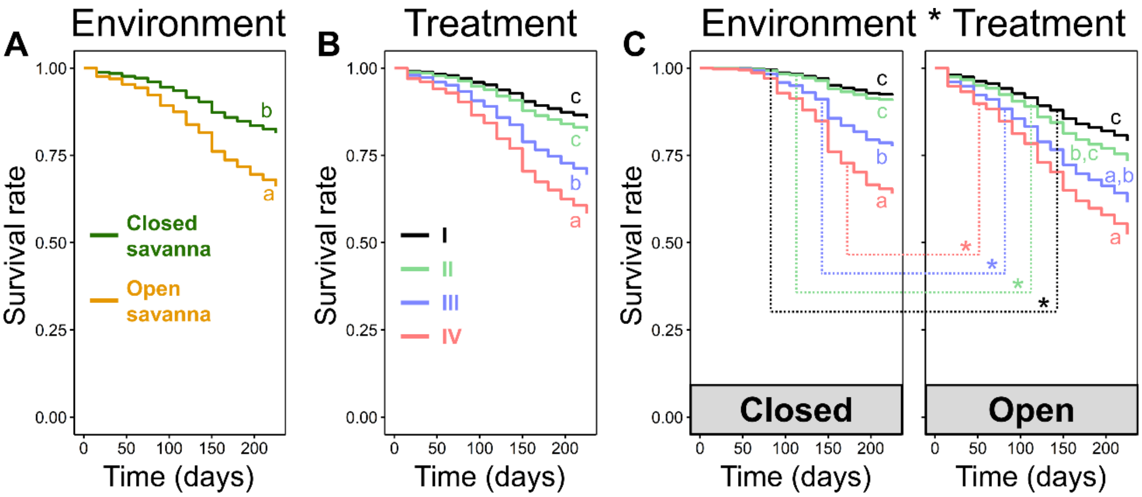


Figure 3. Survival curves showing the effects of the environment (A), herbivory treatment (B), and the interaction between these factors (C) on the survival rates of seedlings over time. Different letters within each frame indicate significant differences at the 0.05 level. Different frames in C are arranged according to the post hoc analysis having environment as a filter. Dotted lines in C refer to contrasts having treatment as a filter (i.e., comparisons between the same treatments), and asterisks indicate respective significant differences at 0.05 level.

Seedling survival according to seed size and re-growth capacity

Considering the influence of seed weight on the proportion of seedling survival, we found that in the closed savanna, there were positive effects only for treatments I and IV (Table 1, Fig. 4). In contrast, in the open savanna, seed weight was related to higher rates of survival across species in all treatments (Table 1, Fig. 4). In addition to having a pervasive effect in all treatments, seed weight effects were stronger on the open savanna, which can be noticed by the higher values of R^2 (Table 1).

Table 1. Statistical results from GLMs with robust fitting testing the influence of seed weight on the proportion of seedling survival according to savanna formation (open and closed) and herbivory levels. The herbivory damage treatments I, II, III, and IV represent the 0%, 50%, 100%, and 100% plus hypocotyl damage, respectively. Significant p-values are expressed in bold and R2 values were calculated only for significant models. DF = 1 in all cases.

Herbivory damage treatments	Open savanna			Closed savanna		
	χ^2	p	R^2	χ^2	p	R^2
I	13.87	**0.001**	0.16	4.32	**0.038**	0.02
II	11.11	**0.001**	0.04	0.54	0.462	—
III	8.53	**0.003**	0.06	2.44	0.118	—
IV	5.99	**0.014**	0.07	7.29	**0.007**	0.01

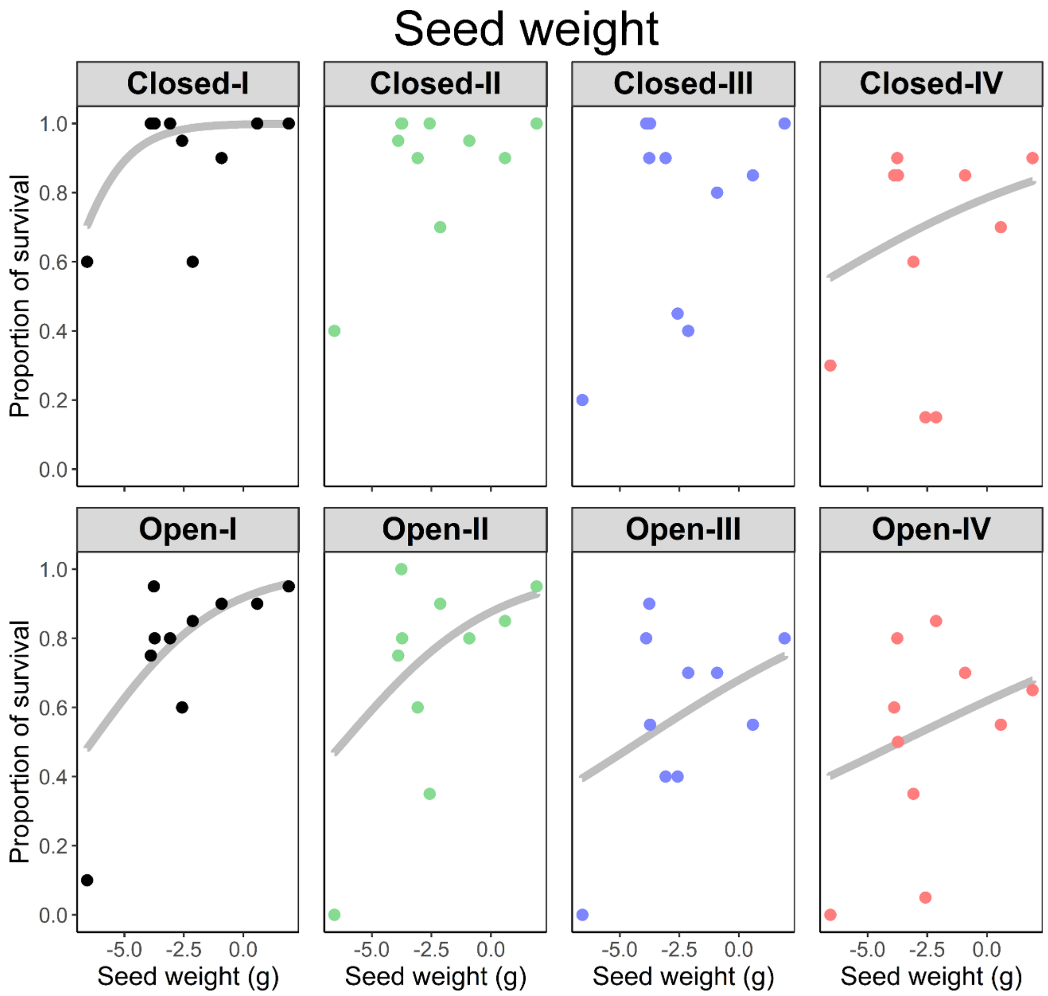


Figure 4. Effects of seed weight (log-transformed) on the proportion of seedling survival of ten different species in closed and open savanna formations according to herbivory damage treatments (I: 0%, II: 50%, III: 100%, and IV: 100% plus hypocotyl). Dots show the observed data points. Model adjusted (predicted) lines in gray are expressed only for significant results (described in Table 1).

Re-growth occurred in all species, having a mean of 48.7 ± 21.20% (±SD) across species, with a minimum of 5% and a maximum of 70%. We found that previous re-growth was positively related to further survival across species. We identified such pattern in plants from species growing in closed savanna being submitted to treatments III ($\chi^2 = 11.35$; df = 1; p = 0.001; $R^2 = 0.12$; Fig. 5) and IV ($\chi^2 = 14.85$; df = 1; $p < 0.001$; $R^2 = 0.04$; Fig. 5). In open savanna, we found a marginally significant result for treatment III ($\chi^2 = 3.81$; df = 1; p = 0.051;

$R^2 = 0.15$; Fig. 5) and a significant result for treatment IV ($\chi^2 = 5.38$; df = 1; $p = 0.020$; $R^2 = 0.17$; Fig. 5).

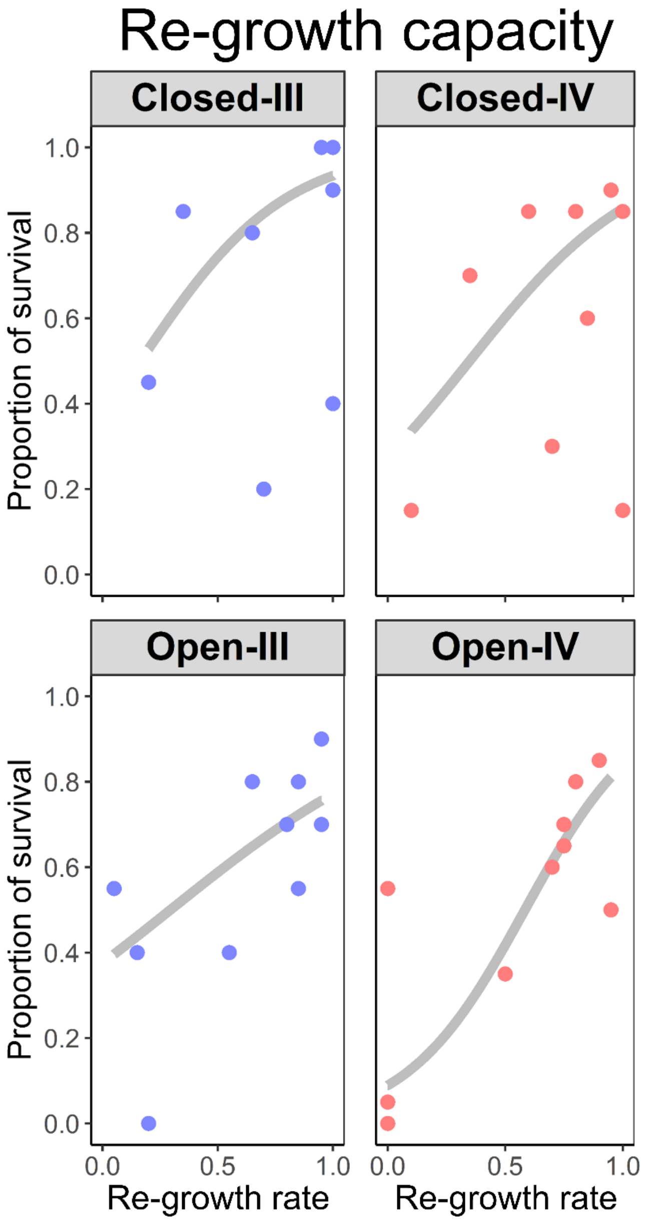


Figure 5. Results showing the effects of the proportion of re-growth per species on the proportion of seedling survival across closed and open savanna formations according to herbivory damage treatments (III: 100%, and IV: 100% plus hypocotyl). Model adjusted (predicted) values are shown in gray lines while dots show the observed data points.

DISCUSSION

Overview

Insect herbivores can determine community composition by changing the abundance and diversity of tree seedlings (Bagchi et al., 2014; Costa et al., 2017; Swamy & Terborgh, 2010). Understanding the combined effects of abiotic and biotic factors with species traits on seedling survival in natural ecosystems is a crucial demand to develop mitigating and conservation strategies in community ecology. This question has particular relevance in the plant communities from the Brazilian savanna that is a global biodiversity hotspot due to their exceptional species richness occurring in threatened environments, which continues to be constantly degraded (Myers et al., 2000; Strassburg et al., 2017). We investigated how leaf-chewing insect, vegetation formation, and species intrinsic traits can influence the survival of initial plant stages in a plant community of the Brazilian Cerrado, which is a baseline study for future conservation and restoration studies of this biodiversity hotspot. Species were segregated according to the higher or lower survival of seedlings for each savanna formation and the treatment of herbivory intensity, consistent with our prediction 1. High levels of herbivory (100% and 100% plus hypocotyl) significantly reduced the seedling survival, mainly in the open savanna formation, consistent with our predictions 2 and 3, respectively. Larger-seeded species generally showed a higher survival rate compared with smaller-seeded species, partially consistent with our prediction 4. Furthermore, re-growth capacity after full defoliation was positively associated with higher survival, consistent with our prediction 5. Overall, our study illustrates how chewing insects impact the survival of tree seedlings of different species in contrasting plant formations, modulating the structure of plant communities. In this context, we emphasize the key importance of leaf-cutter ants in plant establishment, since cutting the hypocotyl of the attacked seedlings drastically reduced their survival at the community level.

Finally, we highlight that our results should be considered in the face of area recovery and management plans in tropical regions.

A general framework of seedling survival across species

Considering the plant diversity represented (10 species from six plant families), the survival variation across species is not surprising (e.g., Barton & Shiels, 2020; Garcia et al., 2020; Lucas et al., 2013). We found survival patterns of seedlings showing species more and less tolerant regarding savanna formation and herbivory treatment (consistent with our first prediction).

Most of the species per environment had a greater tolerance to herbivory (grouped in cluster 1 compared to species in cluster 2). This illustrates a high resilience of the species since multiple abiotic stressors co-occur in savanna ecosystems (Salazar et al., 2012) and especially considering the high levels of damage imposed. After applying 100% defoliation in the tree seedlings of *Colophospermum mopane* (Fabaceae) and evaluating tolerance attributes, Hartnett et al. (2012) found that this species is adapted to high levels of herbivory. Although many species showed stronger tolerance to herbivory (e.g., *H. martiana*, *S. polyphylla*, and *J. cuspidifolia*), all species under the highest level of damage had a decrease in survival in both savanna formations. Also, nine out of ten species exhibited higher survival in closed than open savanna, emphasizing the influence of environmental conditions on the survival of plants. The only species with higher survival in the open savanna was *S. lycocarpum*. This result was expected since this species represents an early successional and light-demanding tree and it is most abundantly found in this savanna formation (Consolaro et al., 2019). This indicates that species have idiosyncratic responses, highlighting the importance of knowing the functional patterns and demands, for example, for an ecologic succession process and restoration purposes. The higher mortality and less resistance of tree seedlings species in open savannas highlight the mechanism that maintains a balance between trees, grasses, and herbaceous plants on different formations in Neotropical savannas and help to explain spatial distribution patterns of fewer woody species in open ecosystems (Salazar et al., 2012; Sankaran et al., 2004).

Seedling survival according to herbivory intensity and savanna formations

We found that the survival rates of undamaged and medium damage seedlings (50%) were not different from each other in open and closed savanna, which is in agreement with many studies reporting resistance of seedlings to moderate leaf loss by chewing insects (Barton & Shiels, 2020; Hinman & Fridley, 2020). However, high levels of damage negatively impacted the survival of seedlings, and the strength of this result was greater in open savanna formations (consistent with our second prediction). It illustrates that environmental conditions can influence the survival response to high herbivory pressure (Barton & Shiels, 2020). In closed savanna, seedlings submitted to 100% plus hypocotyl damage had the lowest survival rates, followed by 100% and 50% removal, which were all different from each other. In open savanna, treatments III and IV were not different from each other, and treatment III was not different from 50% damaged. Altogether, these results demonstrate that the impacts of herbivory intensity can be masked by environmental conditions where seedlings are establishing.

As expected due to losses of the photosynthetic area (Hinman & Fridley, 2020), the higher intensity of herbivory decreased the survival of plants during their establishment phase. Our treatment with the greatest loss of plant tissue resulted in the highest mortality rate — 41.78% for closed and 47.67% for open savanna in contrast to 7.89% and 20.53% of undamaged seedlings. Although few experiments investigated comparatively this high level of herbivory, we can contrast such mortality rates with the study led by Lucas et al. (2013). These authors clipped a one-year plant at 5 cm aboveground and found a survival decrease of 29%. Considering treatment III that had a lower damage intensity than treatment IV, occurred a decrease in mortality to 14.22% and 18.42% in closed and open savanna, illustrating the influence of the loss of vegetal tissues on the survival of seedlings over time in both vegetation formations.

We found that the survival of seedlings was reduced under open than closed savanna (consistent with our third prediction). Overall, open savanna showed 14.69% lowest survival than closed savanna. Specifically comparing the same treatment between closed and open savanna, we found that seedlings occurring in open savanna had 12.64%, 17.23%, 16.84%, and 11.88% lower survival in the treatments I, II, III, and IV, respectively. Since herbivory and abiotic factors can act together as stressors (Lindquist & Carroll, 2004), we emphasize the importance of natural conditions to determine the success of establishments. Thus, our results highlight this stronger effect that abiotic conditions have in open Neotropical savanna formations (Salazar et al., 2012). Comparatively, closed savanna formations have lower irradiance, cooler temperatures, reduced wind speed, and higher litter cover, which is related to soil water deficit reduction and nutrients increase (Salazar et al., 2012) possibly contributing to the establishment and survival of seedlings.

The abiotic conditions in open savanna probably play as a survival barrier for seedlings of most species during all periods (Salazar et al., 2012), as observed by the constant fall in the open survival curves since the beginning of monitoring. However, seedlings in closed savanna showed specific periods of high mortality with an abrupt drop in the survival curves, especially in treatments III and IV. These are concerned with the first month of the dry season (May) and the third consecutive month (July) without rainfall (Brazilian National Institute of Meteorology database). Although we cannot provide direct evidence for limited water availability to seedlings, a study shows that most plants that experience drought and herbivory simultaneously have lower survival when compared with those experiencing the effects of each stressor alone (Barton & Shiels, 2020). Taken together our results of control seedlings and the contrasts between the curves in the two environments we suggested the same consequence. Accordingly, it is important to consider the intensification of climate changes in the world (Collins et al., 2013). For instance, the climate is becoming drier and hotter over the years in the Neotropical savanna where we conducted our study (Hofmann et al., 2021). Further studies and restoration practitioners should consider the interplay between water availability and herbivory pressure as a factor that can negatively influence seedling establishment.

Seedling survival according to species intrinsic traits

We detected a positive effect of seed size of species on the proportion of seedlings' survival for all the herbivory intensity treatments in open and for treatments I and IV in closed savanna (partially consistent with our third prediction). Many studies have shown that larger-seeded species have more chance to survive than smaller-seed species (Armstrong & Westoby, 1993; Green & Juniper, 2004), while other studies found no effect (Baraloto & Forget, 2007; Lucas et al., 2013). Other cases show a negative relationship between seed size and herbivory tolerance, with those small seeds having greater survival (Barton & Shiels, 2020). Since we found no effect only in closed treatments II and III and lower R^2 values in closed treatments I and IV, the seed size seems more determinant to the resistance of seedlings in open savanna. Due to less stressful environmental conditions in closed savanna (Salazar et al., 2012), smaller-seeded species may have better conditions to bypass herbivory and develop in a similar way that larger-seeded under herbivory stress.

Contrary to the community general results of positive seed-size effects found, some smaller-seeded species (e.g., 0.023 g, *J. cuspidifolia*, 0.020 g, *Myracrodruon urundeuva*) showed a higher or similar survival rate compared to larger-seeded species in both environments (e.g., 6.800 g, *H. martiana*; Fig. 2). Overall, small and large-seeded species can adopt different strategies of seed reserve allocation (e.g., root or stem systems) that also may help to explain tolerance to damage and the absence of seed size effect (Myers & Kitajima, 2007). In this sense, an investment in high root biomass may indicate a larger storage reserve and explain further survival after herbivory (Lucas et al., 2013). Nonetheless, we can ponder that the size of the seed positively related to seedling survival (Moles & Westoby, 2004) can be corroborated in the facet of the seedling community but not consider as a rule to determine the stress tolerance of all species.

The resistance of seedlings to herbivory is also linked with the re-growth capacity after herbivory (Hinman & Fridley, 2020). We found that previous re-growth was positively associated with further survival across species for seedlings completely defoliated in both savanna formations (consistent with our last prediction). Re-growth capacity is a typical trait of many savanna trees to recover after disturbances (Zeppel et al., 2015), or even in tropical rainforests, where it is present in all community members (Green & Juniper, 2004). In agreement, we found that re-growth occurred in all species of the savanna tree communities, ranging from 5% to 70%. After a high level of simulated damage such as the removal of the seedlings shoot-system (i.e., clipping the hypocotyl), it is remarkable that 73.16% and 56.31% of all seedlings re-grew in the closed and open savanna, respectively. To support re-growth is necessary to invest energy translocating carbohydrate reserves from the root system (Bellingham & Sparrow, 2000). Our results show that the re-growth effort pays off considering the increase in the survival rate of the seedlings across species.

Atta ants and ecological implications

Atta leaf-cutter ants are indicated as an ecological filter to the establishment of many species (Costa et al., 2017; Garcia et al., 2020; Meyer et al., 2011). Here we also highlight the importance of *Atta* to plant communities at early developmental stages that clipping the hypocotyl of seedlings drastically reduce their survival. Considering that leaf-cutting ants are generalist herbivores (Munique & Calixto, 2018; Wirth et al., 2003) and less selective when foraging on individuals in the early stages of plant growth (Garcia et al., 2020), probably any seedlings species is liable to have the high levels of damage tested here. In addition to the damages imposed, recurrent attacks on seedlings are likely to occur and increase their mortality as indicated in some studies with adult plants (Mundim et al., 2012; Vasconcelos & Cherrett, 1997). Therefore, if the attack is more frequent, the mortality values can be even higher than those found here. In high-impact zones of herbivory, this may result in the reduction of seedling recruitment to zero (Lucas et al., 2013), leading to severe implications for the process of natural succession and ecological restoration (Garcia et al., 2020). Based on this, to succeed in restoring landscapes with the predominance of *Atta* leaf-cutter ants or other chewing herbivores, we indicate: i) to invest in a greater number of individuals of species less resistant to herbivory aiming bypass their mortality rate, as we showed that some species were more resistant than others to damage, and ii) to use the number of seedlings considering the mortality rate depending on the vegetation type.

Final remarks

It is suggested that 31–34% of the remaining Brazilian Cerrado is likely to be cleared by 2050 (Strassburg et al., 2017); therefore, understanding which factors impact the establishment of seedlings is essential to provide information for the success of restoration and mitigation strategies in this region. As we suggest that up to 50% of damage levels do not affect tree seedling survival in the community, we highlight the importance of considering the effects of high herbivory on the establishment of plants for practical restoration projects. Here we reported a specific and poorly studied herbivory type similar to that made by *Atta* leaf-cutter ants, which are dominant herbivores in the Neotropics. We found that the removal of the entire upper part of the seedling by those ants acts as an important ecological filter because can decreases the seedling survival of the tree community to 47.67% regarding the plant formation. In addition, seed size and the re-growth capacity may determine the survival of seedlings after herbivory. Larger-seeded species showed a higher overall survival rate compared with smaller-seeded species, mainly in open savanna, suggesting that seed size is an important plant trait survival predictor under stressful conditions. Furthermore, re-growth capacity after full defoliation was positively associated with higher survival in both vegetations. The results of our experiments indicate that high levels of herbivory together with environmental conditions of savanna formations impose barriers to the establishment of seedlings and may determine the structure of vegetations. Our findings bring important implications for the process of natural succession in Neotropical savannas by helping to explain the natural distribution of trees in contrasting plant formations, and for ecological restoration practices by providing species susceptibility traits and mortality rates for seedlings in tropical vegetations with the presence of chewing herbivores.

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SUPPORTING INFORMATION

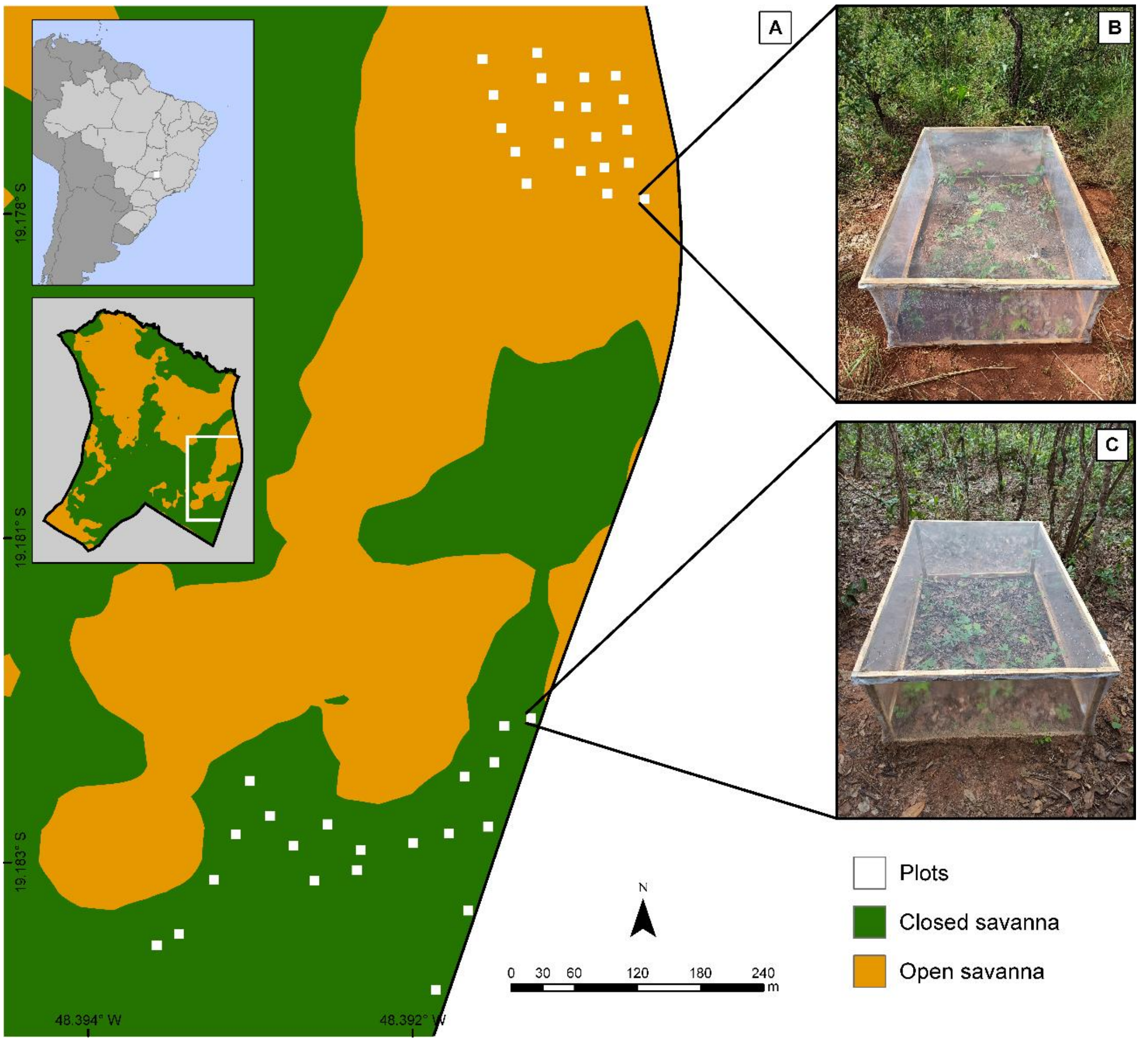

Figure S1. Distribution of experimental plots across the two savanna formations (n = 20 per environment) studied in the Panga Ecological Reserve, Uberlândia-MG, Brazil (A). Protection cage used in open savanna (B), and in closed savanna (C) to avoid seedling access by herbivores and other animals.

CONSIDERAÇÕES FINAIS

Diferentes grupos de insetos herbívoros foram responsáveis por danificar as mudas nas comunidades estudadas. Embora certas guildas de insetos foram mais frequentes que outras nos danos foliares, a quantidade de tecido vegetal removido foi geralmente baixa para a maioria das guildas, possivelmente, como reflexo da especialização alimentar delas. Comparando-se todas as guildas de insetos estudadas, os cortadores causaram maiores danos às plantas. Particularmente, as formigas-cortadeiras-de-folhas foram responsáveis por um nível de herbivoria médio de cerca de 92.5%. Além disso, os diferentes níveis de danos foliares simulados por mastigadores/cortadores refletiram em diferenças na sobrevivência das mudas ao longo das formações savânicas. A perda de metade das folhas presente nas mudas não causou um aumento na mortalidade delas ao nível da comunidade. Entretanto, altos níveis de danos (100% e 100% mais hipocótilo) foram responsáveis por um aumento na mortalidade, que chegou a 47% na savana aberta. Além disso, traços intrínsecos das espécies de mudas (i.e., tamanho de semente e capacidade de rebrota) ajudaram a compreender os padrões de sobrevivência frente as intensidades de danos impostos nas diferentes vegetações do Cerrado.

No primeiro capítulo, ao analisar a frequência de ataque e o nível de dano em 814 folhas de 22 espécies de mudas lenhosas em duas formações vegetais em uma savana Neotropical, foi encontrado que: (i) a frequência de herbivoria foliar foi variável de acordo com a formação da vegetação e a guilda de insetos herbívoros, sendo maior para cortadores em savana fechada e raspadores e mastigadores em savana aberta; (ii) o nível de herbivoria foi maior na savana fechada do que na savana aberta, possivelmente devido aos altos níveis de danos impostos pelos insetos cortadores, incluindo a remoção de praticamente toda a parte aérea das mudas através de um corte nivelar ao hipocótilo, até então nunca estimado; (iii) os níveis de herbivoria foliar ao nível da comunidade foram potencializados pela guilda dos cortadores, indicando sua atuação como um filtro demográfico para a maioria das espécies nas comunidades de plantas da savana tropical; e, (iv) o nível de herbivoria de mineradores, galhadores, sugadores, raspadores e mastigadores foi baixo em comparação aos cortadores, mas, segundo estudos, pode impactar negativamente no crescimento e reprodução das plantas.

No segundo capítulo, ao comparar a sobrevivência de 1520 mudas arbóreas entre quatro tratamentos de herbivoria experimental em duas formações vegetais em uma savana Neotropical, avaliando também um gradiente de tamanho de semente e a capacidade de rebrota dos indivíduos, foi encontrado que: (i) as mudas têm maiores ou menores taxas de sobrevivência de acordo com a espécie, a formação savânica e os tratamentos de intensidade de herbivoria; (ii) altos níveis de herbivoria simulada reduzem significativamente a sobrevivência de mudas, principalmente na savana aberta; (iii) as mudas oriundas de espécies com sementes maiores geralmente apresentam uma taxa de sobrevivência maior comparada com as espécies com sementes menores; e, (iv) a capacidade de rebrota das mudas após altos níveis de dano (i.e., desfolha completa e corte ao nível do hipocótilo) foi positivamente associada a uma maior sobrevivência.

Finalmente, indicamos que a herbivoria na comunidade de mudas das savanas Neotropicais parece ser mais alta do que previamente vem sendo reportada em estudos desenvolvidos em ambientes florestais. Esse resultado provavelmente decorreu da alta densidade de formigas-cortadeiras na área de estudo, as quais foram responsáveis pelos maiores níveis de danos foliares, incluindo a remoção quase completa da parte aérea de 24% das mudas monitoradas (i.e., 74 indivíduos). A maior presença desses insetos na savana fechada drasticamente contribuiu para uma herbivoria 1.4 vezes maior nesse ambiente. Essas formigas causaram danos 6.9 e 1.9 vezes maiores que todas as outras guildas de insetos somadas na savana fechada e aberta, respectivamente. Quando esses níveis de danos causados por formigas-cortadeiras foram simulados em mudas, eles severamente aumentaram a mortalidade delas no nível da comunidade. Com isso, ressaltamos a importância chave das formigas-cortadeiras-de-folhas na sobrevivência de plantas lenhosas que estão no ano inicial de seu desenvolvimento e, portanto, na manutenção da diversidade e estrutura das vegetações no Cerrado. No geral, também fornecemos valores médios de herbivoria (primeiro capítulo) e de sobrevivência de mudas perante diferentes níveis de remoção vegetal (segundo capítulo) para duas formações vegetais na savana Brasileira. Nesse sentido, tais dados podem ser utilizados como uma linha de base para planos de recuperação e manejo de áreas degradadas nesses ambientes. Por exemplo, baseado nos dados de mortalidade das mudas frente aos ambientes e níveis de dano, pode-se selecionar um número adequado de propágulos a serem plantados visando a restauração ou recomposição de uma área com presença de formigas-cortadeiras. Além disso, a escolha das espécies vegetais e o número a ser utilizado de cada espécie para restaurar uma paisagem pode ser tomada com base nas suas características intrínsecas, como no tamanho da semente e na capacidade de rebrota após herbivoria.