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Efeitos da distribuição e diversidade de plantas na comunidade de insetos galhadores e o valor adaptativo da galha ao aumento de temperatura

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Efeitos da distribuição e diversidade de plantas na comunidade de insetos galhadores e o valor adaptativo da galha ao aumento de temperatura

Tese apresentada à Universidade Federal de Uberlândia, como parte das exigências à defesa de Doutorado do Programa de Pós-Graduação em Ecologia e Conservação de Recursos Naturais

Orientador:

Denis Coelho de Oliveira

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Índice

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RESUMO GERAL

A ecologia de insetos galhadores é fascinante, os insetos galhadores são capazes de modificar o desenvolvimento vegetal, criando um ambiente propício para ele se alimentar e desenvolver. A galha, estrutura desenvolvida pelo inseto galhador, oferece proteção ao inseto galhador contra estresses abióticos e bióticos e também um tecido vegetal rico em substâncias energéticas para alimentação. Entretanto, essa habilidade para formação da galha gera também fatores limitantes na sua dispersão e sucesso de desenvolvimento. O inseto galhadores só consegue desenvolver galhas em um ou poucos hospedeiros relacionados geneticamente, por isso, muitas vezes a presença de insetos galhadores em um local depende composição de espécies vegetais do local, e a presença de plantas hospedeiras. Além disso, apesar dos insetos conseguirem se associar a uma ou algumas poucas espécies de plantas, algumas plantas são atacadas por uma grande diversidade de diversidade de insetos galhadores, as chamadas super hospedeiras. Nos estudos a seguir, investiguei como que mudanças na composição e distribuição das espécies arbóreas afetam a população e a diversidade dos insetos galhadores. No primeiro capítulo, investiguei as relações entre a concentração de hospedeiros, e a resistência oferecida a ele por outras espécies e a abundância de galhas. Investiguei também a relação da infestação de galhas na expressão de flores e frutos pelos hospedeiros. No segundo capítulo investiguei o efeito da distribuição geográfica e do número de espécies congêneres ao hospedeiro na vulnerabilidade ao ataque de diferentes galhadores. No terceiro capítulo, investiguei a proteção oferecida pela galha ao inseto galhador frente ao aumento da temperatura crônico e agudo. Concluí que como outros insetos especialistas, as populações de insetos galhadores são beneficiadas pela concentração do recurso, e pela presença de poucas barreiras entre as unidades de recurso. Encontrei também que as plantas hospedeiras mais distribuídas geograficamente são também as mais atacadas por diferentes insetos, e que esse efeito é maior em plantas de gêneros maiores. E que apesar da galha oferecer um microambiente para o desenvolvimento do galhador, o aumento de temperatura por um tempo prolongado supera a capacidade de tamponamento da galha.

Palavras-chave: Ecologia de galhas; Interação inseto-planta; Concentração de recurso; Resistência associada; Tolerância termal

ABSTRACT

The ecology of galling insects is fascinating, galling insects are able to modify plant development, creating a favorable environment for them to feed and develop. The gall, structure developed by the galling insect, offers protection to the galling insect against abiotic and biotic stresses, as well as plant tissue rich in energetic substances for food. However, this ability to form the gall also generates limiting factors in its dispersion and development success. The galling insect can only develop galls on one or a few genetically related hosts, so the presence of galling insects in a location often depends on the composition of plant species in the location, and the presence of host plants. Furthermore, although insects manage to associate with one or a few plant species, some plants are attacked by a great diversity of galling insects, the so-called super hosts. In the following studies, I investigated how changes in the composition and distribution of tree species affect the population and diversity of galling insects. In the first chapter, I investigated the relationships between host concentration, resistance offered to it by other species, and gall abundance. I also investigated the relationship of gall infestation in the expression of flowers and fruits by hosts. In the second chapter, I investigated the effect of geographic distribution and the number of species congenerous to the host on the vulnerability to attack by different gallers. In the third chapter, I investigated the protection offered by the gall to the galling insect against chronic and acute temperature increase. I concluded that like other specialist insects, galling insect populations benefit from the concentration of the resource, and the presence of few barriers between resource units. I also found that the most geographically distributed host plants are also the most attacked by different insects, and that this effect is greater in plants of larger genera. And despite the fact that the gall provides a microenvironment for the development of the galler, the increase in temperature for a prolonged period exceeds the gall's buffering capacity.

Keywords: Ecology of galls; Insect-plant interaction; Resource concentration; Associated resistance; thermal tolerance

INTRODUÇÃO GERAL

Os estudos de galhas dentro da ecologia, como em todas as ciências, têm diversas frentes, anatômicas, fisiológicas e ecológicas e em boa parte deles, as matérias se misturam frente a grande dependência que o galhador tem do desenvolvimento e resposta do hospedeiro. Os insetos galhadores são herbívoros extremamente especialistas, que durante seu desenvolvimento são capazes diferenciar células vegetais para criar um novo tecido vegetal, a galha. Essa alta especificidade na maneira de extrair recurso causa uma forte relação entre o inseto e o hospedeiro, e este, só consegue se desenvolver em um ou poucos hospedeiros relacionados. O inseto galhador também, em muitas vezes está associado a plantas decíduas, e ajusta seu ciclo de vida com as fases de queda e brotamento foliar do hospedeiro. Além disso, a ocorrência do seu hospedeiro pode estar associada a ambientes desfavoráveis a esses insetos. Em ambientes úmidos, as galhas são ambientes propícios para o desenvolvimento de fungos, o que levou a boa parte da diversidade dos insetos galhadores estarem associados a ambientes quentes e secos, como as savanas. Entretanto, a ocorrência da arvore se dá pela plasticidade da mesma em relação as variáveis abióticas, e o inseto pode não estar adaptado para os mesmos climas. O microclima criado pela galha parecer favorável em ambientes secos, por manter uma umidade elevada, que é mantida pelo tecido vegetal da galha. Mas a imobilidade do inseto frente a mudanças no regime de luz durante seu desenvolvimento, ou aumentos súbitos de temperatura podem ser pontos desfavoráveis frente aos seus parentes de vida livre. Sendo assim, o inseto está vulnerável a mudanças em diversos aspectos e níveis da comunidade vegetal, e variáveis fisiológicas, ecológicas e estruturais interagem criando uma complexa interação entre inseto galhador e planta hospedeira. Por isso, esta tese tem o interesse em avaliar a ecologia dos galhadores com um olhar para a comunidade vegetal e os efeitos que podem exercer na comunidade de insetos galhadores e como os insetos pressionam a comunidade vegetal.

Os insetos galhadores são insetos muito pequenos, com alcance de voo curto e capacidade limitada de se mover em condições de vento. Assim, a distribuição dos hospedeiros vegetais pode ser um importante determinante da distribuição desses organismos (Withers & Harris, 1997). A hipótese de concentração de recursos prevê que a agregação de hospedeiros cria oportunidades e condições para que herbívoros especializados com baixa mobilidade aumentem sua abundância (Root, 1973). Portanto, variações no indivíduo hospedeiro, como tamanho e arquitetura, ou no tamanho da sua população, e na composição das espécies de plantas que o rodeiam podem impor uma força de baixo para cima nos insetos galhadores. No entanto, a abundância de galhas tem mostrado relações distintas com a concentração de recursos em escala local (recurso por área). A abundância de galhas em manchas hospedeiras densas (maior concentração de recursos) foi relatada anteriormente como sendo maior devido à capacidade de voo reduzida do inseto galhador (Viana *et al.*, 2013; Avila & Oleques, 2016). No entanto, alguns estudos também encontraram uma menor abundância de galhas por indivíduo hospedeiro em manchas de hospedeiro densas, uma vez que um maior número de hospedeiros pode reduzir a concentração de insetos galhadores por indivíduo (Muiruri & Koricheva, 2017; Coutinho *et al.*, 2019; Ramos *et al.*, 2019). Quando mudamos a perspectiva e reduzimos a escala espacial do recurso e consideramos apenas um único hospedeiro, observamos que a abundância de galhas está positivamente relacionada a um aumento na biomassa do hospedeiro (Leite *et al.*, 2017; Coutinho *et al.*, 2019). Essas diferenças nos resultados da abundância de galhas dependendo da escala dos recursos indicam que outros fatores estão influenciando a abundância de insetos galhadores na população de hospedeiras.

Quando consideramos simultaneamente o hospedeiro e a comunidade de plantas na vizinhança, o aumento na complexidade ambiental pode ser resultado de uma maior diversidade de espécies de plantas, levando a uma mistura mais complexa de voláteis de plantas que podem mascarar o hospedeiro e criar uma resistência associativa (Barbosa et al., 2009). Além disso, uma maior diversidade de espécies também pode aumentar a abundância de predadores, reduzindo indiretamente a abundância de insetos galhadores (Barbosa *et al.*, 2009; Grossman *et al.*, 2019). Além da diversidade de plantas, a estrutura da comunidade de plantas também pode causar uma barreira ou facilitar o estabelecimento dos insetos galhadores. Caules e folhas de plantas hospedeiras podem representar uma barreira física entre o inseto e o hospedeiro (Perrin, 1976; Risch, 1981) e podem interromper as pistas visuais, reduzindo a probabilidade de o inseto galhador encontrar seu hospedeiro (Castagneyrol *et al.*, 2014; Muiruri *et al.*, 2019). Embora estudos tenham demonstrado associações entre diversidade de plantas e abundância de galhas (Grossman *et al.*, 2019; Knuff *et al.*, 2019), o papel da abundância de árvores não hospedeiras ainda não está claro em sistemas naturais. Como os insetos galhadores são insetos muito pequenos, seu voo provavelmente depende do vento (Jermy *et al.*, 1988;

Withers & Harris, 1997) e a perturbação física de indivíduos não hospedeiros pode ter um forte impacto na distribuição das galhas, independentemente da diversidade das espécies.

Os insetos galhadores também podem ter impactos negativos em seus hospedeiros, como abscisão foliar precoce (Williams & Whitham, 1986), danos da copa (Marquis *et al.*, 2002) e depleção de sementes e frutos (Viana *et al.*, 2013), levando a uma força de cima para baixo na comunidade de plantas. Assim como a estrutura da comunidade de plantas afeta a comunidade de herbívoros (Hunter & Price, 1992; Price *et al.*, 2011), os herbívoros também desempenham um papel importante na estrutura da comunidade de plantas (McNaughton, 1983; Karban & Orrock, 2018). Por exemplo, insetos generalistas podem reduzir a cobertura do dossel, permitindo que novos indivíduos e espécies cresçam no sistema e agindo como uma força de cima para baixo que molda a comunidade de plantas (Wilson & Tilman, 2002; Borer *et al.*, 2014). Embora os insetos galhadores ataquem apenas uma pequena parte da planta, eles muitas vezes podem reduzir a área foliar (Silva *et al.*, 2015), causar danos citológicos e metabólicos à planta hospedeira (Nyeko, 2005; Moens *et al.*, 2009; Oliveira *et al.*, 2016), e alterar as relações de fonte-dreno entre os tecidos vegetais (Oliveira *et al.*, 2017; Martini *et al.*, 2020). Essas alterações metabólicas podem levar a mudanças na arquitetura do hospedeiro e reduzir a produção de estruturas reprodutivas (Leege, 2006; Collins & Drummond, 2019). Essas descobertas demonstram a necessidade de esclarecer o impacto dos insetos galhadores na aptidão do hospedeiro, o que pode destacar o papel dos galhadores como um agente na produtividade do ecossistema.

Algumas plantas podem hospedar uma grande diversidade de espécies de insetos galhadores (Formiga *et al.*, 2015). De certa maneira, ficando mais vulneráveis a pressões advindas dos insetos galhadores, e apesar disso, pouco se sabe sobre os fatores que desencadeiam essa vulnerabilidade. É sabido que táxons grandes podem hospedar uma maior diversidade de insetos indutores de galhas em comparação com táxons pequenos (Mendonça, 2007; Araújo, 2011). E a especiação por troca do hospedeiro é reconhecida como um processo provável para a evolução de insetos herbívoros (Drès & Mallet, 2002). No entanto, nenhum estudo forneceu evidências diretas de que a hipótese do tamanho do táxon também está impulsionando a vulnerabilidade do hospedeiro e favorecendo a formação de super hospedeiros. Os insetos galhadores só podem ter a oportunidade de mudar seu hospedeiro se os hospedeiros forem químicos e fenologicamente semelhantes, e os genes do hospedeiro que direcionam a preferência do hospedeiro e contribuem para a sobrevivência da larva devem ser altamente correlacionados (Futuyma & Peterson, 1985). Falhas na oviposição entre hospedeiros filogeneticamente próximos são um importante mecanismo de radiação e especiação em insetos galhadores (Cooley *et al.*, 2003; Stireman III *et al.*, 2006; Egan & Ott, 2007). Um inseto galhador raramente invade duas espécies de hospedeiros (Malenovský *et al.*, 2015) e, quando isso ocorre, uma seleção rápida cria novas populações de insetos galhadores com adaptações regionais (Akimoto, 1990; Craig *et al.*, 1994; Driscoe *et al.*, 2019). Portanto, o táxon poderia aumentar a vulnerabilidade do hospedeiro por meio da troca de hospedeiro entre espécies congêneres em espécies hospedeiras que pertencem a um gênero com maior riqueza de morfotipos.

A distribuição geográfica dos hospedeiros está relacionada com a sua plasticidade para enfrentar diferentes pressões bióticas e abióticas para se estabelecer nos diferentes ecossistemas, como possíveis mudanças na fenologia foliar (Chuine, 2010). Que por sua vez afetam a comunidade local de insetos galhadores (Oliveira *et al.*, 2013; Fagundes *et al.*, 2018), então é esperado que exista diferença entre as associações com insetos em cada local. E isso vem sendo corroborado pela literatura, que mostra maior diversidade de insetos galhadores em uma única planta hospedeira está relacionada ao tamanho da distribuição geográfica do dessa planta. Ou seja, as espécies de plantas com uma distribuição mais ampla hospedam mais espécies indutoras de galhas em comparação com seus parentes (Cornell, 1985; Blanche & Westoby, 1996; Araújo *et al.*, 2013). Embora a riqueza de galhas não seja diferente entre os locais, as espécies de insetos galhadores diferem entre os locais, contribuindo para uma rica comunidade associada ao considerar todos os locais (diversidade beta) (Fagundes *et al.*, 2020). Assim, a distribuição geográfica do hospedeiro pode ser um importante fator direcionando e especiação de insetos galhadores, tanto dentro do hospedeiro quanto na troca do hospedeiro, uma vez que por terem maior distribuição geográfica estão expostos também a diferentes pressões da fauna (Joy & Crespi, 2007; Driscoe *et al.*, 2019).

Por sua vez, a distribuição dos insetos obedece não só a distribuição do hospedeiro, mas também as suas próprias limitações fisiológicas. O inseto galhador usa a estratégia de manipular o fenótipo da planta hospedeira em benefício próprio, induzindo o desenvolvimento de galhas. Os insetos indutores de galhas mostraram uma grande variedade de formas, estruturas e cores que fornecem um diferentes vantagens ao organismo galhador (Stone & Schönrogge, 2003). As hipóteses que discutem o valor adaptativo das morfologias de galhas para a diversificação e distribuição de insetos galhadores são numerosas, mas a hipótese do microambiente pode assumir um papel importante na colonização de ambientes hostis (Fernandes & Price, 1992). O inseto galhador cria a galha, um novo microambiente, que deveria ser termorregulador e protegido da desidratação pelos tecidos da planta hospedeira. No entanto, estudos que quantificam a capacidade da galha de oferecer proteção ao inseto galhador contra a temperatura são muito raros (Martinez, 2009; Miller III *et al.*, 2009), e podem ajudar a entender o papel da galha em fornecer abrigo contra pressões abióticas. O Cerrado possui uma fauna altamente diversa de insetos galhadores, especialmente na savana do Cerrado com áreas abertas de pastagem e alta luminosidade (Fernandes et al., 2005). Portanto, a exposição das plantas à alta temperatura pode ser crônica, ocorrendo em latitudes mais baixas, ou aguda, ocorrendo em copas abertas. E nas galhas foliares a capacidade de dissipar calor é depreciada, principalmente porque as galhas são originadas da rediferenciação dos tecidos vegetais, e reduzem drasticamente o espaço intercelular e a densidade estomática (Oliveira *et al.*, 2017, 2020). O Cerrado está ficando mais quente (Hofmann *et al.*, 2021), e a temperatura crônica pode levar a diferentes cenários alterando o valor adaptativo do microhabitat.

Portanto, nos capítulos a seguir, avaliei os efeitos da comunidade vegetal nos insetos galhadores em uma escala local, verificando como a distribuição do local hospedeiro atua nas populações de insetos, mas também explorei pela primeira vez como o inseto interage com a comunidade vegetal vizinha, e como ela afeta os insetos. Também usamos uma escala macro ecológica para estudar como as comunidades de plantas e insetos do Cerrado interagem, avaliei o efeito distribuição geográfica das plantas e o tamanho do grupo taxonômico em que elas se encontram no número de insetos galhadores que conseguem desenvolver galha nelas. E finalmente, qual é o valor adaptativo da galha em relação a proteção contra o aumento de temperatura, seja ele crônico ou agudo

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Bottom-up and top-down forces in plant-gall relationships: testing the hypotheses of resource concentration, associational resistance, and host fitness reduction

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Abstract. 1. The abundance of insect galls may be influenced by the local host abundance as well as by the structure of the surrounding plant community, while insect galls may reduce host fitness. So far, few studies have been done in order to understand the relationship between galling insect abundance and the surrounding non-host plant community.

2. In the present study, we explored the relationship between galling insect abundance and plant community structure and diversity. We tested the bottom-up forces of host and non-host plant density and non-host diversity on gall abundance. We also tested the top-down force of gall abundance on host fitness.

3. We sampled all trees, measured their basal area, and estimated gall abundance in 25 plots (1 ha in total) located in a Brazilian savanna. In addition, we recorded the presence of flowers and fruits in all trees studied.

4. We found a positive relationship between host basal area per plot and gall abundance and a negative relationship between non-host basal area and species diversity with gall abundance. We also observed a negative association between gall abundance and the probability of the host plant to have flowers or fruits.

5. Our data provide evidence that plant communities in which one host species dominates can be more susceptible to herbivore pressures than communities with less host dominance and more non-host neighbours, and that the non-host species create disruptive cues limiting galling insect infestation.

Key words. Gall ecology, galling insect abundance, host fitness, plant-gall interaction.

Introduction

Plant communities face different kinds of pressures such as those associated with insect feeding. Some insects can be very selective in their feeding while others are more generalist, and all of them could cause drastic impacts on a plant (Karban & Agrawal, 2002). Galling insects are one of the most specialised types of herbivores in nature, being able to manipulate host plant tissues for their own benefit (Shorthouse *et al.*, 2005; Oliveira *et al.*, 2016), using a complex chemical attack to trigger gall formation in their hosts (Zhao *et al.*, 2015). This specific interaction leads to a narrow host range for galling insects, which can exploit only one or few closely related plants (Mendonça, 2007). Due to this specificity, variations in host individual and population size and plant species composition can impose a bottom-up force on galling insects, with gallers responding to the availability of the resource (host plants). Galling insects can also have negative impacts on their hosts, such as early foliar abscission (Williams & Whitham, 1986), canopy damage (Marquis *et al.*, 2002), and seed and fruit

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depletion (Viana *et al.*, 2013), leading to a top-down force on the plant community.

Galling insects are very small insects with a short flight range and have limited capability to move under windy conditions. Thus, the distribution of plant hosts can be an important determinant of the distribution of these organisms (Withers & Harris, 1997). The resource concentration hypothesis predicts that host aggregation creates opportunities and conditions for specialised herbivores with low mobility to increase their abundance (Root, 1973). However, the abundance of galls has shown distinct relationships with resource concentration on a local scale (resource per area). The abundance of galls in dense host patches (higher resource concentration) has been previously reported to be higher due to the reduced flight ability of the galling insect (Viana *et al.*, 2013; Avila & Oleques, 2016). However, some studies also found a lower gall abundance per host individual in dense host patches since a higher number of hosts can reduce the concentration of galling insects per individual (Muiruri & Koricheva, 2017; Coutinho *et al.*, 2019; Fagundes *et al.*, 2019). When we change perspective, and reduce the spatial scale of the resource, and consider only a single host, we observe that gall abundance is positively related to an increase in host biomass ('plant architecture' hypothesis) (Leite *et al.*, 2017; Silva *et al.*, 2017; Coutinho *et al.*, 2019). Therefore, these differences in the results of gall abundance depending on the resource concentration scale indicate that other factors are driving galling insect abundance in the host population.

When we simultaneously consider the host and the plant community in the neighbourhood, the associational resistance hypothesis predicts that a higher plant diversity and structure would have a negative effect on specialist insects by increasing the environmental complexity (Cardoso *et al.*, 2002; Hambäck *et al.*, 2014; Civitello *et al.*, 2015). This increase in environmental complexity could be a result of higher plant species diversity, leading to a more complex blend of plant volatiles, which can mask the host and create an associational resistance (Barbosa *et al.*, 2009). In addition, higher species diversity could also increase the abundance of predators, indirectly reducing galling insect abundance (Barbosa *et al.*, 2009; Grossman *et al.*, 2019). Besides plant diversity, plant community structure can also cause plant associational resistance and susceptibility to specialist insects. Non-host stems and leaves can represent a physical barrier between the insect and the host (Perrin, 1976; Risch, 1981) and can disrupt visual cues and the background, reducing the probability that the galling insect will find its host (Castagneyrol *et al.*, 2014; Muiruri *et al.*, 2019). Although studies have demonstrated associations between plant diversity and gall abundance (Grossman *et al.*, 2019; Knuff *et al.*, 2019), the role of non-host tree abundance is still unclear in natural systems. As galling insects are very small insects, their flight is probably wind dependent (Jermy *et al.*, 1988; Withers & Harris, 1997) and the physical disturbance of non-host individuals could have a strong impact on gall distribution regardless of species diversity.

As the plant community structure affects the herbivore community (Hunter & Price, 1992; Price *et al.*, 2011), the herbivores also play an important role in plant community structure (McNaughton, 1983; Karban & Orrock, 2018). For example generalist insects can reduce canopy cover allowing new individuals and species to grow in the system and acting as a top-down force that shapes the plant community (Wilson & Tilman, 2002; Borer *et al.*, 2014). Although galling insects attack only a small part of the plant, they can often reduce foliar area (de Silva *et al.*, 2015), cause cytological and metabolic damage to the host plant (Nyeko, 2005; Moens *et al.*, 2009; Oliveira *et al.*, 2016), and change the sink and source relationships between plant tissues (Oliveira *et al.*, 2017; Martini *et al.*, 2020). These metabolic changes can lead to shifts in host architecture and reduce the production of reproductive structures (Leege, 2006; Collins & Drummond, 2019). These findings demonstrate a need to clarify the impact of galling insects on host fitness, which may include the gall system as a driver of ecosystem productivity.

We explore here the relationship of the surrounding plant community with gall abundance, and the link between gall abundance and plant host fitness. We measured gall abundance and plant community composition in 25 plots (a total of 1 ha) of a Brazilian savanna, a high-diversity plant, and gall ecosystem. We hypothesised that local (i.e. plant-specific) gall abundance would be positively associated with the host basal area (a correlate of plant biomass) of each individual and with the host basal area per plot. Moreover, we hypothesised that gall abundance would be negatively associated with plant species richness and non-host basal area per plot. Finally, assuming that galling insects impose a strong fitness cost on their hosts, we predicted that the presence of flowers or fruits on plants would be negatively associated with the abundance of galls.

Material and methods

Study area

The study was conducted in November 2017 in the Serra de Caldas Novas State Park (~12 000 ha, 17°48′4.97″S, 48°41′34.54″O), a conservation unit with areas of savanna in Brazil. Although the park is a conservation unit, fire events are frequently recorded there due to the surrounding agricultural lands (Fig. 1). The park has different savanna vegetation types, the most common being the Cerrado 'sensu strictu' (Ribeiro and Walter 1998), a typical savanna vegetation with the presence of an herbaceous layer. In the Cerrado 'sensu strictu' of the park, the herbaceous layer is dominated by native grasses (i.e. *Echinolaena inflexa* (Poir.) Chase.) and the tree layer consists of common savanna species such as *Aspidosperma tomentosum* Mart., *Dalbergia miscolobium* Benth., and *Caryocar brasiliense* Cambess. The region experiences a tropical savanna climate, with rainy summers (October to March, mean temperature 23.4°C and total rainfall 1342 mm) and dry winters (April to September, mean temperature 20.7°C and total rainfall 234 mm) (Alvares *et al.*, 2013) and the predominant soil in the region is Latosol. The 'Cerrado sentido restrito' is a predominantly xeric environment with high plant diversity, harbouring one of the most abundant and diverse gall ecosystems (Price *et al.*, 1998).

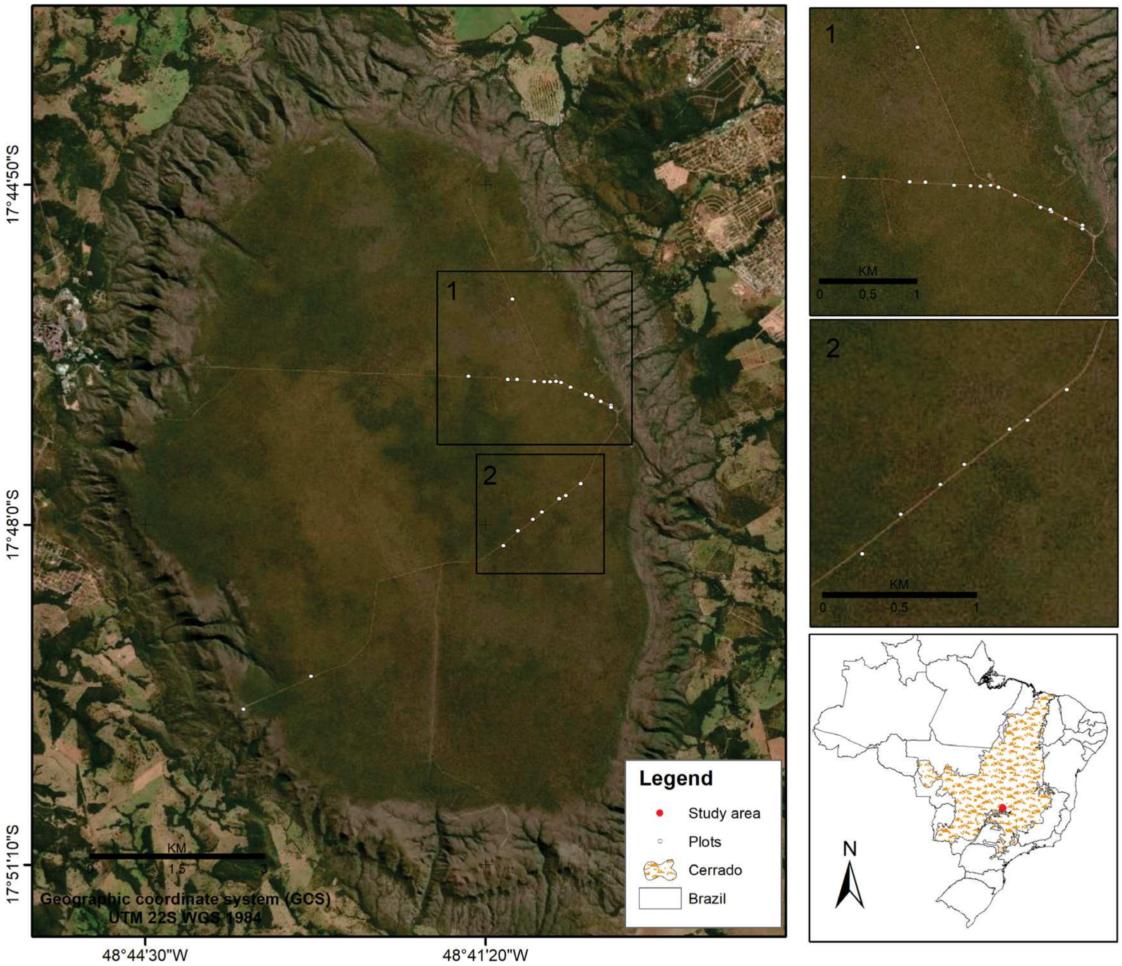

Fig. 1. Map of the study area showing study plot locations (white dots), and the Cerrado biome domain (brown coloured). [Colour figure can be viewed at wileyonlinelibrary.com].

Vegetation sampling

To sample the vegetation community, we established 25 plots (each 20 × 20 m, a total of 1 ha) randomly distributed over the study area. To select the plots, we projected a 20-metre-wide polygon located parallel to and at a distance of 10 m from the principal roads of the park, and we later gridded it into 20 × 20 m plots using geoprocessing tools. Plots were established at a distance of 10 m from the road edge in order to avoid possible road-associated edge effects on the vegetation sampled. We then numbered each grid cell and selected each sample site (= 20 m × 20 m plot) at random (Fig. 1). In each plot, all living trees with a diameter ≥ 5 cm at ground level were sampled; their diameter was measured, their height estimated and the species identified. We sampled only trees with a diameter ≥ 5 cm at ground level in order to account only for the individuals that were already established in the system and had a higher probability to survive and reproduce compared to saplings and seedlings. We further checked for the presence of flower buds, flowers, and fruits in all sampled individuals.

Leaf gall assessment

Leaf gall assessment was carried out on all sampled trees. For tree species that had galls on at least one individual (tagged as hosts), we estimated the gall abundance per individual using a five-point scale: 0) no galls present; 1) low abundance when it was evident that most leaves did not have galls; 2) intermediate abundance when about half the leaves had galls and it was difficult to distinguish if most of the leaves had galls or not; 3) high abundance when it was evident that most leaves had galls, but some leaves did not; and 4) full abundance when all leaves had galls and almost the entire leaf area was affected. To standardise the collection procedure, we searched for galls for 3 min on each tree before considering their absence. Gall assessment was performed by two observers on each tree. Most of the time, their assessments agreed, but when they did not, a third evaluation was performed. Galls were classified into morphotypes since gall morphology is a reliable indicator of galling insect species (Carneiro *et al.*, 2009; dos Isaias *et al.*, 2013). Thus, gall morphotypes were used here as a sampled unit for each galling insect species.

Variables used to assess ecological hypotheses

To study resource concentration, we used the host basal area calculated as pi × R^2, where R = tree circumference radius (stem diameter divided by 2). Host basal area was calculated on a plot scale as the summation of the basal area of each host species per plot, and on an individual scale as individual basal area. For data analysis, we only considered host species that had at least 10 individuals when summed across all 25 plots. Based on these criteria, we used seven tree species as host species in the analyses (*Aspidosperma tomentosum*, *Caryocar brasiliense*, *Dalbergia miscolobium*, *Eremanthus glomerulatus*, *Kielmeyera grandiflora*, *Ouratea spectabilis*, and *Qualea parviflora*), considering each tree host individual as a replicate.

To study associational resistance, we used the basal area of non-host individuals and species diversity per plot. We calculated the basal area of non-host individuals by summing their total basal area in the plot. The non-host basal area per plot differed for each host species since each one had different non-host species in the neighbourhood. As a metric of species diversity, we used rarefied species richness, which accounts for the possible confounding effect of tree density on species richness amongst plots (Gotelli & Colwell, 2001). Rarefied species richness was based on a random sampling of 29 individuals since this was the minimum number of individuals in all plots. The non-host basal area per plot is related to the physical barriers that galling insects have to overcome between hosts (Perrin, 1976; Risch, 1981; Jermy *et al.*, 1988). The species diversity per plot can account for the possible mixture of volatile compounds during host seeking.

As a proxy of host plant fitness, we used species reproductive activity based on the presence of flowers or fruits per individual. We only considered for analysis host species that had at least one individual with flowers or fruits in our sampling. Based on this criterion, we used 11 host species to assess host fitness (*Aspidosperma tomentosum*, *Caryocar brasiliense*, *Dalbergia miscolobium*, *Eremanthus glomerulatus*, *Kielmeyera grandiflora*, *Ouratea spectabilis*, *Qualea parviflora*, *Myrtaceae* sp., *Psidium laruotteanum*, *Qualea multiflora*, and *Qualea grandiflora*).

Data analyses

To evaluate how gall abundance is related to resource concentration (host basal area per plot and per individual) and associational resistance (non-host basal area and species diversity per plot) is related to gall abundance (bottom-up hypotheses), we used a Cumulative Link Mixed Model (CLMM). We included host basal area per plot, host basal area per individual, non-host basal area per plot, rarefied species richness, and their interactions as fixed factors, and host species as a random factor. We included host species as a random factor to account for the species-specific gall interaction with hosts and to test if the patterns occur independent of the gall-host system. We selected the models with the lowest Akaike information criterion (AIC) values and considered models to be equally supported when the difference in AIC was less than two units (Anderson & Burnham, 2004). Among all models that were equally supported (based on AIC), the most parsimonious were selected as most acceptable. The model selections were performed using the dredge function of the 'MuMIn' package (Bartoń, 2019).

To assess whether the presence of reproductive structures on the hosts was related to gall abundance we performed a GLMM with binomial distribution (logistic regression), including gall abundance as a fixed factor and host species and plot as random factors. The data used in all models were standardised prior to the analysis in order to compare different scaled numbers. All mixed models were performed in R 3.1.2 (RStudio Team, 2015) using the glmer function of the 'lme4' package (Bates *et al.*, 2015). Before applying the GLMM models cited above, all variables were tested for colinearity using the Variance Inflation Factor (VIF) and only variables with VIF values <3 were included in the analyses (Craney & Surles, 2002). To calculate VIF, we used the VIF function of the 'vegan' package (Oksanen *et al.*, 2019).

To test for potential spatial autocorrelation among our host individuals, we assessed the degree of spatial autocorrelation in our GLMM residuals using the Moran I test. We used the X and Y coordinates of a central point of each plot to define the location of each host individual. Thus, individuals that were registered in the same plot were considered to have the same geographic location in the model. Moran's I tests were performed with the moran. test function of the 'spdep' package (Bivand, 2015). We found no spatial autocorrelation in any model (Moran's I *P*-value > 0.05) and we continued the analyses using GLMM results.

Results

We sampled 1245 tree individuals of 70 species, 329 of which belonging to 19 species had galls (17 leaf galls, 2 stem galls) (Table 1; Table S1). The most abundant galls were induced by an unidentified Cecidomyiidae on the petiole/leaf of *Dalbergia miscolobium* Benth and by *Pseudophacopteron longicaudatum* (Hemiptera) Male. on leaves of *Aspidorperma tomentosum* Mart. (Table 1). The number of tree individuals in the study plots ranged from 29 to 84 with the total basal area ranging from 0.22 to 0.57 m^2 per plot.

When examining the resource concentration and associational resistance hypotheses (bottom-up forces), three models were used to explain the relationships (Delta AIC < 2, df $<= 8$) (Table S2). The first best model showed that gall abundance was positively related to host basal area per plot (standardised regression coefficient, $\beta = 0.64$; Standard Error, SE = 0.14) (Fig. 2a, Table S3), and the second one added a negative relation to the non-host basal area per plot and rarefied species richness ($\beta = -0.16$; SE = 0.11 and $\beta = -0.21$; SE = 0.11, respectively) (Fig. 2b,c; Table S3). The third-best model showed that gall abundance was positively related to host basal area per plot ($\beta = 0.68$, SE = 0.14) (Fig. 2a; Table S3) and negatively related to non-host basal area and their interaction ($\beta = -0.24$, SE = 0.11 and $\beta = -0.21$, SE = 0.12, respectively) (Fig. 2c; Table S3). Host basal area per individual was not significantly related to gall abundance in the best models. When examining

Table 1. Host plant species and identified galling species sampled in Parque Estadual da Serra de Caldas Novas, central Brazil.

Plant host		Gall systems				
Species	N° of trees (with galls)	Insect taxa order	Family	Host organ	Morphotype	References
Dalbergia miscolobium	122 (91)	Diptera	Cecidomyiidae	Leaf	Globoid	Carneiro *et al.*, 2009
Aspidosperma tomentosum	82 (68)	Hemiptera	Psyllidae	Leaf	Globoid	Malenovský *et al.*, 2015
Kielmeyera grandiflora	97 (62)	–	–	Leaf	Globoid	–
Eremanthus glomerulatus	46 (38)	–	–	Stem	Globoid	–
Ouratea spectabilis	38 (28)	Diptera	Cecidomyiidae	Leaf	Lenticular	Urso-Guimarães and Santos, 2006
Qualea parviflora	19 (10)	Diptera	Cecidomyiidae	Leaf	Globoid	Araújo *et al.*, 2014
Kielmeyera coriacea	105 (9)	–	–	Leaf	Globoid	–
Caryocar brasiliense	13 (5)	Diptera	Cecidomyiidae	Leaf	Globoid	Castro *et al.*, 2012
Andira vermifuga	5 (3)	Diptera	Cecidomyiidae	Leaf	Lenticular	Araújo *et al.*, 2011
Myrtaceae sp1	12 (3)	–	–	Leaf	Globoid	–
Psidium laruotteanum	22 (3)	–	–	Leaf	Globoid	–
Leptolobium dasycarpum	34 (2)	Diptera	Cecidomyiidae	Leaf	Lenticular	Araújo *et al.*, 2011
Aspidosperma macrocarpon	13 (1)	Hemiptera	Psyllidae	Leaf	Globoid	Malenovský *et al.*, 2015
Dimorphandra mollis	23 (1)	–	–	Leaf	Globoid	–
Eriotheca pubescens	3 (1)	Diptera	Cecidomyiidae	Leaf	Globoid	Tuller *et al.*, 2013
Piptocarpha rotundifolia	46 (1)	Diptera	Cecidomyiidae	Stem	Fusiform	Bergamini *et al.*, 2017
Qualea grandiflora	20 (1)	–	–	Leaf	Globoid	–
Roupala montana	1 (1)	–	–	Leaf	Lenticular	–
Styrax ferrugineus	40 (1)	Diptera	Cecidomyiidae	Leaf	Lenticular	Bergamini *et al.*, 2017

Table was sorted by number of galled individuals. (–) indicates no available information about galling insect species.

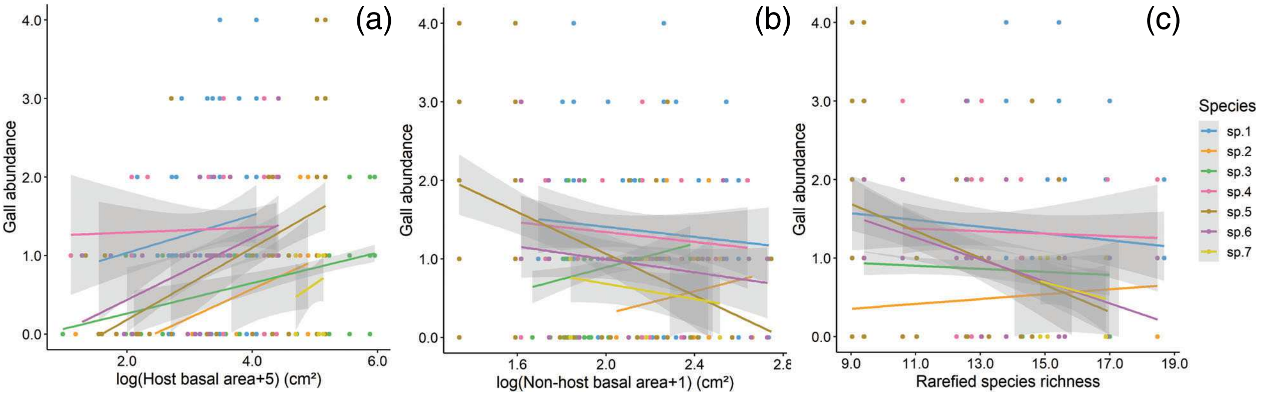

Fig. 2. Bivariate regressions between gall abundance and (a) host basal area, (b) Non-host basal area, and (c) plant species richness. Different colours indicate the relationships by each gall system (i.e. different host species), included as random factor in the model. sp.1 = *Aspidosperma tomentosum*; sp.2 = *Caryocar brasiliense*; sp.3 = *Dalbergia miscolobium*; sp.4 = *Eremanthus glomerulatus*; sp.5 = *Kielmeyera grandiflora*; sp.6 = *Ouratea spectabilis*; sp.7 = *Qualea parviflora*. Note these bivariate relationships are for illustration purposes only and may not necessarily provide the same results as in the multiple regression model (Tables S2 and S3) [Colour figure can be viewed at wileyonlinelibrary.com].

the hypothesis of fitness reduction (top-down forces), the best model showed that the probability of host individuals having reproductive structures was negatively related to gall abundance (β = −0.28, SE = 0.11) (Fig. 3, Table S4).

Discussion

We evaluated how host concentration and associational resistance are related to gall abundance, and how gall abundance is related to one potential measure of plant fitness (the presence of reproductive structures) in a tropical savanna. We found that gall abundance per host was positively related to host basal area per plot and negatively related to the basal area of non-host individuals and species diversity. This indicates that gall abundance is higher in areas with higher resource concentration, but with lower non-hosts plant species between hosts. We also found that the presence of reproductive structures was negatively related to gall abundance, which could indicate that a host individual has a reduced chance of reproduction when it has many galls.

Bottom-up effects on gall abundance

We hypothesised that host concentration (host basal area) at the plot and individual scale would be positively related to gall

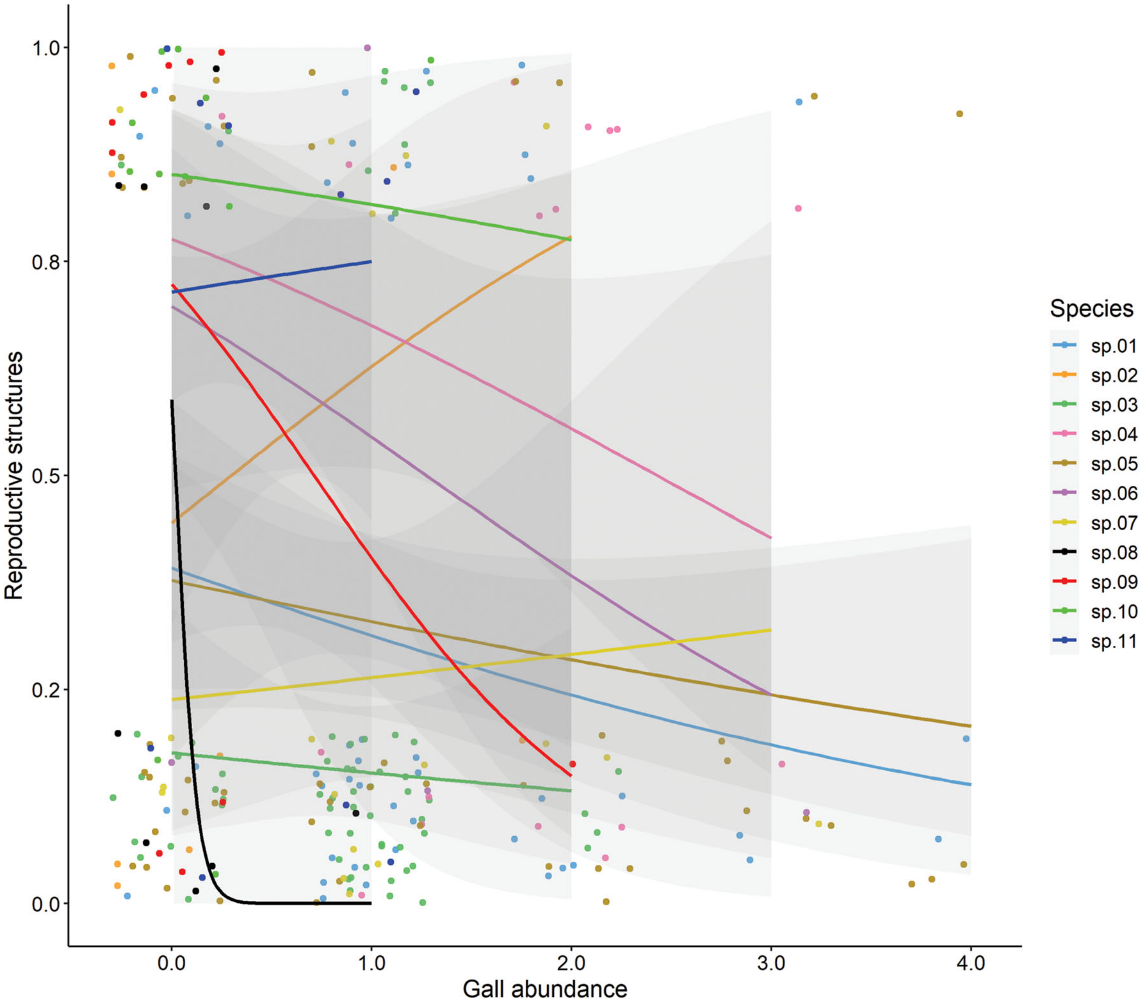

Fig. 3. Mixed logistic regression between the presence of host reproductive structures (flowers and fruits) and gall abundance. The relationships are given by each plant-gall system (included as random factor in the model – Table S4). sp.01 = *Aspidosperma tomentosum*; sp.02 = *Caryocar brasiliense*; sp.03 = *Dalbergia miscolobium*; sp.04 = *Eremanthus glomerulatus*; sp.05 = *Kielmeyera grandiflora*; sp.06 = Myrtaceae sp.; sp.07 = *Ouratea spectabilis*; sp.08 = *Psidium laruotteanum*; sp.09 = *Qualea grandiflora*; sp.10 = *Qualea multiflora*; sp.11 = *Qualea parviflora*. [Colour figure can be viewed at wileyonlinelibrary.com].

abundance per host. When examining resource concentration at the plot scale, we indeed found that gall abundance per host was positively related to host basal area per plot. This could be due to the patterns of galling insect colonisation within the host population; the probability of a host individual becoming infested might be higher if it grows close to an individual that is already infested (Craig, 1984). Our results agree with patterns of nematode galls and other low-mobile insects that show a higher abundance in areas with higher resource concentration (Caballero & Lorini, 2000; Viana *et al.*, 2013; Wenninger *et al.*, 2016). When examining resource concentration on an individual scale, we did not find any significant relationships between host size (basal area) and gall abundance. Large host individuals might suffer less from stresses associated with seasonality due to their deeper root systems, which can provide reliable resources (Veldtman & McGeoch, 2003). Moreover, large hosts offer a large area for galling insect ovipositing, favouring the maintenance of viable populations. Nevertheless, studies that detected positive relationships between tree size and galler abundances were conducted on pastures and degraded areas, where the hosts are often isolated in the landscape (Leite *et al.*, 2017; Cronin *et al.*, 2020). In our study area, the hosts are abundant and grow close to each other, a fact that reduces gall infestation of only one host (Fagundes *et al.*, 2019).

We expected gall abundance to be negatively related to the local abundance of non-host species and to overall species diversity, and our results corroborated these assumptions. The presence of non-host individuals between resources seems to impose a barrier on the galling insect when searching for its host, creating associational resistance (Hambäck *et al.*, 2014). The spatial manoeuvring of small insects is largely based on light-compass orientation, with the host being found by chance (Jermy *et al.*, 1988). A larger non-host basal area creates an environment that makes it less likely for galling insects to encounter host individuals. An example is represented by pine plantations, where the presence of non-host plants causes a reduction of specialist insects due to the difficulties to find paths between hosts (Castagneyrol *et al.*, 2014). In addition, more recent work has shown that plant traits such as leaf size are also important drivers of associational resistance since areas dominated by non-host plants with larger leaves have less gall abundance compared to places dominated by plants with smaller leaves (Muiruri *et al.*, 2019). Similar patterns were also found in experiments using artificial and biotic fences between hosts, where dense fences decreased dipteran immigration to host plants (Wratten *et al.*, 2003).

Besides this relationship with plant community structure, gall abundance was negatively related to plant diversity. A more diverse plant community surrounding the host plant can lead to background complexity that masks the specific host volatiles the insects use in host localisation (Barbosa *et al.*, 2009). Although the use of plant volatiles as cues to predict galling insect distribution is still unknown (Barônio & Oliveira, 2019), these volatile cues are mainly used when the insects are close enough to the resource (as in our study area), where the airflow causes little dilution (Finch, 1980). Since volatile preferences are highly site-adapted, and differ among insects (Doddala *et al.*, 2016), shifts in species composition may confound the galling insect orientation when searching for a host. Furthermore, higher plant species diversity leads to a higher overall diversity of insects (Barbosa *et al.*, 2009) that can prey on the galls. Although we did not measure gall predation, this process can occur and have an impact on the abundance of established galls (László *et al.*, 2014).

Gall abundance reduces the probability of flowers and fruits in host plant

We hypothesised that the presence of flowers or fruits on the host trees would be negatively associated with gall abundance and our data supported this prediction. The presence of reproductive structures is related to reproductive success and could be a good tool for the assessment of plant fitness (Agrawal, 1999; Dukas, 2005), although we know that some plants might have produced fruits and flowers before gall induction when we sampled to test the relationship. However, even in this situation, galls compete for photoassimilates with flowers and fruits (Oliveira *et al.*, 2017), a fact that could lead to the abortion of parts of their reproductive structures (Viana *et al.*, 2013). Galling insects can cause a redifferentiation of host tissue, reducing foliar area and photosynthetic rates (Oliveira *et al.*, 2011). This can change the source-sink relationship and disrupt plant tissue homeostasis, reducing plant resources available for growth and reproduction (Isaias *et al.*, 2015; Oliveira *et al.*, 2017). Among insect galls, leaf galls of psyllid reduce flower production by the host (Leege, 2006). When attacked by bud galling aphids, the host increased the number of branches while fruit production was not affected (Kurzfeld-Zexer *et al.*, 2010). In stem galls induced by Cecidomyiidae, the host reduced the number of flowers in 57% of the crops, but increased the number of flowers during the second flower bloom (Collins & Drummond, 2019).

During gall development, galling insects change the gene expression and hormone content of damaged cells (Zhao *et al.*, 2015; Takei *et al.*, 2017) and can trigger the plant immune response, changing its likelihood of reproduction (Ali & Agrawal, 2012). In addition, gall formation requires the expression of genes related to carpel development (Schultz *et al.* 2019) and thus host plants can receive a fake chemical signalling of fructification, responding to the increase of gall abundance as an ectopic reproductive structure. However, host fitness may depend on other factors rather than just gall abundance, such as nutritional status, water availability, and the influence of other herbivores, which were not measured in this study. However, the present observations show a relationship between leaf galls and the presence of reproductive structures. In future studies, experimental manipulations controlling the gall abundance per host and their growth conditions would clarify whether reproduction is indeed affected by the abundance of galls.

Concluding remarks

In summary, our results suggest that the abundance of galling insects on trees depends on the surrounding plant communities, and that gall abundance might have an effect on host fitness by suppressing reproductive output. Our results strengthen the hypothesis of the resource concentration and associational resistance, signalling that plant communities with species dominance can be more susceptible to specialist herbivore pressures. In addition, the increment of non-host individuals and community species diversity could hamper galling insect colonisation, preventing an increase of gall abundance. Furthermore, our study suggests that galling insects might affect host plant fitness by reducing the probability of hosts to produce reproductive structures. These results reinforce the importance of evaluating the effects of galling insects on their host plants and suggest that, like other plant enemies, galling insects could play an important role in the structuring of the plant community in tropical systems (Gillett, 1962). Considering these ecological interactions, if the galling insects are more abundant where hosts are dominant, and if host fitness is negatively affected by high levels of insect attack (as suggested), this could lead to a situation where host species are at a disadvantage (because of a higher enemy load) and reduce their population over generations. This kind of enemy-mediated negative density-dependence on plant fitness could be important in maintaining plant diversity, affecting plant community distribution (Maron & Crone, 2006; Bagchi *et al.*, 2014). Consequently, there will be a negative feedback since reduced resource availability would lead to a harder establishment of galling insects. Further studies linking patterns of attack by galling insects to the dynamics of plant communities are needed.

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Authors' contribution

VCM and DR designed and conducted the sampling and wrote the manuscript draft. JPJ and DCO helped with data analyses and contributed to writing. All authors critically revised previous drafts and contributed to revisions.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 All sampled trees in the plots established at the Parque Estadual da Serra de Caldas Novas in November of 2017. Reproductive structures (presence of flower or fruit).

Table S2 Model selection with only significant variables/interactions in all models using Gall abundance as response variable.

Table S3 Best models by AICc and parsimony using Gall infestation as response variable.

Table S4 Logistic model using reproductive activity as response variable.

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Capítulo 2 - Host vulnerability to galling insects: Superhost formation in Neotropical savannas

Abstract

The pest pressures on hosts are very different among herbivores guild. Specialist herbivores like galling insects have a very narrow host range, however, some plants species can host a wide diversity of galling insect species. The characteristic shared by these host as wide geographic distribution, taxon size and their interaction may be factors that drive the vulnerability of these superhosts to the galling insects. Using data from trees in the Cerrado biome and galling insect inventories, we created a structural equation model to test if genus taxon size and species geographic distribution increase the tree vulnerability to galling insect attack. The compiled database of tree inventories resulted in 360 tree species belonging to 178 genera and 60 families. Among these tree species, 147 are hosts for 344 galling insect morphotypes. Host geographic range size was the main driver increasing tree vulnerability to galling insects, followed by the number of galls in their genus relatives. Genus taxon size and spatial overlap had an indirect effect on host vulnerability. We argued here that host distribution may increase vulnerability through host ability to colonize different environments so that, at the same time, the hosts suffer different abiotic and biotic pressures. Phenotypic plasticity allows the plant species to adjust their morphology and physiology, creating new resources and a window of opportunity for different herbivores. The increased vulnerability of belonging to a genus with high morphotype richness supports the idea that host shift might be a significant driver of galling insect speciation and radiation.

Introduction

Herbivory has been associated with changing plant fitness via reducing plant productivity and reproduction, and crops can lose about 45% of their yields with a great increase in plant mortality (Hendrix, 1988). Herbivory is a process that shapes plant communities in both cultivated and natural systems and its effects are important for economic and ecological purposes. Plants can increase their vulnerability or resistance to herbivory through changes in morphological traits such as thorns (Milewski, Young, & Madden, 1991), trichomes, leaf toughness and leaf hardness (Agrawal, 1999), or changes in plant community structure and diversity with resource concentration and associational resistance or susceptibility (Hambäck, Inouye, Andersson, & Underwood, 2014; Root, 1973). The selective pressure mediates plant community composition and diversity. In contrast to African savannas where ungulate herbivores have a key role in plant distribution and richness (McNaughton, 1985), in the Brazilian savanna this selective process is done by small animals such as insects, nematodes and others (Costa, Vasconcelos, Vieira-Neto, & Bruna, 2008; Martini, Raymundo, Prado-Junior, & Oliveira, 2021). While the effect of large mammals is to reduce the herbaceous biomass by 3 times and to increase species richness by ~60% of (Jacobs & Naiman, 2008), in the Cerrado, small insects such as cutter ants (Atta genus) are responsible for 15% of foliar biomass consumption (Costa et al., 2008). Therefore, investigating factors that drive plant vulnerability to insect herbivory is essential in order to understand the ecosystem dynamics and to identify possible threats to agriculture such as pests in impacted natural environments.

Galling insects are a specialist guild of herbivores that manipulate the host plant tissues, changing the cellular growth and creating a new structure, the gall (Oliveira et al 2016, Giron et al 2016). The specificity of this interaction results in a narrow host range for each galling insect species (Mendonça, 2007) that can attack one or a few phylogenetically related host plant species (Malenovský, Burckhardt, Queiroz, Isaias, & Oliveira, 2015). However, some plants can be superhosts, hosting a wide diversity of galling insect species (Formiga, Silveira, Fernandes, & Isaias, 2015). The higher host specificity, allied to the possibility of occurrence of superhosts, makes the gall-plant interaction an important system for the assessment of plant vulnerability to herbivory. The presence of the superhost in a plant community can increase the local galling insect richness, and the galling insect richness can be attributed to plant species composition rather than plant diversity (Formiga et al. 2015; Knuff et al. 2019; Araújo et al. 2020). Despite the effect of the superhosts on galling insect diversity, the factors driving the vulnerability of these superhosts to the galling insects are still unclear.

The hypothesis of taxon size or taxonomic isolation has been used to explain the galling insect diversity associated with a plant genus or family (Connor, Faeth, Simberloff, & Opler, 1980). Large plant taxa (number of species) can host a higher diversity of gall-inducing insects compared to small taxa (Walter S Araújo, 2011; Mendonça, 2007). However, no study has provided direct evidence that the hypothesis of taxon size is also driving host vulnerability (formation of superhost). Failures in oviposition between phylogenetically close hosts are an important mechanism of radiation and speciation in galling insects (Cooley, Simon, & Marshall, 2003; Egan & Ott, 2007; Stireman III, O'Hara, & Wood, 2006). The galling insects may only have the opportunity to shift their host if the hosts are chemically and phenologically similar, and the host genes that drive the host preference and contribute to larva survival have to be highly correlated (Futuyma & Peterson, 1985). A galling insect is rarely reported to invade two hosts species (Malenosvki et al. 2017), and when this occurs a quick selection creates new galling insect populations with regional adaptations (Akimoto 1990; Craig et al. 1994, Driscoe et al. 2019). The host-shift speciation is recognized as a likely process for the evolution of herbivorous insects (Drès & Mallet, 2002). Thus, the taxon size hypothesis could increase host vulnerability through an indirect effect of the host shift between congeneric species on host species that belong to a genus with higher morphotype richness.

The higher diversity of galling insects attacking a single host plant is related to the host geographic range size within one plant genus, i.e., the plant species with a wider distribution hosts more gall-inducing species compared to its relatives (Walter Santos Araújo, Scareli-Santos, Guilherme, & Cuevas-Reyes, 2013; Blanche & Westoby, 1996; Cornell, 1985). Although the diversity of hosts usually does not differ between sites, the galling insect species differ between superhost sites, contributing to a rich associated community when considering all sites (beta diversity), resulting in an increase in the number of species associated with a host plant of greater geographic distribution (Marcilio Fagundes et al., 2020). This can be explained by phenological traits of the plant, that vary across their geographical distribution (Chuine, 2010), and by changes in host leaf phenology that affect the local galling insect community (Marcilio Fagundes et al., 2018; Oliveira, Jr, Moreira, Lemos-Filho, & Isaias, 2013). Thus, the host geographic distribution can be an important driver of speciation for galling insect diversity, either within-host or host shift (Driscoe et al., 2019; Joy & Crespi, 2007). In addition, assuming that the galling insects can shift their hosts between congeneric species (Akimoto, 1990) and that the insects have a low ability to migrate among sites (Blanche and Westoby 1996), the host geographical distribution also affects the probability of the host to be found by other galling insect species. The wide distribution of tree species would increase the overlap among congeneric species, favoring the host shift and increasing host vulnerability and formation of the superhosts.

Here, we evaluated the relative importance of host genus size and geographic distribution driving tree species vulnerability to galling insects and superhost formation in the Brazilian savanna, the Cerrado. We have compiled published data from about 360 tree species and 344 gall morphotypes. We hypothesized that host species frequency in the tree inventories (host frequency), the tree species richness in the host genus (genus size), the galling species richness in the genus (genus gall richness), and the geographic distribution overlap between hosts and congeneric species (genus overlap) should be positively related to the richness of galling insect species per host plant. We also tested for indirect effects of the predictor variables and host vulnerability, expecting that in a larger genus with widely distributed species, the species would increase their spatial overlap. Moreover, we expected genus size to have a positive effect on genus gall richness, corroborating the taxon size hypothesis (Fig. 1).

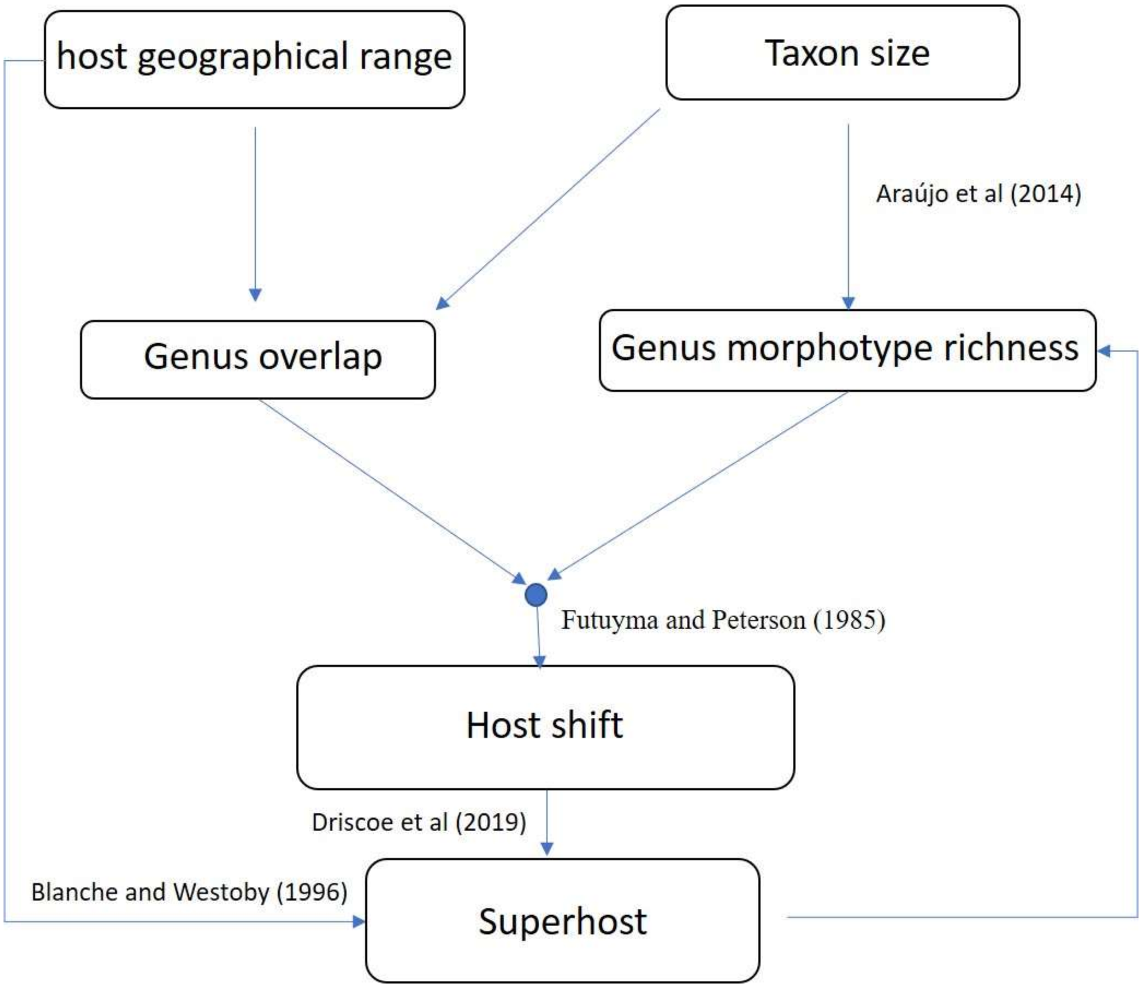

Figure 1 – Metamodel elaborated from the hypothesis used to guide the construction of the structural equation model.

Material and methods

Study area

We compiled data for trees and galling insects from several inventories in a Neotropical Savanna type (the Cerrado sensu strictu) (Fig. 2, A list of data sources used in the study are provided in the Data Source Appendix). The Cerrado Biome includes a wide variety of vegetation types such grassland, savanna and forest formations (see Fagundes et al., 2020a) and has the greatest plant species richness among the world's savannas (Klink and Machado 2005). However, it is highly threatened by deforestation and more than 50% of its area has been transformed to pasture, monoculture crops, and urban areas (Ratter, Ribeiro, & Bridgewater, 1997). Specifically, the Cerrado sensu strictu vegetation is the typical savanna environment, mainly composed of grasses and sclerophyllous trees of medium height (about 6 meters) and few or no canopy connections among trees. The climate has rainy summers (October to March) and dry winters (April to September) (Alvares et al., 2013). The precipitation ranges from 1088 to 2028 mm.year-1 and the mean annual temperature ranges from 19.3 to 26.5 ºC (Alvares et al., 2013). This vegetation type supports a range of deciduous and semideciduous trees (Damascos, Prado, & Ronquim, 2005) and the highest diversity of galling insects in the neotropics (Walter S Araújo, Fernandes, & Santos, 2019).

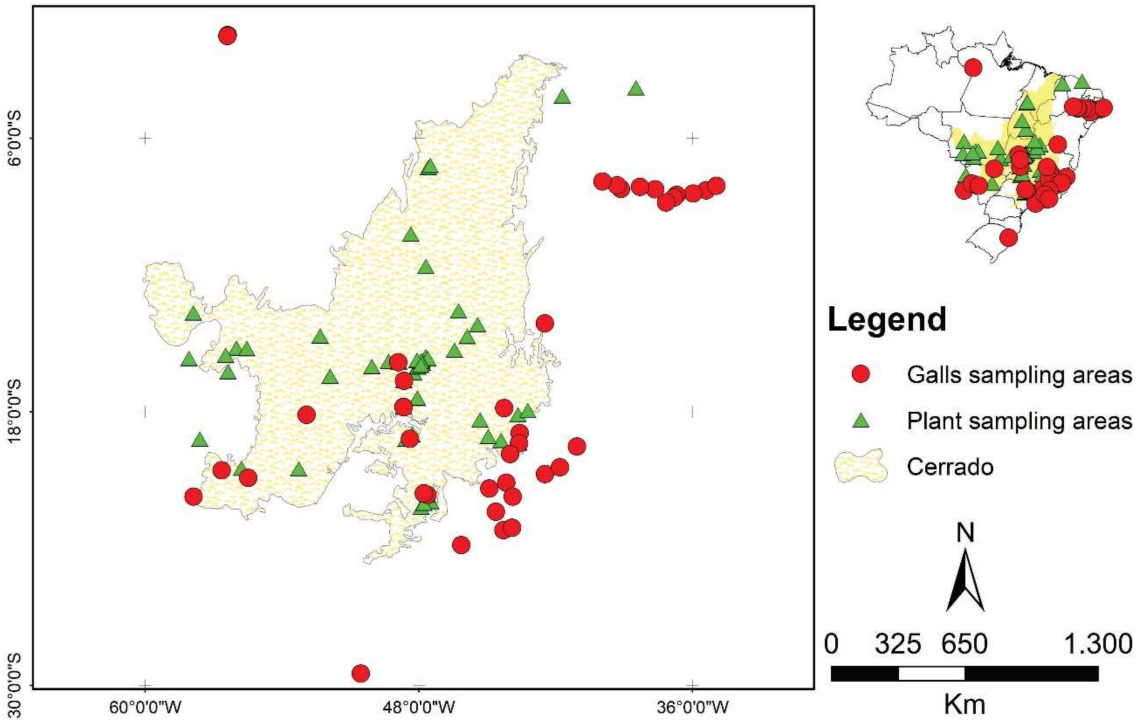

Figure 2 – Map with the limits of the Cerrado biome and the plant ( ) and gall ( ) inventories.

Tree data collection

To collect data about the the composition and distribution of the plant community in order to access data regarding taxon size, genus or species overlap, we compiled data from 56 plant inventories of the Cerrado sensu strictu (Appendix S1). We filtered tree inventories that had at least 1ha of sampled area and individuals with a diameter of more than 3 cm at ground level in order to create a standardized data set. The data were further filtered by species identification and only the tree species identified at the species level were maintained. Next, all plant synonyms were standardized according to the most recent taxonomic data reported in "Flora do Brazil" (http://floradobrasil.jbrj.gov.br/) and we obtained a database with tree species frequency (presence/absence) in each of the 57 inventories along the Cerrado biome.

Galling-insect data collection

To assess gall richness per host plant we compiled data from 28 published galling insect inventories (Appendix S2) collected from all habitats and filtered them to obtain only host species identified at the species level. As galling insects are lacking species identification, each unique morphotype within a single host species was considered a different galling insect species (Carneiro et al., 2009). In addition, we considered galling insect diversity associated only with tree host species, as they represent the most reliable resource for the galling insects (Askew, 1980; Marcilio Fagundes et al., 2020; Veldtman & McGeoch, 2003).

We performed the following sequential filtering of the dataset. First, all morphotype names were standardized according to a published illustrated guide (Isaias, Carneiro, Oliveira, & Santos, 2013). The morphotypes are highly conserved structures and all galling insect individuals from the same species create the same morphological structure in their hosts. We compared the photos available for each gall system in order to determine the diversity of galls in the host. When the photos were not clear enough to distinguish a morphotype as unique, only one of all the conflicting samples was selected to avoid superestimation of gall richness. In summary, galling species determination was based on three criteria: (1) host plant species; (2) morphological characteristics of the galls, morphotypes (e.g., host organ attacked, organ appearance and pubescence); and (3) gall photograph. We did not use color as a filtering factor because color varies widely during gall development or sun exposure (Bomfim, Cardoso, Rezende, Martini, & Oliveira, 2019).

Assessment of plant and galling insect variables

To assess the richness of galling insect species per host plant in the Cerrado, we combined the plant and gall data sets by adding the available morphotypes from gall inventories to all tree species of the tree inventories. Since the gall inventories only report species that are hosting galls, frequent tree species of the tree inventories had no information about the number of gall morphotypes. Therefore, in this study we considered that these species which were not reported in gall inventories were present during gall sampling but did not host any galling insect (e.g., Lafoensia pacari, Tocoyena formosa and Hancornia speciosa) (Martini et al., 2021). The Cerrado sensu stricto has short trees closely accessible to the observers, thus facilitating to search for galls. Moreover, it is the vegetation type with the largest amount of published data about galling insects in Brazil (Walter S Araújo et al., 2019).

To assess genus size, we compiled the number of tree species within each genus in the Cerrado using the "Flora do Brasil" platform (floradobrasil.jbrj.gov.br). A genus with more tree species has a greater probability of raising new hosts due to the phylogenetic relation between species of the same genus. To assess the genus gall richness we used the number of morphotypes associated with each tree species within the host genus for each host species. The genus gall richness represents the possible insects that can shift their hosts within the genus. To assess the host geographic range size (host frequency), we counted the number of different sites where the host species occurred (i.e., frequency) in all tree inventories. We assume that a higher host frequency can represent a wider host geographic distribution since the plant inventory sites were scattered all over the Cerrado biome (Fig. 1). Furthermore, to assess the geographic overlap between species in the same genus, we counted the number of shared plots between a specific species and others of the same genus. We added a value (1) to each congeneric species sharing the plot; for example, if there was a plot with 3 congeneric species, each species of the genus received a value of 2. The geographic overlap between congeneric species would be a proxy to the probability of the galling insect to find a possible new host.

Statistical analyses

To test all the hypotheses, we performed a piecewise Structure Equation Model (piecewise SEM) using generalized linear models (GLM), with the number of gall morphotypes per plant species (superhost) as the last endogenous response variable. Host frequency, genus size, genus gall richness, and genus overlap were included in the framework and acted as response and predictor variables. The hypotheses acted as a framework when designing and optimizing the piecewise SEM to fit the data (Fig. 1). Piecewise SEM was conducted with the R software (Team, 2017) using the "pSEM" function in the 'piecewiseSEM' package (Lefcheck, 2016). We tested for collinearity between variables using the Variance Inflation Factor (VIF) < 3 (Craney & Surles, 2002). To assess VIF, we used the VIF function in the "usdm" package of R (Naimi, 2015), which covered correlations of all parameters measured in the system and investigated in the piecewise SEM model.

We checked the residual of each structural model to find the best distribution needed to adjust our paths, and due to the exponential pattern with a discrete number, we used Poisson distribution when the number of gall morphotypes per plant species (superhost) and genus gall richness were the response variables. To create the generalized linear models we used the lm function of the "lme4" package (Bates, Maechler, Bolker, & Walker, 2015). In the 'piecewise SEM' we used the 'dSep' function for the command of directed separation, which provides information supported by statistical significance to improve the SEM model (Lauritzen, 1996). This function performs a model selection, accounting for missing or incomplete pathways between response and predictor variables for each general linear mixed effect model that makes up the piecewise SEM model. If the tested pathways were statistically significant ($p < 0.05$) and generated a lower Akaike information criterion (AIC score), they were included and the model was further optimized. We selected the models with the lowest AIC using all explanatory variables (Anderson & Burnham, 2004). The final optimized model was tested by chi-square statistics to evaluate its goodness-of-fit (Lefcheck, 2016; Shipley, 2009). If the chi-square was statistically nonsignificant ($p > 0.05$), the model had a good fit to the data.

In addition to the goodness-of-fit for the model as a whole, an R^2 value was calculated for each general linear model. The R^2 value is a measure of the variation in the data explained by a general linear mixed effects model for a particular pathway. Finally, in addition to the direction of the effects found between variables in the piecewise SEM model, a pathway table with estimate values for all direct effects was reported. The effect estimates were used to calculate and compare the strengths of direct and indirect effects between variables in the system. Indirect effects were described as a predictor variable having an effect on a response variable through a simultaneous response of another related predictor variable. We also highlighted the relationship using bivariate analyses in order to visualize the distribution of our samples and the relation between them.

Results

The compiled database of tree inventories resulted in 360 tree species belonging to 178 genera and 60 families (Appendix S3). The most frequent tree species was *Byrsonima coccolobifolia*, occurring in 95% of the plots, followed by *Qualea grandiflora* and *Qualea parviflora*, occurring in 91% and 86% of the plots, respectively. A total of 145 plant species were found only in one site. The genus with higher tree species richness was Eugenia with 34 species, followed by Myrcia and Miconia with 32 and 31 species, respectively. The data had 62 genera with only one tree species. All the genera with a number of species greater than 15 contained rare species with very restricted geographic distribution (frequency = 1).

We also compiled a total of 344 galling insect morphotypes associated with 147 host plant species. We found that 83 plant species hosted two or more galling insect species, 64 plant species hosted only one gall morphotype, and 213 plant species did not host any galling insect. The tree host species with the higher number of galling insect morphotypes was *Copaifera langsdorffii* with 13 morphotypes, followed by *Matayba guianensis* and *Bauhinia rufa* with nine and eight galling insect morphotypes, respectively. The piecewise structural equation model fitted the data (Chi-Squared = 1.33, p = 0.24) and our explanatory variables had direct and indirect effects on the number of gall morphotypes per plant species (i.e., superhost formation) (Fig. 3; Appendix S4).

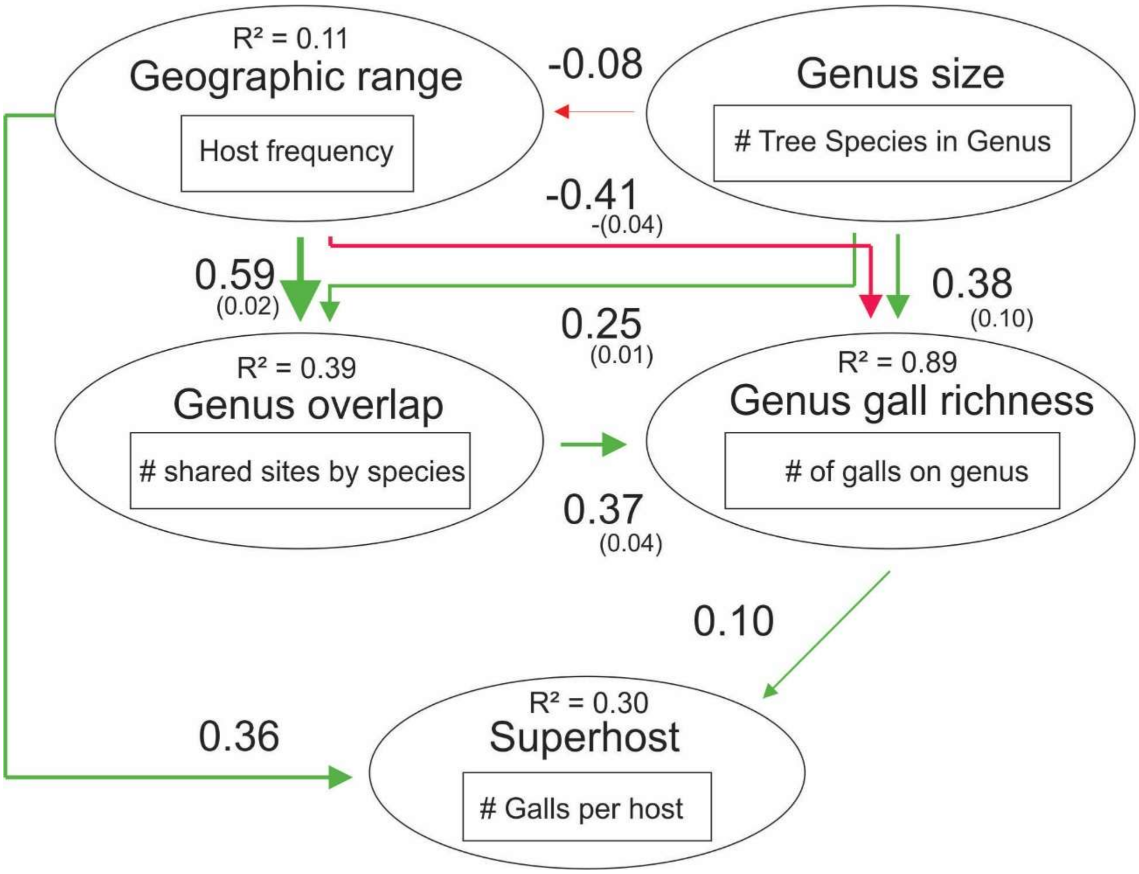

Figure 3 – Piecewise SEM models of host and plant community parameters as predictors of the total number of gall morphotypes per host (Superhosts) in the Cerrado. The arrows represent the unidirectional relationships among the parameters, which either directly or indirectly affect the superhost. Positive and negative paths ($p < 0.05$) are shown as green and red arrows, respectively, with the thickness of the paths

(arrows) scaled to the magnitude of the path strength. Path strengths are designated on the arrows and variables lacking R2 values acted only as predictors. Values in parentheses are the indirect strength effect on the superhost.

The genus gall richness was the most significant parameter, with a Standardized beta coefficient β = 0.10 (Std.e = 0.01) showing a positive direct effect on the number of gall morphotypes per plant species, and mediating the indirect effects of the other parameters ($r^2 = 0.89$). The host geographic range had a stronger positive direct effect on the superhost (β = 0.36; Std.e = 0.004) and also on the genus overlap (β = 0.59; Std.e = 0.04), and negative direct effect on the genus gall richness (β = - 0.41; Std.e = 0.006). The host frequency had an indirect effect on host vulnerability through genus overlap (β = 0.02) and genus gall richness (β = - 0.04). The genus size had positive direct effects on genus gall richness (β = 0.38; Std.e = 0.002) and on genus overlap (β = 0.25; Std.e = 0.05), with only indirect positive effects on the superhost (β = 0.03). The genus species overlap had positive effects on the genus gall richness (β = 0.30), resulting in an indirect effect on the superhost formation (β = 0.04). We found that, despite the effect of host frequency on the genus gall richness, most bivariate relations maintained the pattern described by the pSEM (Fig. 4)

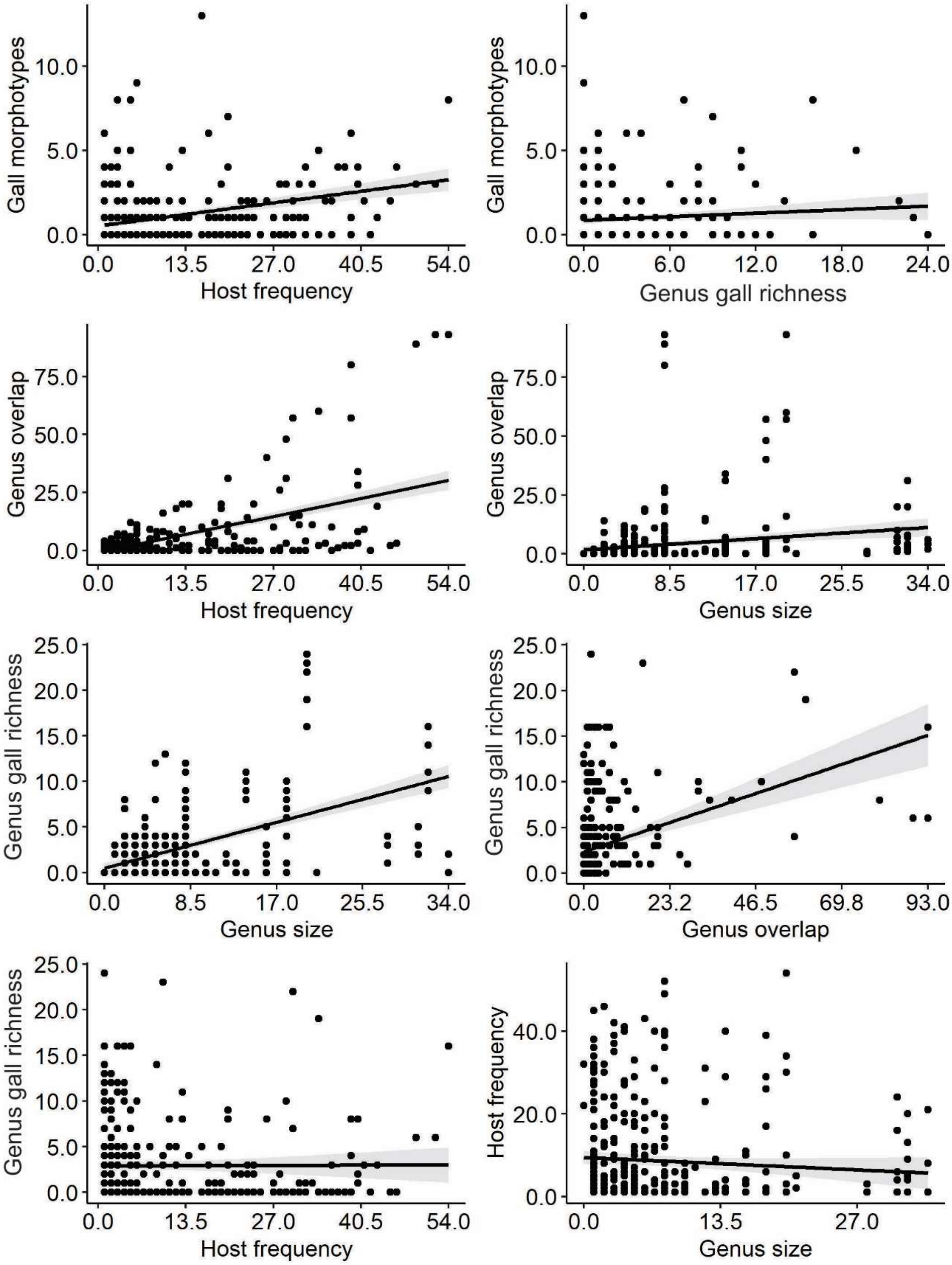

Figure 4 - Bivariate regressions representing each significant path of the piecewiseSEM. We used host plant frequency (Number of plots present), genus gall richness (Number of galls in the other species in the genus), and genus overlap (Total number of shared plots with each congeneric species) as response variables. The predictors were host frequency, genus gall richness, genus overlap, and genus size (Number of plant species). Note that these bivariate relationships are for illustration purposes only and may not necessarily provide the same results as in the piecewiseSEM.

Discussion

Our study is the first combining large scale data on plants and specialist herbivores to evaluate the biotic and geographic drivers of plant vulnerability to galling insects. Our results corroborated the geographic range size hypothesis that predicts increased vulnerability of the plants according to their wide geographic distribution (Blanche & Westoby, 1996). The taxon size hypothesis was partially corroborated since taxon size had both indirect positive and negative effects on plant vulnerability to hosting a rich fauna of galling insects. The genus size increased genus morphotype richness and genus species overlap, which are variables positively related to superhosts, but it also reduced host geographic distribution by diversification of specialized/endemic species, which create species that suffer very little herbivory pressure. The genus species overlap explained 37% of the variation in genus morphotype richness, spreading of morphotypes over the genus species, and it was driven by a positive effect of host frequency and taxon size.

Host frequency had both direct and indirect effects on host vulnerability. The direct positive effect on superhosts could be attributed to an ecological or evolutionary process. From an ecological viewpoint, spatial beta diversity (i.e., species dissimilarity among sites) could be invoked to explain the effect of geographic range size on host gall morphotype total richness. In fact, the composition of gall-inducing insects associated with a single plant species is more related to habitat than to host plant characteristics (Marcílio Fagundes et al., 2020; Ramos, Solar, Santos, & Fagundes, 2019). Thus, a superhost with larger geographic distribution that occurs in environments with different climate harbors different galling species per site, with wide regional diversity considering all different sites (Blanch and Westoby 1996; Araújo et al 2014). From an evolutionary perspective, the direct positive effect of geographic host range on host vulnerability may be related to variation in host plant characteristics such as leaf-sprouting phenology and chemistry. For example, it has been shown that variation in leaf-sprouting time within a plant population can play a relevant role in plant–herbivore interactions (Fagundes, 2014; Forister, 2005). These variations can act as a temporal separation for galling insects, affecting the time of site availability for gall induction or emergence, leading to locally adapted populations with low gene flow (Driscoe et al., 2019). Thus, the geographic range size impacts host phenology and drives within-host speciation (Joy & Crespi, 2007). In

Belonocnema treatae (Hymenoptera: Cynipidae) associated with different Quercus species, a regional speciation though time has been evidenced by molecular analyses (Driscoe et al. 2019).

Host distribution also has an indirect effect on superhosts by increasing genus overlap, with a later increase in genus morphotype richness. We hypothesized that genus overlap would have a positive direct effect on superhosts but we did not find such effect. In fact, genus overlap had a positive effect on genus morphotype richness as a booster of host shift between species within the same genus, which later had a positive effect on host vulnerability. When we tested whether a species in a genus with no galls would increase its number of gall morphotypes by increasing their overlap, we did not find significant relationships. This result strengthens the evolutionary perspective of a superhost formation and the role of host frequency itself, with the number of morphotypes in a species being intrinsically related to the incorporation of galls from distinct groups by colonization (Walter S Araújo, 2011). In this case it is possible that genus overlap would work as a variable driving the probability of galling insects to colonize congeneric species, creating an evenness of morphotype richness across the genus. The galling insects attacking the species closest to their host species is the best scenario for host shift, assuming that the physiology of the hosts is closely related (Futuyma & Peterson, 1985). The host shift on sympatric host species to the galling insect has already been found (Akimoto, 1990; Driscoe et al., 2019). In the Tetraneuraye zoensis galls, eggs from one host species were able to complete their life cycle on another host, but with a change in hatching time and a reduction of gene flow (Akimoto 1990). The maintenance of a plant community with a dominant genus or family could favor the establishment and diversification of the herbivore community.

Genus size had a negative effect on host frequency (geographic distribution), a pattern due to the fact that a larger genus has species with endemism and very limited geographic distribution, called rare species (Schwartz, 2008). We also found a negative direct effect of host frequency on genus morphotype richness as a response to the relation with taxon size. As the larger genus had more galls, but frequency/overlap also matter to the species shift between hosts, these rare tree species had no galls although they belong to a genus with many galls associated with it. Thus, this involves a negative relation not only between taxon size and frequency, but also between frequency and number of galls within the genus. For example, Byrsonima salzmanniana and Byrsonima cydoniifolia are two species in a large genus with 24 associated morphotypes, but both are very rare, and no galls were recorded on them. Although genus size had a positive direct effect on genus morphotypes richness, as already reported in the literature (Walter S Araújo, 2011), there was no direct effect of genus size on the superhost, again due to the rare species, that are from larger taxa but have no galling species associated with them. Since host frequency is a very strong factor driving the formation of superhosts, these rare plants could be suffering low pressures as they are not spread enough to be encountered by choice or luck by any galling insect.

We argued here that species frequency was the parameter with the greatest influence on host vulnerability, resulting in superhost formation. The ability to colonize different environments places the hosts under different abiotic and biotic pressures. In addition, the increased vulnerability of belonging to a genus with great morphotype richness supports the idea that host shift might be a significant driver of galling insect speciation and radiation. We need to point out that, despite all the interactions found here, the galling herbivores species still deserve more systematic identification and sampling. Patterns such as many species having no galls even in a genus with high morphotype richness may be related to a collection bias regarding areas where no one has yet collected or published data. We also found evidence that larger plant taxa have species with shorter geographic distribution but greater spatial overlap between them. Despite the nonsignificant relation between genus size and host vulnerability, our results corroborate the idea that larger taxa have more galls associated with them and have an indirect effect on the probability of superhost formation. Therefore, we conclude that host distribution and genus morphotype richness are the main factors that cause tree vulnerability to galling insects.

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Capítulo 3 - Gall protects the insect against acute temperature rise: testing the microenvironment hypothesis

Abstract

An extreme specialist guild of insects can manipulate the host plant tissues to its own benefit, inducing the development of galls. These galls provide adequate nutrition, protection against natural enemies and environment stressors such as high temperature (microenvironment hypothesis). However, studies testing the ability of the gall to protect the galling insect from temperature stress are very rare. Herein, we evaluated the potential of gall protection against heat-stress using artificial temperature modulation in order to test the microenvironment hypothesis. We collected 422 galling insects from galls induced on leaves of *Qualea parviflora* (Vochysiaceae) and used two treatments (galls with galling insects and galling insects outside the galls) to test if an increase in temperature of one degree Celsius, from 40 to 45 degrees for 10 minutes each, would reduce gall survival. We also tested if the galls can withstand cumulative stress by exposing them to each temperature for additional times, i.e, 20 and 30 minutes. Gall survival increased after a quick flash of 43 and 44 degrees, but was drastically reduced under longer exposures, with more than a 75% increase in mortality. In conclusion, we observed that the gall itself might be a good variable to explain gall adaption to an acute rise in temperature, and that the gall does not protect against long periods of exposure.

Keywords: Critical temperature "maxima"; Heat stress; Gall physiology; Plant-insect interaction

Introduction

Animals modify their immediate surroundings using wood, mud and holes to create domiciles providing shelter against unfavorable temperature and humidity levels. An example of these animals is an extreme specialist guild of insects that can manipulate the host plant tissues to their own benefit by inducing the development of galls. Galls have a huge range of shapes, associated structures and colors that provide an adaptive value to the galling organism (Fernandes and Price 1992). Different hypotheses have been raised about the adaptive value of gall morphologies for galling insect diversification and distribution. Among them, the microenvironment hypothesis can play an important role in harsh environments such as the Savannas, allowing the insect to colonize an environment that would be predator free (Fernandes and Price 1992). The galling insect creates the gall, a new microenvironment that is supposed to be thermoregulated and protected from dehydration using the host plant tissues. However, studies quantifying the ability of the gall to offer the galling insect protection from the temperature are very rare (Martinez 2009; Miller III et al. 2009), and they could help us to understand the role of the gall in providing shelter against abiotic pressures and the distribution of the galling organism. Here we used an experimental approach to evaluate if the gall offers protection to the galling insect against rising temperature.

The Neotropical Savanna, Cerrado "*sensu restrito*" is a vegetation type consisting of a mosaic of closed and open canopy during transitions between forest and savanna (Ribeiro and Walter 1998). Its range is continental and therefore plants are exposed to high variation in chronic temperature ranging from 10 to 35 degrees Celsius (Lehmann et al. 2014), or to acute variation by occurring in open canopies and no shade environments. Despite these variations, the Cerrado has a highly diverse fauna of galling insects (Fernandes et al. 2005) whose adaptation or acclimation to high temperatures occurs over different time scales and at different levels of plant organization (Larkindale et al. 2005). Plants use stomata for gas exchange and lose water molecules, cooling off through evapotranspiration (Sumayao et al. 1980; Monteiro et al. 2016). They also dissipate heat by fluorescence and biochemical reactions (violaxanthin cycle) in order to protect the photosynthetic apparatus (Bilger and Björkman 1991). However, during the day, leaves could reach a temperature even higher than air temperature by 2 degrees Celsius (Quiñones et al. 2019). The ability of leaf galls to dissipate heat is reduced. Galls originate from a redifferentiation of plant tissues, drastically reducing the intercellular space and stoma density (Oliveira et al. 2017; Martini et al. 2020). However, galls of *Andricus quercuscalifornicus* (Aphid) occurring on *Quercus lobata* were able to maintain a lower internal air temperature compared to the air temperature in the plant shade (Martinez 2009; Miller III et al. 2009), corroborating the microenvironment hypothesis.

Animals have evolved different strategies to avoid temperature damage. For example, diet changes can be an important mechanism for the thermal tolerance plasticity of ants since carbohydrates can be used to increase their thermal maxima (Bujan and Kaspari 2017). Carbohydrates improve the synthesis of sorbitol, which is used for protein stabilization in thermal stress (Salvucci, 2000). In addition, sugar can be used to quickly generate ATP (Suarez et al 1996), which is also highly requested during thermal stress (Sokolova 2013). In the galls, the insect increases the sugar content of the cells, as well as antioxidant compounds such as phenolics, photochemical apparatuses and the expression of heat-shock and cold proteins (Takei et al. 2017; Shih et al. 2018), which may help to maintain gall tissue integrity during temperature rise events. Furthermoe, the gall has an increased relative water content (Martini et al. 2020) as well as projections (Bomfim et al. 2019), both characteristics that could help the galls to delay acute luminous stress (Fidelis and Pivello 2011). Water content has been already explored regarding thermal tolerance in honey bees, with the detection of an interaction between these variables (Burdine and McCluney 2019). Despite these features of heat tolerance and resistance, the galls may have no defenses against a chronic temperature rise since they have no ability to move or ventilate their environment. The Cerrado is getting hotter (Hofmann et al. 2021) and the chronic temperature could lead to different scenarios, changing the adaptative value of the gall microenvironment.

Herein we evaluated the potential protection against heat stress using artificial temperature modulation to test the microenvironment hypothesis in galls on *Qualea parviflora*. We used different treatments to test if an increase in temperature of one degree Celsius from 40 to 45 degrees for 10 minutes each reduced gall survival. We additionally tested if the galls couls withstand cumulative stress using long periods of exposure to each temperature. We hypothesized that the gall would offer protection against acute stress and also that, due to the lack of dissipation systems in the larval chamber, the galls would have no effect over long periods of heat exposure.

Methods

Study area, and biological system

The study was conducted in the Neotropical “Cerrado” savanna at the Reserva Ecológica do Panga, in Uberlândia municipality, Brazil, during the during the end of the rainy season in February 2020 and 2021, when the gall and galling insect are well developed. The samples were taken in areas of Cerrado “*sensu stricto”* with discontinuous tree canopy and high luminosity. The region experiences a tropical savanna climate (Cwa), with rainy summers (October to March, mean temperature 23.4ºC and total rainfall 1,342 mm) and dry winters (April to September, mean temperature 20.7ºC and total rainfall 234 mm) (Alvares et al. 2013) and the predominant soil in the region is Latosol. We used in our tests 462 vigorous galls with good tissue expansion.

Qualea parviflora Mart. (Vochysiaceae) is a dominant small to medium-sized tree species found in the Cerrado, mainly with open canopies in grasslands. This plant species hosts galls induced by an unidentified species of Cecidomyiidae (Diptera) (Urso-Guimarães et al. 2003). The gall is globoid shaped (Isaias et al. 2013) with thick cortex and projections (**Fig. 1a**). The galls are resistant to high luminous stress, increasing anthocyanin in response to sunlight and maintaining antioxidant properties (Bomfim et al. 2019). The gall occurs on both abaxial and adaxial leaf surfaces and has a peak of abundance in the summer, when temperatures are higher. The larva is orange and very mobile (**Fig. 1b**) and, when leaving the gall, it actively looks for small pieces of gall to hide. We checked if the larva was alive or dead by poking the larval body with a needle under a magnifying glass, and checking for a reaction. The larva was considered dead if no movement or rigidity was observed after a few tries.

Figure 1. a) Globoid gall induced by a cecidomyiid in *Qualea parviflora*; b) The cecidomyiid insect (red arrow).

Heat treatments

All samples were harvested and submitted to a heat treatment within 30 min using a Digital dry bath (Kasvi K80-S01/02). To test the proposed hypothesis, we carried out two treatments. We compared the temperature and time needed to kill the galling insect outside and inside the gall. In the laboratory, the galls were detached from the leaves and half of them were opened to extract the insect. The insects were then placed inside a 5 ml tube containing a piece of gall cortex to maintain humidity. Next, both the insects and the whole gall were placed inside the bath already pre-heated to the designed temperature. Each group of galls and galling insects was submitted to only one treatment to avoid cumulative stress. We tested an increase in temperature of one degree Celsius from 40 to 45 degrees for 10 minutes each. In the second experiment, to evaluate the effectiveness of the gall's thermal buffer, we tested the galls with cumulative stress using each temperature for additional times of exposures of 20 and 30 minutes (T1 = 10 min, T2 = 20 min and T3 = 30 min). We used a minimum sample of 10 galls per group in all treatments. In each temperature treatment a new set of samples was used since we needed to open the gall to check whether the insect was alive or not. To account for the dead insects that might have been assigned to the gall treatment, we used open control galls (n=20), and all of them were alive. We collected galls at the peak of maturity and always selected "healthy" galls.

Statistical analysis

We used Binomial linear models with the alive or dead status of the galling insect as the response variable. We used the galls, the galling insect treatment, the temperature and their interaction as predictors. All models were performed in R 3.1.2 (Team 2017) using the glm function of the "lme4" package (Bates et al. 2015).

Results

We sampled 422 galls and insects divided into 44 groups of temperature, time of exposure and treatments (insect or insect inside the gall). We evaluated 139 samples exposed for 10 min to each temperature treatment, 68 insects and 71 insects inside the gall. We observed that the gall provided protection against spikes of temperature during heat exposure ($p<0.001$), with the average temperature needed to cause the death of more than half the individuals increasing from 43 to 45 degree Celsius (Figure 2; red lines; T1). For the treatment involving long-term heat exposure, we used 422 samples to test all temperatures and exposures, for a total of 208 and 214 insects inside the gall (Table 1).

We found that the gall did not provide isolation, as shown by the increased mortality in each treatment of time exposure, T1 (est = -1.52 $p < 0.001$), T2 (est = -3.18 $p < 0.001$) and T3 (est = -5.04 $p < 0.001$) (Figure 2; red, green and blue solid lines). Even at temperatures where all insects inside the gall survived the 10 min treatment, the galls started to die if exposed for 30 min. The galls increased insect survival for shorts periods of exposure to 43 and 44 degrees Celsius but showed a drastic reduction in this ability under longer exposures, with more than a 75% increase in mortality. We also found that the insects inside or outside the gall did not show a significant difference in survival at the exposures of 20 and 30 minutes ($p = 0.17$ and $p = 0.84$, respectively) (Figure 2; green and blue lines).

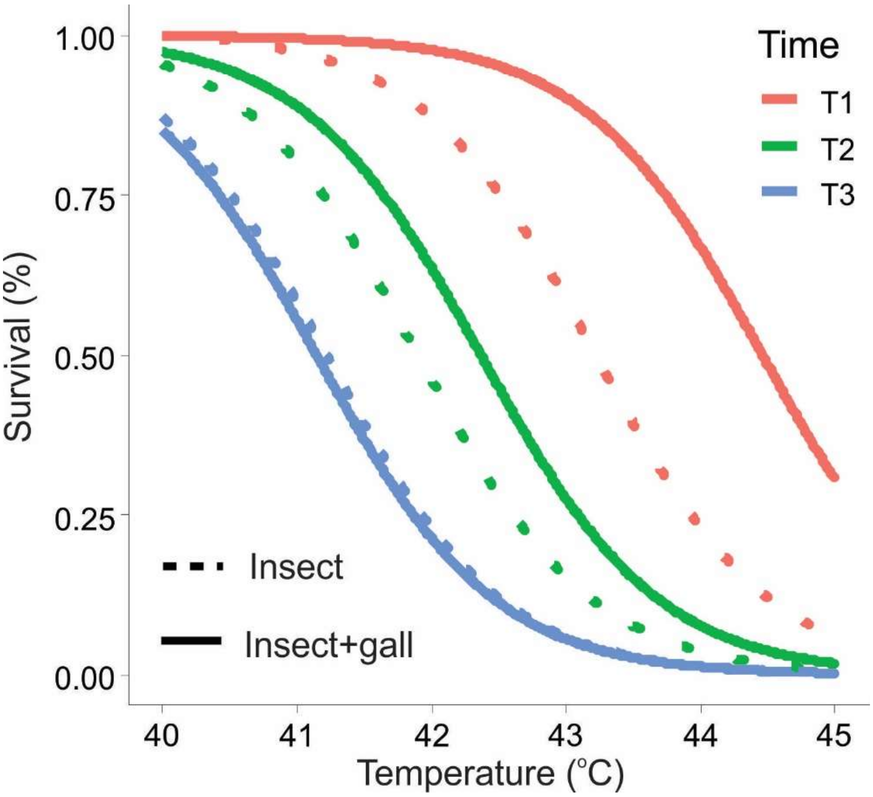

Figure 2 – Binomial generalized linear model (GLM) with the live status as the response variable, temperature exposure, heat exposure, and the insect alone or inside the gall as predictors. T1 = 10 min; T2 = 20min; T3 = 30 min of exposure to heat.

Discussion

We hypothesized that the gall would offer the insect protection against an acute temperature rise. We indeed found that half the insects inside the gall were able to survive a temperature increase of 1 or 2 degrees Celsius more than the insects alone when submitted to a 10 min stress. This reflects the capability of the gall to offer the insect shelter against a quick rise in temperature caused by sunlight passing during the day. Our data corroborate the microenvironment hypothesis that gall induction is, indeed, adaptive versus temperature. However, when submitted to a 30-minute heat stress, the galls had no effect, and both the insects inside or outside the gall started to die during the first treatment.

Plant tissue dissipates energy as heat via the violaxanthin cycle, leading leaves to have higher mean temperatures compared to the air around (Quiñones et al. 2019). Heat dissipation is commonly measured using a photochemical parameter such as Non-Photochemical Quenching (NPQ) (Genty et al. 1989), with the gall being evaluated in terms of tissue vitality (Oliveira et al. 2017; Martini et al. 2020). Thus, although the gall can dissipate heat, the gall tissues maintain the temperature of the leaves and ambient temperature during the day. However, the galls had a delay in reaching this temperature, which was lower inside compared to ambient dtemperature in the morning the temperature (Miller III et al. 2009). In general, plant tissues mainly consist of water and galls, in particular, drastically increase this water content (Oliveira et al 2017, Martini *et al.*, 2020). This feature can help the gall to delay the rise in interior temperature due to the specific heat of water. This, coupled with the induments reflecting the sunlight (Tschan and Denk 2012), and the ability to maintain tissue stability with an antioxidant (Bomfim et al. 2019), creates a window of opportunity for the insect colonize the open areas. In *Andricus quercuscalifornicus* (Hymenoptera) galls, the microclimate was mainly driven by achieving near-saturation levels of relative humidity inside gall tissues (Miller III et al. 2009). However, when the temperature rises for longer periods, this trait does not provide the same protection, with only the morning temperature of galls been colder than the external temperature (Miller III et al. 2009). The galls usually reduce stoma density (Haiden et al. 2012; Tong et al. 2016) with no intercellular space where water can evaporate and exchange heat with the environment, and transpiration by loss of water molecules cools off the plants (Sumayao et al. 1980; Monteiro et al. 2016). Nevertheless, galls of *Baizongia pistaciae* (Hemiptera: Pemphigidae) occurring in *Pistacia palaestina* were able to maintain a lower internal air temperature compared to the air temperature in the plant shade (Martinez 2009).

Analogous to gall metabolism (Isaias et al. 2015), heat stress results in the production of ROS and evokes oxidative stress responses (Potters et al. 2007). Therefore, a rising temperature can pressure the insect not only by the effects on the insect itself but by compromising the interaction between the insect and plant tissue, overloading plant metabolism. For example, flower development depends on ROS signaling (Muñoz and Munné-Bosch 2018), and heat stress induces an early development of tapetal cells (Parish et al. 2012). Higher plants exposed to excess heat exhibit a characteristic set of cellular and metabolic responses required for the plants to survive such as organization of cellular structures, including organelles, cytoskeleton, and membrane functions (Weis and Berry 1988), accompanied by a decrease in the synthesis of normal proteins and the accelerated transcription and translation of heat shock proteins (Bita and Gerats 2013). Changes in hormone regulation occur, such as an increase of abscisic acid (ABA), antioxidants and other protective molecules (Maestri et al. 2002), and a decrease of cytokinin (CK), auxin (AUX), and gibberellic acids (GAs) (Larkindale et al., 2005). Features that could compete with the gall metabolism, especially during the developmental stages, are increases in tissue auxins (Bedetti et al. 2014) and cytokinin concentrations (Mapes and Davies 2001). Moreover, secondary metabolites such as phenolics and anthocyanins are up-regulated during gall development (Takei et al. 2017; Shih et al. 2018), but are also significantly stimulated during heat stress, playing an antioxidative role (Wahid 2007).

In conclusion, we found that galls are an adaptation during an acute rise in temperature, but that long-term exposure surpasses the environmental buffers provided by them. We also highlight here that deforestation and global warming could have a drastic impact on the galling insect community, which are already manipulating plant tissue and dealing with ROS signaling and scavenging (Fernandes 1990; Fernandes et al. 2003; Isaias et al. 2015). The adaptive value of the process of creating a gall to feed vs a free living habitat (Inbar et al. 2004) could be weakened in a scenario of chronic temperature increase; however, we still need studies focusing on the evaluation of the variables that affect the ability of galls to offer buffers against the temperature

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CONSIDERAÇÕES FINAIS

Com os três capítulos, nós renovamos a discussão sobre como a população de instes interage com a comunidade vegetal, e apresentamos a importante participação da comunidade vegetal vizinha ao hospedeiro, na interação inseto-hospedeiro. Também destacamos a importância de se observar o efeito do inseto sobre o desenvolvimento da planta e seu fitness. Assim como as mudanças na comunidade vegetal pode ser um efeito positivo ou negativo para a população, também mostramos aqui pela primeira vez usando a ótica da planta hospedeira, como a sua distribuição e o grupo taxonômico podem ser características que levam a vulnerabilidade a novos ataques de insetos galhadores. E também gerando evidências, das possíveis principais vias de especiação para os insetos, seja ela dentro do mesmo hospedeiro, ou favorecida pela troca de hospedeiros durante um eventual encontro espacial ou fenológico entre as espécies vegetais.

Das hipóteses que testei nos três capítulos, no primeiro capítulo corroborei com a concentração de recuso, e encontrei uma relação positiva entre a área basal da planta hospedeira na parcela e o número de galhas por indivíduo de planta hospedeira. Corroborei com a resistência associada a planta hospedeira proveniente da comunidade vegetal vizinha, onde a área basal e riqueza de espécies próximas a planta hospedeira diminuía a infestação de galha por planta hospedeira na parcela. E também encontrei que a infestação de galhas diminuí a probabilidade da planta hospedeira de estressas caracteres reprodutivos. Colocando assim o inseto galhador como um importante direcionador de produtividade da comunidade vegetal, e também que a própria comunidade vegetal através da distribuição das espécies se ajusta a essa pressão de praga. Nesse contexto, acho que estudos direcionados para avaliar de maneira quantitativa essa perca na capacidade reprodutiva e viabilidade das sementes podem preencher áreas do conhecimento ainda inexploradas.

No segundo capítulo corroborei com a hipótese de que a distribuição geográfica da planta hospedeira colabora para uma maior diversidade de insetos galhadores que induzem galha nela. Encontrei evidências pela primeira vez que as plantas em gêneros hospedeiros maiores também abrigam maior número de galhadores por espécie e por gênero. E que as plantas desses gêneros hospedeiros são mais susceptíveis a sofrer ataque de novos galhadores se tiverem uma maior sobreposição espacial entre elas. Apesar disso, existem gêneros que apesar de apresentarem grande distribuição geográfica e algumas espécies compondo o gênero, ela pode não apresentar galhadores descritos até o momento da coleta de dados. O que destaca a relevância de características taxonômicas e evolutivas da interação planta-inseto. Assim, propus que a distribuição da planta, associada ao número de espécies filogeneticamente próximas são importantes direcionadores da vulnerabilidade de uma planta hospedeira aos ataques de insetos galhadores. Entretanto, estudos de levantamento sistemático dos insetos galhadores, bem como a identificação do inseto são urgentes para melhor a compreensão de padrões de diversificação de insetos galhadores.

No terceiro capítulo, testei a eficiência da galha oferecer uma proteção térmica para o inseto galhador, e corroborei a hipótese onde a galha oferece somente uma proteção contra um aumento de temperatura por um curto período de tempo. Mostrei que em aumentos de temperatura por um tempo de dez minutos são amortecidos pelo tecido da galha, que protegem o inseto galhador, aumentando sobrevivência dele em cenários de aumentos até 1 grau. Apesar disso, se a temperatura permanece alta por mais de 10 minutos a galha perde seu efeito protetor. Os testes foram feitos levando em consideração uma galha de ambiente de savana que acontece em ambas as faces da folha, e é o primeiro estudo a testar quantitativamente essa proteção. Mais estudos nessa área podem ajudar a compreender qual é a vantagem evolutiva contra diferentes estresses abióticos, inclusive relacionando mudanças no formato e tamanho da galha com a mesma variável abiótica.