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CONSERVAÇÃO E BIODIVERSIDADE



GALHAS DE INSETOS: UM MICRO-LABORATÓRIO DE ESTUDOS DE
INTERAÇÕES MULTRÓFICAS E NÃO-TRÓFICAS

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**GALHAS DE INSETOS: UM MICRO-LABORATÓRIO DE ESTUDOS DE INTERAÇÕES
MULTRÓFICAS E NÃO-TRÓFICAS**

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Orientador

Prof. Dr. Denis Coelho de Oliveira

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Resistimos.

RESUMO

Os insetos galhadores são uma das guildas mais especializadas, se alimentando de órgãos vegetais neo-formados e complexos, metabolicamente dependentes de seus estímulos alimentares químicos e/ou mecânicos contínuos. Assim, qualquer mudança no comportamento do galhador por efeito de inimigos naturais pode refletir na configuração anatômica e química da galha. Enquanto alguns insetos utilizam órgãos vegetais maduros para formação de seus microhabitats, os insetos galhadores dependem de sítios reativos à oviposição. A disponibilidade de sítios nas plantas para a indução de galhas, todavia, depende da fenologia da planta hospedeira e, portanto, é necessária uma sincronização das fenofases do inseto galhador para compatibilidade entre surgimento de sítios vegetais reativos e oviposição pelos organismos galhadores. Portanto, todos os organismos que interagem direta ou indiretamente com cada inseto galhador, após o desenvolvimento de suas galhas, dependem desta sincronia de fenofases, e podem também adaptar suas histórias de vida para interação com as galhas em determinados estágios. As galhas oferecem aos inimigos naturais dos insetos galhadores recursos alimentares, funcionando também como microhabitats para uma diversidade de artrópodes que podem escapar de condições abióticas adversas usando a estrutura da galha. Alguns insetos galhadores estabelecem também interações facilitadoras não-tróficas nos ecossistemas em que ocorrem através da disponibilização destes microhabitats após a emergência. As galhas induzidas em locais de gemas axilares, por exemplo, podem permanecer por longos períodos, até anos, nas plantas hospedeiras. Portanto, os táxons de insetos galhadores e o órgão onde as galhas são induzidas determinam suas características histológicas e, como resultado, o tempo de permanência destes microhabitats nas plantas após senescência. Nesta Tese investigamos galhas como microhabitat em diferentes contextos de interações tróficas e não tróficas. Galhas induzidas por Cecidomyiidae em *Piptocarpha macropoda*

(DC.) Baker (Asteraceae) respondem as vespas parasitóides *Platygaster* sp. e, uma vez que um indivíduo de Cecidomyiidae suporta muitos parasitóides, hipotetizamos que um consumo intenso de nutrientes pelo inseto galhador parasitado afetaria o tamanho da galha, o número de células em cada compartimento dos tecidos da galha, bem como o perfil histoquímico. Em um sistema multitrófico estabelecido em *Macairea radula* (Melastomataceae) (envolvendo as galhas induzidas por *Palaeomystella oligophaga* (Lepidoptera: Momphidae) e seus inimigos naturais diretos e indiretos) analisamos como esse sistema é influenciado por condições bióticas. E por fim, também em galhas de *M. radula*-*P. oligophaga* avaliamos porque estas galhas permanecem aderidas à planta hospedeira mesmo após a emergência do inseto galhador, e ainda quais artrópodes oportunistas usam a estrutura destas galhas como micro-habitat.

Palavras-chave: Interações de artrópodes e plantas, histologia vegetal, insetos construtores de abrigo, endoparasitóides, engenharia de ecossistemas, galhas de gema.

ABSTRACT

Galling insects are one of the most specialized guilds, feeding on neo-formed and complex plant organs, metabolically dependent on their continuous chemical and/or mechanical food stimuli. Thus, any change in the behavior of the gall due to the effect of natural enemies may reflect on the anatomical and chemical configuration of the gall. While some insects use mature plant organs to form their microhabitats, galling insects depend on reactive sites for oviposition. The availability of sites in plants for the induction of galls, however, depends on the phenology of the host plant and, therefore, a synchronization of the phenophases of the galling insect is necessary for compatibility between the emergence of reactive plant sites and oviposition by the galling organisms. Therefore, all organisms that interact directly or indirectly with each galling insect, after the development of their galls, depend on this synchrony of phenophases, and can also adapt their life histories to interact with the galls at certain stages. The galls provide the natural enemies of galling insects with food resources, also functioning as microhabitats for a variety of arthropods that can escape adverse abiotic conditions using the gall structure. Some galling insects also establish non-trophic facilitating interactions in the ecosystems in which they occur through the availability of these microhabitats after emergence. Galls induced at axillary bud sites, for example, can remain for long periods, even years, on host plants. Therefore, the taxa of galling insects and the organ where galls are induced determine their histological characteristics and, as a result, the permanency of these microhabitats in plants after senescence. In this Thesis we investigate galls as microhabitat in different contexts of trophic and non-trophic interactions. Galls induced by Cecidomyiidae on *Piptocarpha macropoda* (DC.) Baker (Asteraceae) responds to parasitoid wasps *Platygaster* sp. and, since an individual of Cecidomyiidae supports many parasitoids, we hypothesized that an intense consumption of nutrients by the

parasitized galling insect would affect the gall size, the number of cells in each compartment of gall tissues, as well as the histochemical profile. In a multitrophic system established in *Macairea radula* (Melastomataceae) (involving the galls induced by *Palaeomystella oligophaga* (Lepidoptera: Momphidae) and its direct and indirect natural enemies) we analyzed how this system is influenced by biotic conditions. And finally, also in galls of *M. radula*-*P. oligophaga*, we evaluated why these galls remain attached to the host plant even after the emergence of the galling insect, and also which opportunistic arthropods use the structure of these galls as a micro-habitat.

Keywords: Arthropod-plant interactions, plant histology, shelter-building insects, endoparasitoids, ecosystem engineering, bud galls.

INTRODUÇÃO

Os artrópodes se adaptaram aos mais diversos ambientes no planeta com diferentes histórias de vida, algumas peculiares como as de insetos capazes de construir abrigos em órgãos vegetais, onde passam parte de seu ciclo de vida, principalmente durante o estágio larval quando estão sujeitos à dessecação ou mais suscetíveis à predação (Lill & Marquis 2007, Cornelissen et al. 2016). A construção de abrigos utilizando folhas e fragmentos vegetais é realizada por insetos de diferentes ordens, onde se abrigam nos períodos em que não estão forrageando ou, como no caso de algumas larvas de lepidópteras, se alimentando de fragmentos vegetais destas estruturas (Fukui 2001, Lill & Marquis 2007). Entretanto, alguns grupos de insetos são capazes de manipular os tecidos vegetais e constituir novos órgãos na planta hospedeira com a qual interagem, as galhas (Oliveira et al. 2016). A formação de galhas se dá pelo controle e redirecionamento do crescimento, da diferenciação e alterações fisiológicas nos órgãos da planta hospedeira (Mani 1964; Price, Fernandes & Waring 1987, Oliveira et al., 2016) culminando em diferentes impactos ecológicos (Harris & Pitzschke 2020).

A princípio o hábito galhador surgiu de forma independente e muitas vezes nos insetos. Segundo Price (1987), o hábito galhador surgiu a partir de um ancestral que se alimentava de fungos e não exatamente de tecidos vegetais, como os grupos basais de Cecidomyiidae (Diptera) que seriam micetófagos. Em uma hipótese mais completa para decifrar o caminho evolucionário dos insetos galhadores foi proposto que o hábito evoluiu a partir de minadores, detritívoros, sugadores de floema, comedores de grãos de pólen, micetófagos e até parasitoides (Roskam 2005). O caminho dos parasitoides é fortemente suportado por análises filogenéticas dentro dos Cynipidae (Ronquist et al. 2015). Certamente, as galhas são uma vantagem adaptativa dos insetos galhadores em relação aos seus ancestrais de vida livre e, estas vantagens adaptativas são discutidas com base em três principais hipóteses: nutricional, do microambiente ou microclimática, e do inimigo (Price et al. 1987, Stone & Schönrogge 2003, Lill & Marquis 2003, Price 2005). Estas hipóteses não são mutuamente exclusivas e têm sido alvo de inúmeras investigações sobre microhabitats a pelo menos 20 anos.

Segundo a hipótese nutricional, construtores de abrigo, como insetos galhadores, podem ter acesso a recursos alimentares em quantidade e qualidade superiores aos insetos de vida livre que forrageiam, além de poderem evitar ou tolerar grandes defesas físicas e químicas das plantas (Connor & Taverner 1997, Fukui 2001, Stone & Schönrogge 2003;

Lill & Marquis 2007, Giron et al. 2016). A hipótese do microambiente ou do microclima está fundamentada na possibilidade dos microhabitats promoverem condições de umidade e temperatura mais estáveis em relação ao ambiente externo, fornecendo aos insetos proteção em condições ambientais adversas como temperaturas extremas, baixa umidade, radiação solar e exposição a vento/chuva (Price et al. 1987, Fernandes & Price 1992, Connor & Taverner 1997, Fukui et al. 2001). Assim, considerando que a dessecação é uma pressão seletiva para grande parte dos insetos, principalmente em estágio larval, a menor incidência de raios UV e a baixa exposição ao vento, podem justificar fortemente um fator seletivo para adaptação como construtor de abrigo (Larsson et al. 1997, Lill et al. 2007). A hipótese do inimigo prediz que a alimentação endofítica e oculta em abrigos de plantas confere proteção contra inimigos naturais como predadores, parasitóides e patógenos (Price et al. 1987). Porém, diversos inimigos naturais especializam-se em consumir insetos ou tecidos vegetais destes abrigos (Stone & Schönrogge 2003, Bailey et al. 2009).

Insetos galhadores são alvo de inimigos diretos como predadores e parasitóides ou indiretos como cecidófagos e kleptoparasitas (Luz & Júnior 2019) e, em contrapartida, estes abrigos podem oferecer resistência aos organismos que os atacam. Galhas possuem características secundárias desde parênquimas espessos e rígidos, colorações conspícuas, tricomas tectores e glandulares, até projeções superficiais. Estes traços podem ser respostas às pressões impostas pelos ataques de inimigos generalistas, e/ou de processos coevolutivos com adaptações de inimigos naturais especialistas (Price et al. 1987, Stone & Schönrogge 2003, Hayward & Stone 2005, Inbar et al. 2010). Herbívoros generalistas que invistam em driblar estas características “de defesa” podem obter vantagem ao preda galhas, por seus tecidos nutritivos, consumindo em uma menor porção de tecidos maior concentração de nutrientes em vez de forragear outros órgãos vegetais em maior quantidade (Stone & Schönrogge 2003, Lill & Marquis 2007, Cornelissen et al. 2016).

De fato, os insetos galhadores são uma das guildas mais especializadas (Shorthouse et al. 2005), se alimentando de órgãos vegetais neo-formados (galhas) e complexos, metabolicamente dependentes de seus estímulos alimentares químicos e/ou mecânicos contínuos (Mani 1964, Bronner 1992, Rezende et al. 2019). O desenvolvimento de tecidos específicos de armazenamento, com acúmulo de substâncias nutritivas nas galhas, depende dos comportamentos alimentares dos galhadores (Bedetti et al. 2013, Ferreira et al. 2017, Rezende et al. 2019). Assim, qualquer mudança no

comportamento do galhador (como a interrupção de suas atividades alimentares por paralização corporal sob ataque de parasitóides) pode refletir na configuração anatômica e química da galha (Bedetti et al. 2013, Fujii et al. 2014, Rezende et al. 2019). Embora o controle de hospedeiros por parasitóides seja relatado com mais frequência em interações com hospedeiros de vida livre (Weinersmith 2019), é possível que a manipulação comportamental também ocorra em interações parasitóides-insetos galhadores, e que isso possa refletir em alterações na histologia da galha. Por isso, uma possibilidade de diagnosticar manipulações comportamentais ou impacto do parasitoidismo em insetos galhadores é analisar possíveis alterações nos tecidos e na morfologia das galhas. Um exemplo são as galhas induzidas em *Piptocarpha macropoda* (DC.) Baker (Asteraceae) por Cecidomyiidae (Diptera), que manifestam alterações morfológicas e anatômicas quando os insetos galhadores são endoparasitados por vespas *Platygaster* sp. (Hymenoptera: Platygastriidae). Estas vespas endoparasitóides não paralisam as larvas de Cecidomyiidae que seguem com suas atividades alimentares, e estas galhas aumentam em tamanho e número de camadas de tecido nutritivo comparadas a galhas sem interações com *Platygaster* sp. (Rezende et al. 2022, *aceito para publicação*).

Enquanto alguns insetos utilizam órgãos vegetais maduros para formação de seus microhabitats, os insetos galhadores dependem de sítios reativos à oviposição –com tecidos responsivos aos estímulos, ovos e larvas dos insetos – para estruturação do novo órgão, a galha (Weis et al. 1988, Campos et al. 2010, Castro et al. 2013, Oliveira et al. 2013). A disponibilidade de sítios nas plantas para a indução de galhas, todavia, depende da fenologia da planta hospedeira e, portanto, é necessária uma sincronização das fenofases do inseto galhador para compatibilidade entre surgimento de sítios vegetais reativos e oviposição pelos organismos galhadores (Yukawa 2000, Oliveira et al. 2013, 2016). O comportamento fenológico de plantas hospedeiras pode variar e está relacionado, por exemplo, à disponibilidade de água em seu habitat (Lemos-Filho & Mendonça-filho 2000, Oliveira et al. 2013), algo que em alguns Biomas, como o Cerrado, pode ser uma questão adaptativa importante. Portanto, todos os organismos que interagem direta ou indiretamente com cada inseto galhador, após o desenvolvimento de suas galhas, dependem desta sincronia de fenofases, e podem também adaptar suas histórias de vida para interação com as galhas em determinados estágios e, desta forma, inúmeras cadeias e teias alimentares são estabelecidas a partir das galhas (Hayward & Stone 2005, Forbes et al. 2016, López-Núñez et al. 2019, Weinersmith et al. 2020).

As vantagens das galhas para os inimigos naturais diretos ou indiretos dos insetos galhadores extrapolam os recursos alimentares, funcionando também para estes como microhabitats durante parte dos ciclos de vida (Sanver & Hawkins 2000). Então, organismos em interações tróficas com galhas, e que passam parte de seu ciclo de vida dentro da estrutura, incluindo a maturação de pupa, podem driblar condições abióticas desfavoráveis, evitar a dessecação, exposição à radiação solar, e até mesmo eventos estocásticos como incêndios, comuns nos ambientes de Cerrado (Gonçalves et al. 2021, Rodrigues et al. 2021). Insetos galhadores e a estrutura da galha podem oferecer, a uma diversidade de artrópodes, escape de condições abióticas adversas. Estes insetos são engenheiros de ecossistema alogênicos – organismos capazes de criar ou modificar habitats que afetam direta ou indiretamente a disponibilidade de recursos de abrigo para outras espécies (Jones et al. 1994, 2010) – fornecendo microhabitats dentro das comunidades que ocupam, ainda durante o ciclo de vida nas galhas.

Alguns insetos galhadores estabelecem também interações facilitadoras não-tróficas nos ecossistemas em que ocorrem através da disponibilização de seus microhabitats após a emergência. As galhas senescentes, por exemplo, podem ser utilizadas por artrópodes de diversas classes, como aranhas, formigas e besouros (Cornelissen et al. 2016). Os insetos galhadores investigados como engenheiros de ecossistema podem ser divididos principalmente em dois grupos: aqueles que fazem galhas em regiões das folhas (como nervuras centrais) que promovem a dobradura desses órgãos, formando uma cavidade que funciona como abrigo para artrópodes de diferentes classes (Novais et al. 2020, Pereira et al. 2021); ou galhas que são induzidas em caules ou gemas caulinares (Almeida et al. 2014, Wetzel et al. 2016, Giannetti et al. 2019, Weinersmith et al. 2020), que após a senescência permanecem vazias nas plantas hospedeiras, também formando abrigos. Em comparação, galhas dos caules podem oferecer abrigo por mais tempo do que as galhas das folhas, considerando que as plantas geralmente não desprendem os caules como fazem com as folhas na senescência (e.g. Lenza & Klink 2006, Pfeffer et al. 2018, Costa et al. 2022). Neste contexto, galhas formadas em gemas caulinares são potenciais abrigos resistentes. As galhas induzidas em locais de brotação, podem desenvolver-se morfológicamente mais complexas devido ao maior potencial meristemático de seus tecidos em comparação com galhas induzidas em outros órgãos da planta hospedeira (Weis 1988), ou até desenvolver-se semelhante a um órgão da planta, como um ramo por exemplo, e maximizar características do órgão não-

galhado. Assim, é possível que galhas induzidas em gemas caulinares possam permanecer por longos períodos, até anos, nas plantas hospedeiras

Portanto, os táxons de insetos galhadores e o órgão onde as galhas são induzidas determinam suas características histológicas (Weis 1998, Ferreira et al. 2019) e, como resultado, o tempo de permanência destes microhabitats nas plantas após senescência. As interações facilitadoras não-tróficas, como a engenharia de ecossistemas, podem desempenhar papéis ecológicos e evolutivos relevantes nas comunidades onde ocorrem (Vieira & Romero 2013, Sanders et al. 2014, Romero et al. 2015; Cornelissen et al. 2016). Uma possibilidade é o aumento da abundância e da riqueza de espécies de herbívoros e detritívoros nos habitats modificados como resultado de efeitos “bottom-up”, pelo aumento de locais de oviposição e refúgios e de recursos alimentares de melhor qualidade promovidos microhabitats (Martinsen et al. 2000; Bailey & Whitham 2003; Ohgushi 2005, 2008; Uesugi et al. 2016).

Nesta Tese investigamos galhas como microhabitat em diferentes contextos de interações tróficas e não tróficas. Galhas induzidas por Cecidomyiidae em *Piptocarpha macropoda* (DC.) Baker (Asteraceae) respondem as vespas parasitóides *Platygaster* sp. e, uma vez que um indivíduo de Cecidomyiidae suporta muitos parasitóides, hipotetizamos que um consumo intenso de nutrientes pelo inseto galhador parasitado afetaria o tamanho da galha, o número de células em cada compartimento dos tecidos da galha, bem como o perfil histoquímico. Em um sistema multitrófico estabelecido em *Macairea radula* (Melastomataceae) (envolvendo as galhas induzidas por *Palaeomystella oligophaga* (Lepidoptera: Momphidae) e seus inimigos naturais diretos e indiretos) analisamos como esse sistema é influenciado por condições bióticas. Nossa hipótese é que (i) a ocorrência de galhas deve ser maior durante o período seco, quando artrópodes podem depender mais de microhabitats durante a fase larval, (ii) *P. oligophaga* pode enfrentar diferentes tipos e frequências de inimigos durante os ciclos do ano, e que (iii) eventos estocásticos como o fogo podem reduzir a ocorrência de inimigos naturais e assim aumentar a frequência de insetos galhadores. Também, galhas de *M. radula*-*P. oligophaga* são induzidas nas gemas axilares e, em campo, observamos que essas galhas permanecem aderidas à planta hospedeira mesmo após a emergência do inseto galhador, o que nos levou a investigar (i) se essas galhas induzidas nas gemas se desenvolvem como ramos-caule modificados, com características histológicas que favorecem sua fixação às plantas hospedeiras após a senescência; e (ii) se essas galhas funcionam como

microhabitats secundariamente ocupados por uma diversidade de artrópodes oportunistas em diferentes populações de plantas hospedeiras.

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CAPÍTULO 1

**Endoparasitoidism reflections on the structure of a cecidomyiid-gall on
Piptocarpha macropoda (DC.) Baker (Asteraceae)¹**

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**Endoparasitoidism reflections on the structure of a cecidomyiid-gall on
Piptocarpha macropoda (DC.) Baker (Asteraceae)**

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Abstract

Piptocarpha macropoda (DC.) Baker (Asteraceae) hosts globoid galls induced by cecidomyiid, which may occur as clusters or as solitary stem galls. The cecidomyiid in clustered galls is attacked by a *Platygaster* sp. (Hymenoptera: Platygastriidae), a polyembryonic endoparasitoid. As each individual cecidomyiid supports many parasitoids, we suppose that high consumption of nutrients by the parasitized galling insect will impact gall structural and histochemical profiles. The parasitized and unparasitized galls were morphologically and histochemically evaluated and, in addition, the bodies of parasitized and unparasitized galling larvae were measured. The main consequence of the presence of the endoparasitoids over gall structure is the increase in gall size and nutritive cell layers. Despite the difference in size between galls with and without the endoparasitoids, the parasitized and unparasitized cecidomyiid larvae had similar sizes. This is evidence of a positive impact of the *Platygaster* sp. feeding demand over gall growth, but of a null impact on the cecidomyiid body. This scenario indicates a hyperstimulation on plant cells and tissues by the parasitized cecidomyiid.

Keywords: gall morphology; gall histochemistry; nutritive tissues; polyembryonic parasitoids

18 INTRODUCTION

19 Galls are a new plant organ developed by chemical and/or mechanical stimuli from a
 20 specialized organism, especially insects (Mani 1964). Thus, galling insects manipulate host
 21 plant tissues to develop a microhabitat, the gall, where they remain for part of their life cycle
 22 (Stone and Schönrogge, 2003; Price, 2005; Shorthouse *et al.*, 2005; Oliveira *et al.*, 2016).
 23 Due to their sessile feature, galls are target for the attack of a diversity of natural enemies,
 24 within which the parasitoids stand out as the most frequent (Hanson and Nishida 2014; Mete
 25 and Mergen 2017). The parasitoid species vary regarding the effects they cause on their host's
 26 metabolism and behavior, such as idiobionts or koinobionts (Godfray 2001; Libersat *et al.*,
 27 2009). Among the parasitoid wasps' species, some oviposit on the host body surface and their
 28 larva feeds from the outside as an ectoparasitoid, while others deposit their egg inside the
 29 host insect body and the larva feeds from the inside, as an endoparasitoid (Godfray 2001;
 30 Mayhew 2016). Endoparasitoids can be gregarious (more than one egg is deposited in the
 31 host, and several larvae develop), solitary (one egg deposited in the host, and a single larva
 32 develops), or polyembryonic (one single egg is deposited, and multiple larvae develop)
 33 (Godfray 1994). Endoparasitoids feeding activity may not kill the galling larvae, which
 34 consequently keep gall metabolism operative, but produce subtle alterations that may change
 35 the gall structural profile.

36 The development and morphology of galls depend on the feeding behavior of a
 37 phenotype manipulator, the galling organism, that changes the host plant tissue into own
 38 benefit (Oliveira *et al.* 2016). Thus, any changes in the galling behavior may reflect on the
 39 gall structure. Although host manipulations have been most frequently reported for
 40 parasitoids that attack free-living hosts (e.g. Tanaka and Ohsaki 2006; Libersat *et al.* 2009),
 41 it is possible that behavioral manipulation also occurs in galling insect-parasitoid
 42 interactions, changing the gall morphology providing a possible adaptive significance for the
 43 parasitoid. One specific case was observed in galls induced by *Masakimya pustulae* Yukawa
 44 and Sunose, 1976 (Diptera: Cecidomyiidae) on *Euonymus japonicus* Thunb. and *E. fortunei*
 45 Hand-Mazz (Celastraceae), where the thickness of the gall cortex increases when the galling
 46 insect is parasitized by *Platygaster* sp. (Hymenoptera) (Fujii *et al.* 2014). Once the ability to
 47 induce the development of specific storage and nutritive tissues (with an accumulation of

nutritive substances) in galls depend on galling feeding behavior (Ferreira et al. 2017; Rezende et al. 2019), one possibility of diagnosing behavioral manipulations or the impact of parasitism on galling insects can be analyzing the gall tissues and morphology. In fact, the galls tissues and morphology may respond to any changes in the galling insect stimuli (e.g. Bedetti et al. 2013; Rezende et al. 2019).

In the current study, we evaluate globoid galls induced by Cecidomyiid on *Piptocarpha macropoda* (DC.) Baker (Asteraceae), which may occur as clusters or as solitary stem galls. This host species is typical of seasonal semideciduous forests and works as a pioneer in the colonization of degraded areas. The clustered galls, although arranged in groups, have individual larval chambers, occupied by only one larva of Cecidomyiid, and are isolated among each other by the cortex. Those galls are attacked by *Platygaster* sp. (Hymenoptera: Platygastridae), a polyembryony endoparasitoid, which feeds on the galling insect body fluids. For instance, we did not register any other parasitoid in these galls. As the morphology, histology, and chemistry of galls depend on the galling insects' behavior, any changes induced by the presence of a parasitoid feeding on the galling insects can impact their behavior and gall features. Herein, once an individual of Cecidomyiid supports many parasitoids, we hypothesize that an intense consumption of nutrients by the parasitized galling insect will impact gall size, number of cells in each gall tissues compartment as well as histochemical profile.

MATERIAL AND METHODS

Globoid individual stem galls (n = 83) were collected from a population of *Piptocarpha macropoda* (n = 10 individuals) at Itacolomi State Park (Parque Estadual do Itacolomi), Ouro Preto, Minas Gerais, Brazil (20°26'04.3" S / 43°30'37.4" W). The clustered galls (Fig. 1A) were individualized, dissected, and the organisms encountered inside them recorded and classified in four groups: (1) unparasitized galling larvae (n= 55), (2) parasitized galling larvae (n= 33), (3) unparasitized galling pupae (n= 17) and (4) parasitoid pupae (n= 8). The Platygastridae parasitoids were identified by comparison with specimens deposited in the Zoology Museum of the University of Costa Rica/Costa Rica.

We measured the galls of groups 1 and 2, from the base (point of insertion on the host plant) to the top surface, the height, using a digital caliper (Digimess®). We also measured

the body sizes of the larvae infested by *Platygaster* sp. ($n = 13$) and the unparasitized ones ($n = 16$) to obtain: the distance between the right and left body edge (width) and between the anterior tip to the posterior tip of the body (length), also with a digital caliper. Those larvae measurements were obtained when the larvae were dead and, therefore, with a relaxed body. We performed a Generalized Linear Model (GLM) with Gaussian distribution, to test if gall size differs between the gall groups with unparasitized larvae and the group containing parasitized galling larvae. To compare the length and width of the parasitized and unparasitized galling larvae the data were submitted to ANOVA, Shapiro-Wilk normality test followed by Wilcoxon test for non-parametric data. Statistical analysis was conducted using the R statistical program (R Core Team 2018).

For anatomical analysis, gall fragments ($N=5$ for each of the four considered groups) were fixed in FAA 50 (formalin, acetic acid, 50% ethanol, 1:1:18) (Johansen 1940), dehydrated in an N-ethyl series (70%, 96% and absolute), immersed in isoamyl acetate, and embedded in Paraplast® (Álvarez 2009). The material was sectioned ($18\text{ }\mu\text{m}$) in a rotatory microtome (Leica 2035 BIOCUT®), deparaffinized, and stained in Astra blue-safranin 9:1 (v/v) (Bukatsch 1972, modified to 0.5%). The slides were mounted with varnish Acrilex® (Paiva et al. 2006) and analyzed under a light microscope (Leica DM500®) (Leica, Wetzlar, Germany), and photographed with a coupled digital camera (Leica ICC50 HP®).

For histochemical analyses, free handmade histological sections from different unparasitized and parasitized galls were stained with Sudan red B for detection of lipids (Brundrett et al. 1991), Lugol's reagent for detection of starch (Johansen 1940), and xylydine ponceau for detection of proteins (Vidal 1970). Lipids were stained red, starch in black, and proteins in red. Blank sections were used as control. The sections were mounted on slides with water and analyzed in a light microscope (Olympus Bx41) and photographed with a camera connected to a Tablet x1 (Acer B3-30).

RESULTS

The gall sizes differed according to parasitoid presence ($F_{6,120} = 14.007$, $P = 0.001$). Galls containing parasitized cecidomyiid larvae were larger than galls with unparasitized larvae (Fig. 1). The body size of the galling larvae without parasitoids was similar to the

parasitized larvae in both length ($4.42 \pm 0.32\text{mm}$ and $4.4 \pm 0.35\text{mm}$, $P = 0.77$, respectively) and width ($1.83 \pm 0.20\text{mm}$ and $1.75 \pm 0.22\text{mm}$, $P = 0.79$, respectively)

The globoid galls of *P. macropoda* are light brown and densely covered with trichomes (Fig. 2A). Anatomically, gall wall zonation can be divided into an outer tissue compartment formed by the epidermis with non-glandular trichomes, the cortical parenchyma and fibers, and an inner tissue compartment, formed by the common storage tissue and a nutritive tissue (Fig. 2B). Regarding the nutritive tissue, the galls with unparasitized larvae had 2-4 cell layers (Fig. 2C), whereas the galls with parasitized larvae had 6-8 cell layers (Fig. 2D). Galls with unparasitized pupae had 5-7 cell layers of compact nutritive cells (Fig. 2E), differing from the galls with parasitoid pupae, which had 0-3 cell layers with compact nutritive cells (Fig. 2F), showing the final consumption proportions during the complete cycle of healthy galling's compared to parasitized ones.

Both unparasitized and parasitized galls accumulate lipid droplets in the trichomes, common storage tissue, and nutritive cells (Fig. 3). No starch grains or proteins were detected either in galls with unparasitized larvae or those with parasitized larvae. Based on the number of cell layers, the nutritive tissue was thinner in unparasitized galls (Fig. 3A) than in galls with parasitized larvae (Fig. 3B).

DISCUSSION

The main consequence of the endoparasitoid's presence over the clustered galls on *P. macropoda* is the increase in size, and the number of nutritive cell layers. Usually, each gall, like other developing plant organs, acts as a sink of photoassimilates (Castro et al. 2012; Oliveira et al 2017; Martini et al. 2020). Accordingly, clustered galls can impose a strong sink of photoassimilates, which can be even stronger due to the demand of endoparasitoids in the system, as observed in *P. macropoda*-galling insect-endoparasitoid system.

Despite the difference in size between galls with and without the endoparasitoids, the parasitized and unparasitized cecidomyiid larvae maintain similar body sizes. This is evidence of a positive impact of the *Platygaster* sp. feeding demand over gall development, but of a null impact on the cecidomyiid body. That means, the *Platygaster* sp. infection

stimulates changes in the gall-inducing insect activities, without causing noticeable changes in the infested larvae. Along gall maturation, the maintenance of the host cecidomyiid alive is crucial for the *Platygaster* sp. own survivorship, once the gall tissues depend on the larva feeding behavior (Rezende et al 2009). This scenario of increasing gall size, parasitoid development, and parasitized galling larvae in similar conditions to unparasitized larvae indicates a hyperstimulation on plant cells and tissues by the cecidomyiid. This hyperstimulation results in the increase in lipid-rich nutritive cell layers, from 2-4 to 6-8 layers. The lipidic accumulation is redirected from gall cells toward the galling cecidomyiid body, which intermediates the energetic molecules to the *Platygaster*.

The difference in cell layers of nutritive tissues among galls with larvae and pupae both in parasitized and unparasitized conditions indicates differences in the feeding stimuli. The galling cecidomyiid in the unparasitized condition stimulates the nutritive cells, but due to its feeding consumption, the number of cell layers is constant (2-4 layers). The stimuli of the galling larvae in the parasitized condition over the nutritive cells are higher, which explains the 6-8 layers, and its feeding consumption seems to be similar even under the parasitoid demand, which is assumed by its similarity in body size. The differences in stimuli persist up to the pupal stage both for the galling and the parasitoid insects: In the unparasitized gall condition, with the cecidomyiid pupal stage, the nutritive cells persist but they are smaller and showed symptoms of cellular disruption. However, in the parasitized condition of the cecidomyiid, the stimulus is impaired before *Platygaster* pupation and galling insect death, which explains the decrease of the stimulus in nutritive cell layers.

We can conclude that, the increasing in gall size demands higher energy expenditure to produce new nutritive cells for the galling insect support and the parasitoid survivorship. When the endoparasitoid activity does not interrupt the host cecidomyiid eating, a greater availability of food is guaranteed (Godfray 1994; Mayhew 2016), which can yet compensate the costs for the parasitoid's larvae escape the galling's immune defenses (Fujii et al. 2014). Herein, the effect of *Platygaster* sp. on gall morphology seems to follow the pattern of the increment in cell layers and consequently in gall wall thickness as reported for the *Platygaster* wasp species on *Masakimya pustulae* gall thickness (Fujii et al. 2014). Also, the similar body sizes of the parasitized cecidomyiid compared to the unparasitized ones

indicates that the *Platygaster* sp. infection may stimulate changes in the gall-inducing insect activities, without causing noticeable changes in the cecidomyiid larvae. Currently, the histological analyses evidence that the galling cecidomyiid acts as the intermediate of the *Platygaster* sp. impacts over plant tissues and guarantees these endoparasitoid life cycles.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

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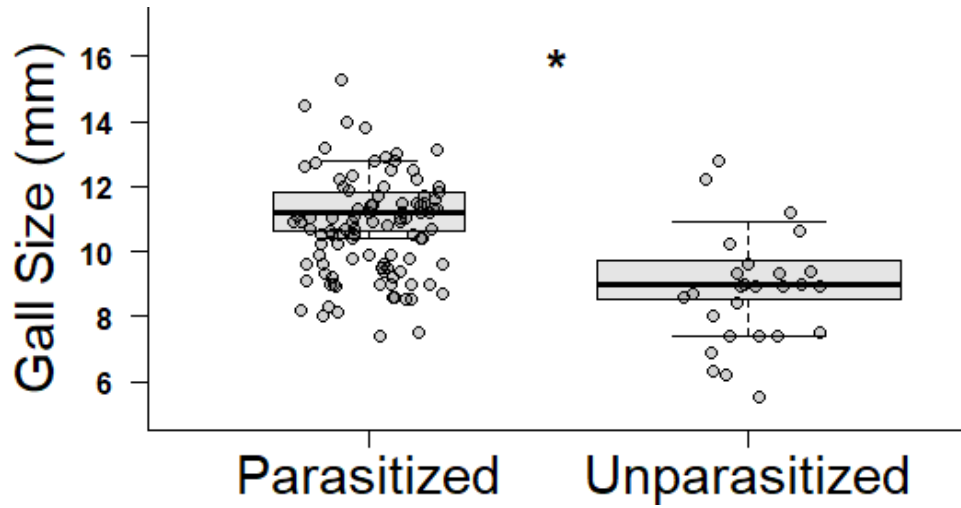
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Figures and captions



253

254 **Figure 1.** Size variation (mm) in galls from clusters on *Piptocarpha macropoda*
 255 (Asteraceae), comparing galls with the galling Cecidomyiid infested by a polyembryonic
 256 *Platygaster* sp. parasitoid versus galls without parasitoids. Galls were larger when occupied
 257 by the parasitized galling insects ($F_{6,120} = 14.007$, $P = 0.001$).

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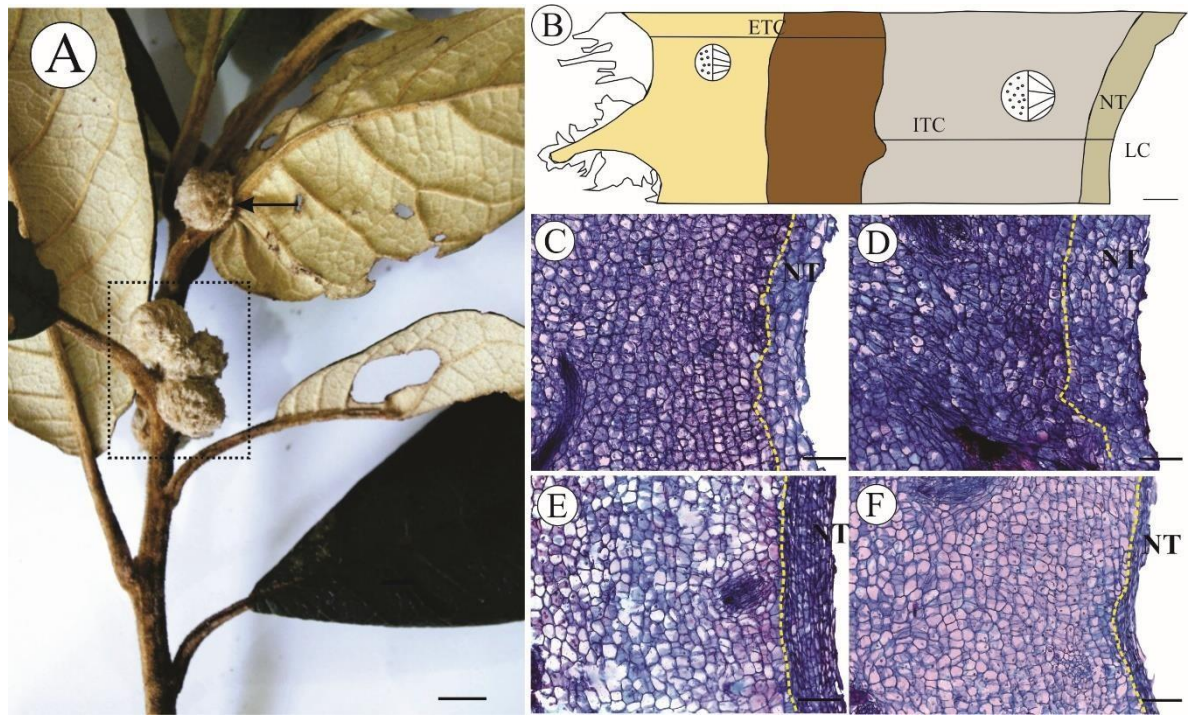


Figure 2. Galls induced by Cecidomyiidae on *Piptocarpha macropoda*. (A) Host plant stem with solitary (arrow) and clustered (square) galls. (B) Diagram of gall anatomical profile, indicating the neoformed tissues compartmentalization. Anatomical differences occurred in the nutritive tissues of galls containing: (C) unparasitized galling larvae, (D) parasitized galling larvae, (E) unparasitized galling pupae, (F) parasitoid pupae. ETC: external tissue compartment, ITC: inner tissue compartment, LC: larval chamber and NT: nutritive tissue. (Dashed lines = nutritive tissue). Scale bars: (A) 5 cm; (B) 50 µm; (C-F) 200 µm.

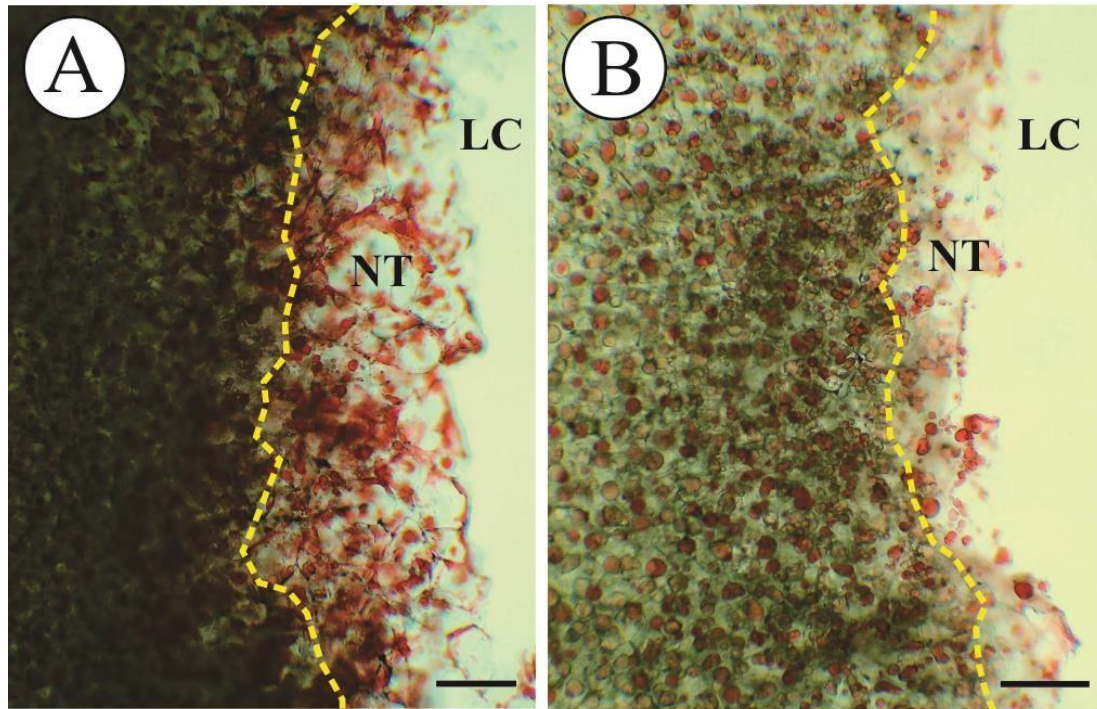


Figure 3. Nutritive tissues from galls induced by Cecidomyiidae on *Piptocarpha macropoda* hosts. Positive histochemical reaction by Sudan Red for lipids in: (A) gall with unparasitized cecidomyiid larva, (B) gall with parasitized cecidomyiid larva. LC: larval chamber, NT: nutritive tissue. (Dashed lines = nutritive tissue). Scale bars: 200 μ m.

CAPÍTULO 2

The multitrophic system configuration on galls of *Macairea radula* (Melastomataceae) induced by *Palaeomystella oligophaga* (Lepidoptera) depends on abiotic events¹

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The multitrophic system configuration on galls of *Macairea radula* (Melastomataceae) induced by *Palaeomystella oligophaga* (Lepidoptera) depends on abiotic events

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Abstract

The galling insects, host plants and all gall interacting organisms may depend on environmental conditions and stochasticity to establish and maintain their life cycles and trophic interactions. The micromoth *Palaeomystella oligophaga* (Lepidoptera: Momphidae) induces galls on *Macairea radula* (Melastomataceae) and its populations suffer mortality caused by direct and indirect negative interactions. Herein, we hypothesized that (i) the occurrence of galls should be greater during the dry period, when arthropods may depend more on microhabitats during the larval stage. However, (ii) *P. oligophaga* can face different kinds and frequencies of enemies during various cycles of the year. In addition, (iii) stochastic events such as fire may reduce the occurrence of natural enemies and thus increase the galling insects' frequency. We quantified the galling survival rates and the proportion and kinds of natural enemies during two life cycles of these gall-inducing insects and after a fire event. We confirmed the bivoltine cycle of *P. oligophaga*, with more galls induced during

the first event but with a higher frequency during the second cycle, followed by the post-fire period. The enemies were more abundant during the first cycle. The bivoltine life cycle may constitute a strategy for *P. oligophaga* to use the galls during different periods of the year because the maturation and emergence of adults depend on the fluctuation of the occurrence of enemies, which varies according to abiotic conditions. In addition, this strategy offers shelter and food resources to all other organisms whose life cycles are synchronized with the availability of this microhabitat.

Keywords

Arthropod conservation, herbivory, natural enemies, plant-insect interactions, trophic relations, population dynamics.

Introduction

The main trophic relationships shaping food chains and webs (at the base) are those between insects and plants, especially herbivore-plant interactions (Schoonhoven et al. 2005; Nakazawa 2020). Some herbivores such as galling insects can manipulate the plant tissues to obtain more than food resources, creating a microhabitat where they can protect themselves against environmental stressors, especially desiccation (Lill et al. 2007) and hide from natural enemies (Price et al. 1987; Stone and Schönrogge 2003, Oliveira et al. 2016). However, these microhabitats usually end up being the sites for tritrophic and even multitrophic relationships among the host plants, galling insects and their direct or indirect enemies (Price et al. 1987, Rezende et al 2021). As a new plant organ, the galls develop by means of chemical and mechanical stimuli of the host tissues by the galling insects (Mani 1964, Shorthouse et al. 2005) and the strategy used by these insects to find available tissues for gall induction

depends on the host phenology and synchronization of both life cycles in the interaction (Yukawa 2000; Oliveira et al. 2013, 2016). The host phenological behavior is related, for instance, to water availability in its habitat (Lemos-Filho and Mendonça-filho 2000) which determines the formation of reactive sites for the galling insect's oviposition (Weis et al. 1988; Campos et al. 2010; Castro et al. 2013; Oliveira et al. 2013). Thus, the huge diversity of galling insects with different life cycles depends on a fine synchronization with events of host plant phenology (Oliveira et al 2016). Likewise, the direct and indirect natural enemies of each galling insect species may depend on this fine synchronization of the use of the food or shelter resources of galls.

The Brazilian savanna biome (Cerrado) has different plant formations (forests, savannas, and grasslands/fields) submitted to seasonal water availability and with a remarkable phenological behavior (Ribeiro and Walter 2008), features that attract a high diversity of galls in the neotropics (Fernandes et al. 2014; Isaias et al. 2014; Martini et al 2021). Nevertheless, stochastic events such as chilling and frost (Filgueira and Pereira 1987, Bannister 2007; De Antonio et al. 2020) or fire can occur during the dry season and may change ecosystem settings (Ramos-Neto and Pivello 2020). Fire can remove the dry biomass and then stimulate a fast recovery rate (Gottsberger and Silberbauer-Gosttsberger 2006; Ribeiro and Walter 2008; Munhoz and Amaral 2010) of plants, thus making available more reactive sites for galling oviposition and gall development. Also, this event may also determine the outcomes of interactions in a community, probably leading to the depletion of the interacting organisms. These abiotic event can be considered to be an evolutionary force having an effect on plants, acting like a consumer analogous to herbivores (Bond and Keeley 2005), and influencing the structure and functionality of insect communities, which vary

73 according to the coupled effects of burn history, plant responses, species interactions (Koltz
74 et al. 2018), and taxon susceptibility (Yekwayo et al. 2018).

75 Therefore, both host plants and galling insects, as well as all gall interacting
76 organisms may depend on environmental conditions such as water availability variations and
77 stochasticity in order to establish and maintain their life cycles and trophic interactions
78 (Bonsignore et al. 2020). For instance, the micromoth *Palaeomystella oligophaga* Becker &
79 Adamski 2008 (Lepidoptera: Momphidae) induces globoid-shaped galls (sensu Isaias et al.
80 2014) on the axillary stem buds of *Macairea radula* Bonpl. (Melastomataceae) (Becker &
81 Adamski 2008) that occurs in wetlands, especially Veredas (wet grasslands), subject to
82 seasonal abiotic variations and fire events during the dry season (Araújo et al. 2013). Rezende
83 et al. (2021) showed the occurrence of at least four insects that have part of their life cycle
84 dependent on these gall formations, using the galling larvae and/or galls tissues as a food
85 resource and the gall structure as shelter until maturity as adults. Consequently, the *P.*
86 *oligophaga* populations suffer mortality caused by direct and indirect negative interactions
87 (Rezende et al. 2021).

88 Herein, we hypothesized that (i) the occurrence of galls should be greater during the
89 dry period, when arthropods may depend more on microhabitats during the larval stage.
90 However, (i) *P. oligophaga* can face different kinds and frequencies of enemies during the
91 cycles of the year, which determine their population variation and a dependence of different
92 species on the formation of these galls. In addition, (iii) stochastic events such as fire may
93 reduce the occurrence of natural enemies and thus increase the galling insects' frequency. To
94 test these hypotheses, we characterized the phenological behavior of the host plant by
95 examining its bud sprouting peaks and the galling life cycle and recording the number of
96 galls formed in each cycle. We then quantified the galling survival rates and the proportion

and kinds of natural enemies during two life cycles of the gall-inducing insects. In addition, we compared these data to those obtained after a fire event in order to test the combined role of seasonal and stochastic effects.

Material and Methods

Study site and multitrophic gall system

The data about gall inductions, host phenophases as well as associated organisms in the multitrophic system during two induction cycles were collected in a wet grassland (Vereda) (19°02'52.9"S 48°20'58.7"W) near Uberlandia municipality, Minas Gerais state, Brazil. In September 2017, a fire burned 62.32 % of the Panga ecological station (19° 10'S, 48°24'O) (Gonçalves RVS *in prep*) including the aerial part of all plants from a population of *M. radula* in a wetland of the reserve. The data after the fire event were collected at the Panga station from the recovered plants of the population. During the three years of duration of the study (2016 to 2018), mean precipitation was 359.51 mm³ (Fig. 1). The climate of the region is characterized as AW with two well-defined periods: a relatively humid one (April – September) and a relatively dry one (October – March) (Álvares et al. 2013).

The studied galls induced on *M. radula* stem buds by *P. oligophaga* are conspicuous (Fig. 2a), with big projections (Rezende et al. 2021) that may contribute to a stable microclimate within these structures (Stone and Schönrogge 2003). The galling insects first feed as larvae on the nutritive tissue cells in the gall center (Fig. 2b), and thus open a wide larval chamber, which they also occupy as pupae (Fig. 2c) until they emerge as adults. Both *P. oligophaga* and the gall microhabitat are resources of food and shelter for different species of arthropods, establishing a multitrophic system (Rezende et al. 2021). As an example of

the use of these galls as microhabitats by insects other than the gallers, the parasitoids *Caliephialtes* sp. (Hymenoptera: Ichneumonidae: Pimplinae), after consuming the galling larvae, form cocoons inside the gall larval chamber using silk and debris, remaining in these vegetal structures through the pupal stage and completing their development in them until adulthood (Fig. 2d, f). Also, the cecidophages Gelechiidae (Lepidoptera) in the early stages of development as larvae before occupying the larval chamber of the gall, dig tunnels in the galls' outermost cortex and form galleries on the surface between the projections, which in some cases are similar in color to these insects (Fig. 2g, h).

Data sampling

Since *P. oligophaga* induces galls on stem buds of *M. radula* (Fig. 2a), we monitored the number of these oviposition sites in a population of the host throughout one year (12 months) (n = 10 individuals) between September 2016 and August 2017. To avoid stem bud sprouting by pruning during field sampling, these ten individuals were used exclusively for phenology monitoring (stem bud appearance). Thus, we recorded the number of buds and galls induced every month. The galls in development were marked and left on the individual, so that it was possible to count only the new galls that emerged monthly.

For the inspections, we collected galls from several different host plant individuals in populations where all insect occupants (either galling or natural enemies) were found in the pupal stage, permitting the collection of adults and taxonomy classification. Here, we considered the individual galls as replicates due to the study goals and the grouped pattern of *M. radula* distribution. Galls induced during the first cycle were collected in April 2017 (n = 356), while those induced during the second cycle were collected in October 2017 (n = 342).

After the fire, the *M. radula* plants recovered, and new galls induced were collected in April 2018 (n = 358).xx

We dissected all samples for separation, identification and, sometimes, a photographic record of the occupying organism using a Leica®500 stereomicroscope coupled to the U-photo system ICC50HD. Insects in the pupal stage were stored in plastic vials until adult emergence for identification. We considered galling predation to have occurred whenever we detected perforation scars on the parenchyma with the galling larva found sucked/dried/dead inside the larval chamber (see Rezende et al. 2021). Galls were classified as having been attacked by fungi only when fungi were found inside the larval chamber together with the dead galling larvae or pupae (*i.e.*, Fig. 1d). For further analyses, we classified the occupants of a gall as: (1) *P. oligophaga* galling, (2) Gelechiidae (cecidophage), (3) Chloropidae (cecidophage), (4) *Caliephialtes* sp. (parasitoid), (5) *Bracon* sp. (parasitoid), and as having been attacked by fungi or predators, according to previous registers and classification for this system by Rezende et al. 2021.

Statistical analyses

We tested if the host stem buds and gall phenological events were evenly distributed across the year. For each period, we considered the average number of buds and the total number of galls. The circumference was divided by 12, and the midpoint of the sector represented the months (Morellato et al. 2010). For both buds and galls we applied the Hermans-Rasson test, which is suitable for data with bi- and multimodality, found in both events. To do so, we used the R-package *CircMLE* (Landler et al. 2019) adjusting 5000 bootstrap replicates to estimate the p-value. The null hypothesis is the presence of uniform (random) distribution throughout the year while the alternative hypothesis indicates that data

are concentrated around specific directions, indicating seasonal patterns. After confirming a bivoltine life cycle (see Results), we tested if the two cycles of induction had equal expected frequencies of the number of galls using a chi-square test. The p-value was computed by Monte Carlo simulation (10,000 iterations) (Hope 1968). We then tested if the co-occurrence of buds and galls was greater than expected by chance by creating a null model. The ROSARIO randomization algorithm was employed since it preserves data temporal autocorrelation (Castro-Arellano et al. 2010). Using the TimeOverlap software (<http://hydrodictyon.eeb.uconn.edu/people/willig/Research/activity%20pattern.html>), we compared empirical values from both Pianka and Czechanowski indices to those from randomizations (10,000 iterations). We then performed a one-tailed test by calculating p-values as the proportions of randomizations that had values equal to or greater than the observed value.

We compared the frequencies of the distinct enemies according to the different ecological conditions (first cycle, second cycle and first cycle post-fire) using a chi-square test (employing Monte Carlo simulation; 10,000 iterations). To aid visualization, we built a mosaic plot using the R-package *vcd* (Zeileis et al. 2007). Next, we performed a correspondence analysis using the R-package *FactoMineR* (Le et al., 2008). This procedure allows the graphic display of columns and rows in a low-dimensional space, thus revealing relationships among variables (Sourial et al. 2010). We drew a contribution biplot (*sensu* Greenacre 2013) considering the proportional contributions of the distinct organisms found in galls. All analyses (except those in TimeOverlap) were conducted using R software version 4.1.0 (R Core Team 2021).

Results

The occurrence of buds ($T = 49.48$; $p < 0.001$; Fig. 3a) and galls ($T = 83.85$; $p < 0.001$; Fig. 3b) was not uniformly distributed throughout the year. Even though *M. radula* produced buds and consequently branch shoots in all months analyzed, the largest production occurred in July (70 buds) during the dry period, and the second peak (67 buds) was in October, at the beginning of the wet period. The months with the lowest stem bud production were August (10 buds) and September (16 buds), at the end of the dry period. The occurrence of galls has a typically bimodal distribution, indicating a bivoltine cycle; the first gall induction cycle starts in February and continues until April, before the end of the wet period. The second occurs from August to October, at the end of the dry period, *i.e.*, the period with lower numbers of available sites on the host. Significantly more galls were induced during the first event than during the second (43 vs. 20; $\chi^2 = 8.40$, $p = 0.004$; Fig. 3b). The overlap between buds and galls was not greater than expected by chance regarding both indexes used ($p_{\text{(Pianka)} \geq \text{observed}} = 0.75$; $p_{\text{(Czechanowski)} \geq \text{observed}} = 0.99$), indicating that the incidence of buds and galls is not coincident.

There were significant differences in the occurrence of occupants according to the period of gall induction ($\chi^2 = 277.83$, $p < 0.001$; Table 1; Fig. 4a, b). During the first cycle, the frequency of the galling *P. oligophaga* surviving/reaching adulthood recorded was 19.10%. Among the natural enemies in the first cycle, we found 5.90% Gelechiidae, 37.64% Chloropidae cecidophages, 16.29% *Caliephialtes* sp. parasitoids, 7.30% *Bracon* sp., 8.99% fungi, and 4.78% signs of predation. The second cycle showed a 61.70% frequency of *P. oligophaga*, even though there were attacks from the same enemies except fungi and predation: Chloropidae (27.78%), Gelechiidae (6.72%), *Caliephialtes* sp. (2.34%) and *Bracon* sp. (1.46%). During the post-fire cycle, *P. oligophaga* galling occurred in 35.20% of the surveyed galls, 12.29% of them Gelechiidae, 19.27% Chloropidae, 1.68% *Caliephialtes*

sp., and 11.45% *Bracon* sp., with a 14.80% rate of occurrence of fungi, and with signs of predation in 5.31% of cases.

When we submitted the results to a correspondence analysis (Fig. 4b), we found that the first axis (CA1) explained 64.08% of data variation while the second (CA2) explained 35.92%. According to environmental conditions, the second cycle was the variable that most explained variance in the CA1 axis (58.61%), followed by the first cycle (40.01%) and then by the post-fire cycle (1.38%). On the other hand, the post-fire cycle was the variable with the highest contribution on the CA2 axis (64.72%), followed by the first cycle (26.27%) and the second cycle (9.01%). Regarding the occurrence on galls, *P. oligophaga* galling insects explained most of the variance in the CA1 axis (47.13%), followed by the natural enemies *Caliephialtes* sp. (22.45%), fungi (13.71%), predators (7.80%), *Bracon* sp. (6.96%), Chloropidae (1.94%), and Gelechiidae (0.00%). In the CA2 axis, *Caliephialtes* sp. (30.91%) explained most of the variance, followed by fungi (23.85%), Chloropidae (17.95%), *Bracon* sp. (13.42%), Gelechiidae sp. (10.33%), predators (3.52%), and *P. oligophaga* (0.02%). Overall, by interpreting both the mosaic plot and the biplot we can infer that some enemies were proportionally more abundant during the first cycle (*i.e.*, *Caliephialtes*, Chloropidae) while others were more abundant during the first cycle/post-fire condition (*i.e.*, Gelechiidae, *Bracon* sp. and fungi). On the other hand, *P. oligophaga* had a higher survival rate during the second cycle.

Discussion

Even with the variations in environmental conditions during the rainy and dry seasons, or the fire effects, the hosts continued to produce stem buds and did not limit the availability of potential oviposition sites. Therefore, the established trophic relations were

not directly limited by the phenophases of the plant hosts (producers), considering that the synchronization between plant-host and galling insect determines the synchrony of the enemies' life cycle with the cycle of their own hosts (e.g., Fei et al. 2014; Bonsignore and Bernardo 2018). Even so, each period of the bivoltine life cycle established by *P. oligophaga* showed different frequencies of the direct and indirect enemies occurring in the galls.

Seasonality does not determine bivoltinism but changes the enemy's frequencies

The bivoltinism of *P. oligophaga* possibly reflects an adaptive pressure imposed by the attacks of the natural enemies related to environmental rainfall conditions. The galling insect larvae - or their induced galls tissues - were attacked by the same morphospecies of natural enemies (except fungi and predators for which we cannot be sure) during both the first and second life cycles and the post-fire cycle, although there were differences in the frequency of attacks. Majeed et al. (2020) reported a seasonal change in arthropod diversity in wetlands, affected by seasonal patterns, climatic conditions, and habitat structure. Although we only sensed arthropods inside the galls, it is possible that the result found here may serve as an indicator of the variation of abundance of arthropods in the Vereda (wetland) community, since the species configuration in the studied multitrophic system changed seasonally.

The frequency of *P. oligophaga* that reached adulthood in the second cycle was three times higher than during the first cycle, whereas the gall induction rates in the second cycle were half those of the first one. This means that during the dry period there were fewer females laying eggs, or each female laid fewer eggs, while more larvae reached the pupal or adult stage. This pattern can be a consequence of several environmental factors, but is probably due to a decrease of food resources caused by drought that affects all the arthropods

involved. It should also be pointed out that this scenario was not due to one or two kinds of enemies since the rainy period (first cycle) involved a higher occurrence of all the natural enemy attacks compared to the second cycle, except for Gelechiidae sp., with emphasis on an increase of both parasitoid species. Since the frequency of *Caliephialtes* sp. decreased from 16.29% to 2.34%, and the frequency of *Bracon* sp. decreased from 7.30% to 1.43%, it seems likely that the populations of these insects are strongly influenced by environmental conditions.

Herein we corroborate the hypothesis that abiotic events interfere with the types and numbers of natural enemies that attack galling insects (Fernandes and Price 1992). The patterns found here indicate that abiotic events influence the survival rates of *P. oligophaga*, reducing the incidence of negative interactions with its natural enemies. Since the diversity and biomass of arthropods have been progressively reduced in all Biomes on a multicontinental scale (Dirzo et al. 2014; Forister et al. 2019; Janzen and Hallwachs, 2019), the wetlands are of fundamental relevance mainly because they are sources of groundwater emergence in the Cerrado (Boaventura 2007), but also because they maintain countless species, including endemic ones (Ávila et al. 2016; Fagundes and Ferreira 2016). Our results reinforce the importance of the wetlands as maintainers of complex and intricate systems of interactions, from producers to the higher trophic levels, adapted to seasonal and stochastic effects on those environments.

Fire event and the configuration of multitrophic system species

The survival rates of *P. oligophaga* were higher during the post-fire cycle compared to the same period of the previous year (first cycle), but lower than during the second cycle due the higher frequencies of *Bracon* sp. parasitoids, Gelechiidae cecidophages and fungi. In

a previous study the authors have shown that *Bracon* sp. and Gelechiidae attack more frequently the galls at higher positions on the plants (Rezende et al. 2021). It is possible that after the aerial part of the vegetation was burned, *M. radula* regrowth maintained a similar height, possibly facilitating the finding of galls induced by *Bracon* sp. and Gelechiidae. While those organisms were favored by stochasticity, the Chloropidae sp. cecidophages suffered a great impact. These flies can pose the greatest danger to *P. oligophaga* survival in galls of *M. radula*, possibly being the most specialized enemies of these galling insects by being the most adapted to circumventing the possible defense traits of these galls (Rezende et al. 2021). Considering that natural enemies are common and intense regulators of herbivore populations (Vidal and Murphy 2018; Welte et al. 2020), in this case the fire acted as a facilitator of the increase of the galling insects' population, reducing the top-down effect.

Here, the plants were burned during the dry season, which allowed us to analyze the recovery effects on the multitrophic relationships during the following rainy season. However, we have no information about what can happen if the plants burn before the second cycle. Based on what we found about the effects of microclimatic variation on the studied system configuration and noting that seasonal fluctuations affect the fire effects on populations dynamics (Just et al. 2016; Dos Santos et al. 2021), we assume that fire can have a more impacting effect on the arthropods if it occurs before or during the second cycle of gall induction. Regarding *P. oligophaga* survival, we believe that periodic burns can decrease their mortality due to consumption by their enemies but can affect plant-hosts that lose top-down regulators of specialist herbivores. *M. radula* sprouted quickly and appeared to be adapted to fire exposure like other species of savanna vegetation that are highly resilient based on different survival strategies (Simon and Pennington 2012; Pilon et al. 2021). Predictable burn regimes are very important for the maintenance of wetland vegetation

structure (Kirkman et al. 2000; Peterson and Reich 2001; Araújo et al. 2013; Heim et al. 2021) and, as reinforced by the present study, fire can influence the maintenance of insect-plant interactions, mostly affecting the population dynamics of natural enemies.

Dynamics of fungi as natural enemies

Another important effect of gall induction over two different periods is that, during the second cycle and dry season, *P. oligophaga* can escape more efficiently the fungal-induced mortality compared with the rainy period. Environmental humidity due to rainfall generates ideal conditions for the proliferation of fungi that may arise from the surface of the galls themselves or inside the insects' bodies - acting as vectors (Currie 1995), whereas the fungi are less active in drier conditions (Preece and Dickinson 1971). How common is this to other galling-plant systems? We surmise that this is an important point to be analyzed in studies of the trophic relations of galls. Here, fungi caused mortality at a proportion similar to or greater than that caused by parasitoids and cecidophages, highlighting the importance of considering fungi as enemies of gall-forming insects.

The impact of fungi on the gall microhabitat is a curious topic of discussion, mainly due to the different roles they can play in trophic relationships and in the configuration of the galls as shelters. For example, there are records of fungi that act as hyperparasitic biological controllers of fungus-induced galls (Byler et al. 1972; Chakravarty and Hiratsuka 1995) or act in association with galling insects in the manipulation of plant tissues (including induction, maintenance and/or development of these structures) (Yukawa and Rohfritsch, 2005; Lebel et al. 2012). Also, some fungi are categorized as symbionts (e.g., Kobune et al. 2012; Zimowska et al. 2017) and others as saprophytes or pathogens that cause the death of gall-forming insects (Batra and Lichtwardt 1963).

Conclusion

The bivoltine life cycle may constitute a strategy for *P. oligophaga* to use its microhabitat, the galls, during different periods of the year, because the maturation and emergence as adults depends on the fluctuation of the occurrence of enemies, which varies according to abiotic conditions. Besides, it offers shelter and food resources to all the other organisms whose life cycles are synchronized with the availability of this microhabitat. The role of galling insect-plant host interactions as a basis for food webs is important throughout rainfall variation, and can be fundamental for all organisms involved, especially for surviving stochastic events such as fires. In this context, it seems likely that *P. oligophaga* establishes microecosystems through the formation of galls in *M. radula*. We conclude that inducing more galls during the dry season may be a strategy for *P. oligophaga* to escape mortality by negative interactions with other insects and fungal infections.

Therefore, even in systems where interacting organisms are in synchronism between their life cycles, climatic and stochastic factors can change the population dynamics within galls, favoring different enemy groups during each period. The present results also reinforce the idea that what happens to the biotic traits of the wetlands, especially the endangered Veredas, determines the dynamics of specific trophic relationships. The wetlands are disappearing (see Gonçalves et al. 2021) and the native species of these environments may be the most affected by anthropization effects (Oliveira et al. 2021). This is very harmful given the importance of these environments mainly for the Cerrado water supply (Boaventura 2007) and considering that, as we presented here, a lot of arthropod species can be maintained by a single plant species that occurs in these environments.

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Declarations

Conflict of interest The authors declare no conflict of interest

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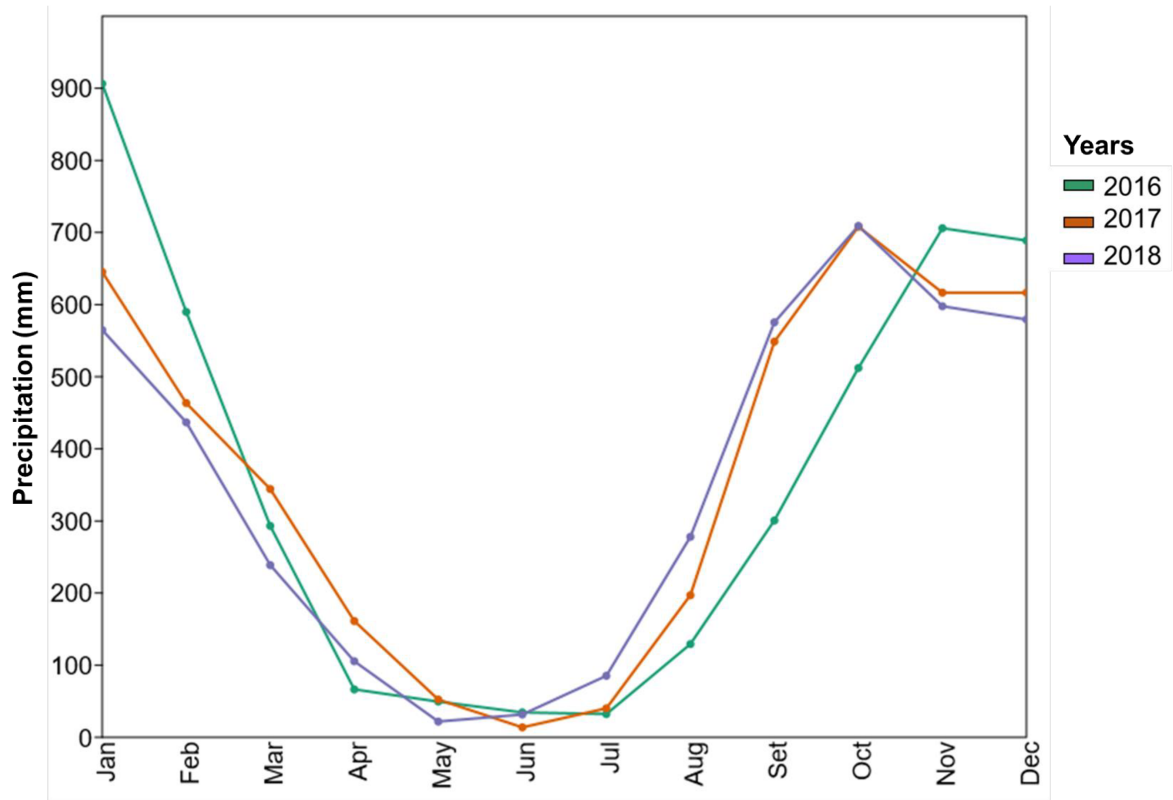


Fig. 1. Mean precipitation at Panga Ecological Station (19° 10'S, 48°24'O) during the three years of duration of the study (2016 to 2018).



Fig. 2. a Galls induced on *Macairea radula* stem buds by *Palaeomystella oligophaga* are conspicuous. **b** The galling insect larvae first feed on gall nutritive tissues and thus open a wide larval chamber, **c** which they also occupy as pupae until emergence as adults. Both *P. oligophaga* and the gall microhabitat are resources of food and shelter for different kinds of organisms **d** such as fungi, for example. The organisms explore these microhabitats in different ways. For example, *Caliephialtes* sp. parasitoids consume the galling larvae and then form cocoons inside the gall larval chamber, **e, f** where they remain and develop as pupae and then complete their development until adulthood. In addition, the cecidophages Gelechiidae are present in the early stages of development as larvae, before occupying the gall larval chamber, **G** dig tunnels in the gall outermost cortex and **h** form galleries on the surface between the projections, which in some cases are similar in color to these insects. GL = galling larva, LC = larval chamber, GP = galling pupa, FGP = fungated galling pupa, PC = parasitoid cocoon, PP = parasitoid pupa.

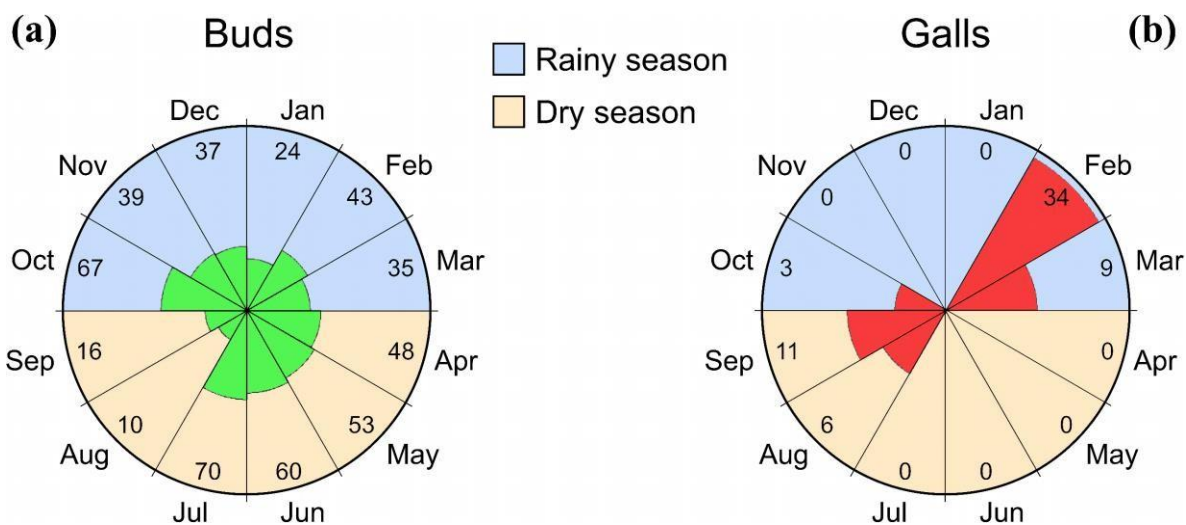


Fig. 3 Rose diagrams demonstrating the distributions of *M. radula* **a** buds and **b** galls across the year and according to season. Raw values for each month are expressed at the periphery of the circle.

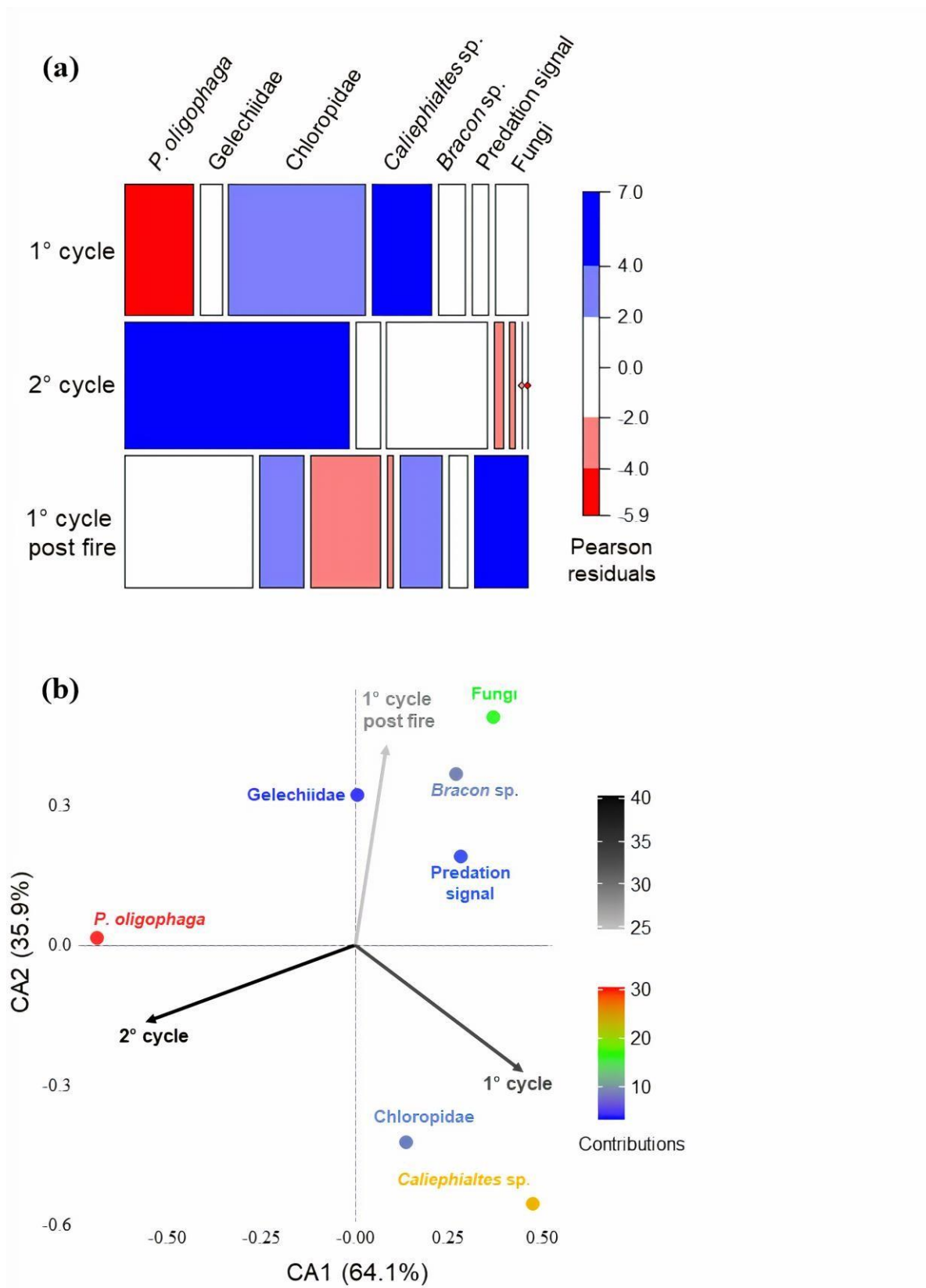


Fig. 4 a Mosaic plot demonstrating the frequencies of the different organisms found in *M. radula* galls according to the distinct environmental conditions. Lower and higher values of Pearson residuals respectively indicate lower (red shades) or higher (blue shades) frequencies than expected. Color intensity indicates the magnitude of the effect. Results in percentages are available in Table 1. **b** Correspondence analysis biplot demonstrating the relationship between organisms and environmental conditions. Grey and color scales refer to the contribution of environmental conditions and to the occurrence of the organisms on galls, respectively.

CAPÍTULO 3

The gall is senescent. Long-life to the plant gall! Histological features of galls induced on *Macairea radula* (Melastomataceae) allow gall's colonization by other organisms after senescence¹

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The gall is senescent. Long-life to the plant gall! Histological features of galls induced on *Macairea radula* (Melastomataceae) allow gall's colonization by other organisms after senescence

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Abstract

Galls is a kind of plant organism interaction defined as a manifestation of the reprogramming of plant cell growth and/or development induced by foreign organisms with different ecological impacts on their host plants. The galling insect taxa and the plant target site for oviposition determine the gall morphology and histological features. For example, galls induced at budding sites can develop morphologically more complex structures due to the greater meristematic potential of their tissues. In addition, these galls could remain attached to the plant body for long periods after the galling insects come out at the senescence phase. Herein we conducted histological analyses to investigate (i) if the galls induced on buds develop as stem-branches-like connections, with histological characteristics that favor their affixation to the host plants after senescence. Also, we

inspected senescent galls to investigate (ii) if the microhabitats within these galls are occupied by opportunistic arthropods in different host-plant populations. The structural features of the studied galls allowed us to diagnose them as stem-like galls. The *M. radula* galls develop as stems with shortened internodes with abundant leaf-like projections. The stem-branch vascular connection allows these galls to remain fixed on the host plants for long periods and work as shelters for different taxa of arthropods in all sampled ecosystems. We showed that *P. oligophaga* activities can involve non-trophic interactions, and their shelters can be functional for the arthropod community. These interactions go beyond impacts on both galling insect and host plant populations, with trophic and non-trophic interactions, which allow us to understand insect *P. oligophaga* as a shelter-builder and ecosystem engineer.

Keywords: arthropod-plant interactions, bud galls, shelter-builders, ecosystem engineer.

Introduction

Galls is a worldwide kind of plant organism interaction defined as a manifestation of the reprogramming of plant cell growth and/or development induced by foreign organisms with different ecological impacts on their host plants (Harris and Pitzschke 2019). In addition, galling insects are defined by Shorthouse and Raman (2005) as “Nature’s most sophisticated guild”, which can induce the development of symmetric and complex structures considered as new plant organs, the galls (Shorthouse and Raman 2005; Oliveira et al. 2016). Gall induction and development depend on the continuous chemical and/or mechanical feeding stimuli on plant tissues by the galling organism (Mani 1964; Bronner 1992; Rezende et al. 2019), which synchronizes its life cycle with the host plant phenology (Oliveira et al. 2016). After the gall inducer recognizes and select its induction site on the host, during the induction phase, it settles and the gall increases its biomass by cell growth and division, which characterizes the growth and

developmental phase. By the end of that phase, the gall reaches its final shape and size, and the galling insect is in the main trophic stage, which characterizes the maturation phase. As the galling organism turns adult, it comes out from the gall structure, and the senescence phase begins (Shorthouse and Rohfritsch 1992).

The structure of galls not only provides nutrition for the galling insects but is also a shelter that provides both a microhabitat with adequate conditions of humidity and temperature and protection against the attack of natural enemies (Stone and Schönrogge 2003, Price et al. 2005). Even though galls are protective structures, different guilds of natural enemies often interact directly or indirectly with them (Luz and Júnior 2019). Consequently, numerous food chains and webs are established having galls and all their developmental phases as the base (e.g., Hayward and Stone 2005; Forbes et al. 2016; López-Núñez et al. 2019; Weinersmith et al. 2020). In this context, galls not only offer food resources for those direct and indirect enemies, but also provide shelter and protection during part of their life cycles (Sanver and Hawkins 2000). For this reason, galling insects and their galls may act as ecosystem engineers, even when galling organisms are still occupying the gall structure. Physical ecosystem engineers are organisms capable of creating or modifying habitats, thus affecting directly or indirectly the availability of resources for other species (Jones et al. 1994, 2010). After galling insects emerges from their galls, their senescent structures often remain attached to the host plant body and can be used as microhabitats by arthropods of several classes, such as spiders, ants, and beetles (Cornelissen et al. 2016).

The galling insects that considered ecosystem engineers can be divided into two main groups: (i) those that make galls by bending, rolling or folding leaves to form a cavity that functions as a shelter for arthropods from different classes (Novais et al. 2020; Pereira et al. 2021); and (ii) galls that are induced in stems or stem buds (Almeida et al.

2014; Wetzel et al. 2016; Giannetti et al. 2019; Weinersmith et al. 2020), whose senescent, hollow structures remain attached to the plant body, also forming shelters. Stem galls can potentially offer shelter for longer periods than leaf galls can, considering that in deciduous plants, stems are long-lasting organs when compared to leaves (Lenza and Klink 2006; Pfeffer et al. 2018; Costa et al. 2022). Thus, galls formed on stem buds can form resistant shelters for long periods of time on host plants, thus functioning as true physical ecosystem engineers.

The galling insect taxa and the host plant organ where galls are induced determine their morphological features and tissue complexity (Weis 1998; Ferreira et al. 2019), which relates to the capacity of a senescent structure to remain attached to the plant body for longer periods. Galls induced at budding sites can develop morphologically more complex structures due to the greater meristematic potential of their tissues when compared to galls induced on other host plant organs (Weis 1988). In non-galled organs, the stem-branch formation occurs through the development of buds, in which the vascular system of the newly formed branch connects to that of the stem. So, the induction of galls on buds can lead to the development stem-branches-like connections, which is responsible for maintaining the gall attached to the host plant, even after the galling insect comes out. Considering this, the oviposition choices as for the selection of induction sites for galls are crucial to determine whether some galling insects are potential ecosystem engineers or not.

Macairea radula (Melastomataceae) was the plant species selected for the current study, once it has globoid galls induced by *Palaeomystella oligophaga* (Lepidoptera: Momphidae) on the axillary buds (Rezende et al. 2019). Field observations showed that senescent *M. radula* galls remain attached to the host plant for long periods after the galling insect leaves, which makes us investigate (i) if these galls induced on buds develop

as stem-branches-like connections, with histological characteristics that favor their affixation to the host plants after senescence; and (ii) if the microhabitats within these galls are occupied by opportunistic arthropods in different host-plant populations, thus allowing to recognize *P. oligophaga* as a physical ecosystem engineer.

Material and Methods

Study area and studied systems

Macairea radula Bonpl. (Melastomataceae) occurs in ecotones between wetlands called “Veredas” and Cerrado *stricto sensu*, both typical phytophysiognomies found in the Cerrado *lato sensu*. *M. radula* hosts globoid-shaped galls (sensu Isaias et al. 2014) induced on the axillary stem bud (Figure 1A) by *Palaeomystella oligophaga* Becker and Adamski (2008) (Lepidoptera: Momphidae). The galling insects have a bivoltine life cycle (Rezende et al., *submitted*) and are attacked by different natural enemies during both life cycles along the year (Rezende et al. 2021; Rezende et al., *submitted*). By the end of each life cycle, the galling insects emerge from the galls and the senescent structure remains attached to the host’s stem (Figure 1B) for two or more years. By the end of that time, the indumentum is usually degenerated, but the gall walls are still conserved (Figure 1C).

Histological analysis

Mature globoid galls of *M. radula* (n = 5; 1.1 cm of height x 0,6 cm of width) containing larvae of *P. oligophaga* were collected from the margins of wetlands at the Panga Ecological Station (19° 10' S, 48° 24' W), municipality of Uberlândia, Minas Gerais state, Brazil. Samples were fixed in FAA (formalin, acetic acid, 50% ethanol, 1:1:18) (Johansen 1940), dehydrated in an ethanol series, immersed in isoamyl acetate,

and embedded in Paraplast[®] (Álvarez et al. 2009). Samples were sectioned (~10 µm thick, transverse and longitudinal sections) in a rotary microtome (Ancap, Brazil), deparaffinized and stained in aqueous Astra blue-safranin 9:1 (v/v) (Bukatsch 1972). Slides were mounted with colorless varnish Acrilex[®] (Paiva et al. 2006) and images were obtained under a photomicroscope (Leica DM 1000; Wetzlar, Germany) coupled with a digital camera (Leica ICC50 HD; Wetzlar, Germany).

Surveys of senescent galls

Three populations of *Macairea radula* with senescent galls, occurring in different ecosystems, were sampled between August and September 2021. We collected 20 senescent galls from 15 host plants (n = 300) from the population located at the margin of a waterfall in Florestina, municipality of Araguari, Minas Gerais state, Brazil (18° 39' 28.8" S 48° 04' 06.8" W); similar sampling (n = 300) was conducted in a Vereda ecosystem, in a rural area (municipality of Uberlândia), Minas Gerais state, Brazil (19° 00' 42.5" S 48° 20' 09.9" W). Additionally, we collected 20 galls from 7 individuals (n = 140) from a population located at the margin of a highway, municipality of Capitólio, Minas Gerais state, Brazil (20° 38' 54.0" S 46° 16' 52.9" W). Thus, a total of 740 galls were collected, conditioned in plastic containers, and taken to the laboratory for dissection. As galls were dissected, all organisms found within the galls chamber were separated, identified to the lowest possible taxonomic level, and, sometimes, photographed using a Leica[®]500 stereomicroscope coupled to the U-photo system ICC50HD.

Results

Histological analyses

The galls induced by *P. oligophaga* on the buds of *M. radula* are globoid, with a single larval chamber, and many leaf-like projections on the surface (Figure 1A; 2A); color varies between red and green and turn to brown after senescence (Figure 1A, B and C). The galls are vascularized by vascular bundles which connect to the vascular system of the non-galled adjacent stem (Figure 2A and B). The leaf-like projections (Figure 2C) develop all around the globoid galls, being vascularized by vascular traces coming from the gall cortex (Figure 2E); numerous glandular trichomes are observed on the leaf-like projections (Figure 2D). Galls induced on the buds of *M. radula* result from a process of internode shortening and over-differentiation of leaf-like projections, which are densely packed together in the nodes (Figures 2 and 3).

Histologically, the globoid galls of *M. radula* have typical nutritive tissue, with a highly vascularized parenchymatous cortex, and leaf-like projections densely arranged at the periphery (Figure 3A). The vascular bundles from the stem are connected to the vascular bundles of the gall peduncle (Figure 3A, B). At the end of this peduncle (base of the gall), a meristematic tissue redifferentiates from the ground parenchyma; sites of cell lignification at the base of the gall can also be observed in some samples (Figure 3C, D). The redifferentiated vascular bundles are dispersed at the base of the gall (nearest to the gall peduncle) and converge to a high density at the uppermost part of the gall (region opposed to the peduncle insertion) (Figures 3E and F). The vascular bundles are distributed across all gall compartments: in the nutritive tissues, in the storage tissues, and in the leaf-like projections (Figure 3F, G, H). In some cases, at the base of the leaf-like projections, a leaf gap or lacuna is observed as the vascular trace connects to vascular bundles in the gall cortex (Figure 3G, H). Further evidence of the “leafy” nature of these projections is the presence of emergences with associated glandular trichomes (Figures 2D and 3H), similar to what is observed on the ungalled leaves of *M. radula* (not shown

herein). In addition, cell divisions seem to be intense at the base of each leaf-like projection, where tissue outgrowths differentiate into new leaf-like projections (Figure 3I), which repeat the pattern of developing new emergencies due to the meristematic activity of epidermis and parenchyma cells (Figure 3H, I).

Occupation of senescent galls by different taxa

The bud galls induced by *P. oligophaga* on *M. radula* (Figure 4A) form a globoid and large gall, whose senescent structure may be occupied by different arthropods in all ecosystems, namely beetles, ants, spiders, and mealybugs (Table 1, Figures 4 and 5). The highest percentage of arthropods occupation (or, signs of such occupation) on galls, was observed for the wetland population, where 40,6% of the senescent galls were occupied; the galls from the highway margin had 25% of occupation, while the waterfall population galls had the lowest percentage of occupation, with 20.3%. We found differences in the assembly of arthropod taxa among the surveyed communities. Galls from the wetland population were occupied by beetles of the Bostrichidae family (Figure 5A), *Cephalotes pusillus* ants (Figure 4B), and unidentified spider's exuvia, ootheca and silk (signs of previous occupation by spiders) (Figure 4B). Galls from the highway margin population were occupied by Bostrichidae beetles, Dactylipiidae mealybugs (Figure 5C), *Crematogaster* sp. (Figure 4B), *Cephalotes pusillus*, and *Pseudomyrmex gracilis* ants (Figure 4B); spider silk was also found in those galls (Table 1). Galls from the waterfall population were occupied by Bostrichidae beetles, Dactylipiidae mealybugs, *Pseudomyrmex gracilis* ants, spiders from the Salticinae subfamily, and unidentified spider's exuvia, ootheca and silk.

The chambers of senescent galls have the mean dimensions of 5mm of height and 17.1mm of width. Nevertheless, the Bostrichidae beetles alter part of the internal structure of the galls by boring tunnels inside the gall walls (Figure 5B). We registered ant nests

(varying among ants' species) in galls from all the ecosystems where we collected. The *Crematogaster* sp. nests were found only on the highway population, *Cephalotes pussilus* nested on the galls of the highway and of the wetland populations, while *P. gracilis* nested on the galls from the highway and the waterfall populations.

Discussion

Long-lasting galls are modified stem-branch structures

Meristematic plant tissues have the capacity to divide and differentiate into new tissues to produce new organs which make plant meristems adaptive sites for gall induction and development (Lev-Yadun 2033; Price 2005; Oliveira and Isaias 2009; Fleury et al. 2015). Galls induced on buds by Lepidoptera are commonly report on different species of Melastomataceae (Isaias et al. 2014) and seem to be a conserved characteristic of different *Palaeomystella* species associated with Melastomataceae (Becker and Adamski 2008; Basilio et al. 2015; Luz et al. 2015). Bud galls have been shown to differentiate into stem-like organs, as reported for galls induced by *Pisphondylia brasiliensis* (Cecidomyiidae) on *Guapira opposita* (Nyctaginaceae) (Fleury et al. 2015), which produce a leaf-like projections in a similar way that is observed for the galls studied herein. Thus, the structural features of galls induced by *Palaeomystella oligophaga* (Lepdoptera: Momphidae) on buds of *Macairea radula* (Melastomataceae) allowed us to diagnose them as stem-like galls, in which a stem-branch vascular connection is clearly established, and leaf-like projections are formed.

The vascularization of non-galled stems of *M. radula* is composed by peripheral vascular bundles with a central parenchymatous pith, which indicate a eustelic organization (sensu Tomescu 2021), similar to the vascularization of the *M. radula* gall

peduncle. In addition, the histological profile showed a similar meristematic tissue on the apex of galls, with cytoplasm dense cells. Gall-inducing organisms can stimulate the reacquisition of new meristematic cells (Carneiro et al. 2017; Ferreira et al. 2019), which allow galls to develop morpho-functional compartments, which in the case of *M. radula* bud galls, the inner tissue compartment relates to a nutritional functionality (Nutritive tissues; see Kuster et al. 2018; Rezende et al. 2018), while the outer tissue compartment has a protective role, with increased number of cell layers and of leaf-like projections, i.e., a thicker gall wall. Such structural increment is classically related to the protection of the gall inducer against natural enemies (Stone and Schönrogge 2003), despite the high frequency of attack by natural enemies on this gall system (Rezende et al. 2021). As the adults leave the galls, triggering senescence, the inner tissue compartment collapses, but the structurally reinforced outer tissue compartment, which lignifies at the base, withstands the senescence process, thus allowing the long-lasting dry galls to be used by other organisms as shelter.

The indumentum covering the galls on *M. radula* were previously referred as emergencies by Rezende et al. (2019), however, the type of vascular connections they establish with the gall cortex, with a typical parenchymatic leaf gap, led us to refer to them as leaf-like projections, similar to those described for the bud galls induced by *Pisphondylia brasiliensis* (Cecidomyiidae) on *Guapira opposita* (Nyctaginaceae) (Fleury et al. 2015). Further evidence of such leafy nature of the gall projections are the glandular trichomes and small emergencies on their surface, which are like those reported for the leaves of *M. radula* produced from the shoot apical meristem (Somavilla and Graciano-Ribeiro 2011). Based on this evidence, we claim that the leaf-like projections on the galls and the non-galled leaves of *M. radula* are, in fact, analogous organs, and even though

galls are induced in totipotent sites (the lateral buds), gall development is still under the morphogenetic restrictions of the host plant (Isaias et al. 2014).

Even though some galls repeat parts of regular plant development programs (Oliveira et al. 2010; Vecchi et al. 2013), the gall structure is considered a new plant organ, with distinct levels of complexity (Shorthouse et al. 2005; Ferreira et al 2019), being the bud galls considered the most complex (Nyman et al. 2000). We suggest that the histological pattern showed here that allows the galls to last longer attached to their host plants, should be similar among the galls induced by *Palaeomystella* spp., as they are all induced on Melastomataceae and are morphologically similar (Becker and Adamski 2008; Basilio et al. 2015; Luz et al. 2015). Therefore, it is plausible to assume that all *Palaeomystella* species play the role of shelter-builders, thus acting as ecosystem engineers.

Long-lasting galls: old structures for new occupants

The senescent *P. oligophaga* - *M. radula* galls are shelters for different taxa of arthropods in all sampled ecosystems, indicating that the ecology of the galling organism habit goes beyond the implications for the trophic interactions among plants, gall inducers, and gall inducers' natural enemies (Rezende et al. 2021), as the gall structure potentially affects the life-history parameters for several arthropods. We showed that *P. oligophaga* activities can involve non-trophic relations among species, and their shelters can be functional for the arthropod community in different ecosystems where they are inserted. Galls as shelters can offer the free-living arthropods improved abiotic conditions, protection against natural enemies, and food availability, increasing resources in the host plants and influencing biotic processes such as predation and parasitism (Baer and Marquis 2020; Fukui 2001). We suppose that at least three trophic levels can benefit from the microhabitat induced by the ecosystem engineer *P. oligophaga*, considering the

273 presence of spiders that are predators and ants as a possible top-down regulation of other
274 herbivores that can attack the host plant *M. radula* (e.g., Wetzal et al. 2015; Pereira et al.
275 2020).

276 Ants dwelling galls were previously reported to establish beneficial interactions
277 with the host plants by removing herbivores or helping avoid pathogens (González et al.
278 2014; Giannetti et al. 2019), thus offering an indirect defense mechanism for the plant
279 against its enemies. In such cases, senescent galls that remain attached to the host plant
280 body are functionally analogous to domatias, acting as myrmecophytic structures, in
281 which ants benefit host plants by decreasing herbivory in exchange for shelter (Heil and
282 McKey 2003) and/or nesting sites (Novais et al. 2020). However, experimental data is
283 needed to support the idea that long-lasting galls such as those on *M. radula* could act as
284 a myrmecophyte structure that benefits the host plant. The ants that we found belong to
285 genera that were previously reported to occupy galls; the assemble of ant species that do
286 so varies according to the size of the gall external opening, the size of the gall internal
287 space, and the gall position on the plant body (Fernandes et al. 1989; Almeida et al. 2014;
288 Giannetti et al. 2019). As we showed here, even for the same population of ant species
289 occupying various galls, more than one type of colony composition may occur in the same
290 host plant, as some galls are occupied by workers, while other are used for nesting, to
291 shelter solitary queens, larvae or pupae (Fernandes et al. 1989; Almeida et al. 2014;
292 Giannetti et al. 2019). Tree ants need nesting sites and, instead of digging, they use plant
293 cavities (Blüthgen and Feldhaar 2010), which may have limited availability for tree ant
294 communities (Novais et al. 2020). This condition makes abandoned insect shelters, e.g.,
295 senescent long-lasting galls, suitable spaces in the hosts that can be used by tree ants,
296 especially in plants that generally do not have functional cavities in their stems and trunks,
297 which is the case of *M. radula*.

Even though ants are commonly found in galls, the most frequently found occupants of *P. oligophaga* galls were Bostrichidae beetles with borer habit as expected for this taxon (Liu et al. 2008), digging tunnels in the senescent galls, despite we believe be an opportunistic relationship of these organisms with *P. oligophaga* - *M. radula* gall system. Besides the non-trophic benefits, the beetles established a trophic relationship with the gall structure, feeding on the senescent tissues of the inner walls; even though the galls are senescent, their higher nutritional quality when compared to non-galled plant tissues (Rezende et al. 2019) can be an attractive food resource for the Bostrichidae beetles. The feeding habit of the beetles may result in increased internal space in the galls, which may favor the occurrence of larger organisms, such as predators; in such sense beetles may also play the role of secondary ecosystem engineers by changing gall structure even after it is senescent. For example, certain larger ant species may only occupy galls after they are secondarily modified, and increased internal space is available (Giannetti et al. 2019). Another example of trophic relationship found herein was the presence of mealybugs inside galls, where they feed during the senescence process. Mealybugs were found mostly at the base of the galls, where they connect to the peduncle and to the stem, probably taking advantage of the metabolites relocated from the gall toward the plant during gall senescence. Possibly, the mealybugs that would feed on other plant structures such as flower buds, causing damage, concentrate on the galls, decreasing negative effects on plant reproduction (Somavilla and Graciano-Ribeiro 2015).

Based on investigations into the impacts of shelters on host plants, we found compelling evidence that the microhabitats investigated here might be important structuring elements with effects on arthropod species richness and abundance on *M. radula* galls (Wetzel et al. 2015; Novais et al. 2020; Pereira et al. 2020; Romero et al. 2021). The effects of galls on their hosts are multiple, from when they are active,

functioning as a sink of metabolites produced by the plant (Castro et al. 2012) with possible deleterious effects (Guimarães et al. 2014, 2015; Martini et al. 2021) or positive effects (Crutsinger et al. 2008; Uesugi et al. 2016). Such positive effect may last even after senescence, with the establishment of possible mutualistic interactions, such as the indirect defense mechanisms against enemies by ants and spiders. In this context, considering the high cost of producing galls, the host plant may have a compensation *a posteriori*, as they obtain secondary advantages from ecological roles played by gall occupants that can benefit the plants in different ways.

Shelter-builders that make long-lasting microhabitats can potentially initiate community-wide indirect effects, probably more than organisms that form ephemeral structures (Cuddington et al. 2007). Few galls are induced by *P. oligophaga* in each *M. radula* along the bivoltine life cycle of the galls (personal observation), but the endurance of these structures for years in the plants promotes the accumulation of shelters in the same host, increasing the supply of shelter resources along the seasons, especially the unfavorable ones (Cuddington et al. 2007). In a review, Cornelissen et al. (2016) pointed out that the efficiency of shelter-building arthropods and their impacts on communities was experimentally tested in several studies, but with microhabitats created by leaf rollers with simulations of shelters manipulating the leaves with objects (e.g., Lill and Marquis 2003, 2004; Crawford et al. 2007; Vieira and Romero 2013). Considering that these shelters are ephemeral (Cornelissen et al. 2016), the durability of the microhabitats of galls induced in buds can represent different impacts on the biodiversity patterns of the communities they occupy when compared to leaf-rollers, and this can be an important field of investigation, although the reproduction of a model that represents the peculiar characteristics of complex bud galls is challenging.

Conclusions

The histological pattern of the bud galls induced on *M. radula*, as the stem-branch vascular connection, allows these galls to remain fixed on the host plants for long periods. The *M. radula* galls develop as stems with shortened internodes with abundant leaf-like projections. So, as the gall remains attached to the host after the senescence, they function as shelters for different taxa of arthropods in distinct ecosystems. These interactions go beyond impacts to both galling insect and host plant populations, with trophic and non-trophic interactions with other organisms, which allow us to understand insect *P. oligophaga* as a shelter-builder and ecosystem engineer.

Table and Figures

Table 1 Arthropod taxa collected in the senescent galls induced by *Palaeomystella oligophaga* on *Macairea radula* populations from the highway, the wetland, and the waterfall ecosystems, in number of occurrence and total frequencies (%).

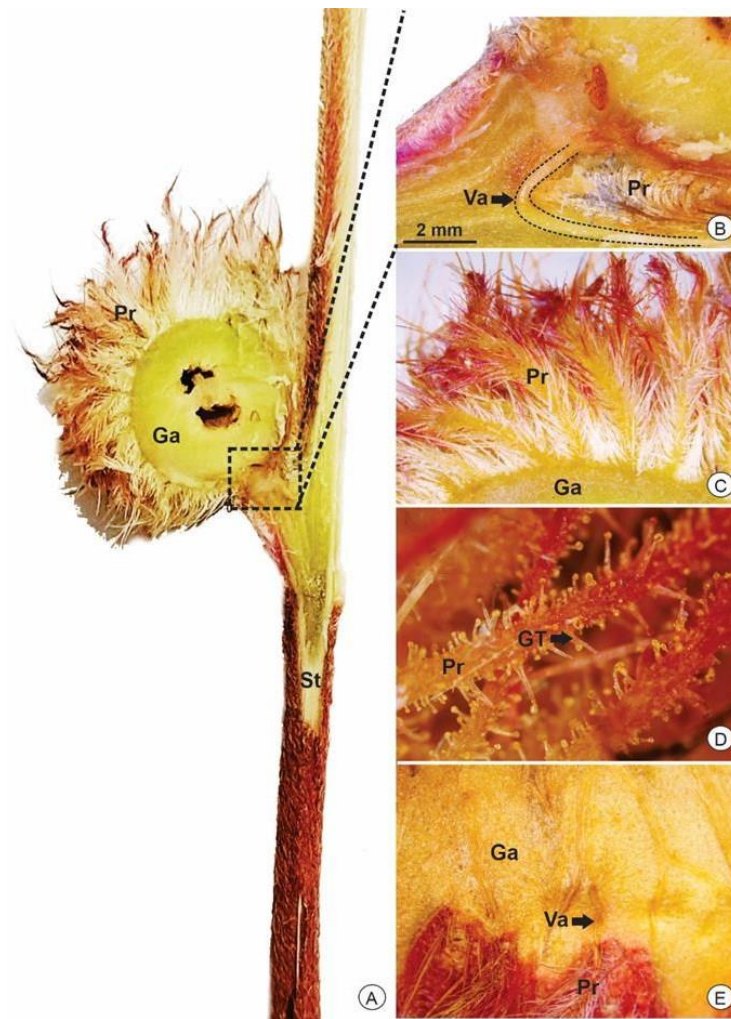
	<i>Location</i>		
	Highway	Wetland	Waterfall
Beetles' total frequency	26,43%	26%	5,33%
Bostrichidae	16	78	34
Mealybugs' total frequency	2,86%	–	2,67%
Dactylipiidae	8	0	4
Ants' total frequency	2,86%	2%	9,67%
<i>Crematogaster</i> sp.	1	0	0
nest – <i>Crematogaster</i> sp.	2	0	0
<i>Cephalotes pusillus</i>	0	14	0
nest – <i>C. pusillus</i>	2	1	0
<i>Pseudomyrmex gracilis</i>	0	0	3
nest – <i>P. gracilis</i>	1	0	1
Spider' total frequency	11,43%	9,67%	1,67%
<i>Salticinae</i>	4	0	2
exuvia	0	8	3
ootheca	0	3	10
silk	1	18	1



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365 **Fig. 1** Galls on *Macairea radula* induced by *Palaeomystella oligophaga*. A- Mature
366 globoid-shaped galls with *P. oligophaga* larvae inside; B- Senescent gall attached to the
367 host's stem; C- Senescent gall with degenerated emergencies, after gall remained attached
368 to the host's stem for a long period.

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Fig. 2 Macroscopical view of galls on *Macairea radula* induced by *Palaeomystella oligophaga*. A- General view of the sectioned gall; B- Detail of the connection between the gall and the stem, showing a continuous and peripheral vascularization; C- Detail of the leaf-like projections on the gall surface; D- Glandular trichomes on the leaf-like projections; E- Detail of vascularization of the leaf-like projections. *Abbreviations:* Ga- Gall; GT- Glandular trichome; Pr- Projection; Va- Vascularization.

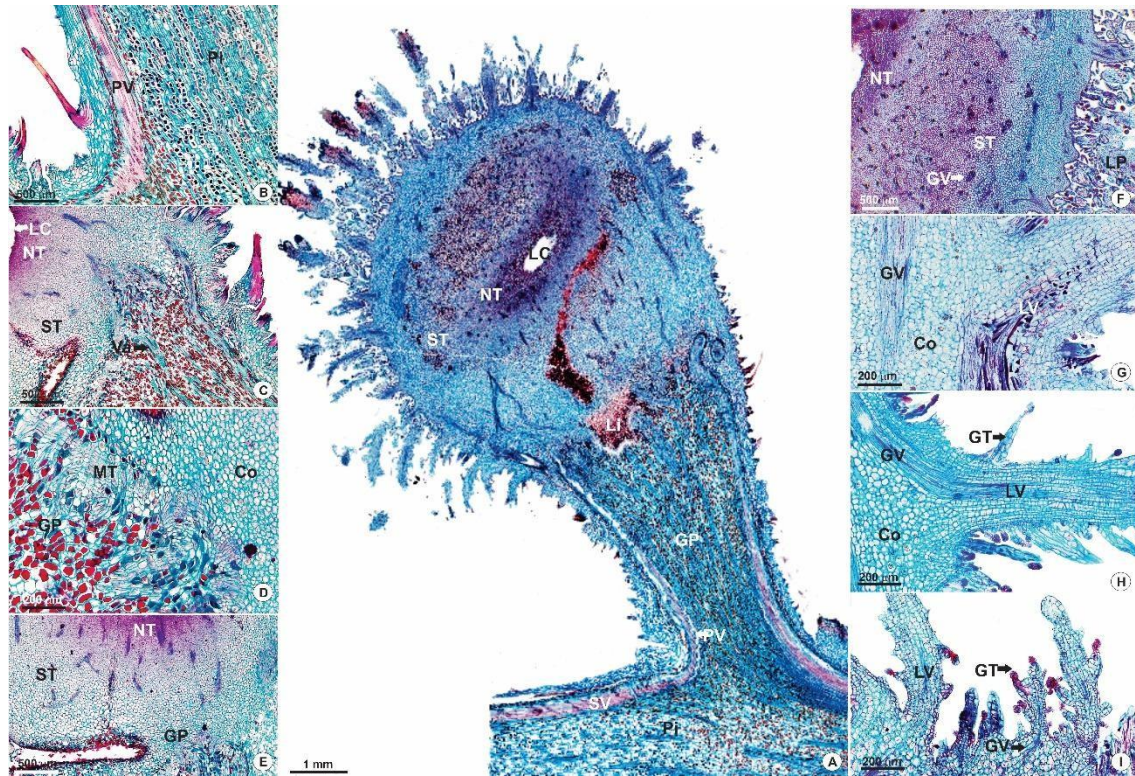


Fig. 3 Anatomical features of *Macairea radula* galls induced by *Palaeomystella oligophaga*. A- General view of the gall, with a single larval chamber, nutritive tissue and storage tissue (parenchymatous cortex); B- Connection between the stem and the gall peduncle, forming a continuous with peripheral vascularization; C, D- End of the gall peduncle, showing new vascular bundles differentiated from newly-formed procambial strands; E, F- Vascular bundles dispersed in gall parenchymatous cortex; G- Presence of a leaf gap at the base of the leaf-like projection; H, I- Detail of the leaf-like projections with glandular trichomes. *Abbreviations:* Co- Cortex; GP- Ground parenchyma; GT- Glandular trichome; GV- Gall vascularization; LC- Larval chamber; Li- Lignification; LP- Leaf-like projection LV- Leaf-like projection vascularization; MT- Meristem tissue; NT- Nutritive tissue; Pi- Pith; PV- Peduncle vascularization; ST- Storage tissue; SV- Stem vascularization; Va- Vascularization.

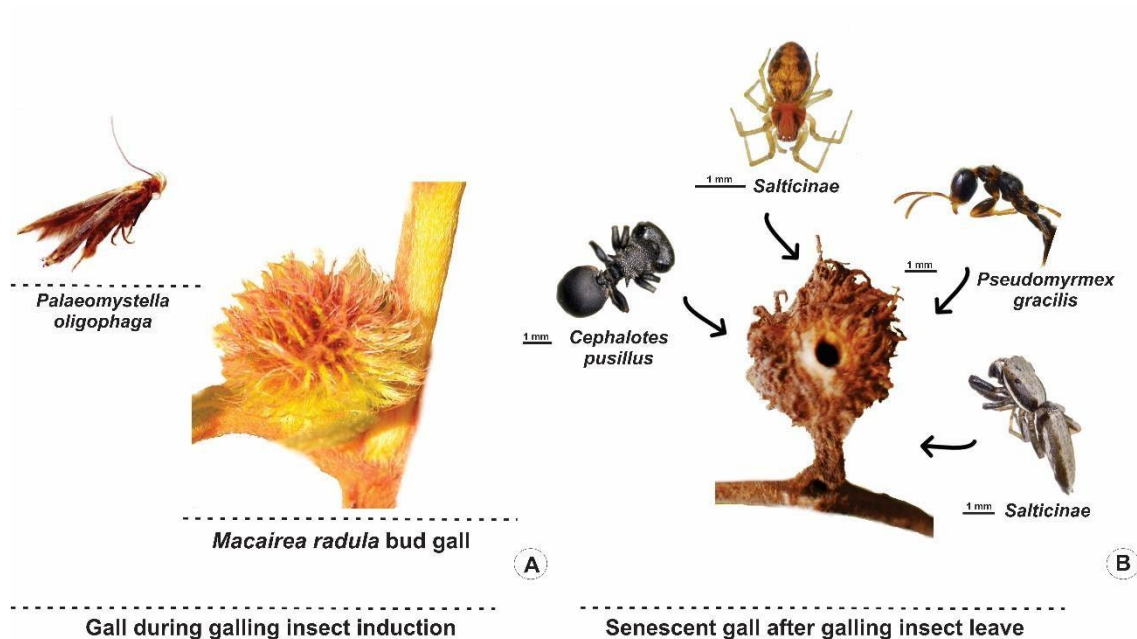


Fig. 4 Features of *Macairea radula* galls occupied by *Palaeomystella oligophaga* (A) and remaining senescent structure after *P. oligophaga* adults leave the galls (B). B- Detail of some arthropods that occupy the larval chamber of senescent galls.

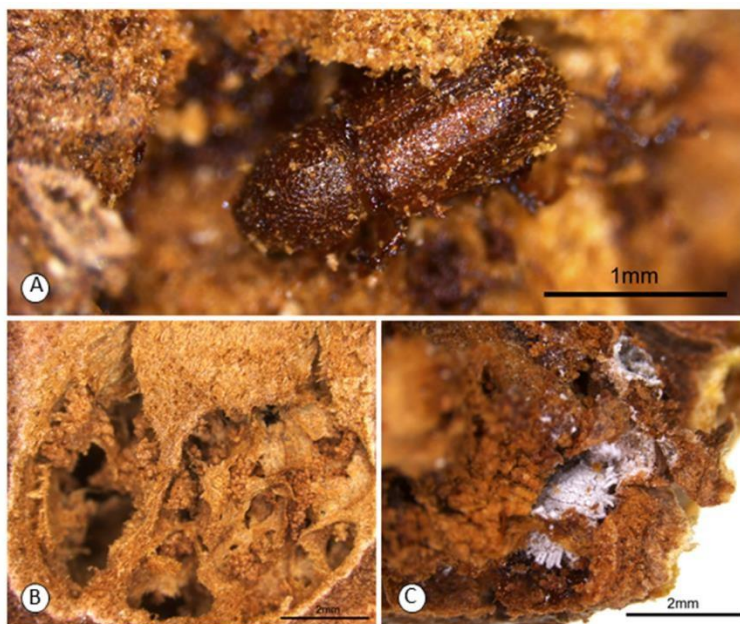


Fig. 5 Senescent galls of *Macairea radula* occupied by Bostrichidae beetles (A) that alter part of the internal structure of the galls by boring tunnels inside the gall walls (B), and Dactylipiidae mealybugs located in a cavity at the gall insertion point on the stem of *M. radula* (C).

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CONSIDERAÇÕES FINAIS E PERSPECTIVAS FUTURAS

Os resultados obtidos nesta Tese expõem algumas particularidades das galhas como microhabitats, no contexto de interações tróficas e não tróficas que nelas se estabelecem. Por exemplo, em uma interação tri-trófica, que envolve uma interação herbívoro-planta, espera-se que a planta se beneficie da interação indireta com o consumidor do herbívoro. Porém, como mostrado aqui, um parasitoide ao consumir o inseto galhador pode gerar o aumento de tecidos nas galhas em que se desenvolvem, ou seja, podem gerar maior dispêndio energético da planta para manter esta estrutura, embora ainda promovam o controle populacional dos insetos galhadores. Diversas interações tróficas podem estar envolvidas com os estágios de desenvolvimento das galhas, e outros insetos que interagem com os insetos galhadores, direta ou indiretamente, podem especializar-se em interagir com esse sistema, e assim sincronizar o ciclo de vida com o desenvolvimento das galhas. Assim, as interações que resultam no desenvolvimento de galhas podem ser recurso para artrópodes de várias espécies das comunidades nos ecossistemas que coabitam.

Os inimigos diretos e indiretos dos galhadores não encontram apenas recurso alimentar ao interagir com as galhas, mas também conseguem abrigar-se usufruindo de um microclima mais estável em relação ao meio externo, escapar de condições adversas incluindo estocasticidades como incêndios, e proteger-se de possíveis inimigos naturais. Ainda, a depender do órgão da planta hospedeira em que as galhas sejam induzidas, estas podem manifestar características estruturais que ofereçam vantagens particulares a comunidade de artrópodes mesmo após a emergência dos insetos galhadores e seus inimigos naturais. A escolha do sítio de oviposição pela fêmea galhadora pode ser determinante no papel que estas estruturas vegetais (as galhas) vão representar a nível de

comunidade e, portanto, de ecossistema. Como no caso das fêmeas de *Palaeomystella oligophaga* induzindo galhas nas gemas axilares de *Macairea radula*, com galhas que se desenvolvem como ramos modificados e na senescência permanecem fixadas nas plantas hospedeiras por anos, fornecendo abrigos para inúmeros artrópodes como formigas e aranhas que podem colonizar e ocupar estas estruturas por longos períodos.

Galhas de insetos são um micro-laboratório de estudos de interações multitróficas e não-tróficas, porém, concluímos que existem muitos desafios quanto ao delineamento amostral para testar conceitos e hipóteses ecológicas, envolvendo galhas. Os sistemas de galhas como modelo de estudos têm algumas peculiaridades em relação a outros herbívoros de vida-livre ou construtores de abrigo como dobradores de folhas. Em alguns casos, é necessário inicialmente compreender o funcionamento do sistema, da interação entre insetos galhadores e plantas hospedeiras (como os processos do desenvolvimento das galhas da indução a senescência, e o sincronismo dos ciclos de vida de ambos) para então pensar e planejar coletas, análises ou modelos experimentais para pesquisar outras questões relacionadas ao entorno biótico e abiótico em que estes sistemas estão inseridos. Portanto, entender a estrutura histológica destes microhabitats, quais organismos ocorrem no sistema galha-planta hospedeira, e quais tipos de interações estão ocorrendo, é um trabalho de história natural fundamental, para formar uma base de conhecimentos que possibilite outras investigações. Como a importância das galhas nos ecossistemas que ocupam na manutenção e incremento das comunidades de artrópodes, indicadores de qualidade ambiental, importância de preservação de plantas hospedeiras de galhas como abrigo para diversos organismos, entre outras.

Os conhecimentos obtidos pelos estudos de história natural às pesquisas experimentais devem ir além das paredes do programa de pós-graduação. Desejo que os conhecimentos discutidos e apresentados nessa Tese possam chegar a outros pós-

graduandos e graduandos do Instituto de Biologia da UFU, a outros de outros cursos, e cidades, e que minha avó possa e compreender também, assim como meus sobrinhos, toda a família, amigos, vizinhos... quero transformar estas pesquisas em histórias contatadas de forma que muitos possam entender o que apresentamos aqui formalmente. Para isso, desenvolveremos vídeos de divulgação científica para redes sociais, onde serão apresentadas as informações dos artigos após publicados, de maneira ilustrada, narrada e legendada para maior alcance. Também, está em construção uma história ilustrada sobre a história de vida dos insetos galhadores *P. oligophaga* nos estágios que se desenvolvem nas galhas em *M. radula*.