Universidade Federal De Uberlândia – UFU
Instituto de Biologia
Programa De Pós Graduação em Ecologia e Conservação de Recursos Naturais

# "INTERAÇÕES MULTITRÓFICAS NO CERRADO: RESULTADOS CONDICIONAIS NAS RELAÇÕES ENTRE PLANTAS, HERBÍVOROS E PREDADORES"

# "MULTITROPHIC INTERACTIONS IN A BRAZILIAN SAVANNA: CONDITIONAL OUTCOMES OF THE RELATIONSHIPS AMONG PLANTS, HERBIVORES AND PREDATORS"

**ESTEVÃO ALVES DA SILVA**

UBERLÂNDIA – MG
FEVEREIRO, 2014

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

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# ÍNDICE

## *RESUMO*

O Cerrado brasileiro compõe a vegetação predominante no Brasil central e a ocorrência de organismos neste bioma é controlada por fatores bióticos e abióticos que podem influenciar as interações multitróficas entre plantas e animais. As condições para a persistência de organismos na natureza é de grande interesse na ecologia de conservação. Entretanto, somente com o conhecimento da história de vida e das interações entre as espécies, podemos fornecer subsídios para a sua preservação em áreas naturais. Nesta tese, apresentamos uma série de estudos originais envolvendo diversas interações entre plantas com nectários extraflorais, herbívoros e seus inimigos naturais, e analisamos os custos e benefícios destas interações para as plantas. Na primeira parte da tese, investigamos como uma variável abiótica (fogo) afetou o mutualismo facultativo entre formigas e plantas e como as formigas e herbívoros responderam ao crescimento das plantas após o fogo. Mostramos que o fogo promoveu o rápido crescimento de *Banisteriopsis campestris* e que isso afetou a qualidade do néctar extrafloral, ao aumentar a concentração de açúcar. Consequentemente, mais formigas visitaram a planta e menor foi a taxa de herbivoria. Porém, em outra planta, *B. malifolia*, pequenos herbívoros eram imunes ao ataque de formigas e provocavam sérios danos às folhas. A segunda parte da tese é dedicada à avaliação do papel de vespas e formigas predadoras na população de herbívoros endofíticos. Mostramos que vespas são mais importantes que as formigas quando se trata de controlar herbívoros que vivem dentro de botões florais. Para concluir, na terceira parte da tese, mostramos como interações inseto-planta são condicionais à variáveis bióticas e físicas e as estratégias que larvas de borboletas mirmecófilas (licenídeos) e tripes usam para escapar do ataque de formigas para se alimentarem das estruturas reprodutivas das plantas. As larvas de licenídeos produzem uma solução açucarada, que serve de alimento para as formigas. Tripes possuem defesas químicas que afastam as formigas. Estes pequenos herbívoros se alimentam e reproduzem em frutos (sâmaras), o que atrapalha a dispersão das sâmaras pelo vento, já que frutos danificados são bastante disformes. Nossos estudos contribuem para um melhor entendimento das interações inseto-planta no cerrado, principalmente no que diz respeito às plantas com nectários extraflorais. O mutualismo facultativo formiga-planta é uma das interações mais interessantes na natureza, porém é altamente condicional e os custos e benefícios destas interações dependem das partes envolvidas (formigas e herbívoros) e de variáveis abióticas.

## *ABSTRACT*

The Cerrado biome (Brazilian savanna) is the predominant natural vegetation of Central Brazil and the occurrence of organisms in this biome is restrained by biotic and abiotic factors which may influence the inter-specific interactions between insects and plants. The conditions for the persistence of organisms in nature are a main concern in conservation ecology. Nonetheless, only with the knowledge of organisms' natural history and ecological interactions we can give support for their preservation in natural areas. In this thesis we present a series of original studies assessing the diverse interactions among extrafloral nectaried plants, herbivores and their possible natural enemies; and the costs and benefits to plants in supporting insect interactions. In the first part of the thesis we show how an abiotic variable (fire) affected the facultative mutualism between ants and plants and how ants and herbivores responded to the resprouting of plants after fire. It was shown that fire promoted a rapid growth of *Banisteriopsis campestris* and affected the quality of extrafloral nectar, by increasing sugar concentration. As a consequence, more ants were attracted to the plants, thus decreasing herbivory rates. Nonetheless, in other plant species, *B. malifolia*, tiny herbivorous were immune to ant attack and inflicted serious damage to leaves. The second part of the thesis is dedicated to evaluate the role of predatory wasps and ants on the deterrence of endophytic herbivores. We show that predatory wasps were more important than ants in the deterrence of endophytic beetles. To conclude, in the third part of this thesis we also show how plant-insect relationships are conditional (to biotic and abiotic factors) and the strategies that caterpillars and thrips use to escape from ant attack while feeding on plant reproductive parts. Florivorous myrmecophilous lycaenid caterpillars release sugared substances to ant-guards and are not attacked. Thrips co-occurred with ants in extrafloral nectaried plants and their herbivory may seem small and negligible in leaves and flower buds, but fruits (samaras) damaged by thrips are distorted and asymmetric, what restrains its dispersive capabilities by wind. Our studies contribute to a better understanding of plant-insect relationships in the cerrado, the largest hotspot in Brazil. The mutualism between extrafloral nectaried plants and ants is one of the most interesting interactions in nature, but it is highly conditional and the costs and benefits involved in these associations depend on the parties involved (ants and herbivores) and abiotic factors.

# INTRODUÇÃO GERAL

## Interações inseto-planta

Nas últimas décadas, tem havido um grande volume de estudos com várias abordagens que exploram as diversas facetas da história natural e as relações entre insetos (em diversas guildas) e suas plantas associadas/hospedeiras. Durante muito tempo, e mesmo atualmente, uma importante questão que ainda permanece sem resposta definitiva refere-se à influência das características químicas, morfológicas e fenológicas das plantas na ocorrência e sobrevivência de herbívoros (Thompson 1988; Power 1992). Diante disso, muitas hipóteses foram levantadas para se tentar sanar, ao menos em parte, este problema, como a "hipótese da diversidade de recursos" (Lawton 1983), a "hipótese do estresse da planta" (White 1984) e a "hipótese do vigor da planta" (Price 1991). Basicamente, estas teorias assumem que as populações dos herbívoros são controladas pela disponibilidade de recursos oferecidos pelas plantas, como tecidos comestíveis, nutrientes e abrigo ou esconderijos. De fato, esta abordagem tem sido extensivamente estudada e têm revelado, por exemplo, que besouros que se alimentam de folhas tenras e macias apresentam melhor desenvolvimento (Raupp 1985), galhadores e minadores possuem preferência por folhas com maior quantidade de nitrogênio livre (Cornelissen & Stiling 2005) e que a ocorrência de tripes em uma determinada planta depende principalmente de fontes abundantes de alimento e locais para reprodução (Mound & Terry 2001).

Entretanto, outro ponto de vista estabelece que as populações de herbívoros são controladas pelos seus inimigos naturais (Nahas et al. 2012). Os exemplos clássicos nos remetem ao uso de espécies predadoras (p. ex. vespas, formigas, hemípteros, ácaros, etc.) como agentes de controle biológico em culturas economicamente importantes (Van Meele 2008). Também não se pode ignorar o papel das formigas e das vespas que atacam ou afugentam os herbívoros que ocorrem em plantas com nectários extraflorais (Nascimento & Del-Claro 2010; Pereira & Trigo 2013).

Em resumo, apesar da disponibilidade de recursos para herbívoros e da presença de inimigos naturais aparentarem serem conjeturas ecológicas conflitantes, tanto as forças base-topo (recursos) e as topo-base (predadores, parasitas e parasitóides) estão intimamente relacionadas e são responsáveis pela manutenção de cadeias alimentares e interações multitróficas (Hunter & Price 1992; Carter & Rysptra 1995).

A herbivoria pode ser uma força evolutiva poderosa em diversos ecossistemas, já que as plantas são atacadas por uma grande variedade de insetos que podem se alimentar de todas as estruturas das plantas, particularmente de folhas, flores e frutos (Del-Claro 2004). As plantas diferem significativamente na capacidade de se desenvolver e reproduzir após o dano, mas geralmente a herbivoria afeta a qualidade, fotossíntese, crescimento, fenologia e capacidade reprodutiva das plantas (Poveda et al. 2003; Badenes-Pérez et al. 2010). Desta forma, é certo assumir que a evolução favoreceu aquelas espécies de plantas com certas defesas ou barreiras contra danos severos (Schneider et al. 2002; Macel *et al.* 2005).

De fato, muitas plantas contam com as mais variadas defesas contra herbívoros, na forma de taninos, látex, tricomas e compostos secundários, entre outros (Coley 1986; Lucas *et al.* 2000; Agrawal & Konno 2009). Além destas defesas estruturais, físicas e químicas, em muitas partes do mundo, notadamente na região Neotropical, muitas espécies de plantas apresentam nectários extraflorais (NEFs) (Machado et al. 2008). Estas estruturas são responsáveis pela atração e manutenção de insetos predadores/carnívoros, os quais comumente são agressivos e atacam herbívoros de forma geral (Bentley 1977; Oliveira et al. 1999). Os NEFs estão presentes em mais de 300 gêneros botânicos (González-Teuber & Heil 2009), ocorrendo em uma grande fração de plantas em diversos biomas (Oliveira & Leitão-Filho 1987; Blüthgen & Reifenrath 2003; Díaz-Castelazo et al. 2004). Estas estruturas liberam uma solução composta de carboidratos e aminoácidos, a qual é uma fonte de alimento valiosa para as formigas (Shenoy et al. 2012). A comunidade de formigas associada à plantas com NEFs é enorme (Blüthgen et al. 2000) e como estas formigas são conhecidas como predadoras, sua ocorrência em plantas com NEFs pode conferir benefícios como aumento no desenvolvimento e capacidade reprodutiva da planta (Nascimento & Del-Claro 2010).

Entretanto, é crescente o número de estudos mostrando que nem sempre as formigas desempenham um papel benéfico para a planta. Isto se deve ao fato de o mutualismo facultativo formiga-planta ser altamente condicional e os resultados desta interação dependerem de uma miríade de fatores como a identidade e abundância das formigas, a localização e estrutura dos NEFs, a qualidade e quantidade do néctar extrafloral, a estratégia de forrageamento e recrutamento das formigas e a identidade e comportamento dos herbívoros (Apple & Feener Jr. 2001; Katayama & Suzuki 2004; Bächtold et al. 2012).

Para se entender o papel de herbívoros e inimigos naturais na ecologia de interações multitróficas, alguns fatores precisam ser investigados, como a regularidade espacial e temporal, a abundância e a história natural das espécies envolvidas (Del-Claro 2004). Sugere-se que a escolha de plantas representativas e abundantes, com ampla distribuição geográfica seja promissora para o estudo de interações, uma vez que pode permitir generalizações e replicações.

O Cerrado é um importante bioma brasileiro cobrindo aproximadamente dois milhões de km$^2$, abrangendo 23% do território brasileiro (Furley & Ratter 1988). O Cerrado se destaca tanto pela diversidade quanto pelo endemismo de espécies (Castro et al. 1999; Klink & Machado 2005). Plantas com NEFs são muito comuns no Cerrado e atraem uma grande diversidade de formigas (Machado et al. 2008; Nascimento & Del-Claro 2010). Porém, somente nos últimos anos têm-se focado esforços em tentar desvendar os diferentes aspectos da ecologia inseto-planta no Cerrado (Oliveira & Marquis 2002), e muito ainda resta a ser investigado, principalmente no que diz respeito às interações entre plantas com NEFs, formigas e herbívoros, pois essas relações envolvem uma parcela importante da entomofauna do Cerrado.

## Malpighiaceae

Malpighiaceae contém aproximadamente 1250 espécies de árvores, arbustos e lianas (em 65 gêneros) que são predominantemente concentradas na região tropical e subtropical, sendo que os neotrópicos respondem por 85% desta diversidade (Anderson 1990; Vogel 1990; Araújo et al. 2010). No Brasil, Malpighiaceae está bem representada, com 300 espécies em 45 gêneros, e é uma das famílias mais diversas no Cerrado, com 126 espécies em 16 gêneros (Conceição 2011). As espécies podem ser encontradas nos mais diversos habitats, apresentando tanto características de plantas de crescimento lento quanto de pioneiras (Schmidt 2005; Medeiros & Miranda 2008; Alves-Silva 2011).

As flores de Malpighiaceae são facilmente reconhecidas no campo, já que suas flores são bastante conservativas em aspecto (Vogel 1990). A corola possui cinco pétalas livres, uma das quais se destaca do plano de simetria das demais e é conhecida como pétala estandarte (Anderson 1979; Johnson 1986). A coloração das flores pode variar de branco, amarela ou azul, mas vermelha é a cor predominante (Anderson 1979; Vogel 1990; Sigrist & Sazima 2004; Mendes et al. 2011). As flores também possuem cinco sépalas que crescem em direção ao centro das flores, formando câmaras florais que servem de microhabitat para insetos herbívoros (Del-Claro et al. 1997). As sépalas podem apresentar de oito a 10 glândulas de óleo, que são o único recurso (além de pólen) oferecido aos polinizadores, já que as flores de Malpighiaceae não produzem néctar (Gaglianone 2003; Sigrist & Sazima 2004; Teixeira & Machado 2009). As glândulas de óleo já estão presentes nos botões florais e persistem até a formação dos frutos (Vicentini & Anderson 1999). Os frutos são morfologicamente diversos; os gêneros *Banisteriopsis* e *Peixotoa* produzem sâmaras com duas ou três asas, que são dispersos pelo vento. *Byrsonima* possui frutos carnosos que são consumidos por aves (Mirle & Burnham 1999; Vicentini & Anderson 1999). As folhas podem ser de vários tipos, como ovadas, lanceoladas e elípticas, com ou sem tricomas. Além disso, em alguns gêneros, como *Peixotoa*, *Banisteriopsis* e *Heteropterys* as folhas possuem um par de nectários extraflorais na base das folhas (Araújo et al. 2010) que são atendidos por formigas e vespas principalmente.

O fato de muitas espécies apresentarem nectários extraflorais é um fator responsável pela ocorrência de formigas nas plantas, as quais podem atuar na remoção de herbívoros que de outra forma poderiam infligir danos à várias estruturas das plantas. Nesta tese, nosso objetivo foi mostrar que Malpighiaceae pode ser usada como modelo para uma série de estudos envolvendo interações multitróficas, especialmente aquelas entre herbívoros e seus inimigos naturais. O entendimento dos efeitos de certas guildas (como herbívoros e formigas que se alimentam de nectários extraflorais) no desenvolvimento e no fitness de Malpighiaceae pode fornecer importantes avançoss para a uma melhor compreensão das interações multitróficas.

## OBJETIVOS GERAIS

**Investigar:**

- o efeito de variáveis ambientais no desenvolvimento e na qualidade dos recursos oferecidos aos insetos associados, e como isto indiretamente afeta as relações formiga-inseto-planta;

- o impacto da ação de um terceiro nível trófico na população de herbívoros endofíticos, que devido à este hábito conseguem escapar do ataque de formigas, mas não de vespas predadoras;

- as relações facultativas condicionais entre formigas e herbívoros e como estes últimos conseguem co-ocorrer com seus possíveis inimigos naturais, enquanto provocam um grande dano na planta hospedeira;

Estes objetivos serão abordados dentro de seis estudos publicados e/ou submetidos à revistas internacionais com corpo editorial. Cada estudo possui particularidades interessantes, porém todos estão interligados a denominadores comuns, como a família botânica (Malpighiaceae), a presença de espécies vegetais com nectários extraflorais e consequentemente, a ocorrência de formigas. Os artigos resultantes dessa tese são os seguintes:

**1** - Fire triggers the activity of extrafloral nectaries, but ants fail to protect the plant against herbivores in a neotropical savanna.

**2** - Effect of post-fire resprouting on leaf fluctuating asymmetry, extrafloral nectar quality, and ant-plant-herbivore interactions. Naturwissenschaften 100:525-532. (2013).

**3** - Foraging behavior of *Brachygastra lecheguana* (Hymenoptera: Vespidae) on *Banisteriopsis malifolia* (Malpighiaceae): Extrafloral nectar consumption and herbivore predation in a tending ant system. Entomological Science 16:162-169. (2013).

**4** - Ant-herbivore interactions in an extrafloral nectaried plant: Are ants good plant guards against curculionids beetles?

**5** - Influence of *Camponotus blandus* (Formicinae) and flower buds on the occurrence of *Parrhasius polibetes* (Lepidoptera: Lycaenidae) in *Banisteriopsis malifolia* (Malpighiaceae). Sociobiology 60:30-34. (2013)

**6** - The role, or the lack thereof, of patrolling ants on the deterrence of herbivore thrips: Effect on plant reproductive outputs?

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# PRIMEIRA PARTE

Objetivo: Investigar o efeito de variáveis ambientais no desenvolvimento e qualidade dos recursos oferecidos aos insetos associados; e como isto indiretamente afeta as relações formiga-inseto-planta

## CAPÍTULOS

**1** - Fire triggers the activity of extrafloral nectaries, but ants fail to protect the plant against herbivores in a neotropical savanna

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

# CAPÍTULO I

Arthropod-Plant Interactions
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ORIGINAL PAPER

## Fire triggers the activity of extrafloral nectaries, but ants fail to protect the plant against herbivores in a neotropical savanna

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# Fire triggers the activity of extrafloral nectaries, but ants fail to protect the plant against herbivores in a neotropical savanna

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## Abstract

Herbivores are attracted to young shoots and leaves due to their tender tissues. However, in extrafloral nectaried plants, young leaves also attract patrolling ants which may chase or prey on herbivores. We examined this scenario in extrafloral nectaried shrubs of *Banisteriopsis malifolia* resprouting after fire, which promoted both the aseasonal production of leaves and the activity of extrafloral nectaries. Results were compared between resprouting (burnt) and unburnt control plants. The aggressive ant species *Camponotus crassus* and the herbivorous thrips *Pseudophilothrips obscuricornis* were respectively rapidly attracted to resprouting plants because of the active extrafloral nectaries and their less sclerophyllous leaves. The abundance of these insects was almost negligible in the control (unburnt) shrubs. Ants failed to protect *B. malifolia*, as no thrips were preyed upon or injured by ants in resprouting plants. Consequently, on average, thirty-seven percent of leaves from resprouting shrubs' had necrosis marks. Upon contact with ants, thrips released small liquid droplets from their abdomen which rapidly displaced ants from the surroundings. This study shows that *P. obscuricornis* disrupted the facultative mutualism between *C. crassus* and *B. malifolia*, since ants received extrafloral nectar from plants, but were unable to deter herbivore thrips.

**Keywords:** *Camponotus* · Extrafloral nectary · Malpighiaceae · Mutualism · Phenology · *Pseudophilothrips*

## Introduction

Plant-herbivore interactions are among the most pervasive interactions on earth and have been the focus of many studies which aimed to understand the factors responsible for the occurrence of herbivore insects in nature (Basset 1991; Marquis et al. 2001; Macel et al. 2005). Some studies point out that insect populations are related principally to the availability of resources such as edible tissues, free nitrogen and shelter for resting and/or hiding (Lill and Marquis 2003; Cornelissen et al. 2008; Jones et al. 2008). Nonetheless another point of view claims that herbivores are also controlled by natural enemies or factors related to their mortality (Power 1992; Carter and Rypstra 1995). In a community context, however, both resources and natural enemies are inter-connected (Santos et al. 2008) and responsible for the maintenance of food chains and multitrophic interactions in various ecosystems (Hunter and Price 1992).

The influence of resources in the occurrence of herbivores has been commonly observed in plants growing after a fire. Quickly resprouting shoots often attract significantly more herbivores than unburnt non-regenerating plants (Seyffarth et al. 1996; Vieira et al. 1996). In the Brazilian savanna, fire is the primary natural disturbance and as such, many plant species have adapted to ensure regrowth of above-ground parts (Medeiros and Miranda

2008; Silva et al. 2009). In some cases fire alters the seasonal phenology of plants (Schmidt et al. 2005) and evidence shows that such plants are more attacked by herbivores (Moreno and Oechel 1991). It occurs because the rapid growth of above-ground structures (leaves, shoots, branches and stems) provides young, more nutritious and less sclerophyllous (hard, tough, leathery) leaves (Reich et al. 1990; Rieske et al. 2002). Nevertheless, herbivores are not the only beings attracted to resprouting plants. As recently shown, fire can also affect the amount of active extrafloral nectaries (EFNs), thus strengthening the outcomes of ant-plant interactions as more ants will be attracted to these nectar producing glands (Alves-Silva and Del-Claro 2013). Furthermore fire may also increase plant structural complexity (i.e. number of shoots and leaves; Lawton 1983) thus increasing the abundance of associated arthropods which feed and/or reproduce on the young tissues (Vieira et al. 1996; Alves-Silva 2011).

Herbivorous thrips (Insecta: Thysanoptera) are very common in complex and heterogeneous habitats (Milne and Walter 2000) and their abundance is primarily influenced by plant resources, such as food and different structures for foraging and reproduction (Moog et al. 2002; Kersch and Fonseca 2005). Despite being ubiquitous among all kinds of growing vegetation (Alves-Silva and Del-Claro 2010), thrips have few known natural enemies, typically leading to massive infestation in several plant species (Morse and Hoddle 2006). Thrips' co-occurrence with aggressive ant species in extrafloral nectaried plants is often observed (Mody and Linsenmair 2004; Alves-Silva 2011), but interestingly, interactions between the parties have been scarcely studied (Del-Claro et al. 1997; Peng and Christian 2004) despite the possible role of ants as biological control agents against pest thrips (Van Mele 2008).

Recently, the thrips species *Pseudophilothrips obscuricornis* (Priesner 1921) (Phlaeothripidae) was observed among regenerating individuals of the extrafloral nectaried shrub *Banisteriopsis malifolia* (Nees & Mart.) B. Gates (Malpighiaceae) after a fire (personal observation). Thrips fed on young leaves and co-occurred with the ant species *Camponotus crassus* (Mayr 1887) (Formicinae). This ant species is commonly observed in extrafloral nectaried plants and is known to be very aggressive towards herbivores and other predatory insects (Oliveira and Freitas 2004; Alves-Silva et al. 2013). In our system, fire occurred in the dry season (July), a period of year when *B. malifolia* EFNs are inactive and leaves are senescent, but resprouting plants presented aseasonal leaf production and fully functional EFNs.

Therefore, in this study we aimed to investigate the influence of the aseasonal phenology of *B. malifolia* on the abundance and interactions of ants and thrips. The study was divided in three objectives: *(i)* to compare the abundance of insects in resprouting and control plants, taking into account the activity of EFNs, plant architecture, and quality; *(ii)* to examine possible antagonistic interactions of ants towards thrips; and *(iii)* to investigate the effect of the latter on plant herbivory.

## Methods

### Study area

Fieldwork was carried out in a *sensu stricto* cerrado area (18º59' S, 48º18' W) in Uberlândia City, Brazil, from May to August 2010. The cerrado covers approximately 230 ha and is dominated by grasses, shrubs (Malpighiaceae, Bignoniaceae, Myrtaceae and Fabaceae) and trees (Caryocaraceae, Ochnaceae, Vochysiaceae) ranging 2–4 m tall. The average rainfall in the region is approximately 1500 mm per year, of which > 90% occurs during the wet season (October - April). Mean monthly temperatures range from 24.8ºC in February to 19.9ºC in June, with an annual mean of 23ºC (Laboratory of Climatology, Federal University of Uberlândia).

## Study system

*Banisteriopsis malifolia* is a much-branched shrub (< 2 m in height). Leaf size and color are related to plant age, with young leaves being small, tender and greenish in color. Fully expanded leaves, which are typically tough and dark green, may reach up to 15 cm in length and 10 cm in width. Leaves possess a pair of EFNs at the base near the petiole, on each side of the midrib (Fig. 1a). *Camponotus crassus* (~ 5 mm in length) is one of the primary EFN feeders in *B. malifolia*, while *P. obscuricornis* is a main sap sucker, feeding on leaves and shoots (Fig.1b). Adults (~ 2 mm in length) and larvae (roughly 1 mm in length) are found in groups of up to 10 individuals under the leaves, where they can remain for prolonged periods while feeding on plant content. Adults are black and winged while larvae are wingless and reddish in color. Thrips herbivory is characterized by brown necrosis spots on the leaf blade (Chen and Willimas 2006), but in high infestations thrips may kill growing tips and shoots (Figure 1c, d).

In May 2010, part of the cerrado area (15600 m²) was burned during an incidental fire. Although all above-ground parts of *B. malifolia* plants were burned, the main stem survived, so new leaves and branches began to regrow from lateral meristems. Leaf production started three weeks after the fire in mid-June. We took this fire as a chance for an experimental manipulation, since nowadays intentional fires in cerrado are prohibited, regardless of scientific purposes. During a field survey in June, all *B. malifolia* plants within the burnt area (*N* = 18) were tagged. These resprouting specimens were considered the treatment group and hereafter referred to as '*resprouting*'. Another 18 specimens were tagged in an adjacent unburnt area to act as control plants. These control specimens did not appear to undergo any resprouting during the study period. This group is referred to hereafter as '*unburnt*'. Plants in both groups were growing in an area characterized by the presence of a fair amount of grasses and shrubs species. All *B. malifolia* in this study were not shaded by the canopy of large trees and received direct sunlight all day long.

## Arthropod abundance in resprouting and unburnt plants

Insect (thrips and ants) sampling was accomplished by visual censuses on each *B. malifolia* plant in the mornings (~ 08:00 to 11:00 h), once a week, during four weeks (from late July to early August). We took advantage of the fact that within this period both resprouting and control plants had insects. However, four weeks after commencement of the fieldwork, unburnt plants advanced in senescence (leaves became crispy and some were abscised) and insects abandoned the plants, so comparative observations were ceased. *Banisteriopsis malifolia* phenology is markedly seasonal, leaf abscission takes place in the dry season (July – August) and the production of new leaves occurs only at the beginning of the rainy season (October). In our study, fire promoted aseasonal leaf production.

The abundance of thrips and ants in both plant groups (resprouting and unburnt) was compared using a Mann-Whitney *U*-test, as data from unburnt plants did not satisfy the assumptions of normal distribution even after transformations. In this and all subsequent tests (see the sections below), the mean number of *P. obscuricornis* and *C. crassus* counted during the study was used. Use of mean insect abundance is suitable because it buffers possible deviations from insect abundances throughout the study, what could otherwise influence statistical tests and provide imprecise results. The relationship between mean abundance of thrips and ants in resprouting *B. malifolia* individuals was examined using a Pearson correlation test (*log* transformed data). This test was not conducted using data from unburnt plants because insect (thrips and ants) sample sizes were small.

### *Banisteriopsis malifolia* architectural complexity

To evaluate the architectural complexity of *B. malifolia*, four variables were measured (adapted from Costa et al. 2010): trunk diameter at 10 cm above the soil, plant height (cm), number of leaves and canopy area ($m^2$). The latter was calculated using the formula for an ellipse given by $A = (\pi \times a \times b)$, where $a$ and $b$ refer to *B. malifolia* canopy width and length, respectively. Such measurements are claimed to account for most aspects of plant size and architectural complexity (Lawton 1983) and influence as well insect abundance (Leather 1986; Araújo et al. 2006). Plant architecture variables (trunk diameter, plant height, number of leaves and canopy area) of each *B. malifolia* group were compared using Student's *t* tests, and the original data of canopy area was logarithmically transformed in order to fit normal distribution (bell-shaped histogram chart and Lilliefor's normality test = $P > 0.05$).

In order to relate the abundance of insects and the architecture of *B. malifolia*, a single index that summarized the architectural complexity of plants was calculated. To generate this index, the principal component analysis (PCA) for multivariate data was performed, using the architecture variables. Data reduction by PCA involves taking scores of a large set of measured variables and reducing them to scores on a smaller set of composite variables that retain as much information from the original variables as possible (Jolliffe 2002). The relationship between plant architectural complexity (PCA index) and insect abundance (mean abundance of *P. obscuriconis* and *C. crassus*) and was tested by Pearson correlation tests, as data fitted normal distribution after *log* transformation.

### Plant quality - leaf sclerophylly and active EFNs

Leaf sclerophylly (hardness, toughness - Read et al. 2006) was used as measure of plant quality. After the fieldwork was concluded, seven leaves from each of the ten burnt and unburnt *B. malifolia* specimens were collected for a total of 140 leaves ($N = 70$ leaves per plant group). In the laboratory, leaves were oven-dried at 80ºC for 24 h to estimate the dry mass (DM, g). Leaves were then photographed in a flat surface and leaf area (LA, $m^2$) was measured with Image J software. The index of sclerophylly, also known as specific leaf mass (DM/LA, g $m^{-2}$; following Bacelar et al. 2004) was compared between resprouting and unburnt controls of *B. malifolia*. The sclerophylly of unburnt and resprouting plants was compared with a Mann-Whitney *U*- test, as original data did not satisfy the assumption of normality (Lilliefor's $P < 0.05$).

The abundance of leaves with active EFNs was estimated in each *B. malifolia* specimen by examining the ten most apical leaves of each plant in the morning (07:00 to 07:30 h), when small drops of extrafloral nectar can be seen being released from EFNs (personal observation). In general, inactive/non-functional EFNs in *B. malifolia* are brownish and flat to concave and contrast to the green and convex shape of active EFNs. Leaves were included in the category of active EFNs whenever at least one EFN was active and releasing nectar; thus susceptible to ant visitation.

The abundance of leaves with active EFNs was almost negligible in unburnt control plants, only 10 of the 180 leaves examined were observed to possess functional EFNs. Thus, between group (resprouting vs unburnt plants) comparisons were not performed. The relationship between the mean abundance of *C. crassus* (*log* transformed data) and leaves with active EFNs (resprouting group) was examined using a Pearson correlation test.

## Herbivory rate

Thrips herbivory intensity (%) in *B. malifolia* was considered only on leaves containing brownish or reddish necrotic spots. Herbivory was estimated as the percentage of injured leaves per individual plant. All *B. malifolia* specimens were deprived of other possible herbivores, by carefully removing them and placing in distant non-experimental plants (following Del-Claro et al. 1996). In unburnt plants, we did not count injured leaves because they were too old and necrotic, which may have originated from either previous herbivory or senescence. Furthermore, leaves also had a reduced surface area indicating the activity of chewing insects. The relationship between mean thrips abundance and herbivory in resprouting plants was examined with a Pearson correlation test after data were *log* transformed.

## Ant attack towards herbivores

To examine *C. crassus* aggressive behavior towards herbivores, live termite workers (*Nasutitermes* sp.) were used as baits on resprouting plants (adapted from Freitas and Oliveira 1996). This procedure was unpractical in unburnt plants because ants were not abundant (see Results section). Two termites were placed on two apical leaves of 12 resprouting *B. malifolia* specimens, and termite attack by *C. crassus* was monitored over a period of 15 minutes. Termites were not fixed, allowing free mobility on leaves. *Camponotus crassus* ants are very susceptible to movements on the leaf blade (Alves-Silva and Del-Claro 2013) and mobile arthropods (beetles, caterpillars, spiders and other ant species) attracts the attention of these ants. Attack was considered successful whenever ants approached and bit termites. In order to verify the behaviour of *C. crassus* towards *P. obscuricornis*, 30 h of field observation were conducted to monitor 10 *B. malifolia* specimens ($N = 3$ h for each specimen). All behavioural acts were recorded following all occurrences sampling method (Altman 1974).

## Results

The occurrence of *C. crassus* and *P. obscuricornis* was common in resprouting *B. malifolia*, while in unburnt plants their abundance was extremely low (ants: $U_{18,18} = 84$; $p < 0.05$; thrips: $U_{18,18}$; $p < 0.0001$) (Fig. 2). The mean abundance of thrips and ants was positively correlated in resprouting plants ($r = 0.5253$; $df = 16$; $p < 0.05$), while in unburnt specimens the low sample size of thrips and ants (Fig. 1) restricted statistical analyses.

*Banisteriopsis malifolia* regenerated rapidly after being burnt, but architectural complexity of resprouting plants was still lower compared to unburnt plants, which were generally taller, had more leaves and larger canopies (Table 1). Nevertheless, the relationship between insect abundance (thrips and ants) and *B. malifolia* architectural complexity was positive and significant in resprouting plants (*C. crassus*: $r = 0.596$; $df = 16$; $p < 0.01$; *P. obscuricornis*: $r = 0.533$; $df = 16$; $p < 0.05$) (Fig. 3). Plant quality was markedly different between *B. malifolia* groups in that leaves from resprouting plants were less sclerophyllous than unburnt control plants, as revealed by leaf specific mass (0.91 ± 0.41 and 1.44 ± 0.28 g $m^{-2}$, respectively, M ± SD; $U_{70,70} = 526$; $p < 0.0001$). The greater part of leaves examined in resprouting *B. malifolia* individuals (68.89% of leaves) possessed active and fully functional EFNs (6.89 ± 1.78, M ± SD, $N = 180$ leaves examined) and the abundance of *C. crassus* and leaves with active EFNs was positively and significantly related ($r = 0.7416$; $df = 16$; $p < 0.001$).

*Camponotus crassus* foraged on branches and leaves, feeding specifically on EFNs. Ants attacked all termite baits placed on *B. malifolia*. When a termite was found, ants bit the termite repeatedly, turning its gaster forward and expelling formic acid. In contrast, no *P. obscuricornis* was preyed upon or injured by ants, despite the fact that thrips were common on leaves, branches and stems. Adult thrips often moved from leaf to leaf while immature thrips were stationary most of the time, feeding on leaves. In these occasions thrips would remain immobile with the legs firmly attached to the leaf. While feeding, thrips were seen moving the head and antennas slightly up and down while its mouthparts were inserted in leaf epidermis. Thrips then fed by sucking up plant sap, leaving brownish necrosis marks on the leaf blade. Thrips also occurred in small cracks among shoots, where they were inaccessible to ants (Fig. 1e). Whenever an ant approached and/or touched thrips (adult or larvae), the latter would raise the abdomen (Fig. 1f), and release a small transparent liquid droplet from the anus causing surrounding ants to rapidly dissipate.

Plants had on average 37% of leaves with herbivory marks consisting of small or extensive brown necrotic spots on the leaf blade (leaves examined per plant: 71.3 ± 52.9, M ± SD, $N = 1284$ leaves; injured leaves per plant: 26.5 ± 18.3, M ± SD, $N = 478$), indicating the places where thrips sucked up leaf contents. Mature leaves frequently became twisted and folded as a consequence of thrips attack. The positive relationship between thrips abundance and herbivory was highly significant ($r = 0.809$; $df = 16$; $p < 0.0001$) (Fig. 4).

## Discussion

Despite having lower architectural complexity, resprouting *B. malifolia* had the highest abundance of ants and thrips because new leaves have active EFNs, which attracted ants; and less sclerophyllous tissues, which were eaten by thrips. Food resources play an important role in the life history of herbivorous thrips and are the most important factors that limit their populations in nature (Mound and Terry 2001; Magalhães et al. 2007). Studies have reported thrips' preference for younger shoots because of the soft tissues and more free nutrients (Kawai 1990; Paine 1992). The influence of plant architecture on the occurrence of thrips has rarely been studied but, in some cases, it may play a more decisive role on thrips' performance than plant nutrients (Varanda and Pais 2006). In *B. malifolia*, the growth of new leaves not only provided food but also space for thrips to forage and reproduce.

The abundance of *C. crassus* was also correlated with the architectural complexity of resprouting *B. malifolia*, as the growth of new leaves was rapid and most EFNs were active. *Camponotus* is a widespread genus in the Brazilian savanna, being one of the most common patrolling ants in extrafloral nectaried plants where they protect the plant against herbivores in general (Nascimento and Del-Claro 2010). In the current study, *C. crassus* attacked all termite baits placed on *B. malifolia* leaves but no thrips at all were preyed upon or expelled from *B. malifolia*. As a consequence of the failure of *C. crassus* to attack the thrips, the injury incurred by *P. obscuricornis* feeding was high. The genus *Pseudophilothrips* contains herbivorous species that cause considerable damage on plant hosts through severe herbivory; in some cases killing growing tips, decreasing plant fitness, and promoting infection by pathogens (Cuda et al. 2008; Manrique et al. 2008). In the current study, shoots and young leaves injured by thrips had extensive necrosis, which expanded from the point where thrips pierced the leaf blade. Occasionally, this necrosis extended to a wide area on the leaves, making the blade fragile and crispy. When thrips fed on young leaves, the leaves became twisted and distorted.

Thrips were found all over young leaves and shoots of *B. malifolia*, occasionally reaching up to the EFNs where *C. crassus* were foraging. However, thrips were left undisturbed even when foraging among ant aggregations. Many invertebrates possess chemical, behavioural, or physical mechanisms that enable them to remain either immune to ants or to avoid predation and/or attack (Gaume et al. 2006; Wcislo and Schatz 2003). Anti-predatory behaviour of thrips includes hiding in small cracks where they are inaccessible to large predators (Del-Claro et al. 1997) and in our study system, *P. obscuricornis* individuals were noticed more than often hiding in between shoots were they were inaccessible to *C. crassus*. Other anti-predatory behaviours of thrips include the secretion of volatile or anal fluids from the upturned abdomen (Howard et al. 1983; Bakker and Sabelis 1989). One type of thrips secretion is composed of mellein, which is an ant repellent and fumigant (Howard et al. 1983; Blum et al. 1992). *Pseudophilothrips obscuricornis* may have been using such a compound to remain immune to the aggressiveness of *C. crassus* during the current study. Other species of thrips manage to live among ant aggregations without being attacked or disturbed (Cavalleri et al. 2010; Alves-Silva and Del-Claro 2011). The co-occurrence of *Pseudophilothrips obscuricornis* with predatory ants on the same host plant can be advantageous for two reasons. First, since *C. crassus* is aggressive towards other herbivores, thrips may gain protection from possible predators. Second, *C. crassus* may displace or prey on herbivores that may otherwise influence the thrips' microhabitat (leaves).

In the current study, we showed that fire had important effects on the enhancement of the insect fauna associated with *B. malifolia*. The resprouting of branches and shoots provided valuable food resources for both *P. obscuricornis* and *C. crassus* during the dry season, a period of the year when *B. malifolia* is not supposed to have young leaves nor be producing nectar in EFNs (as observed in unburnt plants). Investigating the role of *C. crassus* as an indirect guard against thrips is a potential future project. However, generally poor knowledge of natural enemies of thrips will make further investigation difficult. Nonetheless, novel and significant information about interactions between thrips, host plants, and associated arthropods was gathered in the current study.

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## Table 1

Table 1. Comparison of the architectural complexity variables between resprouting and unburnt *Banisteriopsis malifolia* individuals.

| Variables | *Banisteriopsis malifolia* (M ± SD) | | |
|---|---|---|---|
| | Resprouting | Unburnt | *t* test |
| Trunk diameter | 0.40 ± 0.14 | 1.30 ± 0.58 | -6.47 *** |
| Height (cm) | 52.8 ± 30.8 | 99.8 ± 49.2 | -3.44 * |
| Canopy (m²) | 1.28 ± 1.42 | 5.68 ± 5.36 | -4.11 ** |
| Leaves | 71.3 ± 52.9 | 141 ± 77.5 | -3.14 * |

* = $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$ (Student's *t* tests)

## Figures and captions

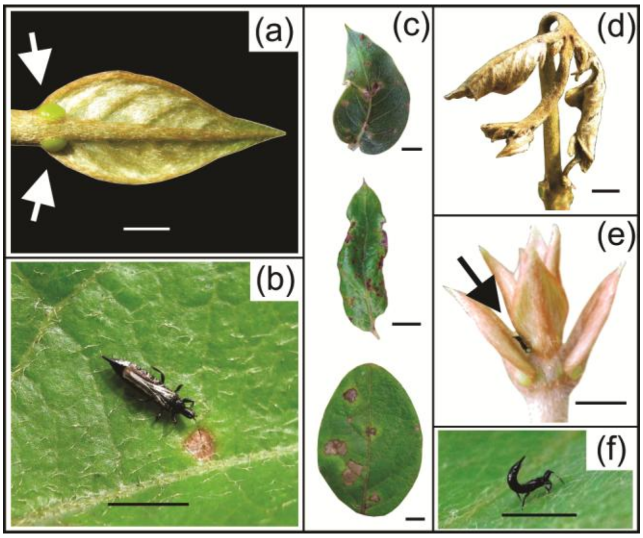

**Fig 1. (a)** *Banisteriopsis malifolia* extrafloral nectaries (arrows); **(b)** *Pseudophilothrips obscuricornis*; **(c)** thrips injury on leaves and **(d)** shoots; **(e)** thrips hid in between leaves (arrow); **(f)** thrips with the upturned abdomen. Scale: a, c, d, e – 5 mm; b, f – 2 mm

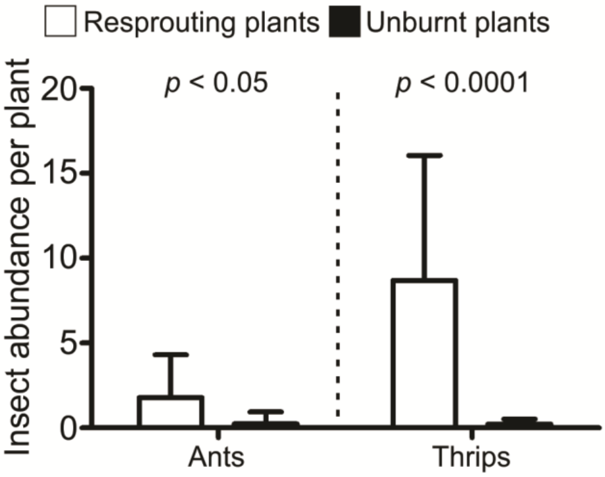

**Fig 2.** Abundance per plant (mean ± SD) of *Camponotus crassus* **(left)** and *Pseudophilothrips obscuricornis* **(right)** on two groups of *Banisteriopsis malifolia* in a neotropical cerrado savanna. Both ants and thrips were significantly more abundant in resprouting plants (Mann-Whitney *U*-tests: $p < 0.05$ and $p < 0.0001$, respectively)

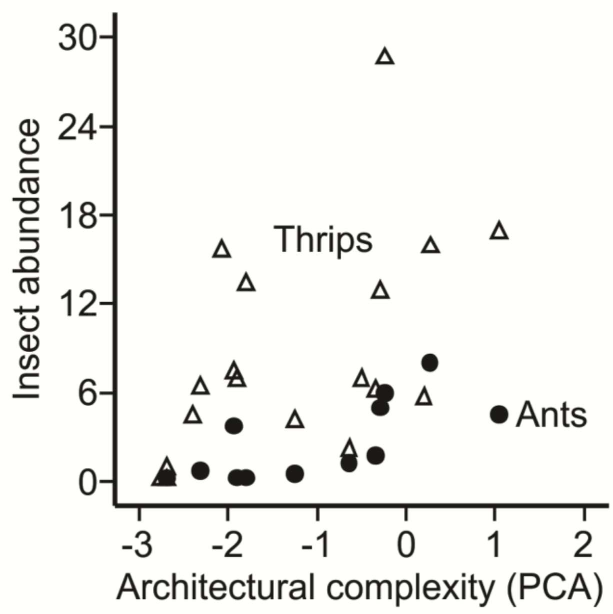

**Fig 3.** Positive relationship between *Banisteriopsis malifolia* architecture (trunk diameter, plant height (cm), number of leaves and canopy area) and the abundance of both *Pseudophilothrips obscuricornis* **(triangles)** and *Camponotus crassus* **(circles)** in a Brazilian savanna. The architectural complexity index was achieved using principal component analysis, which explained 88% of variation in the architecture of specimens

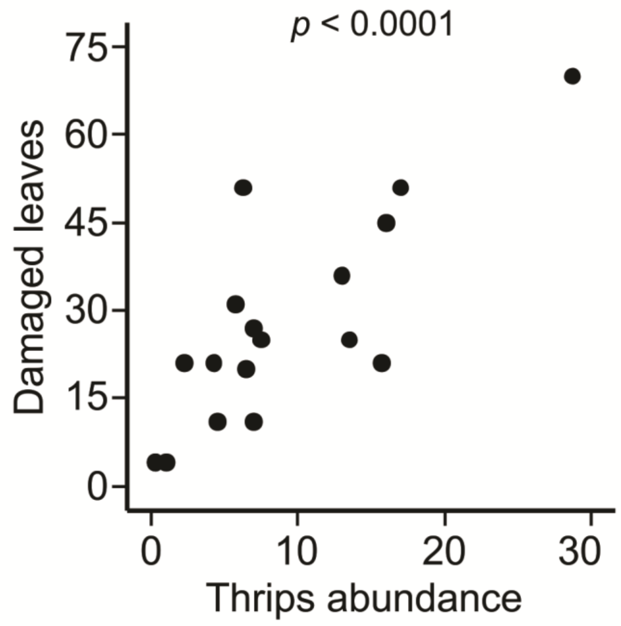

**Fig 4.** Relationship between the abundance of *Pseudophilothrips obscuricornis* and leaves with necrosis marks in *Banisteriopsis malifolia*. Pearson correlation test, $p < 0.0001$

# CAPÍTULO II

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ORIGINAL PAPER

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

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

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## Abstract

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

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

**Abbreviations**

| | |
|---|---|
| FA | Fluctuating asymmetry |
| mg/µL | Milligrams per microliter |
| *Rw* | Right width |
| *Lw* | Left width |

## Introduction

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

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

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

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

## Materials and methods

### Study site

Fieldwork was conducted in a *sensu strictu* cerrado area (18º59'S – 48º18'W; 890 m above sea level) in Uberlândia city, Brazil, from July to November 2010. The Cerrado covers about 400 hectares, of which 230 ha are characterized by *sensu strictu* cerrado, a type of vegetation dominated by herbaceous, shrubs and trees ranging between 2 – 4m tall, and a fair amount of grasses. Malpighiaceae, Bignoniaceae and Myrtaceae are the most abundant and diverse shrubs at the area, while Caryocaraceae, Ochnaceae and Vochysiaceae are the dominant trees. Soils are hydromorphic and acid with a significant amount of aluminum and potassium (Cardoso and Lomônaco 2003). The average rainfall in the region in 2010 was

1248.1 mm, of which 94% was concentrated during the wet season (October to April). Mean monthly temperature ranged from 25.3ºC in February to 20.2º in June, with an annual mean of 23.4ºC (Laboratory of Climatology, UFU, Brazil).

## Study organisms

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

## Sampling

Two groups of *B. campestris* were used in the study. The *resprouting* group contained 29 individuals distributed evenly within a site recovering from fire (≈ 2.5 ha). About three months after the fire, in late September, *B. campestris* developed new shoots and leaves with active extrafloral nectaries. The *control* (unburned) *B. campestris* group contained a further 29 individuals in an adjacent area (≈ 3 ha) which was not affected by fire. All plants at both sites were < 1.5 m in height and had approximately the same phenological status (i.e., young leaves with functional extrafloral nectaries). In addition, all plants belonged to the same population and were located in an area of ≈ 6–7 ha, and therefore were prone to similar biotic and abiotic stresses. Plants were tagged in November and sugar measurements and FA were examined on the ten most apical leaves from each individual plant ($n = 290$ leaves from each group; total $n = 580$ leaves). Within this period, leaves showed no signs of herbivory. Extrafloral nectar was measured with a pocket refractometer and each leaf was measured only once. Sugar quantity (mg) was calculated as "$y = 0.00226 + (0.00937x) + (0.0000585x^2)$", where "$y$" indicates the sugar content in mg and "$x$" is the brix (%) value displayed on the refractometer (*sensu* Dafni et al. 2005). Studies suggest that nectar concentration can be altered by biotic factors, such as herbivory and nectar consumption by insects (Heil et al. 2000; Ness 2003). Therefore, *B. campestris* leaves were bagged in nets to prevent the visits of insects to extrafloral nectaries. Leaves were bagged in the evening and nectar measurements were performed the morning after (07:00–08:00h), as this is the period in which *B. campestris* releases extrafloral nectar and drops of nectar are commonly observed on extrafloral nectaries. The short period of bagging also prevented the nectar from accumulating, which otherwise might influence sugar concentration (see O'Brien et al. 1996). Extrafloral nectar production was asynchronous among leaves within individual plants. Therefore, nectar measurements were conducted over two weeks to obtain as much data as possible. The number of tagged leaves that did not release/produce nectar during the period of the study (inactive extrafloral nectaries) was counted for each individual plant.

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

### Fluctuating asymmetry assessment

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

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

## Statistical analyses

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

## Results

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

Sugar concentration was positively related to leaf FA in resprouting plants ($r_{27}$ = 0.3739; $p$ = 0.0456) and marginally significant in control *B. campestris* individuals ($r_{27}$ = 0.3559; $p$ = 0.0580). The abundance of *C. crassus* ranged from 2 to 12 individuals in resprouting *B. campestris* (6.24 ± 2.32, $n$ = 181) and 3 to 8 individuals in the control group (4.97 ± 1.43, $n$ = 144) ($t_{56}$ = 2.5192; $p < 0.05$). The abundance of *C. crassus* was positively related to sugar concentration in both *B. campestris* groups (resprouting: $r_{27}$ = 0.52539; $p <$ 0.001; control $r_{27}$ = 0.4236; $p < 0.05$) (Fig. 3).

In regards to herbivory, 13 of the 29 *B. campestris* individuals of the resprouting group presented low herbivory levels, moderate herbivory was observed in six individuals and high herbivory in 10 ($n$ = 29 *B. campestris* individuals analyzed). In control plants (n = 29 *B. campestris* individuals analyzed), the scenario was different, low herbivory levels were noticed in four individuals, moderate herbivory in 14, and high herbivory in 11 plants. No individual at the resprouting or control area had extreme herbivory (> 76% of damaged leaves per individual). The abundance of *C. crassus* was associated with low herbivory levels in resprouting *B. campestris* individuals ($F_{2,26}$ = 11.4703; $p < 0.001$). In contrast, ant abundance was not related to herbivory levels in control *B. campestris* plants ($H_2$ = 3.2276; $p$ = 0.1991) (Fig. 4).

## Discussion

### FA and environmental stress due to fire

Results showed that FA in *B. campestris* was influenced by habitat disturbance, as individuals recovering from fire showed elevated FA levels. Positive relationships between habitat disturbance and FA are commonplace and environmental factors are a major source of stress to plants (Kozlov et al. 1996; Puerta-Piñero et al. 2008; Cornelissen and Stiling 2011).

To the best of our knowledge, only one study has assessed the effect of burning on FA (Freeman et al. 2005) and showed significant results. In the cerrado savanna, fire is the foremost form of disturbance, but it still remains widely unknown whether and how it affects the patterns of growth of bilaterally symmetrical traits. In our study, *B. campestris* was shown to be under severe stress as leaf FA levels in resprouting plants were 51.7% higher in comparison to those of control plants. In our study system, several environmental factors might cause FA in *B. campestris*, the more important being differences in sunlight and wind exposure (burned area). Both sunlight (and the lack thereof) and wind exposure are major stress factors for plants (Møller 1995; Puerta-Piñero et al. 2008). For example, *Miconia fallax* DC. (Melastomataceae) is highly susceptible to differences in sunlight and individuals growing in shaded conditions have increased FA levels (Alves-Silva 2012). The opposite might occur in *B. campestris* individuals, as in the field, this plant grows shaded by the canopy of large trees. Thus, open areas and direct sunlight exposure comprise departures from the normal environmental conditions for *B. campestris* and might cause severe stress (high FA) in individuals. The intense exposure of resprouting *B. campestris* individuals to direct sunlight might explain not only the increased FA levels, but also the higher number of leaves with active extrafloral nectaries. As shown by Radhika et al. (2010), extrafloral nectary function might be light-dependent, since light elicits changes in plant physiology, affecting the production of nectar. Additionally, high temperatures might also influence extrafloral sugar quantity and quality (Wyatt et al. 1992; Petanidou and Smets 1996).

### Sugar quality and ant-plant-herbivore interactions

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

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

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**Figures and captions**

**Fig. 1** *Banisteriopsis campestris* leaf. The arrows indicate the location of extrafloral nectaries. Scale bar: 5 mm

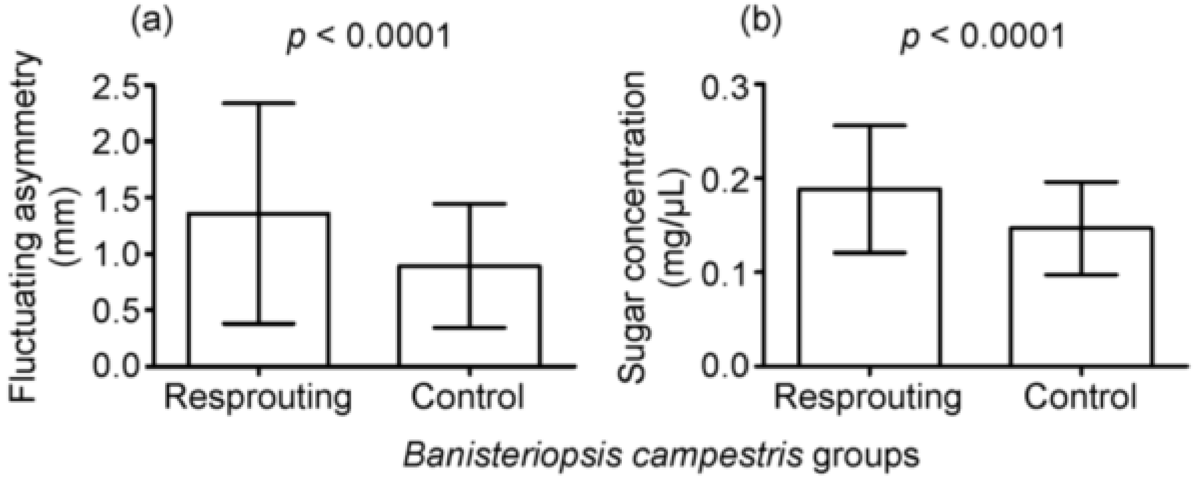

**Fig. 2** **(a)** Fluctuating asymmetry levels between two groups of *Banisteriopsis campestris*. Post-fire resprouting individuals showed higher level of stress. **(b)** Sugar concentration (mg/µL) was higher in resprouting *B. campestris*. Figures show the mean ± SD

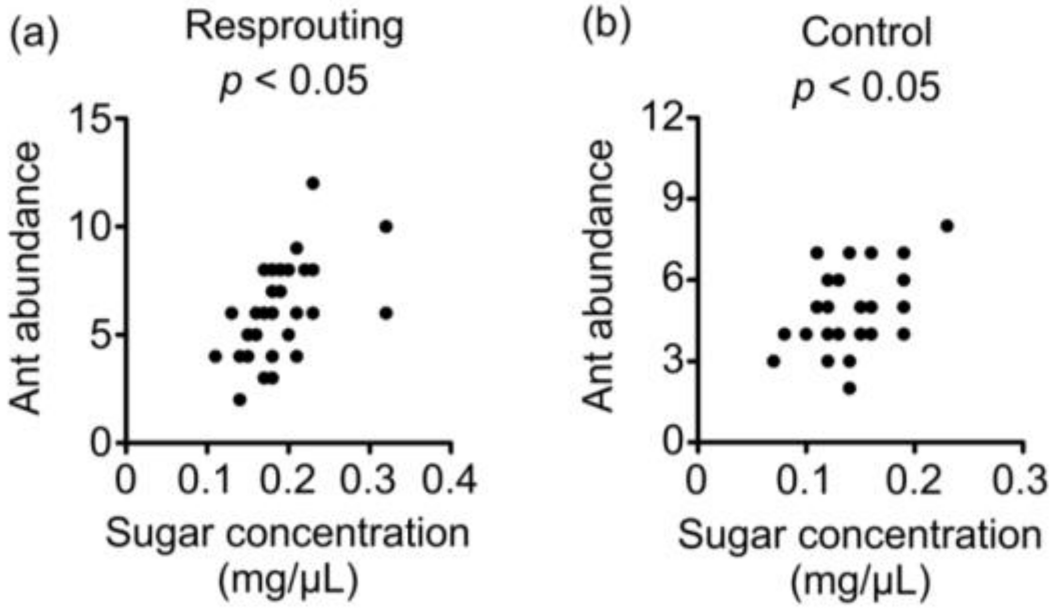

**Fig. 3** Relationship between the abundance of *Camponotus crassus* and extrafloral nectar sugar (mg/µL) in *Banisteriopsis campestris*. The relationship was positive and significant in both resprouting **(a)** and control plants **(b)**

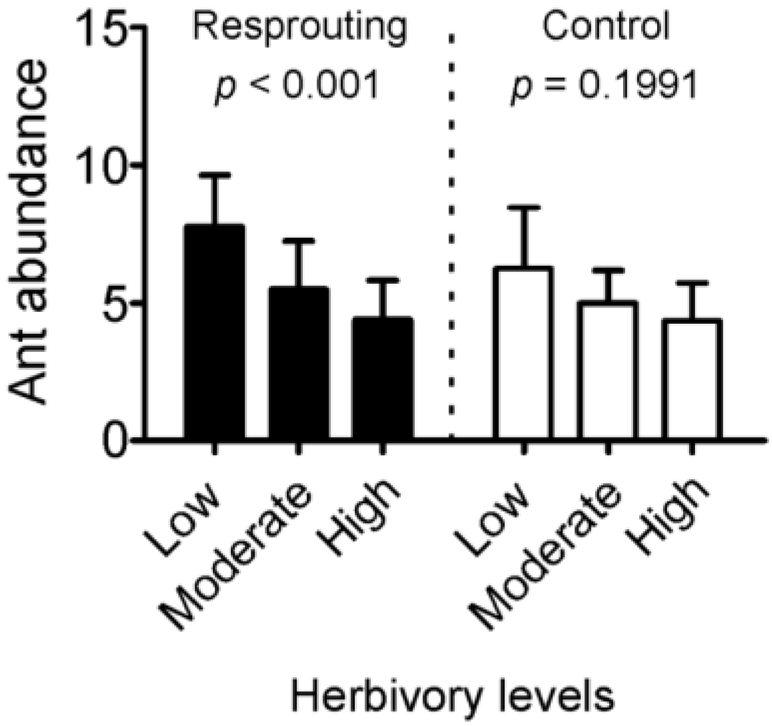

**Fig. 4** Relationship between the abundance of *Camponotus crassus* (mean ± SD) and herbivory levels in *Banisteriopsis campestris*. Ant abundance was related to low herbivory levels in resprouting plants. In control plants, this relationship was not statistically significant. Herbivory levels: low — 1–25% of damaged leaves; moderate — 26–50%; high — 51–75% of damaged leaves per *B. campestris* individual.

# SEGUNDA PARTE

Objetivo: investigar o impacto da ação de um terceiro nível trófico na população de herbívoros endofíticos, que devido à este hábito conseguem escapar do ataque de formigas, mas não de vespas predadoras

## CAPÍTULOS

3 - Foraging behavior of *Brachygastra lecheguana* (Hymenoptera: Vespidae) on *Banisteriopsis malifolia* (Malpighiaceae): Extrafloral nectar consumption and herbivore predation in a tending ant system

4 - Ant-herbivore interactions in an extrafloral nectaried plant: Are ants good plant guards against curculionids beetles?

# CAPÍTULO III

Entomological Science

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ORIGINAL ARTICLE

## Foraging behavior of *Brachygastra lecheguana* (Hymenoptera: Vespidae) on *Banisteriopsis malifolia* (Malpighiaceae): Extrafloral nectar consumption and herbivore predation in a tending ant system

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# Foraging behavior of *Brachygastra lecheguana* (Hymenoptera, Vespidae) on *Banisteriopsis malifolia* (Malpighiaceae): Extrafloral nectar consumption and herbivore predation in a tending ant system

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## Abstract

In the Brazilian savanna many plant species bear regular associations with patrolling ants that are aggressive towards insect herbivores. However, not only ants but also several species of predatory wasps are attracted to plants due to the extrafloral nectaries (EFNs). Such wasps feed on both herbivores and plant exudates. In this study we described the foraging behavior of the social Polistinae wasp *Brachygastra lecheguana* in the extrafloral nectaried shrub *Banisteriopsis malifolia*, and investigated the influence of patrolling ants (*Camponotus blandus*) on the activity of the wasp. *Brachygastra lecheguana* fed on the endophytic larvae of *Anthonomus* (Curculionidae) beetles that developed inside flower buds. The wasp lacerated the bud layers to reach the beetle larvae located at the bud core. The wasp visits to *Ba. malifolia* were statistically related to the abundance of flower buds and beetles. Ant exclusion experiments revealed that the hunting behavior of *B. lecheguana* on beetles was not related to the absence of *C. blandus*. However we found that wasps spent more time consuming extrafloral nectar on branches where ants were excluded. This is the first study reporting extrafloral nectar consumption by *B. lecheguana,* as well as the predation on herbivores in natural areas. In cerrado vegetation ants benefit the plant by reducing insect herbivores, and our study provides evidence that the *B. lecheguana – Ba. malifolia* system represents a potential interaction where the wasp may also benefit the host plant. The value of this wasp species as plant-guard is discussed.

**Key words:** *Anthonomus, Camponotus,* cerrado, Curculionidae, extrafloral nectary, multitrophic interactions.

## INTRODUCTION

Cerrado vegetation is a hotspot that shelters a huge assemblage of plant-animal interactions which have great importance to shape communities and to structure the functioning of the ecosystem as a whole (Oliveira & Marquis 2002; Del-Claro 2004). In this tropical savanna many plant species bear regular associations with patrolling ants that are very aggressive towards herbivores, consequently protecting the plant (Oliveira 1997; Baker-Méio & Marquis 2012; but see Nogueira *et al*. 2012). This ant-plant association is only maintained stable due to the extrafloral nectaries (EFNs), structures present in many vegetative plant parts (Oliveira & Freitas 2004). As these structures occur in more than 90 plant families in the world (Rudgers & Gardener 2004) and up to 17 in the cerrado (Oliveira & Oliveira-Filho 1991), it is not surprising that extrafloral (EF) nectar is consumed by many other arthropods, such as spiders (Ruhren & Handel 1999), parasitoid wasps (Röse *et al*. 2006), beetles (Almeida *et al.*

2011), flies (Heil *et al*. 2004), neuropterans (Limburg & Rosenheim 2001), bees, wasps and others (Keeler 1978). EF nectar is an important source of food which can prolong the survivorship and increase the fecundity of beetles (Lundgren & Seagraves 2011) and heteropterans (Portillo *et al*. 2012). EF nectar also increases the survivorship and enhances the colony growth of patrolling ants (Byk & Del-Claro 2011), which in turn benefit the plant through herbivory reduction (e.g. Oliveira 1997; Körndorfer & Del-Claro 2006).

Nevertheless while several ant-plant relationships are mutualistic, some studies failed to conclude that EF nectar consumption by insects other than ants may benefit the plant to some extent (Rashbrook *et al.* 1992; Heil *et al.* 2004; but see Bentley 1977). Wasps (Vespidae) may be an exception, because some species are predatory while others can also consume nectar and honeydew (Domínguez *et al*. 1989; Sugden & McAllen 1994; Cuautle & Rico-Gray 2003). This feeding flexibility appears to be common in the genus *Brachygastra* (Reyes-Rosas *et al*. 2011). This is the case of the dark paper wasp *B. lecheguana* Latreille (1824), a social Polistinae regarded as a biological control agent in crops, where it feeds on the endophytic larvae of pest leaf-mining butterflies (Gusmão 2000; Fragoso *et al.* 2001). Furthermore evidence indicates that this wasp species can also feed on floral nectar (Mussury *et al*. 2003; Aguiar & Santos 2007).

Despite being a biological control agent in agroecosystems, the impact of *B. lecheguana* upon native plants in natural areas has not been demonstrated. In crops, by preying on the larval stages of herbivores, *B. lecheguana* affects negatively the future populations of its prey, ultimately benefiting the plant (Domínguez *et al*. 1989; Perioto *et al*. 2011; but see Torezan-Silingardi 2011 for comparisons).

Recently, we observed individuals of *B. lecheguana* foraging on the cerrado Malpighiaceae *Banisteriopsis malifolia* (Nees & Mart.) B. Gates, where the wasps visited EFNs and also fed on endophytic larvae of curculionid beetles. In this context, *Ba. malifolia* offers great opportunities to study the foraging and hunting behavior of *B. lecheguana* in a natural system. The system is yet more interesting because *Ba. malifolia* is patrolled by the aggressive ant species *Camponotus blandus* Fr. Smith (1858) which hunts and captures many arthropods, restraining their access to the plant structures. The objective of this paper was to investigate the foraging behavior of *B. lecheguana* on *Ba. malifolia*. We examined the abundance of curculionid larvae consumed by *B. lecheguana*, the existence of changes between beetle and EF nectar consumption and whether there were negative interactions between patrolling ants and the wasp activities or not.

## METHODS

### Study area

Fieldwork was carried out from May to April, 2012, in a cerrado *strictu sensu* area located in Uberlândia city, Brazil (18º59’569’’S - 48º18’351’’W) (see Réu & Del-Claro 2005 for details about the study site).

### Study organisms

*Banisteriopsis malifolia* is a small shrub (less than 3 meters in height) with many branches bearing subdivisions. Leaves may reach up to 15 cm in length and 10 cm in width and possess small trichomes on both sides. Leaves also bear a pair of EFNs at the base near the petiole, at each side of the midrib, which are visited by several ant species, notably *Camponotus* (Fig. 1A) (Alves-Silva 2011). The plant reproductive season starts in late February, when flower buds grow on inflorescences located at the apex of branches and stems. Bud production peaks in April and May and at this period, adult individuals of *Anthonomus* (Curculionidae) (Fig.

1B) are observed foraging on buds and flowers. Its larvae develop inside flower buds (Fig. 1C), consuming internal bud tissues (Fig. 1D). *Banisteriopsis malifolia* supports three *Anthonomus* species: *A. rhinozotis*, *A. sulcatus* and *Anthonomus* sp. All of them are very similar in morphology, behavior and life histories. Therefore, and for the sake of clarity, we will consider the group *Anthonomus* rather than each species separately (description of *Anthonomus* life-histories in Torezan-Silingardi 2011).

### ***Brachygastra lecheguana* visitation pattern**

In order to follow the foraging behavior of *B. lecheguana* we initially tagged 25 *Ba. malifolia* individuals with similar phenological state (almost same height, number of stems, level of leaf herbivory and presence of inflorescences). However we noticed that wasps were far more frequent in seven plant individuals (later we discovered that the other plants had no or few *Anthonomus* endophytic larvae) on which we focused our efforts. All these plant individuals were patrolled by *C. blandus*. These plants were primarily used only to follow the daily cycle of wasp visits to the plant.

The visits of *B. lecheguana* to *Ba. malifolia* were observed for two days from 08:00 to 17:59h. Each plant individual was observed for two minutes in each hour interval. We observed the plants and registered the number of wasps visiting them. *Brachygastra lecheguana* individuals often fly alone in the vicinity of plants, allowing for the observation of each wasp individual separately. So we could distinguish whether the same wasp individual was foraging on the plant or it was another wasp individual. The Kruskal-Wallis test, followed by Dunn’s post hoc test, was performed to analyze temporal differences in the visits of *B. lecheguana* on *Ba. malifolia*. In this analysis we grouped the number of wasps observed on each plant individual in relation to time intervals such as 08:00 – 08:59h, 09:00-09:59h, and so on.

### Factors influencing wasp visitation

To explain the differences on the abundance of *B. lecheguana* visits to the plants, we measured two variables, the flower bud abundance and the number of beetles. Flower bud abundance was estimated by counting the buds at the most apical branch of each *Ba. malifolia* individual. Another variable that might be related to *B. lecheguana* visitation was the abundance of its prey. However we could not collect the flower buds to assess the parasitism rates on these plants, as it might influence the future visits of the wasps. Therefore we counted only the adult *Anthonomus*. These variables were submitted to a multiple linear regression, considering the wasp visits as the dependent variable.

### Temporal variation in beetle predation

To examine the temporal variation of beetle predation by *B. lecheguana* we stood in front of a *Ba. malifolia* individual (the most visited one) and registered all the visits of the wasps to the plant. The choice of using only one plant individual relies on the fact that the other plants were seldom visited by *B. lecheguana* in regards to *Anthonomus* larvae consumption. The census of beetle predation was made on two sunny days, from 08:00 – 17:59h. The hunting behavior was classified as “successful” (*Anthonomus* larva consumed) or “unsuccessful” (bud laceration without larva consumption). In successful predation events, we registered the time that *B. lecheguana* spent opening the flower bud and the time it spent consuming the beetle larva. The abandoned buds (when the wasp quit the laceration and flew away without apparent reason) were collected and opened in laboratory. These buds were opened under a stereomicroscope to verify if they were parasitized by *Anthonomus* larvae.

The influence of ants on the hunting behavior of *B. lecheguana* was examined in two branches from two *Ba. malifolia* individuals (the most visited ones). In this experiment we considered two variables, the presence and absence of ants. To exclude the ants from plant structures, we applied Tanglefoot® (a wax widely used to prevent the access of ants to plant structures - Nahas *et al.* 2012) at the base of a branch (treatment branch). Another branch within the same *Ba. malifolia* individual was left undisturbed (control). As soon as a wasp landed on the branch and started to hunt an *Anthonomus* larva (bud laceration), we observed whether the ants molested the wasp or whether the wasp remained unnoticed. The number of *B. lecheguana* visits on treatment and control branches was also compared.

### Influence of ants on wasp EF nectar consumption

We used the same experimental branches (ants excluded and control) to examine the influence of ants on the EF nectar consumption by *B. lecheguana*. Each plant was observed for two mornings (07:30 – 11:30h). During the wasp landings on the plant, we registered whether it chose the treatment (ants excluded) or the control branch and the time spent on each one. If the wasp landed on the control branch we carefully observed whether it was attacked by *C. blandus*. The number of *B. lecheguana* visits to EFNs on each branch (treatment or control) was compared with a goodness of fit chi-squared test. The time spent on each branch was compared with a Mann-Whitney *U* test.

### Flower bud parasitism rate

*Banisteriopsis malifolia* parasitism rate was investigated by collecting inflorescences and estimating the number of flower buds containing *Anthonomus* endophytic larvae. We chose 20 *Ba. malifolia* individuals (these were not used for wasp behavioral observations) and from each one we collected one inflorescence. In laboratory the flower buds were opened and the presence/absence of *Anthonomus* larvae was registered.

At the end of the fieldwork the abundance of buds with marks of wasp predation were examined in 20 *Ba. malifolia* individuals. In each plant one inflorescence was chosen and the number of buds with scratching marks or holes was counted. This analysis would provide the rate of buds opened by *B. lecheguana* and would permit us to examine to what extent the wasp influenced the *Anthonomus* population. Flower buds opened by *B. lecheguana* presented characteristic scratching and biting marks (Fig 1D, E).

Results are presented as mean ± 1 standard error. The choice of using parametric or non-parametric statistics was based on previous normality tests (Kolmogorov-Smirnov) performed in Bioestat software.

## RESULTS

The wasp *B. lecheguana* foraged on *Ba. malifolia* all day long. Figure 2 shows a slight higher abundance of visits at midday, with a slight decrease of visitations after 15:00h. However, there was no statistical difference between the visits of wasps to *Ba. malifolia* shrubs during the day ($H$ = 6.6799, *d.f.* = 9, $P$ = 0.6704).

Nonetheless we found a significant difference in regards to the number of visits to each *Ba. malifolia* individual ($H$ = 26.3844, *d.f.* = 6, $P$ = 0.0002) and the Dunn's post hoc test showed that two plant individuals received the greater number of visitations (56.25%). The multiple regression revealed a significant relationship between *B. lecheguana* visitation and the abundance of both flower buds and *Anthonomus* ($F$ = 17.9477, $r^2$ = 0.8997, $P$ = 0.0101) (Fig. 3; Table 1). Up to four individuals of *B. lecheguana* were simultaneously observed on each of two plants while each of the other plants bore only one wasp individual at once.

The wasps landed on inflorescences and walked among several flower buds, touching them with alternate and consecutive (up and down) movements of antennas. Migration from inflorescences within the same plant individual was commonplace. When a *B. lecheguana* individual came to a bud containing a larva, the wasp increased the velocity of antennation and started to walk upon the bud more rapidly. The wasp then used its mandibles to lacerate the layers of the bud to reach the *Anthonomus* larva at the bud core. The wasp bit the larva inside the bud and pulled it out, starting to consume it immediately (Fig. 1E). The wasp then chewed the larva and consumed it entirely. During the feeding, the fore legs were not used to manipulate or sustain the larva, only the mandibles were used. The wasps often consumed the *Anthonomus* larva upon the same bud which was opened or sometimes the wasp moved to the adjacent flower bud. After each predation, the wasp instantly started self-grooming, cleaning the antennas and the mandibles with the fore legs.

*Brachygastra lecheguana* hunted and consumed beetles all day long with a marked hunting activity from 11:00 to 15:00h and decreasing greatly after this period (Fig. 4). During the observations in the field, we registered 65 predation attempts of *B. lecheguana* on *Anthonomus* beetles. Attempts were considered as the behavior of flower bud laceration. In 37 (56.9%) of the occasions, the predation was successful (Fig. 4). The causes of failed predation were desistence ($n$ = 18) and molestation by *C. blandus* ($n$ = 10). Desistence was characterized by the behavior of quitting the bud laceration and going to another inflorescence. In the laboratory we found that the abandoned flower buds ($n$ = 10 buds examined) contained no *Anthonomus* larva. The ant molestation occurred whenever *C. blandus* reached the flower buds and attacked *B. lecheguana*. On this occasion the wasp flew away and the ant continued to patrol the entire inflorescence. We did not observe any predation of *C. blandus* upon *B. lecheguana*. None of the wasps observed during the study presented changes from beetle predation to EF nectar consumption.

Among the 37 *Anthonomus* larvae preyed upon, 18 of them were hunted on the experimental branches. Hunting occurred mostly on the branch with free access of ants ($n$ = = 15 (83%)), relative to the branch where ants were excluded ($n$ = 3 (17%)). Therefore we found no evidence that *C. blandus* interferes with the hunting success of *B. lecheguana*.

The time of bud opening was measured on 32 occasions and the time of *Anthonomus* larvae consumption on 31. On one occasion the captured larva was not consumed on the plant, but rather, taken away by the wasp. *Brachygastra lecheguana* spent 365.87 ± 40.66 (range 47 - 913) seconds opening the flower buds. *Anthonomus* larvae consumption was faster, the wasp spent on average 99.42 ± 8.04 ($n$ = 31 records; range 22 – 246) seconds to consume the entire beetle larvae.

We registered 34 visits of *B. lecheguana* to the EFNs (Fig. 1F). The wasp landed on a branch and walked towards young leaves to feed on the EF nectar. The wasps visited many leaves, often flying to reach the leaves at the top of the branches. Out of the 34 EFN visits, 25 (73.5%) were made to branches with ants excluded, while only nine were conducted on branches with free ant access ($\chi^2$ = 7.529, $d.f.$ = 1, $P$ = 0.0101). The time spent on each branch was also markedly different, the wasps stayed on average 1189 ± 228.48 seconds on inflorescences without *C. blandus*, and 161.33 ± 39.6 seconds on inflorescences with ants ($U_{25,9}$ = 26.5, $P$ = 0.0008).

Among the 20 inflorescences collected to investigate the parasitism rate of beetles, 460 flower buds were sampled (23 ± 1.43 buds per inflorescence). We found 95 larvae, indicating a parasitism rate of 20.7%. At the end of the study we counted the number of flower buds in the field with marks of *B. lecheguana* laceration and beetle predation (Fig. 1D). We examined 506 flower buds (25.3 ± 2.05 buds per inflorescences, *n* = 20 inflorescences) and found that 50 of them (9.8%) presented the characteristic marks of *B. lecheguana* beetle predation.

## DISCUSSION

According to our knowledge this is the first study investigating the multiple interactions among a wasp (*B. lecheguana*), an herbivore (*Anthonomus*) and an ant (*C. blandus*) in a Brazilian savanna shrub bearing extrafloral nectaries (*Ba. malifolia*). The dark paper wasp species was shown to feed on both herbivores and plant exudates (EF nectar) and its foraging behavior was often interrupted by patrolling ants. In cerrado vegetation ants and other predators act together ultimately producing complementary effects to reduce herbivory (Nascimento & Del-Claro 2010; Nahas *et al.* 2012). Our study suggests that this ant-wasp-plant system represents a potential interaction where different predators may benefit the host plant.

The *Brachygastra* is recognized as an important predator on many insect species and this genus is even more interesting because it comprises one of the few insects other than bees that produce and store honey (Machado *et al.* 1988; Sugden & McAllen 1994; Reyes-Rosas *et al.* 2011). In addition *Brachygastra* also consume floral nectar from the plants they pollinate (Ish-Am *et al.* 1999; see also Aguiar & Santos 2007). Therefore the EF nectar feeding behavior of *B. lecheguana* is not surprising at all, but the fact that it had not been observed until now is remarkable.

Extrafloral nectar is a major resource for Hymenoptera but this feeding behavior is known mostly for ants, where it is vital for the survivorship of ant colonies (Byk & Del-Claro 2011).

Extrafloral nectar is valuable for several reasons: it is more concentrated than flower nectar (Koptur 1994), may contain up to 12 sugar types and a diverse array of amino acids, proteins, vitamins and a few lipids (Heil *et al.* 1998; Wäckers 2005; Shenoy *et al.* 2012). Therefore it is not unexpected that this resource is also consumed by arthropods other than ants (Rudgers & Gardener 2004).

In our study, to what degree the hunting behavior of *B. lecheguana* is influenced by ants on *Ba. malifolia* was not clearly demonstrated. Of the 65 predation attempts (upon beetles), ants displaced the wasps in 10 occasions (15%), but no wasp was captured by ants. *Camponotus* are known to displace and attack many insect herbivores in cerrado plants (Del-Claro 2004 for a review). Nevertheless, all *B. lecheguana* individuals that foraged on *Ba. malifolia* escaped from ant attack. This result provides an important clue that wasp hunting behavior is not influenced by foraging ants. However, we have obtained the data that are difficult to interpret; they suggest that *B. lecheguana* prefers the branches with free access of ants rather than the branches where ants were excluded (83 % vs 17 %, ant-exclusion experiments). Even if the wasp could make access to herbivores on the EFN-bearing shrubs without any remarkable interference that might possibly be imposed by the predatory ants patrolling on the shrubs, the hunting is expected to equally occur on both of the branches. Since the replication of observations for robust statistics was lacked because of its very low abundance, we decline to discuss this issue but we claim that futures studies with the sufficient number of replications for the similar ant-exclusion experiments would examine more clearly whether patrolling ants on the plant have negative impacts of wasp behavior or not.

*Brachygastra lecheguana* showed a preference to forage on *Ba. malifolia* individuals with more reproductive structures and, consequently, the ones which supported more *Anthonomus* beetles. According to Torezan-Silingardi (2011) wasps can rely on chemical (volatiles), visual and mechanical (vibrations) clues to find parasitized flower buds. Another variable that might influence wasp visitation to *Ba. malifolia* was the density of EFNs per plant. Nevertheless, even the smallest shrub of *Ba. malifolia* has hundreds of leaves and the counting of which ones had active EFNs was impractical. At the period of the study, *Ba. malifolia* leaves had active EFNs, and not only the wasp, but also the *Camponotus* ants visited the EFNs to feed on nectar. In *Ba. malifolia*, the blooming coincides with the production of new leaves, thus allowing the wasps to prey on the beetles (that develops in flower buds) and feed on the EF nectar. We believe that an experimental study with plants with active EFNs and other with EFNs removed/non-functional would answer the question whether the wasps were indeed related to the EFN density, or not.

Our results showed that the parasitism rate of *Anthonomus* larvae in flower buds of *Ba. malifolia* was 20%. Conversely, the number of flower buds with *B. lecheguana* predatory marks in the field was approximately 10%. These results suggest that the predatory behavior of *B. lecheguana* can remove about half of the *Anthonomus* larval population. This reduction of beetle population may be beneficial to *Ba. malifolia*, as wasps are removing the larvae that would otherwise become adult herbivores. By consuming protein sources (predation on herbivores) as well as EF nectar, wasps may have an ecological role similar to ant-guards, as herbivore deterrents (Torres-Hernández *et al.* 2000). The wasp is likely to be an effective enemy of herbivorous insects on the plant, because *B. lecheguana* is undoubtedly a highly predaceous species (Perioto *et al.* 2011), being active all day long (see also Mussury *et al.* 2003).

To date, studies have shown that *B. lecheguana* feeds on crop pests such as leaf miners (Lepidoptera; Perioto *et al.* 2011). The wasp cuts the leaf blade and removes the endophytic lepidopteran larvae (Fragoso *et al.* 2001). The behavior of cutting the plant tissue to capture the larva was also observed in our study. *Brachygastra lecheguana* bit and lacerated the layers of *Ba. malifolia* flower buds to capture the *Anthonomus* larvae at the bud core. However, not all the predation attempts were successful. Wasps aborted the flower bud laceration and left the bud in 18 out of the 28 desistence events. Later we discovered that the abandoned buds contained no *Anthonomus* larva.

The value of *B. lecheguana* as plant-guard requires further studies and it would also be interesting to know whether: i) the *B. lecheguana-Anthonomus* interaction takes place on other natural plants, notably the *Banisteriopsis* community in the area, comprising other four species; ii) *B. lecheguana* foraging behavior is related or not to the identity of ants patrolling different host plants. Such information would be an important tool for the understanding of the ecology of this social wasp and its interactions in the cerrado biome.

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## Table

Table 1. Coefficients and analysis of variance of the relationship among *Brachysgastra lecheguana* visitation (dependent variable), flower bud and *Anthonomus* abundances (multiple regression analysis) in *Banisteriopsis malifolia*. The multiple regressions revealed a significant relationship among the variables. Std. – standard; MS – mean squares; sqr – squared.

**REGRESSION COEFFICIENTS**

| **Variables** | **Coefficient** | **Std. Error** | ***t*-value** | ***P*-value** |
|---|---|---|---|---|
| Intercept (wasp) | -2.2676 | 2.1437 | -1.0578 | 0.3498 |
| Partial regression (flower buds) | 0.0298 | 0.0106 | 2.7955 | 0.0490 |
| Partial regression (beetles) | 1.1069 | 0.4202 | 2.6343 | 0.0579 |

**ANALYSIS OF VARIANCE**

|  | **MS** | ***F*-Ratio** | **Sqr. Multiple *R*** | ***P*-value** |
|---|---|---|---|---|
| Regression | 114.6523 | 17.9477 | 0.8997 | 0.0101 |

**Figures and captions**

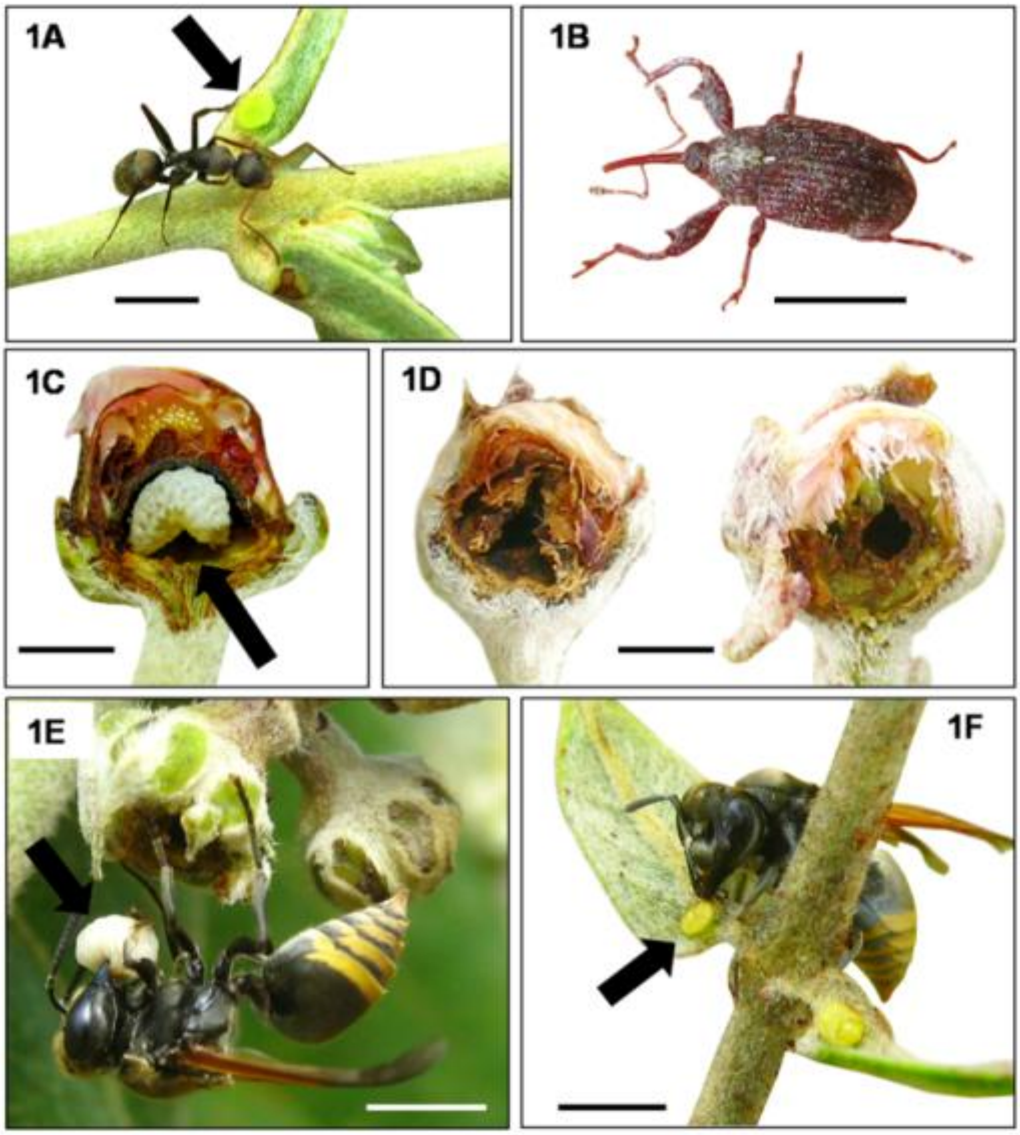


Figure 1. (A) – *Camponotus blandus* foraging on a young leaf of *Banisteriopsis malifolia* with functional extrafloral nectaries (arrow). (B) – adult *Anthonomus*. (C) – An *Anthonomus* larva inside a flower bud (arrow). (D) – flower buds attacked by *Brachygastra lecheguana*, with many lacerations. (E) – *B. lecheguana* feeding on an *Anthonomus* larva (arrow). (F) – *B. lecheguana* visiting the extrafloral nectaries (arrow). Scales: A, B, C, D – 2 mm; E, F – 3 mm. All the photos were edited in Gimp software.

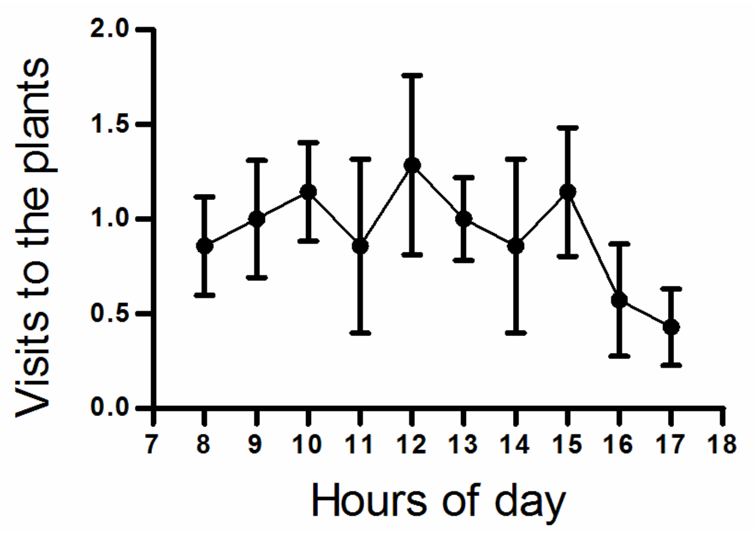


Figure 2. Daytime variation on the number of visits (mean ± 1 SE) of *Brachygastra lecheguana* wasps in *Banisteriopsis malifolia*. The wasp was more abundant at midday and as the day went by, the number of visits decreased. However, these differences were not statistically significant.

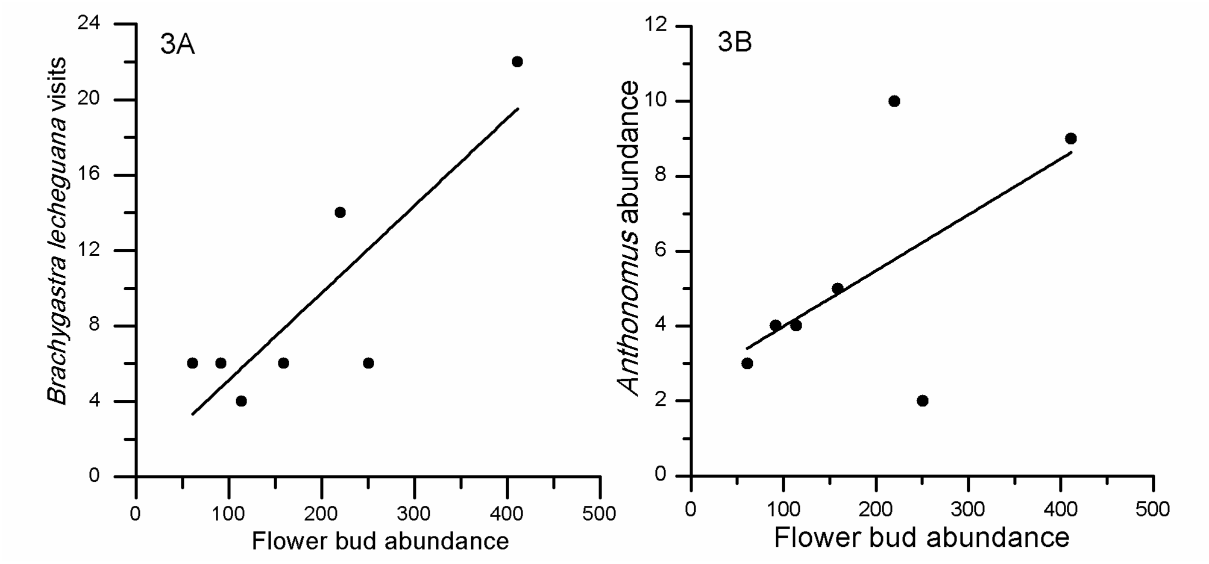


Figure 3. (A) Relationship between *Brachygastra lecheguana* visits to *Banisteriopsis malifolia* and the number of flower buds per plant; and (B) relationship between the number of *Anthonomus* beetles per plant and the number of flower buds per plant. Multiple regression revealed that the wasps' visits to a plant were statistically related to both beetle and flower bud abundances per plant (see the Table 1 for the statistics).

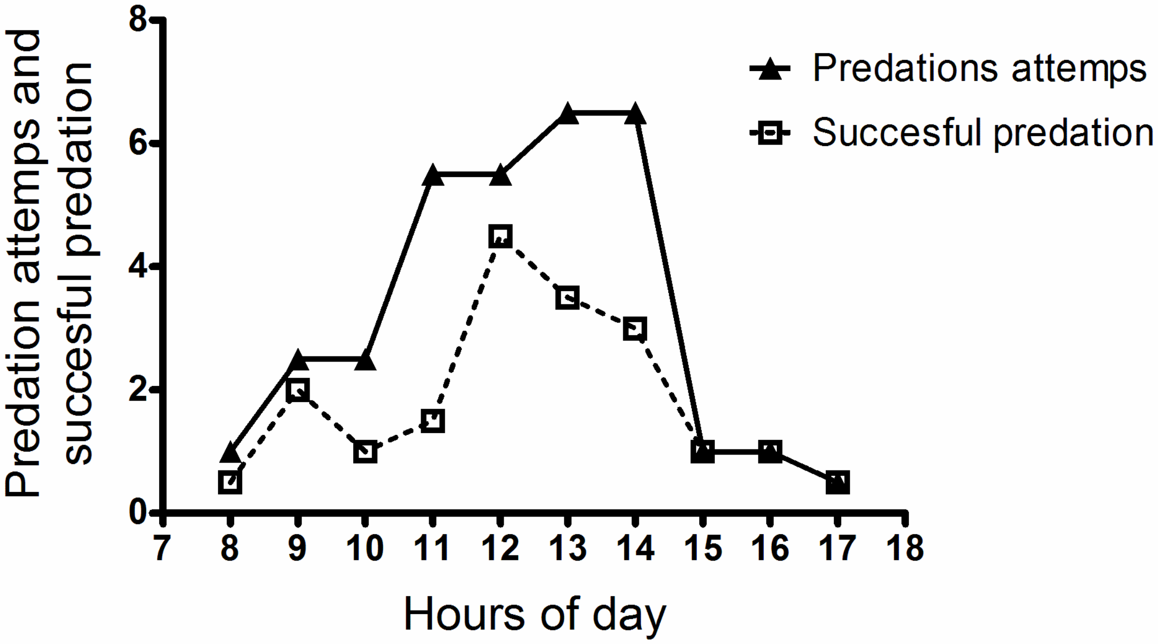
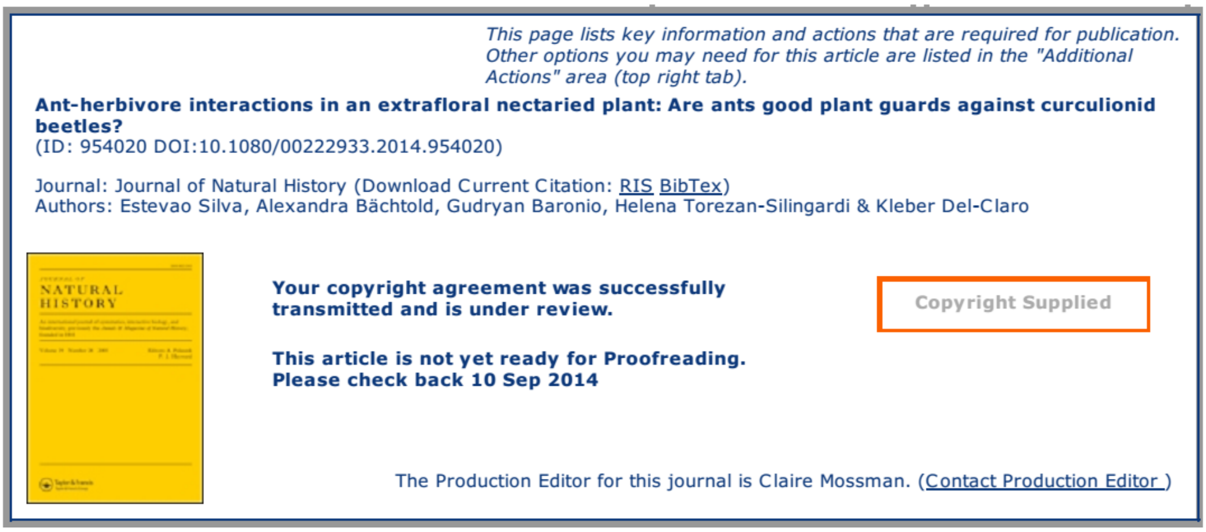


Figure 4. Temporal variation (mean) on the number of predation attempts (flower bud laceration initiated) and successful predation (curculionid larva consumed) of the wasp *Brachygastra lecheguana* on *Anthonomus* endophytic beetle larva in *Banisteriopsis malifolia*. Out of the 65 predation attempts, 37 were successful.

# CAPÍTULO IV



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# Ant-herbivore interactions in an extrafloral nectaried plant: Are ants good plant guards against curculionid beetles?

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## Abstract

Extrafloral nectary drinking ants are known as effective plant guards, but some herbivores may circumvent ant attacks by foraging on different plant parts or presenting adaptations to avoid ant predation. Here we experimentally investigated the effect of *Camponotus blandus* on the florivory of the extrafloral nectaried shrub *Banisteriopsis malifolia*; and a possible spatial segregation between ants and herbivores (leaves and flowers). Flower buds are attacked by *Anthonomus* weevils. Results revealed no significant influence of *C. blandus* on the reduction of florivory. Adult *Anthonomus* are hard-bodied and were immune to ant bites; larvae are endophytic, thus protected from ants. Ants and adult beetles were concentrated in different plant parts (leaves and flowers, respectively) thus restraining the probability of encounters. Our results indicate that the system *C. blandus* – *Anthonomus* – *B. malifolia* is not stable, as ants receive extrafloral nectar, but are unable to protect the plant against weevils.

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**Keywords:** *Anthonomus; Banisteriopsis*; *Camponotus blandus*; Cerrado; extrafloral nectary

## Introduction

In general ant-plant mutualistic relationships are based on reciprocal benefits for both organisms involved. For instance, plants often provide a resource for ants (e.g. extrafloral nectaries, domatia), while ants patrol and defend the plant against herbivore insects (Bronstein et al. 2006). The benefits gained by the plant due to the presence of ants, which are attracted to the nectar-producing structures, have been well documented and include low herbivory rates of both vegetative and reproductive structures, as well as increased vigor, growth and fitness (Fuente & Marquis 1999; Oliveira et al. 1999; Katayama & Suzuki 2004; Kost & Heil 2005). In general, specialized ant-plant interactions, such as the ones involving myrmecophytic species, provides more benefits to the parties involved, as both ants and plants have close relationships of coevolution (Longino 1989; Rocha & Bergallo 1992; Brouat et al. 2001). In contrast, interactions involving extrafloral nectaried plants are not so specific (Blüthgen et al. 2007) and one single plant species can harbor many ant species and the net effect of each ant species in plant performance can be strikingly different (Mody & Linsenmair 2004).

In a broader consensus, ant-plant interactions based on extrafloral nectaries (EFNs) have a positive effect for the plants (Rosumek et al. 2009), but several studies have demonstrated that extrafloral nectaried plants may not benefit from the presence of ants (Rashbrook et al. 1992; Mody & Linsenmair 2004; Nogueira et al. 2012). In fact, aggressive ant behavior towards herbivores may be ineffective in many scenarios, especially those involving endophytic herbivores and/or large, hard-bodied insects (Heads & Lawton 1985; Koptur & Lawton 1988; Riedel et al. 2013). Also, ant attack towards herbivores can be ineffective if both occupy different plant parts (Bächtold & Alves-Silva 2013). For instance, Del-Claro et al. (1997) showed that florivorous thrips co-occurred with aggressive ants in *Peixotoa tomentosa* Adr. Juss. (Malpighiaceae), but thrips lived inside flowers, where they were inaccessible to all but tiny ants.

In extrafloral nectaried plants, ants most likely spend more time foraging on leaves and not in the plant reproductive structures (Wagner & Kay 2002; Junker & Blüthgen 2008), rendering ant protection generally more effective against leaf herbivores (Ness 2003; Dejean et al. 2009). For instance, Koptur (1979) noted that ants reduced foliar damage in *Vicia* (Fabaceae), but found no indication that ants protected the plant against seed predators. In contrast, Oliveira (1997) demonstrated that ants feeding on EFNs were able to remove several florivores from *Caryocar brasiliense* Camb. (Caryocaraceae), which was reflected by increased plant fitness.

A particular scenario of ants visiting both leaves and flowers can be seen in the extrafloral nectaried shrubs of Malpighiaceae. Ants in several species feed on EFNs (Alves-Silva 2011; Bächtold et al. 2013), but they can commonly be observed foraging on inflorescences where they hunt down thrips (Del-Claro 1998) and chase chrysomelid beetles (Reu & Del-Claro 2005), and may significantly reduce herbivore populations. Fernandes et al. (2005) observed a marked reduction of suckers and chewers in inflorescences of *Byrsonima crassifolia* (Linnaeus) H.B.K. (Malpighiaceae) after ants were experimentally removed, indicating that ants patrolled plant reproductive parts. Therefore, the presence of EFN-feeding ants in Malpighiaceae affects not only folivores (Alves-Silva & Del-Claro 2013), but also florivores. However, ant-plant interactions are highly conditional (Bronstein et al. 2006; Marazzi et al. 2013) as ants are not able to protect the plant against all types of herbivores, especially beetles, due to their hard body and strong sclerotization (Koptur & Lawton 1988). Herbivore beetles are pervasive in Malpighiaceae (Del-Claro 2004; Reu & Del-Claro 2005;

Flinte et al. 2006; Torezan-Silingardi 2011; Alves-Silva et al. 2013a; Ferreira & Torezan-Silingardi 2013), but their interactions with patrolling ants are not well understood and require further investigation in order to see whether ants can really deter these herbivores.

In the current study, ant-herbivore interactions in *Banisteriopsis malifolia* (Nees & Martius) B. Gates (Malpighiaceae), an extrafloral nectaried shrub, were investigated. This plant supports the patrolling ant *Camponotus blandus* (Smith 1858) (Formicinae) and the florivore weevil *Anthonomus* spp. (Coleoptera: Curculionidae). Adult beetles forage in the plant and are thus subjected to being found by patrolling *C. blandus*, which is aggressive towards herbivores in general (Oliveira et al. 1987). We specifically examined (i) whether *C. blandus* influenced *Anthonomus* florivory rates; and (ii) spatial segregation between ants and beetles (leaves or flowers, respectively). A positive relationship between ant presence and decreased herbivory might be evidence that ants were protecting the plant against beetles and restraining their oviposition in the plant. Nonetheless we also took into account that *C. blandus* might not be able to deter *Anthonomus*, both because their hard body and a possible spatial segregation. Thus, the effect of ants on herbivory would not be significant.

## Methods

### ***Study area***

The study was conducted in a *stricto sensu* cerrado area (18º59'S – 48º18'W) in Uberlândia City, Brazil, from March to May 2012. This cerrado area covers 230 hectares and is dominated by herbaceous plants, shrubs and trees ranging from 2–4 m tall. The climate is characterized by two well established seasons, a rainy summer and a dry winter. Average rainfall in the region is approximately 1500 mm per year, of which > 90% occurs during the wet season (October to April). Mean monthly temperatures range from 24.8 ºC in February to

19.9 ºC in June, with an annual mean temperature of 23 ºC (Laboratory of Climatology, Federal University of Uberlandia, Brazil, 2011).

### ***Study organisms***

*Banisteriopsis malifolia* is a shrub (< 2 m in height). Fully expanded leaves may reach up to 15 cm in length and 10 cm in width. The leaf blade has tiny trichomes, and the margin is smooth. Leaves possess a pair of prominent EFNs at the base near the petiole on each side of the midrib (Fig. 1a). EFNs are active during the entire rainy season and are frequently visited by *C. blandus* (Alves-Silva et al. 2013a), a ground-nesting generalist ant species that forages on shrubs and trees and is very common in cerrado vegetation. Production of *Banisteriopsis malifolia* flower buds usually begins in late February or March and peaks in mid-April, and they grow on inflorescences located at the apex of branches. On average, buds are 7 mm in diameter, pink in color and surrounded by eight oil glands. Both flower buds and young leaves with active EFNs occur close to each other (Figure 1a). Flowers are pink, have five free petals and bear chambers formed by the S-shaped sepals, which curl towards the center of the flower (Del-Claro et al. 1997). *Anthonomus* (Coleoptera: Curculionidae) is found throughout *B. malifolia* reproductive season, feeding preferentially on buds (Figure 1b), but can also be found on flowers and more rarely on fruits. Its larvae are endophytic and develop inside flower buds. *Banisteriopsis malifolia* hosts three *Anthonomus* species: *A. rhinozotis* (Clark 1988), *A. sulcatus* (Kirsch. 1874) and *Anthonomus* sp., and all of them have a similar natural history and behavior (see Torezan-Silingardi 2011). Thus, in the current study, all *Anthonomus* species were considered as a single group (following Alves-Silva et al. 2013a).

### *Florivory rates*

In March 2012, we tagged 30 *B. malifolia* individuals with approximately the same phenological state (presence of flower buds and leaves with active EFNs) and height (~1.60 m). All study plants supported only *C. blandus* and no other EFN-feeding ant was observed on the plants during the study. Plant specimens were distributed evenly over 10 ha within the study area. In each plant, a control and a treatment branch containing flower buds and leaves with active EFNs were selected. At the base of each treatment branch, atoxic wax (Tanglefoot™) was applied to prevent access of *C. blandus* (Apple & Feener 2001). The control stem was left unaltered, allowing free access of ants to the entire branch. Leaves and other branches that could be used by ants as bridges to climb on experimental branches were removed. Tagged branches were also deprived of other insect herbivores. Prior to application of wax, the number of ants and adult beetles on each individual plant was counted.

We initially counted the total number of buds and the ones with signs of *Anthonomus* florivory (i.e. small punctures on bud surface in control and experimental branches). Florivory rate (%) was calculated as the number of damaged flower buds divided by the total number of buds. At the beginning of the study there was no statistical difference in florivory between branches (control = 7.23 ± 2.32%; M ± SE, *n* = 4881 buds; treatment 7.00 ± 1.69% of damaged buds, *n* = 5321 buds; Wilcoxon test =110.0; *p* = 0.8484), thus discarding study bias. Counting of flower buds (damaged and undamaged) and calculation of the florivory rate was performed in mid-April, just prior to flowering.

### ***Insect spatial segregation***

Spatial segregation of *C. blandus* and *Anthonomus* was examined on control branches from a subsample of 10 *B. malifolia* specimens. The number of ants and weevils in flowers and leaves was counted every 60 minutes from 08h00 to 17h00. In the field, a total of 60 h of observations (*ad libitum*) was devoted to the behavior of *C. blandus* towards adult *Anthonomus*. Each plant was observed once for three hours (20 individual plants, 08h00 to 11h00) on sunny days. Whenever ants encountered beetles, we recorded whether they were attacked or presented any strategy/behavior against *C. blandus* attack.

### ***Statistical analyses***

Quantitative data are shown as mean ± SE. In those cases where data did not satisfy the assumptions of a normal distribution ($p < 0.05$) and transformations were unable to achieve data normality, non-parametric statistical tests were used. Florivory rate (%) in control and treatment branches was compared using a Wilcoxon test. The abundance of ants and beetles per plant (assessed in the beginning of the study prior to application of wax) was compared using a Student's *t*-test (*log10* transformed data). The relationship between *C. blandus* and *Anthonomus* abundances was examined using a Pearson correlation test (*log10* transformed data). The difference in spatial segregation (flowers or leaves) of ant and beetles was examined using a Student's *t*-test. The same test was used to examine which insect was more abundant in flowers during daytime. Statistical tests were performed in Systat 12® and GraphPad Prism 5.0 softwares.

## Results

Florivory by *Anthonomus* in ant-excluded branches was 14% higher than branches with freely roaming ants; however, this difference was not statistically significant (Wilcoxon test = 152.0; $p = 0.3676$) (Figure 2). *Anthonomus* and *C. blandus* abundance per plant was 4.2 ± 0.83 ($n = 126$) and 17.23 ± 2.01 ($n = 517$), respectively ($t = 6.4885$; $df = 58$; $p < 0.0001$). There was no relationship between the abundance of beetles and ants ($r = -0.0430$; $df = 28$; $p = 0.7355$). Both *C. blandus* and *Anthonomus* foraged during the daytime in *B. malifolia*. Ants showed a marked preference for foraging on leaves ($t = 6.3420$; $df = 18$; $p < 0.0001$) (Figure 3a), but they were also observed patrolling flowers (Figure 1c). In contrast, *Anthonomus* foraging activity was concentrated on flowers ($t = 13.1738$; $df = 18$; $p < 0.0001$) (Figure 3b). Even so the abundance of ants on flowers was higher than the abundance of beetles ($t = 2.8271$; $df = 18$; $p < 0.05$; comparison of Figure 3a-b).

*Anthonomus* individuals were very mobile and often migrated from buds to flowers and occasionally fruits. *Camponotus blandus* attacked *Anthonomus* on 17 occasions, with most attacks taking place on the main stem of branches and in one instance on a fruit (Figure 1d). During contact with ants, beetles shrunk their body and clung strongly onto the branch. Ants walked rapidly around and over the *Anthonomus* body, biting and rubbing their abdomen against the beetle to release formic acid. Beetles, however, remained unharmed and attached to the stem. No ant recruiting was noticed. Ants eventually stopped their attack on beetles and backed off in less than a minute. On a single occasion, we observed an *Anthonomus* individual dropping from one inflorescence as it was touched by a *C. blandus*. However, the beetle fell on a flower and continued to forage in the plant. *Anthonomus* individuals were frequently observed in flower chambers. As such, only the dorsal portion of their bodies was exposed to the exterior of the chamber. A single flower was capable of supporting up to three adult *Anthonomus*, but often only one beetle was found in any given flower.

## Discussion

In general, ants play a decisive role in defending extrafloral nectaried plants against herbivores (Marazzi et al. 2013). Nonetheless, beetles may disrupt ant-plant mutualism, as these hard-bodied insects are rarely preyed upon by ants in natural systems, both because they are usually larger than ants and because their strong sclerotization (Messina 1981; Koptur & Lawton 1988; Fiala et al. 1989). In the current study, despite the frequent presence of *C. blandus* in the plants, these ants failed to significantly protect *B. malifolia* from *Anthonomus*. In economically important crops, small-sized weevils can be controlled by predatory ants (Peng & Christian 2007; Abera-Kalibata et al. 2008), but this trend was not observed in *B. malifolia*. *Camponotus blandus* (~5 mm) are larger than *Anthonomus* (~2.5 mm), but in contact with ants, no beetle was injured or preyed upon. *Camponotus blandus* aggressive behavior towards herbivores includes frequent bites, release of acids from the acidopore and the removal of the prey from the plant, thus rendering plant protection (Oliveira et al. 1987; Guimarães Jr. et al. 2006). Nonetheless here we showed that a particular herbivore (*Anthonomus*) was not affected by ants, as no adult beetle was injured by *C. blandus*. The population of *B. malifolia* at the study area supports other patrolling ants, such as *Ectatomma* and *Cephalotes* (Alves-Silva 2011), buy we have never observed these ants attacking and/or preying upon *Anthonomus*. In fact *Cephalotes* was shown to be an ineffective plant guard in *Ouratea spectabilis* Engl. (Ochnaceae), as it did not protect the plant against curculionids (Byk & Del-Claro 2010).

In *B. malifolia*, as in other extrafloral nectaried Malpighiaceae, EFNs are functional during the plant reproductive season, and leaves with EFNs grow next to inflorescences, what might indicate that the foraging behavior of ants would extend to flowers (Del-Claro et al. 1997; Reu & Del-Claro 2005). In fact, Possobom et al. (2010) observed *Camponotus* ants patrolling both vegetative and reproductive structures of *Diplopterys pubipetala* (A. Juss.) W.R. Anderson & C. Cav. Davis (Malpighiaceae), and also exhibiting aggressive behavior. However, in the current study, *C. blandus* foraged mostly on leaves. *Camponotus blandus* is a sugar-dependent ant species (Guimarães Jr. et al. 2006), and in *B. malifolia*, this ant species does not visit the inflorescences very often, except when it tends myrmecophilous insects (Alves-Silva et al. 2013b). Thus the flower-dwelling behavior of *Anthonomus* in *B. malifolia* flowers is advantageous for beetles, as they are seldom disturbed by ants in this structure.

*Anthonomus* in *B. malifolia* can damage up to 20% of flower buds, greatly influencing plant reproductive outputs (Alves-Silva et al. 2013a). Nonetheless, these weevils are preyed upon by the nectar-drinking social Polistinae wasp, *Brachygastra lecheguana* Latreille (1824). As shown by Alves-Silva et al. (2013a) this wasp can reduce up to 50% of the beetle population in *B. malifolia*, acting as an effective plant guard. Nonetheless the authors demonstrated that *C. blandus* may sometimes attack and displace *B. lecheguana* from the plant. Thus, in addition to not protecting *B. malifolia* from *Anthonomus*, *C. blandus* may also interrupt the predatory behavior of wasps, which may otherwise control the weevil population. Antagonistic behavior of ants on predatory wasps was also reported by Cuautle and Rico-Gray (2003), who demonstrated that ant-excluded plants were more frequently visited by wasps, which controlled herbivore populations (see also Mody et al. 2011; Pereira & Trigo 2013). Other studies have also suggested that ants may indirectly benefit herbivore populations by preying on or interfering with the predatory behavior of parasitoids (Pierce & Mead 1981) and spiders (Mody & Linsenmair 2004; Nahas et al. 2012).

Three specific instances in which *C. blandus* was not beneficial to *B. malifolia* were described in the current study. First, no adult *Anthonomus* was preyed upon or expelled from the plant by ants. Second, *Anthonomus* larvae are endophytic, thus protected from ants during the entire immature stage. Third, adult weevils and ants usually occupied different regions of the plant, restraining the probability of encounters. We can also list that *C. blandus* may negatively interfere with the predatory behavior of *B. lecheguana*, which is the main natural enemy of *Anthonomus* (Alves-Silva et al. 2013a). All summed up, in this specific system, it can be suggested that the presence of *C. blandus* was not beneficial for the plant.

Ant-herbivore systems can provide new and unexpected situations to enrich the knowledge of biotic interactions. Asymmetries in ant-plant mutualism (where ants receive nectar but do not protect the plant against herbivores) are still poorly understood (Byk & Del-Claro 2010; Riedel et al. 2013), but can contribute to the advances of ant-plant interactions, especially in the tropics where extrafloral nectaried plants are abundant and diverse (Machado et al. 2008).

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## Figures and captions

Figure 1. Ant-plant-herbivore interactions in *Banisteriopsis malifolia*. (a) Leaf with active extrafloral nectaries, growing close to flower buds. (b) Adult *Anthonomus*. (c) *Camponotus blandus* foraging on a flower. (d) *C. blandus* attacking an *Anthonomus*. Scale: Fig a, b – 10 mm; c,d – 5 mm.

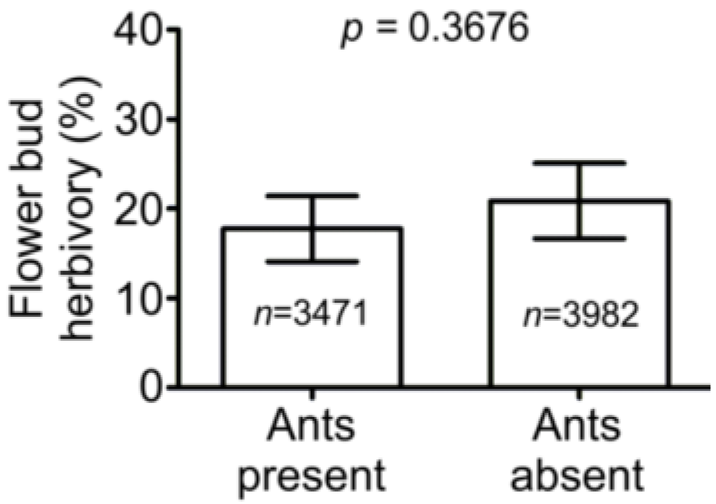

Figure 2. Florivory rates (mean ± SE) in *Banisteriopsis malifolia* based on the presence or absence of *Camponotus blandus*. *Anthonomus* florivory rates were higher in branches without ants, but this difference was not statistically significant. The number of flower buds analyzed in each treatment is given inside bars. $p = 0.3676$ (Wilcoxon test) indicates no statistical significant differences between treatments.

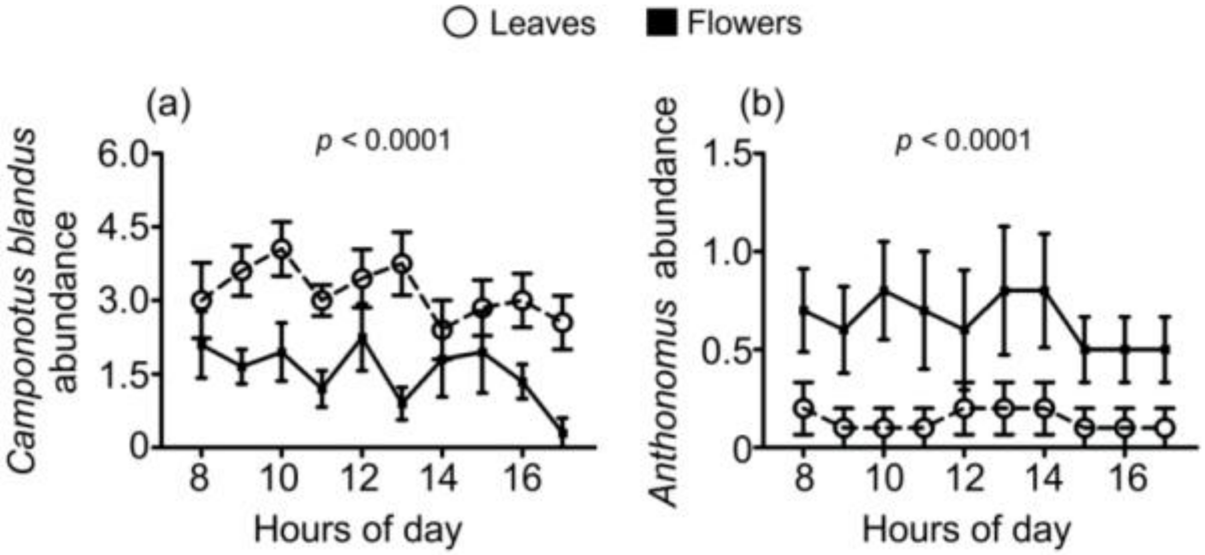

Figure 3. Spatial segregation (mean ± SE) of *Camponotus blandus* and *Anthonomus* in *Banisteriopsis malifolia*. Ants (a) were more abundant on leaves (circles and dotted lines), while beetles (b) were concentrated on flowers (squares and continuous lines). $p < 0.0001$ (Student's *t*-tests) indicates statistical significant differences.

# TERCEIRA PARTE

Objetivo: Avaliar as relações facultativas condicionais entre formigas e herbívoros e como estes últimos conseguem co-ocorrer com seus possíveis inimigos naturais, enquanto provocam um grande dano na planta hospedeira

## CAPÍTULOS

**5** - Influence of *Camponotus blandus* (Formicinae) and flower buds on the occurrence of *Parrhasius polibetes* (Lepidoptera: Lycaenidae) in *Banisteriopsis malifolia* (Malpighiaceae)

6 - The role, or the lack thereof, of patrolling ants on the deterrence of herbivore thrips: Effect on plant reproductive outputs?

# CAPÍTULO V

Sociobiology 60(1): 30-34 (2013)

Sociobiology
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RESEARCH ARTICLE - ANTS

## Influence of *Camponotus blandus* (Formicinae) and flower buds on the occurrence of *Parrhasius polibetes* (Lepidoptera: Lycaenidae) in *Banisteriopsis malifolia* (Malpighiaceae)

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# Influence of *Camponotus blandus* (Formicinae) and flower buds on the occurrence of *Parrhasius polibetes* (Lepidoptera: Lycaenidae) in *Banisteriopsis malifolia* (Malpighiaceae)

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Running title: Influence of ants on the occurrence of lycaenids

**ABSTRACT -** In the Brazilian savanna, myrmecophilous lycaenids are often found in many shrubs feeding on plant reproductive structures while are tended by ants, but only recently has the relative role of both ants and food on the occurrence of lycaenids been given attention. In this study, we investigated the influence of *Camponotus blandus* (Formicinae) and flower bud abundance on the occurrence of *Parrhasius polibetes*, a florivorous lycaenid species that occurs in *Banisteriopsis malifolia* (Malpighiaceae). We also examined to what extent larval florivory was deleterious to plant reproductive outputs. Ant-exclusion experiments revealed that most *P. polibetes* individuals were found on branches with free *C. blandus* access. Nonetheless, the occurrence of larvae was not related to the abundance of ants and flower buds, indicating that the presence, rather than the abundance of mutualistic ants and food, influenced the occurrence of *P. polibetes*. Larvae were attended by *C. blandus*, which antennated frequently the dorsal nectary organ of larvae. Larval florivory was not deleterious to the plant. *Banisteriopsis malifolia* produces thousands of buds simultaneously and larvae feed only on a small portion of flower buds. The occurrence of *P. polibetes* in *B. malifolia* is advantageous for the larvae, since this plant supports mutualistic ants and plenty of food resources.

**Keywords:** Brazilian savanna; larval food; myrmecophily; florivory

## Introduction

Lycaenids are abundant and widely distributed in the Neotropics, accounting for 1200 species distributed in several biomes and vegetation types (Brown Jr., 1993; Robbins & Lamas, 2004). In Brazil, lycaenid records are based mostly on adult individuals, thus little is known about the larval host range (Emery et al., 2006; Francini et al., 2011). Lycaenid larvae generally feed on plant reproductive parts, such as flowers and flower buds (Robbins & Aiello, 1982). However, despite the vast diversity of flora in Brazilian biomes, only recently have the interactions among lycaenid larvae, their host plants, and mutualistic ants been considered in ecological studies (Kaminski & Freitas, 2010; Kaminski et al., 2010a; Silva et al., 2011; Bächtold & Alves-Silva, 2012).

Lycaenidae has one of the most remarkable interactions within the Lepidoptera – the mutualistic associations with ants (Pierce et al., 2002). Larvae have dorsal nectary organs (DNOs) and perforated cupolas organs (PCOs) (Fiedler, 1991). The former releases a sugared substance while the latter pacifies the aggressive behavior of tending ants (Malicky, 1970). In this context, studies have shown that lycaenid female oviposition choices may be ant-mediated (Seufert & Fiedler, 1996; Wynhoff et al., 2008). This trend was observed in *Parrhasius polibetes* (Stoll), a facultative myrmecophilous species that is frequent in the Brazilian cerrado savanna (Silva et al., 2011). Kaminski et al. (2010a) showed that *P. polibetes* female oviposition was mediated by the presence of tending ants. Ants may increase larval performance and survivorship by protecting them from natural enemies (Weeks, 2003). In these cases, females will seek more favorable plants, taking into account not only the presence of ants, but in the case of *P. polibetes*, females will also seek for high quality food (Rodrigues et al., 2010).

The availability of food items also influences the occurrence of lycaenids (Wagner & Kurina, 1997) and by feeding on plant reproductive structures, larvae are supposed to exert negative effects on plant fitness (Oliveira & Del-Claro, 2005). For instance, Badenes-Pérez et al. (2010) discussed the use of lycaenids as biological control agents against the invasive species *Miconia calvescens* DC. (Melastomataceae) in Costa Rica, as larvae were observed to damage up to 30% of the reproductive structures from the plant (see also Jordano et al., 1990).

In the Brazilian savanna (Cerrado biome), the extrafloral (EFN) nectaried shrub *Banisteriopsis malifolia* (Nees & Martius) B. Gates (Malpighiaceae) is patrolled by a wide range of EFN feeding ants (Alves-Silva, 2011). *Camponotus blandus* (Smith) Formicinae is one of the most abundant ant species in *B. malifolia*, being very aggressive towards other arthropods (Alves-Silva et al., 2012). Nonetheless, *C. blandus* has mutualistic relationships with membracids (Oliveira & Del-Claro, 2005). Facultative myrmecophilous lycaenids are found in *B. malifolia* feeding on flower buds and are susceptible to ant contact, but whether larvae are attended by *C. blandus* and the role of ant presence on larval occurrence has not previously been studied.

In this study, we investigated the influence of i) ants (presence and abundance) and ii) flower buds (presence and abundance) on the occurrence of lycaenids in *B. malifolia*. We also examined the quantity of flowers buds consumed by larvae to investigate whether larvae negatively influenced plant fitness. To conclude, we conducted observations of the behavior of *C. blandus* towards *P. polibetes* larvae and towards other herbivores. An appreciation of the factors involved in the occurrence of lycaenid larvae in plants of common occurrence can be a tool for understanding ant-lycaenid mutualisms in the neotropics, especially in the Brazilian cerrado savanna.

## Material and Methods

### Study area

The study was conducted in a *strictu sensu* cerrado area (18º59' S – 48º18' W) in Uberlândia city, Brazil, from March to May 2012, which corresponds to the reproductive season of *B. malifolia*. The cerrado covers about 230 hectares and is dominated by shrubs and trees ranging between 2 - 4m tall. The climate is markedly seasonal with a dry winter (May to September) and a rainy summer (October to April).

### Plant species

*Banisteriopsis malifolia* is a small shrub (< 2 meters high). Leaves have small trichomes on both sides and bear a pair of EFNs at the base, near the petiole. Flower bud production starts in March and peaks in mid-April. Buds are on average 7-10 mm in diameter, pinkish and bear eight oil glands in its circumference. Flower buds grow on inflorescences located at the apex of branches.

### Ant-exclusion experiment

The role of ant presence on the occurrence of lycaenids was examined in 30 individuals of *B. malifolia*, distributed evenly in approximately 10 ha within the study area. All shrubs were patrolled by *C. blandus*. A control and a treatment branch containing flower buds and young leaves with functional EFNs were tagged in each plant individual in late March. At the base of treatment branches (n = 30), a layer of atoxic wax (Tanglefoot® - Grand Rapids, MI, USA) was applied to prevent the access of ants to the plant structures (Nahas et al., 2012). The control branches (n = 30) were left unaltered, allowing the free access of ants to the plant parts. At this occasion, all *B. malifolia* shrubs were carefully examined and no immature lycaenids (egg or larva) were seen.

### Sampling

Lycaenid sampling was performed once a week after wax application, from the first week of April until the end of May. On each occasion, buds, flowers, shoots, and both sides of leaves of the treatment and control branches were examined. *Parrhasius polibetes* larvae found in the field were then collected, individualized in plastic containers (250 ml), and reared in the laboratory until pupation. Other lycaenid larvae that were observed on the plant were also collected and reared in the laboratory. The comparison between the number of *P. polibetes* larvae found in the treatment and control branches was made with a Binomial test. The abundance of *C. blandus* and flower buds was also estimated at the beginning of the study. Flower bud counting was made in a randomly selected inflorescence within each plant. The number of ants foraging on each *B. malifolia* individual was counted once. The difference in the abundance of ants and flower buds in plants with and without *P. polibetes* was made with Student's *t* tests (original data was *log10* transformed to fit normal distribution).

## Ant-lycaenid interactions

In the field, we performed 30 hours of observation (*ad libitum*) on the behavior of *C. blandus* towards *P. polibetes* larvae. Whenever *C. blandus* encountered a larva, we carefully observed whether ants attended the larvae or not. Ant attendance was characterized by quickly and alternate antennation on the dorsal nectary organ, coupled with walking back and forth over or near the larva (Ballmer & Pratt, 1991).

## Ant hostility towards invaders

*Camponotus blandus* hostility towards invaders was examined by placing one live termite worker (*Nasutitermes* sp. - Termitidae) on inflorescences of *B. malifolia* (n = 21 individuals). Each plant received one termite. With this method, we intended to simulate possible wingless lycaenid natural enemies such as spiders, or other insects that might molest lycaenids or interfere with their feeding activity. Termite baits are usually used to investigate the behavior of patrolling ants towards plant invaders (Oliveira, 1997). The termites were followed for 10 minutes and interactions with ants were recorded.

## Larval florivory

The estimation of the daily bud consumption by *P. polibetes* larvae was made under laboratory conditions. Five larvae were fed *ad libitum* with flower buds every 24h. Florivory estimation was conducted in fourth instar larvae only, as in this stage larvae can feed on several flower buds. Florivory rates were compared with the abundance of flower buds in the plants. All quantitative data is presented as mean ± standard error.

## Results

We found eleven (0.37 ± 0.11; n = 30 plants examined) *P. polibetes* in *B. malifolia* and only one larva was found in an ant-excluded branch, while all the other larvae (n = 10) were found in branches with free ant-access (Binomial test Z = 3.0028; $P < 0.01$). Besides *P. polibetes*, other three lycaenid species were found in *B. malifolia*, but in low abundance: *Rekoa* sp. (0.1 ± 0.07; n = 3), *R. marius* (Lucas) (0.03 ± 0.03; n = 1), and *Allosmaitia strophius* (Godart) (0.03 ± 0.03; n = 1).

Each *B. malifolia* had on average 16.42 ± 1.91 *C. blandus* individuals. This plant also presented many flower buds per inflorescence (340.07 ± 35.77). *Parrhasius polibetes* larvae were found in nine *B. malifolia* and these plants had on average 10% more ants and 13% more flower buds. However, none of these variables was related to larvae occurrence (ants: $t_{28} = 0.5523$; $P > 0.05$; flower buds: $t_{28} = 1.0968$; $P > 0.05$; Fig 1).

*Camponotus blandus* attacked all termite baits placed on *B. malifolia* inflorescences. The ants bit the termites several times, often throwing them away from the plant. Nevertheless, no lycaenid larva was preyed upon, attacked or molested by *C. blandus*. In fact ants attended *P. polibetes* larvae. The ants, alone or in groups of two to three individuals, walked over the larva and antennated the whole extension of the *P. polibetes* body, but the antennation was concentrated on the dorsal nectary organ (Fig 2). In the meantime, the larva continued to feed on flower buds. The other lycaenids sampled in *B. malifolia* were not observed being attended by ants.

*Parrhasius polibetes* larvae fed on the whole content of buds, often leaving only an empty shell comprising the external surface of buds. Larvae consumed on average 17.0 ± 5.32 (range 5 – 35; n = 5 *P. polibetes* individuals) flower buds per day. This value refers to the feeding activity of the 4th instar larvae until pupation, which lasted on average seven days. Thus each 4th instar larva can damage on average 119 flower buds, which corresponds to approximately 35% of flower buds per inflorescence (flower bud abundance per inflorescence = 340.07 ± 35.77).

## Discussion

According to Price et al. (1995), cerrado vegetation supports a high diversity, but low abundance, of lepidopterans per species. Concerning lycaenids, Silva et al. (2011) showed that it is necessary large sampling effort to obtain a considerable abundance of larvae in different host plants; and despite considerable field sampling the authors found a low frequency of immature lycaenids in plants of common occurrence. This trend was also observed in our study, as we found a relative high community of lycaenids associated with *B. malifolia*, but the abundance of each species was low.

*Parrhasius polibetes* was the most abundant species (68.75% of individuals) while the other lycaenids were found in low numbers. The occurrence of *P. polibetes* in *B. malifolia* was observed to be mediated by the presence, but not the abundance, of *C. blandus.* Furthermore, *P. polibetes* presence was not related to the abundance of flower buds, but no larvae were found in the end of *B. malifolia* flowering season (May). *Parrhasius polibetes* was the only lycaenid species attended by *C. blandus*. Ant-mediated occurrence seems to be common in *P. polibetes* and Kaminski and Rodrigues (2011) showed that *P. polibetes* tended by *Camponotus* experienced increased performance and survivorship, as ants usually reduced the abundance of lycaenid natural enemies (see also Kaminski et al., 2010a; Rodrigues et al., 2010). In our study, *C. blandus* was very aggressive towards invaders, as demonstrated by the termite bait exposure experiment, suggesting that ants can molest or displace wingless lycaenid natural enemies. *Banisteriopsis malifolia* supports a diverse community of arthropods, including herbivores and predators (Alves-Silva, 2011; Alves-Silva et al., 2012), but in the field no larvae was observed to be attacked or injured by natural enemies, such as spiders or parasitoids, or molested by any other insect.

In our study, despite the limitation of larvae reared in the laboratory, *P. polibetes* fed on approximately 35% of flower buds per inflorescence, but its effect on *B. malifolia* fitness was negligible. *Banisteriopsis malifolia* bears tens of inflorescences, which together may contain more than 5000 flower buds (pers. obs.). Therefore, the reduction of up to 35 flower buds per day (4th instar larvae feeding estimation) will account for only a small portion of flower buds produced by the plant. In this context, the occurrence of *P. polibetes* in *B. malifolia* is unlikely to affect plant fitness to any great extent.

Malpighiaceae are considered as important hosts for *Rekoa* and *A. strophius* in the neotropics (Robbins, 1991; Monteiro, 2000; Kaminski & Freitas, 2010), but in our study the abundance of these lycaenids was low. Both *Rekoa* and *A. strophius* are polychromatic and this characteristic was observed in *B. malifolia*, where larvae turned pink. No *Rekoa* and *A. strophius* larvae were observed in contact with ants in *B. malifolia*, but both species have ant-organs and are facultative myrmecophilous (Robbins, 1991; Monteiro, 2000; Kaminski & Freitas, 2010).

The current knowledge about lycaenid-ant-host systems is based mostly on studies performed in the northern hemisphere. In the neotropics, only recently have the basic aspects of lycaenid life histories such as their distribution, host range, and ant-associations been given attention (Kaminski & Freitas, 2010; Rodrigues et al., 2010; Silva et al., 2011). Given the risk of extinction of some species (see Brown Jr., 1993; Kaminski et al., 2010b), lycaenid studies in the neotropics are imperative. Further studies will aim at unraveling the relationships between lycaenids and Malpighiaceae, as this seems to be an important host for lycaenid larval development (Robbins, 1991; Monteiro, 2000; Kaminski & Freitas, 2010; Bächtold et al., 2013).

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**Figures and captions**

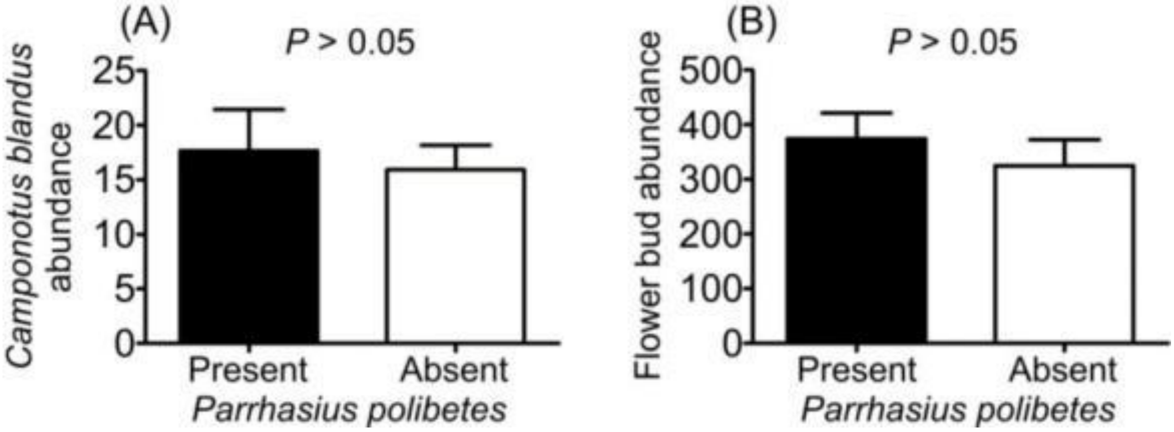

Fig 1. Relationship between the occurrence of *Parrhasius polibetes* according to: A - the abundance of *Camponotus blandus*; and B - the abundance of *Banisteriopsis malifolia* flower buds per inflorescence. Bars show the mean ± standard error.

Fig 2. *Camponotus blandus* tending a *Parrhasius polibetes* larva in *Banisteriopsis malifolia*. The ant is drumming the last body segments of larva. Ant size - 5 mm.

# CAPÍTULO VI

## The role, or the lack thereof, of patrolling ants on the deterrence of herbivore thrips: Effect on plant reproductive outputs?

# The role, or the lack thereof, of patrolling ants on the deterrence of herbivore thrips: Effect on plant reproductive outputs?

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## Abstract

Thrips co-occurrence with ants is commonplace in the neotropics, but their interactions are widely unexplored so as the effect of thrips on plant fitness. Should herbivorous thrips be deterred, a positive effect of ants on plant fitness might be expected. However literature shows that thrips might escape ant attack and feed intermittently on plants. Here we investigated the influence of *Camponotus blandus* on *Pseudophilothrips* abundance and herbivory rates in three extrafloral nectaried species, *Banisteriopsis malifolia, B. stellaris* and *B. laevifolia*). In addition we examined the effect of thrips herbivory on flower set and fruit development and dispersion. The study design accounted for ant-present and ant-excluded treatments. Thrips abundance and herbivory was higher in ant-present branches of *B. malifolia* and *B. laevifolia*, where thrips escape from ants by hiding in between clusters of flower buds. In *B. stellaris*, results were the opposite and thrips were unable to hide from ants as flower bud clusters do not offer hiding places. Despite the damage to flower buds, thrips herbivory had no effect on flower and fruit set. Nonetheless, thrips frugivory affected the development of samaras. Damaged fruits dispersed close to the mother plant, while healthy

fruits dispersed further away. *Pseudophilothris* effect on plant fitness may seem negligible with regards to flower and fruit set, but fruits are severely damaged so as their dispersive capabilities. Therefore thrips do affect plant fitness in the aforementioned plants, and in overall patrolling ants were unable to deter these tiny herbivores.

## Introduction

Thrips are widespread insect herbivores in the neotropics (Mound 2002) where their co-occurrence with patrolling ants in extrafloral nectaried plants is commonplace (Del-Claro et al. 1997). Such co-occurrence might indicate that thrips populations could be maintained at lower levels because of ant’s aggressive behavior towards insects in general. Nonetheless, there are few detailed studies of thrips-ant interactions (Del-Claro et al. 1997), and unfortunately most studies are observational, restraining assumptions of how thrips populations really are affected by ants (see Kranz et al. 1999; Sakai 2001; Peng & Christian 2004). Because of their ubiquity and high abundance in plants, these small insects may kill growing tips and shoots, thus, reducing plant performance, vigor, architectural complexity and fitness (Mound & Zapater 2003; Cuda et al. 2009).

In the case of plants bearing extrafloral nectaries (EFNs), the tending ants are supposed to protect the plant against herbivores in general (Heil & McKey 2003; Rutter & Rausher 2004; Koptur 2005), including thrips (Del-Claro et al. 1997). Nonetheless, in some cases ants fail to protect the plant (Rashbrook et al. 1992; Mody & Linsenmair 2004; Nogueira et al. 2012), as insect herbivores may have morphological and behavioral adaptations to escape from ant contact and attack (Koptur & Lawton 1988; Bächtold et al. 2012; Endo & Itino 2013). With regards to thrips, ants can affect their behavior and reduce their populations (Sakai 2001; Peng & Christian 2004). However, some thrips species can be found co-occurring with aggressive ants without being harmed (Alves-Silva & Del-Claro 2014) as these tiny herbivores can use chemical repellents to discourage ant contact (Suzuki et al. 2004) or hide in places inaccessible to ants (Crespi et al. 1997; Del-Claro 2004). This scenario has a negative consequence for plants, as once free from possible natural enemies (i. e. ants) thrips can feed intermittently and provoke severe damage to their host plants (Alves-Silva & Del-Claro 2014).

In ant-plant systems, authors usually consider fruit set as a good estimator of plant fitness (Nascimento & Del-Claro 2010; Rosumek et al. 2009). Herbivorous thrips have a wide feeding flexibility and consume mostly leaves, flower buds and flowers (Mound & Marullo 1996), but a few species can also feed on fruits, often provoking serious aesthetic damage with distortions and necrosis all over the fruit surface (Rosenheim et al. 1990; Blank et al. 1997). Therefore, it is important to take into account the natural history of frugivore insects in order to verify their real influence on plant reproductive efforts (Ruhren 2003; Pinent 2008).

In the cerrado vegetation several Malpighiaceae shrubs possess EFNs (Araujo et al. 2010), and are support a rich ant fauna together with herbivorous thrips which attack plant reproductive structures (Del-Claro et al. 1997). Therefore, this group of plants is ideal as model to investigate role of herbivorous thrips on plant reproduction, as well as the influence of ant-guards on the deterrence of these small herbivores. Such an approach was examined in three plant species, *Banisteriopsis laevifolia* (A. Juss.) B. Gates, *B. malifolia* (Nees et Mart.) B. Gates and *B. stellaris* (Griseb.) B. Gates. These plants are patrolled by *Camponotus blandus* (Smith 1858) Formicinae, and flower buds and fruits are severely attacked by *Pseudophilothrips* aff. *obscuricornis* ((Priesner 1921) (Phlaeothripidae) (pers. obs). We addressed two main questions: Can ants deter herbivory thrips? To what extent thrips herbivory is detrimental to plant reproduction? Thrips damage on reproductive parts was compared between ant-excluded and ant-present groups. We predicted two contradictory hypotheses: (i) patrolling ants might reduce thrips populations and, thus, herbivory rates (e.g., Peng & Christian 2004); or (ii) thrips would not be molested by ants, thus, inflicting damage to flower buds (e.g., Del-Claro et al. 1997). We also investigated the influence of thrips herbivory on fruit performance and dispersion. During the fruiting season, all *Banisteriopsis* (*laevifolia*, *malifolia*, *stellaris*) have no active EFNs and ants are absent. We hypothesized that (iii) thrips herbivory would affect fruit quality, measured as fruit size and shape, and that herbivory on fruits (samaras) would influence their dispersive capabilities by wind. Given the high frequency and abundance of herbivorous thrips in the vegetation (Pinent et al. 2006), such study may provide a first step towards the understanding of their inter-specific interactions and their effect on plant performance, both of which are still largely unexplored in the neotropics.

## Methods

### Study area

Fieldwork was conducted in a Cerrado biome (Brazilian tropical savanna area) (18º59'S – 48º18'W) in Uberlândia, Brazil. The cerrado covers 230 ha and is dominated by herbaceous and shrubs, such as Malpighiaceae, Myrtaceae and Fabaceae. Trees of Caryocaraceae and Ochnaceae, among other families occur scattered on the area while grasses are common. The wettest season (October to April) concentrates more than > 90% of the annual rainfall (1500 mm per year). Mean monthly temperature ranges from 24.8 ºC in February to 19.9 ºC in June, with an annual mean of 23 ºC (Laboratory of Climatology, UFU, Brazil, 2011).

## Study organisms

*Banisteriopsis laevifolia* and *B. malifolia* are shrubs (< 2 meters high), while *B. stellaris* is a vine. Fully expanded leaves may reach up to 10 cm long and 5 cm wide in *B. laevifolia*; 15 and 10 cm in *B. malifolia*; and 7 and 4 cm in *B. stellaris*. Leaves of all species have a pair of EFNs at the base near the petiole at each side of the midrib (Fig. 1A), but in *B. laevifolia* EFNs are functional only in small leaves (< 2 cm length), which are distributed evenly along the branches with inflorescences. Flower bud production in *B. malifolia* peaks in March and April; in *B. laevifolia* August–September; and in *B. stellaris* in January–February (Table 1). In all these species, flower buds grow on inflorescences located at the apex of branches and stems. Mature flower buds are round and on average 5, 8 and 6 mm in diameter in *B. laevifolia*, *B. malifolia* and *B. stellaris* respectively, and are surrounded by eight oil glands. Fruiting peaks in May, November and March in *B. malifolia*, *B. laevifolia* and *B. stellaris*, respectively (Table 1). All plants produce wind-dispersed *Y*-shaped samaras with crispy wings and tiny trichomes spread all over (Fig. 1B).

*Camponotus blandus* is frequently found in Malpighiaceae and it is very aggressive towards insects in general, including other predatory species (Guimarães Jr. et al. 2006; Alves-Silva et al. 2013). *Pseudophilothrips* aff. *obscuricornis* (hereafter *Pseudophilothrips*) are dominant insect herbivores in *Banisteriopsis*, feeding on leaves, flower buds and fruits, but not on flowers (Figs. 1C–1D). Females lay eggs on shoots, flower buds and fruits, and hatched larvae remain on these structures until pupation, which takes place in the soil. Eggs are kidney shaped and yellowish to brownish in color. Larvae can remain immobile on a given plant structure all day long, and both adult and larvae are found in aggregations of up to 18 individuals, sucking up plant sap from leaves, buds and fruits. Larvae (≈ 1 mm in length) are reddish, wingless and very reticent to migrate between plant structures, while winged adults (≈ 2 mm long and black in color) are more mobile, walking rapidly and migrating from plant parts (pers. obs).

## Flower bud herbivory rates

Flower bud herbivory was assessed in different periods according to the phenology of each plant (Table 1). *Banisteriopsis malifolia* (n = 40 individuals) was studied in March–April 2011; *B. laevifolia* (n = 32 individuals) in July–August 2011 and *B. stellaris* (n = 25 individuals) in January–February 2011. Plants were spread evenly all over 30 ha within the study area and fieldwork was restrained to plants that supported *C. blandus* only.

In each plant, one control and one treatment branch were chosen for the experimental design. The base of 'treatment' branches was banded with a layer of atoxic sticky resin (Tanglefoot™) to prevent the access of ants. To control for the effect of resin, Tanglefoot was also applied on one side of the 'control' branches, allowing the free access of ants to the plant parts (following Nahas et al. 2012). Leaves and other branches that could be used by ants as bridges to climb onto experimental branches were removed or clipped back. Special care was taken with regards to *B. stellaris*, as this plant is a vine. Whenever necessary, plants at the vicinity received resin to avoid the access of ants. Tagged branches of all plants were also deprived of insect herbivores other than thrips.

Right after resin application, we conducted an initial assessment of flower bud herbivory (%), which was calculated as the number of damaged buds divided by the total number of buds produced. Thrips abundance was estimated in each *Banisteriopsis* by visually examining flower buds, shoots and both sides of leaves of the experimental and control branches. In this first sampling, there was no statistical difference in bud herbivory (%) between experimental and control branches ($P > 0.05$ in all cases) (Table 2). Thus, study bias due to differences in herbivory rates was discarded. Thrips were also distributed evenly all over experimental and control branches in all plants (*P* > 0.05 in all cases) (Table 2).

After the beginning of the experimental design (resin application), plants were visited twice a week to ensure that ants were indeed excluded from the experimental branches. During these visits, other herbivores that might influence our study (e.g., beetles, butterfly larvae and grasshoppers) were also removed and placed in distant non-experimental plants. Three weeks after resin application (approximately one week before flowering), flower bud herbivory (%) and thrips abundance were estimated again in experimental and control branches.

### Ant behavior towards thrips

In the field, we conducted 90 hours of observation (*ad libitum*) per plant species of the behavior of *C. blandus* towards *Pseudophilothrips.* Observations were made in 10 individuals of each *Banisteriopsis* species (*laevifolia, malifolia* and *stellaris*) for three hours (0800–1100 h) on consecutive sunny days. Whenever ants came close to thrips we registered whether the latter were attacked, molested or escaped from the ants.

### *Pseudophilothrips* herbivory and flower set

To investigate whether *Pseudophilothrips* herbivory in buds influenced flower set, 20 mature flower buds were tagged in each individual of each plant species (n = 25 *B. malifolia* individuals (500 buds); n = 20 *B. laevifolia* (400 buds); n = 18 *B. stellaris* (360 buds)). Tagged buds belonged all to the same branch and were divided in two groups, 10 flower buds with necrosis spots (see Fig. 1C - hereafter referred to as damaged buds) and 10 healthy buds with no sign of thrips presence or herbivory. Each flower bud was tagged with a fine cloth (2 cm long) tied at its base. Thrips were removed from these branches as they could feed on tagged healthy buds. Plants were also visited twice a week to remove possible florivores. Flower buds were accompanied until flower anthesis.

### Thrips frugivory and fruit development

The influence of thrips herbivory on fruit (samara) development was investigated on different occasions, according to the fruiting phenology of each plant. *Banisteriopsis malifolia* (n = 25 individuals) was studied in May 2011; *B. laevifolia* (n = 20 individuals) in November 2011 and *B. stellaris* (n = 15 individuals) in March 2011. In each individual plant we collected 20 samaras, which were always sampled in pairs, accounting for one damaged samara and the adjacent samara without thrips damage. *Pseudophilothrips* females lay eggs on fruit surfaces, and hatched larvae rarely migrate from fruits, so larvae can spend all the immature stage feeding on a single fruit. As fruits within infrutescences mature at the same time, neighboring fruits are supposed to have the same age. Samara development was examined through measures of length and width (mm) in the laboratory. We expected that healthy samaras were larger and wider than damaged ones.

### Thrips herbivory and fruit dispersion

The influence of thrips herbivory on samara dispersion was examined in 15 *B. malifolia* individuals. All individuals were at least five meters from each other and located in an area containing mostly herbaceous vegetation (< 1 m in height). In each shrub we tagged 20 samaras, divided equally in two groups: one containing samaras damaged by thrips (n = 10 fruits per plant) and the other group with healthy samaras (n = 10 fruits per plant). Both groups of fruits belonged to the same branch, which were located on average 1.62 (± 0.10) cm above the soil and facing east.

Samaras were marked with a red inked pen, and the symbols "*D*" and "*H*" were written in the wings of damaged and healthy samaras, respectively, together with the number of the individual plant (1 to 15). Samaras are dispersed by wind, and we expected that healthy ones would fly farther than the distorted and asymmetric samaras damaged by thrips feeding. Wind speed during the experimental design was measured with a hand anemometer, placed 1.5 meters above the soil and set to measure the mean wind speed in a 10-minute interval during a sunny morning from 0900 to 0910 h. Plants were visited each three days for one month, and the search for dispersed fruits was made on the soil and grasses in the vicinity (≈ 10-m radius) of the plants.

## Statistical analyses

Quantitative data is presented as mean ± standard deviation. In those cases where data did not satisfy the assumptions of a normal distribution ($P < 0.05$) and variance homoscedasticity, and transformations were unable to achieve data normality, we used non-parametric statistical tests. Figures show untransformed data for the sake of clarity. The comparison of the number of thrips in control and treatment branches was made with Student's *t* tests (*B. stellaris* original data was log transformed). Flower bud herbivory (%) in treatment and control branches was compared with Wilcoxon tests, so as the abundance of flowers produced from damaged and healthy buds. The measurements of fruit length and width were compared between healthy and damaged fruits with Student's *t* tests. This test was also used to compare the dispersion distance between healthy and damaged samaras.

## Results

### Flower bud herbivory rates

*Pseudophilothrips* were significantly more abundant in control branches in *B. laevifolia* ($t_{31}$ = 5.6103; $P < 0.0001$) and *B. malifolia* ($t_{39}$ = 2.8613; $P < 0.01$), but in *B. stellaris*, thrips were more abundant in branches without *C. blandus* ($t_{24}$ = 4.0183; $P < 0.0001$) (Fig. 2). In *B. laevifolia* and *B. malifolia*, branches with *C. blandus* (control ones) had on average 33.67 and 31.15% more thrips than ant-excluded branches, respectively. The opposite scenario was observed in *B. stellaris*, where ant-excluded branches had almost twice the number of *Pseudophilothrips* (98.57%) compared to control branches.

Thrips herbivory was markedly different between branches and among plants. In *B. laevifolia* and *B. malifolia*, flower bud herbivory (%) was higher in ant-present branches ($T$ = 133.0; $P < 0.05$ and $T$ = 151.0; $P < 0.005$, respectively), while in *B. stellaris* bud damage was higher in ant-excluded branches ($T$ = 52.0; $P < 0.005$) (Fig. 3).

### Thrips-ant interactions

In the field, ants were observed attacking a large array of arthropods in *Banisteriopsis*, such as spiders, flies, wasps, beetles and grasshoppers. Nonetheless, when ants touched thrips, the latter raised their abdomens and a small drop was noticed in the end of their anus. Ants, then, backed off rapidly and did not return to the thrips. The abdomen raising behavior was noticed in both adults and larvae of *Pseudophilothrips*. Larvae are gregarious and up to six individuals could be found together in a same flower bud, and because of their striking red color, larvae were very conspicuous. In general, the larvae were immobile, feeding on the same bud all day long. In contact with ants, the abdomen-raising behavior of one larva was sometimes followed by the nearest larva. This synergic behavior was sufficient to make ants go away. Adult individuals of *Pseudophilothrips* were frequently found together with larvae, hidden in shoots and undeveloped leaves (especially in *B. laevifolia*) and amongst flower buds. In *B. malifolia* (Fig. 1E) and *B. laevifolia,* buds grow in clusters with each bud touching its neighbor, and the conformation of bud-clusters provides small cracks where thrips were found. In *B. stellaris*, the conformation of flower buds (separate from each other – Fig. 1F) did not permit thrips to hide, and they were very conspicuous in the plant.

## *Pseudophilothrips* herbivory and flower set

Flower buds damaged by thrips presented several black necrosis marks, especially on oil glands (Fig. 1C); nonetheless, thrips herbivory had no effect on mean flower formation per plant and no difference was found on flower set between healthy and damaged buds (*B. laevifolia* $T = 52$; $P > 0.05$; and *B. malifolia* $T = 57$; $P > 0.05$; *B. stellaris* $T = 37$; $P > 0.05$) (Fig. 4).

## Thrips and fruit development

We found a marked significant difference in the size of fruits with and without thrips in all *Banisteriopsis* species. Damaged fruits were on average 7.5, 8.9 and 5.5% shorter than healthy fruits in *B. laevifolia, B. malifolia and B. stellaris*, respectively. The difference in fruit width was 12.4, 10.6 and 24.4% respectively (Table 3).

## Thrips and fruit dispersion

Among the samaras tagged for the investigation of dispersion (n = 300), only 31.3% (n = 94; 6.71 ± 2.46 fruits per plant) were effectively dispersed or recovered. Some samaras were not abscised at all by the plant and became senescent eventually; others were abscised but not found on the ground during the search. In the end of the experiment, we were able to collect 53 healthy and 41 damaged samaras from *B. malifolia*. The wind speed average on the occasion (May) was 3.02 m/s. Damaged samaras were found on average 85.11 ± 5.8 cm away from the mother plant, while healthy samaras were collected 107.67 ± 7.33 cm away from the mother plant ($t_{92}$ = 16.3591; $P < 0.0001$).

## Discussion

### *Pseudophilothrips* herbivory in *Banisteriopsis*

*Pseudophilothrips* (13 spp.) are a poorly studied group (both taxonomically and ecologically) within Thysanoptera (Mound et al. 2010). However, these species are a major concern given their severe herbivory of commercial crops and because thrips feeding facilitates the establishment of fungi in plants (Picanço et al. 2003; Urías-López et al. 2007). In some cases, *Pseudophilothrips* can feed continuously on leaves, provoking serious consequences for plant development, performance and fitness (Del-Claro & Mound 1996; Furmann et al. 2005; Varanda & Pais 2006). All these factors summed with the high reproductive rates of the species (up to four generations per year – Cuda et al. 2006) culminate in severe damage to hosts (Cuda et al. 2008).

In the present study, thrips were shown to inflict necrosis on flower buds of three *Banisteriopsis* species (*laevifolia, malifolia* and *stellaris*); however, damaged buds reached the flower stage, indicating that thrips herbivory did not significantly influenced flower set. *Pseudophilothrips* fed on the external surface of bud tissues and the damage was almost restricted to the oil glands (Fig. 1C). Such damage might affect pollinator visits because oil is a resource provided to *Banisteriopsis* pollinators. Nonetheless, pollinating bees (large-bodied Centridini) reach the flower from above, landing on petals and rubbing their abdomens on anthers and stamens first, and then leaning their body down to reach the oil glands below the flower (see Anderson 1979; Gaglianone 2001; 2003). In this case, cross-pollination is performed before oil consumption, so damaged oil glands are not supposed to affect flower visits; in fact, non-glandular Malpighiaceae are also visited by bees (see also Sigrist & Sazima 2004). Studies have shown that the mere presence of herbivores or the damage in flowers may negatively influence flower visitors and plant fitness, especially when herbivores destroy flower reproductive structures (see Kessler & Halitschke 2009; Botto-Mahan et al. 2011; Lucas-Barbosa et al. 2012). Nonetheless, *Pseudophilothrips* do not occur in flowers in anthesis, therefore, a negative effect of thrips on flower performance, development, pollination and seed set can also be ruled out in *Banisteriopsis*.

Despite the lack of influence in *Banisteriopsis* reproductive outputs, *Pseudophilothrips* damage on samaras was severe and fruits presented necrosis, distortions and deformations. Sucking herbivores may reduce fruit filling and seed set/size (Crawley 1989), but *Pseudophilothrips* feed on the surface of samaras, thus, their influence is more aesthetical (distortions) and functional (dispersion). Samaras are dispersed by wind (Mirle & Burnham 1999), thus, changes in their aerodynamic structure may affect how far from the mother plant samaras are taken (see Augspurger & Franson 1987). The influence of *Pseudophilothrips* on fruit dispersion may negatively affect plant reproductive efforts, as according to Janzel-Connell’s hypothesis, progeny near adults may suffer high mortality rates (Hyatt et al. 2003; Nathan & Casagrandi 2004; Hansen et al. 2008). In this case, even if *Pseudophilothrips* do not affect *Banisteriopsis* flowers or fruit set, their effects on fruit dispersion may restrain plant reestablishment. In *B. malifolia*, damaged fruits were found closer to the mother plant in comparison to healthy fruits, but in general the dispersive capability of these samaras was low (≈ 1-m radius away from the parents). Presumably, the weak winds at the occasion restrained the flight of samaras to further distances (see Greene & Johnson 1992; Nathan et al. 2001). Further studies taking into account seedling establishment and survival may help to understand the real influence of *Pseudophilothrips* on plant reproduction efforts.

## Thrips-ant-plant interactions

Ant-plant interactions mediated by the presence of EFNs have been shown to benefit both species involved, as ants feed on the sugar sources provided by the plant and, in turn, defend the plant against a wide array of insect herbivores (Nascimento & Del-Claro 2010). Nevertheless, in the present study we found evidence that thrips may negatively influence the stability of *C. blandus–Banisteriopsis* interactions, since herbivory rates (in *B. laevifolia* and *B. malifolia*) were higher in ant-present branches, indicating that *Pseudophilothrips* managed to live concomitantly with aggressive ants without being preyed upon. In *B. stellaris*, however, the results were opposite. The explanation for these differences relies on flower bud conformation. In *B. laevifolia* and *B. malifolia*, buds grow in clusters, which provide hiding places for thrips (Fig. 1E); while in *B. stellaris*, buds are distant from each other (Fig. 1F) and thrips are susceptible to ant molestation. In the search for protection, thrips have the ability to enter small cracks where they are inaccessible to natural enemies and protected from harsh environments, and this behavior is known as thigmotaxis (Crespi et al. 1997; Del-Claro et al. 1997). For instance, in *B. laevifolia* and *B. malifolia* thrips are susceptible to extreme temperatures, sometimes peaking at 40 ºC in the cerrado savanna, so the thigmotaxis behavior may protect individuals from desiccation (see Mound & Terry 2001). *Banisteriopsis stellaris* does not provide hiding places for *Pseudophilothrips*, and in the field we noticed that thrips often dropped from the plant under constant disturbances (ant molestation).

No thrips individual was preyed upon by *C. blandus*, but these insects were often molested by these ants. In contact with ants, thrips raised their abdomens and released a small drop from their anus. Ant repellents are commonplace in thrips and several compounds were shown to act as a thrips defense mechanism against natural enemies, especially ants (Blum 1991; Blum et al. 1992; Suzuki et al. 2004). *Camponotus blandus* is very active in *Banisteriopsis*, moving rapidly from plant structures and attacking insects in general (Oliveira & Freitas 2004; Guimarães Jr. et al. 2006). Such behavior may be beneficial to *Pseudophilothrips*, as ants may deter thrips' own herbivores (e.g., hemipterans, neuropterans, beetles and spiders – Mound & Marullo 1996) or competitors. In this case, the pressure exerted by ant molestation towards thrips is counterbalanced by the net benefits of living in an enemy-free environment (see Kaminski et al. 2010).

This study contributes to the knowledge of one of the most ignored insects in the neotropics, the Thysanoptera, and is a first step towards understanding the natural history and ecological interactions among thrips, their possible natural enemies (ants) and host plants. In our study, *C. blandus* failed to protect the plants (*B. laevifolia* and *B. malifolia*) against *Pseudophilothrips*, leading to asymmetries in the stability of the ant-plant mutualism, that is, the ants received food from EFNs, but the plant had no biotic defense against thrips herbivores. Should similar results be found in other study-system involving ants and thrips, it might be evidence that these tiny insects are immune to plant-guards and pervasive herbivores in the vegetation.

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## Tables

**Table 1.** Phenology of three *Banisteriopsis* species (*malifolia*, *laevifolia* and *stellaris*) in a Brazilian tropical savanna. Data were collected in during 2011 through monthly visual inspections in the study plants and is based on the presence and absence of each phenological event.

| **Phenology** | **Jan** | **Feb** | **Mar** | **Apr** | **May** | **Jun** | **Jul** | **Aug** | **Sep** | **Oct** | **Nov** | **Dec** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Banisteriopsis malifolia*** | | | | | | | | | | | | |
| Active NEFs | ■ | ■ | ■ | ■ | | | | | | | | ■ |
| Flower buds | | | ■ | ■ | | | | | | | | |
| Blooming | | | ■ | ■ | ■ | | | | | | | |
| Fruiting | | | | ■ | ■ | ■ | | | | | | |
| ***Banisteriopsis laevifolia*** | | | | | | | | | | | | |
| Active NEFs | | | | | | | ■ | ■ | ■ | | | |
| Flower buds | | | | | | | ■ | ■ | ■ | | | |
| Blooming | | | | | | | ■ | ■ | ■ | | | |
| Fruiting | | | | | | | | ■ | ■ | ■ | ■ | |
| ***Banisteriopsis stellaris*** | | | | | | | | | | | | |
| Active NEFs | ■ | ■ | ■ | | | | | | | | | |
| Flower buds | ■ | ■ | ■ | | | | | | | | | |
| Blooming | | | ■ | ■ | ■ | ■ | | | | | | |
| Fruiting | | | | ■ | ■ | ■ | | | | | | |

**Table 2.** Flower bud herbivory (%), total number of sampled buds and *Pseudophilothrips* abundance in control (ant-present) and treatment (ant-excluded) branches at the beginning of the study in each *Banisteriopsis* species. There was no statistical difference between herbivory and thrips abundance between treatments. The choice of using parametric or non-parametric statistics was based upon normality tests. *T* – Wilcoxon test; *t* – Student's *t* test; *ns* – non-significant.

| **Flower bud herbivory (%)** | | | |
|---|---|---|---|
| ***Banisteriopsis* species** | **Control M ± SD (n)** | **Experimental M ± SD (n)** | **Statistics** |
| *B. malifolia* | 11.10 ± 8.23 (6006) | 9.75 ± 7.74 (6308) | $T = 312^{ns}$ |
| *B. laevifolia* | 8.98 ± 4.61 (7068) | 7.86 ± 5.61 (6545) | $T = 146^{ns}$ |
| *B. stellaris* | 1.67 ± 1.90 (1618) | 1.41 ± 2.15 (1342) | $T = 47^{ns}$ |
| **Thrips abundance** | | | |
| *B. malifolia* | 12.98 ± 8.49 (519) | 11.48 ± 7.94 (459) | $t = 0.8370^{ns}$ |
| *B. laevifolia* | 13.63 ± 4.70 (436) | 14.73 ± 5.40 (473) | $t = 0.9570^{ns}$ |
| *B. stellaris* | 1.04 ± 1.08 (25) | 1.83 ± 1.40 (44) | $T = 82^{ns}$ |

**Table 3.** Difference in fruit size according to *Pseudophilothrips* herbivory in three *Banisteriopsis* species. Damaged fruits were smaller and narrower than healthy fruits. *** - *P* < 0.0001.

| **Malpighiaceae species** | | | |
|---|---|---|---|
| **Fruit length** | **Mean ± SD (n)** | | **Statistics** |
| *Banisteriopsis laevifolia* | 20.00 ± 3.42 (200) | 18.50 ± 3.25 (200) | $t = 4.7282^{***}$ |
| *Banisteriopsis malifolia* | 23.49 ± 3.42 (200) | 21.40 ± 4.22 (200) | $t = 5.1405^{***}$ |
| *Banisteriopsis stellaris* | 18.17 ± 4.01 (150) | 17.09 ± 4.87 (150) | $t = 2.8806^{*}$ |
| **Fruit width** | **Mean ± SD (n)** | | |
| *Banisteriopsis laevifolia* | 17.58 ± 3.40 (200) | 15.33 ± 3.32 (200) | $t = 6.8791^{***}$ |
| *Banisteriopsis. malifolia* | 15.00 ± 3.44 (200) | 13.41 ± 3.30 (200) | $t = 5.0873^{***}$ |
| *Banisteriopsis stellaris* | 18.62 ± 3.93 (150) | 14.08 ± 5.81 (150) | $t = 11.0124^{***}$ |

## Figures and captions

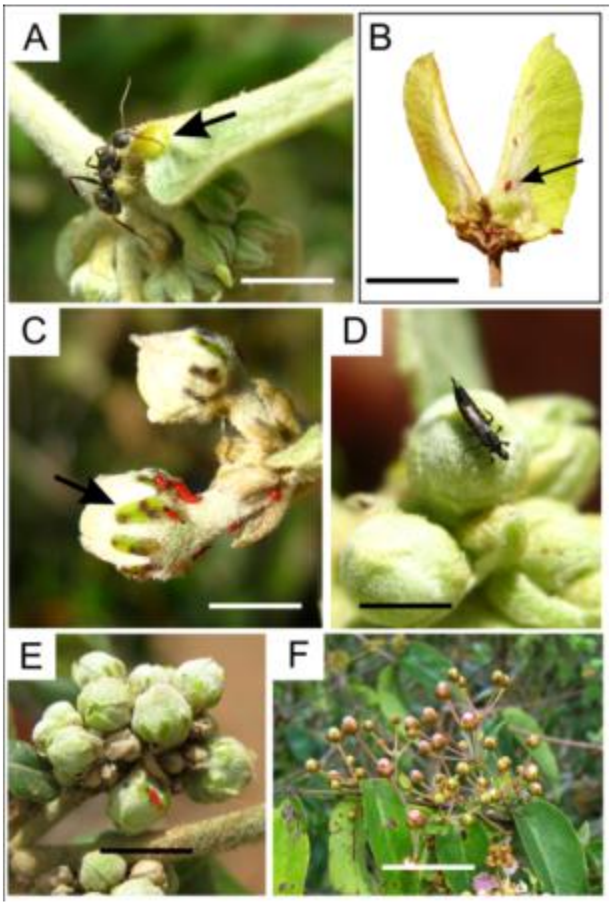

**Figure 1.** (A) *Camponotus blandus* feeding on an extrafloral nectary (arrow) of *Banisteriopsis malifolia*. (B) *B. malifolia* fruits are samaras, which are dispersed by wind. Note that thrips larvae occur all over the fruit (arrow). (C) *Pseudophilothrips obscuricornis* larvae feeding on flower buds. The arrow indicates the necrosis of oil glands. (D) Adult *Pseudophilothrips* foraging on undeveloped flower buds. (E) Conformation of *B. stellaris* and (F) *B. malifolia* flower buds. Note that flower buds are aggregated in *B. malifolia*, while in *B. stellaris* they grow distant from each other. Scales (A), (C) 5 mm; (B) 10 mm; (D) 2 mm; (E) 5 mm; and (F) 120 mm.

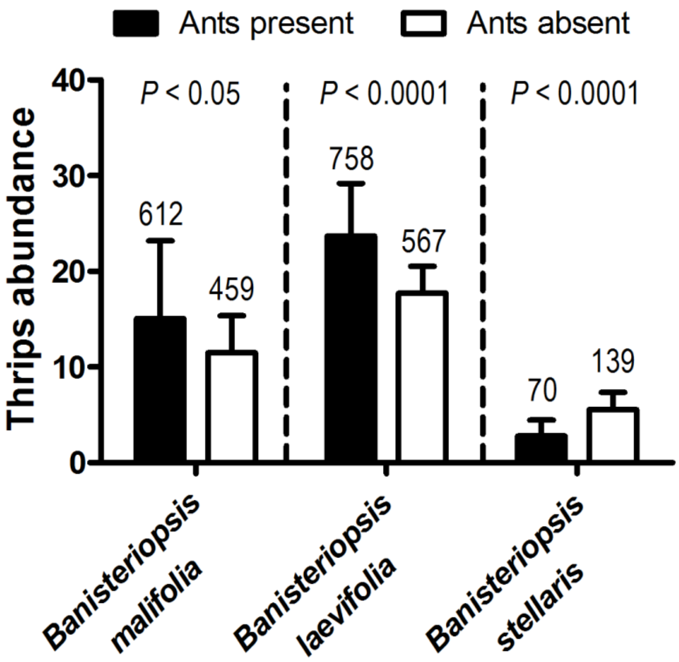

**Figure 2.** Abundance of *Pseudophilothrips* (mean and SD) in three *Banisteriopsis* species, according to the presence or absence of *Camponotus blandus*. In *B. malifolia* and *B. laevifolia*, thrips were more abundant in branches with ants, while in *B. stellaris* thrips were more abundant in branches without ants. Numbers above bars indicate the total abundance of thrips in each treatment. $P < 0.05$ and $P < 0.0001$ indicate statistical significant differences.

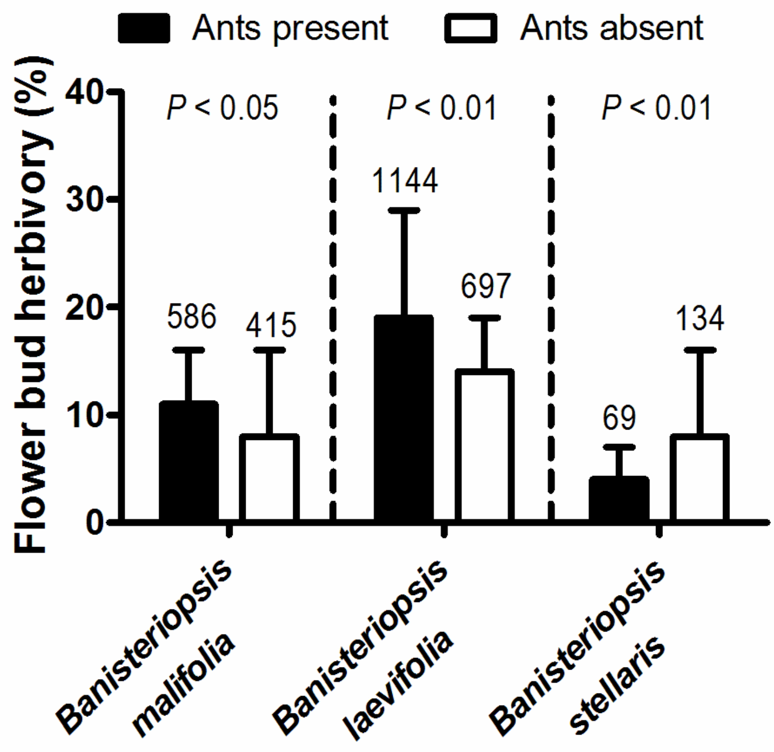

**Figure 3.** Flower bud herbivory (mean and SD in %) in three *Banisteriopsis* species. In *B. laevifolia* and *B. malifolia*, *Pseudophilothrips* feeding damage was higher in ant-present branches, while in *B. stellaris,* ant-excluded branches presented higher herbivory rates in flower buds. Numbers above bars indicate the total abundance of flower buds in each treatment. $P < 0.05$ and $P < 0.0001$ indicate statistical significant differences.

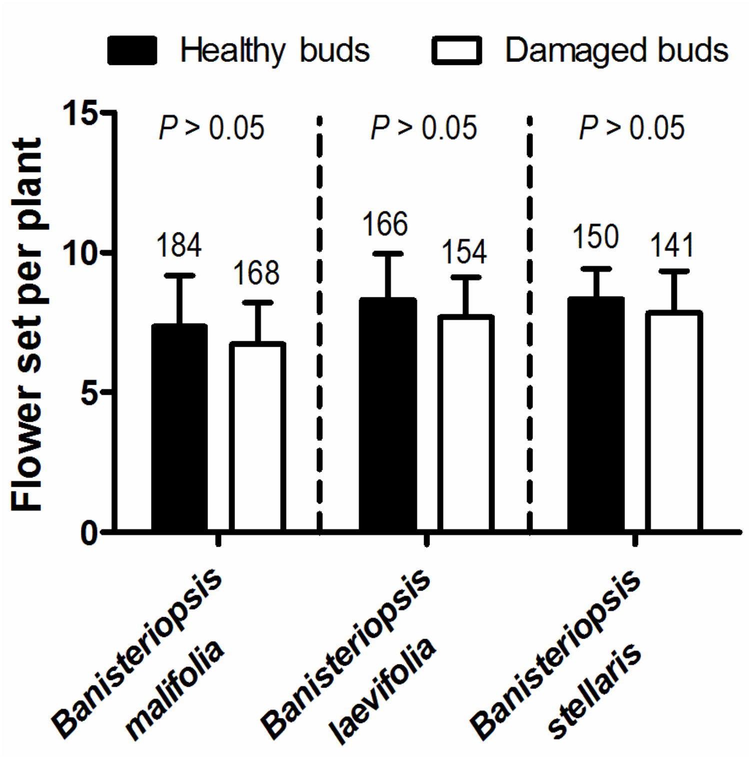

**Figure 4.** Flower set (mean and SD) in three *Banisteriopsis* species according to flower bud herbivory. *Pseudophilothrips* herbivory had no significant influence on flower formation. Numbers above bars indicate the total abundance of flower in each treatment. $P > 0.05$ indicates no statistical significant differences.

## Considerações finais

Ao longo desta tese nós mostramos as particularidades das interações multitróficas em Malpighiaceae, principalmente aquelas envolvendo herbívoros e seus possíveis inimigos naturais, como formigas e vespas. Nós mostramos como certos herbívoros podem afetar negativamente suas plantas hospedeiras, seja pela herbivoria em partes vegetativas e/ou partes reprodutivas. Em Malpighiaceae, tripes são os herbívoros mais frequentes, e por possuir uma flexibilidade alimentar grande, estes pequenos herbívoros podem ser encontrados virtualmente em todas as estruturas da planta, como folhas, botões florais, flores e frutos. O dano provocado à planta é bastante relativo. Por exemplo, foi mostrado que tripes se alimentam intermitentemente de botões florais, porém isto não afeta a produção de flores ou de frutos em espécies de *Banisteriopsis*. No entanto, frutos (sâmaras) danificados por tripes apresentam sérias deformações que restringem sua dispersão pelo vento, pois a herbivoria de tripes afeta a estrutura aerodinâmica das sâmaras. É importante ressaltar que tripes possuem poucos inimigos naturais e aparentemente, estes insetos são imunes às formigas consideradas agressivas, ou seja, aquelas que se alimentam de nectários extraflorais (NEFs) e estabelecem relações mutualistas com as plantas.

Em se tratando de mutualismo, as mesmas formigas que se alimentam nos nectários extraflorais (*Camponotus*), são as mesmas que podem estabelecer relações estáveis com larvas de licenídeos. Malpighiaceae pode suportar uma diversidade considerável de licenídeos, apesar da abundância baixa de larvas encontrada no campo. As larvas de licenídeos secretam uma solução açucarada quando em contato com as formigas, e estas últimas em contrapartida, não atacam as larvas, que continuam a se alimentar de estruturas reprodutivas até o período de pupa. Em nosso estudo, a herbivoria das larvas de licenídeo não afetou significativamente o fitness de sua planta hospedeira, visto que os botões eram produzidos de centenas a milhares. No entanto, chamamos atenção para o fato de que para plantas que produzem poucos botões, a herbivoria de licenídeos pode ser sim custosa para a planta.

Em nossos estudos, herbívoros endofíticos podem ser considerados os mais danosos às plantas, pois podem parasitar até 20% dos botões produzidos. Além disso, devido ao seu desenvolvimento dentro dos botões florais, somente predadores específicos conseguem capturá-los. Neste caso em particular, vespas predadoras foram os principais, senão os únicos, predadores de besouros endofíticos, conseguindo a façanha de remover até 50% da população destes insetos.

Diante de todos estes estudos nós podemos concluir que as Malpighiaceae são um bom modelo de estudo para se investigar interações multitróficas e teias alimentares. Nós focamos nossos estudos nos grupos mais representativos de herbívoros e inimigos naturais, sendo assim, se consideramos grupos menos abundantes de insetos, ainda há muito que ser estudado. A grande abundância e ocorrência de algumas espécies de Malpighiaceae em diversas áreas é um fator que pode facilitar desenhos experimentais mais complexos, como aqueles envolvendo variáveis abióticas. Por exemplo, em dois de nossos estudos nós aproveitamos a rápida rebrota das plantas após queimadas, o que nos permitiu avaliar como o crescimento rápido afetava a ocorrência de formigas e herbívoros, e também se a própria rebrota se traduzia em uma condição estressante para a planta.

Em resumo, podemos concluir que as interações formiga-planta-herbívoros são altamente condicionais, podendo variar de acordo com as espécies envolvidas. Além disso, fatores abióticos não podem ser totalmente ignorados em certos sistemas ecológicos, principalmente quando causam grandes mudanças nas plantas. Nós consideramos esta tese como um esforço para o entendimento das relações de Malpighiaceae com herbívoros e seus inimigos naturais. O fato de as plantas possuírem NEFs é um grande atrativo para estudos de interações, visto que estas estruturas atraem tanto formigas e vespas, que se somam aos herbívoros que ocorrem naturalmente nas plantas, somando-se assim uma grande diversidade de entomofauna. Entender como as interações entre estes níveis tróficos se organizam e quais os custos e benefícios para as plantas será o foco de estudos nos próximos anos.