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INFLUÊNCIA DA FORMIGA MUTUALISTA NO INVESTIMENTO EM DEFESAS DE PLANTAS: UM EXPERIMENTO DE LONGA DURAÇÃO NO SISTEMA *Cecropia-Azteca*

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Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ecologia, para obtenção do título de *Doctor Scientiae*.

Orientador: Ricardo Ildefonso de Campos

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RESUMO

ZORZAL, Gabriela, D.Sc., Universidade Federal de Viçosa, julho de 2024. **Influência da formiga mutualista no investimento em defesas de plantas: um experimento de longa duração no sistema *Cecropia-Azteca*.** Orientador: Ricardo Ildefonso de Campos. Coorientadores: Inácio José de Melo Teles e Gomes.

As plantas demonstram estratégias defensivas diversas contra seus inimigos naturais. Estas defesas podem ser diretas, como compostos químicos e estruturas físicas, e indiretas, como a associação com outros animais. As plantas mirmecófitas, por exemplo, vivem em associação íntima com formigas que desempenham função protetora em troca de alimento e locais de nidificação. Nesse sentido, investir em recursos para atrair formigas pode concorrer, em termos de alocação de energia, com o investimento em defesas diretas, gerando o chamado “trade-off”. Mais especificamente, na relação mutualística entre as formigas do gênero *Azteca* e as árvores do gênero *Cecropia*, já foi demonstrado que as formigas proporcionam múltiplos benefícios às plantas que vão além da proteção. No entanto, não se sabe exatamente como a presença das formigas influencia as características de defesa dessas plantas. A partir dessa lacuna, essa tese foi dividida em dois capítulos: O primeiro deles teve como objetivo testar se a presença de formigas (*Azteca muelleri*) e a herbivoria influenciam o investimento em defesas físicas em *Cecropia glaziovii.* No segundo capítulo foi determinado se a colonização por *A. muelleri* é capaz de mediar a existência de trade-off entre defesas químicas e físicas em *C. glaziovii*. Em relação ao primeiro capítulo, conduzimos dois experimentos, sendo um deles de simulação e outro de proteção de herbivoria em plantas no campo. Já no segundo capítulo, realizamos um experimento manipulativo de longo prazo (oito anos) para avaliar o investimento em defesas químicas e físicas em plantas colonizadas e não colonizadas. Com coletas mensais, acessamos dados de densidade de tricomas foliares (defesas físicas) e compostos fenólicos (defesas químicas) de plantas (*C. glaziovii*) colonizadas e não colonizadas pelas formigas (*A. muelleri*). Descobrimos que em plantas colonizadas por formigas a produção de tricomas é menor, independentemente da herbivoria. Encontramos também que a presença das formigas é um forte mediador do *trade-off* entre as defesas químicas e físicas de plantas; devido a redução na produção de tricomas com um incremento de compostos fenólicos apenas nas plantas não colonizadas. Nossos resultados reforçam a importância das formigas como defensoras, mas também como mediadoras da alocação e na provisão de recursos nessas plantas.

ABSTRACT

ZORZAL, Gabriela, D.Sc., Universidade Federal de Viçosa, July 2024. **Influence of the mutualist ant on investment in plant defenses: a long-term experiment in the *Cecropia-Azteca* system.** Orientador: Ricardo Ildefonso de Campos. Coorientadores: Inácio José de Melo Teles e Gomes.

Plants display diverse defensive strategies against their natural enemies. These defenses can be direct, such as chemical compounds and physical structures, and indirect, such as association with other animals. Myrmecophyte plants, for example, live in intimate association with ants that perform a protective role in exchange for food and nesting sites. In this sense, investing in resources to attract ants can compete, in terms of energy allocation, with investing in direct defenses, generating the so-called "*trade-off*". More specifically, in the mutualistic relationship between ants of the genus *Azteca* and trees of the genus *Cecropia*, it has already been demonstrated that ants provide multiple benefits to plants that go beyond protection. However, it is not known exactly how the presence of ants influences these plants' defense characteristics. Based on this gap, this thesis was divided into two chapters: The first aimed to test whether the presence of ants (*Azteca muelleri*) and herbivory influence investment in physical defenses in *Cecropia glaziovii*. In the second chapter, it was determined whether colonization by *A. muelleri* is capable of mediating the existence of a trade-off between chemical and physical defenses in *C. glaziovii*. Regarding the first chapter, we conducted two experiments, one of which was simulation and the other was protection from herbivory in plants in the field. In the second chapter, we carried out a long-term (eight years) manipulative experiment to evaluate the investment in chemical and physical defenses in colonized and non-colonized plants. With monthly collections, we access data on the density of leaf trichomes (physical defenses) and phenolic compounds (chemical defenses) from plants (*C. glaziovii*) colonized and not colonized by ants (*A. muelleri*). We found that in plants colonized by ants the production of trichomes is lower, regardless of herbivory. We also found that the presence of ants is a strong mediator of the *trade-off* between chemical and physical plant defenses; due to a reduction in trichome production with an increase in phenolic compounds only in non-colonized plants. Our results reinforce the importance of ants as defenders, but also as mediators of resource allocation and provision in myrmecophytes.

SUMÁRIO

INTRODUÇÃO GERAL

As plantas são a base da cadeia trófica e por isso estão envolvidas em diversas interações ecológicas na natureza (Price et al., 1980). Dentre as interações mais notórias na qual as plantas experimentam está a predação, que recebe um nome especial de herbivoria, designada especialmente para o consumo de material vegetal por um herbívoro (Crawley, 1983; Gong & Zhang, 2014). Esta interação é considerada negativa para as plantas, uma vez que o ataque dos herbívoros ocasiona danos a diversos tecidos dos vegetais, afetando a fotossíntese e o estoque energético (McNaughton, 1983). Em alguns casos, dependendo da magnitude da herbivoria, esta pode impactar no fitness da planta e até levar à morte (Hendrix, 1988). O processo evolutivo, frente aos embates entre herbívoros e plantas, selecionou diversas estratégias de defesa e contra defesa que afetaram a dinâmica de populações de plantas e seus herbívoros (Fox, 1981; Fornoni, 2011; Johnson et al., 2015).

Uma das classificações designadas para defesas de plantas aponta um grupo de defesas como constitutivas – sempre presentes nas plantas, independente da pressão de herbivoria – ou induzidas, que são desencadeadas em resposta à herbivoria (Agrawal & Karban,1999; Kaplan et al., 2008). Além disso, outra forma de classificação divide as defesas em diretas, que são produzidas e executadas pela própria planta e indiretas, quando a planta se relaciona com outro organismo que atua em sua defesa (Heil, 2008). Uma terceira classificação aponta essas defesas de plantas como físicas, químicas e bióticas (Agrawal, 1998). Estas defesas químicas são compostos secundários produzidos pelas plantas que têm ação tóxica e afetam a palatabilidade, inibindo a ação dos herbívoros. Já as defesas físicas são estruturas das plantas que atuam como barreira contra os herbívoros. Dentre as mais comuns destacam-se os espinhos, acúleos, tricomas, resinas e dureza foliar (Chaudhary et al., 2018; Hanley et al., 2007). Já as defesas bióticas são realizadas por um parceiro associado à planta que atua na proteção contra os herbívoros (Agrawal & Rutter, 1998; Rosumek et al., 2009). Estes parceiros geralmente são insetos que são atraídos por recompensas como alimento ou abrigo, oferecidos por estruturas naturais ou especializadas da planta (Bronstein et al., 2006; Ness et al., 2009; Koricheva & Romero, 2012).

As plantas sofrem pressão de ataque por diferentes tipos de herbívoros que também contam com diferentes táticas para burlar o sistema defensivo de suas presas (Evans & Schmidt, 1990; Gatehouse, 2012). Neste caso, o investimento em diversos tipos de defesa que atuam simultaneamente pode permitir às plantas se defenderem de uma maior variedade de inimigos naturais (Agrawal, 2007; Rasman & Agrawal, 2009). Porém, investir em várias defesas ao mesmo tempo pode ser inviável à planta em função da quantidade de energia necessária para produzi-las e mantê-las. Assim, devido às relações de custo benefício, é esperado a existência de *trade-off* (relação negativa no investimento da planta entre dois tipos de estratégias de defesa). O *trade-off* entre defesas de plantas é esperado especialmente quando há limitação de recursos (Strauss et al., 2002; Koricheva et al., 2004) ou quando uma das defesas é dispensável devido sua menor eficiência ou especificidade (Karban, 2011; Carmona et al., 2011). Mas vale ressaltar que o *trade-off* pode não ocorrer quando a planta tem energia para investir em várias estratégias de defesa ao mesmo tempo (Coley et al., 1985; Schultz et al., 2013).

As formigas são os insetos mais comumente associados à defesa biótica de plantas (Rosumek, 2009). Neste contexto, a defesa de plantas por formigas é considerada as estratégias de defesa mais eficientes em comparação com as defesas químicas e físicas (Massad, Fincher, Smilanich & Dyer, 2011). As plantas mirmecófitas, encontradas em todas as regiões tropicais, são representadas por cerca de 100 gêneros e têm seu sucesso atribuído à relação com as formigas. Um dos grupos mais conhecidos e estudados de mirmecófitas pertencem ao gênero *Cecropia,* com mais de 180 espécies encontradas em toda a região neotropical. As formigas que majoritariamente são encontradas em associação com plantas de *Cecropia* são do gênero *Azteca* (Formicidae: Dolichoderinae). No sistema *Cecropia-Azteca* a rainha perfura uma região específica de espessura fina do caule (prostoma) e penetra o tronco oco da planta. Uma vez no interior da planta, as formigas emergentes constroem ninhos em câmaras (internós) que servirão de abrigo para a colônia (Janzen, 1969). Além do local de nidificação, a planta oferece os corpúsculos müllerianos, fonte de alimento para as formigas que são produzidas em uma estrutura especializada (trichilium) na base do pecíolo das folhas (Yu & Davidson, 1997). Em contrapartida aos benefícios fornecidos pelas plantas, as formigas também oferecem vantagens na relação. O gênero *Azteca* é composto por espécies de formigas agressivas e com comportamentos territorialistas, combatendo organismos estranhos que por ventura se aproximem da colônia (Zorzal et al., 2021). Sendo assim, as formigas acabam desempenhando papel protetivo contra os inimigos naturais de suas plantas hospedeiras. Além disso, os dejetos provenientes da atividade da colônia são fonte nutricional para as plantas e são absorvidos com o auxílio de um fungo da ordem *Chaetotyriales* cultivado pelas formigas. As formigas, com o comportamento de limpeza, também defendem a planta contra o ataque de fungos e outros patógenos que degradam o pecíolo das folhas. Apesar de muitos estudos acerca dos benefícios da formiga para a planta, pouco se sabe sobre o que rege as estratégias de defesas em plantas mirmecófitas.

Os serviços oferecidos pelas formigas, dando destaque à defesa, fazem das mirmecófitas excelentes modelos para o estudo da atuação de diversas estratégias de defesa nas plantas. Estas plantas, além de possuírem um arsenal de defesas químicas e físicas, se beneficiam do serviço de defesa oferecido por suas formigas mutualistas. Dessa forma, o objetivo geral desta tese é desvendar o possível efeito da formiga mutualista *Azteca muelleri* (defesa biótica) sobre a dinâmica das defesas químicas e físicas em *Cecropia glaziovii*.

Essa tese será dividida em dois capítulos:

Capítulo I: Ants presence, but not herbivory, drives leaf trichome production in a myrmecophyte

(A presença das formigas, mas não a herbivoria, direciona a produção de tricomas em uma mirmecófita)

Este capítulo está formatado em formato de manuscrito para ser submetido à revista *Oecologia*.

Neste capítulo será investigado o papel das formigas mutualistas *Azteca muelleri* e da herbivoria sobre a produção de tricomas pelas plantas *Cecropia glaziovii*. Assim, o objetivo principal foi testar duas hipóteses:

i) Plantas de *Cecropia glaziovii* colonizadas por formigas *Azteca muelleri* produzem menos tricomas porque experimentam menos herbivoria;

ii) Os níveis de herbivoria não influenciam a produção de tricomas em plantas de *Cecropia glaziovii* colonizadas por formigas *Azteca muelleri* .

Se a nossa primeira hipótese for verdadeira, esperamos que as plantas (*C. glaziovii*) com taxas de herbivoria mais baixas produzam menos tricomas, independentemente da presença de formigas. Caso contrário, se corroborada a segunda hipótese, a presença de formigas (*A. muelleri*) induzirá uma menor produção de tricomas, independentemente da herbivoria.

Capítulo II: Are ants capable of mediating defensive trade-offs in myrmecophytes? A long-term exclusion experiment in the *Cecropia-Azteca* system

(Formigas são capazes de mediar *trade-offs* defensivos em mirmicófitas? Um experimento de exclusão a longo prazo no sistema *Cecropia-Azteca*"*)*

Este capítulo está formatado em formato de manuscrito para ser submetido à revista *Plant Ecology.*

Neste capítulo será investigado se a presença de formigas (*Azteca muelleri*) determina o *trade-off* entre as defesas físicas e químicas em uma planta mirmecófita (*Cecropia glaziovii*). Para isso, foram testadas duas hipóteses principais:

i) *C. glaziovii* reduz seu investimento em defesas químicas e físicas após a colonização de formigas;

ii) O *trade-off* entre defesas físicas e químicas está presente apenas em plantas não colonizadas (*C. glaziovii*), e não ocorre em plantas colonizadas.

Se nossas hipóteses estiverem corretas, esperamos uma relação negativa entre a produção de defesas físicas e químicas em plantas não colonizadas e uma relação nula ou positiva em plantas colonizadas por formigas.

CHAPTER ONE: ANTS PRESENCE, BUT NOT HERBIVORY, DRIVES LEAF TRICHOME PRODUCTION IN A MYRMECOPHYTE

ABSTRACT

Plants use defensive strategies against natural enemies that can be direct, such as chemical compounds and physical structures, and indirect, such as association with other animals. Myrmecophytes, for example, live in association with ants that play a protective role in exchange for food and nesting sites. It is known that the presence of *Azteca muelleri* ants provides multiple benefits to *Cecropia glaziovii* (a myrmecophyte plant) that go beyond protection. However, it was not demonstrated how exactly ants' presence influenced these plants' features. In this scenario, we wonder "why do *Cecropia glaziovii* produce less leaf trichomes when colonized by *Azteca muelleri*?". To elucidate this issue, we conducted two field experiments (adding and protecting herbivory) to test the hypothesis that plants colonized by ants produce less trichomes because they experience less herbivory. For the "herbivory adding" experiment, to measure the effect of herbivory on trichomes regardless of ant presence, we subjected naturally colonized (n = 29) and uncolonized plants (n = 28) to three simulated herbivory treatments: control, 10% and 50% loss of leaf area and measured the variation in leaf trichome density after 12 months. For the "ants presence" experiment, to measure the effect of ant presence on trichome production regardless of herbivory, we protected herbivory in naturally colonized (n = 24) and uncolonized plants (n = 21) and calculated trichome production variation after six months. Both of our experiments demonstrate that herbivory does not influence the expression of leaf trichomes in our *Azteca-Cecropia* system. However, the presence of ants was decisive for reducing plant investment in trichomes (510% and 125% decrease in trichome production in colonized plants respectively for the "herbivory adding" and "ants presence" experiments). Contrary to previous results, this is the first experimental work to show a null effect of herbivory on inducing trichome production and sheds light on how the trigger of plant defense strategies are context dependent, especially in myrmecophytes.

Keywords: defense strategies, herbivory, *Cecropia glaziovii*, *Azteca muelleri,* Atlantic forest.

INTRODUCTION

Faced with the existence of countless natural enemies, plants have developed a multiple arsenal of defenses (Letourneau et al., 1983; Agrawal & Fishbein, 2006). Plant defensive strategies can be direct, such as the production of chemical compounds and physical structures (Agrawal, 2004; Walters, 2011; Carmona et al., 2011), and indirect, evolving the protective association with other organisms (Heil, 2008; Massad et al., 2011). Besides, both direct and indirect resistance mechanisms might be presented as constitutive structures related with other functions than defenses (Agrawal & Karban, 1999; Rasmann & Agrawal, 2009) and inducible traits which are enhanced expressed when plants are damage (Schultz, 1988; Karban & Myers, 1989; Kempel et al., 2011). Although a vast literature is dedicated to plant defenses (some reviews on: Freeman & Beattie, 2008; Agrawal, 2011; War et al., 2012; Fürstenberg-Hägg et al., 2013; Gong & Zhang, 2014; Aljbory & Chen, 2018), it is still unclear how herbivory may trigger its production (Frederickson et al., 2013) and especially how plant investment in one strategy will influence the others (Rhoades, 1979; Karban, 2011).

Leaf trichomes are a physical defense strategy that might be considered an ideal plant trait to study herbivory inducibility (Levin, 1973; Karabourniotis et al., 2020). Trichomes mechanically protect plants from herbivores (Levin, 1973; Wang et al., 2021) and at the same time, they are important to plant physiology by reducing leaf evaporation and regulating temperature (Hendrick et al., 2016; Hegebarth et al., 2016). Trichomes can also be considered a good plant characteristic to study mutual interference in plant defense investment. Plant resource allocation in trichomes, which is energetically costly (Cipollini et al., 2018; Kaur & Kariyat, 2020) might ultimately reduce plant energy to other direct and indirect defenses. Previous studies have demonstrated that herbivory by itself can induce an increase in trichome production (Agrawal, 1999; Dalin et al., 2008). Other papers showed that indirect defenses, such as the arrival of mutualistic ants, influenced a decrease in trichome density in an ant-plant (Gomes et al. 2021). However, no studies have been able to unravel the effects of herbivory and the presence of ants on trichome investment in ant plants.

Among the diversity of ant-plants (plants that house and feed ants also called myrmecophytes), the genus *Cecropia* (Cecropiaceae) stands out, with more than 180 species distributed throughout the Neotropical region (Berg et al., 2005). Most *Cecropia* species are myrmecophytes and associated with ants of the genus *Azteca* (Dolichoderinae) (Davidson, 1991; Longino, 1991). *Cecropia-Azteca* system might be considered an ideal system to study trichome induction as it has an abundant presence of leaf trichomes and also presents its mutualistic ant. Besides, *Cecropia-Azteca* one of the most studied myrmecophytic system on earth but there is still much controversy over how *Azteca's* presence influences resource allocation for direct defenses in *Cecropia* (Gianoli et al., 2008; Gomes et al., 2021).

Azteca ants can provide multiple benefits to the *Cecropia* plants that go beyond protection (Oliveira et al., 2015; Gomes et al., 2021). More specifically, it was experimentally demonstrated that after *Azteca muelleri* colonization, *Cecropia glaziovii* experienced an average of 83% less herbivory, 85% increase in foliar nitrogen, and 93% decrease in leaf trichomes (Gomes et al., 2021). However, it was not demonstrated why exactly ants' presence influenced those plants' features. For example: Why do *Cecropia glaziovii* produce less leaf trichomes when colonized by *Azteca muelleri*? The most likely explanation might be that ants' protection against herbivores decreases herbivory, leading to a reduction in trichome production by the plant. However, we could not discard the possibility that trichome display reduction is caused by ants' arrival by itself, regardless of herbivory. Despite the importance of those mechanisms, we are not aware of any paper which tried to disentangle the effects of herbivory and ant presence on trichome inducibility.

To elucidate this issue, we conducted two field experiments with simulated herbivory over 18 months, trying to unravel the effects of ants' presence and herbivory on the production of leaf trichomes in the *Azteca muelleri-Cecropia glaziovii* system. We used these two experiments to test two mutually exclusive hypotheses: i) plants colonized by ants produce less trichomes because they experience less herbivory; ii) herbivory levels do not influence trichome production in ant-colonized plants. If our first hypothesis is true, we expect that plants with lower herbivory rates will produce less trichomes, regardless of ant presence. Otherwise, if we corroborate hypothesis ii), the ant presence will induce a lower production of trichomes, regardless of herbivory.

MATERIAL AND METHODS

Study location

The experiments were carried out at “Mata da Biologia”, an Atlantic Forest fragment located in the Federal University of Viçosa campus, Viçosa, Minas Gerais, Brazil. This area has 75 ha and is composed by secondary semideciduous forest in recovery over 90 years after coffee planting. This region has a subtropical climate with annual rainfall between 1,300 and 1,400 mm and average temperature of 19ºC (Silva et al., 2010).

Biological models

Cecropia glaziovii Snethl. (Urticaceae) is a fast-growing tree commonly found in regenerating forest fragments and is protected to an altitudinal range between 600-1,500 m (Berg et al., 2005). *C. glaziovii* is frequently involved in mutualistic associations with *Azteca muelleri* (Emery, 1893) (Dolichoderinae) ants and this is the only species colonizing *C. glaziovii* trees in the forest fragment studied here (Zorzal et al., 2021). *A. muelleri* is a very aggressive ant species and an effective protector of its host plant. We have studied *C. glaziovii-A. mulleri* for over 10 years and found that the ant offers multiple benefits to plants. Mortality rates of 100% have been found for plants that are not colonized by ants (Gomes et al., 2021). In *C. glaziovii*, plants can also die from severe damage caused by specialist herbivores such as the beetle *Coelomera lanio* (Coleoptera: Chrysomelidae) and the caterpillar *Colobura dirce* (Lepidoptera: Nymphalidae) (Novotny et al., 2003; de Souza Tavares et al., 2015). These herbivores are usually found on uncolonized plants (Rocha & Bergallo, 1992).

Experiment 1 - Effect of herbivory on trichomes regardless of ant presence

To evaluate the influence of leaf herbivory on trichome production *regardless of ants*, we selected 57 plants of *C. glaziovii*, 29 colonized by ants and 28 uncolonized (during the experiment, the ant colonization was protected with Silver Tape® adhesive tape passed over the *prostomes*, see Gomes et al. 2021). Plants were chosen randomly, including plants of different sizes and ages in the experiment. The selection of plants with different characteristics does not affect the results of the experiment, since trichome production in *C. glaziovii* is not related to the plant ontogeny (Gomes et al., 2021; Kariñho-Betancourt et al., 2023). In addition, when choosing plants, we respect a minimum distance of five meters between them to facilitate experimental manipulation.

Plants in each colonization group (colonized and non-colonized) were randomly subjected to three simulated herbivory treatments: control (no simulated cutting), 10% and 50% loss of leaf area. The percentages were established after a pilot survey on the average herbivory level experienced by *C. glaziovii* plants in the study area. In this survey we found an average of 8% of natural herbivory. The highest natural herbivory values found were, approximately 50% of leaf area loss (promoted by leaf cutter ants). So, we defined the values of 10% of simulated herbivory as our first treatment – the one closest to natural herbivory and the second treatment, we defined 50% of leaf area lost – the one simulating extreme damage cases (e.g., leaf-cutting ants). For our third treatment we did not perform any simulated herbivory – this one was defined as control. We selected nine, nine and ten naturally colonized and nine, ten and ten naturally uncolonized plants for the 10% and 50% of simulated herbivory treatment and control group respectively, totalling 57 plants (29 colonized and 28 uncolonized by ants).

We performed herbivory simulation using scissors for the small plants and a trimmer for the tall ones. Therefore, in this experiment the herbivory imposed on the plants was exclusively mechanical (loss of foliar area). To increase the accuracy in the herbivory simulation, we previously collected 150 leaves from other unused plants to analyze how to reach herbivore levels of 10% and 50%. For this, we used images of leaves of different sizes using the Image J software (Rasband, 2006). For the control group, the leaves remained intact. In the first sampling, we carried out a simulation of herbivory (10% or 50%) on all leaves of each plant. Then, once a month, we repeated the herbivory simulation only on the new emerging leaves for 12 months. To indicate the emerging leaves, the youngest leaf of each plant was marked each month. As the levels of herbivory in *C. glaziovii* are homogeneous among plant individuals (Gomes et al., 2021), we did not perform any intervention to control natural damage on leaves submitted to herbivory simulation.

Leaf trichome density was calculated twice along the experiment: the first just before the experiment initiation (September 2021) and the second at the end of the experiment (after 12 months in September 2022). For this, we collected three pieces of 0.25 cm^2 from the three youngest leaves of each plant using scissors, tweezers, scalpels, and rulers. We counted the number of trichomes on the upper surface of each leaf piece, always avoiding the midrib. The leaf pieces were photographed with a camera attached to a stereomicroscope. The photos were taken to Adobe Photoshop CS6 for editing and highlighting the trichomes. Then, the edited images were taken into Python software to count the trichomes. Thereafter, we counted and used the average of the total number of trichomes per leaf and per plant. Finally, we calculated the mean number of trichomes per leaf and the mean per plant. Then, we divided this mean number by the average individual leaf area obtained from three different leaves per plant (to correct for a possible leaf size effect; Gomes et al., 2021). From the same leaves whose trichomes were counted, we also measured total leaf area by photographing them against a whiteboard with 1 cm marks as a reference scale. For this we used the software Image J (Rasband, 2006).

Experiment 2 - Effect of ants on trichomes regardless of herbivory

For the second experiment we performed an herbivory protection experiment using colonized and uncolonized *C. glaziovii* plants. For this, we selected 45 individual plants divided into four groups: 1) uncolonized plants, unprotected against herbivory (n=11), 2) colonized plants, unprotected to herbivory (n=14), 3) uncolonized plants, protected against herbivory (n=10) and 4) colonized plants, protected against herbivory (n=10).

To protect plants against herbivory (groups 3 and 4), we wrapped all the leaves individually with fabric bags (herbivory protected groups). These bags do not prevent ants from accessing the leaves in colonized plants (group iv). For group i and iii (uncolonized plants) we prevented new ant colonization by isolating the *prostomes* with Silver Tape® adhesive tape. We also applied the Silver Tape® outside the *prostomes* region in the ant colonized plants to control the effect of the tape on the plants (groups ii and iv).

Before starting the herbivory protection experiment, we calculated the leaf herbivory levels of four leaves of all 45 plants (two youngest and two oldest leaves). The calculation of the leaf area consumed was performed by taking pictures of the leaves (without removing them from the plants) on a white board with 1 cm marks as a reference scale. We then calculated the percentage leaf damage expressed as means by analyzing photos with the software Image J (Rasband, 2006). After that, we started counting trichomes in the field, selecting three leaves from each of our 45 plants (younger, middle-aged, and older leaves). Then, without removing the leaf from the plant, we cut three pieces of leaf (0.25 cm^2 in size) and took them to the lab. For trichomes density we used the same method as explained in Experiment 1. Trichome counting was performed twice. The first, at the beginning of the experiment (May 2020) and the second, six months later

(October 2020). Finally, at the end of the experiment (just before the second trichome counting), we calculated leaf herbivory levels for the second time using the same methodology as described above. The comparison of herbivory levels on leaves before and after the experiment was necessary to certify the effectiveness of the bags in preventing herbivory.

Data analysis

For experiment 1, we used a two-way ANOVA model to compare variation on trichome density in response to ants' presence and simulated herbivory. We used trichome density as a continuous response variable and ants' presence (colonized and uncolonized plants) and simulated herbivory groups (10% and 50% and control) as categorical explanatory variables (factors). In this analysis, to capture the variation on leaf trichome production along 12 months, we calculated the trichome density delta (final trichome density after 12 months minus initial trichome density calculated at the beginning of the experiment) - averaged (3 leaves) per plant (n=46, as we used only the survived plants after 12 months). Hereafter we will use the Greek letter delta (Δ) to represent trichome density variation in time.

For the second experiment, we used a two-way ANOVA model to test for the possible effect of ants' presence on trichomes density, regardless of herbivory. Here, we used trichome density as a continuous response variable and ants' presence (colonized and uncolonized plants) and herbivory protection groups (protected and unprotected plants) as categorical explanatory variables (factors). Once again, we calculated mean leaf trichome delta (Δ) as: final trichome density after 6 months minus initial trichome density calculated at the beginning of the experiment – averaged (3 leaves) per plant (n=40 plants).

Finally, we performed a two-way ANOVA model to test the efficiency of our herbivory protection experiment. For this model we used herbivory level (percentage of leaf area loss) as a continuous response variable and ants' presence (colonized and uncolonized plants) and herbivory protection groups (protected and unprotected plants) as categorical explanatory variables (factors). Here, we also capture the variation in herbivory level by calculating its delta (Δ): final leaf herbivory level after 6 months minus initial leaf herbivory level calculated at the beginning of the experiment – average (3 leaves) per plant (n=34).

We used the software R 3.4.1 (R Core Team 2017) for all statistical analyses and performed residual analyses for all models and finally checked suitability of error distributions. We used a normal distribution of errors in all analyses.

RESULTS

Effect of herbivory on trichomes regardless of ant presence

After 12 months of experiment, from the 57 plants selected for the experiment, 46 survived. Therefore, from 29 colonized plants, 28 survived and from 28 uncolonized plants, only 18 remained alive until the end of the experiment. From the surviving plants, 17 remained in the control group (11 colonized and 6 uncolonized), 14 in the 10% of simulated herbivory group (9 colonized and 5 uncolonized) and 15 plants in the 50% of simulated herbivory group (8 colonized and 7 uncolonized).

We found no effect of simulated herbivory (neither 10% nor 50%) on trichome density variation over 12 months ($F_{2,34} = 0.119$, $p = 0.888$). On the other hand, we observed a an increase in trichome density over time (positive delta) in uncolonized plants when compared to colonized plants in all treatments of herbivory simulation and the control ($F_{1,36} = 30.32$, $p < 0.001$; Fig. 1). We found no statistical interaction among factors (simulated herbivory and ant colonization – $F_{2,34} = 0.531$, $p = 0.593$). This demonstrates a much stronger increase in the production of trichomes in uncolonized plants over the time (510% of average increase) when compared to colonized plants (139% of average increase) and this result was independent from herbivory simulation.

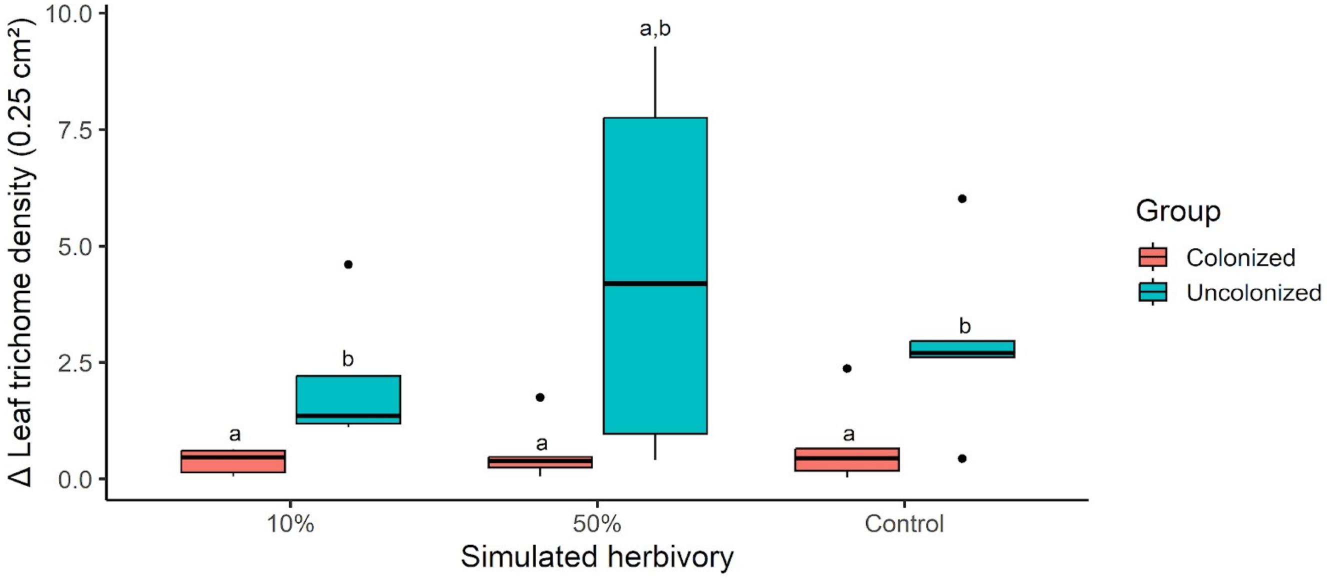

Fig. 1. Trichome density delta over 12 months (mean per plant) in ant colonized and uncolonized plants comparing herbivory simulation treatments. The horizontal line in each box indicates the mean per plant, the boundaries of the box are the standard deviation of the mean, the horizontal lines extend to the most extreme data points, and the dots indicate the 'outliers'.

Effect of ants on trichomes regardless of herbivory

After six months of experiment, 34 plants survived out of the 45 selected. From the surviving plants, 16 remained in the protected group (6 colonized and 10 uncolonized), and 18 plants in the herbivory controlled group (9 colonized and 9 uncolonized). Our plant protection experiment was efficient, as we found a decrease in foliar herbivory over six months in both groups (protected and unprotected), but the herbivory variation was 145 % lower in herbivory-protected plants when compared to unprotect ones (Fig. 2).

Our results showed that uncolonized plants presented stronger and positive trichome density delta (122.5% of average increase) when compared to colonized plants (50% of average increase) (Fig. 3; $F_{1,31} = 10.356$, $p = 0.003$) – for unprotected plants. Like our first experiment, trichome density variation was not affected by herbivory protection (Fig. 3; $F_{1,31} = 0.635$, $p = 0.432$). Finally, we found no statistical interaction between ant presence and herbivory protection groups ($F_{1,28} = 1.29$, $p = 0.265$).

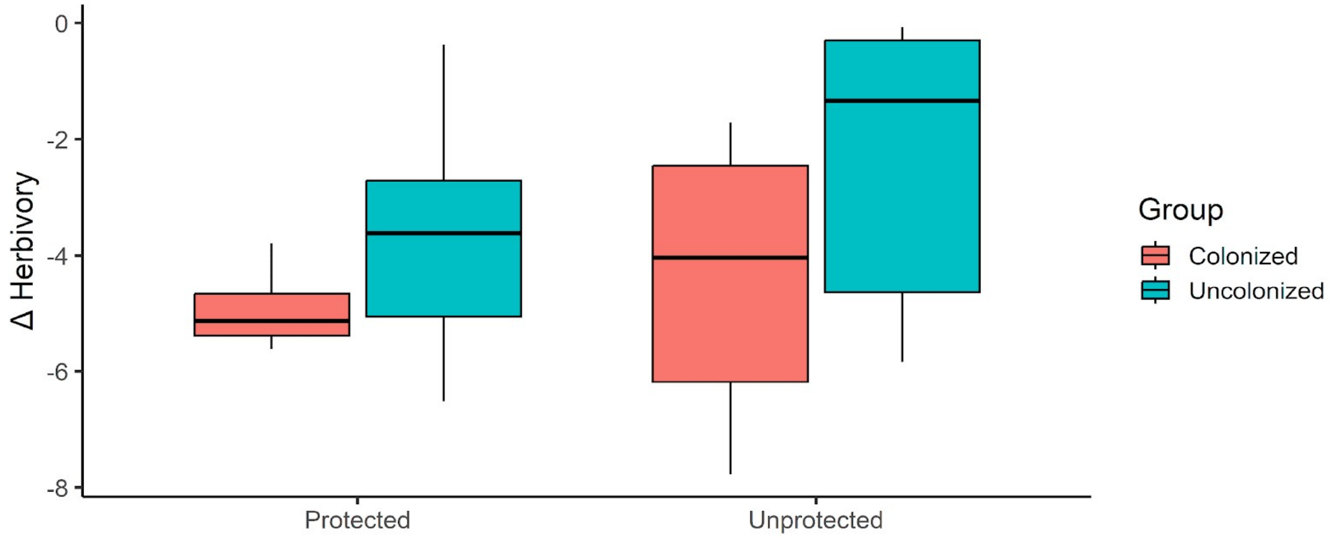

Fig. 2. Herbivory delta after six months (mean per plant - SE) in ant colonized and uncolonized plants, comparing herbivory protected and unprotected plants. The herbivory delta was 145% lower in herbivory-protected plants.

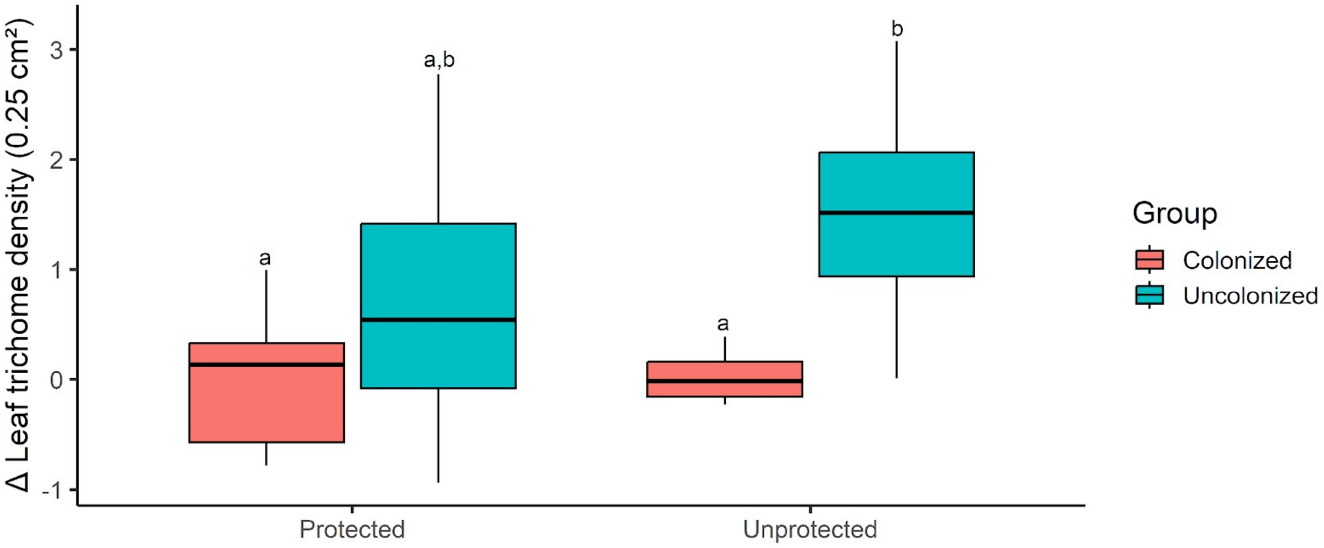

Fig. 3. Trichome density delta after six months (mean per plant + SE) in ant colonized and uncolonized plants, comparing herbivory protected and unprotected plants. Trichome density delta had 122.5% of average increase in uncolonized plants and only 50% of increase in colonized plants.

DISCUSSION

After two field experiments manipulating herbivory performed over 12 and 6 months, we found that the presence of *Azteca muelleri* ants was responsible for the decrease in leaf trichome production of *Cecropia glaziovii* regardless of herbivory. Corroborating our second hypothesis, we found no effect of leaf herbivory on trichome production in both herbivory addition and protection experiments. However, ant colonization once again proved to be an effective switch in turning down trichome production by *C. glaziovii* (Gomes et al., 2021). In our herbivory addition experiment, we showed that after 12 months uncolonized plants showed a 510% mean increase in trichome production compared to colonized plants (139%). In the same way, when we protected plants from herbivory during six months, uncolonized plants experienced an average increment of 122,5% in trichome density while colonized plants showed only 50% increase. Contrary to previous results which found that herbivory positively affect trichome production (Agrawal, 1999; Moraes & Vasconcelos, 2009; Frederickson et al., 2013), this is the first experimental work to show a null effect of herbivory on inducing trichome production and sheds light on how the trigger of plant defense strategies are context dependent.

Is it well described that indirect plant defenses, such as protective ant–plant mutualisms, influences the investment in direct defenses (physical structure like trichomes and chemical compounds as phenolics) (Yamawo et al., 2014; Bartimachi et al., 2015). Heil et al. (2002) suggest that mechanical defenses (such as trichomes) are more costly than chemical defenses and may be subject to strong counter-selection. However, it is still unclear how and when plants will invest in each type of defense. More specifically, despite the scarcity of data on the effect of ant colonization on trichome production in myrmecophytes (but see Moraes & Vasconcelos, 2009; Piovia-Scott, 2011b), the mechanisms behind this seem to be related to plant energy and resource allocation. We found that leaf trichome production is decreased by ant colonization regardless of herbivory and to explain this we rise three hypotheses: i) ants can lead to an increase in nutrient input to the plant through myrmecotrophy (Sagers et al., 2000; Dejean et al., 2012); ii) plants with ants are better protect against pathogens (Leclerc & Detrain, 2018; Gomes et al., 2021); iii) colonized plants presented a strong investment ant food source as Mullerian bodies (Folgarait et al., 1994; Byk & Del-Claro, 2011).We will explain those three hypotheses in the following three paragraphs.

Firstly, although protection against herbivores is the classic ant benefit conferred to the plant (Schupp, 1986; Heil &Mckey, 2003; Oliveira et al., 2015; Zhang et al., 2015), the role of the colony in myrmecophytic plants goes beyond defense. The presence of ants in plant *domacea* can contribute to an increase in nutrients through direct absorption (Sagers et al., 2000; Dejean et al., 2012). In the *C. glaziovii – A. muelleri* system, colonized plants experienced an increase in ^{15}N signature and in total nitrogen content, which not occur in uncolonized plants (Oliveira et al., 2015; Gomes et al., 2021). Thus, food storage, discarded waste and defecation of ants accumulate organic matter in the nest which, through the help of a fungus, can be a source of nutrients for plants (Trimble & Sagers, 2004). In this case, the fungus of the order *Chaetothyriales* is cultivated by the *Azteca* colony inside the plant (Nepel et al., 2016; Mayer et al., 2018), and the fungus is a source of food for the larvae and a component of the card-like nest built inside the *domacea* (Blatrix et al., 2012; Leroy et al., 2017; Mayer et al., 2018). In addition, there are some evidences that the fungus plays a mediating role in the transfer of nutrients from colony residues to the plants (Rico-Gray & Oliveira, 2008; Defossez et al., 2011; Leroy et al., 2011). In this way ant-colonized plants might experience an increase in nutrient input (Fischer et al., 2003; Vidkjær et al., 2015, Gomes 2021). Following the resource availability hypothesis (reviewed in Endara & Coley, 2011) plants with high levels of nutrients tends to decrease investment in defences, as they can easily recovery after herbivory (Bryant et al., 1987; Folgarait & Davidson, 1995). More specifically, it has been shown that plants under conditions of low nutrient content have their trichomes well expressed (Yamawo et al., 2014). This hypothesis may explain why plants with ants showed an increase in trichome expression regardless of herbivory in our study.

Second, colony personality – consistent collective behaviors over time and/or across ecological situations – can be determinant in plant-ant interactions (Sih et al., 2004). The hygiene behavior of the nest and cleaning of the host plant can be effective in combating pathogens that attack both the colony and the plant structures (Scharf et al., 2012; Leclerc & Detrain, 2018). In *C. glaziovii,* an exclusion experiment performed along 4,5 years showed that different from ant-excluded plants,colonized plants were never infected by fungus (Gomes et al. 2021). Likewise, in *Cecropia obtusa*, a pathogenic fungus that produces harmful toxins to plant tissues did not show infection in colonized plants (Roux et al., 2011). Therefore, as the plant response against parasitic fungal infection is energetically costly, this can negatively affect the plant's investment in defense against herbivores such as trichomes.

Finally, ant-colonized plants must increase the energy investment in resources for ants, such as ‘food bodies’ (Piovia-Scott et al., 2011b). In the case of *Cecropia*, an experiment showed that there is a strong increase in Mullerian bodies (MB) production in the presence of symbiotic *Azteca* ants (Folgarait et al., 1994). Thus, as Mullerian Bodies (MBs) are rich in lipids and glycogen (Rickson, 1976) its production involves a high energy cost for the plant. Therefore, it is plausible to infer that if a plant has a high output in MBs to feed ants, it would lead to lower investment in other types of defenses, such as trichomes (Folgarait & Davidson, 1995; Piovia-Scott, 2011).It is known as a *trade-off* between plant defense strategies (revision in Karban et al. 2020). On the other hand, in the case of high energy levels for the plant, they could invest at the same time in indirect (e.g. ant food and housing), and direct defenses such as physical and chemical defenses (Massad et al., 2011, Mendes & Cornellissen, 2017). This last case is recognized as redundancy between defense strategies (Koricheva & Romero, 2012) where the effect of the strategies can be additive and not interactive, depending on the local herbivore population and even the environmental conditions (Kersch & Fonseca, 2005; Abdala-Roberts & Marquis, 2007; Yamawo et al., 2014). Together with our results, this evidence showed how the dynamics of plant defenses can be context-dependent in myrmecophytes.

Contrary to previous results, our two field experiments demonstrate that herbivory does not influence the expression of leaf trichomes in an iconic myrmecophytic system (*Azteca-Cecropia*). This unique evidence is strongly supported by two complementary field experiments, one adding to and the other protecting leaf herbivory over 12 and 6 months respectively. We also demonstrated in both experiments that even without the herbivory effect, the presence of ants is decisive for reducing investment in trichomes by plants. However, it is still unclear if the plant can identify the ant presence and the physiological and molecular mechanisms evolved on the ant identification (González-Teuber & Heil, 2015). In addition, evidence is lacking on how exactly the presence of ants influences plant investment in other defenses in myrmecophytes (Yamawo et al., 2015). More specifically and as an open field of investigation, we raised three hypotheses to explain how ant presence negatively affect plant trichome production regardless of herbivory: i) by increasing nutrient input to the plant; ii) by protecting against pathogens; iii) by increasing plant energy cost to feed ants. Taken together, our results showed how important the presence of ants is for changing the defense strategies of a myrmecophyte plant, which also provides evidence for coevolution between ants and plants. Finally, our results also highlight strong context dependence in mechanisms controlling the expression of plant defenses, especially in myrmecophytes.

REFERENCES

Abdala-Roberts, L., & Marquis, R. J. (2007). Test of local adaptation to biotic interactions and soil abiotic conditions in the ant-tended Chamaecrista fasciculata (Fabaceae). *Oecologia*, *154*(2), 315-326.

Agrawal, A. A., & Rutter, M. T. (1998). Dynamic anti-herbivore defense in ant-plants: the role of induced responses. *Oikos*, 227-236.

Agrawal, A. A. (1999). Induced responses to herbivory in wild radish: effects on several herbivores and plant fitness. *Ecology*, *80*(5), 1713-1723.

Agrawal, A. A., & Dubin-Thaler, B. J. (1999). Induced responses to herbivory in the Neotropical ant-plant association between Azteca ants and Cecropia trees: response of ants to potential inducing cues. *Behavioral Ecology and Sociobiology*, *45*(1), 47-54.

Agrawal, A. A. (2004). Resistance and susceptibility of milkweed: competition, root herbivory, and plant genetic variation. *Ecology*, *85*(8), 2118-2133.

Agrawal, A. A., & Fishbein, M. (2006). Plant defense syndromes. *Ecology*, *87*(sp7), S132-S149.

Agrawal, A. A. (2011). Current trends in the evolutionary ecology of plant defence. *Functional Ecology*, *25*(2), 420-432.

Aljbory, Z., & Chen, M. S. (2018). Indirect plant defense against insect herbivores: a review. *Insect science*, *25*(1), 2-23.

Bartimachi, A., Neves, J., & Vasconcelos, H. L. (2015). Geographic variation in the protective effects of ants and trichomes in a Neotropical ant–plant. *Plant ecology*, *216*(8), 1083-1090.

Berg, C. C., Rosselli, P. F., & Davidson, D. W. (2005). Cecropia. *Flora Neotropica*, 1-230.

Blatrix, R., Djiéto-Lordon, C., Mondolot, L., La Fisca, P., Voglmayr, H., & McKey, D. (2012). Plant-ants use symbiotic fungi as a food source: new insight into the nutritional ecology of ant–plant interactions. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1744), 3940-3947.

Byk, J., & Del-Claro, K. (2011). Ant–plant interaction in the Neotropical savanna: direct beneficial effects of extrafloral nectar on ant colony fitness. *Population Ecology*, *53*, 327-332.

Carmona, D., Lajeunesse, M. J., & Johnson, M. T. (2011). Plant traits that predict resistance to herbivores. *Functional Ecology*, *25*(2), 358-367.

Cipollini, D., Walters, D., & Voelckel, C. (2018). Costs of resistance in plants: from theory to evidence. *Annual plant reviews online*, 263-307.

Dalin, P., Ågren, J., Björkman, C., Huttunen, P., & Kärkkäinen, K. (2008). Leaf trichome formation and plant resistance to herbivory. In *Induced plant resistance to herbivory* (pp. 89-105). Dordrecht: Springer Netherlands.

Davidson, D. W. (1991). Variable composition of some tropical ant-plant symbioses. *Plant-animal interaction*, 145-162.

Defossez, E., Djiéto-Lordon, C., McKey, D., Selosse, M. A., &Blatrix, R. (2011). Plant-ants feed their host plant, but above all a fungal symbiont to recycle nitrogen. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1710), 1419-1426.

Dejean, A., Petitclerc, F., Roux, O., Orivel, J., & Leroy, C. (2012). Does exogenic food benefit both partners in an ant-plant mutualism? The case of *Cecropia obtusa* and its guest Azteca plant-ants. *ComptesRendusBiologies*, *335*(3), 214-219.

Emery, C. (1893). Voyage de MM. Bedot et Pictel dans l'ArchipelMalais. Formicides de l'ArchipelMalais. [Travel of MM. Bedot and Pictel in the Malaysian Archipelago. Formicides from the Malaysian Archipelago]. *Revue Suisse de Zoologie*, *1*, 187-229.

Endara, M. J., & Coley, P. D. (2011). The resource availability hypothesis revisited: a meta-analysis. *Functional Ecology*, *25*(2), 389-398.

Fischer, R. C., Wanek, W., Richter, A., & Mayer, V. (2003). Do ants feed plants? A 15N labelling study of nitrogen fluxes from ants to plants in the mutualism of Pheidole and Piper. *Journal of Ecology*, 126-134.

Folgarait, P. J., Johnson, H. L., & Davidson, D. W. (1994). Responses of Cecropia to experimental removal of Mullerian bodies. *Functional Ecology*, 22-28.

Folgarait, P. J., & Davidson, D. W. (1995). Myrmecophytic Cecropia: antiherbivore defenses under different nutrient treatments. *Oecologia*, *104*(2), 189-206.

Frederickson, M. E., Ravenscraft, A., Arcila Hernández, L. M., Booth, G., Astudillo, V., & Miller, G. A. (2013). What happens when ants fail at plant defense? Cordia nodosa dynamically adjusts its investment in both direct and indirect resistance traits in response to herbivore damage. *Journal of Ecology*, *101*(2), 400-409.

Freeman, B., & Beattie, G. (2008). An overview of plant defenses against pathogens and herbivores.

Fürstenberg-Hägg, J., Zagrobelny, M., & Bak, S. (2013). Plant defense against insect herbivores. *International journal of molecular sciences*, *14*(5), 10242-10297.

Gianoli, E., Sendoya, S., Vargas, F., Mejía, P., Jaffe, R., Rodríguez, M., & Gutierrez, A. (2008). Patterns of Azteca ants' defense of Cecropia trees in a tropical rainforest: support for optimal defense theory. *EcologicalResearch*, *23*, 905-908.

Gomes, I. J. M. T., Solar, R., Blüthgen, N., Vasconcelos, H. L., Silva, L. E. B., Oliveira, K. N., ... & Campos, R. I. (2021). Multiple effects of mutualistic ants improve the performance of a neotropical ant-plant: A long-term study with the Cecropia-Azteca system. *Basic and Applied Ecology*, *57*, 78-90.

Gong, B., & Zhang, G. (2014). Interactions between plants and herbivores: a review of plant defense. *Acta Ecologica Sinica*, *34*(6), 325-336.

González-Teuber, M., & Heil, M. (2015). Comparative anatomy and physiology of myrmecophytes: ecological and evolutionary perspectives. *Research and Reports in Biodiversity Studies*, *4*, 21-32.

Hegebarth, D., Buschhaus, C., Wu, M., Bird, D., & Jetter, R. (2016). The composition of surface wax on trichomes of Arabidopsis thaliana differs from wax on other epidermal cells. *The Plant Journal*, *88*(5), 762-774.

Heil, M., Delsinne, T., Hilpert, A., Schürkens, S., Andary, C., Linsenmair, K. E., ... & McKey, D. (2002). Reduced chemical defense in ant-plants? A critical re-evaluation of a widely accepted hypothesis. *Oikos*, *99*(3), 457-468.

Heil, M., & McKey, D. (2003). Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology, Evolution, and Systematics*, *34*(1), 425-553.

Heil, M. (2008). Indirect defense via tritrophic interactions. *New Phytologist*, *178*(1), 41-61.

Hendrick, M. F., Finseth, F. R., Mathiasson, M. E., Palmer, K. A., Broder, E. M., Breigenzer, P., & Fishman, L. (2016). The genetics of extreme microgeographic adaptation: an integrated approach identifies a major gene underlying leaf trichome divergence in Yellowstone Mimulus guttatus. *Molecular Ecology*, *25*(22), 5647-5662.

.Karabourniotis, G., Liakopoulos, G., Nikolopoulos, D., & Bresta, P. (2020). Protective and defensive roles of non-glandular trichomes against multiple stresses: structure–function coordination. *Journal of Forestry Research*, *31*(1), 1-12

Karban, R., & Myers, J. H. (1989). Induced plant responses to herbivory. *Annual review of ecology and systematics*, *20*(1), 331-348.

Karban, R. (2011). The ecology and evolution of induced resistance against herbivores. *Functional Ecology*, *25*(2), 339-347.

Karban, R. (2020). The ecology and evolution of induced responses to herbivory and how plants perceive risk. *Ecological Entomolo gy*, *45*(1), 1-9.

Kariñho-Betancourt, E., Vázquez-Lobo, A., & Núñez-Farfán, J. (2023). Effect of Plant Defenses and Plant Nutrients on the Performance of Specialist and Generalist Herbivores of Datura: A Macroevolutionary Study. *Plants*, *12*(14), 2611.

Kaur, J., & Kariyat, R. (2020). Role of trichomes in plant stress biology. *Evolutionary ecology of plant-herbivore interaction*, 15-35.

Kempel, A., Schädler, M., Chrobock, T., Fischer, M., & van Kleunen, M. (2011). Tradeoffs associated with constitutive and induced plant resistance against herbivory. *Proceedings of the National Academy of Sciences*, *108*(14), 5685-5689.

Kersch, M. F., & Fonseca, C. R. (2005). Abiotic factors and the conditional outcome of an ant–plant mutualism. *Ecology*, *86*(8), 2117-2126.

Koricheva, J., & Romero, G. Q. (2012). You get what you pay for: reward-specific trade-offs among direct and ant-mediated defenses in plants. *Biology Letters*, *8*(4), 628-630.

Leclerc, J. B., & Detrain, C. (2018). Impact of colony size on survival and sanitary strategies in fungus-infected ant colonies. *Behavioral ecology and sociobiology*, *72*(1), 1-10.

Leroy, C., Séjalon-Delmas, N., Jauneau, A., Ruiz-González, M. X., Gryta, H., Jargeat, P., ... & Orivel, J. (2011). Trophic mediation by a fungus in an ant–plant mutualism. *Journal of Ecology*, *99*(2), 583-590.

Leroy, C., Jauneau, A., Martinez, Y., Cabin-Flaman, A., Gibouin, D., Orivel, J., & Séjalon-Delmas, N. (2017). Exploring fungus–plant N transfer in a tripartite ant–plant–fungus mutualism. *Annals of botany*, *120*(3), 417-426.

Letourneau, D. K. (1983). Passive aggression: an alternative hypothesis for the Piper-Pheidole association. *Oecologia*, *60*(1), 122-126.

Levin, D. A. (1973). The role of trichomes in plant defense. *The quarterly review of biology*, *48*(1, Part 1), 3-15.

Longino, J. T. (1991). Azteca ants in Cecropia trees: taxonomy, colony structure, and behavior. *Ant-plant interactions*, 271-288.

Massad, T. J., Fincher, R. M., Smilanich, A. M., & Dyer, L. (2011). A quantitative evaluation of major plant defense hypotheses, nature versus nurture, and chemistry versus ants. *Arthropod-Plant Interactions*, *5*(2), 125-139.

Mayer, V. E., Nepel, M., Blatrix, R., Oberhauser, F. B., Fiedler, K., Schönenberger, J., & Voglmayr, H. (2018). Transmission of fungal partners to incipient Cecropia-tree ant colonies. *PLoS One*, *13*(2), e0192207.

Mendes, G. M., & Cornelissen, T. G. (2017). Effects of plant quality and ant defense on herbivory rates in a neotropical ant-plant. *Ecological Entomology*, *42*(5), 668-674.

Moraes, S. C., & Vasconcelos, H. L. (2009). Long-term persistence of a Neotropical ant-plant population in the absence of obligate plant-ants. *Ecology*, *90*(9), 2375-2383.

Nepel, M., Voglmayr, H., Blatrix, R., Longino, J. T., Fiedler, K., Schönenberger, J., & Mayer, V. E. (2016). Ant-cultivated Chaetothyriales in hollow stems of myrmecophytic Cecropia sp. trees–diversity and patterns. *Fungalecology*, *23*, 131-140.

Novotny, V., Basset, Y., & Kitching, R. (2003). Herbivore assemblages and their food resources. *Arthropods of tropical forests: spatio-temporal dynamics and resource use in the canopy. Cambridge University Press, Cambridge*, 40-53.

Oliveira, K. N., Coley, P. D., Kursar, T. A., Kaminski, L. A., Moreira, M. Z., & Campos, R. I. (2015). The effect of symbiotic ant colonies on plant growth: a test using an Azteca-Cecropia system. *PloS one*, *10*(3), e0120351.

Piovia-Scott, J. (2011). Plant phenotype influences the effect of ant mutualists on a polymorphic mangrove. *Journal of ecology*, *99*(1), 327-334.

Piovia-Scott, J. (2011b). The effect of disturbance on an ant–plant mutualism. *Oecologia*, *166*(2), 411-420.

R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https ://www.R-proje ct.org.

Rasband, W. S. (2006). *ImageJ*.

Rasmann, S., & Agrawal, A. A. (2009). Plant defense against herbivory: progress in identifying synergism, redundancy, and antagonism between resistance traits. *Current opinion in plant biology*, *12*(4), 473-478.

Rhoades, D. F. (1979). Evolution of plant chemical defense against herbivores. *Herbivores: their interaction with secondary plant metabolites*, 3-54.

Rico-Gray, V., & Oliveira, P. S. (2008). *The ecology and evolution of ant-plant interactions*. University of Chicago Press.

Rickson, F. R. (1976). Anatomical development of the leaf trichilium and Müllerian bodies of *Cecropia peltata* L. *American Journal of Botany*, *63*(9), 1266-1271.

Rocha, C. F. D., & Bergallo, H. G. (1992). Bigger ant colonies reduce herbivory and herbivore residence time on leaves of an ant-plant: Azteca muelleri vs. Coelomera ruficornis on Cecropia pachystachya. *Oecologia*, *91*, 249-252.

Roux, O., Céréghino, R., Solano, P. J., & Dejean, A. (2011). Caterpillars and fungal pathogens: two co-occurring parasites of an ant-plant mutualism. *PLoS One*, *6*(5), e20538

Sagers, C. L., Ginger, S. M., & Evans, R. D. (2000). Carbon and nitrogen isotopes trace nutrient exchange in an ant-plant mutualism. *Oecologia*, *123*(4), 582-586.

Scharf, I., Modlmeier, A. P., Fries, S., Tirard, C., & Foitzik, S. (2012). Characterizing the collective personality of ant societies: aggressive colonies do not abandon their home. *PLoS One*, *7*(3), e33314.

Schupp, E. W. (1986). Azteca protection of Cecropia: ant occupation benefits juvenile trees. *Oecologia*, *70*(3), 379-385.

Schultz, J. C. (1988). Plant responses induced by herbivores. *Trends in Ecology & Evolution*, *3*(2), 45-49.

Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. (2004). Behavioral syndromes: an integrative overview. *The quarterly review of biology*, *79*(3), 241-277.

Silva, C. A., Vieira, M. F., & Amaral, C. H. (2010). Floral attributes, ornithophily and reproductive success of *Palicourea longepedunculata* (Rubiaceae), a distylous shrub in southeastern Brazil. *Brazilian Journal of Botany*, *33*(2), 207-210.

de Souza Tavares, W., de Assis Júnior, S. L., Soares, M. A., & Zanuncio, J. C. (2015). Colobura dirce dirce (Lepidoptera: Nymphalidae) larvae damaging Cecropia hololeuca (Rosales: Urticaceae) in the Zona da Mata, Minas Gerais, Brazil. *Florida Entomologist*, *98*(2), 792-795.

Trimble, S. T., & Sagers, C. L. (2004). Differential host use in two highly specialized ant-plant associations: evidence from stable isotopes. *Oecologia*, *138*(1), 74-82.

Vidkjær, N. H., Wollenweber, B., Gislum, R., Jensen, K. M. V., & Fomsgaard, I. S. (2015). Are ant feces nutrients for plants? A metabolomics approach to elucidate the nutritional effects on plants hosting weaver ants. *Metabolomics*, *11*(4), 1013-1028.

Walters, D. (2011). *Plant defense: warding off attack by pathogens, herbivores and parasitic plants*. John Wiley & Sons.

Wang, X., Shen, C., Meng, P., Tan, G., & Lv, L. (2021). Analysis and review of trichomes in plants. *BMC plant biology*, *21*(1), 1-11.

War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S., & Sharma, H. C. (2012). Mechanisms of plant defense against insect herbivores. *Plant signaling & behavior*, *7*(10), 1306-1320.

Yamawo, A., Tagawa, J., Hada, Y., & Suzuki, N. (2014). Different combinations of multiple defense traits in an extrafloral nectary-bearing plant growing under various habitat conditions. *Journal of Ecology*, *102*(1), 238-247.

Yamawo, A. (2015). Relatedness of neighboring plants alters the expression of indirect defense traits in an extrafloral nectary-bearing plant. *Evolutionary Biology*, *42*(1), 12-19.

Zhang, S., Zhang, Y., & Ma, K. (2015). The equal effectiveness of different defensive strategies. *Scientificreports*, *5*(1), 1-9.Zorzal, G., Camarota, F., Dias, M., Vidal, D. M., Lima, E., Fregonezi, A., & Campos, R. I. (2021). The dear enemy effect drives conspecific aggressiveness in an *Azteca-Cecropia* system. *Scientific reports*, *11*(1), 1-10.

CHAPTER 2: ARE ANTS CAPABLE OF MEDIATING DEFENSIVE TRADE-OFFS IN MYRMECOPHYTES? A LONG-TERM EXCLUSION EXPERIMENT IN THE *CECROPIA-AZTECA* SYSTEM

ABSTRACT

Trade-off indicates competing demands between life history traits that affect ‘fitness’. In plants, it is expected to find a trade-off between herbivory defense strategies in cases of limited resources. Myrmecophytes invest at the same time in resources to attract ants (biotic defense) and photosynthetic products to direct defenses, such as chemical and physical. However, it is still unclear how those investments in direct defenses occurs in ant presence in a myrmecophyte. In this study, we aim to investigate whether ant presence mediated the trade-off between physical and chemical defenses in *Cecropia glaziovii* plants. We hypothesized that 1) physical and chemical traits decrease with time since colonization; 2) there is a trade-off between physical and chemical defenses in plants colonized by ants. To test these hypotheses, we perform a long-term manipulative experiment to evaluate the investment in chemical and physical defenses in colonized and uncolonized plants. Our focus was in total phenolics as chemical compounds and trichomes as physical defensive traits. At the end of the eight-year-long experiment, only 23 out of the 200 planted seedlings survived (colonized by ants, n = 16; uncolonized, n = 7). We found higher trichome densities in uncolonized plants when compared to colonized ones. Furthermore, trichomes decreased in colonized plants over time after colonization and did not differ in uncolonized. Total phenolic concentration increased with colonization time in colonized plants and did not differ in uncolonized plants. We also found a negative correlation between trichomes and phenolics. Trichome density decreased with increasing phenolics in uncolonized plants and did not change in colonized plants. These results bring a new approach about the trade-off between direct defenses and between direct and indirect defenses strategies in an obligate mutualism context. The evidence indicates that the trade-off is context dependent and tightly dependent on some benefits provided by ants. Thus, our study clarifies the way of investment in defenses in myrmecophyte plants.

Keywords: plant defenses, trade-off, trichomes, phenolics, *Cecropia glaziovii*, *Azteca muelleri*

INTRODUCTION

Trade-offs play an important role in limiting the adaptive power of organisms, directly affecting evolution and biodiversity (Futuyma & Moreno, 1988; Meyer & Beardmore, 2015; Farahpour et al., 2018). The term trade-off indicates a negative correlation between two life history characteristics, such as growth, reproduction and defense (Herms e Mattson, 1992; Mole, 1994; Flatt e Heyland, 2011; Tuller et al., 2018). Trade-offs are normally triggered by the lack of energy to invest in two or more life features at the same time (Zera & Harshman, 2001; Jasienska, 2009; Flatt & Heyland, 2011). As ecological trade-offs influenced such crucial life history traits, it has the power to explain the outcome of small scale ecological interactions (Asplen et al., 2012; Turkelboom et al., 2015) and at the same time, large scale patterns of species distribution (Morin and Chuine, 2006). In the case of plants, trade-offs are mainly related to: i) the allocation of resources to grow versus reproduction (Obeso, 2002; Reekie e Avila-Sakar, 2005), ii) to grow versus defense (Herms & Mattson, 1992; Massad et al., 2012), and iii) among different defense strategies (Züst et al., 2012, Moles et al., 2013; Agrawal & Hastings, 2019; Salgado-Luarte et al., 2023). While the first two are better documented, the last is still little known - especially when it comes to defense performed by a mutualist partner.

Plants developed a range of defense strategies to minimize the damage caused by their natural enemies, especially those related to herbivory (Letourneau et al., 1983; Agrawal & Fishbein, 2006). These strategies can be direct, such as chemical (e.g., secondary metabolites) and physical (e.g., trichomes, leaf toughness) defenses (Agrawal, 1998; Hanley et al., 2007; Mithöfer & Boland, 2012; Barton, 2016) or indirect, like those performed by other organisms (e.g., ants), also called biological defenses (Heil, 2008). All those defense strategies demand energy for the plant so trade-offs among them might be expected in cases where resources are limited (Strauss et al., 2002; Koricheva et al., 2004) or where the high level of one defense makes the other dispensable (Rhoades, 1979; Van der Meijden et al., 1988; Karban, 2011). The maintenance of multiple defense strategies may be positively selected when plant resources are high (Hawkes & Sullivan, 2003; Agrawal & Fishbein, 2006) or where specific defenses are effective only against some herbivores groups (Mitra et al., 2008; Carmona et al., 2011). The first challenge when studying trade-offs and plant defense is that they are highly context-dependent (Hakes e Cronin, 2011; Spigler e Woodard, 2019). Second, trade-offs occur among multiple plant defense features at the same time so it can be difficult to find it when relating only two of those characteristics (Agrawal et al., 2011). Another caveat in trade-off studies is that most researchers only demonstrate their presence or absence without testing for possible mechanisms promoting them.

Myrmecophytes are excellent models to study trade-offs in plant defenses, as in addition to physical and chemical, they also present biotic defenses performed by ants (Agrawal & Dubin-Thaler, 1999; Heil & McKey, 2003; Mayer et al., 2014). Considering the resource investment in attract, house and feed ants and the energy allocation to produce chemical and physical defenses, it is reasonable to expect a trade-off among those strategies (Janzen, 1966; Heil, 1997, Kursar & Coley, 2003). Furthermore, the production of chemical and physical defenses may vary with the intensity of the protection performed by ants (Val & Dirzo, 2003; Boege & Marquis, 2005). There are some cases where investment in physical and chemical defenses by a myrmecophyte decreases after ant colonization (Eck et al., 2001; Koricheva & Romero, 2012). On the other hand, defense mechanisms such as secondary metabolites can play other roles in the plant (Bennett & Wallsgrove, 1994; Boeckler et al., 2011; Shalaby & Horwitz, 2015). Therefore, it would be plausible for the plant to maintain the production of those "apparently defensive" compounds, in addition to housing and feeding ants which ultimately break the trade-off. It has been suggested (Heil and Mckey, 2003) that trade-offs among defense features in myrmecophytes seems to be weaker than they were claimed before (e.g. Eck, Fiala, Linsenmair, Hashim & Proksch, 2001; Seigler & Ebinger, 1987).

In summary, it is still unclear how investment in defenses occurs in myrmecophyte plants and in what context there is a trade-off or synergistic action between those strategies. Besides, in the presence of ants and when they properly exercise their defensive role, would there be a need for the plant to regulate investment between direct defenses? Despite the lack of evidence that leads us to a concise conclusion, we believe that in the presence of ants the need for regulation between other forms of defense will be less or will not occur, as ants are capable of defending the plant against a variety of predators (Heil et al.,2001; Massa et al, 2011). Based on this, our main objective is to investigate if ant presence mediates the trade-off between physical and chemical defenses in a myrmecophyte plant. For this, we proposed to test two main hypotheses that 1) *C. glaziovii* reduces their investment in chemical and physical defenses following ant colonization; 2) the trade-off between physical and chemical defenses is only displayed in uncolonized plants, not happening in colonized ones. In case our hypothesis is correct we expect a negative or positive relationship between the production of physical and chemical defenses among uncolonized plants and a null relationship among colonized ones.

Our study is the first one to test if ants mediated defensive trade-offs in myrmecophytes and to strength our findings we performed a long-term (eight years) manipulative experiment with an obligate plant-ant (*Azteca muelleri*) and its host plant (*Cecropia glaziovii*). We consider this as an excellent system as, in exchange for protection and nutrition, *C. glaziovii* offers food and shelter for *A. muelleri* (Gomes et al., 2021) which increases plant resource expenses, probably leading to a trade-off among defense strategies. Our results will help to elucidate the complex mechanisms governing "plant decision making" in the investment on defensive resource allocation, which is a key life history feature defining plant fitness and distribution.

MATERIAL AND METHODS

Study area

We carried out our experiment at the Research, Training and Environmental Education Station "Mata do Paraíso" located in the city of Viçosa, Minas Gerais, Brazil. This 300-ha reserve of the Atlantic Forest biome is dominated by a Montane Semi Deciduous Forest (Meira-Neto & Martins, 2002). According to Köppen, the climate is subtropical with rainy summers and dry winters (Alvares et al., 2013).

Biological models

Our biological models are the tree species *Cecropia glaziovii* Snethl (Cecropiaceae) and the ant *Azteca muelleri* (Emery 1893) (Dolichoderinae), both found in the Brazilian Atlantic Forest biome.We chose the *Cecropia-Azteca* interaction because it is considered one of the most remarkable mutualistic relationships between ants and plants (Janzen, 1969; Longino, 1991) and we also take advantage for the fact that in our study area the individuals of *C. glaziovii* only houses one ant species: *A. muelleri*. In this system, *C. glaziovii* provides shelter and food, while *A. muelleri* offers cumulative benefits by protecting it against natural enemies and providing nutrients to its host plant (Gomes et al., 2021). In addition, most studies on this system have been carried out in species from the Amazon and Central America (Davidson, 2005), with few works performed in the one of the most important hot-spot of Biodiversity the Atlantic Forest biome of Brazil (but see Oliveira et al., 2015; Gomes et al., 2021).

Experimental Design

Seedlings of *C. glaziovii* were obtained from the same parent plant (Flora Londrina®) and the vegetative and reproductive material of the parent plant was identified and deposited in the VIC herbarium under VIC code nº 37.610 at the Federal University of Viçosa, Viçosa, MG, Brazil. We planted 100 six-month-old individuals of *C. glaziovii* seedlings in July 2012 and another 100 in July 2013, totaling 200 monitored individuals. Seedlings were planted in a transverse line (10 m apart) on two lake shores, providing the same type of habitat experienced by natural *C. glaziovii* individuals living in our experimental area. Of the 200 seedlings cultivated in 2012 and 2013, only 23 trees survived until 2020 (colonized by ants, n=14; uncolonized by ants, n=9). Most of these seedlings died within a few months after cultivation, while the remaining trees that did not survive into adulthood succumbed for various reasons, such as: human activity in the area, crushed by the fall of other trees, flooded by heavy rains, and even attacked by leafcutter ants (regardless of colonization by their *Azteca* ants)

Plants were randomly separated into two experimental groups: (i) control group, with plants that were not manipulated and therefore available for colonization by ants (colonized group) and (ii) treatment group, with plants that were prevented from being colonized by ants (uncolonized group). In the uncolonized group, we used 3M Scotch Silver Tape® to cover the prostoma of all plants, with monthly maintenance of the tapes. Any *Azteca* queen found inside a plant of the uncolonized group was eliminated by injecting water into the domatia. To control a possible Silver Tape® effect, the colonized group plants also had their trunks covered with tape, but in regions outside the prostoma. Plant samples for chemical and physical defense analyzes were collected annually from 2012 to 2020, in October 2013, July 2014, May 2015, April 2016, January 2017, May 2018, June 2019 and October 2020. Although collections occur annually, monitoring of these individuals was carried out monthly in order to validate their status and guarantee colonization by ants or their restriction. Data collections were completed in October 2020, totaling eight years of experiment.

Analysis of chemical and physical defenses

We carried out chemical and physical analyzes of leaves of each plant from both treatments. Thus, it was possible to compare the investment in direct defenses between colonized and uncolonized plants. For these analyzes, we collected three leaves from each plant once each year (from 2012 to 2020). As a physical defense, we chose to assess the density of leaf trichomes. For this, we first calculated the leaf area, estimated through photographs of the leaves using the ImageJ software. The number of trichomes was counted in three randomly selected 0.25 mm^2 plots on the upper surface of each leaf, avoiding only the median vein. Next, we averaged the total number of trichomes per leaf and per plant. Finally, the number of leaf trichomes per plant was divided by the average leaf area of the plant to access trichome density.

Our proxy for chemical defense was on total phenolics as this category is widely known within the scope of defensive metabolites and is present in *Cecropia* plants (Latteman et al., 2014). For extraction, we used 1g samples of leaf powder placed in 50 ml screw-capped centrifuge tubes. The tubes received 20 ml of distilled water and were vortexed. Tubes were then placed in an ultrasonic cleaner at 60°C for 30 minutes. Thereafter, the tubes were centrifuged for 10 minutes (2500 xg). The beads were washed twice with 20 ml of distilled water. The supernatants and washes from each sample were combined, brought to 100 ml in volumetric flasks, and aliquots used for the assay. The samples were stored in amber jars at 4°C.

We evaluated total phenolics following the *Folin-Ciocalteu* assay (adapted from Swain & Hillis, 1959), using tannic acid as a standard and read on a spectrometer at 725 nm. Average total phenolics were expressed in milligrams of total phenolics per gram of plant dry mass. We performed three replicates for each sample for each of the assays described.

Data analysis

To test whether plants from different groups (colonized or uncolonized) differ in their investment in physical and chemical defenses over time after colonization, we conducted two linear mixed model (LMM) (for each feature) considering trichome density and total phenolics concentrations as response variables, the colonization status (colonized or uncolonized) as a fixed effect and time after colonization by ants as an explanatory continuous variable. To calculate “time after colonization”, we estimated the time at which plants became receptive to colonization by queen ants. Then, to equalize the time between all sampled plants, we considered the average colonization time of 29 months (found from colonized plants) to estimate a possible colonization time for plants that were not colonized. We then align all plants based on this value and present colonization as happening at time 0 in the results. Plant identity was used as a random effect to avoid temporal and spatial pseudoreplication, as the same individuals were sampled throughout these eight years.

Furthermore, to test the hypothesis of trade-off between chemical and physical traits, we also use a linear mixed model (LMM). In this case, we used trichome density as response variable, total phenolics as explanatory variable and colonization group (colonized or uncolonized) as a fixed effect.

It is important to highlight that although only 23 plants survived until the end of the eight years of experiment, our sampling also covers the remaining cultivated plants, until the moment of their death. In this way, the data analyzed also correspond to those plants that were alive in each collection. We also reaffirm that we took into account the effect of time and the randomization of individuals to mitigate the effect of pseudoreplication on our results.

We used R 3.4.1 software (R Core Team 2016) for all analyses. We also performed residual analyzes and the distribution of errors was verified for all models that followed normal distribution. We performed LMM tests using the package ‘lme4’ (Bates, Mächler, Bolker & Walker, 2015).

RESULTS

As expected, for our first hypothesis, we found a reduction of trichomes on colonized plants over time after colonization ($F_{1,34}$=5.80; p=0.02; Fig. 1), but no effect on uncolonized plants ($F_{1,46}$=3.14; p=0.08). We also observed a higher density of trichomes on uncolonized plants compared to colonized ones ($F_{1,76}$=6.17; p=0.01; Fig. 1). Considering total phenolics, these were affected over time in general ($F_{1,145}$=7,95; p=0.005; Fig. 2). But, contrary to our hypotheses, total phenolic concentration increased with time after colonization in colonized plants ($F_{1,59}$=4.61; p=0.04; Fig. 2) and did not differ in uncolonized plants ($F_{1,84}$=2.49; p=0.12; Fig. 2). Thus, we only corroborate our hypothesis for trichome defenses.

We also corroborate our second hypothesis as we found a negative relationship between trichome density and total phenolics only for uncolonized plants ($F_{1,38}$ = 4.46; p=0.04; Fig. 3). So, we found no relationship between trichomes and phenolics in colonized plants ($F_{1,32}$=0.20; p=0.65; Fig. 3).

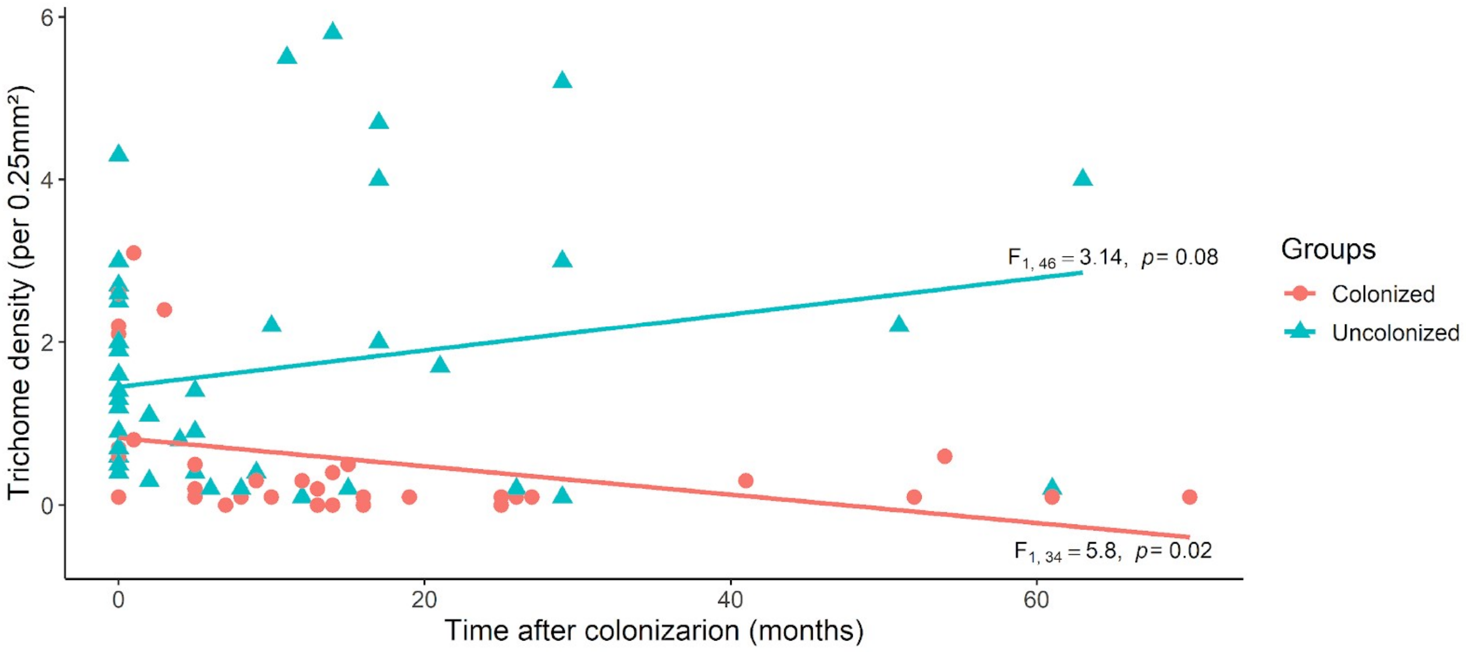

Figure 1: Temporal variation in the density (per 0.25 mm^2) of leaf trichomes in *Cecropia glaziovii* after plants were colonized or not by *Azteca muelleri*.

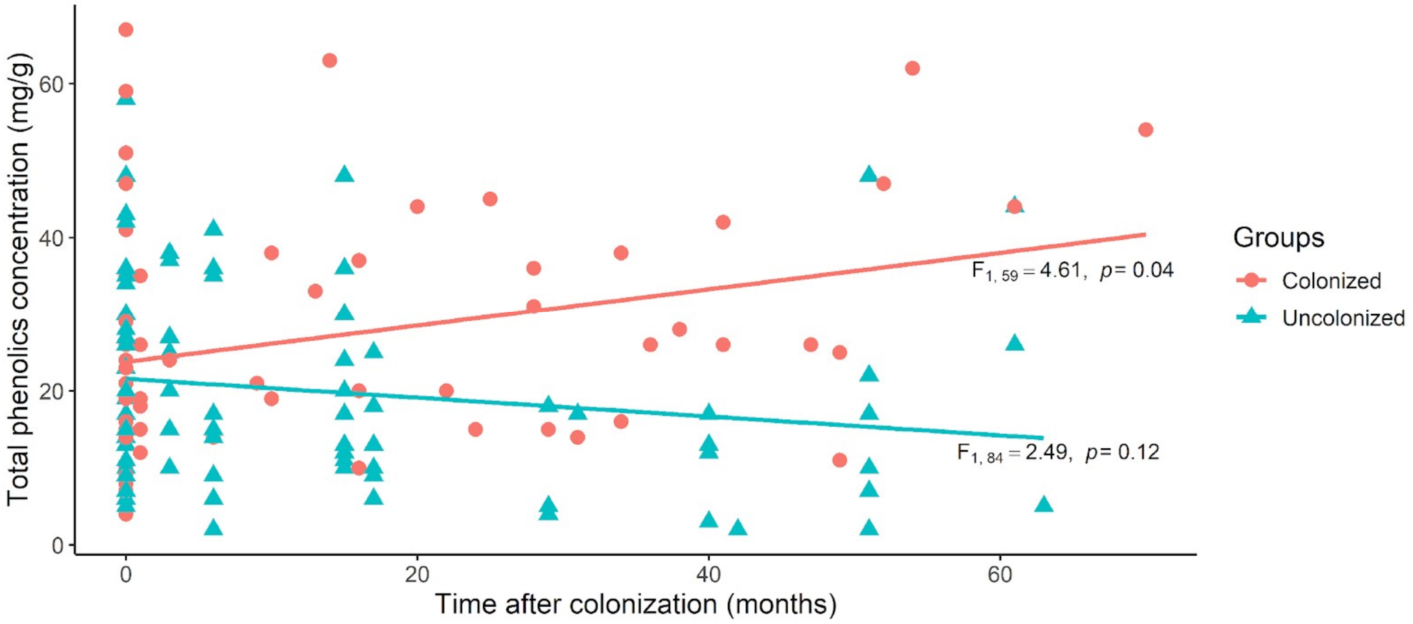

Figure 2: Temporal variation in the total phenolic concentration (mg/g) in *Cecropia glaziovii* after plants were colonized by *Azteca muelleri*.

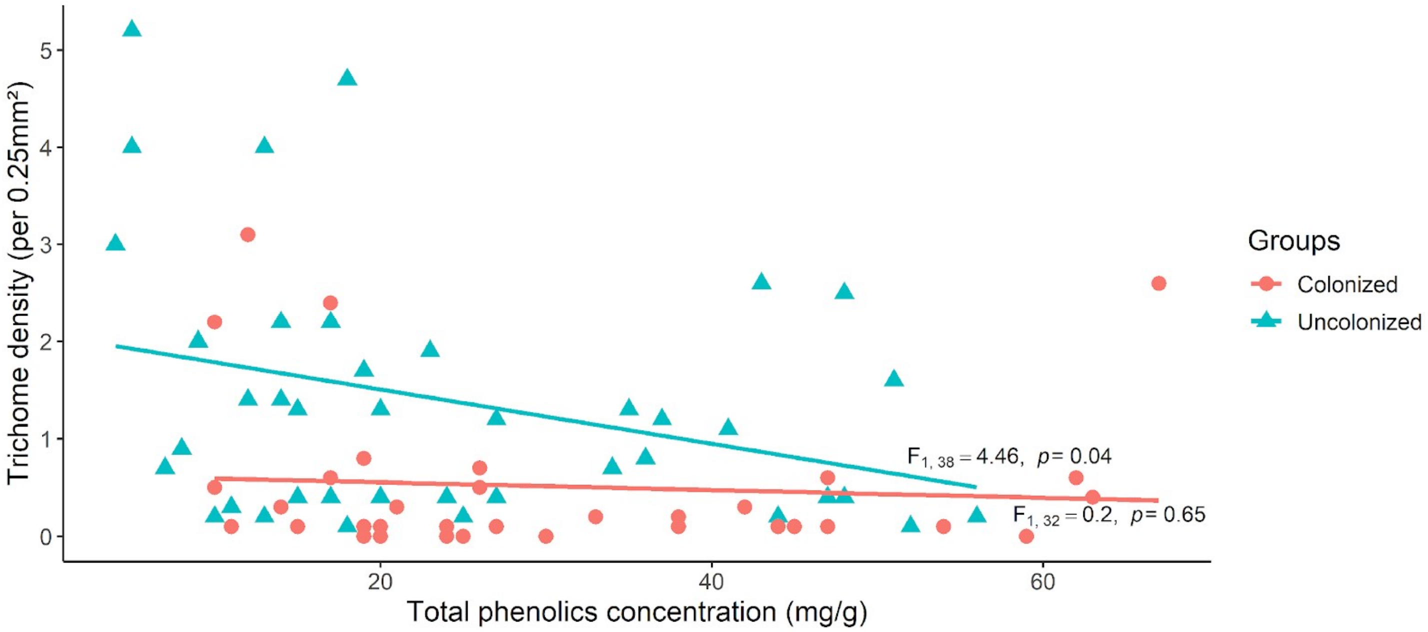

Figure 3: Relationship between density (per 0.25 mm^2) of leaf trichomes and total phenolics concentration (mg/g; log transformed) in plants of *Cecropia glaziovii* colonized (gray circles) or uncolonized (black triangles) by *Azteca muelleri*.

DISCUSSION

Our long-term experiment showed that colonization of *A. muelleri* ants appears as a strong mediator of the trade-off between physical and chemical defenses in *C. glaziovii*. We found that trichomes decreased with the increment in the total phenolics concentration but this trade-off is exclusive to uncolonized plants. It means that ant presence directly influences the direction of resource expenses for *Cecropia* plants. The absence of ants causes an increase in trichomes, while colonized plants experienced a decrease in this physical trait. Contrary to our expectations, high concentrations of phenolics were found in the presence of ants over time following colonization. These results have the potential to shed more light on the trade-off between different defense mechanisms of myrmecophyte plants, considering the role of ants.

It is well known that plants that invest in a wide range of defenses suffer minimal attack from herbivores (Agrawal et al., 2010). However, there is an associated cost for each type of defense, including indirect defenses and maintenance of mutualistic ants. For example, trichomes are structures generally composed of cellulose (Betancur et al., 2010) and the attractive structures for ants are simple carbohydrates (Heil, 1998). The cost of producing cellulose is higher than that of producing simple sugars (Scott, 2008); so food bodies and nectars are cheaper than trichomes. Chemical defenses, in turn, are made up of secondary metabolites (Kumar et al., 2023). These compounds are more expensive, but they can be stocked, recycled and reallocated on other structures or functions (Feeny, 1970). Despite the high cost of producing defenses, the plant can produce a combination of resistance traits rather than increasing its production to a single one when there is an abundance of resources (Yamawo et al., 2014).

Determining the cost-efficient solution between types of defense could be related to the resources needed (or its availability) to produce them. In this way, nutritional input can also influence the investment in multiple defense strategies in myrmecophytes (Wise & Abrahamson 2007; Yamawo et la., 2012; Yamawo et al., 2012a). Some studies evidenced the plant absorption of N^{15} coming from *Azteca* nests (Sagers et al., 2000; Gomes et al., 2021). Regarding phenolics, they are C-based defenses and are indirectly affected by non-constituent compounds (some nitrogen-based) that can pair with carbon to increase carbon fixation in the plant (Bryant et al., 1983; Folgarait & Davidson, 1995). So, plants that receive greater amounts of nitrogen can produce greater amounts of secondary compounds, such as phenolics. This can also be observed in light of the carbon-nitrogen balance hypothesis (Folgarait & Davidson, 1994). It postulates that the composition of the plant's chemical defenses depends on this nutritional balance between C and N (which is generally limiting for the plant (Tilman, 1986)). The nutritional boost provided by ants (mainly nitrogen) can be an extra source of energy for plants and make it possible to invest in more than one defense strategy at the same time (Hawks & Sulivan, 2001; Stamp, 2003). This could also explain our results, as we demonstrated that plants with ants produce more phenolic compounds. Also, nutritionally rich environments (similar to the nutritional enrichment provided by ants) supply an increase in food rewards for ants and lead to a decrease in trichomes in some plants (Yamawo et al., 2014; Tanney et al., 2023).

Considering the trichomes, they are a defensive trait that plays a similar role of ants in defending myrmecophyte plants (Bartimachi et al., 2015). This happens because physical defenses have greater effect in reducing herbivory, just like ants (Bartimachi et al., 2015). Thus, regarding efficiency in defense against herbivory, investing in trichomes and ants could generate redundancy in defenses (investing in defensive mechanisms that achieve the same effect). This way there would be no need for high investment in trichomes while the ants also perform defense. Furthermore, ants benefit plants beyond defense against herbivores (pruning (Davidson, 1988), fertilization (Lucas et al., 2018), asepsis (Offenberg, & Damgaard, 2019) and wound care (Wcislo, 2021)), which would make investing in them more profitable. Despite the costs of maintaining the colony, the other benefits offered by the ant (in addition to defense) can offset the investment. It could explain the decrease in trichome density in the presence of ants; found in our results.

Curiously, in *C. glaziovii*, the decrease in trichomes also seems to be related to the increase in phenolic compounds in uncolonized plants, while the colonized ones had a null effect for this trade-off. If we only consider costs, it seems illogical to invest in a more expensive characteristic (which is chemical defense, using secondary metabolism) than in a cheaper one (such as trichomes or ants). Also as postulated above, in poorer environments (that could be a condition in absence of ants) the plant would invest mainly in physical defenses (Folgarait & Davidson, 1994; Yamawo et al., 2014; Tanney et al., 2023). However, investment in a particular type of defense may be related to the pressure exerted by herbivory (Turley et al., 2013; Gong & Zhang, 2014), so that investment may be associated with a need for more specialized defense against a specific predator, especially in the absence of ants’ defense. Thus, it appears that what governs the production of chemical and physical defenses is not only their cost or the presence of ants, but also the demands of the plant in the face of environmental conditions and its predators - that is, context dependence (about context dependence in plant defenses, see: Heil et al., 2002; Dyer et al., 2003; Agrawal, 2010; Bartimachi et al, 2015).

Regarding chemical defenses, contrary to our expectations, we found that phenolics increase in *C. glaziovii* colonized plants and that this also happens over time after colonization. Other studies show evidence that secondary metabolites can play different roles in the plant beyond defense (Rosenthal & Berenbaum, 1991; Bennett & Wallsgrove, 1994), especially in ant presence (Mellway et al., 2009; Boeckler et al., 2011; Shalaby & Horwitz, 2015). For instance, phenolic compounds may protect plants against oxidative stress caused by UV radiation (Dostálek et al., 2016); also protecting those structures that are attractive to ants. In this sense, our results corroborate with plant defense syndromes hypothesis (Heil et al., 2009 e 2012; Kursar et al., 2009). This hypothesis postulates that given the high diversity of insect herbivores imposing different herbivory impacts, plants should invest at the same time in a wide range of defensive strategies (Rasman & Agrawal, 2009). In our case, the increase in phenolic concentration after ant colonization might be promoted by the high diversity of herbivores experienced by *C. glaziovii* plants (Oliveira et al., 2015). This is also in accordance with the increase in phenolics in the absence of ants, possibly promoted by the pressure exerted by certain herbivores. This should be an explanation for the contradictory results regarding the efficacy of secondary metabolites on plant protection against herbivores (Mitra et al., 2008). Thus, under varying ecological conditions, multiple resistance traits may be adaptive and provide protection against different enemies (Agrawal, 2007; Callis-Duehl et al., 2017). In many systems, multiple defensive traits probably are not redundant or useless and can evolve together (Rasman & Agrawal, 2009; Carmona et al., 2011; Frederickson et al., 2013).

In addition, defense strategies also vary throughout the plant ontogeny and can be affected by abiotic factors such as resource availability (Boege & Marquis, 2005; Barton & Koricheva, 2010; Ochoa-López et al., 2015). Myrmecophytes develop defensive structures over time. Therefore, in their younger stages direct defenses are building up, and ant colonization is not yet possible (Val & Dirzo, 2003; Boege & Marquis, 2005; Frederickson & Gordon, 2009). This temporal availability of resources restricts the ant protection only to the adult plant phase (Fonseca & Benson, 2003). And, besides all that, we also need to consider the possibility of there being an effect of ant activity on plants. Ants could perceive trichomes as obstacles during their movement through the leaves - thus the decrease in trichomes is not related to their production, but rather to the pruning carried out by the ants (Davidson et al., 1989). And, considering the ants' potential to defend their host plants (Schupp, 1986; Bruna et al., 2004; Frederickson, 2005; Gianoli et al., 2008; Dejean et al., 2008 e 2009; Rosumek et al., 2009; Pringle et al., 2012; Queiroz et al., 2020), their effectiveness to the point where trees no longer need to invest in other forms of defense has never been demonstrated. This may be due to multiple factors, such as the effects of plant health or environmental conditions on ant performance. We know that the intensity of ants' behaviors can be affected by the composition of the neighborhood (Zorzal et al., 2021), the ants' ability to perceive plants (Gonçalves-Souza, 2016) and even factors that most directly affect the health of the plant - availability of nutrients, aging or exposure to disturbances (Dejean et al., 2013; Marting, 2018, Marques et al., 2022). Therefore, it is also necessary for the plant to invest in other defenses, in order to generate a greater defensive effect on its predators. Assessing the conditions that affect ant performance and its relationship with other defense mechanisms in plants can further increase understanding of these types of trade-offs.

Our experiment indicates that *A. muelleri* ants influence the trade-off between direct defensive traits in myrmecophytes. Moreover, in colonized *C. glaziovii* trees, ants negatively affect the investment in trichomes and positively the chemical compounds production. However, in the absence of ants, other factors appear to influence the allocation of plant defenses. In this way, ants prove to be extremely important, not only as a plant protection agent. And so, it is also important for the plant to invest in defenses other than ants, in order to expand its ways of combating its herbivores. These results bring a new approach about the trade-off between direct defenses and between direct and indirect defenses strategies in an obligate mutualism system. This evidence indicates that the trade-off is highly context-dependent (as conditions other than the ant can affect it) and also strongly dependent on some benefits provided by ants. We recommend that future studies focus on investigating the role of nutritional benefits of ants and their efficiency in protecting trees in affecting direct plant defenses. Finally, our results might help to clarify the multiple factors triggering myrmecophyte investment on defensive traits. We also emphasize the importance of long-term manipulative studies for greater accuracy in identifying the influence of ants in the defensive pattern of their host plants.

REFERENCES

Agrawal, A. A. (1998). Induced responses to herbivory and increased plant performance. *Science*, *279*(5354), 1201-1202.

Agrawal, A. A., & Dubin-Thaler, B. J. (1999). Induced responses to herbivory in the Neotropical ant-plant association between Azteca ants and Cecropia trees: response of ants to potential inducing cues. *Behavioral Ecology and Sociobiology*, *45*, 47-54.

Agrawal, A. A., & Fishbein, M. (2006). Plant defense syndromes. *Ecology*, *87*(sp7), S132-S149.

Agrawal, A. A. (2007). Macroevolution of plant defense strategies. *Trends in ecology & evolution*, *22*(2), 103-109.

Agrawal, A. A., Conner, J. K., & Rasmann, S. (2010). Tradeoffs and negative correlations in evolutionary ecology In Bell MA, Eanes WF, Futuyma DJ, and Levinton JS, eds. Evolution after Darwin: The First 150 Years. *Sunderland, MA: Sinauer Associates.[Google Scholar]*.

Agrawal, A. A. (2011). New synthesis—trade-offs in chemical ecology. *Journal of chemical ecology*, *37*(3), 230-231.

Agrawal, A. A., & Hastings, A. P. (2019). Trade-offs constrain the evolution of an inducible defense within but not between plant species. *Ecology*, *100*(12), e02857.

Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. D. M., & Sparovek, G. (2013). Köppen's climate classification map for Brazil. *Meteorologische zeitschrift*, *22*(6), 711-728.

Asplen, M. K., Bruns, E., David, A. S., Denison, R. F., Epstein, B., Kaiser, M. C., ... & May, G. (2012). Do trade-offs have explanatory power for the evolution of organismal interactions?. *Evolution*, *66*(5), 1297-1307.

Bartimachi, A., Neves, J., & Vasconcelos, H. L. (2015). Geographic variation in the protective effects of ants and trichomes in a Neotropical ant–plant. *Plant ecology*, *216*, 1083-1090.

Barton, K. E., & Koricheva, J. (2010). The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. *The American Naturalist*, *175*(4), 481-493.

Barton, K. E. (2016). Tougher and thornier: general patterns in the induction of physical defence traits. *Functional Ecology*, *30*(2), 181-187.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., ... & Bolker, M. B. (2015). Package 'lme4'. *convergence*, *12*(1), 2.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal statistical society: series B (Methodological)*, *57*(1), 289-300.

Bennett, R. N., & Wallsgrove, R. M. (1994). Secondary metabolites in plant defence mechanisms. *New phytologist*, *127*(4), 617-633.

Boeckler, G. A., Gershenzon, J., & Unsicker, S. B. (2011). Phenolic glycosides of the Salicaceae and their role as anti-herbivore defenses. *Phytochemistry*, *72*(13), 1497-1509.

Boege, K., & Marquis, R. J. (2005). Facing herbivory as you grow up: the ontogeny of resistance in plants. *Trends in ecology & evolution*, *20*(8), 441-448.

Bronstein, J. L. (1998). The contribution of ant-plant protection studies to our understanding of mutualism 1. *Biotropica*, *30*(2), 150-161.

Bruna, E. M., Lapola, D. M., & Vasconcelos, H. L. (2004). Interspecific variation in the defensive responses of obligate plant-ants: experimental tests and consequences for herbivory. Oecologia, 138, 558-565.

Bryant JP, Chapin III FS, Klein DR. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos. 1983 May 1:357-68.

Callis-Duehl, K., Vittoz, P., Defossez, E., & Rasmann, S. (2017). Community-level relaxation of plant defenses against herbivores at high elevation. *Plant Ecology*, *218*, 291-304.

Carmona, D., Lajeunesse, M. J., & Johnson, M. T. (2011). Plant traits that predict resistance to herbivores. *Functional Ecology*, *25*(2), 358-367.

Davidson, D. W., Longino, J. T., & Snelling, R. R. (1988). Pruning of host plant neighbors by ants: an experimental approach. Ecology, 69(3), 801-808.

Davidson, D. W., Snelling, R. R., & Longino, J. T. (1989). Competition among ants for myrmecophytes and the significance of plant trichomes. *Biotropica*, 64-73.

Davidson, D. W. (2005). Cecropia and its biotic defenses. *Fl Neotrop Monog*, *94*, 214-226.

Dejean, A., Grangier, J., Leroy, C., & Orivel, J. (2008). Host plant protection by arboreal ants: looking for a pattern in locally induced responses. Evolutionary Ecology Research, 10(8), 1217-1223.

Dejean, A., Grangier, J., Leroy, C., & Orivel, J. (2009). Predation and aggressiveness in host plant protection: a generalization using ants from the genus Azteca. Naturwissenschaften, 96, 57-63.

Dostálek, T., Rokaya, M. B., Maršík, P., Rezek, J., Skuhrovec, J., Pavela, R., & Münzbergová, Z. (2016). Trade-off among different anti-herbivore defence strategies along an altitudinal gradient. *AoB plants*, *8*, plw026.

Eck, G., Fiala, B., Linsenmair, K. E., Bin Hashim, R., & Proksch, P. (2001). Trade-off between chemical and biotic antiherbivore defense in the South East Asian plant genus Macaranga. *Journal of Chemical Ecology*, *27*, 1979-1996.

Farahpour, F., Saeedghalati, M., Brauer, V. S., & Hoffmann, D. (2018). Trade-off shapes diversity in eco-evolutionary dynamics. *elife*, *7*, e36273.

Feeny, P. (1970). Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology*, *51*(4), 565-581.

Flatt, T., & Heyland, A. (Eds.). (2011). *Mechanisms of life history evolution: the genetics and physiology of life history traits and trade-offs*. Oxford university press.

Folgarait, Patricia J., and Diane W. Davidson. "Antiherbivore defenses of myrmecophytic Cecropia under different light regimes." Oikos (1994): 305-320.

Folgarait, P. J., & Davidson, D. W. (1995). Myrmecophytic Cecropia: antiherbivore defenses under different nutrient treatments. *Oecologia*, *104*, 189-206.

Fonseca, C. R., & Benson, W. W. (2003). Ontogenetic succession in Amazonian ant trees. *Oikos*, 407-412.

Fredrickson, M. E. (2005). Ant species confer different partner benefits on two neotropical myrmecophytes. Oecologia, 143, 387-395.

Fredrickson, M. E., & Gordon, D. M. (2009). The intertwined population biology of two Amazonian myrmecophytes and their symbiotic ants. *Ecology*, *90*(6), 1595-1607.

Fredrickson, M. E., Ravenscraft, A., Arcila Hernández, L. M., Booth, G., Astudillo, V., & Miller, G. A. (2013). What happens when ants fail at plant defense? C ordia nodosa dynamically adjusts its investment in both direct and indirect resistance traits in response to herbivore damage. *Journal of Ecology*, *101*(2), 400-409.

Futuyma, D. J., & Moreno, G. (1988). The evolution of ecological specialization. *Annual review of Ecology and Systematics*, *19*(1), 207-233.

Gianoli, E., Sendoya, S., Vargas, F., Mejía, P., Jaffe, R., Rodríguez, M., & Gutierrez, A. (2008). Patterns of Azteca ants' defence of Cecropia trees in a tropical rainforest: support for optimal defence theory. Ecological Research, 23, 905-908.

Gomes, I. J. M. T., Solar, R., Blüthgen, N., Vasconcelos, H. L., Silva, L. E. B., Oliveira, K. N., ... & Campos, R. I. (2021). Multiple effects of mutualistic ants improve the performance of a neotropical ant-plant: A long-term study with the Cecropia-Azteca system. *Basic and Applied Ecology*, *57*, 78-90.

Gomes, I. J. M. T., Neves, M. O., & Paolucci, L. N. (2023). Trees harboring ants are better defended than con-generic and sympatric ant-free trees. *The Science of Nature*, *110*(4), 31.

Gong, B., & Zhang, G. (2014). Interactions between plants and herbivores: a review of plant defense. Acta Ecologica Sinica, 34(6), 325-336.

Guerra, P. C., Becerra, J., & Gianoli, E. (2010). Explaining differential herbivory in sun and shade: the case of Aristotelia chilensis saplings. *Arthropod-Plant Interactions*, *4*, 229-235.

Hakes, A. S., & Cronin, J. T. (2011). Environmental heterogeneity and spatiotemporal variability in plant defense traits. *Oikos*, *120*(3), 452-462.

Hanley, M. E., Lamont, B. B., Fairbanks, M. M., & Rafferty, C. M. (2007). Plant structural traits and their role in anti-herbivore defense. *Perspectives in Plant Ecology, Evolution and Systematics*, *8*(4), 157-178.

Hawkes, C. V., & Sullivan, J. J. (2001). The impact of herbivory on plants in different resource conditions: a meta-analysis. *Ecology*, *82*(7), 2045-2058.

Heil, M., Fiala, B., Linsenmair, K. E., Zotz, G., & Menke, P. (1997). Food body production in Macaranga triloba (Euphorbiaceae): a plant investment in anti-herbivore defense via symbiotic ant partners. *Journal of Ecology*, 847-861.

Heil, M., Fiala, B., Maschwitz, U., & Linsenmair, K. E. (2001). On benefits of indirect defense: short-and long-term studies of antiherbivore protection via mutualistic ants. *Oecologia*, *126*, 395-403.

Heil, M., Hilpert, A., Fiala, B., Bin Hashim, R., Strohm, E., Zotz, G., & Linsenmair, K., E. (2002). Nutrient allocation of Macaranga triloba ant plants to growth, photosynthesis and indirect defence. *Functional Ecology*, *16*(4), 475-483.

Heil, M., & McKey, D. (2003). Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology, Evolution, and Systematics*, *34*(1), 425-553.

Heil, M. (2008). Indirect defence via tritrophic interactions. *New Phytologist*, *178*(1), 41-61.

Heil, M. (2009). Damaged-self recognition in plant herbivore defence. *Trends in plant science*, *14*(7), 356-363.

Heil, M., Ibarra-Laclette, E., Adame-Alvarez, R. M., Martínez, O., Ramirez-Chavez, E., Molina-Torres, J., & Herrera-Estrella, L. (2012). How plants sense wounds: damaged-self recognition is based on plant-derived elicitors and induces octadecanoid signaling. *PloS one*, *7*(2), e30537.

Herms, D. A., & Mattson, W. J. (1992). The dilemma of plants: to grow or defend. *The quarterly review of biology*, *67*(3), 283-335.

Janzen, D. H. (1966). Coevolution of mutualism between ants and acacias in Central America. *Evolution*, *20*(3), 249-275.

Janzen, D. H. (1969). Allelopathy by myrmecophytes: the ant Azteca as an allelopathic agent of Cecropia. *Ecology*, *50*(1), 147-153.

Janzen, D. H., & Rosenthal, G. A. (1979). Herbivores: their interaction with plant secondary metabolites.

Jasienska, G. (2009). Reproduction and lifespan: Trade-offs, overall energy budgets, intergenerational costs, and costs neglected by research. *American Journal of Human Biology: The Official Journal of the Human Biology Association*, *21*(4), 524-532.

Karban, R. (2011). The ecology and evolution of induced resistance against herbivores. *Functional Ecology*, *25*(2), 339-347.

Koricheva, J., Nykänen, H., & Gianoli, E. (2004). Meta-analysis of trade-offs among plant antiherbivore defenses: are plants jacks-of-all-trades, masters of all? *The American Naturalist*, *163*(4), E64-E75.

Koricheva, J., & Romero, G. Q. (2012). You get what you pay for: reward-specific trade-offs among direct and ant-mediated defenses in plants. *Biology Letters*, *8*(4), 628-630.

Kumar, S., Korra, T., Thakur, R., Arutselvan, R., Kashyap, A. S., Nehela, Y., ... & Keswani, C. (2023). Role of plant secondary metabolites in defence and transcriptional regulation in response to biotic stress. Plant Stress, 100154.

Kursar, T. A., & Coley, P. D. (2003). Convergence in defense syndromes of young leaves in tropical rainforests. *Biochemical Systematics and Ecology*, *31*(8), 929-949.

Kursar, T. A., Dexter, K. G., Lokvam, J., Pennington, R. T., Richardson, J. E., Weber, M. G., ... & Coley, P. D. (2009). The evolution of antiherbivore defenses and their contribution to species coexistence in the tropical tree genus Inga. *Proceedings of the National Academy of Sciences*, *106*(43), 18073-18078.

Latteman, T. A., Mead, J. E., DuVall, M. A., Bunting, C. C., & Bevington, J. M. (2014). Differences in Anti-Herbivore Defenses in Non-Myrmecophyte and Myrmecophyte Cecropia Trees. *Biotropica*, *46*(6), 652-656.

Letourneau, D. K. (1983). Passive aggression: an alternative hypothesis for the Piper-Pheidole association. *Oecologia*, *60*(1), 122-126.

Longino, J. T. (1991). Azteca ants in Cecropia trees: taxonomy, colony structure, and behavior. *Ant-plant interactions*, 271-288.

Lucas, P. W., Turner, I. M., Dominy, N. J., & Yamashita, N. (2000). Mechanical defences to herbivory. *Annals of Botany*, 86(5), 913-920.

Lucas, J. M., Clay, N. A., & Kaspari, M. (2018). Nutrient transfer supports a beneficial relationship between the canopy ant, Azteca trigona, and its host tree. Ecological entomology, 43(5), 621-628.

MARQUES, Rafael Diniz. Colônias à deriva: efeitos do alagamento no sistema mutualístico Azteca-Cecropia. 2022. 47 f. Dissertação (Mestrado em Ecologia) - Universidade Federal de Viçosa, Viçosa. 2022. Disponível em: <https://locus.ufv.br//handle/123456789/30420>. Acesso em: 04 de setembro de 2024.

Massad, T. J., Fincher, R. M., Smilanich, A. M., & Dyer, L. (2011). A quantitative evaluation of major plant defense hypotheses, nature versus nurture, and chemistry versus ants. *Arthropod-Plant Interactions*, *5*, 125-139.

Massad, T. J., Dyer, L. A., & Vega C, G. (2012). Costs of defense and a test of the carbon-nutrient balance and growth-differentiation balance hypotheses for two co-occurring classes of plant defense. *PloS one*, *7*(10), e47554.

Mayer, V. E., Frederickson, M. E., McKey, D., & Blatrix, R. (2014). Current issues in the evolutionary ecology of ant–plant symbioses. *New Phytologist*, *202*(3), 749-764.

Meira-Neto, J. A. A., & Martins, F. R. (2002). Composição florística de uma floresta estacional semidecidual montana no município de Viçosa-MG. *Revista Árvore*, *26*, 437-446.

Mellway, R. D., Tran, L. T., Prouse, M. B., Campbell, M. M., & Constabel, C. P. (2009). The wound-, pathogen-, and ultraviolet B-responsive MYB134 gene encodes an R2R3 MYB transcription factor that regulates proanthocyanidin synthesis in poplar. *Plant Physiology*, *150*(2), 924-941.

Meyer, J. R., Gudelj, I., & Beardmore, R. (2015). Biophysical mechanisms that maintain biodiversity through trade-offs. *Nature communications*, *6*(1), 6278.

Mitra, S., & Baldwin, I. T. (2008). Independently silencing two photosynthetic proteins in Nicotiana attenuata has different effects on herbivore resistance. *Plant Physiology*, *148*(2), 1128-1138.

Mitra, S. (2008). *Herbivore induced changes in the plant's proteome and the role of atypical herbivore responsive proteins in Nicotiana attenuata* (Doctoral dissertation, Jena, Univ., Diss., 2008).

Mole, S. (1994). Trade-offs and constraints in plant-herbivore defense theory: a life-history perspective. *Oikos*, 3-12.

Moles, A. T., Peco, B., Wallis, I. R., Foley, W. J., Poore, A. G., Seabloom, E. W., ... & Hui, F. K. (2013). Correlations between physical and chemical defenses in plants: trade offs, syndromes, or just many different ways to skin an herbivorous cat? *New Phytologist*, *198*(1), 252-263.

Morin, X., & Chuine, I. (2006). Niche breadth, competitive strength and range size of tree species: a trade-off based framework to understand species distribution. *Ecology Letters*, *9*(2), 185-195.

Mithöfer, A., & Boland, W. (2012). Plant defense against herbivores: chemical aspects. *Annual review of plant biology*, *63*, 431-450.

Obeso, J. R. (2002). The costs of reproduction in plants. *New phytologist*, *155*(3), 321-348.

Ochoa-López, S., Villamil, N., Zedillo-Avelleira, P., & Boege, K. (2015). Plant defence as a complex and changing phenotype throughout ontogeny. *Annals of Botany*, *116*(5), 797-806.

Offenberg, J., & Damgaard, C. (2019). Ants suppressing plant pathogens: a review. Oikos, 128(12), 1691-1703.

Oliveira, K. N., Coley, P. D., Kursar, T. A., Kaminski, L. A., Moreira, M. Z., & Campos, R. I. (2015). The effect of symbiotic ant colonies on plant growth: a test using an Azteca-Cecropia system. *PloS one*, *10*(3), e0120351.

Pereira, Cássio Cardoso, et al. "Are extrafloral nectaries efficient against herbivores? Herbivory and plant defenses in contrasting tropical species." Journal of Plant Ecology 13.4 (2020): 423-430.

Piovia-Scott, J. (2011). The effect of disturbance on an ant–plant mutualism. *Oecologia*, *166*(2), 411-420.

Pringle, E. G., Dirzo, R., & Gordon, D. M. (2012). Plant defense, herbivory, and the growth of Cordia alliodora trees and their symbiotic Azteca ant colonies. Oecologia, 170, 677-685.

Queiroz, A. C., Mendes, C. R., & da Costa, D. B. (2020). Herbivores just need a calm place to feed: Ants cannot help them. Entomological Communications, 2, ec02019-ec02019.

Rasmann, S., & Agrawal, A. A. (2009). Plant defense against herbivory: progress in identifying synergism, redundancy, and antagonism between resistance traits. *Current opinion in plant biology*, *12*(4), 473-478.

Reekie, E. G., & Avila-Sakar, G. (2005). The shape of the trade-off function between reproduction and growth. In *Reproductive allocation in plants* (pp. 189-214). Academic Press.

Rhoades, D. F. (1979). Evolution of plant chemical defenses against herbivores. *Herbivores-Their interaction with secondary plant metabolites*, 3-48.

Rosenthal, G. A., & Berenbaum, M. R. (2012). Herbivores: their interactions with secondary plant metabolites: ecological and evolutionary processes (Vol. 2). *Academic press*.

Rosumek, F. B., Silveira, F. A., de S. Neves, F., de U. Barbosa, N. P., Diniz, L., Oki, Y., ... & Cornelissen, T. (2009). Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. Oecologia, 160, 537-549.

Sagers, C. L., Ginger, S. M., & Evans, R. D. (2000). Carbon and nitrogen isotopes trace nutrient exchange in an ant-plant mutualism. *Oecologia*, *123*, 582-586.

Salgado-Luarte, C., González-Teuber, M., Madriaza, K., & Gianoli, E. (2023). Trade-off between plant resistance and tolerance to herbivory: Mechanical defenses outweigh chemical defenses.

Seigler, D. S., & Ebinger, J. E. (1987). Cyanogenic glycosides in ant-acacias of Mexico and Central America. *The Southwestern Naturalist*, 499-503.

Scott, Peter. Physiology and behaviour of plants. John Wiley & Sons, 2013.

Shalaby, S., & Horwitz, B. A. (2015). Plant phenolic compounds and oxidative stress: integrated signals in fungal–plant interactions. *Current genetics*, *61*, 347-357.

Schupp, E. W. (1986). Azteca protection of Cecropia: ant occupation benefits juvenile trees. Oecologia, 70, 379-385.

Spigler, R. B., & Woodard, A. J. (2019). Context-dependency of resource allocation trade-offs highlights constraints to the evolution of floral longevity in a monocarpic herb. *New Phytologist*, *221*(4), 2298-2307.

Stamp, N. (2003). Out of the quagmire of plant defense hypotheses. *The Quarterly review of biology*, *78*(1), 23-55.

Strauss, S. Y., Rudgers, J. A., Lau, J. A., & Irwin, R. E. (2002). Direct and ecological costs of resistance to herbivory. *Trends in ecology & evolution*, *17*(6), 278-285.

Swain, T., & Hillis, W. E. (1959). The phenolic constituents of Prunus domestica. I.—The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture*, *10*(1), 63-68.

Tanney, C. A., Lyu, D., Schwinghamer, T., Geitmann, A., Ruan, E. D., & Smith, D. L. (2023). Sub-optimal nutrient regime coupled with Bacillus and Pseudomonas sp. inoculation influences trichome density and cannabinoid profiles in drug-type Cannabis sativa. *Frontiers in Plant Science*, *14*, 1131346.

Tilman, D. (1986). Nitrogen-limited growth in plants from different successional stages. *Ecology*, *67*(2), 555-563.

Tuller, J., Marquis, R. J., Andrade, S. M., Monteiro, A. B., & Faria, L. D. (2018). Trade-offs between growth, reproduction and defense in response to resource availability manipulations. *PLoS One*, *13*(8), e0201873.

Turkelboom, F., Thoonen, M., Jacobs, S., & Berry, P. (2015). Ecosystem service trade-offs and synergies. *Ecology and Society*, *21*(1), 43.

Turley, N. E., Godfrey, R. M., & Johnson, M. T. (2013). Evolution of mixed strategies of plant defense against herbivores. New Phytologist, 197(2), 359-361.

Val, E. D., & Dirzo, R. (2003). Does ontogeny cause changes in the defensive strategies of the myrmecophyte Cecropia peltata? *Plant Ecology*, *169*, 35-41.

van der Meijden, E., Wijn, M., & Verkaar, H. J. (1988). Defence and regrowth, alternative plant strategies in the struggle against herbivores. *Oikos*, 355-363.

Wcislo, A., Graham, X., Stevens, S., Toppe, J. E., Wcislo, L., & Wcislo, W. T. (2021). Azteca ants repair damage to their Cecropia host plants. Journal of Hymenoptera Research, 88, 61-70.

Wise, M. J., & Abrahamson, W. G. (2007). Effects of resource availability on tolerance of herbivory: a review and assessment of three opposing models. *The American Naturalist*, *169*(4), 443-454.

Yamawo, A., Katayama, N., Suzuki, N., & Hada, Y. (2012). Plasticity in the expression of direct and indirect defence traits of young plants of Mallotus japonicus in relation to soil nutritional conditions. *Plant Ecology*, *213*, 127-132.

Yamawo, A., Hada, Y., & Suzuki, N. (2012a). Variations in direct and indirect defenses against herbivores on young plants of Mallotus japonicus in relation to soil moisture conditions. *Journal of plant research*, *125*, 71-76.

Yamawo, A., Tagawa, J., Hada, Y., & Suzuki, N. (2014). Different combinations of multiple defence traits in an extrafloral nectary-bearing plant growing under various habitat conditions. *Journal of Ecology*, *102*(1), 238-247.

Zera, A. J., & Harshman, L. G. (2001). The physiology of life history trade-offs in animals. *Annual review of Ecology and Systematics*, *32*(1), 95-126.

Züst, T., Heichinger, C., Grossniklaus, U., Harrington, R., Kliebenstein, D. J., & Turnbull, L. A. (2012). Natural enemies drive geographic variation in plant defenses. *Science*, *338*(6103), 116-119.

CONCLUSÃO GERAL

Por meio de dois experimentos de manipulação em condições de campo, foi demonstrado que a colonização por formigas *Azteca muelleri* influencia a dinâmica de investimento em defesas anti-predação em plantas de *Cecropia glaziovii*. No primeiro experimento que avaliou ao mesmo tempo a influência da herbivoria e da presença das formigas na produção de tricomas em *C. glaziovii*, observamos que as plantas colonizadas apresentaram uma diminuição na densidade de tricomas independente da herbivoria. Já no segundo experimento, onde investigamos o efeito das formigas na demanda conflitante (*trade-off*) entre o investimento em defesas físicas e químicas, os resultados indicaram que apenas as plantas não colonizadas exibem esse trade-off. Esses resultados evidenciam que a presença de *A. muelleri* molda o investimento em defesas diretas em *C. glaziovii.* Isso evidencia a complexidade das interações mutualísticas entre plantas e formigas e destacam a importância das formigas, para além do papel protetivo, na determinação do investimento em estratégias defensivas pelas plantas. Para estudos futuros, é necessário investigar a influência de outros benefícios específicos proporcionados pelas formigas (ex: *input* nutricional, economia de energia, defesa contra patógenos, ou até mesmo a influência de outros parceiros em conjunto com as formigas), na determinação das estratégias de defesas pelas mirmecófitas. Além disso, é importante compreender o efeito da produção de recursos para as formigas na limitação do investimento em defesas diretas. À medida que mostramos outras possibilidades além do clássico *trade-off* entre as defesas das plantas, pesquisas futuras poderão trazer uma abordagem individual dos mecanismos de defesa ou explorar outros mecanismos. Finalmente, este estudo contribui significativamente para o entendimento dos mecanismos de defesa de plantas mirmecófitas e ressalta a necessidade de considerar os aspectos multifacetados das interações mutualísticas na ecologia das plantas.

REFERÊNCIAS BIBLIOGRÁFICAS – INTRODUÇÃO GERAL

Agrawal, A. A. **Induced responses to herbivory and increased plant performance.** Science, 279(5354), 1998. 1201-1202p.

Agrawal, A. A., & Rutter, M. T. **Dynamic anti-herbivore defense in ant-plants: the role of induced responses.** Oikos, 1998. 227-236p.

Agrawal, A. A., & Karban, R. I. C. H. A. R. D. **Why induced defenses may be favored over constitutive strategies in plants.** The ecology and evolution of inducible defenses, 10, 000331745, 1999.

Agrawal, A. A. **Macroevolution of plant defense strategies.** Trends in ecology & evolution, 22(2), 2007.103-109p.

Arimura, G. I., Kost, C., & Boland, W. **Herbivore-induced, indirect plant defences.** Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1734(2), 2005. 91-111p.

Bronstein, J. L., Alarcón, R., & Geber, M. **The evolution of plant–insect mutualisms.** New Phytologist, 172(3), 2006. 412-428p.

Carmona, D., Lajeunesse, M. J., & Johnson, M. T. **Plant traits that predict resistance to herbivores.** Functional Ecology, 25(2), 2011. 358-367p.

Chaudhary, A., Bala, K., Thakur, S., Kamboj, R., & Dumra, N. **Plant defenses against herbivorous insects: a review.** IJCS, 6(5), 2018. 681-688p.

Coley, P. D., Bryant, J. P., & Chapin III, F. S. **Resource availability and plant antiherbivore defense.** Science, 230(4728), 1985. 895-899p.

Crawley, M. J. **Herbivory. The dynamics of animal--plant interactions**. Blackwell Scientific Publications, 1983.

Evans, D. L., & Schmidt, J. O. (Eds.). **Insect defenses: adaptive mechanisms and strategies of prey and predators.** Suny Press, 1990.

Fornoni, J. **Ecological and evolutionary implications of plant tolerance to herbivory.** Functional Ecology, 25(2), 2011. 399-407p.

Fox, L. R. **Defense and dynamics in plant-herbivore systems.** American zoologist, 21(4), 1981. 853-864p.

Fürstenberg-Hägg, J., Zagrobelny, M., & Bak, S. **Plant defense against insect herbivores.** International journal of molecular sciences, 14(5), 2013. 10242-10297p.

Gatehouse, J. A. **Plant resistance towards insect herbivores: a dynamic interaction.** New phytologist, 156(2), 2002. 145-169p.

Gong, B., & Zhang, G. **Interactions between plants and herbivores: a review of plant defense.** Acta Ecologica Sinica, 34(6), 2014. 325-336p.

Hanley, M. E., Lamont, B. B., Fairbanks, M. M., & Rafferty, C. M. **Plant structural traits and their role in anti-herbivore defence.** Perspectives in Plant Ecology, Evolution and Systematics, 8(4), 2007. 157-178p.

Hendrix, S. D. **Herbivory and its impact on plant reproduction.** Plant reproductive ecology: patterns and strategies, 1988. 246-263p.

Janzen, D. H. **Allelopathy by myrmecophytes: the ant Azteca as an allelopathic agent of Cecropia.** Ecology, 50(1), 1969. 147-153p.

Johnson, M. T., Campbell, S. A., & Barrett, S. C. **Evolutionary interactions between plant reproduction and defense against herbivores.** Annual Review of Ecology, Evolution, and Systematics, 46, 2015. 191-213p.

Kaplan, I., Halitschke, R., Kessler, A., Sardanelli, S., & Denno, R. F. **Constitutive and induced defenses to herbivory in above-and belowground plant tissues.** Ecology, 89(2), 2008. 392-406p.

Karban, R. **The ecology and evolution of induced resistance against herbivores.** Functional Ecology, 25(2), 2011. 339-347p.

Koricheva, J., Nykänen, H., & Gianoli, E. **Meta-analysis of trade-offs among plant antiherbivore defenses: are plants jacks-of-all-trades, masters of all?** The American Naturalist, 163(4), 2004. E64-E75p.

Koricheva, J., & Romero, G. Q. **You get what you pay for: reward-specific trade-offs among direct and ant-mediated defences in plants.** Biology Letters, 8(4), 2012. 628-630p.

Massad, T. J., Fincher, R. M., Smilanich, A. M., & Dyer, L. **A quantitative evaluation of major plant defense hypotheses, nature versus nurture, and chemistry versus ants.** Arthropod-Plant Interactions, 5, 2011. 125-139p.

McNaughton, S. J. **Physiological and ecological implications of herbivory. In Physiological plant ecology III: responses to the chemical and biological environment.** Berlin, Heidelberg: Springer Berlin Heidelberg, 1983. 657-677p.

Mithöfer, A., & Boland, W. **Plant defense against herbivores: chemical aspects.** Annual review of plant biology, 63, 2012. 431-450p.

Ness, J. H., Morris, W. F., & Bronstein, J. L**. For ant-protected plants, the best defense is a hungry offense.** Ecology, 90(10), 2009. 2823-2831p.

Price, P. W., Bouton, C. E., Gross, P., McPheron, B. A., Thompson, J. N., & Weis, A. E. **Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies.** Annual review of Ecology and Systematics, 11(1), 1980. 41-65p.

Rasmann, S., & Agrawal, A. A. **Plant defense against herbivory: progress in identifying synergism, redundancy, and antagonism between resistance traits.** Current opinion in plant biology, 12(4), 2009. 473-478p.

Richards, L. A., Glassmire, A. E., Ochsenrider, K. M., Smilanich, A. M., Dodson, C. D., Jeffrey, C. S., & Dyer, L. A. **Phytochemical diversity and synergistic effects on herbivores.** Phytochemistry Reviews, 15, 2016. 1153-1166p.

Rosumek, F. B., Silveira, F. A., de S. Neves, F., de U. Barbosa, N. P., Diniz, L., Oki, Y., ... & Cornelissen, T. **Ants on plants: a meta-analysis of the role of ants as plant biotic defenses.** Oecologia, 160, 2009. 537-549p.

Schultz, J. C., Appel, H. M., Ferrieri, A. P., & Arnold, T. M. **Flexible resource allocation during plant defense responses.** Frontiers in plant science, 4, 2013. 324p.

Strauss, S. Y., Rudgers, J. A., Lau, J. A., & Irwin, R. E. **Direct and ecological costs of resistance to herbivory.** Trends in ecology & evolution, 17(6), 2002. 278-285p.

Val, E. D., & Dirzo, R. **Does ontogeny cause changes in the defensive strategies of the myrmecophyte Cecropia peltata?**. Plant Ecology, 169, 2003. 35-41p.

War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S., & Sharma, H. C. **Mechanisms of plant defense against insect herbivores.** Plant signaling & behavior, 7(10), 2012. 1306-1320p.

Yu, D. W., & Davidson, D. W. **Experimental studies of species-specificity in Cecropia–ant relationships.** Ecological Monographs, 67(3), 1997. 273-294p.

Zorzal, G., Camarota, F., Dias, M., Vidal, D. M., Lima, E., Fregonezi, A., & Campos, R. I. **The dear enemy effect drives conspecific aggressiveness in an Azteca-Cecropia system.** Scientific Reports, 11(1), 6158, 2021.