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Complex interactions in ant–plant mutualisms: The role of multiple ant food resources in mediating plant defence, pollination and fitness in a tropical shrub

Vitor Miguel Costa-Silva[1,2,3] | Gabriela Fraga Porto[1,2] | Carla Vázquez-González[3] | Eduardo Soares Calixto[4] | Xoaquín Moreira[3] | Kleber Del-Claro[1,2]

[1]Graduate Program in Entomology, Department of Biology, Universidade de São Paulo, Ribeirão Preto, Sao Paulo, Brazil

[2]Graduate Program in Ecology and Conservation of Natural Resources, Institute of Biology, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil

[3]Misión Biológica de Galicia (MBG-CSIC), Pontevedra, Galicia, Spain

[4]Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA

Correspondence
Vitor Miguel Costa-Silva, Graduate Program in Entomology, Department of Biology, Universidade de São Paulo, Ribeirão Preto, Sao Paulo, Brazil.
Email: vitormcs146@gmail.com

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Abstract

1. Ants form mutualistic relationships with plants that offer extrafloral nectar (EFN) and with trophobiotic insects such as aphids, treehoppers or caterpillars that secrete energy-rich honeydew. Although these insects may negatively affect plants, their presence—along with EFN—increases ant activity, which enhances herbivore predation, reduces leaf damage and indirectly improves plant growth and reproductive success. However, the same ants attracted to these resources can also interfere with pollinators—by deterring or harming them—thereby potentially reducing pollination efficiency and negatively impacting plant reproductive success. While the roles of ants in plant defence and pollination have been extensively studied, the individual and combined effects of multiple ant-attractive resources, such as EFN and trophobionts, on these ecological services remain poorly understood.
2. In this study, we conducted a field experiment manipulating the presence of EFN and trophobiotic insects (treehoppers or myrmecophilous caterpillars) in the tropical shrub *Banisteriopsis malifolia* and used a structural equation modelling approach to assess the direct and indirect effects of these ant food resources on leaf herbivory, pollinator activity and plant fitness.
3. Our results show that ants reduced leaf herbivory on plants offering both EFN and trophobiotic insects. Although ants did not directly affect pollinator behaviour, leaf herbivory in plants hosting treehoppers indirectly influenced pollination by reducing both visit duration and frequency. Flower production in plants with EFN or treehoppers enhanced pollinator attraction, resulting in greater fruit production and

Xoaquín Moreira and Kleber Del-Claro share the senior authorship.

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fruit mass. However, neither pollinator visit duration nor visit frequency significantly influenced fruit yield across any ant resource treatments. Finally, we detected no indirect effects of ant resource availability on overall plant fitness through changes in herbivory or pollination.

4. These findings suggest that while the presence of multiple ant food resources can enhance ant-mediated plant defence, their effects on pollination and overall plant fitness are more nuanced, highlighting the complex and context-dependent nature of multitrophic interactions in shaping ecological outcomes for plants.

KEYWORDS

Banisteriopsis malifolia, Brazilian cerrado, extrafloral nectar, fruit production and mass, pollinators, trophobiotic insects

INTRODUCTION

Ants are among the most abundant and ecologically influential terrestrial insects, comprising a significant portion of animal biomass in many ecosystems worldwide (Lach et al., 2010). Their ubiquity and diverse foraging behaviours enable them to interact with a wide range of organisms, playing critical roles in nutrient cycling, soil turnover, seed dispersal and biological control (Lach et al., 2010). One of the most ecologically important aspects of ant behaviour is their tendency to form protective mutualisms with plants (Rico-Gray & Oliveira, 2007). In these interactions, plants provide resources such as extrafloral nectar (EFN) and specialised nesting structures (Del-Claro et al., 2016; Mayer et al., 2014; Stadler & Dixon, 2008), while ants defend the plants from herbivores through their omnivorous and aggressive predatory behaviour (Baker-Méio & Marquis, 2012; Rudgers, 2004; Zhang et al., 2015). In addition to plant-based mutualisms, ants form associations with trophobionts—such as hemipterans and myrmecophilous caterpillars—that excrete sugar-rich honeydew (Stadler & Dixon, 2005; Styrsky & Eubanks, 2007). In exchange for this resource, ants protect trophobionts from predators and parasitoids, often leading to increases in their populations (Stadler & Dixon, 2005; Styrsky & Eubanks, 2007). While these interactions attract more ants and can enhance herbivore suppression, trophobionts themselves may negatively impact plants through direct feeding damage and nutrient depletion (Dixon, 1997). Despite these costs, ant activity driven by both EFN and honeydew can reduce foliar damage from other herbivores and indirectly improve plant performance (Anjos et al., 2022; Rosumek et al., 2009; Trager et al., 2010).

However, growing evidence over the past two decades has revealed that ants can also disrupt pollination, both directly and indirectly, with potential negative consequences for plant reproductive success (Calixto et al., 2024; Ness, 2006; Rodríguez-Gironés et al., 2013). Direct interference occurs when ants deter or attack pollinators, reducing visitation rates and impairing pollination outcomes (Ibarra-Isassi & Oliveira, 2018; Rodríguez-Gironés et al., 2013). Indirectly, ants may alter the behaviour or abundance of herbivores, which in turn can affect plant traits relevant to pollinators—such as floral display, scent, nectar quantity or quality (reviewed by Moreira et al., 2019). Herbivory, for example, can reduce visual or chemical cues that attract pollinators, decreasing visitation rates (Kessler et al., 2011; Kessler & Halitschke, 2009). Additionally, the presence of herbivores can shift the plant's resource allocation, affecting flower production or nectar quality, which can further influence pollinator attraction and behaviour (Adler et al., 2012).

These ant-mediated effects are highly context-dependent, shaped by a variety of ecological and biological factors including plant traits, herbivore or pollinator identity and environmental conditions (Chamberlain & Holland, 2009; Del-Claro et al., 2016; Leal et al., 2023). A critical driver of this variability is the type and availability of ant-attractive resources provided by the plant—especially EFN and trophobiotic insects (Gijsman et al., 2021; Moura et al., 2023). For instance, plants offering abundant or high-quality food rewards often attract more ants and elicit more frequent and aggressive patrolling behaviour, resulting in stronger herbivore deterrence (Fagundes et al., 2017). In contrast, when such resources are scarce or patchy, ants may reduce their attentiveness, lower their defensive effort or shift to alternative food sources, thereby weakening their protective effect (Moura et al., 2023). Beyond their role in herbivore deterrence, the type and availability of ant food resources—such as EFN and trophobiotic insects—can also significantly influence pollination dynamics. High levels of ant activity driven by abundant food rewards can have dual disruptive effects on plant fitness. First, ants may interfere with pollination by exhibiting aggressive or territorial behaviour near flowers, reducing pollinator visitation and effectiveness (Villamil et al., 2018). Second, by protecting trophobionts, ants can indirectly support herbivores that cause direct damage to plant tissues (Styrsky & Eubanks, 2007). Conversely, when ant-attractive resources are scarce, lower ant presence around flowers may allow pollinators greater access, potentially enhancing pollination success (Ibarra-Isassi & Oliveira, 2018). Furthermore, the spatial and temporal distribution of these resources can shape how ants interact with both herbivores and pollinators, thereby mediating complex trade-offs between plant defence and reproduction (Moura et al., 2023). Understanding how EFN and trophobiont availability modulates these interactions is essential for predicting the net outcome of ant mutualisms on plant fitness and for clarifying the dual roles ants play as both protectors and potential pollination disruptors.

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In this study, we investigated how the availability of individual and combined ant-attractive resources influences plant defence, pollination and overall fitness in the tropical shrub *Banisteriopsis malifolia* (Nees & Mart.) B. Gates (Malpighiaceae). To address this, we conducted a field experiment in which we manipulated the presence of EFN and trophobiotic insects (treehoppers or myrmecophilous caterpillars). We then used a structural equation modelling (SEM) approach to evaluate the direct and indirect effects of these ant food resources on key ecological processes, including leaf herbivory, flower production, pollinator visitation and reproductive outcomes—measured as fruit production and fruit mass. Our study addresses four central questions: (1) Does herbivory vary with the availability of different ant resources? (2) Do pollination patterns shift in response to variations in ant-attractive resources? (3) How does plant fitness respond to changes in the availability of these resources? (4) Are these effects mediated through direct or indirect ecological pathways? By examining how plant- and insect-provided resources influence ant interactions with herbivores and pollinators, we aim to clarify the dual role of ants as both plant defenders and pollinator disruptors in shaping plant fitness. Understanding these complex dynamics is essential for advancing our knowledge of mutualistic networks and for guiding effective biodiversity conservation and ecosystem management strategies.

MATERIALS AND METHODS

Study area

This study was conducted in a Private Natural Heritage Reserve at Clube de Caça e Pesca Itoroó de Uberândia (CCPIU), in Uberlândia, Brazil (48°17′27.0″ W; 18°58′30.6″ S). The research site covers roughly 200 hectares of *cerrado* sensu stricto vegetation, which includes tree and shrub species ranging from 2 to 15 m in height (Oliveira & Marquis, 2002). The region experiences a distinctly seasonal climate, with a rainy season spanning October to March and a dry season from April to September. Annual mean temperatures range from 18 to 28°C, and rainfall varies between 800 and 2000 mm (Calixto et al., 2021).

Study species

We selected *B. malifolia* as our study model because of its well-documented mutualistic interactions with specialised pollinators and ants, its distinctive extrafloral nectaries that attract ant defenders, and its ecological importance as an endemic tropical shrub with a clearly defined reproductive phenology. These features make it an ideal system for investigating how ant-attractive resources affect plant defence, pollination and overall fitness.

Banisteriopsis malifolia typically grows to heights of 1–3 m. Its leaf production and flowering are closely tied to the rainy season, with a regrowth period from December to January and a reproductive period extending from February to July. The plant's leaves are oppositely arranged, each bearing two extrafloral nectaries, and can reach up to 15 cm in length and 10 cm in width. It produces numerous inflorescences, each with tens of pale white or pinkish flowers clustered at the tips of branches and stems (Alves-Silva et al., 2013). Although self-compatible, *B. malifolia* depends on specific pollinators for successful stigma rupture and pollen deposition (Sigrist & Sazima, 2004). Its flowers provide oil glands at the calyx and pollen as primary rewards, attracting large solitary bees from the tribes Centridini and Tapinotaspidini, including *Centris fuscata*, *Epicharis flava* and *Epicharis bicolor* (Barônio & Torezan-Silingardi, 2017). The peak fruiting period occurs between May and June, with fruits maturing fully by July (Ferreira & Torezan-Silingardi, 2013; Novaes et al., 2022). The fruits are wind-dispersed, with each flower capable of producing up to three fruits, each containing up to three seeds. In this study, we define a 'fruit' as the combined set of fruits and seeds produced per flower (Barônio & Torezan-Silingardi, 2017; Novaes et al., 2020).

In its native range, *B. malifolia* hosts a diverse community of leaf-chewing herbivores. Beetles from the families Curculionidae and Chrysomelidae are primary herbivores, feeding on leaves, buds, flowers and fruits (Alves-Silva et al., 2015). Caterpillars from the Saturniidae family—such as *Pseudautomeris brasiliensis*, *Automeris* sp. and *Automeris bilinea*—also represent significant leaf-chewers. These caterpillars often exhibit gregarious behaviour and consume large amounts of foliage during their feeding period. Collectively, these herbivores can substantially impact plant fitness by defoliating plants within weeks and damaging reproductive structures.

Trophobiont insects, including treehoppers from the Membracidae family (e.g., *Enchenopa gracilis*, *Amastris undulata* and *Guayaquila* sp.) (Ibarra-Isassi & Oliveira, 2018; Moura et al., 2023) and myrmecophilous caterpillars from the Lycaenidae and Riodinidae families (e.g., *Parrhasius polibetes*, *Rekoa marius* and *Synargis calyce*) (Bächtold et al., 2016), are also commonly found on *B. malifolia*. Treehoppers tend to form large aggregations on these plants, feeding primarily on leaf buds and reproductive parts, while myrmecophilous caterpillars are generally solitary and focus on consuming reproductive tissues such as buds and flowers. The presence of these trophobionts, along with the EFN, attracts numerous predatory ant species—such as *Camponotus blandus*, *Camponotus crassus* and *Ectatomma tuberculatum*—that forage on the plant in search of these sugar-rich resources.

Experimental design

In January 2024, we selected 114 adult *B. malifolia* plants, each 1–2 m tall, with 100% young leaves and similar phenological stages. The plants were spaced at least 15 m apart to avoid selecting clones. We mapped ant nests and found that each nest foraged on a single plant, with dominant species nesting nearby. The limited foraging range (maximum 422 cm) suggests minimal overlap between colonies, reducing the risk of spatial autocorrelation in our data. At the beginning of the growing season (February 2024), we assigned these plants to one of five experimental groups based on the type of resources

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available to ants: the first group consisted of plants ($n = 29$) with active extrafloral nectaries hosting both trophobiotic insects (treehoppers and myrmecophilous caterpillars simultaneously) ('all resources' treatment). The second group included plants ($n = 26$) with active extrafloral nectaries but no treehoppers or myrmecophilous caterpillars ('only EFN' treatment). The third group consisted of plants ($n = 23$) hosting treehoppers, but with inactive extrafloral nectaries and no myrmecophilous caterpillars ('only treehoppers' treatment). The fourth group included plants ($n = 15$) hosting myrmecophilous caterpillars, but with inactive extrafloral nectaries and no treehoppers ('only caterpillars' treatment). The fifth group consisted of plants ($n = 23$) with inactive extrafloral nectaries and without treehoppers or myrmecophilous caterpillars ('no resources' treatment). For all plants assigned to treatments with inactive extrafloral nectaries and/or without trophobiont insects, we manually removed any trophobionts (e.g., treehoppers and myrmecophilous caterpillars) every three days and maintained EFN inactivity by covering the nectaries with a thin layer of nontoxic glue, thereby preventing ant access to these resources (Rudgers & Gardener, 2004; Villamil et al., 2019). For plants assigned to the no-resource treatment, we additionally applied a barrier of Tanglefoot resin to prevent ant access. To avoid direct contact with plant tissues, the resin was applied to a tape that was first adhered around the stem. In treatments involving trophobionts, we regulated their abundance throughout the experiment by maintaining consistent densities—approximately 30–50 treehoppers and 1–2 myrmecophilous caterpillars per plant.

During the flowering season, from March to May 2024, we assessed pollinator–plant interactions by conducting 30-min observation sessions per plant (Moreira et al., 2024; Schiestl et al., 2014) carried out three times over the course of the experiment—once in mid-March, once in mid-April and once in mid-May. All observations were performed between 08:00 and 12:00, when pollinator activity was highest, to ensure consistent and representative sampling of floral visitors. During each round, we recorded the duration of pollinator visits, counted the total number of flowers and noted the number of visited flowers on each plant. At the end of the reproductive season (July 2024), we randomly collected 10 fully expanded leaves of similar age (based on their position on the plant, colour and consistency) to assess herbivory. We photographed all leaves and estimated the percentage of leaf area removed by leaf-chewing herbivores (e.g., beetles and caterpillars) using the ImageJ 1.47 software (Schneider et al., 2012). Additionally, we measured fruit production and fresh fruit mass for each plant. For this, we randomly selected one inflorescence per plant to count the number of fruits produced and randomly chose 15 mature fruits to determine their mass. Heavier fruits are generally indicative of higher seed quality, which can positively influence subsequent germination success and seedling vigour (Moles & Westoby, 2004). Thus, fruit mass serves as a useful proxy for assessing reproductive investment and potential plant fitness. We aggregated the pollination data (visit duration, flower count and visited flowers) across all rounds and averaged the herbivory data across all leaves and the fruit mass across all fruits to obtain a single value per plant for statistical analyses. The mean values and standard deviations for each variable across treatments of ant food resources are shown in Table S1.

While we did quantify ant abundance and community composition, these data were collected as part of a separate study (Costa-Silva et al., unpublished data). In that previous work, we found that ant abundance on *B. malifolia* plants varied significantly across the different resource availability treatments. Specifically, plants offering all resources (EFN and both trophobiotic insects) or only treehoppers attracted significantly more ants than those with only myrmecophilous caterpillars or only EFN. The ant community composition also varied across treatments, likely reflecting the distinct nutritional profiles of each food resource. These differences influenced which ant species were attracted to the plants. Nonetheless, some species—such as *Camponotus crassus*, *C. blandus* and *Ectatomma tuberculatum*—were consistently present across all treatments and tended to be more abundant than others.

Statistical analyses

We investigated whether the individual and combined presence or absence of food resources for ants in ant–plant interactions directly and/or indirectly affects plant fitness patterns. To do this, we performed four independent piecewise structural equation models (PSEM), one for each resource type (all resources, only EFN, only treehoppers or only caterpillars) using plant-level data. Specifically, we tested the direct effects of (1) ant resource treatments (coded as a dummy variable; no resource = 0, resource treatments = 1) on herbivory; (2) ant resource treatments on the duration of pollinator visits and visited flowers by pollinators; (3) herbivory on the number of flowers; (4) herbivory on the duration of pollinator visits and visited flowers by pollinators; (5) the number of flowers on the duration of pollinator visits and visited flowers by pollinators; (6) the number of flowers on the number of fruits and fruit mass; and (7) the duration of pollinator visits and visited flowers by pollinators on the number of fruits and fruit mass; (see Table 1 for predictions of each path in the PSEM). We also investigated the indirect effects of resource treatments on flower production (via herbivory); on visit duration and visited flowers (via herbivory and flower production); and on fruit production and fruit mass (via herbivory, flower production, pollinator visit duration and visited flowers by pollinators). Direct effects were estimated as standardised path coefficients for each response variable, while indirect effects were calculated as the product of direct effects along the specified causal pathways. All analyses were conducted using RStudio software version 4.0.1 (R Core Team, 2020). We implemented the PSEM and calculated direct effect coefficients (standardised path coefficients) using the *psem* function from the *piecewiseSEM* package (Lefcheck, 2016). To obtain bootstrapped indirect effects (the product of intervening path coefficients) and their associated 95% confidence intervals, we used the *semEff* function from the *semEff* package.

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TABLE 1 Description of paths (direct effects in Figure 1) and predictions in the piecewise structural equation models (PSEM) analysing the interactions between *Banisteriopsis malifolia* plants, ants, herbivores and pollinators.

Path number	Path	Prediction
1	Ant—Herbivory	Ants decrease leaf damage by suppressing herbivores through predation
2	Ant—Pollination	Ants disrupt pollination by attacking or deterring pollinators, a phenomenon known as pollinator avoidance
3	Herbivory—Flowers	Leaf damage reduces flower production in plants by impairing their ability to photosynthesise and allocate resources efficiently
4	Herbivory—Pollination	Herbivores reduce the duration of pollinator visits and the number of flowers visited by influencing pollinator foraging
5	Flowers—Pollination	A higher production of flowers attracts more pollinators and increases the duration of their visits by creating a more visually and chemically attractive display
6	Flowers—Plant fitness	A higher production of flowers leads to a greater overall number of fertilised ovules, resulting in more and heavier fruits
7	Pollination—Plant fitness	Successful pollination results in larger and heavier fruits

RESULTS

The first PSEM (all resources vs. no resources) revealed a negative direct effect of resource treatment on herbivory ($p < 0.001$; standardised coefficient = −0.47), meaning a decrease in herbivory in the presence of resources. A positive direct effect also was observed between the number of flowers and pollination (duration of visits: $p < 0.001$; standardised coefficient = 0.59 − number of visited flowers: $p < 0.001$; standardised coefficient = 0.61). No direct effects were observed between ant resource treatment, herbivory, the number of flowers, the duration of pollinator visits and the number of visited flowers by pollinators. The number of fruits and fruit mass were not directly affected by the duration of pollinator visits or the number of visited flowers by pollinators (Figure 1a; Table 2). No indirect effects were found in this PSEM (Figure 1a; Table 2).

The second PSEM (only EFN vs. no resources) revealed positive direct effects between the number of flowers and the duration of pollinator visits ($p < 0.001$; standardised coefficient = 0.50), the number of visited flowers by pollinators ($p < 0.001$; standardised coefficient = 0.55), the number of fruits ($p = 0.001$; standardised coefficient = 0.47) and fruit mass ($p = 0.01$; standardised coefficient = 0.41; Figure 1b; Table 2). No direct effects were observed between treatment, herbivory and number of flowers or between duration of visits and number of visited flowers with the number of fruits and fruit mass (Figure 1b; Table 2). No indirect effects were found in this PSEM (Figure 1b; Table 2).

The third PSEM (only treehoppers vs. no resources) indicated a negative direct effect between herbivory and pollination (duration of visits: $p < 0.05$; standardised coefficient = −0.30 − number of visited flowers by pollinators: $p < 0.05$; standardised coefficient = − 0.31; Figure 1c; Table 2). A positive direct effect was observed between number of flowers and duration of visits ($p < 0.001$; standardised coefficient = 0.52), number of visited flowers by pollinator ($p < 0.001$; standardised coefficient = 0.49) and number of fruits ($p < 0.01$; standardised coefficient = 0.41). No direct effects were observed between treatment, herbivory and number of flowers or between duration of visits with the number of fruits and fruit mass (Figure 1c; Table 2). No indirect effects were found in this PSEM (Figure 1c; Table 2).

Lastly, the fourth PSEM (only caterpillars vs. no resources) revealed a positive direct link between the number of flowers and pollination (duration of visits: $p < 0.001$; standardised coefficient = 0.55 − number of visited flowers by pollinators: $p < 0.001$; standardised coefficient = 0.57; Figure 1d; Table 2). No direct effects were observed between ant resource treatment, herbivory, the number of flowers, the duration of pollinator visits and the number of visited flowers by pollinators. The number of fruits and fruit mass were not directly affected by the duration of pollinator visits or the number of visited flowers by pollinators (Figure 1d; Table 2). No indirect effects were found in this PSEM (Figure 1d; Table 2).

DISCUSSION

In ant-plant-trophobiont interactions, the presence of multiple resources often enhances ant protection and reduces herbivory; however, this increased ant activity can also influence other mutualistic interactions, such as pollination, sometimes with negative consequences. Our study found that ants reduced leaf herbivory on *B. malifolia* plants offering multiple resources. While ants did not directly affect pollinator behaviour, herbivory significantly decreased the duration and number of pollinator visits, likely by deterring them. This deterrent effect occurred only in plants hosting treehoppers, suggesting that treehopper presence creates an unfavourable environment for pollinators. Across treatments, more flowers attracted more pollinators and extended their visit duration. The number of flowers positively impacted both fruit production and mass, especially in plants with EFN or hosting treehoppers. However, the number of pollinator visits and visit duration did not significantly affect fruit quantity or mass. Finally, we found no indirect effects of ant resource treatments on plant fitness, either through herbivory or pollination. This study advances our understanding of plant–insect mutualisms by highlighting how multi-resource interactions with ants and treehoppers influence both herbivory and pollination dynamics, revealing that flower production—not pollinator behaviour—is the primary driver of fruit yield. These findings underscore the complexity of indirect effects in mutualistic networks, enriching insights into plant reproductive strategies.

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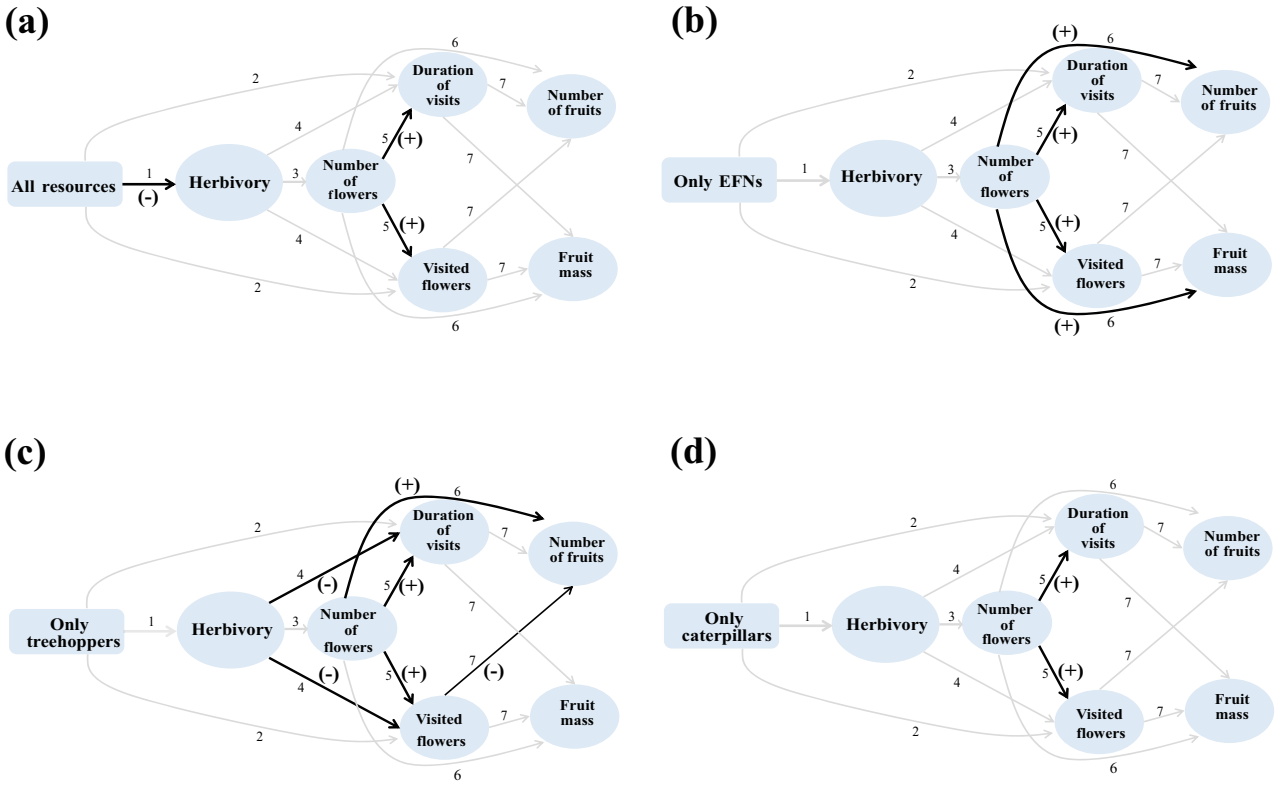


FIGURE 1 Structural equation model showing the direct effects (paths) of presence of resources: (a) all resource, (b) only EFN, (c) only treehoppers or (d) only caterpillars, coded as a dummy variable (resource treatment = 1, no resource = 0) on patterns of plant fitness. Significant paths are in black ($p < 0.05$), while non-significant paths are in grey (both arrows and font). The positive and negative symbols represent the dimension of effects in each variable. In the case of resource treatment, a positive effect would indicate an increase in each response variable in the presence of the resource. The numbers above each arrow describe the route (see Table 2). Explained variance based on marginal R2 in each PSEMs: all resources – herbivory = 22%, number of flowers = 21%, visited flowers = 42%, duration of visits = 33%, number of fruits = 39%, fruit mass = 11%, model's $AIC_c = 2263.569$; only EFN – herbivory = 0%, number of flowers = 4%, visited flowers = 31%, duration of visits = 26%, number of fruits = 57%, fruit mass = 17%, model's $AIC_c = 1587.698$; only treehoppers – herbivory = 3%, number of flowers = 13%, visited flowers = 32%, duration of visits = 30%, number of fruits = 35%, fruit mass = 12%, model's $AIC_c = 1406.070$; only caterpillars – herbivory = 2%, number of flowers = 9%, visited flowers = 32%, duration of visits = 29%, number of fruits = 9%, fruit mass = 11%, Model's $AIC_c = 1150.054$.

Supporting our predictions outlined in path 1 of Table 1, we found that ants significantly reduced leaf herbivory in *B. malifolia* plants that offer multiple resources, highlighting the critical dual defensive role of EFN and trophobionts. When ants have access to diverse food sources, their motivation to patrol and defend these plants from herbivores increases, leading to more effective herbivore suppression (Heil, 2015; Rudgers & Gardener, 2004; Weber & Agrawal, 2014). This finding is consistent with previous studies showing that reliance on EFN alone can result in variable ant defence effectiveness depending on ecological context (Fagundes et al., 2017; Moura et al., 2023; Staab et al., 2023). For instance, a recent study found that predatory ants attracted to both EFN and honeydew from hemipteran in a tropical savanna reduced herbivory more effectively than on plants offering only EFN (Moura et al., 2023). Although trophobionts feed on the plant and can impose substantial costs—sometimes even leading to plant death—they attract ants whose protective role can outweigh these negative effects. These results underscore the importance of resource complementarity in enhancing ant-mediated plant defence and shaping plant–herbivore interactions.

Contrary to our predictions outlined in path 2 of Table 1, our study revealed that ants did not have a direct impact on pollinator behaviour, including the duration of floral visits or the number of flowers visited per bout. This was unexpected, given that trophobiont caterpillars and hemipterans in our system frequently feed on reproductive structures such as buds and flowers—areas where ants also forage, attracted by honeydew secretions. Given this spatial overlap, we anticipated that ants might disrupt pollinators either through direct encounters or by modifying floral traits. Instead, our results indicate that ants effectively reduce herbivory without compromising pollination, consistent with previous research in similar systems (Aguirre et al., 2018; Assunção et al., 2014). For instance, Costa et al. (2023) demonstrated that the yellow crazy ant (*Anoplolepis gracilipes*) and the white-footed ant (*Technomyrmex albipes*) did not deter pollinators from visiting native plants on the island of Mahé, Seychelles.

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TABLE 2 Results from the piecewise structural equation models for direct and bootstrapped indirect effects of plant-based resources for ants (all resources, only EFN, only treehoppers or only caterpillars), coded as a dummy variable (resource treatment = 1, no resource = 0) on herbivory, flower production, duration of pollinator visits, number of flowers visited by pollinators, fruit production and fruit mass.

		All resources		Only EFN		Only treehoppers		Only caterpillar	
Path number		Coef. Sd.	*p*-value	Coef. Sd.	*p*-value	Coef. Sd.	*p*-value	Coef. Sd.	*p*-value
	Direct effects								
1	Herbivory ~								
	Dummy treatment	**−0.474**	**<0.001**	−0.039	0.786	−0.167	0.268	−0.139	0.406
3	Number of flowers ~								
	Herbivory	−0.038	0.788	0.169	0.245	0.197	0.190	0.295	0.073
2–4-5	Visited flowers ~								
	Dummy treatment	0.047	0.741	0.036	0.773	0.094	0.493	−0.066	0.651
	Herbivory	−0.060	0.642	−0.124	0.331	**−0.319**	**<0.05**	−0.193	0.204
	Number of flowers	**0.618**	**<0.001**	**0.556**	**<0.001**	**0.490**	**<0.001**	**0.578**	**<0.001**
2–4-5	Duration of visits ~								
	Dummy treatment	−0.068	0.651	0.062	0.632	−0.009	0.948	−0.060	0.682
	Herbivory	−0.107	0.436	−0.104	0.426	**−0.302**	**<0.05**	−0.193	0.212
	Number of flowers	**0.595**	**<0.001**	**0.509**	**<0.001**	**0.528**	**<0.001**	**0.558**	**<0.001**
6–7	Number of fruits ~								
	Number of flowers	0.299	0.088	**0.473**	**<0.01**	**0.419**	**<0.01**	0.257	0.193
	Duration of visits	−0.159	0.727	0.284	0.667	−0.865	0.064	−0.629	0.479
	Visited flowers	0.323	0.506	−0.046	0.945	**0.974**	**<0.05**	0.633	0.483
6–7	Fruit mass ~								
	Number of flowers	−0.316	0.093	**0.411**	**<0.05**	0.170	0.311	0.279	0.159
	Duration of visits	0.068	0.890	0.061	0.938	−0.227	0.659	0.202	0.820
	Visited flowers	0.211	0.685	−0.172	0.831	0.423	0.410	−0.176	0.845
	Indirect effects								
	Number of flowers ~ Dummy treatment (via herbivory)	0.018	>0.05	−0.007	>0.05	−0.033	>0.05	−0.041	>0.05
	Duration of visits ~ Dummy treatment (via herbivory + number of flowers)	0.053	>0.05	0.001	>0.05	0.032	>0.05	0.004	>0.05
	Visited flowers ~ Dummy treatment (via herbivory + number of flowers)	0.034	>0.05	0.001	>0.05	0.036	>0.05	0.003	>0.05
	Number of fruits ~ Dummy treatment (via herbivory + number of flowers + Duration of visits + Visited flowers)	0.010	>0.05	0.000	>0.05	0.017	>0.05	−0.010	>0.05
	Fruit mass ~ Dummy treatment (via herbivory + number of flowers + Duration of visits + Visited flowers)	0.000	>0.05	−0.003	>0.05	0.009	>0.05	−0.010	>0.05

Note: Analyses were conducted at the individual plant level. Path numbers are described in Table 1. Significant *p*-values are highlighted in bold.

Interestingly, we found no relationship between pollinator visitation and fruit number or mass, suggesting that our observations may not have been sufficiently detailed or frequent to detect subtle treatment effects on pollinator activity. Collectively, these findings underscore the importance of examining specific ecological and evolutionary contexts—such as ant species identity and aggressiveness, pollinator sensitivity, spatial and temporal overlap between ants and flowers and the nature and accessibility of floral rewards—that mediate whether ant activity disrupts or coexists with pollination.

Contrary to our predictions outlined in path 3 of Table 1, our findings revealed that leaf damage did not negatively impact flower production in *B. malifolia*. While previous studies have shown that herbivory can impair flower development by reducing resource availability or damaging meristematic tissues (Boege, 2005; Hanley & May, 2006), other research has demonstrated that many plants exhibit compensatory responses that buffer against these effects (Irwin et al., 2008; Schiestl et al., 2014; Strauss et al., 2001). Such mechanisms may include increased photosynthetic efficiency in remaining

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leaves or reallocation of resources to reproductive structures, allowing floral output to remain stable despite foliar damage (Garcia & Eubanks, 2019). In our study, herbivory levels were relatively low to moderate (<20% leaf area removed), likely below the threshold where compensation breaks down. At higher herbivory levels, nonlinear or threshold effects may become more important. It is important to note, however, that our study did not assess florivory. Unlike leaf herbivory, damage to reproductive structures such as buds and flowers may not be as easily compensated for, potentially exerting stronger effects on reproductive success (McCall & Irwin, 2006). In *B. malifolia*, florivory is common and primarily caused by Curculionidae beetles and Thysanoptera, which consume floral buds and open flowers. Their activity can significantly reduce floral display and potential fruit set (Alves-Silva, 2013; Ferreira & Torezan-Silingardi, 2013). Therefore, future research should investigate florivory and its consequences for plant fitness, as this could provide deeper insight into the resilience of reproductive strategies under multi-trophic herbivore pressure.

Supporting our predictions outlined in path 4 of Table 1, our study demonstrated that herbivory significantly reduced both the duration of pollinator visits and the number of flowers visited—likely due to ants altering their foraging behaviour and attacking or deterring pollinators. This effect was exclusively observed in *B. malifolia* plants hosting treehoppers, indicating that their presence creates an unfavourable environment for pollinators. Ants tending treehoppers in reproductive structures may exhibit aggressive behaviours that interfere with pollinator foraging activities, ultimately disrupting pollination and potentially reducing plant reproductive success (Ibarra-Isassi & Oliveira, 2018). Additionally, in this interaction, ants often concentrate their foraging on reproductive structures, thereby intensifying their impact on pollinators while reducing their influence on herbivores (Moura et al., 2023). In agreement with our findings, Ibarra-Isassi and Oliveira (2018) reported that the presence of treehoppers on the flowers of *Byrsonima intermedia* disrupted pollination by oil-collecting bees, leading to reduced visitation frequency and shorter visit durations. Moreover, treehoppers can impose substantial costs on plants by severely damaging reproductive structures, which may indirectly reduce pollinator attraction and limit ant foraging opportunities (Del-Claro et al., 2016; Ibarra-Isassi & Oliveira, 2018; Moura et al., 2023). These interactions highlight the complexity of trophic relationships within ecosystems, demonstrating that treehoppers can impact not only plant health but also indirectly affect mutualistic partners like pollinators (Del-Claro et al., 2016). Our study contributes to a deeper understanding of how treehoppers presence alters the dynamics of plant–pollinator interactions, emphasising the importance of considering these effects when studying plant reproductive strategies and fitness.

Supporting our predictions outlined in path 5 of Table 1, our study found that a higher production of flowers attracted more pollinators and increased the duration of their visits across all treatments. These findings align with previous research highlighting the significance of floral display size in influencing pollinator behaviour and activity (Barragán-Fonseca et al., 2020; Ferreira & Torezan-Silingardi, 2013; Murakoshi et al., 2024). In addition, supporting our predictions outlined in path 6 of Table 1, our study revealed that the number of flowers significantly increased both fruit production and mass, although this effect was particularly evident in *B. malifolia* plants with EFN or those hosting treehoppers. The presence of EFN plays a critical role in this relationship by providing a nectar source, enhancing the nutritional status of the plant overall. A higher nutrient status could promote better flower and fruit development, resulting in larger fruits with more seeds (Muñoz et al., 2005). Additionally, the interaction with treehoppers may facilitate benefits beyond pollination, such as improved water or nutrient uptake through enhanced root growth (Evans et al., 2011), further contributing to fruit development and mass. This intricate interplay of mutualistic relationships underscores the complexity of plant reproductive strategies and highlights the importance of flower abundance in optimising fruit yield and quality.

Contrary to our predictions outlined in path 7 of Table 1, our study found that the number of flowers visited by pollinators and the duration of their visits did not significantly affect fruit quantity or mass. This unexpected outcome suggests that successful pollination, as indicated by pollinator activity, did not translate into larger or heavier fruits. One possible explanation for this finding could be that while pollinators were active, the overall fertilisation success may have been influenced by factors beyond visitation metrics, such as the genetic compatibility of pollen or environmental conditions affecting seed development (Hirayama et al., 2005; Pearse et al., 2016; Villamil et al., 2022). Additionally, the fruit development process might be more dependent on resource allocation within the plant rather than solely on pollinator interactions (Pearse et al., 2016). Furthermore, it is possible that plants with a higher number of flowers can sustain more ovules and suffer from pollen limitation, leading to similar fruit outcomes regardless of pollinator activity (Larson & Barrett, 1999, 2000; Sun et al., 2018). These results highlight the complexity of plant–pollinator interactions and underscore the importance of considering additional factors influencing fruit development.

This study advances our understanding of multitrophic mutualisms by revealing how the interplay between ants, trophobionts, herbivores and pollinators shapes plant reproductive outcomes. While ant activity reduced herbivory, it did not disrupt pollination, highlighting the potential for mutualists to coexist without trade-offs. Importantly, we show that flower production—not pollinator visitation—is the strongest predictor of fruit yield, emphasising the need to consider plant traits alongside mutualistic interactions. These findings underscore the complexity of indirect effects in ecological networks and contribute to a more nuanced understanding of how multiple mutualists and antagonists influence plant fitness in natural systems.

AUTHOR CONTRIBUTIONS

Vitor Miguel Costa-Silva: Conceptualization; investigation; methodology; validation; visualization; writing – review and editing; formal analysis; writing – original draft; project administration; software; funding acquisition; data curation; resources. **Gabriela Fraga Porto:** Methodology; visualization; investigation; writing – review and editing. **Carla Vázquez-González:** Formal analysis; visualization; validation; software. **Eduardo Soares Calixto:** Conceptualization; methodology.

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Xoaquín Moreira: Conceptualization; funding acquisition; writing – original draft; writing – review and editing; validation; visualization; supervision; resources. **Kleber Del-Claro:** Conceptualization; funding acquisition; writing – review and editing; visualization; validation; supervision; methodology; resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in figshare at https://doi.org/10.6084/m9.figshare.29246108.v1.

ORCID

Vitor Miguel Costa-Silva https://orcid.org/0000-0002-5406-1891
Gabriela Fraga Porto https://orcid.org/0000-0003-4050-7300
Carla Vázquez-González https://orcid.org/0000-0001-6810-164X
Eduardo Soares Calixto https://orcid.org/0000-0003-3617-2464
Xoaquín Moreira https://orcid.org/0000-0003-0166-838X
Kleber Del-Claro https://orcid.org/0000-0001-8886-9568

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SUPPORTING INFORMATION

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

Table S1. Mean and standard deviation values for all variables (herbivory, flower production, duration of pollinator visits, number of flowers visited by pollinators, fruit production and fruit mass) across ant food resource treatments (no resources, only extrafloral nectar (EFN), only treehoppers, only caterpillars and all resources).

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