



## RESEARCH ARTICLE - ANTS

## Negative Effects of Ant-plant Interaction on Pollination: Costs of a Mutualism

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### Abstract

The mutualism of ants and extrafloral nectary-bearing plants is known to reduce rates of herbivory. However, ants may have negative impacts on other mutualisms such as pollination, constituting an indirect cost of a facultative mutualism. For instance, when foraging on or close to reproductive plant parts ants might attack pollinators or inhibit their visits. We tested the hypothesis that ants on EFN-bearing plants may negatively influence pollinator behavior, ultimately reducing plant fitness (fruit set). The study was done in a reserve at Brazilian savannah using the EFN-bearing plant *Banisteriopsis malifolia* (Malpighiaceae). The experimental manipulation was carried out with four groups: control (free visitation of ants), without ants (ant-free branches), artificial ants (isolated branches with artificial ants on flowers) and plastic circles (isolated branches with plastic circles on flowers). We made observations on flower visitors and their interactions, and measured fruit formation as a proxy for plant fitness. Our results showed that pollinators hesitated to visit flowers with artificial ants, negatively affecting pollination, but did not hesitate to visit flowers with plastic circles, suggesting that they recognize the specific morphology of the ants. Pollinators spent more time per flower on the ant-free branches, and the fruiting rate was lower in the group with artificial ants. Our results confirm an indirect cost in this facultative mutualism, where the balance between these negative and positive effects of ants on EFN-bearing plants are not well known.

### Introduction

In tropical areas, 94% of plants require pollinators for reproduction and only a small number of species can reproduce by spontaneous self-pollination or agamospermy (Ollerton et al., 2011). Pollinators distribute pollen from one plant to another for successful cross-pollination; however, this process can often be affected by the presence of other floral visitors, like ants (Toresan-Silingardi et al., 2021). Many plants are engaged in mutualisms with ants (Kuriakose et al., 2018). The main role these mutualistic ants play on plants is the

protection against herbivore attack; in turn plants provide different types of resources, such as places for nesting and food. One of the most common food resources offered by plants to ants is the extrafloral nectar produced by specialized glands known as extrafloral nectaries (EFNs) (Calixto et al., 2018; Del-Claro et al., 2016; Heil, 2015). Indeed, some studies have shown that ants that hunt herbivores present in EFN-bearing plants decrease leaf herbivory (Rosumek et al., 2009; Trager et al., 2010) and/or increase fruit production (Del-Claro and Marquis, 2015; Nascimento and Del-Claro, 2010).



However, mutualistic ants attracted by plant-based resources may forage on entire plant structures, including inflorescences and flowers. Although extrafloral nectar is a sugar-rich reward, ants also forage on plant reproductive parts looking for floral resources (such as nectar and pollen) and potential prey (such as floral herbivores) (Blüthgen et al., 2003; Blüthgen & Fiedler, 2004; Davidson et al., 2003; Sinu et al., 2017; Unni et al., 2021; Hanna et al., 2015; Fuster et al., 2020). The additional foraging behavior on these reproductive plant parts may negatively affect mutualistic plant-pollinator interactions. Although many studies emphasize that ants are important mutualists for plants when defending against herbivores (Calixto et al., 2018; Del-Claro et al., 2016; Rosumek et al., 2009; Trager et al., 2010), there are also cases where they have been shown to negatively impact plant pollination (Assunção et al., 2014; Melati & Leal, 2018; Ness, 2006; Villamil et al., 2018). For example, Byk and Del-Claro (2010) showed that the presence of *Cephalotes pusillus* (Formicidae: Myrmicinae) negatively affects pollination of *Ouratea spectabilis* (Ochnaceae) by feeding on pollen and reducing viability of the pollen grains. Assunção et al. (2014) demonstrated through experimental manipulation in *Heteropterys pteropetala* (Malpighiaceae) that ants, which feed on EFNs but also visit leaves and inflorescences, may have a negative impact on the plant fruit production by attacking or chasing pollinators.

Some studies have shown that shape and odor are important ant traits that can be detected by pollinators, who will avoid visiting these flowers (Assunção et al., 2014; Ballantyne & Willmer, 2012; Adam R. Cembrowski et al., 2014). For example, using dummy ants on flowers, Assunção et al. (2014) showed that pollinators easily recognize ant shape and color, which significantly reduced floral visitation rate and fruit production. When ants are present, floral visitors may approach a flower, but can also change their behavior by reducing the time spent on flowers (Aguirre-Jaimes et al., 2018; Assunção et al., 2014; Ness, 2006; Sousa-Lopes et al., 2020; Villamil et al., 2018; Sinu et al., 2017; Unni et al., 2021; Hanna et al., 2015; Fuster et al., 2020), or they will not come into contact with the reproductive parts, becoming ineffective in pollination. In the Brazilian tropical savannah, smaller bees tend to spend more time analyzing flowers with tending ants before approaching, since they are more affected by ant presence on flowers than larger bees (Assunção et al., 2014; Barônio and Del-Claro, 2017). Thus, considering that pollinators can recognize ant shape and/or odor (Ballantyne and Willmer 2012; Cembrowski et al. 2014), the ant presence on flowers can negatively affect the pollination, ultimately decreasing fruit production.

*Banisteriopsis malifolia* (Malpighiaceae) is an abundant EFN-bearing plant species in the Brazilian savannah (henceforth “Cerrado”), which is commonly visited by several ant species (Torezan-Silingardi, 2007). In addition, large bees represent the main pollinators of this species (Torezan-Silingardi, 2007).

It is therefore an advantageous system to investigate indirect effects of ant-plant mutualisms on plant reproduction. The aim of this study was to investigate the impact of ants on floral visitor behavior and fruit production of an EFN-bearing plant, *B. malifolia*. We tested the hypothesis that in ant-plant mutualisms mediated by EFNs, the ant presence on flowers will negatively interfere with floral visitor behavior and fruit production. Thus, the two following hypotheses were evaluated: *Hypothesis 1* – the presence of ants on flowers decreases the total number of visits and the time spent on the flower by pollinators; *Hypothesis 2* – the presence of ants on flowers decreases the fruit set by influencing the pollinator behavior.

## Material and Methods

### Study area

The fieldwork was carried out from March to May 2018 in the Brazilian Cerrado, within the ecological reserve of the Clube de Caça e Pesca Itororó de Uberlândia in the city of Uberlândia, state of Minas Gerais, Brazil (18°58'30"S, 48°17'27"W). This region has two well-defined seasons (Aw-type Köppen climate): rainy, with a hot and humid climate from October to March, monthly rainfall of  $270 \pm 50$  mm (average  $\pm$  SD) and monthly temperature of  $23 \pm 5^\circ\text{C}$  (average  $\pm$  SD); and dry, with a cold and dry climate from April to September, precipitation of  $22 \pm 20$  mm (average  $\pm$  SD) and temperature of  $19 \pm 3^\circ\text{C}$  (average  $\pm$  SD) (Alvares et al., 2013; Calixto et al., 2021b; Vilela et al., 2017). The reserve's Cerrado vegetation ranges from open areas with grasses and small shrubs, to more closed-canopy areas, with trees reaching up to 12 meters in height (Del-Claro et al., 2019). Pastures and properties with different levels of natural area conservation surround the ecological reserve.

### Plant species

*Banisteriopsis malifolia* (Nees & Mart.) B. Gates (Malpighiaceae) is a common Cerrado shrub, which blooms from March to June (Calixto et al., 2021b; Vilela et al., 2014). This species has EFNs (located on the underside of the leaves, at the junction with the petiole) that attract ants which feed on their secretion (Barônio & Del-Claro, 2017). It has paniculate inflorescences with flowers that offer oil and pollen to floral visitors, which have five long-pedunculate petals and its reproduction depends on pollinators, with cross-pollination (Vogel, 1990). The flower can produce one to three schizocarpic fruits of the samara type (Barroso et al., 2004) which are wind dispersed. Leaves show alternating phyllotaxis. Voucher specimens were deposited in the Herbarium Uberlandensis under number HUFU 00013413.

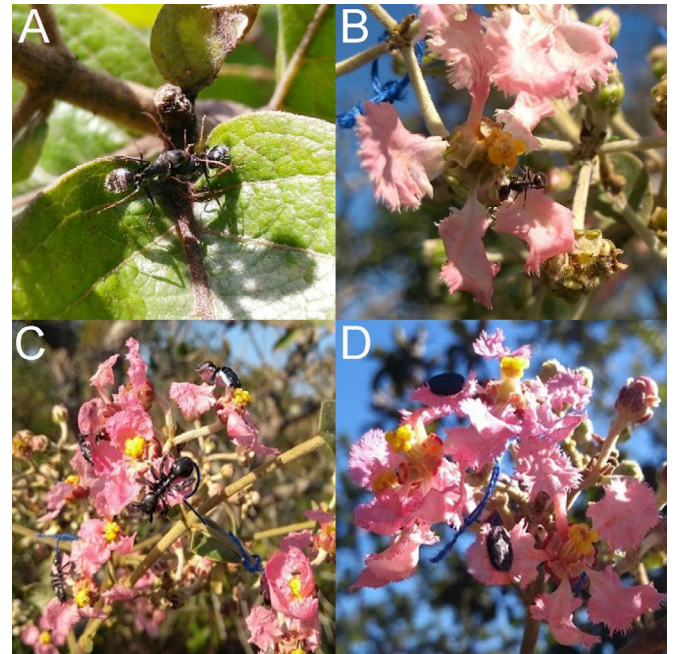
### Experimental set-up

To test our hypothesis, 48 individuals of *B. malifolia* with similar phenological characteristics (height, number of branches, number of inflorescences, number of flower buds

per inflorescence, active EFNs and reproductive stage) and 10 meters apart were marked along a 3 km transect. We did not use a block design, since the constant presence of plastic ants (see below) on the inflorescence could dissuade potential visitors of other treatments. Individuals were randomly and equally divided into four treatments: control, artificial ants (hereafter “AA”), plastic circles (hereafter “PC”), and without ants (hereafter “WA”). In all treatments, in each individual plant, five flower buds were marked to ensure that the anthesis of the flower only occurred after the start of the experiment. Flowers remain open about 2-3 days after the anthesis. Pre-anthesis floral buds were bagged with Voile bags and pollinator visitation was only allowed at the time of field observations, when the floral anthesis had already occurred, from 08:00 am to 12:00 pm (since bees’ activity is concentrated at this time (Assunção et al., 2014), from which the flowers were bagged again. Each morning one plant from each treatment was observed for four hours (08:00 to 12:00), totaling 192 hours of observation (4 hours per plant x 48 plants). In total, for each treatment we analyzed 60 flowers, resulting in a total of 240 flowers equally distributed across 48 plants. It was observed that the abundance of pollinators in the afternoon is much lower than in the morning. Therefore, the collections were all concentrated in the morning period (an average of 1.3 visits in the afternoon and an average of 6.6 visits in the morning).

In the control group, a Tanglefoot resin (Tree Tanglefoot® pest barrier – Rapids, Michigan, USA) was applied 10 cm from the soil to half of the circumference of the stem base to control for a possible effect of the resin on the other treatments. In this way, access of ants and other arthropods was at least partly allowed. The treatment WA underwent the same procedure as the control group, however the application of Tanglefoot was made around the entire circumference of the stem, to prevent any ants from accessing the plant. Any ants present prior to the application of tanglefoot were removed manually from these plants and the surrounding vegetation to avoid contact bridges. The treatment AA received the same experimental manipulation as WA, but here artificial ants made of black plastic (based on the morphology of the most common ants that forage on this plant, ants of the genus *Camponotus*, as shown in Alves-Silva, 2011; Fagundes et al., 2017) (Fig 1A and B) were glued onto the petals of all flowers open on a particular day of observation using white paper glue (Fig 1C). All plastic ants and circles were glued in the same position on the flowers. To control for a possible effect of glue on the other treatments, a little drop was also applied to petals in the other treatments. Five plastic ants were used on each plant to simulate an average ant colonization rate on each plant. Artificial ants were removed at the end of each observation period. The last treatment, PC, underwent the same procedures as the AA group, but instead of artificial ants, black plastic circles made of the same material as the artificial ants were placed on the flower petals (Fig 1D), and removed at the end of each observation.

Furthermore, 5 pieces of plastic were also used in each plant. The number of visits was quantified by counting the number of times that floral visitors, mostly bees, were observed collecting resources such as oil and pollen. The time spent visiting flowers was determined using a timer. The hesitation was scored by counting the number of floral visitors that approached a flower but did not actually visit it (it approaches a flower hovering over it and moves away without making the floral visit) (Villamil et al., 2018). After all observation periods had finished, the flowers were bagged again, and after ca. 20 to 30 days, the fruits formed in each group were counted.



**Fig 1.** The black ant (*Camponotus crassus*) frequently visitant of *Banisteriopsis malifolia* foraging on the plant A); Flower with a real ant on the petals of the flower B); Flowers with examples of the treatments with artificial ants and plastic circles C) and D) respectively.

### Statistical analysis

All statistical analyses were performed in RStudio 4.0.0 (R Core Team, 2020) at 5% of significance level. Model suitability, homoscedasticity and overdispersion (when applicable), were checked in all models. When overdispersion was detected for counting response variable, it was used the negative binomial distribution through package “MASS” (Venables & Ripley, 2002). GLM analyses were conducted with the package “stats” and “glmmTMB” (Brooks et al., 2017) followed by Likelihood-Ratio test with the package “car” (Fox & Weisberg, 2011). Pairwise comparisons were conducted using Estimated Marginal Means with the package “emmeans” (Lenth, 2020).

To verify if there was no disturbance on floral display (number of open flowers per inflorescence) caused by opened flowers from different treatments, the total number of

approaches (the sum of the number of visits and hesitations) per plant was compared between treatments. Flower plant display did not influence the results since there was no significant difference in the number of approaches between treatments (GLM:  $\chi^2 = 2.699$ ,  $p = 0.440$ ; Fig S1).

*Hypothesis 1* – the presence of ants on flowers negatively influences pollinator behavior

To evaluate if pollinators present a different number of visits, and hesitations as well, among treatments, we used a GLM with negative binomial error distribution controlling for overdispersion. Treatments were fit as fixed factor and the total number of visits or hesitations per plant as count response variable. To evaluate the proportion of visited flowers per total flowers selected per plant, we used a GLM with Beta distribution and “logit” link function. Treatments were considered as fixed factor and proportion of visits as the response variable. The results of this test (proportion of visited flowers per total flowers selected per plant) show the real impact that ants have on pollinators after a first visit. After landing on a flower with ant, the pollinator may not visit another flower in the same inflorescence due to ant presence in this first visited flower. To evaluate if pollinators differ in the time spent per flower per treatment, it was used a GLM. Treatment was considered as fixed factor and the average time spent visiting flowers per plant as the response variable.

*Hypothesis 2* – the presence of ants on flowers decreases plant reproductive success

To verify if fruit production differs between treatments, first the number of fruits produced was divided by the number of selected flowers (fruit proportion). Then, it was used a GLM with Beta distribution and “logit” link function. Treatments was used as fixed factors and the fruit proportion per plant as the response variable. Since we found a significant difference between the number of visits and the number of fruits produced among groups (see *Results*), we then fit a GLM with Poisson distribution, evaluating the influence of the number of visits (predictor variable) in the number of fruits produced (response variable).

## Results

*Hypothesis 1* – the presence of ants on flowers negatively influences pollinator behavior

There was a significant difference in the number of visits between treatments (GLM:  $\chi^2 = 29.553$ ,  $p < 0.001$ , Fig 2a). The AA group presented the lowest number of visits when compared to the other groups. The PC and WA groups had the highest number of visits, but did not differ statistically amongst themselves. The control group placed in the middle (Table 1), presenting more visits than the AA group and fewer visits than the WA and PC groups, but with no statistical difference between WA and PC (Fig 2a).

**Table 1.** Mean and Standard Error of each variables per treatment.

	Mean $\pm$ SE				
	Number of visits	Number of hesitations	Proportion of visits	Time (sec)	Proportion of fruits
Control	7.33 $\pm$ 1.31	1.25 $\pm$ 0.44	0.74 $\pm$ 0.10	4.29 $\pm$ 0.83	0.70 $\pm$ 0.05
Artificial Ants	1.08 $\pm$ 0.84	6.41 $\pm$ 1.17	0.12 $\pm$ 0.08	2.68 $\pm$ 0.52	0.32 $\pm$ 0.04
Plastic Circles	11.33 $\pm$ 3.38	0.00 $\pm$ 0.00	0.99 $\pm$ 0	5.55 $\pm$ 0.76	0.64 $\pm$ 0.09
Without Ants	9.91 $\pm$ 1.85	0.00 $\pm$ 0.00	0.99 $\pm$ 0	6.66 $\pm$ 0.94	0.63 $\pm$ 0.08

The number of hesitations also showed a significant difference between treatments (GLM:  $\chi^2 = 109.28$ ,  $p < 0.001$ , Fig 2b). The AA group had the highest number of hesitations, differing statistically from the other three treatments. On the other hand, the control, PC and WA groups did not present significant difference between them (Fig 2b).

The proportion of visits showed a significant difference between treatments (GLM:  $\chi^2 = 38.522$ ,  $p < 0.001$ , Fig 2c). AA group was the least visited, statistically differing from the other three groups (Fig 2c). The control, PC and WA did not statistically differ among themselves.

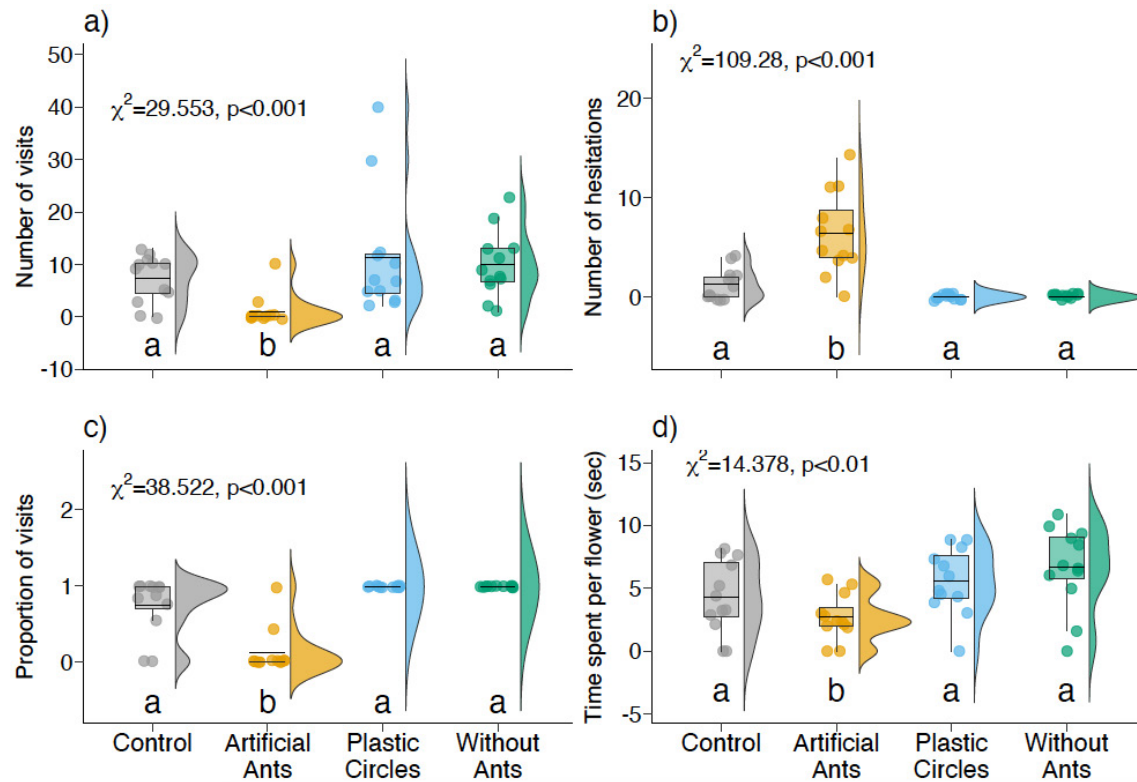
The time spent visiting flowers statistically differed between treatments (GLM:  $\chi^2 = 14.378$ ,  $p < 0.01$ , Fig 2d). The AA group differed from control, PC and WA, showing that pollinator spent less time when ants are constantly

present than in control, PC and WA treatments (Fig 2d). However, control, PC and WA groups did not differ from each other (Fig 2d).

*Hypothesis 2* – the presence of ants on flowers decreases plant reproductive success

The proportion of fruits produced significantly differed between treatments (GLM:  $\chi^2 = 13.329$ ,  $p < 0.01$ , Fig 3). The AA group showed the lowest production of fruits, which significantly differed from the other three groups. Control, WA, and PC groups showed a similar proportion of fruits produced. (Fig 3). We found a positive influence of the number of visits in the number of fruits produced (GLM:  $\chi^2 = 11.753$ ,  $p < 0.001$ , Fig 4).





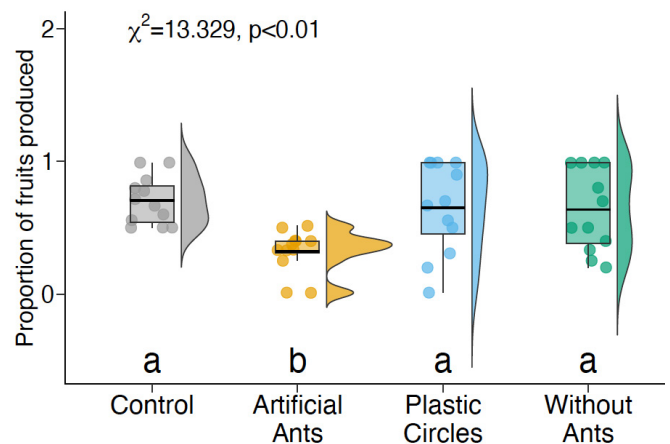
**Fig 2.** Number of visits (a), Number of hesitations (b), Proportion of visits (c), and Time spent per flower (d). The boxes represent mean (horizontal bars), maximum and minimum, raw data (points), and “violin plot” based on Kernel density function. Letters represent statistic difference among treatments by Estimated Marginal Means.

## Discussion

This study showed that artificial ants present on flowers have negative impacts on flower visitors' behavior of *B. malifolia*, resulting in reduced fruit production; however, with no significant difference for the control group (natural conditions). In particular, the results demonstrated that artificial ants are responsible for a significant reduction in number and proportion of visits, time spent per flower by floral visitors, and a reduction in the number of fruits produced. Although there was no significant difference in the variables analyzed between the control group and the isolation group, the considerable negative impact of artificial ants demonstrates that the ants' visual cue alone is enough to drive away possible pollinators and negatively affect the fruiting of plants.

The negative effects caused by artificial ants in this study is related to ants' display, which is interpreted as threat to some visually oriented animals (Aguirre-Jaimes et al., 2018; Dáttilo et al., 2016), such as bees. The fact that ants tend to attack all plant visitors and do not distinguish between herbivores and pollinators (Tsuji et al., 2004; Willmer et al., 2009) means that the presence of ants is an important factor for floral visitors (Assunção et al., 2014; Barônio & Del-Claro, 2017; Galen, 1999; González et al., 2013; Ibarra-Isassi & Oliveira, 2017; Junker et al., 2007; Tsuji et al., 2004; Wagner, 2000; Willmer et al., 2009). The first effect caused

by ants on floral visitors is the reduction in their floral visit frequency and an increase in their number of hesitations (Aguirre-Jaimes et al., 2018; Assunção et al., 2014; Junker et al., 2007; Sousa-Lopes et al., 2020). A study developed by Assunção et al. (2014) in the same study area showed negative effects of artificial ants on visitors' behavior in *Heteropterys pteropetala*, decreasing frequency of visits and fructification.



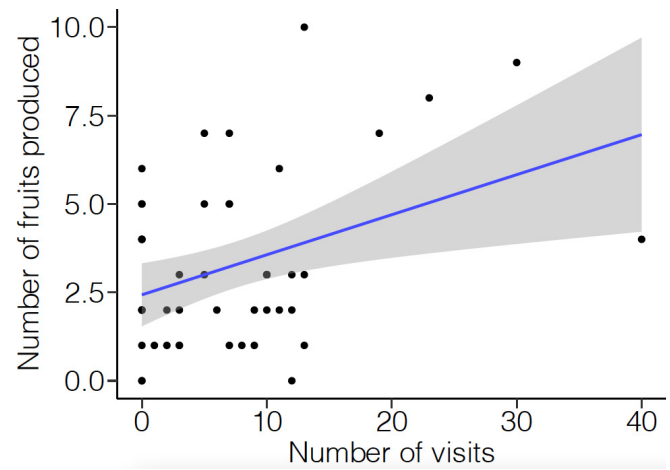
**Fig 3.** Proportion of fruits production among treatments. The boxes represent mean (horizontal bars), maximum and minimum, raw data (points), and “violin plot” based on Kernel density function. Letters represent statistic difference among treatments by Estimated Marginal Means.

Similar results were also reported by Barônio and Del-Claro (2017), who showed negative effects of natural ants on bee behavior in the same plant studied, *B. malifolia*, and in *Banisteriopsis campestris*. However, in Barônio and Del-Claro' (2017) study, there was no guarantee of the presence of the image of an ant at the time that bees were visiting flowers. The authors used only two treatments manipulating the presence and absence of natural ants (control and isolation). In our study, as plastic ants were fixed, there was always an image of an ant at the time of visitation, even if it did not move. This shows something unprecedented in relation to the study by Barônio and Del-Claro (2017), testing how the image of ants affects pollination. In addition, Barônio and Del-Claro (2017) did not bag flowers before and after field observations, as our study did, guaranteeing the result of pollination after the visitation. Finally, our study quantified the time of visitation in all groups used in the study, which was done only indirectly in the study by Barônio and Del-Claro (2017). With all these improvements in the methodology of our study, we have significantly increased our understanding of how ant image cues can be important for pollinators behavior and plant pollination.

On the other hand, ants can have no or positive effects on pollination of plants. For instance, Almeida and Figueiredo (2003) showed that in certain plant species, ants do not interfere with the activity of pollinators, and may even have a positive impact on pollination rates. In another study, Santos and Leal (2019) also found no negative impact of visiting ants of *Turnera subulataem* on pollination rates or on plant reproductive success, suggesting that the ecological costs of the presence of ants may depend on the characteristics of the pollination system of the plant, being higher in plants that depend on pollinators more sensitive to ants (that demonstrate more hesitation behavior). Finally, Holland et al. (2011) showed that the presence of ants was beneficial to the pollination rates of *Pachycereus schottii*, a species of Sonoran Desert cactus, when the abundance of ants in plants was higher. In this context, the results of these studies together with ours show how context-dependent the outcomes of this ant-plant-pollinator system can be.

Besides the number of visits and hesitations, the proportion of visits (flowers visited/total experimental flowers) can be an important variable for plant fitness (Fig 4). Studying the ant effects on flowers, Altshuler (1999) showed that pollinators that were dissuaded by ants were discouraged to make successive visits to the next flowers on the same plant individual. As a result, even if floral visitors initially visit a flower on a plant, they may tend to concentrate their subsequent visits on other plant individuals without ants. In this way, the remaining flowers on the plant or inflorescences are unlikely to be visited, which can lead to a reduction to fruit set in individuals with ants on flowers (Romero & Koricheva, 2011). In our study, the AA group had the lowest proportion of visits, statistically different from the other three groups.

This result reinforces the impact that visual clues from plastic ants can have on plant pollination rates, causing pollinators look for plants without these visual clues.



**Fig 4.** Positive influence of the number of visits made by pollinators on the number of fruits produced in *Banisteriopsis malifolia*. GLM:  $\chi^2 = 11.753$ ,  $p < 0.001$ .

Visitors spent in average less time per flower in AA than in the other three treatments. Time is another important variable related to plant fitness, leading to a decrease in pollen removal and fruit set (Calixto et al. under review) or an increase in cross-pollination, which can lead to an increase of fruit set (Villamil et al., 2020). For instance, Calixto et al. (under review) showed that more aggressive ants can significantly decrease the time spent by pollinators when visiting flowers, resulting in a significant decrease of fruits produced in *Qualea multiflora* (Vochysiaceae). On the other hand, Aguirre-Jaimes et al. (2018) showed that the effective pollinator of *Vigna luteola* (Fabaceae) spent less time visiting flowers in order to avoid ant predation, which might result in an increase of fruit set; and Villamil et al. (2020) observed in *Turnera velutina* (Passifloraceae) that ants decreased pollinator foraging time and flower visit duration, but increased outcrossing rates. Given that, it seems that the time spent by pollinators can have different effects depending on the system that they are involved, resulting in negative, neutral, or positive effects to plants (González et al., 2013; Sousa-Lopes et al., 2020).

Artificial ants can negatively interfere with the proportion of fruits. This is likely a direct consequence derived from the other three effects (reduction of number of visits, proportion of visits, and time of visit) caused by the presence of artificial ants on flowers. Similarly, as found by other studies, foraging ants on flowers are responsible to reduce fruit and seed set (Assunção et al., 2014; Barônio & Del-Claro, 2017; Ibarra-Isassi & Oliveira, 2017). For instance, Ness (2006) showed that ants interfered with pollinator behavior and not only reduced the number of fruits, but also the fruit and seeds weight.

The ant species used as model for designing artificial ants (*Camponotus crassus*) is often found foraging on *B. malifolia*, as well as on other EFN-bearing plants (Lange et al., 2019, 2017), protect them from herbivore attack (Calixto et al., 2021a), resulting in a positive effect for plants. However, due to their presence and aggressive behavior towards pollinators, these ants can cause negative effects on pollinator behavior, leading to some cost of the mutualism. Nonetheless, it is not clear to what extent or how the context can change the outcomes of these interactions. For instance, González et al. (2013) showed that weaver ants deter less effective pollinators of *Melastoma malabathricum* flowers, attracting more *Xylocopa* bees, the more effective pollinators, ultimately resulting in a higher number of fruits produced. This result from González et al. (2013) can be one of the explanations for the higher number of fruits produced in Control compared to AA treatment. Larger bees like *Bombus* and *Xylocopa* represent the main pollinators of *B. malifolia* and usually are not affected by *C. crassus* presence (Barônio & Del-Claro, 2017; Cembrowski et al., 2014; Junker et al., 2007; Calixto et al. under review). It was also observed the presence of *Trigona* bees, smaller than *Bombus* and *Xylocopa*, which are considered the most effective pollinators of *B. malifolia*. The difference in behavior between bees of different sizes in relation to artificial ants was not analyzed in this study. However, Barônio and Del-Claro (2017) verified the difference in the behavior of bees of different sizes in relation to natural ants. Given that, we suggest that *C. crassus* might be deterring other smaller bees that could not pollinate the plant, as shown in our Fig 2, but “allowing” visits by larger bees. However, it is also important to take into account that the fact that bees visit fewer flowers in the same individual and then move to another can favor the rate of cross-pollination.

## Conclusion

The presence of artificial ants on a flower could negatively affect plant pollination rates, reducing the frequency of visits, increasing the frequency of hesitations, reducing the time spent by the pollinators in the flower, and decreasing fruiting rates, affecting the competitive ability of plants, an essential factor in an environment that is rapidly being degraded, such as the Cerrado. This demonstrates the importance that visual cues from a predatory ant on the plant can have on the behavior of pollinating bees, and ultimately on plant fitness. Studies such as this, which investigate protective mutualisms and their costs, are needed to increase our understanding of multi-tritrophic interactions and their role in ecosystem biodiversity (Bronstein, 2021).

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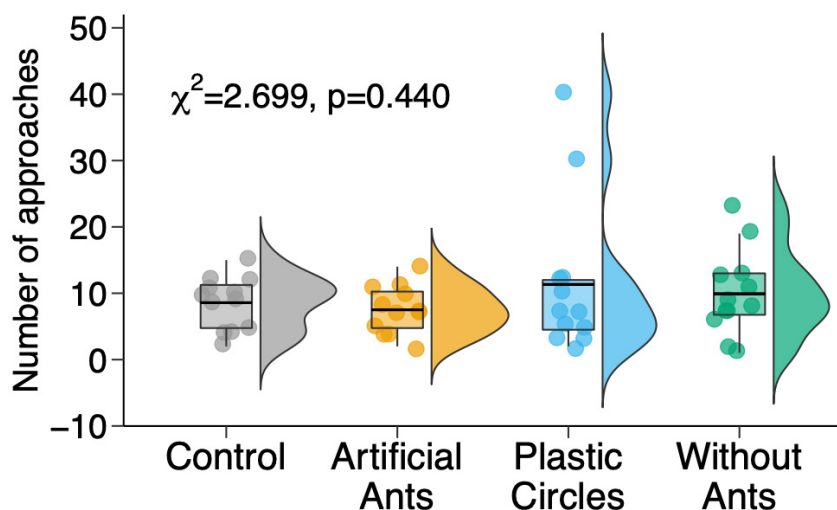
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**Figure Supplementary S1:** Number of approaches among treatments (GLMM:  $\chi^2 = 1.300$ ,  $p = 0.728$ ). The boxes represent mean (horizontal bars), maximum and minimum, raw data (points), and “violin plot” based on Kernel density function. Letters represent statistic difference among treatments by Estimated Marginal Means.