



Temporal variation in the effect of ants on the fitness of myrmecophilic plants: seasonal effect surpasses periodic benefits

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Received: 19 May 2022 / Revised: 19 May 2022 / Accepted: 20 June 2022 / Published online: 28 June 2022
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Abstract

Plants provide extrafloral nectar, which is a food resource taken by ants, especially aggressive species that may act as plant guards. To our knowledge, no study has been conducted to concurrently investigate the fluctuation of plant fitness over its whole reproductive season, recording and comparing both short periods (different samplings during the plant's reproductive season) and the season/pooled data (all fruits produced during the reproductive season). Here, by assigning plants to either ant-present or absent treatments, we investigated the influence of the protective foliage-dwelling ant, *Camponotus crassus*, on the flower bud and fruit production of four extrafloral nectaried plants (*Ancistrotropis firmula*, *Bionia coriacea*, *Cochlospermum regium*, and *Peixotoa tomentosa*) throughout their annual reproductive season. Periodic samples in the field revealed a large variation in plant reproduction throughout the season; the increases in buds and fruits were not constantly higher in plants with ants, and in fact, plants without ants had more reproductive structures sometimes. Nonetheless, the examination of the pooled data, i.e., cumulative number of flower buds and fruits produced during the reproductive season, revealed the plants with ants produced more flower buds and fruits (e.g., up to two-fold greater in *A. firmula*) compared to ant-absent treatments. Our results indicate the effects of ants on plant reproduction are not constant over time, but the net benefits to plants with ants are reflected in increased fruit production. Therefore, the investigations of the benefit of ants on plants should consider the whole plant's reproductive season rather than single samplings within plant reproduction period.

Keywords Biotic defense · *Camponotus* · Cerrado · Extrafloral nectar · Fabaceae · Myrmecophily

Introduction

Thousands of plant species have evolved a mutualistic interaction with ants mediated by the presence of extrafloral nectaries (EFNs) (Weber et al. 2015). Despite being a facultative mutualism, the interaction between ants and plants must provide net benefits to both parties to maintain its stability (Bronstein 1998; Del-Claro et al. 2016). In this relationship,

ants gain a carbohydrate-rich food resource responsible for colony growth, while in turn, plants experience less herbivore damage and increased reproductive output (Trager et al. 2010; Pereira et al. 2020).

In ant-plant studies, authors tend to examine plant reproduction only during specific periods and overlook the full length of the plant's reproductive season. In fact, most of what we know about the effects of ants on plant reproduction comes from data either collected on a single occasion or from results pooled (summing the values of reproductive structures produced across the entire reproductive season) (Del-Claro et al. 1996; Sobrinho et al. 2002; Leal et al. 2006; Aranda-Rickert et al. 2017; Sanz-Veiga et al. 2017; Melati and Leal 2018; Jiang et al. 2019). The reproductive season of many plants in the neotropics lasts several weeks or even months, and the production of flowers, buds, and fruits (and the activity of EFNs) is constant and not restricted to a single occasion (Vilela et al. 2014). Thus, conclusions about the effects of ants on plant reproduction over short periods may be either overestimated or underestimated; in contrast,

Communicated by: Sean O'Donnell

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examining the pooled fitness of plants may hide fluctuations in the production of reproductive structures throughout the reproductive season.

To the best of our knowledge, no study has been conducted to concurrently investigate the fluctuation of plant fitness over its whole reproductive season, recording both short periods (different samplings during the plant's reproductive season) and the season/pooled data (all fruits produced during the reproductive season). Thus, there has never been a discussion of which approach is the most appropriate to investigate the effect of ants on plant reproduction. For instance, when it comes to temporal effects of ants on leaf herbivory, the results vary a lot; in some scenarios, plants with ants regularly experience low levels of herbivory (Nascimento and Del-Claro 2010), while in others, the herbivory is either high or low depending on the time of sampling (Kelly 1986; Fuente and Marquis 1999). For instance, Nogueira et al. (2012) showed that ants had either a constant positive or constant negative effect on the reproduction of two Bignoniaceae species over two sampling periods (but the pooled effect was not examined). Kelly (1986) also demonstrated that herbivory varied according to time and the presence of ants. Ant-plant interactions are an established field of study so it might be the time to provide advances on the knowledge of these relationships by examining in detail whether the effect of ants on plant's reproduction is constant over time (i.e., the fitness will always be higher in plants with ants?) or if there are important variations that will reflect in the net production of structures.

In this study, we investigated the influence of ants on the reproductive output of four EFN-bearing plant species in a Brazilian tropical savanna. Both the EFN and reproductive season take place at the same period in all plants (Bächtold et al. 2017; Anjos et al. 2017). This is common in several tropical botanical families and has been considered an example of the optimal defense theory (EFNs are expected to be functional in periods when the plant is most susceptible to herbivores; Calixto et al. 2021a, b). Thus, it is expected that ants remain on plants as long as the reproductive period (flowers until fructification) takes (Alves-Silva and Del-Claro 2016).

Here we addressed two main questions: (i) Is the relationship between ant presence and plant reproductive success constant throughout the plants' reproductive season, or does it vary with time? (ii) What are the net benefits for plants in this association with ants? We hypothesized that, just like the herbivory, the effect of ants on plant reproduction varies in time, depending on the sampling occasion (Kelly 1986); however, the pooled data (sum of all plant structures produced per plants all over their reproductive season) could show that plants with ants will have greater fitness than plants where ants are excluded (Del-Claro et al. 1996). The study was conducted with four extrafloral nectaried plants in

a Brazilian savanna, namely, *Ancistrotropis firmula* (Mart. Ex. Benth.) A. Delgado (Fabaceae), *Bionia coriacea* (Nees & Mart.) Benth. (Fabaceae), *Cochlospermum regium* (Mart. ex Schrank) Pilg. (Bixaceae), and *Peixotoa tomentosa* A. Juss. (Malpighiaceae). Our working hypothesis is in line with discussions about the stability of ant-plant mutualisms, in that plants may benefit from ant presence despite variations in these benefits (Heil and McKey 2003; Bronstein et al. 2006; Thompson 2013). Overall, this study builds toward a better understanding of how the ant-plant mutualistic relationship can vary over time.

Material and methods

Study area

The study was carried out from late April to early October 2018 in a sensu stricto Cerrado vegetation (14° 42' 56" S–52° 21' 36" W, 300 a.s.l.) (Brazilian tropical savanna, Cerrado biome) at the Bacaba city park (city of Nova Xavantina), located at the eastern edge of the state of Mato Grosso, Brazil. The park as a whole (~450 ha) lies in a Cerrado-Amazonia continuum; the climate is characterized by two well-defined periods, a hot-rainy (October to late April) and a dry season (May to September); the annual precipitation is up to 1500 mm, and the mean monthly temperature is 25 °C, with daily peaks above 40 °C. The study plots covered 24 ha.

Study species

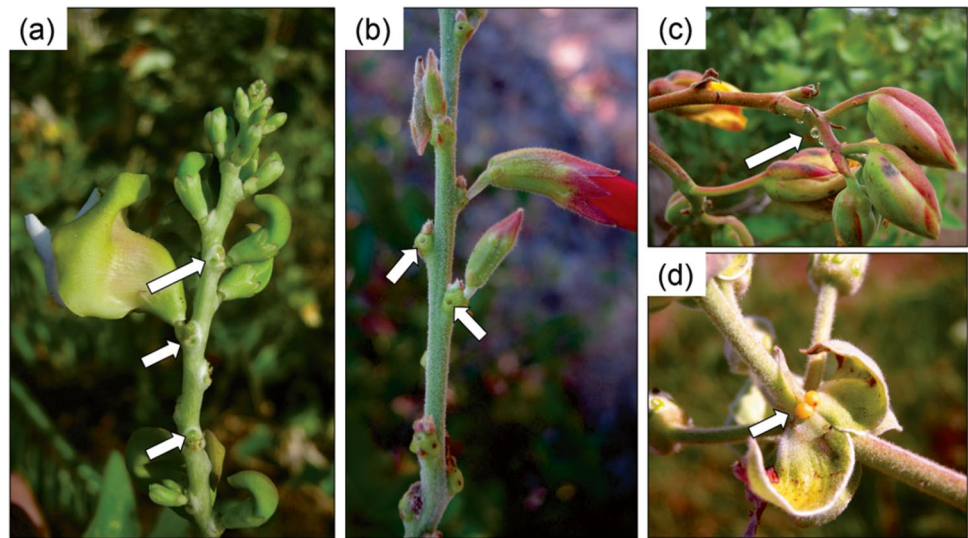
The main characteristics of the four study plants, *A. firmula*, *B. coriacea*, *C. tomentosa*, and *P. tomentosa*, are summarized in Table 1 (see also Fig. 1 and the Online Resource 1). In summary, all these plants species are shrubs, rarely exceeding 1.5 m tall, and all possess EFNs, which are active during blooming and not located on leaves, but rather along the inflorescences (*P. tomentosa* does have EFNs on leaves, but at the period of the study, the dry season, the EFNs are inactive, and leaves are senescent). All shrubs have a single stem, and their canopy seldom touches nearby plants. Their reproductive season takes place in the dry period of Cerrado (May to September), and by the end of September, the fruiting season ends (personal observation). These species were chosen because of their abundance in the study area and their relationship with mutualistic ants (see below).

The reproductive season of all plant species is impaired with the leaf flush period. During the dry period of the Cerrado, these plants undergo flowering, and by late September, all of them ceased the production of fruits; leaves, on the other hand, are scarce, crispy, senescent, and are thus ignored by ants that forage on the EFNs located on the inflorescences. Leaves are produced at the onset of the rainy

Table 1 Characteristics of four extrafloral nectaried plant species of a Brazilian savanna

Characteristics	Plant species			
	<i>Ancistrotropis firmula</i>	<i>Bionia coriacea</i>	<i>Cochlospermum regium</i>	<i>Peixotoa tomentosa</i>
Size	< 2 m	< 1.5 m	< 1.5 m	< 2 m
EFN location	At the base of flower buds	At the base of flower buds	Stipules near flower buds	Base of leaves and stipules near flower buds
Reproduc. season	May to September	April to June	May to July	May to August
Infloresc. type	Raceme	Spike	Simple cyme	Panicle
Flower buds	Hook-shaped, purple	Red, fusiform	Brownish, curvilinear triangle	Yellow, round
Flowers	Purple, zygomorphic, petals fused	Red, zygomorphic, tubular	Yellow, actinomorphic, separate petals	Yellow, actinomorphic, separate petals
Fruits	Lanceolate, green pods	Flat brown pods	Brown, cotton-like	Red samara with three wings

Fig. 1 Extrafloral nectary plants from a Brazilian tropical savanna. **a** *Ancistrotropis firmula*, **b** *Bionia coriacea*, **c** *Cochlospermum regium*, and **d** *Peixotoa tomentosa*. The arrows indicate the location of extrafloral nectaries



season. The *C. regium* is the only plant to have some individuals producing leaves in the dry period, but even so, just like the other species, the EFNs were not located on leaves. For these reasons, the leaves were not a variable in our study.

The study plants sustained individuals of *Camponotus crassus* Mayr, 1862 (Formicinae), and in fact, this was the only ant species with sufficient frequency to be studied, as it was recorded in most plants of each species. Other ant species, such as *Ectatomma*, *Cephalotes*, and *Crematogaster*, were seldom noted. *Camponotus crassus* is widespread in the Cerrado biome and is found almost exclusively on EFN-bearing plants (Anjos et al. 2017; Lange et al. 2019). The frequency and abundance of *C. crassus* on EFN plants, along with its aggressive behavior and high sensitivity to herbivores (Alves-Silva et al. 2014; Lange et al. 2019), make this ant an excellent study model to investigate the potential benefits to plants (Calixto et al. 2021b). This ant species is dominant on EFN plants, monopolizing the resources and rarely allowing the presence of other subordinate or non-dominant ant species (Fagundes et al. 2017). In this context,

by focusing only on *C. crassus*, we were able to isolate the plant reproductive output from the presence of confounding effects of other ants and concentrate the effort on this nearly ubiquitous ant species.

Plant phenology and fitness

All study plants were located in the strictu sensu Cerrado, and none was under the canopy of trees or in rocky soils, thus minimizing possible confounding effects of environment on plant performance. In addition, the large extension of the study area allowed us to tag plants at least 10 m distant of each other.

To investigate the role of ants in plant reproduction, we initially tagged 43 individuals of *B. coriacea*, 41 of *C. regium*, 42 of *P. tomentosa*, and 47 of *A. firmula*, which were (in terms of sample size, almost equally) randomly assigned ant-present or ant-absent treatments. Before conducting the experiment, plants were visited for 2 weeks in order to ensure that all were indeed visited by ants, and that

C. crassus was the only ant species on plants. To prevent ants from accessing plant parts in the “ant-absent” treatment, we surrounded the plant stem with a layer of atoxic resin (Tanglefoot®) at approximately 10 cm above the soil. This procedure has been constantly used in studies that aim to evaluate the role of ants as antiherbivore defense (Jones et al. 2017). All study plants are single-stemmed shrubs, so a band with Tanglefoot in the main stem was just enough to isolate the plants from ants.

The foraging ants of the “ant-absent” treatment were removed with the aid of a small paint brush. More resin was applied whenever necessary to ensure that it was a barrier against ants. In addition, we removed any surrounding vegetation that could act as an ant bridge (Nogueira et al. 2012). The resin was also applied to a small portion of control plants stems, in order to control for the effect of the resin.

During the study, some plants perished due to unknown causes. By the end of the fieldwork, the actual sample size was 39 ($N=19$ ant-present and 20 ant-absent) *B. coriacea*, 34 ($N=15$ ant-present and 19 ant-absent) *C. regium*, 27 ($N=11$ ant-present and 18 ant-absent) *P. tomentosa*, and 22 ($N=11$ ant-present and 11 ant-absent) *A. firmula* individuals.

Two weeks after applying the resin, we started to count the plant structures. Flower buds and fruits were periodically counted by visual estimation (see dates in Figs. 2 and 3) from the beginning until the end of each plant’s reproductive

season. Flowers were not counted because they do not last as long as flower buds, and sometimes, they lose the petals rapidly, making them inconspicuous and hard to notice; thus, we might have underestimated the number of flowers in each sampling. The counting of flower buds and fruits was therefore more reliable (Alves-Silva and Del-Claro 2016).

Regarding herbivores, we attempted to collect and identify insects throughout the study period, but surprisingly, we noticed only a few resident florivorous caterpillars ($n=3$ lycaenids in ant-present *Peixotoa* and $n=4$ riodinids in ant-present *Cochlospermum*), and sporadic beetles and orthopterans, that flew away as we approached the plants. Thus, we decided to not sample and include herbivores into the analyses as this might cost us time and resources, and we might also disturb ants (*C. crassus* is very sensitive; Alves-Silva and Del-Claro 2013) during the process of scanning the plant to search herbivores. However, this does not mean that herbivores were not there, because flowers and flower buds still showed signs of chewing herbivores (some herbivores are active during the nighttime; Silva et al. 2017).

Statistical analyses

In this study, we do not aim to compare the reproductive output among plants, but rather describe the pattern for each plant species alone. All statistical analyses and figures were performed using the R statistical software version 3.6.2 (R

Fig. 2 Production of flower buds in four extrafloral nectaried plants — **a** *Ancistrotropis firmula*, **b** *Bionia coriacea*, **c** *Cochlospermum regium*, and **d** *Peixotoa tomentosa* — according to the absence and presence of ants, and time of sampling. The miniature figures show the cumulative number of flower buds. The figure shows the median, quartiles, and the maximum and minimum values. Statistical results depicted in Tables 2 and 4

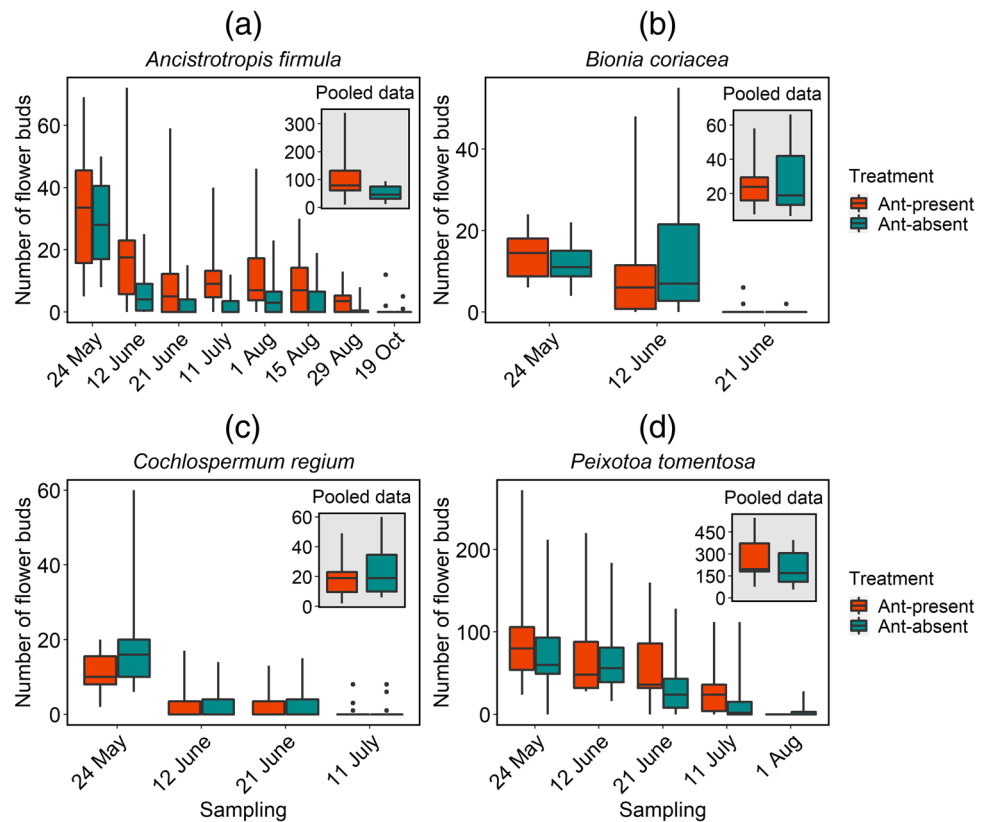
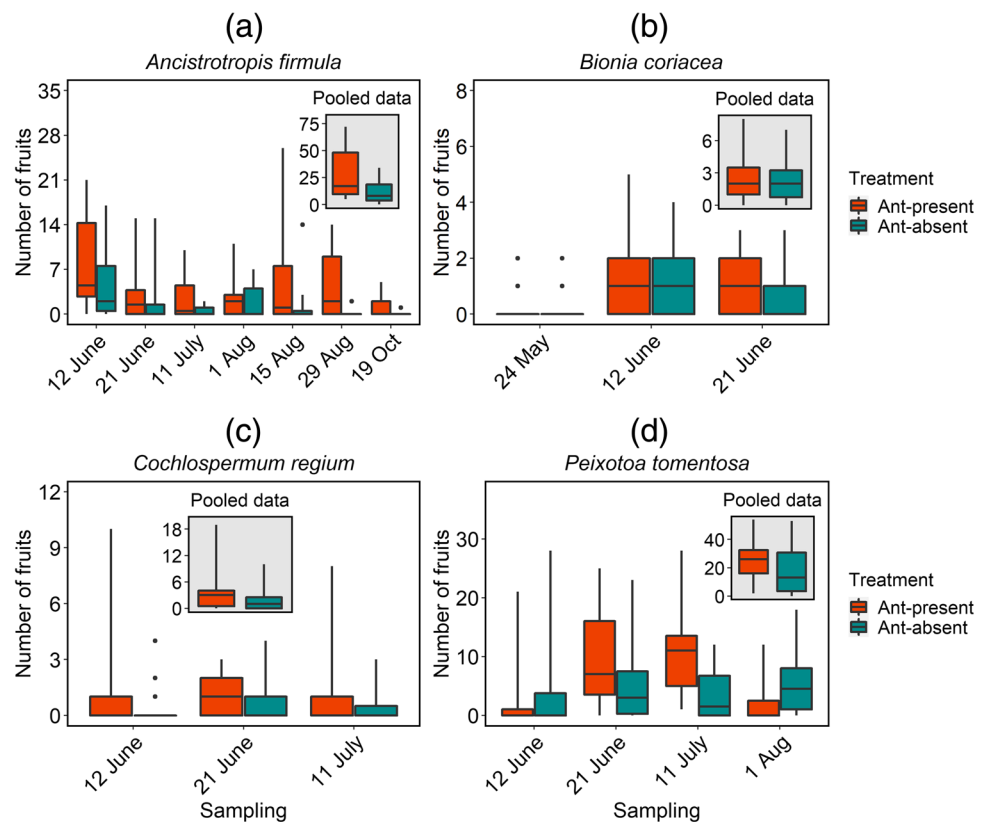


Fig. 3 Number of fruits in four extrafloral nectaried plants — **a** *Ancistrotropis firmula*, **b** *Bionia coriacea*, **c** *Cochlospermum regium*, and **d** *Peixotoa tomentosa* — according to the absence and presence of ants, and time of sampling. The figure shows the median, quartiles, and the maximum and minimum values. The miniature figures show the cumulative number of flower buds. Statistical results depicted in Tables 3 and 4



Team 2019), with the alpha (probability) set as 0.05. We verified model fit by checking the distribution of residuals, heteroskedasticity, and overdispersion.

To analyze the relation between the presence of ants and the periodic production of flower buds and fruits, we conducted a generalized mixed effect model (GLMM) followed by Wald test using the packages “glmmTMB” and “car,” respectively (Fox and Weisberg 2011; Brooks et al. 2017). Our models for flower buds and fruits were fitted with either Poisson or negative binomial distribution errors whenever appropriate, in order to decrease overdispersion and AIC values. The interaction of plant groups (ant-present or absent) and weekly censuses were fitted as fixed factors, and censuses were also fitted as random factor to control for temporal repeated measures.

To evaluate the net benefits of ants on the production of flower buds and fruits, we estimated the Hedges’s g effect size (standardized mean difference; difference between means divided by the pooled standard deviation) (Nakagawa and Cuthill 2007; Fritz et al. 2012) for each plant species. We summed all the flower buds and fruits produced per species and per treatment; then, we calculated the mean and standard deviation values (Viechtbauer 2010). We assumed values of $g < 0.49$ as small; $0.5 < g < 0.79$ as medium and $g > 0.8$ as high (Cohen 1988). We then created a random effects model to calculate the mean effect size and confidence intervals for buds and fruits. For this, weighted data

of all species (mean, standard error, and sample size) were examined simultaneously, and an estimate and 95% confidence intervals were provided as an overall estimation of the effect of ants on plants (Schwarzer et al. 2015). The mean effect size is considered statistically significant if the confidence interval does not cross zero.

Results

We found no effect of the interaction between treatment (ant presence or absence) and weekly censuses on the number of flower buds produced in any plant species (Table 2; Fig. 2). Analyzing the simple main effects, we observed that the presence of ants significantly increased the number of flower buds in *A. firmula* only. In both *B. coriacea* and *C. regium*, we noted (non-significant) variations in the number of flower buds depending on sampling and ant-presence/absence (in *B. coriacea* and *C. regium*); in *P. tomentosa*, the ant-present treatments had more flower buds in all but the last sampling, but results were also non-significant (Fig. 2). The number of buds significantly decreased throughout the reproductive season in all species (Table 2; Fig. 2), as expected from the phenological process given that these plants bloom only in specific periods.

We found no effect of the interaction between treatment (ant presence or absence) and weekly censuses on the

Table 2 GLMM results for flowers buds influenced by treatment (ant presence/absence) and time (sampling periods) in four extrafloral nectaried plant species in a neotropical savanna. Significant *P*-values are in bold

Flower buds			
Variables	Wald's chi-square	D.F	<i>P</i> -value
<i>Ancistrotropis firmula</i>			
Treatment	10.71	1	0.0011
Time	117.27	7	< 0.0001
Treatment × time	8.40	7	0.2984
<i>Bionia coriacea</i>			
Treatment	0.21	1	0.6392
Time	52.41	2	< 0.0001
Treatment × time	3.82	2	0.1480
<i>Cochlospermum regium</i>			
Treatment	1.53	1	0.2160
Time	123.51	3	< 0.0001
Treatment × time	1.76	3	0.6232
<i>Peixotoa tomentosa</i>			
Treatment	1.67	1	0.1961
Time	36.22	4	< 0.0001
Treatment × time	0.26	4	0.9919

number of fruits produced by all plant species, except for *P. tomentosa* (Table 3; Fig. 3). In this species, the number of fruits was higher or lower in plants with ants depending

Table 3 GLMM results for fruits produced according to treatment (ant presence or absence) and time (sampling periods) in four extrafloral nectaried plant species in a neotropical savanna. Significant *P*-values are in bold

Fruits			
Variables	Wald's chi-square	D.F	<i>P</i> -value
<i>Ancistrotropis firmula</i>			
Treatment	8.37	1	0.0038
Time	24.84	6	0.0003
Treatment × time	10.11	6	0.1197
<i>Bionia coriacea</i>			
Treatment	0.43	1	0.5076
Time	15.20	2	0.0004
Treatment × time	0.72	2	0.6942
<i>Cochlospermum regium</i>			
Treatment	3.61	1	0.0572
Time	3.53	2	0.1706
Treatment × time	0.61	2	0.7361
<i>Peixotoa tomentosa</i>			
Treatment	4.52	1	0.0334
Time	15.95	3	0.0011
Treatment × time	12.24	3	0.0066

on the sampling time (Fig. 3). Evaluating the simple main effects, the presence of ants was positively and significantly related with the number of fruits in *A. firmula* and *P. tomentosa* (Table 3; Fig. 3). In *C. regium* and *B. coriacea*, the variation in fruits was not significantly influenced by ants (Table 3; Fig. 3). There was a significant variation in fruit production over the reproductive season of all species but *C. regium* (Table 3; Fig. 3).

The cumulative mean number of buds produced throughout the reproductive season was on average two-fold greater in *A. firmula* with ants; in *P. tomentosa* plants with ants produced on average roughly 30% more buds in comparison to ant-excluded treatments. Nonetheless, in both *B. coriacea* and *C. regium*, plants with ants had on average less buds than treatments (the median was higher for *B. coriacea* with ants, but the mean was the opposite) (Online Resource 2; Fig. 2, miniature figures). Most effect sizes of flower buds were low, except for *A. firmula*. Although plants with ants generally showed increased bud production, as shown by the positive mean effect size, results were not significant (Table 4).

The mean cumulative number of fruits produced by each species was higher in plants with ants (Fig. 3, miniature figures; Online Resource 2). In *A. firmula*, for instance, plants with ants produced on average 150% more fruits than their counterparts without ants. The effect size for fruits was significant, indicating that plants with ants produced significantly more fruits than plants without ants (Table 4).

Table 4 Effect size and mean effect size comparing the flower buds and fruits of four extrafloral nectaried plants according to the presence or the absence of ants. CI=95% confidence interval. The mean effect size is considered statistically significant if the CI interval does not cross zero

Plant species	Effect size	Variance	Relative size
Flower buds			
<i>Ancistrotropis firmula</i>	0.83	0.19	Large
<i>Bionia coriacea</i>	-0.14	0.10	Small
<i>Cochlospermum regium</i>	-0.43	0.12	Small
<i>Peixotoa tomentosa</i>	0.42	0.10	Small
Mean effect size=0.12; CI= -0.41 to 0.65			
Heterogeneity: $Q=6.22$, $df=3$; $p=0.1014$			
Fruits			
<i>Ancistrotropis firmula</i>	0.88	0.19	Large
<i>Bionia coriacea</i>	0.19	0.10	Small
<i>Cochlospermum regium</i>	0.44	0.12	Small
<i>Peixotoa tomentosa</i>	0.45	0.15	Small
Mean effect size=0.44; CI=0.08 to 0.80			
Heterogeneity: $Q=1.55$, $df=3$; $p=0.6709$			

Discussion

To be considered as a mutualism, the interaction between ants and EFN-bearing plants must provide net benefits to both organisms (Bronstein 1998). For plants, the best approach for evaluating these benefits is by quantifying the structures related to the reproductive output, such as flower buds, flowers, fruits, and seeds (Trager et al. 2010). Nonetheless, researchers usually tend to evaluate plant reproduction during specific periods; thus, overestimating or underestimating the conclusions about the net benefits that ants provide to plants throughout the reproductive season. In this context, it is paramount to understand the impact of ant presence on plant fitness periodically over its reproductive season, as well as considering the pooled data.

In this study, we found that *C. crassus* was related to increases in plant reproductive output, especially the number of fruits. This result was expected since the role of *C. crassus* as plant-guard has been shown in studies of protective mutualisms (Oliveira et al. 1987; Calixto et al. 2021b). Nonetheless, it was unknown how the effect of ants varied in time. Periodic samples in the field revealed a large variation in the production of buds and fruits throughout the season; however, the cumulative number of fruits was higher for each plant species with ants.

Herbivory itself might not be related to plant investment in fruits (Trager et al. 2010), so the effect of ants on plant reproduction (flowers, fruits, seeds) is a better predictor of fitness (Sobrinho et al. 2002; Leal et al. 2006). Nevertheless, there is no pattern in the temporal investigation of plant fitness, and most results range from pooled data to specific periods within plant blooming (Oliveira et al. 1999; Sobrinho et al. 2002; Leal et al. 2006; Del-Claro and Marquis 2015). More rarely, the fitness fluctuations within a period are shown (Nogueira et al. 2012). This is surprising, given the long time that ant-plant interactions are being investigated (Heil and McKey 2003). There is such an amount of data showing the temporal (and spatial) fluctuation of herbivory, herbivores, and extrafloral nectar (Heil et al. 2000; Wirth and Leal 2001; Nascimento and Del-Claro 2010; Nogueira et al. 2020; Pereira et al. 2020), but temporal fluctuations of plant reproduction have been overlooked. This could have shown that the effect of ants on plant fitness varies as much as their effect on herbivory. In fact, our investigation of plants in repeated periods throughout their reproductive season indicated a great variation in the production of buds and fruits.

Except for *A. firmula*, where control plants had increased performance, the other plants had significant variations in the number of flower buds and fruits. In *C. regium*, the number of flower buds was higher in plants without ants; however, these plants produced more fruits

in ant-present treatments. This shows that despite producing fewer flower buds, plants with ants produced substantially more fruits than plants without ants. In *P. tomentosa* and *A. firmula*, both the number of buds and fruits were regularly higher in plants with ants. The importance of such periodic assessment was to show that the effects of ants on plant reproduction are not constant over time.

The number of flower buds and fruits in control plants was not statistically higher throughout plant's reproductive season. The only significant result for flower buds was in *A. firmula*, as control plants had more flower buds. This plant species sustained more ants in comparison to the other species, and this might have directly influenced the number of flower buds and fruits (Giusto et al. 2001; Katayama and Suzuki 2004; Martins et al. 2020). Regarding fruits, results were significant for *A. firmula* and *P. tomentosa*, as both had more fruits in control plants in most samples. The examination of the pooled data (average number structures produced per plant and per treatment) yielded contrasting effect sizes; thus, the mean effect size for buds was not significant. The number of fruits was higher in all control plants (particularly in *A. firmula* and *P. tomentosa*), where the effect sizes were all positive and the mean effect size was significant.

Plant reproductive output varied a lot in our data, raising several questions: To what extent do EFN plants benefit from associating with ants? How many fruits should an EFN plant produce to balance the energetic cost of producing EF nectar? EF nectar is assumed to require low inputs from plants (O'Dowd 1979; Rutter and Rausher 2004), and in general, the costs associated to antiherbivore deterrence are not totally understood. In a meta-analysis, authors found no concluding evidence that plant defenses incur fitness costs (Koricheva 2002). Even if there are costs to maintaining EFNs, they are compensated by the benefits plants gain from ant presence in terms of increased fitness (Sugiura et al. 2006; Trager et al. 2010).

Unlike EF nectar production, sexual reproduction requires a high investment in energy, nutrient allocation, and potential trade-offs (Obeso 2002; Aragón et al. 2009). Therefore, we may assume that the mutualism with ants is highly advantageous for plants, as it requires low investment in exchange for increased reproduction. But how high should the fitness of EFN plants be when ants are present? *Bionia coriacea*, for instance, produced 20% more fruits in plants with ants, but the difference was not statistically significant. If we look only at the raw data (and overlook statistical tests), we might assume this is a relevant result for plants, because the low investment in EFNs (which produce nectar regardless of ant presence, as noted in plants where ants were excluded) and the supporting effects of ants reflected in the production of more fruits in comparison to ant-excluded treatments (Rudgers and Gardener 2004; Rutter and Rausher 2004).

In the neotropics, where ant-plant interactions are ubiquitous, such a mutualistic system has been widely explored in many scenarios, but still there are gaps to be filled. Here, we showed that the effect of ants on plant reproduction can vary in time, but despite this, the net benefits, such as the production of fruits, tend to be higher in plants with ants. The detailed investigation of the effect of EF nectar-drinking ants throughout the season of plant reproduction might increase our understanding of the spatiotemporal outcomes of facultative ant-plant interactions in the neotropics, where the reproductive phenology of plants may last several months.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00114-022-01805-w>.

Acknowledgments We are grateful to Eva Colberg for English proof-reading; CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), Universidade do Estado de Mato Grosso; the Programa de Pesquisa Ecológica de Longa Duração — PELD — Transição Cerrado-Amazônia: bases ecológicas e socioambientais para a conservação (etapa III); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior — Brasil (CAPES) — Finance Code 001 (ESC).

Author contribution EAS and KM conceived the ideas and designed methodology; EAS, KM, and GRS collected the data; EAS and ESC analyzed the data; EAS, KM, GRS, and ESC led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. Should the manuscript be accepted for publication, the data supporting the results will be archived in an appropriate public repository and the DOI will be included accordingly.

Declarations

Ethical responsibilities of authors This work is not under consideration in another journal.

Ethics approval N/A.

Consent to participate N/A

Consent for publication N/A

Competing interests The authors declare no competing interests.

References

- Alves-Silva E, Del-Claro K (2013) Effect of post-fire resprouting on leaf fluctuating asymmetry, extrafloral nectar quality, and ant-plant-herbivore interactions. *Naturwissenschaften* 100:525–532. <https://doi.org/10.1007/s00114-013-1048-z>
- Alves-Silva E, Del-Claro K (2016) On the inability of ants to protect their plant partners and the effect of herbivores on different stages of plant reproduction. *Austral Ecol* 41:263–272. <https://doi.org/10.1111/aec.12307>
- Alves-Silva E, Bächtold A, Barônio GJ et al (2014) Ant-herbivore interactions in an extrafloral nectaried plant: are ants good plant guards against curculionid beetles? *J Nat Hist* 49:841–851. <https://doi.org/10.1080/00222933.2014.954020>
- Anjos DV, Caserio B, Rezende FT et al (2017) Extrafloral-nectaries and interspecific aggressiveness regulate day/night turnover of ant species foraging for nectar on *Bionia coriacea*. *Austral Ecol* 42:317–328. <https://doi.org/10.1111/aec.12446>
- Aragón CF, Méndez M, Escudero A (2009) Survival costs of reproduction in a short-lived perennial plant: Live hard, die young. *Am J Bot* 96:904–911. <https://doi.org/10.3732/ajb.0800223>
- Aranda-Rickert A, Fracchia S, Yela N, Marazzi B (2017) Insights into a novel three-partner interaction between ants, coreids (Hemiptera: Coreidae) and extrafloral nectaries: implications for the study of protective mutualisms. *Arthropod Plant Interact* 11:525–536. <https://doi.org/10.1007/s11829-016-9487-z>
- Bächtold A, Alves-Silva E, Del-Claro K (2017) Ant-related oviposition is not associated to low parasitism of the myrmecophilous butterfly *Allosmaitia strophius* in an extrafloral nectaried shrub. *Acta Oecologica* 83:15–21. <https://doi.org/10.1016/j.actao.2017.06.007>
- Bronstein JL (1998) The contribution of ant-plant protection studies to our understanding of mutualism. *Biotropica* 30:150–161. <https://doi.org/10.1111/j.1744-7429.1998.tb00050.x>
- Bronstein JL, Alarcón R, Geber M (2006) The evolution of plant-insect mutualisms. *New Phytol* 172:412–428. <https://doi.org/10.1111/j.1469-8137.2006.01864.x>
- Brooks ME, Kristensen K, van Benthem KJ et al (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J* 9:378–400
- Calixto ES, Lange D, Bronstein J et al (2021a) Optimal defense theory in an ant-plant mutualism: extrafloral nectar as an induced defense is maximized in the most valuable plant structures. *J Ecol* 109:167–178. <https://doi.org/10.1111/1365-2745.13457>
- Calixto ES, Lange D, Moreira X, Del-Claro K (2021b) Plant species specificity of ant-plant mutualistic interactions: differential predation of termites by *Camponotus crassus* on five species of extrafloral nectaried plants. *Biotropica* 1–9. <https://doi.org/10.1111/btp.12991>
- Cohen J (1988) *Statistical power analysis for the behavioral sciences*, 2nd edn. Routledge, New York
- Del-Claro K, Marquis RJ (2015) Ant species identity has a greater effect than fire on the outcome of an ant protection system in Brazilian Cerrado. *Biotropica* 47:459–467. <https://doi.org/10.1111/btp.12227>
- Del-Claro K, Berto V, Réu W (1996) Effect of herbivore deterrence by ants on the fruit set of an extrafloral nectaried plant, *Qualea multiflora* (Vochysiaceae). *J Trop Ecol* 12:887–892
- Del-Claro K, Rico-Gray V, Torezan-Silingardi HM et al (2016) Loss and gains in ant-plant interactions mediated by extrafloral nectar: fidelity, cheats, and lies. *Insectes Soc* 63:207–221. <https://doi.org/10.1007/s00040-016-0466-2>
- Fagundes R, Dáttilo W, Ribeiro SP et al (2017) Differences among ant species in plant protection are related to production of extrafloral nectar and degree of leaf herbivory. *Biol J Linn Soc* 122:71–83. <https://doi.org/10.1093/biolinnean/blx059>
- Fox J, Weisberg S (2011) *An R companion to applied regression*, second. SAGE, California
- Fritz CO, Morris PE, Richler JJ (2012) Effect size estimates: current use, calculations, and interpretation. *J Exp Psychol Gen* 141:2
- Fuente MAS, Marquis RJ (1999) The role of ant-tended extrafloral nectaries in the protection and benefit of a Neotropical rainforest tree. *Oecologia* 118:192–202. <https://doi.org/10.1007/s004420050718>
- Giusto B, Anstett MC, Dounias E, McKey DB (2001) Variation in the effectiveness of biotic defence: the case of an opportunistic ant-plant protection mutualism. *Oecologia* 129:367–375. <https://doi.org/10.1007/s004420100734>

- Heil M, McKey D (2003) Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annu Rev Ecol Evol Syst* 34:425–553. <https://doi.org/10.1146/annurev.ecolsys.34.011802.132410>
- Heil M, Fiala B, Baumann B, Linsenmair KE (2000) Temporal, spatial and biotic variations in extrafloral nectar secretion by *Macaranga tanarius*. *Funct Ecol* 14:749–757. <https://doi.org/10.1046/j.1365-2435.2000.00480.x>
- Jiang X, Deng H, Qu J et al (2019) The function of extrafloral nectaries in the pollination and reproduction of *Sambucus javanica*. *Plant Species Biol* 34:53–60. <https://doi.org/10.1111/1442-1984.12234>
- Jones IM, Koptur S, Peña JE (2017) Exploring whether and how ants (Hymenoptera: Formicidae) affect reproductive fitness in *Senna mexicana* var. *chapmanii* (Fabaceae). *Florida Entomol* 100:539–545. <https://doi.org/10.1653/024.100.0308>
- Katayama N, Suzuki N (2004) Role of extrafloral nectaries of *Vicia faba* in attraction of ants and herbivore exclusion by ants. *Entomol Sci* 7:119–124. <https://doi.org/10.1111/j.1479-8298.2004.00057.x>
- Kelly CA (1986) Extrafloral nectaries: ants, herbivores and fecundity in *Cassia fasciculata*. *Oecologia* 69:600–605. <https://doi.org/10.1007/BF00410369>
- Koricheva J (2002) Meta-analysis of sources of variation in fitness costs of plant antiherbivore defenses. *Ecology* 83:176–190. [https://doi.org/10.1890/0012-9658\(2002\)083\[0176:MAOSOV\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[0176:MAOSOV]2.0.CO;2)
- Lange D, Calixto ES, Rosa BB et al (2019) Natural history and ecology of foraging of the *Camponotus crassus* Mayr, 1862 (Hymenoptera: Formicidae). *J Nat Hist* 53:1737–1749. <https://doi.org/10.1080/00222933.2019.1660430>
- Leal IR, Fischer E, Kost C et al (2006) Ant protection against herbivores and nectar thieves in *Passiflora coccinea* flowers. *Ecoscience* 13:431–438. [https://doi.org/10.2980/1195-6860\(2006\)13\[431:APAHAN\]2.0.CO;2](https://doi.org/10.2980/1195-6860(2006)13[431:APAHAN]2.0.CO;2)
- Martins J, Moreira A, Assunção M et al (2020) Trade-off in plant-ant interactions: seasonal variations. *Braz J Biol*. <https://doi.org/10.1590/1519-6984.229848> (in press)
- Melati BG, Leal LC (2018) Aggressive bodyguards are not always the best: preferential interaction with more aggressive ant species reduces reproductive success of plant bearing extrafloral nectaries. *PLoS ONE* 13:1–13. <https://doi.org/10.1371/journal.pone.0199764>
- Nakagawa S, Cuthill IC (2007) Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biol Rev* 82:591–605
- Nascimento EA, Del-Claro K (2010) Ant visitation to extrafloral nectaries decreases herbivory and increases fruit set in *Chamaecrista debilis* (Fabaceae) in a Neotropical savanna. *Flora* 205:754–756. <https://doi.org/10.1016/j.flora.2009.12.040>
- Nogueira A, Guimarães E, Machado S, Lohmann L (2012) Do extrafloral nectaries present a defensive role against herbivores in two species of the family Bignoniaceae in a Neotropical savanna? *Plant Ecol* 213:289–301. <https://doi.org/10.1007/s11258-011-9974-3>
- Nogueira A, Baccaro FB, Leal LC et al (2020) Variation in the production of plant tissues bearing extrafloral nectaries explains temporal patterns of ant attendance in Amazonian understory plants. *J Ecol* 108:1578–1591. <https://doi.org/10.1111/1365-2745.13340>
- O'Dowd DJ (1979) Foliar nectar production and ant activity on a neotropical tree, *Ochroma pyramidale*. *Oecologia* 43:233–248. <https://doi.org/10.1007/BF00344773>
- Obeso JR (2002) The costs of reproduction in plants. *New Phytol* 155:321–348
- Oliveira PS, da Silva AF, Martins AB (1987) Ant foraging on extrafloral nectaries of *Qualea grandiflora* (Vochysiaceae) in cerrado vegetation: ants as potential antiherbivore agents. *Oecologia* 74:228–230. <https://doi.org/10.1007/BF00379363>
- Oliveira PS, Rico-Gray V, Díaz-Castelazo C, Castillo-Guevara C (1999) Interaction between ants, extrafloral nectaries and insect herbivores in Neotropical coastal sand dunes: herbivore deterrence by visiting ants increases fruit set in *Opuntia stricta* (Cactaceae). *Funct Ecol* 13:623–631. <https://doi.org/10.1046/j.1365-2435.1999.00360.x>
- Pereira CC, Boaventura MG, de Castro GC, Cornelissen T (2020) Are extrafloral nectaries efficient against herbivores? Herbivory and plant defenses in contrasting tropical species. *J Plant Ecol* 13:423–430. <https://doi.org/10.1093/jpe/rtaa029>
- R Team (2019) R: a language and environment for statistical computing (3.5.0). R Foundation for Statistical Computing
- Rudgers JA, Gardener MC (2004) Extrafloral nectar as a resource mediating multispecies interactions. *Ecology* 85:1495–1502. <https://doi.org/10.1890/03-0391>
- Rutter MT, Rausher MD (2004) Natural selection on extrafloral nectar production in *Chamaecrista fasciculata*: the costs and benefits of a mutualism trait. *Evolution (N Y)* 58:2657–2668. <https://doi.org/10.1111/j.0014-3820.2004.tb01619.x>
- Sanz-Veiga PA, Ré Jorge L, Benítez-Vieyra S, Amorim FW (2017) Pericarpial nectary-visiting ants do not provide fruit protection against pre-dispersal seed predators regardless of ant species composition and resource availability. *PLoS ONE* 12:e0188445
- Schwarzer G, Carpenter JR, Rucker G (2015) Meta-analysis with R. Springer, Switzerland
- Silva JO, Leal CRO, Espírito-Santo MM, Morais HC (2017) Seasonal and diel variations in the activity of canopy insect herbivores differ between deciduous and evergreen plant species in a tropical dry forest. *J Insect Conserv* 21:667–676. <https://doi.org/10.1007/s10841-017-0009-9>
- Sobrinho TG, Schoederer JH, Rodrigues LL, Collevatti RG (2002) Ant visitation (Hymenoptera: Formicidae) to extrafloral nectaries increases seed set and seed viability in the tropical weed *Triumfetta semitriloba*. *Sociobiology* 39:353–368
- Sugiura S, Abe T, Makino S (2006) Loss of extrafloral nectary on an oceanic island plant and its consequences for herbivory. *Am J Bot* 93:491–495. <https://doi.org/10.3732/ajb.93.3.491>
- Thompson JN (2013) Relentless evolution. University of Chicago Press, Chicago
- Trager MD, Bhotika S, Hostetler JA et al (2010) Benefits for plants in ant-plant protective mutualisms: a meta-analysis. *PLoS ONE* 5:e14308. <https://doi.org/10.1371/journal.pone.0014308>
- Viechtbauer W (2010) Conducting meta-analyses in R with the metafor package. *J Stat Softw* 36:1–48
- Vilela AA, Torezan-Silingardi HM, Del-Claro K (2014) Conditional outcomes in ant-plant-herbivore interactions influenced by sequential flowering. *Flora* 209:359–366. <https://doi.org/10.1016/j.flora.2014.04.004>
- Weber MG, Porturas LD, Keeler KH (2015) World list of plants with extrafloral nectaries. www.extrafloralnectaries.org. Accessed 19 Sept 2018
- Wirth R, Leal IR (2001) Does rainfall affect temporal variability of ant protection in *Passiflora coccinea*? *Ecoscience* 8:450–453. <https://doi.org/10.1080/11956860.2001.11682674>

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