Extrafloral nectar secretion does not mediate ant predatory activity on *Pityrocarpa moniliformis* plants in a seasonally dry forest

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ABSTRACT

Plants bearing extrafloral nectaries (EFNs) and their attendant ants are part of a mutualistic, facultative and generalized interaction. The final balance of this interaction can vary greatly over time and space, making the outcome context-dependent. In this study, we investigated how nectar volume and concentration mediate ant attraction and ant predatory activity on a legume species, as well as evaluate their daily variation in a seasonally dry tropical forest in northeastern Brazil. Our study model was the tree species *Pityrocarpa moniliformis* (Benth.) Luckow & R. W. Jobson (Fabaceae), and we used simulated herbivores (termites) to assess the predatory activity of ants. We found that ants interacted more on the EFNs of plants that produce higher nectar volumes, but not to those producing higher nectar concentrations. Ant predatory activity was not influenced by the volume or concentration of extrafloral nectar. Regarding the daily variation in nectar secretion, we found higher volumes secreted at nighttime, but no difference in nectar concentrations between day and night. Ant predatory activity was similar throughout the day and night. Our results indicate that ants are more sensitive to variations in nectar volume than concentration and that the volume secreted is higher at night; both probably due to the water restriction of the Caatinga. However, ant predatory activity is not related to nectar secretion volume or concentration, highlighting the complexity of mechanisms mediating the interaction between EFN-bearing plants and ants.

1. Introduction

Mutualisms are reciprocal and positive interactions between species, where both partners involved provide and receive resources or services (Schemske and Horvitz, 1988), implying costs for both partners (Noë and Hammerstein, 1995). However, interactions between potentially mutualistic partners are far from altruistic as the individuals involved tend to maximize their benefits, regardless of the consequences for their partner (Jones et al., 2015). An interaction is mutualistic when the final balance of the interaction has a positive net effect (for example, increased fitness) for both partners; that is, when the benefits received exceed the costs of providing the resources or services (Bronstein, 2015). The net outcome of such interactions, and the degree of dependence, is widely variable in space and time (Cushman and Addicott, 1991; Chamberlain et al., 2014). Thus, mutualisms can vary due to changes in the composition and behavior of mutualistic partners (Gorlic, Atkins and

Losey, 1978; Câmara et al., 2018), as well as variations in abiotic factors (e.g., Mooney et al., 2016; Oliveira et al., 2021). These sources of variation can affect the underlying mechanisms, regulating the costs and benefits to each partner and the ultimate outcome of the interaction (Bronstein, 1994).

One of the most studied examples of a mutualism is that of the association between ants and plants bearing extrafloral nectaries (EFNs) (Marazzi, 2013; Del-Claro et al., 2016). In this interaction, the plants produce extrafloral nectar to attract ants, which, when foraging on the host plant, eat or chase away herbivores, potentially decreasing the herbivory rates and increasing plant growth and/or reproduction (Rosumek et al., 2009; Trager et al., 2010). Extrafloral nectar is a widely available resource for ants and is easily collected by most arboreal and various terrestrial ant species (Law and Koptur, 1986; Blüthgen and Fiedler, 2004). Therefore, the availability of extrafloral nectar is an essential source of variation in the outcome of interactions between

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plants and ants (Heil et al., 2001), affecting the pattern of ant attendance to EFNs and the organization of EFN-visiting ant communities (Blüthgen et al., 2000). Thus, changes in the quantity and quality of extrafloral nectar result in changes in the identity of partners and may influence the protection services they offer (Ness et al., 2006; Rico-Gray and Oliveira, 2007; Fagundes et al., 2017; but see Oliveira et al., 2021). However, interactions between EFN-bearing plants and ants are facultative and generalized (Rico-Gray and Oliveira, 2007). Hence, individuals of different species of plants and ants can interact with different partners at different frequencies over time and space (Dáttilo et al., 2013; Chamberlain et al., 2014; Dáttilo et al., 2016). Therefore, as the outcomes of such interactions are widely variable, ant species can act as protectors, defending plants against herbivores, or opportunists, collecting nectar but not protecting the plant (Del-Claro et al., 2016).

Although the interaction between EFN-bearing plants and protective ants is widely studied, the mechanisms behind the variation in their outcomes are still poorly understood (Fagundes et al., 2017; Lange et al., 2017). Comprehending these mechanisms is particularly important in dry environments since plant species with EFNs are remarkably pioneers, occurring mainly in open, sunnier (Schupp and Feener, 1991) and drier environments (Aranda-Rickert et al., 2014). In drier environments, the higher temperatures and lower humidity during the daytime compared to nighttime makes extrafloral nectar secretion particularly expensive and, therefore, not continuous (Pyke, 1991; Heil et al., 2000; Pringle, 2015). Thus, secretion can be regulated over time, consequently, affecting ant-plant interactions. For example, some plants can attract ants by secreting greater amounts of nectar at times of day when herbivore pressure is greatest (Holland et al., 2010; Dáttilo et al., 2015; Lange et al., 2017). Despite evidence of temporal variation in EFN secretion and ant attendance, most studies have investigated how nectar modulates ant attendance in just one period of the day, which has led to a significant gap in our knowledge on nectar secretion and ant dynamics (but see Koptur, 1984; Dáttilo et al., 2015; Lange et al., 2017; Anjos et al., 2017). Given the daily variation in the nectar secretion, which can determine the frequency and identity of ant partners (Lange et al., 2017), it is necessary to improve our understanding of nectar secretion patterns and their effects on the predatory activity of ants on the same focal plant during both daytime and nighttime.

Here, we investigated the daily variation in extrafloral nectar production and its role in attracting ants and predatory activity in the Caatinga, a seasonally dry tropical forest located in Brazil. A large proportion of the Caatinga flora is composed of EFN-bearing plants (Melo et al., 2010; Leal et al., 2017; Câmara et al., 2018), which are an important resource for ant communities (Silva et al., 2019). Previous studies of ant-plant interactions in the Caatinga have shown that spatial variation in anthropogenic disturbance and aridity have the potential to negatively affect these interactions (Leal et al., 2015; Câmara et al., 2018, 2019; Oliveira et al., 2021). However, these studies observed ants attending EFN-bearing plants only during the daytime. Additionally, only one of these studies evaluated whether EFN traits modulate the interaction and protections services offered by attendant ants (Oliveira et al., 2021). Finally, ant species composition in the Caatinga differs between daytime and nighttime (Silva et al., 2019), which can lead to different patterns of ant attraction to EFN-bearing plants and protection services. Thus, we address the following questions: (1) What is the role of extrafloral nectar production in ant attraction and predatory activity? (2) Does nectar production vary throughout the day (i.e., day vs night)? and (3) Is there a daily variation (i.e. day vs night) in ants' predatory activity? We expected that an increase in nectar quantity (i.e., nectar volume) and quality (i.e., sugar concentration) would lead to an increased in the number of ant interactions to EFNs and in ant predatory activity. Furthermore, we also expected plants to secreting more and higher-quality nectar (i.e., volume and sugar concentration) and attracting more aggressive ants at nighttime compared to daytime.

2. Material and methods

2.1. Study area

We conducted this study in the Catimbau National Park (8°24′00″ - 8°36′35″ S and 37°09′30″ - 37°14′40″ W), a 607 km^2 protected area of Caatinga dry forest located in the state of Pernambuco, Northeastern Brazil. The annual rainfall in the region varies between 480 and 1100 mm, and a rainy season between January and July (Alvares et al., 2014), but rainfall is more concentrated between May and July, with an average annual temperature of around 23 °C (Rito et al., 2017). The predominant soils are quarzitic sands, occurring in approximately 75% of the area, supporting a low-stature dry forest with a predominance of Fabaceae, Euphorbiaceae and Myrtaceae species (Rito et al., 2017). EFN-bearing plants are very diverse and locally abundant in the Catimbau, where 15% of woody species and 40% of individuals bear these glands (Leal et al., 2017). The richest families with EFNs are Fabaceae and Euphorbiaceae, but species from Anacardiaceae, Cactaceae, Capparaceae, Passifloraceae, and Turneraceae are also reported as bearing EFNs (Câmara et al., 2018).

2.2. Study species

Our EFN-bearing plant model, *Pityrocarpa moniliformis* (Benth.) Luckow & R. W. Jobson (Fabaceae), is an endemic tree from Brazil (Morim, 2015), widely distributed and abundant in the study area (Rito et al., 2017). The EFNs of *P. moniliformis* are enclosed-concave glands located in the rachis between the first pair of pines (Melo et al., 2010, Fig. 1A) and are responsible for most interactions with ants in our study area (Câmara et al., 2018).

2.3. Experimental design

2.3.1. Nectar volume and concentration, ant visitation and predatory activity

To evaluate whether individuals who secrete more nectar attract more ants, we first selected 39 adult plants of *P. moniliformis* with similar phenological stage, size, and architecture (i.e., height 1–3 m, and with a diameter at soil level >3 cm, *sensu* Rodal et al., 1992), to control ontogenetic effects on nectar production (Oliveira et al., 2021). Plants were separated from each other by a minimum of 10 m to ensure that most ants observed were from different colonies (Agosti and Alonso, 2000). First, to evaluate nectar volume and concentration of *P. moniliformis*, for each one of the 39 individuals, we systematically chose three apical branches with fully expanded leaves, totaling 117 EFNs, and we washed each EFN with distilled water and dried them with a paper towel (Oliveira et al., 2021). EFNs were isolated for 24 h from nectar-feeding insects by covering the leaves with a standard-size nonwoven bag and applying Tanglefoot® around each branch of the plant (according to Blüthgen, Gottsberger & Fiedler, 2004; Bixenmann et al., 2011). After 24 h, we collected all the extrafloral nectar that had been secreted with a microsyringe (Hamilton 10 μl). We diluted the nectar samples with distilled water in an amount proportional to the volume collected (Dutton et al., 2016). We then measured the sugar concentration using a Kasvi 0–32% Brix refractometer (the Brix degrees correspond to 1g of sugar/100g of solution). Thus, the concentration was estimated with the following equation: [$C1 = (V2C2)/V1$], where: V1 is the collected volume; V2 is the volume after the addition of distilled water; C1 is the initial concentration and; C2 is the concentration obtained by the addition of distilled water (Díaz-Castelazo et al., 2005; Oliveira et al., 2021).

We recorded ant visits to EFNs on the same *P. moniliformis* individuals one day before nectar collection and we used the visits as a measure of plant attractiveness to ant. We selected one apical branch from each of the 39 individuals and observed the ants for 5 min (following Leal et al., 2015). We considered the interaction when ants
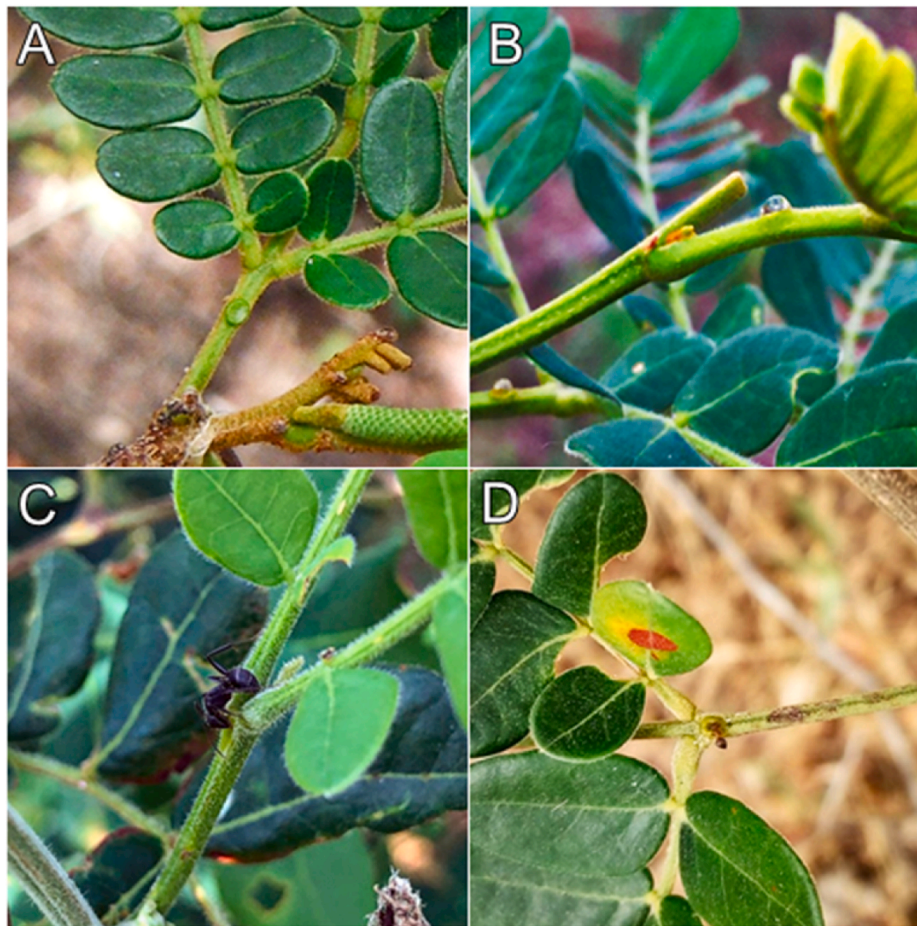
Fig. 1. (A) Extrafloral nectary structure, (B) drops of extrafloral nectar, (C) *Camponotus crassus* and (D) *Dorymyrmex thoracicus* visiting the extrafloral nectary of *Pityrocarpa moniliformis* (Fabaceae) in the Catimbau National Park, Northeast Brazil.

touched mouthparts in EFNs (Câmara et al., 2018; Oliveira et al., 2021). Thus, interactions were defined according to the frequency with which each ant species interacted with the EFNs, regardless of the number of workers foraging on the plants (Câmara et al., 2018; Silva et al., 2020; Oliveira et al., 2021). Hence, for each plant, we recorded the number and species of ants that interacted with EFNs.

Finally, in the same plant individuals, we selected another apical branch to assess whether individuals that secrete nectar in higher quantity and quality benefited from increased predatory ant activity. For this, we conducted bioassays with termites as simulated herbivores (Oliveira et al., 1987). The use of termites simulating natural herbivores allowed us to compare the protection capacity of various ant species using a standardized protocol, with a high number of replicates (Fagundes et al., 2017). Our protocol involved gluing five live termite workers (*Syntermes* sp.) by the dorsum onto the apical part of the branch and/or leaves with non-toxic white glue. We carried out all the handling, as well as the gluing of termites on the plants, using tweezers so as not to cause disturbances that would interfere with the activity of the ants on the plants. After the last termite was placed, we observed them for 10 min (Apple and Feener Jr., 2001; Oliveira et al., 2021), checking the number and species of ants involved in the attack and/or removal. We stored ants that could not be identified in the field in 70% alcohol for later identification in the laboratory. We collected extrafloral nectar samples as well as carrying out all ant visitation to EFNs observations and ant-termite attack experiments between 7:00 a.m. and 10:00 a.m., from January to April 2018. These months correspond to the rainy season in the Caatinga, which is when *P. moniliformis* has leaves and produces nectar. We evaluated each plant only once, both for nectar

measurements, for the number of interactions and for ant attacks on simulated herbivores.

2.3.2. Daily variation of nectar production and ant predatory activity

To verify how the secretion of extrafloral nectar varied during the daytime and nighttime, we selected 10 adult *P. moniliformis* individuals (different from the individuals selected to the previous experiments). In each plant, we chose five apical branches with fully expanded leaves. We than washed each EFN with distilled water and dried them with a paper towel (Oliveira et al., 2021). The same individuals and branches were isolated for 5 h in the morning (from 6 a.m. to 11 a.m.) and 5 h in the night (from 6 p.m. to 11 p.m.). Then we measured the volume and concentration of extrafloral nectar using the same methods described above. According to our previous observations from pilot experiments, 5 h was considered enough time for nectar accumulation. We collected extrafloral nectar from all plants in a single day.

To assess whether ant predatory activity varies between the day and night, we conducted bioassays using termites as a simulated herbivore (with the same methods described above) on 53 plants during the day (7–9 a.m.) and at night (7–9 p.m.). We evaluated the daily variation in ant predatory activity only once per plant, during the day and night. We collected ants that carried out attacks and preserved them in 70% alcohol for later identification in the laboratory.

2.4. Data analysis

We used a generalized linear model (GLM) to verify how nectar quantity (volume) and quality (concentration) influenced ant visitation

to EFNs. We used the average volume and concentration per individual as independent variables, and the number of interactions was our response variable. Since our response variable was a count variable, we used a Poisson error distribution (log link function). We performed a GLM with a negative binomial distribution (MASS package in R; Venables and Ripley, 2002) to assess whether individuals who secrete nectar in greater quantities and concentrations experience greater ant protection against simulated herbivores. For this, we used the average nectar volume and concentration as independent variables and the accumulated number of ant attacks against each termite as response variables. We used linear mixed models (LMMs; nlme package; Pinheiro et al., 2021) to verify if there were differences in the nectar volume and concentration and the number of ant attacks between the daytime and nighttime. In these analyses, we included the plant individuals as a random factor, in order to avoid pseudo-replication. In cases where there was no sugar concentration (i.e., when there was no secretion of nectar), we just used the volume in the analysis (Lange et al., 2017). Finally, we also performed a Pearson correlation test to assess whether there was a correlation between the volume of secreted nectar and the concentration of sugars. We performed residuals analysis and checked for overdispersion in all models to evaluate model fit. We performed all analyzes with the R, version 4.0.5 (R Development Core R Core Team, 2021).

3. Results

3.1. Nectar volume and concentration, ant visitation and predatory activity

Pityrocarpa moniliformis nectar secretion volume ranged from 0.1 μl to 1.0 μl (0.30 mean ± 0.42; mean ± SD) and nectar concentration from 3.33 Brix to 27.66 Brix (18.18 Brix ± 8.46 Brix). Nectar volume and concentration were not correlated ($r = -0.19$; df = 29; p = 0.18).

We observed 122 interactions between ants and EFNs, corresponding to eight ant species, seven genera and five subfamilies (Table 1). The most common ant species feeding on EFNs were *Camponotus crassus* (56 interactions, 46% of all interactions), followed by *Dorymyrmex thoracicus* (22 interactions, 18%) and *Crematogaster evallans* (17 interactions, 14%). The number of interactions was positively related to nectar volume (Table 2; Fig. 2). However, nectar concentration did not influence the number of interactions (Table 2). Regarding the number of attacks on termites, we recorded eight attacks (4.10% of termites), which were carried out by *C. crassus* (four attacks), *Cr. evallans* (three attacks) and *Azteca* sp. (one attack). The number of attacks was not related to either nectar volume or nectar concentration (Table 2).

Table 1

Ant species that interacted with *Pityrocarpa moniliformis* (Benth.) Luckow & R. W. Jobson (Fabaceae) extrafloral nectaries and their total number of interactions, as well as the number of attacks on termites in the Catimbau National Park, Northeast Brazil.

Subfamily	Ant species	Nº of interactions	Nº of attacks
Dolichoderinae	*Azteca* sp.	5	1
	Dorymyrmex thoracicus Gallardo, 1916	22	0
Ectatomminae	*Ectatomma muticum* Mayr, 1870	5	0
Formicinae	*Camponotus crassus* Mayr, 1982	56	4
	Camponotus sp.	7	0
Myrmicinae	*Cephalotes pusillus* Klug, 1824	6	0
	Crematogaster evallans Forel, 1907	17	3
Pseudomyrmicinae	*Pseudomyrmex gracilis* Fabricius, 1804	4	0

Table 2

Effect of nectar volume and concentration on the number of interactions and number of attacks on termites on *Pityrocarpa moniliformis* (Benth.) Luckow & R. W. Jobson (Fabaceae) in the Catimbau National Park, Northeast Brazil.

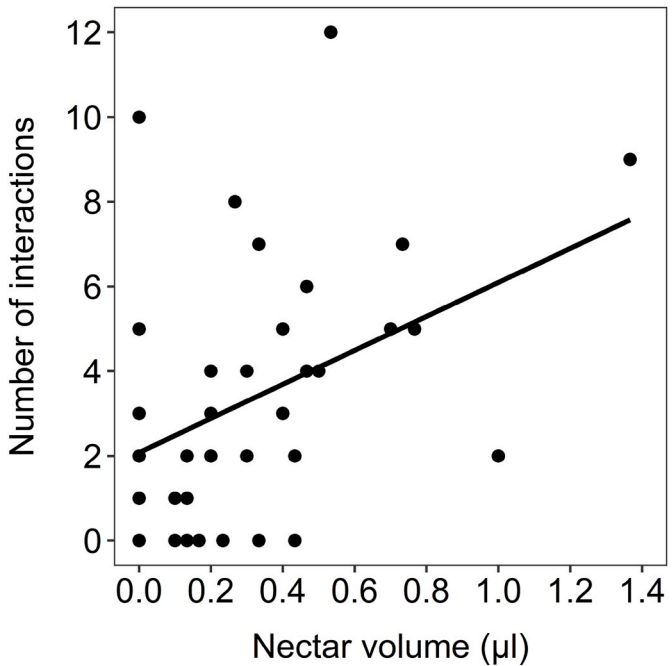| Response variables	Explanatory variables	DF	F	P
Extrafloral nectar volume	Number of interactions	37	5.76	**0.01**
	Number of attacks	37	3.29	0.06
Nectar concentration	Number of interactions	28	0.52	0.25
	Number of attacks	28	5.98	0.17

Fig. 2. Effect of nectar volume (μl) on the number of interactions in *Pityrocarpa moniliformis* (Fabaceae) in the Catimbau National Park, Northeast Brazil.

3.2. Daily variation of nectar secretion and predatory ant activity

Out of the 100 nectaries evaluated in the 10 individuals of *P. moniliformis*, 17% secreted nectar during the daytime and 28% during the nighttime; the remaining did not secrete nectar during the day (83%) or night (72%). The nectar volume secreted at night ranged from 0.1 to 4.5 μl (0.23 ± 0.29; mean ± SD) and was approximately twice higher than the nectar volume secreted during the daytime (LMM: t = 2.27; df = 9; p = 0.04; Fig. 3), ranging from 0.1 to 5.8 μl (0.14 ± 0.28). The nectar concentration did not vary between day and night (daytime: 18.09 Brix ± 15.15; nighttime: 9.23 Brix ± 2.07 Brix; LMM: t = −1.63; df = 7; p = 0.16).

Seven ant species attacked the termites, with *Azteca* sp. attacking exclusively during the daytime, while *C. cingulatus*, *C. fastigatus*, *C. vittatus* and *Ectatomma muticum* exclusively during the nighttime. *C. crassus* and *Cr. evallans* attacked the termites during both day and night (Fig. 4). We counted a total of 16 ant attacks on termites (6.4% of all termites) during our experiment; 31.25% occurred during the daytime and 68.75% during the nighttime, although this difference was statistically insignificant (LMM: t = 1.72; df = 77; p = 0.08).

4. Discussion

Our study examined the potential role of *P. moniliformis* extrafloral nectar secretion in ant visitation and attack to simulated herbivores and the importance of daily variation in nectar secretion in mediating ant predatory activity. Our findings indicate that: (1) higher extrafloral
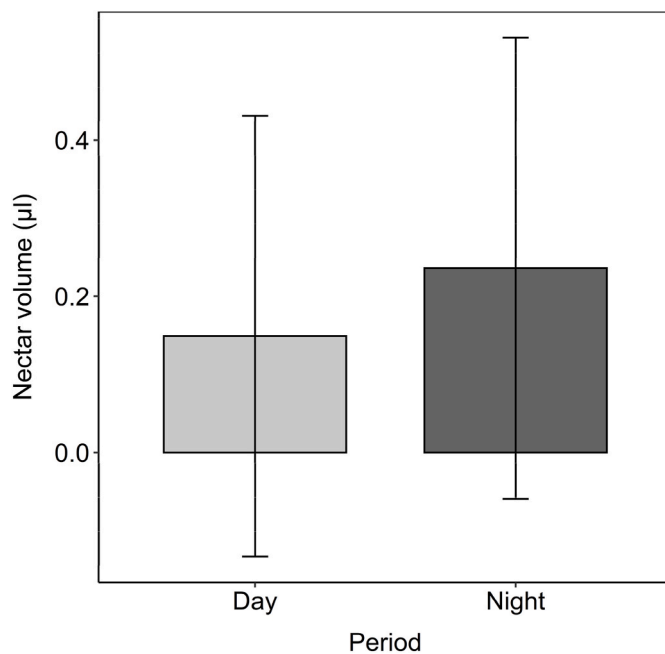
Fig. 3. Mean volume (μl) produced by the extrafloral nectaries of *Pityrocarpa moniliformis* (Fabaceae) during the daytime and nighttime in the Catimbau National Park, Northeast Brazil. The errors bars corresponds to the standard deviation of the mean.
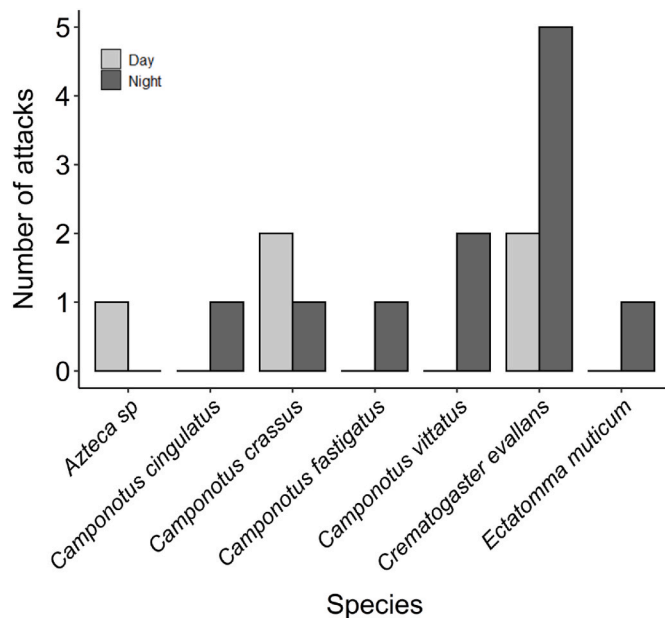
Fig. 4. Number of ant attacks on simulated termite prey on *Pityrocarpa moniliformis* (Fabaceae) individuals during the day and night in the Catimbau National Park, Northeast Brazil.

nectar volume increases the number of feeding ants, but ant attendance is not affected by nectar concentration; (2) ant attacks to simulated herbivores (termites) are low (~5% of offered termites) and are not related to either nectar volume or concentration; (3) there is a daily variation (daytime vs nighttime) in nectar secretion with higher volumes secreted during the nighttime, which is not related to nectar concentration and; (4) ant attacks on simulated herbivores are similar in both periods of the day, thus are not influenced by the volume and/or concentration of nectar secreted. Taken together, these results indicate

that *P. moniliformis* extrafloral nectar secretion does not mediate ant predatory activity.

Many studies have shown that an increase in nectar production is able to attract more ants, which, consequently, can lead to an increase in the probability of ants finding insect herbivores on host plants (Rosumek et al., 2009; Bixenmann et al., 2011; Del-Claro and Marquis, 2015; Flores-Flores et al., 2018). Here, we found that ant visitation to EFNs is higher on plants producing higher nectar volumes but not on plants producing nectar in higher sugar concentrations. Since nectar is a source of water and sugar for ants and we conducted this study in a dry forest, which is limited mainly by water, higher quantities of nectar should increase the relative value of nectar for ants (Leal and Peixoto, 2017), increasing ant foraging per plant. In fact, the water contained in nectar is an important resource for ants in drier environments (Ruffner and Clark, 1986). High temperatures are responsible for the increase in the metabolic rate of insects, increasing the frequency of ant pumping and the ingestion rate of more dilute nectar (Falibene and Josens, 2014). For example, ants in the Arizona desert that visit the EFNs of the species of *Ferocactus acanthodes* (Cactaceae) increase their activities in warmer months and are more likely to consume more water and less sugar per mg/nectar at higher temperatures (Ruffner and Clark, 1986). This pattern is also true for other nectarivorous insects. For example, in an experimental study, moths exposed to environments with low humidity prefer to consume more diluted nectar than when exposed to environments with higher relative humidity (Contreras et al., 2013). We, therefore, suggest that the increase in EFN-attendant ants as nectar volume increases may represent a strategy by the ants to satisfy their need for water in these dry environments. In this way, ants can change their food preferences in relation to nectar according to their physiological demands.

The two most frequent EFN-visiting ant species on individuals of *P. moniliformis* were *Camponotus crassus* (46% of all ant visits; Fig. 1B) and *Dorymyrmex thoracicus* (18% of all ant visit; Fig. 1C). In a study carried out in the same study area, *C. crassus* and *D. thoracicus* ants were found to be highly dependent on extrafloral nectar, establishing their nests closer to EFN-bearing plants than other ant species (Silva et al., 2019). Both ant species are known to have morphological, physiological, and behavioral attributes that allow them to carry larger quantities of nectar (Eisner, 1957) and to exhibit dominant behavior (Eisner, 1957; Belchior et al., 2016; Fagundes et al., 2012, 2017). However, despite the higher ant visitation in plants that secrete nectar in greater volumes, and *C. crassus* and *D. thoracicus* being the main species visiting the EFNs of *P. moniliformis,* neither the volume nor the concentration of extrafloral nectar were related to the predatory activity of ants. The few studies investigating the effect of nectar volume on ants' defensive activity also did not detect a relationship (Fagundes et al., 2017; Oliveira et al., 2021; but see Calixto et al., 2021). On the other hand, many studies have shown that a high sugar concentration in nectar induces an aggressive behavior in ants against natural enemies (Fagundes et al., 2017; Flores-Flores et al., 2018). Thus, our results suggest that because ants are attending plants with higher nectar volume but not plants with higher sugar concentration, this preference for nectar in more volume does not trigger an aggressive response in the ants (see Ness et al., 2009; Alves-Silva and Del-Claro, 2013).

The volume of extrafloral nectar secreted by *P. moniliformis* was greater at nighttime, however, the concentration did not vary between these periods. It is not uncommon for nectar volume to vary without being accompanied by the variation in concentration (Izaguirre et al., 2013; Oliveira et al., 2021). Some factors can modulate the daily responses of nectar secretion in these plants. Extrafloral glands are generally active throughout the day (Bentley, 1977), but in arid and semi-arid ecosystems EFNs are more active at nighttime (Dáttilo et al., 2015). During the daytime, the temperature is very high, and the water contained in the nectar can evaporate (Nicolson et al., 2007). Additionally, this daily variation in the volume of secreted nectar was not accompanied by a daily variation in ant predatory activity. However,

four out of the seven ant species that attacked the termites on *P. moniliformis* attacked exclusively at night and only *Azteca* sp. attacked exclusively during the day (Fig. 4). In general, in our study area, ants visit EFN-bearing plants more frequently at night (Silvino & Leal, unpublished data). Thus, this fact of most species attacking at night must be related to the increase in the probability of having more attacks due to a greater visitation of ants in the night, mainly because at nighttime the temperature in Caatinga areas is milder.

In general, ants attacked few termites in both attack experiments performed in our study (i.e., 4.1% only during the day, and 6.4% in the day-night comparison). This pattern is consistent with another study carried out in the same study area, where ants attacked only 5.5% of offered termites (Oliveira et al., 2021). In fact, plants with EFN in dry areas seem to receive less benefit than plants in more humid environments (Nogueira et al., 2012; Oliveira et al., 2021; but see Leal and Peixoto, 2017). In areas of Caatinga, plants are not limited by light availability and increase photosynthetic rates at least during the rainy season and, consequently, increase the production of carbohydrates available for nectar (Lüttge, 2013). Thus, contrary to previous speculation (e.g., Pyke, 1991; Heil et al., 2000), nectar production may be cheaper, even in the absence of an efficient protection service. In this scenario, ants can act in an opportunistic way, feeding on nectar but not giving any benefits in return (Bronstein, 1994; Jones and Callaway, 2007), which may result in higher levels of herbivory in plants (Del-Claro et al., 2016).

In synthesis, our study contributes to our understanding of the context-dependence of interactions between EFN-bearing plants and ants. Ants' physiological demand for water in dry environments, such as in the Caatinga dry forest, presumably influences their preference for greater nectar volume, rather than higher sugar concentration. However, this preference did not result in increased predatory ant activity, and ants act opportunistically, consuming nectar but not providing an anti-herbivory service. In addition, time of day (i.e., day vs night) affects the volume of nectar produced by individuals of *P. moniliformis*, but does not influence the sugar concentration of nectar, nor does it affect the predatory activity of ants. Our findings reinforce the conditional and complex nature of this interaction. Further studies are needed to assess the natural history and the costs and benefits for each partner involved in these interactions.

Credit author statement

C.S., E.M.S.R. and I.R.L. conceived the project idea and developed the experiment methods; C.S. collected the extrafloral nectar, interaction and ant predatory activity data; C.S., E.M.S.R. and C.H.F.S. collected daily variation in the secretion of extrafloral nectar data; F.M. P.O. and J.I.F.D. collected daily variation in ant predatory activity data. C.S. conducted the statistical analysis and the writing of the manuscript. I.R.L. supervised the development of the project and the writing of the manuscript and provided funds. All authors contributed to the manuscript and approved its submission.

Declaration of competing interest

The authors declare that they have no financial conflict of interest and/or personal relationships that have influenced the execution of this manuscript.

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