The Distraction Function of Extrafloral Nectaries: Keeping Ants Away From Flowers and Preventing Disruption of Pollination

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ABSTRACT

In exchange for extrafloral nectar, ants deter herbivores from the plants, reducing the amount of herbivory they suffered. However, this defensive mutualism can sometimes have negative effects on plants, as ants may also visit flowers, deterring pollinators and reducing plant fitness. The Distraction Hypothesis posits that extrafloral nectaries (EFNs) have the function of attracting ants and preventing them from visiting flowers and disrupting pollination. In the present study, we tested this hypothesis in the field by conducting an ant-exclusion experiment in *Ferocactus recurvus* plants. First, we evaluated the effect of ants on pollination. Then, we tested the predictions of the Distraction Hypothesis. Specifically, we hypothesized that ants have a negative effect on plant pollination and that EFNs function to distract ants, keeping them away from flowers and preventing them from disrupting pollination. According to our hypothesis, we found that ant visits to flowers resulted in a decrease in seed production and overall plant fitness. Flowers with ants had fewer seeds and were smaller in size compared to ant-excluded flowers. In accordance with the Distraction Hypothesis, we found that a higher number of EFNs reduced the probability of finding ants on flowers. To minimize ant-pollinator conflict, *F. recurvus* plants produce EFNs with higher sucrose concentrations, which effectively keep ants away from the flowers. Plant width was found to be positively correlated with the number of EFNs and flowers, and there is an “optimal” number of EFNs that attracts a higher number of ants. Overall, our findings highlight the complex and dynamic nature of interactions between plants, ants, and pollinators, and the potential trade-offs that exist between ant protection and pollinator attraction.

Keywords: *Ferocactus recurvus*, Distraction Hypothesis**,** ant-pollinator conflict, pollination disruption, ant-plant interactions, extrafloral nectar.

INTRODUCTION

Certain plant species have developed strategies to mitigate the harmful impacts of herbivores: which can be categorized as direct defenses and indirect defenses. Direct defenses refer to plant traits that directly affects the physiology and behavior of the herbivores, encompassing chemical defenses and spines (Walters, 2010). Indirect defenses are plant traits that attract natural predators of herbivore insects, such as extrafloral nectaries (EFNs) which are secretory structures that provide insects like ants or wasps with sweet secretions as a reward (Bentley, 1977; Rico-Gray & Oliveira, 2007b). The most studied function of EFNs is the defensive function, in which EFNs attract natural predators of herbivore insects such as ants (Del-Claro et al., 1996; Oliveira & Freitas, 2004). In exchange for this reward, ants protect the plants against insect herbivores, reducing the amount of herbivory suffered (Janzen, 1966); and increasing plant performance in terms of growth and reproduction (Chamberlain & Holland, 2009). However, sometimes this mutualism between ants and plants could have negative effects for plants, since ants may also visit the flowers, deterring pollinators and reducing plant fitness, resulting in an indirect cost of mutualism (Assunção et al., 2014; Ness, 2006). Therefore, it has been suggested that EFNs also have the function of distracting ants away from flowers. The Distraction Hypothesis posits that EFNs have the function of attracting ants and preventing them from visiting flowers and disrupting pollination. This hypothesis was first proposed by Kerner (1878), but has received little attention and surprisingly it has been tested in only a few studies (Chamberlain & Holland, 2008; Galen, 2005; Holland et al., 2011; Villamil et al., 2019; Wagner & Kay, 2002). However, only few a studies have considered the role of EFN on pollinator behavior and even fewer on plant fitness (Ness 2006, Nicklen & Wagner 2006, Holland et al. 2011).

Ants visiting EFNs can interfere with pollination in several ways. First, their presence on flowers may reduce the visitation rate or the time pollinators spend on flowers due to their aggressive behavior or simply because their presence could be perceived as a danger for pollinators (Assunção et al., 2014; Junker et al., 2007; Villamil et al., 2019). Second, ants can damage reproductive structures such as pistils when searching for floral nectar (Ashman & King, 2005). Third, they may consume floral nectar without providing pollination services, reducing the attractiveness of flowers to pollinators (Rico-Gray & Oliveira, 2007a), and potentially reducing pollen viability due to cuticular secretions (Beattie et al., 1984; Wagner, 2000).

Ant-plant mutualisms involve costs and benefits, so natural selection should favor plant traits that help mitigate the negative effects of ants, while retaining the anti-herbivory benefits that ants provide to the plants. Some of these mechanisms may include providing high-quality rewards or spatial segregation of EFNs and flowers. For example, plants are known to modify the nectar composition and volume, producing more nutritious nectar or nectar with a higher sugar content (Bixenmann et al., 2011; Heil, 2004; Heil et al., 2001), that could keep ants away from the flowers. Another strategy is to place EFNs away from flowers to avoid potential conflict between ants and pollinators (Raine et al., 2002).

In *Ferocactus recurvus* (Cactaceae), EFNs are located very close to the flowers, and sometimes ants visit the flowers which could increase the chances of a conflict between ants and pollinators. Here we tested the Distraction Hypothesis in the field by conducting an ant-exclusion experiment and observations in *F. recurvus.* We evaluated the ecological implications of this hypothesis addressing the following questions: (a) What is the effect of ants on *F. recurvus* pollination? (b) Do EFNs have the function to distract ants from visiting the flowers? (c) What are the mechanisms that plants of this species evolved to avoid ant-pollinator conflict? We hypothesized that 1) rewarding ants with extrafloral nectar reduce their visitation of flowers, reducing ant-pollinator conflict; 2) plants with more flowers will have more EFNs to distract ants and reduce the negative effects of ant visitation to flowers.

Finally, 3) extrafloral nectar should have a higher concentration of sucrose than floral nectar, making it more attractive to ants.

MATERIALS AND METHODS

Study species

Ferocactus recurvus (Fig. 1a) is an endemic plant that is distributed in the semiarid region of the Tehuacán-Cuicatlán valley, in the states of Puebla and Oaxaca, México. The plant can grow between 10 to 50 cm in height, with a spiral arrangement of ribs and curved red spines. The hermaphrodite flowers have yellow or purple tepals (Arias et al., 2012). *F. recurvus* plants have extrafloral nectaries on the upper part of the plant near the base of the flowers that secrete drops of nectar (Fig. 1b,c), which commonly attract ants feeding on these sweet secretions (Marazzi et al., 2013; Sandoval-Molina et al., 2023). The blooming period starts in October and finishes in March, and mature fruits can be found between March and May (Arias et al., 2012). Flowers have diurnal anthesis and remain open for 2-5 days. They are self-incompatible, and rely on pollinators to produce seeds (Córdova-Acosta et al., 2017). Flowers of *F. recurvus* are visited by flies (Order: Diptera), trips (Order: Thysanoptera), hummingbirds (Trochilidae: Apodiformes), bees (Order: Hymenoptera) and occasionally by ants (Fig. 1d, Order: Hymenoptera). However, only native bees (i.e., *Diadasia* sp. and bees from the Augochlorini tribe) make contact with reproductive structures of the flowers, acting as the effective pollinator of this cactus (Córdova-Acosta et al., 2017).

Study area and experimental design

This research was conducted at the Botanical Garden Helia Bravo Hollis (18°19’54” N, 97°27’21” W) located in the municipality of Zapotitlan Salinas, in the State of Puebla, México. The Botanical Garden is situated in the Tehuacán-Cuicatlán Biosphere Reserve and has an annual average rainfall of 376.4 mm, with two well-defined seasons: the rainy season (June to September) with high levels of inter-annual predictability, and the dry season (October to May). The average annual temperature in the study site is 20.7º C (Valiente, 1991). The vegetation in the study site mainly consists of crassicaule scrub, which is dominated by *Neobuxbaumia tetetzo*, *Ferocactus recurvus* and spiny shrubs such as *Prosopis laevigata*, *Mimosa luisiana,* and *Mamillaria collina* (Zavala-Hurtado, 1982).

We delimited two 50 m transects along the semi-path inside the botanical garden, and selected and labeled 17 individuals of *Ferocactus recurvus*, separated by at least 5 m each other. This design ensured that there were different ant colonies on each selected plant.

Ant activity patterns and plant traits

To examine the activity patterns of ants feeding on the EFNs and visiting the flowers of *F. recurvus*, we conducted three censuses: one in the morning (8 to 10 hrs), one in the afternoon (12 to 16 hrs), and one at night (19 to 23 hrs). During each census, we counted the number of worker ants feeding on the extrafloral nectaries for 60 seconds, and we also recorded the presence or absence of ants on flowers. The presence of ants inside the flowers was represented by 1, and the absence of ants was represented by 0, generating a binary response variable. We used this data to determine the probability of finding ants in flowers, as described in the statistical analysis below. To determine the ant species composition, we collected ants from each plant using entomological tweezers and placed them in 1.5 ml Eppendorf tubes with 70% ethanol for preservation. We identified the ants in the laboratory with the aid of taxonomic keys (Fisher et al., 2007; Mackay & Mackay, 1989), and with the assistance of the entomological collection IEXA at the Instituto de Ecología, A.C. Xalapa, where specimens were deposited.

We also measured the heigh and width of each plant selected and counted the number of flowers, and the number of extrafloral nectaries active per plant. We considered as active extrafloral nectaries those in which ants were observed feeding directly on them.

Impact of ants on plant fitness

To determine the effect of ants visiting flowers on plant fitness, we conducted an ant-exclusion experiment. We selected 2 to 4 flowers per plant, randomly assigned as control and treatment each of them. Ant-exclusion treatment consisted in surround the flower with a tiny band of Tanglefoot (Tanglefoot Co., MI, USA) at the base to avoid the entrance of ants but allowing pollinators visitation. Control flowers were left intact, allowing the access of ants and pollinators. Prior to the application of the treatment, we removed ants, as well as natural object from the plants (e.g., sticks, adjacent herbaceous plants, spines) that could be used by ants as a bridge to the excluded flowers. We preferred this method instead of destroying EFNs because damage can lead to changes in EF nectar composition and volume (Heil et al., 2001). Additionally, occluding the EFNs is challenging due to the plant's architecture. Three months after, we collected the ripe fruit that survived (N=30) in plastic bags and transported them to the laboratory. Then fruits were dissected to extract, wash, and dry the seeds. We obtained the wet and dry weight of the seeds using a balance with a precision ± 0.1 μg. The dried seeds were then scanned with a multifunctional office scanner (Hewlett Packard, USA) at higher resolution possible (1200 dpi), and the images obtained were analyzed using the software SmartGrain (Tanabata et al., 2012). This software allows to count the number of seeds of each fruit and calculate seed area, length, and width of each individual seed.

Floral and extrafloral nectar sucrose concentration

In order to analyze for differences in sucrose concentration of both floral and extrafloral nectar, samples were collected directly from plants in the field and in a greenhouse. For floral nectar (N=10), early in the morning we covered the flowers with fabric to exclude pollinators and allowed nectar to accumulate. Later, in the afternoon, we collected nectar from these flowers using 2-µL microcapillary tubes (Drummond Scientific, USA). For extrafloral nectar (N=6), we isolated EFNs using Tanglefoot and allowed nectar to accumulate for 24 hours. Then we collected the nectar from each plant using 2-µL microcapillary tubes (Drummond Scientific, USA). Due to the low volume of extrafloral nectar, we pooled all nectar secreted by each plant for 7 days to estimate the sucrose concentration in the laboratory. Microcapillary tubes with nectar were placed in eppendorf tubes in an ice-filled cooler and transported to the Laboratorio de Ecología de Comunidades y Poblaciones at Universidad Autónoma del Estado de México (UAEMex). The eppendorf tubes were stored at -20°C until they were analyzed using high-performance liquid chromatography (HPLC). Sucrose was quantified using standard curves, with pure standards purchased from Sigma-Aldrich (St. Louis, MO) using a Waters 717 liquid chromatograph with autosampler, Waters 2487 HPLC Absorbance UV-Vis Detector, Waters 1525 Binary HPLC Pump, Waters control module with SAT/IN Bus (Waters, Milford, MA, USA), and an HPLC carbohydrate column (Aminex HPX-87N, BioRad, Hercules, CA, USA). The chromatographic method was isocratic with 100% HPLC-grade water at a flow rate of 0.6 mL/min for 10 min.

Statistical analysis

All statistical analyses were performed using R (RCoreTeam, 2022; RStudioTeam, 2022). We fitted three different models: two generalized linear mixed models (GLMMs, i-ii), and one generalized linear model (GLM, iii). For the number of ants foraging on EFNs: (i) we used a negative binomial distribution with a “log” link function. For the probability of visiting ants to flowers: (ii) we predicted the probabilities using a binomial error distribution with a “logit” link function, and modeled these probabilities using beta regression, which is useful for proportional data (Douma & Weedon, 2019). For the number of EFNs: (iii) we used a negative binomial with a “log” link function. We included the plant ID number as random component on each GLMM previously listed.

We fitted all models using the *glmmTMB* package in R (Brooks et al., 2017). We then checked the goodness of fit of the models using the scaled residuals created with the R package *DHARMa* (Hartig, 2018). We generated the plots using *ggeffects* (Lüdecke, 2018) and *ggplot* (Wickham, 2016). Pairwise comparisons were made using the *emmeans* package (Lenth, 2016).

To test for differences in nectar concentration between floral and extrafloral nectar we used a t-test. For the number of seeds, dry weight, area, length and width of the seeds between treatments, we performed the t-tests. Prior to the paired comparison test, we checked for normality of each variable using a Shapiro-Wilk test and an F test for homogeneity of variances. Due to error detection of *SmartGrain* software (very small objects), some morphological seed traits were deemed as outliers and were removed from the dataset prior the analysis.

RESULTS

Ant foraging activity, EFNs and flowers

Ants foraging on EFNs: We observed a turnover in ant species composition foraging on extrafloral nectaries between day and night. During daylight, we found three ant species associated with EFNs: *Camponotus rubrithorax*, *Crematogaster distans*, and *Brachymyrmex musculus*. During the night we found the same species, but we also found *Camponotus atriceps*. During our surveys, we found that some plants were visited by two ant species foraging on EFNs at the same time.

We found strong evidence that the number of EFNs influences the abundance of ants foraging (Fig. 2a, $X^2 = 21.03$, $P < 0.001$). Plants bearing from 30 to 40 EFNs had a greater number of ants than those with 0 to 20 ($P = 0.01$), and 40 to 51 extrafloral nectaries ($P = 0.004$). Plants bearing from 0 to 20 EFNs tended to have fewer ants than those with 20 to 30 active EFNs ($P = 0.07$). There was no evidence supporting the effect of the number of flowers on the number of ants foraging on EFNs.

We found strong evidence that the number of ants foraging on EFNs was influenced by the time of day, as it varied throughout the sampling hours (Fig. 2b, $X^2 = 29.62$, $P = 0.003$). During the daytime when anthesis occurs, we observed two peaks of ant activity at 10 and 15 hrs, whereas at night, the peak was observed at 20 hrs.

Probability of ants visiting flowers

We found strong evidence that the time of day influences the probability of ant visitation of flowers, being lower for most of the time during anthesis (Fig. 2b, $X^2 = 270.08$, $P <0.001$), but this probability was higher in the morning (8 hrs) and afternoon (16 hrs). We found strong evidence that the probability of ants visiting flowers increases with the number of flowers (Fig. 2c, $X^2 = 27.58$, $P <0.001$). We found strong evidence that the probability of ant visitation decreases when plants had a greater number of active EFNs ($X^2 = 9.12$, $P = 0.003$; Fig. 2d).

Effect of ants on seed set

Flowers that were excluded from ants produced 54.47% more seeds than control flowers with ants (mean ± SD: Control, 1376.8 ± 615.34; Ant-excluded: 2126.8 ± 589.54; t = -3.07, $P = 0.004$; Fig. 3a). Seeds of fruit from flowers without ants had greater dry weight (t = -2.65, $P = 0.01$; Fig. 3b), area (t = -2.25, $P = 0.02$; Fig. 3c). Seeds of fruit from flowers without ants had a greater width (t = -15.81, $P <0.001$; *S1* Fig. 1a) than those visited by ants.

However, seeds from ant-excluded flowers were smaller in length than those from flowers accessed by ants (t = 4.97, *P <0.001; S1* Fig. 1b).

Nectar sucrose concentration

We found that extrafloral nectar was 3.46 times more concentrated in sucrose than floral nectar (Mean ± SD: EFN: 0.52 ± 0.1; Floral: 0.15 ± 0.08; t = 7.28*, P <0.001;* Fig. 4a*).*

EFNs, flowers and plant size

Number of EFNs and flowers: We observed a trend towards an increase in the number of active EFNs with the increasing number of flowers, which was marginally significant (X^2 =3.46, P = 0.06; Fig. 4b).

Number of EFNs and plant height/width: We found strong evidence of a significant positive association between the number of EFNs (X^2 =6.62, P = 0.01) and the number of flowers (X^2 =691, P = 0.009; Fig. 4c) with plant width. We found a marginally significant positive association between the number of EFNs and plant height (X^2 =3.72, P = 0.054; Fig. 4d), but we found no significant association between the number of flowers and plant height.

DISCUSSION

Ants provide an effective defense against insect herbivores, but can also interfere with plant-pollinator interactions (Cembrowski et al., 2014; Ness, 2006; Unni et al., 2021). Thus, plants with EFNs must balance the benefits of ant protection against herbivores with the potential costs of ant interference with pollinators. One potential solution is to distract ants away from flowers by increasing the number of EFNs or modifying their reward production. However, the distraction function of EFNs has received little attention in the literature, and only a few studies have tested. The Distraction Hypothesis states that EFNs attract ants away from flowers, thus preventing them from interfering with pollination (Chamberlain &

Holland, 2008; Galen, 2005; Villamil et al., 2019; Wagner & Kay, 2002). Our results support this hypothesis, indicating that the EFNs of *F. recurvus* reduce the probability of finding ants on flowers by increasing EF nectar quality, preventing them from disrupting plant-pollinator interactions. Additionally, the number of EFNs and flowers per plant is linked and depends on the width of the plant. Therefore, produce an optimal number of flowers in response to the number of active EFNs to reduce the conflict between ants and pollinators.

Impact on plant pollination and fitness

Ants visiting EFNs can interfere with plant pollination, reducing plant fitness. This interference may be particularly important in plants with a higher degree of pollinator specialization, such as some *Ferocactus* species, whose flowers are pollinated almost exclusively by cactus-specialist bees like *Diadasia rinconis* (McIntosh, 2005). Our study found that the reduction in the number and size of seeds produced by flowers visited by ants may be associated with a lower rate of bee visits, resulting in less pollen deposition on flower stigmas and ultimately reducing the number of seeds produced, as well as their size and weight, traits associated with seed germination and survival (Saeed & Shaukat, 2017).

Although the precise mechanisms by which ants interfere with pollination remain unclear, it is possible that their presence on flowers deters bees, resulting in a decrease in their visitation rate and the time spent on the flowers. Similar effects have been observed in other *Ferocactus* species. For instance, in *F. viridescens*, where the ant species *Linepithema humile* were present on the flowers, the effective pollinating bees *Diadasia* sp. spent less time on the flowers and produced fewer seeds per fruit (LeVan et al., 2014). Similarly, in *F. wislizeni,* plants attended by the more aggressive ant species *Solenopsis xyloni* were visited less frequently, had shorter visitation times, and produced fewer and lighter seeds than those visited by other ant species (Ness, 2006).

Another possibility for the negative effect of ants on flower pollination is the physical damage caused by ants while searching for nectar. Ashman & King (2005) reported that ants can damage the pistils of flowers during their foraging activities. In addition, ants have cuticular secretions that can reduce the viability of pollen (Beattie et al., 1984; Wagner, 2000). The antibiotics produced by ants in their metapleural glands can render pollen unviable (Beattie et al., 1986; Wagner, 2000), which could explain the reduction in pollen viability and subsequent decrease in seed production observed in our study. Further research is necessary to explore the specific mechanisms by which ants interfere with flower pollination, including examining ant behavior and assessing any damage caused to reproductive structures by ants.

Mechanisms to reduce plant-pollinator conflict

Plants must deal with a possible trade-off between the benefits of ant protection and the indirect costs of ants foraging on EFNs. Therefore, natural selection should favor plant traits that minimize the negative effects of ants while retaining the anti-herbivory benefits. Several mechanisms could reduce the ant-pollinator conflict, such as changes in reward quality or spatial segregation of EFNs and flowers. Producing EF nectar with a higher sugar content (Bixenmann et al., 2011; Heil, 2004; Heil et al., 2001), may keep ants away from the flowers, reducing the ant-pollinator conflict and enhancing the defensive protection of ants against herbivores (González-Teuber et al., 2012; Heil, 2013). Although a manipulative experiment on sugar concentrations in the field would be more appropriate to reveal how sugar concentrations play a role in the distraction hypothesis, we found that the sucrose concentration in the EF nectar was higher than in the floral nectar. This suggests that during the flowering season, *F. recurvus* plants secrete more concentrated nectar to attract ants, preventing them from visiting the flowers and increasing their defensive response against herbivores. Further experiments varying the concentration of sugars are needed to understand whether varying sucrose concentrations lead to a reduction in ant visitation to flowers.

Another strategy adopted by plants is to place EFNs away from flowers to avoid possible conflicts between ants and pollinators (Raine et al., 2002). However, this is not the case for *F. recurvus,* in which EFNs are located at the top of the plant where the flower buds grow. The Optimal Defense Theory posits that plants should allocate more resources to defend their most valuable parts, such as reproductive tissues, since they are directly related to reproductive success and their loss is important in terms of fitness (McKey, 1979; Rhoades, 1979). The fact that EFNs are located close to valuable and vulnerable reproductive structures supports the assumptions of the Optimal Defense Theory. Thus, it is possible that for *F. recurvus,* EFNs could also act as defense and distraction at the same time. Although the defensive function of EFNs has been widely documented in different ant-plant systems (Chamberlain & Holland, 2009; Koptur, 2005; Rico-Gray & Oliveira, 2007b), the defensive function of the nectaries in this species of cactus has not been proven yet.

Natural selection should also favor plants with a higher number of EFNs to reduce ant visitation to flowers as Wagner & Kay (2002) suggest. Our results show that plant width was positively correlated with the number of EFNs and flowers. Interestingly, we also found a positive association between the number of EFNs, and the number of flowers produced per plant. This evidence suggests that *F. recurvus* plants allocate their resources to produce flowers depending on the number of active EFNs in the plant and the resources available in their environment. This way, plants can reduce the negative effect of ants on flowers, retain the defensive benefits of ants foraging on EFNs, and increase their reproductive capacity.

Our findings suggest that there is an optimal number of EFNs that attract a higher abundance of ants foraging on the plant. Producing many EFNs may be energetically costly for the plant and may attract more ants than necessary, leading to increased competition for resources and potential negative effects on pollination. Therefore, plants may balance the number and quality of their EFNs to optimize their anti-herbivore defense and minimize the negative effects of ants on pollination. This trade-off between anti-herbivore defense and pollinator attraction is a complex process and is likely influenced by various ecological and environmental factors.

In this study, we tested the Distraction Hypothesis and analyzed the effect of EFNs on plant-pollinator-ant interactions in the cactus species *Ferocactus recurvus*. We found that ant visits to flowers reduced the number and size of seeds produced, impacting plant fitness. Therefore, plants must adopt mechanisms to reduce ant-pollinator conflict. One solution adopted by *F. recurvus* plants is producing EFNs with a higher quality or higher sugar concentration to keep ants away from the flowers. In accordance with the Distraction Hypothesis, we found that a higher number of EFNs reduced the probability of finding ants on flowers. Plant size was positively associated with the number of EFNs and flowers, with stronger evidence for plant width than height. The proximity of EFNs to reproductive structures supports the prediction of the Optimal Defense Theory, since flowers are directly related to reproductive success, and their loss can have a significant impact on fitness. Overall, these findings highlight the complex and dynamic nature of interactions between plants, ants, and pollinators, and the potential trade-offs that exist between ant protection and pollinator attraction in extrafloral nectaries bearing plants.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTIONS

Mario A. Sandoval Molina, and Mariusz Krzysztof Janczur conceived the ideas and designed methods; Mario A. Sandoval Molina, Jessica Rosas-Sánchez, and Mariusz Krzysztof Janczur collected the data; Emilio-Gonzalez Camarena performed laboratory analysis; Mario A. Sandoval Molina analyzed the data; Mario A. Sandoval Molina, and Mariusz Krzysztof Janczur led the writing of the manuscript; Mario A. Sandoval Molina, and Mariusz Krzysztof Janczur reviewed and edited the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

All data used in this research are available without restriction in the Harvard Dataverse database: Sandoval, Mario, 2023, "Replication Data for: The Distraction Function of Extrafloral Nectaries: Keeping Ants Away From Flowers and Preventing Disruption of Pollination in *Ferocactus recurvus*", https://doi.org/10.7910/DVN/OUF6WT, Harvard Dataverse.

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Table 1. Effects of the number of flowers, number of EFNs and time of day on the number of ants, the probability of ant visiting the flowers and the number of EFNs. Significance levels were calculated with an ANOVA type III. Significant values are shown in bold.

Analysis of Deviance Table (Type III tests)

Number of ants foraging			
Variables	*Chisq*	*Df*	*P.value*
Number of flowers	0.01	1	0.919
Number of EFNs	21.03	3	**<0.001**
Time of day (hours)	29.62	12	**0.003**
Probability of ants visiting the flowers			
Number of flowers	27.58	1	**<0.001**
Number of EFNs	9.12	1	**0.003**
Time of day (hours)	270.08	7	**<0.001**
Number of EFNs			
Number of flowers	3.46	1	0.063
Plant width	6.62	1	**0.01**
Plant height	3.72	1	0.054
Number of flowers			
Plant width	691	1	**0.009**
Plant height	0.63	1	0.42

Figure 1. *Ferocactus recurvus* and extrafloral nectaries. (a) An individual with flower buds in the apical part, with spiral arrangement of ribs and spines. (b) Drops of nectar on the tip of extrafloral nectaries. (c-d) Ants of *Camponotus rubrithorax* and *Crematogaster distatus* feeding on extrafloral nectar.

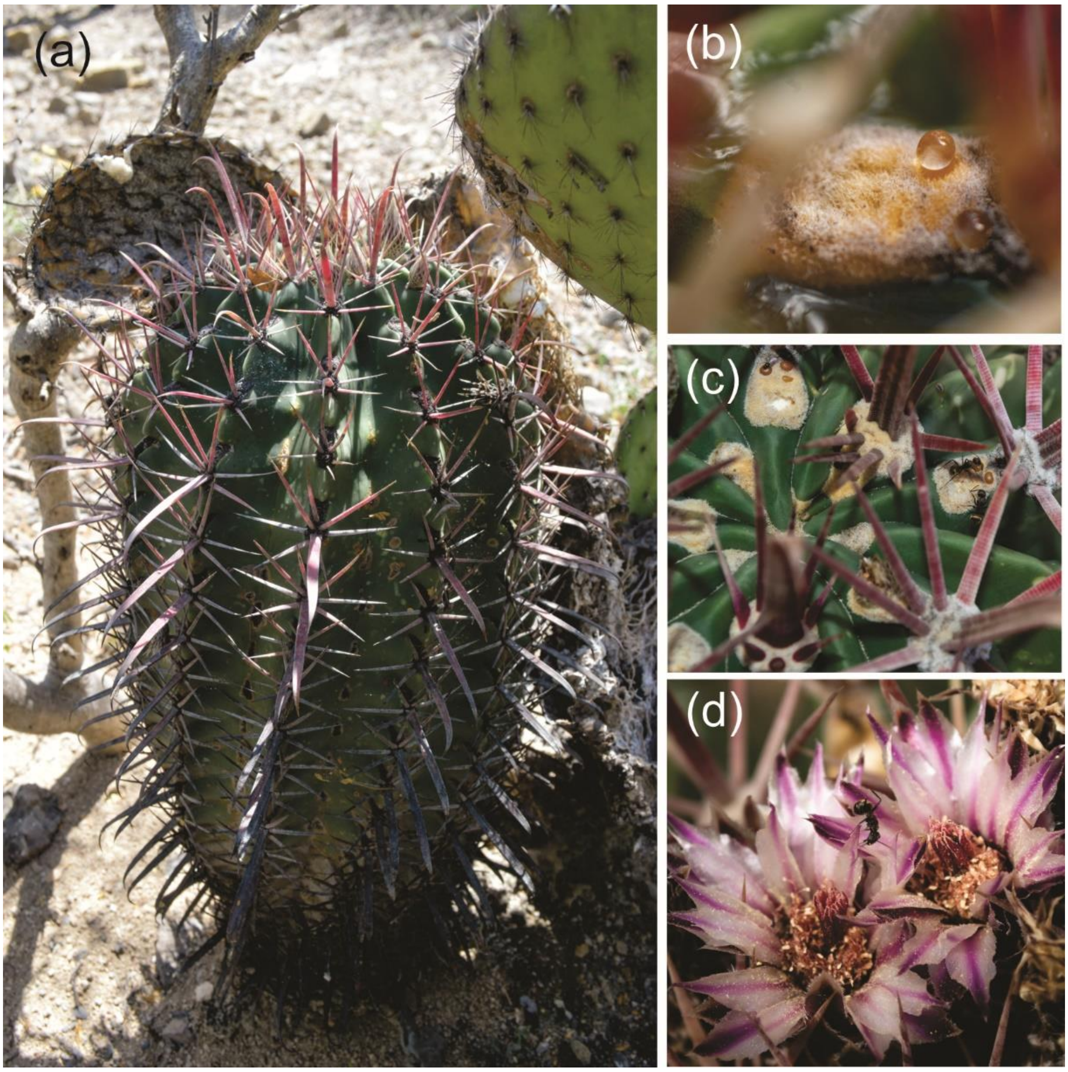


Figure 2. Number of ants foraging on extrafloral nectaries and probability of ants visiting flowers of *Ferocactus recurvus*. (a) Number of ants foraging in association with the number of EFNs. (b) Activity patterns of foraging ants and the probability of finding ants in the flowers through the day. Dashed line: number of ants foraging on EFNs. Continuous line: Probability of ants visiting flowers. (c) Probability of ants visiting flowers in relation to the number of flowers on the plant. (d) Probability of ants visiting flowers in relation to the number of EFNs on the plant. In Fig. (a), values represent mean ± SE. The shading in (c) and (d) represents the 95% confidence intervals.

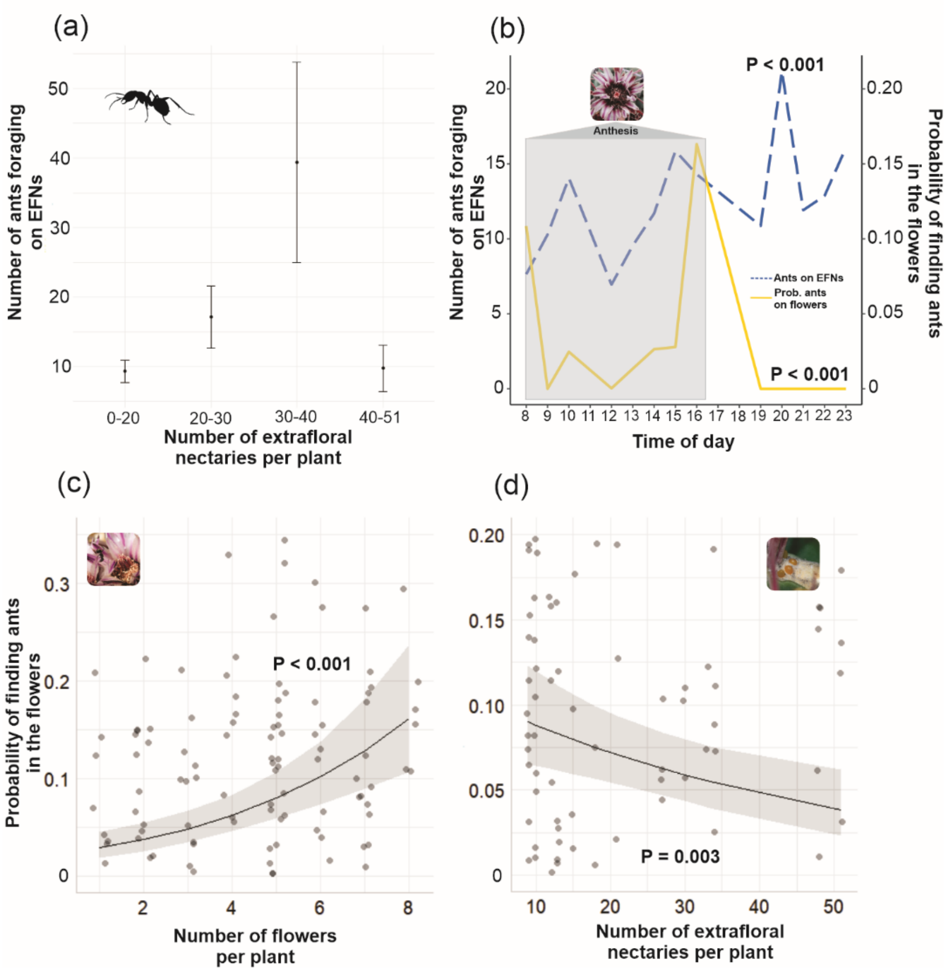


Figure 3. Seeds of *Ferocactus recurvus* obtained after an ant-exclusion experiment. (a) Number of seeds per fruit, (b) dry seeds weigth, and (c) seed area between ant-excluded and control treatments. Values represent mean ± 95% confidence intervals.

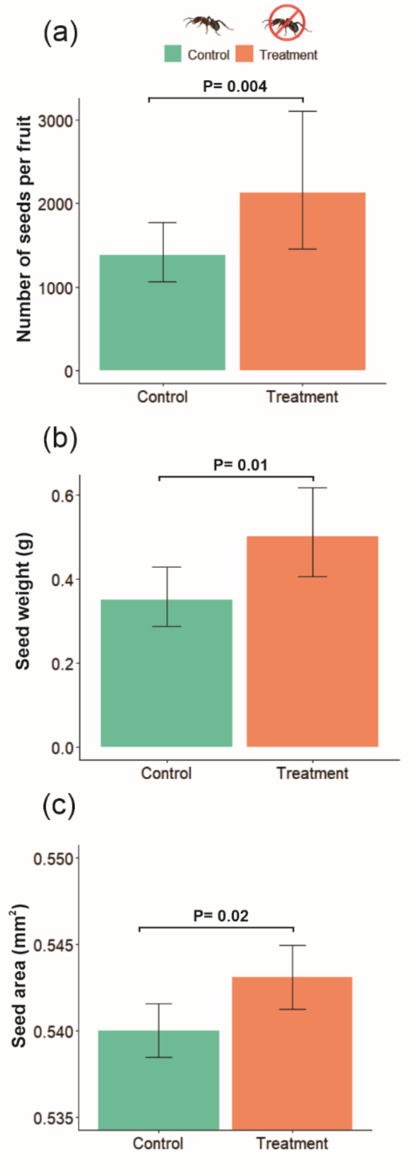
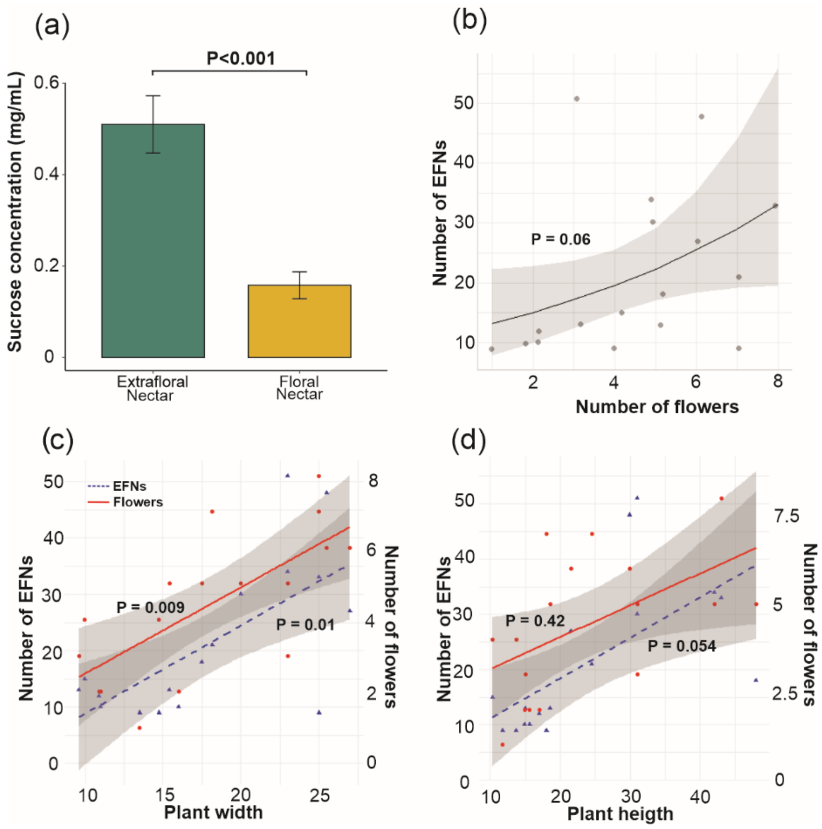


Figure 4. Sucrose nectar concentration and plant traits. (a) Difference in sucrose concentration between extrafloral and floral nectar of *Ferocactus recurvus.* Values represent mean ± SE. (b) Relationship between EFNs and flowers. (c-d) Effect of plant width and height on the number of EFNs (dashed line) and number of flowers (continuous line).



S1 Supporting information.

Table 1. Model statistics showing the effect of number of flowers, extrafloral nectaries per plant, on the number of ants foraging in *Ferocactus recurvus* plants. We showed the post-hoc contrasts for number of extrafloral nectaries, but we only showed significant differences between hours. Significant values are shown in bold.

	Number of ants			
Predictors	*Incidence Rate Ratios*	*std. Error*	*Statistic*	*p*
(Intercept)	2.209	0.264	8.362	**<0.001**
Number of flowers	-0.007	0.065	-0.101	0.919
Number of EFNs [20-30]	0.716	0.293	2.443	**0.015**
Number of EFNs [30-40]	1.595	0.377	4.227	**<0.001**
Number of EFNs [40-51]	0.175	0.365	0.479	0.632
Observations	237			
Marginal R^2 / Conditional R^2	0.356 / 0.546			

Post-hoc contrasts

Number of extrafloral nectaries

contrast	*ratio*	*SE*	*df*	*t.ratio*	*p.value*
0-20 / 20-30	0.488	0.143	230	-2.440	0.072
0-20 / 30-40	0.202	0.076	230	-4.220	**<0.001**
0-20 / 40-51	0.839	0.306	230	-0.470	0.963
20-30 / 30-40	0.415	0.163	230	-2.220	0.119
20-30 / 40-51	1.717	0.672	230	1.382	0.511
30-40 / 40-51	4.137	1.743	230	3.369	**0.004**

Table 2. Model statistics for the logistic regression showing the effect of the number of EFNs and number of flowers on the probability of ants visiting the flowers of *Ferocactus recurvus*. Significant values are shown in bold.

	Probability of ant visitation			
Predictors	*Estimates*	*std. Error*	*Statistic*	*p*
(Intercept)	-3.447	0.253	-13.646	**<0.001**
Number of flowers	0.265	0.051	5.252	**<0.001**
Number of extrafloral nectaries	-0.022	0.007	-3.021	**0.003**
Observations	168			
Marginal R^2 / Conditional R^2	0.968 / 0.998			

Table 3. Model statistics for the logistic regression showing the probability of ants visiting the flowers of *Ferocactus recurvus* during daylight when anthesis occurs. Significant values are shown in bold.

	Probability of ant visitation			
Predictors	*Estimates*	*std. Error*	*Statistic*	*p*
(Intercept)	-2.106	0.305	-6.908	**<0.001**
Hour [9]	-8.731	0.270	-32.389	**<0.001**
Hour [10]	-1.573	0.044	-35.879	**<0.001**
Hour [12]	-5.994	1.007	-5.954	**<0.001**
Hour [13]	-2.192	0.040	-54.986	**<0.001**
Hour [14]	-1.503	0.035	-43.339	**<0.001**
Hour [15]	-1.447	0.034	-42.469	**<0.001**
Hour [16]	0.471	0.031	15.429	**<0.001**
Observations	168			
Marginal R^2 / Conditional R^2	0.789 / 0.998			

Table 4. Model statistics showing the effect of the number of flowers on the number of EFNs active on *F. recurvus* plants. Significant values are shown in bold.

	Number of EFNs			
Predictors	*Log-Mean*	*std. Error*	*Statistic*	*p*
(Intercept)	2.450	0.327	7.487	**<0.001**
Flower nr	0.131	0.067	1.970	**0.049**
Observations	17			
R^2 Nagelkerke	0.263			

Table 5. Model statistics showing the effect of plant height and width on the number of flowers and number of EFNs active on *F. recurvus* plants. Significant values are shown in bold.

	Number of EFNs				Number of flowers			
Predictors	*Log-Mean*	*std. Error*	*Statistic*	*p*	*Log-Mean*	*std. Error*	*Statistic*	*p*
(Intercept)	1.526	0.323	4.719	**<0.001**	0.402	0.326	1.234	0.217
Plant width	0.052	0.019	2.686	**0.007**	0.048	0.018	2.655	**0.008**
Plant height	0.020	0.009	2.153	**0.031**	0.007	0.009	0.799	0.424
Observations	17				17			
R^2 Nagelkerke	0.863				0.599			

S1 Fig 1. Seeds of *Ferocactus recurvus* obtained after an ant-exclusion experiment. (a) seed width, and (b) seed length between ant-excluded and control treatments. Values represent mean ± 95% confidence intervals.