



# Consequences of ants and extrafloral nectar for a pollinating seed-consuming mutualism: ant satiation, floral distraction or plant defense?

J. Nathaniel Holland, Scott A. Chamberlain and Tom E. X. Miller

*J. N. Holland (jhollandiii@gmail.com), S. A. Chamberlain and T. E. X. Miller, Dept of Ecology and Evolutionary Biology, Rice Univ., 6100 S. Main St., Houston, TX 77005, USA.*

Non-pollinating consumers of floral resources, especially ants, can disrupt pollination and plant reproductive processes. As an alternative food resource to flowers, extrafloral nectar (EFN) may distract and satiate ants from flowers, thereby reducing their antagonistic effects on plants. Yet, EFN may actually attract and increase ant density on plants, thus increasing the disruption of pollination and/or their defense of plants. In this study, we tested the effects of ants and EFN on pollinating seed-consuming interactions between senita cacti and senita moths in the Sonoran Desert. Prior study of senita showed that EFN can distract ants from flowers, but consequences for plant–pollinator interactions remain unstudied. In our current study, ant exclusion had no effect on pollination or oviposition when moths were abundant (>85% flower visitation). Yet, in an ant by EFN factorial experiment under lower moth abundance (<40% visitation), there was a significant effect of ant exclusion (but not EFN or an ant  $\times$  EFN) on pollination and oviposition. In contrast with our predictions, ant presence increased rather than decreased pollination (and oviposition) by moths, indicating a beneficial effect of ants on plant reproduction. While ant density on plants showed a saturating response to continuous experimental variation in EFN, in support of ant satiation and distraction, the probability of pollination and oviposition increased and saturated with ant density, again showing a beneficial effect of ants on plant reproduction. Ants showed no significant effect on fruit set, fruit survival, or fruit production of oviposited flowers in the ant exclusion experiment. Ants did not affect the survival of moth larvae, but there was a marginally significant effect of ants in reducing wasp parasitism of moths. We suggest that EFN may not only distract ants from disrupting plant–pollinator interactions, but they may also enhance plant–pollinator interactions by increasing pollination and reducing wasp parasitism. Though often considered dichotomous hypotheses, ant distraction and plant defense may be synergistic, though the mechanism(s) for such positive ant effects on plant–pollinator interactions needs further study.

Ants interact with plants in a variety of ways, ranging from parasitism to mutualism and including herbivory, seed dispersal, and plant defense (Rico-Gray and Oliveira 2007). In many ant–plant interactions, extrafloral nectar (EFN) is a key food resource that mediates pairwise and community-level interactions of many diverse plants (Koptur 1992, Heil and McKey 2003, Rudgers and Gardner 2004). The production of EFN occurs in >25% of angiosperm families and in more than 330 plant genera throughout terrestrial ecosystems of the world (Koptur 1992). While the presumably repeated, convergent evolution of extrafloral nectaries suggests a common function among plants, multiple hypotheses occur for their utility. The defense (or protection) hypothesis proposes that EFN resources serve as an indirect plant defense that attracts and rewards ants (and other arthropods) for their resistance against natural enemies (e.g. herbivores, florivores, pre-dispersal seed predators, plant competitors). Extension of optimal defense theory for plant secondary

compounds to EFN (Wäckers and Bonifay 2004, Holland et al. 2009), along with recent meta-analyses of ant effects on plants (Chamberlain and Holland 2009, Rosumek et al. 2009), is providing ever greater support for the generality of ant–plant defensive mutualisms. Yet, for studies in which ants do not have a strong effect on plant defense, alternative hypotheses have been sought to explain the utility of EFN. The nutrition hypothesis proposes that, if ants build their nests in or near plants supplying EFN, then nutrients from the nests (e.g. ant debris, waste products) may enhance plant nutrition and performance (Wagner 1997, Sagers et al. 2000, Wagner and Nicklen 2010). The distraction hypothesis, on the other hand, proposes that EFN resources divert ants and other non-pollinating insects away from flowers, thereby reducing the disruption of pollination and plant reproduction (Wagner and Kay 2002, Galen 2005). While EFN may have evolved in the past for one particular function, EFN may well have an alternative function under current ecological

conditions (Galen 2005). Moreover, these hypotheses for the current function of EFN are not mutually exclusive.

Central to the distraction hypothesis are the well-established antagonistic effects of ants on plant reproduction, including pollen consumption, reduction in pollen viability, pollinator interference, the consumption of floral nectar, and florivory (Galen 1983, Beattie et al. 1984, Rico-Gray 1993, Visser et al. 1996, Puterbaugh 1998, Galen 1999, Ness 2006). As an alternative resource, EFN can decrease ant exploitation of flowers and their negative effects on pollination and plant reproduction by diverting and distracting individual ants away from foraging on floral resources and by satiating ants with EFN. Reduced exploitation of flowers, however, depends not only on the satiation of individual ants, but also on a saturating density response of ants to the supply of EFN resources. If ant density increases linearly or in some otherwise non-saturating way with EFN resources, then EFN may not reduce antagonistic effects of ants on pollination and plant reproduction, as the aggregative density of ants on plants will simply be proportional to available resources (EFN and flowers). While support occurs for ant distraction and satiation in artificial ant–plant systems (Wagner and Kay 2002, Galen 2005), empirical studies of natural systems are needed that examine the effects of EFN and ant–plant interactions on plant–pollinator interactions, including whether the aggregative density response of ants varies with the supply of EFN resources, and as a result, how plant–pollinator interactions vary with ant density.

In this study, we examined the consequences of EFN-mediated ant–plant interactions for plant–pollinator interactions using the pollinating seed-consuming mutualism between senita cacti and senita moths in the Sonoran Desert of North America (Holland and Fleming 1999, Holland 2002). Senita moths not only actively pollinate stigmas during flower visitation, but they also lay a single egg on flowers, larvae of which then consume developing ovules and fruit. Prior study of senita ant–plant interactions have shown that flowers do not have secondary compounds that deter ant interactions; EFN reduces ant contacts with and time spent in flowers; ant–flower interactions decrease with EFN; and, ants are largely commensalistic with plants as they did not increase bud and fruit survival in the years and populations studied (Chamberlain and Holland 2008). While these results support the distraction (rather than plant defense) hypothesis for EFN in senita, studies need to explicitly test the consequences of EFN and ants for plant–pollinator interactions. For the senita system in particular, how do pollination and oviposition rates by senita moths vary with the presence and absence of ants? Does the aggregative density response of ants saturate with the supply of EFN resources and, in turn, do ant effects on plant–pollinator interactions co-vary with the ant density response to EFN? Do ants increase fruit production and moth larval survival, and do ants deter wasp parasitism of moth larvae? We addressed these questions using a series of experiments ranging from categorical (with, without) manipulations of ants and EFN to experimental continuous variation in EFN. We evaluated ant density, pollination, oviposition, larval survival, and wasp parasitism in response to these experiments.

## Methods

### Study system

Our studies occurred near Bahia de Kino, Sonora, Mexico (28°53'N, 111°57'W) during the spring and summer flowering months of 2007. Senita cactus *Pachycereus schottii*, Cactaceae is a long-lived (>75 years), multi-stemmed columnar cactus reaching heights of 2 – 5 m. Plants produce thousands of buds, flowers and fruits from spine-bearing areoles during each flowering season from April to August. Hermaphroditic flowers have an inferior ovary with 100 – 400 ovules, 100+ anthers, one pistil, and a lobule stigma (Holland and Chamberlain 2007). Senita flowers open at sunset and typically close prior to sunrise, but not more than 12 h later. Only ~50% of flowers produce any nectar at all, with an average 0.5 µl in nectar-producing flowers (Holland and Fleming 1999). Ants do exploit floral resources, especially in the absence of EFN (Chamberlain and Holland 2008). Mature fruits are berry-like, with ~180 seeds per fruit. Senita cacti rely on an obligate pollinating seed-eating moth (the senita moth, *Upiga virescens*; Lepidoptera: Cambidae) for pollination and sexual reproduction, as senita are self-incompatible and co-pollinating diurnal (halictid) bees are rarely influential (Holland and Fleming 2002). Females actively pollinate by rubbing their pollen-covered abdomens directly onto stigmas. After active pollination, female moths usually lay a single egg within the open corolla, such that moth eggs are uniformly distributed among flowers (Holland and Fleming 1999, Holland et al. 2004a). Larvae consume developing seed and fruit tissue before exiting the base of fruits and pupating within cactus stems. Not all eggs survive to produce larvae. Anecdotal observations indicate that ants can occasionally remove eggs from open flowers. Also, 12 – 17% of larvae succumb to a koinobiont endoparasitoid wasp (*Temelucha* sp.; Hymenoptera: Ichneumonidae) (Holland and Fleming 1999). Only ~50% of flowers initiate fruit due to limited resources, but pollen limitation can occur (Holland 2002, Holland et al. 2004b, Holland and Chamberlain 2007). Fruit set occurs within 6 d after anthesis, larvae consume fruit between 8 – 15 d after anthesis, and fruit mature in ~30 d. In sum, ~15 – 30% of immature fruit are lost to moth larvae and other herbivores (Holland and Fleming 2002, Holland et al. 2004b).

Buds, flowers and immature fruits are susceptible to a diverse range of herbivores and florivores, as they largely lack the secondary alkaloid and silica compounds that defend stem tissues (Gibson and Nobel 1986). Buds, flowers and immature fruits produce EFN from the tips of their tepals (see photos in Chamberlain and Holland 2008), which may serve as an indirect plant defense by attracting and rewarding a guild of 14+ species of ants for their resistance against natural enemies (Chamberlain and Holland 2008, Holland et al. 2009). The ant species largely do not nest in senita. EFN production and ant consumption occur mostly during nocturnal hours, which is the time period when flowers are open (Holland et al. 2010).

### Ant exclusion experiment

We conducted an ant exclusion experiment to examine whether the presence or absence of ants altered moth

pollination and oviposition of flowers of senita cacti. We haphazardly chose four stems within each of 31 plants, two of which were assigned to a control (with ant access) treatment and the other two to an ant exclusion (removal) treatment. Data from each of the two stems within each treatment were pooled for analyses. We applied Tanglefoot to the base of each ant exclusion stem, which is successful in removing ants from senita (Chamberlain and Holland 2008). Ant access stems had more ants than ant exclusion stems early in the flowering season (mean  $\pm$  SE; ant access:  $82.2 \pm 14.9$ , exclusion:  $1.1 \pm 0.4$ ,  $t = 25.6$ ,  $DF = 30$ ,  $p < 0.0001$ ), in the middle of the flowering season (ant access:  $43.5 \pm 8.2$ , exclusion:  $0.5 \pm 0.4$ ,  $t = 19.0$ ,  $DF = 30$ ,  $p < 0.0001$ ), and toward the end of the flowering season (ant access:  $47.1 \pm 10.8$ , exclusion:  $1.4 \pm 0.7$ ; Wilcoxon signed-rank test,  $Z = 232.5$ ,  $DF = 30$ ,  $p < 0.001$ ). Given that senita have 50 – 100+ stems per plant, it is unlikely that ant removal on the two exclusion stems artificially created higher ant abundance on the two ant access stems.

Following moth activity at ~23:00 h (Holland and Fleming 1999), we censused all possible flowers (977 flowers among 31 plants over two nights) for their pollination and oviposition status. These data were collected early in the flowering season with high moth densities. Pollination status of flowers was readily assessed by inspecting stigmas for pollen grains with a 10 $\times$  hand lens. Oviposition of flowers was also easily assessed by inspecting corollas for eggs. Due to insufficient flowering in some plants, our sample size was reduced to 28 plants. As both the control with ants and ant exclusion treatments were applied to each replicate plant, we used paired difference t-tests to evaluate the effects of ants on moth pollination and oviposition. To assess if ants were consuming eggs laid in flowers, we compared the difference between the proportion of flowers pollinated and the proportion of flowers with eggs for the control and ant exclusion treatments using a t-test.

### **Ant exclusion effects on fruit set, fruit survival, fruit production, larval survival and wasp parasitism**

In the above described ‘ant exclusion experiment’ we also examined the consequences of ant presence and absence on the fate of flowers and larvae of senita moths. We labeled 716 flowers containing a moth egg across 27 plants and followed the fates of the flowers and larvae in terms of fruit set (flowers initiating fruit developing within 6 d of anthesis), fruit survival (immature fruits surviving from 6 – 22 d), fruit production (ripe fruit), larval survival, and the emergence of an adult moth or a wasp parasitoid. Every 2 d we censused the labeled flowers, and between 22 – 25 d we collected stem tissue to census for a moth or wasp pupa. These data were collected later in the flowering season when moths were intermediate in abundance. Wasp parasitism was assessed via differences in pupal form and rearing moth larvae for adult moths or wasp pupae. We evaluated the effects of ant treatments (control, ant exclusion) on fruit set, fruit survival, fruit production, and larval survival using paired difference t-tests, as both treatments occurred within each plant. We used a  $2 \times 2$   $\chi^2$ -test to examine the effects of control and

ant exclusion on the emergence of reared moths versus parasitoid wasps ( $n = 152$ ).

### **Ant/EFN factorial experiment**

We conducted a  $2 \times 2$  factorial experiment to assess if the effects of ants on pollination and oviposition were conditional upon the presence or absence of EFN. The two factors, ants (control with ant access, experimental ant exclusion) and EFN (control with EFN, experimental EFN removal), were fully crossed, resulting in four treatments: (1) control with ant access, with EFN; (2) ant exclusion, with EFN; (3) with ant access, EFN removal; (4) ant exclusion, EFN removal. We assigned each of the four treatments to one stem within each of 31 plants. We used Tanglefoot to exclude ants. Because EFN occurs on buds and immature fruits, we were eliminated EFN from treatment stems by removing buds and fruits. Other methods of EFN removal (e.g. glue on nectary) are not feasible in the senita system. On the EFN removal stems, ~15 mature buds were left intact to census for pollination and oviposition at anthesis. By leaving approximately the same number of flowers per treatment stem, variation in the responses of moths to variation in flower number were minimized. While some buds and thus some EFN remained on treatment stems to measure pollination and oviposition, the treatment did reduce EFN resources, as stems typically have hundreds of buds and immature fruits. Ant–plant interactions were given two days to equilibrate to the treatments, which is sufficient for ants to respond to treatments (Chamberlain and Holland 2008). These data were collected later in the flowering season when moths were intermediate in abundance. We tested if the proportion of flowers pollinated, the proportion of flowers with eggs, and the difference between these (indicating ant egg removal) varied with ant and EFN treatments using a PROC MIXED model with plant as a random factor (SAS Institute 2004). For this analysis, our sample size was reduced to 15 plants for statistical analyses due to low flowering in some treatment stems. However, we also conducted GLMM analyses of a more complete data set (110 observations among 29 plants) assuming a binomial distribution using lmer of the lme4 package of R (R Development Core Team 2009). We report the F-statistics of the PROC MIXED analysis, though qualitative results did not differ between the two different analyses.

We first wish to point out a caveat of our methodology of removing buds and fruits as an EFN treatment. EFN removal by knocking off buds and fruit could potentially induce a defense pathway in senita cacti and in turn influence secondary chemistry or volatile production. Either one of these responses could actually increase ant abundance, though not likely localized to a particular stem within a plant. Our results do not support this caveat (Fig. 2a). Also, prior studies of senita have shown that stems behave independently (Holland and Chamberlain 2007), so the effects of EFN removal on one stem likely do not influence secondary chemistry and volatile production of another stem. Moreover, while removing buds and fruits could influence re-allocation of resources within stems, removing buds and fruit as a way to manipulate EFN is not likely to alter resource allocation between stems of whole plants (Holland and Fleming 2002).

## Ant density responses to continuous variation in EFN and pollination and oviposition responses to ant density

To examine if the aggregative density response of ants varies and in particular saturates with EFN resources, we conducted an experiment in which we manipulated EFN resources along a continuum, rather than categorically as in the ant/EFN factorial experiment. We randomly assigned 66 plants to 17 EFN treatments ranging from 0 – 500 EFN units (buds and fruits): eight replicate plants of 0 EFN; four replicates for each of 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 EFN; and three replicate plants for each of 250, 300, 350, 400, 450 and 500 EFN. EFN abundance was pruned to the treatment level of buds and fruits. Plants with low EFN units could not be assigned to high EFN treatments. Ant-plant interactions were given two days to equilibrate to the treatment, which is sufficient for ants to respond to changes in EFN availability (Chamberlain and Holland 2008). We measured how ant density on plants varied with this continuous variation in EFN resources, and how pollination and oviposition varied with resulting ant densities. These data were collected later in the flowering season when moths were intermediate in abundance.

We examined whether ant density responses were a linear or saturating function of the supply of EFN resources using maximum likelihood estimation (MLE) and Akaike information criteria (AIC) using R and the *bbmle* package (Burnham and Anderson 2002, Crawley 2007, Bolker 2008, R Development Core Team 2009). Ant abundance was described best by a negative binomial distribution (rather than Poisson), as variance was greater than the mean. Using the negative binomial density function, we used MLE of model parameters for linear ( $a + bx$ ) and saturating ( $c + (ax/(b + x))$ ) functions of ant density with EFN resources ( $x$ ), and compared their fits to the data using AIC corrected for sample size ( $AIC_c$ ), as  $n/K < 40$ , where  $K$  is the number of parameters in the model (Burnham and Anderson 2002). We used MLE and  $AIC_c$  to examine whether the probabilities of pollination and oviposition were best described as a constant value, or as a linear or saturating function of ant density. We used a logit transformation,  $y = e^{f(x)} / (1 + e^{f(x)})$ , where  $y$  is the probability of pollination or oviposition,  $f(x) = a$ ,  $f(x) = a + bx$ , or  $f(x) = ax/(b + x)$ , and  $x$  is the empirically observed ant densities of the continuous EFN experiment. Pollination and oviposition models were fit using a binomial density function, where each plant had a number of trials (flowers) and successes (with pollen or eggs), where number of trials ranged from 5 – 20 per plant. Sample sizes for the pollination and oviposition analyses were reduced from 66 to 45 plants due to insufficient flowering in some replicates.

## Results

### Ant exclusion experiment

At the beginning of the flowering season, when moth abundance was high, the mean ( $\pm$  SE) proportion of flowers pollinated was high and not significantly different ( $t = 0.843$ ,  $DF = 27$ ,  $p = 0.407$ ) between control ( $0.868 \pm 0.026$ ) and

ant exclusion ( $0.846 \pm 0.034$ ) treatments. Likewise, mean ( $\pm$  SE) proportion of flowers with eggs was high and not significantly different ( $t = 0.4314$ ,  $DF = 27$ ,  $p = 0.670$ ) between control ( $0.822 \pm 0.030$ ) and ant exclusion ( $0.809 \pm 0.037$ ) treatments. The difference between the proportion of flowers pollinated and the proportion of flowers with eggs between the control and ant exclusion treatments was not significant ( $t = 0.3521$ ,  $DF = 27$ ,  $p = 0.7275$ ), indicating that ants were not entering into flowers and consuming eggs. Consistent with the distraction hypothesis, these results indicate that, at least under high moth densities, ants were not significantly altering and in particular disrupting plant-pollinator interactions in the presence of extrafloral nectar.

### Ant exclusion effects on fruit set, fruit survival, fruit production, larval survival and wasp parasitism

The mean ( $\pm$  SE) proportion of flowers with eggs setting fruit was high, but not different ( $t = 1.1221$ ,  $DF = 26$ ,  $p = 0.2721$ ) between control ( $0.823 \pm 0.034$ ) and ant exclusion ( $0.785 \pm 0.033$ ) treatments. The proportion of flowers with eggs surviving as immature fruit was high, but not different ( $t = 0.5889$ ,  $DF = 26$ ,  $p = 0.561$ ) between control ( $0.657 \pm 0.035$ ) and ant exclusion ( $0.629 \pm 0.049$ ) treatments. The proportion of flowers with eggs producing mature fruit was lower than fruit set and fruit survival, but not different ( $t = -0.0659$ ,  $DF = 26$ ,  $p = 0.948$ ) between control ( $0.290 \pm 0.030$ ) and ant exclusion ( $0.292 \pm 0.042$ ) treatments. These results are consistent with a prior study in which ants did not benefit flower-to-fruit survival (Chamberlain and Holland 2008). While larval survival did not differ significantly ( $t = -0.5667$ ,  $DF = 26$ ,  $p = 0.5758$ ) between control ( $0.252 \pm 0.035$ ) and ant exclusion ( $0.275 \pm 0.039$ ) treatments, there was a marginally significant effect ( $\chi^2 = 3.20$ ,  $DF = 1$ ,  $p = 0.074$ ) of ant exclusion

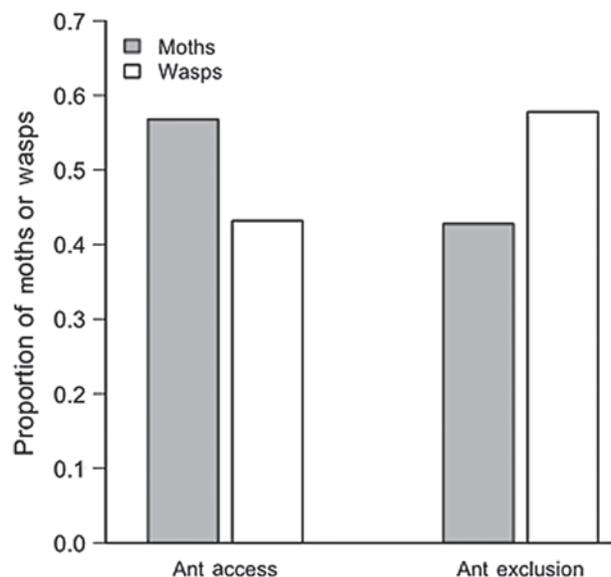


Figure 1. Proportion of moths or wasps emerging from senita moth larvae, for the control, with ant access treatment versus the experimental ant exclusion treatment. There was a marginally significant effect ( $\chi^2 = 3.20$ ,  $DF = 1$ ,  $p = 0.074$ ;  $n = 152$  moths and wasps) of ant exclusion on decreasing wasp parasitism rates of larvae.

on wasp parasitism rates of larva. In particular, when ants were excluded from stems, higher wasp parasitism rates of senita larvae occurred, as indicated by fewer moths emerging and more wasps emerging from senita moth larvae (Fig. 1;  $\chi^2 = 3.20$ ,  $DF = 1$ ,  $p = 0.074$ ).

### Ant/EFN factorial experiment

At the time of this experiment, flower visitation rates were lower (<40% of flowers) than the above ant exclusion experiment. We predicted a significant ant  $\times$  EFN interaction, such that ants disrupted plant–pollinator interactions in the absence of alternative EFN resources. In contrast with our predictions, there was no significant ant  $\times$  EFN interaction, nor a significant effect of EFN, on pollination or oviposition (Table 1, Fig. 2). There was a significant effect of ants on both pollination and oviposition, but rather than disrupting plant–pollinator interactions, ants increased pollination and oviposition, indicating a beneficial effect on plant–pollinator interactions. There was not a significant effect of ant, EFN, or ant  $\times$  EFN on the difference between pollination and oviposition (Table 1), indicating that ants did not enter into flowers and consume eggs.

### Ant density responses to continuous variation in EFN and pollination and oviposition responses to ant density

Variation in the aggregative density response of ants to EFN resources was best described by a saturating function, rather than a linear function (Table 2, Fig. 3a). While the saturating model was supported over the linear model, such support was more significant (i.e.  $\Delta AIC_c > 2$ ) when one or both of two extreme outliers were removed (i.e. 533 and 850 ants, both of which were statistical outliers, as defined as  $2 \times$  greater than the interquartile range above the third quartile; Zar 1999, Crawley 2007). In the saturating model,  $a$  is the saturation density of ants,  $b$  is the half-saturation abundance of EFN, and  $c$  is the  $y$ -intercept, indicating that some small, but positive density of ants occurs on plants in the absence of EFN. Based on AIC and MLE of parameters of the saturating model, ant density does not increase linearly (indefinitely) with increasing supply of EFN resources, but rather saturates between 100 – 130 ants with  $\sim 110$  EFN resource units (i.e. buds, fruits).

Table 1. Results of mixed model analyses of a fully crossed factorial experiment of ant and extrafloral nectar (EFN) effects on the pollination (proportion of flowers pollinated), oviposition (proportion of flowers oviposited), and the difference between pollination and oviposition of senita cactus flowers *Pachycereus schottii* by senita moths *Upiga virescens*, with plant as a random block. Significant F- and p-values are in bold. These qualitative results of PROC MIXED (SAS Institute 2004) are consistent with lmer of R (R Development Core Team 2009).

Effect	Pollination			Oviposition			Poll. - Ovi.		
	DF	F	p	DF	F	p	DF	F	p
EFN	1,80	0.13	0.722	1,80	0.02	0.807	1,80	0.04	0.837
Ant	1,80	5.67	<b>0.019</b>	1,80	5.16	<b>0.026</b>	1,80	0.06	0.801
EFN $\times$ Ant	1,80	1.11	0.295	1,80	1.47	0.229	1,80	0.06	0.810

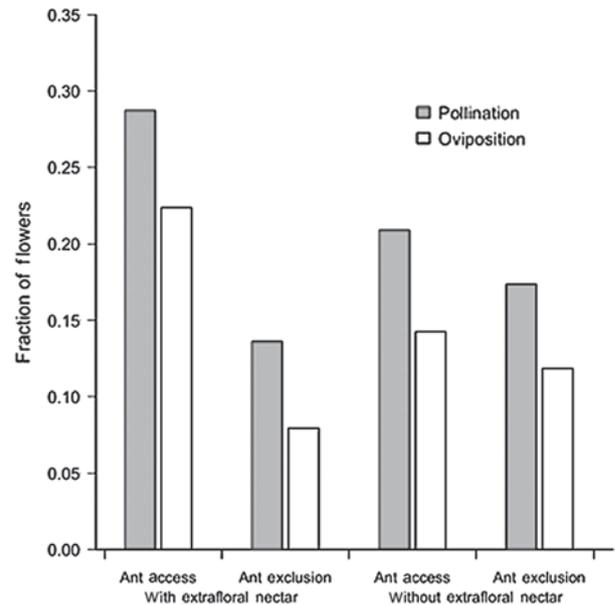


Figure 2. Results of the fully-crossed ant (with ant access, without ant access) and extrafloral nectar (EFN) (with EFN, without EFN) factorial experiment on the pollination (fraction of flowers pollinated) and oviposition (fraction of flowers) of senita cactus flowers *Pachycereus schottii* by obligate pollinating seed-consuming senita moths *Upiga virescens*. Statistical results are reported in Table 1.

As ant density varies with EFN resources, so may the disruption of plant–pollinator interactions. We predicted that as EFN resources decrease, ant disruption of plant–pollinator interactions would increase via ant exploitation of floral resources. In contrast to our predictions, the probability of pollination and oviposition increased with ant density (Table 2, Fig. 3b–c). Specifically, the strongest support was for a saturating increase in the probability of pollination and oviposition, instead of a linear increase or constant probability (Table 2). Moderate increases in ants (up to  $\sim 100$ ) increased successful plant–pollinator interactions but, because the function saturates, further increases in ants did not yield yet greater probabilities of pollination or oviposition.

## Discussion

Plants are most commonly thought to produce extrafloral nectar (EFN) to attract and reward ants that provide resistance against natural enemies that are detrimental to plant growth and reproduction. Indeed, meta-analyses and optimal defense theory are providing broad support for the plant defense hypothesis (Wäckers and Bonifay 2004, Chamberlain and Holland 2009, Holland et al. 2009, Rosumek et al. 2009). Yet, not all EFN-mediated ant–plant interactions are (always) mutualistic. In such cases, other hypotheses may help explain the function of EFN and ant–plant interactions (Wagner 1997, Sagers et al. 2000, Wagner and Kay 2002, Galen 2005, Wagner and Nicklen 2010). The distraction hypothesis predicts that EFN distracts ants from flowers and satiates them with an alternative resource, minimizing ant disruption of pollination and plant reproduction (Wagner and Kay 2002). We conducted a series of

Table 2. Model fitting and selection for ant density responses to extrafloral nectar, and for pollination and oviposition responses to ant density. Analyses of the linear and saturating models of ant density are presented for the complete data set and for the data set less the two extreme statistical outliers ( $2\times$  interquartile range above the third quartile). Three models were tested for pollination and oviposition probabilities: a constant value, linearly with ant density, and saturating with ant density.  $K$  is the number of parameters in the model;  $AIC_c$  is the corrected AIC for sample sizes of  $n / K < 40$ ;  $\Delta AIC_c$  is the difference in  $AIC_c$  values, and  $\omega_i$  is the Akaike weight (relative probability) of the models. Parameter  $a$  for pollination and oviposition has been back transformed from the logit to have meaning in regard to real probabilities.

Model	K	$AIC_c$	$\Delta AIC_c$	$\omega_i$	Parameter values			
					a	b	c	p
Ant density (complete data set, $n = 66$ )								
Saturating	4	751.4	0.0	0.582	133.7	20.7		18.9
Linear	3	752.0	0.7	0.418	49.3	0.412		
Ant density (without two extreme outliers, $n = 64$ )								
Saturating	4	707.1	0.0	0.977	94.6	1.0		18.4
Linear	3	714.5	7.5	0.023	55.7	0.279		
Pollination ( $n = 45$ )								
Saturating	2	110.4	0.0	0.633	0.890	51.5		
Linear	2	111.9	1.5	0.298	0.811	0.004		
Constant	1	114.8	4.4	0.070				0.773
Oviposition ( $n = 45$ )								
Saturating	2	147.4	0.0	0.877	0.770	33.0		
Constant	1	152.0	4.6	0.088				0.691
Linear	2	153.8	6.5	0.035	0.718	0.0007		

experiments to test the effects of ants and EFN on pollinating seed-consuming interactions between senita cacti and senita moths. Contrary to our predictions based on our prior work (Chamberlain and Holland 2008), our results not only continue to support the distraction hypothesis, but they also support the plant defense hypothesis. Below, we discuss the results of our experiments, and how multiple functions of EFN are not necessarily mutually exclusive and may in fact be synergistic.

As an alternative food resource, EFN may divert ants from exploiting floral resources, and thus prevent ant disruption of pollination and plant reproduction. The utility of distraction may arise in two different, though not necessarily mutually exclusive ways (Wagner and Kay 2002, Galen 2005). First, distraction is effective if the aggregative density response of ants is a saturating function of EFN resources, thereby decreasing per capita exploitation of flowers via the satiation of ants. In accord, the aggregative density response of ants was a saturating function of senita's EFN resources (Fig. 3a). If ants had increased proportionally (linearly) with EFN (and flower resources), then per capita exploitation by ants would not decline with EFN (Wagner and Kay 2002, Galen 2005). Because the aggregative density response of ants did saturate with EFN resources, EFN can aid in distracting ants from floral resources. Second, if EFN is a higher quality reward, a more readily accessible resource, and/or a numerically more abundant resource, then EFN may simply divert and distract individual ants away from foraging on flowers. There was not a significant treatment effect of EFN or ant  $\times$  EFN interaction on pollination or oviposition rates in the factorial experiment. Such insignificance was likely due to the lack of ants on stems of the EFN removal

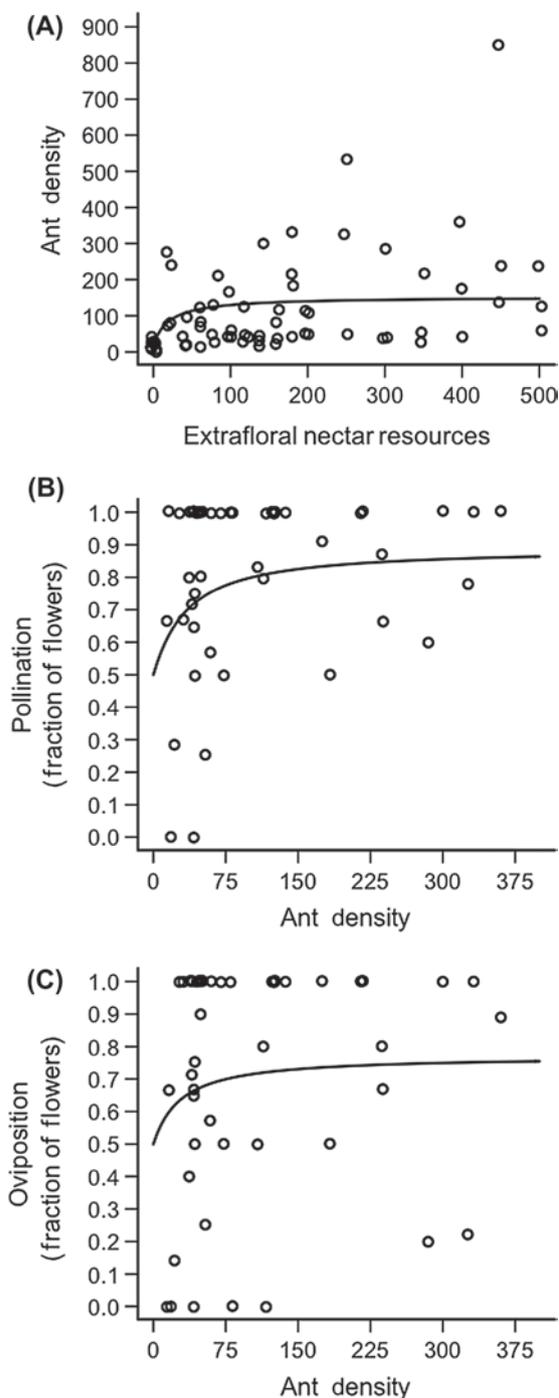


Figure 3. Results of ant density responses to experimental, continuous variation in extrafloral nectar (EFN) (A), and pollination (B) and oviposition (C) responses to ant density. The best fit model of the statistical results reported in Table 2 are plotted for each figure. (A) The aggregative density response of ants to the experimental manipulation of EFN resources. (B) The probability of pollination and (C) the probability of oviposition expressed as the fraction of flowers versus ant density.

treatment without Tanglefoot. While the ant exclusion treatment was effective in eliminating ants, EFN removal did not assure the maintenance of ants on control with ant stems but without EFN. In other words, in the absence of EFN, which consequently reduced the abundance of ants, Fig. 3a,

an ant  $\times$  EFN effect on plant–pollinator interactions would not be discernable. In this way, EFN removal likely confounded the results of the factorial experiment via the aggregative density response of ants to the EFN removal treatment. Nevertheless, our prior studies of ant interactions with senita cacti clearly demonstrated that EFN does divert and distract ants from exploiting floral resources (Chamberlain and Holland 2009). In sum, EFN can function to prevent ants from disrupting plant–pollinator interactions both by diverting ants from floral resources and by satiating ants through a saturating aggregative density response to EFN.

There was a significant effect of ants on pollination and oviposition by senita moths, but rather than disrupting plant–pollinator interactions, ants increased pollination and oviposition rates in the factorial experiment (Table 1, Fig. 2), indicating that, in addition to EFN distracting ants from flowers (Chamberlain and Holland 2008), ants have a beneficial effect on plant–pollinator interactions. Consistent with these results, the probability of pollination and oviposition also increased and saturated with ant density in the experiment with continuous variation in EFN (Table 2, Fig. 3b–c). Results of these two experiments are in contrast with other recent studies that show ants disrupting pollination (Ness 2006, Galen and Geib 2007, Lach 2007). Nonetheless, in two independent experiments of our studies, ants were beneficial to plant–pollinator interactions by increasing pollination and oviposition rates of senita moths, though the exact mechanism of these positive effects remains unclear.

Despite ants increasing the probability of pollination and oviposition by moths, ants had no significant effects on fruit set, survival of immature fruit (and thus pre-pupal larval survival), or fruit production. These results are consistent with a prior study which concluded that ants were largely commensalistic due to the lack of such effects on plant reproduction (Chamberlain and Holland 2008). Although ants did not influence the survival of immature fruit of senita, ants did appear to provide a benefit to moths by decreasing wasp parasitism and increasing pupal survival. It is unclear at what point wasps oviposit into larvae, but it is likely when larvae are within immature fruit tissue prior to their boring into the cactus stem and then pupating.

In the senita cactus/moth/ant system, EFN-mediated ant–plant interactions appear to have several beneficial effects on the interacting species. EFN distracts ants from exploiting flowers and disrupting pollination (Chamberlain and Holland 2008). Ants increased the probability of pollination and oviposition (Fig. 2, Fig. 3b,c). Ants also increased moth pupal survival by reducing wasp parasitism (Fig. 1). Prior work on the distraction hypothesis has considered it as an alternative hypothesis to the more commonly recognized plant defense hypothesis (Wagner and Kay 2002, Galen 2005, Chamberlain and Holland 2008). In the senita system, there is support for both the distraction and defense hypotheses for the production of EFN in the senita system, as EFN distracted ants away from exploiting floral resources and ants increased pollination, though the mechanism of the latter remains unclear. These processes likely act in concert with one another to the benefit of plants, as both plant defense and floral distraction can increase plant growth, survival, and reproduction. While the cactus/moth mutualism has been studied primarily in terms of its pairwise interactions, as we

integrate other species (e.g. ants, wasps) into our studies we learn more of how the community context can influence the outcomes and dynamics of the pairwise interactions.

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