DENSITY-MEDIATED, CONTEXT-DEPENDENT CONSUMER–RESOURCE INTERACTIONS BETWEEN ANTS AND EXTRAFLORAL NECTAR PLANTS

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Abstract. Interspecific interactions are often mediated by the interplay between resource supply and consumer density. The supply of a resource and a consumer's density response to it may in turn yield context-dependent use of other resources. Such consumer-resource interactions occur not only for predator-prey and competitive interactions, but for mutualistic ones as well. For example, consumer-resource interactions between ants and extrafloral nectar (EFN) plants are often mutualistic, as EFN resources attract and reward ants which protect plants from herbivory. Yet, ants also commonly exploit floral resources, leading to antagonistic consumer-resource interactions by disrupting pollination and plant reproduction. EFN resources associated with mutualistic ant-plant interactions may also mediate antagonistic ant-flower interactions through the aggregative density response of ants on plants, which could either exacerbate ant-flower interactions or alternatively satiate and distract ants from floral resources. In this study, we examined how EFN resources mediate the density response of ants on senita cacti in the Sonoran Desert and their context-dependent use of floral resources. Removal of EFN resources reduced the aggregative density of ants on plants, both on hourly and daily time scales. Yet, the increased aggregative ant density on plants with EFN resources decreased rather than increased ant use of floral resources. including contacts with and time spent in flowers. Behavioral assays showed no confounding effect of floral deterrents on ant-flower interactions. Thus, ant use of floral resources depends on the supply of EFN resources, which mediates the potential for both mutualistic and antagonistic interactions by increasing the aggregative density of ants protecting plants, while concurrently distracting ants from floral resources. Nevertheless, only certain years and populations of study showed an increase in plant reproduction through herbivore protection or ant distraction from floral resources. Despite pronounced effects of EFN resources mediating the aggregative density of ants on plants and their context-dependent use of floral resources, consumer-resource interactions remained largely commensalistic.

Key words: ant-plant interaction; antagonism; commensalism; consumer-resource interaction; context dependent; density mediated; distraction; extrafloral nectar (EFN); mutualism; Pachycereus schottii; protection; senita.

INTRODUCTION

Central to the study of interspecific interactions is understanding the interplay between the availability and supply of resources and consumer density. The supply of resources, whether prey of predators or limiting resources of competitors, is essential to a consumer's density response to interspecific interactions, and resulting dynamics of the interacting species (Tilman 1982, Murdoch et al. 2003, Turchin 2003). Density responses of consumers to the supply of resources may manifest as functional or numerical responses, or as an aggregative density response, that is a short-term behavioral change in the number of consumers aggregating with the supply of resources (Turchin 2003). The supply of and a consumer's density response to one resource may in turn yield context-dependent use of and

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effects on another resource, such as that exemplified by apparent competition (Holt 1977, Abrams et al. 1998). Such consumer-resource interactions are well recognized to form the basis of both predation and competition (Murdoch et al. 2003, Turchin 2003), but little consideration has been given to mutualism as a consumer-resource interaction. Yet, nearly all mutualisms are consumer-resource interactions: one species functioning as a consumer and the other as a resource (Holland et al. 2005). Mutualists produce resources that mediate their interactions by attracting and rewarding the consumers with which they interact. For example, plants produce nectar and fruit resources which attract and reward pollinators and seed dispersers; mycorrhizal fungi and rhizobial bacteria obtain carbohydrate resources from plants and supply nutrients to them; and, food excretions by hemipterans and sugar secretions by lycaenid caterpillars attract and reward ants in exchange for protection from natural enemies. Because mutualism, like predation and competition, is a consumerresource interaction, it too may depend critically on the interplay between the supply of resources and density responses of consumers, both of which may be key factors contributing to the well-established context dependency of their interactions (Bronstein 1994, Agrawal et al. 2007).

Consumer-resource interactions between ants and plants abound in nature, ranging from mutualistic protection to antagonistic florivory, herbivory, and granivory (Hölldobler and Wilson 1990). Mutualistic ant-plant interactions are mediated by a wide variety of resources produced by plants, such as beltian bodies, domatia, eliasomes, and extrafloral nectar. Of particular ubiquity in nature are ant-plant interactions mediated by extrafloral nectar (EFN) resources (nectar not associated with pollination), with species of >25% of angiosperm families, including 332 genera, bearing EFN resources (Koptur 1992). Consumer-resource interactions between ants and EFN plants are often mutualistic, as EFN resources attract and reward ants which protect plants from herbivory (Bronstein 1998, Heil and McKey 2003, Bronstein et al. 2006). However, ants also commonly exploit floral resources, which results in antagonistic interactions by disrupting pollination and plant reproduction through florivory, pollen consumption, pollinator interference, reduced pollen viability, and depletion of floral nectar (Galen 1983, 2005, Beattie et al. 1984, Rico-Gray 1993, Visser et al. 1996, Puterbaugh 1998, Junker et al. 2006, Ness 2006).

Although commonly associated with mutualistic interactions, EFN resources may also mediate antagonistic ant-flower interactions through the behavioral, aggregative density response of ants on plants. If the aggregative density response of ants on plants increases with EFN resources, then ant-flower interactions may also increase simply due to their greater abundance on plants. Alternatively, the supply of EFN resources may distract and satiate ants, thereby reducing their use of floral resources (Wagner and Kay 2002). Yet, flowers may have properties that deter and repel ants from using them as resources (Ghazoul 2001, Junker et al. 2006, Ness 2006). Thus, ant use of floral resources may depend contextually on the supply of EFN resources, the aggregative density responses of ants, and whether flowers repel ants. If floral deterrents do not occur, then both mutualistic ant-plant interactions and antagonistic ant-flower interactions may depend on the interplay between the supply of EFN resources and aggregative density responses of ants on plants. Even though plant protection and ant distraction resulting from the supply of EFN resources are not mutually exclusive, both feasibly operating in concert with one another, little attention has been given to whether antagonistic antflower interactions depend contextually on the supply and availability of EFN resources.

Using a guild of ants interacting with EFN-bearing senita cacti in the Sonoran Desert, we studied how the supply of EFN resources mediates the behavioral, aggregative density response of ants on plants and their context-dependent use of floral resources. Specifically, we addressed the following questions: Do floral chemicals deter ants from utilizing floral resources? Does the behavioral, aggregative density response of ants on plants vary with the availability of EFN resources? Do ant–flower interactions increase with the supply of EFN resources, or alternatively, do EFN resources distract ants from interacting with floral resources? Do ants increase plant reproduction through herbivore protection and/or reduce plant reproduction by interacting with flowers and disrupting pollination processes?

Methods

Study system.—We studied interactions between senita cacti (Pachycereus schottii Engelmann) and a guild of ants at Organ Pipe Cactus National Monument (OPCNM) in Arizona, USA during the spring and summer flowering season of 2006. Senita is a multistemmed columnar cactus attaining heights of 2-4 m and can live for >75 years. Senita produce 10-40 flowers per night from April through July at OPCNM. Hermaphroditic flowers have an inferior ovary containing many ovules, 100s of anthers, one pistil, and a lobule stigma. At sunset, flowers open and anthers dehisce; flowers close <12 h later, typically before sunrise. Mature fruits are berry-like, with ~ 180 seeds per fruit. Senita cacti rely on the obligate pollinating seed-eating senita moth (Upiga virescens Hulst) for pollination, as senita is entirely self-incompatible and co-pollinators are typically unimportant (Holland and Fleming 2002).

Extrafloral nectar (EFN) is produced from the tips of tepals on buds and flowers, and the tips of withered tepals on immature fruits. Such EFN associated with reproductive structures of senita cacti, and ant use of EFN, are illustrated in Fig. 1. In addition to EFN associated with reproductive structures, senita also have extrafloral nectaries just below areoles of new growth on stems, but extrafloral nectar production from these sub-areole nectaries usually occurs after the flowering season when new stem growth occurs. Extrafloral nectar production from tepals can vary within and among buds, flowers, and fruits, as well as with the relative size of such reproductive structures (S. A. Chamberlain and J. N. Holland, *unpublished data*).

Over a 24-h time period, mean \pm SE (range, *n*) secretion of extrafloral nectar for buds and immature fruit were 1.21 \pm 0.32 µL (0–8.1 µL, *n* = 31 plants) and 0.73 \pm 0.20 µL (0–5.6 µL, *n* = 31 plants), respectively. Over ~9 hours for which flowers are open, secretion of extrafloral nectar was 0.84 \pm 0.26 µL (0–3.3 µL, *n* = 16 plants). Buds and fruit are the dominant EFN resource, as flowers are only open for one night. Insects using EFN on senita include flies, beetles, and parasitoids, but ants are the most common consumers, including 12 species in three subfamilies at OPCNM: *Crematogaster depilis, C. larreae, Monomorium* sp., *Pheidole obtusospinosa, P. vistana, Solenopsis xyloni,* and *Tetramorium hispidum* (Myrmicinae); *Camponotus fragilis, C. ocrea*

tus, and *Myrmecocystus placodops* (Formicinae); and *Dorymyrmex insanus* and *Forelius mccooki* (or possibly *F. pruinosis*; Dolichoderinae; Mackay and Mackay 2002). No ant species were observed to live on or within senita. All ants except *Forelius* were only active on senita after sunset (when flowers open). Buds, flowers, and immature fruit are all susceptible to a diverse range of herbivorous insects.

Floral repulsion of ants.-If flowers of senita cacti have chemicals that deter ants, then EFN resource supply may not mediate ant-flower interactions. We conducted behavioral assays to assess if flowers have ant repellents using similar protocols to Ness (2006). One hemisphere of a petri dish was a control and the other was wiped with a freshly opened senita flower. As pollen may be a source of ant repellency (Nicklen and Wagner 2006), we only used newly opened flowers with dehisced anthers. All petri dishes, flowers, and individual ants were used only once. We measured percentage of time spent in each hemisphere for 15 ants of each of four species (Camponotus ocreatus, Crematogaster depilis, Forelius mccooki, and Pheidole obtusospinosa) collected from multiple plants within an hour prior to the experiment. Ants were introduced to the petri dish immediately after dishes were wiped with flowers. After a 30 second acclimation period, ants were observed for 300 seconds. At 150 seconds, we rotated petri dishes 180° to account for any orientation effect. We used nonparametric (Wilcoxon) paired-difference tests (due to nonnormal residuals) to examine whether the mean percentage of time in the floral hemisphere differed from the null hypothesis of 50%. We tested for differences among ant species using an ANOVA (SAS Institute 2004).

EFN-mediated ant-plant interactions.-First, we conducted observational studies to examine whether EFN resources mediate the naturally occurring aggregative density of ants on plants and their interactions with flowers. On 28 randomly chosen plants for 14 census nights we quantified the abundance of ants on plants, the proportion of flowers per plant that contained ants within flowers (ants consume EFN on the outside of flowers [see Fig. 1], so we only counted ants inside flowers), and the supply of EFN resources. The supply of EFN resources was estimated by the surrogate variable of the abundance of buds, flowers, and fruits. We censused three stems per plant as a proxy for whole plants, as thousands of buds, flowers, and fruits per plant were not feasibly countable (nor their EFN secretion rates measured). Flower number on the three stems was strongly correlated with whole-plant flower number among the census nights (n = 392, r = 0.42, P <0.0001). We recorded the proportion of flowers with ant species, but pooled species for analyses. Averaging among nights within plants to avoid pseudoreplication, we used simple linear regression to test whether the aggregative density of ants on plants varied with EFN resources, and the proportion of flowers with ants varied

with EFN resources (SAS Institute 2004). Ant abundance, proportion of flowers with ants, and EFN resources were ln-transformed prior to analysis to meet assumptions of normality and homoscedacity.

Second, we experimentally tested if the aggregative density of ants on plants depended on the availability of EFN resources. We haphazardly chose two flowering stems per plant (n = 29 plants) with similar levels of EFN resources, as estimated by the number of buds, flowers, and fruit. We randomly assigned each stem to a control or EFN elimination treatment. Control stems were not manipulated, and we removed all buds, flowers, and fruit from EFN removal stems. Although removal of EFN with glue was the preferred protocol (Rudgers 2004), it was not feasible due to the large number of buds, flowers, and fruits. Treatments were established in the afternoon when ants were inactive on senita. Abundance of ant species was censused both diurnally and nocturnally on each of the two stems the day prior to experimental manipulation and for three days following. On the first day of the treatment, ants were censused once by day (18:30-19:30 hours) and four times by night (20:00, 21:00, 22:00, and 23:00 hours). On the next two days, ants were censused once by day (18:30 hours) and twice by night (20:00 and 21:00 hours). We used repeated-measures ANOVAs to test treatment effects on the aggregative density response of ants (PROC GLM; SAS Institute 2000). The first analysis was for the hourly time scale immediately following treatment establishment; the second was for the daily time scale. Ant species were lumped for statistical analyses; observations during censuses indicated that species responded similarly, such that statistical effects were not driven solely by the response of one abundant species. Ant abundance was In-transformed to meet statistical assumptions. Although analyses were performed on In-transformed abundance, for clarity we report results as percentage change in ant abundance standardized to pre-manipulation ant abundance.

Third, we experimentally tested whether ant-flower interactions are mediated by EFN resources by randomly assigning two flowering stems per plant (n = 23) plants) to a control or EFN removal treatment. We quantified EFN resources (buds, flowers, and fruits) for the two stems prior to manipulation to assess any differences in pre-manipulation resource levels on ant responses. Prior to ant activity on plants (18:00 hours), all but one flower was removed from both control and EFN-removal stems; all buds and fruit were also removed from EFN-removal stems. Each flower on control and EFN-removal stems was observed for five minutes every 30 minutes for the two hours (20:00-22:00 hours) of peak flower visitation by senita moths (Holland and Fleming 1999). We measured the time flowers were occupied by ants and the frequency of ant interactions inside of flowers on control and EFNremoval stems. We summed data across five-minute time periods, as no differences occurred in response variables



FIG. 1. Extrafloral nectar production, and ant consumption thereof, associated with buds, flowers, and immature fruit of senita cacti (*Pachycereus schottii*). The left-hand panels show droplets of extrafloral nectar emerging from (a) the tips of tepals of multiple buds and (b) a single magnified bud. The right-hand panels show individuals of *Crematogaster* consuming nectar (c) from the tips of tepals on an immature fruit and (d) at the base of an open flower. Photo credits: J. N. Holland.

among time periods. Due to lack of normality in the data, analyses were performed using nonparametric (Wilcoxon) paired difference tests (SAS Institute 2004). We were unable to examine ant species effects due to small sample sizes for individual species.

Ant effects on plant reproduction.-We conducted antexclusion experiments to examine how ants influence plant reproduction through protection from herbivores and interactions with flowers. Plant reproduction is predicted to increase when ants are present due to their deterrence of herbivores, but plant reproduction could also decrease due to ant use of floral resources and their disruption of pollination. We established control and ant-exclusion treatments on each of 42 replicate plants. To improve our measure of treatment effects and avoid idiosyncrasies of particular stems, we used multiple flowering stems for each treatment (131 control and 150 ant-exclusion stems among the 42 plants). Although applying treatments to whole plants was the preferred design, it was not logistically feasible and would have limited our sample sizes relative to a paired design. We excluded ants using Tanglefoot (Tanglefoot, Grand Rapids, Michigan, USA) applied to tape on the base of each stem. We did not apply tape to control stems, as prior studies showed no tape effect on plant response variables (H. Passmore and J. N. Holland, unpublished data). The effect of the exclusion treatment on ant abundance was examined twice during the experiment. Both analyses showed significant differences in ant abundance between control and exclusion stems (first census: control, 41.3 ± 7.2 ants [all data are reported as mean \pm SE]; exclusion, 5.9 \pm 3.6 ants; two-tailed paired t test, t = 4.90, df = 41, P < 0.0001) (second census: control, 22.1 \pm 5.5 ants; exclusion, 7.6 \pm 1.7 ants; twotailed paired t test, t = 2.52, df = 41, P = 0.016). Although mean ant abundance on exclusion stems was not 0, this was largely driven by just a few stems on which ants gained access. On the first census of treatment effectiveness, 142 of 159 Tanglefoot stems (89%) had no ants on them whatsoever; 10 of the 17 stems that did have ants on them had fewer than 10 ants each. On the second census, 128 of 161 stems (80%) had no ants on them: 19 of the 33 stems that had ants on them had fewer than 10 ants each.

Senita's long flowering season allowed us to conduct several trials of this experiment for multiple plant



FIG. 2. Percentage of time (mean \pm SE) that four ant species spent in the floral hemisphere of the ant behavioral assays. The null hypothesis of no preference (50%) for the control vs. floral hemisphere of petri dishes is depicted by a dashed line. Bars sharing the same letter are not significantly different (Tukey's hsd test, P > 0.05).

reproduction variables. Multiple times throughout senita's flowering season a set of buds, flowers, and fruits were marked individually on control and treatment stems. Buds, flowers, and fruit of individual stems within each treatment of each plant were pooled in calculating each response variable, thereby treating plants as replicates instead of individual stems. Response variables included bud survival (proportion of buds surviving to anthesis), fruit set (proportion of flowers initiating fruit six days after anthesis), immature fruit survival (proportion of set fruit surviving to mature fruit), fruit maturation (proportion of flowers surviving to 20 days after anthesis), flower-to-fruit longevity (age to which flowers and fruit survived), and seed production (seeds per fruit). We also include unpublished results of a comparable experiment conducted at another site in another year (1998, Bahia de Kino, Sonora, Mexico; H. Passmore and J. N. Holland, unpublished data). Senita do not appear to reallocate resources among stems in response to experimental manipulations (Holland and Fleming 2002, Holland and Chamberlain 2007), which may have otherwise confounded our measures of plant reproductive response variables given the paired design of treatments within each individual replicate plant. Plant response variables (exclusion minus control) were analyzed with paireddifference t tests with plants as replicates. When parametric assumptions were not met, we used nonparametric (Wilcoxon) paired-difference tests (SAS Institute 2004). Although our statistical inference is based on paired (exclusion minus control) differences, for clarity and ease of interpretation we present means and error estimates for the two treatments separately.

RESULTS

Floral repulsion of ants.—Flowers showed no obvious repulsive properties to four different ant species (Fig. 2). No ant species spent significantly more time in the control vs. floral hemispheres of the behavioral assays (Wilcoxon paired-difference test, df = 15, P > 0.20 for each species). Moreover, time in control vs. floral hemispheres did not differ among the four ant species (one-way ANOVA, $F_{3,60} = 0.5493$, P = 0.651). Though not explicitly tested, observations of other ants in flowers on senita (*S. xyloni* and *C. fragilis*) that were not included in behavioral assays suggest they too were not averse. Thus, there were no obvious confounding effects of floral deterrents on EFN resources mediating ant–flower interactions.

EFN-mediated ant-plant interactions.-Observational studies of naturally occurring ants on plants showed that their behavioral, aggregative density response increased with the supply of EFN resources (simple linear regression, $r^2 = 0.32$, $F_{1,26} = 12.1$, P = 0.0018; Fig. 3a). Although the aggregative density of ants on plants increased with EFN resources, observations of naturally occurring ant-flower interactions showed that ant use of floral resources, as measured by the proportion of flowers containing ants, decreased rather than increased with EFN resources (simple linear regression, $r^2 = 0.52$, $F_{1,12} = 12.9$, P = 0.0037; Fig. 3b). Of all ants observed in flowers, 4%, 6%, 7%, 17%, and 66% were D. insanus, S. xyloni, Crematogaster depilis, Camponotus fragilis, and P. obtusospinosa, respectively. The decrease in antflower interactions with EFN resources was not simply a result of variation in ant abundance among plants, as the proportion of flowers with ants was not related to ant abundance per plant (simple linear regression, $r^2 =$ 0.04, $F_{1,12} = 0.42$, P = 0.5288).

Like the observational studies (Fig. 3a), experiments also showed that the aggregative density response of ants on plants was mediated by the availability of EFN resources (Fig. 4). The aggregative density of ants was unaltered on stems for which EFN remained intact. Yet, when EFN resources were eliminated, aggregative ant density on treatment stems decreased by 54% within hours of the manipulation ($F_{1,28} = 21.61, P < 0.0001$) and remained low for the following three days of observation ($F_{1,27} = 34.74$, P < 0.0001). There was a significant interaction between control and EFN-removal stems with time, for both the hourly (Pillai's trace =0.53, $F_{3,26} = 9.88$, P = 0.0002; Fig. 4 inset) and daily analyses (Pillai's trace = 0.54, $F_{3,25} = 9.92$, P = 0.0002; Fig. 4), indicating that the behavioral, aggregative density response of ants on plants was mediated by EFN resources. Damaging plants by removing reproductive structures with EFN may have activated the senita cactus's octadecanoid pathway and induced EFN secretion (Heil et al. 2001), thereby contributing to aggregative ant density on control vs. EFN-removal stems. Yet, EFN secretion is likely not inducible, but



FIG. 3. (a) Mean ant abundance per plant and (b) mean proportion of flowers with ants in them as a function of the quantity of EFN (extrafloral nectar) resources, as estimated by the abundance of buds, flowers, and fruits per stem (see *Methods*). Each data point is (a) a mean of 14 census dates for each of 28 plants or (b) a mean of 28 plants for each of 14 census dates. All variables were In-transformed prior to analysis and are presented as In-transformed values here. Lines are simple linear regression with 95% confidence bounds. Note that these results should be interpreted as a qualitative (i.e., positive) relationship between ant abundance and EFN resources, rather than specific functional responses given that data are averages among nights and plants.

rather constitutive, as EFN accumulates in the absence of ants and conspicuous herbivory (S. A. Chamberlain and J. N. Holland, *unpublished data*). Thus, because EFN is secreted constitutively, removing buds, flowers, or fruits did not likely induce EFN secretion (cf. Heil et al. 2004). Moreover, if the aggregative density response of ants on plants resulted from induced EFN production through plant damage, then we should have seen increases in ants on control stems, which we did not (Fig. 4).

Experimental studies also support EFN resources mediating antagonistic ant-flower interactions (Fig. 5). In comparing ant interactions with flowers for stems with and without EFN resources, ants showed a greater use of floral resources when EFN resources were removed. Ants occupied flowers for longer time periods on stems without EFN resources compared to stems with EFN resources (Wilcoxon paired-difference test, Z = 51.5, df = 22, P = 0.036; Fig. 5a). Ants also interacted more frequently with flowers on stems without EFN resources compared to flowers on stems with EFN resources (Wilcoxon paired-difference test, Z = 50.0, df = 22, P = 0.023; Fig. 5b). Increased time spent in flowers and increased ant-flower interactions for stems without EFN resources occurred despite the aggregative density of ants decreasing on stems without EFN resources (Wilcoxon paired-difference test, Z = -113, df = 23, P <0.0001; Fig. 5c). These results were not confounded by



FIG. 4. Proportional change in ant abundance (mean \pm SE) over four days on control and experimentally reduced EFN stems; 18 June is pre-manipulation, and hence the standard reference point for proportional change. The inset represents change on an hourly time scale of the night immediately following the afternoon manipulation.

the pre-manipulation differences in the supply of EFN resources between control and treatment stems, as no significant difference occurred (0.04 \pm 4.84 buds, flowers, and fruits per stem; mean \pm SE) between them prior to manipulation (two-tailed paired *t* test, *t* = -0.0086, df = 23, *P* = 0.993; Fig. 5d). Thus, the supply of

EFN resources increases the aggregative density of ants on plants while simultaneously reducing ant-flower interactions.

Ant effects on plant reproduction.—In 2006 for the OPCNM population, seed production did increase in the presence of ants in one trial, but sample size was small (*n*



FIG. 5. Ant response to EFN (extrafloral nectar) reduction as an experimental test of the distraction hypothesis. Although a paired design, separate means (\pm SE) for control vs. EFN reduction are presented (n=23 plants for all comparisons) for (a) the time (seconds) ants spent in flowers once ants entered flowers; (b) number of times ants contacted flowers; (c) ant abundance post-manipulation, during the experiment; and (d) pre-manipulation EFN resource levels, as estimated by the abundance of buds, flowers, and fruits per plant (see *Methods*).

* P < 0.05; ns, non-significant.

Plant variable, year	п	Control	Ant exclusion	P^{\dagger}
Bud survival (proportion)				
1998	18	0.65 ± 0.04	0.62 ± 0.03	0.59
2006	23	0.64 ± 0.05	0.71 ± 0.04	0.16
Fruit set (proportion)				
2006. trial 1	36	0.20 ± 0.03	0.22 ± 0.03	0.61
2006, trial 2	9	0.22 ± 0.04	0.18 ± 0.07	0.63
Immature fruit survival (proportion)				
2006	21	0.68 ± 0.06	0.62 ± 0.08	0.58
Fruit maturation (proportion)				
1998	9	0.78 ± 0.04	0.86 ± 0.04	0.03
2006, trial 1	36	0.12 ± 0.02	0.13 ± 0.02	0.81
2006, trial 2	19	0.71 ± 0.03	0.75 ± 0.05	0.54
Flower-to-fruit longevity (days)				
2006	36	5.70 ± 0.53	5.85 ± 0.58	0.86
Seed production (number of seeds)				
2006. trial 1	8	188.3 ± 21.3	145.0 ± 25.3	0.01
2006, trial 2	37	159.1 ± 8.5	154.3 ± 7.5	0.54

TABLE 1. Effects (mean \pm SE) of control and ant exclusion treatments for six plant reproductive variables.

Notes: See *Methods* for a description of variables. P values are for paired-difference tests using mean difference between treatments (plants as replicates), with a null hypothesis of 0. Sample sizes (n, the number of plants) vary among response variables because we were not able to quantify variables for some plants.

 \dagger Parametric paired-difference *t* tests were used in all cases, except "Seed production 2006, trial 1," in which a nonparametric (Wilcoxon) paired-difference test was used.

= 8 plants). Yet, the other trial with a larger sample size (n = 37 plants) showed no effect on seed production. In 1998 for the Bahia de Kino population, ant exclusion increased fruit maturation, suggesting that ant-flower interactions can reduce plant reproduction (Bahia de Kino, Sonora, Mexico; H. Passmore and J. N. Holland, unpublished data; Table 1). Even though the particular plant responses to ants in certain years and populations of study did occur, ants did not have an overwhelming effect on plant reproduction, either through plant protection or ant-flower interactions, as no effects were observed on bud survival, fruit set, fruit survival, fruit maturation, or flower-to-fruit longevity (Table 1). Thus, ants did not appear to have sustained mutualistic or antagonistic effects on plants, though certain years and populations did show that plant reproduction can be altered via herbivore protection and ant-flower interactions.

DISCUSSION

Recognition that both predation and competition are consumer–resource interactions has provided a mechanistic basis for understanding the consequences of interspecific interactions for the dynamics of ecological systems (Tilman 1982, Murdoch et al. 2003, Turchin 2003). Species interactions are often mediated by the interplay between resource supply and consumer density, and the supply of and a consumer's density response to one resource may in turn yield context-dependent use of other resources. The point has generally been missed, however, that mutualism is also a consumer-resource interaction (Holland et al. 2005). We studied interactions between a guild of ants and extrafloral-nectarbearing senita cacti to examine how the supply of one resource mediates a consumer's density response and its context dependent use of another resource. Specifically, we examined how the supply of extrafloral-nectar (EFN) resources mediates the behavioral, aggregative density response of ants on plants, and in turn whether ant use of floral resources is context dependent upon floral chemical deterrents, EFN resource supply, and density responses of ants. Even though empirical support occurs for each of floral deterrents (e.g., Ghazoul 2001), EFNmediated ant-plant protection (e.g., Bronstein et al. 2006), and EFN-mediated use of floral resources by ants (e.g., Ness 2006), studies have not examined the context dependency of each of them on one another. This is despite the fact that EFN-mediated distraction of ants from floral resources may be contingent upon floral chemical deterrents and that EFN-mediated ant-plant protection interactions may simultaneously mediate ant use of floral resources.

First, and possibly foremost, the context dependent use of EFN and floral resources by ants may be contingent on whether flowers have properties that deter and repel ants (Ghazoul 2001). Moreover, ant species may vary in their deterrence from flowers by floral chemical deterrents (Ness 2006). Our behavioral assays showed no indication that senita cacti had floral properties that deterred any of multiple ant species from using them as resources (Fig. 2). Because antflower interactions were not contingent upon floral chemical deterrents, consumer-resource interactions between ants and floral resources may be mediated by the supply of EFN resources. In the senita system, antflower interactions can be particularly detrimental for plant reproduction by disrupting their sole, obligate pollinator, whose peak flower visitation coincides with nocturnal ant activity (Holland and Fleming 1999).

The supply of EFN resources by plants did mediate the behavioral, aggregative density response of ants on plants (Figs. 3a, 4, and 5c) and their interactions with flowers (Figs. 3b, 5a, b). The aggregative density response of ants on plants increased with the supply of EFN resources, but this did not increase ant-flower interactions. Instead, ant-flower interactions decreased with the supply of EFN resources, which likely satiated and distracted ants from floral resources. However, when EFN resources were absent, ant interactions with flowers and the duration of those interactions increased compared with when EFN resources were available. Thus, ant use of floral resources did not appear to be context dependent upon the aggregative density of ants on plants, as ant-flower interactions did not increase with the density of ants on plants. In fact, ant interactions with flowers increased on plant stems without EFN resources despite their aggregative density decreasing compared to stems with EFN resources (Fig. 5). In this regard, ant use of floral resources was context dependent on the supply of EFN resources. Hence, the aggregative density response of ants on plants was mediated by, and context dependent upon, the supply of EFN resources. To this end, the supply of EFN resources can mediate the potential for both mutualistic ant-plant interactions and antagonistic ant-flower interactions by increasing the aggregative density response of ants protecting plants, while simultaneously reducing ant use of floral resources.

Interactions between ants and EFN-bearing plants are often mutualistic, as EFN is a food resource that attracts and rewards ants that in turn protect plants from herbivory (Bronstein et al. 2006). However, ants also often exploit floral resources (e.g., Galen 1983), which commonly leads to antagonistic ant-plant interactions through their interference with plant reproduction and pollination processes (e.g., Ness 2006). Although we did not explicitly quantify ant effects on herbivores and pollinators, our ant exclusion studies are consistent with ants increasing plant reproduction through plant protection and decreasing plant reproduction through disruption of pollination, depending upon the particular year and population of study (Table 1). Note that all plant reproduction variables in Table 1 relate to female fitness, and male fitness variables may also be affected by ants, as ants are known to reduce pollen viability when they contact dehisced anthers (Nicklen and Wagner 2006). While we did not quantify the effects of ants on male fitness, ants did not show any sustained

positive or negative effects on female fitness as measured through multiple plant reproductive variables. In addition, it is worth noting that, as with any ant-plant study employing Tanglefoot, such an ant exclusion treatment may also affect herbivore access to plants, though we do not suspect this confounds the lack of significant effects of ants on plant reproduction. Flightless herbivores do indeed feed on senita, but most herbivores can access senita in the presence of Tanglefoot. Despite these caveats and the pronounced effects of EFN resources in mediating the aggregative density of ants on plants and their interactions with flowers, consumer-resource interactions between ants and plants were largely commensalistic. Other studies have shown great spatiotemporal variation in and context dependency of mutualistic interactions of EFN-mediated antplant interactions (Horvitz and Schemske 1990, Rashbrook et al. 1992, Bronstein 1994, Zachariades and Midgley 1999, Wirth and Leal 2001, Mody and Linsenmair 2004, Rudgers and Strauss 2004, Moya-Raygoza 2005).

In our studies, EFN resources did mediate the aggregative density response of ants on plants and their interactions with floral resources, such that interplay between resource supply and consumer density of potentially mutualistic interactions can indeed be crucial to the ecological consequences of their interspecific interactions. Other consumer-resource systems that are often mutualistic may too depend critically on the supply of resources, and often mutualistic consumers may become commensalistic or antagonistic by exploiting their partners beyond those resources supplied as mutualistic rewards, like that of ant use of floral resources of senita cacti. For example, hemipterans (e.g., coccids, aphids) produce food excretions consumed by ants. In tending hemipterans and consuming food excretions, ants protect them from natural enemies. Depending on hemipteran excretion rates, ant density, and among other factors, hemipteran density, ants can shift to consuming individuals rather than tending them and only consuming their excretions, thereby leading to commensalistic or antagonistic ant-hemipteran interactions (Stadler and Dixon 2005). Similarly, lycaenid caterpillars produce food secretions that attract and reward ants in exchange for their protection from natural enemies. Yet, ants may go beyond consuming the food secretions produced by lycaenid larvae to eating individual larva (Pierce et al. 2002). Thus, consumer-resource interactions comprised of typically mutualistic consumers may be prone to conditional outcomes depending on the supply (and quality) of resources provided as mutualistic rewards. The supply of resources may be critical to the establishment of other consumer-resource mutualisms. For example, the quantity and constituents (sugars, amino acids) of floral nectar and pollen resources of plants can be key in determining which pollinators interact with which plants (Potts et al. 2003). Likewise, ambient levels of soil nutrients (e.g., phosphorus, nitrogen) and their supply rate to plants by mycorrhizae or rhizobia may be crucial in the establishment of plant-microbial mutualisms (Douglas 1994, Smith and Read 1997). Although these and many other interactions are often consumerresource mutualisms, they may become antagonistic if the consumer exploits a species beyond those resources supplied as mutualistic rewards, which may commonly depend on the supply of the reward resource and density responses of consumers to them.

In sum, our results have shown that resource-supply and consumer-density responses of potentially mutualistic interactions, like that of predator-prey and competitive interactions, can be crucial contextual factors in mediating their interspecific interactions. Examining mutualisms through the lens of consumerresource interactions can lead to otherwise unrecognized principles more commonly appreciated in predator-prey and competitive systems. Indeed, context dependency is increasingly a central component of understanding the strengths, outcomes, and dynamics of species interactions (Agrawal et al. 2007). Explicitly considering the supply rates of resources provisioned by one mutualist and a consumer's density response to them may provide new insights into the well-established context dependency and conditionality of mutualistic interactions (Bronstein 1994).

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LITERATURE CITED

- Abrams, P. A., R. D. Holt, and J. D. Roth. 1998. Apparent competition or apparent mutualism? Shared predation when populations cycle. Ecology 79:201–212.
- Agrawal, A. A., et al. 2007. Filling key gaps in population and community ecology. Frontiers in Ecology and the Environment 5:145–152.
- Beattie, A. J., C. Turnbull, R. B. Knox, and E. G. Williams. 1984. Ant inhibition of pollen function: a possible reason why ant pollination is rare. American Journal of Botany 71:421– 426.
- Bronstein, J. L. 1994. Conditional outcomes in mutualistic interactions. Trends in Ecology and Evolution 9:214–217.
- Bronstein, J. L. 1998. The contribution of ant-plant protection studies to our understanding of mutualism. Biotropica 30: 150–161.
- Bronstein, J. L., R. Alarcon, and M. Geber. 2006. The evolution of plant–insect mutualisms. New Phytologist 172: 412–428.
- Douglas, A. E. 1994. Symbiotic interactions. Oxford University Press, New York, New York, USA.
- Galen, C. 1983. The effects of nectar thieving ants on seedset in floral scent morphs of *Polemonium viscosum*. Oikos 41:245–249.
- Galen, C. 2005. Catching ants with honey: an experimental test of distraction and satiation as alternative modes of escape from flower-damaging ants. Oecologia 144:80–87.

- Ghazoul, J. 2001. Can floral repellents pre-empt potential antplant conflicts? Ecology Letters 4:295–299.
- Heil, M., S. Greiner, H. Meimberg, R. Krüger, J.-L. Noyer, G. Heubl, K. E. Linsenmair, and W. Boland. 2004. Evolutionary change from induced to constitutive expression of an indirect plant resistance. Nature 430:205–208.
- Heil, M., T. Koch, A. Hilpert, B. Fiala, W. Boland, and K. E. Linsenmair. 2001. Extrafloral nectar production of the antassociated plant, *Macaranga tanarius*, is an induced, indirect, defensive response elicited by jasmonic acid. Proceedings of the National Academy of Sciences (USA) 98:1083–1088.
- Heil, M., and D. McKey. 2003. Protective ant-plant interactions as model systems in ecological and evolutionary research. Annual Review of Ecology, Evolution, and Systematics 34:425–453.
- Holland, J. N., and S. A. Chamberlain. 2007. Ecological and evolutionary mechanisms for low seed : ovule ratios: need for a pluralistic approach? Ecology 88:706–715.
- Holland, J. N., and T. H. Fleming. 1999. Mutualistic interactions between *Upiga virescens* (Pyralidae), a pollinating seed-consumer, and *Lophocereus schottii* (Cactaceae). Ecology 80:2074–2084.
- Holland, J. N., and T. H. Fleming. 2002. Co-pollinators and specialization in the pollinating seed–consumer mutualism between senita cacti and senita moths. Oecologia 133:534–540.
- Holland, J. N., J. H. Ness, A. L. Boyle, and J. L. Bronstein. 2005. Mutualisms as consumer-resource interactions. Pages 17–33 *in* P. Barbosa and I. Castellanos, editors. Ecology of predator-prey interactions. Oxford University Press, New York, New York, USA.
- Hölldobler, B., and E. O. Wilson. 1990. The ants. Harvard University Press, Cambridge, Massachusetts, USA.
- Holt, R. D. 1977. Predation, apparent competition, and the structure of prey communities. Theoretical Population Biology 12:197–229.
- Horvitz, C. C., and D. W. Schemske. 1990. Spatiotemporal variation in insect mutualists of a neotropical herb. Ecology 71:1085–1097.
- Junker, R., A. Y. C. Chung, and N. Blüthgen. 2006. Interaction between flowers, ants and pollinators: additional evidence for floral repellence against ants. Ecological Research 22:665– 670.
- Koptur, S. 1992. Extrafloral nectary-mediated interactions between insects and plants. Pages 81–129 in E. A. Bernays, editor. Insect–plant interactions. CRC Press, Boca Raton, Florida, USA.
- Mackay, W. P., and E. E. Mackay. 2002. The ants of New Mexico. Edwin Mellen Press, Lewiston, New York, USA.
- Mody, K., and K. E. Linsenmair. 2004. Plant-attracted ants affect arthropod community structure but not necessarily herbivory. Ecological Entomology 29:217–225.
- Moya-Raygoza, G. 2005. Relationships between the ant *Brachymyrmex obscurior* (Hymenoptera, Formicidae) and *Acacia pennatula* (Fabaceae). Insectes Sociaux 52:105–107.
- Murdoch, W. M., C. J. Briggs, and R. M. Nisbet. 2003. Consumer-resource dynamics. Princeton University Press, Princeton, New Jersey, USA.
- Ness, J. H. 2006. A mutualism's indirect costs: the most aggressive plant bodyguards also deter pollinators. Oikos 113:506–514.
- Nicklen, E. F., and D. Wagner. 2006. Conflict resolution in an ant–plant interaction: *Acacia constricta* traits reduce ant costs to reproduction. Oecologia 148:81–87.
- Pierce, N. E., M. F. Braby, A. Heath, D. J. Lohman, J. Mathew, D. B. Rand, and M. A. Travassos. 2002. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). Annual Review of Entomology 47:733–71.
- Potts, S. G., B. Vulliamy, A. Dafni, G. Ne'eman, and P. Willmer. 2003. Linking bees and flowers: How do floral

communities structure pollinator communities? Ecology 84: 2628–2642.

- Puterbaugh, M. N. 1998. The roles of ants as flower visitors: experimental analysis in three alpine plant species. Oikos 83: 36–46.
- Rashbrook, V. K., S. G. Compton, and J. H. Lawton. 1992. Ant-herbivore interactions: reasons for the absence of benefits to a fern with foliar nectaries. Ecology 73:2167– 2174.
- Rico-Gray, V. 1993. Use of plant-derived food resources by ants in the dry tropical lowlands of Veracruz, Mexico. Biotropica 25:301–315.
- Rudgers, J. A. 2004. Enemies of herbivores can shape plant traits: selection in a facultative ant–plant mutualism. Ecology 85:192–205.
- Rudgers, J. A., and S. Y. Strauss. 2004. A selection mosaic in the facultative mutualism between ants and wild cotton. Proceedings of the Royal Society of London B 271:2481– 2488.
- SAS Institute. 2000. SAS version 8.1. SAS Institute, Cary, North Carolina, USA.
- SAS Institute. 2004. JMP version 5.1.2. SAS Institute, Cary, North Carolina, USA.

- Smith, S. E., and D. J. Read. 1997. Mycorrhizal symbiosis. Academic Press, New York, New York, USA.
- Stadler, B., and A. F. G. Dixon. 2005. Ecology and evolution of aphid–ant interactions. Annual Review of Ecology Evolution and Systematics 36:345–72.
- Tilman, D. 1982. Resource competition and community structure. Princeton University Press, Princeton, New Jersey, USA.
- Turchin, P. 2003. Complex population dynamics. Princeton University Press, Princeton, New Jersey, USA.
- Visser, D., M. G. Wright, and J. H. Giliomee. 1996. The effect of the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), on flower-visiting insects of *Protea nitida* Mill. (Proteaceae). African Entomology 4:285–287.
- Wagner, D., and A. Kay. 2002. Do extrafloral nectaries distract ants from visiting flowers? An experimental test of an overlooked hypothesis. Evolutionary Ecology Research 4: 293–305.
- Wirth, R., and I. R. Leal. 2001. Does rainfall affect temporal variability of ant protection in *Passiflora coccinea*? Ecoscience 8:450–453.
- Zachariades, C., and J. J. Midgley. 1999. Extrafloral nectaries of South African Proteaceae attract insects but do not reduce herbivory. African Entomology 7:57–76.