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Short Communication

# Temporal variation in extrafloral nectar secretion by reproductive tissues of the senita cactus, *Pachycereus schottii* (Cactaceae), in the Sonoran Desert of Mexico

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ABSTRACT

Plant/ant interactions mediated by extrafloral nectar (EFN) are common in nature. EFN is produced by plant species of >330 genera across 25% of all angiosperm families. Despite natural history observations suggesting its widespread occurrence within the Cactaceae, few studies have quantified EFN production by cacti. In this study, we conducted ant-exclusion experiments to examine temporal variation in, and ant consumption of, EFN produced by buds and fruits of the senita cactus (*Pachycereus schottii*) in the Sonoran Desert. EFN production by both buds and fruits was greatest at night and nearly absent by day. EFN remaining on buds and fruits was lower (and nearly absent) with ants than without ants. These results suggest the need for further studies of senita and other cacti that examine the ability of EFN production to attract and reward, but not necessarily oversupply ant consumers that provide them with herbivore resistance.



Many ant–plant interactions are mediated by resources produced by plants, including beltian bodies, domatia, and extrafloral nectar (Heil and McKey, 2003). These resources are what attract ants to plants and hence mediate interactions between the two species. Such ant–plant interactions are often mutualistic, as the resources attract and reward ants for their protection of plants against natural enemies (Bronstein, 1998; Bronstein et al., 2006; Heil and McKey, 2003). Ant–plant mutualisms with extrafloral nectar (EFN) (nectar not associated with pollination) are particularly common in nature, occurring in >330 genera of plant species across 25% of all angiosperm families (Koptur, 1992). Despite such widespread recognition among angiosperms, few studies have quantified EFN in species of Cactaceae in arid environments (Ruffner and Clark, 1986). As arid environments are often limited in the availability of water and sugar resources, EFN may be a generally important bottom-up resource in deserts. In this study, we conducted ant-exclusion experiments to examine when and how much EFN is produced by buds and fruits of senita cacti (*Pachycereus schottii* Engelmann; Cactaceae), and its consumption by ants that defend the plants against natural enemies. We do not examine ant effects on herbivore deterrence, nor the inducibility of senita's EFN, both of which have been examined elsewhere (Chamberlain and Holland, 2008; Holland et al., 2009).

We studied senita cacti near Bahia de Kino, Sonora, Mexico (28°53′N, 111°57′W). Senita is a multi-stemmed columnar cactus attaining heights of 2–4 m and living for >75 years. Plants can produce thousands of buds, flowers, and fruits from their spine-bearing areoles during flowering seasons, though not all buds and flowers survive to produce flowers or fruits. Flowers open at sunset and usually close prior to sunrise. Senita cacti rely on a seed-eating senita moth (*Upiga virescens* Hulst) for pollination, as plants are self incompatible and co-pollinating bees are rarely important (Holland and Fleming, 2002). Only ~50% of flowers initiate fruit due to limited resources, such that low fruit set (fraction of flowers initiating fruit maturation) results from trade-offs in resource (water) allocation between flower production and fruit set, although pollen limitation can occur (Holland, 2002; Holland et al., 2004). Not all immature fruit survive the 20–25 days of development (Holland, 2002; Holland et al., 2004), as some (15–29%) are lost to moth larvae and others to herbivores.

As reproductive tissue of cacti largely lack defensive alkaloids and silica compounds common in stem tissue (Gibson and Nobel, 1986), the buds, flowers and immature fruits of senita are all susceptible to herbivory from a diverse range of chewing and sucking insects, including larvae of a pyralid moth (*Cactobrosis fernaldialis*), longhorn beetles (e.g., *Moneilema gigas*), leaf-footed bugs, lace wings, aphids, and among others, mirid bugs. Buds,

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flowers and immature fruits produce extrafloral nectar (EFN) from the tips of their tepals (see photos in Chamberlain and Holland, 2008), which can be consumed by flies, wasps, and beetles, though by far the most dominant consumer is a guild of 14 ant species: *Crematogaster depilis* (Wheeler), *Monomorium* n. sp. *'desert'*, *Pheidole obtusospinosa* (Pergande), *P. vistana* (Forel), *Solenopsis xyloni* (McCook), *Tetramorium hispidum* (Wheeler), and *Cephalotes rohweri* (Wheeler) (Myrmicinae); *Camponotus fragilis* (Pergande), *Camponotus atriceps* (Smith)/*sayi* (Emery) (not differentiated in the field), and *Myrmecocystus mimicus* (Wheeler) (Formicinae); *Pseudomyrmex pallidus* (Smith) and *P. gracilis* (Santschi) (Pseudomyrmicinae); *Dorymyrmex bicolor* (Wheeler) and *Forelius mccooki* (McCook)/*pruinosus* (Roger) (not differentiated in the field) (Dolichoderinae). Species were identified using the museum collection at Harvard University, with taxonomy following Bolton's Catalogue of Ants of the World. Except for *Forelius*, activity patterns of the ants on senita are largely limited to dawn and nocturnal hours. In addition to reproductive tissues, senita cacti have extrafloral nectaries just below their areoles (sub-areole nectaries), but secretion from them has been largely observed after flowering seasons on new stem growth.

We measured EFN on one bud and one immature fruit on each of two randomly chosen stems on each of 31 randomly chosen plants. Each stem was assigned to either a control with ants or an ant-exclusion treatment. Placing each treatment within each replicate plant facilitated larger sample sizes and the control of individual plant effects. Ant-exclusion was established 2 days prior to measurements by applying Tanglefoot (Tanglefoot, Grand Rapids, MI, USA) to the base of stems, as in a prior study of senita (Chamberlain and Holland, 2008). We measured EFN secretion on the marked buds and fruits in 8 h time increments over a total of 48 h, during May 25th–27th, 2007. These 8 h increments were established to coincide with nocturnal (20:00–04:00 h) and rough diurnal (04:00–12:00 h, 12:00–20:00 h) time periods. EFN was measured at the end of each 8 h increment using 2 μL micropipettes. EFN volume was calculated as the length of the pipette filled with EFN, divided by the pipette length multiplied by 2 μL. By measuring EFN every 8 h, we were able to estimate both standing stocks and rates of EFN production. We censused ant abundance on control stems to relate temporal patterns in EFN production with ant activity and abundance.

Including each treatment within each replicate plant is reasonable for senita, as stems behave largely independent of one another and senita does not appear to re-allocate resources among reproductive units (Holland and Chamberlain, 2007; Holland and Fleming, 2002). All analyses were performed using non-parametric statistics, because normality could not be met after transformation due to many zeros in the data. We examined within and between day variation in EFN production by buds and fruits using the ant-exclusion stem. We used Wilcoxon paired difference tests to compare EFN production between nocturnal and diurnal time periods within a 24-h cycle. We repeated this analysis for each 24-h cycle of the 48 h experiment. We compared EFN between the two 24-h cycles (Day 1, Day 2) using a Wilcoxon paired difference test with the ant-exclusion stem of replicate plants. We tested for differences between standing stocks of EFN for control with ant and ant-exclusion stems using Wilcoxon paired difference tests (paired within replicate plants).

For both buds and fruits, there was a strong temporal pattern in EFN production within each of the two 24-h cycles (Day 1, Day 2) (Fig. 1, Table 1). EFN production was significantly greater during nocturnal hours than diurnal hours (Fig. 1, Table 1). In fact, nocturnal rates of EFN secretion were orders of magnitude larger than the nearly absent diurnal rates (Table 1). These 24-h cycles of nocturnal production and more-or-less diurnal cessation in EFN

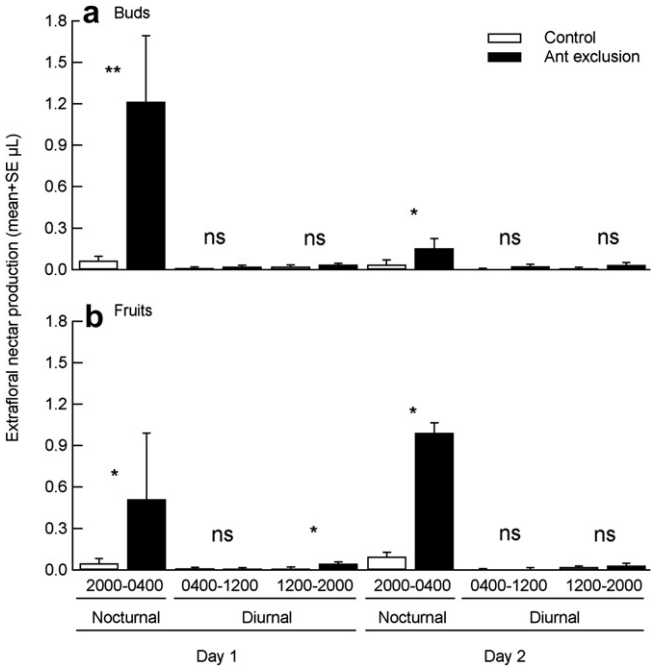


**Fig. 1.** Extrafloral nectar (EFN) production (mean ± SE) for control with ants and ant-exclusion treatment stems of senita by buds (a) and fruits (b) over a 48-h time period. EFN is reported for 8 h time increments. Significant differences in standing stocks of EFN between control with ants and ant-exclusion treatments are represented by: $^{**}P < 0.001$; $^{*}P < 0.05$; ns, not significant (Wilcoxon paired difference test).

corresponded with daily patterns of ant activity. Typical daily patterns of ant abundance on *P. schottii* (mean ± SE, $n = 31$ plants) were 4.0 ± 1.5, 2.7 ± 0.8, and 17.0 ± 3.3 ants per plant at 09:15, 14:00, and 20:30 h, respectively. Despite the qualitatively consistent pattern between the two 24-h cycles, some quantitative differences did occur in EFN production between Day 1 and Day 2 (Fig. 1, Table 1). Total rates of EFN production were greater on Day 1 (first 24-h cycle) than Day 2 for buds, but no difference occurred for fruits (Table 1). The difference was largely driven by the decrease in the nocturnal EFN production by buds on Day 2.

EFN production for buds and fruits did differ between control with ants and ant-exclusion stems (Fig. 1). For both buds and fruits, nocturnal standing stocks were significantly less on control with ant than ant-exclusion stems. Diurnal standing stocks were nearly absent, and did not differ between control with ant and ant-exclusion stems, with the one exception of lower standing stocks on control with ant than ant-exclusion stems for buds on Day 1 from 12:00 to 20:00 h (Fig. 1). Consistent with nocturnal ant activity, consumption of EFN by ants was largely limited to nocturnal hours, as indicated by the >90% difference in standing stocks of EFN for both buds and fruits between control with ant and ant-exclusion stems (Fig. 1).

Before discussing a few possible implications of these results, we identify two important caveats of this study. First, we were only able to measure nectar volume in the field at the time of this study, despite the advantages of measuring nectar content (e.g., sugars, amino acids, or total soluble solids). Second, evaporation of water from the EFN in the arid desert may contribute to the diurnal/nocturnal patterns in EFN, and possibly contribute to ant activity. Though feasible, several observations suggest otherwise. First, capillary action was a necessary pre-requisite to measure EFN volume with the 2 μL micropipettes. If evaporation substantially reduced the already small volumes of EFN, then increased viscosity would have prevented its uptake into the micropipettes

**Table 1**
Rates of extrafloral nectar production (mean $\mu L\ h^{-1} \times 10^{-2}$) for buds and fruits of senita by day (Diu) and night (Noc) for Day 1 and Day 2, and total daily rates for ant-exclusion stems. Sample sizes ($n$) are plants. Significant differences (Wilcoxon paired difference test, $Z$) are denoted by superscripts: $^{**}P < 0.001$; $^{*}P < 0.05$; ns, not significant.

| | Day 1 | | | | Day 2 | | | | Day 1 vs. Day 2 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Diu | Noc | $n$ | $Z$ | Diu | Noc | $n$ | $Z$ | Day 1 | Day 2 | $n$ | $Z$ |
| Buds | 0.36 | 15 | 30 | 94** | 0.28 | 1.53 | 30 | $20^{ns}$ | 5.3 | 0.7 | 30 | −105** |
| Fruits | 0.34 | 6.4 | 30 | 65* | 0.21 | 12.4 | 30 | 47* | 2.4 | 4.3 | 30 | $-38^{ns}$ |

via capillary action. We are familiar with this, as increased viscosity did occur for a small total of four buds and fruits on the last day of the study, which were then removed from analyses. Moreover, if substantial evaporation of EFN was occurring, then crystallized sugars of the EFN would have been observed on the buds and fruits during the time of the study, as more commonly occurs in hotter times of late June and July at the Bahia de Kino field site. Second, if evaporation was reducing EFN volume from nocturnal through diurnal time periods, then we should have observed gradual and marked declines in EFN volumes from the nocturnal through the diurnal time periods. For example, EFN volumes during the cooler morning times of 04:00–12:00 h should be measurably greater than from 12:00 to 20:00 h. Yet, such patterns did not occur. Temperatures in the area of Bahia de Kino remain cool and often less than 28–30 °C through May and into June (Holland and Fleming, 2002), as the field sites are situated near the Gulf of California with frequent cool sea breezes and high humidity. Lastly, our results for temporal variation in EFN, like any study of EFN, may be unavoidably confounded by artificial effects that sampling EFN may have on later EFN production (Heil et al., 2000), though any such sampling effects would likely be similar among study plants.

Despite these caveats, our results suggest that senita may minimize their EFN production through the quantity and daily timing of EFN production. Indeed, plants should not over-produce EFN to attract ants for their defense against herbivores. Senita showed nocturnal production and diurnal cessation of EFN. Nocturnal and nearly absent diurnal production of EFN correlates with daily activity patterns of most ants that consume EFN of senita (Chamberlain and Holland, 2008). Other ant consumers of Cactaceae EFN are active by day or night (Blom and Clark, 1980; Ness, 2006). When ants had access to EFN, only small quantities of EFN remained, suggesting little over-production. Ants are likely the primary consumers, as other non-flying insects were rare and flying insects that could access EFN on ant-exclusion stems depleted EFN little compared to that attributable to ants (i.e. EFN production on exclusion vs. control stems). Any potential effects of flying insects on EFN would likely only change the magnitude and not the direction of the results. Overall, our estimates of ant consumption of EFN are conservative, particularly if plants increase EFN production when consumed by ants, or plants reduce EFN production when not consumed. Although few studies have explicitly examined EFN production by species of Cactaceae (see Ruffner and Clark, 1986), our results do suggest that plants may temporally match EFN production with that necessary to attract and reward but not oversupply ant consumers providing the herbivore resistance. We have evaluated the production of extrafloral nectar (EFN) by both buds and fruits of senita cacti (*P. schottii*) to attract and reward mutualistic ants as a defense against herbivores. We have shown that EFN production is largely restricted to nocturnal hours. Further studies of EFN of additional species of cacti are needed, especially given their dominance in plant communities in the Sonoran Desert.

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