**Interaction between ants, extrafloral nectaries and insect herbivores in Neotropical coastal sand dunes: herbivore deterrence by visiting ants increases fruit set in *Opuntia stricta* (Cactaceae)**

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**Summary**

1. This study examines the anti-herbivore effect of ants visiting the extrafloral nectaries (EFNs) of *Opuntia stricta* (Cactaceae) and its possible influence on the plant’s reproductive output in Mexican coastal sand dunes. *Opuntia*’s EFNs are located in the areoles of the developing tissue of emerging cladodes and flower buds.
2. Ants visited the EFNs of *O. stricta* on a round-the-clock basis. The associated ant assemblage was formed by nine species distributed in four subfamilies, and the species composition of the principal ant visitors changed markedly from day to night period.
3. Cladodes of control (ants present) and treatment (ants excluded) plants of *Opuntia* were equally infested by sucking bugs and mining dipterans. Damage to buds by a pyralid moth, however, was significantly higher on treatment than on control plants. Ant visitation to *Opuntia*’s EFNs translated into a 50% increase in the plant’s reproductive output, as expressed by the number of fruits produced by experimental control and treatment branches. Moreover, fruit production by ant-visited branches was positively and significantly associated with the mean monthly rate of ant visitation to EFNs.
4. This is the first demonstration of ant protection leading to increased fruit set in the Cactaceae under natural conditions. Although the consequences of damage by sucking and mining insects remain unclear for *Opuntia*, the results show how the association of EFNs with vulnerable reproductive plant organs can result in a direct ant-derived benefit to plant fitness.

**Key-words:** Ant foraging schedule, ant–plant mutualism, ant protection, cactus, herbivory, reproductive output


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**Introduction**

Insect herbivores may eat virtually all types of plant tissue and herbivore damage may occur at any stage of a plant’s life cycle (Crawley 1983). However, because herbivores consume both vegetative and reproductive tissue, the impact of herbivory on plant fitness may depend largely on the type of tissue being consumed (Marquis 1992). A number of plant characteristics are hypothesized to have evolved as responses to selective pressure exerted by herbivores, including structural, chemical, physiological and life-history traits (reviewed by Marquis 1992). Mutualistic associations with ants constitute one such defence strategy, and hundreds of plant species produce domatia (structures that house ant colonies) and/or food rewards (food bodies, extrafloral nectar) to attract ants which in turn provide the plant with some protection against herbivores (see Beattie 1985; Davidson & McKey 1993; Whitman 1994).

Extrafloral nectaries (EFNs) are nectar-secreting organs not directly involved in pollination which are found on virtually all above-ground plant parts (Bentley 1977a; Elias 1983; Koptur 1992; Oliveira & Pie 1998). Plants bearing EFNs are widely distributed around the world, and available evidence supports the general contention that they are more common in tropical than in temperate environments (Bentley 1977a; Oliveira & Leitão-Filho 1987; Pemberton 1988; Oliveira & Oliveira-Filho 1991; Schupp & Feener 1991; Coley & Aide 1991). Although EFNs attract a variety of nectar-feeding insects (Koptur 1992), ants are by far the most frequent visitors to...
EFN-bearing plants both in temperate and tropical habitats (Oliveira & Brandão 1991; and included references). In the past two decades, a number of experimental field studies have demonstrated that ant visitation to EFNs may increase plant fitness by deterring leaf herbivores (Koptur 1979; Stephenson 1982; Smiley 1985), bud or flower herbivores (Schemske 1980; Rico-Gray & Thien 1989; Oliveira 1997) and seed predators (Inouye & Taylor 1979; Pickett & Clark 1979; Keeler 1981). Some studies, however, have found no apparent benefit to the plant from ant visitation (e.g. O'Dowd & Catchpole 1983; Whalen & MacKay 1988). As stressed by Schemske (1983), ant–plant mutualisms mediated by EFNs are facultative and non-specialized, as indicated by the wide variety of associated ant visitors (see also Thompson 1988; Bronstein 1998). In fact, ant-derived benefits to EFN-bearing plants can be conditioned by factors such as time (Tilman 1978), habitat type (Barton 1986), aggressiveness of ant visitors (Horvitz & Schemske 1984; Oliveira, Silva & Martins 1987; Rico-Gray & Thien 1989), as well as the capacity of herbivores to overcome ant predation (Koptur 1984; Heads & Lawton 1985; Freitas & Oliveira 1996).

The present study examines the anti-herbivore effect of ants visiting the EFNs of *Opuntia stricta* Haw. (Cactaceae) and its possible influence on the plant’s reproductive output in Mexican coastal sand dunes. Although Pickett & Clark (1979) showed that herbivore deterrence by visiting ants can increase fruit production by plants of *Opuntia acanthocarpa* in greenhouse conditions, the authors were not able to show such an effect in the species’ natural habitat (Sonoran Desert, AZ). In this study we use field observations and controlled ant-exclusion experiments to test the hypothesis of ant protection leading to reproductive benefits in *O. stricta* under natural conditions. Three questions were addressed: (1) which are the ant visitors to the EFNs of *O. stricta*; (2) does ant visitation reduce the infestation levels or damage by the plant’s principal herbivores; (3) does ant presence, and ant density on plants, affect the reproductive output (i.e. fruit set) of *O. stricta*?

**THE PLANT, AND THE STUDY SYSTEM**

*Opuntia stricta* is a succulent cactus (up to c. 2 m tall) that commonly occurs along the coastal sand dunes of Veracruz, México (Novelo 1978). *Opuntia* flowers can be pollinated by bees and birds (see Grant & Grant 1981), and in México the fruits are consumed by several birds, rodents and other mammals (González-Espinoza & Quintana-Ascencio 1986; Janzen 1986). Ants actively visit the plant both day and night, and gather around the EFNs located in the areoles of the developing tissue of emerging cladodes, as well as in the areoles of developing flower buds (Fig. 1; see also Rico-Gray 1993). *Opuntia*’s extrafloral nectar contains sugars and amino acids at average concentrations of 11 m and 279 mm, respectively (Pickett & Clark 1979; for *O. acanthocarpa*). *Opuntia stricta* is attacked by four principal herbivores in the sand dunes of Veracruz: (1) *Narnia* sp. (Hemiptera: Coreidae) adults mate on the plant and egg batches (eight to 14) are laid on the spines; nymphs and adults suck plant juice from cladodes and produce typical white rings around punctures (Mann 1969); (2) *Hesperolabops* sp. (Hemiptera: Miridae) egg batches not seen but nymphs and adults suck plant juice from cladodes and punctures are detectable by white dots; (3) mining insects’ (Diptera) mining/feeding activity by developing larvae leave easily detectable tunnels within infested cladodes; (4) bud-destroying moth (Lepidoptera: Pyralidae, Phycitinae) eggs are laid on floral buds and developing cladodes, and larval burrowing/feeding activity within the plant organ leaves characteristic external marks (see also Mann 1969).

**Study site and methods**

Field work was carried out from April 1997 to March 1998, at the Centro de Investigaciones Costeras La Mancha located in the state of Veracruz on the coast of the Gulf of México (19 ° 36 ´ N, 96 ° 22 ´ W; elevation < 100 m). The climate is warm and sub-humid. Mean annual temperature is 24–26 °C, with total annual precipitation ranging from 1100 to 1500 mm, and a rainy season occurring between June and September. The
Ant–plant–herbivore interaction in sand dunes

The protective role of visiting ants against herbivores of *O. stricta* was tested with ant-exclusion experiments. By early June 1997, before the beginning of the wet season, 19 experimental plant pairs of *O. stricta* (0–6–20 m tall) were tagged along nearly 500 m of stabilized dune vegetation. Plants within a pair were approximately the same height, 0·5–20 m apart from each other, and in the same phenological stage (no buds, flowers or fruits). Each plant in a given pair was randomly designated by the flip of a coin as a treatment or control plant, and had one branch selected for the monthly records of herbivore and ant activity, as well as fruit production (see below). Experimental branches in a given plant pair had equal numbers of cladodes (one to four). Ants were prevented from climbing on experimental branches of treatment plants by applying to their base a sticky barrier of tree Tanglefoot® (Tanglefoot Co., Grand Rapids, MI, USA). Grass bridges providing aerial access of ants to treated plants were regularly pruned. Ants had free access to control individuals of *O. stricta*.

Herbivore activity on experimental branches of control and treatment plants was measured monthly from June 1997 to March 1998 (no record in December). Infestation levels by *Narnia* and *Hesperolabops* sucking bugs were determined by counting the number of nymphs and adults present on the branches. Damage by mining dipterans to each cladode of experimental branches was estimated visually prior to ant treatment (June 1997) and after 10 months of ant-exclusion (March 1998). Intensity of miner attack is expressed as the percentage surface area covered by miner tunnels. Four categories were established: none (0%), low (≤30%), medium (30–60%), and high (≥60%). Infestation by the pyralid moth was evaluated by counting the number of external marks left by caterpillars on cladodes and flower buds of experimental branches. Pyralid damage was measured only in February and March 1998, when bud emission by *O. stricta* and egg-laying activity by the moths were most intense.

The number of ants visiting the EFNs of experimental branches of control plants was counted monthly, simultaneously with the records of herbivore activity (see above). Fruit production by control and treatment branches was determined at the end of *O. stricta*'s fruiting period at La Mancha (March 1998). To account for unknown effects of size among experimental branches, the number of ripe fruits in a given branch was divided by the number of cladodes that formed it.

**STATISTICAL ANALYSES**

Hemipteran activity (pooled for *Narnia* and *Hesperolabops*) on experimental plant pairs along successive months was analysed by repeated-measures ANOVA after square-root transformation of the data (to normalize the distribution). Damage by mining insects was compared using chi-square tests (Yates correction) on the proportion of experimental cladodes falling under different categories of miner attack before (June 1997) and after 10 months of ant-exclusion (March 1998). Because the data on pyralid damage were not normally distributed, larval external marks to experimental plants pairs were compared using a Mann–Whitney *U*-test. Data on fruit production by control and treatment branches were analysed by a two-tail paired *t*-test after passing the normality assumption.

**Results**

**ANT VISITATION TO *O. STRICTA*’S EFNs**

Ants visited the EFNs of *O. stricta* on a round-the-clock basis and at any given sampling period 9·5 ± 1·1 plants were occupied by ants (mean ± SD; 10 plants checked at 1 h intervals). Average visitation rate ranged from 1·4 to 7·9 ants per plant, and the activity rhythm of nectarivorous ants was negatively and significantly related with temperature (Fig. 2). The ant assemblage associated with the EFNs of *O. stricta* was formed by...
nine species distributed in four subfamilies, as follows: Formicinae, Camponotus planatus, Camponotus abdominalis, Camponotus sp. 10, Paratrechina longicornis; Myrmicinae, Crematogaster brevispinosa, Monomorium cyaneum; Dolichoderinae, Forelius aff. pruinosus; Pseudomyrmecinae, Pseudomyrmex sp. 5 (pallens group), Pseudomyrmex sp. 7 (pallidus group).

Although some ant species were seen at EFNs both day and night, the species composition of the principal ant visitors changed markedly from one period to the other (Fig. 3). Camponotus planatus, P. longicornis, C. brevispinosa and Pseudomyrmex sp. 5 were the most frequent species on plants during daytime samplings, whereas the dominant C. abdominalis was by far the most frequent visitor at night, followed by the less common and exclusively nocturnal Camponotus sp. 10 (Fig. 3a). The contrasting activity rhythms of the two most dominant ants at EFNs, C. planatus and C. abdominalis, are shown in Fig. 3b for the entire census period. No aggressive interaction between EFN-gathering ants was ever observed on O. stricta.

ANT-EXCLUSION EXPERIMENTS AND HERBIVORE INFESTATION LEVELS

Control and ant-excluded plants of O. stricta were equally infested by Narnia and Hesperolabops sucking bugs throughout the field experiment (Fig. 4; repeated-measures ANOVA, $F_{1,36} = 0.067, P = 0.797$). Visiting ants were never observed attacking the hemipterans and seemed to ignore them on control plants. Similarly, ant visitation to EFNs had no effect on the infestation levels by mining insects; the proportion of infested cladodes was similar for either experimental plant class, both before ($\chi^2 = 1.279, df = 2, P = 0.734$) and after ant treatment ($\chi^2 = 0.973, df = 2, P = 0.807$) (Fig. 5). On the other hand, damage by the pyralid moth was significantly higher on treatment than on control plants. External marks left by caterpillar burrowing activity within cladodes and flower buds were more numerous on ant-excluded than on ant-visited plants (mean $\pm$ SD = 0.84 ± 1.92 vs 0.10 ± 0.3, respectively; Mann–Whitney U-test, $U = 893.0, P < 0.001, N = 19$).

ANT EFFECTS ON PLANT REPRODUCTION

Ant visitation to O. stricta’s EFNs translated into a 50% increase in the plant’s reproductive output: the mean number ($\pm$ 1 SD) of fruits per cladode produced by experimental branches was 3.62 ± 1.80 for ant-visited plants vs 2.40 ± 0.34 for ant-excluded ones (paired t-test, $t = 2.564, df = 18, P = 0.0195$). Moreover, fruit production by control branches was positively and significantly associated with the mean rate of ant visitation to EFNs along the successive months of the study (Fig. 6).

Discussion

Extrafloral nectar is regarded as a general ant reward capable of attracting to the plant a wide diversity of ants (Carroll & Janzen 1973; Beattie 1985). Surprisingly, however, most studies dealing with ant–plant systems mediated by EFNs have focused on the role of ‘key’ ant species, and relatively few investigate the associated ant assemblage through daytime and night censuses (see Oliveira & Brandão 1991; and included references). In general the emerging pattern from 24 h censuses at EFNs reveals a clear turnover of ant species through time, with quite distinct diurnal and nocturnal assemblages of visiting ants (Bentley 1977b; Blom & Clark 1980; Oliveira & Brandão 1991; Oliveira, Klitzke & Vieira 1995). In some cases, however, little daily turnover in ant species composition has been reported, with nectar sources being continually visited by one or a few dominant ant species (e.g. O’Dowd 1979; Beckmann & Stucky 1981). The segregation of daily foraging schedules among sympatric ant species is especially common in tropical areas and generally results from distinct humidity and
temperature ranges tolerated by different species (Levings 1983; Hölldobler & Wilson 1990; Cerdá, Retana & Cros 1998). The round-the-clock census of the ant assemblage at EFNs of *O. stricta* revealed a clear species turnover within the 24 h period and a significant relationship between ant activity and temperature. The pattern here reported for coastal sand dunes has also been registered for nectarivorous ant assemblages living in other similarly exposed environments such as deserts (Blom & Clark 1980) and savannahs (Oliveira & Brandão 1991; Oliveira et al. 1995), where ant foraging schedules are apparently more closely linked with marked daily oscillations in temperature (see Bernstein 1979; Moreno-Casasola 1982). Although such differences in daily foraging rhythms may be proximately adjusted by the species’ physiological properties, they can allow temporal partitioning of persistent liquid resources such as extrafloral nectar (Oliveira & Brandão 1991), and have also been reported for ant assemblages harvesting homopteran honeydew (Del-Claro & Oliveira 1999) and lepidopteran secretions (DeVries 1991).

Two species of *Camponotus*, *C. planatus* and *C. abdominalis*, comprised most of the ant observations at *O. stricta*’s EFNs, followed by *P. longicornis* and *C. brevispinosa*. These four ant species, but most especially *C. planatus* and *Cr. brevispinosa*, are very common in Mexican coastal sand dunes, where they forage for several types of plant-derived food resources, including floral and extrafloral nectar from many plant species, as well as homopteran and lepidopteran secretions (Rico-Gray 1989, 1993; Rico-Gray & Thien 1989; Rico-Gray & Castro 1996; Rico-Gray et al. 1998). These data confirm the dominance of *Camponotus* and *Crematogaster* ants on tropical foliage (Wilson 1987), and on plants bearing EFNs worldwide (see Oliveira & Brandão 1991; and included references).

Although ant visitation to EFNs of Cactaceae has long been reported in the botanical literature (Lloyd 1908), only more recently has this interaction been studied in greater detail (Pickett & Clark 1979; Blom & Clark 1980; Ruffner & Clark 1986; Pemberton 1988). Cacti extrafloral nectar may represent an extremely rich food source for ants because it contains not only sugars (fructose, glucose and sucrose) but also several amino acids which are thought to be essential for insect nutrition (Pickett & Clark 1979; Ruffner & Clark 1986). The current study involving *O. stricta*, visiting ants and insect herbivores extends earlier reports by other authors by providing a more precise picture of the species interactions within the system through ant-exclusion experiments. The field observations and experiments in the coastal sand dunes of La Mancha demonstrated that round-the-clock visitation by ants to *O. stricta*’s EFNs can reduce herbivore damage to plant reproductive structures and increase by 50% the plant’s fruit set under natural conditions. Moreover, the level of ant visita-

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**Fig. 3.** (a) Daily turnover in species composition of the principal ant visitors to the extrafloral nectaries of *O. stricta* in Veracruz, México. Frequency is expressed as the mean number of plants (± 1 SE) on which the species was recorded during diurnal (07.00–19.00 h) and nocturnal (20.00–06.00 h) samplings. Ten plants were checked at 1 h intervals. The ants *Monomorium cyanenum* and *Pseudomyrmex* sp. 5 (pallens group) were each recorded just once during daytime and are not represented in the graph. (b) Daily species replacement by the two dominant ants at the extrafloral nectaries of *O. stricta* in Veracruz. Occurrence is expressed as the number of plants on which the species was recorded at hourly samplings.

**Fig. 4.** Infestation levels by *Narnia* and *Hesperolabops* sucking bugs (nymphs and adults) on experimental plant pairs of *O. stricta* through time. Herbivore activity is not affected by ant treatment (repeated-measures *ANOVA*, $F_{1,36} = 0.067, P = 0.797$). Data are means ± 1 SE.
tion to the plants was also shown to positively affect fruit production. Although similar results were obtained in other EFN-mediated ant–plant systems (see Bentley 1977a,b; Inouye & Taylor 1979; Koptur 1979; Schemske 1980; Keeler 1981; Rico-Gray & Thien 1989; Del-Claro, Berto & Réu 1996) this is the first demonstration of ant protection leading to increased fruit set in the Cactaceae under natural conditions.

The results from the ant-exclusion experiments further corroborated other studies showing that the deterring capacities of visiting ants may not be equally effective against all types of herbivores infesting a plant and this may be owing to several factors. First, some herbivores may possess mechanisms to overcome ant predation and can feed on the plant despite the ants (e.g. Horvitz & Schemske 1984; Koptur 1984; Heads & Lawton 1985; Freitas & Oliveira 1992, 1996). Second, variation in the abundance, aggressiveness or size of ant visitors can affect their protective abilities against a particular herbivore species (Bentley 1977b; Inouye & Taylor 1979; Horvitz & Schemske 1984; Oliveira et al. 1987; Rico-Gray & Thien 1989). Third, herbivore vulnerability to ant predation can depend strongly on the type of plant tissue being consumed relative to the spatial and temporal distribution of extrafloral nectar within the plant crown (Bentley 1977a,b; Tilman 1978; Koptur & Lawton 1988; Oliveira 1997).

Although Pickett & Clark (1979) reported that Crematogaster opuntiae ants attack/kill captive Chelinidea sp. sucking bugs on O. acanthocarpa, the observations of Blom & Clark (1980) on Ferocactus gracilis confirm ours on O. stricta in that visiting ants (including Crematogaster) do not disturb Narnia or Hesperolabops sucking bugs on the plants. Foraging ants are known to attack and dislodge bud/fruit-feeding hemipterans on plants bearing EFNs near reproductive structures (Oliveira 1997). Although Narnia bugs are reported to feed on buds and fruits (Mann 1969), their feeding marks on O. stricta were mostly confined to well-developed cladodes (as well as those of Hesperolabops), where they presumably are less vulnerable to interference from the ants gathering at the EFNs of apical developing buds and cladodes (Fig. 1).

Insect larvae feeding on internal plant tissue, such as miners and borers, may live within their ‘shelters’ relatively safe from ants (Heads & Lawton 1985; Costa, Oliveira-Filho & Oliveira 1992). However, internal plant feeders can be negatively affected by ants if ant activity at a plant’s food source is likely to disturb ovipositing adults, as repeatedly shown for plants bearing EFNs on reproductive organs (e.g. Bentley 1977b; Inouye & Taylor 1979; Schemske 1980; Horvitz & Schemske 1984; Oliveira 1997). Results from the ant-exclusion experiments with O. stricta are clear-cut with respect to the two types of internal feeders tested: damage to cladodes by mining...
dip terans was not affected by ant treatment, whereas the damage by pyralid burrowing larvae to flower buds was significantly higher on ant-excluded plants. The observed pattern most likely results from the more intense ant activity at the EFNs of floral buds than on cladodes. While ovipositing insects are known to be negatively affected by ant activity near a nectar source (Inouye & Taylor 1979; Freitas & Oliveira 1996), the spatial distribution of extrafloral nectar in Opuntia probably makes cladode mining dipterans less vulnerable to ants during oviposition. Similar results with ants vs internal feeders on another nectary plant are reported by Oliveira (1997).

In conclusion, the present study with O. stricta has shown that round-the-clock ant activity at EFNs can reduce damage to reproductive structures and increase the plant’s reproductive output. Differential deter rence by ants toward the insect herbivores of Opuntia may result from several factors, but the spatial distribution of extrafloral nectar within the plant is possibly the most likely one. The fitness consequences of her bivore damage to vegetative tissue are frequently less clear and more difficult to measure than direct damage to plant reproductive structures (Marquis 1992). Although the consequences of damage by sucking and mining insects remain unclear for Opuntia, this study clearly shows how the association of EFNs with vulner able reproductive plant organs can result in a direct ant-derived benefit to plant fitness.

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