

Effects of ant behaviour and presence of extrafloral nectaries on seed dispersal of the Neotropical myrmecochore *Turnera ulmifolia* L. (Turneraceae)

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This paper describes the myrmecochory system of *Turnera ulmifolia* in a coastal sand dune matorral in Mexico. *Turnera ulmifolia* has elaiosome-bearing seeds and extrafloral nectaries (EFNs). In ten quadrants (4 × 15 m) ant–seed interaction was monitored, and an interaction intensity index calculated and correlated with the number of seedlings. Seed removal rates by ants were surveyed every 2 h for 24 h, the ants being observed both on and beneath the plants. The role of the elaiosome in seed removal was evaluated by offering seeds with and without elaiosomes, and elaiosomes only. Finally, the effect of ant manipulation in seed germination was evaluated. There were 25 ant species associated with seeds and/or EFNs, the most frequently recorded being *Monomorium cyaneum* and *Forelius analis*. There was a positive correlation between the intensity index and seedling number per quadrant. There was significantly higher mean seed removal during the day than during the night (19.3% and 12.3%, respectively), and from beneath than on the plant (21.9% and 9.5%, respectively). The preference for elaiosomes only was also greater during the diurnal period, and when gathered on, rather than beneath, the plant. Seed manipulation by *F. analis* enhanced germination by *T. ulmifolia*. Seed removal, dispersal distances, seed predation and germination were largely determined by ant behaviour. The presence of EFNs may be influencing seed removal on the plant by attracting a specific assemblage of omnivorous ants. Among such assemblages associated with *T. ulmifolia* we encountered a variety of behaviours, with ant species either good at defending plants but bad at dispersing seeds, or vice versa. We discuss the way in which these two rewards, and the processes involved (defence and dispersion), could have interacted with each other and evolved. © 2005 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2005, 86, 67–77.

ADDITIONAL KEYWORDS: defence – elaiosome – Mexico – myrmecochory – sand dune matorral – seed removal rate.

INTRODUCTION

The coevolution of plants and ants involving systems of rewards (e.g. specialized food bodies, nest sites, extrafloral nectar, elaiosome-bearing seeds) and services (e.g. defence, dispersion) has resulted in a variety of elaborate and complex mutualistic interactions (Beattie & Hughes, 2002). Plants may offer only one kind of reward, but any combination of them may be present. The provision of a variety of rewards may be one solution to the variable levels of protection, such as those elicited by extrafloral nectaries, as it may

lead to the evolution of specialization in the ants associated with them, generating a shift from facultative to obligate interactions (Beattie & Hughes, 2002)

Extrafloral nectar is a reward produced by secretory glands usually found on leaves (blades, petioles), but also found on reproductive structures (e.g. inflorescence spikes, sepals, buds, fruit) (Bentley, 1977). Extrafloral nectaries (EFNs) are common structures in many plant species (93 flowering plant and five fern families; Beattie & Hughes, 2002). It has been suggested that EFNs have evolved as a generalist plant defence, attracting ants that in turn repel or remove herbivores (Koptur, 1991; but see Becerra & Venable, 1989). However, little is known about the evolution of ant–plant/EFNs interactions (Beattie, 1985; Pember-

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ton, 1992), and other visitors of EFNs, such as spiders and wasps, have proved to be effective plant defenders (Ruhren & Handel, 1999; Cuautle & Rico-Gray, 2003).

The elaiosome is a lipid-rich structure attached to seeds of plants dispersed by ants (Ridley, 1930). Elaiosomes are highly attractive to many ant species, and are generally considered to be a specific adaptation to promote removal by ants and subsequent dispersal. Ants typically carry the intact diaspore (seed + elaiosome) to their nests, where the elaiosome is removed and eaten. The seed may then be discarded within the nest or deposited on a waste midden (Beattie & Hughes, 2002). Ant behaviour has been shown to be an important factor in the fate of seeds of myrmecochorous plants (Hughes & Westoby, 1992; Gorb & Gorb, 2003). In general, the plant is associated with an ant species assemblage, in which not all the ants have the same behaviour, and not all behaviours result in a successful dispersion of the seeds. Ant behaviour may have been an important selective force in the evolution and maintenance of myrmecochory (Hughes & Westoby, 1992).

Turnera ulmifolia L. (Turneraceae) is a herbaceous plant that grows in sand dunes. *T. ulmifolia* offers two kinds of reward for ants: extrafloral nectar and elaiosome-bearing seeds. These rewards and the interactions mediated by them (defence and dispersion) have been discussed separately (Beattie & Hughes, 2002), even though there is evidence that suggests that they can influence each other (Horvitz & Schemske, 1984). Here we describe the dispersal system of *T. ulmifolia*, evaluating the importance of ant behaviour, with particular attention paid to the role of EFNs in influencing seed dispersion. Four questions are addressed: (1) Which ant species are attracted to *T. ulmifolia* seeds? (2) Does ant behaviour correlate with seedling recruitment? (3) What are the rates of seed removal, and what is the impact of elaiosome presence on seed removal by ants? (4) Does seed handling by ants affect germination? Finally, we use a multispecies approach to discuss the role of ants in the defence and dispersion of *T. ulmifolia*.

METHODS

STUDY SITE

The study was carried out during March 2000 to August 2002, in the coastal sand dune scrub system at Centro de Investigaciones Costeras La Mancha (CICOLMA), located on the coast of the state of Veracruz, Mexico (19°36'N, 96°22'W; elevation <100 m). The climate is warm and subhumid; the rainy season occurs between June and September. Total annual precipitation is 1100–1500 mm, mean annual temperature is 24–26 °C, and minimum temperature is 15 °C.

Tropical deciduous forest, tropical dry forest, sand dune matorral and mangrove forest are the major vegetation types in the study area (Moreno-Casasola *et al.*, 1982).

In the matorral, vegetation composition varies according the stability of the dune and the protection offered against wind and sea. The more common species are *Caesalpinia crista* L., *Chamaecrista chamaecristoides* Green (Leguminosae), *Hibiscus tiliaceus* L. (Malvaceae), *Opuntia stricta* Haw. (Cactaceae), *Randia laetevirens* Standl. (Rubiaceae) and *Turnera ulmifolia* (Turneraceae) (Moreno-Casasola *et al.*, 1982; Castillo-Campos & Medina-Abreo, 2002).

DESCRIPTION OF THE SYSTEM

Turnera ulmifolia L. (Turneraceae) is a polymorphic, polyploid complex of herbaceous, perennial weeds bearing extrafloral nectaries; it is native to much of the Neotropics (Gama, Narave & Moreno, 1985; Barrett & Shore, 1987). It shares a variety of vegetation associations and exhibits two contrasting patterns of floral morphology, with either dimorphic or monomorphic populations for a range of floral traits (e.g. style length, stamen height, pollen size; Barrett & Shore, 1987). At the study site, *T. ulmifolia* grows on semi-stabilized and stabilized sand dunes; it is monomorphic and self-compatible with long styles and a range of stamen heights. It flowers and fruits year-round, with a peak in the summer (rainy season) (Torres-Hernández *et al.*, 2000). Branches grow continuously from an apical meristem, regularly producing leaves. Flowers are axillary, and 1–3 open per day (Gama *et al.*, 1985; Torres-Hernández *et al.*, 2000). Flowers last less than a day, and the associated leaf remains throughout fruit development, which lasts 2–3 weeks. EFNs are located at both sides of the petiole, close to its insertion.

Ants [*Camponotus planatus* (Roger), *C. atriceps* (Fabricius), *Monomorium cyaneum* (Wheeler), *Crematogaster brevispinosa* (Mayr), *Dorymyrmex bicolor* (Wheeler), *Forelius analis* (Andre), and *Pseudomyrmex* spp.), and wasps [*Polistes instabilis* (Olivier) and *Polybia occidentalis* (Saussure)] forage for the nectar produced by EFNs (Torres-Hernández *et al.*, 2000; Cuautle & Rico-Gray, 2003). *T. ulmifolia* fruits produce a mean of 56 seeds (SE = 2.04, *N* = 40 fruits). Seeds are *c.* 3 mm long and have attached a soft elaiosome.

T. ulmifolia differs from the myrmecochorous type described by Beattie & Lyons (1975), in that the peduncle is not prostrate during desiccation in order to maintain the capsule on or near the substrate. The fruits are dehiscent capsules. The seeds remain attached to the valves, although those not removed by ants eventually fall to the ground. The mean percent-

age of seeds that fall to the ground is 67% (B. Salazar-Rojas, pers. comm.). Seeds do not remain viable for long periods; for example, 21-month-old seeds no longer germinate (M. Cuautle, unpubl. data). The main leaf herbivore is a caterpillar (*Euptoieta hegesia* Cramer, Lepidoptera: Nymphalidae) that is most active from June to August, although it can be found year-round (Cuautle & Rico-Gray, 2003).

Previous studies have shown that *T. ulmifolia* individuals associated with the largest ants visiting this plant (*C. atriceps*, Torres-Hernández *et al.*, 2000; Cuautle & Rico-Gray, 2003) or wasps (*P. instabilis* and *P. occidentalis*), had higher fruit production than plants without ants or wasps (Cuautle & Rico-Gray, 2003). Torres-Hernández *et al.* (2000) also showed that the small ant species did not protect the plant, resulting in a lower fruit yield.

ANT SPECIES, ANT BEHAVIOUR AND CORRELATION WITH SEEDLING NUMBER

In order to establish the identity and behaviour of the ants attracted to *T. ulmifolia* elaiosome-bearing seeds, we marked a transect (perpendicular from the beach to the dune scrub interior) divided into ten 4 m × 15 m quadrants, 3 m apart. In each quadrant all *T. ulmifolia* plants were recorded and numbered. We counted the number of branches, reproductive branches (with fruit) and fruits per plant. Beneath each plant we placed a Petri dish (with a 1.5 cm opening to allow ants in and out) containing 40 seeds, and for 11 min we registered all ant–seed interactions (categories: 0 = no interaction; 1 = ant approaching a seed; 2 = ant touching a seed; 3 = ant trying to lift a seed; 4 = ant carrying a seed; and 5 = ant successfully placing a seed in nest). We registered the species of ant and the distance that the seed was moved (interactions 4 or 5). This was done for all plants ($N = 63$; plants per quadrant ranged from 0 to 13) within the quadrants, and the sampling order was established at random (observations between 09:00 and 12:00 h or between 16:00 and 20:00 h). This was repeated for the three main seasons of the zone: dry (May), wet (August) and cool (November, same marked transect and plants for all seasons).

We calculated an index of the intensity of ant–seed interaction, based on ant behaviour, and related it to seedling recruitment. This index was calculated by adding the values (0–5) of all interactions recorded during the three seasons in the respective quadrant, weighted by the number of plants per quadrant. Interactions with *M. cyaneum* were not considered because the ant only removes the elaiosome and does not carry the seed. All new seedlings within the transect were registered and assigned to the respective quadrant; those outside the quadrants were assigned to the

nearest one. Even though we surveyed the area for new seedlings throughout the year, most were found during the rainy season. The ant's spatial distribution pattern, although dynamic with regard to nest entrances – the entrance to a nest could change from one week to the next – remained constant with regard to the ant species dominating the area of the selected quadrant.

We used Pearson correlations to test whether or not there was an association between: (1) the intensity of interaction and the number of seedlings per quadrant; (2) the number of seedlings and total number of branches; (3) the number of seedlings and reproductive branches, and (4) the number of seedlings and total number of fruits.

SEED REMOVAL RATES AND IMPORTANCE OF ELAIOSOME PRESENCE ON SEED REMOVAL

To determine the removal rate of *T. ulmifolia* seeds by ants, as well as establish the importance of elaiosomes to seed removal, we designed two experiments. Seed removal rate was determined as the number of seeds taken by ants per 2-h period. Two hours was selected because removal can be very rapid. For both experiments we used 2-cm diameter plastic boxes, each with 40 seeds. Those placed on the plant were attached to an apical leaf of a branch using double-sided masking tape. Fruits that had already opened were removed to avoid interfering with seed removal from the plant boxes. Similar boxes were placed on Petri dishes with an opening on the ground beneath the plant. All seeds were collected the day before the experiment; boxes with fresh seeds were used for each new period. As we needed 4800 seeds/day/experiment, the experiments were only done during peak seed production in the wet season. Experiments were performed during the summer of 2003 on days when climatic conditions were most favourable (i.e. no rain or strong wind).

For the first experiment, seed removal rates, both on and beneath the plant, were determined for a 24-h period over three different days ($N = 5$ boxes/treatment/2 h). Every 2 h we registered the ant species, if present, which were removing seeds from boxes as well as foraging nectar from EFNs. To prevent ants from learning to search for seeds at a particular plant location (i.e. within the plant crown, or beneath the plant) we used the following sample design. We employed the same transect mentioned above, where we marked points 8 m apart. We then allocated the nearest *T. ulmifolia* individuals to each point, giving us 20 focal plants. For the first sampling period, boxes containing seeds were placed alternately (on, then beneath) the plants marked with even numbers. For the second period, we retrieved the boxes used for the previous period and then placed new boxes on plants

marked with odd numbers. Similarly, for the third period, we retrieved previous boxes and placed new ones on plants marked with even numbers, although we reversed the treatments (now the order was beneath, then on) so that the same plant/treatment combination was only repeated after 8 h.

For the second experiment we compared removal rates of complete seeds, seeds without elaiosomes, elaiosomes only, placed on or beneath selected plants (six treatments, $N = 5$ boxes/treatment/2 h). As in the first experiment, 30 focal plants were selected. This was done for two diurnal (9:00–11:00 and 17:00–19:00 h) and two nocturnal periods (21:00–23:00 and 5:00–7:00 h) over the course of 3 days.

For both experiments, the response variable was the mean percent of removed seeds/2 h/treatment. It was arcsine transformed in order to cover assumptions of normality and homoscedasticity (Zar, 1996). Two wider periods, diurnal and nocturnal, were considered. Partially nested ANOVAs were done (Sahai & Ageel, 2000) with the factor 'shift' (diurnal, nocturnal) nested on 'day', and the factor 'position' (on/beneath) crossed with 'shift'. For the second experiment, the factor 'elaiosome' (complete seeds, seeds without elaiosome, elaiosomes only) crossed with 'position' and 'shift', was included. All computations in this study were done using Statistica (1998).

IMPACT OF ANT BEHAVIOUR ON SEED GERMINATION

To explore the effect of manipulation by ants on *T. ulmifolia* germination, seeds were subjected to different treatments: (1) seed manipulation by the ant, (2) mechanical removal of elaiosome, and (3) control. The seeds used in the experiment were collected on 27 July 2001. For the first treatment, seeds were marked with fluorescent dyes and put near the entrance of four *F. analis* nests. For the subsequent treatments, seeds of the same stock were stored in paper bags (at ambient temperature) until used. Most of the seeds placed near the nests ($N = 173$ –200) were taken inside; seeds not retrieved by the ants were removed by us. After 1 week, the seeds inside the nest were removed. Taking the nest entrance as the centre, a 1 m² square was marked off and digging executed to a depth of 6 cm, except in the case of nest 1, in which it continued to a depth of 50 cm, where most marked seeds were found. To recover all seeds, the excavated soil was sieved. The recovered seeds that lacked an elaiosome were put to germinate on 23 August 2001 (27 days after collection) along with seeds from the same collection lot that belonged to the other two treatments: seeds with elaiosomes (control) and seeds without elaiosomes (removed with tweezers when they were sown).

The germination experiment was conducted in a greenhouse on the study site (CICOLMA). The sub-

strate used was ultra fine vermiculite (QUIMIL Co.). Seeds were sown in plastic pots; and a layer of Tangle-foot was applied to their rims to prevent ants from removing the seeds. Ten seeds were placed in each pot at a depth of 2 cm (seven pots/treatment). The pots were placed on plastic trays that were filled with water to 2–3 cm. This level was maintained for 7–10 days, after which time the water was removed; no watering followed for 7 days (J. Shore, pers. comm.). Afterward, the pots were watered every other day and germinated seeds were sampled once a week. Pots with differing treatments were intermixed in the greenhouse area to prevent results from reflecting microenvironmental differences. The response variable was the number of emerged seedlings after 1 month had elapsed.

RESULTS

ANT SPECIES, ANT BEHAVIOUR AND CORRELATION WITH SEEDLING NUMBER

In total, at least 25 ant species associated with *T. ulmifolia* were found. Fourteen were associated with EFNs and seeds, eight with EFNs only, and three with seeds (Table 1). Of the total events recorded ($N = 225$), 53% did not belong to category 0 (no interaction). Of those that were different from zero, 39%, 38% and 23% of the events took place in the dry, rainy, and cool seasons, respectively. The mean distance that seeds were transported before reaching the nest was 83.9 cm (± 13.11 SE, $N = 39$), taking into consideration all ant species (range of variation 10–356 cm). On average, *F. analis* transported seeds for the longest distance (107.3 cm ± 27.18 SE, $N = 16$). The most frequent ants and number of events recorded per species appear in Figure 1. Of these ants, *M. cyaneum* is the

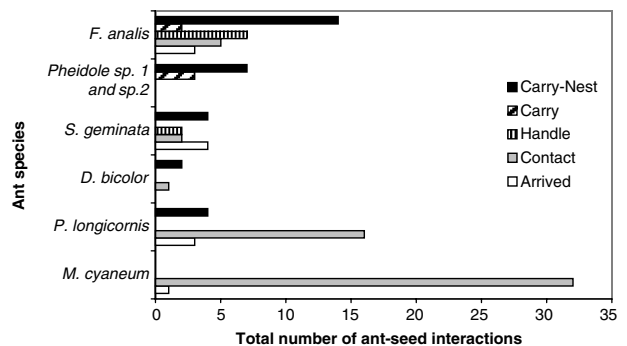


Figure 1. Total number of ant–seed interactions by category (carry-nest, carry, handle, contact and arrived) for the more frequent ant species, registered in 11 min seed removal experiment. At the upper part of the graph there are the ant species which more ant–seed interactions corresponded to carry-nest category; at the lower part, those which ant–seed interactions were mainly contacts.

Table 1. Ant species associated with the extrafloral nectaries (EFNs) and/or seeds (S) of *Turnera ulmifolia* (most frequently recorded species are in bold) found during the day (D) and/or night (N), on (O) and/or beneath (B) the plant, with indications of food habits (FH). 'XX' indicates a strong association with that resource

Subfamily	Species	EFNs	S	D	N	O	B	FH	Reference
Dolichoderinae	<i>Azteca</i> sp.	X	X	X	X	X	X	Omnivore	Rico-Gray pers. comm.
	<i>Dolichoderus lutosus</i> (Smith)	X		X	X	X	X	Omnivore	Mackay (1993)
	<i>Dorymyrmex bicolor</i> (Wheeler)	X	X	X	X	X	X	Predator	Rojas pers. comm.
	<i>Forelius analis</i> (Andre)	XX	XX	XX	XX	XX	XX	Omnivore	Rojas & Fragoso (2000)
Formicinae	<i>Brachymyrmex</i> sp.	X	X	X	X	X	X	Nectarivore	Rojas pers. comm.
	<i>Camponotus atriceps</i> (Fabricius)	X	X		X	X	X	Omnivore	Rojas pers. comm.
	<i>C. coloratus</i> (Forel)	X			X	X	X	Omnivore	Rojas pers. comm.
	<i>C. longinoi</i> (Mackay)	X			X	X	X	Omnivore	Rojas pers. comm.
	<i>C. planatus</i> (Roger)	XX	X	X	X	X	X	Omnivore	Rojas pers. comm.
	<i>Paratrechina longicornis</i> (Cantreille)	X	X	X	X	X	X	Omnivore	Rojas & Fragoso (2000)
Myrmicinae	<i>Cephalotes minutus</i> (Fabricius)	X		X		X		?	Lund (1831)
	<i>Crematogaster brevispinosa</i> (Mayr)	XX	X	X		XX	X	Omnivore	
	<i>Crematogaster</i> sp.	X	X		X	X	XX	Omnivore	
	<i>Leptothorax echinatinodis</i> (Forel)	X		X		X		Omnivore	Rojas & Fragoso (2000)
	<i>Leptothorax wilda</i> (Smith)	X				X		Omnivore	MacKay (2000)
	<i>Monomorium cyaneum</i> (Wheeler)	XX	XX	XX	X	XX	XX	Granivore	Hölldober & Wilson (1990)
	<i>Pheidole</i> sp. 1	X	XX	XX	XX	XX	X	Granivore	Hölldober & Wilson (1990)
	<i>Pheidole</i> sp. 2	X	XX	XX	XX	XX	X	Granivore	Hölldober & Wilson (1990)
	<i>Solenopsis geminata</i> (Fabricius)	X	X	X	X	X	XX	Granivore	Hölldober & Wilson (1990)
	<i>S. (Diplorhoptum) aff. carolinensis</i> 1		X	X			X	Plesiobiotic	Rojas pers. comm.
	<i>S. (Diplorhoptum) aff. carolinensis</i> 2		X	X			X	Plesiobiotic	Rojas pers. comm.
	<i>Solenopsis</i> sp.		X		X	X	X	?	
	<i>Tetramorium spinosum</i> (Pergande)	X		X	X	X	X	Omnivore	Rojas & Fragoso (2000)
Pseudomyrmecinae	<i>Pseudomyrmex brunneus</i> (F. Sm.)	X		X	X	X		Predator/Nectarivore	Rico-Gray unpubl. obs.
	<i>Pseudomyrmex gracilis</i> (Fabricius)	XX	X	X	X	X		Predator/Nectarivore	Rico-Gray unpubl. obs.

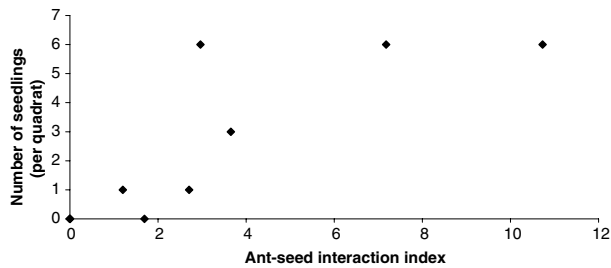


Figure 2. Relation between the ant–seed interaction index and the seedling number, in ten sampling quadrants. A greater index value means a greater number of ant–seed interactions registered or a greater intensity in this interaction. The correlation between these variables was significant ($r = 0.83$, $P = 0.003$, $N = 10$). (Three of the points fall on the origin).

only one that never dispersed seeds; it just consumed the elaiosome. In contrast, *F. analis* took the seed to the nest 45% of the recorded times. Diets of ants associated with EFNs or elaiosomes are shown in Table 1.

A significant and positive correlation (Fig. 2) was noted between the ant–intensity interaction and the number of seedlings in each quadrant ($r = 0.83$, $P = 0.003$, $N = 10$). No significant correlation existed between the number of seedlings and any of the other variables, indicating the productive stage of plants during the three seasons: sum of branches produced ($r = 0.090$, $P = 0.80$), sum of reproductive branches ($r = 0.009$, $P = 0.98$), and total fruits produced ($r = -0.003$, $P = 0.99$).

SEED REMOVAL RATES AND IMPORTANCE OF ELAIOSOME PRESENCE TO SEED REMOVAL

Seed removal rates

There was significantly higher mean seed removal in the diurnal than in the nocturnal period (Table 2, mean diurnal = $19.3\% \pm 3.2$ SE, nocturnal = $12.3\% \pm 2.7$ SE). The factor position was also significant (Table 2), with higher mean seed removal beneath than on the plant (beneath = $21.9\% \pm 3.6$ SE; on = $9.5\% \pm 1.6$ SE). One interesting finding was that in 81% of cases ($N = 21$) the ant species removing seeds on the plant also visited the EFNs. Beneath the plant, this occurred only 17% of the time ($N = 29$). Figure 3 shows how the more frequent ant species were distributed both in terms of the position and shift in which they were found.

Elaiosome importance for seed removal

Three interactions were significant (Table 3). Significant differences in elaiosome preference arose only during the diurnal period (Fig. 4), when the mean percentage of elaiosomes removed was significantly

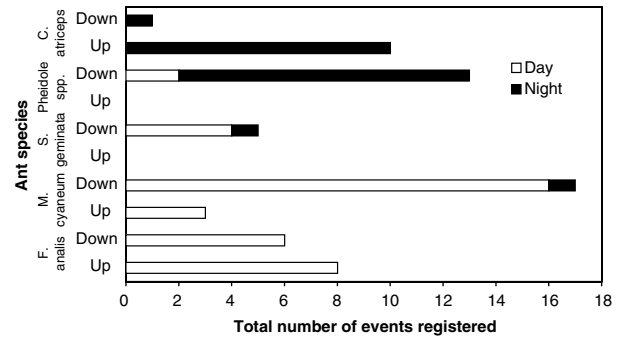


Figure 3. Total number of events registered for the main ant species, during diurnal or nocturnal periods and, on or beneath *Turnera ulmifolia* individuals.

Table 2. Results of the ANOVA computed to analyse the effect of shift (diurnal/nocturnal) and position (on/beneath plant), on seed removal rates by ants during a 24-h period ($P < 0.05$ are in bold)

Factor	d.f.	<i>F</i>	<i>P</i>
Day	2	3.52	0.036
Shift	3	3.94	0.012
Position	1	7.88	0.007
D*P	2	1.37	0.261
S*P	3	0.04	0.989
Error	60		

Table 3. Results of the ANOVA computed to analyse the effect of shift (diurnal/nocturnal), position (on/beneath plant) and elaiosome (complete seeds/seeds without elaiosome/elaiosome only) on seed removal rates by ants, in elaiosome importance experiment ($P < 0.05$ are in bold)

Factor	d.f.	<i>F</i>	<i>P</i>
Day	2	1.16	0.325
Shift	3	28.7	<0.001
Position	1	5.09	0.030
Elaiosome	2	9.16	<0.001
D*P	2	1.89	0.165
S*P	3	1.27	0.299
D*E	4	1.54	0.212
S*E	6	2.43	0.045
P*E	2	5.02	0.012
D*P*E	4	3.12	0.026
S*P*E	6	1.52	0.201
Error	36		

higher ($P < 0.004$, simple contrast) than that of complete seeds (i.e. with elaiosome attached) and of seeds lacking elaiosomes, with no significant differences between the last two treatments ($P = 0.38$, simple contrast).

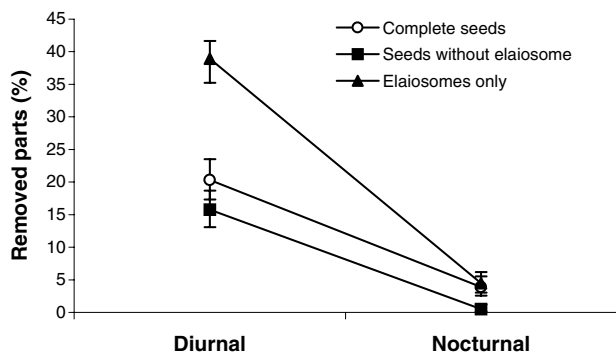


Figure 4. Removed seed parts (either complete seeds, seeds without elaiosome or elaiosomes only), at two periods (diurnal, nocturnal). There were a significant interaction ($P = 0.045$) due to a higher removal of elaiosomes only, during the day. The percentages shown are the arc sin retransformations, bars correspond to the confidence limits (Zar, 1996).

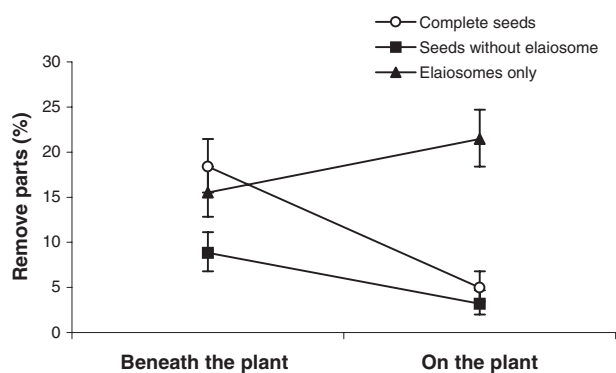


Figure 5. Removed seed parts (either complete seeds, seeds without elaiosome or elaiosomes only), at two different positions (on, beneath) with respect to *Turnera ulmifolia* individuals. There was a significant interaction ($P = 0.012$), due to a higher removal of elaiosomes only, on the plant. Mean while, complete seeds were preferred over seeds lacking elaiosome, by ants foraging on the soil beneath the plant. The percentages shown are the arc sin retransformations, bars correspond to the confidence limits (Zar, 1996).

Elaiosome*Position interaction (Fig. 5) showed that preferences for the elaiosome alone only exist on the plant. There, mean percentage of removal of elaiosome alone was significantly higher ($P < 0.005$, simple contrast) than that of complete seeds and of seeds lacking elaiosomes, with no significant differences between the last two treatments ($P = 0.5$, simple contrast). Beneath the plant, complete seeds were to a significant extent more frequently removed ($P = 0.042$, simple contrast), than those without elaiosomes.

Day*Elaiosome*Position was also significant, suggesting differences among days.

ANT EFFECTS ON SEED GERMINATION

A significantly higher germination percentage was found for seeds obtained from nests (26%), than for seeds with (4%) and without (6%) elaiosomes ($\chi^2 = 16.89$, d.f. = 2, $P < 0.001$).

DISCUSSION

BEHAVIOUR OF ANT SPECIES AND ITS ASSOCIATION WITH SEEDLING RECRUITMENT

Myrmecochory has led to the development of special anatomical, biochemical and phenological adaptations in the plant, enhancing ant attraction and thus increasing the effectiveness of seed dispersal (Gorb & Gorb, 2003). Although there is an impression of asymmetry in the levels of adaptation among the ants and plants, the presence of particular colony organizations and morphological and behavioural traits is also required in order for an ant species to be considered an effective disperser. In this study we show that ant behaviour determines the number of removed seeds, dispersal distances, the number of predated seeds, and germination success in *T. ulmifolia*. Our results suggest that ants may also determine the establishment of seedlings. Previous studies have stressed the importance of ant behaviour in seed dispersal (Hughes & Westoby, 1992; Gorb & Gorb, 2003), or demonstrated a spatial relationship between ants and seedlings of myrmecochorous plant species (Davidson & Morton, 1981; Gorb, Gorb & Punttila, 2000). Here we present the first report using a behavioural variable (ant–seed interaction) correlated with seedling presence, an association that has proved to be significant. Contrary to expectations, no correlation existed between fruit production and seedling number per quadrant, which would appear to indicate that the ant with which the plant interacts is more important than the amount of seeds available for dispersal.

This is the first indication that ants might play an important role as *T. ulmifolia* seed dispersers. However, what could be the specific advantage for seeds that are found, transported and manipulated by ants? Our results show that when seeds are exposed to *F. analis* there is an increase in germination percentage. This effect appears to be the result of something else besides simple mechanical removal. Seeds from which elaiosomes had been experimentally removed did not yield higher germination percentages than those which retained them. Other research has suggested that seed manipulation by ants enhances germination rates (Horvitz & Beattie, 1980; Horvitz, 1981; Pizo & Oliveira, 1998).

SEED REMOVAL RATES

It is clear that seed removal by ants is higher during the day. The differences found between the diurnal and nocturnal periods could result from the species diel turnover, a phenomenon previously noted by other authors (Levings, 1983; Oliveira *et al.*, 1999). The higher daytime removal rate could be due more to particular species behaviour, rather than to differences in species number alone, though fewer species were recorded during the day (5 species, vs. night, 9 species). Fruiting and seed release in myrmecochorous plants can be synchronized with periods of higher ant activity or lower predator activity (Gorb & Gorb, 2003). In *T. ulmifolia*, the time of day at which seeds are released would determine seed availability to certain ant species. *Camponotus atriceps* is more active at night, whereas *F. analis*, which removes a higher number of seeds than *C. atriceps*, is more common during the day.

There was a higher seed removal rate beneath than on the plant. A greater number of ant species was found beneath the plant (9 species, vs. 5 on the plant). Two – *F. analis* and *M. cyaneum* – were found both on the plant and beneath. Greater numbers of granivorous ant species (e.g. *Pheidole* spp. and *S. geminata*; Hölldobler & Wilson, 1990) were observed beneath the plant. We suggest that these differences are related to the presence of EFNs (see beneath).

ELAIOSOME IMPORTANCE TO SEED REMOVAL

Several studies have found that the elaiosome plays a determinant role in seed removal. For example, ants remove large seeds with large elaiosomes more quickly (Gorb & Gorb, 1995), as well as revealing a preference for seeds with an elaiosome compared to those without (O'Dowd & Hay, 1980; Hughes & Westoby, 1992; Quilichini & Debussche, 2000; Garrido *et al.*, 2002). In our study, ants did show a preference for elaiosomes, but only during the day and on the plant. On the other hand, complete seeds were preferred by ants foraging beneath the plant. These results can be explained based on the behaviour of five ant key species in the system: *F. analis*, *M. cyaneum*, *Pheidole* sp.1 and sp.2, and *S. geminata*. The first two are found predominantly during the day, and are often associated with EFNs. It is possible that *F. analis*, an omnivorous species, could prefer elaiosomes or complete seeds over seeds without elaiosomes. *M. cyaneum* only collects elaiosomes. The reason why there was no preference for elaiosomes beneath the plant can be attributed to the presence of the three other ant species. *Pheidole* sp.1 and sp.2 and *S. geminata* are all considered granivorous and probably prefer complete seeds. Our results are similar to those of Wolff &

Debussche (1999), who evaluated dyszoochory by ants and myrmecochory along a successional gradient and found a preference for seeds with an elaiosome in all successional stages except the earliest, due to the presence of *Messor structor*, a granivorous ant.

PRESENCE OF EFNS AND SEED REMOVAL

The results indicate that removal percentage and elaiosome preferences differ according to location (beneath or on the plant), as also do ant species richness and behaviour. We suggest that these differences are associated with the presence of EFNs, which could be acting like a 'selective sieve' (Thompson, 1994) attracting omnivorous ants instead of granivorous ants, influencing the composition of the ant assemblage on the plant and making it different from the fauna beneath the plant. Omnivory may have been an important character in myrmecochorous evolution, as shown by the fact that the fatty acid composition of elaiosomes appears to be convergent with insect prey, attracting omnivorous ants that would not normally remove and eat plant material (Beattie & Hughes, 2002). Thus, EFNs may exercise an indirect influence on seed removal, as ants foraging on EFNs could have better access to *T. ulmifolia* seeds.

We suggest that the presence of EFNs could have influenced the evolution of myrmecochory in this system. The effectiveness of seed removal is enhanced by the location of EFNs at those microsites most frequently visited by ants (Gorb & Gorb, 2003). Since different ant species have different foraging niches (Dlussky, 1967 in Gorb & Gorb, 2003), seed deposition by plants strongly influences ant visits. Seed deposition might have been a character under selection in myrmecochory evolution, as is shown by the fact that two strategies for seed presentation have evolved. In the herbaceous myrmecochores of the northern hemisphere, the peduncle prostrates during dehiscence to maintain the capsule on or near the substrate, a characteristic that allows ants to discover seeds easily. In shrubs of the southern hemisphere, seeds usually fall to the ground singly, often preceded by a ballistic expulsion. *Turnera ulmifolia* does not present any of these strategies, but the presence of EFNs in itself encourages frequent visitation by omnivorous ants. However, to determine if the presence of EFNs influences seed removal, a specific experiment comparing seed removal in the presence/absence of EFNs is required.

DEFENCE AND DISPERSION

In general, plant–animal interaction studies have focused on a single type of interaction. This leads to oversimplification, as most plant populations and indi-

viduals presumably 'perceive', over ecological and evolutionary time, the composite result of their interactions with a broad array of animal interactors (Herrera, 2000). When a multispecies or multitrophic approach is taken, it becomes clear that different groups of organisms with which a plant can interact combine to exert upon it an influence that is seldom additive (Juenger & Bergelson, 1998; Herrera, 2000; Cuautle & Rico-Gray, 2003).

In studies of ant-plant interactions, plant rewards and services offered by the ants, and the interactions involved, are often discussed separately, despite evidence that they might influence each other. For example, Horvitz & Schemske (1984) worked with *Calathea ovoidensis* Matuda, an acaulescent perennial herb with EFNs. They found that the ant *Wasmania auro-punctata* Roger, associated with EFNs, defended *C. ovoidensis* from herbivores. They also mention, however, that the ant might have a negative impact on seed dispersal, since it actively gathers the lipid reward from the seed aril without displacing it.

The two roles that ants play in the *T. ulmifolia* system – defence (mediated by EFNs) and dispersal (mediated by elaiosome-bearing seeds) – are analysed jointly. From this perspective, we understand why *T. ulmifolia* sustains tiny ants that do not fulfil a defence function for the plant, i.e. they appear to play a vital role in seed dispersal. We also find that the presence of EFNs could influence seed dispersion by attracting a certain ant community. This scenario leads us to ask about the way in which these two rewards, or others offered in ant-plant systems, could have been influencing each other in their evolution.

Ant-plant systems that are species-specific have evolved, but are rare. Examples include Janzen's (1966) *Acacia-Pseudomyrmex* system, in which all ant requirements are fulfilled by the plant. However, it has been suggested that a way to produce fidelity could be the provision of a variety of rewards and a constant supply of them (not variable in time and space) (Beattie & Hughes, 2002). *Turnera ulmifolia* offers two rewards that cover some of the nutritional requirements of ants. These resources are produced in a predictable way (throughout the year), although not in the same quantity (Torres-Hernández *et al.*, 2000; Díaz-Castelazo *et al.*, 2004). Twenty-five ant species make use of these resources, a high number for only one plant species. Moreover, a quarterly census (Díaz-Castelazo *et al.*, 2004) in the same study site showed that *T. ulmifolia* was the plant species most frequently visited by ants, compared with seven other EFN-bearing plant species. In the same study, after *Crotalaria indica* L. (12 related species), *T. ulmifolia* had the most ant species associated with EFNs (11 related species) (Díaz-Castelazo *et al.*, 2004). Thus, *T. ulmifolia* seems to be a key resource for ant com-

munities in this sand dune scrub. However, it is unlikely that this, or other similar systems, will lead to the evolution of a species-specific ant-plant interaction; rather it will lead to the acquisition of an ant assemblage with ideal characters, specially behaviour, for defence and dispersion.

Among the ant assemblage associated with *T. ulmifolia* we have *Camponotus atriceps*, good at defending but bad at seed dispersal, *F. analis* bad at defending and good at dispersal, and even the 'parasite' *M. cyaneum* (*sensu* Gómez & Espadaler, 1998). Is this an ideal ant assemblage for *T. ulmifolia*? Cogni, Raimundo & Freitas (2000) found a similar assemblage associated with *T. ulmifolia* individuals in south-east Brazil. They found species of *Camponotus*, *Crematogaster* and *Pheidole*. However, they also found a species belonging to the genus *Ectatomma*, which was observed removing seeds along with *Camponotus* species. It is probable that the ideal assemblage could turn out to vary within a geographical mosaic (Thompson, 1994). It is hoped that this study will encourage discussion of the way in which interactions mediated by several plant rewards and ant services could influence each others' evolution.

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