Short communication

Removal of *Pilosocereus leucocephalus* (Cactaceae, tribe Cereeae) seeds by ants and their potential role as primary seed dispersers

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A B S T R A C T

Myrmecochory sensu stricto is uncommon in Neotropical forests. In these ecosystems the role of ants as secondary dispersers of non-myrmecochorous seeds is well known, however, the primary dispersal of this type of seed by ants has been poorly documented. Only a few anecdotal observations scattered throughout the literature report the removal of these seeds from fruit on the plant by ants. We show experimentally that ants are able to remove more pulp and seeds from fruit on the branches than do flying vertebrates in *Pilosocereus leucocephalus*, a columnar cactus bearing typically ornithochorous fruit. In addition, experiments emulating seed cleaning revealed that cleaned seeds germinate earlier. These results suggest a possible role of ants as primary cactus dispersers; one that requires more extensive research on seed fate and seedling establishment success.

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Myrmecochory sensu stricto is uncommon in Neotropical forests (Rico-Gray and Oliveira, 2007) where 90% of the plants bear fleshy fruits and presumably rely on vertebrates for seed dispersal (Frankie et al., 1974). However, the lack of elaiosomes on the seeds does not preclude ant–seed interactions. Seeds primarily dispersed by vertebrates eventually reach the forest floor where ants collect them and carry them to their nest (Pizo et al., 2005). After feeding their larvae with the pulp, ants put the seeds, undamaged, in refuse piles where seedlings find suitable growing conditions (Horvitz, 1981; Beattie, 1985; Pizo and Oliveira, 2001). Ants also prevent pathogen infestation by removing the fleshy coat from the seed (Ohkawara and Akino, 2005; Christianini et al., 2007). Although the role of ants as secondary dispersers of non-myrmecochorous seeds is widely recognized at present, the primary dispersal of these seeds by ants has received scant attention. The few anecdotal reports available only describe ants removing seeds from fruit on the plants without any quantification (Wheelwright, 1985; Silvius, 1995; Montiel and Montaña, 2000).

During spring and summer 2006 and 2007, in addition to birds we observed ants climbing and taking seeds from fruit on the branches of the *Pilosocereus leucocephalus* columnar cactus. We found this behaviour interesting for several reasons: the seeds do not have any elaiosomes, the fruit morphology of this cactus species suggests bird dispersal, and all previous studies done on columnar cacti report primary seed dispersal by flying vertebrates (Silva, 1988; Wendelken and Martin, 1988; Silvius, 1995; Sosa, 1997; Soriano et al., 1999; Godínez-Alvarez and Valiente-Banuet, 2000; Godínez-Alvarez et al., 2002; Soriano and Ruiz, 2002). From our field observations three main questions arose: (1) how important are ants in the removal of seeds from fleshy fruit on these plants, relative to other dispersers? (2) Is fruit pulp an important factor in ant attraction, and (3) does manipulation by ants (specifically seed cleaning) enhance seed germination?

The study area is located at Loma de Rogel, Veracruz, Mexico (19°34′N, 96°47′W). The natural vegetation is a moderately disturbed tropical dry forest with a discontinuous canopy. The soil is scarce and its distribution heterogeneous: it is absent from canopy gaps, and moderately developed in rocky cavities and beneath trees. Both of these microhabitats are suitable for *P. leucocephalus* establishment (Munguía-Rosas and Sosa, 2008). *P. leucocephalus* has three fruiting pulses from April to September. Fruits are spheroid (6 × 5 cm diameters and 60–100 g), split open when ripe and have red fleshy pulp in which an average of 830 small seeds (2.5 × 1.5 mm, 1.4 mg) is embedded. We quantified the lipid concentration of seed and pulp using the extraction, purification and quantification protocols of Christie (1993), Folch et al. (1957), and Ahlgren and Merino (1991), respectively. The lipid concentration in seeds was 123 mg g^{-1} and 21.8 mg g^{-1} in pulp.

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To identify the guild of frugivorous vertebrates, we sampled volant vertebrates during spring and summer 2007 in the study area and its surroundings. Birds and bats were captured with four mist nets (9×2 m) from 06.00 to 10.00 and from 20.00 to 24.00, respectively. Total sampling effort was 180 net-h m^{-2} for birds and 270 net-h m^{-2} for bats. We identified ten birds belonging to nine species and 18 bats belonging to four species. Seven of the bird species and two of the most abundant bat species feed on fruit (see Appendix). There was no previous record of the species composition of the ant community, so we documented the identity of ants visiting fruits and seeds when carrying out the experiments described below.

We compared fruit mass depletion by different fruit visitors in a field exclusion experiment. During early summer 2007 we collected 34 ripe fruits from 15 different cacti. When ripe, the fruit split open and expose their pulp to dispersers, so to simulate natural conditions collected fruits were split into two or three pieces of similar volume and fixed on cactus branches. Three pieces, placed 20 cm apart and 150–210 cm above ground, were assigned to only one external branch per individual. Each branch with three pieces of fruit represented a statistical block. Each piece in a block was randomly assigned to one of three treatments: (I) removal by ants, excluding vertebrates and other animals with a wire cage (mesh opening = 5 mm); (II) removal by vertebrates, excluding ants with Tanglefoot, a natural and odourless resin, spread around the fruit; and (III) control, no removal, protecting the fruit with a plastic net (opening < 0.5 mm). We have used these exclusion devices in other experiments and they do not deter birds or ants from visiting the experimental plants. The number of replicates, 24, was given by the number of blocks (one branch with three pieces of fruit). Each piece of fruit was weighed at the beginning and then every 12 h over the 72 h of the experiment. The decrease in fruit mass was considered to be a measure of seed removal (fruit mass explains 61.72% of variance in seed number; $n = 33$ fruit portions, $P < 0.001$). Mass lost in the control treatment was attributed to manipulation, rainfall, wind or evaporation. When weighing the fruit, we recorded vertebrate visits on a focal block for 1 h with a Sony Handycam video camcorder model DCR-TRV280, using infrared light at night. We filmed six different blocks for a total of 6 h: 3 h in the morning and 3 h at night. The exclusion experiment was conducted during a fruiting peak in the dry season. Naturally attached fruits were removed in order to maintain a constant fruit number among the experimental plants. Fruit number on the experimental cacti and on the neighbouring cacti was similar (mean number of fruit on non-experimental plants is five). Our experiment lasted three days, similar to the period that ripe fruit remains on the cactus under natural conditions before being completely eaten. We statistically compared fruit mass depletion among treatments by fitting a linear mixed-effects model (LME). Treatments were specified as the fixed effect, and the blocks and repeated measures as the random effects of the model (Pinheiro and Bates, 2000). To detect any differences among treatments we performed a posteriori contrasts (Crawley, 2002).

To assess whether fruit pulp stimulates interest by ants in seeds, we placed 20 cleaned seeds in each of 25 Petri dishes (stations), and a variable number of seeds with pulp in each of another 25 Petri dishes, all on the forest floor (2 treatments, 25 replicates). Seed number was not fixed in the second treatment because we used seeds embedded in fresh pulp; however we did count the initial number of seeds in each station by spreading the pulp out in the dish. This initial seed number was used in the statistical analyses. The stations were placed at least 5 m apart and protected from vertebrates with plastic cages (opening: 8 mm). Instantaneous counts of the number of ants feeding at the stations were done

during the morning (09.00–10.00) 24 h after the start of the experiment. The seeds remaining in the dishes after 96 h were counted. We realize that a more realistic experimental setup would have used the cactus branches as the seed location, but it was virtually impossible to place seeds without pulp on the almost-vertical branches of the cactus. The number of ants visiting the stations and the proportion of seeds removed were compared between treatments using deviance analyses (generalized linear models). A Poisson error distribution was used for the number of visiting ants, and a binomial error distribution for the proportion of removed seeds.

The effect of seed cleaning on germination was tested using two treatments: cleaned seeds and seeds embedded in pulp. Five fruits were cut in two: one half was used to obtain cleaned seed (washed with tap water and dried at room temperature for 2 d) and the other as a source of seeds with pulp. Groups of 30 cleaned seeds and approximately the same number of seeds with pulp (mean number per dish was 34) were put into 60 randomly placed Petri dishes (30 per treatment) lined with moist filter paper under greenhouse conditions. We calculated the proportion of germinated seeds every three days over 15 d and compared it between treatments by fitting a LME, after an arcsine-square root-transformation of the data. The treatment was the fixed factor; the fruit and the repeated measures, the random factors. All the statistical analyses described in this and the previous sections were done using S-Plus 2000.

Fruit mass depletion differed statistically among treatments ($F_{1, 334} = 5.44$, $P < 0.01$). A posteriori comparison revealed differences between ant removal and the control and vertebrate removal treatments; the latter two treatments did not differ. Removal by ants was always greater than removal in the other two treatments; however, the magnitude of the differences varied over time (Fig. 1). Filming recorded six visits of the hummingbird *Amazilia tzacatl* sipping juice from six different pieces of fruit. The Varied Bunting, *Passerina versicolor*, visited one fruit twice. Even though the camera did not have high enough resolution to record ants, we directly observed *Solenopsis aurea* and two species of *Pheidole* ants climbing the cactus branches and taking seeds with pulp (a colour photo is available as Supplementary material). Although we saw the ants taking and transporting the seeds, we were unable to identify the final destiny or how far the seeds are transported by ants because ants carrying seeds disappeared in small crevices or beneath spiny vegetation.

Our estimates of fruit mass removal by ants are highly conservative because the exclusion mesh was an obstacle that reduced the rate of removal. Even so, the evidence suggests that ants are able to remove more fruit mass than vertebrates can. As we have both caught frugivorous flying vertebrates and seen birds feeding on fruits in this and other cactus populations, it is too risky to conclude that this situation can be generalized in space and time. During our study the fleshy fruit of two other columnar cactus species (*Neobuxbaumia euphorbioides* and *Stenocereus griseus*) was available; it is likely that competition for dispersers may have influenced the visiting rate of flying vertebrates to experimental fruits. As no research has been done in the study area, we do not know the population status of birds and bats. The important point is that at certain locations and times ants are able to use resources which were previously thought to be almost exclusively consumed by vertebrates. Other authors have also observed ants climbing and taking the seeds from cactus fruit in other places (e.g. *Opuntia rastera* in the Chihuahuan Desert; Montiel and Montaña, 2000). In fact, Silvius (1995) realized that the fruit of *S. griseus* can be depleted by ants if vertebrates are absent. However, in these studies the authors only reported direct seed removal from fruit on plants by ants without any quantification.
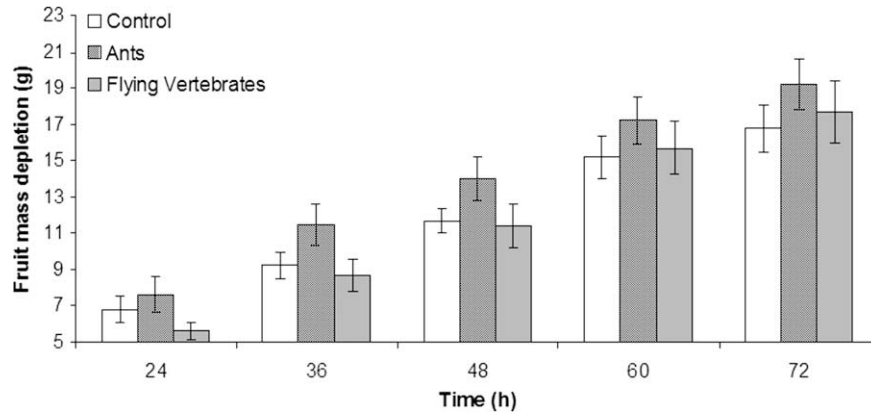
Fig. 1. Fruit mass depletion under three experimental treatments: (I) fruit accessible only to ants, vertebrates excluded with a wire cage, (II) fruit accessible only to flying vertebrates, insects excluded with Tanglefoot resin, (III) the control, with fruits covered with mosquito netting to exclude all visitors. The time when the fruit was weighed is given on the *X*-axis. Bars show mean values ± SE.

We encourage others to research the contribution of ants to seed dispersal in other species even though seed dispersal syndrome suggests non-myrmecochorous seed dispersal.

Significantly more ants fed on pulpy seeds (6.48 ± 2.14 ants per station; mean ± SE) than on cleaned seeds (0.24 ± 0.24 ants per station; $\chi^2_1 = 187$, $P < 0.001$); initial seed number had no effect ($\chi^2_1 = 2.65$, $P = 0.10$). On the other hand, ants removed slightly more cleaned seeds (93.6 ± 3.3%) than seeds with pulp (74.6 ± 7.2%) from stations. Although the difference was small, it was significant ($\chi^2_1 = 118$, $P < 0.001$). The number of seeds initially present had a positive influence on removal ($\chi^2_1 = 43.3$, $P < 0.001$). No seeds were found close to the stations, we infer ants carried the seeds farther a field than the area we searched for seeds outside the stations (60–70 cm). The ant species manipulating or carrying seeds on the forest floor were *Crematogaster* sp., *Ectatomma* sp., *Monomorium* sp., two *Pheidole* sp., *S. aurea*, *Tapinoma* sp., and *Trachymyrmex* sp. However, we saw only *Pheidole* and *S. aurea* ants climbing *P. leucocephalus* branches.

Seeds in their pulp attracted more ants than cleaned seeds on the forest floor. However, seeds of *P. leucocephalus* are rarely found on the forest floor with pulp on them because the fruit of this cactus species rarely fall to the ground spontaneously. Therefore, ants interested in the pulp must climb the cactus branches to reach the seeds with pulp. *Pheidole and Solenopsis*, the species we observed climbing *P. leucocephalus* and removing the seeds from

fruit on the branches, also climb other cactus species to feed on nectar produced by extrafloral nectaries (Blom and Clark, 1980). Similar to ants climbing plants in search of extrafloral nectaries, the ants that climb *P. leucocephalus* obtain sugar and water from pulp (fleshy fruits are rich in these; Baker et al., 1998); two resources that are highly valuable in arid regions (Ruffner and Clark, 1986). Therefore, fruit pulp is probably one factor attracting ants to *P. leucocephalus* fruit.

The proportion of germinated seeds was statistically greater for artificially cleaned seeds than for seeds enveloped in pulp (Fig. 2; $F_{1, 54} = 33.2$, $P < 0.01$) during the first ten days. Although by the end of the experiment both treatments showed similar proportion of germinated seeds, seedlings from cleaned seeds emerged earlier and therefore have a greater likelihood of surviving (Verdú and Traveset, 2005). Therefore, in addition to removing seeds from fruit on the plant, ants consume the fleshy coats and this enhances seed germination and probably seedling survival. Our results on germination enhancement after artificial seed cleaning are conservative since, in addition to cleaning the seeds, ants also secrete antifungal substances (Ohkawara and Akino, 2005). We saw both *Pheidole* and *Ectatomma* ants taking seeds from the forest floor and only the former taking the seeds from the fruits on cactus branches. We were able to follow two ants until they abandoned the seeds in rocky cavities. We recognize that more observations and experimental work are needed to reach more conclusive results on the final destiny of seeds and the role of ants as primary seed dispersers. However, it is well known that ants (even those considered granivorous) carry or abandon some seeds in safe microsites, such as ant nests (Rico-Gray and Oliveira, 2007). Because of the predominance of bare rock, suitable sites for ant nesting are restricted to those with soil; the latter accumulates in rocky cavities where *P. leucocephalus* is preferentially recruited (Munguía-Rosas and Sosa, 2008).

In conclusion, we show that ants remove *P. leucocephalus* seeds directly from fruit on the plant and take them from the parental plant; also, that seed cleaning enhances seed germination. How far ants are transporting the seeds and the final fate of the seeds requires further observations. We hope this contribution triggers interest in the primary dispersal of non-myrmecochorous seeds by ants in other plant species.
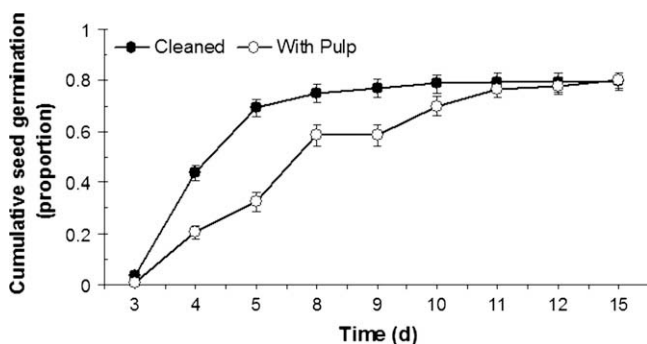
Fig. 2. Effect of seed cleaning on seed germination. Curves represent the cumulative germination of *P. leucocephalus* seeds subjected to two treatments: artificially cleaned seeds (filled circles) and seeds immersed in pulp (open circles). Symbols and bars represent the mean ± SE. The experiment lasted 15 d. As online Supplementary material we provide a colour photo showing a *Pheidole* sp. ant carrying a seed of *P. leucocephalus* down a cactus branch. The seed has pulp on it.

Appendix. Supplementary material

Supplementary material associated with this article can be found in the online version, at doi:10.1016/j.jaridenv.2008.12.017.

Appendix. Flying vertebrates caught in the cactus population under study and three contiguous populations, April–July 2007. The main food in the diet is given in the last column. *N* indicates the number of individuals captured.

Site	Species	N	Main food in diet
Study population	Bats		
	Artibeus jamaicencis	5	Fruit
	Choeroniscus godmani	3	Nectar
	Birds		
	Arremonops rufivirgatus	2	Omnivorous
	Columbina passerina	1	Seeds
	Campylorhynchus gularis	1	Insects + fruit
	Campylorhynchus rufinucha	1	Insects
	Icterus gularis	1	Insects + fruit
	Passerina cyanea	1	Seeds
	Passerina versicolor	1	Seeds
	Pitangus sulphuratus	1	Omnivorous
Contiguous populations	Bats		
	Artibeus intermedius	12	Fruit
	Artibeus lituratus	5	Fruit
	Chiroderma salvini	1	Fruit
	Chiroderma villosum	1	Fruit
	Glossophaga morenoi	2	Nectar
	Glossophaga soricina	3	Nectar + insects
	Leptonycteris curasoae	2	Nectar
	Leptonycteris nivalis	3	Nectar
	Birds		
	Camptostoma imberbe	1	Insects + fruit
	Cyanocompsa parellina	1	Seeds
	Dives dives	2	Insects + fruit
	Euphonia hirundinacea	1	Fruit
	Empidonax sp.	3	Insects
	Pipilo fuscus	1	Seeds
	Turdus grayi	1	Insects + fruit
	Vireo belli	2	Insects

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