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Mechanical defence in seeds to avoid predation by a granivorous ant

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Abstract Harvester ants have traditionally been considered as seed predators that negatively affect plants. In some cases, however, they can also act as positive seed dispersers. During field observations, we noted that a portion of Psoralea bituminosa seeds that were collected and carried to the nest by the granivorous harvester ant Messor barbarus were discarded intact in refuse piles outside the nest. We analyzed and compared the physical characteristics of size, mass and toughness in P. bituminosa seeds from two different origins: intact seeds found in the ant's refuse piles and seeds collected directly from the plants. Seeds from refuse piles were similar in width but lighter and tougher than seeds from the plant. Our results point to a mechanical defence based on seed toughness to avoid predation by M. barbarus and suggest that an elevated proportion ($\sim 69\%$) of the seeds produced by P. bituminosa could be too tough to be consumed by this ant. These transported but uneaten seeds could benefit by being moved far from the mother plant and this could act as a selective evolutionary pressure towards tough seeds.

Keywords *Messor barbarus* · *Psoralea bituminosa* · Seed predation · Seed toughness

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Introduction

Harvester ants have traditionally been considered as seed predators that negatively affect plant populations. However, there is also evidence that they can act as seed dispersers with positive effects for the plants because, in some cases, the ants may not eat some of the seeds they collect (Rissing 1986; Levey and Byrne 1993; Detrain and Tasse 2000). In these cases, the mere distancing from the parent plant can have positive effects for the seed (Beattie 1985; Andersen 1988; Whitney 2002), by reducing density-dependent or distance-responsive seed predators, pathogens, or seedling competition near the parent plant (escape hypothesis; Howe and Smallwood 1982). Moreover, the seeds are sometimes rejected in a refuse pile, which in some cases can be considered a safe site rich in nutrients and poor in competitors that can favour germination and seedling emergence (directed dispersal hypothesis; Howe and Smallwood 1982). Various fates have been described for seeds collected by harvester ants that are finally not eaten: being dropped during transport on the way to the nest (Detrain and Tasse 2000), rejected on refuse piles around the nests (O'Dowd and Hay 1980; Rissing 1986; Danin and Yom-Tov 1990; Levey and Byrne 1993; Boyd 1996; Detrain and Tasse 2000) and neglected in the granaries in the case of nest relocation (Hughes 1990). This behaviour has usually been viewed as a mistake made by the ants (MacMahon et al. 2000). However, in some cases, these surviving seeds could be discarded by ants deliberately due to mechanisms that prevent seed consumption, such as the presence of toxic compounds on the seed coat (Majer and Lamont 1985), a smooth surface that makes handling the seeds difficult (Pulliam and Brand 1975), or a strong seed coat as a mechanical defence (Rodgerson 1998). Seeds infected by fungi (Knoch et al. 1993) or empty seeds (Baroni Urbani 1992) may also be detected by particular individuals and subsequently discarded.

In the present study, we investigate the relationship between the granivorous seed harvester ant *Messor barbarus* L. and seeds of *Psoralea bituminosa* L. (Papilionaceae). *M. barbarus* includes *P. bituminosa* seeds in its diet. Seeds are collected and transported to the nest where they are eaten, and then seed scraps are discarded in a refuse pile outside the nest. However, we can also observe intact, uneaten *P. bituminosa* seeds in these piles. Here, we analyze if the *P. bituminosa* seeds that are collected and transported but refused intact by *M. barbarus* in middens outside the nests have any particular trait to explain why they were not consumed by the ants. We consider two possibilities for seeds that are not predated: a large seed size that prevents ants from handling the seeds or a toughness that prevents ants from breaking them.

Materials and methods

Study area and plant species

The study was carried out from May to July in both 2002 and 2006. Sampling was done in a suburban area near Rubí (NE Spain; 41° 28' N 2° 1' E). The site is an old field on former agricultural arable land, abandoned 15 years ago. Vegetation is mainly a herbaceous community of *Inulo-Oryzopsietum miliacea* (Bolós 1962). *P. bituminosa* is an annual herb that grows up to 1m high and is present in shrublands and waysides of the Mediterranean basin. The seeds of *P. bituminosa* are winged and the *M. barbarus* worker ants use the wings as handles to transport the seeds easily.

Plant seeds vs. midden seeds

To look for specific traits in the seeds collected but finally discarded by M. barbarus, seeds were obtained from two different origins: directly from the plant (100 seeds proportionally collected from ten different plants) and from the refuse piles of *M. barbarus* nests (five nests checked once a week for a month, during the seed dispersal period). In the refuse piles, we found consumed and unconsumed seeds but only the second type was selected. Among these, nonviable seeds (i.e., parasitized empty coats or coats with a nonviable embryo, which were easily broken with light pressure applied with forceps) were excluded. For each seed (plant origin n = 100; refuse pile origin n = 50), the mass (\pm 0.01 mg) was measured with a precision balance (Precisa 80A-200M); the minimum width (\pm 0.01 mm) was measured with an electronic digital caliper (Australian Measuring Instruments), and the toughness (in newtons, ± 2 N) was measured with a Chatillon Universal Force Tester (Amtek/Chatillon, Largo, FL, USA). The toughness, i.e., the amount of force required to fracture the seed, was measured electronically by placing the seed on a fixed plate and lowering an upper plate onto the seed at a constant speed until the seed fractured. These characteristics were compared for the seeds from the two different origins (plant vs. refuse pile) with a single-factor analysis of variance.

Seed ageing

The effect of ageing on seed characteristics was tested. Seeds were collected from five different *P. bituminosa* plants and were left to dry uncovered in the laboratory. From time to time, during a 4-month period (from June to October), ten seeds of each plant were randomly chosen and the mass, the width and the strength were measured. The effect of ageing was evaluated by testing the null hypothesis that the slope of the linear regression for each variable was equal to zero.

Seed collection by ants

We looked for *M. barbarus* ant nests in the study zone and, when located, ten P. bituminosa seeds were placed close to the ants' foraging trail at about 1 m from the nest entrance. The ten seeds placed nearby had been previously measured and selected so that there were five smaller, lighter seeds and five larger, heavier seeds. This was done to test if worker ants made a selection of the harvested seeds. When a worker gathered a seed and transported it to the nest, both the worker and the seed were captured and, later in the laboratory, the mass, the width and the toughness of the seed and the head width of the worker ant were measured. Head width offers a precise estimation of ant size, especially for the subfamily Myrmicinae (Kaspari and Weiser 1999). This was done at ten different M. barbarus nests. The correlations between the ants' sizes (head width) and the measured characteristics of the seeds harvested by them were analyzed using Pearson's correlation coefficient.

At the same time, the mandible gap of *M. barbarus* was measured. This was done using metallic sheets of different gauge widths. The mandible gap was measured by holding a worker by its thorax with soft forceps and offering it different sheet gauges in increasing widths until the ant could not grasp one. This was performed using hands-free binocular lenses (Optivisor, \times 7, Donegan Optical Company, KS, USA). A total of 50 *M. barbarus* workers were measured in this way.

Statistical analysis

All statistical analyses were run with the SPSS package for Windows, version 14.0 (SPSS Inc.).

Results

Plant seeds vs. midden seeds

Seeds collected from the ant refuse piles were tougher than seeds obtained from the plants ($F_{1, 148} = 12.085$; P < 0.001; Fig. 1). While toughness in seeds from the plants ranged from 36 to 158 N (mean ± SE = 89.24 ± 2.90 N), all seeds from refuse piles had toughness higher than 70N (104.76 ± 2.50 N). However, seeds from the refuse piles were lighter (mean ± SE = 12.84 ± 0.37 mg) than those collected from the plants (15.12 ± 0.26 mg; $F_{1, 148} = 25.722$; P < 0.001; Fig. 1). Seed width did not differ in the two origins (plant origin = 1.96 ± 0.02 mm; refuse pile origin = 1.92 ± 0.03 mm; $F_{1, 148} = 1.007$; P = 0.317; Fig. 1).

Seed ageing

Ageing did not cause variations in seed toughness, seed width, or seed mass during the studied period (Fig. 2). The slopes of linear regressions did not differ significantly from zero in any of the three seed variables (P > 0.1; Table 1).

Seed collection by ants

All the seeds placed near the ant nests (both the small, light ones and the large, heavy ones) were gathered and transported by worker ants within a few minutes. The size (head width) of the harvester ants did not correlate with any of the seed characteristics measured (mass: r = 0.087, P = 0.390, n = 100; width: r = 0.083, P = 0.413, n = 100; toughness: r = 0.136, P = 0.179, n = 100; Fig. 3).

The mandible gap of *M. barbarus* workers ranged from 0.80 to 2.80 mm (mean \pm SE = 1.64 \pm 0.07 mm). This wide gap range is explained by the high level of polymorphism by this species.

Discussion

Our results point to seed toughness as the main factor responsible for their refusal by *M. barbarus* workers. When ants discover that they cannot break and eat a seed, it is ejected from the nest and discarded outside in the refuse pile. As seed mass is unlikely to limit the transport of *P. bituminosa* seeds by *M. barbarus*, the lightness of seeds from the refuse piles could be explained in part by the significant negative correlation (r = -0.347, P < 0.001, n = 100; although the low coefficient denotes a high dispersion of data) between toughness and mass found in the seeds (plant origin). An ageing effect, such as seed dehydration over time, can be discarded as an option for this loss of mass, as demonstrated in this study. Moreover, the tested

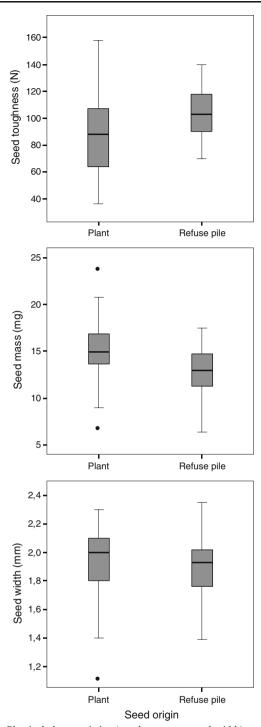
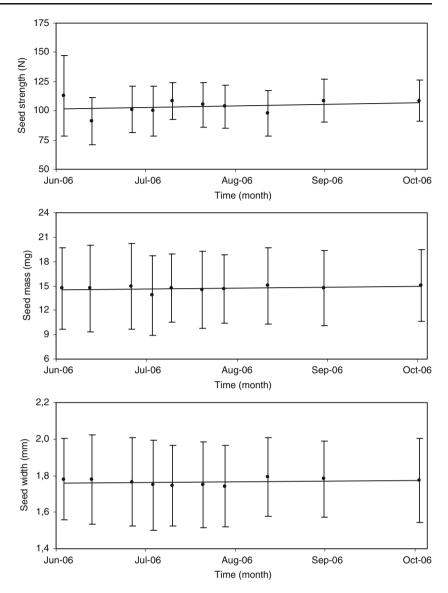


Fig. 1 Physical characteristics (toughness, mass and width) measured in *P. bituminosa* seeds obtained from the plants (n=100 seeds) and from the refuse piles (n=50) of *M. barbarus* nests. *Box plots* denote the median and interquartile range; *vertical bars* denote minimum and maximum data values and *circles* denote atypical values

seeds were collected from middens during the dehiscence period of *P. bituminosa* to insure they had only been exposed for a few days and thus minimize the possible effects of ageing. Furthermore, our results demonstrate that seed ageing does not cause changes in either seed width or Fig. 2 Monitoring of *P. bituminosa* seed characteristics (toughness, mass and width) over time. *Error bars* denote standard deviation from the mean (n=50). Regression line is presented



seed toughness. The width range of *P. bituminosa* seeds is not a limiting (or preferential) factor for their transport and cracking by *M. barbarus*, as no differences were found between seeds from the two origins. Moreover, seed width (range 1.10 to 2.35 mm) is below the maximum mandible gap of *M. barbarus* (2.80 mm). However, our results demonstrate that *M. barbarus* does not selectively harvest *P. bituminosa* seeds based on seed mass, width, or toughness. Moreover, the size of *M. barbarus* workers does not result in a selection of the harvested *P. bituminosa* seeds based on any of the three seed variables. Therefore, the differences found between

 Table 1
 The effect of ageing on seed characteristics. Simple linear regression for seed characteristics (toughness, mass and width) during the monitoring period. The *t*-test evaluates the significance of the slope's deviation from zero

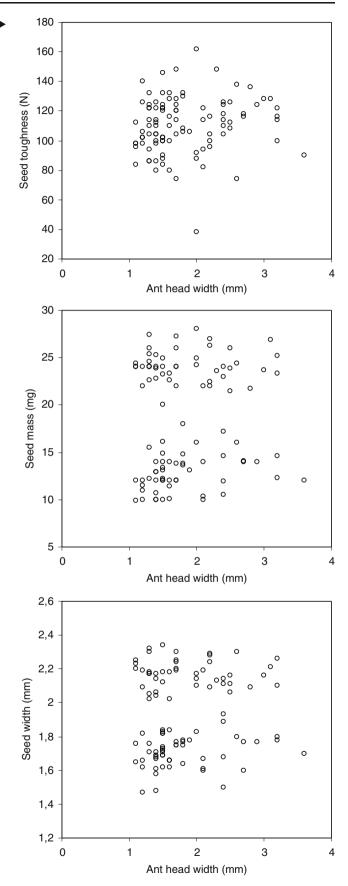
	Ν	R^2	Coefficient		Std. Err.	Т	Р
Seed toughness	500	0.005	Origin	101.569	0.029	1.620	0.106
G 1	500	0.001	Slope	0.047	0.007	0.500	0.5(1
Seed mass	500	0.001	Origin Slope	14.502 0.004	0.006	0.582	0.561
Seed width	500	0.000	Origin Slope	1.761 0.000	0.000	0.338	0.735

Fig. 3 Bivariate plots connecting the ants' size (head width) with each \blacktriangleright of the three measured characteristics (toughness, mass and width) of the seeds they collected

the plant seeds and the midden seeds are not the result of a selective harvest carried out by ants.

A number of studies suggests that harvester ants can act as dispersers of nonmyrmecochorous species when some collected seeds are finally not consumed but rather deposited far from the parent plant (Rissing 1986; Levey and Byrne 1993; Detrain and Tasse 2000). However, the exact reasons why seeds are not predated are not well known and it is usually considered to be a mistake made by the ants. In the present study, rather than contradict the ant mistake hypothesis, we prove that in some cases this ant behaviour may be deliberate and is probably due to the ants being unable to eat the seed. Some works have suggested seed toughness as a characteristic that prevents harvester ants from using the seed as food (O'Dowd and Hay 1980; Danin and Yom-Tov 1990; Boyd 1996; Rodgerson 1998). In P. bituminosa, seed toughness seems clearly decisive in determining which seeds are not eaten by *M. barbarus*. Moreover, this suggests that the maximum strength that these ants can apply with their mandibles could be around 70N, which is within the toughness range of P. bituminosa seeds. Other studies have demonstrated the influence of seed toughness on the predation of nuts by orangutans (Lucas et al. 1994) and the lack of predation by rodents (Janzen 1982) with harder seeds being dispersed and weaker seeds eaten, much like in P. bituminosa.

A measure of the collecting potential of *M. barbarus* is given by Detrain and Tasse (2000) who estimated that around 50,000 seeds are retrieved on average per foraging trail. We do not know the exact number of P. bituminosa seeds colleted by M. barbarus, but from our field observations we think it could be significant (unpublished observation). If the mandible strength limit of *M. barbarus* is around 70 N (as all seeds from their refuse piles were over this limit), then 69% of the P. bituminosa seeds measured (plant origin) were over this level. Therefore, we expect that a large number of seeds may eventually not be eaten. Boyd (1996) described a similarly high level of seeds deposited in middens (45%) in Fremontodendron decumbens and an even more extreme "seed escape" was described by Danin and Yom-Tov (1990) for Silvbum marianum, in which nearly all the seeds were relocated in the midden after being transported to the nest (seed toughness 83.4 N; unpublished observation). Another instance when seeds may not be eaten is when they are temporarily left in caches (Wilcock and De Almeida 1988; Reyes and Fernández-Haeger 1999). The escape rate in temporary caches was estimated by Detrain and Tasse (2000) to be as low as 0.01%, which corresponded to seeds that were left, dropped and not recovered, by M.



barbarus. The fate of that small fraction of seeds was not assessed and therefore it is still open to debate whether or not they participate in plant recruitment. Furthermore, the authors show that all seeds dropped on the way (not in caches) to the nest were subsequently recovered by other ants from the same nest. Thus, if the low number of seeds dropped during transport could result in beneficial dispersal and increase the probability of germination and emergence, then in the present case of seeds left in middens this benefit would probably be greater, and the role of *M. barbarus* as a dispersing agent of *P. bituminosa* could influence the spatial distribution of this plant.

Given that predation occurs, the mechanical defence of P. bituminosa seeds is not completely effective against M. barbarus. However, the loss of a number of weaker seeds retrieved and eaten by this ant could be the price paid by P. bituminosa to achieve dispersal benefits for some tougher seeds. Further work is still needed on the ability of these refused seeds to germinate, survive and develop into reproducing adults. Nonetheless, the broad range of seed toughness presented by P. bituminosa, with seeds that can be eaten by harvester ants but others that are tougher than the ants' mandible strength, results in an evolutionarily dynamic system that could allow dispersion of this plant by this granivorous ant. An analysis of variation of seed coat toughness in F. decumbens (Boyd 1996), S. marianum (Danin and Yom-Tov 1990) and Datura discolor (O'Dowd and Hay 1980) from both plants and refuse heaps would shed light on this possibility.

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