Seed predation of the tussock-grass *Stipa tenacissima* L. by ants (*Messor* spp.) in south-eastern Spain: the adaptive value of trypanocarpy

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Abstract

Seed predation by animals has been shown to be an important factor in the evolution of dispersal mechanisms and diaspore morphology of plants. We studied seed production and predation of the perennial tussock-grass *Stipa tenacissima* L. in “El Desierto”, a semi-desert area in south-eastern Spain. As almost all other species in the genus, this plant produces diaspores with a long awn capable of hygroscopic movements that may lead to a drilling of the basal part of the diaspore into the soil substrate, to inserting into crevices or to lodging under rock fragments (trypanocarpy). Three species of granivorous ants (*Messor barbarus*, *M. timidus*, *M. bouvieri*) consume a substantial proportion of the seed production of *S. tenacissima*. We show in detail that the presence of the awn and the drilling mechanism of *S. tenacissima* reduce the intake rate of diaspores by *Messor* spp. dramatically. The long awn (mean overall length 56.8 mm) makes the diaspore cumbersome and heavier, so that it can only be retrieved much more slowly than a diaspore without the awn. Cutting of the awn by an ant requires a long time (mean 9.35 min) and small workers (head width < 1.3 mm) are incapable of it altogether. Diaspores drilled into the ground can still be excavated by workers. However, once the awn breaks off by weathering at the distinct preformed breaking point at

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the base of the awn, diaspores have practically escaped predation because ants cannot locate them underneath the soil surface. These results are consistent with the hypothesis that trypanocarpy is a very effective mechanism against seed predation in *S. tenacissima*. We also evaluate how seed predation by *Messor* spp. affects the population structure of *S. tenacissima* and caution against categorizing this grass as a mast species as previously suggested. Finally, we discuss the implications of pre-dispersal seed predation by *Messor* spp. for the interaction with *S. tenacissima* and other plants.

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1. Introduction

In semi-arid and arid regions of the world, the low amount of rainfall and the irregular distribution in time of rainfall events make living conditions for plants extremely harsh. Their reproductive success crucially depends on whether their seeds germinate at the right place and at the right time (Gutterman, 1994). On the other hand, seeds are subject to intense predation by animals (primarily rodents, ants and birds) for whom they represent a high-quality and nutrient-rich resource that can also be stored. Thus, an effective dispersal mechanism not only has to ensure that a diaspore gets to a place favourable for both germination and establishment, but also must reduce risk of seed predation. Granivory has indeed been shown to be an important factor in the evolution of dispersal mechanisms and diaspore morphology of plants (Janzen, 1971; Elliott, 1974; Benkman, 1995; Benkman et al., 2001).

Granivorous ants often destroy a large proportion of the diaspore production (e.g. Brown et al., 1979; Andersen, 1989; Crist and MacMahon, 1992) and certain diaspore characteristics and dispersal strategies have been shown to reduce seed losses to granivorous ants. For example, a hard seed coat makes seeds less attractive for granivorous ants and/or protects them from destruction so that they are often discarded unharmed in the refuse piles (O’Dowd and Hay, 1980; Danin and Yom-Tov, 1990; Boyd, 1996; Rodgerson, 1998). Gutterman (1994) suggested that small seed size might be an effective way of avoiding seed predation, since ants cannot locate tiny seeds, which have fallen into crevices (“escape strategy”). Espadaler and Gómez (1996, 1997) showed that the presence of an elaiosome leads to the removal by non-granivorous ants and thus seeds can get effectively dispersed to safe sites. An exceptionally smooth seed coat makes handling and transport difficult for granivorous ants (Pulliam and Brand, 1975; Davison, 1982; Rodgerson, 1998). Pulliam and Brand (1975) found that foragers of *Pogonomyrmex barbatus* harvest seeds with a smooth outline like those of *Amaranthus* spp., *Chenopodium* sp., *Rumex* sp., and *Lepidium lasiocarpum* less frequently than they are available. Myxospermy is another dispersal mechanism effective against seed predation. Seeds of species such as *Plantago coronopus* and *Carrichtera annua*, for instance, adhere to the soil crust when wetted by a mucilaginous layer. This significantly reduces the intake rate by ants and allows some seeds to remain on the soil surface until they germinate.
(Gutterman and Shem-Tov, 1997). High predation rates may also be avoided by dispersing the seeds during times of low ant activity (Pulliam and Brand, 1975), or shortly before they germinate so that seeds are available to ants only for a short period of time (“aestatirophy”, Gutterman, 1993; Hensen, 1999).

Trypanocarpy is a dispersal mechanism that has evolved convergently in the Geraniaceae (e.g. in the genera Erodium, Pelargonium, Sarcocaulon) and the Poaceae (e.g. Arrhenaterum, Avena, Aristida, Stipagrostis, Stipa) (van Rheede van Oudtshoorn and van Rooyen, 1999). Trypanophorous diaspores are characterized by a long appendage that consists of two parts—a basal part which is capable of spiral twisting by hygroscopic movements, and an upper part which is always straight and at right angles to the basal part. Trypanocarpy (in Stipa and in other grasses) has been interpreted as a mechanism to move the diaspore into suitable microsites and to facilitate germination (Ghermandi, 1995; Weiglin, 1995). van Rheede van Oudtshoorn and van Rooyen (1999) and Hensen (1999) suggested that trypanocarpy serves yet another, potentially equally important, purpose—the prevention of seed predation.

Here we examine the interaction between the tussock-grass *Stipa tenacissima* and granivorous ants of the genus *Messor* in south-eastern Spain with the objective to elucidate which selective advantages are associated with trypanocarpy in this plant. In particular, the following questions are addressed: Which proportion of *S. tenacissima* diaspores is lost through seed predation? How does the presence of the awn affect the retrieval rate of diaspores by *Messor* foragers? Does the presence of the awn make diaspores less attractive for *Messor* foragers? Does trypanocarpy influence the germination process in *S. tenacissima*?

2. Material and methods

2.1. Study sites and organisms

The study was conducted in “El Desierto” de Tabernas, a semi-desert area located approximately 25 km north of Almería, south-eastern Spain (37°05′N, 2°25′W, 200–700 m altitude). The climate is semi-arid, with a mean annual temperature of 17.8°C and a mean annual rainfall of 235.4 mm (Lázaro et al., 2001) falling mainly between October and April. The hill plateaus are typically covered by grassland, which is dominated by the perennial *S. tenacissima* L. (Freitag, 1971). This grass occurs in the southern and eastern parts of the Iberian Peninsula, on the Balearic Islands and over large parts of north-western Africa (Freitag, 1971; Le Houérou, 2001). It is characterized by an extensively ramified system of subterranean axes forming large, circular, densely tufted tussocks often wider than 50 cm in diameter. Inflorescences often rise more than 1 m above the surrounding vegetation. In south-eastern Spain diaspores are dispersed mainly between May and June and germinate with the first autumn rains when soil temperatures have dropped below 25°C (Hensen, 1999).

The long-awned diaspores of *S. tenacissima* (Fig. 1) are trypanophorous, i.e. the basal part of the awn twists when dry and straightens when wet. The basal part of the
awn has an average length of 16.2 mm (±1.8 mm S.D., n = 20), the distal part is 40.2 mm (±2.3 mm) long, the diaspore without awn 10.1 mm (±0.8 mm). Mass investment into different parts of the diaspore for *S. tenacissima* and the two other *Stipa* species occurring in El Desierto, *S. capensis* Thun. and *S. parviflora* (n = 100 in all cases) is summarized in Table 1.

In the *Stipa* grassland, six different ant species predominantly collect seeds as food: *Messor barbarus* L., *M. timidus* Espadaler, *M. bouvieri* Bondroit, *Goniomma compressisquama* Tinaut, *G. blanci* Andrê, and *Oxypommyrmex sauleyi* Emery. However, due to their small body size, the latter three are incapable of retrieving *S. tenacissima* diaspores and were therefore not further examined in this study. The three *Messor* spp. show different degrees of polymorphism: body length in the worker caste varies between 4.1–12.65 mm (*M. barbarus*), 4.1–10.4 mm (*M. timidus*),

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### Table 1

Descriptive statistics for mass investment into different diaspore parts for *S. tenacissima*, *S. capensis*, and *S. parviflora* (n = 100 in all cases)

<table>
<thead>
<tr>
<th>Species</th>
<th>Mass (mg) Mean ± S.D. (Min–Max)</th>
<th>Diaspore</th>
<th>Diaspore without awn</th>
<th>Caryopsis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. capensis</em></td>
<td>4.87 ± 1.13 (2.18–7.79)</td>
<td>1.52 ± 0.33 (0.84–2.21)</td>
<td>1.01 ± 0.23 (0.59–1.55)</td>
<td></td>
</tr>
<tr>
<td><em>S. parviflora</em></td>
<td>1.93 ± 0.30 (1.22–2.85)</td>
<td>1.01 ± 0.16 (0.58–1.46)</td>
<td>0.84 ± 0.16 (0.46–1.15)</td>
<td></td>
</tr>
<tr>
<td><em>S. tenacissima</em></td>
<td>9.03 ± 1.32 (6.08–12.82)</td>
<td>5.83 ± 1.08 (3.16–8.45)</td>
<td>3.73 ± 0.91 (1.62–5.73)</td>
<td></td>
</tr>
</tbody>
</table>

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![Fig. 1. Long-awned diaspore of *S. tenacissima* showing the breaking (disarticulating) point.](image)
and 4.0–8.15 mm (M. bouvieri). All of them display group foraging with conspicuous trails, but may flexibly switch to single foraging when resource density is low as has been described for other Messor species (Hahn and Maschwitz, 1985; Neumayer, 1994). Since maximum width of the head capsule excluding the eyes (HW) is a very good indicator of dry body mass (DM) in all three species (unpublished data, C.S.) and can be measured very quickly, this variable was used as a measure of overall body size.

Seed production and predation were measured within a homogenous S. tenacissima—area (10,700 m², ~40% plant cover) on a hill plateau in the core area of El Desierto, about 500 m south of the N 340 (hereafter referred to as study site A). All Messor colonies were mapped and their distribution pattern was examined using the “Nearest-neighbour’s test” (Clark and Evans, 1954). In 2000 and 2001, experiments with M. timidus and M. bouvieri were carried out here and in adjacent Stipa grassland areas. Colonies of M. barbarus were absent on study site A, so that additional observations and experiments with this species were made at Sartenilla, near Tabernas, in the plains towards the Sierra Alhamilla (study site B).

2.2. Seed production, predation and seedling establishment

Seed production on study site A was measured in 1999, 2000, and 2001. Each year in May, the number of spikes was counted in ten (nine in 2000) randomly selected 5 × 5 m² quadrates. The number of S. tenacissima seedlings (defined as those individuals with a height of less than 30 cm and without old leaf blades) was also counted. The number of diaspores per spike was counted by collecting all the spikes of 20 tussocks along a transect on a neighbouring hill plateau covered by S. tenacissima grassland with similar density of Stipa tussocks and similar plant cover at the end of April, when no seeds had yet been dispersed. Seed intake and the activity of two colonies each of M. timidus and M. bouvieri were monitored from the end of April to mid-June in 1999 in order to calculate the number of seeds retrieved by Messor colonies. Once a week, 100 foragers returning to the nest were collected and robbed of their prey items, which were later identified in the laboratory. Activity was assessed every 10 days by counting the workers returning to the nest within 3 min every hour during a 24 h period from 3 p.m. until 2 p.m. on the following day (April 30th and May 1st; May 10th and 11th; May 20th and 21st; May 30th and 31st; June 9th and 10th; June 16th and 17th). On June 9th and 10th, only one of four colonies was active, on June 16th and 17th, none of them showed foraging activity. These 2 days were not included in the calculation of the mean number of foraging trips per day. On all days when activity was monitored, we also checked for all Messor colonies on the study site whether it showed foraging activity or not. To estimate the number of S. tenacissima diaspores retrieved by each focal colony we multiplied separately the average number of diaspores collected per one hundred foragers by the average number of daily foraging trips, the average number of foraging days per week and the duration of observations, 8 weeks.
2.3. Effect of diaspore characteristics on handling, transport and preference by workers of different sizes

The diaspores of *S. tenacissima* are cumbersome and foragers need to cut off the awn sooner or later in order to carry the fruits into the nest entrance. Entire diaspores were presented at a distance of 5–10 cm from an active *M. barbarus* foraging trail 15 m away from the nest entrance and fixed by placing small pebbles on the distal part of the awn. Cutting time was measured from the moment the forager made a first attempt to cut until the awn was completely removed. The entire diaspore and the forager were subsequently collected in alcohol. We also collected such individuals whose efforts remained unsuccessful. This data set was obtained from a single colony on study site B over the course of 2 days in May 2001. HW was measured later in the lab using a dissecting microscope fitted with an ocular micrometer.

The transport velocity of *S. tenacissima* diaspores by *Messor* foragers of different sizes was assessed by measuring the time an individual carrying a diaspore back to the nest required to traverse a 1 m section of trunk trail on an even surface. The forager was then carefully collected and stored in alcohol, while the seed was kept dry in a separate vial. We selected foragers in such a way as to cover the entire size range. Later in the laboratory the diaspore was weighed and the dry mass of the worker (48 h in a 60°C oven) was measured in order to estimate its live mass (LM) using the logarithmic equation: \( \text{LNLM} = 1.235 + 0.906 \times \text{LN DM} \) \( (r^2 = 0.996, n = 105 \text{ workers of all size classes}) \). This data set was obtained from a single *M. barbarus* colony on an overcast afternoon in May 2001 with temperature ranging between 23°C and 26°C.

To analyse the effect of burden (defined as \( (M_a + M_i)/M_a \), where \( M_a \) is the mass of the transporting ant and \( M_i \) is the mass of the *S. tenacissima* diaspore with or without awn) on running speed (in m/s), we used ordinary least-squares regression of LN-transformed data as the purpose was prediction (LaBarbera, 1989). Since *M. timidus* and *M. bouvieri* have highly similar body proportions as *M. barbarus* for a worker of a given dry mass (unpublished data, C.S.), we assume that these results are representative for all three species.

Observations of workers on the foraging trail suggested that smaller workers were unable to retrieve diaspores of *S. tenacissima*. To confirm this assumption two random samples of workers were taken for one colony each of both *M. barbarus* and *M. timidus*: (1) about 150 outbound foragers on a foraging trail at a distance of 1 m from the nest entrance, and (2) about the same number of foragers that returned to the nest carrying a *S. tenacissima* diaspore. Additionally, samples of outbound foragers (N approximately 150) were collected in May 1999 and May 2000 at a distance of 50 cm from the nest entrance on a fully established foraging trail from three colonies each of *M. barbarus, M. timidus,* and *M. bouvieri* to examine the mean, maximum and minimum size of workers and their size frequency distribution. For all ants, HW was measured as described above.

We often observed *Messor* colonies harvesting fruits of a variety of plant species directly from the mother plant (pre-dispersal seed predation). In colonies of
M. pergandei, larger workers cut “difficult” seeds from their stalks, dropping them to the ground to be picked up and transported homeward by the smaller workers (S.A. Gordon, cited in Oster and Wilson, 1978, p. 255). To test the hypothesis that such division of labour also exists in M. timidus, two random samples of workers were taken for each of three colonies in May 2000: (1) 80 outbound foragers on a foraging trail at a distance of 1 m from the nest entrance, and (2) 80 foragers trying to harvest fruits from the spikes of S. tenacissima-plants. HW was later measured as described above.

In order to assess the effect of the presence of the awn on retrieval speed and to evaluate whether workers show preferences for diaspores with or without awn, laboratory experiments were carried out in Germany. For that purpose, two colonies of M. timidus (hereafter referred to as Mti A and Mti B) were completely excavated and transported to the University of Würzburg where they were kept in a gypsum nest (following the method of Cerdan, 1989) at room temperature. The diet consisted of natural seeds from south-eastern Spain (S. tenacissima, Helianthemum almeriense, Euzomodendron bourgaeanum) and additionally two freshly killed locusts (Locusta migratoria) or cockroaches (Periplaneta americana), once a week. Water was always freely available.

In a first laboratory experiment, we measured running speed of M. timidus workers carrying S. tenacissima diaspores with/without awn. The nest of colony Mti A was connected to a foraging arena (1.50 m × 0.60 m) by a plastic tube. After workers had been given one day to explore the area, the experiment was started by presenting S. tenacissima diaspores with and without awn at the end of the foraging arena opposite to the nest entrance. Diaspores were chosen randomly so that both types of diaspore differed in their average mass. The time an individual worker needed to pick up (handling time, HT), and to carry the diaspore over a distance of 1 m (transport time, TT) were measured with a stop watch. Then the worker and its Stipa diaspore were collected in order to measure ant head width as well as ant mass (LM) and diaspore mass (ED) to the nearest 0.1 mg. Only data on foragers that followed a straight line back to the nest entrance were included in the analysis. 100 foragers in the foraging arena were collected and measured, in order to find out whether foragers of all sizes participated in the transport of S. tenacissima diaspores. HT, transport speed (V, defined as 1 m/TT) and LM were compared between both groups of foragers using the Student’s t-test or—whenever appropriate—the Mann–Whitney U-test. Despite enormous seed stores in the nest, foraging motivation of the workers did not decline and data were gathered each day between 17:00 and 01:00 h over a period of a week. Room temperature was almost constant at 21°C and varied only between 20°C and 23°C, so that its effect on running speed was neglected during the data analysis.

Using the same arrangement as in the previous experiment, we conducted a second experiment with Mti B and S. parviflora to examine whether foragers show a preference for diaspores with or without awn. The two diaspores offered (one with and the other without awn) were positioned at a steep angle with the basal tips touching each other, so as to guarantee that a forager would detect both simultaneously and make therefore a real choice. To control for the effects of mass
on preference, we selected diaspores in such a way that each of the diaspores offered had a similar total mass of 1.4 mg. HT, TT and LM were measured as in the experiment previously described. Only the data of foragers who followed a straight line back to the nest entrance were included in the analysis. It soon became obvious that only few foragers selected the complete diaspore. In order to obtain data on HT and transport speed of workers carrying diaspores with awn, additional data was recorded for foragers carrying diaspores of *S. parviflora*, which is also common in El Desierto and has a similar morphology as *S. tenacissima*. This allowed us to investigate foragers of all size classes, as small foragers often did not manage to transport the much heavier diaspores of *S. tenacissima* (see results). HT, V, and LM were compared between both groups of foragers using the student’s *t*-test or—whenever appropriate—the Mann–Whitney *U*-test. To evaluate whether one kind of diaspore was preferred, the *χ²*-test was employed.

2.4. *Germination of S. tenacissima with and without awn*

In order to examine the importance of trypanocarpy with regard to the germination process, we recorded the germination success in a greenhouse experiment of both complete diaspores and those from which the awn had been cut off. Plastic dishes (27 × 45 cm²) equipped with drainage holes were filled with a 1 cm gravel layer and a 4 cm soil medium layer that was composed of 15 parts compost, 2 parts sand, 3 parts clay and 5 parts peat, previously sieved to remove larger organic debris. The soil medium was then pressed with a wooden board to simulate the densely packed soil typical for the *Stipa* grassland in El Desierto. Twenty diaspores per dish were laid on the soil surface. For both diaspore types (with and without awn) there were three replicates each. At an interval of 2–3 days we simulated a rainfall event by spraying about 400 ml water onto each of the dishes. The interval between watering allowed an entire cycle of unwinding and winding up again of the awn. Room temperature varied between 15°C and 25°C during the experiment. The number of germinated seeds was counted once a week. After 8 weeks the number of germinated diaspores per dish was compared between both treatments using the Mann–Whitney *U*-test.

### 3. Results

#### 3.1. *Seed production, predation and seedling establishment*

Seed production varied by a factor of 9.7 between years (Table 2). The number of seedlings, however, stayed constant in all 3 years (comparison between 1999 and 2000: *p* > 0.8; between 1999 and 2001: *p* > 0.1). In 1999 the mean number of diaspores per spike was 113 (±21.5 S.D.). In the period between the end of April and mid-June 1999 the two *M. timidus* colonies harvested 25,191 and 64,933 *S. tenacissima* diaspores, respectively, while the two colonies of *M. bouvieri* gathered 15,245 and 22,240 diaspores. These figures equal the seed production of areas
between 426 and 1816 m². Altogether we found 19 *Messor* colonies on the study site, so that the foraging area of one colony would theoretically on average cover 560 m². The mean distance of a colony to the nearest neighbour was $r_a = 14.9$ m, while the mean expected distance at random distribution was $r_e = 11.9$ m. The deviation from random distribution $R = r_a / r_e = 1.257$ is statistically significant (two-tailed $t$-test, $p < 0.05$). Thus, all *Messor* colonies together show a regular distribution, i.e. “overdispersion”. This indicates that foraging by *Messor* workers may be of similar intensity throughout the study area.

3.2. Effect of diaspore characteristics on handling, transport and preference by workers of different sizes

Cutting off the awn took between 2.7 and 17.0 min ($9.4 \pm 4.3$ min, $n = 22$). Successful foragers all had a HW larger than 1.5 mm (range 1.55–2.85 mm), while all those that gave up had a HW smaller than 1.3 mm ($n = 5$, range 0.97–1.24 mm).

The largest forager (LM 29.7 mg) carried a *S. tenacissima* diaspore at 0.05 m/s, i.e. her speed was seven times as high as the speed of the smallest one (LM 1.8 mg). The *S. tenacissima* diaspores weighed between 2.79 and 8.13 mg. From 96% of them, the awn had been cut off partly or completely. Burden had a very strong negative effect of running speed ($LN V = -3.14 - 1.11 LN Burden$, $r^2 = 0.706$, see Fig. 2).

In the *M. barbarus* colony, outbound foragers had an average HW of 1.84 mm ($\pm 0.57$ mm, $n = 223$), whereas *Stipa* foragers had a HW of 1.96 mm ($\pm 0.50$ mm, $n = 148$). In the *M. timidus* colony, outbound foragers had an average HW of 1.57 mm ($\pm 0.27$ mm, $n = 153$), whereas *Stipa* foragers had a HW of 1.69 mm ($\pm 0.26$ mm, $n = 145$). In both *M. barbarus* and *M. timidus*, foragers transporting *S. tenacissima* diaspores were on average larger than normal foragers ($p < 0.05$ and $p < 0.001$, respectively). This effect is due to a rarity of foragers with a HW < 1.3 mm in the two groups of *Stipa* foragers. The descriptive statistics of the sizes of foraging workers has been summarized in Table 3.

In none of the three *M. timidus* colonies tested were foragers attempting to harvest diaspores from spikes larger than normal foragers. Foragers on spikes had a mean HW of 1.68 mm ($\pm 0.33$ mm), 1.66 mm ($\pm 0.25$ mm), and 1.57 mm ($\pm 0.23$ mm),...
opposed to a mean HW of outbound foragers of 1.63 mm (±0.38 mm), 1.61 mm (±0.31 mm), and 1.51 mm (±0.30 mm). The differences were not significant (in all cases p > 0.1).

In the first laboratory experiment, diaspores without the awn were lighter than entire diaspores (LM 6.3 ± 1.0 mg, n = 50 vs 9.7 ± 1.3 mg, n = 50, p < 0.001), and on average carried at a speed 1.4 times higher than that of entire diaspores (0.011 ± 0.002 m/s vs 0.008 ± 0.002, p < 0.001). HT was much longer for entire diaspores (1.15 ± 0.61 vs 0.53 ± 0.29 min, p < 0.001). Foragers carrying entire diaspores (LM 7.8 ± 2.1 mg, HW 1.74 ± 0.21 mm) and those carrying diaspores without awn (LM 7.6 ± 2.8 mg, HW 1.71 ± 0.28 mm) did not differ in size (p > 0.70.

Table 3
Maximum width of the head capsule excluding the eyes (HW) for foraging workers of nine Messor colonies

<table>
<thead>
<tr>
<th>Colony</th>
<th>Mean HW (mm) (± S.D.)</th>
<th>n</th>
<th>Max (mm)</th>
<th>Min (mm)</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. barbarus 1</td>
<td>1.84a ± 0.57</td>
<td>154</td>
<td>3.30</td>
<td>0.9</td>
<td>30.91</td>
</tr>
<tr>
<td>2</td>
<td>1.73a ± 0.51</td>
<td>135</td>
<td>3.15</td>
<td>0.85</td>
<td>29.16</td>
</tr>
<tr>
<td>3</td>
<td>1.58b ± 0.54</td>
<td>151</td>
<td>3.25</td>
<td>0.85</td>
<td>34.35</td>
</tr>
<tr>
<td>M. timidus   1</td>
<td>1.75a ± 0.31</td>
<td>168</td>
<td>2.77</td>
<td>1.16</td>
<td>23.24</td>
</tr>
<tr>
<td>2</td>
<td>1.57b ± 0.26</td>
<td>153</td>
<td>2.35</td>
<td>1.05</td>
<td>16.78</td>
</tr>
<tr>
<td>3</td>
<td>1.57b ± 0.25</td>
<td>160</td>
<td>2.3</td>
<td>1.00</td>
<td>16.15</td>
</tr>
<tr>
<td>M. bouvieri  1</td>
<td>1.36c ± 0.26</td>
<td>142</td>
<td>1.95</td>
<td>0.85</td>
<td>19.19</td>
</tr>
<tr>
<td>2</td>
<td>1.21d ± 0.20</td>
<td>113</td>
<td>1.9</td>
<td>0.8</td>
<td>16.91</td>
</tr>
<tr>
<td>3</td>
<td>1.18d ± 0.24</td>
<td>177</td>
<td>2</td>
<td>0.8</td>
<td>20.21</td>
</tr>
</tbody>
</table>

Values sharing the same letter are not statistically different from each other (p > 0.05, t-test or—when appropriate—Mann-Whitney U-test).

Fig. 2. Relationship between running speed (in m/s) and burden (defined as \((M_a + M_i)/M_a\), where \(M_a\) is the mass of the transporting ant and \(M_i\) is the mass of the S. tenacissima diaspore with or without awn) for loaded foragers of M. barbarus. LN-transformed data were linear regressed: LNSpeed = -3.14 – 1.11LNBurden, n = 105, \(r^2 = 0.706\), p < 0.001.
for LM, \( p > 0.90 \) for HW), but were larger than the average forager (1.52 ± 0.31 mm; \( p < 0.001 \) and \( p < 0.002 \), respectively).

In the second laboratory experiment, \( M. \) timidus foragers preferred the diaspora without awn (67 diaspores without vs 16 with awn; \( \chi^2 = 15.67; \text{df.}= 1; \ p < 0.001 \)). While foragers carrying complete \( S. \) parviflora diaspores were not heavier than those carrying \( S. \) parviflora diaspores without awn (4.4 ± 2.03 mg, \( n = 43 \) vs 5.0 ± 2.6 mg, \( n = 65, \ p = 0.22 \)), they needed longer HT (51.2 ± 26.5 vs 16.5 ± 14.6 s, \( p < 0.001 \)) and transported their diaspores at only about half the speed (0.013 ± 0.005 vs 0.026 ± 0.008 m/s), which indicates that differences in manoeuvrability between diaspores with or without awn, and not differences in mass, were responsible for the observed pattern.

3.3. Germination experiment

Entire \( S. \) tenacissima diaspores needed between 6 and 14 cycles of unwinding and winding up again of the awn for drilling themselves into the ground. They crept on the ground until locating a crevice in the soil or until they reached the dish wall. Only in such places diaspores drilled themselves into the ground. The number of germinated seeds after 8 weeks was higher for the replicates with entire diaspores (10.3 ± 1.9, MIN–MAX 9–13, \( n = 3 \) vs 1.3 ± 1.2, 0–3, \( n = 3; \ p < 0.05 \)).

4. Discussion

4.1. Seed production and predation

In 1999, a year of low seed production, \( Messor \) colonies retrieved a very high percentage of the \( S. \) tenacissima diaspores on study site A. If not all seeds of \( S. \) tenacissima collected by ants were destroyed, then the overall effect of seed collection by ants for the plant might be beneficial instead of detrimental (dyszoochory). Seeds might be lost along the long foraging trails, discarded unharmed into the refuse pile or be able to germinate in the nest chambers after the colony dies or moves to a new nest site (e.g. Rissing, 1986; Vorster et al., 1994). However, there was no seedling establishment of \( S. \) tenacissima on nest chaff piles. Furthermore, we never observed that diaspores were discarded along foraging trails. Detrain and Tasse (2000) showed that only 0.1% of seeds discovered by \( M. \) barbarus foragers get lost on the way to the foraging trail and that all diaspores dropped on the trail were subsequently recovered within 24 h. \( Messor \) colonies are generally sessile (but see Brown, 1999), so that seedling establishment in abandoned nests is also not an important phenomenon here. Thus, it can be concluded that in El Desierto seed retrieval of \( S. \) tenacissima diaspores by \( Messor \) equals seed predation.

Measuring seed intake according to the method used in 1999 is a very time-consuming procedure and could thus not be repeated in the other years. Nevertheless, several lines of argument indicate that the percentage of \( S. \) tenacissima seeds harvested may also be substantial in other years. First of all, these ant species
store seeds in underground chambers and should harvest as many seeds as possible, since they cannot predict the length of the following winter and the amount of seed production in the next year. In the laboratory, the colonies maintained a high foraging motivation despite immense amounts of seeds stored in the nest. In an ad hoc experiment in the laboratory, the Mti B colony collected 150g of *Amaranthus* seeds within a single day. If more *S. tenacissima* seeds had been available, the *Messor* colonies would most probably have continued harvesting after the beginning of June when there was a steep decline in ant activity on the study site (C.S., unpublished data). In a year of extraordinarily high seed production Haase et al. (1995) found that harvesting by *Messor* spp. continued throughout the summer. Because seed predation by other animals (rodents and birds) was not investigated, the estimated losses through seed predation are still rather conservative.

4.2. Trypanocarpy—a mechanism against seed predation?

The morphology of *S. tenacissima* diaspores provides several advantages with respect to the interaction with the *Messor* spp. The laboratory experiment with *S. parviflora* showed that the presence of the awn makes these diaspores less attractive for foragers and increases the handling time drastically. Complete diaspores of *S. parviflora* were transported at about half the speed as diaspores without an awn but of the same mass. In the laboratory experiment with *S. tenacissima*, transport speed of entire diaspores was also greatly reduced compared to that of diaspores without the awn and handling took more than twice as long. The reduced transport speed of complete *S. tenacissima* diaspores is probably not only due to their higher mass, but also to the unfavourable forward shift of the centre of mass resulting from the enormous length of the awn (Zollikofer, 1994; Röschard and Roces, 2002). From the perspective of a *Messor* colony, the retrieval of complete diaspores appears to be very inefficient as only 41% of the carried mass would be nutritionally available. However, in the natural habitat the foragers have to cut off at least the distal part of the awn, because otherwise the diaspores often get stuck at obstacles and workers do not manage to move the cumbersome diaspore into the nest entrance. In fact, only about 4% of the *S. tenacissima* diaspores that we collected from foragers were complete (figure for transport speed experiment). In *M. barbarus*, the cutting of the awn lasts on average 9.35 min. This means that a *M. barbarus* forager carrying a *S. tenacissima* diaspore at a speed of 0.025 m/s (average value observed in experiment) could cover a distance of 14 m during the same time span that is required to remove the awn. Thus, when harvesting diaspores from this distance, the retrieval rate would be reduced to about half compared to a situation where foragers would harvest diaspores without awn. This long handling time is highly remarkable also within another context. According to optimal foraging theory, foragers should select food items that allow the highest energy intake rate per unit time. However, in studies addressing questions of optimal foraging in harvester ants, seed mass has often been used as an equivalent of energy intake rate (e.g. Willott et al., 2000). In situations, where handling and transport times differ strongly for different seed types, this can lead to wrong conclusions. In
contrast to the long handling time for *S. tenacissima*, small seed types can be picked up by *Messor* workers very quickly (e.g. 0.27 and 0.55 min for *E. bourgaeanum* and *H. almeriense*, respectively).

Foragers with a head width of less than 1.3 mm did not succeed in removing the awn in our experiment and thus could not carry the diaspore to the nest. The physical inability of small foragers to cut off the awn explains that foragers retrieving *S. tenacissima* diaspores were on average larger than normal outbound foragers. As the percentage of foragers with a head width of less than 1.3 mm was quite large in the *Messor* colonies (19–34%, 4–16%, 38–74% for *M. barbarus*, *M. timidus*, and *M. bouvieri*, respectively), the presence of the awn essentially makes the diaspore unavailable for a substantial part of the forager force. This problem could be solved if larger workers specialize in cutting awns, and diaspores are picked up and transported homeward by the smaller workers. We never observed such task specialization, neither did we find division of labour according to size in the harvesting process directly from *Stipa* plants. Even without the awn, *S. tenacissima* diaspores are quite heavy so that the transport speed especially of smaller foragers is fairly low. For them, carrying a diaspore is much more difficult not only due to the higher burden, but also because their mandible gap is not sufficiently wide for grabbing it in the middle at the widest circumference (C.S., pers. obs.). Grabbing it close to the callus tip results in an unfavourable forward shift of the centre of mass and thus in a reduced running speed (see above). Diaspores not detected by *Messor* foragers can eventually drill themselves into crevices in the ground, underneath pebbles or sometimes even into the soil substrate if this is sufficiently loose and if the diaspore finds an obstacle like organic matter to anchor itself. Very little rainfall or even the morning dew seem to be sufficient for the drilling effect to take place, because diaspores were found drilled into the ground in May 1999 despite very little rainfall during that period. That *Messor* foragers can still excavate such diaspores, was observed twice (in both cases the process took several minutes). However, it is almost impossible for foragers to locate such diaspores drilled into the ground whose awn has broken off by weathering. Interestingly, there seems to be a preformed breaking point (disarticulating point) at the base of the awn (see Fig. 1). With the basal part of the diaspore (i.e. the caryopsis enclosed by the glumes) in a fixed position, the awn always breaks off at the base when bent. So, we would argue that there are two different requirements for the design of the awn in *S. tenacissima*: on the one hand the diaspore has to be drilled into crevices or into the ground, and on the other hand the awn should break off soon after that, so that they can no longer be found by *Messor* foragers. How these two requirements can be reconciled, remains a very interesting question for future studies on the biomechanics of trypanocarpy in *Stipa*.

The diaspores of all species in the genus *Stipa* s.l. show trypanocarpy, except for those in the sections Aristella and Orthoraphium, where the awn is straight and untwisted (Freitag, 1985). Besides enabling diaspores to escape predation, trypanocarpy brings other selective advantages. In the germination experiment, complete diaspores of *S. tenacissima* germinated more rapidly than those whose awn had been removed. This effect, which is probably due to increased water uptake
of diaspores drilled into the ground, had also been found by Ghermandi (1995) for *S. speciosa*, and has crucial importance in semi-arid environments like El Desierto. The presence of the awn also improves the capacity of diaspores for wind dispersal. In some species of the genus there are hairs on the basal (as in *S. tenacissima*) or the distal part of the awn (e.g. *S. pulcherrima*, *S. tirsa* and *S. lessingiana*). Trypanocarpy in *Stipa* may yet have an additional function: diaspores of *S. capensis* for instance, which represents the only annual in the entire genus, get attached to or even penetrate clothes and human skin easily and therefore seem well suited for epizoochorous dispersal. Silberbauer-Gottsberger (1984) reported epizoochorous dispersal for several Brazilian Cerrado grass species (e.g. *Aristida* spp., *Gymnopogon* spp., *Trachypogon spicatus*), all of which have trypanophorous diaspores.

Since all selective advantages of trypanocarpy discussed here are complementary it is difficult to come up with a scenario of its evolutionary origin. This is further complicated by taxonomic difficulties. Barkworth and Everett (1986) regard the generic interpretations within the Stipeae as artificial and proposed several species groups within the genus *Stipa*, which they consider to be monophyletic. Nevertheless, when comparing the biogeography of both *Messor* (Cerdan, 1989; Bolton, 1995; Cagniant and Espadaler, 1997) and *Stipa* (Freitag, 1985; Barkworth and Everett, 1986; Le Houérou, 2001), it becomes evident that both groups may have interacted with each other for long periods of time in arid and semi-arid regions at least from Inner Asia to the western Mediterranean. Besides the present study there are few published data on predation levels of *Stipa* spp. by *Messor* spp. in these regions. Wilby and Shachak (2000) found intense predation of *S. capensis* by *M. arenarius* in the Negev Desert. As diaspores of *S. tenacissima* have to survive the dry summer months without being destroyed by *Messor* before they start germinating after the first autumn rains, the escape from predation intuitively appears to be an equally important function as the facilitation of germination. If trypanocarpy evolved under selection pressure from granivorous ants, we would assume that populations of *S. tenacissima* or different species in the genus differ in the structural design of the awn. Since we cannot think of another advantage of losing the awn after the diaspore has been drilled into the ground other than to avoid the detection of the diaspore by a seed predator, we predict that species occurring in areas of high predation levels should have an awn that breaks off just above the caryopsis whereas in areas without or little predation by *Messor* spp. the awn will lack a distinct breaking point. This hypothesis is currently being studied in more detail.

4.3. Effect of seed predation by ants on the population structure of *S. tenacissima*

The population structure of this grass is difficult to analyse, because it grows clonally and sometimes even shows clonal reproduction (Freitag, 1971). Tussocks often overlap in dense stands and therefore it remains unclear whether they are actually individuals. Also, even if two tussocks right next to each other are not linked by rhizomes and can then be considered as individual plants, fingerprinting techniques would be necessary to determine if they became established from seeds or
by clonal fragmentation. Because of these difficulties, an analysis of the population structure by means of a size-class histogram (Hutchings, 1997) of tussocks was not applicable. We found that the number of seedlings germinated in 1998, 1999, and 2000 was the same despite enormous (9.8-fold) differences in seed production. According to Andersen (1989), seed predation has no impact on the population structure of long-lived perennial plants as long as predation does not reduce the seed density on the ground below the density of safe sites. It can then be concluded that either S. tenacissima is safe-site limited or that the number of seeds was reduced by *Messor* predation below the number of available safe sites in all 3 years.

Haase et al. (1995) reported heavy seed production (31,500 seeds per 25m²) of *S. tenacissima* at a study site about 15km to the north of the El Desierto in 1993. They hypothesized that mast fruiting in this plant may serve to satiate seed predators and to ensure recruitment by seeds. Since no long-term data on the coefficient of variation of seed production are available, we caution against categorizing *S. tenacissima* as a mast species (sensu Kelly, 1994). There does not seem to be a population wide synchronization of flowering and fruiting, and the varying reproductive output may simply a result of abiotic factors, i.e. water availability. In 1999—despite the low percentage of tussocks that produced fruit stands—there were tussocks in less dense stands close to study site A showing enormous seed production. On that study site, *S. tenacissima* seed production was on average 894 seeds per 25 m² in 1999, 8726 seeds per 25 m² in 2000 and 4486 seeds per 25 m² in 2001 (assuming that the 1999 figures for the number of diaspores per spike are representative for all 3 years). In 1999 *Messor* spp. collected a very high percentage of the seed production, while the proportion of seeds taken in 2000 and 2001 is not known. During separate experiments in May 2000 and May 2001 (C.S., unpublished data) *Messor* foragers harvested almost exclusively *S. tenacissima* seeds. In 2000 there was virtually no seed production by the small shrubs like *H. almeriense, Thymus* spp., *Anthyllis cytisoides* and *Launaea* spp. on study site A. As *Messor* spp. are generalist seed predators, the reproductive output of *S. tenacissima* would have to be synchronized with that of other plants in the habitat in order to reduce the population density of *Messor* spp. with years of low seed production and to satiate them in years of strong seed production. As granivorous ants store seeds in their nest, it is not clear whether their population density would actually decrease in years of low seed production. Tevis (1958) reported that colonies of *M. pergandei* even survived several years of drought and resulting low seed availability in California. To our knowledge, true masting has so far only been reported for plants that suffer high predation levels by specialist flower or seed predators rather than by generalists (e.g. Kelly et al., 2000). Only long-term exclusion experiments can help to answer the question of how seed predation by *Messor* spp. influences the population structure of *S. tenacissima*. However, even if seed predation of *Messor* spp. does not have any effect on the population structure, it may nevertheless represent an important selection pressure for the evolution of diaspore morphology as *Messor* would then still determine the identity of the occupants of the safe sites (Andersen, 1989).
4.4. Pre-dispersal seed predation by *Messor* spp.

Foraging workers of the three *Messor* spp. very often climb plants of many species and cut the fruits directly from the mother plant, irrespectively of whether they are ripe or not. One observation in May 1999 may illustrate the enormous harvesting capacity of these ants: a single colony of *M. timidus* harvested all but 14 of the 1100 fruits of a *S. tenacissima* tussock from the fruit stands within a single night. Pre-dispersal seed predation by *Messor* has been reported from various locations and for various species (Went et al., 1972; Gordon, cited in Levieux and Diomande, 1978; Oster and Wilson, 1978; Hahn and Maschwitz, 1985; Rissing, 1988, Steinberger et al., 1992; Guttermann, 1993; Cerdá and Retana, 1994; Neumayer, 1994; Reyes and Fernández Haeger, 2001). Pre-dispersal predation circumvents various dispersal mechanisms by which diaspores could otherwise escape. When harvesting *S. tenacissima* fruits from the spikes, *Messor* colonies prevent diaspores from drilling themselves into the ground. In a similar way harvesting fruits of *Thymus hyemalis* and *H. almeriense* ensures that these small seeds (mean seed mass 0.68 and 0.08 mg respectively, \( n = 100 \) in both cases) cannot fall into cracks in the soil, where they may not be reached by workers. In the dry river beds of El Desierto (“ramblas”) the elaiosome-bearing diaspores of *C. aspera* cannot get dispersed by the ant *Tapinoma nigerrimum*, when *Messor* workers collect them directly from the plant. Other dispersal mechanisms like myxospermy and aestatiphory, which may principally be helpful in reducing seed losses through predation (see Introduction), are also not effective under these circumstances. Throughout our field work, only a single plant species was found that effectively prevents harvesting of its fruits from the mother plant by *Messor*: at the beginning of June 1999 foragers of *M. barbarus* were observed trying to cut fruits of *Euphorbia characias* in the Sierra de los Filabres. They got entangled by the milky sap and most of them died.

5. Conclusion

In *S. tenacissima* and other members of the genus, the selective advantages of trypanocarpie have been associated with improved germination and better dispersal by both wind and animals. Our study is the first in providing strong evidence for the hypothesis that trypanocarpie reduces seed losses through ant predation, both by substantially reducing retrieval rates by granivorous ants and by making diaspores unavailable to ants when they drill into the ground. Since *Messor* ants were shown to consume a substantial proportion of seed production of the tussock-grass *S. tenacissima*, they may therefore represent a significant factor in the evolution of its diaspore morphology.

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