

The role of dung beetles in seed dispersal in an arid environment

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ABSTRACT. Dung beetles can influence seedling emergence and survival. However, the direction and magnitude of this effect will depend on the functional traits of the dung beetle community and on the prevailing environmental conditions. We studied the role of dung beetles in seed dispersal of *Prosopis flexuosa* DC. in an arid environment. We conducted an experimental study to assess how different dung beetle species differ in their effectiveness as secondary seed dispersers. To evaluate this question, we selected four species belonging to three functional groups: lifters, tunnelers, and rollers. Dung beetles removed 0.7%-7.5% of the seeds embedded in cow dung. The net effect of beetles on seedling emergence and survival varied markedly among species: *Malagoneia puncticollis* (roller) had neutral effects on seedling emergence and positive in their survival. *Sulcophanaeus imperator*, *Digitonthophagus gazella* (tunneler) and *Eucranium arachnoides* (lifters) species had positive effect on seedling emergence. However, the effect of *S. imperator* on seedling survival was negative, and those of *D. gazella* and *E. arachnoides*, neutral. Our results indicate that although dung beetles remove few seeds of *P. flexuosa*, their main role consisted in producing changes in micro-environmental conditions for the seeds that remained in the dung pile.

[Palabras clave: dung beetles, seeds, dispersal, aridity, desert, grazing]

RESUMEN. El rol de los escarabajos estercoleros en la dispersión de semillas en un ambiente árido. Los escarabajos estercoleros pueden influenciar la emergencia y supervivencia de plántulas. Sin embargo, la dirección y la magnitud de este efecto dependerá de los atributos funcionales de la comunidad de escarabajos estercoleros y de las condiciones ambientales dominantes. Estudiamos el rol de los escarabajos estercoleros en la dispersión de semillas de *Prosopis flexuosa* DC. en un ambiente árido. Realizamos un estudio experimental para evaluar la efectividad de cuatro especies de escarabajos estercoleros en la dispersión secundaria de semillas. Para ello, seleccionamos cuatro especies de escarabajos estercoleros que pertenecen a distintos grupos funcionales: levantadores, cavadores y rodadores. Los escarabajos estercoleros removieron entre 0.7%-7.5% de las semillas embebidas en el estiércol de vaca. El efecto neto de los escarabajos sobre la emergencia de plántulas y su supervivencia varía marcadamente entre especies: *Malagoneia puncticollis* (rodador) tuvo un efecto neutro sobre la emergencia de plántulas y positivo en la supervivencia. *Sulcophanaeus imperator*, *Digitonthophagus gazella* (cavadores) y *Eucranium arachnoides* (levantadores) tuvieron un efecto positivo sobre la emergencia de plántulas; sin embargo, el efecto de *S. imperator* sobre la supervivencia de plántulas fue negativo, y los efectos de *D. gazella* y *E. arachnoides*, neutros. Nuestros resultados indican que, aunque los escarabajos estercoleros remueven pocas semillas de *P. flexuosa*, su rol principal consistió en cambiar las condiciones microambientales para las semillas que permanecen en el estiércol.

[Palabras clave: escarabajos estercoleros, semillas, dispersión, aridez, desierto, pastoreo]

INTRODUCTION

Dung beetles are an essential component of biodiversity in many ecosystems. Because dung beetle larvae need vertebrate dung to develop, adult beetles transport the dung to their nests, which are usually burrowed underground (Nichols et al. 2008). By engaging in these activities, dung beetles accelerate the growth of bacteria involved in nitrogen fixation (Kazuhira et al. 1991), modify soil morphology (bioturbation), thus diminish its compaction and increase the flow of air and water (Nichols et al. 2008), improve soil fertility, control parasites (especially flies) (Ridsdill-Smith and Hayles 1987) and facilitate nitrogen absorption in plants (Huerta et al. 2013), enhancing their growth. Moreover, when dung beetles transport dung to their nests they may also move seeds embedded in dung. Transported seeds have the opportunity of germinating, as larvae and adults do not feed on seeds (Nichols et al. 2008). However, larger seeds may be discarded by dung beetles because they may be perceived as undesirable material (Andresen 2002). Therefore, dung beetles may conduct secondary seed dispersal (diplochory), although their effectiveness as seed dispersers depends on multiple intrinsic and extrinsic factors (Andresen and Feer 2005) and is thus difficult to predict.

Previous studies have shown that secondary seed dispersal by dung beetles may be complementary to seed dispersal by frugivorous and herbivore animals (Vander Wall and Longland 2005; Koike et al. 2012). Studies in tropical regions of the Americas (Andresen 2003; Culot et al. 2018) and Africa (Shepherd and Chapman 1998) indicate that secondary dispersal by dung beetles facilitates seed germination and seedling emergence and survival due to seed burial in an environment protected from desiccation (Chambers and MacMahon 1994) and less variable microclimate than soil surface (Vander Wall and Longland 2004), thus decreasing seed predation probability and increasing germination rate. Seed dispersal by dung beetles also reduces seed agglomeration in dung (Andresen 2003; Andresen and Levey 2004; Urrea-Galeano et al. 2019), which decreases intraspecific competition among seedlings, as well as pathogen and herbivore attacks (Estrada and Coates-Estrada 1991; Andresen 2002; Chapman et al. 2003). However, little is known about the role of dung beetles as secondary seed dispersers in arid and semiarid ecosystems (e.g., Verdú et al.

2009; Ardali et al. 2016) where their role may be relevant as they can decrease seed mortality due to adverse climatic conditions.

The probability of dispersal of seeds harbored in dung depends on seed type and size, dung beetle abundance and composition, and dung quantity and quality (Andresen 2002; Braga et al. 2017). Dung beetle species differ in the way in which they transport dung, which could influence their effectiveness as secondary seed dispersers (Estrada and Coates-Estrada 1991; Vulinec 2002). There are four functional groups that are defined according to the dung transport behavior: rollers, tunnelers, dwellers and lifters (Doube 1990; Ocampo and Hawks 2006). Rollers transport dung by molding it into a ball, which they roll with their hind legs to a variable distance (6-15 m) and vertically to a variable depth; in contrast, tunnelers bury dung without transporting it under the dung pat mainly vertically below the dung pat (12-35 cm of depth) (Halffter and Matthews 1966; Heymonds and von Lengerken 1929; Andresen 2002) either including or excluding the seeds along the tunnel (Andresen and Feer 2005). In turn, dwellers build the nest inside the dung pile or very close to it, and thus are unlikely to have a significant role in seed dispersal (Andresen and Feer 2005). Lifters, which transport dung without molding it into a dung ball, grasping the dung with the fore tibiae and running forward using middle and hind legs (Halffter and Matthews 1966; Ocampo and Hawks 2006); lifters transport dung horizontally also to variable distances and depths (5-60 cm) (Monterresino and Zunino 2003).

Seeds benefit from dung beetle activities only if they are buried to an appropriate depth for germination and emergence, allowing stem elongation to reach the soil surface (Fenner 1987) and access light for photosynthesis. For most seeds, emergence success declined drastically when they were buried deeper than 3 cm (Andresen and Feer 2005). In particular, we studied *Prosopis flexuosa* DC, one of the dominant tree species in the Central Monte ecoregion, whose seeds are consumed by cattle have low germination probability and most seedlings do not survive longer than a week (Campos et al. 2011). In a previous trial with seeds from the leguminous tree *P. flexuosa*, we observed that the probability of emergence increased substantially in seeds, whose size is 6 mm, immersed in dung and buried 2 cm below ground compared to seeds in dung on the soil surface (Maldonado 2017).

In this context, we hypothesized that dung beetles are effective seed dispersers in arid environments, carrying the seeds to more stable underground environmental conditions, thus mitigating the high evapotranspiration and the low humidity predominant in this type of climate, also to avoid de temperature fluctuations, wind and the runoff. Thus, we expected a positive net effect of dung beetles on seedling emergence and survival when seeds are buried at moderate depths (up to 3 cm). Moreover, according to the dung transport behavior, we expected that *E. arachnoides* (lifters) and *M. puncticollis* (rollers) transported seeds embedded in dung horizontally and vertically, while *S. imperator* and *D. gazella* (tunnelers) transported seeds mainly vertically.

MATERIALS AND METHODS

Study species

We selected the most representative species of our study area, the dryland Monte desert, where these ones are among the most abundant species (Maldonado 2017). The four species selected belonged to three different functional groups: an exotic, invasive tunneler, *Digitonthophagus gazella* (Fabricius 1781); a native tunneler, *Sulcophanaeus imperator* (Chevrolat 1844); a native roller, *Malagoniella (Megathopomina) puncticollis* (Blanchard 1846); and a native lifter, *Eucranium arachnoides* (Ocampo 2007).

Moreover, we studied the effect of dung beetles on the most abundant tree species of the Monte desert biogeographic region: *Prosopis flexuosa* (Fabaceae, Mimosoideae) (Ffolliott and Thames 1983) has high interannual variability, a key species for the region since many other species, including pollinators, birds, mammals interact with this species (Villagra et al. 2002; Chacoff et al. 2012). Seed production of *P. flexuosa* has high interannual variability and is consumed by many rodent, grazer and insect species (Campos and Velez 2015). Domestic cattle, which are highly abundant in the region, also frequently consume *P. flexuosa* (Aschero et al. 2016). In fact, 100 g of cow dung may contain up to 200 seeds of this species (Campos et al. 2011).

General experimental design

We conducted the experiments in semi-open greenhouse conditions, which consisted of a 15

m by 4 m enclosure placed in the experimental campus of CONICET Mendoza. We made the observations in February 2015, which corresponds to the middle of summer in our study area. The mean, maximum and minimum temperatures during this period were 25.5 °C, 32.3 °C and 18.7 °C, respectively. We excluded rain precipitation with a plastic roof. For the enclosure walls we used a garden-shade net, so as to exclude dogs and other animals that could interfere with our experiment, while enabling full ventilation.

We collected the beetles in Ñacuñán Nature Reserve during 10 nights (19 to 29 January 2015). We used three complementary methods to catch the beetles: dung baited pitfall traps without liquid for non-flying beetles (*E. arachnoides*) during 24 h, hand collection in and below cow dung (*S. imperator* and *D. gazella*) and light trap. We kept beetles individually in plastic containers with soil and covered with porous cloth bags until the start of the experiment. We fed them with fresh cow dung. The experiment started immediately after the beetles arrived at the experimental campus of CONICET Science and Technology Center in Mendoza. We collected *P. flexuosa* seeds one year before the start of the experiment and assessed germinability whose result was 100%. We scarified seeds mechanically with sandpaper to simulate the scarification caused by cow digestion. After scarification, we haphazardly embedded seeds in a dung pile in the centre of each experimental pot. In all the experimental pots with beetles, we put only one individual, except for *D. gazella*, for which we included a female-male pair, because in a pilot experiment we had observed that single individuals did not remove any dung. Only with *D. gazella* and *S. imperator* were we able to identify females and males.

On day 6 after the experiment started, we counted the number of seedlings that emerged without destroying them so avoid perturbing the experiment. Thirty-four days after the experiment started we measured: the number of seeds removed from the dung pile, the distance between the removed seeds and the dung pile, the depth of buried seeds, the number of emerged seedlings, and the proportion of living seedlings. To this end, we broke the dung pile and dug it into a pot to look for seeds, recording the depth and horizontal distance from the pile. After this point of the experiment, all beetles were dead, so we ended the experiment.

Experimental design

Effects of *S. imperator*, *E. arachnoides* and *D. gazella* on seeds. For this experiment, we built circular pots of 23 cm in diameter and 27 cm in height, filled with 10.5 kg of dry sandy soil up to 24 cm in height. The pots were made with rigid polypropylene plastic bags and fine insect netting on top to prevent the beetles from escaping. There was a variable number of pots per treatment, determined by the number of individuals collected for each dung beetle species: *S. imperator*, 21; *E. arachnoides*, 11; *D. gazella*, 14; and control, 15 (Figure 1). Each pot for *S. imperator* contained either one male (n=8) or one female (n=13), pots for *E. arachnoides* contained one individual of unidentified sex, and pots for *D. gazella* had one male and one female. All the pots contained 150 g of cow dung with 10 *P. flexuosa* seeds embedded.

Effects of *M. puncticolis* on seeds. For this experiment, we built larger pots to provide more space for these beetles to roll the dung. The circular pots measured 40 cm in diameter and 40 cm in height and were filled with 40 kg of dry sandy soil, manually compacted until they reached a uniform height of 25 cm in all the pots. The pots were made with a metallic, flexible mesh, covered with an inner black nylon sheet ~30 cm high, and fine insect netting on top to prevent the beetles from escaping. The experimental design consisted of 20 pots with one individual of *M. puncticolis* and 8 control pots, both with 150 g of cow dung homogenized with 10 *P. flexuosa* seeds embedded.

Statistical analyses. To test whether seedling emergence is facilitated by dung beetles, we used generalized linear mixed effects models (GLMM) for proportional data (emerged seedlings/total seeds) with a binomial family using the lme4 package of R statistical software (Bates et al. 2004). Different dung beetles species and control were a fixed factor, and random factors were the number of beetles (0 for control pots, 1 for all the pots with beetles except for the pots with *D. gazella* that contained 2 beetles) and the pot size (small or large). Moreover, we tested the effect of different species on seedling survival (34 days after the experiment started) using a GLM for proportional data (emerged seedlings/total seeds) with a binomial family using the lme4 package as previously, with dung beetle species and control were explanatory variables. All the analyses were performed using R software version 3.2.2. We used the visreg package (Breheny and Burchett 2017) to visualize the regression models (R Development Core Team 2015).

RESULTS

Effects of *S. imperator*, *E. arachnoides* and *D. gazella* on seeds

E. arachnoides removed 7.5% of the seeds. This removal did not differ from those accidentally removed by water or gravity in the control pots ($\chi^2=10.11$, $P=0.37$). Seeds manipulated by *E. arachnoides* beetles were buried at 13-24 cm depth ($\bar{x}=18$ cm, $SE=3.2$), and only one out of

Table 1. Effect of dung beetles over seedlings emergence of *P. flexuosa*, at day 6 since the beginning of the experiment. The significance level is indicated in the column (P-value).

Tabla 1. Efecto de los escarabajos estercoleros sobre la emergencia de plántulas de *P. flexuosa* al día 6 luego del comienzo del experimento. El nivel de significancia está indicado en la columna P-value.

Fixed effect: Species	Coef.	SE	P-value	Confidence interval
<i>D. gazella</i>	1.2	0.41	0.004	0.4-2.07
<i>E. arachnoides</i>	1.5	0.42	0.0004	0.7-2.4
<i>M. puncticolis</i>	-0.07	0.25	0.76	-0.6-0.42
Random effect: Pot size	Variance	SE	P-value	
	2.2	1.48	<0.001	

Table 2. Effect of dung beetles over seedlings survival of *P. flexuosa*, at day 34 since the beginning of the experiment. The significance level is indicated in the column (P-value).

Tabla 2. Efecto de los escarabajos estercoleros sobre la supervivencia de plántulas de *P. flexuosa*, al día 34 luego del comienzo del experimento. El nivel de significancia está indicado en la columna P-value.

Species	Coef.	SE	P-value	Confidence interval
<i>D. gazella</i>	0.038	0.21	0.86	-0.38-0.46
<i>E. arachnoides</i>	0.17	0.23	0.45	-0.3-0.63
<i>M. puncticolis</i>	0.76	0.19	0.0001	0.4-1.15
<i>S. imperator</i>	-0.95	0.2	<0.0001	-1.37-(-0.55)

110 seeds was horizontally transferred at 3 cm away from the dung pile. On the other hand, *S. imperator* removed 6.6% ($\chi^2=9.7$, $P=0.32$) the of seeds, and six seeds of them were buried at 5-24 cm ($\bar{x}=15.7$ cm, $SE=1.9$, $n=6$), and three seeds were horizontally transferred ($\bar{x}=5$ cm, $SE=1.15$, $n=3$). Finally, two of the nine seeds relocated by *S. imperator* did not germinate until day 34. The presence of *E. arachnoides* facilitated the probability of the emergence of seedlings (Table 1) but was neutral after 34 days (Table 2).

The presence of *S. imperator* also facilitated seedling emergence (Table 1), but thirty-four days after the start of the experiment seedling survival was lower than in control pots (Table 2). In the analysis performed for *S. imperator*, we did not distinguish between females and males because they did not present significant differences in the amount of dung buried ($t=0.30$; $g.l.=17.4$; $P=0.8$). The presence of *D. gazella* in the dung was negligible, as this beetle species increased the probability of emergence in the seedlings and did not have a significant effect on the survival in the seeds that remained in the dung (Tables 1 and 2). *D. gazella* buried only one seed at 5 cm of depth (0.7%, $\chi^2=0.81$, $P=0.32$).

Effects of *M. puncticollis* on seeds

Although *M. puncticollis* was not effective as a remover, it increased the probability of survival as an indirect effect. Most seeds were not removed by roller beetles; in fact, they transferred only one seed just 1 cm away from the dung pile. *M. puncticollis* did not increase the probability of emergence in the seedlings that remained in cow dung (Table 1), but significantly increased the probability of seedling survival (Table 2).

The effect of the number of beetles on the seedlings was not significant on day 6 and on day 34 of the experiment ($P=1$), hence it was not included in the selected models. The effect of the pot size on seedlings was significant on day 6 ($P=<0.05$) but not on day 34 ($P=1$); therefore, it was included only in the model selected for day 6 (Figures 1 and 2).

DISCUSSION

The main objective of this study was to experimentally assess the effect of different dung beetle on the secondary seed dispersal of the tree *P. flexuosa* after a first dispersal phase by cows, and to evaluate this effect

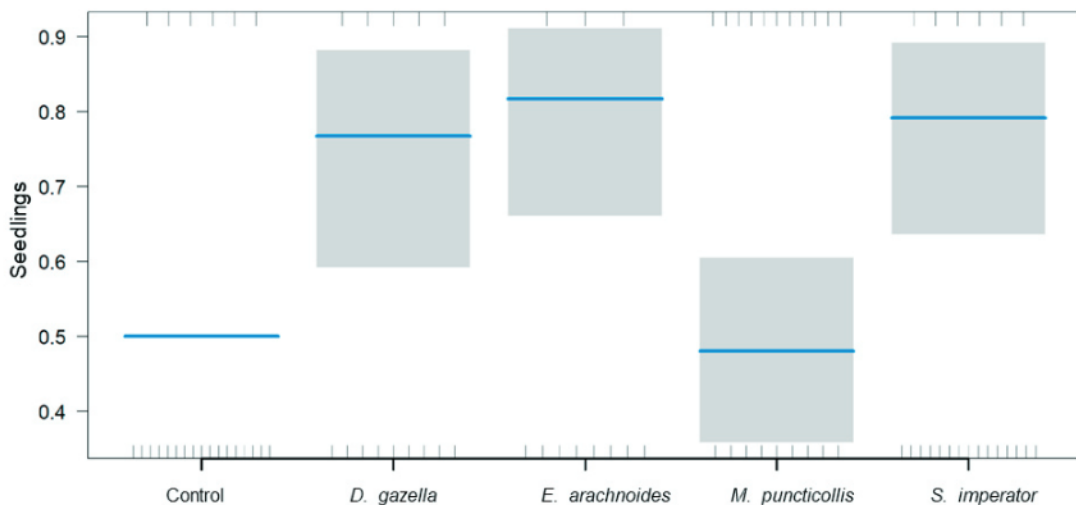


Figure 1. Effect of dung beetle species over the emergence of seedlings at 6 days the experiment startup. Treatments, from left to right: control, *D. gazella*, *E. arachnoides*, *M. puncticollis* and *S. imperator*. The blue lines represent estimated median values. Separate vertical lines on the top represent observations with positive residuals and on the bottom for observations with negative residuals. Gray boxes represent 95% confidence intervals. X axis: dung beetles species. Y axis: refers to the proportion of living seedlings modelled.

Figura 1. Efecto de los escarabajos estercoleros sobre la emergencia de plántulas de *P. flexuosa* al día 6 luego del comienzo del experimento. Tratamientos, de izquierda a derecha: control, *D. gazella*, *E. arachnoides*, *M. puncticollis* y *S. imperator*. Las líneas azules muestran los valores medianos estimados. Para cada una de las especies evaluadas, las líneas verticales superiores representan las observaciones con residuos positivos y las líneas verticales inferiores representan las observaciones con residuos negativos. Las cajas grises representan un intervalo de confianza de 95%. Eje X: especies de escarabajos estercoleros. Eje Y: se refiere a la proporción de plántulas vivas modeladas.

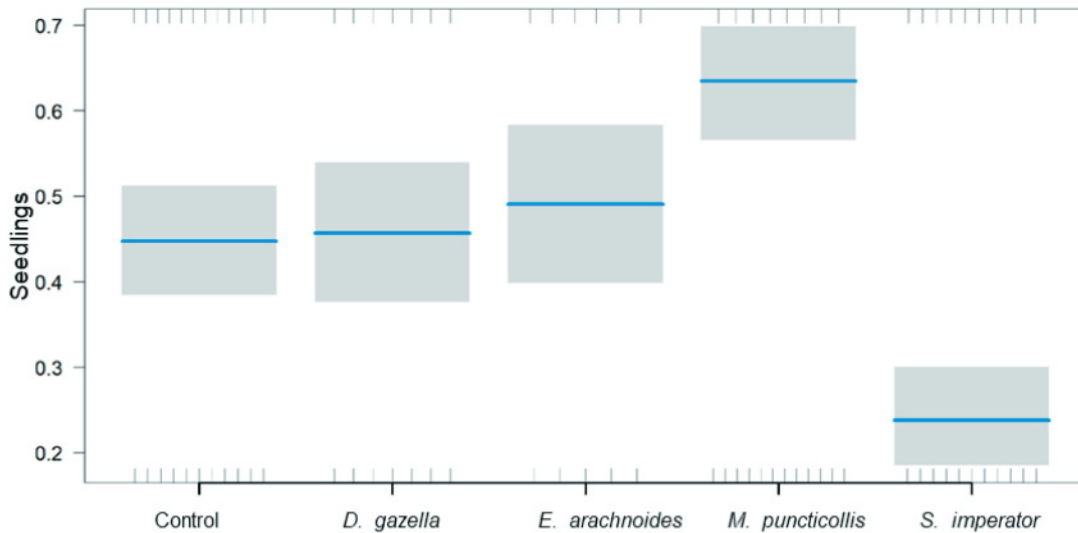


Figure 2. Effect of dung beetle species seedlings survival at 34 days the experiment startup. Treatments, from left to right: control, *D. gazella*, *E. arachnoides*, *M. puncticollis* and *S. imperator*. The blue lines represent estimated median values. Separate vertical lines on the top represent observations with positive residuals and on the bottom for observations with negative residuals. Gray boxes represent 95% confidence intervals. X axis: dung beetles species. Y axis: refers to the proportion of living seedlings modelled.

Figura 2. Efecto de los escarabajos estercoleros sobre la supervivencia de plántulas de *P. flexuosa* al día 34 luego del comienzo del experimento. Tratamientos, de izquierda a derecha: control, *D. gazella*, *E. arachnoides*, *M. puncticollis* y *S. imperator*. Las líneas azules muestran los valores medianos estimados. Para cada una de las especies evaluadas, las líneas verticales superiores representan las observaciones con residuos positivos y las líneas verticales inferiores representan las observaciones con residuos negativos. Las cajas grises representan un intervalo de confianza de 95%. Eje X: especies de escarabajos estercoleros. Eje Y: se refiere a la proporción de plántulas vivas modeladas.

in an arid environment: The Monte desert. We found that the role of dung beetles in the emergence and survival varied between species: *M. puncticollis* had neutral effects on seedling emergence and a positive effect on their survival, and both tunnelers (*S. imperator* and *D. gazella*) had positive effects on seedling emergence, although *S. imperator* had a negative effect on seedling survival, while the invasive tunneler had a neutral effect. Furthermore, *E. arachnoides* had positive effects on emergence and neutral effect on seedling survival. Additionally, the magnitude of the direct effect of seeds dispersed out of the dung pad appears to be much less relevant than the indirect effect. Indeed, we found that even though the beetles did not remove the majority of the seeds that were embedded in dung, in some cases we observed differences in seed emergence and survival with respect to control conditions. This is probably due to the fact that dung disintegration carried out by dung beetles can facilitate the seedling emergence and reduce seed clumping (Urrea-Galeano et al. 2019). Such dung disintegration produced a change in micro-environmental conditions for the seeds that remained in the dung, or in

those that were removed horizontally some centimeters, but not due to their vertical translocation.

It is remarkable that in the cases whereby we observed an early positive effect on emergence (with *S. imperator*, *D. gazella* and *E. arachnoides*), we observed a neutral or negative effect on seedling survival, and in other case (*M. puncticollis*) at the beginning of the experiment, the effect was neutral but positive on the seedling survival. The negative effect of dung beetles on establishment was also reported by Urrea Galeano et al. (2019) in a warm and humid zone for two other tree species, as we observed with *S. imperator*. The positive effects of dung beetles on seedling emergence seem important in arid field conditions because early germination could provide a greater opportunity for seedlings to receive early seasonal rain pulses. These rain pulses are essential factors for seedling establishment in arid ecosystems (López et al. 2008), in comparison to seedlings that germinate later and temporally further from the rainy season (i.e., early summer). On the other hand, we observed two potentially

positive effects provided by lifters and rollers. One effect was an individual of the lifter functional group burying a seed without dung, which could contribute to seedling survival. Because seeds buried without dung may prevent fungus proliferation and rotting (Andresen and Feer 2005), they are also more difficult to spot by predators such as other insects or rodents because they are attracted to the smell of dung (Andresen and Feer 2005). The second effect was the strong effect on seedling survival by roller beetles, possibly because they removed small amounts of dung, mainly from the edges of the dung pad.

One relevant functional trait of dung beetles is their body size because larger beetles need more food to feed and reproduce, then the larger beetles bury more and larger seeds (Feer 1999; Vulinec 2002; Andresen 2002, 2003; Morales-Alba et al. 2022). Our results indicate that the body size also determined the effects observed on the seeds because the smaller beetles removed a negligible quantity of seeds (*D. gazella* and *M. puncticolis* whose body size is 10-13 mm), while the larger beetles removed more seeds (*S. imperator* and *E. arachnoides* whose body size is 23-28 mm, and 18.4-30.4 mm respectively), in agreement with recent findings of Morales-Alba et al. (2022).

It is important to recognize some limitations of the present study. First, the experiments were conducted in pots and not in field conditions, and consequently, we did not consider other factors that could also influence seedling emergence and survival, such as the reduction of viability by cattle (Campos y Ojeda 1997) and the effect of shading. In this regard, the result of dung beetle activity could be more positive in more shaded sites that promote early seedling survival, avoiding in those cases the higher probabilities of removal and perhaps seed predation by rodents that occur under shrub cover (Velez et al. 2016). However, in a longer period (10 months), higher rates of

seedling establishment have been reported in areas with intensive transit of cows, around adult individuals of *P. flexuosa* (Campos et al. 2011). A second limitation of our study has to do with the different space requirements of the studied dung beetle species, which prevented us from using pots of the same size for all treatments. For this reason, comparisons between different species should be made with caution. Finally, the large number of seeds that were not removed by dung beetles could be a consequence of the relatively large size (6 mm) of *P. flexuosa* seeds, as suggested by the results of a previous experimental study (Braga et al. 2017) in which dung beetles removed small beads (3.5 mm) more than larger beads (8.6 and 15.5 mm). Even though our experiment had limitations, it is also important to emphasize that an experimental approach offers insights difficult to obtain under field conditions.

Our study contributes to understanding of the role of dung beetles as seed dispersers in arid environments. We have shown that in these systems, dung beetles can facilitate seedling emergence, disintegrating the dung pad, because its desiccation occurs quickly and constitutes a physical barrier that prevents seedlings from emerging out of the dung, as it has been suggested in Ishikawa (2011) for a dry season in temperate grasslands. Moreover, our study is the first that attempts to assess the effect of an abundant and cosmopolitan invasive dung beetle species (*D. gazella*) on seed dispersal.

ACKNOWLEDGEMENTS. This research was funded by CONICET (PIP-2014-11220130100395CO), FONCYT (PICT 2014-3168 and PICT 2015-0582). MBM and AMS were supported with doctoral fellowships from CONICET. DPV and NPC are career researchers with CONICET. We thank Mariana Allasino and Gualberto Zalazar for their help during fieldwork and Hugo Debandi for his help with the setup of the experiments.

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