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INVASION NOTE

Food habits of European rabbit and its role as seed dispersal of two Mosqueta roses: Facilitation among non-native species in a semiarid protected area of Argentina?

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Abstract Herbivore and plant invasions can modify the structure and functioning of ecosystems through positive interactions that facilitate their mutual establishment. An important consequence of the feeding behaviour of rabbits is that they can play a key role in seed dispersal by endozoochory. We examined the diet and potential for dispersing *Rosa rubiginosa* and *R. canina* (*Rosa* spp.) of European rabbits introduced in a semiarid protected area of Argentina. We found entire seeds in 100% of the samples of *Rosa* spp. analysed (n = 11, 187 pellets). Our results indicate that the passage of *Rosa* spp. seeds through the digestive tract of rabbits (64%) did not diminish their viability in comparison to the seeds taken directly from fruits (66%) (χ^2 = 0.09, $p > 0.05$). This study has identified new positive interactions between an invasive mammal and two invasive plants, a finding that suggests that these invader complexes could have a reciprocal effect on one another, which would potentially aid their invasive process in a semiarid protected area of Argentina.

Keywords *Rosa rubiginosa* · *R. canina* · *Oryctolagus cuniculus* · Endozoochory · Invasional meltdown

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Introduction

The presence of a new species in the system can cause various effects on a native community such as changes in interactions or appearance of new ones (Blackburn et al. 2014). Positive interactions occur frequently among non-native species, suggesting that mutualisms are important in several invasion scenarios (Braga et al. 2018). For example, introduced herbivorous mammals launch interactions with non-native plants (plant-disperser interactions), which promotes species integration in the invaded community. Thus, if the interacting species are both introduced and prove a beneficial outcome to each other, they could be contributing to a process by which non-native species facilitate one another's invasion (invasional meltdown) (Traveset and Richardson 2006, Simberloff and Van Holle 1999). The fruit and seed consumption by herbivores may benefit the plant, not only, due to viable seeds eliminated in faeces at suitable sites for germination and survival, but also, because the plant escape of the high mortality rates close to the parent plant (dispersal by endozoochory; Howe and Smallwood 1982; Janzen 1980). Hence, invader complexes created among non-native plant–seed dispersers may contribute to success of their invasion and, therefore, to their expansion in the invaded range (Traveset and Richardson 2006). Particularly, lagomorphs may disperse viable seeds of grasses and forbs consumed incidentally while feeding on foliage and, of fleshyfruited plants whose fruits are intentionally consumed (Schupp et al. 1997).

Aridlands are water-controlled ecosystems with infrequent discrete largely unpredictable water inputs, where most of the plants have low nutritional value and where herbivores are dependent on vegetation for food and water (Noy-Meir 1973). Three invasive species that occur in arid and semiarid areas of Argentina, coexist in our study area: the mosqueta roses, *Rosa rubiginosa* and *R. canina* (hereafter *Rosa* spp.) and the European rabbit, *Oryctolagus cuniculus* (Dalmasso et al. 2011, Cuevas et al. 2011). *Rosa* spp. have a set of attributes that make them a good invader. These shrubs possess a broad range of reproductive strategies to produce fruits and seeds (e.g. production of a large mass of seeds, longevity of seeds, more fruits availability in periods of food shortage) which enable them to colonize new areas through dispersal vectors, such as birds and mammals (Mazzolari et al. 2017). Another invasive component of this arid ecosystem is the European rabbit, native to the Iberian Peninsula and the south of France. The European rabbit is a generalist herbivore with high plasticity in selecting food (Gálvez-Bravo et al. 2008). Rabbits act as endozoochorous seed dispersers of different plant species in their area of origin, as well as in areas where they have been introduced (Castro et al. 2008; Salas-Pascual et al. 2009). As a result of their feeding behaviour and their role as seed dispersers, rabbits can affect the structure, composition, and functioning of plant communities in invaded environments.

Based on the invasional meltdown hypothesis (Simberloff and Van Holle 1999), European rabbit and *Rosa* spp. have a reciprocal positive effect on one another. In particular, we hypothesize that European rabbit plays a major role as potential endozoochoric seed dispersers of *Rosa* spp., so it could be expected that the rabbits will consume fruits of *Rosa* spp. and they will eliminate its viable seeds through faeces. Therefore, the aim of our study was to explore the trophic ecology of the European rabbit, particularly its food habits, and the role as an endozoochorous seed disperser of *Rosa* spp. in a semiarid protected area of Argentina.

Materials and methods

The study area was located on the semiarid Andean foothills in the Natural Reserve of Villavicencio, Mendoza province, Argentina (32° 31′ S–69° 1′ W, Fig. 1). The reserve occupies 62,000 ha and its altitudinal range varies from 900 to 3200 m a.s.l. Annual precipitation is between 120 and 300 mm. The reserve protects three important phytogeographic arid biomes: Puna, Cardonal and Monte (Dalmasso et al. 1999). Sampling was conducted during the wet season 2009–2010 (December to March). We established 24 strip transects (100 × 10 m) from the site where rabbits were intentionally released in 2006 (Cuevas et al. 2011) following the topography of the area (Fig. 1). Transects were placed randomly within two geomorphological units, hillsides (n = 12) and riverbeds (n = 12), of the three ravines covering the greater environmental heterogeneity: (1) Hornillos; (2) Darwin and (3) Toro (Fig. 1). These sites present a characteristic vegetation of the Cardonal Phytogeographic province dominated by shrubs of the genera *Lycium*, *Baccharis*, *Artemisia* and *Adesmia*, and by grasses of the genera *Stipa*, *Poa* and *Aristida*. Riverbeds are characterized by the presence of non-native plant species including *Rosa* spp. (Dalmasso et al. 1999).

We collected fresh rabbit faeces along the transects checked one time during wet season. All faeces collected in one transects constituted one sample for diet analysis. At this transects, plant cover was measure through point quadrat method (Passera et al. 1983) for food availability data. We also collected *Rosa* spp fruits and fruits from several plants to make a reference collection. To determine the rabbit's diet, we used fresh faeces from each transect, which were evaluated through microhistological techniques and analysis that permit identification of leaf epidermis, stems, seed teguments and arthropod body parts (Dacar and Giannonni 2001). For each sample, we prepared microscope slides and systematically examined 50 fields under 40X microscope. Food items in the faecal samples were identified by comparison to reference material. Presence of a food item was recorded, and its relative frequency per slide was determined by dividing the number of microscope fields in which this item occurred by the total number of microscope fields observed × 100 (Holechek and Gross 1982).

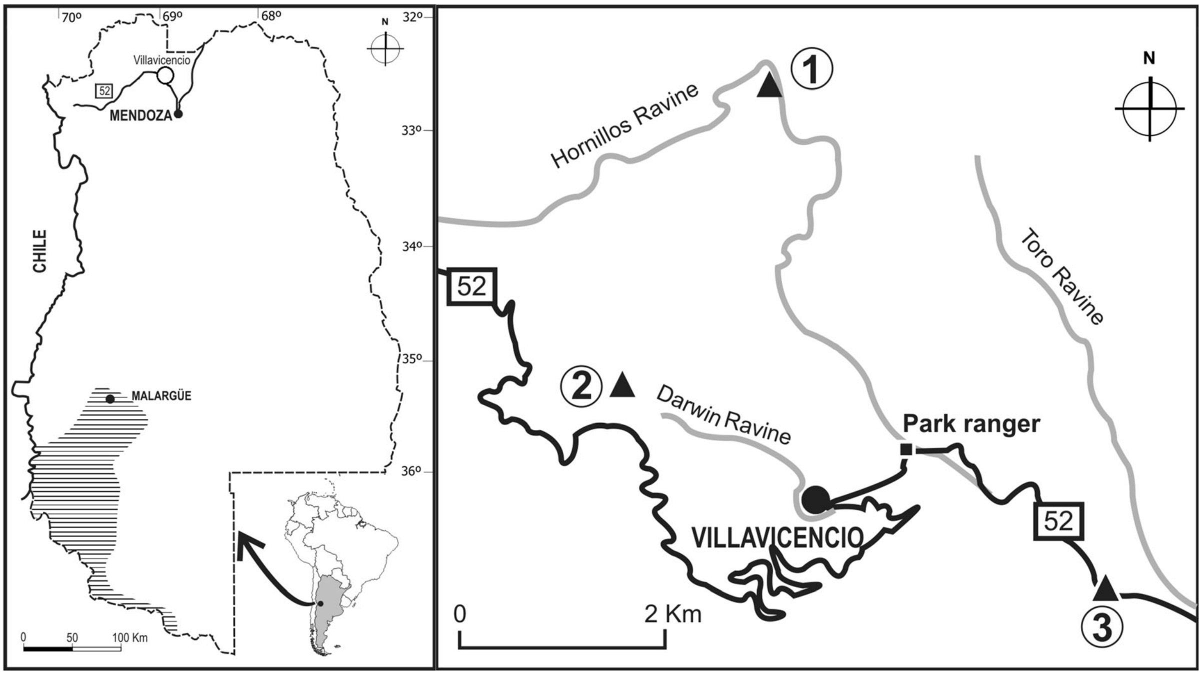

Fig. 1 Left: Distribution of European rabbit (*Oryctolagus cuniculus*) in Mendoza province (dashed line) and study area (white circle). Right: Potential dispersal corridors: Hornillos Ravine (1), Darwin Ravine (2) and the third path towards the Monte desert foothills (3) in the Natural Reserve of Villavicencio. Map extracted and modified from Cuevas et al. 2011

Plant species were clustered into four categories according to life form: herbs, grasses, sub-shrubs (shrubs less than 1 m tall) and shrubs, and we estimated the proportion of each food category. Food availability was calculated from vegetation data obtained from transects. Selectivity of food items was estimated using Manly's Selectivity Index (Manly et al. 2002): $\alpha_i = (Pu_i/Pa_i)/\Sigma(Pu_i/Pa_i)$, where Pu_i is the observed proportion of item i in the rabbit's diet and Pa_i is the available proportion of item i in the environment. If α_i is greater than 1/k, being k the number of food items, indicate selection (greater than random). If α_i is less than 1/k, indicate avoided, and, $\alpha_i = 1/k$ indicate a consumption in accordance to availability (Manly et al. 2002). To test the reliability of Manly's index, we resampled rabbit faeces 1000 times by bootstrapping. We then calculated the average values and the 95% confidence intervals of Manly's index. All analyses were performed with the package Quantmod (Ryan et al. 2018) in environment R 3.5.1 software (R Development Core Team 2018).

To evaluate the viability of the entire sweet briar seeds found in rabbit faeces, we used a standard bioindicator (2, 3, 5 triphenyltetrazolium chloride (TTC)) that detects seed viability by staining the embryo tissue pink/red. Conditions for the tetrazolium test for the genus *Rosa* was taken from the International Rules for Seed Testing (2005). Viability of seeds was determined by their coloration, using microscope amplification in comparison to positive control seeds collected from the study site. A Chi square statistic test of homogeneity (Zar 2010) was used to analyse differences in the proportion of viable seeds obtained from faeces and the control treatment.

Results and discussion

We collected rabbit faeces from almost half the sampled transects (n = 11) where the 75% was recorded on riverbed transects and on only 2 hillside transects. The presence of rabbit signs in riverbed transects could be explained by the influence of factors like vegetation, soil, and places with streams or higher moisture (Cuevas et al. 2011). Hundred percent of the samples contained 135 entire seeds of Rosa *spp.* (0.7 ± 0.9 *roses seeds. pellet*$^{-1}$), whereas 36.4% of the samples were found also damaged seeds (Table 1).

Approximately, 27% of the 135 digested entire seeds were non-productive, that is, empty seeds, with a poorly developed embryo, infected by fungi or by wasp larvae (Table 1). The proportion between seed damaged/entire was low, suggesting that European rabbit plays a less important role as seed predator than as disperser to *Rosa* spp.

The rabbit's diet was composed of different parts (leaves and seeds) of 30 plant species. Seed teguments represented 6% of the diet and all corresponded to *Rosa* spp. The European rabbit consumed mainly herbs (50%), followed by subshrubs (17.9%), shrubs (16.5%) and grasses (15.5%) (Fig. 2). The bulk of the diet (more than 70%) was composed of: *Medicago lupulina* (Fabaceae) (32.6%), *Poa resinulosa* (Poaceae) (11.7%), *Lycium* sp. (Solanaceae) (10.3%), *Spartium junceum* (Fabaceae) (6.5%), *M. sativa* (6.4%) and seeds teguments of *Rosa* spp. (Rosacea) (5.7%) (Table 2). Previous reports on the diet of rabbits in Argentina's Patagonia showed that grasses represent the main category in the diet throughout the year whereas herbs had moderate participation in the spring and summer diets (Bonino and Borelli 2006). In its native Mediterranean ecosystem, the rabbit has been shown to accommodate its diet to the resource availability resulting from interactions with the other herbivores coexisting in the area (Soriguer 1983). Thus, when herbaceous plants are scarce by the presence of other herbivores, rabbits consume mainly grasses whereas, if grazing alone, they prefer herbs (Soriguer 1988). This could be happening inside the protected area, where the rabbit is one of the primary herbivores along with the non-native European hare (*Lepus europaeus*) and native guanaco (*Lama guanicoe*), and there is no great grazing pressure by livestock on herbaceous vegetation.

Trophic selection for food categories was positive for herbaceous plants, while grasses, sub-shrubs and shrubs were avoided (Fig. 3a). Selection for food items was positive for four out of the 13 species of herbs consumed (Fig. 3b and Table 2). Rabbits consumed a higher proportion of the species they selected (about 50%), mainly the non-native *Medicago lupulina*. This species is considered a good forage resource due to its nutritional value (Passera et al. 1983). Consuming nourishing food could be important especially in arid and semi-arid ecosystems where the majority of plants have low nutritional value (Noy-Meir 1973). Even if the European rabbit did not select seeds of *Rosa* spp., showed a consumption greater than 5% in the diet during the wet season being the 6th item more consumed (Fig. 3b and Table 2). Previous studies have shown that the rabbit consumption of the woody species increases when the availability of herbaceous decreases by seasonality variation (Bonino and Borelli 2006). For example, comparison with dry season could showed a great consume fruits of *Rosa* spp. because constitute a nutritious, accessible and abundant food in this period. Therefore, the relative importance of rabbit–*Rosa* spp. interaction may

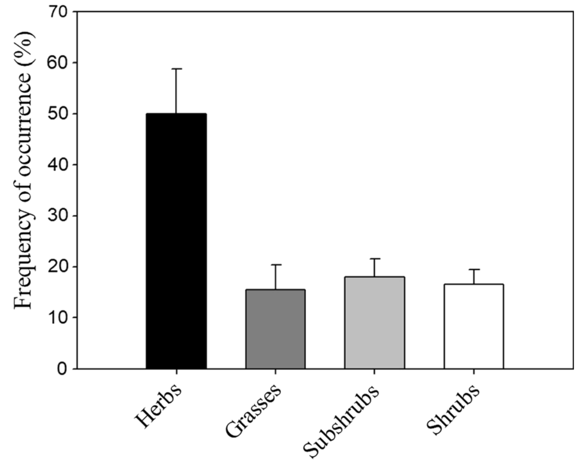

Fig. 2 Frequency of occurrence (mean ± SE) of each food categories in European rabbit diet during wet seasons in Reserve Villavicencio, Argentina

Table 1 Number of seeds found in the 187 pellets analysed (n = 11) collected throughout the sampling in Villavicencio Reserve. Number of viable seeds over total number of seeds tested for digested and control seeds (χ^2 = 0.09, df = 1, $p > 0.05$)

Number of seeds				Viable	
Damaged	Entire	Nonproductive seeds	Healthy embryo	Digested seeds	Control seeds
24	135	37 (9 larvae)	98/135 (72.6%)	63/98 (64%)	65/98 (66%)

Table 2 Mean and standard error (SE) of percent frequency of occurrence and selectivity for items present in European rabbit's diet in Villavicencio Reserve, Argentina

Category	Food item	Status	Frequency (%)		Selection
			Mean	SE	
Herbs	*Antirrhinum majus*	Non-native	0.7	1.1	–
	Artemisia mendozana	Native	0.1	0.4	–
	Centaurea calcitrapa	Non-native	0.2	0.6	–
	Conyza chilensis	Native	2.7	3.1	+
	Dichondra sericea	Native	1.1	1.5	–
	Medicago lupulina	Non-native	32.6	29.5	+
	Medicago sativa	Non-native	6.4	13.1	+
	Lactuca sp.	Non-native	1.6	3.0	0
	Phacelia secunda	Native	0.1	0.3	–
	Plantago lanceolata	Non-native	0.6	0.8	–
	Sisyrinchium chilensis	Native	0.2	0.3	–
	Sphaeralcea sp.		0.1	0.3	–
	Trigonella monspeliaca	Non-native	3.7	5.7	+
Grasses	*Bromus brevis*	Native	1.4	2.0	–
	Cynodon dactylon	Non-native	0.1	0.2	–
	Poa resinulosa	Native	11.7	7.6	0
	Schismus sp.	Non-native	1.5	4.0	0
	Stipa sp.		0.8	1.1	–
Subshrubs	*Acantholippia seriphioides*	Native	0.7	1.5	–
	Condalia micropolylla	Native	0.8	2.6	+
	Junellia juniperina	Native	0.1	0.4	–
	Junellia scoparia	Native	5.2	9.0	–
	Menodora sp.	Native	0.1	0.3	–
	Monnina dyctiocarpa	Native	0.1	0.2	–
	Lycium sp.		10.3	10.6	+
	Senecio subulatus	Native	0.7	2.3	0
Shrubs	*Adesmia* sp.		4.2	5.4	0
	Buddleja sp.		0.1	0.2	–
	Rosa spp.	Non-native	5.8	8.5	–
	Spartium junceum	Non-native	6.5	9.3	0

largely depend on fruits and seeds availability in the invaded community in a given season.

Seventy-three percent of the entire seeds presented healthy embryos (Table 1). We found no significant viability differences ($\chi^2 = 0.09$, df = 1, $p > 0.05$) between seeds obtained from faeces (digested; 64% viable) and seeds obtained from fruits (control; 66% viable) (Table 1). Our results suggest that the passage of seeds of *Rosa* spp. through the digestive tract of rabbits does not affect their viability. The relatively large number of viable seeds after ingestion by rabbits could provide an advantage if seeds are deposited at suitable sites, far from the parent plant (Schupp et al. 2010). Further research regarding these new interactions should consider another aspect such as plant benefits resulting from seed deposition patterns, field germination success, and abundance, feeding strategies and movement of seed dispersers (Schupp et al. 2010).

Our study identifies new interactions between an introduced mammal and two invasive plants, and suggests that these invader complexes could have a reciprocal effect on one another, potentially aiding their invasive process (and subsequent impacts) in the semiarid foothills in the Andes of Argentina. Further studies should consider other components to

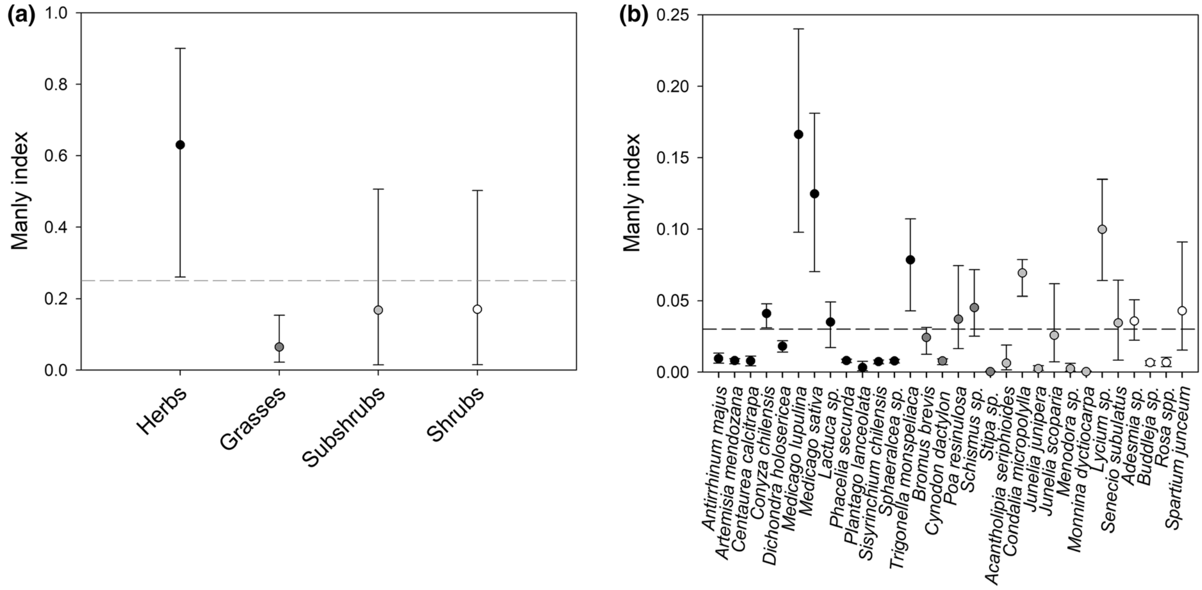

Fig. 3 Manly's selectivity index (± 95% CI) for food categories (**a**) and to food items (**b**). Dotted line indicates 1/k = 0.25 and 1/k = 0.03 for a use proportional to the availability respectively. Black circle = Herbs; dark gray circle = Grasses; light gray circle = Subshrubs and white circle = Shrubs

understand the effectiveness of *European rabbit* as a seed disperser of *Rosa* spp. such as quality of treatment of seed by germination tests (legitimacy), quality of seeds deposition (efficiency) or quantitatively through the number of seeds dispersed per plant. We reinforce the importance of studies that may detect new positive interactions between non-native species, such as the other invasive herbivorous mammal *Lepus europaeus* inside of areas by high conservation value. These positive interactions would potentially cause an invasional meltdown and leading to an accelerating increase in number of introduced species and their impact on native community.

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