

Endozoochory seed dispersal by goats: recovery, germinability and emergence of five Mediterranean shrub species

D. Grande¹, J. M. Mancilla-Leytón^{2*}, M. Delgado-Pertiñez³ and A. Martín-Vicente²

¹ *Área de Sistemas de Producción Agropecuarios. División de Ciencias Biológicas y de la Salud. Universidad Autónoma Metropolitana Iztapalapa. Avda. San Rafael Atlixco 186. Col. Vicentina. 09340 Iztapalapa (México DF), Mexico*

² *Departamento de Biología Vegetal y Ecología. Universidad de Sevilla. Apdo. 1095. 41080 Sevilla, Spain*

³ *Departamento de Ciencias Agroforestales. Escuela Técnica Superior de Ingeniería Agronómica. Universidad de Sevilla. Ctra. Utrera, km 1. 41013 Sevilla, Spain*

Abstract

Herbivores can play an important role as seed dispersal vectors, ungulates constituting potential seed dispersal agents of Mediterranean grasses and shrubs. We evaluated the role of domestic goats as seed dispersers of five representative Mediterranean forage shrub species [*Cistus albidus* L., *Phillyrea angustifolia* L., *Calicotome villosa* (Poir.) Link., *Rhamnus lycioides* L. and *Atriplex halimus* L.]. Following seed ingestion by goats, total faeces were collected at 24-h intervals for five days. The total number of seeds recovered varied among species, with *R. lycioides* showing the minimum recovery percentage (1.3%) and *C. albidus* (35.8%), the maximum. Seed recovery was significantly related to seed hardness and length. In most species, the maximum amount of seeds recovered occurred 48-72 h after ingestion. The passage through the goat gut significantly depressed seed germination in *C. albidus*, *C. villosa* and *A. halimus*; inhibited it in *R. lycioides* and increased it in *P. angustifolia*. Seedling emergence was significantly lower in intact dung pellets than in broken-down ones, and both significantly lower than in uneaten seeds (control). The results of this study show that goats can potentially favor or inhibit seed dispersal of browsed Mediterranean shrub species. Therefore, goat grazing could be a potential management tool for expanding target shrub species populations or preventing shrub encroachment in undesired areas.

Additional key words: endozoochory; shrubland; seed hardness; seed recovery.

Introduction

Endozoochory has been extensively studied in the past, and has recently gained new momentum. The role of herbivorous mammals as endozoochorous seed dispersers has been recently highlighted (Willson, 1993; Pakeman *et al.*, 2002; Myers *et al.*, 2004), and several studies have proved the presence of seeds in the dung of wild and domestic herbivores (Sánchez & Peco, 2002; Manzano *et al.*, 2005; Ramos *et al.*, 2006; Kuiters & Huiskes, 2010; De la Vega & Godínez-Álvarez, 2010; Mancilla-Leytón *et al.*, 2011, 2012). In addition, several seed characteristics such as size, hardness, shape, and the fruit in which they are contained are very im-

portant factors that also affect seed dispersal by animals (Janzen, 1986). Since herbivorous mammals have a long seed residence time in guts (24 to 72 h) (Olson & Wallander, 2002; Mancilla-Leytón *et al.*, 2011) and make long-distance routes (Cory, 1972; Klein, 1981), they can promote the rapid dispersal of plant populations. Due to the particular feeding habits and ranging behavior of browsers (Morand-Fehr *et al.*, 1983; Devendra, 1990; Milne, 1991), herbivores such as domestic goats can be seed dispersers.

It has been documented that goats can disperse the seeds of shrub species in arid and semiarid regions of Mexico (Baraza & Valiente-Banuet, 2008; Giordani, 2008), and various herbaceous species in New Zealand

* Corresponding author: jmancilla@us.es
Received: 26-10-12. Accepted: 13-05-13.

Abbreviations used: MTE (mean time to emergence); MTG (mean time to germination); PE (percentage of seedlings established); PR (percentage of seed recovery).

(Harrington *et al.*, 2011) or the United States (Lacey *et al.*, 1992). In Spain, its dispersal role has been studied for legume shrubs such as *Adenocarpus decor-ticans* and *Retama sphaerocarpa* (Robles *et al.*, 2005), or Mediterranean shrubs such as *Cistus salvifolius*, *Halimium halimifolium*, *Myrtus communis* and *Pistacia lentiscus* (Mancilla-Leytón *et al.*, 2011). Nevertheless, the available information related to other common Mediterranean shrubs that goats eat is limited, and little attention has been given to its role as seed dispersers of the browsed shrub species.

The aims of this study were to determine if goat gut passage affected the germination of seeds from five common Mediterranean shrub species, and if seed inclusion in faeces had effects on seedling emergence and growth. The following questions were therefore addressed: i) which is the percentage of recovery seed after the digestive process?, ii) which seed properties influence recovery?, iii) what is the temporal pattern of seed recovery after ingestion?, and iv) how does ingestion by goats affect the recruitment of the studied species?

Material and methods

Seed selection and biometric characteristics

Five common Mediterranean shrub species of high forage value and widely spread in the Mediterranean region (Valdés *et al.*, 1987) were selected: *Cistus albidus* L. (Cistaceae), *Phillyrea angustifolia* L. (Oleaceae), *Calicotome villosa* (Poir.) Link. (Fabaceae), *Rhamnus lycioides* L. (Rhamnaceae) and *Atriplex halimus* L. (Chenopodiaceae). The seeds were obtained from a forestry nursery (Semillas Silvestres, S.L.) in Cordoba, Spain.

In order to determine average seed volume, 100 seeds of each species were measured with an electronic vernier caliper. Rupture strength (hardness) of each seed was also measured with a digital force gauge (PCE-FM50).

Seeds retrieved after gut passage

Six adult female Payoya goats of similar size and age (3-year old, 40-45 kg average weight) were individually housed at the Teaching and Experimental Farm of the Faculty of Agriculture, University of Se-

ville. Goats were kept in individual metabolic pens with a collector system for faeces, where they were fed. At the beginning of the experiment, each goat was offered a high number of seeds from each species (21,388 seeds: 5,000 seeds of the species *C. albidus*, *P. angustifolia*, *R. lycioides* and *A. halimus*, plus 1,388 seeds of *C. villosa*) in order to ensure that some passed the goats guts. Seeds were mixed with barley grains (250 g) to facilitate intake (Mancilla-Leytón *et al.*, 2011), and offered for roughly one hour until fully consumed by goats. From then on, goats were fed alfalfa hay and barley grain, and had free access to water. All dung pellets produced by each goat were collected every 24 h for five days (0-24, 24-48, 48-72, 72-96 and 96-120 h after seed ingestion), and dried at room temperature for 72 h in a bell jar with silica gel to avoid seed fermentation and damage. The dung was then broken down for seed retrieval: 20 subsamples of 4 g of dung were daily taken per goat, and the number of seeds recorded. The average number of seeds retrieved from daily subsamples was extrapolated to the total daily dung amount per animal, thus providing an estimate of the daily percentage of seed recovery (PR) through the following expression: $PR = [(Mf \cdot Sr) / 4S] * 100$, where *Mf* is the total daily mass of faeces (time interval), *Sr* is the average number of seeds retrieved per subsample (4 g of pellet), and *S* is the number of seeds ingested per goat (Mancilla-Leytón *et al.*, 2012).

Seed germination after gut passage

The germination of seeds retrieved from goat dung during the two time intervals of greatest seed recovery rates was compared to the germination of seeds that remained uneaten. Each species seeds ingested during the same time interval (day) by every goats were pooled for this experiment. Seeds from *C. albidus*, *C. villosa* and *A. halimus* were classified under three categories: (1) control, seeds that were not eaten; (2) 24-48 h, seeds retrieved between 24 and 48 h after ingestion; (3) 48-72 h, seeds retrieved between 48 and 72 h after ingestion. In the case of *R. lycioides* and *P. angustifolia* seeds, categories were: control, 48-72 h and 72-96 h. Very few seeds were retrieved during the other time intervals, and thus were not tested.

Some of the seeds retrieved were partially broken, missing part of the cotyledons but with an intact embryo. Since the number of broken seeds was very low in all species (<0.02% retrieved seeds), they were not

tested for germination. Only seeds with no evidence of apparent external damage after examination under a microscope were used for the germination experiment.

Seeds of all treatments were disinfected by a 2-min immersion in a 1% sodium hypochlorite solution, and thoroughly rinsed with sterile distilled water (10 min). Each species seeds were then placed on filter paper in 5-cm Petri dish. Each Petri dish contained 25 seeds, with 4 replicates per treatment. Distilled water (3 mL) was added to each dish, which were then wrapped with parafilm and placed in a germinator (ASL Aparatos Científicos M-92004, Madrid, Spain) for 60 days with a regime of 12 h of light (25°C, 35 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 400-700 nm) and 12 h of darkness (12°C). This temperature regime was chosen to resemble the end of autumn temperatures in Mediterranean climates, when these species germinate. The dishes were inspected daily and germinated seeds were counted and removed. We considered that seeds had germinated after root emergence (1-2 mm). The water level was also adjusted daily with distilled water.

Three germination parameters were determined: final germination percentage, time of first germination and mean time to germination (MTG), calculated as: $MTG = \sum_i (n_i \cdot d_i) / N$, where n_i is the number of seeds germinated at day i , d_i the incubation period in i days, and N is the total number of seeds that germinated in the whole treatment (Brenchley & Probert, 1998).

Viability test

The tetrazolium test was applied to four 20-seed replicates of each species collected both from pellets and from uneaten seeds (control), to determine the viability of the embryos (MacKay, 1972). Seeds were kept in water during 16 h at a constant 25°C temperature, and then submerged in a 1% aqueous solution of 2,3,5-triphenyl-tetrazolium chloride, pH 7, in darkness for 24 h at a constant temperature of 25°C. Seeds were then dissected and embryo analysed with a magnifying glass (Bradbeer, 1998).

Seedling emergence from dung

The effect of being contained in dung for seedling emergence was tested in a greenhouse experiment. Three treatments were applied: 1) seeds retrieved from intact dung, 2) seeds retrieved from broken-down dung,

simulating crumbling of pellets under natural conditions (*i.e.* rainfall or animals trampling the pellets) (Mancilla-Leytón *et al.*, 2012) and 3) seeds not ingested by goats. In each of the dung treatments (1 and 2), 16 pots with 12 g of dung were placed on the surface of a sand/vermiculite mixture (1:1), eight pots with dung collected in the first time interval of greatest seed recovery, and another eight with dung collected during the second greatest seed recovery time interval (24-48 and 48-72 treatments for *C. albidus*, *C. villosa* and *A. halimus*; 48-72 and 72-96 h treatments for *R. lycioides* and *P. angustifolia*). In the third treatment (control group), eight pots each containing 20 seeds which had not passed through the goats guts were also placed on the surface of the sand/vermiculite mixture. All pots were randomly placed in the greenhouse with day/night temperature of 25/15°C and watered by imbibition with tap water, providing the same amount of water to all replicates. Emergence of seeds was daily monitored for 60 days. The biomass and height of the seedlings emerged in each treatment were measured at the end of the experiment.

The percentage of seedlings established (PE) at the end of the sampling period was estimated for each species by a similar procedure to seed recovery: $PE = 100 \cdot Se / 3 \cdot Sr$, where Se is the average number of seedlings established of a species, and Sr is the average number of seeds found in 4 g of pellets (based on 20 subsamples).

Data analysis

To assess whether seed recovery is related to characteristics of the seeds (length, width, volume, hardness), a multiple regression model was performed. Differences among biometric characteristics, total number of seeds retrieved from dung between treatments, total number of germinated seeds, time to first germination, mean germination time, seed viability and number and sizes of seeds emerged from uneaten seeds, and retrieved from intact and crumbled dung collected at different time intervals were statistically evaluated through performance of one-way ANOVA analysis. After testing the variables for normality using the descriptive statistics of asymmetry and kurtosis, ANOVAs were performed with either log-transformed data (mean time of germination or MTG, mean time of emergence or MTE) or raw data (rest of variables). Tukey test was performed to evaluate significant differences among

treatments. All statistical analyses were carried out with SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Seed biometric characteristics and recovery after gut passage

The characteristics of seeds are listed in Table 1. All biometric variables showed significant differences between species. *R. lycioides*, *P. angustifolia* and *C. villosa* seeds were significantly longer, wider and showed larger volumes than the rest of the seeds ($F = 54.58$, $F = 26.97$ and $F = 17.64$, $p \leq 0.05$, respectively). *C. salvifolius* and *C. villosa* seeds displayed the greatest resistance to breakage ($F = 56.292$, $p \leq 0.05$).

The total percentage of seeds retrieved from goat faeces widely varied among species with *R. lycioides* registering the lowest recovery rate (1.3%) and *C. albidus*, the highest (35.8%) (Fig. 1). The largest total amounts of retrieved seeds correspond to *C. albidus* (1795) and *C. villosa* (330), accounting for 35.8 and 23.7% of the seeds eaten by the goats, respectively, while the amounts of seeds retrieved of *A. halimus* (4%), *P. angustifolia* (2.7%) and *R. lycioides* (1.3%) were significantly lower (Fig. 1). The number of seeds retrieved per time interval differed among all species, with most species registering seed recovery between 48-72 h after ingestion, although goats defecated seeds of *C. albidus*, *P. angustifolia* and *C. villosa* until the end of the experiment (fifth day of sampling).

After verifying the normality, homoscedasticity and absence of autocorrelation, the results of multiple regression model showed the existence of a significant relationship between seed recovery and hardness and length of the same ($R^2 = 0.71$, $F = 28.14$; $p \leq 0.001$) described by the following equation: $\% \text{ recovery} = 9.64 + (1.34 \cdot \text{hardness}) - (4.92 \cdot \text{length})$.

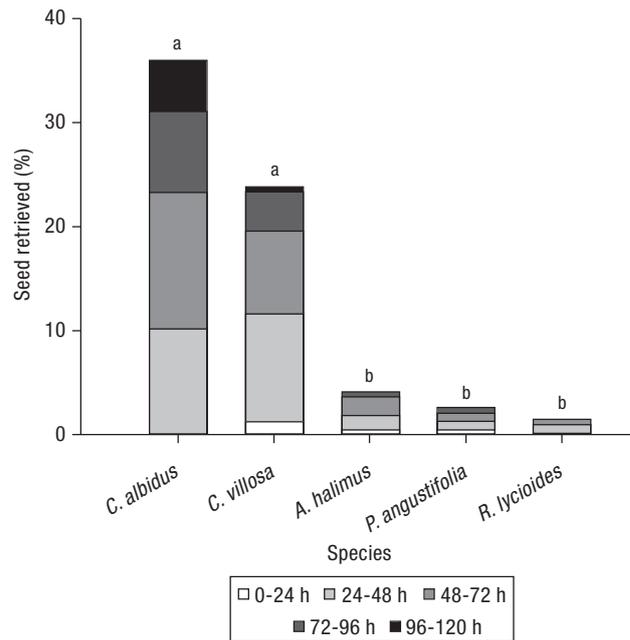


Figure 1. Percentage of seeds (mean, $n = 6$) retrieved from goat faeces 24, 48, 72, 96 and 120 hours after consumption in the five species studied. Different letters indicate significant differences among species in the total number of seeds retrieved (Tukey test; $p \leq 0.05$).

Seed germination after gut passage

Passage through the goat gut only significantly increased seed germination in *P. angustifolia* (Tukey test, $p \leq 0.05$) (Fig. 2). In contrast, it decreased seed germination in *C. villosa*, *A. halimus* and *C. albidus*, although no statistical differences were found (Tukey test, $p \geq 0.05$). Finally, none of the *R. lycioides* seeds eaten by goats germinated as opposed to the control seeds (4%), proving that goat gut passage had an inhibitory effect in seed germination of this species (Fig. 2).

The number of days to first germination and mean time of germination (MTG) and viability are shown in Table 2. Only seeds retrieved from *A. halimus* (24-48 h

Table 1. Seed biometric characteristics of the five species studied (mean \pm SE, $n = 100$)

Species	Length (mm)	Width (mm)	Volume (mm ³)	Hardness (N)
<i>Cistus albidus</i>	1.36 \pm 0.07 ^a	0.95 \pm 0.08 ^a	0.63 \pm 0.09 ^a	25.60 \pm 2.59 ^a
<i>Calicotome villosa</i>	3.31 \pm 0.06 ^b	2.64 \pm 0.08 ^b	11.59 \pm 0.93 ^b	20.48 \pm 1.38 ^a
<i>Atriplex halimus</i>	1.34 \pm 0.12 ^a	1.24 \pm 0.15 ^a	1.03 \pm 0.03 ^a	3.08 \pm 0.38 ^c
<i>Phillyrea angustifolia</i>	4.75 \pm 0.02 ^c	2.98 \pm 0.02 ^c	22.01 \pm 2.56 ^c	16.03 \pm 0.66 ^d
<i>Rhamnus lycioides</i>	4.47 \pm 0.03 ^c	2.30 \pm 0.11 ^b	11.88 \pm 0.91 ^b	7.83 \pm 0.34 ^c

Different letters indicate significant differences among treatments within each species (Tukey test; $p \leq 0.05$).

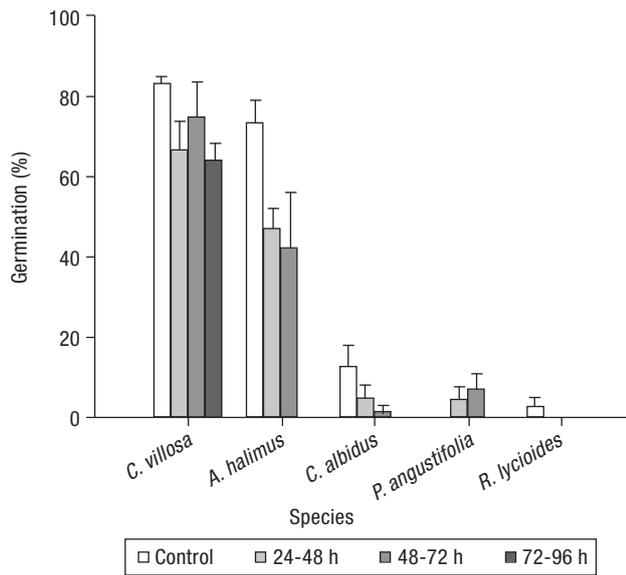


Figure 2. Germination percentage (mean \pm SE, $n = 4$) of control seeds and defecated seeds 24-48, 48-72 and 72-96 hours after ingestion of the five species studied.

and 48-72 h) and *C. villosa* (24-48 h) showed significantly lower time to first germination than control seeds. Also, passage through the goat gut significantly shortened germination time of retrieved seeds from *A. halimus* and had no effect in rest of species (Table 2). Finally, the tetrazolium test showed no significant differences in the viability percentages among control

seeds and seeds retrieved from goat faeces in *C. albidus* and *C. villosa*. In the case of *A. halimus*, *P. angustifolia* and *R. lycioides*, control seeds were significantly more viable than defecated ones ($p \leq 0.05$) (Table 2).

Seedling emergence

The seedling emergence rate and MTE are shown in Table 3. There was seedling emergence from control seeds of all species except *P. angustifolia*. The lowest percentage of emergence in the control treatment was found in *C. albidus* seeds (6.2%), while the largest was registered in *A. halimus* seeds (57.5%). There was no seedling emergence in intact faeces for any species (Table 3). Seedling emergence in crumbled faeces was only registered in *C. villosa* and *A. halimus* seeds retrieved 24-48 and 48-72 h after ingestion.

Seedling emergence in *C. villosa* seeds retrieved from crumbled faeces at the 24-48 h interval (55%) was slightly higher than control seeds (51.2%), although no significant differences were found (Tukey test; $p \geq 0.05$). Meanwhile, this species showed a significantly lower emergence of seedlings in seeds retrieved at the 48-72 h interval (7.9%) (Table 3). On the other hand, seedling emergence was significantly lower in *A. halimus* seeds retrieved at the 24-48 h (6.9%) and 48-72 h (3.7%) intervals when compared to control seeds (57.5%) (Table 3).

Table 2. Number of days to first germination, mean time of germination (MTG) and viability of the control and defecated seeds. Values are mean \pm SE ($n = 4$)

Species	Treatment	1 st Germination (d)	MTG (d)	Viability (%)
<i>Cistus albidus</i>	Control	30.0 \pm 6.8 ^a	28.3 \pm 7.7 ^a	82.0 \pm 2.6 ^a
	Retrieved 24-48 h	30.2 \pm 12.4 ^a	35.2 \pm 10.7 ^a	80.1 \pm 2.7 ^a
	Retrieved 48-72 h	36.0 \pm 8.0 ^a	42.0 \pm 10.0 ^a	80.2 \pm 6.1 ^a
<i>Calicotome villosa</i>	Control	8.0 \pm 1.7 ^a	23.9 \pm 0.7 ^a	94.1 \pm 1.2 ^a
	Retrieved 24-48 h	12.0 \pm 0.5 ^b	26.8 \pm 1.0 ^a	91.6 \pm 4.2 ^a
	Retrieved 48-72 h	8.2 \pm 1.0 ^a	27.5 \pm 1.2 ^a	91.6 \pm 3.2 ^a
<i>Atriplex halimus</i>	Control	4.0 \pm 0.2 ^a	6.4 \pm 1.4 ^a	86.1 \pm 2.0 ^a
	Retrieved 24-48 h	1.0 \pm 0.1 ^b	2.3 \pm 0.5 ^b	60.7 \pm 5.8 ^b
	Retrieved 48-72 h	1.7 \pm 0.7 ^b	4.5 \pm 1.1 ^{ab}	42.5 \pm 13.8 ^b
<i>Phyllirea angustifolia</i>	Control	NG	NG	94.3 \pm 2.0 ^a
	Retrieved 48-72 h	4.7 \pm 2.4 ^a	9.5 \pm 2.4 ^a	65.9 \pm 6.5 ^b
	Retrieved 72-96 h	5.2 \pm 6.3 ^a	10.5 \pm 5.2 ^a	60.7 \pm 3.6 ^b
<i>Rhamnus lycioides</i>	Control	4.7 \pm 3.8	9.5 \pm 3.8	89.0 \pm 1.9 ^a
	Retrieved 48-72 h	NG	NG	35.7 \pm 1.7 ^b
	Retrieved 72-96 h	NG	NG	33.3 \pm 2.7 ^b

Different letters indicate significant differences among treatments within each species (Tukey test; $p \leq 0.05$). NG: not germinated.

Table 3. Seedlings emergence rate, mean time of emergence (MTE), biomass and height or length of seedlings emerged from intact and crumbled faeces collected 24-48 and 48-72 hours after ingestion. Values are mean \pm SE (n = 8)

Species	Treatment	Emergence (d)	MTE (d)	Aerial portion		Roots	
				Biomass (mg)	Height (cm)	Biomass (mg)	Length (cm)
<i>Cistus albidus</i>	Control	6.2 \pm 2.3	28.1 \pm 12.0	10.0 \pm 0.0	1.6 \pm 0.4	9.3 \pm 0.0	2.8 \pm 0.4
	24-48 h intact faeces	NE	NE	NE	NE	NE	NE
	24-48 h crumbled faeces	NE	NE	NE	NE	NE	NE
	48-72 h intact faeces	NE	NE	NE	NE	NE	NE
	48-72 h crumbled faeces	NE	NE	NE	NE	NE	NE
<i>Calicotome villosa</i>	Control	51.2 \pm 4.2 ^a	28.1 \pm 4.1 ^a	29.2 \pm 2.9 ^a	7.4 \pm 2.6 ^a	12.2 \pm 1.2 ^a	17.6 \pm 1.3 ^a
	24-48 h intact faeces	NE	NE	NE	NE	NE	NE
	24-48 h crumbled faeces	55.0 \pm 10.3 ^a	23.7 \pm 2.0 ^a	37.2 \pm 6.2 ^a	11.5 \pm 0.8 ^a	15.7 \pm 1.7 ^a	12.7 \pm 0.9 ^a
	48-72 h intact faeces	NE	NE	NE	NE	NE	NE
	48-72 h crumbled faeces	10.8 \pm 3.3 ^b	17.4 \pm 7.6 ^a	37.5 \pm 9.6 ^a	7.4 \pm 2.2 ^a	12.5 \pm 2.5 ^a	11.2 \pm 4.0 ^a
<i>Atriplex halimus</i>	Control	57.5 \pm 9.2 ^a	4.5 \pm 0.4 ^a	16.7 \pm 4.1 ^a	3.9 \pm 0.4 ^a	4.6 \pm 0.9 ^a	14.8 \pm 2.3 ^a
	24-48 h intact faeces	NE	NE	NE	NE	NE	NE
	24-48 h crumbled faeces	6.9 \pm 3.4 ^b	3.5 \pm 1.0 ^a	29.3 \pm 4.4 ^a	2.0 \pm 1.1 ^a	6.0 \pm 2.9 ^a	13.8 \pm 1.9 ^a
	48-72 h intact faeces	NE	NE	NE	NE	NE	NE
	48-72 h crumbled faeces	3.7 \pm 4.9 ^b	1.5 \pm 1.8 ^a	26.8 \pm 5.9 ^a	4.5 \pm 1.9 ^a	5.8 \pm 2.2 ^a	14.0 \pm 0.9 ^a
<i>Phyllirea angustifolia</i>	Control	NE	NE	NE	NE	NE	NE
	48-72 h intact faeces	NE	NE	NE	NE	NE	NE
	48-72 h crumbled faeces	NE	NE	NE	NE	NE	NE
	72-96 h intact faeces	NE	NE	NE	NE	NE	NE
	72-96 h crumbled faeces	NE	NE	NE	NE	NE	NE
<i>Rhamnus lycioides</i>	Control	5.0 \pm 3.3	9.3 \pm 1.3	4.4 \pm 0.6	2.6 \pm 0.2	4.4 \pm 0.6	8.8 \pm 1.1
	48-72 h intact faeces	NE	NE	NE	NE	NE	NE
	48-72 h crumbled faeces	NE	NE	NE	NE	NE	NE
	72-96 h intact faeces	NE	NE	NE	NE	NE	NE
	72-96 h crumbled faeces	NE	NE	NE	NE	NE	NE

Different letters indicate significant differences among treatments within each species (Tukey test; $p \leq 0.05$). NE: no emergence.

The MTE in seedlings of *C. villosa* emerged from seeds retrieved 24-48 h (23.7 days) and 48-72 h (17.4 days) after ingestion were shorter than that of seedlings from control seeds (28.1 days), although no significant differences were found. The same tendency was observed in seedlings of *A. halimus* at 24-48 and 48-72 h intervals, including the lack of significant differences with the control treatment (Table 3).

In *C. villosa* seedlings emerged from seeds recovered at the 24-48 and 48-72 h intervals, a tendency to higher biomass and height of the aerial portion was registered when compared with control seeds, with no significant differences found; the same pattern was also observed in seedlings of *A. halimus* emerged from seeds retrieved from crumbled faeces 24-48 and 48-72 h after ingestion (Table 3). On the other hand, the biomass and length of the roots of *C. villosa* seedlings emerged from seeds retrieved at the same sampling ti-

mes were also generally larger than those of the control treatment, although less prominently than in the aerial part case. Contrastingly, the biomass and length of the roots of *A. halimus* seedlings showed a clear tendency to increase along the subsequent recovery intervals when compared with the control treatment, although no significant differences were found ($p \geq 0.05$) (Table 3).

Discussion

The percentages of *C. albidus* seeds retrieved (35.8%) from goat dung are similar to those obtained for *C. salvifolius* (30%) by Mancilla-Leytón *et al.* (2011). This can be related to the hardness of their seeds, characteristic of Cistaceae family, in addition to its small size (see Table 1), which increased their resistance to mastication and rumination (Castro & Robles,

2003; Mancilla-Leyton *et al.*, 2011). Moreover, the percentages of *C. villosa* seeds retrieved (23.7%) are similar to those obtained for the legumes *Cajanus cajan* (23.8%) and *Lotononis bainesii* (29.9%) consumed by cattle (Gardener *et al.*, 1993). The hard coat of these seeds was undoubtedly the main factor favoring recovery or otherwise, its large size could have favored its destruction during mastication and rumination, and since a negative relationship between seed size and percentage of seeds recovered after gut passage has been documented for goats (Simao Neto *et al.*, 1987), rabbits and sheep (Staniforth & Cavers, 1977; Russi *et al.*, 1992; Pakeman *et al.*, 2002). The percentages of seeds retrieved of *A. halimus* (4%), *P. angustifolia* (2.7%) and *R. lycioides* (1.3%) were lower than those obtained for other herbaceous and shrub species eaten by domestic ruminants such as sheep (10-72%, Ghassali *et al.*, 1998; 2.3-74%, Razanamandranto *et al.*, 2004; 10-23%, Manzano *et al.*, 2005), goats (7-30%, Mancilla-Leytón *et al.*, 2011) or cattle (6.2-80%, Gardener *et al.*, 1993). These low retrieval percentages are similar to those obtained for commercial seeds of fodder species (*Moricandia arvensis*, *Vicia ervilia* and *Medicago sativa* cv. Aragón) fed to sheep (below 5%, Ramos *et al.*, 2010), seeds of *Lolium perenne* (1.9%) and *Trifolium repens* (3.9%) (Harrington *et al.*, 2011) and commercial seeds of *Trifolium subterraneum* (1.5%) and *Lolium rigidum* (2%) eaten by goats (Mancilla-Leytón *et al.*, 2012). In the case of *P. angustifolia*, *R. lycioides* and *A. halimus*, seed size was more important than hardness in the recovery success: retrieval was favored in small size seeds (*A. halimus*) and jeopardized in large seeds (*P. angustifolia* and *R. lycioides*) (see Table 1).

The largest amount of seed recovery from faeces occurred during the first 48 h, coinciding with the retrieval times found in various shrub seeds consumed by goats (Harrington *et al.*, 2011; Mancilla-Leytón *et al.*, 2011, 2012), sheep (Manzano *et al.*, 2005) and cattle (Gardener *et al.*, 1993). The presence of seed of several species in the faeces recollected the third, fourth and fifth day after ingestion, is a similar finding to that obtained for *Silybum marianum* seeds eaten by goats (Harrington *et al.*, 2011), or *Cytisus scoparius* seeds found in the faeces of sheep up to 160 h after intake (Manzano *et al.*, 2005).

The passage through the goat gut did not enhance seed germination in *C. albidus*, as other authors have also found for other species of Cistaceae (Manzano *et al.*, 2005; Ramos *et al.*, 2006). This can be partly ex-

plained by the primary seed dormancy (physical dormancy) imposed by the hard seed coat (Thanos *et al.*, 1992; Baskin *et al.*, 2000), characteristic of this botanical family. The passage through the goat gut did not soften *C. villosa* seeds coat, therefore not improving germination, but neither caused damage to the embryo which would have adversely affected seed germination, as evidenced by the viability percentages of seeds obtained in the tetrazolium test (Table 2). On the contrary, the passage through the goat gut did soften the seed coat of *A. halimus*, decreasing the viability of ingested seeds.

The germination enhancement registered in *P. angustifolia* seeds after goat gut passage is remarkable, since this species seeds show a primary dormancy (physical dormancy) imposed by a hard seed coat (Table 1 and Takos & Efthimiou, 2003). As a consequence, various pre-germination treatments have been suggested to promote this species germination including sulfuric acid or hot water immersion, or a combination of either methods, or stratification in wet sand for several months (Takos & Efthimiou, 2003). In the case of *R. lycioides* seeds, goats behaved as seed predators, this finding also being reported for other shrub species such as *P. lentiscus* (Mancilla-Leytón *et al.*, 2011).

The complete absence of seedling emergence in the intact faeces could be explained by the tight structure of goat dung pellets, which can act as a mechanical barrier to seedling emergence, and fades out when pellets are broken-down. Consequently, trampling by grazing animals, rainfall and coprophagous insects that break-down the dung are necessary for seedling establishment, especially for species that cannot emerge from intact dung (Mancilla-Leytón *et al.*, 2012). The clear tendency to higher biomass and height of the *C. villosa* and *A. halimus* seedlings emerged from crumbled faeces compared with control seedlings, could be related to the higher nutrient content of organic matter in faeces than in the vermiculite substrate and to a higher water retention capacity of crumbled faeces (Malo & Suárez, 1998; Traveset *et al.*, 2001; Traveset & Verdú, 2002).

As conclusion, the size and hardness effect of seeds, the goat's gut passage effect and the mechanical effect of pellets, are important criteria to consider the dispersion of seeds, and these have been evident in this study. Unravelling zoochorous dispersal mechanisms in a semi-natural environment may therefore offer both fundamental and necessary applicable ecological knowledge. The incorporation of wild plant Mediterranean

species into the diet of domestic grazers can be an important step to disperse those species in Mediterranean areas. Goats can potentially favor or inhibit seed dispersal of the plants that they eat. Goat grazing could also be used as a management tool for spreading populations of target shrub species or to prevent shrub encroachment in undesired areas. Therefore, these results should be considered when developing conservation and restoration plans of natural vegetation after major disturbances (fire, tillage, crop abandonment, etc).

Acknowledgements

The authors thank Mr. Jose Ardila and Dehesa de Gatos S.L. for their help and collaboration. Special thanks to Ms. Yolanda Paz for her great support and dedication in processing the samples in the laboratory, and Mr. Óscar Andrade for field assistance. This study was partially supported by the Consejería de Medio Ambiente (Junta de Andalucía, OG-052/07). J.M. Mancilla-Leytón gratefully acknowledges an FPMI grant (Junta de Andalucía). Dra. Raquel Fernández lo Faso revised the English version of the manuscript.

References

- Baraza E, Valiente-Banuet A, 2008. Seed dispersal by domestic goats in a semiarid thorn scrub of Mexico. *J Arid Environ* 72: 1973-1976.
- Baskin JM, Baskin CC, Li X, 2000. Taxonomy, anatomy and evolution of physical dormancy in seeds. *Plant Spec Biol* 15: 139-152.
- Bradbeer JW, 1998. Seed dormancy and germination. Chapman & Hall, NY.
- Brenchley JL, Probert RJ, 1998. Seed germination responses to some environmental factors in the seagrass *Zostera capricorni* from eastern Australia. *Aquatic Bot* 62: 177-188.
- Castro J, Robles AB, 2003. Dispersión endozoócora por ganado ovino de las semillas de seis especies de Cistáceas. *Actas de la XLIII Reunión Científica de la Sociedad Española para el Estudio de los Pastos: pastos-desarrollo y conservación* (Robles AB *et al.*, eds). Consejería de Agricultura y Pesca, Junta de Andalucía, Granada-Sevilla (Spain). pp: 645-650.
- Cory VL, 1972. Activities of livestock on the range. *Agricult Exp* 367: 18-23.
- De la Vega GS, Godínez-Álvarez H, 2010. Effect of gut passage and dung on seed germination and seedling growth: donkeys and a multipurpose mesquite from a Mexican inter-tropical desert. *J Arid Environ* 74: 521-524.
- Devendra C (ed), 1990. The use of shrubs and tree fodders by ruminants. *Proc Workshop Shrubs and Tree Fodders for Farm Animals*. International Development Research Centre, Denpasar, Indonesia. pp: 42-60.
- Gardener CJ, McIvor JG, Jansen A, 1993. Passage of legume and grass seeds through the digestive tract of cattle and their survival in faeces. *J Appl Ecol* 30: 63-74.
- Ghassali F, Osman AE, Cocks PS, 1998. Rehabilitation of degraded grasslands in North Syria. The use of awassi sheep to disperse the seeds of annual pasture legumes. *Exp Agric* 34: 391-405.
- Giordani L, 2008. The role of goats in germination and dispersal of *Mimosa luisana* Brandege (Leguminosae-Mimosoideae) seeds in Tehuacán-Cuicatlán valley, Puebla State, Mexico. Master thesis. Norwegian University of Life Sciences, Noragric, Norway.
- Harrington KC, Beskow WB, Hodgson J, 2011. Recovery and viability of seeds ingested by goats. *New Zealand Plant Protec* 64: 75-80.
- Janzen DH, 1986. Mice, big mammals and seeds: it matters who defecates what and where. In: *Frugivores and seed dispersal* (Estrada A, Fleming TH, eds). W Junk, Dordrecht, NL. pp: 251-271.
- Klein J, 1981. *La Mesta*. Alianza Editorial, Madrid.
- Kuiters AT, Huiskes HPJ, 2010. Potential of endozoochorous seed dispersal by sheep in calcareous grasslands: correlations with seed traits. *App Veg Sci* 13: 163-172.
- Lacey JR, Wallander R, Olson-Rutz K, 1992. Recovery, germinability, and viability of Leafy Spurge (*Euphorbia esula*) seeds ingested by sheep and goats. *Weed Tech* 6: 599-602.
- MacKay DB, 1972. The measurement of viability. In: *Viability of seeds* (Roberts EH, ed). Chapman & Hall, London. pp: 172-208.
- Malo JE, Suárez F, 1998. The dispersal of a dry-fruited shrub by red deer in a Mediterranean ecosystem. *Ecography* 21: 204-211.
- Mancilla-Leytón JM, Fernández-Alés R, Martín Vicente A, 2011. Plant-ungulate interaction: goat gut passage effect on survival and germination of Mediterranean shrub seeds. *J Veg Sci* 22: 1031-1037.
- Mancilla-Leytón JM, Fernández-Alés R, Martín Vicente A, 2012. Low viability and germinability of commercial pasture seeds ingested by goats. *Small Rum Res* 107: 12-15.
- Manzano P, Malo JE, Peco B, 2005. Sheep gut passage and survival of Mediterranean shrub seeds. *Seed Sci Res* 15: 21-28.
- Milne JA, 1991. Diet selection by grazing animals. *Proc Nutr Soc* 50: 77-85.
- Morand-Fehr P, Bourbouze A, Le Houérou HN, Gall C, Boyazoglu JG, 1983. The role of goats in the Mediterranean area. *Livest Prod Sci* 10: 569-587.
- Myers JA, Vellend M, Gardescu S, Marks PL, 2004. Seed dispersal by white-tailed deer: implications for long distance dispersal invasion and migration of plants. *Oecologia* 139: 35-44.
- Olson BE, Wallander RT, 2002. Does ruminal retention time affect leafy spurge seed of varying maturity? *J Range Manage* 55: 65-69.
- Pakeman RJ, Engelen J, Attwood JP, 2002. Ecological correlates of endozoochory by herbivores. *Funct Ecol* 16: 296-304.

- Ramos ME, Robles AB, Castro J, 2006. Efficiency of endozoochorous seed dispersal in six dry-fruited species (Cistaceae): from seed ingestion to early seedling establishment. *Plant Ecol* 185: 97-106.
- Ramos M, Robles A, González-Rebollar J, 2010. Ley-farming and seed dispersal by sheep: two methods for improving fallow pastures in semiarid Mediterranean environments? *Agric Ecosyst Environ* 137: 124-132.
- Razanamandranto S, Tigabu M, Neya S, Odén PC, 2004. Effects of gut treatment on recovery and germinability of bovine and ovine ingested seeds of four woody species from the Sudanian savanna in West Africa. *Flora* 199: 389-397.
- Robles AB, Castro J, González Miras E, Ramos ME, 2005. Effects of ruminal incubation and goat's ingestion on seed germination of two legume shrubs. *Opt Mediterr Ser A* 67: 111-115.
- Russi L, Cocks PS, Roberts EH, 1992. The fate of legume seeds eaten by sheep from a Mediterranean grassland. *J App Ecol* 29: 772-778.
- Sánchez AM, Peco B, 2002. Dispersal mechanisms in *Lavandula stoechas* subsp. *pedunculata*: autochory and endozoochory by sheep. *Seed Sci Res* 12: 101-111.
- Simao Neto M, Jones RM, Ratcliff D, 1987. Recovery of pasture seed ingested by ruminants. Seed of six tropical pasture species fed to cattle, sheep and goats. *Aust J Exp Agr* 27: 239-246.
- Staniforth RJ, Cavers PB, 1977. The importance of cottontail rabbits in the dispersal of *Polygonum* spp. *J App Ecol* 14: 261-267.
- Takos IA, Efthimiou GS, 2003. Germination results on dormant seeds of fifteen tree species autumn sown in a northern Greek nursery. *Silvae Genet* 52: 67-71.
- Thanos CA, Georghiou K, Kadis C, Pantazi C, 1992. Cistaceae: a plant family with hard seeds. *J Bot* 41: 251-263.
- Traveset A, Bermejo T, Willson M, 2001. Effect of manure composition on seedling emergence and growth of two common shrub species of Southeast Alaska. *Plant Ecol* 155: 29-34.
- Traveset A, Verdú M, 2002. A meta-analysis of the effect of gut treatment on seed germination. In: *Seed dispersal and frugivory: ecology, evolution and conservation* (Levey DJ, Silva WR, eds). Univ of Florida, Gainesville (USA) and Universidad Estadual Paulista, Sao Paulo (Brazil). pp: 339-350.
- Valdés B, Talavera S, Galiano F, 1987. Flora vascular de Andalucía Occidental. Vols I-III. Ed. Ketres, Barcelona.
- Willson MF, 1993. Mammals as seed-dispersal mutualists in North America. *Oikos* 67: 159-176.