1 Short communication

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4 **POTENTIAL OF RED DEER (*Cervus elaphus*) TO DISPERSE VIABLE SEEDS BY SPITTING**
5 **THEM FROM THE CUD**

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Abstract

Most studies and reviews on endozoochorous seed dispersal by ungulates overlook that ruminants spit viable seeds from the cud, i.e. after they were ingested and carried out inside the rumen. To assess the importance of this modality of endozoochory, we performed feeding trials with three captive red deer (*Cervus elaphus*) and four plant species differing in fruit and seed traits. Our deer both regurgitated from the rumen and ejected, and also defecated, seeds of the four species, at a ratio of about 6:10. If this ratio is representative, about 37% of the released seeds would be spat and consequently ignored by studies considering only seeds contained in feces.

Key words: endozoochory, plant-ungulate interactions, ruminants, seed regurgitation, long-distance seed dispersal

Wild ungulates have ecological influence nearly on every biome except the Antarctica. Their potential to impact on the structure and functioning of ecosystems is thus remarkable. For instance, ungulates condition the diversity and dynamics of plant communities (e.g. Hegland and Rydgren 2016), either affecting directly plant demography through grazing, browsing and seed dispersal, or indirectly by modifying the plant environment, such as edaphic conditions, nutrient flows and water cycle (Hobbs 1996). Because of this, understanding plant-ungulate interactions has been long an important challenge for ecology and conservation (Danell et al. 2006). Traditionally, the antagonistic component of ungulate herbivory (predation of seeds and adult plants) has received more attention than its mutualistic one (seed dispersal). Interestingly, however, a number of investigations have documented the pervasive beneficial effects of seed dispersal by ungulates on plant population dynamics and community assemblage (e.g. Malo and Suárez 1995; Boulanger et al. 2011), which could even compensate the negative effect of herbivory (Bodmer and Ward 2006; but see Lecompte et al. 2016).

A large proportion of wild ungulates are ruminants, i.e. members of the order Artiodactyla which have a rumen and employ foregut fermentation to digest food. At present, about 200 species of wild ruminants do exist, distributed almost worldwide and living across many habitats and climates (Hackmann and Spain 2010). Besides, in areas of temperate Eurasia and North America the populations of wild ruminants (e.g. deer, wild goats) are increasing because of habitat changes and under-harvesting, exacerbating their impact on ecosystems (e.g. Coté et al. 2004). Ungulate ruminants disperse seeds both attached to their bodies (epizoochory) and inside their guts (endozoochory). Usually, seeds dispersed via epizoochory are transported while adhered to the fur and the hooves, and seeds dispersed via endozoochory are defecated after passing intact throughout the digestive tract (Albert et al. 2015). However, ruminants have an additional form of endozoochory directly linked to the peculiar way they process the ingested food. Because food fermented at the rumen (known as cud) must be regurgitated and re-chewed (*ruminare* in Latin means to chew again), ruminants can disperse seeds by spitting them from the cud while ruminating (Feer 1995).

Evidence of seed dispersal from the cud by Cervidae and Bovidae has been reported in tropical areas of Africa (e.g. Feer 1995), America (e.g. Janzen 1985; Yamashita 1997) and Asia (e.g. Prasad et al. 2006; Brodie et al. 2009), but it has been surprisingly overlooked in temperate areas, where endozoochory is ordinarily associated only to seeds contained in dung samples (e.g. Pakeman et al. 2002; Albert et al.

2015). Quoting Janzen, some authors just mentioned (without investigating it) the possibility of white-tailed deer (*Odocoileus virginianus*) spitting large seeds while ruminating in North America (e.g. Myers et al. 2004), and Delibes et al. (2016) have recently reported domestic goats spitting from the cud seeds of several plant species in Spain and Morocco. In spite of information shortage, we suspect that seed dispersal from the cud is widespread among ruminants also in Eurasia, with important and increasing ecological consequences. To illustrate its potential, we performed feeding trials with captive red deer (*Cervus elaphus*), a species with a wide distribution that in the wild consumes regularly fruits, at least in autumn (Gebert and Verheyden-Tixier 2001). We supplied deer with a variety of plants differing in fruit and seed sizes and structures, predicting that deer will ingest and spit out while chewing the cud a significant part of offered seeds. This portion is regularly ignored in most studies of seed dispersal by ruminants.

Because stressful conditions are known to affect rumination activity (e.g. Silanikove 1992) we avoided the separation of the animals and the use of metabolic cages, trying to make the experiments as “natural” as possible. Trials were made with three female deer at the Iberian Deer Experimental Farm of University of Castilla-La Mancha in Albacete, Spain (38°57’33.11’’N and 1°52’44.62’’W). By logistic reasons we did not use any male. The animals were kept together in a 24 m² outdoor facility. After fasting 12 hours, 300 single-seeded fleshy fruits of three species differing in fruit size and type (dwarf palm, *Chamaerops humilis*, Arecaceae; hawthorn, *Crataegus monogyna*, Rosaceae; hackberry, *Celtis australis*, Ulmaceae; Table 1) and 30 pods (approx. 300 seeds in all) of carob, *Ceratonia siliqua* (Fabaceae) were offered to them, mixed with their usual fodder. Given that our aim was not to investigate the functional role of red deer at Mediterranean ecosystems, but to prove the general ability of the species to disperse a variety of seeds while ruminating, we chose seeds with different sizes and shapes (Table 1), without considering if the plant species were previously reported at the diet of the species in Iberia; they were not, in our knowledge (e.g. Bugalho and Milne 2001; Martínez 2009) but we have observed wild red deer eating at least dwarf palm and carob fruits in Doñana and Sierra Morena, respectively (South Spain). Fruits were provided once a day and immediately afterwards deer behavior was continuously observed during two consecutive hours. Then, we collected and counted the seeds fallen during ingestion (i.e. short-distance dispersal) and removed all the intact (not handled) fruits, which were offered early in the morning the following two days. Thus, we counted the ingested fruits and the ejected seeds during food manipulation along a 72 h period. In addition, observations were conducted each 4-5 hours to locate the specific sites

90 where deer lied down and ruminated. In these sites small piles of cleaned seeds spat from the cud were
91 often found. Overall, we collected all regurgitated seeds and feces delivered by deer during 12
92 consecutive days (i.e. excluding the first one). Feces were broken and sieved under a constant water flux
93 at the laboratory to recover delivered seeds.

94 Deer harvested most offered fruits and seeds (on average 84.5%; $n = 1200$; Tab. 1). From the harvested
95 seeds ($n = 1014$), 79.9% were ingested and 20.1% released during mastication before ingestion. Larger
96 seeds (those from dwarf palms) and seeds contained in multi-seeded pods (carob) were more frequently
97 dropped during mastication (about 40-30%), while smaller ones (those from hawthorn) were rarely
98 ejected (1%; Table 1). From the ingested seeds ($n = 810$), 77.9% disappeared (i.e. were completely
99 digested or missed), 13.8% were defecated, and 8.3% were spat from the cud (Table 1). Most spat seeds
100 were released in 1-4 days after fruit ingestion. Besides, a few seeds were spat until 8 (dwarf palm), 9
101 (carob) and 10 (hawthorn) days after the last ingestion, suggesting that some of the disappeared seeds
102 could have been released after the end of our experiment. The documented long retention times indicate a
103 high potential of this mechanism to lead to long-distance seed dispersal. As expected, larger seeds (dwarf
104 palm) were delivered more frequently from the cud, while smaller ones (hawthorn) were mostly
105 defecated. In the middle-sized hackberry and carob, approximately 40% of the seeds released after
106 ingestion were spat while ruminating and 60% were defecated. Most of the seeds regurgitated from the
107 cud were red-stained (i.e. were alive) when tested with tetrazolium chloride, suggesting they were viable
108 (Hartmann and Kester 1983). Besides, we sowed, under controlled conditions, 10 spat seeds of hackberry
109 and 15 of carob; four seeds of the former and two of the last species germinated in the subsequent year.

110 Apparently, most of the seeds were completely digested, confirming the ability of ungulates to depredate
111 seeds (Bodmer and Ward 2006). However, as a rule of thumb, seeds of any of the four species could be
112 dropped while ingesting, spat while chewing the cud, defecated, or digested. This means that deer could
113 act for many plant species as short-distance seed dispersers (seed dropping), endozoochorous long-
114 distance seed dispersers (spat from the cud and defecated seeds), and seed predators (digested seeds).

115 Future research should focus in identifying the many factors that in nature could influence seed fate after
116 ingestion, some intrinsic to dispersers (e.g. hunger, attraction for the food, stress), other intrinsic to the
117 plant (e.g. size of the fruits and the seeds, fruits abundance) and some ones extrinsic to both but
118 influencing foraging behavior (e.g. habitat, risk of predation, competition).

Due to factors mentioned above, results from new trials with other species and/or experimental conditions are likely to differ quantitatively of those presented here; however, our qualitative results are strong enough to emphasize the importance of spitting from the cud as a mechanism of seed dispersal and to suggest some important biases in previous studies of endozoochory by ruminants in temperate areas:

1. Spitting viable seeds from the cud is not exclusive for tropical areas, but seems to be generalized also in temperate ones. Thus, the number of dispersed seeds by wild ruminants is being underestimated in field studies based exclusively on dung collection. According to our results, the average proportion of defecated to spat seeds would be close to 10:6 (i.e. for each 100 seeds detected in feces, there will be 60 missed seeds spat from the cud).

2. Seeds of some plant species with particular traits can be (almost) exclusively dispersed from the cud, being missed when studying seed dispersal only through feces. For instance, Janzen (1985) stated that white-tailed deer “regurgitate the nuts (of *Spondias mombin*) while chewing their cud” and that “nuts never pass into the lower digestive tract”. In our case, the large dwarf palm seeds were spat three times more often than defecated.

3. Seeds collected from manure likely will mix those spat from the cud and defecated. Then, results will not be comparable to those merely based on feces collected in the field. Besides, studies about germinability using these seeds could be misleading because of the contrasting treatments received by spat and defecated seeds, presumably mixed at the samples.

As conclusion, research on the potential of wild ungulates as seed dispersers cannot longer overlook the importance of spitting from the cud as a relevant mechanism of long-distance dispersal by ruminants. Certainly, rumination is carried out usually in hidden places and it is thus difficult to observe, especially in wild ruminants. In addition, clean seeds can be collected quickly by seed predators or secondary dispersers. Nevertheless, by reducing analyses to seeds contained in dung samples, researchers are likely to miss an important proportion of dispersed seeds, thus limiting the potential of their investigations concerning ungulate effects on ecosystem functioning.

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Plant species	Seed diameters (mm)	# offered seeds	# harvested seeds	# ejected seeds before ingestion	# spat seeds from the cud	# defecated seeds	# disappeared seeds
<i>Chamaerops humilis</i>	16.3 x 11.0	300	228	92	10	3	123
<i>Crataegus monogyna</i>	6.1 x 4.8	300	260	3	5	41	211
<i>Celtis australis</i>	6.7 x 6.3	300	226	26	10	13	177
<i>Ceratonía siliqua</i>	7.5 x 5.3	300 ^a	300	83	42	55	120
All species pooled	-	1200	1014	204	67	112	631

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205 Table 1. Results of the feeding trials with three female red deer (*Cervus elaphus*) performed at the
206 University of Castilla-La Mancha during 12 consecutive days. ^a Number of offered *Ceratonía* seeds is
207 approximate.

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