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Mancilla-Leytón J.M., Martín Vicente A.

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# The role of goats in improving crop substrate: Post-ingestion germination and survival of two Mediterranean herbaceous forage species

J.M. Mancilla-Leytón<sup>1</sup> and A. Martín Vicente

Departamento de Biología Vegetal y Ecología de la Universidad de Sevilla  
Apartado 1095 Sevilla 41080 (Spain)

<sup>1</sup>e-mail: jmancilla@us.es

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**Abstract.** We assessed the ability of Payoya goats to disperse two endozoochorous Mediterranean herbaceous forage species: *Trifolium subterraneum* (legume) and *Lolium rigidum* (grass). Seeds of these two species were fed to six individually housed goats. For five days following ingestion, the faeces were collected every 24 hours. Following examination of the contents of the faeces the percentage of seed loss (chewing and digestion) was determined as well as the distribution of seed recovery over time, germination efficacy and survival of seedlings after passage through the digestive tract. The percentage of seeds recovered was 1.5% in *T. subterraneum* and 2% in *L. rigidum*, whilst germination was between 33-38% in *T. subterraneum* and 35-37% in *L. rigidum*. In the study species, biomass and herbage height was clearly higher in the seedlings emerged from the faeces than in controls. Despite the low values obtained, we can consider that the use of livestock can be a cheap and effective method to introduce new pasture species.

**Keywords.** Endozoochory – Goats – *Trifolium* – *Lolium* – Seed.

**Le rôle des chèvres dans l'amélioration des pâtures : Germination après l'ingestion de deux espèces fourragères herbacées méditerranéennes**

**Résumé.** On a évalué la capacité de dispersion par les chèvres de deux espèces herbacées d'intérêt fourrager : *Trifolium subterraneum* (Fabaceae) et *Lolium rigidum* (Poaceae). Les semences de ces espèces ont été mélangées à la nourriture de six chèvres logées individuellement. Après leur ingestion, on a récolté les selles pendant cinq jours chaque 24 heures, on a examiné les pourcentages de perte de semences (par mastication et digestion), la distribution des graines récupérées dans le temps, la germination et l'efficacité de la survie des semis après le passage dans le tube digestif. Le pourcentage de graines récupérées a été de 1,5% pour *T. subterraneum* et de 2% pour *L. rigidum*, alors que la germination se situe entre 33-38% pour *T. subterraneum* et 35-37% pour *L. rigidum*. Pour les deux espèces étudiées, la biomasse et la hauteur des individus ont été de significativement plus élevées dans les plantes issues de matières fécales que chez les témoins. Malgré les faibles valeurs obtenues, on peut considérer que l'utilisation du bétail peut être un outil bon marché et efficace pour introduire de nouvelles espèces fourragères d'intérêt.

**Mots-clés.** Endozoochorie – Chèvres – *Trifolium* – *Lolium* – Semence.

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## I – Introduction

In plant-animal interaction, seed dispersal (epi and endozoochory) has gained prominence in recent years. It is currently necessary to know which species are dispersed by herbivores, and whether the seeds are carried near or far from the parent plant, but also to know whether the dispersed species are viable (disperser legitimacy), if dispersal improves their germination ability and if they are deposited in appropriate places (disperser effectiveness). This study aims to answer these questions.

The objective of this study was to evaluate the dispersal ability of Payoya goats, which are endozoochorous of two Mediterranean herbaceous forage species: *Trifolium subterraneum* (legume) and *Lolium rigidum* (grass). We determined the percentage of loss of seeds (through

mastication and digestion), distribution of seed recovery in time, the efficiency of seed germination following passage through the digestive tract and the survival and biometric characteristics of the emerging seedlings.

## II – Materials and methods

We selected cultivated varieties of two Mediterranean grass forage species: *Trifolium subterraneum* var. *campeda* and *Lolium rigidum* var. *wimmera*. These species were fed only once to six individually housed Payoya goats. The number of seeds consumed per animal was: 10,000 *Trifolium subterraneum* and 30,000 *Lolium rigidum*. To test the loss of seeds through mastication and digestion, an experiment was conducted with three treatments: (i) goats were fed with seeds alone; (ii) seeds were mixed with 250 g of concentrate (37.1% barley, 24.8%, maize 14.9% oats, 10.9% soybean, 10.9% pea, 0.5% sunflower oil, 1% corrector); and (iii) seeds were mixed with 200 g of wholemeal sunflower pellets. The animals had unlimited access to alfalfa and water. Goat dung was collected every 24 hours for five days and dried at room temperature for 72 hours in a bell jar with silica gel. The dung was then broken up to search for seeds: for each day and each animal, 20 subsamples of 4 g of dung were taken, and the number of potentially viable seeds recorded. The average number of seeds recovered in 4 g was extrapolated to the total dung per animal and day, thus providing an estimate of the percentage of recovery.

Seed viability was determined using some of the recovered seeds and the tetrazolium viability test. The remainder were placed in a germinator (ASL Scientific Apparatus M-92004, Madrid, Spain) with a photoperiod of 16 h light (25°C, 35 mol/m<sup>2</sup>/s, 400-700 nm) and 8 hours dark (12°C), for a period of 60 days. Moreover, to test the ruminant effect of the goat itself, four replicates of 25 control seeds of each species, which had not passed through the digestive tract, were also tested.

The ability of seeds to emerge and establish as seedlings in the interior of the dung was assessed by a greenhouse experiment. Two treatments were included in the experiment: (i) seeds placed within intact dung; and (ii) seeds placed within lightly broken up dung. In both cases 12 g samples of dung were placed on the surface of a sand/vermiculite mixture contained in a pot. A control group was created in the same way but with seeds that had not passed through the digestive tract. Germination and emergence of seeds was monitored daily. Final biomass and height was measured for each treatment.

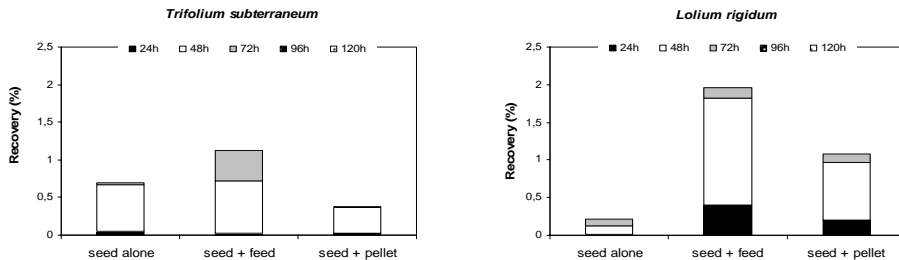
The data were analyzed with an analysis of variance using SPSS v 17.0.

## III – Results and discussion

The recovery rate of seeds varied according to how they were supplied to the goats, with treatment 2 (seeds + feed) producing the highest recovery values. For both plant species, treatment 2 clearly showed a higher recovery rate, with the greatest recovery rates observed at 48 hours after ingestion (Fig. 1). Small, round and hard-coated seeds often dominate in mammalian herbivore faeces (Myers *et al.*, 2004; Pakeman *et al.*, 2002; Heinken *et al.*, 2001; Malo and Suarez 1995). These seeds are more likely to escape mastication and to withstand the effects of acids, enzymes and bacteria in the gut. However, our data indicate that the seeds of the two species used, with different shapes and sizes, presented similarly high rates of destruction. This may be because the seeds used for this experiment are cultivars selected for their reduced seed hardness, a feature that promotes germination in the field but which can also increase seed destruction in the digestive process.

The percentage germination of the control seeds of *T. subterraneum* (97%) was significantly higher than the germination of seeds recovered from dung ( $F = 1476.32$ ,  $P < 0.05$ ). The percentage germination of seeds recovered at 48 hours (30.00%) was slightly higher, although

not significantly ( $P>0.05$ ) than that of seeds recovered after 72 hours (33.34%). In the case of *L. rigidum*, the results were very similar: the percentage germination of control seeds (90.00%) was significantly higher than that of seeds recovered from dung ( $F = 1146.91$ ,  $P < 0.05$ ). There was no significant difference ( $P>0.05$ ) between the percentage germination of seeds recovered at 48 hours (58.33%) and at 72 hours (67.39%) (Table 1).



**Fig. 1. Mean rate of recovery of *T. subterraneum* and *L. rigidum* seeds after ingestion by goats, every 24 h.**

The Tetrazolium viability test revealed significant differences between the *T. rigidum* control seeds (97%) and those recovered from dung at 48 h (41.25%) and 72 h (37.5%) ( $F = 380.92$ ,  $P < 0.05$ ), with no significant differences found between the viability of the recovered seeds ( $P>0.05$ ). The pattern was similar for *L. rigidum*: control seeds presented a significantly higher viability (94%) than those recovered at 48 h (68.72%) and 72 h (69.56%) ( $F = 285.71$ ,  $P < 0.05$ ), there were no significant differences between the recovered seeds ( $P>0.05$ ). This loss of viability may be due to damage to the embryo caused by the fermentation of the dung. In this way, phenolic compounds and fatty acids present in dung can act as germination inhibitors in some species (Traveset *et al.*, 2001) (Table 1).

**Table 1. Percentage germination and proportion viable seeds by Tetrazolium test. Mean  $\pm$  standard error. Different letters indicate significant difference (Tukey Test,  $P < 0.05$ )**

	Germination (%)	Viability (%)
<i>T. subterraneum</i>		
Control	97 $\pm$ 1.65a	97 $\pm$ 1.65a
48 h	30 $\pm$ 10.6b	41.25 $\pm$ 7.18b
72 h	33.3 $\pm$ 4.56b	37.5 $\pm$ 3.58b
<i>L. rigidum</i>		
Control	90 $\pm$ 2.58a	94 $\pm$ 1.15a
48 h	58.33 $\pm$ 7.6b	68.78 $\pm$ 3.98b
72 h	67.39 $\pm$ 9.9b	69.56 $\pm$ 8.19b

The percentage of emergence in *T. subterraneum* was clearly higher (93.75%) in the control seeds, than in the seeds recovered from dung, in both the intact and broken up dung treatments. Thus, the emergence of the seeds included in the broken up dung, and recovered at 48 h (33.00%), was significantly higher than the percentage germination of seeds contained in the intact dung (11.25%) ( $F = 1410$ , 12;  $P < 0.05$ ). However, in the 72 h treatment, seedlings only emerged from the broken up dung (16.67%). In the case of *L. rigidum*, emergence in the control group (90.00%) was significantly higher than in the rest of treatments. No seedlings

emerged in the intact dung treatment (48 and 72 h). Emergence from the broken up dung, recovered at 48 h (36.67%) and at 72 h (8.33%) was significantly lower than the emergence found in the control group ( $F = 1686.09$ ,  $P < 0.05$ ).

Regarding the biomass of seedlings emerging from the dung, for *T. subterraneum* we found that aboveground biomass was significantly higher in those seedlings that emerged from the dung (0.08 g MS), than in the control (0.05 g MS) ( $F = 845.25$ ;  $P < 0.05$ ). Likewise, the final height achieved in the seedlings emerged from the dung (4.5 cm) was significantly higher than in the control (3.2) ( $F = 235.45$ ;  $P < 0.05$ ). Below-ground biomass showed no significant differences ( $P > 0.05$ ).

In the same experiment repeated with *L. rigidum*, the shoots of the seedlings emerged in the dung (0,065 g MS) was significantly higher than the control (0.01) ( $F = 578.01$ ;  $P < 0.05$ ). The final height reached in the seedlings emerged from the dung (8.5 cm) was significantly higher than in the control (2.4 cm) ( $F = 261.34$ ;  $P < 0.05$ ). Below-ground biomass showed no significant differences.

Although the percentages of recovery and subsequent emergence found in this study are low in absolute terms, we can say that a significant number of seeds can become established from the dung (approximately 0.5% of ingested seeds for both species), despite being commercial seed. This number can be increased by increasing the number of seeds ingested by livestock. In addition, faecal material can confer more vigour to seedlings due to the release of nutrients (N, P and organic C), having a fertilizing effect on the soil and plant as we have been able to see (Traveset *et al.*, 2001; Traveset and Verdú, 2002).

## IV – Conclusions

Given that each established seedling can produce hundreds or thousands of seeds, we can conclude that the use of grazing goats for the implantation of cover crops in arable crops could be a viable and sustainable practice that benefits the soil, the crop itself, the quality of pasture, livestock and the economy of the farmers and/or livestock producers.

Although the above data and arguments may be optimistic in the implementation of this "seeding method", it stands to reason that under natural conditions the emergence and establishment of seedlings will be less than that expected under greenhouse conditions. It is therefore intended to pursue this line of research, specifically to carry out a comparative study of wild seed, as well as the field establishment of these species.

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