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Relationship between seed survival and seed characteristics of nine Mediterranean legumes after ingestion by sheep

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SUMMARY – The present work examines the relationship between different seed characteristics (seed size: length and width, seed form, seed volume, seed mass and hardseededness) and seed survival of nine legumes adapted to Mediterranean environments after ingestion by sheep (*Segureña* breed). Species used in the present study include: *Trigonella polyceratia* L., *Bituminaria bituminosa* (L.) C.H. Stirt, *Lathyrus filiformis* (Lam.) J. Gay, *Vicia peregrina* L., *V. ervilia* (L.) Willd, two *V. sativa* L. (a commercial cultivar and a wild one) and two *Medicago sativa* L. (a commercial cultivar and a wild one). Our results show that apart from hardseededness (R^2=0.83), none of the chosen seed attributes were able to explain the relationship between survival and seed characteristics. For legumes and other species with physical dormancy the proportion of hardseededness could be a useful approach to assess endozoochorous seed dispersal potential.

Keywords: Forage species, endozoochory, gut passage, hardseededness.

***RESUME* –** *"Rapport entre la survie et les caractéristiques des semences pour neuf légumineuses méditerranéennes après leur ingestion par des ovins". Le présent travail examine le rapport entre la survie et les différentes caractéristiques des semences (la longueur, la largeur, la forme, le volume et la masse des graines, et le taux de graines dures) de neuf légumineuses adaptées aux environnements méditerranéens après l'ingestion par les moutons (race* Segureña*). Les espèces utilisées dans la présente étude incluent :* Trigonella polyceratia *L.,* Bituminaria bituminosa *(L.) C.H. Stirt,* Lathyrus filiformis *(Lam.) J. Gay,* Vicia peregrina *L.,* V. ervilia *(L.) Willd, deux* V. sativa *(espèce spontanée et cultivar commercial), et deux* Medicago sativa *(espèce spontanée et cultivar commercial). Nos résultats montrent que sauf pour le taux de graines dures (R^2=0.83) aucun des attributs de la semence choisis n'était capable d'expliquer le rapport entre les caractéristiques de la semence et la survie. Pour les légumineuses et d'autres espèces à dormance physique, la proportion du taux de graines dures pourrait être une approche utile pour évaluer le potentiel de dissémination des graines par les animaux (endozoochorie).*

Mots-clés : *Espèces fourragères, semence, endozoochorie, passage par le tube digestif, taux de graines dures.*

Introduction

Seed survival after ingestion by ruminants is an important dispersal mechanism both in wild and cultural landscapes with implications to conservation, biology and restoration ecology. A critical phase of dispersal by ruminants is the survival of seeds after chewing, rumination and the action of acids, enzymes and bacteria inside the digestive tract. Attributes such as shape, size, mass, volume and hardseededness (hard seed cover) could influence the degree to which seeds survive the ruminant ingestion and ruminant gut passage.

Legume species are highly preferred by domestic and wild herbivorous. Endozoochorous dispersal of their seeds by ruminants is common (Baskin and Baskin, 1998), a feature that may be helpful in the management and improvement of pastures. Examples of seed dispersal studies in the Mediterranean region are frequent in literature (Malo and Suárez, 1995) but scarce information exists on the relationship between seed characteristics and seed survival after ingestion and gut passage of a ruminant. The present work examines this relationship considering different seed characteristics (seed size: length and width, seed form, seed volume, seed mass and hardseededness) after ingestion and gut passage of sheep (*Segureña* race) of nine legumes adapted to Mediterranean environments. Species used in the present study include: *Trigonella polyceratia* L, *Bituminaria bituminosa (L.)* C.H. Stirt, *Lathyrus filiformis* (Lam.) J. Gay, *Vicia perergrina* L., *V. ervilia* (L.) Willd, two *V. sativa* L. (a commercial cultivar and a wild one) and two *Medicago sativa* L. (a commercial cultivar and a wild one).

Materials and methods

Wild and commercial cultivars seeds of nine legumes adapted to the Mediterranean climate were used (Table 1). Seed mass, size (length and width), volume, and form were obtained to each legume. Seed mass (mg) was determined by weighing 5 replicates of 50 seeds. Volume was obtained in millilitres by the displacement of ethanol (94% strength) by 100 seeds (FAO, 1988). Seed size (mm) of 25 seeds were measured parallel (length) and opposite (width) to the hillum (FAO, 1988). Seed shape was classified either as round or not.

Table 1. Legume species used and the weight and number of seeds of each legume fed to sheep

Species	Type/Supply	Seeds fed per replication	
		Weight (g)	Number
Lathyrus filiformis	Wild	250	10157
Medicago sativa	Wild	120	43181
	Comercial	120	39583
Vicia sativa	Wild	600	7216
	Comercial	600	8554
Vicia peregrina	Wild	90	949
Vicia ervilia	Comercial	510	11154
Trigonella polyceratia	Wild	100	239207
Bituminaria bituminosa	Wild	130	6888

To determine seed recovery from faeces, only whole seeds with a seed coat were used. Each of the seed samples were mixed with barley hay and fed to 3 adult sheep (Table 1). Sheep were kept in stables and faeces were collected 24, 48, 72 and 96 hours after seed supply. Each faecal sample was air dried, weighed, and 10 subsamples, each of 10 g, were used to estimate seed recovery. Seeds were manually separated from faeces. Only whole seeds with a seed coat were taken into account. Percentage of seed recovery was determined by referring the mean number of seed found in 10 g to the total mass of dung.

To determine seed survival twenty-five seeds per five replications for each species were allowed to germinate on a double layer of filter paper in sterile Petri dishes and incubated in a growth chamber (20ºC/15ºC; 12h light/12h darkness). The dishes were regularly watered with distilled water to maintain moisture conditions. The number of germinated seeds was recorded every two days for thirty days. Germination was defined as the emergence of the radicule. Ungerminated seeds were tested for viability via the tetrazolium test (AOSA, 2000) and the latter viable seeds classified as hard (hardseededness). Survival of seeds was calculated by adding both germinated and hard seeds percentage.

The data on seed recovery and seed survival were transformed (arcsine square root) and the variance analysed. Non normal data were analysed for variance by the Kruskall-Wallis procedure. Seed survival data were plotted against the independent variables: seed mass (mg), seed length (mm), seed width (mm), seed volume (ml) seed shape and hardseededness (%). A linear regression analysis tested how seed survival was related to these independent variables. Statistical analysis was performed using Statgraphics plus 5.1.

Results and discussion

Table 2 shows seed shape, size, volume and weight of the legumes studied. Seeds from the legume species ranged in length from 2.3 mm to 6.7 mm; in width from 1.0 mm to 6.7 mm; in weight from 0.93 mg to 67.95 mg; in volume from 0.001 ml to 0.180 ml.

Only seeds of *T. polyceratia* were sufficiently recovered 96 h after feeding to perform analysis. No seeds of *V. sativa, V. ervilia* and *M. sativa* commercial survived ingestion and gut passage. The amount of hard seeds and survival of seeds (Tables 3 and 4) depended on the amount of hard seeds in the original seed lot.

Table 2. Shape, dimensions, volume and weight of nine legumes

	Shape	Lenght (cm)	Width (cm)	Vol (ml)	Weight (mg)
M. sativa Wild	Not round	0.30±0.1	0.17±0.1	0.038	2.20±0.9
M. sativa Comercial	Not round	0.32±0.1	0.24±0.1	0.037	2.40±0,7
B. bituminosa	Not round	0.54±0.1	0.27±0.1	0.180	18.53±1.0
T. polyceratia	Not round	0.23±0.1	0.1±0.1	0.001	0.93±0.3
L. filiformis	Round	0.32±0.1	0.30±0.1	0.044	44.72±3.0
V. ervilia	Round	0.60±0.1	0.60±0.1	0.113	59.30±5.0
V. peregrina	Round	0.39±0.1	0.40±0.1	0.080	46.15±4.0
V. sativa Wild	Round	0.67±0.1	0.67±0.1	0.137	67.95±6.0
V. sativa Comercial	Round	0.60±0.1	0.60±0.1	0.130	56.93±6.0

Table 3. Hard seed content of nine legumes before feeding sheep (*Segureña* race) and 24, 48, 72, 96 h after excretion

	Hardseededness				
	Control	24h	48h	72h	96h
M. sativa Wild	29.6a	46.8b	77.6c	88.0d	-
M. sativa Comercial	8.0b	0.032a	0a	0a	-
B. bituminosa	36.8a	47.2bc	52.8c	44.8b	-
T. polyceratia	59.2a	82.4b	88.0bc	91.2c	90.4c
L. filiformis	82.0a	85.6a	89.6a	80.76a	-
V. ervilia	0a	0a	0a	0a	-
V. peregrina	53.6a	-	91.2b	-	-
V. sativa Wild	0a	0a	0a	0a	-
V. sativa Comercial	0a	0a	0a	0a	-

*Means in each row that are not followed by the same letter differ at $P<0.05$.

Table 4. Survival of seeds after 24, 48, 72 and 96h after ingestion by sheep (*Segureña* race)

		Survival of seeds after feeding			
	Control	24h	48h	72h	96h
M. sativa Wild	97.6c	16.0a	89.6b	100c	-
M. sativa Comercial	40.0b	0.032a	0a	0a	-
B. bituminosa	85.6d	34.4a	49.6b	34.4a	-
T. polyceratia	87.2a	92.0ab	87.2a	96.0b	88.0a
L. filiformis	91.0a	96.8b	99.2b	92.6a	-
V. ervilia	91.2b	0a	0a	0a	-
V. peregrina	96.8b	0a	96.0b	0a	-
V. sativa Wild	97b	0a	0a	0a	-
V. sativa Comercial	96.8b	0a	0a	0a	-

*Means in each row that are not followed by the same letter differ at $P<0.05$.

It has been shown that small seeds are usually found in the dung of large herbivores (Malo and Suárez, 1995; Pakeman, *et al.,* 2002). Janzen (1984) proposes that in order to be able to survive gut passage, seeds should be small, round and hard. Furthermore, Moussie (2004) explains that seed size and seed mass are fair predictors of seed survival after ingestion and gut passage. However, our results show that seed attributes such as mass, volume, size and shape were not significantly correlated to survival (Table 5). This lack of correlation could be explained in terms that (i) seeds of the different species differ in their attributes thus challenging a distinction of the influence of any specific attribute on seed survival and (ii) a seed attribute that may help the survival of a seed may be overcome by another attribute that could be disadvantageous.

On the other hand, hardseededness (R^2=0.83) was able to explain seed survival. In fact, only those legumes presenting a hard seed content survived ingestion and gut passage. Seed coats may be eroded and softened after feeding (Izhaki and Ne'eman, 1997). However, a hard seed cover might provide a defence against bacteria and chemical attack inside the digestive tract of the sheep.

Table 5. Relationship between seed survival and seed characteristics of nine legumes

	R^{2*}					
	Shape	Lenght	Width	Volumen	Mass	Hardseedness
Survival of seeds	0.065	0.178	0.129	0.132	0.078	0.83

* $P < 0.05$.

Although some legumes produce virtually no hard seeds (eg. commercial cultivars chosen for lack of hardseededness and hence, rapid germination), hardseededness is a common feature and the main mechanism of seed dormancy in legumes. For legumes and other species with physical dormancy (e.g. *Cistaceae* or *Convolvulaceae*) a determination of the percentage of hard seeds in a seed lot could be an useful approach to assess the ability of seeds to survive the ingestion and gut passage of sheep and thus, endozoochorous seed dispersal potential.

Conclusions

Our results suggest that seed survival can not be derived from seed mass, seed volume, seed size and seed shape. On the other hand, hardseededness is a fair predictor of seed survival after ingestion and gut passage of sheep.

References

AOSA (2000). *Tetrazolium Testing Handbook. Contribution No. 29*. Association of Official Seed Analysts. New Mexico.

Baskin, C.C. and Baskin, J.M. (1998). *Seeds. Ecology, biogeography and evolution of dormancy and germination*. Academic Press, San Diego.

FAO (1988). *Evaluation of seed legumes*. FAO. Rome.

Izhaki, I. and Ne'eman G. (1997). Hares (*Lepus* spp.) as seed dispersers of *Retama raetam* (Fabaceae) in a sandy landscape. *J. Arid Environ.,* 37: 343-354.

Janzen, D.H. (1984). Dispersal of small seeds by big herbivores: Foliage is the fruit. *Amer. Nat*, 123: 338-353.

Malo, J. E. and Suárez, F. (1995). Herbivorous mammals as seed dispersers in a Mediterranean dehesa. *Oecol.*, 104: 246-255.

Moussie, M. (2004). *Seed dispersal by large herbivores: Implications for restoration of plant diversity*. PhD Thesis. University of Groningen.

Pakeman, R.J., Digneffe, G. and Small, J.L. (2002). Ecological correlates of endozoochory by herbivores. *Funct. Ecol.*, 16: 296-304.