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in

Molina Alcaide E. (ed.), Ben Salem H. (ed.), Biala K. (ed.), Morand-Fehr P. (ed.).
Sustainable grazing, nutritional utilization and quality of sheep and goat products

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 67

2005
pages 111-115

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SUMMARY – *Adenocarpus decorticans* Boiss. and *Retama sphaerocarpa* (L.) Boiss. are two leguminous shrubs native of the Mediterranean Basin. Both species are common in the shrublands of the Mediterranean mountains and are highly selected as feed by livestock such as sheep and goats. Furthermore, the two species are useful plants for recovering degraded lands. Here, we analyse the effect of different treatments on seed germination in *A. decorticans* and *R. sphaerocarpa*, considering: (i) scarification of the coats; (ii) incubation in the rumen of goats during 24, 48 and 72 h; and (iii) passage through the digestive tract of goats, testing germination of seeds from faeces collected 2, 3 and 4 days after ingestion. Mechanical scarification boosted germination (*A. decorticans*: 89.0% *vs* 5.0% for control treatment; *R. sphaerocarpa*: 90.0% *vs* 5.0% for control treatment), indicating that seeds have a potentially high germination rate when the coat is eroded. Seeds recovery, after animal ingestion, was 7.4% for *A. decorticans* and 17.4% for *R. sphaerocarpa*. Ruminal incubation as well as animal ingestion improved germination in *A. decorticans* (up to 68.0%). Incubation in rumen and animal ingestion, however, had no effect on *R. sphaerocarpa* seed germination. However, *R. sphaerocarpa* seeds, partially broken by chewing but not destroyed, showed a high germination percentage (average of 50.0%). The results suggest that livestock may be an effective disperser of these two legume species.

Key words: Leguminous, goats, endozoochory, ingestion, rumen, digestion, germination.

RESUME – *"Effets de l'incubation ruminale et de l'ingestion par des chèvres sur la germination de semences de deux légumineuses arbustives :* Adenocarpus decorticans *et* Retama sphaerocarpa *(L.)".* Adenocarpus decorticans *Boiss. et* Retama sphaerocarpa *(L.) Boiss. sont des légumineuses arbustives natives du Bassin Méditerranéen. Les deux espèces sont fréquentes dans les maquis des montagnes méditerranéennes et sont très consommées par les moutons et les chèvres. Par ailleurs, ces arbustes peuvent être utilisés pour la réhabilitation des parcours dégradés. Nous avons étudié dans ce travail l'effet des traitements suivants sur la germination des semences de* A. decorticans *et* R. sphaerocarpa*: (i) scarification des graines, (ii) incubation dans le rumen des chèvres pendant 24, 48 et 72 h et, (iii) le passage par l'appareil digestif de la chèvre pendant 2, 3 et 4 jours après l'ingestion. La scarification mécanique a amélioré la germination des semences (*A. decorticans*: 89.0% vs 5.0% pour le traitement témoin;* R. sphaerocarpa*: 90% vs 5% pour le traitement témoin) indiquant que les semences ont un haut pourcentage de germination lorsqu'elles sont érodées. Après ingestion par les chèvres 7.4 % des semences de* A.decorticans *et 17.4 % des semences de* R. sphaerocarpa ont été récupérés*. L'incubation dans le liquide ruminal et l'ingestion par les animaux ont amélioré la germination des grains de* A. decorticans *(jusqu'à 68.0%). Cependant, pour* R. sphaerocarpa *l'incubation dans le rumen et l'ingestion par les chèvres avaient un faible effet sur la germination des semences. Néanmoins, les semences de* R. sphaerocarpa, *partiellement concassées par la mastication, ont montré un haut pourcentage de germination (moyen 50.0%). Ces résultats suggèrent que le bétail peut être considéré un moyen efficace pour disperser ces deux légumineuses dans le parcours.*

Mot-clés : *Légumineuses, chèvres, endozoochorie, ingestion, rumen, digestion, germination.*

Introduction

Adenocarpus decorticans Boiss. and *Retama sphaerocarpa* (L.) Boiss. (Leguminosae) are two common shrubby species native of the Mediterranean Basin. These species are highly consumed by sheep and goats (Robles and Boza, 1993; Fernández *et al.*, 1997) and are useful plants for recovering degraded lands, given their abundance in communities, large root system, and high canopy cover. As a result, these species have been proposed for the recovery of degraded Mediterranean shrublands and for pasture improvement (Le Houérou, 2001).

Leguminosae is a family where endozoochorous ungulate dispersal is common (Baskin and Baskin, 1998), a feature that may be useful in the management of livestock as well as both shrub cover and regeneration. However, information on the effect of ungulate ingestion on seed dispersal of Leguminosae pasture species of the Mediterranean region is restricted to a few scrubby species (Malo and Suarez, 1995; Malo *et al*., 2000). In addition, studies on endozoochorous mammal dispersal in the Mediterranean Basin generally focus on the identification of dung-borne seeds or on the retention time in the gut (Malo and Suarez, 1995), but information on the effect of gut passage on seed germination is scarce. In this study, we seek to determine the effectiveness of seed dispersal by domestic livestock of *A. decorticans* and *R. sphaerocarpa*. For this, we specifically address the following questions: (i) which is the percentage of seed recovery after the digestive processes, from seed consumption to defecation?; (ii) which is the temporal pattern of dispersal?; and (iii) which is the effect of gut passage on seed germination?

Materials and methods

Study species

A. decorticans, a shrub roughly 2 m high, inhabits degraded Mediterranean forests (mostly *Quercus* and *Abies* forests) in the southern Iberian Peninsula and NW Africa, between 1000-2200 m a.s.l. The fruit is a dehiscent pod, with seeds (2-5 per pod) 4-5 mm long and around 25 mg in weight. *R. sphaerocarpa,* an aphyllous, erect, multi-branched leguminous shrub some 2-3 m high, is common in open, dry sunny habitats between 0-1400 m a.s.l. in the Iberian Peninsula and NW Africa, forming the main canopy cover in many semiarid areas. The fruit is an indehiscent, ovoid monosperm legume (7-9 mm long) with an around 90 mg ovoid seed.

Ripe *A. decorticans* pods were collected at 1600 m a.s.l. in Sierra Nevada National Park (SE Spain, 36°56'N, 3°24'W), in open habitat of degraded *Quercus pyrenaica* forests. *R. sphaerocarpa* pods were collected at 950 m in the Guadix-Baza Basin (SE Spain, 37°26'N, 3°05'W), in degraded semiarid shrublands. Fruits were harvested from at least 20 plants per species. Seeds of *A. decorticans* and pods of *R. sphaerocarpa* were manually extracted and stored in paper bags at room temperature until the start of the experiments.

Seed treatments and germination experiments

Germination experiments were performed in 2002 in a growth chamber at 20 ºC under darkness conditions, which have proved appropriate for these genera (López *et al.,* 1999). Seeds were subjected to the following treatments:

(i) *Mechanical scarification.* Seeds coats were slit with a cutter. This treatment provides valuable information on seed viability of hard-coated seeds, being a reference to compare the effectiveness of other treatments.

(ii) *Ruminal incubation.* Seeds were placed inside a Daisy Incubator with ruminal goat liquor (malagueña x granadina race) diluted 4 times with a McDougall's Buffer Solution and incubated for 24, 48 and 72 h (5 bags per treatment, containing 25 seeds each one). These are the standard times used in experiments to test seed germination and food degradability in ruminants (Molina Alcaide et al., 2000; Robles et al., 2002), and match the usual food-retention times in the digestive tract of these animals (Castro and Robles, 2003). After ruminal incubation, each bag and its content was thoroughly washed with sterile distilled water. The animals were fed with alfalfa hay at maintenance level.

(iii) *Animal ingestion.* Seeds were supplied to penned goats with the usual diet (alfalfa hay). One goat was fed with 100 g of *A. decorticans* seeds (equivalent to 3602 seeds), and another goat with 100 g of *R. sphaerocarpa* pods (equivalent to 1031 seeds). Feces were collected at 48, 72 and 96 h after ingestion. We quantified (i) seed recovery (proportion of seeds recovered with respect to seeds consumed); (ii) pattern of seed dispersal (proportion of seeds recovered after each sampling period); and (iii) germination percentage (estimated for each recovery period). Some of the recovered *R. sphaerocarpa* seeds were partially broken, missing part of the cotyledons but with an intact embryo. These seeds were separated in germination experiments as another treatment ("broken") only for this

species. The number of partially broken recovered seeds for *A. decorticans* was too low to carry out a new test.

(iv) Control. Intact seeds.

Seeds were germinated in glass Petri dishes of 12 cm diameter containing filter paper disks resting on a single layer of 5 mm glass beads. Dishes were initially moistened with 10 ml of distilled water and 5 ml of a suspension of Benomyl fungicide at 0.05%, being thereafter watered as needed with sterilised distilled water. Petri dishes were randomly repositioned within the chamber every 5 days. Germination, identified as visible radicle protrusion, was recorded at intervals of 2-4 days for an overall period of 100 days (32 sampling dates in total). When possible, we used 10 dishes per treatment containing 10 seeds each. Nevertheless, for animal ingestion, some of the treatments reported a lower number of seeds (25 seeds after 96 h in *A. decorticans*, 70 seeds after 72 h and 40 seeds for "broken" treatment in *R. sphaerocarpa*), with Petri dishes and seeds per dishes being adjusted to seed availability (5 to 10 dishes). To prevent fungal attack, seeds were disinfected by immersion in a 1% sodium hypochlorite solution for 10 min before germination, followed by thorough rinsing with sterile distilled water. Non-germinated seeds at the end of the assays were tested for viability with the tetrazolium test (0.1% 2,3,4 triphenyltetrazolium chloride).

Data analysis

Germination was analysed with a Cox's Proportional Hazards semiparametric model using the maximum partial likelihood as the estimation method (Fox, 1993; Allison, 1995). In addition, cumulative germination was compared among treatments with a contingency analysis in order to explore the final result without influence of the shape of the survival curve. Finally, among-treatment differences for cumulative germination were identified by subdividing the chi-square analysis (Zar, 1996). Analyses were performed using JMP 5.0 software. Throughout the paper, means are shown ±SE (calculated per Petri dish).

Results and discussion

Seed recovery after gut passage was 7.4% for *A. decorticans* (all days pooled, 266 seeds). For *R. sphaerocarpa*, seed recovery was 17.4% (179 seeds); of those, 69.8% were undamaged seeds (coats intact), whereas the remaining 30.2% (i.e. 5.3% of recovered seeds) were partially broken but still with undamaged embryo (broken seeds, hereafter; very few seeds appeared broken for *A. decorticans*). Seeds were recovered mostly between 48 and 72 h (39.1 and 50.4% for *A. decorticans*, respectively; 32.8 and 57.6% for *R. sphaerocarpa*, respectively), whereas only a small proportion was recovered after 92 h (11.5% for *A. decorticans*; 9.6% for *R. sphaerocarpa*), with significant differences between days (χ^2analysis, d.f.=2, P<0.05 for the two species).

Germination curves differed among treatments for the two species (*A. decorticans*: L-R χ^2=197.92, d.f.=7, P<0.0001; *R. sphaerocarpa*: L-R χ^2=315.28, d.f.=7, P<0.0001). Similarly, cumulative germination differed among treatments (*A. decorticans*, χ^2=153.30, d.f.=7, P<0.0001; *R. sphaerocarpa*, χ^2=312.45, d.f.=7, P<0.0001). Mechanical scarification boosted the germination of the two species (Fig. 1), with 89.0±2.8% *vs.* 5.0±1.7% for control treatment in *A. decorticans*, and 90.0±4.2 *vs.* 5.0±2.2% in *R. sphaerocarpa*. Incubation in the rumen increased the germination percentage in *A. decorticans*, although without differences among incubation times (average of 36.7%, Fig. 1). Animal ingestion also increased the germination percentage in *A. decorticans*, particularly for seeds collected 92 h after ingestion (68%, Fig. 1). Ruminal incubation or animal ingestion had no significant effect on the germination of non-broken *R. sphaerocarpa* seeds (Fig. 1). However, damage by chewing boosted the germination (60.0% for broken seeds collected 48 h after ingestion, 40.0% after 72 h, and 50.0% after 96 h; see Fig. 1, all data pooled for analysis due to low sample size). Viability of non-germinated seeds was around 95% in all the cases, except for broken treatment, where a fungal infection forced to end of the experiment at day 40.

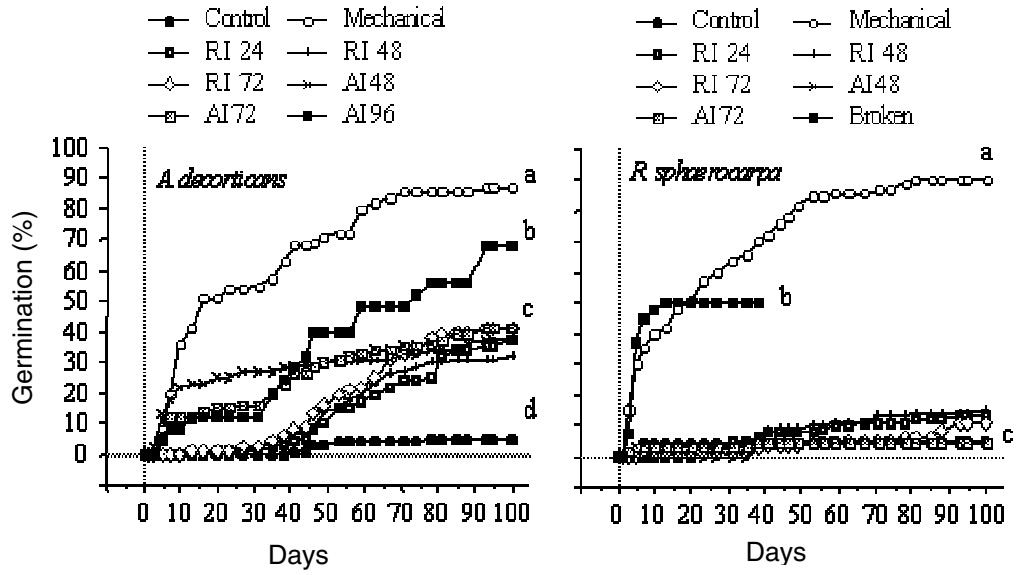

Fig. 1. Percentage of *Adenocarpus decorticans* and *Retama sphaerocarpa* seeds germination at different days from the start of the experiment. Different letters indicate among-treatment differences located at α-level of 0.05 by subdividing the χ^2 test. Mechanical: Mechanical scarification; RI: Ruminal incubation at times 24, 48 or 72 h; AI: Animal ingestion at times 48, 72 or 96 h; Broken: Seeds broken by chewing but with intact embryo. See text for details on treatments.

Our results indicate that the germination of *A. decorticans* and *R. sphaerocarpa* seeds was low (5%) when no treatment was applied (Angosto and Matilla, 1993; López *et al*., 1999). On the contrary, germination was boosted after mechanical scarification (López *et al*., 1999), indicating that seed germination of these two species relies on some mechanisms breaking their coats. In fact, hardseededness is a common feature in Leguminosae species (Baskin and Baskin, 1998; Grouzis and Danthus, 2001).

Mechanisms breaking the dormancy imposed by hard coats are diverse in nature (Mayer and Poljakoff-Mayber, 1989; Baskin and Baskin, 1998), consumption by herbivores being particularly well documented in some families producing dry fruits, including the Leguminosae (Baskin and Baskin, 1998). Seed coats may be eroded and softened during the digestive processes (Izhaki and Ne'eman, 1997), as occurred with *A. decorticans*. Alternatively, seed coats may be broken during chewing, as occurred in *R. sphaerocarpa*, where coats seem to be hard enough to resist digestion as indicated by lack of greater germination after ruminal incubation or for not destroyed seeds after digestion (Izhaki and Ne'eman, 1997). In any case, this gives to animals a potential role as endozoochorous seed dispersers if a fraction of the seeds remains not destroyed (Stiles, 1992). In this sense, our results show that around 5% of the seeds either remained intact and with increased germinability (*A. decorticans*) or lost only a fraction of their content, thereby retaining viability and germinability (*R. sphaerocarpa*). In addition, germination time after animal ingestion or ruminal incubation was reduced considerably. Given the scant germination percentage of untreated seeds and the long time required for germination, our results support the idea that domestic livestock can be an effective mechanism of dispersal in these two Leguminosae species. In addition, all retention times increased the germination percentage of intact or broken seeds, signifying that germinable seeds may be dispersed by domestic livestock throughout a wide area. The fact that a considerable proportion of recovered *R. sphaerocarpa* seeds were broken whereas broken seeds were negligible in *A. decorticans* might be related both to a possible greater hardness of *R. sphaerocarpa* seeds (potential broken seeds of *A. decorticans* being totally destroyed during the digestive processes) as well as to the larger size of *R. sphaerocarpa* seeds, which may imply higher residence in the rumen and thus higher probability of being re-chewed during rumination (Fredrickson *et al*., 1997).

Conclusions

Our results suggest that (i) livestock may be an effective disperser of *A. decorticans* and *R. sphaerocarpa* in Mediterranean shrublands, and (ii) seed germination could be enhanced by herbivore ingestion not only by digestion process but also by chewing.

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