ELSEVIER

Available online at www.sciencedirect.com

SciVerse ScienceDirect

South African Journal of Botany 79 (2012) 19–24

SOUTH AFRICAN JOURNAL OF BOTANY

www.elsevier.com/locate/sajb

Survival and germination of three hard-seeded *Acacia* species after simulated cattle ingestion: The importance of the seed coat structure

P. Venier *, C. Carrizo García, M. Cabido, G. Funes

Instituto Multidisciplinario de Biología Vegetal (UNC-CONICET), Casilla de correo 495, 5000, Córdoba, Argentina

Received 15 August 2011; received in revised form 12 November 2011; accepted 14 November 2011

Abstract

Endozoochory has been suggested as an important mechanism for long-distance plant dispersal that facilitates seed arrival to new and more favorable habitats. The hardness (mechanical strength) and thickness of the seed coat are important characteristics for seed survival and germination after passage through an herbivore gut. We studied the effects of seed passage through cattle gut on seed coat structure in three hard-seeded *Acacia* species that have physical dormancy, *A. aroma*, *A. atramentaria* and *A. caven*, occurring in the semiarid woodlands and shrublands of central Argentina. Histology of the seed coat was examined and a simulated cattle consumption experiment was conducted. Only *A. aroma*, which has the thinnest seed coat (in terms of epidermis and sclerified parenchyma) of the three species, showed a high germination percentage after seed passage through the digestive tract of cattle, whereas seeds of *A. atramentaria* and *A. caven* remained hard and viable. We conclude that the structure of the seed coat in hard-seeded species is crucial in determining the success of endozoochory.

Keywords: *Acacia*; Endozoochory; Germination; Hard seeds; Seed coat

1. Introduction

Endozoochory is an important mechanism for long-distance plant dispersal, and in influencing plant distribution patterns (Fenner and Thompson, 2005; Haarmeyer et al., 2010) by facilitating seed arrival to new and sometimes more favorable habitats (Grubb, 1977; Traveset et al., 2007; Zobel et al., 2000). Successful dispersal by endozoochory depends on seed survival after animal digestion, followed by seed germination and seedling establishment after feces deposition. Seed survival and germination after passage through an animal's digestive tract are critical phases during which ingested seeds are subjected to several damaging processes (D'hondt and Hoffmann, 2011; Gardener et al., 1993a; Peco et al., 2006; Varela and Bucher, 2006). The acidic conditions and the different kinds of enzymes present in the rumen and large intestine can scarify the seed surface (Gardener et al., 1993a) and in some cases can also affect the embryo (Campos et al., 2008).

The probability of seed survival after ingestion by herbivores has been related to seed mass, shape and coat thickness (Peco et al., 2006; Traveset et al., 2007). In legume species, seed survival after cattle digestion has been associated with traits such as coat hardness (mechanical strength) (Gardener et al., 1993a, 1993b; Miller and Coe, 1993), which has in turn been related to water impermeability (physical dormancy — sensu Baskin and Baskin, 1998). It has been shown that physical dormancy of seeds can be broken after passage through an animal's digestive tract (Peco et al., 2006) by the action of both microorganisms that trigger a strong microbial fermentation, and by digestive enzymes (Gardener et al., 1993b). Both processes can alter the seed coat and promote mechanical or chemical scarification, increasing germination probability when physical dormancy is broken (Peco et al., 2006; Van Staden et al., 1989), or seed death when the embryo is damaged (Campos et al., 2008; Samuels and Levey, 2005; Traveset et al.,

* Corresponding author. Tel./fax: +54 351 4331056/2104.
E-mail address: paulavenier@gmail.com (P. Venier).

0254-6299/$ -see front matter © 2011 SAAB. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.sajb.2011.11.005

2007). Although hardness and thickness of the seed coat are important characteristics for seed survival and germination after digestion by large herbivores, the actual effects of passage through the gut on seed coat structure has been little explored in the literature.

Most studies on endozoochory have evaluated the influence of consumption by cattle on species with "hard" vs. "soft" seeds, i.e., seeds with and without physical dormancy, respectively. No studies have explored whether hard-seeded species differ in the degree of seed coat hardness (epidermis height and thickness of the sclerified parenchyma, among other measures) or germination characteristics following ingestion. The genus *Acacia* sens. lat. (Fabaceae: Mimosoideae) includes over 1200 species with a pantropical distribution (Ross, 1981), inhabiting tropical and subtropical regions of the Americas, Australia, Africa, and southern Asia. Twenty species of *Acacia* occur in Argentina, mainly in arid and semiarid regions (Zuloaga and Morrone, 1999). *Acacia* species occurring in Argentina are both trees and shrubs, 2–6 m in height, and their pods are indehiscent in some species and dehiscent in others. Among five *Acacia* species co-occurring in xerophytic woodlands of central Argentina, three species have indehiscent pods (*A. aroma*, *A. atramentaria* and *A. caven*) and two have dehiscent pods (*A. gilliesii* and *A. praecox*). *Acacia aroma*, *A. caven* and *A. atramentaria* are shrubs frequently occurring in open areas within secondary woodlands of Córdoba, Argentina. Seeds of the three species with indehiscent pods are hard, i.e. they have mechanical strength and physical dormancy (Funes and Venier, 2006; Funes et al., 2009; Venier, 2011); their fruits, especially of *A. aroma* and *A. caven*, are consumed by ungulates during the dry season when other food sources are scarce (Abraham de Noir et al., 2002; Aronson, 1992; Gutierrez and Armesto, 1981; Pensiero et al., 2003). Therefore, the regenerative stage of these species could be favored by the presence of cattle in those areas because cattle could facilitate range expansion through fruit consumption and seed dispersal (Grubb, 1977).

In the present study we explored the effects of seed passage through cattle gut on the seed coat structure of three *Acacia* species with indehiscent pods (*A. aroma*, *A. atramentaria* and *A. caven*) occurring in the semiarid woodlands and shrublands of central Argentina. The aims of this study were to: (1) describe the characteristics of the undamaged seed coat in the three hard-seed *Acacia* species; (2) analyze seed survival and germinability after passage through the digestive tract of cattle; and (3) analyze the responses of the seeds in the three species to cattle ingestion in terms of seed coat structure.

2. Materials and methods

2.1. Study area and seed collection

The study area is located in Córdoba province, central Argentina, at about 700 m a.s.l., at the southern extremity of the Gran Chaco, one of the most widely extended seasonally dry subtropical forest formations in South America (Zak et al., 2008). The vegetation is a mosaic of seasonally dry forests dominated by *Aspidosperma quebracho-blanco* and open secondary woodlands and shrublands that can be the result of fires or land clearing (Cabrera, 1976; Cabido et al., 1994; Morello et al., 1985). The climate is predominantly semiarid and monsoonal. Mean annual rainfall is 500 mm, with a long dry season from April to October, and mean annual temperature is 19.9 °C (Zak et al., 2008).

Mature dry hard seeds, i.e. seeds that have mechanical strength and physical dormancy, of *A. aroma* Guilles ex Hook. & Arn., *A. caven* (Molina) Molina, and *A. atramentaria* Benth. were collected from at least 15 individuals and after-ripened in paper bags at room temperature (±22 °C) for 30 days before the start of the experiments.

2.2. Seed coat structure

Mature seeds were scarified with sandpaper and soaked in a 20% HCl solution for ±24 h to soften the seed coats (D'Ambrogio de Argüeso, 1986). Seeds were then cut in thick slices, washed with distilled water, dehydrated in an ethanol-xylene series, and embedded in Paraplast (Sigma, USA). Serial cross-sections 10–12 μm thick were made using a rotatory microtome, stained with 0.05% Toluidine Blue, and observed under a light microscope. Four digital micrographs (1 photo = 1 seed) were taken to measure some histological features of the seed coat (Tables 1 and 2) using the program ImageJ (Rasband, 1997–2009). Two radial lines (from the cuticle to the inner layer of the seed coat) were traced on each micrograph to measure histological characters (Tables 1 and 2).

2.3. Simulated cattle consumption experiment

2.3.1. Ruminal and acid digestion

Seeds of each *Acacia* species were subjected to a simulated herbivore consumption treatment by exposing them to mechanical and chemical attack by both ruminal and acid digestion, and then set to germinate. Seeds were removed from the pods before storage and treatment because the studied seeds are

Table 1
Structural features (mean values ± SE) of the seed coat of three *Acacia* species studied. Different letters indicate significant differences ($p \leq 0.05$) between species.

	A. atramentaria	*A. caven*	*A. aroma*	*P*
Epidermis height (μm)	156.03 ± 1b	131.8 ± 1.1ab	118.62 ± 1.5a	0.0002
No. layers of sclerified parenchyma	35.13 ± 1.3b	33 ± 0.9ab	27.5 ± 0.7a	0.0327
Mean thickness of sclerified parenchyma (μm)	523.9 ± 18b	544.2 ± 10.3b	373.5 ± 12.5a	0.0066
Light line-surface epidermis distance (μm)	54 ± 1.6a	67.8 ± 3b	65 ± 1.2ab	0.0327
Mean diameter of sclerified parenchyma cells (μm)	34.1 ± 1.1b	34.5 ± 1.02b	24 ± 0.5a	0.0132
Mean thickness of sclerified parenchyma cell walls (μm)	3.9 ± 0.15a	5.3 ± 0.1b	4.4 ± 0.1ab	0.0014

Table 2
Statistical significance (p) and values of the F and H statistics for the Analysis of Variance and Kruskal Wallis, respectively, performed to compare seed coat variables between species.

Variable	F	p
No. layers of cells in sclerified parenchyma	111.84	<0.0001
Mean thickness of sclerified parenchyma	157.79	<0.0001
Distance from light line to epidermal surface	155.18	<0.0001
Mean diameter of sclerified parenchyma cells	4.02	0.0207
Mean thickness of sclerified parenchyma cell walls	78.74	<0.0001
	H	
Epidermis height	17.89	0.0013

most likely to pass unharmed through the digestive tract of ruminants when they eat the fruits, as occur in other legumes with similar fruits (Casado et al., 2001; Cecconello et al., 2003; Peinetti et al., 1993). In most cases, when the pods are eaten by cattle, they are digested because they are the fraction that is more nutritive for the cattle. While seeds pass unharmed through the digestive tract because they are not destroyed by chewing, hence they can be frequently encountered in cattle dung fruits (Casado et al., 2001; Cecconello et al., 2003; Peinetti et al., 1993). Moreover, we used seeds out of the pods because our aim was to evaluate the direct mechanical and chemical effects of passage through the digestive tract on seed coat (Samuels and Levey, 2005).

Three replicates (40 seeds each) of the three species were placed in heat-sealed nylon mesh bags. The mesh allowed free passage of microorganisms. All bags were incubated for 48 h inside the rumen of three fistulated cows (Gardener et al., 1993b; Peco et al., 2006) in the "Estación Experimental Manfredi", National Institute of Agricultural Technology (INTA).

Seeds are usually retained in the most acid part of the gut (abomasum and duodenum) for 2–4 h (Warner, 1981); hence, after removal from the rumen, each bag and its contents were rinsed with tap water and then loose seeds (without the bag) were immediately placed for 2 h in a 0.1 N pepsin-hydrochloric acid solution in an oven at 40 °C. The solution was prepared by dissolving 1 g of pepsin in 1 l of HCl 0.1 N (Peco et al., 2006; Pérez et al., 2005). After both ruminal and acid digestion, and before the incubation in germination chamber, the number of germinated, imbibed (permeable, ungerminated seeds that lost physical dormancy), hard (intact, firm, ungerminated, impermeable seeds -still with physical dormancy-), and dead (rotten, digested) seeds were recorded.

2.3.2. Incubation in germination chamber

After both ruminal and acid digestion, imbibed and seeds still hard, and three control seed replicates (each replicate consisting of 20 seeds that had not been subjected to the digestive processes) of the three *Acacia* species were set to germinate for 30 days in growth chambers with 12/12 h light/dark at 25/15 °C, the optimal temperature regime for germination of these species (Funes et al., 2009). Replicates were set in Petri dishes on filter paper saturated with 5 ml of distilled water. After incubation at 25/15 °C, the number of germinated, still hard, and rotten (or digested) seeds was recorded. All seeds that remained hard after incubation at 25/15 °C were scarified with a razor blade and returned to growth chambers for 15 days. Germination of scarified seeds was recorded at the end of the experiment.

2.4. Statistical analysis

An Analysis of Variance (ANOVA) and a *post-hoc* LSD Fisher test were performed to compare continuous variables of seed coat characteristics among species. Epidermis height was analyzed through a non-parametric Kruskal–Wallis

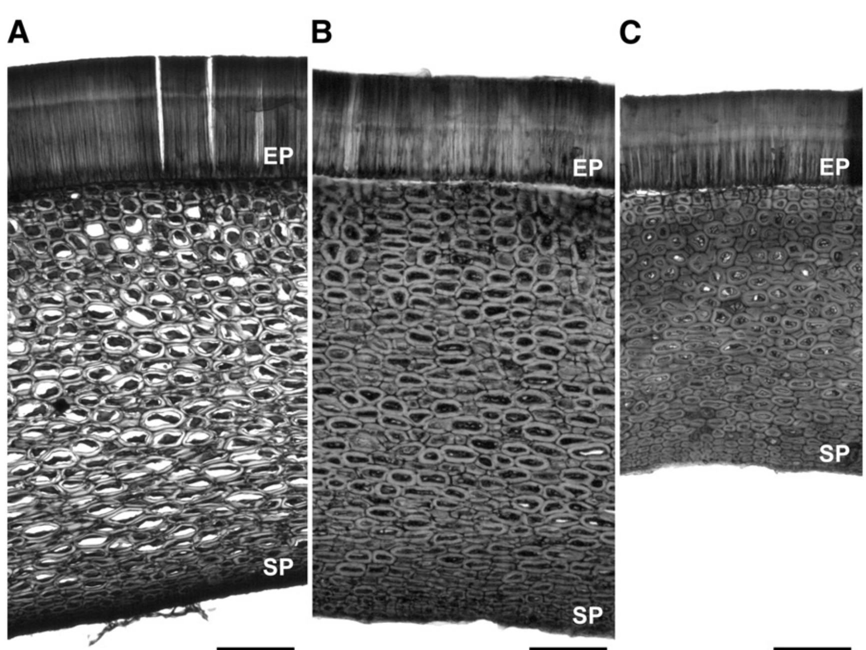

Fig. 1. Micrographs of seed coat of A) *A. atramentaria*, B) *A. caven* and C) *A. aroma*. EP: epidermis; SP: sclerified parenchyma. Scale bars: 100 μm.

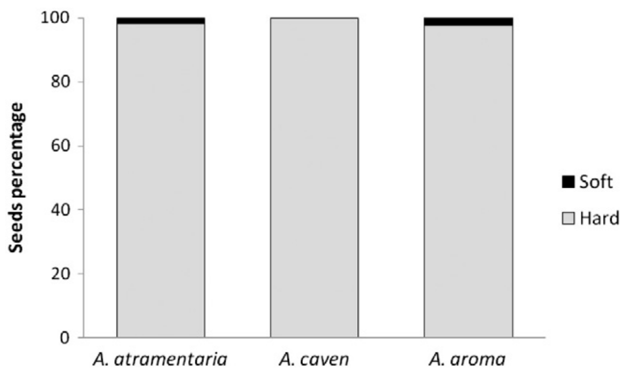

Fig. 2. Percentage of permeable ['soft'] (black) and impermeable ['hard'] (gray) seeds of the three *Acacia* species after simulated cattle ingestion (ruminal digestion + acid digestion).

analysis because this variable does not meet the assumptions of normality and homogeneity of variances (Sokal and Rohlf, 1995). After the simulated herbivore consumption experiment, a Friedman test was used to compare the percentage of hard (intact) and soft (imbibed) seeds between species. A *T*-Test was performed to compare final germination percentages between treated and control seeds within each species (Sokal and Rohlf, 1995).

3. Results

3.1. Seed coat structure

The mature seed coat of the three species exhibited a similar general structure: a single-layered epidermis (palisade-like cells with sclerified walls) and a variable number of sclerified parenchyma layers below it (isodiametric cells in cross section) (Fig. 1). *A. aroma* had the thinnest seed coat (±491 μm) with the shortest epidermal cells, lowest mean thickness of sclerified parenchyma, smallest mean diameter of the sclerified parenchymatic cells and lowest number of cell layers in the sclerified parenchyma. Both *A. atramentaria* and *A. caven* has seed coats of similar thickness (±680 μm) but differed significantly from one another in the dimensions of the component parts (Fig. 1; Tables 1 and 2).

3.2. Simulated cattle consumption experiment: seed survival and germination

3.2.1. Ruminal and acid digestion

After simulated cattle ingestion (ruminal digestion + acid digestion) more than 95% of the seeds of all three species remained hard ($T^2 = 3.25$; $p = 0.1451$) (Fig. 2), with no seeds of *A. caven* and only a few seeds of *A. aroma* and *A. atramentaria* softening ($T^2 = 1.60$; $p = 0.3086$) (Fig. 2). None of the seeds had germinated.

3.2.2. Incubation in germination chamber

Only *A. aroma* exhibited a statistically significant germination percentage (71.5%) after the entire treatment (ruminal digestion + acid digestion + incubation chamber) (Table 3). This contrasts with the control germination of 6.7%. Of the remaining seeds, 9.19% died and 19.36% remained impermeable. Seeds of both *A. atramentaria* and *A. caven* remained almost entirely impermeable after incubation in germination chamber. In addition, the seeds of *A. atramentaria* that were soft (imbibed) after both ruminal and acid digestion died during incubation in the germination chamber. The controls for *A. atramentaria* and *A. caven* showed a very low germination percentage, which was not statistically different from the null germination percentage of the treated seeds (Table 3). For all three species, both control and treated seeds that failed to germinate and were then scarified reached high germination percentages (Table 3), confirming high seed viability after treatment.

4. Discussion

Our results showed that the three *Acacia* species with hard seeds and physical dormancy exhibit two different responses to endozoochory, with seed of *A. aroma* responding positively to simulated ruminal + acid scarification and those of *A. atramentaria* and *A. caven* remaining entirely unaffected. Seed coat thickness has been identified as a predictor trait of the fate of seeds consumed by ruminants (Doucette et al., 2001; Razanamandranto et al., 2004).

Seeds of *A. aroma*, which were stimulated to germinate after simulated passage through the digestive tract of ruminants, had the thinnest seed coat among the tested species, i.e., shortest epidermal cells, smallest mean thickness of sclerified parenchyma, smallest mean diameter of the sclerified parenchymatic cells, and lowest number of cell layers in the sclerified parenchyma. These characteristics are likely to be linked to the greater experimental sensitivity of this species to the action of the digestive acid fluids of cattle in the breaking of physical dormancy, as it has been reported for other species (Peco et al., 2006). The lignified epidermis and epidermis height have been proposed as the main barriers to water uptake for seeds of the three *Acacia* species (Venier et al., 2012).

Table 3
Differences in germination percentage (mean ± standard error) between treated (simulated cattle ingestion) and control seeds among the three *Acacia* species. Results of the *T*-Test are shown for non-scarified seeds (left box). In the right box it shown the differences between treated and control seeds in germination percentage of persistently impermeable seeds that were then scarified. S: scarified seeds.

	% germination				% germination (S)	
	Control	Treatment	*t*	*P*	Control	Treatment
A. atramentaria	1.67 ± 1.67	0	1	ns	88.14 ± 6.09	88.33 ± 5.07
A. caven	1.67 ± 1.67	0	1	ns	100	100
A. aroma	6.67 ± 4.41	71.45 ± 3.23	−11.85	0.0001	96.43 ± 3.57	100

The positive germination response observed in *A. aroma* is in contrast to the pattern shown by *A. atramentaria* and *A. caven*, in which the hard seed coat of these species were not scarified and softened by the action of the microorganisms or of digestive enzymes nor by the raised temperature in the rumen. We assume that the thicker epidermis and parenchyma layers in the seed coat of these two species resist scarification (Baskin and Baskin, 2000; Razanamandranto et al., 2004). Similar results were found in woody species of the African savanna, such as *Acacia seyal*, *Prosopis africana* and *Burkea africana*, in which gut treatment did not improve germination capacity of dormant seeds (Razanamandranto et al., 2004) like in *A. caven* and *A. atramentaria*. Nevertheless, Miller (1995) emphasizes the important role that large hervibores can play in the dispersal of intact seeds.

Our results indicate that the ecological consequence of the effect of endozoochory by cattle will depend on the species ingested. Although seed passage through the digestive tract is unlikely to damage seeds of the three *Acacia* species studied, the effect on germination will differ. In the three *Acacia* species endozoochory is likely to facilitate seed dispersal to different microsites and far away from the mother plant (Grubb, 1977; Traveset, 1998) but only in *A. aroma* is endozoochory by cattle likely to have the additional advantage of breaking physical dormancy and therefore of increasing germination potential in this species. An additional benefit of endozoochory is that cattle feces can provide a suitable microenvironment (temperature and humidity) for germination and seedling survival during the dry and cold winter season in semiarid central Argentina, where this species occurs (Gokbulak and Call, 2004). Scarification during passage through the digestive tract of domestic livestock has been reported for other Fabaceae species, like *Prosopis caldenia* (Peinetti et al., 1993; Peláez et al., 1992) and other species of *Acacia* (Miller, 1995; Van Staden et al., 1989).

Similarly, Renison et al. (2010) suggested that *Prosopis alba* and *P. nigra* may improve their rates of germination after passage through the gut of Greater Rhea (*Rhea americana*). In contrast, Campos et al. (2008) found that seed mortality in *P. flexuosa*, a tree species that coexists with the *Acacia* species studied in this work, increased following passage through the gut of two large herbivores.

Several studies have demonstrated that the effects of the seed passage through the digestive tract of large herbivores vary with the permeability of the seed coat (Gardener et al., 1993a; Razanamandranto et al., 2004; Traveset, 1998). Thus, species with soft, permeable seed coats are usually damaged, whereas species with hard, impermeable seeds coats usually benefit (D'hondt and Hoffmann, 2011). Our study demonstrated that the effects of endozoochory will differ even among hard-seeded species. In the species of *Acacia* studied here, these differences are correlated with the histological characteristics of the seed coats, especially epidermal cell height and parenchyma thickness.

We studied loose seeds because our interest was to analyze the effect of endozoochory directly on the seed coat structure of each species. However, since the seeds of *Acacia* species, including *A. aroma*, may be ingested by large herbivores when they are still inside the pods, further studies should be conducted to evaluate the effect of passage of the entire mature pod through an herbivore digestive tract (Samuels and Levey, 2005) in order to fully understand the role of endozoochory on the dispersion and distribution patterns of the *Acacia* species in central Argentina.

Acknowledgments

Funding for this work was provided by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET — PIP 01286), SECyT (Universidad Nacional de Córdoba), IAI CRN II 2005, and MinCyT Córdoba — Préstamo BID-PID No. 013/2009. We are thankful to Dr. Arnaldo Mangeaud for advising in statistical analysis and to Jorgelina Brasca for her English revision of the manuscript.

References

Abraham de Noir, F., Bravo, S., Abdala, R., 2002. Mecanismos de dispersión de algunas especies de leñosas nativas del Chaco Occidental y Serrano. Quebracho 9, 140–150.

Aronson, J., 1992. Evolutionary biology of *Acacia caven* (Leguminosae, Mimosoideae): intraspecific variation in fruit and seed characters. Annals of the Missouri Botanical Garden 79, 958–968.

Baskin, C.C., Baskin, J.M., 1998. Seeds. Ecology, Biogeography and Evolution of Dormancy and Germination. Academic Press, San Diego.

Baskin, J.M., Baskin, C.C., 2000. Evolutionary considerations of claims for physical dormancy-break by microbial action and abrasion by soil particles. Seed Science Research 10, 409–413.

Cabido, M., Manzur, A., Carranza, L., González Albarracin, C., 1994. La vegetación y el medio físico del Chaco Árido en la provincia de Córdoba, Argentina Central. Phytocoenologia 24, 423–460.

Cabrera, A., 1976. Regiones Fitogeográficas de Argentina. Enciclopedia Argentina de Agricultura y Jardinería 2 (1), 1–85 (2da. Edición. Ed. ACME. Bs. As., Argentina).

Campos, M.C., Peco, B., Campos, V.E., Malo, J.E., Giannoni, S.M., Suárez, F., 2008. Endozoochory by native and exotic herbivores in dry areas: consequences for germination and survival of *Prosopis* seeds. Seed Science Research 18, 91–100.

Casado, C., Benezra, M., Colmenares, O., Martínez, N., 2001. Evaluación del bosque deciduo como recurso alimenticio para bovinos en los llanos centrales de Venezuela. Zootecnia Tropical 19, 139–150.

Cecconello, G., Benezra, M., Obispo, N., 2003. Composición química y degradabilidad ruminal de los frutos de algunas especies forrajeras leñosas de un bosque seco tropical. Zooctenia Tropical 21, 149–165.

D' hondt, B., Hoffmann, M., 2011. A reassessment of the role of simple seed traits in mortality following herbivore ingestion. Plant Biology 13, 118–124.

D'Ambrogio de Argüeso, A., 1986. Manual de Técnicas en Histología Vegetal. In: Hemisferio Sur, S.A. (Ed.), (Buenos Aires, Argentina).

Doucette, K.M., Wittenberg, K.M., McCaughey, W.P., 2001. Seed recovery and germination of reseeded species fed to cattle. Journal of Range Management 54, 575–581.

Fenner, M., Thompson, K., 2005. The ecology of seeds. Cambridge University Press, Cambridge.

Funes, G., Venier, P., 2006. Dormancy and germination in three *Acacia* (*Fabaceae*) species from central Argentina. Seed Science Research 16, 77–82.

Funes, G., Díaz, S., Venier, P., 2009. La temperatura como principal determinante de la germinación en especies del Chaco seco de Argentina. Ecología Austral 19, 129–138.

Gardener, C.J., McIvor, J.G., Jansen, A., 1993a. Passage of legume and grass seeds through the digestive tract of cattle and survival in faces. Journal of Applied Ecology 30, 63–74.

Gardener, C.J., McIvor, J.G., Jansen, A., 1993b. Survival of seeds of tropical grassland species subjected to bovine digestion. Journal of Applied Ecology 30, 75–85.

Gokbulak, F., Call, C.A., 2004. Grass seedling recruitment in cattle dungpats. Journal of Range Management 57, 649–655.

Grubb, P.J., 1977. The maintenance of species-richness in plant communities: the importance of the regeneration niche. Biological Reviews 52, 107–145.

Gutierrez, J.R., Armesto, J., 1981. El rol del ganado en la dispersión de semillas de *Acacia caven* (Leguminosae). Ciencia e Investigación Agraria 8, 3–8.

Haarmeyer, D.H., Bösing, B.M., Schmiedel, U., Dengler, J., 2010. The role of domestic herbivores in endozoochorous plant dispersal in the arid Knersvlakte, South Africa. South African Journal of Botany 76, 359–364.

Miller, M.F., 1995. *Acacia* seed survival, seed germination and seedling growth following pod consumption by large herbivores and seed chewing by rodents. African Journal of Ecology 33, 194–210.

Miller, M.F., Coe, M., 1993. It is advantageous for *Acacia* seeds to be eaten by ungulates? Oikos 66, 364–368.

Morello, J.H., Protomastro, C., Sancholuz, L., Blanco, C., 1985. Serie del Cincuentenario de la Administración de Parques Nacionales 5, 1–53.

Peco, B., López-Merino, L., Alvir, M., 2006. Survival and germination of Mediterranean grassland species after simulated sheep ingestion: ecological correlates with seed traits. Acta Oecologica 30, 269–275.

Peinetti, R., Pereyra, M., Kin, A., Sosa, A., 1993. Effects of cattle ingestion of viability and germination rate of caldén (*Prosopis caldenia*) seeds. Journal of Range Management 46, 483–486.

Peláez, D.V., Bóo, R.M., Elia, O.R., 1992. Emergence and seedling survival of caldén in semiarid region of Argentina. Journal of Arid Environments 32, 173–179.

Pensiero, J., Muñoz, J., De, D., Martínez, V., 2003. Proyecto de invetigación aplicada a los recursos forestales nativos (PIARFON). Alternativa de sustentabilidad del bosque nativo del Espinal. Área etnobotánica. Proyecto Bosque Nativo y Áreas Protegidas-Argentina Banco Mundial-Nº 4085 AR.

Pérez, A.G., Abadía, B., Arreaza, L.C., 2005. Aplicación de una metodología para cuantificar la digestibilidad intestinal proteica en rumiantes. Revista Corpoica 6, 43–47.

Rasband, W.S., 1997–2009. Image J. U.S. National Institutes of Health, Bethesda, Marylandhttp://rsb.infonih.gou//ij.

Razanamandranto, S., Tigabu, M., Neya, S., Odén, P.C., 2004. Effects of gut treatment on recovery and germinability of bovine and ovine ingested seeds woody species from the Sudanian savanna in West Africa. Flora 199, 389–397.

Renison, D., Valladares, G., Martella, M.B., 2010. The effect of passage through the gut of the Greater Rhea (*Rhea americana*) on germination of tree seeds: implications for forest restoration. Emu 110, 125–131.

Ross, J.H., 1981. An analysis of the African *Acacia* species; their distribution, possible origins and relationships. Bothalia 13, 389–413.

Samuels, I.A., Levey, D.J., 2005. Effects of gut passage on seed germination: do experiments answer the questions they ask? Functional Ecology 19, 365–368.

Sokal, R.R., Rohlf, F.J., 1995. Biometry. Freeman, New York.

Traveset, A., 1998. Effect of seed passage through vertebrate frugivores' guts on germination: a review. Perspectives in Plant Ecology. Evolution and Systematics 1 (2), 151–190.

Traveset, A., Robertson, A.W., Rodríguez-Pérez, J., 2007. A review on the role of endozoochory in seed germination. In: Dennis, A.J., Green, R.J., Schupp, E.W., Westcott, D.A. (Eds.), Seed dispersal: theory and its application in a changing world. CABI Publishing, Wallingford, pp. 78–103.

Van Staden, J., Manning, J.C., Kelly, K.M., 1989. Legume seeds: the structure: function equation. In: Stirton, C.H., Zarucchi, J.L. (Eds.), Advances in legume biology. Missouri Botanical Garden, St Louis, MO. Monographs in Systematic Botany 29, 417–450.

Varela, O., Bucher, B.H., 2006. Pasaje time, viability, and germination of seeds ingested by foxes. Journal of Arid Environments 67, 566–578.

Venier, P., 2011. ¿Pueden los caracteres regenerativos explicar la coexistencia de especies de *Acacia* (Fabaceae) en los bosques xerófilos de la Provincia de Córdoba? Tesis de Doctorado en Cs. Biológicas. Facultad de Cs. Exáctas, Físicas y Naturales. Universidad Nacional de Córdoba. Argentina.

Venier, P., Carrizo García, C., Cabido, M., Funes, G., 2012. Physical dormancy and histological features of seeds of five *Acacia* species (Fabaceae) from xerophytic forests in central Argentina. Flora. 207, 39–46

Warner, A.C.I., 1981. Rate of passage of digesta through the gut of mammals and birds. Nutrition Abstracts and Reviews (Series B) 51, 789–820.

Zak, M.R., Cabido, M., Cáceres, D., Díaz, S., 2008. What drives accelerated land cover change in central Argentina? Synergistic consequences of climatic, socioeconomic, and technological factors. Environmental Management 42, 181–189.

Zobel, M., Otsus, M., Liira, J., Moora, M., Mols, T., 2000. Is small species richness limited by seed availability or microsite availability? Ecology 81, 3274–3288.

Zuloaga, F., Morrone, O., 1999. Catálogo de las plantas vasculares de la República Argentina II. Fabaceae-Zygophyllaceae (Dicotyledoneae). : Monogr. Syst. Bot, vol. 74. Missouri Botanical Garden Press, St. Louis, Missouri.

Edited by JC Manning