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

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**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.,

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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 |