

Structural anti-herbivore defense reduction of two Patagonian spiny shrubs in response to long time exclusion of large herbivores



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ABSTRACT

This work focuses on the structural defenses of the two dominant spiny shrub species of the Patagonian shrub-grass steppe. We compared the amount of structural defenses (spines and thorns) of *Mulinum spinosum* (Cav.) Pers. (Apiaceae) and *Adesmia volckmannii* Phil. (Fabaceae) between grazed and long term non-grazed plots. *M. spinosum* showed a higher spinescence than *A. volckmannii*. Moreover, both species plants located in grazed plots showed higher spinescence than those located in non-grazed ones. Therefore, we can conclude that (1) *M. spinosum* has higher spinescence than *A. volckmannii*, probably because sheep prefer *M. spinosum* flowers than *A. volckmannii* leaves; and (2) both *M. spinosum* and *A. volckmannii* showed an increase of spinescence induced by herbivory. These responses may be evidences of co-evolution between these shrub species and large wild browsers that guarantee an adequate capacity to tolerate herbivory disturbance. Such capacity would be important to maintain community functioning, because shrubs provide secure sites for seed germination and, in some cases, are able to set symbiotic interactions with N-fixing bacteria.

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1. Introduction

Domestic animal husbandry may cause severe degradation and reduction of species richness and diversity in ecosystems that have not co-evolved with large herbivores (Freeland, 1990; Milchunas et al., 1988). In these systems, desertification and loss of most preferred plant species are commonly seen as consequence of exotic herbivores overgrazing (Milton et al., 1994). Instead, many plants that evolved under herbivore selection developed several defense traits (Strauss and Agrawal, 1999).

Many plants exhibit structural anti-herbivore defenses; i.e. morphological or anatomical traits that confer a fitness advantage to a plant by deterring herbivores from feeding on it (Hanley et al., 2007). Moreover, some well adapted plants are able to increase their defenses in response to herbivory (Karban and Baldwin, 1997) and to achieve considerable regrowth rates after normal levels of herbivory (Strauss and Agrawal, 1999). For example, the density of

thorns of *Acacia* trees is higher below than above 4 m, the maximum height at which giraffes usually feed in African savannahs (Milewski et al., 1991), and in browsed than in non-browsed trees at Negev desert (Rohner and Ward, 1997). Moreover, thorn length of species from the same genera was reduced in 40% after five years of herbivore exclusion (Young et al., 2003). As aridity and herbivory are convergent selection factors (Milchunas and Lauenroth, 1993), we use the term “defense” in a broad sense, since plants spinescence may have not been selected only in response to herbivory but could be an exaptation from traits developed as aridity adaptations (Gould and Vrba, 1982).

In Patagonia, domestic animal husbandry caused severe changes in soil and plants across many environments (Golluscio et al., 1998). There, the history of grazing with domestic herbivores is short, because sheep husbandry began in the late XIX century (Milchunas et al., 1988). However, the region has been grazed by wild guanaco (*Lama guanicoe* Müller) for the past 10,000 years (Lauenroth, 1998). This grazing pressure must have been relatively high, taking into account the XIX century reports of the existence of more than 1000 individual guanaco troops (Moreno, 1879). Such co-evolution between vegetation and wild herbivores could explain the common anti-herbivore defenses found in plants of the Patagonian steppe,

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which include structural and chemical strategies (Lauenroth, 1998). Supporting that, in north-western Patagonia, three species of *Berberis* showed increased density and length of thorns in response to a higher risk of herbivory after being affected by fire (Gowda and Raffaele, 2004). In the Western district of Patagonian phytogeographical province, the two dominant spiny shrubs are *Mulinum spinosum* (Cav.) Pers. (Apiaceae) and *Adesmia volckmannii* Phil. (Fabaceae). In spite of being well defended plants, their cover decreases as grazing pressure increases (Perelman et al., 1997) because both species are browsed by large herbivores (wild and domestic) during short periods of time in spring and summer (Bonvissuto et al., 1983).

M. spinosum has foliar spines and *A. volckmannii* has stem thorns (Burkart et al., 1971; Constance, 1971). Both species are important protein and phosphorus sources (Somlo et al., 1985) but are differentially preferred by sheep. They mainly browse on *A. volckmannii* twigs and *M. spinosum* young leaves at the beginning of the growing season (October–November), before these become lignified, and on the inflorescences of *M. spinosum* and leaves of *A. volckmannii* during the rest of spring and summer (Bonvissuto et al., 1983). During this season, ewes are in the second or third lactating month and their phosphorus requirements are the highest in the whole year (NRC, 1985). Probably, sheep prefer *M. spinosum* because the phosphorus content of its flowers is higher than that present in *A. volckmannii* leaves towards the end of the growing season (Somlo et al., 1985). This could be the reason for a higher spinescence in *M. spinosum*. The flowers and fruits of *M. spinosum* and *A. volckmannii* are spineless, but they are surrounded by spines and thorns, respectively (Burkart et al., 1971; Constance, 1971). Possibly, as stated for *Acacia tortilis* (Gowda, 1996), protection of flowers and fruits would be positively correlated with spine and thorn density in neighboring branches. Although it was proven that secondary compounds did not change in *M. spinosum* and *A. volckmannii* in response to herbivory (Cavagnaro et al., 2003), no information exists on the effect of herbivory on structural defenses in these two species.

The aim of this work is to show that *M. spinosum* and *A. volckmannii* are able to cope with large domestic herbivores impact by increasing their inducible structural defenses. Therefore, we will characterize the constitutive and inducible structural anti-herbivore defenses of these two spiny shrubs. Our first hypothesis proposes that *M. spinosum* will have higher spinescence than *A. volckmannii* in both grazed and non-grazed sites, and our second hypothesis proposes that both species will show higher spinescence in grazed than in non-grazed sites.

2. Material and methods

2.1. Site description

The experiment was conducted at Río Mayo INTA (Instituto Nacional de Tecnología Agropecuaria) experimental field (SW Chubut - Argentina; lat 45° 25'S, long 70° 20'W), during the summer 2004. We compared plants of both species located within and outside an 8 ha, 20-years old enclosure settled in 1983. The dominant plant community is a grass-shrub steppe characterized by *Pappostipa speciosa* (Trin. et Rupr.) Romaschenko; Poaceae (coirón amargo), *Adesmia volckmannii* Phil.; Fabaceae (mamuel choique), *Berberis heterophylla* Juss.; Berberidaceae (calafate) and *Poa lanuginosa* Poir.; Poaceae (pasto hilo). Soils are Calciorthids (Golluscio et al., 1982) of coarse texture with low water holding capacity and without alkalinity or salinity problems. They cover uniformly all the study site so nutrients and water availability were the same for plants in both treatments. The average annual precipitation is 150 mm, and 70% of it falls in autumn-winter, when mean monthly

temperature varies from 2 °C to 7 °C (Sala et al., 1989).

2.2. Experimental approach

To study induced responses to large browsers impact, we selected plants grown before the enclosure was installed (>20 years old). There is no information about dendrochronology for *A. volckmannii* and *M. spinosum* show no growth rings since the stem has a wire-like structure (Ancibor, 1986). Consequently we used plant diameter as an age indicator (Damascos and Ghermandi, 2007; Fernández et al., 1991; Oñatibia et al., 2010). Twenty plants of each species located along a 20 m transect in the non-grazed area and another twenty situated in moderated grazed areas were measured in order to determine the most common plant size (modal size) present in the community. Afterwards, we ensured that modal-sized plants corresponded to individuals more than 20 years old (Fernández et al., 1991, 1992; Oñatibia et al., 2010). Plants modal size analysis revealed that most common plant diameter class was 0.61–0.65 m for *M. spinosum* individuals located in grazed and non-grazed sites. Most common diameter class for *A. volckmannii* individuals was 0.85–1 m in grazed and non-grazed sites. Considering those values, we estimated age of plants according to data presented by Cipriotti and Aguiar (2010) who measured *M. spinosum* and *A. volckmannii* plants growing in a plot where shrubs had been removed 15 years before. They found that *M. spinosum* and *A. volckmannii* grew, approximately, 1.8 and 4.2 cm in diameter per year, respectively (data reprocessed from Cipriotti and Aguiar, 2010), in Río Mayo Experimental Field. Thus, we concluded that *M. spinosum* and *A. volckmannii* shrubs included in the most common diametric classes were older than 20 years old. *M. spinosum* is a typical compact cushion-shaped shrub (Ancibor, 1986; Constance, 1971; Damascos and Ghermandi, 2007) and *A. volckmannii* is an inverted cone-shaped shrub with long and sparse branches (Burkart et al., 1971) (Fig. 1).

M. spinosum density of spines is higher than *A. volckmannii* density of thorns (0.77 vs. 0.54 spines/thorns.cm⁻², respectively; $F = 26.92$; $P = 0.0001$; no differences between grazed and ungrazed sites). However, the effectiveness of these structural defenses depends not only on spines/thorns density but also on their spatial distribution and orientation. Therefore, to take into account not only spine and thorn density but their spatial distribution and arrangement, we developed a perforation density measuring device which simulated an adult sheep mouth approaching the shrub to browse on it (Fig. 2 a and b).

This device measures the density of perforations per area unit produced by spines and thorns on a sensitive membrane. The device consists in a 7.5 × 7.5 × 7.5 cm one-side open cube containing a piston that holds a 6.5 × 6.5 × 2.5 cm piece of foam rubber covered with a sensitive membrane (freezer film). The open side of the cube is surrounded by a metal plate that restricts the device approximation to the plant. Once the device is set beside the plant, the piston moves forward slowly, by means of a screw, till the plant spines or thorns perforate the film (Fig. 2 b and c). After measurement, the film is unfolded from the piece of foam rubber and the perforations left on it are counted.

We chose 10 modal-sized plants of each species along a transect line within the enclosure and 10 modal-sized plants of each species in another transect line situated in a moderately grazed (0.3 sheep.ha⁻¹) neighboring area historically grazed by ewes and lambs during growing season (November–January). We could not avoid a pseudoreplicated design (Hurlbert, 1984) because it was impossible to achieve true replications of such an old and big enclosure (Hargrove and Pickering, 1992). In the grazed area, as not all plants of any species are usually defoliated (Golluscio et al., 1998, 1999), we chose individuals showing clear signs of have being



Fig. 1. *Mulinum spinosum* (left) and *Adesmia volckmannii* (right) plants.

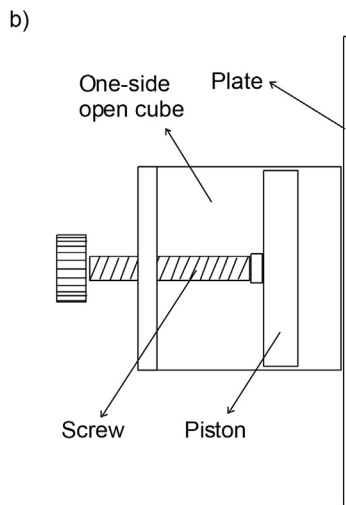
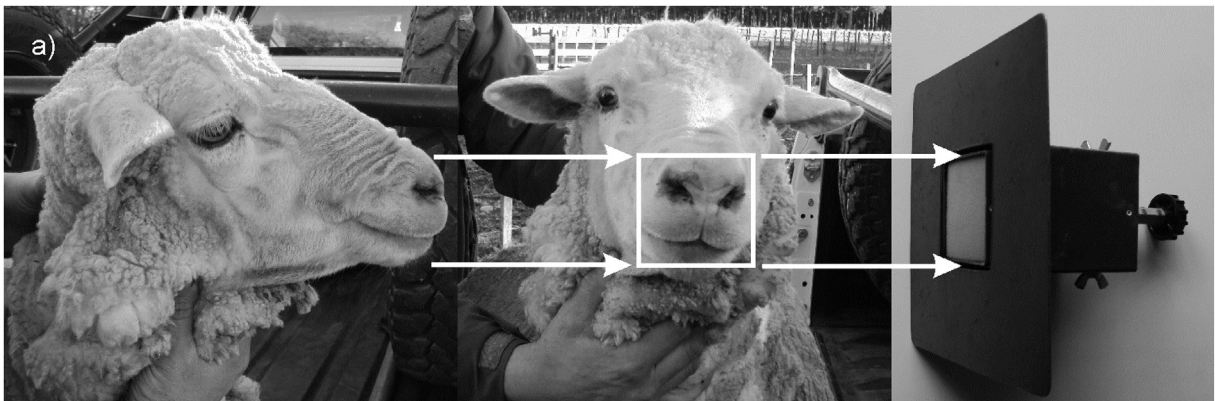


Fig. 2. (a) Comparative view of the device and sheep mouth, (b) Perforation density measuring device (device side view) and (c) Measuring density of perforations on a *Mulinum spinosum* shrub.

browsed. The 40 individuals were situated 5 m apart from each other and were taken as experimental units, so every measurement

performed on each plant was a subsample.

Before beginning the experiment, we performed several test

measurements per plant and the standard deviation found within each individual was very low ($SD = 0.04$). At the beginning of summer, we performed two measurements per plant (subsamples), at a constant height of 20 cm above soil level, the one at which ewes usually feed, because plants are tall and wide enough to avoid ewes from feeding on their top (Cavagnaro and Golluscio, pers. obs.). We aimed to record changes in spinescence only in plant parts effectively browsed by sheep (Milewski et al., 1991). Lambs do not graze or even browse in this season: They are one to three months old and still depend on mother's milk (Helman, 1951), since their rumens are not completely developed yet (Leat, 1970).

2.3. Statistical analyses

To analyze data of perforation density, we used a two-way factorial species (*M. spinosum* vs. *A. volckmannii*) \times grazing (grazed vs. non-grazed) analysis of variance (ANOVA), with a completely randomized design. All statistical tests were performed using InfoStat statistical package (InfoStat, 2002).

3. Results

M. spinosum showed higher level of structural defenses (0.11 perforations.cm⁻²) than *A. volckmannii* (0.07 perforations.cm⁻²) ($F = 14.86$; $P = 0.0005$; Fig. 3). *M. spinosum* and *A. volckmannii* showed higher perforation density in grazed than in non-grazed treatments (0.13 vs. 0.09 perforations.cm⁻² and 0.09 vs. 0.06 perforations.cm⁻², respectively) ($F = 14.13$; $P = 0.0006$; Fig. 3). We didn't find any significant grazing \times species interaction ($F = 0.11$; $P = 0.741$; Fig. 3).

4. Discussion

A proposed theory supporting the co-evolution of wild large herbivores and plants predicts that plants and their predators are involved in a constant adaptive dynamic (Berenbaum and Zangerl, 1998) and that, sometimes, herbivores are capable of overcoming plant resistance (Thompson, 2005). Our results suggest that ewes (and even guanacos) prefer *M. spinosum* because of its higher level of phosphorus in flowers and fruits than that in leaves of *A. volckmannii* during lactating months.

The structural responses to grazing supported our hypothesis stating that *M. spinosum* and *A. volckmannii* would show higher

spinescence in grazed than in non-grazed plots. Plants that have been excluded from grazing during 20 years relaxed their structural defenses, showing inducible responses in the face of tissue loss. According to our preliminary experiment, individuals chosen were there before the enclosure was built. Therefore, observed changes in spinescence of these individuals should necessarily be induced by sheep grazing. We can exclude guanaco and choique (*Pterocnemia pennata* d'Orbigny) as possible large browsers today because their presence in the area of study is only occasional (Cavagnaro pers. obs.). Finally, European hare (*Lepus europaeus* L.), although abundant, feeds basically on grasses like those from *Pappostipa* and *Poa* genera and on graminoids like *Carex* and *Juncus* genera (Somlo et al., 1997).

We can conclude that (1) *M. spinosum* has higher spinescence than *A. volckmannii*, probably because sheep prefer *M. spinosum* flowers than *A. volckmannii* leaves; and (2) both *M. spinosum* and *A. volckmannii* showed an increase of spinescence induced by herbivory. However, the way in which *M. spinosum* and *A. volckmannii* vary their spinescence is not very clear and merit new studies since they vary their spinescence without varying their spines/thorns density. In the case of *M. spinosum*, every leaf is transformed in a three-pointed spine and its total spinescence is extremely high (Ancibor, 1986; Constance, 1971). Therefore, the induced response found in this experiment might be a consequence of a spatial rearrangement of spines, resulting from branches with similar spinescence but growing closer (Pisani and Distel, 1999) or spines changing their insertion angle, as a consequence of large browsers herbivory. In the case of *A. volckmannii*, plants are inverted cones with sparse and dispersed branches (Fernández et al., 1991; Oñatibia et al., 2010). As a consequence, the induced increase of effective thorns in response to grazing could be due to an indirect effect, similar to the proposed for *M. spinosum*, resulting from a spatial rearrangement of thorns (Pisani and Distel, 1999) or it could also be the result of a reduced growth rate on browsed plants similar to that stated by Milewski et al. (1991) for *Acacia seyal* which produced shorter internodes in conditions of heavy browsing.

M. spinosum has a high arbuscular micorrhizal fungi colonization (Fontenla et al., 2001), *A. volckmannii* has rhizobial nodules (Golluscio et al., 2006, 2014), and both species have a crucial role in the installation of new individuals of grasses (Soriano et al., 1994). As a consequence, their browsing resistance would be important to maintain the community functioning, both from biogeochemical and demographic points of view.

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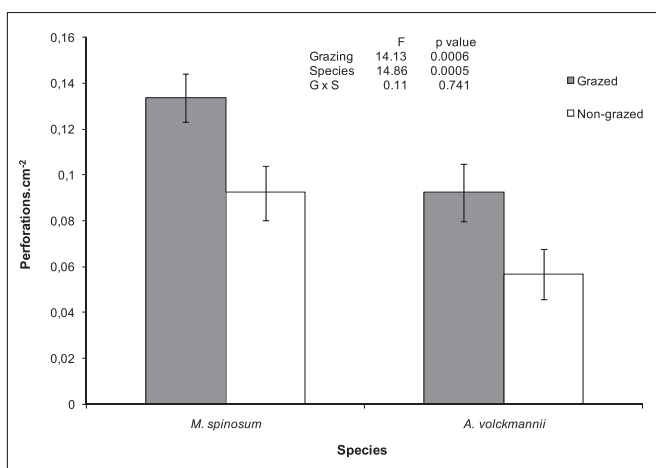


Fig. 3. Perforation density (perforations.cm⁻²) for the two species at the two grazing levels. Bars represent Standard Errors. The table consigns the F values obtained in ANOVA test, with P levels.

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