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Long-term plasticity in growth, storage and defense allocation produces drought-tolerant juvenile shrubs of *Prosopis alpataco* R.A. Philippi (Fabaceae)

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ABSTRACT

Evaluation of phenotypic plasticity of plants is important to predict the long-term fate of populations exposed to environmental change. Climate scenarios predict a decrease in rainfall and increase in temperature for Northern Patagonia (Argentina). The long-term assessment of the effect of water shortage on allocation patterns of *Prosopis alpataco* provides insights into how climate change could affect this dominant shrub of the Monte Desert. A single-factor (water supply) field experiment was conducted. Phenotypic plasticity in biomass partitioning and allocation to storage and defense was assessed over the course of pre-reproductive growth during five years. Water-effect and size-dependent effects were sorted out. Our results indicate that as plants grow larger, root:shoot ratio increases, as well as total non-structural carbohydrates pool, irrespective of water treatment. Increasing belowground allocation through partitioning to reserves instead of allocation to non-storage mass, favors carbohydrate forms that later can be mobilized. Spine mass ratio increased 3-fold in response to drought. These conservative strategies might facilitate the persistence of *Prosopis alpataco* in a novel and drier environment, through the production of drought-tolerant juvenile individuals.

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Introduction

Individual organisms can alter their morphology, development, physiology and life history depending on environmental conditions (Hautekèete et al., 2009; Sultan, 2000). Increasing evidence indicates that plasticity can play a major role in the ecological distribution and evolution of a species because it permits individual genotypes to produce versatile phenotypes able to grow and reproduce in several different environments (Hegazy et al., 2010; Pfennig et al., 2010; Sultan, 2003; Weiner, 2004). The evaluation of this plasticity is important to predict the long-term fate of populations exposed to environmental change since it may allow a species to buffer short-term changes and maintain viable populations (Ghalambor et al., 2007).

Climate scenarios predict decrease in rainfall and increase in temperature for most semiarid regions in the world (IPCC, 2007). Particularly for Northern Patagonia, linear trends in regionally averaged annual and seasonal temperature and precipitation records indicate significant warming and drought over the last century (Masiokas et al., 2008). Water availability is the main limiting factor for plant growth in the Patagonian steppe, because the wet season (winter and early spring) and the growing season do not coincide. As a consequence, water availability accounts for 22–56%

of the variability in the cover of the main species (Jobbágy, 1993). Therefore, to examine the effect of water availability on dominant shrubs is not only important for a better understanding of species, but also for providing insights into how rainfall decrease and climate change could affect semiarid ecosystems (Padilla et al., 2010). All together, studies of phenotypic plasticity in response to environmental changes are transferable into distribution modeling (Thuiller et al., 2008).

In this paper we evaluate the response of *Prosopis alpataco* R.A. Philippi (Fabaceae), a dominant shrub in the Monte Desert of Argentina, to higher aridity by manipulating water supply in a long-term field experiment. This species withstands high variation in water availability throughout its distribution area, from La Rioja (Lat. 28°S) to Patagonia (Lat. 43°S; Cabrera, 1994). Mean height of plants decreases from North to South, ranging from 3 m in the northern populations to 0.3 m in the southernmost area of distribution (Palacios and Brizuela, 2005). We tested phenotypic plasticity in biomass partitioning and allocation to storage and defense over the course of pre-reproductive growth, during five years.

Environmental influences can cause significant shifts in investments towards needed structures or functions (de Kroon et al., 2009) such as storage and defense. Total non-structural carbohydrates (TNC), as regulators of physiological adjustments to sub-optimal environments (Bond and Midgley, 2001; Kabeya and Sakai, 2005; Palacio et al., 2006; Tursun et al., 2011; Wang et al., 2007), constitute a key factor influencing carbon balance and survivorship of juvenile perennials under resource limitation (i.e. Imaji

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Table 1
Mean values (±S.E.) of traits of juvenile *Prosopis alpataco* grown under field conditions in the Chubut River Valley ("mean" columns). For each trait, General Linear Model (GLM) was applied for the effects of treatment, with total biomass (ln transformed) as a covariate. A significant treatment effect indicates a response to water availability and a significant biomass effect indicates that the trait changes as plants are growing. A significant interaction between them ($B \times T$) indicates that the trajectory of development differs between the treatments. GLM statistics are *F*-values. Shrubs were subjected to full irrigation (control) and drought-stressed. LMA, leaf mass area. ns, non-significant.

Trait	Mean		GLM (covariate: ln total biomass)		
	Control	Drought-stressed	Biomass	Treatment	$B \times T$
Total plant mass (g D.W.)	47.31 (9.1)	22.60 (5.5)	–	–	–
Allocation to roots (% total mass)	46.74 (19.7)	42.72 (18.2)	9.33***	0.03ns	0.18ns
Structural root mass (% total mass)	35.46 (1.34)	33.62 (1.27)	2.79ns	0.03ns	0.31ns
TNC$_{root}$ mass (% total mass)	11.29 (0.77)	9.10 (0.63)	17.19***	0.64ns	0.48ns
Root TNC concentration (% root mass)	23.93 (0.90)	21.07 (0.78)	12.89***	1.22ns	0.17ns
Allocation to stems (% total mass)	53.25 (19.7)	57.28 (18.2)	9.33***	0.03ns	0.18ns
Structural stem mass (% total mass)	42.36 (1.45)	47.56 (1.55)	9.34***	1.56ns	0.02ns
TNC$_{stem}$ mass (% total mass)	10.98 (0.79)	9.72 (0.42)	8.70***	7.04***	2.14ns
Stem TNC concentration (% stem mass)	20.33 (1.02)	16.99 (0.53)	0.22ns	7.52***	2.42ns
Root:shoot ratio	0.92 (0.06)	0.76 (0.06)	7.01*	0.47ns	0.09ns
Spine mass ratio (g g^{-1} total mass)	0.08 (0.02)	0.25 (0.03)	12.02***	53.18***	11.66***
Root TNC concentration (% root D.W.)	21.07(0.78)	23.94(0.09)	8.47***	1.45ns	1.21ns

* $P < 0.05$.
*** $P < 0.01$.

and Seiwa, 2010; Kobe et al., 2010; Kobe, 1997; Myers and Kitajima, 2007). The TNC pool of the stems is particularly important for growth since withdrawal of TNC from stems precede withdrawal from roots (McPherson and Williams, 1998) and wood formation requires the supply of large amounts of nonstructural carbohydrates to produce cellulose, hemicelluloses and lignin (Luo et al., 2006). Therefore, it is our first prediction (1) that control plants of *Prosopis alpataco* growing under high watering treatment will accumulate more biomass, have a higher stem TNC concentration, and consequently, TNC$_{stem}$ mass than drought-stressed plants. Our second prediction (2) is that water shortage will provoke an increase in roots TNC concentration of juvenile plants.

Allocation to defense is another key sink for perennials growing in low-resource environments. Leaves and branches of *Prosopis alpataco* are defended by conspicuous spines (Burkart, 1976). This species is noticeably green and attractive to domestic and wild herbivores (i.e. sheep, guanaco and insects) during summer. Considering that the replacement of resources lost to herbivores becomes more costly as potential growth rate becomes more limited by water availability (Coley et al., 1985), browsing by animals is usually reduced by the presence of spines (Gowda, 1996) and relative allocation of biomass to spines in *Prosopis* species appears to be affected by resource availability (Pisani and Distel, 1996). Our third prediction (3) is that allocation to spines (i.e. spine mass ratio) will be higher in plants subjected to drought-stress during the juvenile period.

Given the potentially large intra-specific variability of *Prosopis* in size, habit and life history (Burkart, 1976; Vilela et al., 2003; Villagra and Cavagnaro, 2006), we expect watering treatments to provoke differences in relative growth rate and consequently, size disparity between groups (i.e. plants subjected to drought-stress will be smaller than control plants). Therefore we incorporated the effect of plant size to test the watering effect on allocation patterns (Moriuchi and Winn, 2005).

Materials and methods

Plant material and experimental design

Prosopis alpataco is a spiny shrub, with aerial shoots thin, erect, and flexuous, with pairs of stipule-derived spines at the basis of the drought-deciduous pinnate leaves. Bulk seeds were collected from a native population located in Neuquén, Argentina (39°15′28″S; 68°47′58″W). Mean annual precipitation of this area is 310 mm. Shrubs of this population are of 2.10 ± 0.20 m height.

Mechanical scarification was applied to the seeds to promote germination (Vilela and Ravetta, 2001). Seeds were sown in germination trays filled with peat moss and sand in equal proportions in October 2000. One year later, seedlings were transplanted to the field. The experiment was conducted in the ecotone between the Monte Desert and Patagonia phytogeographical regions, in a farm named Bod Amlwg, located in Treorci, Chubut River Valley, Patagonia Argentina (43°16′57″S; 65°24′47″W). In this area, temperature is similar to the place where seeds were collected (mean minimum temperature of the coldest month is 1 °C; absolute minimum air temperature is −10.8 °C; Cabrera, 1994) but water availability is lower (mean annual precipitation 179 mm vs. 310 mm). The northern part of Chubut has the greatest proportion of sunshine hours of Patagonia (50%) and the annual average solar radiation is 180 W m^{-2} (Paruelo et al., 1998; www.inta.gov.ar/chubut).

A single-factor (water supply) field experiment, with a completely randomized design was conducted. Experimental units consisted of plots (3 plots per treatment, 25 plants per plot, for a total of 75 shrubs per treatment). Rows and plants within a row were 1.5 m apart. Plants were randomly assigned to a plot and watering levels were randomly applied to plots. Two watering levels were applied: low watering (drought-stress) and high watering (control). Control plots were irrigated to field capacity (approximately 15 mm) every seven days, from transplant to final harvest. Plants in the low watering treatment were irrigated approximately every 21 days, when the rate of CO_2 assimilation of drought-stressed plants was significantly lower than that of control plants (3.8 ± 1.37 μmol CO_2 s^{-1} m^{-2} vs. 18.07 ± 1.13 μmol CO_2 s^{-1} m^{-2}; $P < 0.01$). The frequency of water application for the low watering treatment was determined by the rate of CO_2 assimilation, used as a physiological indicator of plant stress (Baker et al., 2007). For this is purpose, assimilation was measured weekly in early spring (3 repetitions in September). Briefly, CO_2 uptake was recorded using a portable infrared analyzer open gas exchange system (ADC, Hertfordshire, UK). Measurements were recorded during mid-morning (10–12 am) in the last three fully expanded leaves per plant (3 plants per plot; 3 plots per treatment; Vilela et al., 2003). Each measurement took ca.10 s. Watering was applied, for both treatments, from September to April, when plants are in full-leaf. During the wintertime, dormant plants were not irrigated. Weed control was done by hand pulling. To examine allometric trajectories, plants were harvested 18, 30, 42 and 54 months after sowing (3 repetitions/plot; 3 plots/treatment). Harvested plants were chosen at random. All plant material was oven-dried to constant weight at 60 °C before weighing.

Morphological and allocation variables

The following variables were measured/calculated: (a) growth variables: total biomass; structural root mass (% total mass); structural stem mass (% total mass) and root to shoot ratio (root mass/stem mass; R: S); allocation to roots and to stems (structural mass + TNC mass expressed as a function of total plant mass); (b) storage variables: total non-structural carbohydrates content in root (TNC_{root} mass) and stems (TNC_{stem} mass); root TNC concentration and stem TNC concentration; (c) defense variable: spine mass ratio (spine mass/stem mass).

Leaf mass was not included in the analysis because *Prosopis alpataco* is a deciduous shrub and the timing of leaf fall depends on environmental conditions. Summer drought, wind, or early freezing temperatures provoke unexpected partial or total leaf shedding. As this is a long-term study, in order to homogenize measurements among years, leaves were not considered. Spine mass ratio was calculated according to Hanley et al. (2007).

Total non-structural carbohydrates were determined by autoclaving (0.1 MPa, 15 min) 50 mg of biomass in 100 ml of distilled water. Polysaccharides (including starch) by this way are hydrolyzed into simple sugars. Dissolved sugars were determined by the anthrone method (Sadasivian and Manickam, 2010; Yemm and Willis, 1954).

Data analysis

Biomass variables were transformed to their natural logarithms before analysis to meet the assumptions of normality and homoscedasticity associated to General Linear Models. For each trait included in Table 1, we performed a generalized linear model for the effects of treatment with total biomass as a covariate. A significant treatment (water) effect indicates a plastic response to resource availability and a significant biomass effect indicates that the trait changes as plants grow (size-dependent change). Non-significant interactions indicate that the trajectories of development do not differ between treatments (Moriuchi and Winn, 2005). Natural log transformed plant biomass data were regressed on defense, storage and structural variables to describe the ontogenetic trajectory within each treatment. Treatment comparisons are based on differences in mass partitioning to different plant organs (i.e., organ mass as a function of total plant mass; Kobe et al., 2010) and are not based on absolute differences in organ mass.

The allometric relationship ln shoot mass vs. ln root mass best fitted a linear regression model. Regression coefficient (slope) of this relationship was tested in each treatment using a Student's *t*-test to determine if they were statistically different from 1 (McConnaughay and Coleman, 1999). The slopes of fitted curves were statistically compared between treatments using *F*-test (Fig. 2a).

Results

### Water effect on allocation variables

Prosopis alpataco showed a significant plasticity in growth rates in response to water availability: drought-stressed plants accumulated half the biomass of control plants (Table 1). TNC_{stem} mass and stem TNC concentration as a function of whole plant mass differed between treatments (Fig. 1a and b). This supports our first prediction, plants growing under high watering show a higher total plant mass and stem TNC concentration than drought-stressed plant, which leads to a higher proportion of stem carbohydrate reserves (TNC_{stem} mass; Table 1) in the former.
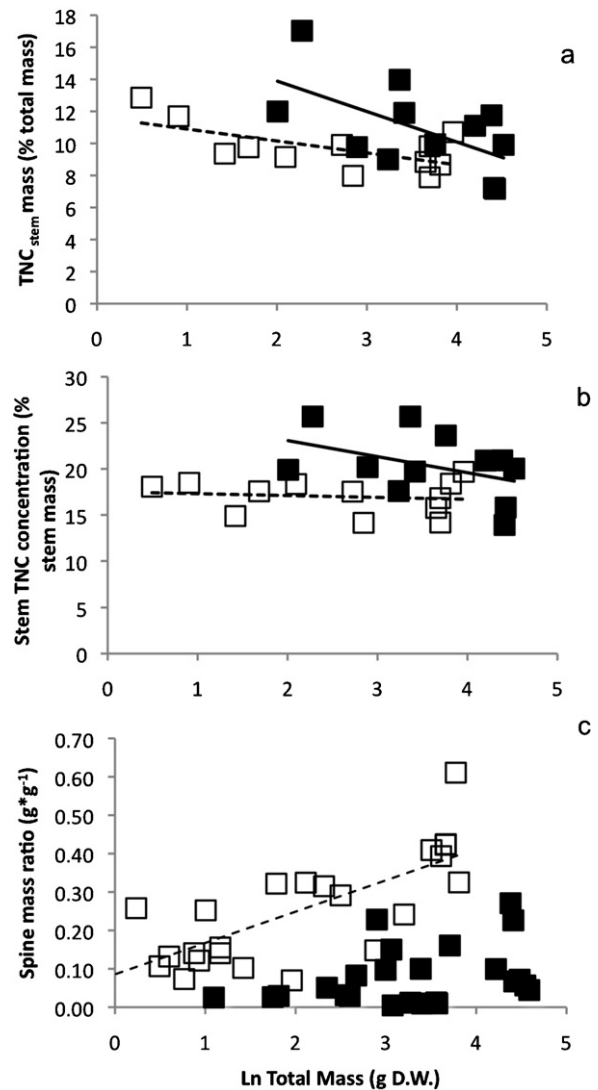
Fig. 1. Watering effect on storage and defense variables. (a) TNC_{stem} mass ($Y = 17.7 - 1.9X$; $R^2 = 0.35$ and $Y = 11.6 - 0.75X$; $R^2 = 0.39$) for control and drought-stressed plants, respectively. Slopes' comparison: $F = 2.14$; $p > 0.05$; (b) stem TNC concentration ($Y = 26.6 - 1.75X$; $R^2 = 0.18$ for control plants and $Y = 17.5 - 0.20X$; non-significant for drought-stressed plants) and (c) spine mass ratio ($Y = 0.05 - 0.0008X$, non-significant for control plants and $Y = 0.14 - 0.007X$; $R^2 = 0.63$ for drought-stressed plants) as a function of whole plant mass for *Prosopis alpataco* individuals, over a 54 months period, in response to two treatments of water availability. Levels of resources are designated as follows: open symbols and dashed line, drought-stressed; closed symbols and solid line, control.

In agreement to our third prediction, spine mass ratio was greater in drought-stressed plants than in control plants at a given total plant mass (Fig. 1c). A significant interaction was found between treatment and biomass (Table 1) indicating that treatments followed different allometric trajectories: as growth proceeded, spine mass ratio increased in drought-stressed plants while this proportion remained constant in control plants (Fig. 1c).

Contrary to expectation under prediction 2, the effect of watering treatment on root TNC concentration (% root mass) was negligible. Drought-stressed plants did not show a higher root TNC concentration than control plants.
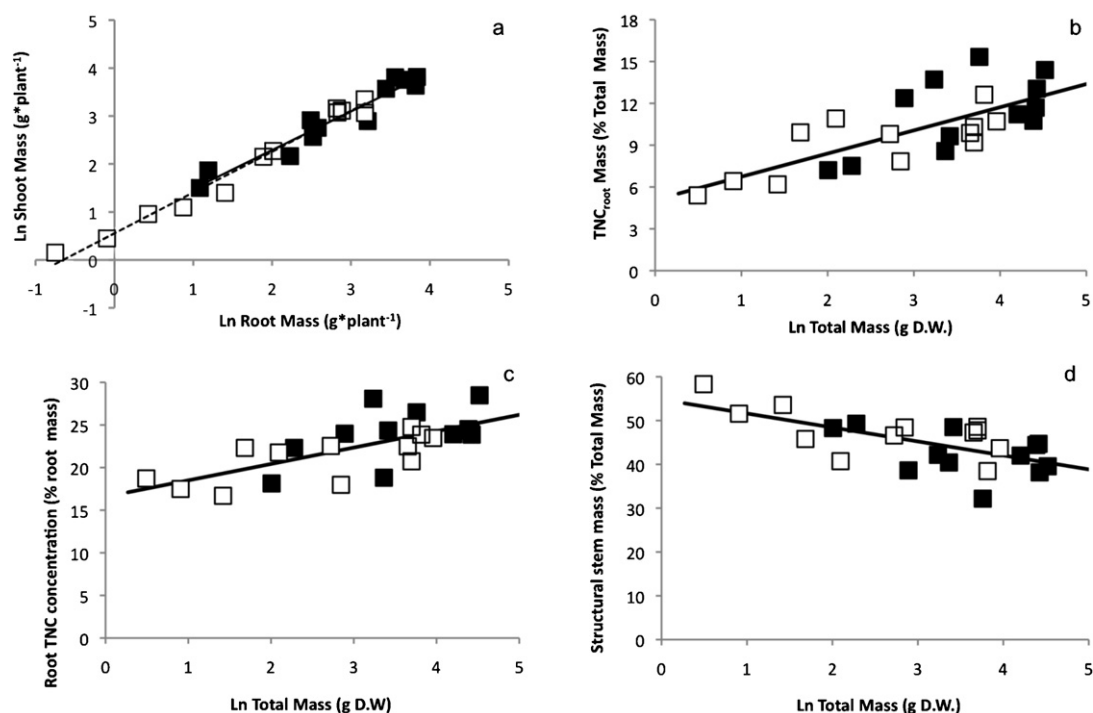
Fig. 2. Size-dependent changes in biomass partitioning and allometric trajectories: (a) allometric plot of shoot vs. root mass for *Prosopis alpataco* individuals; (b) TNC root mass; (c) root TNC concentration; and (d) structural stem mass as a function of whole plant mass over a 54 months period, in response to two treatments of water availability. Levels of resources are designated as follows: open symbols and dashed line, drought-stressed; closed symbols and solid line, control. Each point corresponds to one plot (experimental unit) and is the mean of data from three plants.

Size-dependent changes in biomass partition and allometric trajectories

As plants grow resulting in bigger size resources are allocated preferentially to roots. Root:shoot ratio increased with size in both groups (i.e. significant biomass effect; Table 1). Comparison of the response curves (slopes of the allometric plot of ln root mass vs. ln stem mass > 1, $t_{10} = 1.81$, $p < 0.01$, as depicted in Fig. 2a) indicates that changes occur along a fixed trajectory (i.e. fitted curves did not differ between treatments). This higher proportion of root mass arises from greater partitioning to TNC_{root} mass (Fig. 2b). Root TNC concentration increased with size (Fig. 2c), resulting in a higher TNC_{root} mass while structural root mass proportion remained constant throughout the course of plant growth (Table 1).

The proportion of biomass allocated to stems declined, as a consequence of growth, at expense of structural stem mass (Fig. 2d). Stem TNC concentration remained constant (non-significant biomass effect; Table 1) and TNC_{stem} mass as a proportion of total plant mass slightly increased (10%; $p < 0.01$).

Discussion

Climate change has been identified as the largest forthcoming threat to biodiversity across most biomes (MEA, 2005). In an attempt to understand and predict the *Prosopis alpataco* potential to cope with increasing drought stress, expected to occur in Patagonia with the ongoing global climatic changes (IPCC, 2007), we tested the long-term phenotypic plasticity in biomass partitioning and allocation to storage and defense in response to water shortage over the course of pre-reproductive growth during five years. During the juvenile phase, perennials are expected to allocate resources to two primary functions: (1) production of new tissues and (2) protection of already acquired resources (Lerdau and Gershenzon, 1997). Overall, our data indicate that juvenile *Prosopis*

alpataco plants exhibit two conservative strategies in growth and defense allocation:

(a) Root to shoot ratio and TNC pool increase with size

An increase of root to shoot ratio throughout the vegetative phase of *P. alpataco* was provoked by a simultaneous decrease of allocation to structural stem mass (−11%) and an increase of root reserves (+20%). Increasing belowground allocation through partitioning to reserves instead of allocation to non-storage mass, is a conservative strategy that favors carbohydrate forms that later can be mobilized (Kobe et al., 2010). This is consistent with the high levels of carbohydrates required for leaf flushing, vegetative growth, frost tolerance and achievement of the adult stage (i.e. reaching the reproductive phase; Chapin et al., 1990) without threatening survivorship of the individual (Kobe, 1997; Marquis et al., 1997). Increased TNC concentrations have been reported for woody perennials during periods of drought (Körner, 2003). Contrary to expectations, we had to reject our second prediction, because TNC concentrations did not increase in roots in response to drought. At similar plant mass, root TNC concentration and TNC_{root} mass were comparable in plants growing under both treatments (i.e., control and drought-stressed individuals occupy different regions on the same allometric trajectory; Fig. 2a). A lack of response of root TNC concentration to drought has been previously reported for other *Prosopis* shrub species (Vilela et al., 2003).

On the other hand, drought provoked a decrease in stem TNC concentration. There are at least two possible explanations for this result: reserves are not stored in aboveground organs during periods of drought, as reported by Larcher and Thomaser-Thin (1988) or, during a summer drought period the non-structural carbohydrate reserves become depleted (Meletiou-Christou et al., 1998).

(b) Allocation to spines increases under drought

Prosopis alpataco develops constitutive defenses (i.e. geminate spines are produced before the occurrence of herbivory; Burkart, 1976; Karban and Baldwin, 1997), protecting the highly nutritious foliage consumed predominantly by vertebrate grazers (Vilela et al., 2009). The efficacy of spines as an herbivore deterrent has been demonstrated in a number of studies (Cooper and Ginnett, 1998; Gowda, 1996; Obeso, 1997; among others). This strategy is optimal where the probability of herbivory is high (Ito and Sakai, 2009; Ronel et al., 2010), as it is the case in the Monte Desert. In our experiment, spine mass ratio increased 3-fold in response to drought, which is consistent with the Growth-Differentiation Balance hypothesis (Herms and Mattson, 1992) and with the idea that poor resource environments will favor plants that invest a high fraction of resources into structural defense (Hanley et al., 2007). Coley et al. (1985) suggested that defense investment increases as the potential growth rate of the plant decreases (holding herbivore pressure constant) because even though the construction cost of leaves does not increase (Villar and Merino, 2001), replacement of resources lost to herbivores becomes more costly as potential growth rates become more limited by resource availability. This adjustment might probably enhance plant fitness by reducing the probability of herbivore attack through a restriction of leaf accessibility and a decrease of the potential rate of nutrient ingestion by the browser (Wilson and Kerley, 2003).

This defense strategy in response to environmental changes would facilitate the persistence of this perennial plant in its area of distribution, which depends on its ability to survive during the long juvenile phase, reaching successfully the adulthood and becoming able to reproduce (Thuiller et al., 2008).

Acknowledgements

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