



# Drought and herbivory as modulators of intraspecific differentiation in seedlings of a mountain tree

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## Abstract

In mountain ecosystems, plant regeneration might be constrained by multiple factors that change along elevation gradients and promote traits differentiation. Drought is a strong filter for seedling establishment that might be modified by herbivory co-occurrence. Populations of the tree *Maytenus boaria* support lower soil moisture and higher herbivory pressure at low elevations than at mid-elevations in Córdoba Mountains, central Argentina. Consequently, we expect that populations from the low elevation perform better in response to drought than populations from mid-elevations and that herbivory modifies these responses. Seedlings from the two elevation origins were exposed to two levels of simulated drought and herbivory in a greenhouse experiment. The selected elevations corresponded to the lowest edge of species distribution (with driest soils and highest herbivory pressure) and the central mid-elevation. Performance-related variables, biomass allocation patterns and several morphological and physiological traits were measured. Mortality patterns and most of morphological and physiological variables showed that drought is a stressful factor at the regeneration stage of *M. boaria*. The drought effect was increased by simulated herbivory in some variables (LMF, RM:SM and SPAD). In most variables, origin did not influence seedling performance, suggesting that drought response of seedlings is independent of populations' elevation. Only leaf number and water potential were in line with our predictions and showed an origin response to drought.

**Keywords** Functional traits · Biomass allocation · *Maytenus boaria* · Regeneration

## Introduction

Natural gradients offer great opportunities to assess intraspecific differentiation of plant traits and predict how species may cope with climate change (Körner 2012). In mountain ecosystems, plant regeneration might be constrained

by multiple factors that change along elevation gradients (Körner 2012; Moreira et al. 2018). While the effects of temperature on seedling regeneration of woody species have been extensively studied (Harsch et al. 2009; Körner 2012), the effects of other stressors that vary along the elevation gradient, as drought, have received less attention (Engelbrecht et al. 2007). Especially in semiarid mountains, where precipitation increases with elevation, drought is expected to be the main hazard for seedling establishment at the warmer, lower edge of species' distribution (Engelbrecht et al. 2007; Kitajima and Fenner 2000), and to be an important selective pressure (Larter et al. 2017; Varela et al. 2010). Consequently, populations along environmental gradients continuously exposed to different levels of drought, might express differences in morphological or physiological traits improving their performance under the environmental conditions of origin (Halbritter et al. 2018; López Goldar and Agrawal 2021). Additionally, the seedling responses to drought might be modified by the interaction with other factors that also change along elevation gradients as herbivory. Herbivory often increases toward low elevations where temperatures

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and plant productivity are higher than in high elevations (Anderegg et al. 2015; Rodríguez-Castañeda et al. 2010; but see Moreira et al. 2018). While the herbivory effects on seedlings have been extensively characterized (e.g., Barton 2013, 2016; Thomson et al. 2003), how herbivory affects drought responses, especially among populations that differ in natural herbivory pressure has been less studied (Barton and Shiels 2020).

Survival and growth are important aspects of seedling performance during establishment (Connolly and Wayne 1996). Regarding functional traits, drought may promote an increase in the minimum water potential (Bhaskar and Ackerly 2006) and a reduction in leaf number (LN) to maintain physiological activity (Gianoli and González-Teuber 2005; Liu et al. 2011). In terms of biomass allocation patterns, drought might promote higher investment in roots to increase water uptake (Barton et al. 2020; Taeger et al. 2015). After herbivory, the growth priority are new leaves, while root biomass decreases in damaged plants (Barton 2016; McNaughton 1983). Seedlings may also respond by increasing photosynthetic rate (Thomson et al. 2003). Finally, drought and herbivory may promote similar morphological changes, such as reduction in LN and area (LA), and specific leaf area (SLA; Stevens et al. 2008; Thomson et al. 2003). Despite the large number of studies about drought and herbivory effects on adult plants, how herbivory might modulate plant response to drought, especially on seedlings, have been scarcely studied (Barton 2013; 2016).

The South American tree *Maytenus boaria* is distributed along a broad elevation range, where populations show clinal variation in functional traits (Marcora et al. 2013). Seedlings grown in a common garden have shown differentiation in their functional traits among four elevation origins, suggesting adaptations to local conditions. Some attributes of performance-related traits (i.e., large leaves and high sapling height and biomass) that indicate high growth were mostly observed in saplings from lower or intermediate elevations, whereas the opposite was observed in saplings from higher elevations (Marcora et al. 2017). In these mountains, soil water content decreases towards low elevations, whereas air temperature increases; therefore, seedlings in lowlands are exposed to drought, even in the wet season (Tecco et al. 2016). Additionally, livestock load and arthropod diversity and abundance increase towards low elevations of these mountains (Marcora et al. 2013; Ramos 2018). Consequently, herbivory pressure increases toward lower elevations, where temperatures and productivity are generally higher (Anderegg et al. 2015; Rodríguez-Castañeda et al. 2010). In this context, we postulate that contrasting levels of drought will generate differential expression in plant functional traits and seedling recruitment between elevations and that herbivory pressure might affect these drought responses differentially. Two populations of *M. boaria* from two

elevations with contrasting conditions of drought and herbivory were thus selected to assess intraspecific differences in their offspring. Since seedlings from the lower elevation come from individuals historically exposed to both stressors, we predict that the offspring of the population from the drier and warmer low elevation will be less affected by drought than the offspring from the cooler and moisture mid-elevation, and that these drought responses will be less affected by herbivory in the seedlings from the lower elevation. Most predictions on species response to climate change are based on evidence from northern temperate regions (Chambers et al. 2017; Halbritter et al. 2018). Such predictions might not hold true in dry seasonal climates, which are widely represented in South America (Kuemmerle et al. 2017), as well as in other southern regions that remain poorly studied (Barton et al. 2020; Varela et al. 2010).

## Materials and methods

### Species and study area

*Maytenus boaria* Molina (Celastraceae) grows in the mountains of central Argentina, hereafter Córdoba Mountains, along an elevation gradient from ~900 m a.s.l. to the highest elevation (about 2700 m a.s.l.). Mean annual temperatures decrease from 15.7 to 10.6 °C (Marcora et al. 2008), while annual precipitation increases from 700 to 1000 mm (García 2013). Soil moisture increases with elevation (Tecco et al. 2016). We selected two populations growing at contrasting elevations to obtain the seeds for the experiment (32° 03' S; 64° 51' W): populations growing under maximum water stress and herbivory pressure (i.e., lowest edge of species distribution at 1200 m a.s.l.; low origin) and populations growing at the central elevation of this species in Córdoba mountains (1700 m a.s.l.), where maximal seedling survival was previously observed (i.e., mid-origin) (Marcora et al. 2013).

### Experimental design

Seeds were collected from 20 mature trees at each elevation in summer (February–March). Seeds were set to germinate in germination chambers at 25/15 °C (12/12 h light/dark photoperiod). Emerged seedlings were transplanted to 0.68-L plastic pots (one per seedling) containing a mixture of sand and soil in a 2:3 proportion. During the experiment, plants were grown in a greenhouse under an alternating photoperiod of about 12 h light and 12 h darkness (natural photoperiod of spring between 20/09 and 22/11). Mean air temperature and humidity in the greenhouse were 22.12 °C ( $\pm 0.15$  SD) and 65.36% ( $\pm 0.56$  SD), respectively. This greenhouse mean temperature is below the absolute

maximum temperatures experienced in the field by low and mid populations during summer (32.8 and 29.5 °C at 1200 and 1800 m a.s.l., respectively; Marcora et al. 2008). Pots were automatically watered until the start of the experiment. To minimize the influence of initial height, seedlings were grouped in blocks of eight pots. Each block included seedlings from both origins in four treatment combinations: control, drought, simulated herbivory, and drought + simulated herbivory. A total of 15 blocks were established. An additional block was included, which contained only four pots with seedlings from the low origin. Blocks were rotated weekly. The complete experimental design consisted of 124 seedlings that were distributed in two drought treatments  $\times$  two simulated herbivory treatments  $\times$  two elevation origins  $\times$  16 or 15 replicates (for the low and mid-origin, respectively).

Pots were manually watered every 2–3 days, with half of them receiving 5 ml and the other half, 50 ml. The two water levels chosen were expected to mimic differences in soil moisture content measured at each elevation under field conditions (Tecco et al. 2016). Soil water content was 8.26% ( $\pm 4.11$  SE) for the drought condition and 26.99% ( $\pm 3.89$  SE) for the well-watered condition (Moisture Probe Meter 160-B of ICT International Pty Ltd).

The simulated herbivory treatment was performed one week after the start of the differential watering treatment. We manually clipped with scissors half of the seedlings under each water regime level and removed all the biomass above cotyledons (Lorca et al. 2019). The severity of this simulation mimics the actual herbivory pressure experienced by wood species' seedlings under field conditions in the study area (Teich et al. 2005; Giorgis et al. 2010; Marcora et al. 2013). After the two months of experiment, all surviving seedlings were harvested.

### Seedling measurements

Seedling performance was assessed in terms of mortality, final biomass and relative growth rate (RGR). The RGR was calculated as the difference between the final and initial height ( $\log_{10}$  transformed) divided by time (2 months) (Sánchez-Gómez et al. 2008). The initial height of seedlings was measured after applying the simulated herbivory treatment.

Leaf number (LN) was recorded and one leaf was taken from each seedling and scanned to calculate leaf area (LA;  $\text{mm}^2$ ) using the ImageJ program (Schneider et al. 2012) and specific leaf area (SLA;  $\text{mm}^2 \text{mg}^{-1}$ ). Total leaf chlorophyll concentration was estimated using the SPAD502 chlorophyll meter (Konica Minolta). SPAD was measured only in seedlings with at least one true leaf ( $n=88$ ), avoiding cotyledons, which always had higher SPAD values. Water potential ( $W_{p_{\text{shoot}}}$ ; MPa) was measured in a subsample of 32 seedlings

(4 replicates  $\times$  2 origins  $\times$  2 drought levels  $\times$  2 herbivory levels). This was performed within two minutes after harvest of each seedling using a pressure chamber (Scholander et al. 1965). Water potential measurements were taken inside the greenhouse at midday on sunny days (ca. from 12.00 to 14.00 h) using the principal shoot of each seedling. Harvested seedlings were separated into leaves, stem and roots, oven-dried at 70 °C for 48 h and weighed. Final biomass, leaf, stem and root mass fraction (RMF; SMF; LMF, respectively), and root mass to shoot mass ratio (RM:SM) were calculated.

### Data analysis

The response variables (Table 1) were analyzed with a full linear mixed-effects model (function `lme`; package `lme4`), in which drought, herbivory, origin, and their double and triple interactions were included as fixed factors. The block was included as random factor. A systematic model simplification was applied (i.e., top-down model simplification beginning with a model with all the desired factors included) based on the AIC values. During this process, we compared all potential models with the null model and among them. Consequently, the random factor was also assessed and included only when its effect was significant. The structure of the final models was obtained through the application of the likelihood ratio test (Zuur et al. 2009), using the `anova` function (Ferenc et al. 2021). The final model was analyzed including only the selected factors and the specific assumptions of each assessed model. After this process, generalized linear mixed-models (function `lme`; package `lme4`), including the random factor (i.e., block) were finally run only for the variables final biomass, LMF, LN and SLA. Leaf number (LN) was analyzed using a Generalized Linear Mixed Model (`glmer` function, `lme4` package) with a Poisson error distribution and overdispersion was checked following Zuur et al. (2009). For the remaining variables, after the model reduction process, generalized linear model were used (i.e., random effect was not included): mortality, RGR, RMF, SMF, RM:SM, LA, SPAD, and  $W_{p_{\text{shoot}}}$ . Final biomass, LMF, SMF, LA, SLA, and  $W_{p_{\text{shoot}}}$  were  $\log_{10}$  transformed to meet assumptions of normality. Seedling mortality was included as a binomial variable in a Generalized Linear Model (`glm` function, `stats` package) and p values were obtained using an Analysis of Deviance (`Anova` function, `car` package). In this case, overdispersion was checked (residual deviance < freedom degrees). All analyses and graphs were performed with RStudio ver. 1.1.463 (2009–2018).

**Table 1** Response of seedlings of the tree species *Maytenus boaria* from two elevation origins (O) to drought (D) and herbivory (H)

Response variables		(Intercept)	Drought (D)	Herbivory (H)	Origin (O)	D*H	D*O	H*O	H*O*D
Performance	Mortality		$\chi^2=41.14$ <b><math>p &lt; 0.001</math></b>	$\chi^2=2.71$ $p=0.10$	$\chi^2=5.55$ <b><math>p = 0.02</math></b>				
	RGR	$F=51.81$ $p < 0.0001$		$F=4.40$ <b><math>p = 0.03</math></b>					
	Final biomass	$\chi^2=233.63$ $p < 0.0001$	$\chi^2=0.99$ $p=0.32$	$\chi^2=18.07$ <b><math>p &lt; 0.0001</math></b>		$\chi^2=0.15$ $p=0.69$			
Biomass allo- cation	LMF	$\chi^2=395.52$ $p < 0.0001$	$\chi^2=0.6.93$ <b><math>p &lt; 0.01</math></b>	$\chi^2=1.49$ <b><math>p &lt; 0.01</math></b>		$\chi^2=4.36$ <b><math>p = 0.04</math></b>			
	SMF	$\chi^2=35.85$ $p < 0.0001$	$\chi^2=1.83$ $p=0.17$	$\chi^2=0.21$ $p=0.64$	$\chi^2=0.35$ $p=0.55$	$\chi^2=0.51$ $p=0.47$	$\chi^2=1.29$ $p=0.25$	$\chi^2=0.14$ $p=0.7$	$\chi^2=0.7$ $p=0.47$
	RMF	$\chi^2=393.66$ $p < 0.0001$	$\chi^2=9.76$ <b><math>p &lt; 0.001</math></b>	$\chi^2=2.65$ $p=0.10$		$\chi^2=1.61$ $p=0.20$			
	RM:SM	$F=137.77$ $p < 0.0001$	$F=7.31$ <b><math>p &lt; 0.01</math></b>	$\chi^2=2.67$ $p=0.10$		$\chi^2=5.24$ <b><math>p = 0.02</math></b>			
Morphological traits	LN	$\chi^2=77.89$ $p < 0.0001$	$\chi^2=20.74$ <b><math>i &lt; 0.0001</math></b>	$\chi^2=2.49$ $p=0.11$	$\chi^2=0.77$ $p=0.38$	$\chi^2=0.57$ $p=0.45$	$\chi^2=19.63$ <b><math>p &lt; 0.0001</math></b>	$\chi^2=0.5$ $p=0.47$	$\chi^2=1.6$ $p=0.21$
	LA	$F=182.06$ $p < 0.0001$	$F=9.35$ <b><math>p &lt; 0.01</math></b>	$F=0.09$ $p=0.76$		$F=2.63$ $p=0.10$			
	SLA	$\chi^2=4592.197$ $p < 0.0001$		$\chi^2=14.169$ <b><math>p &lt; 0.0001</math></b>					
Physiological traits	SPAD	$F=303.11$ $p < 0.0001$	$F=4.38$ <b><math>p = 0.04</math></b>	$F=2.53$ $p=0.11$		$F=7.96$ <b><math>p = 0.01</math></b>			
	Wp <sub>shoot</sub>	$F=0.02$ $p < 0.88$	$F=9.86$ <b><math>p &lt; 0.01</math></b>		$F=1.32$ $p=0.25$		$F=5.23$ <b><math>p = 0.03</math></b>		

Statistics and  $p$  values are provided only for variables included in the final models after backward model reduction process (see methods). RGR (relative growth rate), LMF (leaf mass fraction), SMF (shoot mass fraction), RMF (root mass fraction), LN (leaf number), LA (leaf area), SLA (specific leaf area), SPAD (estimation of chlorophyll content), Wp<sub>shoot</sub> (water shoot potential). Random effects (block) were included in final models of final biomass (SD=0.44), LMF (SD=0.18), LN (SD=0.68) and SLA (SD=0.63)

Bold type indicates a statistically significant difference between treatments for a factor

## Results

At the end of the experiment, 100 seedlings (80.65%) were alive. Both drought and simulated herbivory had negative effects on most of the assessed variables (Table 1). For some variables, the effect of drought differed between elevation origins, whereas the effect of simulated herbivory was always independent of seedling elevation origin.

### Performance and biomass allocation patterns

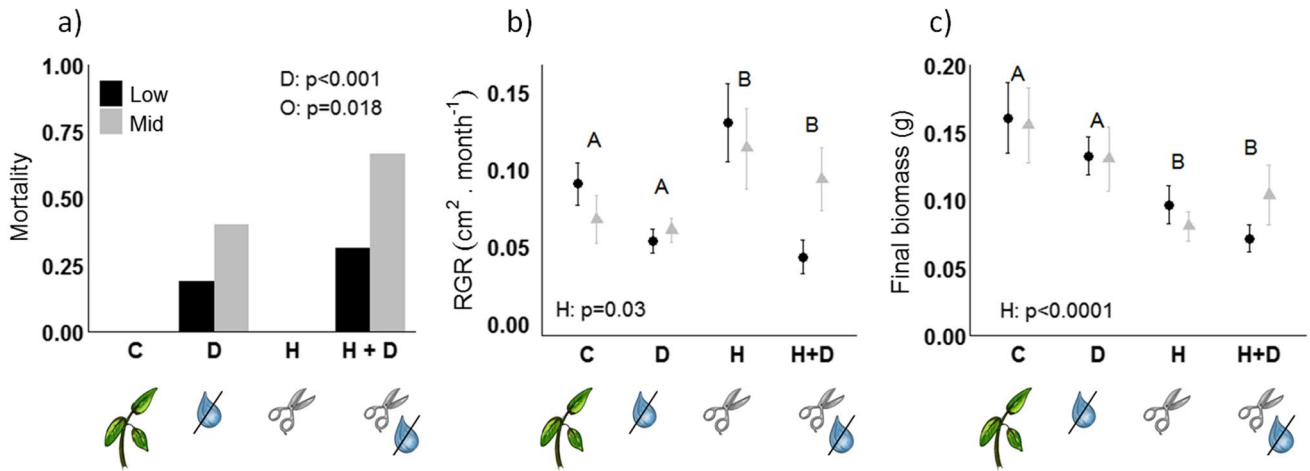
Mortality of *M. boaria* seedlings differed between drought levels and between elevation origins, whereas there was no effect of simulated herbivory on mortality (Table 1). Mortality was higher in seedlings from mid-origin, and drought increased mortality for seedlings from both elevations (Fig. 1a). Drought did not have an impact on seedlings' relative growth rate (RGR), whereas simulated herbivory had a positive effect on it (Fig. 1b). Drought did not affect the final biomass of seedlings whereas simulated herbivory significantly reduced it (Fig. 1c; Table 1).

None of the biomass allocation components were affected by origin. Drought decreased leaf mass fractions (LMF), increased root mass fractions (RMF) and increased seedling root-to-shoot ratios (RM:SM). This effect on LMF and RM:SM was increased significantly under the combined effects of drought and simulated herbivory (Fig. 2d). SMF was not affected by any treatment (Fig. 2b).

### Seedling traits

Most morphological and physiological traits were affected by drought, and some of them were affected by the interaction between drought and simulated herbivory or elevation origin (Table 1). Leaf number (LN) was reduced by the effect of drought in seedlings of the low elevation (Fig. 3b). Drought reduced seedlings' leaf area (LA) but had no effect on their specific leaf area (SLA), which was increased by herbivory (Fig. 3c).

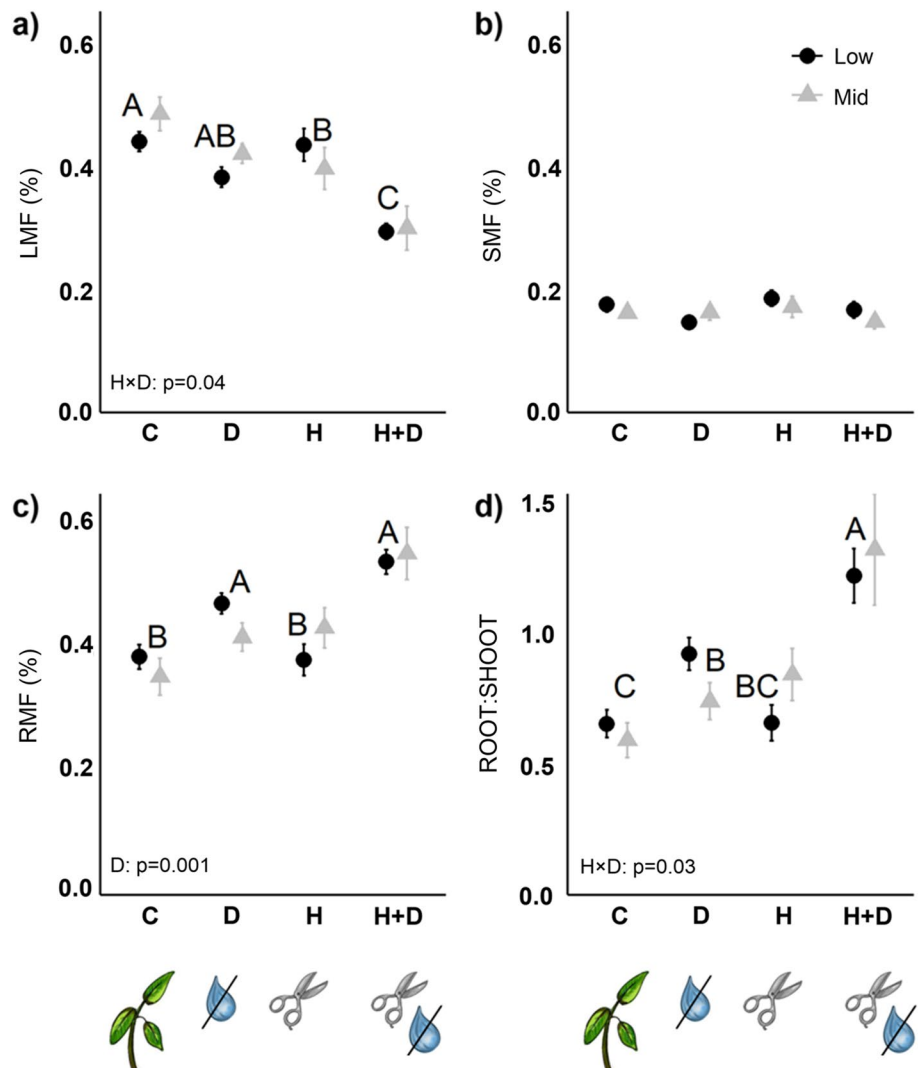
Seedlings' SPAD (i.e., leaf chlorophyll concentration) increased under combined effect of drought and simulated herbivory (Fig. 4a). Finally, seedlings' water potential

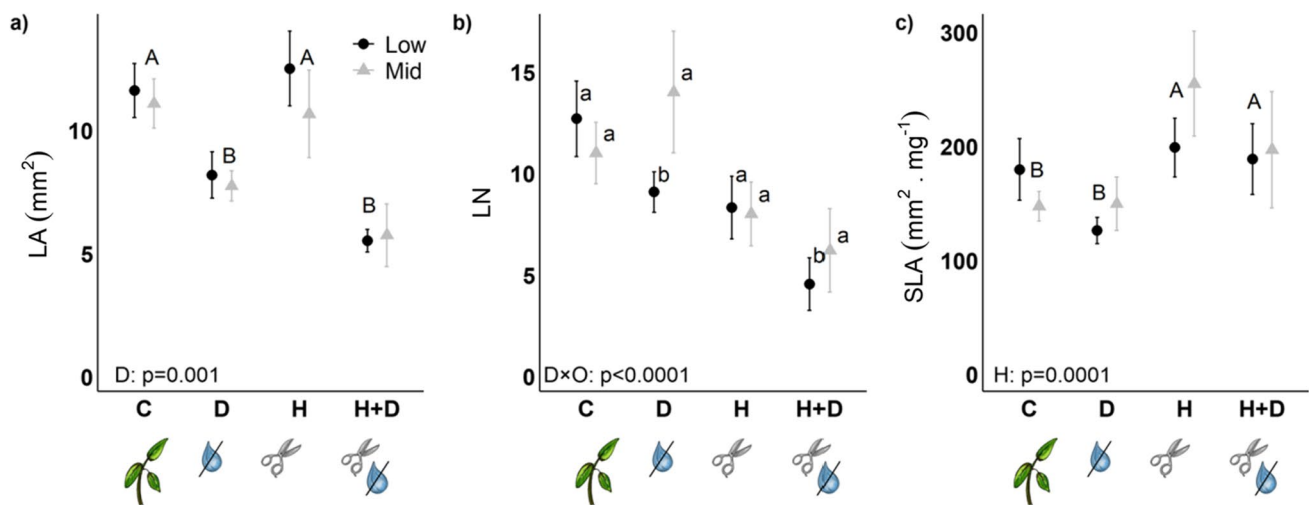


**Fig. 1** Predictors of performance of *Maytenus boaria* seedlings of low (black bars and points) and mid- (white bars and points) elevation origins in response to drought and herbivory. **a** Seedling mortality (proportion); **b** relative growth rate (RGR; cm/month); **c** final biomass (g). Four treatments were applied to seedlings: control (C),

drought (D), simulated herbivory (H), drought and simulated herbivory (DH). Points represent the mean  $\pm$  SE. Different letters indicate differences among levels of factors when the interaction between two factors was significant (see Table 1)

**Fig. 2** Biomass allocation patterns of *Maytenus boaria* seedlings from low (black points) and mid- (white points) elevation origins in response to drought and simulated herbivory. **a** Leaf mass fraction (LMF); **b** Stem mass fraction (SMF); **c** Root mass fraction; and **d** Root to shoot ratio (RM:SM). Points represent mean  $\pm$  SE

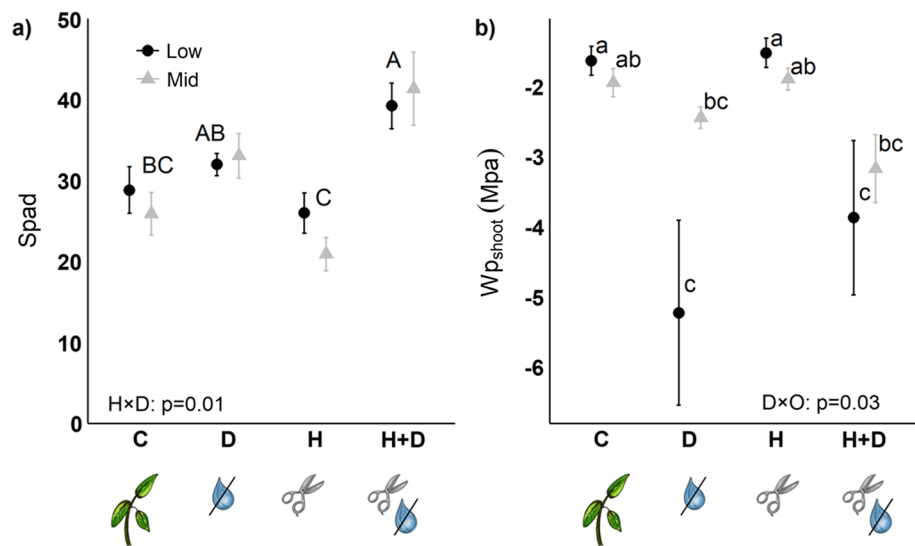




**Fig. 3** Morphological traits of *Maytenus boaria* seedlings of low (black points) and mid- (white points) elevation origins in response to drought and simulated herbivory. **a** Leaf area (LA;  $\text{cm}^2$ ); **b** Leaf number (LN); **c** Specific leaf area (SLA;  $\text{cm}^2/\text{g}$ ). Points represent

mean  $\pm$  SE. Capital letters indicate statistically significant differences of the main effect factors and interactions between factors by small letters

**Fig. 4** Physiological traits of *Maytenus boaria* seedlings of low (black points) and mid- (white points) elevation origins in response to drought and simulated herbivory. **a** SPAD; **b** Water seedling potential of shoots ( $W_{p_{\text{shoot}}}$ ; MPa). Points represent mean  $\pm$  SE



( $W_{p_{\text{shoot}}}$ ) was affected by the interaction between drought and elevation origin, showing a decrease in response to drought that was significantly lower in seedlings from the low origin (Fig. 4b).

## Discussion

Mortality patterns and most morphological and physiological variables showed that drought is a stressful factor of *Maytenus boaria* at the regeneration stage and that drought effect was exacerbated by simulated herbivory

(LMF, RM:SM and SPAD). In most variables, origin did not influence seedling performance, suggesting that drought response of *M. boaria* seedlings is independent of the elevation origin of their population. Only leaf number (LN) and water potential ( $W_{p_{\text{shoot}}}$ ) were in line with our predictions and showed an origin response to drought.

## Drought effects

Among the assessed traits, seedlings from the low origin showed a reduction in LN in response to drought. This response is a well-recognized desiccation-avoidance

adaptation of woody plants (Gurevitch et al. 1986; Yin et al. 2005). Additionally, while seedlings from the cool and moist elevation (mid-origin) showed small differences in  $W_{p_{shoot}}$  between watering levels, those from the low origin had more negative values in response to drought, i.e., three times lower than well-watered seedlings (Table 1; Fig. 4b). These lower values suggest a higher degree of tolerance to physiological drought in plants from the low than those from the mid-origin. The minimum  $W_{p_{shoot}}$  value reached by a plant has been related to high capacity to cope with water shortage (Bhaskar and Ackerly 2006; Soliani et al. 2021). Indeed, the  $W_{p_{shoot}}$  indicate the maximum water deficit that leaves and xylem can tolerate to maintain physiological activity (Bhaskar and Ackerly 2006). This physiological response to drought of seedlings from the low population could be reflected in subsequent changes in morphological variables and even in mortality. In this sense, drought increased mortality in seedlings of both populations (Fig. 1a), with those from the mid-population showing higher mortality than those from the low population. This could relate to the mid-population having a lesser exposure history to drought compared to the low population. It is also possible that the environmental conditions of the greenhouse (located at 400 m a.s.l.) were more contrasting to the conditions experienced at field by the mid-population (e.g., temperature, atmospheric pressure, etc.), thereby increasing their mortality under drought.

Finally, a suite of attributes related to a stress resistance syndrome (i.e., high RMF, RM:SM, SPAD, and low LMF, LN, LA and  $W_{p_{shoot}}$ ; sensu Chapin et al. 1993) also indicated that the species has certain ability to respond to drought at both elevations. Seedlings' SPAD and RMF increased in response to drought (Table 1). The variation in SPAD values has been found to be positively correlated to chlorophyll content, photosynthetic rate, absorption of photosynthetic active radiation, and carbohydrate content in leaves (Fotovát et al. 2007; He and Sun 2016). In turn, biomass allocation to roots enhances the root surface area available for water uptake. Accordingly, both, SPAD and RMF have shown to be related traits in response to multiple stressors (He and Sun 2016), expected to improve seedling survival and growth under drought (Khurana and Singh 2001). This tolerance response has been found in species adapted to moisture-deficit conditions (Chapin et al. 1993; Wright et al. 1992).

### Joint effects of drought and simulated herbivory

Our results showed that drought effects were increased by herbivory. Accordingly, under the combined effects of both stressors, LMF, RMF, RM:SM ratio and SPAD followed the expected trend in response to drought (He and Sun 2016; Keefover-Ring et al. 2016) and opposite to that produced by simulated herbivory (Gassmann 2004; Thomson et al.

2003). That is, under both stressors seedlings would be allocating more biomass to the organ through which the most limiting resource is acquired, i.e., roots (Barton and Shiels 2020). In addition, the higher increase in chlorophyll content (SPAD) would allow seedlings to compensate the effects of both stressors and their consequences (He and Sun 2016; Khurana and Singh 2001).

Unlike predicted, simulated herbivory did not drive differential responses between elevation origins, both in drought-stressed and well-irrigated seedlings. This could be driven by the absence of intraspecific differences in the response to this factor between populations. However, many herbivory-related traits were not measured in this study and complementary simulation approaches are needed to confirm this inference. When simulated herbivory was the only factor applied, seedlings exhibited an increase in RGR and a decrease in final biomass. Rapid vertical growth can allow seedlings to escape apical meristem damage by mammalian herbivores often resulting in greater survival and reproduction (Keefover-Ring et al. 2016). While seedlings resprouted after simulated herbivory, it is possible that they need more time to achieve full tolerance. Further studies are needed to assess intra-specific trait differentiation in the capacity to compensate for lost biomass under different browsing intensities (e.g., percentage of aboveground biomass removed) or as an induced response to herbivory (e.g., saliva, Keefover-Ring et al. 2016; Rooke 2004). Additionally, traits related to plant defenses in response to herbivory should be measured to better understand the mechanisms of defense and tolerance of this species (Keefover-Ring et al. 2016; Rooke 2004). Finally, it would be important to discriminate whether the maternal effects affects the species and population response to drought and herbivory (Agrawal 2002).

### Final remarks

In many regions of the world, the frequency and intensity of droughts is increasing due to global climate change (IPCC 2014), with a significant influence on forest regeneration (Anderegg et al. 2015). Similarly, the increase in rainfall heterogeneity is promoting local drought in Córdoba Mountains (García 2013). Our results suggest that *M. boaria* seedlings from both populations are affected by drought and this effect increases when herbivory co-occurs. Thus the scenarios proposed by climate change may particularly affect those populations of the areas that may exhibit higher chances of increasing aridity, such as low land areas. In addition, seedlings of *M. boaria* from the low elevation showed lower mortality and seem better capable to deal with drought by adjusting LN and  $W_{p_{shoot}}$  than populations from the cooler and moister elevation. This suggests that intraspecific differentiation might exist in this species but further studies in

other ontogenetic stages and including greenhouse conditions similar to both origins are needed to confirm this trend (Cotado and Munné-Bosch 2020).

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**Author contributions** PM and SZ contributed to study conception and design. Material preparation and data collection were performed by SZ, GA and PM. The analysis data were performed by AF, SZ and PM. The first draft of the manuscript was written by PM and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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## Declarations

**Competing interest** The authors have no relevant financial or non-financial interests to disclose.

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