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Responses to water scarcity in six sclerophyllous species established in a dry and burned site in central Chile

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Abstract

In this study, we investigated the responses to water scarcity after the post-transplant shock in six sclerophyllous species established in a Mediterranean-type climate site severely burned by fire. Seedlings of the species *Acacia caven*, *Lithraea caustica*, *Quillaja saponaria*, *Escallonia pulverulenta*, *Peumus boldus* and *Colliguaya odorifera* were cultured in the nursery on 140 mL pots and then planted in the field under tree shelters and submitted to two irrigation treatments during summer (2 L⁻¹ week⁻¹ seedling⁻¹ cf. no watering). Species differed by irrigation treatment in survival, stomatal conductance, and transpiration, with *P. boldus* and *E. pulverulenta* exhibiting the lowest values in the treatment with no watering. This suggests that young seedlings of both species have not the capacity to survive during dry summers in Mediterranean-type climates unless irrigations are increased in frequency and quantity.

Keywords: Outplanting survival; Mediterranean-type climate; gas exchange; forest restoration; burn severity

Introduction, scope and main objectives

In Mediterranean-type ecosystems young seedlings must tolerate long periods of drought after been established and develop mechanisms to survive the post-transplant shock (Kaushal and Aussenac 1989; South and Zwolinsky 1997). In these ecosystems, the survival of young seedlings mainly depends on the morphological attributes of the planting stock at establishment and the adaptation strategies to cope with drought (Margolis and Brand 1990; Grossnickle 2005). High quality of the planting stock can help newly established seedlings to overcome the post-transplant shock because it positively influences outplanting performance (Tsakalidimi et al. 2005; Jacobs et al. 2012).

In the Mediterranean ecosystems of central Chile, most of vegetation types are dry xerophytic and mesic communities, with the latter being dominated by sclerophyllous species (Arroyo et al. 1993). Among these species, the pioneer *Acacia caven* (Mol. Mol.), the mid-successional *Lithraea caustica* (Mol.) Hook. & Arn., and the late-successional *Quillaja saponaria* (Mol.) are characterized by a deep root system as an important adaptation to survive the long summer periods (Hoffman and Kummerow 1978). However, there is lack of information about other species in this ecosystem and adaptation mechanisms of newly established seedlings to cope with the post-transplant shock and face drought. In this context and given the different behavior and growth habit among Chilean sclerophyllous species against drought, we have undertaken a comparative field experiment to investigate the responses followed by six species to cope with water stress. We assessed morphological (growth and survival) and leaf-level physiological responses (gas exchange, fluorescence, and water potential) among the species *A. caven*, *L. caustica*, *Q. saponaria*, *E. pulverulenta* (Ruiz et Pav.) Pers., *Peumus boldus* Mol., and *Colliguaya odorifera* Mol. one year after outplanting in a dry site.

Methodology/approach

The trial was established at an experimental station property of the Universidad de Chile located in central Chile (35°34' S, 72°06'W, 254 m a.s.l.) with Mediterranean-type climate. In 2017, the total area of the experimental station was consumed by fire. Seeds of the six species under study were soaked in distilled water for 24 h and, after excluding those that floated, were set to germinate. Seedlings were grown in 140 mL pots filled with composted bark of *P. radiata* combined with the slow-release fertilizer Basacote 9M® at a dose of 3 g L⁻¹ and cultured under ambient conditions of temperature and light until June 2019. In July the same year we established a trial in a split plot design with five blocks. Two levels of watering (2 L plant⁻¹ week⁻¹ from November 2019 to March 2020 (W+), and no watering (W-)) were the whole plot treatment, and the six species were the split-plot treatment. The total number of seedlings was 600 (i.e., 2 watering treatments × 6 species × 5 blocks × 10 seedlings per plot). Seedlings were hand planted in 40 × 40 × 30 cm planting holes at a spacing of 1 × 1 m (i.e., tree stocking of 10,000 stems ha⁻¹).

One year after establishment all seedlings were measured for height (H) and root collar diameter (D) using a stainless-steel ruler and a digital caliper, respectively. The slenderness index was estimated as the height to diameter ratio (H/D). During January 2020 we measured light-saturated photosynthetic rate (A_{sat} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration (E, $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), intrinsic water use efficiency ($\text{WUE}_i = A_{\text{sat}}/g_s$) using a portable gas exchange system LI 6800 (LICOR Inc., Lincoln, NE, USA) in fully-developed leaves located on the upper third of the seedlings. We also measured midday stem water potential (Ψ_{stem}) by using a Scholander pressure chamber (PMS Instrument, Albany, USA), and the quantum efficiency of photosystem II in light-adapted samples upon application of a saturating flash ($\Phi_{\text{PSII}} = \Delta F/F_m'$), with the LI 6800.

Prior to the analyses, the data were examined and conformed to the normality and homogeneity of variance assumptions required for the analysis of variance. To meet the assumption of normality and constant variances, the data were transformed according to the Box-Cox transformation when appropriate. In the case of survival, categorical values (i.e., 1, 0), were expressed in percentages at the plot level and transformed by the arcsine square root transformation. All the statistical analyses were performed with SPSS version 18.0 software (SPSS Inc, Chicago, Illinois, USA).

Results

One year after outplanting, *P. boldus* and *A. caven* were the lowest seedlings with the slimmer diameters, but they exhibited positive and significantly higher increments in H as also did the species *L. caustica* (Table 1). The species *A. caven* exhibited the highest increment in H. Contrarily, *Q. saponaria* and *C. odorifera*, despite having the tallest seedlings with the thickest diameters of all species prior to planting (data not shown), they experienced negative increments in H and medium increments in D. In terms of survival, it was observed that *P. boldus* had a survival c.a. two-fold lower than that of *L. caustica*, which exhibited the highest survival of the experiment.

Table 1: Mean \pm standard error for growth, increments and survival according to species and watering treatment. Lowercase and uppercase letters indicate statistical differences among species and between watering treatments respectively ($P < 0.05$). D: root collar diameter; H: total height; INCd: increment in D; INCh: increment in H; SUR: survival

Trait					
	D (mm)	H (cm)	INCd (mm)	INCh (cm)	SUR (%)
Species					
<i>P. boldus</i>	3.5 \pm 0.1c	20.0 \pm 0.9bc	0.0 \pm 0.2c	4.9 \pm 1.2b	33 \pm 9c
<i>E. pulverulenta</i>	4.4 \pm 0.1b	24.0 \pm 0.8bc	0.9 \pm 0.1ab	6.3 \pm 1.4ab	63 \pm 9b
<i>Q. saponaria</i>	5.3 \pm 0.1a	33.8 \pm 0.8a	0.8 \pm 0.1ab	-2.1 \pm 1.1c	78 \pm 5ab
<i>L. caustica</i>	5.7 \pm 0.3a	24.5 \pm 1.3b	1.4 \pm 0.1a	6.4 \pm 1.0ab	89 \pm 3a
<i>C. odorifera</i>	5.2 \pm 0.1a	33.4 \pm 1.1a	0.8 \pm 0.1b	-0.8 \pm 0.8c	81 \pm 8ab
<i>A. caven</i>	2.9 \pm 0.1c	19.4 \pm 1.2c	0.9 \pm 0.1ab	9.5 \pm 1.0a	83 \pm 3ab
Watering treatment					
W+	4.8 \pm 0.2A	25.9 \pm 1.0A	0.8 \pm 0.1A	3.8 \pm 0.9A	82 \pm 3A
W-	4.3 \pm 0.2B	26.8 \pm 1.6A	0.9 \pm 0.1A	4.3 \pm 1.1A	60 \pm 6B
F-value and significance level					
Watering (W)	14.4**	0.9ns	0.4ns	0.3ns	22.6**
Species (S)	57.2**	34.9**	7.9**	21.5**	14.7**
W \times S	1.8ns	1.1ns	0.7ns	1.0ns	3.5**

(**): $p < 0.01$; (ns): non-significant.

Watering treatments only affected A_{sat} and Ψ_{stem} and both traits were drastically reduced in the W- treatment (Table 2). No differences were observed for WUE_i among species, and *A. caven* had the highest A_{sat} , g_s , and E. Like survival, the lowest performance in gas exchange traits was observed in *P. boldus*, which exhibited A_{sat} , g_s , and E 98, 23, and 4 times lower than that of *A. caven*. A_{sat} was similar among *E. pulverulenta*, *Q. saponaria*, and *L. caustica*, but g_s and E were lower in *L. caustica*. The traits g_s and A_{sat} were significantly low in *P. boldus*, whereas A_{sat} was not severely affected in *L. caustica* despite both species had similar g_s . Similarly, *Q. saponaria* and *C. odorifera* had similar g_s , but A_{sat} was slightly higher *C. odorifera*. In terms of fluorescence, ΦPSII was higher in *A. caven* and lower in *P. boldus* and *L. caustica*. The hydric status of seedlings follows a similar trend to that of gas exchange and fluorescence. The species *E. pulverulenta* had higher gas exchange parameters and fluorescence than *P. boldus* but was the most water stressed. Average midday stem water potential in this species was close to -4.0 MPa. In the case of *L. caustica*, *C. odorifera*, and *A. caven*, those species had the lowest Ψ_{stem} of the experiment, but g_s was higher in the latter species.



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Table 2 - Mean \pm standard error for gas exchange traits and water potential according to species and watering. Lowercase and uppercase letters indicate statistical differences among species and between watering treatments respectively ($P < 0.05$). A_{sat} : light-saturated photosynthesis; g_s : stomatal conductance; E: transpiration; WUE_i : intrinsic water use efficiency; Φ_{PSII} : quantum efficiency of photosystem II; Ψ_{stem} : midday water potential.

Trait	A_{sat} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	g_s ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	E ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	WUE_i (A_{sat}/g_s)	Φ_{PSII} ($\Delta F/F_m'$)	Ψ_{stem} (MPa)
Species						
<i>P. boldus</i>	0.1 \pm 0.4c	0.01 \pm 0.00b	0.000 \pm 0.00b	95 \pm 91a	0.11 \pm 0.02c	-2.8 \pm 0.6ab
<i>E. pulverulenta</i>	2.9 \pm 0.5bc	0.07 \pm 0.02ab	0.001 \pm 0.001ab	49 \pm 7a	0.18 \pm 0.02bc	-3.8 \pm 0.4c
<i>Q. saponaria</i>	4.7 \pm 1.4bc	0.10 \pm 0.02ab	0.001 \pm 0.001ab	73 \pm 24a	0.15 \pm 0.02bc	-2.2 \pm 0.5ab
<i>L. caustica</i>	4.6 \pm 0.5bc	0.05 \pm 0.01b	0.001 \pm 0.001b	125 \pm 18a	0.11 \pm 0.08c	-1.3 \pm 0.1a
<i>C. odorifera</i>	7.4 \pm 1.7ab	0.13 \pm 0.04ab	0.002 \pm 0.001ab	54 \pm 25a	0.20 \pm 0.01ab	-1.9 \pm 0.5a
<i>A. caven</i>	9.8 \pm 1.9a	0.23 \pm 0.07a	0.004 \pm 0.00a	77 \pm 22a	0.29 \pm 0.02a	-1.8 \pm 0.2a
Watering treatment						
W+	6.4 \pm 0.9A	0.10 \pm 0.02A	0.002 \pm 0.00A	89 \pm 12A	0.17 \pm 0.01A	-1.9 \pm 0.2A
W-	3.5 \pm 0.7B	0.09 \pm 0.02A	0.001 \pm 0.00A	68 \pm 29A	0.17 \pm 0.01A	-2.7 \pm 0.3B
F- value and significance						
Watering (W)	9.1**	0.0ns	0.1ns	0.2ns	0.0ns	5.6*
Species (S)	8.1**	3.7**	3.9**	0.5ns	10.4**	4.9**
W \times S	1.5ns	2.6*	2.6*	0.8ns	0.1ns	1.9ns

(**): $p < 0.01$, (*): $p < 0.05$; (ns): non-significant.



Discussion

Studies on sclerophyllous species have shown that survival and growth improved using larger seedlings (Puértolas et al. 2003; Villar-Salvador et al. 2004; Cuesta et al. 2010), whereas the opposite is expected when planting small seedlings due to the reduced volume exploration by roots and consequently the insufficient water uptake and survival (Oliet et al. 2009). In our study, however, we observed a different trend in the species *Q. saponaria* and *C. odorifera*. Both species were the tallest at the beginning of the experiment, but they were not the best survivors. On the contrary, the species *A. caven* and *P. boldus* were among the shortest seedlings prior to planting and exhibited positive increments in height after outplanting; however, survival in the W- treatment was dramatically different each other. *A. caven* had a survival 10 times superior to *P. boldus*. Similarly, *L. caustica* had a middle H prior to planting, but the species exhibited the highest survival in both watering treatments. It seems that in the study site, characterized by low soil water availability and high temperatures during summer, the survival is not related with seedling H, and it might probably be related to seedling H/D. In the case of *A. caven* and *L. caustica*, both species exhibited H/D ratios near 4.5 prior to planting (data not shown). While the optimum range for H/D that signifies good quality seedlings is unknown for the species under study, the establishment of seedling with excessive low or high H/D values (i.e., stocky or spindly seedlings, respectively), it is not recommended as they have a low chance to survive in dry and windy sites (Grossnickle and MacDonald 2018). The survival of *P. boldus* seems to be more associated to physiological traits because the species had H/D similar to that of *A. caven* and *L. caustica* but its SUR was drastically reduced in the W- treatment.

Photosynthesis and stomatal conductance varied considerably among species, and results compared well with other studies in the same species under moderate to severe water restriction (Donoso et al. 2015; Magni et al. 2018; Espinoza et al. 2020). Considering the responses in stomatal regulation of water status after the post-transplant shock and during the summer season, the six species under study can be grouped according to their drought avoidance strategy as “water savers” and “water spenders” (Guehl and Aussenac 1987; Lo Gullo and Salleo 1988; McDowell et al. 2008; Nolan et al. 2017). On the one hand, the species *P. boldus* and *E. pulverulenta* seems to exhibit to the “water-saver” strategy (isohydric behavior) i.e., they close stomata to prevent damage but increase water potential (less negative) at the risk of carbon depletion (particularly *P. boldus*), whereas *L. caustica*, *C. odorifera*, *Q. saponaria*, and *A. caven* seems to follow the “water-spender” strategy (anisohydric behavior) i.e., they open stomata and decrease water potential, without negatively affecting carbon assimilation. The high survival and carbon assimilation of *A. caven*; associated to high stomatal conductance and low water potentials in comparison to the other species, might be also related to the small leaf area of this species, which implies lower water demands. The species was able to maintain a high survival in the W- treatment even with half the stomatal conductance reported in the W+ treatment. According to Grassi and Magnani (2005) g_s lower than $0.1 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$ are indicative of severely water stressed seedlings, but *A. caven* was the only species able to maintain $g_s > 0.1 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$ in the W- treatment. However, the species *L. caustica* had the highest survival in the W- treatment with only $0.04 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$ of g_s .

Conclusions/ wider implications of findings

The six species under study possessed different responses to cope with water stress. *A. caven* showed high stomatal conductance and relatively low water potential. In contrast, *L. caustica* maintained higher water potentials with important stomatal closure, demonstrating a drought tolerance response. None of the species under study were able to sustain both high water potential and stomatal activity and the least drought-resistant species was *P. boldus*. This species developed low water potentials despite having a very low

stomatal conductance. We conclude that seedlings of *P. boldus* and *E. pulverulenta* do not possess adequate responses to water stress that confer the capacity to survive the post-transplant shock during arid summers in Mediterranean-type climates.

This investigation was financed by a Chilean governmental agency with the purpose that small landholders were involved in the restoration of burned areas. In this first stage we have corroborated which native species can be used for active restoration of severely burned and drought-prone sites, but the involvement of small landholders remains a challenge because of their current demands (i.e., home, water, fodder, etc.) and their preference for the establishment of fast-growing species (i.e., *Eucalyptus* and *Pinus* plantations) with higher values for end products (i.e., posts, logs, pulpwood, etc).

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