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Interactive effects of water stress, container size and fertilizer on survival, gas exchange and morphological traits of *Quillaja saponaria* seedlings

Efectos combinados de la restricción hídrica, el tamaño de contenedor y la dosis de fertilizante en la supervivencia, intercambio gaseoso y atributos morfológicos en plantas de *Quillaja saponaria*

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SUMMARY

Quillaja saponaria is a valuable commercial and ecological Chilean native species that has suffered considerably degradation countrywide; however, the determination of the optimum characteristics of the planting stock, an important aspect in restoration projects, has receive little attention. The aim of this study was to evaluate the effect of container size and dose of fertilizer on morphological and physiological responses of *Q. saponaria* seedlings cultivated in a nursery under well-watered and water restricted conditions. After 27 days growing under contrasting watering regimes, growth, biomass allocation, and gas exchange were measured in 6-month-old seedlings. We found that survival, height and diameter increment, and root dry weight were higher in the water stressed seedlings cultivated in large containers. The water stressed seedlings cultivated in large containers also had superior stomatal conductance, however when the fertilizer dose was decreased, this trait decreased considerably. Thus, a superior performance in a water-restricted environment could be expected in those seedlings cultivated in larger containers with low fertilizer.

Key words: biomass allocation, water stress, gas exchange, quillay, survival.

RESUMEN

Quillaja saponaria es una especie nativa del bosque nativo chileno valiosa por sus cualidades económicas y ecológicas y que ha sufrido una importante degradación a lo largo del país; sin embargo, la determinación de las características óptimas del material de plantación, un aspecto importante en proyectos de restauración, ha recibido poca atención. El objetivo de este estudio fue evaluar el efecto del tamaño de contenedor y la dosis de fertilizante en la respuesta morfológica y fisiológica de plantas de *Q. saponaria* cultivadas bajo dos regímenes de riego en vivero (riego normal vs. restricción hídrica). Después de 27 días de crecimiento bajo condiciones de riego contrastantes, se evaluó el crecimiento, la asignación de biomasa y variables de intercambio gaseoso en plantas de seis meses de edad. Los resultados indican que en el tratamiento de restricción hídrica las plantas cultivadas en contenedores grandes mostraron la mayor supervivencia, incremento en diámetro y altura y biomasa radical. Las plantas del tratamiento de restricción hídrica cultivadas en contenedores grandes también mostraron tasas superiores de conductancia estomática, sin embargo, cuando se disminuyó la dosis de fertilizante, esta variable disminuyó considerablemente. Se podría esperar que, aquellas plantas cultivadas en contenedores de mayor volumen y con dosis baja de fertilizante, tuvieran un mejor desempeño en ambientes con restricción hídrica.

Palabras clave: asignación de biomasa, restricción hídrica, intercambio gaseoso, quillay, supervivencia.

INTRODUCTION

Quillaja saponaria Mol. is a sclerophyllus evergreen tree species widely distributed in Mediterranean central Chile, which has suffered substantial degradation due to harvesting and conversion of land to agricultural uses (INFOR 2000). In addition to its ecological importance (Cruz *et al*. 2013), *Q. saponaria* is valuable for its commercial bark saponin extract (Pelah *et al*. 2002). Although restoration activities have been developed in Chile, little is known about seedling quality and nursery cultivation in containers, both key aspects for the success in the restoration of Mediterranean ecosystems (Chirino *et al*. 2008) as they modify characteristics of the planting stock, by affecting the availability of water and nutrients (McConnughay and Bazzar 1991) and influencing field performance (Villar-Salvador *et al*. 2004).

The container type determines the morphological characteristics of the root system (Landis *et al*. 1990, Domínguez-Lerena *et al*. 2006, Chirino *et al*. 2008). Larger containers usually result in larger root systems which are linked to the capacity of seedlings to avoid lethal water stress since roots reach deeper moist soil horizons (Villar-Salvador *et al*. 2012a), while a small container physically limits root growth (Aphalo and Rikala 2003) due to the low quantity of soil and the reduction in water availability and nutrient content (Poorter *et al*. 2012). Fertilization also determines plant functional attributes, enhancing growth and outplanting survival in conifers from mesic environments (Luis *et al*. 2009) and in Mediterranean evergreen sclerophyllus trees (Villar-Salvador *et al*. 2004); however, fertilization can increase water uptake by a species in some cases or reduce its drought tolerance in others (Cortina *et al*. 2013). The intensity of drought, site conditions and species' response can explain these differences (Villar-Salvador *et al*. 2012b). Ovalle *et al*. (2016) found that high fertilized *Q. saponaria* seedlings established in Mediterranean field conditions (*i.e*., dry period of seven months) did not show a superior performance when contrasted to unfertilized ones. In extremely harsh Mediterranean environments, low fertilized seedlings are considered more resistant to drought because they reduce leaf area and increase root biomass (Hernández *et al*. 2009), both characteristics of a drought tolerant genotype.

Seedling quality and nursery culture regimes for *Q. saponaria* seedlings have received little attention in Chile (*e.g.*, Ovalle *et al*. 2016). Thus, in an effort to contribute to the understanding of *Q. saponaria* nursery culture, we initiated a preliminary study aimed at determining the influence of watering, container size and fertilization level on the early growth, survival and physiological responses of the species. We hypothesized that seedlings cultivated in larger containers with low fertilizer will have a superior performance in water stress conditions.

METHODS

Nursery experiment. We applied two watering treatments (well-watered (WW) and water-stress (WS)), two container sizes (140 and 280 mL) and three fertilizer doses (2, 4 and 6 g L^{-1} of substrate) to 1,620 seedlings in total (2 watering regimes (W) × 2 container sizes (C) × 3 fertilizer doses (F) × 3 replicates × 45 seedlings per replicate). The substrate consisted of a mixture of composted bark and perlite (7:3 v) combined with the slow release fertilizer Basacote 9 M® at the proposed doses. Seeds were sown on 09/15/2013 (day 1). Following germination, seedlings were arranged in a split-plot design, with watering regime as the whole plot and container size crossed with dose of fertilizer as the sub-plots. After 150 days of growth under watering to container capacity (*i.e*., until 02/11/2014), seedlings were submitted to WW and WS treatments for 27 more days (*i.e*., until 03/10/2014, totalizing 177 days growth). The severity of the watering regimes was based on predawn water potential (Ψ_{pd}). In the WW treatment seedlings maintained a Ψ_{pd} of -0.5 MPa, as measured with a pressure chamber (PMS Instruments Co., Corvallis, OR, USA). In the WS treatment, three 7-day cycles of withholding of water, plus two days of watering between each cycle, were applied. Ψ_{pd} was -2.0 MPa on average.

Morphological assessments. We measured height (H, cm) and root collar diameter (D, mm) in days 150 and 177. Increments in D and H (INC_{DH}, mm) were calculated as $INC_{DH} = 177_{DH} – 150_{DH}$. Seedlings were harvested on day 177 and oven-dried at 65 °C for 48 h. Three sections of each seedling (*i.e.*, roots, leaves and stems) were weighed (± 0.01 g), giving the root dry weight (RDW, mg), the leaves dry weight (LDW, mg) and the stem dry weight (SDW, mg). The root:shoot ratio was derived as RSR = RDW/(SDW+LDW). Survival (SUR, %) was measured as a categorical trait (*i.e.*, 1 = alive seedling, 0 = dead seedling).

Gas exchange and xylem water potential measurements. After the water stress cycles had finished, net photosynthesis (A_n, µmol CO_2 m^{-2} s^{-1}), transpiration (E, mmol H_2O m^{-2} s^{-1}) and stomatal conductance (g_s, mmol H_2O m^{-2} s^{-1}) were measured in five seedlings per treatment. Intrinsic water use efficiency (IWUE, µmol CO_2 mol H_2O^{-1}) was calculated as the ratio between the net photosynthesis and the stomatal conductance. Measurements were taken between 11:00 and 15:00 (local time) with a portable photosynthesis system (Li-6400XT, LI-COR Inc., Lincoln, NE, USA) under 1,300 mmol m^{-2} s^{-1}. After that, stems were excised near the substrate surface and predawn water potential (ψ_{pd}) was measured in five seedlings per treatment.

Statistical analyses. All traits were analyzed with the general linear model approach (GLM) to analysis of variance, with type III sum of squares, using SPSS software version 18 (SPSS Inc, Chicago, Illinois, USA). Comparisons for the categorical trait survival were performed using a Chi-square test.

RESULTS

Morphological response of Q. saponaria *seedlings*. Interaction of watering and container size had a strong influence on most seedling attributes (table 1). Those water-stressed seedlings cultivated in larger containers showed the highest survival, height and diameter increment, and root dry weight, while those water-stressed seedlings cultivated in small containers showed the opposite tendency as they were larger and with slightly more biomass allocated to leaves (figure 1). Interactions between watering and fertilizer, and container with fertilizer also affected plant development; well-watered and high fertilized seedlings were larger but with less shoot biomass (figure 2A, 2B), while larger container and medium fertilized seedlings showed a superior height increment (figure 2C). Triple interaction of fertilizer dose with watering and container were only significant for root collar diameter ($P < 0.05$).

Physiological performance of Q. saponaria *seedlings*. Interactions between watering and container affected gaseous interchange traits (table 2). The water-stress seedlings cultivated in larger containers had a superior transpi-

Table 1. Significance level for factors and morphological traits under study. *Statistically significant values ($P < 0.05$).

Nivel de significancia para los factores y variables morfológicas analizadas. *Valores estadísticamente significativos ($P < 0,05$).

Traits	Significance level					
	W	C	F	W×C	W×F	C×F
D	0.309	0.119	0.064	0.056	0.166	0.061
H	0.001*	0.002*	0.081	0.001*	0.000*	0.146
INC_D	0.000*	0.283	0.097	0.005*	0.414	0.914
INC_H	0.394	0.000*	0.005*	0.000*	0.073	0.022*
RDW	0.632	0.162	0.832	0.000*	0.056	0.259
LDW	0.298	0.005	0.466	0.001*	0.059	0.596
SDW	0.519	0.003	0.449	0.000*	0.013*	0.737
RSR	0.146	0.124	0.764	0.222	0.714	0.880
SUR	0.000*	0.000*	0.153	0.000*	0.263	0.161

D = root collar diameter, H = height, INC_D = diameter increment, INC_H = height increment, RDW = roots dry weight, LDW = leaves dry weight, SDW = shoot dry weight, RSR = root:shoot ratio, SUR = survival. No significant effect for W × C × F interaction was found in most traits, so for simplicity, data are not shown.

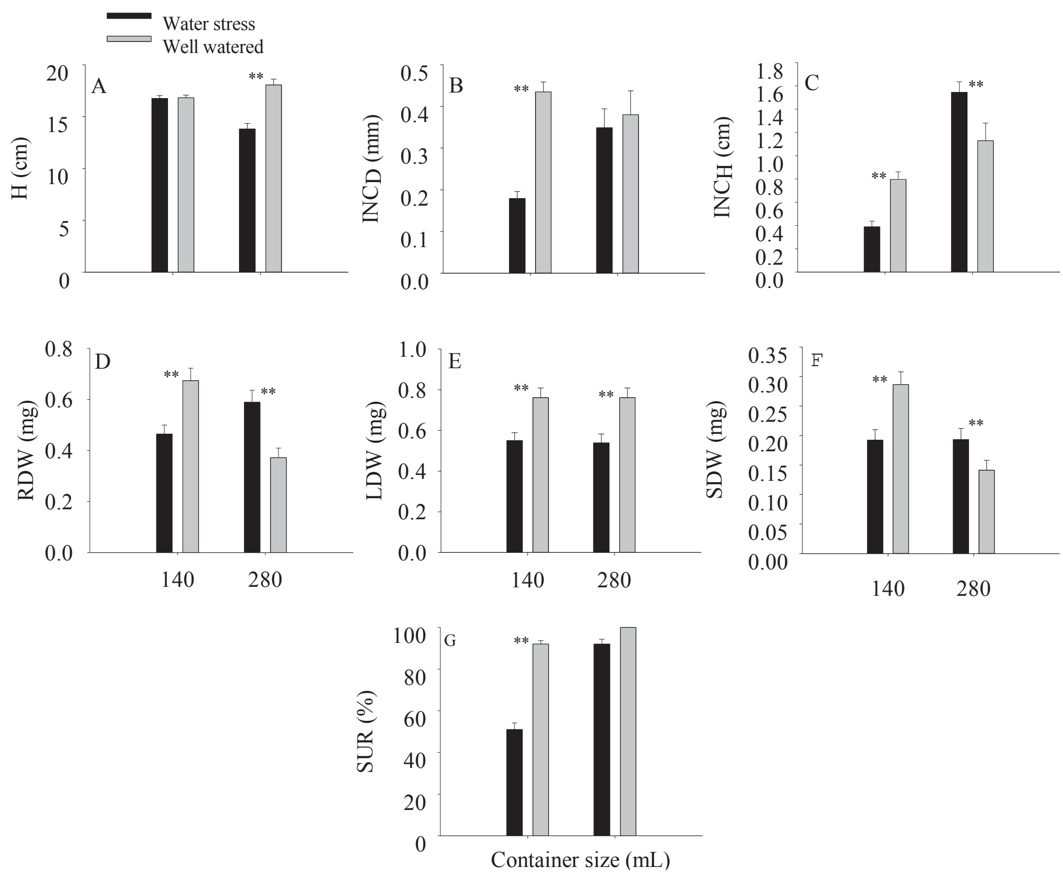

Figure 1. Interactive effect of the watering (WW vs WS) and container (140 vs 280 mL) treatments on growth, biomass and survival of *Q*. *saponaria* seedlings. H = height, INC_D = diameter increment, INC_H = height increment, RDW = roots dry weight, LDW = leaves dry weight, SDW = shoot dry weight, SUR = survival. ** indicate significant differences within water treatments at the 0.01 level. The error bars indicate the standard error.

Efecto de los tratamientos de riego (WW vs WS) y contenedor (140 vs 280 mL) en el crecimiento, biomasa y supervivencia de *Q*. *saponaria*. H = altura, INC_D = incremento en diámetro, INC_H = incremento en altura, RDW = peso seco raíces, LDW = peso seco hojas, SDW = peso seco tallo, SUR = supervivencia. ** indica diferencias significativas al interior de los tratamientos de riego al nivel del 0,01. Las barras representan el error estándar.

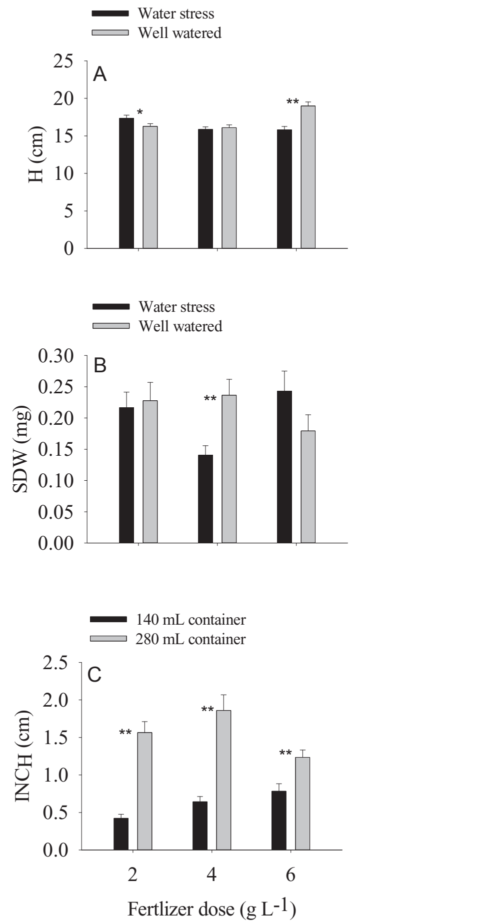

Figure 2. Interactive effect of the watering (WW vs WS) and fertilizer (2, 4 and 6 g L^{-1}) treatments (A, B), and between container (140 vs 280 mL) and fertilizer (C) on growth and biomass of *Q. saponaria* seedlings. H = height, SDW = shoots dry weight, INC_H = height increment. * and ** indicate significant differences within water and container treatments at the 0.05 and 0.01 levels, respectively. The error bars indicate the standard error.

Efecto de los tratamientos de riego (WW vs WS) y fertilizante (2, 4 y 6 g L^{-1}) (A, B), y entre el contenedor (140 vs 280 mL) y fertilizante (C) en el crecimiento y biomasa de plantas de *Q. saponaria*. H = altura, SDW = peso seco tallo, INC_D = incremento en diámetro. * y ** indican diferencias significativas al interior de los tratamientos de riego y contenedor al nivel del 0,05 y 0,01 respectivamente. Las barras representan el error estándar.

ration and stomatal conductance (figure 3A, 3B), although they were less water use efficient (figure 3C) contrasted to small container seedlings. The interaction of watering and fertilizer also affected seedlings physiological status. In the water-stressed treatment photosynthesis and stomatal conductance were higher for seedlings cultivated with higher fertilizer (figure 3D, 3E). Container and fertilizer also had significant effects; low fertilized seedlings, indistinctly of container, were more water use efficient (figure 3F); however, a high variability for small seedlings was observed, thus, these results must be interpreted with caution.

DISCUSSION

Growth, biomass and survival. The higher survival of the water stressed seedlings cultivated in large containers is explained by their small size with the consequent more water per gram of plant. Donoso *et al*. (2011) found that water stressed *Q. saponaria* saplings reduced 55 % of leaf biomass, which in turn decreased the transpiration rate. In contrast, small container seedlings were taller and probably depleted the pot water more quickly as they had slightly more leaf biomass. According to Poorter *et al*. (2012), water is depleted quickly when large plants are cultivated in small containers. Another explanation for the higher survival of water stressed seedlings cultivated in large containers is their higher root biomass which suggests that increased root biomass may have enlarged *Q. saponaria* root capacity to supply water per unit of leaf area and to maintain higher water potential in a water restricted environment (*i.e*., -1.6 MPa). By contrast, the small water-stressed container seedlings had a water potential of -2.4 MPa.

Physiological performance. The lowest gas exchange values for water stressed seedlings reflect the extent that low water potential, as that experienced by the stressed seedlings, can reduce stomatal conductance. This effect tended to be more pronounced in low fertilized seedlings, which indicates that fertilization improves gaseous interchange variables during the drydown of *Q. saponaria*. Trubat *et*

Table 2. Significance level for factors and physiological traits under study. *Statistically significant values ($P < 0.05$).

Nivel de significancia para los factores y variables fisiológicas analizadas. *Valores estadísticamente significativos ($P < 0,05$).

Traits	Significance level					
	W	C	F	W × C	W × F	C × F
A_n	0.000*	0.587	0.000*	0.540	0.005*	0.225
E	0.000*	0.009*	0.001*	0.000*	0.292	0.067
g_s	0.000*	0.090	0.058	0.019*	0.042*	0.065
IWUE	0.000*	0.228	0.013*	0.002*	0.186	0.026*

A_n = net photosynthesis, E = transpiration, g_s = stomatal conductance, IWUE = intrinsic water use efficiency. No significant effect for W × C × F interaction was found in all traits, hence for simplicity, data are not shown.

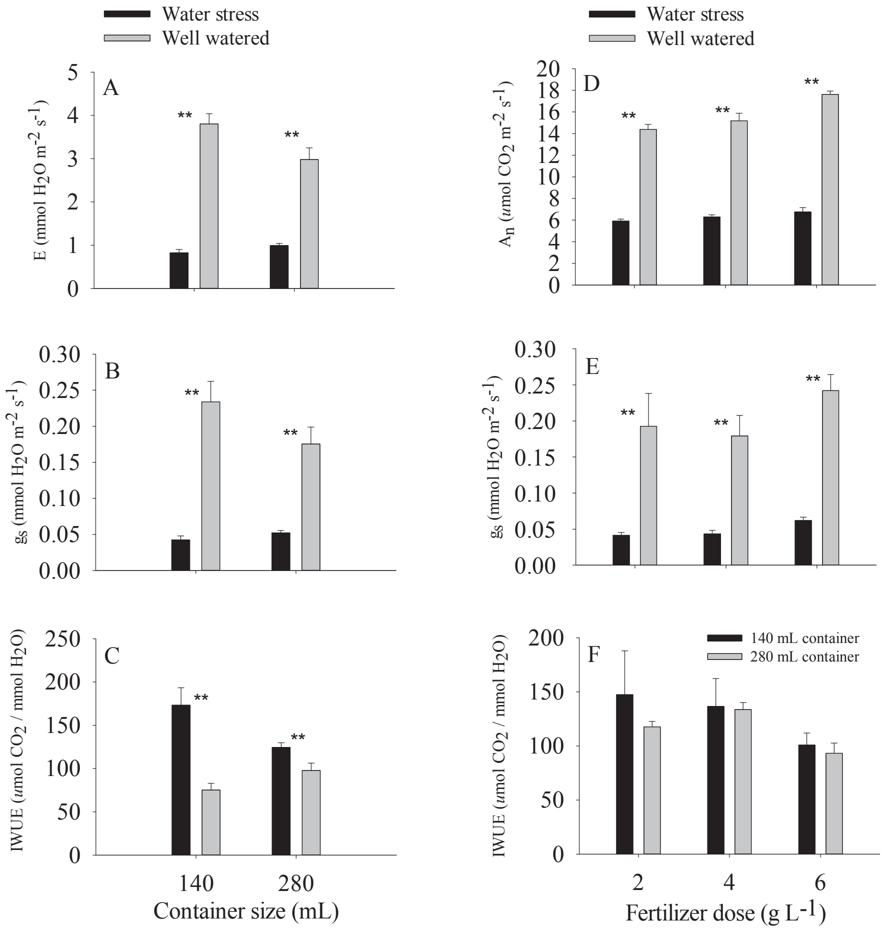

Figure 3. Interactive effect of the watering (WW vs WS) and container (140 vs 280 mL) treatments (A, B, C); between watering and fertilizer (2, 4 and 6 g L^{-1}) treatments (D, E), and between container and fertilizer (F), on gas exchange of *Q*. *saponaria* seedlings. A_n = net photosynthesis, E = transpiration, g_s = stomatal conductance, IWUE = intrinsic water use efficiency. ** indicate significant differences within water and container treatments at the 0.01 level. The error bars indicate the standard error.

Efecto de los tratamientos de riego (WW vs WS) y contenedor (140 vs 280 mL) (A, B, C); del riego y fertilizante (2, 4 y 6 g L^{-1}) (D, E), y entre el contenedor y fertilizante (F) en el intercambio gaseoso de plantas de *Q*. *saponaria*. A_n = fotosíntesis neta, E = transpiración, g_s = conductancia estomática, IWUE = eficiencia intrínseca del uso del agua. ** indica diferencias significativas al interior de los tratamientos de riego y contenedor al nivel del 0,01. Las barras representan el error estándar.

al. (2008) pointed out that the suppression of nitrogen inputs has shown promising results in semi-arid areas. A low nutrient availability reduces transpiration (Evans 1996) and increases water use efficiency (Hernández *et al*. 2009). Our results corroborate those findings because higher intrinsic water use efficiency was found in the water stressed seedlings cultivated with low fertilizing. Negative effects of high fertilization on the drought tolerance of *Quercus ilex* L. were observed by Villar-Salvador *et al*. (2004). This is because increased nitrogen nutrition produces bigger plants that can deplete water more rapidly (due to a higher leaf biomass and rate of transpiration) compared to plants with lower nitrogen nutrition (Lloret *et al*. 1999).

Seedlings cultivated in larger containers with low fertilizer had a superior growth and survival, and low stomatal conductance in the water-stress condition. These preliminary results allow us to speculate that those type of seedlings could have more probability of surviving outplanting in Mediterranean ecosystems; however, this hypothesis needs further research.

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REFERENCES

Aphalo P, R Rikala. 2003. Field performance of silver-birch planting-stock grown at different spacing and in containers of different volume. *New Forests* 25(2):93-108.

Cruz P, C Schulz, P Honeyman, A Cabello. 2013. *Quillaja sapo-*

naria Mol. Quillay Familia: Quillajaceae. *In* Donoso C ed. Las Especies Arbóreas de los Bosques Templados de Chile y Argentina. Autoecología. Valdivia, Chile. Marisa Cuneo Ediciones. p. 546-556.

Cortina J, A Vilagrosa, R Trubat. 2013. The role of nutrients for improving seedling quality in drylands. *New Forests* 44(5): 719-732.

Chirino E, A Vilagrosa, EI Hernández, A Matos, VR Vallejo. 2008. Effects of a deep container on morpho-functional characteristics and root colonization in *Quercus suber* L. seedlings for reforestation in Mediterranean climate. *Forest Ecology and Management* 256(4): 779-785.

Donoso S, K Peña, C Pacheco, G Luna, A Aguirre. 2011. Respuesta fisiológica y de crecimiento en plantas de *Quillaja saponaria* y *Cryptocarya alba* sometidas a restricción hídrica. *Bosque* 32(2): 187-195.

Domínguez-Lerena S, N Herrero, I Carrasco, L Ocaña, JL Peñuelas, JG Mexal. 2006. Container characteristics influence *Pinus pinea* seedlings development in the nursery and field. *Forest Ecology and Management* 221(1): 63-71.

Evans JR. 1996. Developmental constraints on photosynthesis: effects of light and nutrition. *In* Baker NR ed. Photosynthesis and the environment. Kluwer, Dordrecht. p. 281-304.

Hernández EI, A Vilagrosa, VC Luis, M Llorca, E Chirino, RV Vallejo. 2009. Root hydraulic conductance, gas exchange and water potential in seedlings of *Pistacia lentiscus* L. and *Quercus suber* L. under different conditions of fertilization and light radiation. *Environmental and Experimental Botany* 67(1): 267-276.

INFOR (Instituto Forestal, CL). 2000. Quillay: Una alternativa multipropósito para la zona central. Santiago, Chile. INFOR. 12 p.

Landis TD, RW Tinus, SE McDonald, JP Barnett. 1990. Containers and growing media, vol. 2. The container Tree Nursery Manual. Agric. Handbook, 674. Washington, DC, USA. U.S. Department of Agriculture Forest Service. 88 p.

Lloret F, C Casanovas, J Peñuelas. 1999. Seedling survival of Mediterranean shrubland species in relation to root:shoot ratio, seed size and water and nitrogen use. *Functional Ecology* 13(2): 210-216.

Luis VC, J Puértolas, J Climent, J Peters, AM González-Rodríguez, D Morales, MS Jiménez. 2009. Nursery fertilization enhances survival and physiological status in Canary Island pine (*Pinus canariensis*) seedlings planted in a semiarid environment. *European Journal of Forest Research* 128(3): 221-229.

McConnughay KDM, FA Bazzar. 1991. Is physical space a soil resource? *Ecology* 72 (1): 94-103.

Ovalle JF, EC Arellano, JA Oliet, P Becerra, R Ginocchio. 2016. Linking nursery nutritional status and water availability post-planting under intense summer drought: the case of a South American Mediterranean tree species. *iForest Biogeosciences and Forestry* 9(5):758-765.

Pelah D, Z Abramovich, A Markus, Z Wiesman. 2002. The use of commercial saponin from *Quillaja saponaria* bark as a natural larvicidal agent against *Aedes aegypti* and *Culex pipiens*. *Journal of Ethnopharmacology* 81(3):407-409.

Poorter H, J Bühler, J van Dusschoten, J Climent, JA Postma. 2012. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Functional Plant Biology* 39(11): 839-850.

Trubat R, J Cortina, A Vilagrosa. 2008. Short-term nitrogen deprivation increases field performance in nursery seedlings of Mediterranean woody species. *Journal of Arid Environments* 72(6):879-890.

Villar-Salvador P, R Planelles, E Enríquez, J Peñuelas-Rubira. 2004. Nursery cultivation regimes, plant functional attributes, and field performance relationships in the Mediterranean oak *Quercus ilex* L. *Forest Ecology and Management* 196(2): 257-266.

Villar-Salvador P, J Puértolas, B Cuesta, J Peñuelas, M Uscola, N Heredia-Guerrero, JM Benayas. 2012a. Increase in size and nitrogen concentration enhances seedling survival in Mediterranean plantations. Insights from an ecophysiological conceptual model of plant survival. *New Forests* 43(5-6): 755-770.

Villar-Salvador P, JL Peñuelas, DF Jacobs. 2012b. Nitrogen nutrition and drought hardening exert opposite effects on the stress tolerance of *Pinus pinea* L. seedlings. *Tree Physiology* 33:221-232.

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