



Production and establishment techniques for the restoration of *Nothofagus alessandrii*, an endangered keystone species in a Mediterranean forest

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Abstract

Ruil (*Nothofagus alessandrii*) is an endangered keystone species from the Mediterranean climate zone of Chile. Ruil's fragile state of conservation urges development of restoration programs, but specific protocols for nursery production and field establishment that ensure plant survival are largely unknown. Therefore, we examined the effect on nitrogen (N) fertilization and container size during nursery production in combination with the use of mesh shelters after outplanting on survival and growth during the first growing season in the field. First year outplanting survival of nursery-grown container seedlings was enhanced when seedlings were given nitrogen (N) during nursery production and deployed with mesh tree shelters in the field. The volume of the container had no effect on outplanting survival and growth. Increasing N from zero to 200 mg N L⁻¹ provided sufficient N levels, resulting in increased seedling height, root-collar diameter, shoot biomass, and total seedling N and phosphorus concentrations. Additional N provided by the 400 and 600 mg N L⁻¹ treatments underwent luxury consumption by the seedlings with no further benefits in field performance. Improved growth in the nursery, along with the use of mesh tree shelters after outplanting, especially during the typical summer drought, may be responsible for increased survival during the first growing season. Increasing the performance of nursery-grown ruil seedlings is essential to restoring this endangered, vulnerable, and foundation species within the highly biodiverse, yet seriously threatened endemic Maulino Costero Forest of the Mediterranean climate of central Chile.

Keywords Container volume · Mediterranean climate · Nitrogen fertilization · Outplanting performance · Ruil · Tree shelter

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Introduction

Acute loss and chronic degradation of forests is a worldwide problem. Minnemeyer et al. (2011) estimated that nearly two billion ha of forestland are degraded; conversion to agriculture, poor silvicultural practices, and losses to urban population growth are important factors (Ahrends et al. 2010; DeFries et al. 2010). This degradation has led to loss of biodiversity and other ecosystem services (Duffy 2009; Butchart et al. 2010). Worldwide, forests within Mediterranean climate zones have not been immune to this problem (Scarascia-Mugnozza et al. 2000).

Outplanting seedlings is often desired for restoration programs because high-quality seedlings can ensure successful establishment and a rapid restoration trajectory (Pinto et al. 2011; Stanturf et al. 2014). Successful establishment is particularly challenging in regions with Mediterranean climates because of a dry summer season that occurs simultaneously with high temperatures that together impose physiological drought during a 1–5 months period (Quezel 1985; Valladares et al. 2004). Unfortunately, several climate change models predict Mediterranean regions will experience increasing temperatures and decreasing precipitation (Santibañez et al. 2014), intensifying the length and magnitude of droughts (Sheffield and Wood 2008) and exacerbating the challenge of restoring Mediterranean forests. In addition, reforestation success in Mediterranean forests can be limited by poor management during nursery production, which yields seedlings of low quality that lack the ability to obtain sufficient water during the summer drought (Becerra et al. 2011). Thus, nursery managers aim to produce high-quality plants with phenotypic characteristics linked to water stress avoidance, such as reduced leaf area, greater root-collar diameter (RCD), low shoot-to-root ratio, reduced height, and large root volume (Trubat et al. 2006). Nursery production techniques need to achieve specific morpho-physiological characteristics for seedlings destined for Mediterranean conditions are, however, highly species-specific.

A seedling's ability to thrive after outplanting is influenced by nursery practices. The Target Plant Concept assists land and nursery managers in producing the correct seedling stocktype toward enhancing outplanting survival and growth despite expected stresses incurred in the field (Dumroese et al. 2016). Nursery practices can improve the morphological and physiological properties of that stocktype (i.e., its quality), particularly those related to water stress tolerance (van den Driessche 1984; Puttonen 1997; Vilagrosa et al. 2003). Morphological properties, the traditional basis for describing seedling quality (Grossnickle 2012), are positively correlated with container volume (Landis et al. 2010) and fertilization is one of the most critical aspects in developing high plant quality (Landis 1989).

Container attributes, which include characteristics paramount for restoration (Landis et al. 2010), and fertilization have been the focus of seedling quality research for species found in Mediterranean regions such as Canary Island pine (*Pinus canariensis*) (Luis et al. 2009), ponderosa pine (*Pinus ponderosa*) (Pinto et al. 2011), and several oak (*Quercus*) species (Villar-Salvador et al. 2004; Trubat et al. 2010; Mariotti et al. 2015). In Chile, while exotic plantation species have been investigated (Coopman et al. 2008; Monsalve et al. 2009; González-Rodríguez et al. 2011), research regarding production and quality of native Mediterranean species for restoration is scarce and is mainly focused on the effects of slow release fertilizer on growth during the nursery stage for species such as ulmo (*Eucryphia cordifolia*), coigüe (*Nothofagus dombeyi*), huala (*Nothofagus leonii*), raulí (*Nothofagus nervosa*), and quillay (*Quillaja saponaria*) (Bustos et al. 2008; Donoso et al. 2009; Santelices et al. 2015; Espinoza et al. 2017).

Fertilization is the most important nursery practice influencing plant quality for restoration, especially for plants produced in containers where root volume is limited (Landis 1989), but work with Mediterranean species has generated contrasting results. On one hand, research has shown positive correlations among nursery fertilization, plant size, and outplanting survival, suggesting that nutritional state (mainly nitrogen, N) provides an important explanation for enhanced performance during plantation establishment (Puértolas et al. 2003; Villar-Salvador et al. 2004; Luis et al. 2009; Oliet et al. 2009). This response could be linked to an increase in N and carbon that promotes a positive feedback cycle, where root growth sustains photosynthesis and photosynthesis promotes root and stem growth in return, ensuring plant establishment (Grossnickle 2005; del Campo et al. 2010; Trubat et al. 2011). On the other hand, Trubat et al. (2008) and Cortina et al. (2013) found that higher N fertilization during nursery production could produce a morphological imbalance when stem biomass exceeds root biomass. Under water stress conditions, such an imbalance could increase transpiration rates and reduce stem water potential and root hydraulic conductivity (Hernández et al. 2009).

In addition to container type and N fertilization, some on-site practices can also be implemented to promote seedling establishment. One such practice is the use of tree shelters (hereafter “shelters”), installed to protect seedlings against animal browsing (e.g., Taylor et al. 2006), as a complement to weed control (e.g., McCreary et al. 2011), and to improve survival and growth in areas with seasonal drought (e.g., Oliet et al. 2005; Luis et al. 2009; Cuesta et al. 2010; Oliet et al. 2019). Shelters, available in a variety of designs including solid or mesh, constitute a very low cost, easy-to-use, effective practice (Bainbridge 1994; Ponder 2003). Shelters can ameliorate air temperature, radiation, relative humidity, vapor pressure deficit (VPD), CO₂ concentration, and wind within them, thereby reducing evapotranspiration (Bergez and Dupraz 2000, 2009; Jiménez et al. 2005; Navarro-Cerrillo et al. 2005; Puértolas et al. 2010).

Seedling performance within shelters appears to be species-specific, especially in dry environments (Marques et al. 2001; Oliet et al. 2019), and because of species’ shade tolerance levels. In Mediterranean climates, Puértolas et al. (2009) observed increased field mortality and decreased root growth when shade-intolerant Aleppo pine (*Pinus halepensis*) was grown in deep shade, while opposite results were found for holm oak (*Quercus ilex*), a shade-tolerant species. Despite being classified as a shade-intolerant species (Bustamante and Castor 1998), ruil (*Nothofagus alessandrii*) grew poorer during nursery production under full sunlight than in moderate shade (Santelices et al. 2014), suggesting that it might benefit from the shading effects afforded by shelters when outplanted.

The need to restore Mediterranean forests in Chile is great. Chile’s historical, natural isolation promoted an exceptionally high level of endemism and biodiversity (Smith-Ramírez 2004; Armesto et al. 1996). Natural forests of Chile’s Mediterranean region comprise one of the world’s 25 biodiversity hotspots (Myers et al. 2000) but have been declared one of the world’s most threatened habitats (Dinerstein et al. 1995). Specifically, the Maulino Costero Forest of Chile’s central region is characterized by high floristic species richness with one of the highest numbers of tree species among Chilean forests (Echeverría et al. 2006). Ruil is an endemic, keystone species within this forest association (Santelices and Riquelme 2007). In less than a century, nearly all of the Maulino Costero Forest and its ruil were converted to monoculture plantations of radiata pine (*Pinus radiata*) and blue gum (*Eucalyptus globulus*) (Armesto et al. 1992; Clapp 1995; Nahuelhual et al. 2012). The remaining stands are increasingly fragmented (Echeverría et al. 2006). By 1991, ruil forest was relegated to 185 highly isolated, small fragments (≤ 12 ha; 95% were 1–2 ha each) (Bustamante and Castor 1998). In response to this detrimental state, ruil was declared a

national monument and considered an endangered species, hence its logging forbidden (Chilean Agriculture Ministry 1995; Barstow et al. 2017). The remaining Maulino Costero Forests was severely affected by forest fires in 2017 (Valencia et al. 2018). Because of its dominance in the Maulino Costero Forest and its critical state of conservation, ruiil has high-priority in restoration programs (Santelices et al. 2018).

Recognizing that the specific morpho-physiological characteristics plants need to withstand water stress under Mediterranean climate conditions are highly species-specific (Rubio et al. 2003); considering the urgency for restoration of the threatened, Mediterranean, Maulino Costero Forest, its diverse flora and fauna, and in particular its endangered, keystone species, ruiil; and the paucity of information for restoring the native Mediterranean forest ecosystems of Chile, we hypothesized that nursery produced ruiil seedlings grown in larger containers, fertilized with higher doses of N, and outplanted with mesh shelter protection would have increased performance during the first growing season. To test this hypothesis, we had two objectives. Our first objective was to evaluate the effect of container size and N fertilization on morpho-physiological attributes of nursery produced ruiil plants. Our second objective was to explore field performance of those morpho-physiological attributes with an added factor of mesh shelters to elucidate favorable conditions for ruiil restoration.

Methods

We employed two completely randomized factorial designs replicated three times. In the nursery, our independent factors were container volume (two levels) and N rate (four levels). After assessing plant quality, seedlings were outplanted in a field stage, where we added a third independent factor, shelter (two levels).

Nursery stage

Ruiil seedlings were grown at the Centro Tecnológico de la Planta Forestal, Instituto Forestal (lat -36.84° , long -73.13°), Biobío Region, Chile. Seeds were collected (March 2012) from the Curepto commune, Maule Region (lat -35.08° , long -72.03°), within ruiil's natural range, and promptly stored (8°C). Immediately before sowing (September 2012), we soaked seeds 48 h in Giberplus (10% w:w gibberellic acid; Anasac, Chile) at a concentration of 200 mg L^{-1} of gibberellic acid. Germination capacity was 64%. We sowed seeds into two types of expanded polystyrene containers having cavities 14 cm deep: (1) 84 cavities, $130\text{ mL cavity}^{-1}$, $336\text{ cavities m}^{-2}$; (2) 60 cavities, $280\text{ mL cavity}^{-1}$, $240\text{ cavities m}^{-2}$. Containers were filled with composted *P. radiata* bark medium having total, aeration, and water retention porosity of 49, 25, and 24%, respectively. The analysis of total nutritional composition of this substrate indicated 0.49% total N, 0.09% phosphorous (P_2O_5), 0.1% potassium (K_2O), 0.5% magnesium (MgO), and 0.7% calcium (CaO). Once germination was complete, we transplanted sufficient seedlings to ensure 12 full trays of each container type.

The N rate treatment commenced 60 days after sowing (November 2012) using four N concentrations: 0 (control), 200, 400, and 600 mg L^{-1} . To each N solution (0, 200, 400, and 600 mg L^{-1} ; the proportion of nitrate and ammonium was 1:1), we added phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) at constant rates of 150, 100, 80, 40, and 60 mg L^{-1} , respectively, using these soluble salts: urea ($\text{CO}(\text{NH}_2)_2$),

magnesium sulfate (MgSO_4), potassium sulfate (K_2SO_4), potassium carbonate (K_2CO_3), potassium nitrate (KNO_3), potassium phosphate monobasic (KH_2PO_4), monoammonium phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$), diammonium phosphate ($(\text{NH}_4)_2\text{HPO}_4$), ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$), calcium oxide (CaCO_3), and sodium nitrate (NaNO_3). Micronutrients were added according to Landis (1989). We randomly assigned three trays of each container type to each of the four N concentrations (each tray was a replicate). Irrigation need was monitored with soil moisture sensors (ECH₂O EC-5; Decagon, Pullman, WA USA); sensor values for volumetric water content ($\text{m}^3 \text{m}^{-3}$) were calibrated with gravimetric mass to estimate the percentage of available water (Dumroese et al. 2015). When the substrate lost 50% of the available water, fertigation (fertilizer mixed with water) was alternated with clear-water irrigation until June 2013. Fertigation and irrigation were administered by hand.

In June 2013 we randomly selected (from the interior of the containers) 32 seedlings from each container type (2) × N rate (4) × replicate (3) combination (768 total) and measured height (cm) and root-collar diameter (RCD; mm). Two additional seedlings were randomly selected from each replicate (6 seedlings per treatment; 48 total), separated into leaves, stems, and roots, and oven-dried at 65 °C until constant weight. We calculated biomass partitioning by dividing each plant component by total biomass to obtain ratios of leaf mass, stem mass, and root mass. Finally, the biomass seedlings from the 130-mL containers were composited within replicate, re-dried at 45 °C, ground, and analyzed for whole seedling N, P, K, and Ca concentrations using colorimetric (N and P) or atomic absorption (K and Ca) methods according to Temminghoff and Houba (2004).

Field stage

Our study site was located within the Curepto commune (lat -35.08° , long -72.03° ; 170 m.a.s.l) in Maule region, Chile. It is characterized by a Mediterranean climate with a summer drought of at least four months (usually December through March) and a mild winter rainy season (Amigo and Ramírez 1998). Average annual temperature and precipitation is 13.8 °C and 753 mm. January is typically the harshest month with mean maximum and absolute maximum temperatures of 24.4 and 30.3 °C and mean precipitation of 8 mm (Luebert and Plissock 2006). Previously a *P. radiata* plantation, the site was harvested 2 years before this experiment. Only a few, highly decomposed trunks were observed at outplanting, at which time scattered shrubs from 1 to 4 m in height were pruned to ground line. The soil is of metamorphic origin. Soil samples collected from six equally distributed points across the site showed an average pH of 6.1 and an organic matter content of 3.6%. Levels of soil nitrate, P, and K were 3.3 ± 0.6 , 4.2 ± 1.3 , and $137 \pm 62 \text{ mg kg}^{-1}$, respectively, and for Ca and Mg the values were 4.2 ± 2.3 and $2.6 \pm 1.2 \text{ cmol kg}^{-1}$, respectively.

In July 2013 we established three blocks (30 × 30 m; corresponding to the nursery replicates) perpendicular to the slope, each with 16 plots (7.5 × 7.5 m). In each block, we randomly assigned each container type × nitrogen rate combination to two plots, of which one was randomly assigned shelters. Each plot had 25 seedlings at a density of 4444 plants ha^{-1} (1.5 × 1.5 m; 25 seedlings × 2 container types × 4 nitrogen rates × 2 levels of shelter × 3 blocks = 1200 seedlings). We planted each seedling into a 40 × 40 × 40 cm area where the soil was loosened with a shovel. Each shelter consisted of three wooden posts 3–5 cm in diameter and 1.2 m long, forming an equilateral triangle about 15 cm from the seedling, covered to a height of 1 m with plastic mesh (Raschel, Marienberg, Chile). Photosynthetically active radiation (PAR) was measured with a LI-250A light meter (LI-COR, Lincoln,

Nebraska, USA) coupled to a LI-190R sensor (LI-COR, Lincoln, Nebraska, USA) inside and outside the mesh. Calculating the proportion of PAR inside shelters compared to full-sun exposure, we determined that the mesh shelters provided 60% shade.

Three months after outplanting (November 2013; onset of summer drought), we assessed survival ($n=1200$) and measured height (cm) and RCD (mm) on surviving seedlings. Survival was re-assessed monthly until the end of the first field season (May 2014) when height and RCD of all surviving seedlings was re-assessed.

Data analysis

For the nursery stage, our independent variables were container type and N rate in three replications. For the dependent variables [height (cm) and RCD (mm)], $n=32$ per container type \times N rate \times replication combination. For biomass, biomass partitioning, and total seedling concentration of N, P, K, and Ca, $n=2$ per container type \times N rate \times replication combination. For the field stage, our independent variables were container type, N rate, and shelter in three replications. The dependent variables of survival (%), height (cm), and RCD (mm) (initial $n=1200$ for each metric) were analyzed with a repeated measurement model (Kuehl 2001), modeling the variance and covariance structure with a 95% level of confidence. Statistical differences between means were performed with Tukey's HSD test for multiple comparisons. Statistical analyses were performed with R statistical software (version 3.4.3) using the nlme package (version 3.1-137).

Results

Nursery stage

We detected no neither an interaction of container type and N rate (all $p>0.21$) nor a container volume effect (all $p>0.14$) on final height, RCD, or biomass partitioning; only N rate was significant (Table 1). Applying 200–400 mg N L⁻¹ significantly increased height (~300%) compared to the non-N control ($p<0.0001$). These two N rates were similar and significantly shorter (~20%) than the 600 mg N L⁻¹ rate (Table 1). Although N rates ≥ 200 mg N L⁻¹ had no effect on RCD, they averaged significantly more RCD (~37%) than the control ($p=0.0006$). The pattern of significant differences for biomass partitioning of leaves and roots was similar to that of RCD, but leaf partitioning increased with increasing N rate ($p<0.0001$) whereas higher biomass partitioning toward roots occurred in the control ($p<0.0001$). Stem partitioning tended to increase with each increase in N rate (Table 1; $p<0.0001$). We observed no interaction of container type and N rate on biomass of seedling components (all $p\geq 0.51$). Compared to the control, N rate ≥ 200 mg L⁻¹ significantly affected the biomass of leaves ($p<0.0001$) and stems ($p<0.0001$) but not roots ($p=0.0636$) (Fig. 1a). The container with 280-mL cavities yielded plants with significantly more leaf ($p=0.0434$), stem ($p=0.0053$), and root ($p=0.0008$) biomass than those grown in 130-mL cavities (Fig. 1b).

Recalling that nutrient concentration was only evaluated for seedlings produced in 130-mL containers, we found that for total seedling N concentration, seedlings receiving ≥ 400 mg N L⁻¹ had significantly higher concentrations (~50%) than those receiving ≤ 200 mg N L⁻¹ (Table 1; $p=0.0081$). For total seedling P concentration, N rates ≥ 200 mg L⁻¹ resulted in 300% higher concentrations than the control ($p=0.0002$).

Table 1 Mean (\pm standard error) height; root collar diameter (RCD); ratio of leaf (LMR), stem (SMR), or root (RMR) mass to total seedling mass; and total seedling nitrogen and phosphorous concentration for ruii seedlings at the end of the growing season of nursery production having been grown with two container types and four rates of nitrogen (N)

Treatment	mg N L ⁻¹	Height (cm)	RCD (mm)	Biomass partitioning			Nutrient concentration (%)**	
				LMR	SMR	RMR	Nitrogen	Phosphorous
Nitrogen (N)	0	15.4 \pm 3.0 c*	3.84 \pm 0.20 b	0.11 \pm 0.05 b	0.36 \pm 0.06 c	0.53 \pm 0.04 a	0.61 \pm 0.04 b	0.11 \pm 0.02 b
	200	44.8 \pm 7.7 b	5.19 \pm 0.22 a	0.21 \pm 0.03 a	0.49 \pm 0.04 b	0.29 \pm 0.06 b	0.64 \pm 0.02 b	0.33 \pm 0.02 a
	400	44.8 \pm 8.6 b	5.14 \pm 0.28 a	0.19 \pm 0.05 a	0.53 \pm 0.04 ab	0.28 \pm 0.04 b	0.93 \pm 0.16 a	0.31 \pm 0.04 a
	600	54.3 \pm 6.4 a	5.51 \pm 0.15 a	0.18 \pm 0.04 a	0.55 \pm 0.05 a	0.27 \pm 0.03 b	0.94 \pm 0.07 a	0.28 \pm 0.02 a

*Different letters for each variable indicate significant differences at $p < 0.05$ according to Tukey HSD

**Nitrogen and phosphorous concentrations correspond to plants growing in 130-mL containers. Remaining variables include data from both types of container

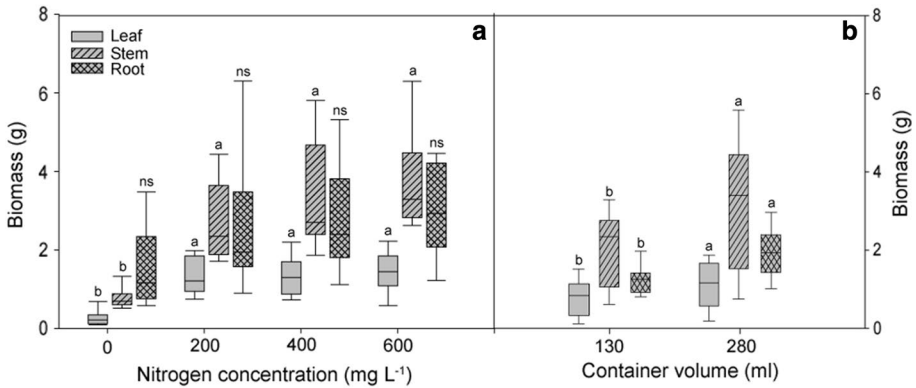


Fig. 1 Leaf, stem, and root biomass (g) of ruil seedlings at the end of the nursery stage according to increasing nitrogen fertilization (a) and container volume (b). Tops and bottoms of boxes represent the 25th and 75th percentiles, respectively. Error bars above and below the boxes indicate the 90th and 10th percentiles. The solid line in the center of each box marks the median value. For each tissue type across nitrogen fertilization rates, different letters indicate significant differences according to Tukey’s HSD at $p < 0.05$; ns: non-significant. For each tissue type, different letters indicate significant differences between container volumes according to Tukey’s HSD at $p < 0.05$

Potassium and Ca were unaffected by N rate ($p=0.0813$ and 0.2330 , respectively; data not shown).

Field stage

All seedlings were alive at the onset of the dry season when the first measurement was taken (November 2013). Survival decreased significantly from November 2013 through May 2014, the end of the first growing season (Fig. 2). Shelter significantly affected survival (Table 2); shelters yielded 2.5× seedling survival compared to that without shelters. Survival was significantly affected by N rate (Table 2); seedlings provided N averaged $48 \pm 8\%$ survival, a rate significantly higher than the $28 \pm 7\%$ of the non-N control treatment. Container volume had no effect on survival (Table 2).

Fig. 2 Field survival (%) of ruil seedlings with (black circles) and without (white circles) mesh shelters during the first growing season (November 2013 through May 2014). Circles indicate means \pm standard error. Significant differences according to Tukey’s HSD (ns = non-significant; *significant at $p < 0.05$; ***significant at $p < 0.0001$) correspond to differences between shelter treatments (with or without) at each measurement time

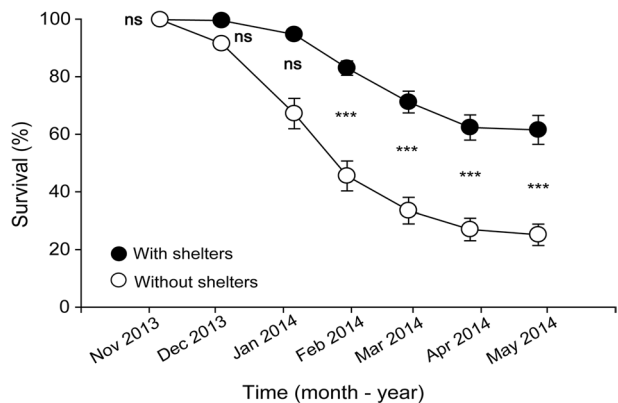


Table 2 Mean (\pm standard error) values for morphological parameters, source of variation, and *p* values of ruii seedlings after one field season according to increasing rates of nitrogen fertilization, shelter, and container volume

Treatment	Height (cm)	Root-collar diameter (mm)	Survival (%)	
Nitrogen (N)				
0 mg L ⁻¹	22.9 \pm 2.7 c*	2.73 \pm 0.09 c	28.3 \pm 7.88 b	
200 mg L ⁻¹	57.3 \pm 2.7 b	4.61 \pm 0.19 b	49.0 \pm 9.15 a	
400 mg L ⁻¹	52.9 \pm 3.1 b	4.50 \pm 0.17 b	50.7 \pm 7.63 a	
600 mg L ⁻¹	62.5 \pm 3.3 a	4.89 \pm 0.16 a	45.3 \pm 7.12 a	
Shelter (S)				
Shelter	58.8 \pm 1.9**	3.68 \pm 0.12 b	61.5 \pm 5.02 a	
No shelter	49.9 \pm 1.9	4.21 \pm 0.18 a	25.1 \pm 3.72 b	
Container volume (C)				
130 mL	47.9 \pm 1.8**	4.22 \pm 0.13 b	39.7 \pm 5.7 a	
280 mL	56.3 \pm 2.1	4.47 \pm 0.16 a	37.8 \pm 5.9 a	
Source of variation	<i>df</i>	<i>p</i> values		
Block	2	0.3167	0.0190	0.2718
N	3	< 0.0001***	< 0.0001	0.0054
S	1	0.0017	0.0109	< 0.0001
C	1	< 0.0001	0.0023	0.6260
N \times S	3	0.5733	0.1437	0.3459
N \times C	3	0.5415	0.1879	0.2144
S \times C	1	0.0269	0.3169	0.1644
N \times C \times S	3	0.3267	0.7129	0.8881

*Different letters with each main effect and seedling parameter indicate significant differences at $p < 0.05$ according to Tukey's HSD

**Significant interaction described in the text

****p* values in bold indicate significant differences for each variable at $p < 0.05$ according to Tukey's HSD

Shelter and container volume interacted to significantly affect height (Table 2). Addition of a shelter to the 280 mL seedlings significantly increased their heights (58.1 \pm 2.5 cm) compared to those without shelters (45.8 \pm 1.3 cm), whereas heights among the 130 mL containers were similar regardless of shelter treatment (47.9 \pm 1.9 cm with shelter and 44.1 \pm 1.7 cm without shelter). Height was significantly affected by N rate (Table 2); the 200 and 400 mg N L⁻¹ treatments averaged 55 cm in height, significantly different than the control, and significantly different than the 600 mg N L⁻¹ rate that averaged 62 cm in height.

Although none of the dependent variables interacted to affect RCD, shelter, container volume, and N rate significantly affected RCD (Table 2). Sheltered seedlings had significantly less RCD than those without shelters. Seedlings originating from the larger volume container (280 mL) had significantly more RCD than those from the 130-mL containers. Finally, seedlings produced with 600 mg N L⁻¹ had significantly more RCD than those from the 400 and 200 mg N L⁻¹ treatments, which had significantly more RCD than the control (Table 2).

Discussion

For ruil, we demonstrated that adding N increases seedling height, RCD, and shoot biomass compared to withholding N, similar to the results of Villar-Salvador et al. (2005) for several Mediterranean species, including kermes, holm, and cork oaks (*Quercus cocifera*, *Q. ilex*, and *Quercus suber*), Italian stone pine (*Pinus pinea*), and Spanish juniper (*Juniperus thurifera*). Within the treatments applied in our study, N addition was also the main factor affecting biomass and biomass partitioning. Despite the paradigm that larger containers yield larger seedlings (Pinto et al. 2011), we found no significant effect on height or RCD for ruil grown in different container types but did observe increases in leaf, stem, and root biomass, but not their partitioning. This concurs with research on other Mediterranean species (Puértolas et al. 2012; Mariotti et al. 2015; Uscola et al. 2015).

Increasing N fertilization increases N and P concentrations at the whole plant level (Miller and Timmer 1994; Ovalle et al. 2016), and Villar-Salvador et al. (2005) observed this for a variety of nursery-grown Mediterranean species specifically regarding N concentration. Moreover, increasing N fertilizer rates for several Mediterranean species has shown, despite yielding seedlings thought to have morphological attributes more vulnerable to drought (Puértolas et al. 2003; Luis et al. 2009), improved survival and growth after outplanting. This is consistent with research showing that larger plants have better field survival and growth (Puértolas et al. 2003; Villar-Salvador et al. 2004, 2012; Cuesta et al. 2010; Oliet et al. 2013; Tsakalidimi et al. 2013). Here, our results with ruil are similar: increasing N fertilization increased N and P concentrations and seedling size, which had a positive effect on field survival. Root-collar diameter is recognized as an important seedling quality attribute of nursery seedlings because it promotes field survival (Ritchie et al. 2010), particularly under water stress conditions (South et al. 2001). Notably, increasing N supply enhanced ruil RCD in the nursery, field survival, and RCD growth after outplanting.

In contrast, some researchers advocate that smaller seedlings with N and P deficiencies may fare better after outplanting because these deficiencies increase exploitation efficiency, which in turn favors resource uptake efficiency (Rubio et al. 2003; Trubat et al. 2006). Accordingly, Vileta et al. (2003) indicated that outplanting seedlings with higher root mass ratio is a better strategy to enhance water and nutrient absorption toward avoiding water stress. Trubat et al. (2008) and Cortina et al. (2013) suggested that increasing aboveground biomass could promote transpiration rates and reduce water potential leading to enhanced plant vulnerability to water stress. Despite that our control plants (0 mg N) had significantly lower N concentrations in shoots and presented significantly higher root mass ratio and lower aboveground biomass partitioning than N-fertilized seedlings, they also displayed significantly lower RCD growth in the nursery and subsequently lower field survival and RCD growth, reinforcing the hypothesis that for Mediterranean species, larger plants with higher N concentration have better field performance (Cuesta et al. 2010).

Haase and Rose (1995) define nutrient sufficiency as the point where an increase in biomass is observed without an increase in plant N concentration, indicating that all N is used for growth. Luxury consumption occurs when plant N concentration increases without a significant increase in biomass. Our data showed that the 200 mg N L⁻¹ fertilization rate provided enough N to reach a sufficiency level of this element, whereas the 400 and 600 mg N L⁻¹ rates revealed luxury consumption without reaching toxic

N levels. According to several authors (Timmer 1996; Oliet et al. 2013; Schott et al. 2016), luxury consumption increases the ability of plants to form new tissue at outplanting, although this was only observed in the 600 mg N L⁻¹ treatment with an increase in height and RCD. Also, luxury consumption levels were not translated into increased survival at outplanting.

Despite our observation that the larger container volume increased ruil seedling biomass compared to those growing in the smaller container volume, we observed no improvement in seedling survival. Although this seems contradictory to results with other species such as red (*Quercus rubra*) and white oak (*Quercus alba*) (Salifu et al. 2008) and slash pine (*Pinus elliottii*) (South and Mitchell 1999) where enhanced field survival occurred with larger plants, those studies were focused mainly on temperate species. Our results with ruil, adapted to a Mediterranean climate, are similar to results for other Mediterranean species *P. halepensis* and *P. pinea* (Puértolas et al. 2012) and *Ziziphus lotus* (Oliet et al. 2012); Puértolas et al. (2012) found no differences in field performance for these pines across a wide range of container volumes that generated a variety of seedling sizes.

Among the results found in this research, the use of mesh shelters is essential for increasing survival of ruil seedlings in a Mediterranean climate during the first growing season in the field. Researchers have noted that sheltering seedlings with mesh buffers them from extreme climatic conditions associated with temperature, relative humidity, and VPD (Bellot et al. 2002). By reducing maximum temperatures and VPD, shelters reduce transpiration losses, thereby slowing the rate of soil water depletion (Jiménez et al. 2005; del Campo et al. 2006; Padilla et al. 2011), which leads to improved performance. Specifically for mesh shelters in Mediterranean climates, Oliet et al. (2019) reported that shelters with 40% transmissivity, similar to those used in this study, provided a decrease in temperature and VPD. Indeed, shelters have increased survival of several Mediterranean species including *Q. coccifera* (Bellot et al. 2002), *Q. suber* (Chaar et al. 2008), and *Q. ilex* (Navarro-Cerrillo et al. 2005; Oliet and Jacobs 2007; Puértolas et al. 2010). Moreover, Santelices et al. (2012) found improved survival of sheltered ruil seedlings 3 years after outplanting; all non-sheltered seedlings died. While the effect of shelter protection on survival depends largely on species and shelter type (i.e., plastic tube or meshcloth), the main trait explaining plant responses to tree shelters is their degree of shade-tolerance, with shade-tolerant species benefiting more from protection (Puértolas et al. 2010; Oliet et al. 2019). Even though ruil has been classified as a shade-intolerant species (Bustamante and Castor 1998), it clearly benefits from a decrease in PAR provided by mesh. Thus, further research of the physiological responses of ruil towards shade is needed to understand its degree of shade-tolerance. Shelters have been found to increase height growth of other Mediterranean species, especially when those seedlings were produced in larger containers (Bellot et al. 2002; Chaar et al. 2008). Moreover, an increase in height was found in biara (*Banksia attenuata*), djarraly (*Eucalyptus marginata*) (Close et al. 2009), and Oregon white oak (*Quercus garryana*) (Devine and Harrington 2008) when protected with mesh shelters. Our results with ruil are consistent with these findings. This shoot elongation of sheltered seedlings could be a response to reduced radiation that allocates more resources toward elongation rather than support structures, such as secondary xylem and diameter growth (Sharpe et al. 1999). Indeed, we observed that our ruil in shelters were taller but had reduced RCD compared to those without shelters.

Conclusions

To the best of our knowledge, this is the first study to take a more holistic approach to nursery cultural practices for ruiil and its subsequent outplanting performance in conjunction with tree shelter use. We found that N fertilization of 200 mg N L⁻¹ provided sufficient N, whereas higher N concentrations (400 and 600 mg N L⁻¹) led to luxury consumption that did not yield additional survival benefit on the outplanting site. Despite ruiil being classified as shade-intolerant, using shelters that provided 60% shade dramatically increased first-year survival, probably by providing atmospheric conditions more conducive to seedling growth. Although differences in container volume failed to provide any benefit to ruiil field performance, a wide range of container volumes and types remain to be tested, so we cannot unequivocally conclude that container volume has no effect on ruiil seedling survival and growth. Similarly, rates between 0 and 200 mg N L⁻¹ may also provide sufficient N more efficiently. Therefore, despite our observations that a sufficient N rate coupled with use of a mesh shelter improved ruiil field survival, we suggest that further research is needed to refine optimum fertilization levels and understand the specific physiological mechanisms by which these factors improve ruiil survival.

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