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Effects of shading and slow release fertilizer on early growth of *Nothofagus leonii* seedlings from its northernmost distribution in Central Chile

Efecto de distintos niveles de sombra y dosis de fertilizante en el cultivo en vivero de plantas de *Nothofagus leonii* procedentes de su distribución más septentrional en Chile Central

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SUMMARY

Nothofagus leonii (huala) is an endemic species of the Mediterranean area of Chile for which available information for the propagation and nursery cultivation is conspicuously lacking. In the present study we analyzed the effect of shading and fertilization in the early development of seedlings during one growing season. Three shade treatments (0, 50 and 80 %) and three doses of a slow release fertilizer (3, 6 and 9 g of Basacote ® plus 9M per liter of substrate) were studied for effects on *N. leonii* growth (root collar diameter, height), biomass (dry masses of leaves, shoots and roots) and physiological (chlorophyll *a* content, chlorophyll fluorescence) responses. Analyses showed a significant effect of the shading. Plants treated with 0-50 % shade are significantly superior to those under 80 % shade. No differences were found for fertilizer applications. *Nothofagus leonii* grows more efficiently under 50 % and it would be possible to produce plants ~ 25.5 cm long stem and ~ 3.2 mm diameter root collar. Results suggest that *N. leonii* could require some degree of protection from direct sunlight in their early stages of development.

Key words: huala, early growth, nursery, shading, slow-release fertilizer.

RESUMEN

Nothofagus leonii (huala) es una especie endémica de la zona mediterránea de Chile de la cual aún existe insuficiente información sobre el cultivo de plantas en vivero. En el presente estudio se analizó el efecto de la cobertura y de la fertilización en el desarrollo inicial de las plantas durante una savia. Se ensayaron tres niveles de sombra (0, 50 y 80 %) y tres dosis de fertilizante de entrega lenta (3, 6 y 9 g de Basacote® plus 9M por litro de sustrato). Se evaluó la respuesta morfológica (diámetro de cuello, altura, biomasa por componentes) y fisiológica (clorofila *a*, fluorescencia de la clorofila) de *N. leonii*. Los resultados muestran que hubo un efecto significativo de la sombra. Aquellas plantas tratadas con 0-50 % de sombra se diferencian significativamente de las sometidas a 80 %, presentando un mejor desempeño en vivero. No se registraron diferencias para el fertilizante. Se puede concluir que es más eficiente cultivar plantas de *N. leonii* en vivero bajo una cobertura de 50 % siendo posible producir plantas con ~ 25,5 cm de altura y ~ 3,2 mm de diámetro de cuello. Los resultados sugieren que *N. leonii* sigue una estrategia de regeneración por claros y que la especie requiere de un grado de protección a la luz en los primeros estadio de su desarrollo.

Palabras clave: huala, crecimiento inicial, vivero, sombra, fertilizante de liberación lenta.

INTRODUCTION

Most native forests in Chile are dominated by *Nothofagus* species, contributing to one of the most important natural resources of the country (Ramírez 1987). Maule Region has *ca*. 188,000 hectares of native forests classified under the type Roble-Hualo forest (CONAF-CONAMA-BIRF 1999). Most of these forests have decreased mainly due to anthropogenic pressure, *i.e.* logging and burning to clear land for cereal cultivation, the use of forests as energy sources and the massive replacement by plantations with exotic species, mainly *Pinus radiata* D. Don, *Eucalyptus globulus* Labill and *Eucalyptus nitens* (Dean *et* Maiden) Maiden (Donoso and Landaeta 1983, Bustamante and Castor 1998). This region has a unique forest ecosystem in the world because it is the only one in which *Nothofagus* spp. forests grow in a predominantly Mediterranean climate (Donoso 1982). Some of the species present in these forests are endemic while others have conservation priorities (Benoit 1989). One is *Nothofagus leonii* Espinosa (huala), an endemic species of the central region of Chile, which corresponds to a natural hybrid between *Nothofagus glauca* (Phil.) Krasser and *Nothofagus obliqua* (Mirb.) Oerst (Donoso and Landrum 1979). The natural distribution of *N. leonii* has been dramatically reduced and restricted. Despite its presence is limited to areas where it coexists with *N. glauca* and *N. obliqua, N. leonii* holds a high scientific value as a natural hybrid and for its strict endemism (Donoso 2013).

Nothofagus leonii forests are distributed across a highly fragmented landscape in the Maule Region of Chile. This situation has caused significant changes in abiotic and biotic conditions as well as a reduction or change in the diversity and abundance of this species, with the resultant fragments being isolated from each other with loss of connectivity (Bustamante *et al*. 1995). This fragmentation and isolation will cause a reduction in the genetic variability of *N. leonii* (Ausdesirk *et al*. 2003) and will negatively affect the quantity and quality of seeds, which in turn will affect the natural regeneration of the species (Henríquez 2004).

In this context, it is necessary to promote the restoration of *N. leonii* forests, and because *Nothofagus* spp. species have low dispersal ability and short seed viability, artificial plantations offer one option to achieve regeneration. To do so, it is necessary to understand the main aspects of nursery plant production, such as container size, irrigation, shadow and fertilization regimes, in order to know which conditions are the most favorable for the propagation of the species. Fertilization and shade seem to be two important factors in *Nothofagus* spp. nursery cultivation (Santelices *et al*. 2011a). It has been recognized that seedlings of the *Nothofagus* genus grow under the protection of adult trees and shaded by the understory (Coopman *et al.* 2008, Reyes-Días *et al*. 2009, Santelices *et al.* 2011a). According to Cortina *et al*. (2013) the level of fertilization is one of the most important factors to consider in the production of seedlings to be established in Mediterranean drylands.

Shadecloth is used to protect susceptible species from high radiation and heat and has been adopted for the propagation of some northern *Nothofagus* species (Santelices *et al*. 2011a, 2013). Fertilization directly affects plant growth, improves rooting capacity after transplantation and may increase resistance to biotic and abiotic stresses (Oliet *et al*. 2005). Also, fertilization is one of the most important cultural practices for plant quality in reforestation, especially for seedlings produced in containers in which the limited volume seriously hinders their growth (Landis 1989). A slow-release fertilizer is characterized by gradually supplying mineral nutrient elements over time, and, in some cases, it might reduce leaching losses (Jacobs *et al*. 2005).

Functional attributes determine the survival and growth of planted seedlings in reforestation projects in drought-prone sites, hence determining the optimum features of the planting stock for such conditions is crucial. Defining seedling quality comes from measurements of seedling properties that describe material (*i.e.* single point measures of individual plant parameters such as shoot height, stem diameter, root mass, etc.) and performance (*i.e.* plant measurements reflecting an integrated response of many material attributes to defined environmental conditions, such as physiological responses) attributes (Ritchie 1984). To date, no studies have been found that examined the single or combined effects of shading and fertilization on *N. leonii* seedling growth. It is expected that both shading and fertilization would significantly affect the initial development of the species, with an increase in growth when decreasing the shade level and when increasing the fertilization dose, as happens with other *Nothofagus* species (Santelices *et al*. 2013). This study aims at analyzing the effects of different levels of shade and doses of a slow release complex fertilizer on early growth and physiological responses of container-grown nursery *N. leonii* seedlings.

METHODS

Plant and cultivation material. Nothofagus leonii seeds were collected during 2009 from “El Colorado” locality in the San Clemente Municipal district (UTM coordinates 295836 E, 6058432 S, 650 m a.s.l.), in Maule Region of Central Chile. Dominant or codominant trees with clear bole and well developed crown were selected for seed collection. Five mother plants with ripen fruits were randomly selected, according to the nearest neighbor method, ensuring a minimum distance of at least 100 m between mother plants. Fruits were collected directly from the branches and transported to the Universidad Católica del Maule nursery on the same day and kept at 4 °C. Based on findings of Santelices *et al*. (2013) on *N. glauca* and to overcome dormancy, seeds were soaked for 24 h in gibberellic acid (Giberplus®) solution containing 400 mg L^{-1}.

The seeds were sown in rigid plastic containers of 140 mL (Termomatrices ®) at an approximate depth of 0.5 cm. The substrate consisted of a mixture of composted bark of *P. radiata* and perlite (7:3 v), which was combined with the slow release fertilizer Basacote ® 9 M (16 % N, 8 % P_2O_5, 12 % K_2O, 12 % SO_3, 2 % MgO, 0.02 % B, 0.05 % Cu, 4 % Fe, and 0.06 % Mn), at three different doses: 3, 6 and 9 g L^{-1} of substrate. During their cultivation, the plants were protected by a plastic sunshade mesh (Raschel®). Treatments applied were: (1) control with 0 % shading, which received 100 % of the photosynthetically active radiation (PAR), (2) 50 % shadow (Raschel®) (41 % of PAR), and (3) 80 % shadow (Raschel®) (20 % of PAR). Since a factorial experiment was used, PAR value for fertilizer treatment was averaged. PAR levels in each treatment were determined by using the EARS PPM200 portable pulse-modulated fluorometer (EARS, Kanaalweg 1, 2628EB, Delft, The Netherlands) for which dark adaptation is not required for certain measurements. In the germination process, seedlings were watered daily using micro-sprinklers. Later, once the seedlings emerged, the substrate was maintained at field capacity. For this, a subsample of trays was weighed after water has drained away and afterward periodically weighed taking care to keep this weight.

Experimental design and measurements. A split-plot design with shading as the whole factor (0 %, 50 % and 80 % Raschel® mesh) and slow-release fertilizer dose as the split factor (Basacote® Plus 9M dose of 3, 6 and 9 g L^{-1}) was applied to three replicates of 45 seedlings per replicate, *i.e.* 1,215 seedlings in total (3 shading levels × 3 fertilizer doses × 3 replicates × 45 seedlings per replicate = 1,215 seedlings). After that, the development of the plants in the nursery after a single-season growth period (*i.e.* eight months) was assessed. Twenty-five seedlings located in the center of the block were evaluated for morphological attributes stem length (L, cm), root collar diameter (D, mm), aboveground biomass (AB, g), belowground biomass (RB, g), and total biomass (TB, g). For dry mass estimation, seedlings were oven-dried at 60 °C for 24 h and then weighed. With this information, the following indices were calculated: slenderness index (SI) [1], shoot:root index (SRI) [2] and Dickson index (DI) [3], according to the following formulas:

$$SI = \frac{L\ (cm)}{D\ (mm)} \qquad [1]$$

$$SRI = \frac{AB\ (g)}{RB\ (g)} \qquad [2]$$

$$DI = \frac{TB\ (g)}{\frac{L\ (cm)}{D\ (mm)} + \frac{AB\ (g)}{RB\ (g)}} \qquad [3]$$

Specific leaf area (SLA, $cm^2\ g^{-1}$) was also measured. For this, five plants per treatment were randomly selected and three leaves per plant were excised and subsequently scanned, dried and weighed. Individual leaf surface was measured by using a digital planimeter, and dry weight was estimated with a 0.01 g precision balance.

Before the morphological measurements, chlorophyll fluorescence was measured in 20 plants per treatment during January 2010. Plants were randomly selected from the center of the blocks and measurements were made with an EARS PPM200 portable pulse-modulated fluorometer between 12:00 and 15:00 h. Three measurements were made at equal intervals over the whole length of the plant, and the average recorded. Chlorophyll *a* content (Chl *a*) was also determined by using the method of Gitelson *et al*. (2003), *i.e.*, acetone extraction followed by a spectrophotometry analysis, in the same 20 plants used for chlorophyll fluorescence measurements.

Data analyses. All traits were analyzed through 2-Way ANOVA using shading and fertilizer as fixed factors. The general linear model approach (GLM) to analysis of variance, with type III sum of squares, was used. Prior to the analyses, data were examined and conformed to the normality and homogeneity of variance assumptions required for the analysis of variance. When the assumptions were not met, standard transformations, such as log (x), square root (x), sine (x) and cosine (x), were carried out. The mean values showing significant differences were compared with the Tukey test at a 5 % level. The SPSS v.18 software was used for all statistical analyses.

RESULTS

At 32 weeks of growth, an average of 25.5 ± 0.12 cm in height and 3.2 ± 0.02 mm in diameter was observed in *N. leonii* seedlings. There were no interactions among the studied factors. Shading resulted in significant differences in all morphological and physiological attributes. No differences were found between fertilizer treatments, either alone or in combination with shading. Seedlings cultivated with three fertilizer doses showed similar growth, biomass and physiological response.

In general, with the increase of shade, height growth was higher, while root collar diameter experienced a decline. Biomass in the different fractions was also different only for the shading percentage. Exposure to full light conditions increased seedlings body mass and diminished foliar area, while in the shaded condition the opposite tendency was observed. Changes were also observed in quality plant indices, with seedlings growing in shade treatment allocating more biomass to the shoots, as evidenced by the higher shoot:root ratio. Seedlings subjected up to 50 % shade made a more important investment in belowground biomass at the expense of aboveground biomass, unlike plants subjected to 80 % shade, which presented a large investment in aboveground biomass. Seedlings also showed differences in slenderness when grown at different shade levels. As expected, shaded seedlings were more slender than full light seedlings, as they are higher and with slimmer diameters.

Shading not only changed plant growth and biomass, but also affected plant physiological properties. As expected, shaded seedlings showed significantly lower chlorophyll fluorescence. Chlorophyll *a* content differed among the shading treatments, with shaded seedlings having higher chlorophyll *a* content than full light seedlings.

DISCUSSION

Effect of light intensity. At low irradiance, leaves retain more of the limiting amount of photosynthates, leaving less carbon for root growth. At low soil nutrient availability, roots use relatively more of these resources, leaving less for the shoots (leaves), *i.e.* more root biomass allocation (Poorter and Nagel 2000). This allocation behavior was observed in this study. Seedlings treated with 80 % shade have on average 2.1 g shoot biomass, in contrast to the 1.2 g of root biomass. Results obtained for the shoot:root ratio were close to 1 in 0-50 % shade treatments, *i.e.* a balance between the transpiring and absorbing surface, while the 80 % shade treatment presented a higher value, *i.e.* more biomass allocated to shoots. According to Villar-Salvador (2007), a shoot:root ratio near 1 implies a superior chance of seedling survival in drought prone sites. The core

Table 1. Effect of shading and fertilizer on morphological attributes of container-grown *Nothofagus leonii* seedlings. Mean values ± S.E. (values with the same letter are not significantly different, $P < 0.05$).

Efecto de diferentes tratamientos de sombra y fertilizante en los atributos morfológicos de plantas de *Nothofagus leonii* cultivadas en vivero. Medias ± error estándar (valores medios con la misma letra no son significativamente diferentes entre sí, $P < 0,05$).

Treatment	Photosynthetically active radiation ($u$mol m^{-2} s^{-1})	Root collar diameter (mm)	Stem height (cm)	Specific leaf area (cm^2 g^{-1})	Biomass (g)		
					Aboveground	Belowground	Total
Shading (%)							
0	1,600	3.4 ± 0.16 a	22.5 ± 1.6 b	104.9 ± 10.5 b	2.1 ± 0.2 a	1.9 ± 0.1 a	4.1 ± 0.4 a
50	500	3.1 ± 0.11 ab	23.5 ± 1.0 b	125.1 ± 25.8 b	1.4 ± 0.2 b	1.4 ± 0.1 ab	2.8 ± 0.3 b
80	100	3.1 ± 0.12 b	30.5 ± 1.6 a	168.1 ± 30.3 a	2.1 ± 0.2 a	1.2 ± 0.1 b	3.4 ± 0.2 ab
Fertilizer dose (L^{-1})							
3	730	2.9 ± 0.1 a	24.4 ± 1.2 a	138.5 ± 39.9 a	1.8 ± 0.2 a	1.5 ± 0.1 a	3.4 ± 0.3 a
6	730	3.4 ± 0.1 a	25.8 ± 1.6 a	128.8 ± 39.2 a	1.7 ± 0.1 a	1.5 ± 0.1 a	3.2 ± 0.3 a
9	730	3.4 ± 0.1 a	28.2 ± 1.9 a	134.8 ± 26.7 a	2.1 ± 0.2 a	1.5 ± 0.1 a	3.6 ± 0.4 a

Table 2. Effect of shading and fertilizer on quality indices of container-grown *Nothofagus leonii* seedlings. Mean values ± S.E. (values with the same letter are not significantly different, $P < 0.05$).

Efecto de diferentes tratamientos de sombra y fertilizante en los índices de calidad de plantas de *Nothofagus leonii* cultivadas en vivero. Medias ± error estándar (valores medios con la misma letra no son significativamente diferentes entre sí, $P < 0,05$).

Treatment	Photosynthetically active radiation (%)	Quality index		
		Slenderness	Shoot:root	Dickson
Shading (%)				
0	1,600	7.1 ± 0.4 b	1.1 ± 0.0 b	0.5 ± 0.06 a
50	500	8.0 ± 0.4 b	1.0 ± 0.4 b	0.3 ± 0.03 a
80	100	11.0 ± 0.5 a	2.1 ± 0.3 a	0.2 ± 0.02 b
Fertilizer dose (L^{-1})				
3	730	8.9 ± 0.5 a	1.5 ± 0.3 a	0.3 ± 0.05 a
6	730	8.5 ± 0.6 a	1.3 ± 0.2 a	0.3 ± 0.04 a
9	730	8.9 ± 0.8 a	1.5 ± 0.1 a	0.4 ± 0.05 a

of this theory is that plants shift their allocation towards shoots if the carbon gain of the shoot is impaired by a low level of above-ground resources, such as light and CO_2. Similarly, plants shift allocation towards roots when there is a low level of below-ground resources, such as nutrients or water. These shifts could be seen as adaptive, as they enable the plant to capture more of those resources that most strongly limit plant growth (Poorter and Nagel 2000). Trubat *et al*. (2011) suggested that nutrient-deficient seedlings may be better prepared to withstand transplant shock and summer drought. As functional attributes will determine the survival and growth of planted *N. leonii* seedlings in reforestation projects in Mediterranean drought-prone sites, determining the optimum characteristics features of the planting stock for such conditions is crucial; however, this is beyond the scope of this paper.

The slenderness ratio, in which low values (*i.e.* 4-7 for Mediterranean species in Navarro *et al*. (2006)) tend to reflect a better biomass distribution and better seedling status (Bustos *et al*. 2008), is in an appropriate range for the 0-50 % shade levels, unlike the 80 % shade level in which a high biomass allocation to shoots was observed. This implies higher transpiration rate and water consumption. The Dickson index has been successfully used to predict field performance of various conifer species (Ritchie 1984); however, Gonzalez *et al*. (1996) observed a poor fitness of this index as a predictor of *Nothofagus alpina* (P. *et* E.) Oerst. (raulí) plant quality. The values obtained in this study are comparable to those obtained by Santelices *et al*. (2011b), where Dickson index values for *N. alessandri* vary from 0.12 to 0.47 depending on factors such as fertilization rate, sowing time, and pregermination treatments.

Specific leaf area was larger with increasing shade level, consistent with Santelices *et al*. (2012), who obtained values between 177 and 200 $cm^2 g^{-1}$ (with 50 and 80 % shade, respectively) in a *N. alessandrii* plantation. Minotta and Pinzauti (1996) reported similar results in *Fagus sylvatica* L. A highly specific leaf area value implies a higher number of chloroplasts and enzymes and enhances photosynthetic capacity per unit leaf area (Evans and Poorter 2001). In addition plants grown in environments with high light intensities have thick leaves (Björkman 1981), due in part to long palisade cells. In *N. leonii* this may involve a high efficiency to capture and use limited amounts of light and to consume carbohydrates efficiently. On the other hand, Sims *et al*. (1994) modeled the impact of changes in specific leaf area on the plant growth rate and found that this was more significant at low light intensities.

In the physiological response, the study showed that the shading treatment directly affects the chlorophyll fluorescence (table 3). In the case of chlorophyll *a* content, differences were only observed for the shading treatments. In general, shade plants have more chlorophyll, which is coincident with results of this study. Plants treated with moderate shade (*i.e.*, 41 % photosynthetically active radiation) have more chlorophyll *a* content than that found in those treated with 100 % photosynthetically active radiation. Evans and Poorter (2001) found similar results (*i.e.*, an increase in chlorophyll content with decreasing light intensity) when evaluating 10 forest species. This would indicate a more important amount of photosystem II reaction centers and increased photosynthetic efficiency in *N. leonii* for this shade level. Plants that receive high irradiance develop a thicker palisade parenchyma to protect the mesophyll from a very high radiation. This finding is consistent with the chlorophyll fluorescence values obtained in this study because, in the 100 % photosynthetically active radiation treatment, larger values were observed. Thus, with high radiation levels, *N. leonii* plants develop strategies to prevent photooxidation (*i.e.*, greater fluorescence emission) and are able to survive and grow in low light intensities.

Effect of fertilizer doses. There were no significant differences in seedling growth resulting from differences in fertilizer doses, which agrees with Santelices *et al*. (2011ab) for *N. alessandrii.* Nutrient availability affects plant biomass allocation, and an increased amount of nutrients often decreases root biomass allocation (Poorter and Nagel 2000). However, in this study and similar to Klooster *et al*. (2012) in *Quercus rubra* L., there were no changes in growth, biomass allocation or specific leaf area when increasing the fertilizer dose. As we did not measure tissue nutrient concentration we speculate that *N. leonii* appears to take up available nutrient beyond their current needs and store it in stems and roots (*i.e.* a luxury consumer), and that extra high fertilization or nutrient loading induces luxury uptake. Thus, it may be possible that increased internal nutrient reserves resulting from nutrient loading in *N. leonii* seedlings may be readily exploited later to facilitate new growth at outplanting; however, this needs further field experimentation. We did not find a decrease in plant growth while increasing nutrient content, thus our results also suggest that the toxicity level described by Salifu and Jacobs (2006) was not reached.

Table 3. Effect of shading and fertilizer on physiological attributes of container-grown *Nothofagus leonii* seedlings. Mean values ± S.E. (values with the same letter are not significantly different, $P < 0.05$).

Efecto de diferentes tratamientos de sombra y fertilizante en los atributos fisiológicos de plantas de *Nothofagus leonii* cultivadas en vivero. Medias ± error estándar (valores medios con la misma letra no son significativamente diferentes entre sí, $P < 0,05$).

Treatment	Photosynthetically active radiation (%)	Chlorophyll *a* content ($\mu mol\ m^2\ s^{-1}$)	Chlorophyll fluorescence (μmol photon $m^2\ s^{-1}$)
Shading (%)			
0	1600	2.2 ± 0.2 b	3,006.2 ± 90.5 a
50	500	2.9 ± 0.2 a	2,320.3 ± 117.8 b
80	100	2.8 ± 0.2 a	2,221.6 ± 141.2 b
Fertilizer dose (L^{-1})			
3	730	2.5 ± 0.1 a	2,713.9 ± 105.9 a
6	730	2.5 ± 0.2 a	2,198.5 ± 171.2 b
9	730	2.8 ± 0.3 a	2,729.5 ± 88.6 a

The chlorophyll *a* content gives an indirect estimation of plant nutritional status because much of the leaf nitrogen is incorporated in chlorophyll (Moran *et al*. 2000). All three fertilizer doses analyzed in this study give an adequate amount of nitrogen to be incorporated into the chlorophyll *a* production; however, 6 g L^{-1} could be a suitable dose in *N. leonii* and other Chilean fagaceae, such as *N. alessandrii* (Santelices *et al*. 2011b), since fluorescence decreases. This could imply an increase in the photosynthetic efficiency.

CONCLUSIONS

We concluded that shading affects morphological and physiological responses of container-grown *N. leonii* in the nursery. Better development is achieved with an intermediate level of light intensity. With 50 % shadow, *i.e.*, 41 % of photosynthetically active radiation and according to their morphological attributes and quality indices, seedlings with an acceptable quality were obtained. There was no clear effect on seedlings growth due to the different doses of slow-release fertilizer.

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