Field Establishment Techniques for Guindo Santo, an Endemic Species from Central Chile

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Abstract

First-year outplanting performance was measured in guindo santo (*Eucryphia glutinosa* (Poepp. & Endl.) Baill.), a Chilean endemic tree species in the Mediterranean climate zone, which is catalogued as a near-threatened species. The effects on survival of initial plant size, fertilization at establishment, and shade (with or without nurse trees) were evaluated during the first growth season. Planting guindo santo under nurse trees was the most important treatment to increase survival, compared with trees planted in full sun. We believe that the positive effects of nurse trees on survival are linked to a decrease in plant drought stress during summer, in response to higher levels of soil water content and a decrease in incident irradiation. We strongly suggest the use of shade, like nurse trees or tree shelters, during guindo santo establishment in a Mediterranean climate.

Introduction

The success of many restoration programs relies on the field establishment, survival, and growth of nursery-produced plants. Transplant shock often impairs plant performance during the first growing season (Close et al. 2005), especially under adverse site conditions such as poor soil fertility or severe climatic conditions such as those present in Mediterranean-type climates (Valladares et al. 2004). Thus, several post-outplanting treatments such as fertilization (Fox et al. 2006, Li et al. 1999), shading (Bellot et al. 2002, Puértolas et al. 2010), and weed control (Fleming et al. 1998, Navarro-Cerrillo et al. 2005, Nilsson and Orlander 1999) have been shown to enhance survival and growth of plants during the first few seasons after outplanting.

First-year fertilization of outplanted seedlings has the main objective of increasing survival and short-term productivity (Fox et al. 2006). In Chile, the effects of early plantation fertilization treatments have been mainly focused on exotic species such as Monterey pine (*Pinus radiata* D. Don) and *Eucalyptus* spp. (Rubilar et al. 2008, Schönau and Herbert 1989), which are subjected to intensive forest management programs. We know of only one field fertilization experiment with native species under restoration programs, namely coigüe (*Nothofagus domeyi* Mirb.) (Donoso et al. 2009). Although fertilization can improve early performance of several species (Barros et al. 1992, Drechsel and Schmall 1990, Li et al. 1999, Mhando et al. 1993), benefits of this treatment depend on several factors, such as soil characteristics and species-specific nutrient requirements (Rodríguez 1993), for which this information is nonexistent for native species under restoration programs in Chile.

In Chile, evidence is mounting that manipulating micro-environmental conditions on the planting site can increase field survival and performance (Soto et al. 2017, Valenzuela et al. 2016). The use of shelters, mesh guards, or outplanting under nurse trees are the most-used treatments to decrease environmental stresses on seedlings (Jiménez et al. 2005, Navarro-Cerrillo et al. 2005, Puértolas et al. 2010). These treatments improve micro-site conditions and facilitate seedling establishment, especially in open, deforested areas with stressful environmental conditions (Oliet et al. 2015, Padilla and Pugnaire 2006). Specifically, the use of adult nurse plants can ameliorate extreme environmental stresses by reducing soil water evaporation, lowering air and soil temperature, and decreasing the amount of radiation reaching the plants (Padilla and Pugnaire 2006). In Chilean native species, mesh guards and shelters have been successfully used for quillay (*Quillaja saponaria* Molina), Roble (*Nothofagus obliqua* Birb.), laurel (*Laureliopsis phillipiana* Looser.), and olivillo (*Aextoxicon punctatum* Ruiz & Pavon.) (INFOR, unpublished data). Likewise, the native conifer ciprés de las guaitecas (*Pilgerodendron uviferum* D. Don) had a 200-percent growth increase when outplanted under a nurse canopy (Bannister 2015).

Fertilization and outplanting under nurse trees are silvicultural treatments that can be applied during restoration programs on Mediterranean sites of Central Chile, which has been declared one of the world's most threatened habitats (Dinerstein et al. 1995). Despite its vulnerability, little is known about applying cultural treatments during outplanting restoration species. Such is the case of guindo santo (*Eucryphia glutinosa* (Poepp. & Endl.) Baill.), a Chilean endemic species whose habitat extends from Linares province (-36°05´S, -71°10´W) to Malleco province (-38°14´S, -71°45´W) at elevations between 200 and 1,400 m (650 to 4,600 ft) and is only found near rivers and streams west of the Andes (Muñoz 1966). Guindo santo is a small, deciduous tree that reaches only 5 m (16 ft) tall. The tree has white flowers up to 6 cm wide with red stamens and foliage that turns orange or purple during autumn; such characteristics confer high ornamental value and importance for honey production (Hechenleitner et al. 2005) (figure 1). Guindo occurs in relatively separated subpopulations of native forest (Echeverría and Rodríguez 2014), indicating high levels of fragmentation. The International Union for Conservation of Nature (IUCN) has categorized guindo santo as a near-threatened species due to excessive clearing to establish exotic species plantations and construct hydroelectric plants.

The objective of our study was to evaluate effects of fertilization and outplanting under nurse trees on the survival and growth of guindo santo during the first growing season in a Mesomediterranean area of Central Chile.

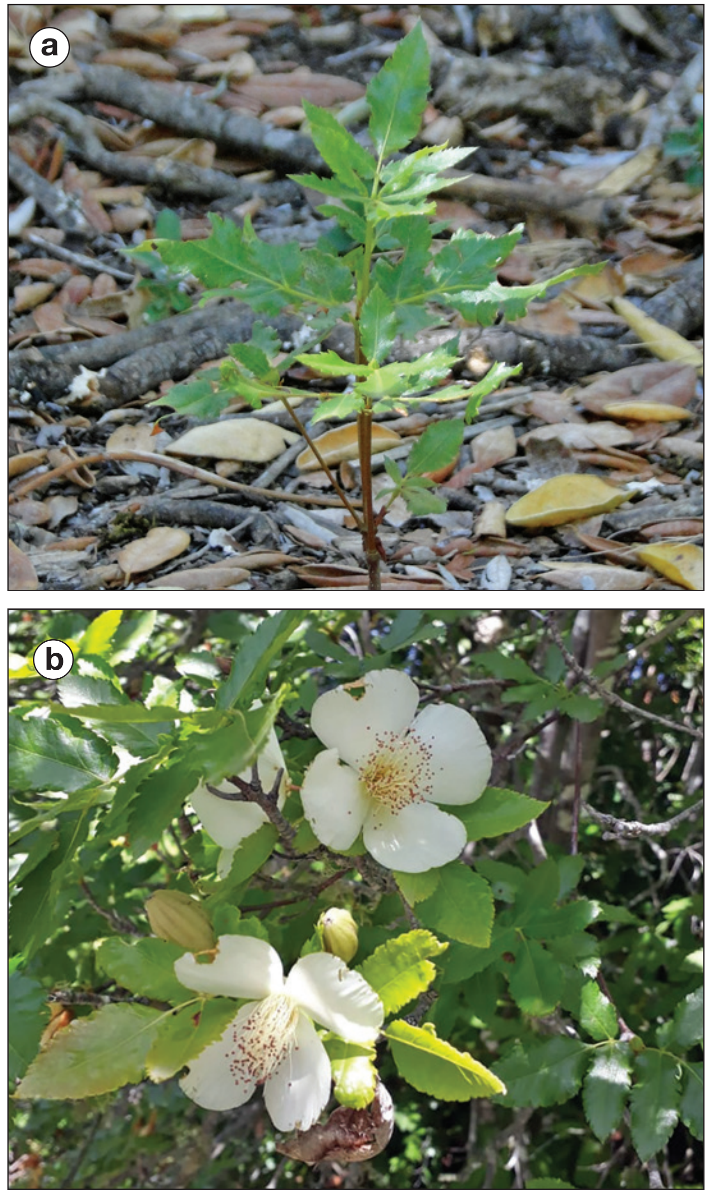

Figure 1. (A) Guindo santo seedling and (B) a close-up of the flowers known for their high value as an ornamental for honey production. (Photos by Hernán Soto, Instituto Forestal, 2012).

Methods

Nursery Production Stage

We collected guindo santo seeds in April 2011 in San Fabián de Alico commune, Biobío region (-36°35´S, -71°28´W), which is within the species' natural range. Seeds were stored in zipper plastic bags and refrigerated at 4 °C until October 2011, when they were soaked in water 24 hours prior to sowing. Seeds were sown into 20 expanded polystyrene containers, each having 84 cavities (130 ml cavity^{-1} [4.39 fl oz cavity^{-1}]) and 336 cavities m^{-2}), and germination occurred under greenhouse conditions. Containers were filled with composted Monterey pine bark having total, aeration, and water retention porosity of 49, 25, and 24 percent, respectively. The germination period lasted 61 days after sowing (DAS) from October through November 2011, during which we irrigated 5 min per day. Once germination was complete and until the end of the growing season, irrigation was applied daily to return the amount of water in the medium to container capacity.

After the germination phase, we fertilized seedlings following a three-stage scheme. Initially, between 61 DAS until 123 DAS, fertilization was applied using Ultrasol Inicial fertilizer 15-30-15 (N-P-K) (SQM, Chile) applied at 2 g L^{-1} (0.265 oz gal^{-1}) or 300 mg L^{-1} N (0.037 oz gal^{-1}) with sprinklers once a week to return the water volume to container capacity. The second fertilization stage lasted from 123 DAS until 242 DAS using Ultrasol Crecimiento 25-10-10 (N-P-K) (SQM, Chile) alternating with Ultrasol Desarrollo (18-6-18) (SQM, Chile), both in application rates of 3 g L^{-1} (0.397 oz gal^{-1}) of fertilizer (500 and 180 mg L^{-1} N, respectively [0.064 oz gal^{-1} and 0.023 oz gal^{-1}]), in the same manner as the previous stage. The third stage of fertilization lasted between 242 DAS until 303 DAS. During this stage, fertilization was applied using Ultrasol Producción (13-6-40) (N-P-K) (SQM, Chile) twice a week, at a rate of 2 g L^{-1} (0.265 oz gal^{-1}) of fertilizer (260 mg L^{-1} N [0.034 oz gal^{-1}]). Additionally, calcium nitrate was also applied twice a week during this stage in doses of 2 g L^{-1} (0.265 oz gal^{-1}) and Coldkiller® (AQM, Chile) was applied once a week in a dose of 2 ml L^{-1} (0.462 in3 gal^{-1}) to prevent frost damage.

Plant height varied widely at the end of the nursery stage. Thus, seedlings were sorted into two height categories before outplanting: 10 to 20 cm (approximately 4 to 8 in; H1) and 25 to 35 cm (approximately 10 to 14 in; H2).

Field Stage

The field study was installed at the Bullileo sector (-36°35´S, -71°28´W), San Fabián de Alico commune in Biobío region, Chile. This location is the southern limit of the Mediterranean climate and is in the Andean foothills of south-central Chile. The climate is Mesomediterranean with perhumid conditions, mild winters, and dry summers (Donoso 1996, Amigo and Ramírez 1998). Almost 80 percent of the annual precipitation occurs between May and September. During the first growing season after outplanting (from November to April), the accumulated precipitation was 1,041 mm (41 in), the mean temperature was 19.2 °C (66.56 °F), and the maximum air temperature in summer was 30.9 °C (87.62 °F). The soils are shallow with a predominance of volcanic material, including andesitic and basaltic materials (Donoso 1996).

In August 2012, we established the seedlings under two contrasting sun exposure conditions corresponding to two neighboring fields: one at full sun exposure (figure 2A) and the other with boldo (*Peumus boldus* Molina.) trees (4 to 6 m tall [13 to 20 ft]) as nurse trees (shade condition) (figure 2B). Herbaceous plants were removed from both fields before outplanting holes (30 by 30 by 30 cm [approximately 12 by 12 by 12 in]) were dug with a planting shovel at a 1 by 1 m (approximately 3 by 3 ft) spacing equivalent to 10,000 plants ha^{-1} (approximately 4,000 plants ac^{-1}). Two field fertilization treatments were applied: a control treatment with no fertilizer application and a fertilization treatment with a single application of 115g (4.05 oz) of Vitra 8-20-7 (N-P-K) plus 1 percent B (sodium borate). Fertilizer was applied around the plant in a groove of 15-cm (5.9-in) diameter and 5-cm (approximately 2-in) depth (figure 3).

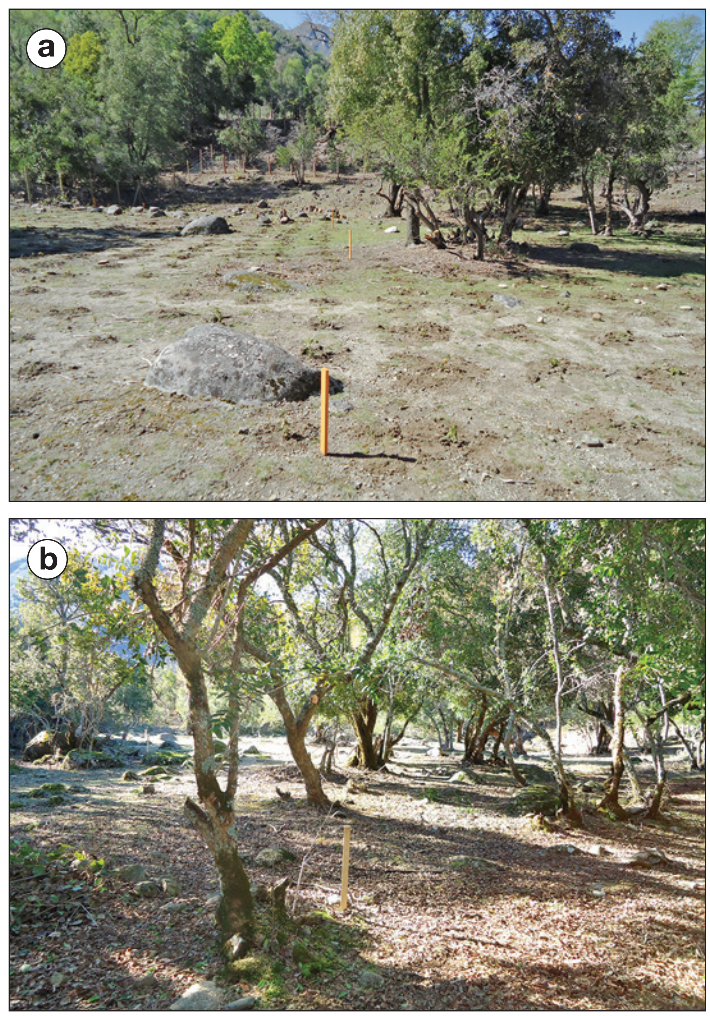

Figure 2. Guindo santo seedlings were outplanted near Bullileo with either (A) full sun or (B) shade provided by boldo nurse trees. (Photos by Manuel Acevedo, Instituto Forestal, 2012)

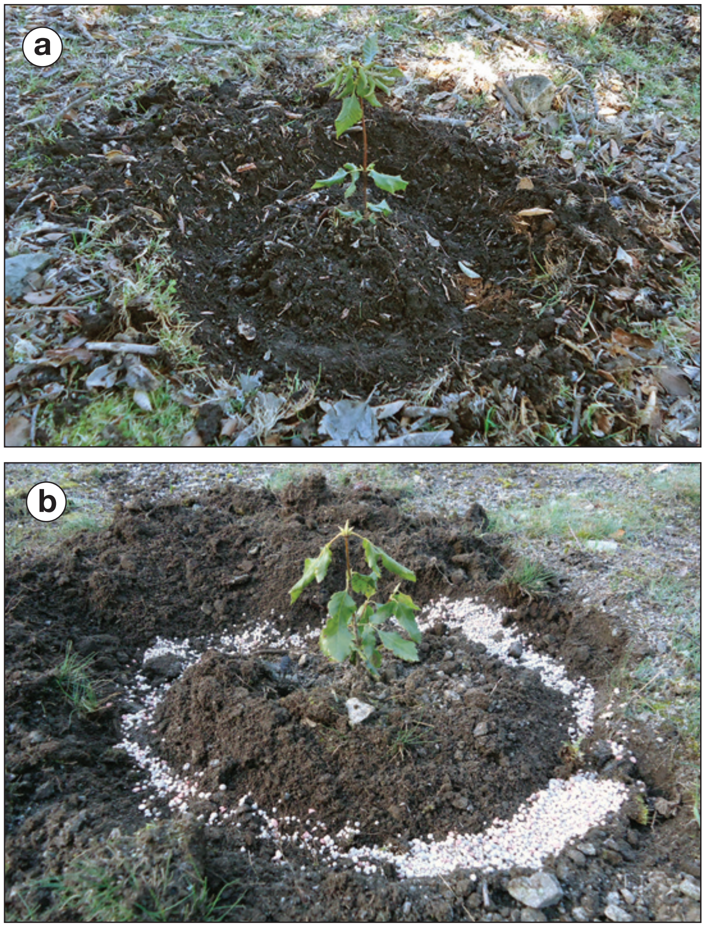

Figure 3. At outplanting, control seedlings of guindo santo were either (A) not fertilized or (B) had a ring of fertilizer applied in a 15-cm-diameter circle. (Photos by Manuel Acevedo, Instituto Forestal, 2012)

Survival and Growth Increment Measurements

Survival was evaluated monthly during the first growing season (October 2012 through April 2013). Each seedling was measured for stem diameter at ground line and height at plant establishment (October 2012) and at the end of the first growing season (April 2013). Stem diameter and height growth increments were calculated by subtracting the first measurement from the last measurement.

Pre-Dawn Water Potential Measurements

Pre-dawn water potential was measured monthly in the upper third leaves of 72 seedlings (3 plants per treatment combination replicate). During the evening before each measurement, leaves were wrapped in aluminum foil until pre-dawn at which time leaves were collected and kept on ice until the determination of leaf water potential using a Scholander pressure chamber (PMS Instruments, Albany, OR).

Gravimetric and Volumetric Soil Water Content

Gravimetric soil water content was determined monthly. Three soil samples from each treatment combination replicate (72 samples total) were collected with a shovel from the top 20 cm (7.9 in) of the soil profile, then weighed, dried in a forced ventilation oven for 48 hours at 65 °C (149 °F), and reweighed. Soil water content was calculated according to the following formula:

$$\%\, soil\ water = \frac{(weight\ of\ wet\ soil(g) - weight\ of\ dry\ soil\ (g))}{weight\ of\ dry\ soil\ (g)}\ x\ 100$$

Also, for field characterization purposes, continuous volumetric water content was determined using soil moisture sensors (EC-5, METER Group, Pullman, WA) installed 20 cm (7.87 in) below the soil surface and recorded hourly (Em-50, METER Group) from January 2013 through April 2013.

Photosynthetic Photon Flux Measurements

Monthly determinations of photosynthetic active radiation (PAR) were performed with a quantum sensor (LI-190, LI-COR, Lincoln, NE) attached to a light meter (LI-250A, LI-COR). Five measurement points were distributed randomly in each experimental unit. These measurements were performed at midday on clear days at plant level.

Experimental Design and Data Analyses

Our field experiment was laid out in a split-plot design consisting of two sun exposure conditions (whole plots) by two outplanting fertilization levels by two seedling height categories with three replications, each having 49 seedlings per treatment combination for a total of 1,176 plants. Variance analysis of repeated measurements was performed by modelling the structure of the variance and co-variance. Multiple comparisons were performed using the Tukey-Kramer test with a 95-percent confidence to test the effects of light condition, fertilization, plant size, and time on plant survival. Average stem diameter and height growth increments were obtained for each experimental unit and used for variance analysis. All statistical analyses were performed with Infostat software (V.2011p) and R extension (V.2.15.0).

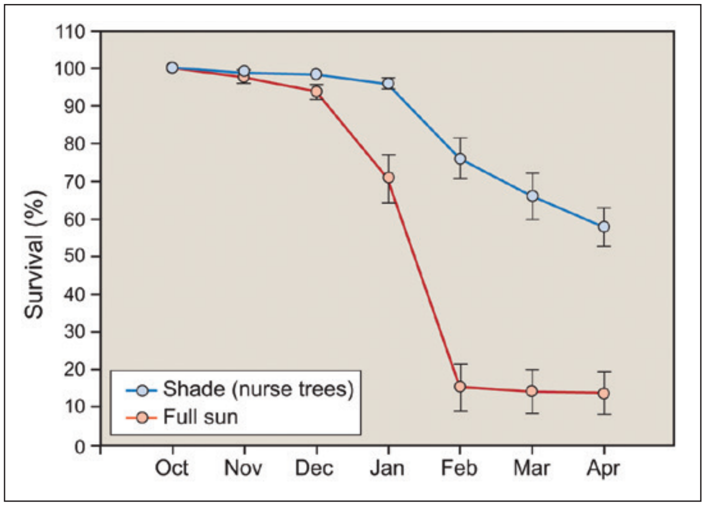

Figure 4. Survival (± standard deviation) of guindo santo seedlings during the first growing season (October 2012 through April 2013) differed over time between those grown under full sun exposure and those that were shaded by nurse trees.

Results

The interaction of time and level of sun exposure significantly affected survival at the end of the first growing season. Survival decreased for seedlings grown in full sun as well as in shade, but the magnitude of the decrease was greater for seedlings grown in full sun (figure 4). Both exposure treatments had the steepest declines in survival during the warmest summer temperatures in January (figure 4).

No significant interactions were observed for height growth, but the main effects of sun exposure, initial plant height, and field fertilization were significant (table 1). Exposure to full sun reduced seedling height growth compared with those that were planted under nurse trees. Short seedlings (10 to 20 cm [approximately 4 to 8 in]) had less height increment than did tall seedlings (25 to 35 cm [approximately 10 to 14 in]). Fertilization increased the height increment.

Table 1. Effect of initial seedling height, shade exposure condition, and fertilization on guindo santo height growth and root collar diameter growth during the first growth season. For each variable, means followed by different letters indicate significant differences between treatment according to Tukey at $p<0.05$.

Treatment		Mean height growth (cm	Root collar diameter (mm)
Initial seedling height	10 – 20 cm	9.26 b	2.04 a
	25 – 35 cm	15.18 a	2.20 a
Sun exposure	Full sun	5.34 b	2.06 a
	Nurse plants	14.14 a	2.14 a
Fertilization	No	10.25 b	2.04 a
	Yes	14.51 a	2.23 a

Stem diameter increment was unaffected by treatment and averaged 2.12 mm (0.083 in) for all treatments (p=0.338).

Pre-dawn water potential was significantly affected by the interaction between time of measurement and exposure condition. Similar to survival results, pre-dawn water potential was maintained until January 2013 (figure 5) and then decreased significantly during February 2013, although seedlings under full sun exposure had a significantly lower pre-dawn water potential (higher stress) than seedlings grown under shade. During the next month (March 2013), an increase in water potential values was observed in both treatments, although plants never reached the values observed during field establishment.

Gravimetric soil water content in both sun exposure conditions decreased from the time of outplanting until April 2013 and was significantly higher in the shaded plots than in those under full-sun exposure (figure 6A). While the gravimetric water content indicated a steady decrease in soil water during the first growing season, the continuous monitoring of the volumetric water content using soil water sensors indicated an increase in this parameter on February 17, 2013, due to a precipitation event (figure 6B), especially in the soil under full sun exposure. After this event, volumetric soil water content steadily decreased in both exposure conditions, reaching values in April 2013 found previous to the precipitation event.

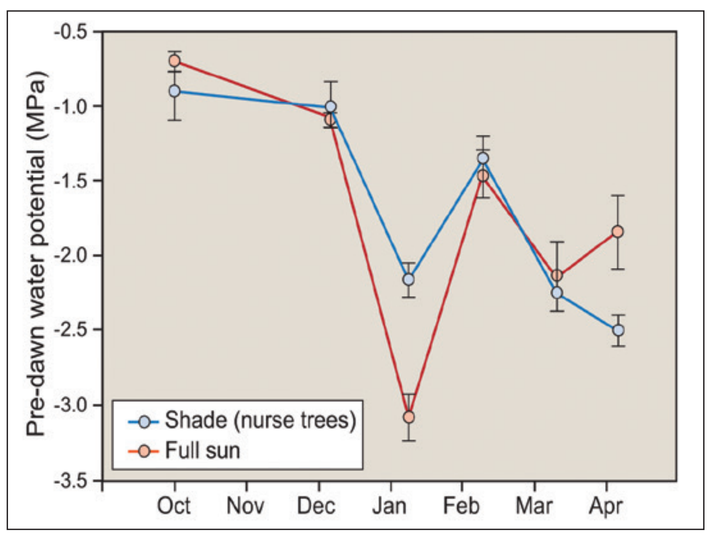

Figure 5. Pre-dawn water potential (MPa) of guindo santo seedlings varied during the first growing season but tended to be higher for seedlings grown under shade condition compared with those grown under full sun exposure.

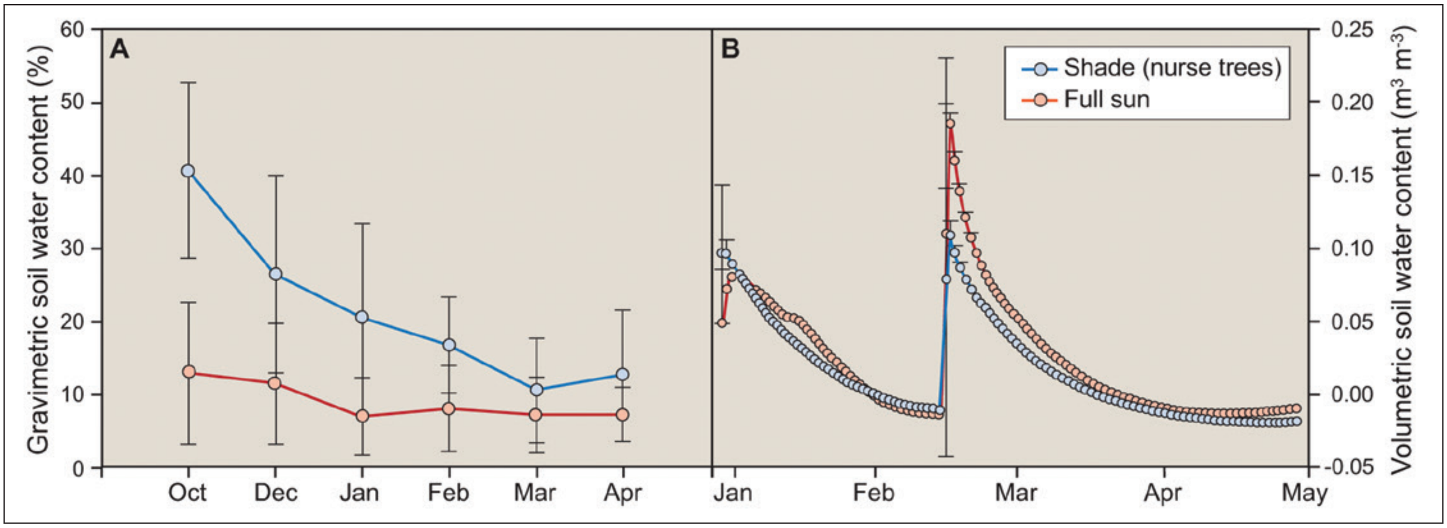

Figure 6. (A) Gravimetric and (B) volumetric soil water content during the first growing season under nurse tree shading and under full sun exposure.

PAR was significantly higher in the field under full sun exposure, compared to the field shaded with boldo nurse trees. This difference was maintained through all the first growing season (figure 7).

Discussion

One of the main constraints for restoration programs in Mediterranean climates is seedling survival during the first growth season, which is often hampered by highly stressful conditions such as water scarcity during summer (Becerra et al. 2011). Thus, research efforts should focus on treatments with potential to reduce environmental pressure on seedlings and thereby increase survival. Such factors or treatments include the use of nurse plants and fertilization during establishment.

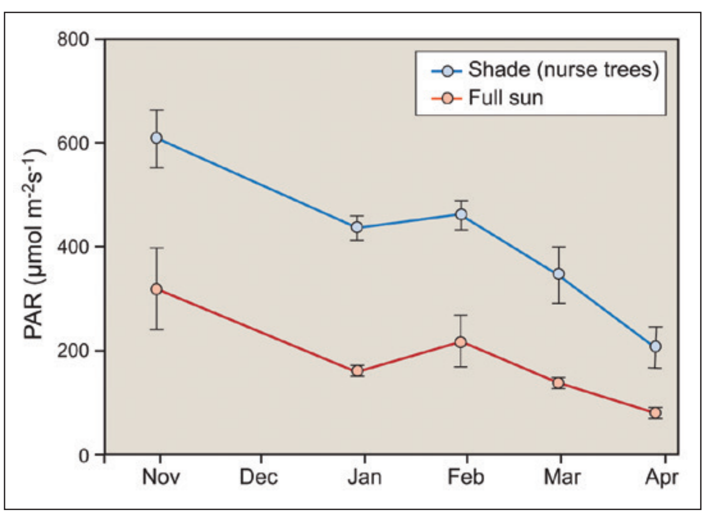

Figure 7. Photosynthetic active radiation (PAR) for guindo santo plants during the first growing season under nurse trees and under full sun exposure.

Our results show that the use of nurse plants for establishment of guindo santo modifies aboveground and belowground environments to improve seedling survival. These beneficial effects are consistent with other reports with Mediterranean species, such as longleaf pine (*Pinus palustris* Mill.) (Knapp et al. 2013, Rodriguez-Trejo et al. 2003), black pine (*Pinus nigra* Arnold) and scots pine (*Pinus sylvestris* L.) (Castro et al. 2002), and English yew (*Taxus baccata* L.) (Peragón et al. 2015). In our study, the boldo nurse trees resulted in gravimetric soil water content and decreased solar radiation at plant level, common shade effects known to be beneficial to plant growth and survival (Valladares et al. 2008). Indeed, in our study, outplanted seedlings under the nurse trees had consistently higher pre-dawn water potential compared with seedlings grown in full sun, suggesting that shaded plants were less water-stressed during summer (February 2013).

The use of nurse trees or shrubs has not always been favorable to plant survival and growth, however, especially under high-density situations when the nurse plants have intercepted rainfall (Valladares and Pearcy 2002), leading to lower water availability and increased drought stress. In our study, volumetric water content increased equally with or without nurse trees, suggesting that the boldo canopy density was ideal for decreasing irradiation but not impairing precipitation from recharging the soil. Shade can also have adverse, species-specific effects on plant growth, especially under drought conditions (Valladares and Pearcy 2002).

Although soil organic matter (OM) content was not measured, research regarding the use of nurse trees for restoration in Mediterranean species found higher OM content under nurse trees, such as kermes oak (*Quercus coccifera* L.) and mastic tree (*Pistacia lentiscus* L.) (Arévalo et al. 1993, De la Torre and Alías 1996), and grasses (Maestre et al. 2001). Soil OM increases water retention and positively affects soil structure, nutrient cycling, and nutrient availability (Sánchez et al. 2006) and increases cation exchange capacity (Page-Dumroese et al. 2000). Removal of OM from the soil surface can have a negative impact on seedling growth as observed in other species such as western white pine (*Pinus monticola* Douglas ex D. Don) and Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) (Page-Dumroese et al. 1997).

Field fertilization improved growth but not survival in our study, which agrees with studies published in Mediterranean species. In ponderosa pine (*Pinus ponderosa* Douglas ex Lawson), Fan and Moore (2002) indicated that fertilized seedlings had greater height and stem diameter at the time of outplanting compared with unfertilized control plants, although higher fertilizer rates increased mortality in this species. Sloan and Jacobs (2013) found that neither controlled-release fertilizers nor immediately available fertilizers significantly affected field survival of white spruce (*Picea glauca* (Moench) Voss) and aspen (*Populus tremuloides* Michx.), though growth of white spruce was positively affected by both types of fertilization. Sutton (1975) also found a growth response to fertilizer in white spruce. Seedling responses to field fertilization depend on field factors such as competing vegetation (Brockley 1988), soil nutrient availability (Nilsson and Allen 2003), and soil moisture (Everett et al. 2007, Rubilar et al. 2008), but, in general, many studies with conifers (Brockley 1988, Fan and Moore 2002, Fan et al. 2004, Haase et al. 2006) and hardwoods (Jacobs et al. 2005) have shown an increase in height and root-collar diameter growth when planted with fertilizers.

Although it has been reported that larger nursery plants perform better under field conditions (Villar-Salvador et al. 2012) and are preferred for reforestation in Mediterranean climates, we found that nursery plant size had no effect on field survival during the first growth season. For silvicultural purposes, seedling uniformity is a desired trait (Basey et al. 2015), but for ecological restoration, non-uniformity and high variability in morphological and growth traits indicate genetic diversity (Smith et al. 2007). Thus, using nursery stock of varied sizes may be suitable for restoration programs, especially in species such as guindo santo, in which plant height variability has no negative effect on field survival.

Conclusions

Our results show that growth and survival of guindo santo plants in a Mediterranean climate, regardless of their initial size, was enhanced by the use of boldo nurse trees, which increased soil gravimetric water content and decreased incident irradiation, and which, in turn, decreased plant stress as noted by higher predawn water potential values during the summer. Fertilization also increased plant growth but had no effect on survival. Similar results may be obtained with other nurse tree species or with tree shelters; more research is required to discern the best approach.

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