Use of alternative containers for promoting deep rooting of native forest species used for dryland restoration: the case of *Acacia caven*

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The size of a container determines the development and quality of root systems. In the case of taprooted forest species used for dryland reforestation, deeper containers may favour early root development and, consequently, better soil profile colonization after outplanting. Although research on container design for worldwide tree species has been developed in the last decades, technical solutions for containerized forest species with a taproot system have been poorly documented. We present a case study using *Acacia caven* (Mol.) Mol., which has fast-growing taproots and long lateral and superficial roots. The aim of this study is to evaluate the effects of different containers on rooting volume in the early morphological development of *A. caven* seedlings. Ten day-old seedlings were cultivated in five different PVC container types varying in volume, width and length (T440-Short, T440-Long, T880-Short, T880-Long, and T440-C), in a completely randomized design for one growing season. At the end of the study, whole seedling samples were destructed to assess taproot length, lateral root biomass, and total root/shoot dry biomass. To evaluate the potential plant capacity for developing new roots, a subsequent experiment using the root growth potential test was performed successfully. Results showed that change in root volume distribution (short vs. elongated containers) had the greatest influence on seedling quality, whereas the size of container (small volume vs. large) was of minor importance. Elongated containers (35 cm to 40 cm in length) with self-pruning basal roots produced seedlings with smaller shoot/root ratios, longer root systems, and a greater ability to restart new root growth in deeper container strata. Elongated containers also prevented taproot deformation. The present study suggests that it would be appropriate to rethink container design for seedlings of deep-rooted xerophytic species destined for water-limited transplanting conditions.

**Keywords:** Native Tree Domestication, Root Growth Potential, Root Morphology, Seedling Quality

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**Introduction**

*Acacia* trees (Leguminosae) are commonly used around the world in the restoration of degraded forest ecosystems (Dumroese et al. 2011, Jeddi & Chaieb 2012, Hernández et al. 2015). Among these, *Acacia caven* is one of the most widespread Neotropical South American tree species (latitude 18° to 37° S – Aronson 1992). It has been commonly used for the reforestation of degraded arid regions (Donoso et al. 2015), as its massive and deep-rooted system allows it to cope with a sudden abundance of water (Aronson 1992). This species generates positive interactions (facilitation) with co-occurring tree species that have lower water-stress resistance (Armento & Pickett 1985), as it improves both the soil nutritional level due to its high nitrogen fixation potential and the micrometric conditions under the canopy (Aronson 1992).

Deep roots, or taproots, defined as roots that grow to depths greater than 1 m (Maeght et al. 2013), are functionally important for drought-adapted woody species (Schenk & Jackson 2002a, Comas et al. 2013, Ovalle et al. 2015). Their main functions are hydraulic redistribution, resistance to embolism and to physical-chemical weathering, and C sequestration in deep soil strata, among others (Bleby et al. 2010, Johnson et al. 2014). As for early seedling survival and establishment, taproots allow rapid deep growth toward deep water sources available in soils, particularly before the first dry season (Canadell & Zeder 1995, Padilla & Pugnaire 2007). Indeed, a number of studies have demonstrated a positive relationship between root length and early seedling survival in arid (Löhn et al. 2011), semi-arid (Ovalle et al. 2015), and tropical dry forest ecosystems (Marksteijn & Poorter 2009). From a silvicultural point of view, a main challenge for nurseries is to produce containerized seedlings that mimic the root size and architecture of naturally gener-
ated seedlings. However, in most cases, containers used for nursery propagation result in seedlings with restricted root development and a completely different root architecture compared to natural conditions (Tsakaldimi et al. 2009, Dumroese & Landis 2015). This is the case with small containers (< 400 mL), as broadly described in the literature (Romero et al. 1986, Del Campo et al. 2010, Ritchie & Landis 2010, Aghai et al. 2014, Walsh et al. 2015). Chilean nurseries commonly use small containers such as black polyethylene bags, which are quite shallow with typical volumes lower than 400 mL (Ovalle et al. 2015). In addition, black polyethylene bags absorb solar radiation and increase substrate temperatures, which retards root growth at temperatures greater than 30 °C. Reduced volume in containers does not promote lateral root self-pruning at early growth stages, and this often results in root deformation, which further restricts growth (Dominguez-Lerena et al. 2006). Limitations of small containers result in serious morphological constraints and higher shoot/root ratios. This has a strong and negative impact on seedlings’ water economy after they are outplanted, particularly under arid and semiarid climate conditions (Romero et al. 1986, Tsakaldimi et al. 2005).

In the last decade, there has been an increasing interest in improving seedling quality of deep-rooting Mediterranean tree species, including the redesign of propagation containers (Chirino et al. 2008, Mariotti et al. 2015a, Ovalle et al. 2016). Traditional nursery cultural practices meant to improve the quality of seedlings have been developed mostly for tree species belonging to North American boreal forests (Haase 2005, Grossnickle 2012), which tend to develop shallow root systems (Schenk & Jackson 2002b). By contrast, the plant quality in xeric environments is related to the development of drought-tolerant traits (Cortina et al. 2013) such as low specific leaf area, large stem diameter, low shoot/root ratio, and large root volume (Trubat et al. 2006). These traits need to be achieved at early propagation stages. In this context, large-volume containers with better volume distribution would facilitate tap-root growth and improve whole plant quality for xerophytic species, assuring proper root functionality for the seedlings’ survival and establishment in soils with low water availability (Bengough et al. 2011). Several studies have reported on a positive relationship between large-volume containers, root systems (Mariotti et al. 2015a) and seedlings’ total height (Akpo et al. 2014). An increase of up to 40% in root biomass has been obtained by doubling container volume (Poorter et al. 2012), and significant improvements in root growth capacity, root hydraulic conductance, and stomatal conductance have been obtained with containers above 500 mL in volume and 25 cm in depth (Chirino et al. 2008). Furthermore, spiral root prevention, generation of lateral roots, and improvements in root distribution and fibrosity have resulted from the inclusion of container walls to promote self-pruning of basal and lateral roots (Ritchie & Landis 2010).

No documented studies provide evidence concerning the quality of plant attributes of A. caven seedling production, which are commonly used in the reforestation of highly degraded dry land. It is necessary to evaluate the application of nursery practices commonly used for other productive tree species (Quiroz et al. 2012) to improve nursery management of deep-rooted xerophytic species. In the present study, we evaluate the effect of container size and volume distribution on A. caven seedlings during early plant development.

Materials and methods

Experimental conditions and plant material

Acacia caven seedlings were cultivated in a growth room at Centro de Investigación Minera y Metalúrgica (CIMM) in Santiago, Chile (33° 26′ S – 74° 40′ W). Inside the controlled growth room, the established mean temperature was 23 ± 2 °C, relative humidity was 46%, and light intensity was 273 µmol s⁻¹ m⁻², with a 12/12 h photoperiod. Seedlings were produced from seeds collected from individual trees growing in Elqui Province, north-central Chile (29° 49′ S – 70° 48′ W). The seeds were subjected to chemical scarification with concentrated sulfuric acid (technical quality) for 120 minutes before they were used. They were then germinated in 1-L containers filled with A-6 perlite substrate (Harborl tế®). The average final seedling size (length) before transplantation to experimental containers was 3 cm for shoots, 5 cm for root systems, and a pair of well-developed true leaves. No root malformation at the tap-root was observed. Seedlings were individually transplanted to experimental containers filled with substrate (described below – Fig. 1). Water content of the containers was daily checked and when it reached 40% field capacity, demineralized water was added up to 80% field capacity. Seedlings were fertilized twice along the assay with 40 mL of a modified Hoagland’s solution containing 150 mg L⁻¹ N, 80 mg P L⁻¹, and 100 mg K L⁻¹. Seedlings were grown for six months until control seedlings reached 60 cm in height, which is the average size equivalent to a plant of this species after one nursery season in central Chile.

Experimental design and treatments

The study was conducted in two stages. The first stage consisted of an experiment with five treatments distributed randomly over the benches in the plant growth room, using containers of different sizes and with varying volume distributions: T440-Short (440 mL, 10 cm in length, 7.5 cm in diameter), T440-Long (440 mL, 35 cm in length, 4.0 cm in diameter), T880-Short (880 mL, 20 cm in length, 7.5 cm in diameter), T880-Long (880 mL, 45 cm in length, 5.0 cm in diameter), plus the control treatment T440-C (440 mL, 10 cm in length, 7.5 cm in diameter – Fig. 1). To obtain different sizes and rooting volume distributions, containers with the same volume (440 mL or 880 mL) but different lengths (10 cm to 45 cm in length) were used (Fig. 1). Experimental containers were made of PVC (polyvinyl chloride) tubes. The control
treatment was a 12 × 15 cm black polyethylene bag of 440 mL, which is the most commonly used container in local tree nurseries (Ovalle et al. 2015). To promote self-pruning of basal roots and good drainage, all PVC containers were cut vertically in their bases (3 cm long every 3 cm), and a 2 × 2 mm plastic mesh opening was glued to the base. To favour lateral self-pruning of secondary roots, side slots were made along each PVC container. PVC containers were left suspended (in contact with air) along each PVC container. PVC containers were used to fill the containers was A-6 perlite (Horborlite®), one-part peat (Kekkilä DSM), and two parts loam soil. To maintain constant substrate density in all treatments, the correlation between weight of the substrate and container volume was considered. The substrate volume used for the treatments T440-C, T440-Short, and T440-Long was 385 mL, while a volume of 770 mL was used for the treatments T880-Short and T880-Long.

Seedlings’ morphological measurements

From each treatment, six randomly chosen seedlings were assessed for morphology measurements (Trubat et al. 2010). Morphological variables measured were shoot height, root collar diameter (RCD), shoot dry biomass, root dry biomass, shoot height/RCD ratio, and shoot/root dry biomass ratio. Shoot height was measured with a graduated metal ruler from the substrate surface to the apex of the main stem. RCD was measured with a digital caliper below the insertion of the cotyledons. To determine dry biomass, plant tissues were washed with tap water and dried at 45 °C in an air-fired oven until constant weight was reached. Taproot length was measured with scanned images obtained from the root system, after being thoroughly washed with tap water. For taproot length, container depth and the presence of spiral roots were considered. Shoot height and RCD were evaluated once a month for all plants. The remaining variables were evaluated only at the end of the assay, six months after establishment.

Root growth potential tests

The second stage of the experiment was performed to assess the potential capability of roots to produce new growth after growing in different containers. Therefore, the root growth potential (RGP) test was performed following the methodology used by Ritchie (1985) and Trubat et al. (2010). Six plants per treatment were randomly selected at the end of the first stage of experimentation and transplanted into a larger PVC container (70 cm wide). PVC tubes were longitudinally cut into two parts to facilitate the final evaluation. The size of containers varied according to the initial treatment, but the plants had equal space left for further growth (3 cm to each side of the original root plug and 20 cm to the base). The experimental substrate used to fill the containers was A-6 perlite (Horborlite®). The containers were irrigated twice a week with demineralized water up to 100% field capacity. RGP tests lasted for 28 days (Ritchie 1985), as assessed from a pre-test with the objective of determining the number of days required for A. caven roots to grow in the container without reaching the bottom. After cultivation, each container was longitudinally opened and divided into transverse 10-cm-deep sections; the number of roots larger than 1 cm (Chirino et al. 2008) per section was then determined. New root growth in terms of dry biomass was also analysed; new roots were cut by hand, washed, and dried at 45 °C in an air-fired oven until constant weight was reached.

Data analysis

One-way analysis of variance (ANOVA) was used to specify significant differences (P<0.05) among treatments for each response variable evaluated in the first stage of the experiment. For the RGP test, one-way ANOVA was used to determine significant differences (P<0.05) among treatments for each substrate depth section of the containers. Fisher’s LSD test was used to discriminate between treatments (P<0.05). Prior to the ANOVA, normality was verified by the Shapiro-Wilk test and the homogeneity of variances by Levene’s test. If requirements were not met, data was transformed or analysed using the nonparametric Kruskal-Wallis test. All statistical analyses were performed with InfoStat® software v. 2010.

Results

Morphological parameters

The different container types resulted in significant effects (P<0.05) on A. caven seedlings’ shoot height, RCD, shoot height/RCD ratio, and taproot length (Tab. 1). In general, containers with higher volume (T880-Short and T880-Long) did not exhibit a clear pattern for morphological parameters when they were compared to containers with lower volume (T440-Short, T440-Long and T440-C – Fig. 1). Seedlings that were grown in 440-mL containers had significantly higher shoot height and shoot height/RCD ratio (P<0.05) for the control treatment compared to the short and elongated containers. RCD was significantly higher (P<0.05) for seedlings grown in short containers (T440-C, T440-Short, and T880-Short), independent of container volume. No significant differences were found between type of container for shoot biomass, root biomass, shoot/root dry biomass ratio, or Dickson quality index (DQI – Tab. 1). Treatment with the largest container (T880-Long) promoted significantly longer taproot length (P<0.05) in relation to the other treatments and control. By contrast, short containers (T440-C, T440-Short, and T880-Short) showed small taproot growth (Tab. 1) and root deformation at the container base. Therefore, container depth influenced taproot length. In addition, the taproot in T440-C was three times longer than that in T440-Short. In T440-C, 67% of the seedlings had spiral roots, while those grown in containers with side holes (T440-Short, T440-Long, T880-Short, and

<table>
<thead>
<tr>
<th>Morphological parameter</th>
<th>T440-C</th>
<th>T440-Short</th>
<th>Treatments</th>
<th>T440-Long</th>
<th>T880-Short</th>
<th>T880-Long</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot height (cm)</td>
<td>63.4 ± 3.7</td>
<td>50.0 ± 3.8</td>
<td>Treatments</td>
<td>41.0 ± 2.6</td>
<td>55.5 ± 4.4</td>
<td>52.7 ± 3.4</td>
<td>5.13</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>RCD (mm)</td>
<td>4.0 ± 0.1</td>
<td>3.9 ± 0.2</td>
<td>Treatments</td>
<td>3.3 ± 0.1</td>
<td>4.0 ± 0.1</td>
<td>3.6 ± 0.1</td>
<td>7.10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Shoot height/RCD ratio</td>
<td>157.6 ± 8.1</td>
<td>126.2 ± 7.4</td>
<td>Treatments</td>
<td>125.7 ± 6.8</td>
<td>139.6 ± 8.9</td>
<td>147.9 ± 8.2</td>
<td>3.06</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Shoot biomass (g)</td>
<td>1.6 ± 0.3</td>
<td>1.5 ± 0.2</td>
<td>Treatments</td>
<td>0.8 ± 0.1</td>
<td>1.9 ± 0.4</td>
<td>1.6 ± 0.2</td>
<td>2.44</td>
<td>0.073</td>
</tr>
<tr>
<td>Root biomass (g)</td>
<td>1.1 ± 0.1</td>
<td>1.2 ± 0.3</td>
<td>Treatments</td>
<td>0.7 ± 0.1</td>
<td>1.4 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>1.80</td>
<td>0.161</td>
</tr>
<tr>
<td>Shoot/root ratio</td>
<td>1.4 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>Treatments</td>
<td>1.1 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.01</td>
<td>0.422</td>
</tr>
<tr>
<td>Taproot length (cm)</td>
<td>18.8 ± 3.5</td>
<td>6.9 ± 0.3</td>
<td>Treatments</td>
<td>26.1 ± 0.9</td>
<td>12.6 ± 0.5</td>
<td>33.7 ± 0.5</td>
<td>24.20</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Dickson Quality Index (DQI)</td>
<td>0.18 ± 0.02</td>
<td>0.19 ± 0.04</td>
<td>Treatments</td>
<td>0.12 ± 0.01</td>
<td>0.20 ± 0.03</td>
<td>0.18 ± 0.02</td>
<td>1.50</td>
<td>0.231</td>
</tr>
</tbody>
</table>
T880-Long) did not have spiral roots (Tab. 1).

Root growth potential test
No significant differences concerning root biomass or total number of new roots were found among treatments for A. caven seedlings after the RGP test (Tab. 2). However, significant effects (P<0.05) were found in terms of root length/container length (%), as expected. Seedlings of the T880-Long treatment tended to produce greater root biomass than the other treatments, including the control. By contrast, the T440-Long treatment tended to produce the lowest root biomass. Comparing seedlings of different sizes, we observed that root biomass of T880-Short was 25% larger than that of the T440-Short treatment, because the former has twice the volume. A similar tendency was detected for elongated containers where T880-Long produced twice the biomass of the T440-Long treatment. In terms of the number of new roots, larger containers (T880-Short and T880-Long) tended to produce larger amounts compared to small containers and the control.

Although there were no significant differences in root biomass and number of new roots, the distribution of these parameters varied with container depth (Fig. 2). Biomass and new roots were mainly concentrated at the location of the base of the first-stage root plug, except for T440-Short and T440-Long, in which the biomass and new roots were concentrated under the dotted line (Fig. 2). In Fig. 2, the point where the biomass curve intercepts the root number curve corresponds to 0.75 mg of biomass per root. When the root biomass curve is located to the left of the root number curve, the roots have a value less than 0.75 mg, which we define as “thin roots”. On the other hand, when the biomass curve is located to the right of the root number curve, the roots have a value greater than 0.75 mg, which we define as “thick roots”. Furthermore, it was observed that thick roots were concentrated under the dotted line and thin roots dominated above the dotted line (Fig. 2).

Seedling roots of T440-C and T440-Short treatments colonized and were concentrated up to 30 cm in depth, while seedling roots of T880-Short and T880-Long treatments colonized up to 40 cm and 70 cm in depth, respectively. Seedling roots of T440-Long, which had half the volume of T880-Short and T880-Long, colonized up to 60 cm in depth (Fig. 2).

Discussion
The present study confirms that container characteristics have important implications on seedling morphology of deeprooting species such as A. caven. Among the morphological variables influenced by type of container, a change in rooting volume distribution (short versus elongated container) was the most important, whereas, as container size (small versus large volume) had a minor influence on taproot length and RCD. Different plant responses to container type have been described by other authors for Mediterranean tree species (Chirino et al. 2008, Mariotti et al. 2015a), and in many cases the differences depend on root growth strategies (Schuch et al. 2000).

Field and experimental evidence has shown that direct sowing of tree species with a taproot system produces trees that can cope with water restrictions more effectively than those grown in nurseries and outplanted in field conditions, because of greater development of taproots (Zadworany et al. 2014). Therefore, elongated containers guarantee higher rates of seedling survival under semiarid conditions (Vallejo et al. 2012) as the root development mimics seedling growth under natural conditions. According to Peman et al. (2006), water

Tab. 2 - Root morphology characteristics of Acacia caven seedlings after the root growth potential (RGP) tests. Mean ± standard error values are given. Different letters indicate significant differences among treatments according to one-way ANOVA and Fisher’s LSD tests (P<0.05). Treatments: T440-C (440 mL, 10 cm in length); T440-Short (440 mL, 10 cm in length); T440-Long (440 mL, 35 cm in length); T880-Short (880 mL, 20 cm in length); T880-Long (880 mL, 45 cm in length).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatments</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root biomass (mg)</td>
<td>T440-C</td>
<td>115.9 ± 29.7</td>
<td>77.0 ± 12.2</td>
</tr>
<tr>
<td></td>
<td>T440-Short</td>
<td>87.8 ± 11.8</td>
<td>94 ± 16</td>
</tr>
<tr>
<td></td>
<td>T440-Long</td>
<td>87.8 ± 11.8</td>
<td>94 ± 16</td>
</tr>
<tr>
<td>Root number</td>
<td>T880-Short</td>
<td>71 ± 5</td>
<td>22.3 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>T880-Long</td>
<td>71 ± 5</td>
<td>22.3 ± 3.9</td>
</tr>
<tr>
<td>Root length (cm)</td>
<td>T440-C</td>
<td>22 ± 3.9</td>
<td>25.6 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>T440-Short</td>
<td>22 ± 3.9</td>
<td>25.6 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>T440-Long</td>
<td>22 ± 3.9</td>
<td>25.6 ± 1.7</td>
</tr>
<tr>
<td>Root length/Container length (%)</td>
<td>T440-C</td>
<td>22.3 ± 40</td>
<td>66 ± 6</td>
</tr>
<tr>
<td></td>
<td>T440-Short</td>
<td>22.3 ± 40</td>
<td>66 ± 6</td>
</tr>
<tr>
<td></td>
<td>T440-Long</td>
<td>22.3 ± 40</td>
<td>66 ± 6</td>
</tr>
</tbody>
</table>

Fig. 2 - Distribution of new root biomass (white circles and lowercase letters) and new roots number (filled circles and capital letters) of Acacia caven seedlings for the root growth potential (RGP) test. Different letters indicate significant differences among treatments for each section of the container. The dotted line indicates the depth where the base of the first stage assay root plug was located.
uptake efficiency could be seriously diminished by the confinement effect that plant roots suffer in short containers (20 cm in length). In addition, the same authors suggest that restrictions to vertical root growth of Quercus ilex result in total lower root lengths. In our study, we observed that elongated containers (35 to 45 cm in length), independent of their volume, promoted larger increases in taproot length than shorter containers. In fact, the largest container type (T880-Long) quintupled the root lengths. On the other hand, growth of seedlings was observed by Quiroz et al. (2009), Aghai MM, Pinto JR, Davis AS (2014). Container volume and growing density influence western larch (Larix occidentalis Nutt.), seedling development during nursery culture and establishment. New Forests 45: 199-213. - doi: 10.1007/ s11056-013-9402-8


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The authors would like to thank the Chilean Research Center for Mining and Metallurgy (CIMM) and the CONICYT FB 0002 (2014) for funding the study; Professors Eduardo Olate and Paulina Fernández for their contribution to the present study; Elisabeth Trangolao, Elena Bustamante, Margaret Opazo and Marcela Jimenez for their help with the experiments; and Consuelo Gazitúa for helping with graphical presentations.

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Improving container design for developing taproots


