



# Does woody species with contrasting root architecture require different container size in nursery?

## ¿Especies leñosas con contrastante arquitectura radical requieren tamaños de contenedor diferentes en vivero?

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### ABSTRACT

Small containers used in seedlings production of tree species with different root architecture can result in serious constraints for root growth and unsuitable plant quality, with potential impacts on field performance. We compare responses of root morphology to changes in container size and shape in both deep-rooting (*Acacia caven*) and shallow-rooting (*Baccharis linearis*) species, both commonly used in active restoration plans in central Chile. For both species, seedlings were cultivated in four different PVC container-types varying in size (440 mL and 880 mL) and shape ([10, 20, 35, and 45] cm in length), and a control treatment that consists in the commonly container-type used in Chilean nurseries (440 mL black polyethylene bag). Seedlings were cultivated for one growing season under controlled conditions and a set of above- and belowground morphological parameters were evaluated. A second short experiment was performed afterwards to evaluate potential seedling quality through the root growth potential (RGP) test. Results showed that an increase in container volume differently affected morphological parameters of *A. caven* and *B. linearis* seedlings. The control treatment had high presence of spiral roots for the deep-rooting species, restricting normal root growth. For both species, elongated containers (>35 cm) produced seedlings with smaller shoots with longer root systems and without deformations in the taproot or main root. Elongated containers also restarted growth of new roots more deeply in the container as result of the RGP test. We concluded that contrasting root architecture of native species require different container size in nursery to promote an adequate root development and growth.

KEYWORDS: container design, pioneer plants, root growth, seedling quality.

### RESUMEN

El uso de contenedores pequeños en producción de especies arbóreas con diferente arquitectura radical puede generar serias restricciones para el crecimiento de las raíces y producir plantas de pobre calidad y bajo desempeño en campo. El objetivo del estudio fue comparar respuestas morfológicas radicales al cambiar el tamaño y la forma del contenedor en especies de enraizamiento profundo (*Acacia caven*) y superficial (*Baccharis linearis*), ambas de alto interés para la restauración activa en Chile central. Las plántulas fueron cultivadas en cuatro tipos de tubos de PVC, que variaban en tamaño (440 mL y 880 mL) y forma ([10, 20, 35 y 45] cm de longitud vertical), más el control definido por la bolsa negra tradicional de polietileno de 440 mL (contenedor tradicional). Las plántulas fueron cultivadas durante una temporada de crecimiento bajo condiciones controladas. Al final del periodo de estudio se evaluaron altura y diámetro de tallo, longitud de raíz principal o pivotante, biomasa de raíces laterales y relación biomasa aérea/radical. Posteriormente, se aplicó la prueba de potencial de crecimiento radical (PCR) a un conjunto de plántulas seleccionadas al azar. Los resultados mostraron que un incremento en el volumen del contenedor afectó de forma diferenciada los parámetros morfológicos de *A. caven* y *B. linearis*. Se observó un alto porcentaje de raíces en forma de espiral en la especie con raíz pivotante (*A. caven*) cultivada en contenedores tradicionales. En ambas especies, los contenedores alargados (>35 cm) produjeron plantas con vástagos más pequeños, raíces más largas, sin deformaciones y con mayor capacidad de recuperar el crecimiento de raíces nuevas en zonas profundas del contenedor. Este estudio concluye que especies arbóreas con diferente arquitectura radical requieren de contenedores diferentes durante la fase de producción en vivero para promover el adecuado desarrollo y crecimiento de raíces.

PALABRAS CLAVE: diseño de contenedores, especies pioneras, crecimiento radical, calidad de plántula.

## INTRODUCTION

Experiences in dryland reforestation recognize the importance of seedling quality, and particularly, belowground components (root architecture and morphology) of woody species for water stress resistance (Padilla and Pugnaire 2007; Negreros-Castillo, Apodaca-Martinez and Mize, 2010; West *et al.*, 2012; Ovalle, Ginocchio, Arellano and Valenzuela, 2017). In xerophytic species, the ability of seedlings to survive drought periods after outplanting largely depends on roots developed during the nursery phase. Therefore, nursery practices should be designed to promote proper root development to optimize the acquisition of limited site resources after outplanting (Luis *et al.*, 2009). Suitable plant containers for proper root system development are a key aspect in nursery production (Chirino, Vilagrosa, Hernández, Matos and Vallejo, 2008; Mariotti *et al.*, 2015). Seedling root development is affected by shape, size, color, and container material (Dumroese and Landis, 2015). For example, a gain up to 40% in root biomass has been found when container volume is doubled (Poorter, Buhler, Van Dusschoten, Climent and Postma, 2012). In general, the propagation of native woody species in semiarid ecosystems utilizes black polyethylene bags with volumes as low as 400 mL (Aghai, Pinto and Davis, 2014). These containers do not promote lateral root self-pruning at early growth stages; therefore result in root deformation and restricted shoot growth and root depth (Dominguez-Lerena *et al.*, 2006) and unsuitable shoot/root biomass ratios that negatively impact the water economy of seedlings (Tsakalimi, Zagas, Tsitsoni and Ganatsas, 2005). This is particularly relevant in tree species adapted to dry environments that have fast growing taproots and long lateral and superficial roots (Ovalle, Arellano and Ginocchio, 2015). Therefore, to obtain adequate morpho-type seedlings, a container with basal root pruning and suitable size (volume) and shape (diameter and depth) should be used to prevent spiral taproot formation and to improve both generation of lateral roots, and root distribution (Chirino *et al.*, 2008; Ovalle, Arellano, Ginocchio and Becerra, 2016; De la Fuente, Ovalle, Arellano and Ginocchio, 2017).

## OBJECTIVES

The aim of the present study is to compare responses of root morphology to changes in size and shape of containers in both a

deep-rooting (*A. caven*) and shallow-rooting species (*B. linearis*), two pioneer and nurse plants frequently used in reforestation programs in the semiarid Mediterranean-type conditions of central Chile.

## MATERIALS AND METHODS

### Plant material and experimental growing conditions

Native woody species *A. caven* (Mol) Mol. (Fabaceae) and *B. linearis* (Ruiz *et Pav*) Pers. (Asteraceae) were chosen for their contrasting root morphologies: *A. caven* has a deep root system with a pivotal taproot that allows access to groundwater and *B. linearis* has a dimorphic root system, which uses both groundwater (main root) and rainfall (Donoso, 1982). Seedlings were produced from seeds collected from individuals growing at the Elqui Province, north-central Chile (29° 49' S–70° 48' W). Seeds of *A. caven* were subjected to chemical scarification using concentrated sulfuric acid (technical quality) for 120 minutes. Seeds of *B. linearis* do not need pregerminative treatments. The seeds were sown in A-6 perlite (Harborlite ®) followed by transplantation to container. The experimental containers were filled up to 88% of the total volume with a standard propagation mixture of one part A-6 perlite (Harborlite ®), one-part peat (Kekkilä DSM), and two parts of sandy soil. Average final seedling size (height), before transplant to experimental containers, was 3 cm for shoot, 5 cm for root system, and a pair of well-developed true leaves. Water content of containers was daily checked. They were watered with demineralized water up to 80% field capacity each time the substrate reached 40% field capacity. Seedlings were fertilized twice during the assay with 40 mL of a modified Hoagland's solution containing 150 mg N L<sup>-1</sup>, 80 mg P L<sup>-1</sup>, and 100 mg K L<sup>-1</sup> (Harper, Smith and Macnair, 1997). Seedlings were grown for six months, until control seedlings reached 60 cm in height, an average size equivalent to a plant of this species after one nursery season in central Chile. Environmental conditions of the plant growth room were: mean temperature of 23 °C ± 2 °C, relative humidity of 46%, and light intensity of 273 μmol s<sup>-1</sup>m<sup>-2</sup>, with a 12/12 h photoperiod.

### Experimental design and treatments

A randomized experimental design was conducted with five treatments consisting of different types of containers for seedling



production (Fig. 1). Sixteen replicates per treatment and species were considered. The control treatment (C) was a 12 cm × 15 cm black polyethylene bag (440 mL), commonly used in Chilean nurseries. The remaining four containers (T1, T2, T3, and T4) were made with PVC tubes (polyvinyl chloride) (Table 1). To favor basal and lateral air pruning of primary and secondary roots, a 2 mm × 2 mm plastic mesh was glued in the bottom of the PVC tube and slots were made along each PVC container (2 cm below and above the end of the tubes). PVC containers were left suspended (in contact with air) on a plastic structure. Seedling density was 82 plants per square meter. Distribution of treatments in the plastic structure was completely randomized. More details of the experimental design were presented by De la Fuente *et al.* (2017).

## Measurements

Shoot height and stem collar diameter were measured once a month in every seedling ( $2 \times 5 \times 16 = 160$  seedlings). Six randomly selected plants per treatment were assessed after six months ( $2 \times 5 \times 6 = 60$  seedlings). Seedling shoot total height, stem collar diameter, shoot and root dry biomass, height/stem collar diameter ratio, and shoot/root dry biomass ratio were measured. To determine dry biomass, the seedlings were carefully separated from substrate and thoroughly washed with tap water and then dried at 45 °C in a forced air oven until constant weight. Root morphology was evaluated based on scanned images (Epson Perfection 4490 Scanner, Nagano, Japan) of the root system. Taproot (*A. caven*) or main root (*B. linearis*) length considered the container depth and the presence of spiral roots. Spiral root was defined as that root that had an angle less than 90° (Ortega *et al.*, 2006).



FIGURE 1. Photographs of the different size and shape of experimental containers used in the present study. All photos taken in November 2012 by Luz M. de la Fuente

TABLE 1. General characteristics of experimental containers.

Container		Volume (mL)	Length (cm)	Diameter (cm)
Treatment	Material			
C	Black polyethylene bag	440	10	7.5
T1	PVC	440	10	7.5
T2	PVC	440	35	4.0
T3	PVC	880	20	7.5
T4	PVC	880	45	5.0

### Root growth potential (RGP) test

The ability of roots to form new roots (longer than 1 cm) after seedling outplanting was tested according to the Ritchie (1985) procedure. After the first assay, six randomly selected seedlings from each treatment ( $2 \times 5 \times 6 = 60$  seedlings) were transplanted into larger PVC tubes filled with A-6 perlite (Harborlite®) and watered twice a week to 100% field capacity with demineralized water. After 28 days of growth under the same plant growth conditions of the first stage assay, each PVC tube was longitudinally opened and divided into 10 cm depth sections. The new root number and biomass (dry weight) produced per section of the A-6 perlite substrate were recorded.

### Statistical analysis

Data normality was verified by the Shapiro-Wilks test and homogeneity of variances by the Levene's test. If requirements were not met, the data was either transformed or analyzed using the nonparametric Kruskal–Wallis test. Independent one-way analysis of variance was performed to evaluate the effect of types of containers and the RGP test on shoot and root growth. For each analysis, treatments with significant differences ( $P < 0.05$ ) were identified with the Fisher's LSD test. Statistical analyses were carried out using InfoStat® version 2010 (Di Rienzo *et al.*, 2012).

## RESULTS

After six months of propagation, *A. caven* seedlings showed significant differences between containers in shoot height, stem collar diameter, and length of the taproot (Table 2; Fig. 2). Specifically, root length, shoot height and height/stem collar diameter ratio were significantly larger in C compared to T1 (containers of equal shape (10 cm depth) and volume (440 mL)). The taproot in C was three times longer than in T1, but 67% of the plants showed spiral roots. When comparing T1 to T2 (same volume but different depth), T2 had a smaller stem collar diameter and longer taproot. The same pattern was observed when comparing T3 to T4 (Table 2). When comparing plants grown in T1 (440 mL) and T3 (880 mL), no significant differences were observed. However, in thinner containers (T2 and T4) shoot height significantly increased when volume and depth increased (T4) (Table 2).

*Baccharis linearis* seedlings showed significant differences between containers in all morphological parameters evaluated (Table 2). When comparing C and T1, C was significantly greater in root biomass. Comparing T1 and T2 (both 440 mL, but T2 had shorter depth), the stem collar diameter, shoot biomass, and root biomass were greater in the T1 container. The same occurred when comparing T3 to T4 (Table 2). However, these differences were more evident in plants from smaller containers. T1 seedlings produced approximately 100% more biomass than T2, while T3 plants only produced 50% more biomass than T4. Regarding the main root length, significant differences were also observed; plants from the deepest containers, T2 (35 cm) and T4 (45 cm), presented the longest roots, with no significant differences between them (Table 2). When comparing T1 (440 mL) to T3 (880 mL), the plants grown in T3 were taller, had greater stem collar diameter, increased biomass, and increased length of the main root. Seedlings grown in T3 generated 85% more shoot biomass and 50% more root biomass than plants grown in T1. Shoot biomass in T4 increased 150% more than those in T2 (Table 2).

### Root growth potential test

Seedlings of *A. caven* showed no significant differences in production of total root biomass ( $F = 1.26$ ;  $P = 0.3112$ ) and in total number of new roots ( $F = 1.50$ ;  $P = 0.2328$ ) (Table 3). Although there were no significant differences in total root biomass production and number of roots, distribution of these parameters varied with container depth (Fig. 3). The roots in shallower containers (C and T1) colonized and concentrated at shallower depths, reaching 30 cm. Roots of T3 plants colonized up to 40 cm and T4 up to 70 cm, while roots of T2 plants, which had half the volume of T3 and T4, colonized up to 60 cm in depth (Fig. 3). For *B. linearis*, total biomass ( $F = 9.15$ ,  $P < 0.05$ ) and total number of new roots ( $F = 8.35$ ,  $P < 0.05$ ) showed significant differences among treatments (Table 3). The lowest total root biomass and lowest number of roots were produced in plants of smaller containers (C, T1, and T2), with no significant differences among them. The largest increases in total root biomass and total number of roots were observed in larger containers, T3 and T4; T3 biomass was significantly greater than T4 (Fig. 3).

Table 2. Morphological parameters evaluated in *Acacia caven* and *Baccharis linearis* seedlings after six months of propagation in different containers (treatments).

Morphological parameter	Treatment *					F; P-value
	C	T1	T2	T3	T4	
<i>Acacia caven</i>						
Shoot height (cm)	63.41 ± 3.69 c	49.96 ± 3.76 ab	40.99 ± 2.60 a	55.50 ± 4.37 bc	52.69 ± 3.37 b	F = 5.13 P < 0.05
Stem collar diameter (mm)	4.02 ± 0.11 b	3.91 ± 0.16 b	3.25 ± 0.09 a	3.96 ± 0.13 b	3.55 ± 0.11 a	F = 7.10 P < 0.05
Shoot height/Stem collar diameter	157.63 ± 8.10 b	126.19 ± 7.38 a	125.69 ± 6.79 a	139.25 ± 8.92 ab	147.88 ± 8.19 ab	F = 3.06 P < 0.05
Shoot biomass (g, dry weight)	1.65 ± 0.31	1.47 ± 0.21	0.82 ± 0.11	1.93 ± 0.38	1.57 ± 0.22	F = 2.44 P = 0.073
Root biomass (g, dry weight)	1.11 ± 0.14	1.20 ± 0.27	0.72 ± 0.10	1.38 ± 0.24	1.32 ± 0.17	F = 1.80 P = 0.161
Shoot biomass/Root biomass	1.44 ± 0.15	1.34 ± 0.16	1.14 ± 0.09	1.41 ± 0.14	1.19 ± 0.08	F = 1.01 P = 0.422
Taproot length (cm)	18.78 ± 3.53 b	6.87 ± 0.28 a	26.13 ± 0.85 bc	12.57 ± 0.52 ab	33.65 ± 0.51 c	F = 24.20 P < 0.05
Plants with spiral roots (%)	67	0	0	0	0	
<i>Baccharis linearis</i>						
Shoot height (cm)	47.88 ± 1.57 bc	43.92 ± 1.55 ab	39.91 ± 1.22 a	55.94 ± 2.07 d	53.16 ± 3.40 cd	F = 31.73 P < 0.05
Stem collar diameter (mm)	4.04 ± 0.13 b	3.97 ± 0.12 b	3.04 ± 0.08 a	4.71 ± 0.13 c	3.79 ± 0.11 b	F = 31.74 P < 0.05
Shoot height/Stem collar diameter	120.50 ± 5.66 ab	111.69 ± 4.42 a	132.00 ± 4.65 bc	120.56 ± 6.13 ab	140.81 ± 8.92 c	F = 3.39 P < 0.05
Shoot biomass (g, dry weight)	1.92 ± 0.20 bc	1.71 ± 0.22 b	0.93 ± 0.08 a	3.15 ± 0.22 d	2.30 ± 0.17 c	F = 19.37 P < 0.05
Root biomass (g, dry weight)	1.08 ± 0.09 cd	0.83 ± 0.03 b	0.45 ± 0.07 a	1.25 ± 0.08 d	0.89 ± 0.06 bc	F = 17.46 P < 0.05
Shoot biomass/Root biomass	1.82 ± 0.23 a	2.08 ± 0.29 ab	2.16 ± 0.15 abc	2.54 ± 0.13 bc	2.58 ± 0.10 c	F = 3.06 P < 0.05
Main root length (cm)	5.94 ± 0.74 a	5.28 ± 0.27 a	28.80 ± 1.27 c	11.98 ± 0.65 b	30.47 ± 0.87 c	F = 217.56 P < 0.05
Plants with spiral roots (%)	0	0	0	0	0	

Mean ± standard error values are presented; different letters indicate significant differences among treatments.

\*Treatment codes according to table 1.

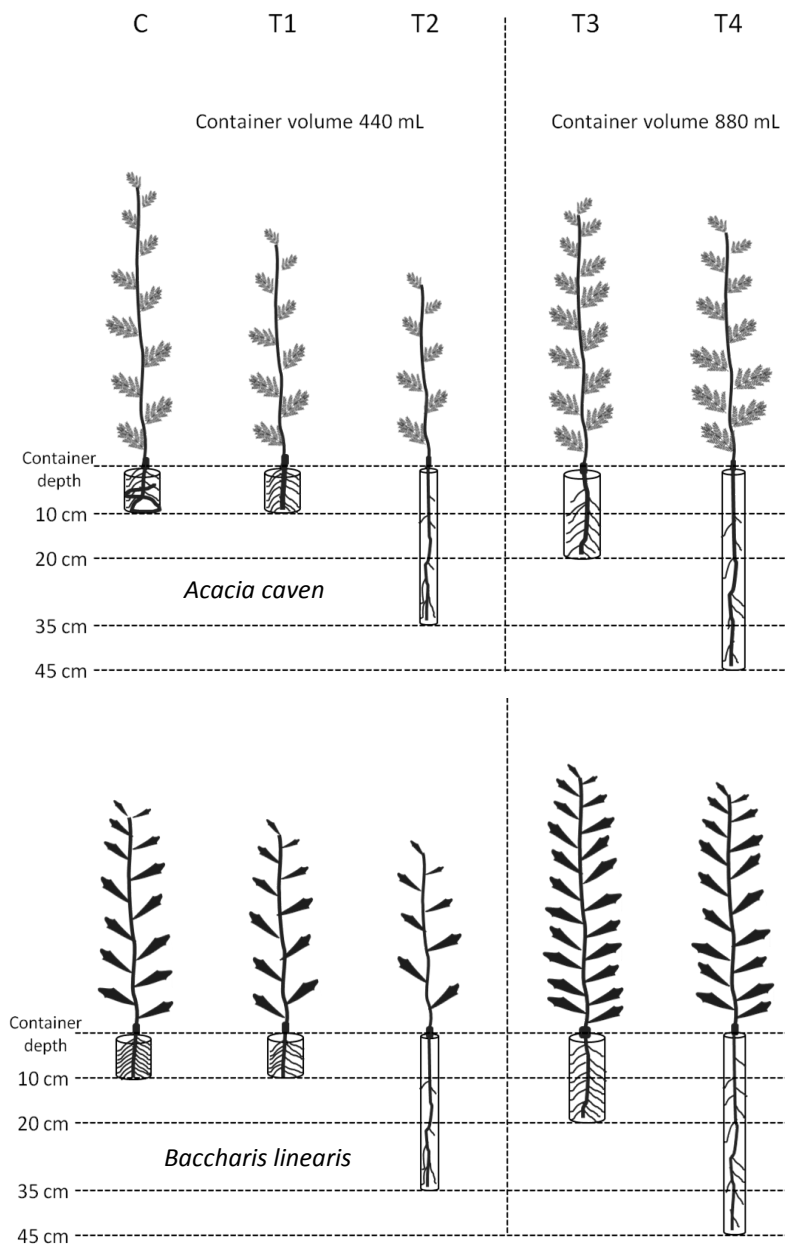


FIGURE 2. Scaled graphical representation of some morphological parameters obtained on seedlings of *Acacia caven* and *Baccharis linearis* grown in different types of containers at the end of experimental propagation stage (treatment codes according to table 1).

## DISCUSSION

Results of this study demonstrate that change in size and shape of containers has significant impact on morphological responses of seedlings of *A. caven* and *B. linearis*, with greater influence on *B. linearis*. For *B. linearis*, the increased volume of containers had a

positive effect on shoot biomass, which may be associated with increased nutrients and water availability because there exist a strong relation between the container size and the resources availability into the container (Landis, Steinfeld and Dumroese, 2010).



TABLE 3. Total dry root biomass (mg) and new roots after the root growth potential tests (RGP). Mean  $\pm$  standard error values are presented; different letters indicate significant differences among treatments.

Treatment *	<i>Acacia caven</i>		<i>Baccharis linearis</i>	
	Root biomass	Root number	Root biomass	Root number
C	115.9 $\pm$ 29.7	71 $\pm$ 15.0	160.9 $\pm$ 25.9 a,b	102 $\pm$ 17.0 a
T1	87.8 $\pm$ 11.8	71 $\pm$ 5.0	190.8 $\pm$ 19.9 a,b	149 $\pm$ 15.0 a
T2	77 $\pm$ 12.2	60 $\pm$ 9.0	112.4 $\pm$ 21.5 a	141 $\pm$ 20.0 a
T3	109.9 $\pm$ 28.0	94 $\pm$ 16.0	334.2 $\pm$ 32.7 c	268 $\pm$ 35.0 b
T4	147.1 $\pm$ 31.2	111 $\pm$ 29.0	233.7 $\pm$ 35.3 b	240 $\pm$ 29.0 b
F and P-values	F = 1.26; P = 0.311	F = 1.50; P = 0.233	F = 9.15; P < 0.05	F = 8.35; P < 0.05

\* Treatment codes according to table 1.

The plant growth differential response to containers type and size has been broadly described by a number of authors in woody species of semiarid environments, and is related to different root growth strategies presented by each particular species (Romero, Fisher and Mexal, 1986, Dominguez-Lerena *et al.*, 2006; Chirino *et al.*, 2008; Mariotti *et al.*, 2015). However, also container size itself could determine changes in plant growth through decrease in photosynthesis activity per unit leaf area, independently of the woody species (Poorter *et al.*, 2012).

When a plant has a taproot it does not normally restart its growth in depth once outplanted and in some cases the plant simply dies (Dominguez-Lerena *et al.*, 2006). Then, the importance of growing seedling with longer taproot, through adequate container design, led to an increase in root hydraulic conductance and number of new roots colonizing the deepest layers and, consequently improve the plant ability to avoid water-stress after outplanting (Chirino *et al.*, 2008). In the case of *B. linearis*, spiral roots were not detected, as it does not have a strong taproot, but a dual system, which has a rooting pattern that depends on the ease of penetration into the substrate.

Diverse approaches have been proposed for recommending better plant containers. Dominguez-Lerena *et al.* (2006) suggested a minimum volume of 300 mL for seedlings destined to dry environmental conditions, while Poorter *et al.* (2012) suggested a container size which the plant biomass does not exceed 1 g L<sup>-1</sup>. In our study, all treatments exceeded that threshold, resulting 3.5

g L<sup>-1</sup> for large containers (880 mL) and 6.5 g L<sup>-1</sup> for small containers (440 mL). This later implies the need to further progress in improving container design to reach an adequate balance between size and plant quality.

Another important operational issue is the cost of production of seedlings in large containers (Puértolas, Jacobs, Benito and Peñuelas, 2012; Dumroese and Landis, 2015). From this point of view, smaller containers (440 mL) could be more appropriate for the cultivation of *A. caven* and *B. linearis*; of these, T2 has the greatest advantages in that it has smaller diameter and therefore uses less space in the nursery. Smaller container also results in less planting cost due to reduced soil movement (Puértolas *et al.*, 2012). Plants of elongated containers could present root plug disintegrate (Peñuelas and Ocaña, 1996); however, these containers did not evidence this problem during the process of transplantation to the PCR assay.

Both *A. caven* and *B. linearis* species are commonly used for restoration plans of drylands due their strong water-resistance ability (Donoso, 1982). This implies that under dry conditions is more convenient to get a larger seedling size (Cuesta *et al.*, 2010), which is achieved using large containers (Poorter *et al.*, 2012; Mariotti *et al.*, 2015). The ecophysiological basis that explains the better responses of large seedlings under limited water availability is because larger seedlings had higher nitrogen remobilizable rates, which promote higher growth of new roots for water absorption (Cuesta *et al.*, 2010).

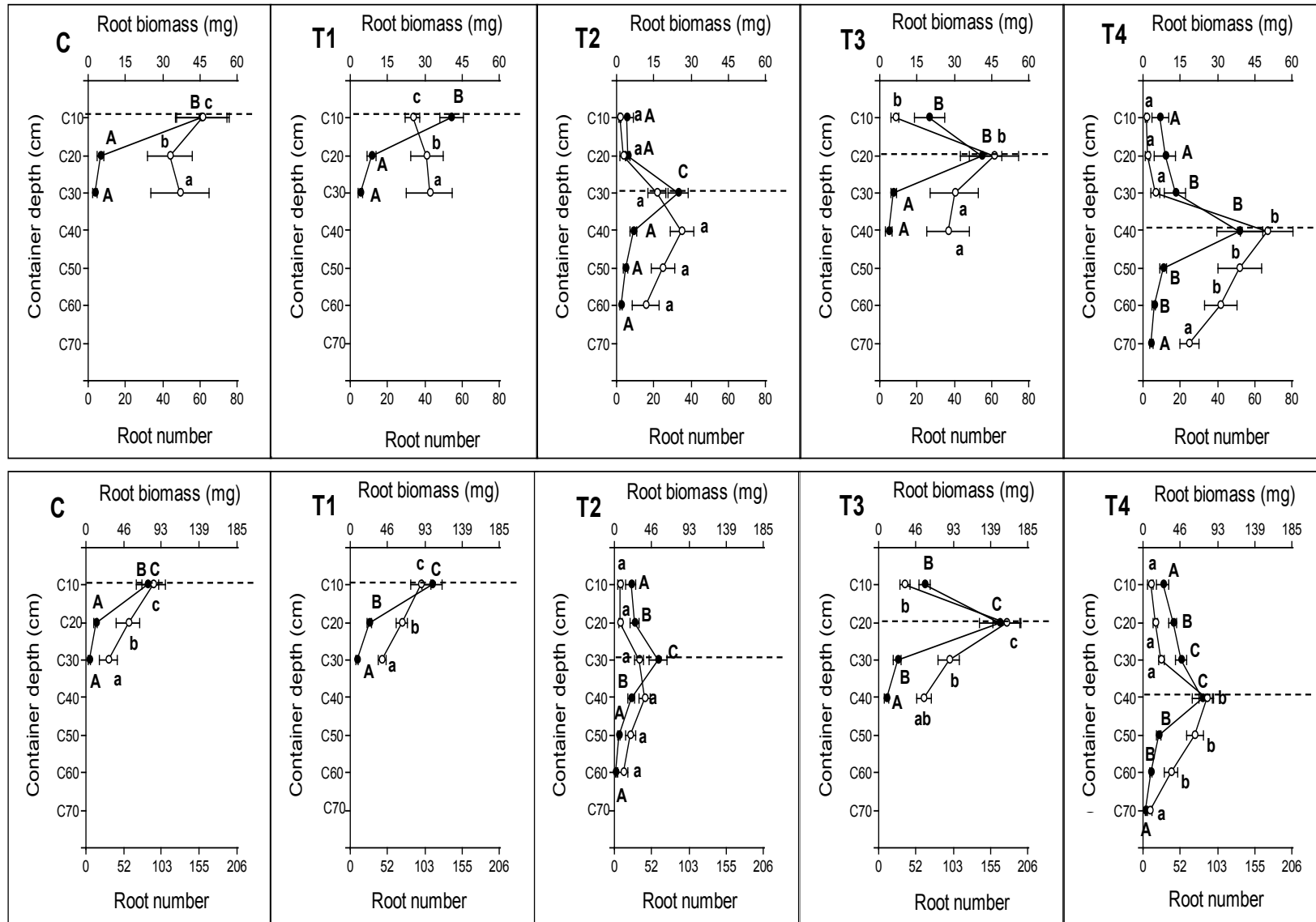


FIGURE 3. Distribution of new root biomass (white circles and lowercase letters) and new root number (filled circles and capital letters) as result of the RGP test. Upper graphs: *Acacia caven*; Lower graphs: *Baccharis linearis* (codes according to Table 1).

Different letters indicate significant differences among treatments for each section of the containers. The dotted line indicates the depth where the base of the first stage assay root plug was located





Container shape, at constant volume, was evaluated by comparing T1 versus T2 (both 440 mL) and T3 versus T4 (both 880 mL). Elongated containers caused a decline in stem collar diameter in both species tested. In *B. linearis*, elongated containers caused decreased shoot and root biomass; this may be due to the longer openings in these types of containers. However, this behavior has been also observed in elongated containers that do not have slots (Korndörfer, Mosena and Dillenburg, 2008). Also, this response has been observed in other woody species where the root length is determined by the container length (Dominguez-Lerena *et al.*, 2006; Peman, Gil-Pelegrin and Voltas, 2006). There is evidence that the root plug of an elongated container has greater contact surface with the soil, and consequently could promote a quick restart of root growth (Grossnickle, 2005).

Both species showed the capacity to develop new roots (RGP test). This capacity is essential for improving seedling survival after outplanting (Tsakalidimi *et al.*, 2005; Trubat, Cortina and Vilagrosa, 2010). In addition, the RGP test showed that new roots were developed on sides of the root plug, which are potentially better surfaces for water absorption and nutrient uptake. Instead, thicker root growth restarted at the base of the root plug, which improves anchorage and absorbs more water from deeper soil layers. The RGP test suggested that seedling roots grown in elongated containers (T2 and T4) have advantage over shorter containers (C and T3). The reason is that under field conditions, the plants from elongated containers would colonize the deepest soil strata quicker than plants from short containers. This would help the "thick" roots to reach the deep-water reserves (Canadell and Zedler, 1995), which represent a safer water source in semiarid environments (Ehleringer and Dawson, 1992).

## CONCLUSIONS

The main conclusion of the present study is that both container size and shape, in combination with the species, affect the initial plant growth. Elongated containers produced seedlings with smaller shoots but with a longer taproot or main root, a proper morphological characteristic for dryland plants. Containers that favor basal root pruning avoid taproot deformation in *A. cavendishii* seedlings, a practice that is not necessary for *B. linearis* due to its dual root system. The increase in container volume (size) only

increased the biomass of *B. linearis*, therefore, using a container of 440 mL and deeper than 35 cm seems more suitable for growing *A. cavendishii* and *B. linearis* seedlings.

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