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Effects of a deep container on morpho-functional characteristics and root colonization in *Quercus suber* L. seedlings for reforestation in Mediterranean climate

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ABSTRACT

In the last decades, reforestation and afforestation programs are being carried out mainly with containerized seedlings. Container design determines the morphological and physiological characteristics of seedlings. However, container characteristics are often the same for plant species with very different growth strategies. The most commonly used nursery containers are relatively shallow and limit tap root growth; consequently, species relying on the early development of a long tap root to escape drought, such as those of the *Quercus* genus, might need to be cultivated in deep containers. The aim of this paper was to compare the morphological and physiological characteristics of *Quercus suber* L. seedlings cultivated in shallow containers (CCS-18, depth 18 cm) with seedlings cultivated in deep containers (CCL-30, depth 30 cm). Both container types used were made of high-density polyethylene, cylindrical in shape, open-bottomed, with a diameter of 5 cm, two kinds of vertical ribs on the inside wall showing a cultivation density of 318 seedlings/m^2. At the end of nursery culture, the seedlings cultivated in the CCL-30 deep container presented a longer tap root, higher shoot and root biomass and higher Dickson Quality Index (DQI). Moreover, the CCL-30 seedlings showed a higher root growth capacity (RGC), they reached deep substrate layers faster and they presented higher root hydraulic conductance. These morpho-functional advantages improved the CCL-30 seedling water status, which was expressed by higher stomatal conductance during an imposed drought period.

1. Introduction

In the Mediterranean region, the success of forest restoration actions is frequently related to the annual rainfall, soil type and seedling quality (Tsakaldimi et al., 2005; Cortina et al., 2006). Planting seedlings in drylands and degraded soils is often discouraging because of high mortality rates and poor growth (Vilagrosa et al., 1996; Cortina et al., 2004). On the other hand, direct seeding is generally not successful due to water limitations and animal predation (Leyva and Fernández-Alés, 1998; Merouani et al., 2001). Thus, the use of seedlings produced in containers or forest trays is the most common technique for introducing native species in dry and semiarid ecosystems (Cortina et al., 2004).

Forest restoration through the planting of resprouting shrubs and trees on degraded lands is recommended to increase the resilience of Mediterranean degraded ecosystems (Vallejo et al., 2000; Vilagrosa et al., 2003a). Forest restoration is a complex technique that covers various fields of activity, such as species selection, nursery culture and soil preparation among others. In this context, the characteristics of the nursery culture, and particularly the container type, are among the main factors to consider in the production of seedlings of quality; thus, the container utilized should be according to morpho-functional characteristics of the species. Moreover, plant species show different root system development patterns due to different biotic and abiotic factors (Cairns et al., 1997), and abiotic factors can often be critical in the plantation establishment phase. Therefore, nursery cultivation should try to favour the particular root development strategy of a given species in order to optimise its potential for establishment in harsh field conditions.

Container design determines the morphological and physiological characteristics of seedlings, mainly in terms of their root systems (Landis et al., 1990; Aphalo and Rikala, 2003; Domínguez-Lerena et al., 2006). The literature offers frequent reports on the

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effects of container volume and seedling density (Howell and Harrington, 2004; South et al., 2005; Tsakaldimi et al., 2005; Domínguez-Lerena et al., 2006), but very few articles deal with the effects of container depth (Chirino et al., 2005; Pemán et al., 2006). Container depth can determine root system growth and tap root length, and thus it can modify soil colonization in deep soil horizons. Growth of new roots out of the root plug for deep soil colonization is a critical factor for seedling survival, especially under Mediterranean climate, where root system size and distribution, root–soil contact, and root hydraulic conductivity can affect the capacity of the seedling to take up water after outplanting (Simpson and Ritchie, 1997; Grossnickle, 2005). On the other hand, new advances in container design have been carried out in the last decades. In general, forest trays commonly used in reforestation programs in subhumid, dry and semiarid Mediterranean ecosystems have cell volumes between 250 and 400 cm^3, and a maximum depth of 18 cm (Peñuelas and Ocaña, 1996). Containers and forest trays with depths close to 30 cm have very large volumes (more than 1000 cm^3), which are necessary in these cases to prolong the culture time (Howell and Harrington, 2004).

Cork oak (*Quercus suber* L.) is a typical resprouting Mediterranean species of great interest for restoration in fire-prone ecosystems (Pausas, 2004; Vallejo et al., 2006; WWF, 2006). This evergreen oak develops a strong tap root during the nursery culture period (Tsakaldimi et al., 2005; Chirino et al., 2005). The upper part of the tap root (about 10–15 cm) is considered a lignotuber, a specialized structure with dormant buds that have resprouting capacity after perturbations (Verdaguer et al., 2001; Pascual et al., 2002). The true root system in this species starts under this lignotuber structure. Consequently, the use of shallow containers with a maximum depth of 18 cm for cork oak cultures will limit the seedling capacity to develop an adequate root system.

The hypothesis of this work was that the root system of cork oak seedlings cultivated in deep containers would develop a long tap root which could quickly reach deep soil horizons in the field after outplanting and thus have a higher probability of finding some soil moisture at the onset of the dry season. This pattern of growth would imitate the development strategy of this species for establishment in natural conditions. A seedling with a long tap root that colonises deep soil layers would have an adequate morphology and biomass distribution for this species, and this should improve its physiological response to water stress conditions. To test this hypothesis, the aim of this paper was to compare the morphological and physiological characteristics of *Q. suber* L. seedlings cultivated in shallow containers (depth of 18 cm), with respect to seedlings cultivated in deep containers (depth of 30 cm). For this purpose, we analyze biomass fractions, root system development and its capacity for water transport and the responses of seedlings to imposed drought conditions.

2. Materials and methods

2.1. Container design and nursery culture

Cork oak (*Q. suber* L.) seedlings were grown for a 1-year period in two kinds of containers manufactured by CETAP-Antonio Matos Lda. (Forestry Containers Manufacturer Company, Espinho, Portugal). The shallow container (CCS-18), with a depth of 18 cm and a volume of 353 cm^3, represented the container commonly used for cork oak culture in the nursery, while the deep container (CCL-30), with a depth of 30 cm and a volume of 589 cm^3, constituted a technological innovation (Fig. 1). Both container types used were made of high-density polyethylene, cylindrical in shape, open-bottomed, with a diameter of 5 cm and show a cultivation density of 318 seedlings/m^2. Inside, these containers have two kinds of vertical ribs (smaller section and larger section) to prevent root spiraling. At the bottom of each, there is a 3 cm-wide net to favour air root pruning. The CCL-30 deep container is patented by CETAP-Antonio Matos Lda. (No. ref. 9976. Boletim Propiedade Industrial no. 11-2004, Portugal).

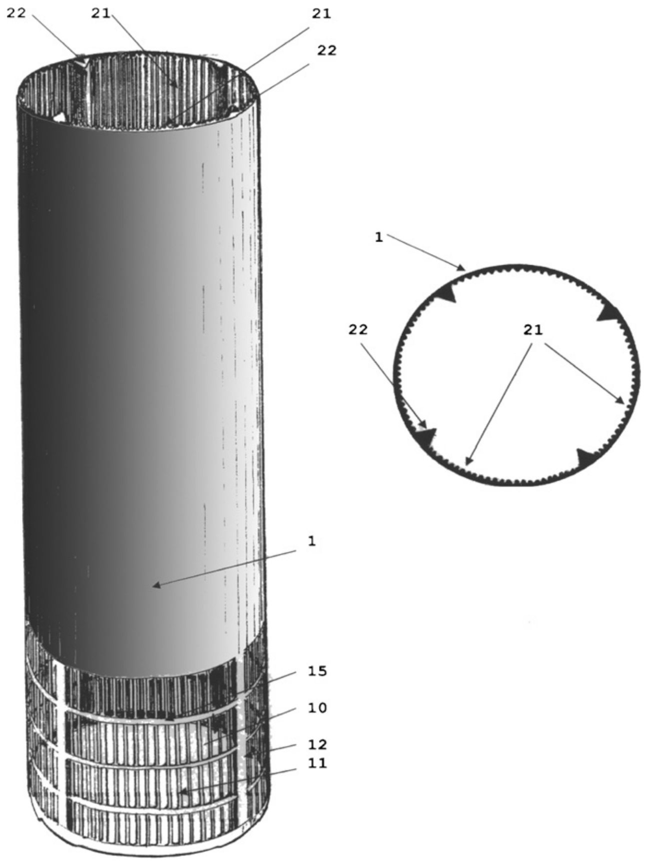

Fig. 1. Drawing of CCL-30 deep container (front and cross section). Legend—1: cylindrical shape, 21: vertical ribs of smaller section, 22: vertical ribs of greater section, 10: network in the lower part composed by horizontal hoops (15) and extensions of vertical ribs (11 and 12).

The culture activity was carried out at the Public Nursery of Santa Faz, Alicante Forest Service, Spain (38°23′N, 0°26′W; 80 m a.s.l.; 240° SW facing) with a mean annual rainfall of 353 mm and a mean annual temperature of 18 °C (Pérez Cueva, 1994). Cork oak acorns from the Espadàn mountain range (Castellón, Spain) were supplied by the Regional Government Forest Service (Banc de Llavors, Quart de Poblet) and were seeded in April 2004. The substrate was limed peat and coconut peat (1:1, v/v) fertilized with 57 mg NO$_3$; 69 mg NH$_4$; 60 mg P; 344 mg K per litre of substrate. Additional slow-release fertilizer (Osmocote plus©, N-P-K: 14-8-14; approximates longevity of 12 months at a mean temperature of 21 °C) was mixed with the substrate at a dose of 1 g/L of substrate. The watering regime was moderate in accord with the seedling water demand and to avoid excessive seedling development (15 mm in autumn, winter and spring, applied 2 days/week, and 25 mm in summer, applied 3 days/week).

2.2. Seedling morphology and biomass

At the end of nursery culture, at 10 months of cultivation, morphological characterization was carried out. Fourteen seedlings per treatment were randomly sampled, and shoot height (H_s) and root collar diameter (RCD) were measured. Seedlings were cut at the cotyledon insertion point and separated into four fractions:

leaves, stem, fine roots (diameter <2 mm) and tap root (diameter >2 mm). Dry weight of each fraction was determined after oven drying at 65 °C for 48 h. Three morphological indices were calculated. The slenderness index (SI) was determined as the relation between shoot height and root collar diameter [$SI = H_s/RCD$], root/shoot ratio [DW_R/DW_S] where DW_R is root dry weight and DW_S is shoot dry weight and Dickson Quality Index (DQI) (Dickson et al., 1960). The Dickson Quality Index uses two quality indices (slenderness index and shoot/root ratio) and was calculated according to the equation [$DQI = SM_T/(SI + (DW_S/DW_R))$] where SM_T is seedling dry weight.

2.3. Root growth capacity (RGC) test

In order to analyze how the new container (CCl-30) can influence the capacity of new roots to colonize soil, a root growth capacity (RGC) test was carried out. This test was implemented 2 weeks after the end of the nursery culture. Seven seedlings per treatment were randomly selected and transplanted to 5.7 L PVC tubes (diameter = 11 cm and long = 60 cm) filled with silica sand. These PVC tubes had previously been cut lengthwise into two sections to facilitate the extraction of new roots at the end of the RGC test. The water regime applied was 20 mm every 10 days. The RGC test had a duration of 45 days and was carried out in full sunlight at an average temperature of 17.4 ± 0.2 °C and a mean air humidity of 82.1 ± 0.8%. At the end of the RGC test, all new roots growing outside the root plug were classified by depth. At 10 cm intervals down to the bottom of the container, the silica sand was carefully removed and the new roots were counted, cut and their dry weight determined. Seedlings were separated into leaves, stem, fine roots (diameter <2 mm) and tap root (diameter >2 mm), and the dry weight of each of these fractions was determined after drying the samples in an oven (65 °C for 48 h).

2.4. Drought period

Another set of seven seedlings per treatment were randomly selected for an imposed drought period. The aim was to measure seedling water status and its response to drought conditions. Seedlings were transplanted to PVC tubes similar to those used in the RGC test (5.7 L), filled with silica sand and placed in full sunlight. They were then grown for 70 days and watering with 20 mm of water every 10 days. The night before the beginning of the drought period, the seedlings were watered to field capacity. Seedling response to drought conditions was monitored by measuring stomatal conductance (G_S; mmol H_2O m^{-2} s^{-1}) using a Porometer AP4 (Delta-T Devices Ltd., Cambridge, UK). Measures were carried out every 3 or 4 days for 16 days. The mean temperature during the drought period was 16.5 ± 0.3 °C (mean maximum temperatures 23.9 ± 0.8 °C) and the mean air humidity was 62.9 ± 1.6%. G_S was measured in five leaves per seedling at 2-h intervals from 8:00 to 18:00 h solar time. In addition, air temperature (T^a; °C), air humidity (H; %) and photosynthetic active radiation (PAR; mmol m^{-2} s^{-1}) were monitored every 10 min by means of HOBO© sensors (Onset Computer Corporation). Vapour pressure deficit (VPD; kPa) was calculated from T^a and H data.

2.5. Root hydraulic conductance

Another set of seedlings transplanted into PVC tubes (similar to the RGC test) were used to measure the water transport capacity of root systems by means of a High Pressure Flowmeter (HPFM, Dynamax Inc., Houston). Measures were carried out 90 days after outplanting. The night before the measurements, seedlings were watered to field capacity. The following day, seedling stems were cut underwater to about 10–30 mm above the substrate surface and then connected to the HPFM. Root hydraulic conductance (K_R) was measured using the method described by Tyree et al. (1995). Both water flow into roots (F) and applied pressure (P) were measured every 3 s while the applied pressure was continuously increased from 0.1 to 0.5 MPa at a constant rate. K_R was calculated as the slope of the plot of F versus P [$K_R = dF/dP$] and corrected for water and room temperature. Morphological parameters were used to normalize root hydraulic conductance (K_R) in terms of leaf area ($K_{R\text{-}LA}$; kg m^{-2} s^{-1} MPa^{-1}), root surface area ($K_{R\text{-}RS}$; kg m^{-2} s^{-1} MPa^{-1}) and root length ($K_{R\text{-}RL}$; kg m^{-1} s^{-1} MPa^{-1}). At the end of the K_R measures, seedlings were extracted as in the RGC test. Seedlings were separated into leaves, stem, fine roots (diameter <2 mm) and tap root (diameter >2 mm). Leaves and fine roots were scanned on a professional scanner (Epson Expression 1680 Pro, Seiko Epson Corporation, Nagano, Japan) with transparency adapter and analyzed with specialized software (WinRhizo, Regent Inst., Canada) in order to obtain the leaf area, root length and root surface area for each seedling.

2.6. Statistical analysis

Statistical analysis to compare the seedlings produced in the two containers was carried out with SPSS© statistical software v. 13.0 (SPSS Inc., Chicago, IL, USA). Data on seedling morphological characteristics, root growth capacity test and root hydraulic conductance were compared by means of analysis of variance (one-way ANOVA). The relationship between water flow and pressure applied in root hydraulic conductance measurements was verified by means of linear regression analysis. Daily stomatal conductance during the drought period was compared by means of General Lineal Model (GLM) repeated measures. Data were transformed when necessary to assure the assumptions of the analysis of variance.

3. Results

3.1. Morphological characteristics of seedlings and biomass allocation patterns

Seedlings cultivated in both container types showed similar shoot height, root collar diameter and slenderness index at the end of the nursery period. However, seedlings cultivated in deep containers (CCL-30) had a longer tap root than seedlings in shallow containers CCS-18 (Table 1). They also showed higher leaves dry weight, stem dry weight, tap root dry weight, and fine roots dry weight. Consequently, the seedlings cultivated in CCL-30 showed higher above-ground and below-ground biomass than the CCS-18 seedlings (Table 1). In spite of the differences found in biomass, seedlings cultivated in both types of containers showed similar allocation patterns to roots and shoots, with no significant differences in the root/shoot ratio (Table 1). Nevertheless, CCL-30 showed a tendency to allocate more biomass to roots than CCS-18 (root dry weight: seedling dry weight ratio, $P = 0.084$). In both cases, the tap root accounted for more than 50% of the seedling dry weight. Fine roots biomass represented a smaller fraction (<10% of seedling dry weight) while the leaves and stem fractions represented about 30% of seedling dry weight. Seedlings in deep containers reached a higher plant quality than seedlings in shallow containers, as expressed by the Dickson Quality Index value (Table 1).

Table 1
Seedlings morphological characteristics at the end of the nursery period

Seedlings morphological characteristics	Container type		F-value
	CCS-18	CCL-30	
Shoot height (cm)	36.11 ± 2.45	40.75 ± 2.55	0.274 ns
Root collar diameter (mm)	5.28 ± 0.35	5.19 ± 0.60	0.041 ns
Sturdiness Index (cm mm^{-1})	7.18 ± 0.44	7.12 ± 0.36	0.002 ns
Tap root length (cm)	18.71 ± 0.11	29.29 ± 0.90	381.191^{***}
Leaves dry weight (g)	1.97 ± 0.17	2.80 ± 0.23	8.185^{**}
Stem dry weight (g)	1.86 ± 0.22	2.51 ± 0.83	4.435^{*}
Shoot dry weight (g)	3.83 ± 0.35	5.31 ± 0.42	7.323^{*}
Tap root dry weight (>2 mm) (g)	6.62 ± 0.58	10.48 ± 0.63	20.274^{***}
Fine root dry weight (<2 mm) (g)	0.77 ± 0.10	1.34 ± 0.10	16.681^{***}
Root dry weight (g)	7.47 ± 0.63	11.89 ± 0.65	23.892^{***}
Seedlings dry weight (g)	11.30 ± 0.93	17.20 ± 0.99	19.030^{***}
Root/shoot ratio (g g^{-1})	2.02 ± 0.15	2.31 ± 0.11	2.500 ns
Dickson Quality Index (g cm^{-1} mm^{-1})	1.48 ± 0.40	2.35 ± 0.66	17.665^{***}

Mean ± standard error; $N = 14$; ns: not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3.2. Root growth capacity

Forty-five days after being transplanted to PVC containers, seedlings showed aboveground and belowground characteristics similar to those found at the end of the nursery period (data not shown). Some differences were detected with respect to patterns of new roots growth (Fig. 2). CCL-30 showed deeper soil colonization than CCS-18 containers ($P = 0.003$), with an average of 40.3 ± 2.0 and 29.6 ± 2.1 cm for CCL-30 and CCS-18, respectively. In the shallow layers (0–10 cm depth) both seedling containers presented the same root growth. The number of new roots developed was higher ($P = 0.010$) in the CCS-18 containers in the 10–20 cm depth range, whilst this same variable was higher in the CCL-30 containers at depths of both 20–30 cm ($P = 0.019$) and 30–40 cm ($P \leq 0.001$). The biomass of new roots by depth followed a similar pattern: it was higher for CCS-18 containers in the 10–20 cm depth range ($P = 0.011$; Fig. 2) and higher for CCL-30 containers in the 30–40 cm depth range ($P = 0.013$). At the 40–50 cm depth, new roots were detected only in the CCL-30 seedlings; CCS-18 seedlings did not reach this depth.

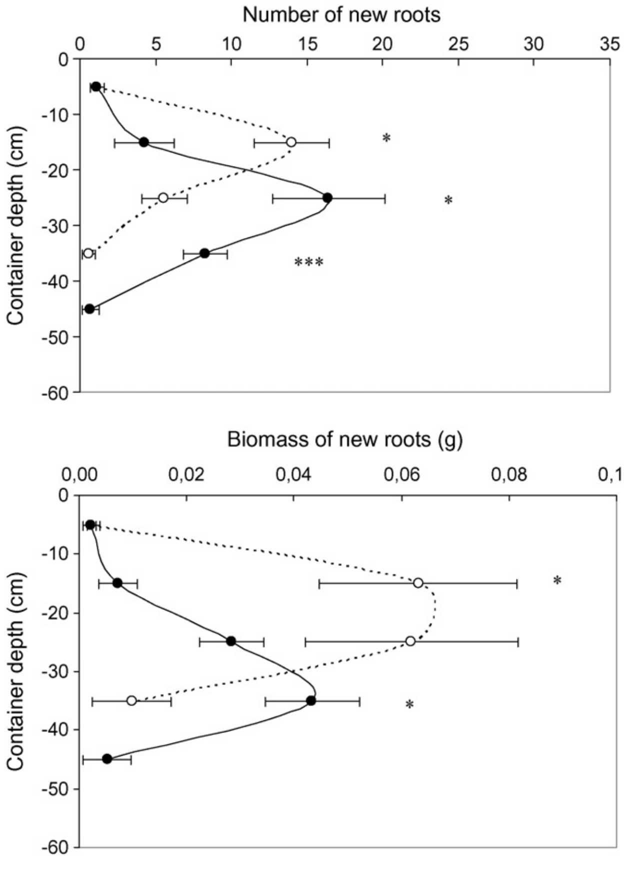

Fig. 2. Root growth capacity test. Number of new roots and biomass of new roots colonizing the substrate. Extraction at 45 days after transplanting (mean ± standard error; $N = 7$; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; white circle and dashed line: seedling cultivated in CCS-18; black circle and solid line: seedling cultivated in CCL-30).

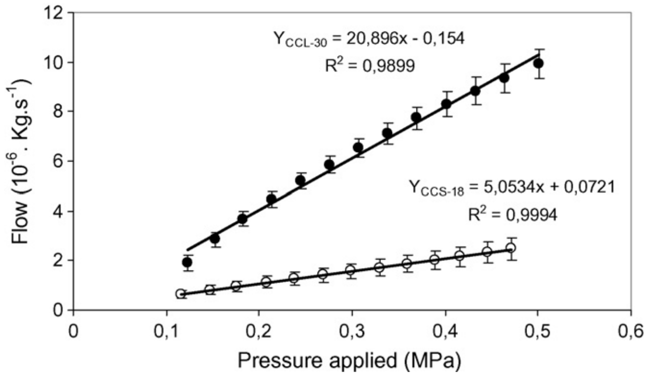

Fig. 3. Water flow through the root system for different pressures applied in roots from the two container types. Mean ± standard error, $N = 7$; white circle: seedling cultivated in CCS-18 and black circle: seedling cultivated in CCL-30.

3.3. Root hydraulic conductance

The relationship between F and P was linear in both container types ($R^2 = 0.99$). The slope of linear regression was lower in CCS-18 than in CCL-30, indicating higher water flow in deep containers for the same pressure value (Fig. 3). When root hydraulic conductance was expressed per unit of leaf area or in terms of root variables, statistical differences were found between both container types (Fig. 4). Seedlings cultivated in CCL-30 containers showed almost two times higher root hydraulic conductance per unit of leaf area than CCS-18 containers ($K_{R\text{-}LA}$, $P = 0.035$). Differences were higher when hydraulic conductance was

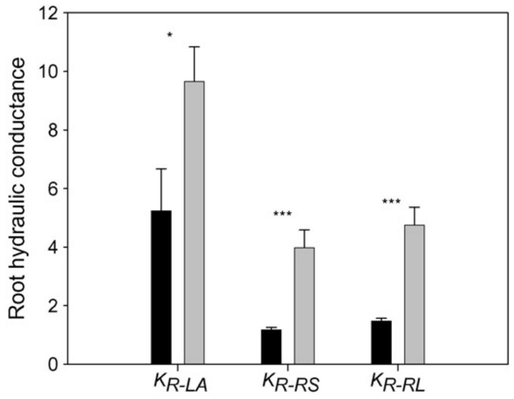

Fig. 4. Root hydraulic conductance per leaf unit area ($K_{R\text{-}LA}$ = kg m^{-2} s^{-1} MPa^{-1} × 10^{-4}), per root unit surface area ($K_{R\text{-}RS}$ = kg m^{-2} s^{-1} MPa^{-1} × 10^{-4}), and per root unit length ($K_{R\text{-}RL}$ = kg m^{-1} s^{-1} MPa^{-1} × 10^{-7}). Mean ± standard error, $N = 7$. Grey bar: seedling cultivated in CCL-30, black bar: seedling cultivated in CCS-18. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

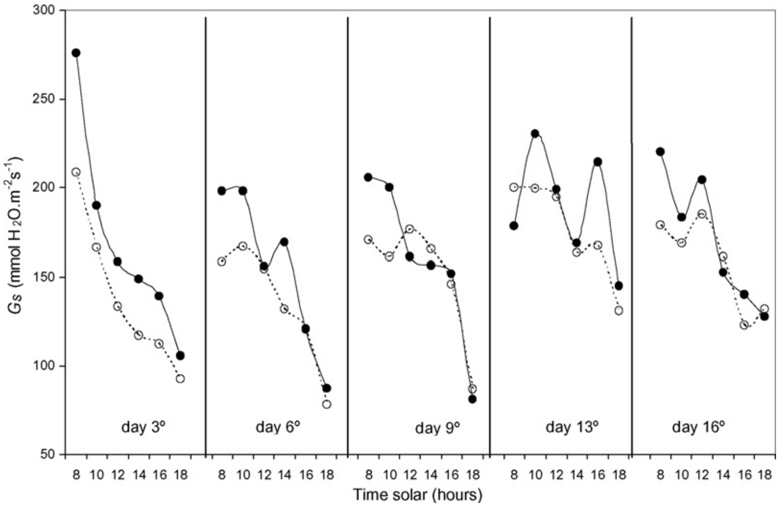

Fig. 5. Stomatal conductance on each measuring day in 2-h intervals from 08:00 to 18:00 h solar time ($N = 7$; white circle and dashed line: G_S in CCS-18 seedlings container; black circle and solid line: G_S in CCL-30 seedlings container).

expressed per root surface area ($K_{\text{R-RS}}$, $P \leq 0.001$) or root length ($K_{\text{R-RL}}$, $P \leq 0.001$).

3.4. Stomatal conductance in drought period

Maximum values of stomatal conductance were registered between 08:00 and 10:00 h UTC with a reduction during midday in seedlings of both types of containers (Fig. 5). CCL-30 containers reached higher G_S values than CCS-18 containers during the hours of maximum G_S (early in the morning and afternoon); while at midday, in both container types G_S decreased and registered similar values. Daily mean G_S values were significantly higher in CCL-30 than in CCS-18 seedlings, with values ranging from 155 to 198 mmol H_2O m^{-2} s^{-1} and 135 to 176 mmol H_2O m^{-2} s^{-1} for CCL-30 and CCS-18, respectively (Fig. 6). These differences were mainly observed during the first 9 days (Anovar, $G–G' = 0.005$) and were reduced later (Anovar, $G–G' = 0.045$). The last days of the drought period were affected by unstable weather conditions. The air humidity showed high values which favoured the descent of VPD and produced an increase in G_S.

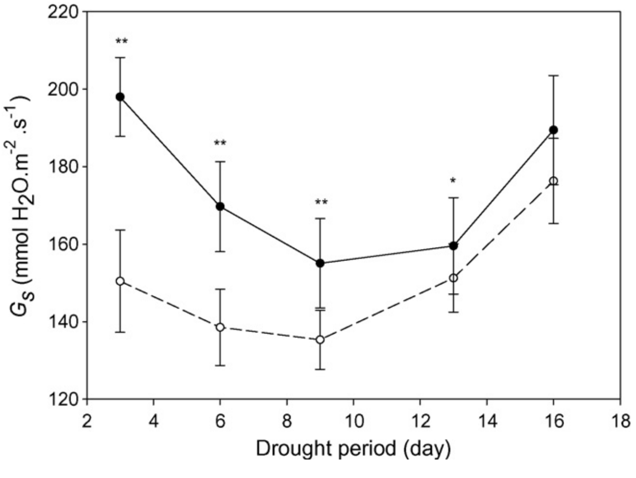

Fig. 6. Mean daily stomatal conductance by container type in drought cycle test ($N = 7$; $^{*}P < 0.05$; $^{**}P < 0.01$; white circle and dashed line: G_S in CCS-18 seedlings container; black circle and solid line: G_S in CCL-30 seedlings container).

4. Discussion

4.1. Effects of a deep container on seedling morphology and root system growth

The effect of container size has been the object of several studies on plant stock quality. In general, a relationship has been observed between container size or shape and seedling growth (Aphalo and Rikala, 2003; Domínguez-Lerena et al., 2006). In the present study, deep containers favoured the growth of the tap root. This result agrees with those obtained by Chirino et al. (2005) and Pemán et al. (2006). Adequate root development is very important for Mediterranean species like *Q. suber*; in fact, one of the main strategies of this species is to develop a deep tap root during the early stages of plant development (Tsakaldimi et al., 2005). This tap root enables it to access deep soil water reserves, a factor considered critical for plant survival during summer drought (Canadell and Zedler, 1995; Canadell et al., 1996). Moreover, a well-developed root system improves the capacity of *Q. suber* to resprout after perturbations (Canadell and López-Soria, 1998; Verdaguer et al., 2001; Pascual et al., 2002).

The use of deep containers in the nursery does not necessarily produce excessive height growth. Adequate control of watering and fertilization could modulate seedling height and RCD growth (Landis et al., 1990). In this study, height and RCD of seedlings were not affected by the type of container. These results are similar to observed in other Mediterranean evergreen oaks that develop a deep tap root, such as *Quercus coccifera* and *Q. suber* seedlings reported by Chirino et al. (2005) and *Quercus ilex* by Pemán et al. (2006).

In spite of showing no differences in shoot height and RCD, the seedlings in CCL-30 containers developed higher stem, leaves and root biomass those in CCS-18 containers. This response was probably a consequence of the higher volume of the container, resulting in higher water and nutrient availability for seedling growth in the nursery. Small volume containers can restrict water and nutrient availability and impose physical limitations to root system growth (Aphalo and Rikala, 2003; Domínguez-Lerena et al., 2006). Although CCL-30 seedlings developed higher biomass, the allocation patterns between above- and belowground resources (root to shoot ratio) were unaffected by the type of container. Root/shoot ratios showed values around 2, which were relatively higher than other Mediterranean oak seedlings (Ksontini et al., 1998; Tsakaldimi et al., 2005). The relative allocation of resources to roots or shoots has been considered a key factor in plant strategies regarding water use (Leyva and Fernández-Alés, 1998) and is very important for seedling performance and survival in the field (South, 2000). Although both types of containers showed similar slenderness index and shoot/root ratio, CCL-30 seedlings showed higher Dickson Quality Index mainly due to higher root dry weight. The DQI is considered an index of morphological development to predict seedling field performance (Dickson et al., 1960) and has been successfully used in several species (Roller, 1976; Ritchie, 1984; Hunt, 1990; Luis et al., 2004; Marques et al., 2006).

The depth of the container determines the length of the tap root and thus the depth of root system positioning in the soil (Peñuelas and Ocaña, 1996). Seedlings in CCL-30 containers showed a longer tap root, allowing the tip of the tap root to reach deeper layers than CCS-18 containers. This favours the colonization of the deepest horizons by new roots due to the amount of roots located at the bottom of the root plug (Biran and Eliassaf, 1980; Ortega et al., 2006). At outplanting, this deeper positioning of the root system may help to avoid or reduce the competition for water with herbaceous plants in the top soil horizons, which can be an important factor limiting seedling performance in the field (Grossnickle, 2005).

Root growth capacity has been considered a measure of seedling vigour (Simpson and Ritchie, 1997) and often predicts seedling field performance in relation to overcoming planting stress (Grossnickle, 2005). In this study, the CCL-30 seedlings were the first to reach the deepest substrate layers and showed a higher number of new roots and more biomass at lower depths. These results would seem to indicate that, under field conditions, the root system of seedlings cultivated in deep containers would reach quickly the deeper soil horizons, where the soil moisture is more stable, even in the summer drought period (Chirino, 2003). Thus, the early colonization of deep soil horizons by root systems would probably contribute to a better physiological status and seedling performance during the summer. In fact, it has been reported that the highest seedling mortality rate is generally produced during the first summer period after outplanting (Vilagrosa et al., 1996; Vallejo and Alloza, 1998).

4.2. *Effects of a deep container on seedling water status*

A linear relation between F and P with high determination coefficients has been reported by Nardini et al. (1998) and Pemán et al. (2006), with the slope being higher in deep containers (Pemán et al., 2006), just as we reported in our results. Therefore, the deep container favoured a higher root system water transport capacity, expressed in a higher K_{R}. Moreover, CCL-30 seedlings showed higher $K_{\mathrm{R\text{-}LA}}$, $K_{\mathrm{R\text{-}RS}}$ and $K_{\mathrm{R\text{-}RL}}$ which coincides with the results reported by Pemán et al. (2006). These authors suggest that the deep container favours a more efficient root system for water transport than the shallow container. The range of $K_{\mathrm{R\text{-}LA}}$ data shown in this work is similar to those presented in other studies on young *Q. suber* plants by Nardini et al. (1999). Several works have pointed out that any root restriction on water conduction can affect plant vegetative growth and morphological characteristics (Tschaplinski and Blake, 1985; Webster et al., 2000). In fact, seedlings developed in CCS-18 presented lower shoot and root biomass than seedlings in CCL-30. Moreover, previous studies have indicated that xylem efficiency to transport water can limit transpiration and photosynthesis (Hubbard et al., 2001; Zweifel et al., 2007). In CCS-18 seedlings, the lower root colonization in deep soil horizons combined with the reduced capacity to supply water to leaves would produce important limitations for competing for resources when these seedlings are planted in the field (Tyree, 2003).

The G_{S} values shown by *Q. suber* in this paper are similar to those observed in other Mediterranean evergreen oaks such as *Q. coccifera* by Vilagrosa et al. (2003b), and *Q. ilex* by Villar-Salvador et al. (2004). Maximum stomatal conductance values were observed in the morning, which agrees with Sturm et al. (1998) and Vilagrosa et al. (2003b). The lower water content in the substrate during the last days of the drought period decreased G_{S}, reducing the differences between seedlings in both container types; which agree to Leyva and Fernández-Alés (1998), Vilagrosa et al. (2003b) and Villar-Salvador et al. (2004). Weather conditions (sunny or cloudy days) affect stomatal conductance. Urban et al. (2007) indicated that cloudy days show PAR values that are not stressful with respect to sunny days, as well as T^{a}, H and VPD values that favour higher G_{S}. In our case, the high H and low VPD at the end of the drought period test could be the cause of the high G_{S} values. *Q. suber* is a species that is well-adapted to drought (Nardini and Tyree, 1999) and maintains a favourable ratio between water loss and water uptake during the dry period (Nardini et al., 1999). The morpho-functional advantages observed in this paper for seedlings cultivated in deep containers would contribute to a better water status, as expressed by higher stomatal conductance during the drought period test. These results are related to a longer tap root, a greater root hydraulic conductance, and a higher number of new roots colonizing the deepest layers offering higher water availability.

5. Conclusions

This study demonstrates that the nursery cultivation of *Q. suber* in deep containers improves the morpho-functional attributes and seedling quality. Deep containers produce seedlings with a longer tap root that can quickly reach the deeper soil horizons by means of higher growth in the number and biomass of new roots. The morpho-functional advantages observed in deep containers favour a higher root water transport capacity of the root system, which contributes to a better water status under drought stress conditions. Our results indicate that deep containers could be used in other Mediterranean oaks that develop an important tap root when planted in dry conditions; however, we consider that this research needs to be continued at the scale of experimental plots in the field for a definitive assessment of the technique.

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