Morphological and functional variability in the root system of *Quercus ilex* L. subject to confinement: consequences for afforestation

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Abstract – We examined root morphological and functional differences caused by restrictions imposed to vertical growth in the root system of holm oak (*Quercus ilex* L.) seedlings to assess the consequences of using nursery containers in the development of a confined root system for this species. Thus, root morphological, topological and functional parameters, including hydraulic conductance per leaf unit area (KRL), were investigated in one-year seedlings cultivated in three PVC tubes differing in length (20, 60 and 100 cm). Longer tubes showed greater projected root area, root volume, total and fine root lengths, specific root length (SRL) and KRL values than did shorter tubes. On the other hand, the length of coarse roots (diameter > 4.5 mm) and the average root diameter were greater in shorter tubes. The strong positive correlation found between KRL and SRL (r = +0.69; P < 0.001) indicated that root thickness was inversely related to water flow through the root system. We concluded that root systems developed in longer tubes are more efficient for plant water uptake and, therefore, changes in root pattern produced in standard forest containers (i.e. about 20 cm length) may in fact prevent a proper establishment of the holm oak in the field, particularly in xeric environments.

*Quercus ilex* L. / root hydraulic conductance / root morphology / afforestation

Résumé – Variabilité morphologique et fonctionnelle du système racinaire de *Quercus ilex* L. soumis au confinement : conséquences pour les reboisements. Nous avons examiné les différences morphologiques et fonctionnelles qui ont été induites par des restrictions imposées au développement vertical des racines de plantules de chêne (*Quercus ilex* L.). Les paramètres morphologiques, topologiques et fonctionnels du système racinaire, et aussi la conductivité hydraulique par unité de surface foliaire (KRL), ont été recherchés sur des plantules d’une année cultivées dans des tubes de PVC de différentes longueurs (20, 60 et 100 cm). Les tubes les plus longs présentent des surfaces projetées de racines, des volumes racinaires, des longueurs de racines fines et totales, des longueurs spécifiques SRL et des KRL supérieurs à ceux des tubes courts. Inversement, dans les tubes courts se trouvent des racines de plus fortes sections avec les longueurs les plus grandes de grosses racines (diamètre > 4,5 mm). La forte corrélation positive trouvée entre KRL et SRL (r = +0.69; P < 0.001) indique que la grosseur de la racine est inversement proportionnelle au flux d’eau transporté. On conclut que les systèmes racinaires développés dans des tubes plus longs sont plus efficaces dans l’extraction de l’eau et, par conséquence, les modifications dans le modèle du développement des racines qui ont été produites dans les conteneurs standards (e.g. environ 20 cm de longueur) pouvaient empêcher l’établissement approprié du chêne in situ, surtout dans des environnements à fortes contraintes hydriques.

*Quercus ilex* L. / conductance hydraulique racinaire / morphologie racinaire / reboisements

1. INTRODUCTION

About one million hectares of agricultural land were afforested in the European Union from 1994 to 1999. The holm oak (*Quercus ilex* L.) was the most extensively used species [22] mainly due to its wide ecological amplitude. Indifferent to lithological substratum, the holm oak is found in the thermo, meso and upper Mediterranean thermotypes and in semiarid, dry and humid climates [27]. It stands among the deepest-rooted plant species [10], developing a strong taproot that usually grows several centimetres in length within a few weeks of germination. By the end of the first growing season, the taproot can easily reach a length of 50 cm or even one meter [11, 24]. This feature allows for deep water uptake during drought episodes [12, 13]. The taproot in the holm oak is highly orthogeotropic, though this characteristic may not be present in mesic environments [3], and several zones can be distinguished showing unequal development of lateral roots [3, 24]. In semiarid climates, such a differential development may have important consequences for the dynamics of water extraction during a soil-drying cycle, as described for *Quercus coccifera* [23].

In afforestation programmes, the holm oak is established either through seeding or planting (traditionally, both methods have been recommended), provided that acorn predators are controlled [11, 15]. Although similar experimental results have been obtained with regard to survival [1, 4, 9], shoot growth patterns clearly differ for both methods. Indeed, one-year seedlings often discontinue their shoot elongation shortly after transplanting, especially under drought or competition. At this time, a new taproot and fine lateral roots are formed (Pemán and Gil, unpublished results). This observation suggests that the seeding and planting techniques may...
bear different consequences with regard to root system development, which may ultimately affect seedling establishment [34].

When container seedlings are used for planting, the container characteristics affect the plantlet root system. For example, ribs or slits on the cell inner walls prevent root spiralling, and holes in the base facilitate drainage and encourage air pruning of roots. Continual air pruning induces a limitation to the main root growth by shortening its length to the depth of the container and preventing the development of replacement taproots. The confining of lateral roots in the container and their downward growth lead to the generation of an orthoglotropic lateral root system rather than the more usual plagiotropic one that originates from the taproot air pruning [24].

Fitter has proposed topological models for characterising root systems [6]. In particular, the herringbone and dichotomous structures are extreme cases of a wide range of topologies. The herringbone system appears when branching is confined to the main axis, hence resulting in the most ordered possible root pattern. The dichotomous structure develops when branching is equiprobable at all links. Theoretical considerations suggest that herringbone-type root systems are more efficient for resource acquisition, but more expensive to produce and maintain because they support the greatest proportion of high-magnitude links [6]. Simulation models confirm that the herringbone architecture would be favoured in environments with low soil-resource availability [7, 8].

Roots impose the greatest resistance to liquid water flow in the soil-plant-atmosphere continuum (SPAC) [26]. Thus, the concept of root hydraulic conductance (K_R) has been the subject of numerous studies. K_R (i.e. the inverse of hydraulic resistance) is defined as the ratio that measures water movement through the roots relative to an external driving force controlling the water flow. The adequacy of the root system to supply water to leaves can be estimated by the root hydraulic conductance per leaf unit surface area (K_{RL}) [16]. According to the composite transport model [32], root water supply to the shoot may change according to the shoot demand owing to an adjustment of root hydraulic conductance. Water deficit reduces root growth and the capacity of roots to take up water by suberisation [20, 26, 33]. Therefore, the root hydraulic conductance may vary in response to external (drought or salinity) or internal (nutritional state, water status, demand of water) factors [32], but the extent by which changes in root morphology influence root hydraulic conductance still needs to be determined.

The aim of this study was to describe and compare morphology, topology and functional differences of root system types developed within containers that vary in total length, as an indirect approach to reproduce the holm oak root characteristics generated under either the seeding or planting techniques for afforestation. A better understanding of such differences may thus be relevant for defining the most suitable afforestation method for this species in drought-prone Mediterranean areas.

2. MATERIALS AND METHODS

The study used acorns from “La Mancha” (Spain) provenance region (altitude: 500–1 000 m; annual precipitation: 312–539 mm; climate: semiarid to subhumid). The acorns were cultivated in three types of PVC tubes differing in length in order to evaluate differences in morphology and functional responses caused by restrictions to growth in the holm oak root system. The shortest tube (ST) was 20 cm long, which is the recommended container length for holm oak cultivation under Mediterranean conditions [9]. The largest tube (LT) was 100 cm long, and was aimed at obtaining a root system developed without vertical restriction to growth. An intermediate length of 60 cm (hereafter, MT) was also used. A galvanized mesh was placed at the bottom of the tubes to prevent substrate movement and to facilitate root air pruning. As container volume has a clear influence on root system morphology [9, 21, 28], this parameter was kept nearly constant by selecting the most appropriate tube diameter allowed by the commercial offer: diameters chosen were 105 (ST), 59.5 (MT) and 43 (LT) mm. This yielded volumes of 1 700 cm^3 (ST, MT) and 1 500 cm^3 (LT) respectively. The substrate employed was a mixture of sand and silt (2:1 v/v) to facilitate root extraction and cleaning. A slow-release fertiliser (OSMOCOTE® Mini 18+6+11) was incorporated into the bulk substrate in a dose of 3.5 g L^{-1}. Forty seedlings were cultivated in each container type according to a completely randomised design and using one seed per container. Seedlings were kept well watered during the growing period and were cultivated in a shade house to tone down light intensity to 50% of the external solar radiation. The study was carried out in the experimental fields of CITa (Zaragoza, Spain) for one vegetative period in 1999.

2.1. Shoot morphology and root morphology and topology

For the morphological characterisation of shoots and roots and the topological characterisation of roots, 22 seedlings were taken randomly per type of container at the end of the vegetative period (mid-November). Plant height and root collar diameter were recorded for each seedling using a Vernier calliper, and total leaf area (A_L) was estimated using an electronic planimeter (Delta-T, Cambridge, England). Measurements were not performed on roots with a diameter smaller than 0.5 mm. The following root variables were obtained using the image analysis software WinRhizo® v.4.1 (Regent Ltd., Canada): projected root area (A_R), total root length (L_R), average root diameter (D_R), projected area relative to total length (A_R / L_R), root volume (V_R), total length of roots with a diameter greater than 0.5 mm (L_R), length by diametric classes (0.5 to 2 mm, L_R0.5<2; 2 to 4.5 mm, L_R2<4.5; > 4.5 mm, L_R2<4.5), and the ratio of the length for each dynamic class to the total length. Dry root weight (DRW) was calculated after drying the roots in an oven at 60 °C for 48 h. L_R and DRW values were used to calculate the specific root length index (L_R/DRW) (hereafter SRL). This index is used as an indicator of root thickness [26] and has been applied to study variations in root morphology in relation to different nutrient levels, water content and soil types [6].

Root topology was assessed by estimating the following parameters using the software WinRhizo® v.4.1: number of exterior root links (or magnitude, μ); number of root links in the longest unique path from the base link to an exterior link (or altitude, a); and sum of links in all possible unique paths from the base link to all exterior links (or total exterior path length, p_e). The following topological
indexes were then calculated: altitude-slope (the regression slope of $\log_{10}a$ on $\log_{10}h$) and pathlength-slope (the regression slope of $\log_{10}p_r$ on $\log_{10}h$). High values of these indexes (with a theoretical maximum equal to one for the altitude-slope index) represent root systems with a herringbone structure, in which branching is largely confined to a main axis. Low values (with a theoretical minimum equal to zero for both indexes) represent a dichotomous pattern, in which all exterior links join another exterior link [6].

2.2. Root hydraulic conductance

At the end of the vegetative period, ten seedlings per tube type were randomly taken. Root hydraulic conductance was estimated using a pressure chamber [5] adapted to the container size. In particular, seedlings were cut at 80 mm above the substrate so that about 20 mm of stem protruded from the pressure chamber [17]. Water flow (F) was then measured on the stem cut-surface at different constant pressures. Firstly, the chamber pressure was increased at a rate of 0.07 MPa min$^{-1}$ up to 0.7 MPa. After the first 10 min at 0.7 MPa, flow was measured ten times (every two minutes) by placing Eppendorf® tubes filled with an absorbent sponge in contact with the stem cut surface. The tubes were then weighed on a digital balance. Afterwards, the pressure was decreased at intervals of 0.175 MPa using a rate of 0.07 MPa min$^{-1}$, and flow measurements were repeated ten times every two minutes at constant pressure levels of 0.525, 0.350 and 0.175 MPa. Water flow was approximately stable at any pressure (coefficient of variation $\leq 7.5\%$) and, therefore, measurements were quasi-steady state. Root hydraulic conductance ($K_R$) was calculated from the slope of the straight line relating water flow (F) to pressure applied (P). In addition, the root hydraulic conductance per leaf unit surface area ($K_{R\ell}$) and the root hydraulic conductance per root unit surface area ($K_{R\ell R}$) were obtained by dividing $K_R$ by $A_{\ell}$ and $A_R$, respectively.

2.3. Statistical analysis

In order to evaluate differences in root and shoot morphology between tube types, data on morphological variables were subjected to analysis of variance (ANOVA) for a completely randomised design. A discriminant canonical analysis was also performed to determine which root variables showing significant differences in the ANOVA were more effective to differentiate between root structures as affected by tube type. An analysis of covariance was used to detect differences between tube types for Log$_{10}$ indexes. In each case, Log$_{10}h$ was used as covariate, with container length being the factor for analysis. Differences on Log$_{10}a$ and Log$_{10}p_r$ were tested by the interaction between the factor and Log$_{10}h$. The degree of correlation between $K_{R\ell R}$ and the root morphological variables was calculated using Pearson’s correlation coefficients. All analyses were performed using standard SAS/STAT procedures [29].

3. RESULTS

There were no significant differences in shoot morphological variables between tube types. As regards root morphology, significant differences were found in $D_R$, $A_R$, $V_R$, and DRW (Tab. I), and in root length parameters (Tab. II). Particularly, $A_R$ and $V_R$ were about 38% and 88% higher, respectively, in LT than in ST, whereas $D_R$ was about 50% higher in ST than in LT and DRW was about 50% higher in LT and ST than in MT. Besides, $L_R$ was about two-fold higher in LT than in ST (Tab. II). A similar trend was observed for $L_{R\ell 0.5<D<2}$. However, seedlings grown in ST showed a greater length of coarse roots ($L_{R\ell D>4.5}$) than in LT and MT, which displayed similar $L_{R\ell D>4.5}$ values. In particular, $L_{R\ell D>4.5}$ was around two-fold higher in ST than in LT (Tab. II). On the other hand, the root length of the intermediate diametric class ($L_{R\ell 2<D<4.5}$) showed no significant variation between tube types. For ST, about 88% of total root length corresponded to $L_{R\ell 0.5<D<2}$, whereas 5% was related to $L_{R\ell D>4.5}$. For LT, values were 94% and 1% for $L_{R\ell 0.5<D<2}$ and $L_{R\ell D>4.5}$, respectively. Significant differences between tube types were also detected for SRL, with LT having around two-fold higher values than ST (Tab. II). There were no significant differences in topological indexes between tube types. Average values for altitude-slope and pathlength-slope were 0.58 and 1.25, respectively.

A discriminant canonical analysis was performed in order to obtain an overall differentiation between tube types given measurements on root morphological variables (Fig. 1). The first two canonical variables contributed to differentiate among tube types, although the grouping of individual seedlings corresponding to each tube type was mainly observed along the first canonical axis, which accounted for 86% of the total between-group variance. According to the eigenvector positions, the most informative variables were $L_{R\ell D>4.5}$ and $V_R$, together with other root characteristics such as $L_R$ and $A_R$. However, $V_R$ and the root length variables provided somewhat redundant information according to their partially overlapping position in the biplot, with overall higher values for all these variables characterising the shorter (ST) tube type. On the other hand, the information given by $A_R$ was less related to that provided by the aforementioned traits.
Table I. Root morphology. Projected area, volume, dry root weight average diameter and DSW/DRW ratio.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Projected area (Ar) (cm²)</th>
<th>Volume (VR) (cm³)</th>
<th>Dry root weight (DRW) (g)</th>
<th>Average diameter (DR) (cm)</th>
<th>DSW/DRW</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>36.8 b</td>
<td>1.1 b</td>
<td>1.2 a</td>
<td>0.20 a</td>
<td>1.4</td>
</tr>
<tr>
<td>MT</td>
<td>37.4 b</td>
<td>1.5 ab</td>
<td>0.8 b</td>
<td>0.15 b</td>
<td>1.1</td>
</tr>
<tr>
<td>LT</td>
<td>50.9 a</td>
<td>2.0 a</td>
<td>1.2 a</td>
<td>0.13 b</td>
<td>0.9</td>
</tr>
<tr>
<td>SE</td>
<td>3.87</td>
<td>0.16</td>
<td>0.09</td>
<td>0.006</td>
<td>0.19</td>
</tr>
</tbody>
</table>

SE: mean standard error. Different letters denote significant differences (p < 0.05), Tukey Test.

Table II. Root length. Total length of roots with diameter greater than 0.5 mm (Lr), length of diametric class (0.5 < d ≤ 2 mm) (LR0.5<d≤2), length of diametric class (2 < d ≤ 4.5 mm) (LR2<d≤4.5), length of diametric class (d > 4.5 mm) (LRd>4.5), specific root length (SRL), LR0.5<d≤2/LR and LRd>4.5/LR ratio.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Lr (cm)</th>
<th>LR0.5&lt;d≤2 (cm)</th>
<th>LR2&lt;d≤4.5 (cm)</th>
<th>LRd&gt;4.5 (cm)</th>
<th>SRL (m g⁻¹)</th>
<th>LR0.5&lt;d≤2/LR</th>
<th>LRd&gt;4.5/LR</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>191.5 b</td>
<td>169.1 b</td>
<td>13.4</td>
<td>9.0 a</td>
<td>1.92 b</td>
<td>0.86 b</td>
<td>0.05 a</td>
</tr>
<tr>
<td>MT</td>
<td>252.5 b</td>
<td>229.8 b</td>
<td>18.3</td>
<td>4.3 b</td>
<td>2.85 ab</td>
<td>0.90 a</td>
<td>0.02 b</td>
</tr>
<tr>
<td>LT</td>
<td>388.7 a</td>
<td>365.2 a</td>
<td>19.2</td>
<td>4.3 b</td>
<td>3.47 a</td>
<td>0.93 a</td>
<td>0.01 b</td>
</tr>
<tr>
<td>SE</td>
<td>28.10</td>
<td>26.73</td>
<td>2.07</td>
<td>0.69</td>
<td>0.274</td>
<td>0.011</td>
<td>0.004</td>
</tr>
</tbody>
</table>

SE: mean standard error. Different letters denote significant differences (p < 0.05), Tukey Test.

Figure 2. Water flow through the root system for different pressures applied in roots from different container types: 20 cm depth (circle), 60 cm depth (triangle) and 100 cm depth (square) (n = 10).

The relationship between flow measured (F) and pressure applied (P) (Fig. 2) was linear for each pressure interval between 0.17 and 0.7 MPa (R² = 0.99), irrespective of container type. The slope of the linear regression of F to P was significantly smaller in ST than in LT and MT, indicating that lower flows were obtained in ST for a particular pressure value. KRL and KRR (Fig. 3) had significantly lower values in ST as compared with LT and MT. In particular, KRL was about three-fold higher in LT than in ST (3.19 × 10⁻⁶ versus 1.18 × 10⁻⁶ kg s⁻¹ m⁻² MPa⁻¹). Differences in KRL between LT and MT were not significant. Overall, KRR and KRL took similar values for a particular tube type due to the similarity between leaf surface and root surface areas. The correlation analysis showed negative association between KRL and D_R (r = -0.55; P < 0.05), and positive relationships between KRL and SRL (r = 0.69; P < 0.001), VR (r = 0.47; P < 0.05), LR0.5<d≤2 (r = 0.49; P < 0.05) and LR (r = 0.47; P < 0.05).

4. DISCUSSION

Although variation in tube length among container types was considerable, it did not modify significantly shoot morphology of one-year holm oak seedlings. This observation can be attributed to the comparable total volume of all tube types.
employed, since seedling size has been directly related to container volume in many studies [2, 21, 28]. On the contrary, our results indicate the development of different root morphologies owing to the influence of tube length. Overall, the deepest tube showed the highest values of root length parameters, except for \( L_R = 4.5 \). In this case, higher \( L_R = 4.5 \) and \( D_R \) values for ST than for LT, probably caused by a more intense root pruning in the former, are consistent with results described by Riedacker and Belgrand for Quercus robur [25]. Those authors found that lateral roots became thicker when vertical downward growth of taproots was physically restrained. Since SRL is often used either as an overall index of root thickness or as an estimator of the benefit (length) to cost (dry weight) ratio of the root system [26], the root pattern generated in LT containers could be considered as more efficient than that obtained in ST. The discriminant canonical analysis on morphological variables, on the other hand, succeeded in differentiating among groups of root morphologies belonging to each tube type. In this regard, the root system generated by ST could be distinguished from that of LT mainly through the gradient of the first canonical function, in which the variables \( L_R = 4.5 \) and \( A_R \) provided rather complementary information.

Notably, the average value across tube types (0.58) obtained for the slope of the regression line between the parameters altitude (\( \hat{a} \)) and magnitude (\( \hat{\mu} \)) shows that the different root systems can be classified as being of the herringbone-type, the most efficient structure for exploring and exploiting soil resources [7]. On the other hand, the impossibility of differentiating between root systems produced by different containers, according to the topological models of Fitter [6], is noteworthy. In particular, the root system obtained in ST could not be ascribed to a dichotomous model, as initially expected. This finding suggests that the root system produced by a standard container would not bear any of the associated structural advantages of such a root model (e.g., lower cost and greater efficiency in water conduction as compared to the herringbone model) [7, 8].

The relation between water flow and pressure applied was linear, irrespective of tube type, as also reported by other authors both for the holm oak and other species [17–19, 26]. For ST, \( K_{RL} \) values were extremely low; on the contrary, \( K_{RL} \) values for LT were high, in agreement with those reported in other studies for this species [16, 17, 19]. Such differences between tube types suggest a greater efficiency in water uptake from roots to leaves in LT and MT than in ST. Seasonal \( K_{RL} \) changes reported by Nardini [17] for holm oak indicates that this species presents a maximum efficiency in water uptake during the spring, when the soil is still wet. Therefore, low \( K_{RL} \) seedlings, such as those produced in standard containers, may have their establishment compromised shortly after planting under the harsh summer conditions typical of semiarid areas.

The strong positive correlation between \( K_{RL} \) and SRL indicates that the root systems characterised by less massive roots per unit length have a higher hydraulic conductance [20, 26, 31]. Thus, our results would be in agreement with the view that hydraulic architecture follows the ‘energy minimization’ principle introduced by the West et al. model (WBE) [14, 35], since the root system developed in LT shows the lowest root hydraulic resistance for a given investment (DRW).

According to the composite transport model [32], the radial resistance to water flow was higher in the ST root system as compared to the LT system, probably due to a higher suberisation rate [26, 32, 33]. However, further research would be needed to confirm this point. In fact, a low permeability of coarser roots, together with a limited root-to-shoot ratio, is one of the main causes of transplanting stress, which may affect seedling establishment in field conditions [30].

In summary, our results reveal that there are morphological and functional differences among root systems developed in containers of different length. Particularly, root systems developed in larger tube types were more efficient in water uptake. This outcome suggests that the modification of root growth pattern brought about by commercial forest containers may influence holm oak establishment in the field. Therefore, the use of direct seeding, which allows for a non-restricted development of the root system, may be the recommended choice in afforestation programmes for the holm oak, particularly in xeric environments where the incidence of recurrent drought episodes compromises growth and survival.

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