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Can the use of large, alternative nursery containers aid in field establishment of *Juglans regia* and *Quercus robur* seedlings?

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Abstract Over the last 30 years in Italy, a high percentage of tree plantations have failed to achieve the objective of enhancing the quality of forest products, and also restoring/rehabilitating abandoned or degraded agricultural lands. In this study, we evaluated the effects of nursery cultivation in large, novel containers and duration of cultivation on early field establishment of 2-year-old seedlings of *Quercus robur* L. and *Juglans regia* L. Two sizes (9800 and 15,500 cm^3) of a new container (Superoots Air-pot®) and one size (4900 cm^3) of a traditional container (Plastecnic®) were tested and seedlings were sampled for shoot and root growth and biomass allocation. Prolonging the cultivation period to 2 years had a positive effect on both species, with a marked increase in above- and below-ground biomass, maintaining a desirable balance between shoot and root systems. Both sizes of Air-pots for *Q. robur* and the bigger Air-pot for *J. regia* produced seedlings that were taller than 1.5 m, with a low branch component combined with a high frequency of apical dominance. The quantity and size of first order lateral roots varied between years within containers, and increased in deeper substrate layers during the second year. Early field results did not show marked signs of transplanting stress, but with low height growth in all treatments. Seedlings grown in both Air-pots exhibited well-developed and well-structured root and shoot systems, thus showing promise in the establishment of high quality timber plantations of fine hardwoods; such attributes can be beneficial wherever

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proper seedling shoot and/or root sizes confer an advantage in reforestation and restoration scenarios.

Keywords Persian walnut · Pedunculate oak · Nursery stock quality · Shoot-to-root ratio · First order lateral roots · Field performance

Introduction

In Central and Southern European countries, plantations for producing commercial timber and for other agroforestry systems have steadily increased during the recent decades (Eichhorn et al. 2006) and have been established in ex-cultivated lands due to the financial incentives available through the EU Regulation. Such plantings have also important concurrent functions in restoring over-exploited and poor or marginal lands due to the reintroduction of forest ecosystems functions and services, such as: increasing biodiversity, carbon sequestration and enhancing landscape aesthetics (Eichhorn et al. 2006). In Italy, tree farming and agroforestry systems, including fine hardwoods oriented to high-quality products (veneer), have been most actively promoted (Colletti 2001).

Over the years, practices designed to improve productivity of fine hardwoods plantations have evolved. The rotation period has been shortened and pruning methods refined to provide high quality products (Balandier and Dupraz 1999; Bohanek and Groninger 2003; Ravagni and Buresti Lattes 2006). At the same time, establishment of plantations at relatively lower densities have been encouraged (Balandier and Dupraz 1999; Ravagni and Buresti Lattes 2006; Buresti Lattes et al. 2014). Nevertheless, in Italy, a high percentage of plantations designed over the last decades have failed to achieve the objective of enhancing forest resources of high quality (Cappelli et al. 2009), as well as restoring/rehabilitating abandoned or degraded agricultural lands, with the aim of providing a greater ecological balance in countryside management, and mitigating greenhouse effects (Stavi and Lal 2013). One of the reasons has been the lack of high-quality seedlings (Tani et al. 2007a; Maltoni et al. 2010), which has been often linked to unsuccessful hardwood afforestation and reforestation plantings (Jacobs et al. 2005; Salifu and Jacobs 2006). Preliminary plantation surveys (Tomat et al. 2005; Cappelli et al. 2009) have highlighted two critical phases: (1) delayed plantation establishment, implying a longer rotation, and (2) the lack of formative pruning from an early age, which is detrimental for tree form and value, downgrading the quality of the boles (required for veneer). In this framework, whatever the stock type, qualified seedlings should be tall and with suitable architectural features of the shoot (i.e., straightness and apical dominance) and root systems, such that the seedlings have a good probability of establishing and competing successfully in the field (Armand 1992; Drenou 2000; Fennessy and MacLennan 2003; Maltoni et al. 2010).

Nursery stocktypes (e.g., distinguished by bareroot/container systems and stocktype variations therein) have a large effect on resulting seedling quality (Wilson and Jacobs 2006; Pinto et al. 2011b). Containers currently used to grow seedlings for forest plantings have a wide range of shape and size (Landis 2009; Pinto et al. 2011a). Containers for hardwood seedlings generally have a volume ranging from 250 to 450 cm^3 (Landis et al. 1990; Chirino et al. 2008), occasionally higher (to 800 cm^3), and rarely deeper than 18–20 cm (Chirino et al. 2008; Morrissey et al. 2010). Usually, containers with larger volume or higher depth are used to prolong the cultivation time beyond 1 year (Howell and

Harrington 2004; Chirino et al. 2008; Morrissey et al. 2010). In Italy, hardwoods are mostly container grown, using a range of types and sizes, generally from 400 to 1200 cm^3 and the cultivation currently lasts 1 year.

Many studies have highlighted the need to examine the effect of container type and size on seedling structure, survival, and growth (NeSmith and Duval 1998; Pinto et al. 2011a). Containers influence growth and biomass allocation (Tsakaldimi et al. 2005; Gilman et al. 2010; Dumroese et al. 2011), root system development and architecture (Oddiraju et al. 1994; Heiskanen and Rikala 1998; Chirino et al. 2008). Therefore, container type can influence success and cost of planting programs (NeSmith and Duval 1998; Pinto et al. 2011a). According to the Target Plant Concept (Rose et al. 1990; Landis 2011), container type is an important factor in determining seedling quality (Ritchie and Landis 2010; Pinto et al. 2011b) and to refine quality assessments (Aphalo and Rikkala 2003; Wilson and Jacobs 2006; Pinto et al. 2011a). Studies have often shown positive effects of larger containers on seedling growth (Aldhous and Mason 1994; Ritchie and Landis 2010; Poorter et al. 2012). However, past research has generally emphasized conifer species under reforestation scenarios or use of hardwoods to restore degraded lands in arid or harsh conditions, while studies examining container effects on development of fine hardwoods meant for plantings designed to produce timber yielding high quality forest products (e.g., veneer) and/or for forest restoration are lacking (Wilson and Jacobs 2006; Maltoni et al. 2010).

The objective of this study was, therefore, to assess the influence of new and large containers, in combination with a cultivation period of 2 years, on seedling quality and early field development of *Quercus robur* L. and *Juglans regia* L. We hypothesized that the quality of seedlings propagated with new container types and after a longer cultivation period than usual (1 year) would favor successful early phases of plantation. The assessment included the analysis of root system morphology and shoot system characteristics that affect subsequent pruning operations and bole quality. Mariotti et al. (2015) compared the effects of a wide range of different sizes and shapes of forest nursery containers on quality indices of 1-year-old seedlings to be used in productive plantations of fine hardwoods. We took advantage of these results, evaluating two large sizes of the new container type (Superoots Air-pot®) in comparison with a traditional large container (Plastecnic®).

Materials and methods

Nursery stock characterization

The studied species were *Quercus robur* (pedunculate oak) and *Juglans regia* (Persian walnut), which are used in multipurpose plantations for wood production in Europe (Ducousso and Bordacs 2004; Voulgaridis and Vassiliou 2005; Mohni et al. 2009; Bolte and Löf 2010). The stocktype for both the nursery and field trials included in this study were produced in the forest nursery "Centro Biodiversità Vegetale e Fuori Foresta" located in Montecchio Precalcino (45°39′20″N; 11°32′40″E) and managed by Veneto Agricoltura Regional Administration (Northeastern Italy). The site characteristics were described in Mariotti et al. (2015). Three container types were studied: two big sizes of the new container Superoots Air-pot® (AIR-3: 9800 cm^3, 40 cm deep and 19 cm wide; and AIR-4: 15,500 cm^3, 60 cm deep and 19 cm wide) and one big size of traditional container

Plastecnic® (PL-2: 4900 cm^3, 20 cm deep and 19 cm wide at top section). The Superoots Air-pot is a cylindrical container with a new air-pruning system that consists of perforated cones along its sidewalls and a grid at the bottom (Mariotti et al. 2015). There are no flat surfaces to deflect roots and promote the development of fine roots while also inhibiting root circling and spiraling (Amoroso et al. 2010; Gilman et al. 2010). In Italy, this container is frequently used to grow ornamental plants. The Plastecnic is a frustum of pyramid shape; the bottom is tapered with regularly distributed circular holes to minimize root deformation.

Seeds were obtained from Veja (Veneto, Italy) for *Juglans regia* and Palù di Moriago (Veneto, Italy) for *Quercus robur*. Seeds were sown at the end of winter 2012 in seedling trays into a substrate described in Mariotti et al. (2015). All containers were watered to field capacity at sowing. In both years, the containers were irrigated daily with 20 mm of water, from the sowing date to the end of June (from the end of March, the 2nd year), 40 mm from end of June to the beginning of September, and 20 mm from September to mid-October. During both years, substrate moisture was checked at different container depths (using Soil moisture meter PCE-SMM1) to ensure that seedlings were well watered. A total of 272 seedlings were grown, of which 162 were used in the nursery trial and 110 in the field trial.

Nursery trial

The nursery study included 27 seedlings, per species and container type, in a completely randomized design within the species. Twelve seedlings per each species-container combination were randomly sampled at the end of the first year (2012), and the remaining 15 at the end of the second year (2013), for morphological assessment. During both growing seasons (from March to leaf abscission in 2012, and from time of bud burst to leaf abscission in 2013), stem height was measured monthly on each plant. Year-end morphological assessment included the following: root collar diameter (RcD), first year and second year height (H), height/root collar diameter ratio (H/RcD), number of growing flushes (only for *Q. robur*), number of internodes (only for *J. regia*), and dry weight of stem and branches. The root system was divided into main root and first order lateral roots (FOLR), which were grouped according to the diameter at the junction with main root (<0.1 cm, 0.1–0.3 cm, and >0.3 cm). Moreover, FOLR were separated according to three 20-cm depth layers (from root collar to 20 cm, 20–40 cm, and >40 cm). The dry mass of the each below-ground portion was recorded; FOLR > 0.3 in biomass was calculated by adding the first order main root weight and its second and higher order roots. The number of FOLR > 0.3 and FOLR 0.1–0.3 was also counted (at each layer). The ratios between each measured portion of seedling biomass to the total biomass and between the root system biomass and the container volume were calculated.

Field trial

The field trial included a sample of 80 seedlings for *J. regia* and of 30 seedlings for *Q. robur*. For *J. regia*, the experimental design was a randomized incomplete block design due to lower number of AIR-3 seedlings. It consisted of 8 blocks with 4 plants x treatment x plot: 4 blocks were complete with the three stocktypes (AIR-3, AIR-4, PL-2) and 4 blocks where the AIR-3 treatment was missing. Thus, this experiment included 16 AIR-3, 32 AIR-4, and 32 PL-2 seedlings. A total of 30 seedlings of *Q. robur* (10 per each stocktype AIR-3, AIR-4, PL-2) were included in a completely randomized block design.

The lower number of oak seedlings and the unbalanced experimental design for walnut were due to varying availability of seeds and to some seedlings mortality. Thus, the field experiment was planned differently for the two species, according to the number of available plants.

At the beginning of April 2014 seedlings were transplanted at a site managed by the Research Centre CRA-PLF (45°08′53″N, 8°30′55″E) located in the Po Valley of North-western Italy. The area was fenced to protect plants from wildlife. The mean annual temperature is 12.3 °C (1970–2005); the coldest month is January (average 3.1 °C), while the warmest is July (average 25.5 °C). The average annual precipitation is 772.2 mm and its monthly distribution follows a regime with minimum rainfall in June (average 7.0 mm), a maximum in May (average 154.4 mm), and with a secondary peak in October (average 71.2 mm). Dry periods typically occur from mid-June to the end of July. The lithological substrate is recent and results from gravelly-sandy or silty Holocene floods (*Alluvium medium*), (Servizio Geologico d'Italia 1969). Soil is loamy sand in the surface layers and sandy loam more in depth, and limestone is absent. The site phyto-sociological association is *Querco (roboris)—Ulmetum minoris* (Mondino 2007).

Stem height increments and physiological attributes (Chlorophyll *a* fluorescence and Chlorophyll Content Index) were measured on four different dates during the growing season (9 June, 27 June, 22 July, 2 September). Chlorophyll *a* fluorescence properties were assessed on the first two fully expanded leaves from the apex of all plants included in the field trial. For each date, sampling was conducted between 12:00–14:00 h on sunny days. Measurements were taken on each of two leaves per plant exposed to dark (30 min), using leaf clips placed on the middle part of abaxial leaf blades. Chlorophyll *a* fluorescence was induced by red actinic light (with energy of 1500 μmol m^{-2} s^{-1}), and the first 3 s of transient fluorescence were registered with time intervals increasing from 10 μs within the first 300 μs of the measurement up to 100 ms intervals for times longer than 0.3 s; these data were analyzed and the so-called JIP-test was conducted using Biolyzer v.3.0.6 software (both developed in the Laboratory of Bioenergetics, University of Geneva, Switzerland) (Strasser et al. 2000). The fluorimeter used was a Plant Efficiency Analyser (Hansatech Instruments Ltd., King's Lynn, UK). Measured parameters were: F_o, chlorophyll fluorescence intensity measured when all PSII reaction centers are assumed to be open (minimal fluorescence, $\approx F_{50}$ μs)—the measured value may be affected by several other parameters (at $t = 0$); Fm, maximal chlorophyll fluorescence intensity measured when all photosystem II (PSII) reaction centers are closed (=Fp); T_{FM}, time needed to reach F_m; Area, the area above the chlorophyll fluorescence curve between F_o and F_m (reflecting the size of the platoquinone pool). Calculated parameters were: F_v, variable chlorophyll fluorescence ($F_m - F_o$); F_v/F_m, a value that is related to the maximum quantum yield of PSII; RC/ABS, density of reaction centres per PSII antenna chlorophyll; F_v/F_o, a value that is proportional to the activity of the water-splitting complex on the donor side of the PSII; $(1 - V_j)/V_j$, measure of forward electron transport, where V_j is the relative variable fluorescence at time *J* (relative variable fluorescence at phase *J* of the fluorescence induction curve); PI_{ABS}, the performance index that is calculated as (RC/ABS) × $(\Pi_{Po}/(1 - \Pi_{po}))$ × $(\Psi_o/(1 - \Psi_o))$, where, RC is for reaction centre, ABS is for absorption flux, Π_{po} is for maximal quantum yield for primary photochemistry, and Ψ_o is for the quantum yield for electron transport, $\Pi_{Po}/(1 - \Pi_{po})$ is a 'conformation' term for primary photochemistry, and $\Psi_o/(1 - \Psi_o)$ is a 'conformation' term for thermal reactions (non-light dependent reactions).

The values of parameters characterizing PSII functioning were shown in a "spider plot", which enables to easily identify deviation (in positive or negative) from the typical shape. If fluorescence parameters overcome the threshold for undamaged PSII, this indicates that significant and permanent damage to the photosynthetic system has probably occurred (e.g., Ugolini et al. 2014).

Chlorophyll Content Index (CCI) was measured by CCM-200 Plus (Opti-Sciences Inc., Hudson, NH, USA) on the same leaves.

At the end of September, the plants were accurately excavated and stem height, root collar diameter, presence of apical dominance, and stem and branches dry weight, for the

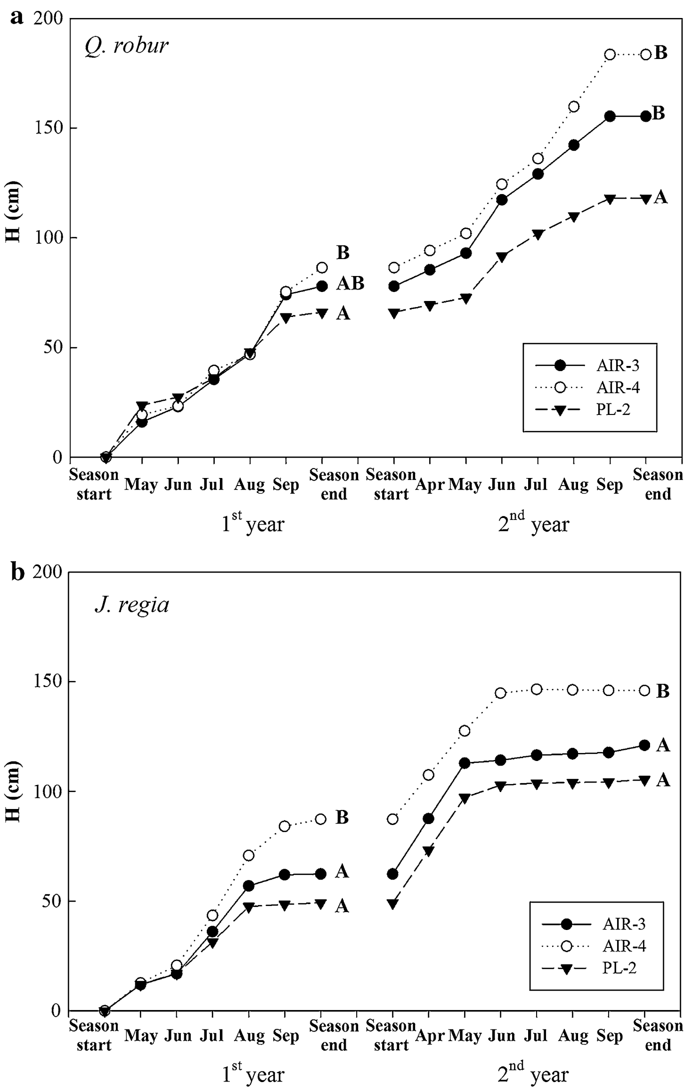


Fig. 1 Height (H) recorded during the two nursery growing seasons for *Q. robur* (**a**) and *J. regia* (**b**). Duncan post hoc test for height at the end of nursery cultivation is shown (PL-2: Plastecnic 4900 cm^3; AIR-3: Air-pot 9800 cm^3; AIR-4: Air-pot 15,500 cm^3)

shoot system, and root depth, width, and dry mass separating main roots from FOLR, for the root system, were measured.

Statistical analysis

In the nursery trial the analysis of variance (ANOVA) was performed to determine differences among stocktypes, for each species separately, in shoot growth and in shoot and root biomass of the seedlings, considering treatments as source of variation (containers:

Table 1 ANOVA test results (p values) of above- and below-ground system main variables among containers for both species, at the end of the 1st and 2nd year in the nursery (in bold $p \leq 0.05$)

ABOVE-GROUND								
	Height		Height increment		RcD		H/Rcd	
	1st	2nd	1st	2nd	1st	2st	1nd	2nd
Q. robur	**0.0432**	**0.0005**	–	**0.0012**	0.4200	**0.0350**	0.1251	**0.0072**
J. regia	**0.0020**	**0.0051**	–	0.9785	0.1496	0.1902	**0.0010**	**0.0015**
	Total biomass		Shoot system		Stem		Branches	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Q. robur	0.8232	**0.0042**	0.4714	**0.0046**	0.3638	**0.0063**	0.9572	**0.0062**
J. regia	0.9933	**0.0033**	0.2401	**0.0018**	0.2304	**0.0035**	0.2198	**0.0462**
	Shoot/total		Stem/total		Branches/total		Shoot/root system	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Q. robur	0.3773	0.2643	0.8206	0.4337	0.5597	0.4339	0.3358	0.1088
J. regia	**0.0025**	0.2110	**0.0016**	0.3270	0.1446	0.2740	**0.0143**	0.1664
BELOW-GROUND								
	Root system		Main roots		FOLR > 0.3		FORL 0.1–0.3	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Q. robur	0.1164	**0.0067**	0.0755	**0.0010**	0.5447	0.4128	0.5887	0.2932
J. regia	0.7815	**0.0076**	0.8631	**0.0039**	**0.0293**	0.9663	**0.0217**	**0.0100**
	FORL < 0.1		Root system/total		Main roots/total		FOLR > 0.3/total	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Q. robur	0.9677	0.3079	0.3773	0.2643	0.2572	0.1862	0.9168	0.8076
J. regia	**0.0015**	0.4340	**0.0025**	0.2110	**0.0489**	0.5329	**0.0026**	0.2923
	FORL 0.1-0.3/total		FORL < 0.1/total		Root sytem/container volume			
	1st	2nd	1st	2nd	1st	2nd		
Q. robur	0.1361	0.1879	0.1820	0.0104	**0.0029**	0.4126		
J. regia	**0.0003**	0.1280	**0.0161**	0.5488	0.7815	**0.0009**		

AIR-4, AIR-3, PL-2). In the field trial, for *Q. robur*, ANOVA considered treatments in nursery cultivation (containers) as a source of variation; whereas, for *J. regia*, the sources of variation were blocks and treatments (containers). In case of significant results ($p \leq 0.05$), Duncan's post hoc test was used for multiple comparisons ($\alpha = 0.05$). Percentage data were transformed using arc sine square root transformation to meet ANOVA assumptions, according to distribution of residuals. StatSoft Statistica 9 (Tulsa, Oklahoma, USA) was used to process all data.

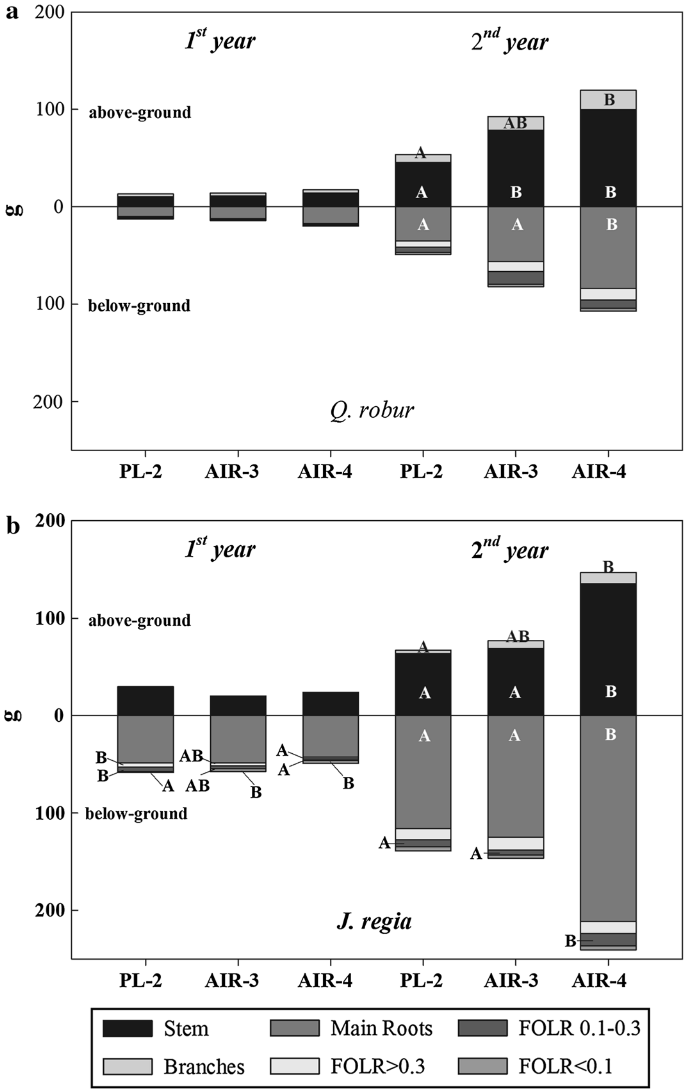


Fig. 2 Seedling above and below-ground biomass at the end of the 1st and 2nd year of nursery cultivation for *Q. robur* (**a**) and *J. regia* (**b**). Duncan post hoc test is shown. Percentages on 2nd year bars show the total increase in biomass (PL-2: Plastecnic 4900 cm^3; AIR-3: Air-pot 9800 cm^3; AIR-4: Air-pot 15,500 cm^3 FOLR: first order lateral roots)

Results

Nursery trial

For *Q. robur*, after 2 years of nursery cultivation, mean seedling height in both Air-pots exceeded 150 cm (Fig. 1a), and was significantly higher than in PL-2; greater increments at the end of the 2nd year were also observed in these containers (AIR-4 100.3 cm; AIR-3

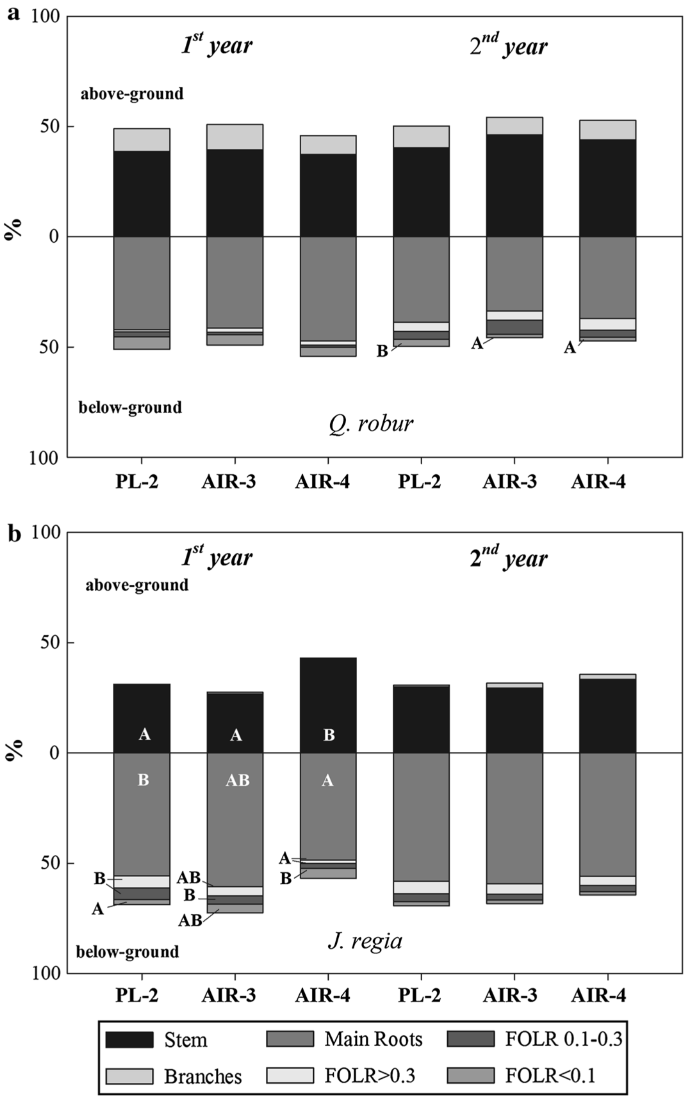


Fig. 3 Seedling above and below-ground biomass proportions relative to total plant biomass (%) at the end of the 1st and 2nd year of nursery cultivation for *Q. robur* (**a**) and *J. regia* (**b**). Duncan post hoc test is shown (PL-2: Plastecnic 4900 cm^3; AIR-3: Air-pot 9800 cm^3; AIR-4: Air-pot 15,500 cm^3; FOLR: first order lateral roots)

78.9 cm; Table 1). Differences in seedling growth among stocktypes were evident from August of the 1st year, while during the 2nd growing season the gap began to increase from May onwards (Fig. 1a). Seedlings grown in Air-pots achieved also greater RcD and H/RcD (AIR-3, 2.1 cm and 82.8, and AIR4, 2.2 cm and 81.7, respectively) than PL-2 (1.7 cm and 68.6, respectively). In *J. regia*, only AIR-4 resulted in significantly higher seedlings that were taller than 150 cm at the end of the 2nd growing season (Table 1; Fig. 1b) and they had lower H/RcD values (AIR-4, 62.6 > AIR-3, 52.5, and PL-2, 43.2). RcD was not affected by stocktype. Container type influenced walnut seedlings growth beginning in July of the 1st year; however, in the 2nd year, growth rate dramatically slowed since June, similarly in all treatments (Fig. 1b; Table 1).

After the 1st year, the container type did not affect oak biomass, while differences occurred in FOLR for walnut (Table 1; Fig. 2b). In the 2nd year, biomass sharply increased in all above- and below-ground parts, in both species (Fig. 2 a, b). After two growing seasons (Table 1; Fig. 2a), *Q. robur* seedlings in AIR-4 had higher biomass than those in PL-2 in all variables excluding all FOLR; in *J. regia*, seedlings in AIR-4 had more stem, main root, and FOLR 0.1–0.3 biomass than the other two treatments.

The container type did not affect plant biomass ratios in *Q. robur* except for the fine FOLR fraction in the 2nd year (Table 1; Fig. 3a). Oak seedlings, in general, had shoot/root ratio close to one in both years. Within-container comparison of biomass fractions between years showed notable differences in biomass allocation in both Air-pots (Table 2). In *J. regia*, container type influenced biomass ratios in the 1st year (Table 1; Fig. 3b); the seedlings grown in bigger Air-pot showed more changes in biomass fractions between years (Tables 2, 3). The shoot/root ratio in walnut was significantly higher in AIR-4 (0.63)

Table 2 ANOVA test results of the comparisons of biomass fractions between the 1st and 2nd year seedlings within the containers (in bold $p \leq 0.05$)

	PL-2		AIR-3		AIR-4	
Q. robur						
Stem/total	0.5912		**0.0418**	2nd > 1st	**0.0138**	2nd > 1st
Branches/total	0.7595		0.1278		0.9624	
Main roots/total	0.3324		**0.0378**	1st > 2nd	**0.0206**	1st > 2nd
FOLR > 0.3/total	**0.0210**	2nd > 1st	**0.0204**	2nd > 1st	**0.0168**	2nd > 1st
FOLR 0.1–0.3/total	0.3858		**0.0019**	2nd > 1st	**0.0008**	2nd > 1st
FOLR < 0.1/total	**0.0032**	1st > 2nd	**0.0001**	1st > 2nd	**0.0001**	1st > 2nd
Root system/container volume	**0.0098**	2nd > 1st	**0.0001**	2nd > 1st	**0.0000**	2nd > 1st
J. regia						
Stem/total	0.6683		0.6161		**0.0260**	1st > 2nd
Branches/total	**0.0022**	2nd > 1st	0.0761		**0.0006**	2nd > 1st
Main roots/total	0.4886		0.8266		0.1011	
FOLR > 0.3/total	0.8641		0.9855		**0.0167**	2nd > 1st
FOLR 0.1–0.3/total	**0.0033**	1st > 2nd	0.0522		0.5058	
FOLR < 0.1/total	0.7139		**0.0002**	1st > 2nd	**0.0001**	1st > 2nd
Root system/container volume	**0.0000**	2nd > 1st	**0.0025**	2nd > 1st	**0.0002**	2nd > 1st

PL-2: Plastecnic 4900 cm^3; AIR-3: Air-pot 9800 cm^3; AIR-4: Air-pot 15,500 cm^3

Table 3 Mean value and SD of for the variables after one year in the field for both species

ABOVE-GROUND						
	H increment (cm)	RcD (cm)	Shoot system (g)	Stem (g)	Branches (g)	Total biomass (g)
Q. robur						
PL-2	12.8 ± 7.9 A	1.5 ± 0.8 A	75.7 ± 92.2 A	59.9 ± 72.6 A	15.8 ± 18.0 A	130.2 ± 155.9 A
AIR-3	26.0 ± 11.7 B	2.4 B	190.7 ± 89.0 B	145.8 ± 72.7 B	45.0 ± 28.1 B	340.4 ± 137.7 B
AIR-4	21.1 ± 10.7 AB	2.4 B	185.2 ± 127.5 B	151.0 ± 77.8 B	34.3 ± 36.1 AB	324.4 ± 222.5 B
p treatments	**0.0011**	**0.0005**	**0.0425**	**0.0456**	**0.0468**	**0.0280**
J. regia						
PL-2	16.0 ± 10.2	3.1 ± 0.5	146.3 ± 57.1 A	126.8 ± 50.8	15.3 ± 19.8	376.5 ± 151.7 A
AIR-3	17.7 ± 11.1	3.1 ± 0.8	176.9 ± 92.6 B	157.7 ± 87.3	18.8 ± 23.7	504.9 ± 404.4 AB
AIR-4	11.2 ± 8.9	3.2 ± 0.6	197.1 ± 89.6 B	147.9 ± 84.6	15.1 ± 16.2	568.8 ± 249.5 B
p treatments	0.1790	0.5145	**0.0158**	0.3943	0.9813	**0.0471**
p blocks	0.1965	**0.0176**	0.2322	0.0771	0.1346	**0.0220**
BELOW-GROUND						
	Width (cm)	Depth (cm)	Root System (g)	Main roots (g)	FOLR (g)	Shoot/root system
Q. robur						
PL-2	29.5 ± 13.3 A	40.9 ± 12.2 A	54.4 ± 64.2 A	31.7 ± 35.0 A	22.7 ± 29.2	1.3 ± 0.8
AIR-3	42.6 ± 17.4 B	55.9 ± 12.1 B	149.6 ± 55.8 B	89.4 ± 32.3 B	60.2 ± 27.7	1.4 ± 0.3
AIR-4	45.7 ± 18.1 B	65.4 ± 11.8 C	139.1 ± 99.4 B	87.7 ± 46.9 B	51.4 ± 57.4	1.6 ± 0.8
p treatments	**0.0079**	**0.0000**	**0.0217**	**0.0046**	0.1287	0.5023
J. regia						
PL-2	58.0 ± 13.8	47.5 ± 8.3 A	230.2 ± 102.5 A	143.0 ± 69.7 A	87.2 ± 44.3	0.61 ± 0.2
AIR-3	59.9 ± 12.1	62.6 ± 10.8 B	390.5 ± 333.6 B	272.6 ± 325.7 B	99.1 ± 42.6	0.58 ± 0.2

Table 3 continued

BELOW-GROUND						
	Width (cm)	Depth (cm)	Root System (g)	Main roots (g)	FOLR (g)	Shoot/root system
AIR-4	60.2 ± 11.9	72.5 ± 13.8 C	353.0 ± 205.4 AB	254.2 ± 19.2 B	73.8 ± 45.2	0.66 ± 0.2
p treatments	0.4757	**0.0000**	**0.0471**	**0.0429**	0.5589	0.4867
p blocks	0.4411	0.8498	0.2323	0.1498	**0.0101**	0.2581

Results of ANOVA (in bold $p \leq 0.05$) among stocktypes and post-hoc test (homogeneous groups in capital letters). PL-2: Plastecnic 4900 cm^3; AIR-3: Air-pot 9800 cm^3; AIR-4: Air-pot 15,500 cm^3

Table 4 Mean values and SD (g) of different sizes of FOLR biomass grouped according to container depth sections for both species, at the end of the 1st and 2nd year in the nursery (FOLR > 0.3—only first order root; secondary—including further orders roots—on FOLR > 0.3; FOLR0.1-0.3; FOLR < 0.1)

	Container depth sections	FOLR > 0.3	Sec on FOLR > 0.3	FOLR 0.1–0.3	FOLR < 0.1
PL-2	1st year				
Q. robur	0–20	0.28 ± 0.52	0.09 ± 0.23	0.68 ± 0.67	1.32 ± 0.40
	20–40	–	–	–	–
	40–60	–	–	–	–
J. regia	0–20	1.91 ± 1.54	2.47 ± 1.90	4.29 ± 2.75	1.11 ± 0.66
	20–40	–	–	–	–
	40–60	–	–	–	–
	2nd year				
Q. robur	0–20	4.25 ± 5.03	2.37 ± 3.07	5.24 ± 5.76	2.04 ± 1.78
	20–40	–	–	–	–
	40–60	–	–	–	–
J. regia	0–20	5.47 ± 3.04	6.24 ± 3.51	7.17 ± 3.91	4.21 ± 3.22
	20–40	–	–	–	–
	40–60	–	–	–	–
AIR-3	1st year				
Q. robur	0–20	0.34 ± 0.83	0.04 ± 0.09	0.29 ± 0.60	0.61 ± 0.36
	20–40	0	0	0.16 ± 0.22	0.67 ± 0.62
	40–60	–	–	–	–
J. regia	0–20	1.46 ± 1.21	1.57 ± 1.11	1.79 ± 1.10	1.59 ± 0.72
	20–40	0.02 ± 0.08	0.01 ± 0.04	1.05 ± 1.00	1.14 ± 0.67
	40–60	–	–	–	–
	2nd year				
Q. robur	0–20	4.43 ± 6.06	3.52 ± 5.57	5.32 ± 3.41	1.01 ± 0.57
	20–40	1.10 ± 1.19	0.85 ± 1.29	2.53 ± 1.83	1.31 ± 1.13
	40–60	–	–	–	–
J. regia	0–20	6.60 ± 9.06	5.37 ± 7.86	3.05 ± 2.05	1.71 ± 1.04
	20–40	0.49 ± 0.76	0.35 ± 0.58	2.27 ± 1.88	1.47 ± 1.76
	40–60	–	–	–	–
AIR-4	1st year				
Q. robur	0–20	0.60 ± 1.06	0.15 ± 0.32	0.27 ± 0.32	0.52 ± 0.27
	20–40	0.02 ± 0.08	0.01 ± 0.03	0.19 ± 0.33	0.51 ± 0.38
	40–60	0	0	0.03 ± 0.12	0.33 ± 0.27
J. regia	0–20	0.90 ± 1.33	0.59 ± 0.69	1.05 ± 0.63	1.37 ± 0.91
	20–40	0	0	0.46 ± 0.37	1.06 ± 0.57
	40–60	0.01 ± 0.02	0	0.40 ± 0.42	0.42 ± 0.30
	2nd year				
Q. robur	0–20	6.31 ± 6.57	1.94 ± 2.32	2.90 ± 2.44	1.08 ± 0.83
	20–40	1.45 ± 2.60	0.89 ± 2.10	3.61 ± 2.68	0.94 ± 0.53
	40–60	0.62 ± 1.36	0.64 ± 1.87	2.17 ± 1.75	0.91 ± 0.78

Table 4 continued

	Container depth sections	FOLR > 0.3	Sec on FOLR > 0.3	FOLR 0.1–0.3	FOLR < 0.1
J. regia	0–20	5.26 ± 6.48	4.47 ± 3.91	4.51 ± 3.14	1.90 ± 1.67
	20–40	1.64 ± 2.35	0.99 ± 1.34	5.35 ± 4.84	1.48 ± 0.74
	40–60	0.12 ± 0.24	0.15 ± 0.32	2.46 ± 1.97	1.02 ± 0.66

PL-2: Plastecnic 4900 cm^3; AIR-3: Air-pot 9800 cm^3; AIR-4: Air-pot 15,500 cm^3

than in PL-2 (0.47) and AIR-3 (0.39) in the 1st year, while no differences occurred in the 2nd year (about 0.50 across all stocktypes).

In all container types and for both species, the main root reached the bottom of the container at the end of the 1st year and the distribution of FOLR biomass along substrate layers (Table 4) highlighted the scarce presence of larger FOLR in the deepest layers (>20 cm) of both Air-pots. After 2 years of nursery cultivation, the proportional amount of the two bigger FOLR sizes passed in all substrate layers (Table 4). In the 2nd year, in *Q. robur,* root biomass increased significantly in the two Air-pots, both for the main roots and, more steeply, for the FOLR; in *J. regia*, only AIR-4 had a sharp increase in main roots and total FOLR biomass.

In *Q. robur*, the root biomass/container volume ratio showed differences (PL-2 > Air-pots) in the 1st year, (Table 1), the opposite result occurred in *J. regia* (PL-2 > Air-pots in 2nd year). In the 1st year, in walnut, this ratio was more than double than that of oak.

Field trial

In *Q. robur* and *J. regia*, fluorescent transient analysis showed similar values of all parameters characterizing PSII functioning throughout the whole study period (Fig. 4). In particular, the maximal quantum efficiency of PSII (calculated from F_v/F_m) and the efficiency of the water-splitting complex on the donor side of PSII (as inferred from F_v/F_o) did not significantly decrease as the season progressed. The absence of marked stress on the photosynthetic efficiency of these seedlings was highlighted by the very few significant differences between averaged fluorescence parameters across the three container types. F_o measured at first sampling (with decreasing values from new to traditional containers in *Q. robur*, and $(1 - V_j)/V_j$ in the second sampling (with decreasing values from new to traditional containers), in *J. regia*. ANOVA highlighted significant differences for CCI in the third sampling, in *Q. robur*, with increasing values from traditional to new containers (AIR-4 > PL-2) and in the first and second sampling, in *J. regia* (higher in AIR-4).

No mortality occurred for both species. In *Q. robur*, AIR-3 seedlings grew significantly more than those in PL-2, while no differences due to container type occurred in *J. regia* (Table 3). Oak seedlings in both Air-pots showed significantly higher values than those in PL-2 for almost all above-ground variables, with more than double the shoot biomass (i.e., by 216.3 % in AIR-4 and 251.8 % in AIR-3) by the end of nursery cultivation. Values greater than 1.3 were recorded for the shoot/root ratio, with no differences among stocktypes. Apical dominance was recorded in 22.2 % of the plants grown in PL-2, 45.5 % in AIR-3, and 66.7 % in AIR-4. Biomass increase was also observed in the root system for seedlings grown in both Air-pots, particularly in FOLR (at least +118.7 %); seedlings grown in Air-pot containers had also a greater root exploration capacity, in terms of width and depth, than PL-2; however, in relation to container dimension, the root system grew deepest in plants grown in PL-2 (+104.3 %), while the widest was recorded in both Air-containers (at least +124.2 %). Nevertheless, seedlings in AIR-4 had the deepest root system in absolute value (Table 3).

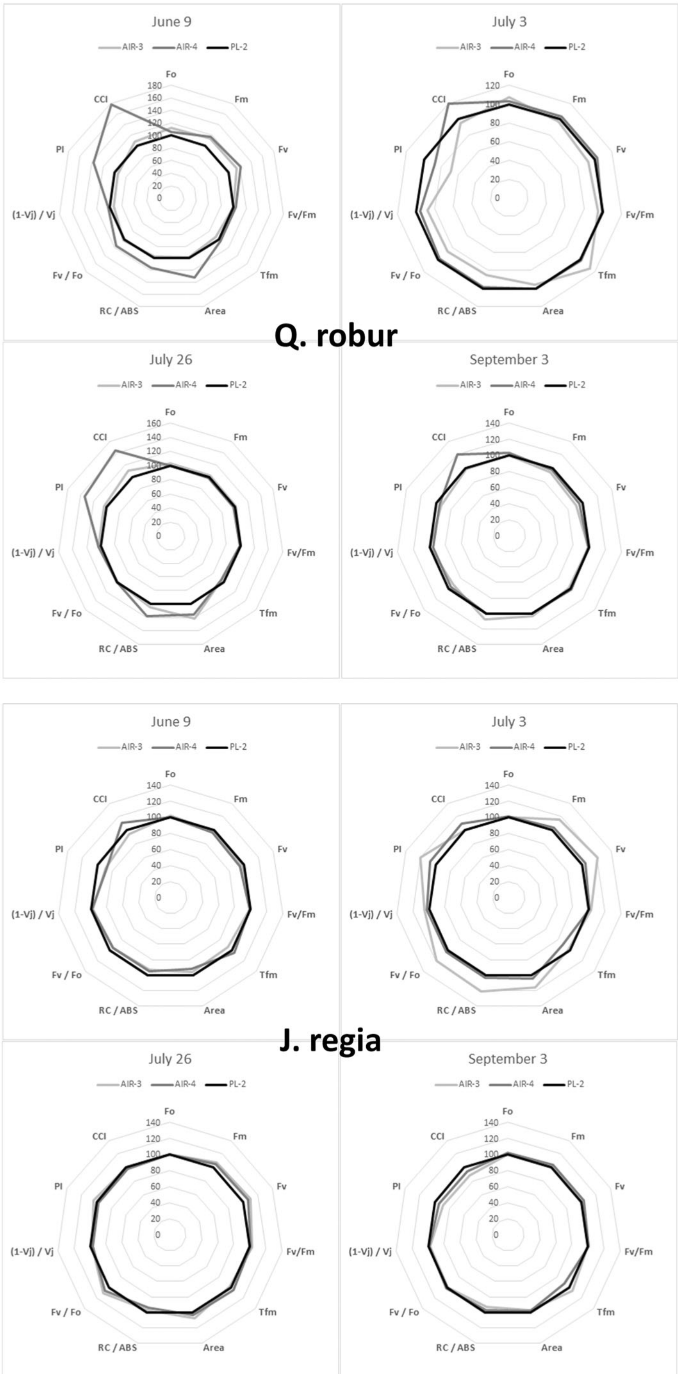
J. regia plants grown in the traditional container had the lowest values in biomass (Table 3). After 1 year in the field, above-ground biomass generally increased, slightly in AIR-4 (by 36.3 %) and dramatically in the other two types (by 122.0 % in PL-2, and by 140.0 % in AIR-3). Similar results occurred also in root biomass; concerning FOLR, the increase was by 151.3 % in AIR-4, 266.7 % in PL-2, and 365.2 % in AIR-3. All stocktypes showed similar root exploration capacity, with root systems being at least three times wider than the container width; concerning root depth, the highest increase in comparison with container size was observed in PL-2 (+137.5 %). Despite the positive response to planting of seedlings grown in PL-2, the deepest root system occurred for those grown in AIR-4 (Table 3). Apical dominance was present in 76.7 % of walnut plants grown in PL-2, 73.3 % in AIR-3, and 65.6 % in AIR-4.

Discussion

Nursery trial

The expectation that bigger and deeper containers would have a positive effect on seedling growth was confirmed in this study. For both species, in the traditional bigger container, the height after the 1st year in the nursery was similar or slightly greater than current one-year-old bareroot stock produced in Italy in the past decades (Calvo and D'Ambrosi 1995; Ciccarese 1998; Tani et al. 2007b) and generally in line with the standards indicated by Armand (1992) and Calvo and D'Ambrosi (1995) for bareroot stocktype for tree-farming purposes. The noteworthy results of both Air-pots proved that the larger volume size was effective in increasing: (a) the duration of the growing season through early October, which is an advantage in Mediterranean conditions where early frosts are uncommon; (b) the growth rate of seedlings, and thus (c) their final height and biomass. One-year-old seedlings in Air-pots were much taller than those observed in traditional and common container types for both species at the same age (Johnson 1981; Aldhous and Mason 1994; Brønnum 2004; Schmal et al. 2011). Chirino et al. (2008) observed that deeper containers (30 cm) produced taller seedlings in *Q. suber*. Pronounced effects of large containers have been observed in other hardwood species (Hathaway and Whitcomb 1977; Funk et al. 1980; Omari 2010; Morrissey et al. 2010; Dumroese et al. 2011).

Both Air-pots supported the semi-determinate growth pattern of *Q. robur*, which continued flushing until the early fall, as in favorable environmental conditions (Borcher 1975; Hanson et al. 1986; Harmer 1989). By contrast, in *J. regia* shoot elongation slowed or ceased growth in mid-summer, consistently with its monocyclic pattern of shoot growth (Solar and Štampar 2003). In this species, only the seedlings grown in the biggest Air-pot showed a considerably greater development of their height and biomass; notably, about 2/3 of seedlings sampled in this container were taller than average, and a few seedlings skewed the overall result. Often, the shape and size of containers exert serious constraints on the growth of roots and their function, especially in hardwood species (Wilson et al. 2007), adversely affecting seedling development. In walnut, which is characterized by a vigorous taproot, a smaller container volume would probably limit root development and plant growth (Le Dizes et al. 1997; Mohni et al. 2009). The bigger Air-pot strongly influenced



seedling structure and development from one to two years of nursery cultivation, with the longer cultivation period resulting in a mean stem height that met the minimum standard of the highest quality veneer in Italy (Ravagni and Buresti Lattes 2006). On the other hand, in

◀**Fig. 4** A 'spider plot' of selected parameters characterizing behavior of Photosystem II of *Q. robur* and *J. regia* seedlings after 1 year in the field (*See text* for Nomenclature for the meaning of the symbols and the parameters). All values are shown as percent of PL-2 (PL-2: Plastecnic 4900 cm^3; AIR-3: Air-pot 9800 cm^3; AIR-4: Air-pot 15,500 cm^3)

Q. robur, both Air-pots were effective in promoting growth and biomass accumulation. In both species, longer nursery cultivation resulted in a marked increase of biomass production over plant height; nevertheless, the relative biomass allocation to different plant portions was not affected by container size. The strong positive relationship between seedling biomass and container volume was expected for these uncommonly large containers (Poorter et al. 2012).

Our results highlighted that 1 year of nursery cultivation was enough for the main root of *Q. robur* and *J. regia* to reach the bottom of deeper containers, as observed by Chirino et al. (2008). Many studies have reported positive effects of container depth on root growth in other hardwoods (Chirino et al. 2005; South et al. 2005; Morrissey et al. 2010). The longer cultivation period was useful to enrich FOLR in all depth layers, in both species, highlighting that during the second year the root system colonized the container in width and, thus, in volume. Chirino et al. (2008) observed that in 30 cm deep containers *Q. ilex* seedlings developed deeper and more functional roots. Wilson et al. (2007) observed that, in small containers, *Quercus rubra* one-year-old seedlings developed FOLR both along the length of the main root and at the base of the main root, in a greater number than bare-root stocktype, which is partially in contrast with our results. The importance of a well-articulated root system is widely emphasized (Ruehle and Kormanik 1986; Davis and Jacobs 2005; Wilson and Jacobs 2006). FOLR are key aspect in seedling quality assessment because they play an important role in field establishment of hardwoods; FOLR are related to seedling transport functions, water and nutrient uptake (Wilson et al. 2007) and, thus, to the success in overcoming transplant stress (Kormanik 1986; Ruelhe and Kormanik 1986; Dey and Parker 1997). In both species, seedlings maintained a similar balance between shoot and root system during both years of nursery cultivation, with minor effects of container type. Walnut seedlings produced relatively more below-ground biomass, confirming the potential of this species for developing a vigorous root system, as also observed by Becquey (1997) and Picon-Cochard et al. (2001). Oak seedlings showed a good shoot–root balance, generally in line with values found in the literature (Lyr and Garbe 1995; Ammer 2003). Shoot/root ratio is an important attribute for hardwood seedling quality assessment (Wilson and Jacobs 2006), being linked to field performance in semi-arid environments (Leiva and Fernández-Alés 1998; Villar-Salvador et al. 2004).

Field trial

The absence of marked stress on the photosynthetic efficiency of all stocktypes in the early phases of field establishment was highlighted by very few differences between fluorescence parameters across the three container types. Chlorophyll fluorescence allows for rapid evaluation of seedling quality (Wilson and Jacobs 2012). In our study, planting stress, poor root-soil contact and limited root development did not deter the establishment of taller seedling, as reported in other cases (Burdett 1990). Seedling establishment is highly dependent on microhabitat, particularly in Mediterranean environments, where plant mortality during summer is the main factor limiting regeneration of many woody species (Villar-Salvador et al. 2012). In this sense, alternatively, or in parallel, to the use of large containers and plants (with operational difficulties), other cost-effective techniques (e.g., nurse crops and soil preparation) may support the establishment of the desired stocktype.

Despite the absence of evident stress, height increment was not fully satisfactory, especially for *J. regia.* In intensive hardwood plantings aimed toward high-quality timber production, about 50 cm of shoot growth is considered a threshold index for overcoming establishment stress (Ravagni and Buresti Lattes 2006). Height increments were also generally lower than those observed in the Po valley for fine hardwood bareroot stocktype (Tani et al. 2007b, c, 2008a, b). Among late-successional trees, stress-sensitive species (*J. regia* more than *Q. robur*) must withstand disturbances for a long time during field establishment. In particular, seedling size at planting has been found to influence responses to vegetative competition and plant survival in several oak species (Kormanik et al. 1998; Na et al. 2013). Proper functional balance and structural quality of plants grown in AIR-3 might have helped the seedlings of both species to overcome transplanting stress. The unexpected less satisfactory growth of seedlings in AIR-4 was probably related to the lower biomass of first order roots in relation to above-ground system development, which warrants further study. Adequate shoot–root balance is, indeed, an important issue in water uptake capability at the time of planting to avoid stress, especially from drought (Burdett 1990).

Positive results were obtained in relation to shoot system structure for growing high quality, productive plants; the branch biomass component was limited in both species, which is relevant to reduce early pruning and favor apical dominance. *Quercus* spp. tend to develop a shrubby structure in the first year (Buck-Sorling and Bell 2000; Drenou 2000) and, thus, seedlings lose apical dominance after transplanting (Harmer 1989; Drenou 2000; Barthélémy and Caraglio 2007). Root system establishment effects were particularly evident for *Q. robur* grown in both Air-pots, which showed increased FOLR; whereas, as much FOLR biomass increase was observed in *J. regia* grown in AIR-3. Root growth and soil exploration are critical to physiological performance during field establishment and longer FOLR have been associated with higher leaf gas exchange rates (Gazal and Kubiske 2004). In particular, well-developed FOLR in bigger seedlings would affect the capability of plants to absorb water and nutrients, increasing root surface (Davis and Jacobs 2005; Dey and Parker 1997; Grossnickle 2005; Thompson and Schultz 1995). Moreover, seedlings of both species grown in Air-pots provided soil exploration deeper than 56 cm and, therefore, they were able to reach more humid soil layers during the first year in the field. Sagrera et al. (2013) observed positive field performances in terms of root system development in *Salix* spp. grown in containers with similar volume and depth.

To provide a more complete evaluation of the tested containers we conducted a rough assessment of cultivation costs for nursery management. Cost per seedling, though preliminary and tentative, was maximum 20 times higher (about 0.25 € for one year current production in 1200 cm^3 container *versus* about 4.5 € for two years of cultivation in AIR-4). Thus, this stocktype may have limited utility in practice, likely where production targets are focused on a limited number of high value plants per hectare (e.g., superior genotypes). A more complete economic assessment should consider the nursery stock market prices and entire planting rotation, including reduced pruning operations and a shortened rotation period due to the growth and structure of such a stocktype. Currently, EU funds are available for tree-farmers to sustain the purchase of the stocktype. Growing trees is a long-term investment for forest landowners and, thus, cost-share assistance for planting trees is critically important to many landowners. Indeed, most landowners converting land to tree plantations have taken advantage of government subsidies, which may facilitate the use of large and relatively expensive container types, as long as EU and the local government maintain direct and indirect subsidies.

Overall, results obtained using new bigger containers in combination with longer nursery cultivation provided an encouraging and relevant initial data set to implement indicators for successful plantings aimed at high quality timber. Plants grown in the nursery, in both new containers for *Q. robur* and in bigger Air-pot for *J. regia*, showed a good balance between the shoot and root system, as well as many potentially promising characteristics for future high-quality wood production. Seedlings taller than 1.5 m, with fewer branches and more apical dominance are suitable for reducing both the expected rotation length and the frequency of early pruning. Even though height growth in the field was not fully satisfying, early performance results did not show marked signs of transplanting stress. Three-year-old plants had reached a stem length close to 2 m and had well-structured root and shoot systems. It must be pointed out that, in tree farming in Mediterranean climates, 2 m is the maximum height generally reached by these species after at least 4 years in field. Although only early establishment data was reported here, the present results and additional sampling on plant performance during subsequent years should provide good initial information required to examine the relationships among nursery cultivation, outplanting techniques and seedling structure/function in the production of veneer quality hardwood timber. Nevertheless, the influence of such large containers on shoot and root system attributes can be beneficial wherever seedling shoot and/or root sizes confer an advantage in other reforestation and restoration scenarios, such as for resistance to competition and/or drought stress.

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