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REGULAR ARTICLE

A comparison of root architecture and shoot morphology between naturally regenerated and container-grown seedlings of *Quercus ilex*

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Abstract We explored the different mechanisms developed by naturally regenerated seedlings of *Quercus ilex* L. (Holm oak) under Mediterranean conditions compared to container-seedlings commonly used in plantations. We examined the differences in root architecture (including topology and morphology) and shoot parameters. The results showed that there are many differences in the architecture of the root system as well as in the shoot morphology between the two types of seedlings. The naturally regenerated seedlings were smaller with regard to most of the shoot and root parameters, but they developed a longer taproot, only first order lateral roots, and presented a more herringbone-like root system compared to the container seedlings. Conversely, all types of container seedlings, were larger and had a more extended root system with many orders of lateral roots, while their taproot length was restricted within the container's depth. The quotient log (α)/ log (μ) for all seedlings, showed a tendency to decrease with plant size. A strict herringbone root system with an elongated taproot may be the optimal root architecture for *Quercus ilex* L. seedlings in order to survive under Mediterranean conditions.

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Introduction

Quercus ilex L. (Holm oak) is among the most commonly used oak species in western European Mediterranean restoration projects (Vallejo et al. 2000). This species is a slow-growing, sclerophyllous evergreen oak with resprouting capabilities and is a major structural component of the natural forests and woodlands in western European and northern African Mediterranean regions, and thus a valuable species for restoration of abandoned cropland and other deforested areas (Rey Benayas and Camacho-Cruz 2004; Villar-Salvador et al. 2004b). However, the two important factors that limit the establishment and growth of woody seedlings in Mediterranean environments, particularly in abandoned cropland and in deforested areas, are excessive irradiation and reduced water availability (Rey Benayas and Camacho-Cruz 2004; Valdecantos et al. 2006).

Q. ilex regeneration is facilitated by co-occurring woody plants, since wild oak seedlings appear mostly under shrubs or trees in most ecosystems where they have the highest emergence and survival (Puerta-Piñero et al. 2006; Retana et al. 1999).

The establishment of container planted seedlings of woody species in degraded Mediterranean environments may be aided by nursery treatments that promote the development of a deep and well-structured root system. The rapid development of such root systems will improve water uptake and transport (Chirino et al. 2008; Green et al. 1999; Tsakaldimi et al. 2005; Vallejo et al. 2000). For example, *Q. ilex* container seedlings developed in long tubes showed the highest values of root length parameters and were more efficient for plant water uptake (Peman et al. 2006). For oak seedlings, the large number of primary first order lateral roots and the high root system fibrosity (root system with a relatively high root surface area and with a large number of root apices) are considered parameters that improve field survival and early growth of seedlings (Schultz and Thompson 1997; Wilson et al. 2007). However, the field survival and growth of *Q. ilex* planted seedlings, as well as other Mediterranean oaks, are frequently very low (Hatzistathis et al. 1999; Pausas et al. 2004; Navarro Cerrillo et al. 2005; Tsakaldimi et al. 2005; Valdecantos et al. 2006; Vilagrosa et al. 2003; Villar-Salvador et al. 2004a). The poor survival of *Quercus* plantations in the field has, in most cases, been attributed to the low quality of the planted seedlings and the limited resources (Retana et al. 1999; Tsakaldimi et al. 2005, Valdecantos et al. 2006). Nevertheless, it is still unknown why Mediterranean oak container-seedlings with well-developed root systems, possess low survival rates in the field. Information concerning survival mechanisms in naturally regenerated *Q. ilex* seedlings under Mediterranean conditions as compared to container-seedlings would therefore be highly useful.

Root architectural analysis allows a formal description of root systems and has important ecological applications since architecture reflects root plasticity responses to environmental heterogeneity and edaphic constraints to plant productivity and determines the function of roots in mechanical support of the shoots (Fitter and Stickland 1991; Lynch 1995; McPhee 2005).

Root architecture of oak seedlings has been the subject of several studies (Collet et al. 2006; Tamasi et al. 2005; Wilson et al. 2007). However, with the exception of Peman et al. (2006) and Navarro Cerrillo et al. (2005) contributions on root characteristics of containerized *Q. ilex* seedlings, no study has analyzed the differences in root architecture and shoot morphology between containerized and naturally regenerated seedlings of *Q. ilex*.

Therefore, in this study we had four specific goals: (i) to determine shoot morphological characteristics of 1 year old naturally regenerated and container seedlings of *Q. ilex* (ii) to describe root architecture (including topology and morphology) of the above seedlings, (iii) to find any correlations between most of the root and shoot characteristics and finally (iv) to explore any differences in mechanisms developed by *Q. ilex* naturally regenerated seedlings compared to container-seedlings. This study used the measurements into shoot and root morphology and biomass of 1 year old container-seedlings from another investigation (Tsakaldimi et al. 2005), concerning the same species.

Materials and methods

Study area for the naturally regenerated seedlings

The study site of naturally regenerated seedlings of *Quercus ilex* L. was located in the “Kassandra” Peninsula (Chalkidiki) and in the “Stavros” area (northeast Chalkidiki), northern Greece. The Kassandra Peninsula is located 60 km south-east of Thessaloniki at 25^{o} 30΄E and 40^{o}N. It is the first of the three peninsulas of Chalkidiki from west to east. This area covers 35,000 ha. Land use is agricultural (57%) and forest (40%), covered mainly by *Pinus halepensis* Miller forests. According to data from the nearest meteorological station at Kassandria, the climate of the area is of the Mediterranean type with mild winters and hot dry summers. The mean annual rainfall of the last decade is 560 mm and the mean annual air temperature is 16.5°C. The dry period begins in the middle of April and lasts until the middle of September (Tsitsoni and Karagiannakidou 2000). The soils are mainly loamy-clay, with a high pH (7–8.4) and the organic matter ranges from 2–4% (Tsitsoni 1997). *Q. ilex* appears mainly on slopes exposed north, north-east and north-west and is intermingled with *P. halepensis* (which dominates in the overstorey), in a mosaic landscape accompanied with tall shrubs *Fraxinus ornus* L., *Phillyrea latifolia* L., *Quercus coccifera* L., *Pistacia lentiscus* L and sub-shrubs *Asparagus acutifolius* L., *Rubia peregrina* L and *Smilax aspera* L, (Tsitsoni and Karagiannakidou 2000).

In northeastern Chalkidiki, the study site is located in the “Stavros” area. The area is 80 km east of the city of

Thessaloniki, and elevation ranges from 40–120 m with an aspect of NE. The climate is thermo-mediterranean. Metamorphic rocks (gneisses, schists) prevail as geological substrates. Soils are medium deep, sandy-clay and light acid. Phytosociologically the area belongs to the broadleaf evergreen zone (*Quercetalia ilicis*) and particularly to *Quercion ilicis* subzone. The woody vegetation consists of *Q. ilex, Quercus frainetto* Ten.*, Quercus pubescens* Willd.*, Q. coccifera, Carpinus orientalis* Miller*, F. ornus, Olea europaea* var. *sylvestris* Brot., *Arbutus unedo* L.*, P. latifolia, Erica arborea* L.*, Sorbus domestica* L.*, Laurus nobilis* L.*, P. lentiscus, Juniperus oxycedrus* L.*, Pistacia terebinthus* L. *and Sorbus torminalis* (L.) Crantz (Zagas et al. 2004). The main characteristic of this vegetation is the loss of thermophilic pines (*P. halepensis* and *Pinus brutia* Ten.) which shows that there was an intensive exploitation of the vegetation in the past (Koutrakis and Lazaridou 1999).

Selection and extraction of the naturally regenerated seedlings

In November 2007, the 1-year-old naturally regenerated seedlings of *Q. ilex* were randomly harvested from the two study areas. Seedlings were collected from different sites in *Q.ilex* stands: from the understorey, from edges and from gaps. Most seedlings were found near canspesific adults. We randomly selected seedlings from different sites because of the spatial variation of holm oak regeneration and the difficulty in finding an adequate number of 1 year old seedlings (non-resprouted plants) in one specific site. It must be noted that almost all holm oak stands in Greece are coppiced as a result of a long history of human management and they regenerate from resproutings (Zagas et al. 2004).

The root systems were excavated according to the method described by Martinez-Sanchez et al. (2003); i.e. digging a deep enough trench at a given distance (about 0.3 m radius) from the excavated individual so as not to damage its root system. Then small hand tools and hands were used to remove soil from roots and finally the entire seedling was removed. The extracted seedlings were then put in plastic bags on which the stand conditions were recorded.

Nursery seedlings

Container-grown 1 year old seedlings of *Q. ilex* were produced in a previous study of Tsakaldimi et al. (2005), at an open-air forest nursery, N. Chalkidona, near Thessaloniki, northern Greece. The container seedlings were produced using three types of containers with different features: *paper-pot* FS615, *quick-pot* T18 and *plantek* 35F (the last two are plastic pots) with a cell cavity in each container type 482×10^3, 650×10^3 and 275×10^3 mm^3 in volume and 150 mm, 180 mm and 130 mm in depth, respectively. The growing medium consisted of peat and coarse perlite in ratio 3:1 (v/v) and incorporated mixed fertilizer. Acorns (provenance Chalkidiki northern Greece) were sown in March 2001 and the seedlings were lifted after they had become fully dormant in early November. Further details for the production method of these seedlings and the experimental design followed are given in Tsakaldimi et al. (2005).

Root and shoot measurements

Shoot height, root collar diameter, number of leaves, biomass and root system morphology and topology were measured in 27 naturally regenerated seedlings, excavated from the field. The root balls of the excavated seedlings were washed after being soaked for 1 hr in water and the whole root system was extracted under running water and a sieve used to collect any root fragments that detached from the system.

Intact root systems were spread to minimize overlaps in a tray (A4 size) with a transparent glass bottom which was placed on a scanner and covered with a light-proof white cover. Subsequently, the root systems were scanned to produce digital images in uncompressed tagged image file (TIF) format by a scanner (ScanJet 4c, Hewlett-Packard Co.). The scanner resolution was set at 300 dpi which is sufficient if all roots are thicker than 0.1 mm (Kirchhof and Pendar 1993). An image analysis software (Delta-T Devices Ltd. Cambridge, England) was used to analyze the images and to estimate: total root length, taproot length, root area and the total root volume of each root system. The total root volume was calculated by the Length Sin θ function (this uses trigonometry to calculate the length of the hypotenuse of a right angled triangle formed by the length of the horizontal and vertical intercepts in order to calculate the object thickness) which is considered the most accurate procedure (Kirchhof and Pendar 1993). The software calculates the total root surface area using the equation RSA = $\pi * 2R * L$ (R = radius and L = length), since it assumes that root objects are cylindrical (Kirchhof and Pendar 1993).

For biomass measurements, shoots and root systems (after scanning) were oven-dried at 70°C for 48 h and then weighed (Sorgona et al. 2005). Based on dry weight measurements the shoot to root (S:R) ratio was calculated. Also, the specific root length (SRL cm g^{-1}) was calculated as the total root length divided by the root biomass for each individual seedling (Sorgona et al. 2005; Trubat et al. 2006). SRL is considered a good indicator of the absorptive surface produced per volume of root (Nicotra et al. 2002), and it has been applied to study variations in root morphology in relation to different nutrient levels, water content and soil types (Fitter 1985).

For shoot height, number of leaves, root collar diameter, biomass and root morphology (total root length, root surface area and total root volume) of 1 year old *Q. ilex* container-seedlings, data were obtained from the previous study of Tsakaldimi et al. (2005). Furthermore, in this study new measurements were taken of taproot length and root topology (magnitude (μ), altitude (α), and topological indices) of these seedlings, and the specific root length (SRL cm g^{-1}) was also estimated.

The topology (the pattern of root branching) along with the overall size of the root system, determines the in situ space-filling properties of the root system within the soil and thus is an important component of resource uptake capacity and anchorage (Berntson 1997). The topology of the root systems was analyzed using Fitter's terminology (Fitter 1985; 1987). The scanned root system of each seedling was measured for i) magnitude μ (number of external links or the number of root tips) and ii) altitude a (number of links in the longest path from base to tips), counted manually, and the topological indices: the quotient $\log a / \log \mu$, the slope of the linear regression between $\log(\alpha)/\log(\mu)$ and the ratio $a/E(a)$ (ratio of altitude to the expected altitude) were calculated according to Werner and Smart (1973), Fitter et al. (1988) and Fitter and Stickland (1991). The quotient $\log(a)/\log(\mu)$ is very easy to calculate, it is relatively size-independent and gives extreme values similar to those given by the slope of regression $\log(a)/\log(\mu)$, 1 for herringbone-like root systems (with a single main root and one order of laterals) and about half that value (depending on the magnitude) for dichotomous root systems (all external links form new branches with equal probability), (Glimskar 2000). The ratio $a/E(a)$ indicates, for any given root system, the extent to which branching deviates from the random growth model (value =1), either towards a herringbone (values>1) or a dichotomous (value <1) pattern (Fitter et al.1988; Roumet et al. 2006).

Data analysis

The results were analyzed using one-way ANOVA and for multiple comparisons of the means the Waller Duncan Test was used. A Student's t-test was performed for the differences among the naturally regenerated seedlings and "average" container seedlings. The homogeneity of variances was tested by Levene's test. When the homogeneity of variances was not assumed the non parametric Mann–Whitney test was used, comparing two groups in each analysis. Regression and correlation analysis were performed to explore the relationship between seedling characteristics. Statistical analysis was performed with SPSS (SPSS Inc., version 14).

Results

Naturally regenerated seedlings were significantly shorter with a smaller root collar diameter and a lower leaf number than all types of container seedlings (Table 1). The biomass also varied among the type of seedlings (Table 2) as a result of differences in seedling size. All types of container seedlings had more biomass allocated to roots and shoots than naturally regenerated seedlings. Container seedlings also invested more biomass in shoots than in roots, leading to higher S/R ratios (mean = 1.5±0.06), whereas the naturally regenerated seedlings had S/R ratios close to 1, (Table 2).

Container seedlings had significantly greater total root length, root surface area and total root volume than naturally regenerated seedlings (Table 3). However, the taproot was significantly longer (19.4±1.1 cm) in naturally regenerated seedlings compared to container seedlings (14.5±0.4 cm), as the taproot was restricted within the container. The naturally regenerated seedlings, although they were shorter in shoot height, had a longer taproot (Fig. 1) and the main axis of root represented the highest proportion of the total root

Table 1 Shoot parameters according to the type of *Q. ilex* seedlings

Type of seedling	n	Shoot height (cm)	Root collar diameter (mm)	Number of leaves
Container seedlings				
Paper-pots	72	40.1 (1.21)^{a}	5.1 (0.12)^{a}	48 (8.0)^{a}
Quick-pots	72	20.8 (0.9)^{b}	4.3 (0.09)^{b}	28 (2.3)^{a}
Plantek-F	72	24.0 (0.8)^{b}	4.2 (0.09)^{b}	35 (4.0)^{a}
Mean (pooled data)		29.3 (0.8)*	4.56 (0.07)*	37 (4.0)*
Naturally regenerated seedlings	27	13.17 (0.99)^{c*}	2.45 (0.1)^{c*}	5 (0.3)^{b*}

Values are means ± standard error (in brackets). Within a column, means followed by different letters in superscript are significantly different ($P<0.05$, Waller Duncan test). When there was not homogeneity of variances the non parametric Mann–Whitney test was used

n number of seedlings in sample

*significant differences ($P<0.05$), between average container seedlings and naturally regenerated seedlings (Student t- test)

system (Fig. 2). No significant differences were found for SRL between naturally regenerated and "average" container seedlings (90.2 cm g^{-1} and 81.1 cm g^{-1} respectively). However, among all types of seedlings, the *quick-pot* seedlings exhibited the greatest SRL, (Table 3).

Concerning root topology, the μ and a were considerably higher in the container seedlings (means 178±14 and 62±4.6, respectively), (Table 4), because of their more extended root system (many first and second order laterals). The topological index $a/E(a)$ was not significantly affected by the type of seedling and fluctuated between 1.28 and 1.54, values that show a marked tendency toward herringbone branching. The quotient $\log(\alpha)/\log(\mu)$ ranged from 0.77 to 0.95, also indicating the trend to herringbone pattern of all root systems but it was significantly greater in naturally regenerated seedlings with a value near to the theoretical maximum 1, which illustrates a strict herringbone root system. The plot of $\log(\alpha)$ to $\log(\mu)$ for both types of seedlings (naturally regenerated vs. container seedlings) showed a strong close-linear relationship over the whole range of magnitude (Fig. 3). The slope of the linear model, in the case of naturally regenerated seedlings, did not differ significantly from that corresponding to strict herringbone configuration (Fig. 3).

Among all types of seedlings, the root and shoot dry weights were the variables which were significantly correlated with the most root and shoot characteristics (Table 5). Total root length was also correlated with many root and shoot characteristics. Magnitude was negatively correlated with the length of taproot. In all seedlings, root surface area was

Table 2 Shoot and root biomass and shoot to root ratio according to the type of *Q. ilex* seedlings

Type of seedling	n	Shoot biomass (g)	Root biomass (g)	Total biomass (g)	Shoot to Root ratio
Container seedlings					
Paper-pots	12	8.3 (0.80)^{a}	4.6 (0.43)^{a}	12,9 (1.2)^{a}	2.0 (0.08)^{a}
Quick-pots	12	4.4 (0.44)^{b}	3.5 (0.30)^{b}	7.9 (0.7)^{b}	1.2 (0.07)^{bc}
Plantek-F	12	3.8 (0.24)^{b}	2.9 (0.23)^{b}	6.7 (0.4)^{b}	1.3 (0.07)^{b}
Mean		5.5 (0.42)*	3.7 (0.21)*	9.2 (0.61)*	1.5 (0.06)*
Naturally regenerated seedlings	27	0.4 (0.04)^{c*}	0.4 (0.03)^{c*}	0.8 (0.07)^{c*}	1.1 (0.06)^{c*}

Values are means ± standard error (in brackets). Within a column, means followed by different letters in superscript are significantly different ($P<0.05$, Waller Duncan test). When there was not homogeneity of variances the non parametric Mann–Whitney test was used

n number of seedlings in sample

*significant differences ($P<0.05$), between average container seedlings and naturally regenerated seedlings (Student t- test)

Table 3 Root morphological characteristics according to the type of *Q. ilex* seedlings

Type of seedling	n	Taproot length (cm)	Total root Length (cm)	Root surface area (cm^2)	Total root volume (cm^3)	SRL (cm g^{-1})
Container seedlings						
Paper-pots	5	13.9 (0.4)^{c}	737.6 (70.1)^{a}	131.68 (11.1)^{a}	6.63 (0.9)^{a}	50.2 (4.4)^{c}
Quick-pots	5	15.8 (0.4)^{b}	814.4 (86.3)^{a}	118.06 (15.3)^{a}	4.22 (0.6)^{b}	121.9 (13.7)^{a}
Plantek-F	5	13.4 (0.4)^{c}	444 (50.2)^{b}	80.57 (8.9)^{b}	4.24 (0.6)^{b}	71.1 (6.6)^{bc}
Mean		14.5 (0.4)*	665.3 (49.1)*	110.1 (7.9)*	5.03 (0.4)*	81.1 (7.6)
Naturally regenerated seedlings	27	19.4 (1.1)^{a*}	66.5 (6.5)^{c*}	47.19 (4.1)^{c*}	1.57 (0.2)^{c*}	90.2 (8.4)^{b}

Values are means ± standard error (in brackets). Within a column, means followed by different letters in superscript are significantly different ($P<0.05$, Waller Duncan test). When there was not homogeneity of variances the non parametric Mann–Whitney test was used

n number of seedlings in sample

*significant differences ($P<0.05$), between average container seedlings and naturally regenerated seedlings (Student t-test)

significantly positively correlated with total root length and root dry weight ($R=0.89$ and $R=0.86$ respectively, $P<0.01$), (Table 5).

Container seedlings had a greater rate of root surface area production per unit of root length and root dry weight and also a greater rate of root length per unit root dry weight (Fig. 4a–c) than naturally regenerated seedlings. However, significant linear regressions were observed only between root surface area and total root length for both container ($y=21.27+0.13\times$, $R^2=0.70$, $P<0.01$) and naturally regenerated seedlings ($y=11.94+0.53\times$, $R^2=0.70$, $P<0.01$) (Fig. 4a).

The quotient log (α)/ log (μ) was strongly negatively correlated ($P<0.01$) with root surface area ($R=-0.72$), total root length ($R=-0.81$) and shoot and root dry weight ($R=-0.73$ and $R=-0.83$ respectively) for all seedlings (Table 5), indicating a tendency to decrease with plant size. The relation between this quotient and root dry weight was more linear when logarithmic values of root dry weight were used. When log (α) /log (μ) was plotted against log (root dry weight) the model derived was $y=-0.152\times+0.87$ with $R^2=0.74$ and $P<0.01$ (Fig. 5).

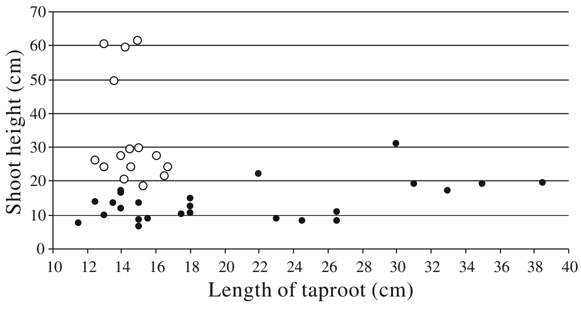

Fig. 1 Scatter plot of shoot height vs. length of taproot for naturally regenerated (●) and container seedlings (○)

Fig. 2 Scanned images of two representative root systems of **a** container- and **b** naturally regenerated *Q. ilex* seedlings (scale 1: 4.5). In **a**, some lateral roots were detached in order to flatten the root system for better viewing

Table 4 Mean magnitude (μ), altitude (a) and topological indices (log (a)/log (μ) and $a/E(a)$) of the root system according to the type of *Q. ilex* seedlings

Type of seedling	n	magnitude (μ)	altitude (a)	log (a)/log (μ)	$a/E(a)$
Container seedlings					
Paper-pots	5	200 (28.7)^{a}	58 (2.2)^{a}	0.77 (0.01)^{c}	1.28 (0.06)^{ns}
Quick-pots	5	194 (13.0)^{a}	69 (10.5)^{a}	0.79 (0.02)^{bc}	1.51 (0.18)^{ns}
Plantek-F	5	138 (25.9)^{a}	57 (7.0)^{a}	0.82 (0.01)^{b}	1.54 (0.08)^{ns}
Mean		178 (14.0)*	62 (4.6)*	0.8 (0.01)*	1.45 (0.08)
Naturally regenerated seedlings	27	24 (3.4)^{b*}	18 (2.1)^{b*}	0.95 (0.01)^{a*}	1.38 (0.05)^{ns}

Values are means ± standard error (in brackets). Within a column, means followed by different letters in superscript are significantly different ($P<0.05$, Waller Duncan test). When there was not homogeneity of variances the non parametric Mann–Whitney test was used

n number of seedlings in sample, *ns* non significant differences

*significant differences ($P<0.05$), between average container seedlings and naturally regenerated seedlings (Student t-test)

Discussion

We found that 1 year old *Q. ilex* seedlings grown in containers under favourable nursery conditions demonstrated significant differences in shoot morphology and root architecture with regard to the naturally regenerated seedlings grown in the field. These results confirm the view that the ability of seedlings to survive and grow in different environments depends on the integrated response of the above- and belowground organs to resource availability (Paz 2003).

Compared to naturally regenerated seedlings, all types of container seedlings had significantly greater shoot height, root collar diameter, leaf number, shoot and root biomass, root surface area, total root volume and total root length. However, among the containerized seedlings, the *paper-pot* seedlings had the greatest shoot height, diameter, biomass allocation and total root volume. These results can be attributed to the permeable walls of the *paper-pots* which allowed moisture and soluble nutrients to move between the cells, thus these resources were more easily available for seedlings (Tsakaldimi et al. 2005). All container-seedlings also had greater rate of root surface area production per unit of root length and root dry weight, and a greater rate of root length per unit root dry weight than the naturally regenerated seedlings. Similar linear relationships concerning above root traits were found by Anderson et al. (2007) who studied potted plants of two grass species (*Digitaria macroblephara* S. and *Themeda triandra* F.). These results showed that container seedlings were enhanced by the favorable nursery conditions. However, no significant differences were found in SRL between "average" container—and naturally regenerated seedlings. The highest SRL values were found in the biggest containers (*quick-pots*). Peman et al. (2006) also found that *Q. ilex* seedlings grown in deeper pots possessed higher values of SRL. Higher SRL implies thinner roots and a better exploitation efficiency (Hodge 2004; Nicotra et al. 2002; Trubat et al. 2006). Nevertheless, the interpretation of this index is rather confusing. For example, Trubat et al. (2006) reported increases in SRL of some Mediterranean woody species in response to water limitation, while Wright and Westoby (1999) and Nicotra et al. (2002) reported lower SRL values in species in low rainfall environments.

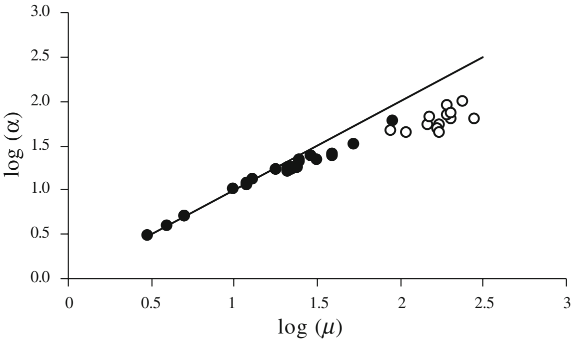

Fig. 3 Altitude vs. magnitude of root systems, (●) naturally regenerated seedlings, (○) container seedlings. Reference line: strict herringbone topology (—)

Table 5 Correlation among root and shoot characteristics of *Q. ilex* seedlings (pooled data from naturally regenerated and container seedlings)

Related characteristics	Root dry weight (g)	Root surface area (cm2)	Total root length (cm)	Length of taproot (cm)	Shoot dry weight (g)
Root dry weight (g)		0.862**	0.856**	ns	0.932**
Root surface area (cm2)	0.862**		0.887**	ns	0.812**
Root volume (ml)	0.639**	0.776**	0.467**	ns	0.637**
Length of taproot(cm)	−0.381*	ns	−0.328*		−0.347*
Total root length (cm	0.856**	0.887**		−0.328*	0.775**
SRL (cm g−1)	−0.286*	ns	ns	ns	−0.362**
Quotient log(α)/log(μ)	−0.827**	−0.717**	−0.811**	0.463**	−0.727**
Magnitude (μ)	0.879**	0.759**	0.937**	−0.385*	0.786**

ns not significantly different

*Statistically significant at $P<0.05$, **Statistically significant at $P<0.01$

The naturally regenerated seedlings presented a more balanced S/R ratio and a significantly longer taproot than container seedlings whose taproot was restricted within the container's depth. Taking into account the Mediterranean climatic conditions, the elongation of the taproot at the expense of aboveground growth, root collar diameter and other root traits, may be a mechanism of naturally regenerated seedlings to reach deeper moist soil layers and survive. Drought stress frequently alters the allocation of resources, for example promoting root growth at the expense of aboveground parts (Stewart and Lieffers 1993). Water availability tends to increase with soil depth, especially in habitats with a prolonged dry season. In such habitats, species may develop deeper roots to supply a unit of productive tissue (Fitter et al. 1991; Paz 2003).

Root topology was also modified in relation to the type of seedlings, reflecting the root plasticity responses of *Q. ilex* seedlings to environmental heterogeneity. The container seedlings had a more extended root system with many first and second order laterals and thus they presented significantly higher values of magnitude and altitude with regard to naturally regenerated seedlings. However, all root systems of all seedlings showed a marked tendency towards herringbone branching ($\alpha/E(\alpha) > 1$). Nevertheless, only the naturally regenerated seedlings presented a strict herringbone root system (log (α)/log (μ) near to 1, with a single root axis and first order laterals (Fig. 2). The main axis of these root systems contained the largest proportion of total root system. This result is also confirmed by the negative correlation between taproot length and magnitude. Thus, naturally regenerated seedlings invested more in taproot length as they did not develop many orders of lateral roots. Similar results were found by Nicotra et al. (2002), who studied root architecture and morphology of Australian perennial plants. Peman et al. (2006) also reported a trend to a herringbone type root system in containerized *Q. ilex* seedlings, but they found lower values of log (α)/log(μ) (0.58) compared to that of container seedlings in our study.

Despite the variations in other traits, there seems to be a similar relationship between log(α) and log(μ), independent of seedling type (Fig. 3). The quotient log (α)/log(μ) was strongly negatively correlated with root surface area, total root length and shoot and root dry weight for all studied seedlings, indicating that there was a tendency to decrease with plant size. These results are confirmed by the linear relationship between this quotient and log root dry weight which also shows that altitude and magnitude are both strongly negatively correlated with root biomass, as suggested by Fitter (1987).

Root system architecture can play an important role in seedling establishment, as it has a strong influence on the efficiency of soil resource capture (Spanos et al. 2008). Less branched root systems (as herringbone structures where lateral roots are constrained to the main axis) are more efficient than highly branched systems at acquiring water and

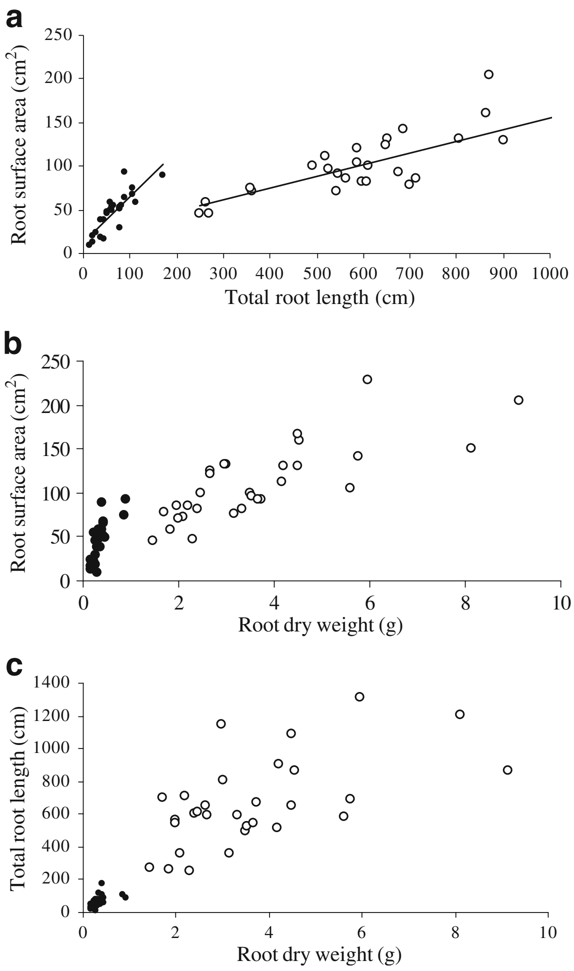

Fig. 4 Scatter plots of root surface area vs. total root length (**a**), root surface area vs. root dry weight (**b**) and total root length vs. root dry weight (**c**) for container (○) and naturally regenerated (●) seedlings. *Trend lines* fitted to linear regression

nutrients, because they exploit more soil volume per unit of root volume (Berntson 1994; Fitter et al. 1991; Martinez-Sanchez et al. 2003). Therefore, less branched root systems, e.g. in the naturally regenerated seedlings from our study, may be characteristic of plants adapted to low water or nutrient poor conditions (Fitter et al. 1988; Fitter and Stickland 1991). Nicotra et al. (2002) found that low rainfall-adapted species produced simpler root systems that increased rooting depth at the expense of branches (therefore with lower values of magnitude and altitude) and root diameter, compared to species from higher rainfall habitats, but there was no consistent effect of precipitation on branching index, though all species had indices (α/ E(α)) > 1, indicating herringbone topology.

The strict herringbone topology of the small (naturally regenerated) seedlings could be interpreted as a need for these seedlings to quickly reach lower soil layers for less risk of desiccation and better anchorage (Glimskar 2000) and better water conditions (Martinez-Sanchez et al. 2003), whereas the larger (container) seedlings have more to gain water and nutrients from the nutrient rich growing medium close to their roots. However, according to Tsakaldimi et al. (2005), when these container-seedlings were planted in the field, their mortality was higher after the dry summer period. The survival during the first year was greatest in *paper-pot* seedlings (73%) and a possible explanation for this is because of their initial morphological characteristics. Nevertheless, the establishment failure of *Q. ilex* seedlings, which has been reported in many previous studies (Hatzistathis et al. 1999; Tsakaldimi et al. 2005; Villar-Salvador et al. 2004a), cannot be attributed only to the morphology of above- and belowground organs. It is important to bear in mind that the artificial establishment of *Q. ilex* usually takes place in very different environments (abandoned cropland, deforested and degraded areas) with increased incident radiation and low water availability than that where natural regeneration of the species occurs (Rey Benayas and Camacho-Cruz 2004; Valdecantos et al. 2006). During the early establishment phases of *Q. ilex,* the survival of naturally regenerated seedlings was enhanced in sites with intermediate light levels and medium to high water levels (Retana et al. 1999). But even for the naturally regenerated seedlings, we cannot decisively suggest that those with a strict

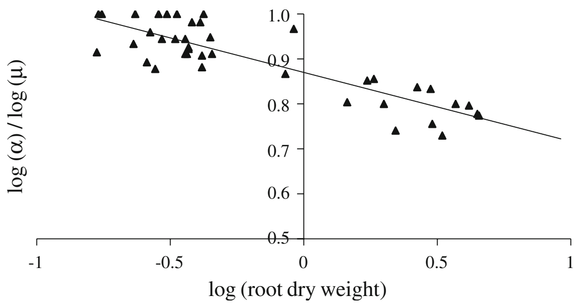

Fig. 5 The quotient log(α)/ log(μ) vs. log(root dry weight) for all studied seedlings. *Trend line* fitted by the regression (y=−0.152×+0.87, R^2=0.74, P<0.01)

herringbone configuration and an elongated taproot will survive, because no field research was conducted either on the root architecture of the dead seedlings or for the other factors that may contribute to the survival e.g. local biotic and abiotic conditions. Therefore, further research on the establishment success of container and naturally regenerated seedlings under the same field conditions, could contribute to a better understanding of how *Q. ilex* adapts to harsh Mediterranean conditions.

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