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Root morphology, stem growth and field performance of seedlings of two Mediterranean evergreen oak species raised in different container types

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Abstract

Outplanting container-grown oak seedlings with undesirable shoot and root characteristics result in poor establishment and reduced field growth. The objective of this study was to determine the influence of container type on both above-and below-ground nursery growth and field performance of one-year old tap-rooted seedlings *Quercus ilex* L. and *Quercus coccifera* L. The experiment was conducted in an open-air nursery and the seedlings were grown in three container types. At the end of the nursery, growth period seedlings' shoot height, diameter (5 mm above root collar), shoot and root biomass, root surface area, root volume and total root length were assessed. Then the seedlings were planted in the field and their survival and growth were recorded for two growing seasons after outplanting. The results showed a difference between the *Quercus* species in the effect of container type. *Q. ilex* seedlings raised in *paper-pot* had significantly greater height, diameter, shoot and root biomass and root volume than those raised in the other two container types. Similarly, *Q. coccifera* seedlings raised in *paper-pot*, had significantly greater above-and below-ground growth than those raised in the other two container types. Both oak species showed relatively low survival in the field; the mortality was mainly observed the first year after outplanting, especially after the summer dry period. However, 2 years after outplanting, the *paper-pot* seedlings of the two oak species showed better field performance.

Introduction

In ecological studies, the evergreen sclerophylls are regarded as one of most typical components of the Mediterranean type vegetation (Saleo and LoGullo, 1990). Many restoration projects have established plantations of these evergreen resprouting species (Vallejo et al., 2000; Vilagrosa et al., 2003). Despite the great efforts in oak regeneration research, the successful planting of oaks is still fraught with uncertainty (Pope, 1993). Early attempts to introduce broad-leaved resprouting species to the Mediterranean basin (e.g., *Quercus* species) faced high seedling mortality, and until recently, nursery and field techniques were poorly developed for these two species (Pausas et al., 2004). In eastern Spain as well as in Greece, the field survival and growth of planted Mediterranean oaks are frequently very low (Hatzistathis et al., 1999; Pausas et al., 2004; Tsakaldimi, 2001; Vilagrosa et al., 2003; Villar-Salvador et al., 2004).

The poor development of *Quercus* seedlings plantations, in some cases, could be attributed to the low quality of the planted seedlings.

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Nursery cultivation regimes can strongly determine the functional characteristics of seedlings and their field performance (Landis et al., 1990; Simpson, 1995; Villar-Salvador et al., 2004). For instance, during the container seedling production, container size, growing density and design characteristics of the containers are important determinants of seedling quality (Landis et al., 1990). The volume of the cavity is one of the most obvious and important characteristics of a container because in general, the larger the container the larger the seedling that can be produced. However, the optimum container size varies according to many different factors, including species, growing density, environmental conditions and length of the growing season. Pine species that are tolerant of crowding, such as loblolly pine, could be produced in small-volume containers with a high growing density. In contrast, broad-leaved species should be produced at lower growing density because their leaves intercept more water and nutrients and generate more shade (Landis et al., 1990). One of the most serious problems in containers, especially in the case of seedlings with tap roots such as oaks, is the tendency of seedling roots to spiral around the inside of the container or to concentrate at the base of the container (Biran and Eliassaf, 1980; Landis et al., 1990). Root spiraling is most serious in round, smooth-walled plastic containers and can seriously reduce seedling quality after outplanting. In contrast, well-developed and well-structured root systems with numerous first order laterals are one of the most essential attributes of high quality oak seedlings (Day and Parker, 1997; Thompson and Schultz, 1995).

However, the influence of container type on seedling quality and the outplanting performance of Mediterranean oak species has received almost no attention and to the best of our knowledge, no study on root morphology of seedlings of these species has been reported.

Thus, the objective of this study was to determine the influence of container type on both above- and below-ground nursery growth and field performance of two tap-rooted seedlings, *Quercus ilex* and *Quercus coccifera*.

Materials and methods

Nursery phase

Experimental treatments The experiment was conducted in an open-air nursery of Forest Service (N. Chalkidona, North Greece). Acorns of *Quercus ilex* L. and *Quercus coccifera* L. were sown in mid-March. Three container types were selected to provide a wide range in container volumes, density of plants, and design characteristics as these have been shown to have a strong influence on the morphology and field performance of seedlings (Jones et al., 2002; Landis et al., 1990; Salonius and Beaton, 1994). The container types used for the tap-rooted seedlings production were: (a) *paper-pot* FS 615; made of biodegradable paper, planted with the seedling, each cavity is hexagonally shaped, bottomless, 482×10^3 mm^3 in volume and 150 mm in depth, and (b) and (c) two rigid re-usable plastic containers from which the seedlings are removed before planting: (b) quick pot T18; each cavity is of square shape, tapered from top to bottom, has interior vertical anti-spiralling ribs and open crossed base and is 650×10^3 mm^3 in volume and 180 mm in depth, and (c) *plantek* 35F; each cavity has similar design features to *quick-pot* but air root pruning is achieved from the sides of the walls and from the base, and is 275×10^3 mm^3 in volume and 130 mm in depth. All cavities were filled with sphagnum Lithuanian peat of medium structure and coarse perlite (3:1, v/v). This potting medium is commonly used in Greek forest nurseries. The potting medium was fertilized with 1.3 kg mixed fertilizer (N:P:K 15:30:15 + micronutrients), 0.6 kg potassium sulfate, 1.0 kg superphosphate (0-20-0), 0.4 kg magnesium sulfate and 2 kg lime (CaO) per m^3 of peat.

The three treatments were arranged in a randomized complete block design with three replications for each of 2 species $\times$ 3 container types. There were 24 seedlings per container type, in each block (total 216 seedlings per species) and all seedlings were identified with a number. All seedlings were irrigated with an overhead irrigation system, as needed.

Growth measurements and destructive sampling At the end of the growth period in the nursery, on

November, the shoot height, the diameter (measured 5 mm above the root collar) of all seedlings were measured with an accuracy of 1 and 0.1 mm, respectively. Twelve randomly selected seedlings per treatment (4 seedlings × 3 replications) of each species, were collected for destructive sampling and they were transferred to the Laboratory for biomass measurements. From these selected seedlings, five random root samples per treatment were used for the root morphology estimations prior to biomass measurements. The root system was separated from the soil, under a gentle water jet, using a sieve to collect any root fragments detached from the system. Then, each root system was put into a glass box and covered with a white plastic sheet to keep it in a fixed position and improve the contrast of the root image. The box was placed on a scanner (Hewlett Packard, ScanJet 6100C) connected to a computer, and an image analysis system (DT-Scan, Delta T-Devices) was used to determine the total root length, the root surface area and the total root volume (Barnett and McGilvray, 2001; Fitter et al., 1991). For biomass measurements the seedlings were divided into two parts: shoot (stem + needles) and root system. Both parts were oven-dried at 70 °C for 48 h and then they were weighed (Thompson, 1985).

Field experiment

In early December, eight-month-old *Q. ilex* and *Q. coccifera* seedlings were outplanted to the field in 'Kassandra' Peninsula, Chalkidiki (North Greece), which is located 80 km south-east of Thessaloniki at 25°30′ E and 40° N. According to the climatic data (period 1978–1997) from the meteorological station of the Forest Service, the climate of the area is of the Mediterranean type with mild winters and dry hot summers. The mean annual rainfall reaches 581 mm, while the mean annual air temperature goes up to 16.3 °C and the mean maximum air temperature of the warmest month (July) is 30.1 °C. The dry period begins in the middle of April and lasts until the middle of September (Tsakaldimi, 2001; Tsitsoni, 1997). The vegetation of the area belongs to *Quercetalia illicis* floristic zone.

For each species, twenty seedlings per treatment per replication were planted in a randomized complete block design with three replications; the identity of nursery blocks was maintained in the field. Experimental blocks (500 m^2 each) were located on three independent sites of W, NW and N aspects and of moderate slopes (15–30%) and they were not irrigated. The distance between the sites was approximately 300 m. The soil of the three sites, where the experiment was conducted, is characterized as deep, sandy-clay loam, neutral to moderate alkaline and rich in organic matter at the surface horizons (Tsakaldimi, 2001).

The seedlings being hand planted in pits (0.30 × 0.30 m) and they were spaced 2 m apart. The survival was recorded for each seedling for two successive years after planting. Furthermore, 2 years after planting, height and diameter growth of each seedling were assessed (with an accuracy of 1 and 0.1 mm, respectively). The relative growth rates (RGR) for both height and diameter, after a period of 2 years, were calculated as the difference between the natural logarithms of final and initial height or diameter respectively, divided by time between the beginning and the end of field experiments (in years) (Elvira et al., 2004; Villar-Salvador et al., 2004).

Statistical analysis

All statistics were calculated with SPSS software. Distribution was tested for normality by Kolmogorov--Smirnov criterion and the homogeneity of variances was tested by Levene's test. The percentages were transformed to arsine square root values, before analysis. Significant differences between treatment means were tested using analysis of variance (one-way ANOVA). Wherever treatment effects were significant, the Duncan's Multiple Range Test was carried out to compare the means (Norusis, 1994; Snedecor and Cochran, 1988).

Results

Nursery performance

Both species were affected by the type of container. *Q. ilex* seedlings grown in *paper-pot* were significantly taller, had greater diameter and shoot biomass than seedlings grown in *quick-pot* and *plantek* (Table 1). Also, the root biomass, the shoot/root mass ratio and the total root volume found to be significantly greater in seedlings

Table 1. Effects of container type on *Q. ilex* seedling characteristics at the nursery phase

	Container type		
	Paper-pot (FS 615)	*Quick-pot* (T18)	*Plantek* (35 F)
Above-ground seedling characteristics			
Shoot height (mm)	401 (12.1)[a]	208 (9.1)[b]	240 (8.1)[b]
Root-collar diameter (mm)	5.1 (0.12)[a]	4.3 (0.09)[b]	4.2 (0.09)[c]
Shoot dry weight (g)	8.3 (0.80)[a]	4.2 (0.44)[b]	3.8 (0.24)[b]
Below-ground seedling characteristics			
Root dry weight (g)	4.6 (0.43)[a]	3.5 (0.30)[b]	2.9 (0.23)[b]
Root surface area (mm^2)	13 168 (1110)[a]	11 806 (1536)[a]	8057 (896)[b]
Root volume (mm^3)	6630 (890)[a]	4220 (570)[b]	4240 (610)[b]
Total root length (mm)	7376 (701)[a]	8144 (863)[a]	4440 (502)[b]
Shoot dry weight/Root dry weight	2.0 (0.1)[a]	1.2 (0.1)[b]	1.3 (0.1)[b]

Values are means ± standard error (in parenthesis). Within a row, means followed by different letters, are significantly different ($P < 0.05$).

Table 2. Effects of container type on *Q. coccifera* seedling characteristics at the nursery phase

	Container type		
	Paper-pot (FS 615)	*Quick-pot* (T18)	*Plantek* (35 F)
Above-ground seedling characteristics			
Shoot height (mm)	283 (15.4)[a]	136 (11.5)[b]	139 (9.2)[b]
Root-collar diameter (mm)	4.2 (0.12)[a]	3.1 (0.11)[b]	3.1 (0.09)[b]
Shoot dry weight (g)	4.5 (0.69)[a]	2.1 (0.25)[b]	1.6 (0.11)[b]
Below-ground seedling characteristics			
Root dry weight (g)	3.6 (0.49)[a]	2.8 (0.29)[a]	1.7 (0.14)[b]
Root surface area (mm^2)	12 306 (1996)[a]	7950 (888)[b]	5839 (614)[b]
Root volume (mm^3)	6410 (1290)[a]	4260 (460)[ab]	3300 (480)[b]
Total root length (mm)	6233 (963)[a]	4148 (462)[b]	2973 (353)[b]
Shoot dry weight/Root dry weight	1.3 (0.08)[a]	0.7 (0.05)[c]	0.9 (0.07)[b]

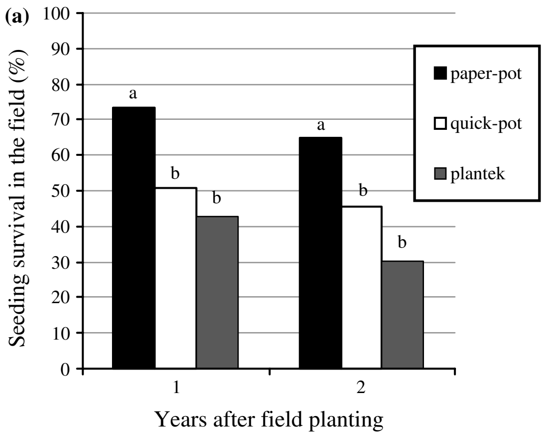
Values are means ± standard error (in parenthesis). Within a row, means followed by different letters, are significantly different ($P < 0.05$).

grown in *paper-pot* than in seedlings grown in *quick-pot* and *plantek*. The total root surface area and root length did not show significant differences among the *paper-pot* and *quick-pot* seedlings, but were significantly greater than those of *plantek* seedlings.

Similarly to *Q. ilex* seedlings, *Q. coccifera paper-pot* seedlings exhibited the greatest height, diameter and shoot biomass (Table 2). On the contrary, the seedlings grown in *quick-pot* did not differ from those grown in *plantek* but both were found significantly smaller than *paper-pot* seedlings. The container type significantly affected the root morphology. *Paper-pot* seedlings had a more extended root system; their root surface area and the total root length were significantly greater than that of seedlings raised in plastic containers, and were twice or more greater than those of *plantek* seedlings. The root volume, and the root biomass allocation did not differ between *paper-pot* and *quick-pot* seedlings but remained greater than that of *plantek* seedlings. The shoot/root mass ratio was significantly greater in seedlings grown in *paper-pot*.

Field survival

One year after outplanting (on November), the survival rate presented significant differences among the treated seedlings of *Q. ilex*, and it was



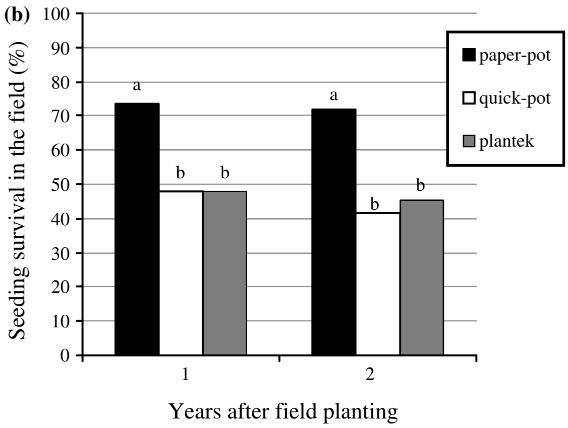


Figure 1. Effect of container type on *Q. ilex* (a) and *Q. coccifera* (b) seedling survival in the field; the first and the second year after outplanting. For the same year, means followed by different letter are significantly different ($P < 0.05$). Error bars are not shown because they are too small.

Table 3. Container type effects on height, root collar diameter and relative growth rates (RGR) for *Q. ilex* and *Q. coccifera* seedlings, 2 years after field planting

Field growth	Container type		
	Paper-pot (FS 615)	*Quick-pot* (T18)	*Plantek* (35 F)
Q. ilex			
Height (mm)	473 (22.5)^{a}	315 (21.4)^{b}	362 (26.6)^{b}
Height RGR (mm mm^{-1} year^{-1})	0.9 (0.12)^{b}	1.8 (0.26)^{a}	1.6 (0.39)^{a}
Root-collar diameter (mm)	8.4 (0.37)^{a}	6.3 (0.28)^{b}	6.4 (0.37)^{b}
Diameter RGR (mm mm^{-1} year^{-1})	0.25 (0.02)^{ns}	0.20 (0.03)^{ns}	0.21 (0.03)^{ns}
Q. coccifera			
Height (mm)	367 (23.6)^{a}	278 (28.7)^{b}	273 (24.2)^{b}
Height RGR (mm mm^{-1} year^{-1})	1.6 (0.23)^{b}	2.7 (0.35)^{a}	3.1 (0.53)^{a}
Root-collar diameter (mm)	6.6 (0.32)^{ns}	5.8 (0.31)^{ns}	5.7 (0.47)^{ns}
Diameter RGR (mm mm^{-1} year^{-1})	0.24 (0.03)^{ns}	0.28 (0.03)^{ns}	0.29 (0.05)^{ns}

Values are means ± standard error (in parenthesis). Within a row, means followed by different letters, are significantly different ($P < 0.05$).

negatively affected by the summer drought period (Figure 1a). Seedlings grown in *paper-pot* presented significantly greater survival rate (73.3%) than those grown in *quick-pot* (50.9%) and *plantek* (42.9%). During the second year after outplanting, the summer drought period caused a further reduction of survival rate; 8.3% for *paper-pot* seedlings, 5.3% for *quick-pot* seedlings and 12.5% for *plantek* seedlings.

Q. coccifera seedlings had also difficulties surviving in the field (Figure 1b). The first year after outplanting, the survival rate significantly reduced. The survival of *paper-pot* seedlings was 73.6%, while the survival recorded in the *quick-pot* and *plantek* seedlings reduced at half, and it was 47.9 and 47.7%, respectively. At the end of the second growth period in the field, the survival rate reduced to 71.7% for *paper-pot* seedlings, 41.7% for *quick-pot* seedlings and 45.5% for *plantek* seedlings.

After recording survival rates we excavated five dead seedlings of each treatment and species and found that their roots were restricted to the space of the nursery root plug and none of them had developed new roots out of it.

Growth in the field

At the end of the second year in the field, 23 months after outplanting, the *Q. ilex* seedlings shoot height and diameter presented significant differences among the treatments and followed the same trend as in the nursery (Table 3). The larger and thicker seedlings were those that had grown in *paper-pot* and the smaller seedlings were those that had grown in *quick-pot* and *plantek*. However, *quick-pot* and *plantek* seedlings showed significantly greater height RGR than *paper-pot* seedlings, while the diameter RGR did not differ among treated seedlings.

Similarly, the larger *Q. coccifera* seedlings had grown in *paper-pot* while the smaller ones had grown in *quick-pot* and *plantek*. The *quick-pot* and *plantek* seedlings again showed significantly greater height RGR than the *paper-pot* seedlings while their diameter and diameter RGR did not show significant differences among treated seedlings (Table 3).

Discussion

The seedlings of both oaks produced in the three container types, were healthy, none of them showed root spiraling and all of them approximately reached the appropriate dimensions for planting. According to EU legislation (Council Directive 71/161/EEC, 1971) *Quercus* seedlings, 1 or 2 years old, are considered suitable for planting when their height is 150–250 mm and root-collar diameter is 4 mm. Concerning the Mediterranean oaks, Nardini et al. (2000) found that two-year old *Q. ilex* seedlings, raised in containers, were much smaller than those of our study; their stem diameter was only 2.7 mm, height 420 mm, total root dry weight 0.5 g and root surface area was 3680 mm^2. Also, Villar-Salvador et al. (2004), found that 10-month-old *Q. ilex* seedlings, grown in forest-pot 300 containers and fertilized with slow-release fertilizer N:P:K (15:7:15), 1 kg m^{-3} peat, were only 141 mm in height and they allocated shoot dry weight 1.72 g and root dry weight 3.39 g. In our study, seedling dimensions of the two oak species were much differentiated among the container types used. In both oak species, seedlings raised in *paper-pot* were superior to seedlings raised in the other two plastic containers. Contrary to what has been reported for larger containers with lower growing densities (Aphalo and Rikala, 2003; Landis et al., 1990; Tanaka and Timmis, 1974), the *paper-pot* although it is smaller in size and create higher seedling densities than *quick-pot*, it increased shoot height, diameter, biomass allocation and enhanced the root morphology of the oak seedlings. A possible explanation for this, is the construction material of the *paper-pot*; the paper is permeable and allow water and soluble salts to move laterally between the cavities of the container. This positively affected the water and nutrient availability for each seedling and thus enhanced the seedlings' growth (Tsakaldimi, 2001). Moreover, although there are no measurements, the *quick-pot* and *plantek*, which are plastic and black-colored containers, may absorb more solar radiation which can increase the root temperature. High soil temperatures were especially reported for black plastic containers (Whitcomp, 1989). The high root temperatures can inhibit root growth and may even result in seedling mortality (Landis et al., 1990).

In the field, both oak seedlings had difficulties to survive, but the mortality was much higher in *Q. ilex* seedlings. Similarly, Villar-Salvador et al. (2004) report that *Q. ilex* seedlings have lower survival and growth when compared with other Mediterranean woody species. This indicates that this species is more susceptible to stress factors during its early life stages and especially during the first summer period. In this study also, the mortality was mainly observed at the end of the first year after outplanting and after the summer dry period, and varied considerably among the container seedlings. The survival rate of *paper-pot* seedlings was much greater (73.3% for *Q. ilex* and 73.6% for *Q. coccifera*) than that of the other container seedlings, while the survival rate of the *plantek* seedlings was only 42.9% for *Q. ilex* and 47.7% for *Q. coccifera*. At the end of the second year after outplanting, there was a further reduction of seedling survival. However, the survival of seedlings grown in *paper-pot* remained higher (65 and 71.7% for the *Q. ilex* and *Q. coccifera*, respectively) while the survival recorded for *plantek* seedlings was 45.5% for *Q. coccifera* and only 30.4% for *Q. ilex*. Villar-Salvador et al. (2004) found that 2 years after outplanting, the mortality of *Q. ilex* seedlings reached to 42% and tended to occur during the summer period. Hatzistathis et al. (1999) found that *Q. ilex* grown in *paper-pot*, had very low survival (33.7%), 18 months after outplanting in the Kassandra, northern Greece.

The better survival of *paper-pot* seedlings can be attributed to their initial morphological characteristics. Villar-Salvador et al. (2004) reported that, *Q. ilex* seedlings with largest shoots and with a higher S/R ratio had lower mortality than those with opposite attributes, 2 years after outplanting. Cortina et al. (1997) found that shoot height was also positively correlated with field survival of *Q. ilex* seedlings. Also, in a previous study, Tsakaldimi (2001) found that diameter was a good predictor for field survival of *Q. coccifera* seedlings; the thicker the seedlings the higher the survival. Similarly, in our study, the *paper-pot* seedlings of both oak species, that exhibited the lower mortality, had much greater shoot height, root-collar diameter, shoot dry weight and S/R ratio at the time of planting. The poor performance of smaller seedlings may be due to an unbalanced carbon economy during their establishment phase and the summer period (Villar-Salvador et al., 2004). Root characteristics may also have contributed to the better survival of *paper-pot* seedlings. The greater root volume and root surface area (as well as the greater total root length only in the case of *Q. coccifera*) of *paper-pot* seedlings, may have resulted in a better water and nutrient uptake during their early stages after outplanting and especially during the summer drought. When growth or survival is limited by water (as is observed in the Mediterranean basin) or nutrient availability, immediately after outplanting, roots play a more important role in the performance of container seedlings (Aphalo and Rikala, 2003). Furthermore, it may be important that *paper-pot* seedlings were planted with pots, thus, they had their roots protected not only during the planting work but the whole first year after outplanting until the roots increased and penetrated the soil. In contrast, *quick-pot* and *plantek* seedlings, which were planted without the cavity, had their roots unprotected and moreover their roots had difficulty crossing a textural discontinuity from a light, friable growing medium to natural soil (Tinus, 1986). According to Ruehle and Kormanik (1986), oak seedlings must develop new roots soon after planting if they are to survive and grow. This seems to be confirmed in our study since all the excavated dead seedlings had developed no roots out of the nursery plug.

The differences in seedlings size in the nursery phase, of both oak species, persisted 2 years after the outplanting in the field. *Paper-pot* seedlings remained significantly taller than the other container seedlings, although the height relative growth rate (RGR) was greater in *quick-pot* and *plantek* seedlings. *Q. ilex* seedlings raised in *paper-pot* also had the greatest field diameter, while their diameter relative growth rate (RGR) did not differ from that of the other container seedlings. In the case of *Q. coccifera*, although *quick-pot* and *plantek* seedlings had smaller diameter at planting they grew as well as the larger *paper-pot* seedlings. However, Villar-Salvador et al. (2004), reported that *Q. ilex* seedlings with larger shoots and with a higher S/R ratio had larger stem volume increase, 2 years after outplanting. In contrast, studies among many other species concluded that, differences in seedlings size at planting disappeared after one or two growth periods in the field (Jones et al., 2002; Simpson, 1995). Actually, growth following outplanting is more complex than mere survival and is related to the planting environment, the genetic potential and the physiological and morphological status of the seedlings, at the time of outplanting (Mexal and Landis, 1990).

Conclusions

The results of this study suggest that the container type has a strong influence on seedling quality and outplanting performance of *Q. ilex* and *Q. coccifera* seedlings. The *paper-pot* contributes to the production of taller, thicker and heavier seedlings with a more extended root system. The better quality of these seedlings in combination with the fact that the *paper-pot* seedlings have their roots protected from transplanting shock, results in better field performance. Also, it is suggested that larger oak seedlings have better survival and they remain greater 2 years after outplanting. Grading criteria for oak seedlings' shoot height and root-collar diameter will be important for sites where environmental stress may be high.

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