

Physiological and transplanting performance of *Quercus ilex* L. (holm oak) seedlings grown in nurseries with different winter conditions

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Abstract

Resistance to drought and frost is a requisite for seedling survival and growth in Mediterranean continental areas. Environmental conditions in the nursery influence the functional characteristics of seedlings. We addressed if cultivation in nurseries that experience different winter conditions affected cold hardiness and other functional attributes relevant for transplanting performance of *Quercus ilex* (holm oak) seedlings. It was hypothesised that plants grown at cold-winter sites have higher stress resistance, vigour, and transplanting performance than the seedlings cultivated in mild-winter sites. We also studied if current root growth activity, determined as the number of white roots on the plug surface, can be used to predict frost hardiness. Seedlings from the same seed lot were cultivated at an inland and a coastal nursery, the former having colder winters than the latter. Cultural practices in both nurseries were similar. On seven dates from mid-summer 2000 to mid-spring 2001, frost damage was assessed by shoot mortality and electrolyte leakage after freezes to either $-12\text{ }^{\circ}\text{C}$ or $-19\text{ }^{\circ}\text{C}$. Potential drought tolerance, root growth capacity, plant morphology, shoot nutrient and soluble sugar concentration were determined in late fall and in mid-winter. Seedlings were transplanted at an inland site in late fall and in mid-winter and their survival and growth was measured for 2 years. Coastal plants cold hardened later in the fall and dehardened earlier in the spring than inland seedlings. In the fall and in early spring, coastal plants had greater frost damage and shoot mortality than inland seedlings at both -12 and $-19\text{ }^{\circ}\text{C}$. In mid-winter, frost damage and shoot mortality after $-12\text{ }^{\circ}\text{C}$ did not differ between nurseries but was greater in coastal plants after $-19\text{ }^{\circ}\text{C}$. Inland plants had lower osmotic potential either at full turgor or at the turgor loss point suggesting they were potentially more drought-tolerant. Inland seedlings were bigger, concentrated more shoot N and soluble sugars, and had higher specific leaf mass and root growth capacity than coastal seedlings. Current root growth activity had the same seasonal variation pattern at both locations and did not correlate with frost hardiness. Nursery location did not affect transplanting mortality. However, inland seedlings had greater growth than coastal seedlings when planted in mid-winter planting but not in late fall. This study demonstrates that differences in winter conditions in the nursery have a strong effect on the functional and transplanting performance of *Q. ilex* seedlings and that current root growth activity cannot be used as a predictor of the frost hardiness in this oak.

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1. Introduction

Drought has an important role in the functioning of Mediterranean woodlands. However, cold stress in Mediterranean continental areas also imposes severe restrictions to plant life (Mitrakos, 1980). Afforestation in Mediterranean climate is done during the cold season. Therefore, it is an important requisite to use cold-resistant seedlings when plantations are made in Mediterranean continental areas, especially if ever-green species are used.

Environmental conditions and cultural practices in the nursery have a strong influence on the functional characteristics

Abbreviations: ϵ , modulus of elasticity (MPa); FD_{-12} , frost damage after a frost cycle to $-12\text{ }^{\circ}\text{C}$ (%); FD_{-19} , frost damage after a frost cycle to $-19\text{ }^{\circ}\text{C}$ (%); Ψ_{mtp} , osmotic potential at the turgor loss point (MPa); Ψ_{m100} , osmotic potential at full water saturation (MPa); SLM, specific leaf mass (mg cm^{-2}); SVI, stem volume increase (ml); T_{m} , mean air temperature ($^{\circ}\text{C}$); T_{min} , minimum air temperature of the coldest month ($^{\circ}\text{C}$)

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of plants and consequently on their transplanting performance (van den Driessche, 1991; Villar-Salvador et al., 2004a). Air temperature and daylength affect seedling growth in the nursery, and their reduction at the end of summer triggers cold acclimation in many temperate species (Sakai and Larcher, 1987; van den Driessche, 1991; Arnott et al., 1993). The rate of temperature diminution during cold acclimation determines the degree of cold hardiness of plants (Fernández et al., 2003; Greer and Robinson, 1995). In parallel to cold acclimation, many plant species also increase their drought tolerance by changes in the cell wall elasticity and the osmotic potential at full turgor (van den Driessche, 1989; Valentini et al., 1990). These variations may increase the potential of plants to maintain photosynthesis and growth under water stress conditions (Turner, 1986).

Reduction in temperature depresses root growth (Lopushinsky and Max, 1990). The number of white roots, which is a measure of current root growth activity, declined during the coldest months in *Pinus pinaster* seedlings overwintering in the nursery (Fernández and Pardos, 1995). In *Quercus ilex* seedlings, the seasonal variation in the number of white root roots on the plug surface was positively correlated with air temperature (Corchero de la Torre, 1998). These previous studies suggest that current root growth activity might be a estimator of cold hardiness in container-grown seedlings.

Winter temperature in the nursery depends on its location. Frost hardiness in *Pinus radiata* and *Pseudotsuga menziesii* seedlings was greater in nurseries that experienced more frost days than nurseries located in mild-winter areas (Menzies et al., 1981; Schuch et al., 1989). Pardos et al. (2003) found differences in several functional attributes between *Quercus ilex* seedlings cultivated inside a glasshouse and outdoors and they suggested that temperature was the main factor influencing these differences. Many Spanish forest nurseries are located in mild-winter sites like those close to the Mediterranean Sea. Reforestation projects of inland Iberian Peninsula, where frosts are frequent and severe, frequently use plants grown at these nurseries. It has been suggested that low cold hardiness of the seedlings produced in mild-winter areas could be one of the reasons for the poor performance of many plantations in Mediterranean continental areas. However, this topic has received little attention (Pardos et al., 2003).

Quercus ilex L. (holm oak) is a late succession evergreen tree that dominates many types of woodland in the western Mediterranean basin. It is widely used for the afforestation of both abandoned croplands and woodlands but it often has poor transplanting performance compared with other forest species (Baeza et al., 1991). In this study, we examined if (1) *Q. ilex* seedlings cultivated in sites with different winter temperatures have distinct functional and transplanting performance, and (2) the seasonal variation of root growth activity is correlated with frost hardiness. To achieve these objectives we grew seedlings in two nurseries placed in sites with different winter temperatures. At both nurseries, we used the same seed lot, container type, growing medium and fertilisation regime, and the seedlings were well watered during the study period. Frost hardiness, shoot water relations, root growth capacity,

morphology, and transplanting performance were assessed. We tested the hypothesis that plants grown in a nursery that experiences cold winters would have higher stress resistance, vigour, and transplanting performance than the seedlings cultivated in a nursery that experiences mild-winter conditions.

2. Material and methods

2.1. Study sites and plant cultivation

Two nurseries with different winter conditions were selected. The first nursery (inland nursery) was located in the Centro Nacional de Mejora Forestal “El Serranillo” (Guadalajara, centre of Spain, 40°39'N, 3°19'W) at 650 m a.s.l. The second nursery (coastal nursery) was located in the Centro de Investigaciones sobre la Desertificación (Albal, eastern Spain, 39°20'N, 0°26'W), at 20 m a.s.l. and close to the Mediterranean Sea. The inland nursery has a typical continental Mediterranean climate with cold winters and very hot and dry summers. The coastal nursery experiences milder winter and summer conditions than the inland nursery. Historical weather records indicate that the inland nursery has 73 days of frost per year and a mean minimum air temperature in January (T_{\min}) of -1.4°C . The coastal nursery typically has 9 days of frost and T_{\min} is 4.9°C . During the study period (2000–2001), winter season was milder than average historical record but differences between locations still remained (Fig. 1). During the summer, mean air temperature (T_m) at the inland nursery and coastal nursery were similar. Main T_m differences between locations occurred in winter and spring. The inland nursery had lower T_{\min} than the coastal nursery during all the studied period.

In early March 2000, acorns of *Q. ilex* subsp. *ballota* from La Mancha provenance (inland Spain) were sown in Forest Pot 300 containers[®] (Nuevos Sistemas de Cultivo S.L., Girona,

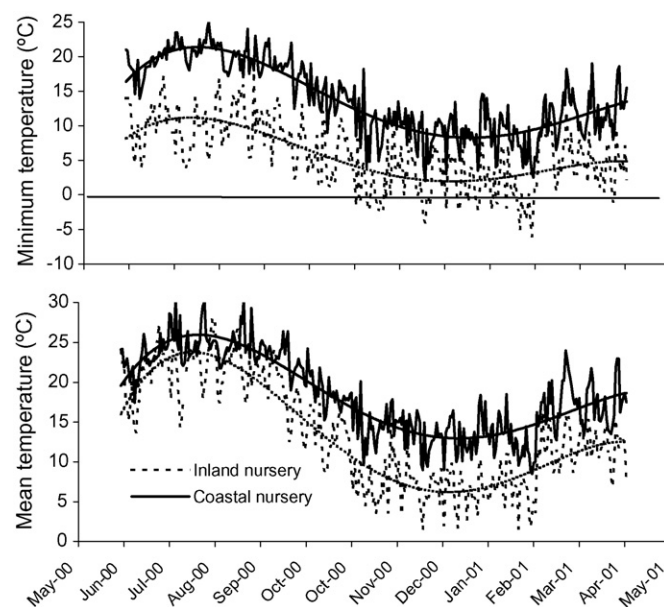


Fig. 1. Time course of daily minimum and mean air temperature during the study period (July 2000–April 2001) in the coastal and inland nursery.

Spain). This container has 50 cavities of 300 ml and it is 18 cm deep with a growing density of 387 plants m^{-2} . Three days after sowing half of the containers were taken to the coastal nursery. Growing medium was a fertilized light peat (Original Kasper B6). Nutrients were supplied as a N–P–K (15–7–15) slow release fertiliser mixed in the peat (1 kg per m^3 of peat), which provided 34 mg N, 16 mg P and 34 mg K per seedling. Plants did not receive any extra fertilisation during the rest of the experiment. At both nurseries, seedlings were irrigated when the weight loss of four randomly selected containers exceeded 20–25% their maximum weight at saturation. Inland seedlings were grown in a greenhouse until late May 2000 to avoid frost damage and then raised outdoors under full sun for the rest of the experiment. This common practice in many Spanish inland nurseries extends the growing period permitting plant stock to achieve optimum size for afforestation. Coastal seedlings were cultivated outdoors during all the study.

2.2. Frost damage

On seven dates from July 2000 to April 2001, we compared the resistance to frost of the seedlings grown in both nurseries. On each date, two groups of eight seedlings per nursery location were randomly selected. The first group was subjected to a frost of $-12\text{ }^{\circ}\text{C}$ and the second group was subjected to a frost of $-19\text{ }^{\circ}\text{C}$. Seedlings were transplanted into Styrofoam containers to isolate root systems from frost and placed in a freezer. Temperature was reduced at a rate of $3\text{ }^{\circ}\text{C h}^{-1}$ to either $-12\text{ }^{\circ}\text{C}$ or $-19\text{ }^{\circ}\text{C}$ where it remained for 3 h and then it was raised to $10\text{ }^{\circ}\text{C}$ at a rate of $4\text{ }^{\circ}\text{C h}^{-1}$. Frost damage was measured by electrolyte leakage (Earnshaw, 1993) and shoot mortality. For electrolyte leakage two leaves per plant were cut in square pieces of 2–3 mm and washed twice in distilled water for 10 min. They were then placed in a vial with 20 ml distilled water, placed on an illuminated bench at laboratory temperature and periodically shaken. Frost damage was determined as:

$$\frac{EC_i}{EC_f} \times 100$$

where EC_i is the electroconductivity of the water bathing the leaf pieces after 24 h and EC_f , the electroconductivity of the same water after autoclaving the vials for 10 min at $110\text{ }^{\circ}\text{C}$. Autoclaving produces tissue destruction and complete release of cell solutes. After frost damage assessment, plants were left in a glasshouse to recover for 3 months and the number of plants with dead shoots was recorded. A plant was considered to have a dead shoot when foliage was completely brown and no resprouting occurred from the stem. Temperature inside the glasshouse was maintained above $5\text{ }^{\circ}\text{C}$.

2.3. Shoot water relations

Difference in potential drought tolerance were assessed by measuring the osmotic potential at the turgor loss point ($\Psi_{\pi\text{tlp}}$), the osmotic potential at water saturation ($\Psi_{\pi 100}$), and the modulus of elasticity (ϵ). These parameters were derived in late fall (beginning December 2000) and in mid-winter (mid-

February 2001) from pressure–volume (P – V) curves following the free-transpiration method (Koide et al., 1989). On each date, seven randomly sampled plants per nursery location were watered the afternoon before and maintained in the dark until morning shoot sampling. Shoot xylem water potential was determined with a pressure chamber (SKPM 1405/80, Skye Instruments Ltd., Powys, UK). Shoot weight at full saturation was calculated following the method described in Kubiske and Abrams (1990).

2.4. Plant morphology and tissue nutrient and soluble sugar concentration

In early December 2000 and again in mid-February 2001, 16 plants per nursery and date (four groups of four plants) were randomly sampled and immediately frozen to $-25\text{ }^{\circ}\text{C}$ until processing. Once defrosted, shoots were cut at the cotyledon insertion point and separated into leaves and stems. Total leaf area per plant was measured with an image analysis system (Dias, Delta-T Devices LTD, Cambridge, UK). Root plugs were washed from the media, rinsed in distilled water, and dried at $60\text{ }^{\circ}\text{C}$ for 48 h together with leaves and stems. Specific leaf mass (SLM) was calculated as the ratio of plant leaf mass to plant leaf area. Shoots of plants in each group were pooled separately and ground. N concentration was determined by the standard Kjeldahl procedure, while P and K were determined by vacuum inductively coupled plasma emission spectroscopy (Spectroflame D, Spectro Analytical Instruments GmbH & Co. KG, Kleve, Germany) after sample humid digestion. For shoots, soluble sugar concentration was determined in mid-February 2001 following methodology of Spiro (1966).

2.5. Root growth

We determined two features of root growth. On the one hand, we measured the capacity of roots to egress from the plug after transplanting under optimal conditions. On the other hand, we measured the seasonal root growth activity in the plug of the seedlings that remained in each nursery. For root growth capacity, in early December and in mid-February 16 seedlings from each nursery were transplanted into 3-liter pots (one plant per pot) containing perlite. Plants were randomly arranged and placed in a greenhouse. Seedlings were kept well watered by irrigating them every other day. After 21 days, plants were lifted, cleaned from the potting medium and all the new roots longer than 1 cm protruding out of the root plug were cut and oven dried at $60\text{ }^{\circ}\text{C}$ for 48 h. Root growth capacity of each plant was determined as the total mass of new roots. The seasonal root growth activity was determined on 7 days from July 2000 to April 2001 in 16 plants per nursery, by counting the number of white root tips longer than 1 cm growing on the surface of the plug.

2.6. Transplanting performance

To compare the out-planting performance of the seedlings cultivated in both nurseries we transplanted plants in early

December 2000 (fall plantation) and in mid-February 2001 (winter plantation). Planting was done at Santorcaz (Madrid, centre of Spain, 40°29'N, 3°12'W) on a flat site 850 m a.s.l. The plot had a deep clay loamy soil and was an 11-year-old abandoned wheat cropland. The historical mean annual rainfall and temperature is 490 mm and 13.1 °C, respectively. The average maximum and minimum temperatures of the hottest and coldest months are 31.5 and 0.1 °C, respectively, and summer drought typically lasts 3–4 months. The coldest frost during the winter of 2000–2001 was –10 °C and it occurred at the end of February 2001. The outplanting design consisted of four complete random blocks, each treatment (planting date × nursery location) being hand planted as a 15-seedling row within each block. Soil preparation consisted of 60 cm deep holes made with an excavator. Mortality and growth were recorded at the end of the first and second growing seasons. Stem volume increment (SVI) was used as a measure of plant growth in the field. SVI was calculated from the height and diameter of the stem as the product of plant height and 1/3 basal area. Diameter was measured immediately above the cotyledon insertion points. Height and diameter were measured up to the nearest 0.1 and 0.01 cm, respectively. The plot was not irrigated and weeds around each plant were controlled by hoeing and herbicides.

2.7. Data analyses

We used a two-way general linear model ANOVA to analyse differences between nursery locations with the main factors as nursery location and date. Mortality frequency differences between locations after frost cycles were analysed by the Yates corrected Chi-square test. Field transplanting mortality and

growth were analysed by a repeated measures ANOVA where the sum of squares of interactions between block and the other factors (planting date and nursery location) were pooled with the error sum of square. Relationship between frost damage and the number of white root tips was analysed by correlation. A stepwise multiple regression analysis was done to analyse the contribution of the mean air temperature the preceding 15 days and daylength to frost damage variation. Non-linear regression was used to analyse the relationship between frost damage after –12 °C and the mean air temperature in each location. We considered results significant when P -values were ≤ 0.05 . Statistical analyses were performed with STATISTICA 6.0 (StatSoft, Inc., Tulsa, OK, USA).

3. Results

3.1. Frost damage

Frost damage after freezing to –12 °C (FD₋₁₂) was less than following freezing to –19 °C (FD₋₁₉), especially in mid fall and in winter. For both nursery locations, FD₋₁₂ and FD₋₁₉ declined through the fall to a minimum value in early January (Fig. 2). Plants cultivated in the coastal nursery had significantly higher FD₋₁₂ and FD₋₁₉ during the fall and winter than the seedlings raised in the inland nursery, but no differences were observed either in summer or in mid-spring (location × date, $P < 0.001$). Inland seedlings reduced FD₋₁₂ and FD₋₁₉ earlier in the fall than coastal plants. In the spring, FD₋₁₉ of coastal seedlings increased earlier than in inland plants but this response was not observed with FD₋₁₂.

In July and April, seedlings from both locations had very high mortality (>90%) when subjected to either –12 °C or

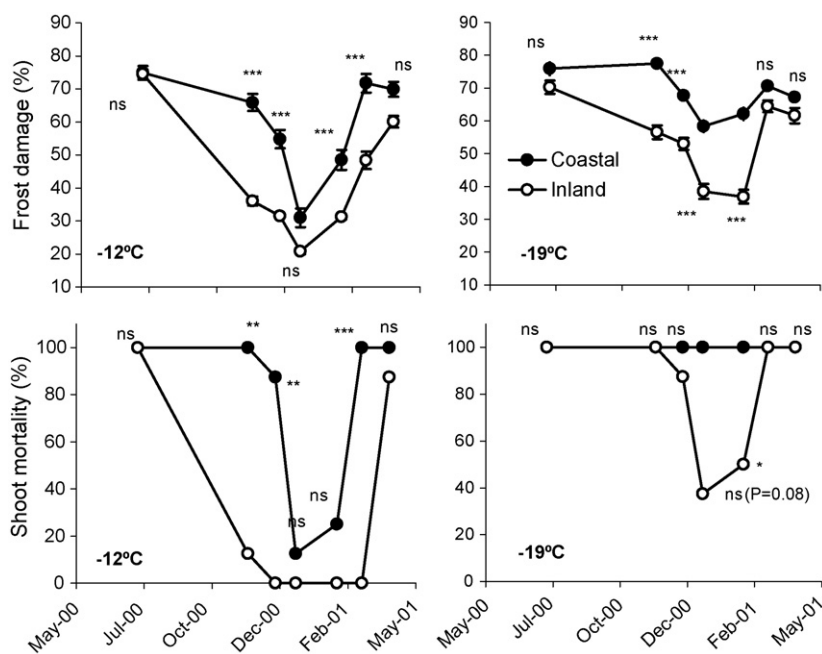


Fig. 2. Variation of frost damage (upper figures) and shoot mortality (lower figures) in *Quercus ilex* seedlings cultivated in a coastal and an inland nursery after subjecting them to frost cycles of –12 °C (left figures) and –19 °C (right figures). The datum from each point has obtained from eight plants. Vertical bars are ± 1 standard error.

–19 °C. Time course of mortality after –12 °C freezing followed a similar trend as for FD_{-12} , being very high in summer and in spring, and minimum in winter (Fig. 2). Shoot mortality after freezing of the inland seedlings declined earlier in autumn and increased later in spring than did the mortality of coastal plants. After freezing to –12 °C, coastal seedlings had significantly higher mortality than inland seedlings in the fall and in early spring. Inland plants had no mortality from December to mid-February and mortality in coastal plants remained lower than 30% from early January to mid-February. In mid-winter, no significant mortality differences after –12 °C freezing existed between nurseries.

After freezing to –19 °C, coastal plants had 100% mortality on all dates. Mortality in the inland seedlings was 100% in summer, in early fall and in spring. However, it remained less than 50% in mid-winter and the difference between nurseries for this period was statistically significant (Fig. 2).

Stepwise multiple regression showed that the mean air temperature of the preceding 15 days but not daylength had a significant contribution to explain FD_{-12} variation ($\beta = 0.68$, $P = 0.006$ and $\beta = 0.26$, $P = 0.21$ for temperature and daylength, respectively, model $r^2 = 0.81$, $F = 23.1$, $P > 0.001$). However, the relationship between FD_{-12} and the mean air temperature of the preceding 15 days was different in both nurseries. Reduction in FD_{-12} with temperature decrease was steeper in the coastal nursery than in the inland nursery. In both cases, no reduction in FD_{-12} was observed until mean temperature of the 15 preceding days was lower than 17 °C (Fig. 3).

3.2. Shoot water relations

$\Psi_{\pi 100}$ and $\Psi_{\pi tlp}$ were lower in mid-February than in early December. In both dates, inland seedlings had significantly lower $\Psi_{\pi tlp}$ and $\Psi_{\pi 100}$ than coastal nursery seedlings. Maximum difference in $\Psi_{\pi tlp}$ and $\Psi_{\pi 100}$ were 0.38 and 0.25 MPa,

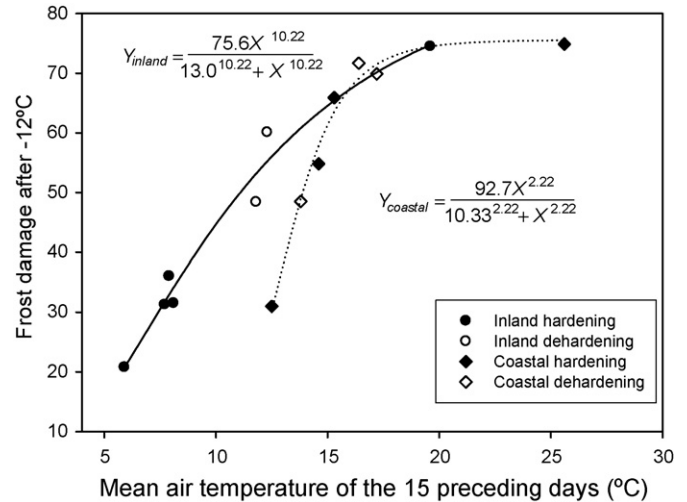


Fig. 3. Relationship between the frost damage measured at –12 °C and the mean air temperature of the 15 preceding days in *Quercus ilex* seedlings cultivated in a coastal (dotted line) and an inland nursery (solid line). Frost damage in each point is the mean of eight plants. Determination coefficients of both fitted models were 0.99, $P < 0.001$.

respectively. Nursery location and measuring date did not affect ε (Table 1).

3.3. Morphology and tissue nutrient concentration

Plant mass and SLM increased from early December to mid-February. Inland plants had greater mass and SLM than the coastal seedlings. Plant mass differences between locations were attributed to differences in root mass but not in shoot mass (data not shown). Nursery location and measuring date did not affect mean leaf size (Table 1).

Inland plants concentrated more N in shoots than coastal plants, these differences being greater in mid-February than in

Table 1

Water relations, plant morphology, shoot nutrient concentration, and root growth capacity in *Quercus ilex* seedlings cultivated in an inland and in a coastal nursery. Parameters were determined on two dates along the fall and winter. Data are means ± 1 standard error. F -values and significance from the ANOVA are given in the three columns of the right. On each sampling date, statistically significant differences ($P \leq 0.05$) between locations are indicated with an asterisk. $\Psi_{\pi 100}$: osmotic potential at water saturation, $\Psi_{\pi tlp}$: osmotic potential at the turgor loss point, ε : modulus of elasticity, SLM: specific leaf mass, ns: $P > 0.05$

	Early December		Mid-February		Nursery	Date	Nursery \times date
	Inland	Coastal	Inland	Coastal			
$\Psi_{\pi 100}$ (MPa)	-2.68 ± 0.06	-2.57 ± 0.05	-2.99 ± 0.08	-2.74 ± 0.07	10.1**	184.4***	2.21 ns
$\Psi_{\pi tlp}$ (MPa)	-3.50 ± 0.09	-3.34 ± 0.10	-3.91 ± 0.09	-3.53 ± 0.10	7.53*	39.9***	0.078 ns
ε (MPa)	14.3 ± 1.30	13.0 ± 1.61	13.8 ± 1.29	14.6 ± 1.14	0.01 ns	0.35 ns	0.25 ns
Plant mass (g)	7.06 ± 0.26	6.41 ± 0.36	8.82 ± 0.31	7.33 ± 0.55	7.57*	11.93**	1.17 ns
Mean leaf area (cm ²)	3.57 ± 0.16	3.28 ± 0.21	3.11 ± 0.45	3.74 ± 0.10	3.03 ns	0.001 ns	0.39 ns
SLM (mg cm ⁻²)	19.1 ± 0.19	18.8 ± 0.41	21.7 ± 0.19	19.4 ± 0.84	7.25*	9.54**	3.69 ns
Shoot N (mg g ⁻¹)	8.87 ± 0.47	7.90 ± 0.44	9.00 ± 0.29	6.92 ± 0.24	31.9***	0.64 ns	7.58*
Shoot P (mg g ⁻¹)	0.57 ± 0.05	0.60 ± 0.01	1.02 ± 0.07	1.02 ± 0.02	1.1 ns	106.1***	1.9 ns
Shoot K (mg g ⁻¹)	4.65 ± 0.59	5.95 ± 0.30	2.85 ± 0.50	3.00 ± 0.27	1.22 ns	21.0***	2.31 ns
Shoot soluble sugars (mg g ⁻¹)			94.5 ± 3.2	64.9 ± 1.4	t -Student = 8.41***		
Root growth capacity (mg)	35.4 ± 9.4	8.9 ± 2.66	46.7 ± 19.9	7.5 ± 2.6	8.61**	0.19 ns	0.32 ns

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

early December (interaction nursery location × date, Table 1). Nursery location had no effect on shoot P and K concentration, but shoot P concentration was higher in February than in December while shoot K was lower. Soluble sugars in shoots were 46% higher in the inland seedlings than in the coastal plants.

3.4. Root growth

Root growth capacity of the inland plants was, on an average, 5.1 times higher than that of the coastal seedlings. No differences in root growth capacity were observed between December and February (Table 1).

Number of white root tips on the surface of the root plug changed over the study period ($P < 0.001$). It increased from summer to mid-November and then declined to early January, remaining very low through early spring. Nursery location had no influence on the number of white root tips and FD_{-12} was not correlated with the number of white roots in any location (Fig. 4). Similarly, the number of white roots was not correlated with either the mean temperature of the preceding 15 days (Inland: $r = 0.21$ $P = 0.65$, Coastal: $r = 0.17$ $P = 0.72$).

3.5. Transplanting performance

Transplanting mortality and SVI increased over the first 2 years after planting ($P = 0.001$). Seedlings transplanted in

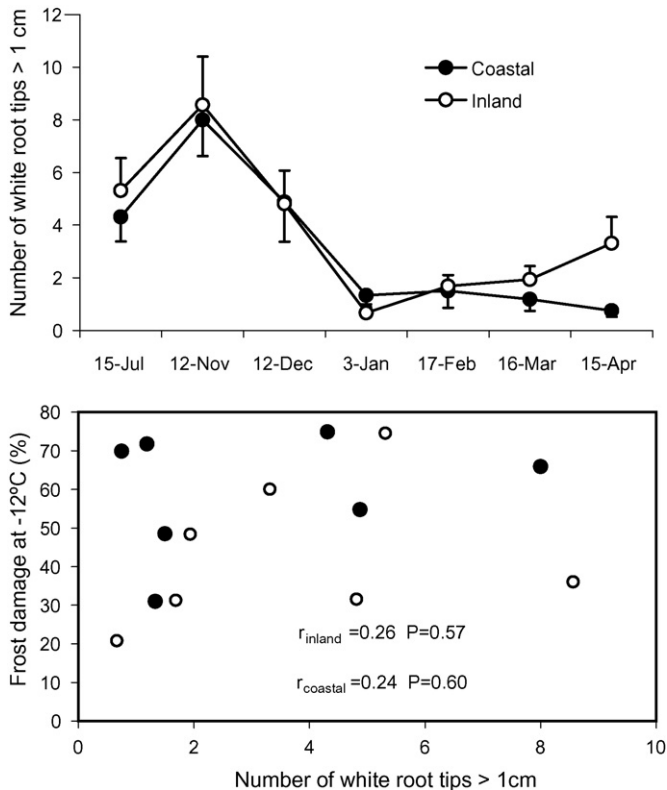


Fig. 4. Seasonal variation in the number of white root tips on the surface of the root plugs (upper figure) and relationship between the number of white root tips and frost damage measured at -12°C (lower figure) in *Quercus ilex* seedlings cultivated in a coastal and an inland nursery. Each point is the mean of 15 plants. Vertical bars are 1 standard error.

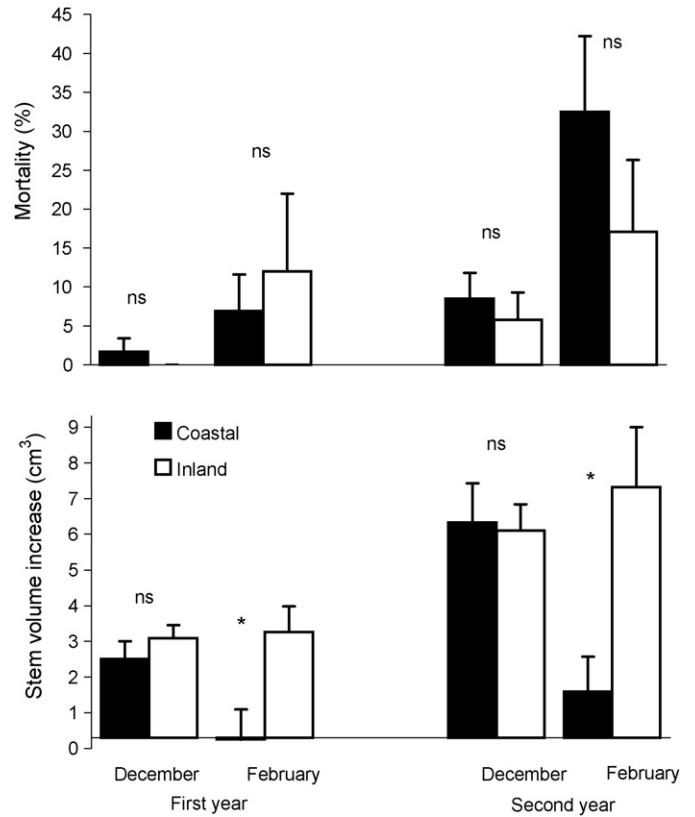


Fig. 5. Shoot mortality and stem volume increase at the end of the first and second year after transplanting in *Quercus ilex* seedlings cultivated in a coastal and an inland nursery. Plants were transplanted in early December and in mid-February. Data are means ± 1 standard error.

mid-February had significantly higher mortality ($P = 0.02$) than seedlings transplanted in December, independently of nursery origin. After the second growing season, inland plants had, on an average, 11% less mortality than the coastal plants but this difference was not significant (Fig. 5). However, SVI of the inland plants was significantly greater than the SVI of the coastal seedlings in the mid-February planting but not in the early December planting (Location × Planting date, $P = 0.048$). After the first growing season, SVI of the coastal nursery plants transplanted in February was negative and after the second growing season, it remained 4.2 times lower than the SVI of the inland nursery plants. At the end of the first growing season, 21% of coastal seedlings had partial shoot dieback while partial shoot dieback only affected 4% of inland plants ($P = 0.01$). Consequently, shoot size of coastal plants was at least 20% smaller than their initial shoot size at transplanting.

4. Discussion

Nursery location had an important influence on several functional traits of *Q. ilex* seedlings. In accordance with our hypothesis, plants cultivated in the inland nursery had higher frost resistance, were potentially more drought-tolerant and had greater root growth capacity than the plants grown in the coastal nursery. Besides, inland nursery seedlings concentrated more N and soluble sugars than coastal nursery plants.

4.1. Physiological differences between locations

The seasonal pattern of cold acclimation in the seedlings of both nurseries was similar to the pattern followed by other temperate species (see Sakai and Larcher, 1987; Gansert et al., 1999; Bigras et al., 2001). Cold hardening increased in the fall reaching a maximum level in the winter and then it decreased through the spring. However, cold acclimation of the two locations differed in two aspects. First, inland seedlings hardened earlier in the fall and dehardened later in the spring than coastal plants. The cold hardening delay of coastal seedlings was greater than the cold dehardening delay of inland seedlings. Second, maximum cold hardiness of inland plants was greater than the maximum cold hardiness of coastal plants. Consequently, mid-winter mortality of coastal plants after a -19°C frost was 100% whereas it was only 40% in the inland seedlings (see Fig. 2). Many studies have shown that soluble sugars have a prominent role in the frost resistance of plants, higher sugar concentration being associated with greater cold hardiness (Ögren et al., 1997; Uemura and Steponkus, 2003). Consistent with these studies, we observed that inland seedlings had 46% more soluble sugars than coastal plants.

Cold hardening differences related to nursery location have been also reported in *Pinus radiata* and *Pseudotsuga menziesii* (Menzies et al., 1981; Schuch et al., 1989). In these studies temperature, daylength and number of frosts appeared to be the main environmental factors explaining the differences among locations. Sakai (1970) observed that *Salix babylonica* cuttings from the same tree had greater cold hardiness when transplanted to locations with colder winters along a latitudinal gradient. We consider that the cold hardiness differences between nurseries in our study can be largely attributed to the strong differences in winter temperature between locations. This idea is supported by the strong relationship between FD_{-12} and mean air temperature of the 15 previous days (Fig. 3). However, sensitivity of FD_{-12} to temperature variation was greater in the coastal nursery than in the inland nursery. Bearing in mind that both locations experience chilling temperature at different dates and therefore at different daylength, our results suggest that the effectiveness of temperature on cold hardening in *Q. ilex* might depend on daylength. Christersson (1978) observed that short days and mild temperature had similar cold hardening effects in *Pinus sylvestris* and *Picea abies* than long days and low temperature. Daylength differences among locations in our study were probably too small to explain the frost hardiness differences in our study. Maximum difference between locations was 12 min in summer and winter solstice. Nursery cultural practices can also influence cold acclimation (Arnott et al., 1993; Bigras et al., 2001). However, we consider that cultural practices have not accounted for the cold acclimation differences because they were similar in both locations and plants remained outdoors under full sun during most of the year.

Low soil temperature can induce water stress due to limited root growth and higher water uptake resistance (Pavel and Fereres, 1998). Therefore, seedlings planted in cold sites should be drought tolerant. Nursery location not only affected cold

hardiness but also affected potential drought tolerance. Inland seedlings had lower $\Psi_{\pi\text{tlp}}$ and $\Psi_{\pi100}$ than coastal nursery seedlings (see Table 1). Plants with lower $\Psi_{\pi\text{tlp}}$ have the potential to maintain gas exchange and cell elongation at lower water potentials (Bennett and Sullivan, 1981; Turner, 1986). Similarly, protoplasm dehydration tolerance in *Q. ilex* is enhanced with $\Psi_{\pi100}$ reduction (Villar-Salvador et al., 2004b). Therefore, the lower $\Psi_{\pi\text{tlp}}$ and $\Psi_{\pi100}$ in inland seedlings should confer them an advantage over coastal seedlings if they experience water stress immediately after transplanting. Similar to our findings, *Pseudotsuga menziesii* seedlings had lower $\Psi_{\pi\text{tlp}}$ and tolerated greater dehydration when cultivated at 8°C than when cultivated at 25°C (van den Driessche, 1989). Reduction of root temperature during cultivation produced similar changes in water relations in *Chamaecyparis nootkensis* (Grossnickle and Russell, 1996). Changes in $\Psi_{\pi\text{tlp}}$ can be attributed to variations in $\Psi_{\pi100}$ or ε or both (Turner, 1986). However, nurseries did not differ in ε so the differences observed in $\Psi_{\pi\text{tlp}}$ can be attributed to differences in $\Psi_{\pi100}$. Accumulation of K, soluble sugars and organic compounds like aminoacids contribute to the reduction in $\Psi_{\pi100}$ (Épron and Dreyer, 1996; Hare et al., 1998). In this study, inland plants had higher shoot N and soluble sugar concentration than the coastal plants, which might explain their differences in $\Psi_{\pi100}$. Villar-Salvador et al. (2004b) found no relationship between $\Psi_{\pi100}$ and shoot K in drought-conditioned *Q. ilex* seedlings, suggesting that K has no role in $\Psi_{\pi100}$ variation. The lack in shoot K differences between locations in this study supports this contention.

4.2. Morphological and root growth differences between locations

Seedlings of the inland nursery were bigger than the plants of the coastal nursery in spite of that the growing season in the inland location is shorter than in the coastal location. This result could be partially explained by the cultivation of the inland seedlings in a greenhouse the first 3 months, a practice that is common in several inland Spanish nurseries. SLM also was higher for the inland seedlings (see Table 1). In a within-species comparison, Körner et al. (1986) observed greater SLM in plants thriving under colder conditions at higher altitude than plants living at milder conditions at lower altitude. Differences in SLM can be attributed to differences in leaf thickness or density, or both (Witkowski and Lamont, 1991). Because low temperature hinders shoot growth in winter, differences in SLM probably reflect changes in leaf density rather than an increase in leaf thickness. In our study inland nursery seedlings had higher N and soluble sugar concentration than coastal plants (see Table 1), which likely have contributed to increase tissue density.

Plants of both nurseries had similar current root activity in spite of the great temperature differences between locations. Moreover, root activity in both nurseries was not correlated with either frost hardiness or air temperature. In contrast to our study, Corchero de la Torre (1998) found that the number of white roots was positively correlated with the minimum air

temperature of the previous 15 days. The lack of correlation between current root growth activity and air temperature in our study explains the absence of relationship between root growth activity and frost hardiness and suggests that root growth and cold hardiness in this species do not have the same environmental controls.

In contrast to current root activity, root growth capacity differed between nursery locations, which might be explained by the distinct environmental conditions experienced by the plants in the root growth capacity tests and in the nurseries. Root growth capacity has been used as a predictor of the transplanting performance of forest species because it measures the functional vigour of seedlings and it is frequently positively correlated with their cold hardiness (see Ritchie and Dunlap, 1980; Simpson, 1990). Our results agree with these previous records as inland plants, which had greater frost resistance than coastal seedlings, had also greater root growth capacity. Similar to our findings, Pardos et al. (2003) observed that *Q. ilex* seedlings had greater root growth after exposure to low temperature. However, it is unclear if there is causal relationship between frost hardiness and root growth capacity (Simpson, 1990). The differences between locations in root growth capacity could be due to their differences in shoot N, soluble sugar concentration, or plant size.

4.3. Transplanting differences between locations

After two growing seasons, out-planting performance of plants from the two nurseries differed. Although mortality differences were not significant, inland-nursery seedlings grew more than coastal plants, which accords with their greater cold hardiness, potential drought tolerance, and root growth capacity. Growth difference in the field between nurseries occurred for the mid-winter planting but not in the late-fall planting (Fig. 4). Growth difference was significant after the first growing season and it remained after the second growing season. In three conifer species, reduction in temperature during their cultivation tended to increase their transplanting survival but no clear trend was observed in transplanting growth (van den Driessche, 1991). The lack of differences in mortality and growth between nursery locations in the fall planting may be attributed to the mild temperatures in the first part of the winter after transplanting. The lowest temperature recorded from early December 2000 to mid-February 2001 was -5.6°C , which is not low enough to damage holm oak seedlings, even for plants grown in a coastal nursery. The lowest temperature recorded in the field plot was -10°C on February 26, 5 days after the winter planting. This frost might have damaged coastal plants more than inland seedlings in the winter planting, reducing their growth in spring. This assertion is supported by the higher shoot dieback in coastal plants than in inland seedlings. Coastal plants transplanted in fall were not affected by this frost because they had probably acclimated to cold in the field.

Our conclusions are that *Q. ilex* seedlings of the same seed lot but grown in nurseries that experience mild conditions during the fall and winter have less vigour (lower root growth

capacity) and are less frost resistant and potentially less drought tolerant than plants cultivated in nurseries placed in cold winter areas. This can impair their transplanting performance when planted to cold winter areas. Finally, the number of white root tips on the plug surface cannot be used as a predictor of the cold hardiness of *Q. ilex* seedlings.

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