

Effects of acorn storage duration and parental tree on emergence and physiological status of Cork oak (*Quercus suber* L.) seedlings

Hachemi Merouani*, Carmen Branco, Maria Helena Almeida and João S. Pereira

Instituto Superior de Agronomia, Departamento de Engenharia Florestal,
Tapada da Ajuda, 1399 Lisboa Codex, Portugal

(Received 10 August 2000; accepted 12 January 2001)

Abstract – This study was conducted to evaluate how parental trees and seed storage duration influenced subsequent seedling physiological status and growth. Seedling emergence rate was higher than 90% independently of the duration of seed storage or parental trees. Seed storage shortened significantly the time and increased the uniformity of seedling emergence. Consequently, the delayed seedling emergence from fresh seeds could be explained by epicotyl dormancy. Seed size varied with parental tree. Seedling growth rate was greatly affected by seed size, independently of storage treatment. Seedlings originating from large seeds (>5 g) had the fastest growth rates and seedlings from the smallest seeds (<4 g) had the slowest. Final shoot height, however, depended on the duration of seed storage. The seed size and the duration of storage had a great effect on the initial rate of leaf production, but did not affect the final number of leaves. Leaf chlorophyll concentration was reduced as the duration of seed storage increased but was independent of parental tree (i.e., seed size). Seedling biomass was positively related to seed size. The duration of seed storage reduced the shoot/root ratio, but no significant effect was observed among parental trees. The shoot/root value of seedlings from stored seed was about 1.5 and the one of seedlings from fresh seed was about 2.

seed storage / seed size / seedling growth / shoot / root ratio / *Quercus suber*

Résumé – Effet de l'arbre producteur et de la durée de conservation des glands sur l'état physiologique des plants de chêne liège (*Quercus suber* L.). Quel que soit l'âge des glands ou l'arbre producteur, l'émergence des plants est supérieure à 90 %. La durée et l'uniformité de l'émergence des plants sont significativement affectées par la conservation des glands ; par conséquent le retard dans l'émergence des plants issus des glands frais peut être expliqué par l'existence d'une dormance épicotyloire. La croissance des plants est rythmique : elle est caractérisée par une alternance de périodes d'allongement et de périodes de repos. Le rythme de croissance est fortement affecté par la taille des glands quel que soit leur âge. En effet, la croissance des plants issus des gros glands (>5 g) est plus rapide que celles des plants issus des petits glands (<4 g), mais la hauteur finale dépend de l'âge des glands. La taille des glands et leur conservation affectent fortement le rythme d'apparition des feuilles mais pas le nombre final. La concentration en chlorophylle des feuilles diminue chez les plants issus des glands conservés quel que soit l'arbre producteur. La biomasse des différentes parties du plant est réduite pour les petits glands conservés. La conservation des glands influe sur le rapport système aérien/système souterrain, mais aucun effet de l'arbre producteur n'est observé. Sa valeur est de 1,5 pour les plants issus des glands conservés et de 2 pour ceux issus des glands frais.

conservation des glands / taille des glands / croissance des plants / rapport système aérien/système souterrain / *Quercus suber*

* Correspondence and reprints
Tel. +351 21 365 33 84; Fax. + 351 21 364 50 00; e-mail: hmerouani@isa.utl.pt

1. INTRODUCTION

Cork oak (*Quercus suber* L.) has great social and ecological importance in the Mediterranean region. In many cases, however, natural regeneration is impeded by the biotic and abiotic factors of the forest environment [2, 21, 25, 38] as well as by grazing and management practices of the agro-forestry systems, where they exist. Due to this difficulty, the artificial regeneration may be an important alternative for the rejuvenation of Cork oak stands. In the Mediterranean region seedling establishment from direct sowing of acorns is often poor [18, 34, 40, 45] due to damage caused by rodents, for example. Other techniques were suggested for the regeneration of Cork oak stands. For example, Croizeau and Roget [21] suggested that sowing in spring pre-germinated acorns collected from the ground at the end of winter might be a solution. Nevertheless, the frequency of artificial regeneration by planting is increasing.

In Portugal, a considerable effort has been made during the last 10 years to increase the area of Cork oak stands by planting both in forestland and in abandoned arable land [34]. The rate of success has been quite variable. For example, an evaluation of the afforestation/reforestation with Cork oak by planting in Southern Portugal (Algarve) showed a large seedling mortality (higher than 50% [34]. In experimental plantations carried out to evaluate nursery techniques in southern Portugal, seedling survival varied between 40 and 93% [20, 48] as function of drought, site characteristics, seedling handling [34] and nursery practices [8].

Several studies [7, 8, 26, 31, 32, 36, 41, 44, 47] with other species indicated that the morphological and physiological quality of seedlings is one of the criteria conditioning growth and seedling performance in the field. A positive relationship between seed size and seedling establishment and growth was reported for a variety of species [23, 46], including oaks [14, 15]. A large variability in seed size is common in oak species [3, 39] and could affect seedling quality. On the other hand, it has been shown that seed storage may be a way to palliate the irregular acorn production and to maintain a regular supply of acorns to nurseries [39]. In Cork oak, however, up to now no attempt was done to explore the relationship between parental tree (often associated with seed size) and seedling growth and the effect of seed storage on the physiological status of seedlings. The objectives of this study were to evaluate how parental tree and the duration of acorn storage would influence seedling emergence and subsequent growth and physiological status.

2. MATERIALS AND METHODS

At the end of November 1998, morphologically mature acorns were collected from 12 trees at Herdade da Palma (South of Portugal). The details of the site, harvest technique and seed treatment, were described by Merouani et al. [39]. After acorn collection, the seedlots were slightly dried for 1 week at 20 °C and then stored separately in polyethylene bags (30 µm thick) at 0 °C for 6 months. The moisture content of acorns at the beginning of storage ranged between 38% to 45%. Seed size varied between parental trees and the average seed weight are shown in *table 1*.

The seeds with different storage periods, i.e., freshly collected seeds (control) and seeds with 2, 4, and 6-month storage, were sown as described by Merouani et al. [39]. After pre-germination (radicle length of 2–4 cm) the seeds were transferred to plastic containers (37 × 28 × 24 cm) filled with sand and peat (1V/1V) added with 1.5 g L⁻¹ thym solution. For each tree, 3 replicates with 4 acorns per replicate were placed in a controlled-environment growth chamber (Fitoclima 700 EDTU, ARALAB, Portugal) with temperature, light, humidity and CO₂ control. Daytime temperature was 25 °C and 18 °C at night. Photoperiod was 10 h light and 14 h dark. The relative humidity was about 65% and 350 ppm CO₂. Irradiance was on average 900 µmol m⁻² s⁻¹ at substratum level and 1300 µmol m⁻² s⁻¹ at maximum plant height. The substratum was watered every second day. The duration of the experiment was 8 weeks.

To evaluate seedling vigour and status, several morphological, physiological and biometric parameters were measured on seedlings from each seed physiological status (fresh and stored). Epicotyl emergence was recorded daily and the sowing date was considered as day 0. For each seedling, shoot height and total number of leaves were monitored weekly. At the end of the growing process and before seedling destruction for biomass analysis, two leaf discs per leaf and one leaf per seedling were removed from the young fully expanded leaves of 4 or 6 seedlings for chlorophyll concentration. Chlorophyll was extracted in the dark from leaf discs ground in a mortar with 80% acetone. The absorbencies were read at 645 and 663 nm respectively in a HITACHI U 2001 spectrophotometer.

The 8-week-old seedlings were harvested for biomass determination. Shoot length, number of leaves, stem diameter and the length of primary roots, were measured. Each seedling was separated into leaves, stem, primary root and lateral (fine) roots, oven dried for 48 h at 80 °C

Table I. Effect of parental trees and seed storage duration on the total emergence rate and the emergence precocity of seedlings.

Parental trees	Seed storage duration (months)	% of total emergence	% of emergence at different time interval after seed sowing:			
			15–20 days	20–25 days	25–30 days	> 30 days
1 (6.1 g)	0 (Fresh)	100			50	50
	2	100		50	16.7	33.3
	4	100	41.7	33.3	8.3	16.7
	6	91.7	36.4	63.6		
2 (5.6 g)	0 (Fresh)	83.3		10	70	20
	2	83.3		70	10	20
	4	100	25	25	25	25
	6	100	50	41.7		8.3
3 (3.2 g)	0 (Fresh)	100		16.7	58.3	25
	2	91.7		54.4	9.1	36.4
	4	100	8.3	66.7	25	
	6	100	25	66.7	8.3	
4 (7.0 g)	0 (Fresh)	83.3			80	20
	2	91.7		54.5	27.3	18.2
	4	100	16.7	58.3	16.7	8.3
	6	100	33.3	66.7		
5 (2.7 g)	0 (Fresh)	100		8.3	66.7	25
	2	75		22.2	33.3	44.4
	4	100	8.3	75	8.3	8.3
	6	100	58.3	41.7		
6 (3.7 g)	0 (Fresh)	100		16.7	66.6	16.7
	2	100		50	25	25
	4	83.3		40	60	
	6	75	11.1	77.8	11.1	
7 (6.6 g)	0 (Fresh)	83.3		50	40	10
	2	75		33.3	22.2	44.4
	4	100	50	25	16.7	8.3
	6	100	91.7	8.3		
8 (6.4 g)	0 (Fresh)	83.3		20	40	40
	2	100		41.7	16.6	41.7
	4	91.7	72.7	9.1	18.2	
	6	100	33.3	50	16.7	
9 (7.6 g)	0 (Fresh)	100		25	58.3	16.7
	2	91.7		45.4	36.4	18.2
	4	100	8.3	75	16.7	
	6	100	16.7	75	8.3	
10 (5.3 g)	0 (Fresh)	100			16.7	83.3
	2	100		91.7	8.3	
	4	91.7		27.3	63.6	9.1
	6	100	16.7	66.7	8.3	8.3
11 (4.5 g)	0 (Fresh)	100			83.3	16.7
	2	83.3		60	10	30
	4	66.6		62.5	25	12.5
	6	100	16.7	75	8.3	
12 (5.8 g)	0 (Fresh)	100			58.3	41.7
	2	91.7		18.2	72.7	9.1
	4	100	16.7	58.3	16.7	8.3
	6	100	25	66.7	8.3	

The value between parentheses (column 1) corresponds to the seed fresh weight.

and the dry weight of each plant part was then determined. Shoot/root ratio and root/total seedling biomass, were calculated.

A two-way analysis of variance (ANOVA) was performed to determine the effects of seed size and the duration of cold storage on the different parameters evaluated. To compare time of emergence, total stem and primary root length, total number of leaves, basal diameter, chlorophyll concentration and biomass of seedlings from the 2, 4 and 6 months stored seed with those of seedling from fresh seed, the Dunnett's test versus control was used. The Tukey's multiple comparison procedure was used to distinguish effects of parental trees.

3. RESULTS

3.1. Seedlings emergence

The rate and time of emergence of seedlings from fresh and stored seeds of the 12 parental trees are shown in *tables I and II*. Total seedling emergence was higher than 90% for all parental trees and seed physiological status (fresh or stored), except in the cases where some seedlings died just after emerging (*table I*). For all paren-

tal trees seedling emergence from fresh seed was higher than 25 days, whereas in the 4 and 6 months stored seed a high emergence rate was already observed between 15 and 20 days after sowing (*table I*). The time of epicotyl emergence was significantly reduced in stored seeds in comparison to the fresh seeds (*table II*). Although no correlation was observed between seed weight and the seedling emergence time ($r^2 = 0.008, 0.006, 0.009$ and 0.02 respectively for fresh seed and for 2, 4 and 6 months stored seed), it appears that seedling emergence varied between parental trees (*table II*). The later emergence of the seedlings from the fresh seed of tree No. 10 was significantly different ($P < 0.05$) from that of trees No. 7, 9, 3 and 6 (*table II*). Most of the seedlings (83.3%) from fresh seed of tree No. 10 emerged only after 35 days after sowing, whereas half of the seedlings of tree No. 7 emerged at 20–25 days (*table I*). However, this variability in emergence disappeared when the seeds were stored. The differences between trees after 2 ($P = 0.190$) and 4 months storage ($P = 0.298$) were not significant (*table II*).

3.2. Seedling growth and number of leaves

Figure 1 illustrates the growth rhythm of the 8-week-old seedlings. Four growth phases were distinguished

Table II. Effect of parental trees and seed storage duration on seedling emergence time (days).

Parental trees	Seed storage duration (months)			
	0 (fresh)	2	4	6
1	31.2ab (8.0)	32.2a (10.9)	25.7a* (11.7)	22.2ab* (2.4)
2	28.0ab (2.7)	27.5a (7.5)	26.9a (7.7)	22.0ab* (3.9)
3	27.3a (2.9)	31.5a (14.2)	24.2a* (2.9)	23.3ab* (2.7)
4	29.3ab (6.6)	30.9a (12.6)	24.7a* (5.1)	22.0ab* (2.6)
5	28.3ab (3.1)	30.7a (5.9)	25.7a (7.4)	20.6ab* (3.1)
6	27.4a (3.1)	31.1a (11.8)	26.3a (2.5)	21.6ab* (2.2)
7	26.2a (4.0)	33.9a (9.4)	24.7a (10.1)	18.6a* (2.2)
8	28.2ab (3.2)	34.6a (15.5)	30.8a (13.9)	22.9ab* (4.0)
9	27.5a (4.4)	30.0a (10.4)	24.4a (1.9)	23.3ab* (2.6)
10	35.8b (7.0)	24.6a* (2.0)	29.2a* (7.7)	23.8b* (3.2)
11	28.3ab (3.7)	32.5a (14.9)	24.7a (3.7)	23.2ab* (1.9)
12	29.5ab (3.6)	28.4a (2.7)	24.5a* (4.2)	23.0ab* (2.1)

The value between parentheses represents the standard deviation.

* Significant differences in seedlings emergence from stored seed with the one from fresh seed. In the same column values sharing the same letter are not significantly different.

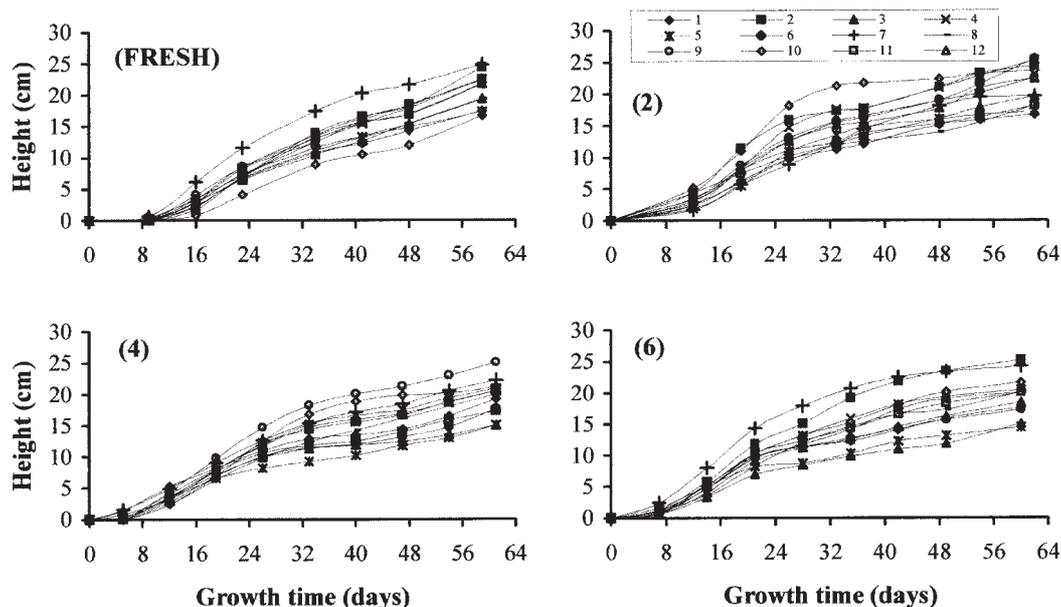


Figure 1. Growth rhythm of seedlings from pre-germinated fresh seed and stored seed for 2, 4 and 6 months. Each curve refers to the parental tree (1 to 12).

during the time of the experiment: the first, corresponding to seedling emergence (5–10 days after the start of the experiment), the second was characterised by the relative fast growing, lasting about 2 weeks. A third phase of slow growth was followed by the last phase of rapid growth, well defined for seedlings from fresh seed (*figure 1*). The growth rhythm and the duration of the third phase appear to be dependent on seed storage duration and parental tree. The increase in the number of leaves showed the same patterns described above (data not shown).

Figure 1 shows that growth in height was greatly affected by parental tree. Seedlings from seeds of trees No. 5 and 3 (small seeds) had the slowest growth rates and the ones from seeds of tree No. 7, 4, 9 and 2 (large seeds) had the highest growth rates (*figure 1*). However, seedlings from fresh seeds of tree No. 10 showed the slowest growth rate. The increment in leaf number followed the same pattern (data not shown). Therefore, the effect of parental tree on the final shoot height appears to be dependent on the seed physiological status (fresh or stored). Even though there were small differences between parental trees in the case of fresh seeds, the variability in height among parental trees increased with the duration of seed storage (*table III*). The final shoot height of seedlings originating from seeds of trees No. 5, No. 3 and No. 6 (small acorns) was significantly lower than in

seedlings issued from large acorns. In the case of fresh seed, there were differences only between trees No. 10 and No. 7 (*table III*). On the other hand, even though the seeds from trees No. 1 and No. 12 were large, they produced the shortest seedlings after 6 months of storage. For the final number of leaves, however, no significant differences were observed among parental trees as a consequence of a large variability within the population of seedlings originating from the each individual. This variation was less pronounced in seedlings from fresh seeds than from those issued from seeds stored for 6 months (*table III*).

3.3. Chlorophyll Concentration

The leaf chlorophyll concentration of seedlings from fresh and stored seed showed a non-significant variation between parental trees ($P = 0.128$), but for most parental trees it decreased significantly with the duration of seed storage especially after 4 and 6 months storage (*figure 2*).

3.4. Primary root length and stem diameter

Figure 3 shows an increase in primary root length with seed storage duration. After 6 months of storage this increase in primary root length became significant for

Table III. Effect of parental trees and seed storage duration on the final shoot height (cm) and the final number (No.) leaves of 8-week-old seedlings.

Parental trees	Seed storage duration (months)							
	0 (Fresh)		2		4		6	
	Height	No. Leaves	Height	No. Leaves	Height	No. Leaves	Height	No. Leaves
1	17.5ab (5.7)	22.8a (11.1)	15.2ab (5.0)	16.7a (5.2)	19.3ab (8.6)	22.7a (15.9)	17.6 (3.9)	17.5ab (3.4)
2	24.4ab (9.0)	28.7a (12.5)	17.7ab (6.9)	24.4a (9.9)	20.6ab (11.7)	27.0a (16.4)	25.3 (5.2)	22.1ab (4.6)
3	19.4ab (5.7)	22.3a (3.9)	16.0ab (5.5)	19.6a (6.6)	15.1a (5.1)	19.2a (5.1)	15.0 (3.4)	17.8ab (5.3)
4	24.5ab (7.8)	34.9a (20.7)	17.2ab (7.3)	25.4a (10.9)	21.0ab (6.4)	29.3a (10.5)	20.5 (4.7)	24.7a (10.5)
5	17.3ab (5.5)	21.1a (11.0)	10.6*a (5.8)	17.2a (7.5)	15.1a (2.8)	17.3a (3.9)	14.5 (3.6)	16.6b (2.7)
6	21.7ab (7.3)	25.4a (9.2)	18.3ab (5.9)	25.5a (13.8)	17.7ab (6.1)	20.2a (5.8)	17.3 (3.4)	19.9ab (3.0)
7	26.9a (6.9)	30.6a (16.8)	17.4*ab (4.1)	19.7a (4.1)	22.2ab (8.5)	23.6a (10.5)	24.2 (4.3)	22.0ab (6.4)
8	19.9ab (8.2)	22.5a (6.2)	16.4ab (7.1)	18.3a (5.2)	20.0ab (7.4)	23.6a (10.2)	20.1 (5.1)	22.2ab (7.6)
9	22.5ab (8.5)	25.8a (14.2)	21.1b (7.3)	22.6a (8.2)	25.0b (7.3)	29.2a (9.4)	20.0 (4.7)	19.5ab (4.1)
10	16.7b (7.9)	21.8a (13.9)	21.2b (5.7)	23.7a (11.1)	21.2ab (8.0)	28.8a (12.7)	21.6 (6.0)	21.3ab (6.2)
11	22.4ab (6.4)	31.9a (11.6)	17.0ab (6.6)	18.8*a (6.2)	17.3ab (4.4)	20.0*a (5.2)	20.0 (4.8)	21.3*ab (3.4)
12	21.8ab (7.7)	31.9a (17.3)	18.4ab (4.7)	24.6a (8.7)	20.5ab (4.9)	27.9a (9.9)	18.5 (3.3)	20.7ab (6.4)
Significant differences in final shoot height among parental trees						→	2 # 5, 3, 6, 1, 12; 10 # 5, 3;	7 # 5, 3, 6, 1 9 # 5

The value between parentheses represents the standard deviation.

* Significant differences in final shoot height or number of leaves of seedlings from stored seed with the ones from fresh seed. In the same column values sharing the same letter are not significantly different.

Table IV. Effect of parental trees and seed storage duration on shoot/root ratio of 8-week-old seedling.

Parental trees	Storage duration (months)							
	0 (Fresh)		2	4	6			
1	1.42b	(0.19)	1.02a*	(0.26)	1.66a	(0.46)	1.09a	(0.22)
2	1.88bcd	(0.28)	1.48a	(0.32)	1.63a	(0.60)	1.13a*	(0.17)
3	2.38bcd	(0.63)	1.43a*	(0.74)	1.86a	(0.52)	1.47a*	(0.46)
4	1.65bcd	(0.59)	1.45a	(0.36)	1.94a	(0.57)	1.41a	(0.26)
5	2.28acd	(1.09)	1.37a	(0.60)	1.76a	(0.47)	1.60a	(0.39)
6	1.48bcd	(0.35)	1.14a	(0.41)	1.71a	(0.55)	1.43a	(0.36)
7	1.94bcd	(0.16)	1.19a	(0.31)	1.40a	(0.58)	1.18a*	(0.35)
8	2.55abcd	(0.52)	1.34a*	(0.65)	2.06a	(0.89)	1.62a*	(0.42)
9	2.60a	(1.05)	1.31a*	(0.27)	1.64a*	(0.57)	1.08a*	(0.31)
10	2.13bcd	(1.01)	1.20a	(0.20)	1.69a	(0.45)	1.76a	(0.47)
11	2.16acd	(0.28)	1.14a*	(0.58)	1.79a	(0.60)	1.59a*	(0.25)
12	1.82bcd	(0.85)	1.44a	(0.33)	1.60a	(0.38)	1.41a	(0.27)

The value between parentheses represents the standard deviation.

* Significant differences in shoot/root ratio of seedling from stored seed with the one from fresh seed. In the same column values sharing the same letter are not significantly different.

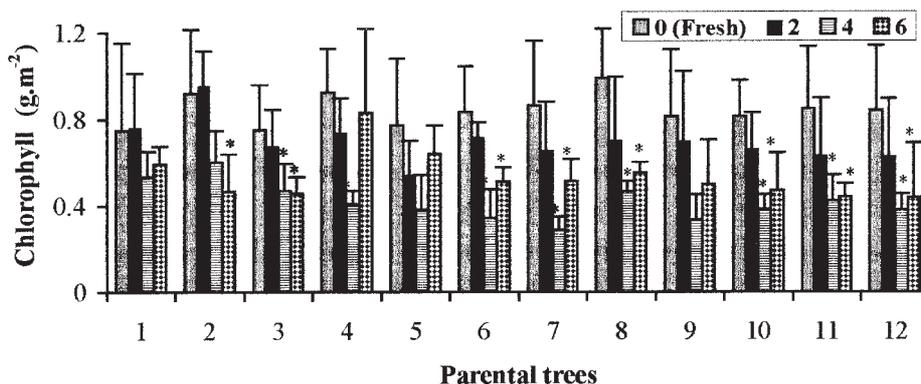


Figure 2. Effect of parental trees and seed storage duration on chlorophyll concentration of 8-week-old seedlings.
* Significant differences in leaf chlorophyll concentration of seedlings from stored seed with the one from fresh seed.

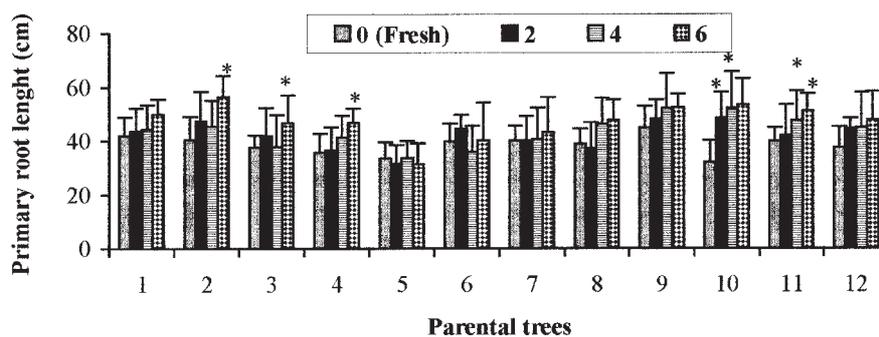


Figure 3. Effect of parental trees and seed storage duration on the primary root length of 8-week-old seedlings.
* Significant differences in primary root length of seedlings from stored seed with the one from fresh seed.

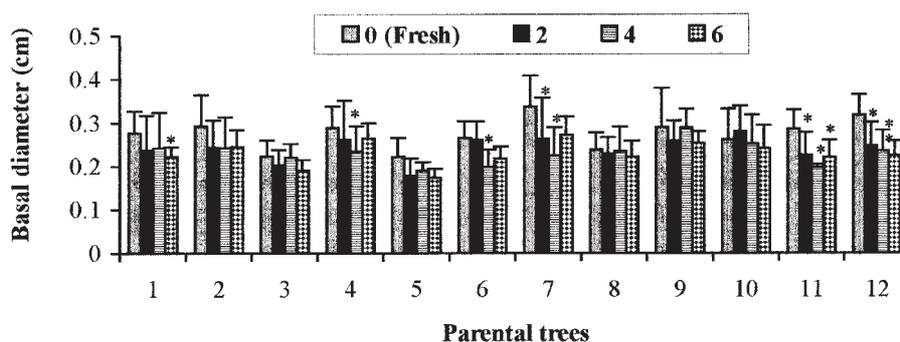


Figure 4. Effect of parental trees and seed storage duration on basal diameter of 8-week-old seedlings.
* Significant differences in basal diameter of seedlings from stored seed with the one from fresh seed.

many parental trees. Seedlings from seeds stored for 6 months of tree No. 5 and No. 6 (small seeds) had the shortest primary root and those from seeds of trees No. 2, No. 10 and No. 9 the longest (figure 3). The variation among parental trees became more important as storage duration increased.

Although, the duration of seed storage led to a decrease in seedling stem diameter for all parental trees but became significant only for trees No. 1, No. 11 and No. 12 at 6 months storage (figure 4). In general, the seedlings from the smallest seed (trees No. 5 and No. 3) had significantly lower stem diameter independently of storage time (figure 4).

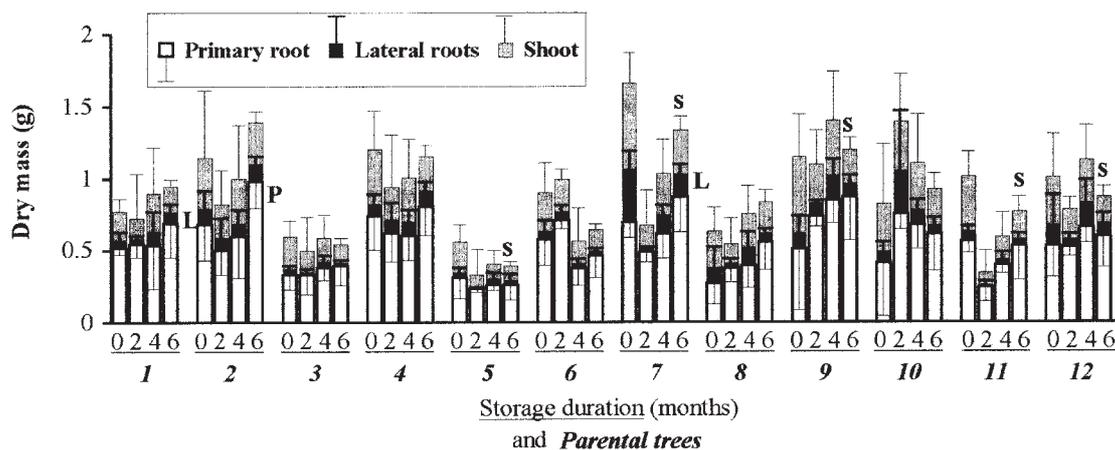


Figure 5. Effect of parental trees and seed storage duration on primary root and lateral roots and shoot biomass of 8-week-old seedlings. S, P and L represents the significant differences in shoot, primary root and laterals roots, respectively of seedlings from seeds stored for 6 months to those from fresh seeds.

Table V. Effect of parental trees and seed storage duration on Root/Total biomass ratio of 8-week-old seedling.

Parental trees	Storage duration (months)			
	0 (Fresh)	2	4	6
1	0.42a (0.04)	0.50a* (0.06)	0.38a (0.06)	0.48a* (0.05)
2	0.35ab (0.04)	0.41a (0.05)	0.40a (0.11)	0.47a* (0.04)
3	0.31ab (0.07)	0.46a (0.20)	0.36a (0.07)	0.42a (0.08)
4	0.40ab (0.11)	0.42a (0.06)	0.36a (0.10)	0.42a (0.04)
5	0.33ab (0.11)	0.44a (0.10)	0.37a (0.06)	0.39a (0.06)
6	0.41ab (0.05)	0.49a (0.13)	0.39a (0.09)	0.42a (0.06)
7	0.34ab (0.02)	0.46a (0.06)	0.44a (0.12)	0.47a (0.07)
8	0.29b (0.04)	0.47a (0.18)	0.36a (0.12)	0.39a (0.07)
9	0.30ab (0.10)	0.44a* (0.05)	0.39a* (0.08)	0.49a* (0.06)
10	0.35ab (0.12)	0.46a* (0.04)	0.38a (0.06)	0.37a (0.06)
11	0.32ab (0.03)	0.50a* (0.17)	0.37a (0.07)	0.39a (0.04)
12	0.38ab (0.10)	0.42a (0.05)	0.39a (0.06)	0.42a (0.05)

The value between parentheses represents the standard deviation.

* Significant differences in root/total biomass ratio of seedling from stored seed with the one from fresh seed. In the same column values sharing the same letter are not significantly different.

3.5. Seedling biomass

For many parental trees the seedling stem biomass decreased significantly with the duration of seed storage. On the contrary, little change was observed for the below-ground biomass (primary root and lateral roots) (figure 5). For the primary root biomass the differences

among parental trees became more important as storage duration increased, and no differences were found for stem and lateral roots biomass. In seedlings issued from seeds stored for 6 months, the primary root biomass decreased significantly for trees No. 5, No. 3, No. 6 and No. 11 (small seed) and for trees No. 10 and No. 12 (large seed).

The seedlings from fresh seed of most parental trees showed higher values of the shoot/root ratio (about 2), but those originating from stored seeds, the ratio was 1.5 in average, over all seed storage periods (*table IV*). Moreover, the seedling shoot/root ratio decreased as seed storage duration increased and became significant after 6 months storage for at least half of parental trees (*table IV*). The differences in shoot/root ratio among parental trees occurred only in seedlings from fresh seeds (*table IV*). Concomitantly, the root/total seedling biomass increased with seed storage but no significant differences were found between parental trees except the differences between trees No. 1 and No. 8 for the fresh seeds (*table V*).

4. DISCUSSION

The success of afforestation/reforestation programmes often depends upon availability and viability of seeds and seedling quality. The latter may be defined as the integration of morphological and physiological characteristics, which control the possibilities of survival and growth [8, 30]. According to Mattsson [35] however, there are still no seedling attributes predicting field performance. On the other hand, the rate and the uniformity of seedling emergence are important issues in nursery practice. In our study we found that seedling emergence rate and parameters such as shoot/root ratio, often related with growth and survival after planting, were influenced seed storage duration and parental trees in Cork oak. In this study, acorn size varied mostly with parental trees. This variation among trees of the same population is common in *Quercus* species [3, 15]. Acorn size may influence growth and survival of seedlings. Brookes and Wigston [15] showed that large acorns of *Q. petraea* and *Q. robur* have greater amounts of nutrients. Studies, on *Quercus rugosa* and *Q. laurina* showed that seedling size was significantly affected by the amount of reserves originally available in the cotyledons [14]. Therefore, the decrease in final shoot height and in stem diameter of seedlings from smallest seeds and from large seeds (trees No. 1 and No. 12) at 6 months of seed storage, could be explained by the initial amount reserves in one case, and their depletion during storage in the other case. It is known that soluble carbohydrates generally decline with seed ageing [42].

Although the percentage of seedling emergence was very high (more than 90%) and independent of seed storage duration and parental trees, the non-emergence and

the precocious mortality of some seedlings (see *table I*) was probably due to the deficiency of reserves in the acorns (cotyledons). Bonfil [14] concluded that a low amount of reserves after excision of cotyledons affect greatly the seedling survival.

The duration of seed storage affected significantly seedling emergence time and uniformity. The delay in the emergence of seedlings from fresh seeds as compared to stored seeds can be explained by the existence of epicotyl dormancy, which progressively breakdown as seed storage duration increased. This epicotyl dormancy may be related to high seed moisture content, as observed for fresh seed of tree No. 10, which was very high (about 52.84%) [39].

Cork oak seedlings grow rhythmically: after emergence the shoot elongation occurs by rapid growing lasting about 2 weeks, which alternate with resting periods. This characteristic is already known for almost all Temperate Zone species [33, 43] including oak species [4, 9, 10] in the juvenile phase.

The seedling growth rate was greatly affected by seed size, both just at harvest time (fresh seed) and after seed storage. Seedlings from large seeds (>5 g) had the highest and seedlings from smallest seeds (<4 g) the lowest growth rates. Bonfil [14] showed the same effect of acorn size on the seedling growth. However, the consequences of growth rate on final shoot height depended on duration of seed storage (see *figure 1*). In fact, the final shoot height of seedlings issued from the smallest seeds was only significantly reduced for stored seeds, even though the growth rate of seedlings from fresh seed was low. The relatively longer resting period of the seedlings from stored seed may be responsible for the reduction of their final shoot height. For many authors, growth inhibition is related to the metabolism regulation and to the mechanisms of transport of nutrient [5, 11, 12, 43]. In *Castanea sativa*, the diffusion of the acid 5,5'-dimethylxazolidin 2,4-dione (DMO) and its accumulation in the meristematic zone of the apical bud favoured shoot elongation [43]. Excision of the young leaves, causing a continuous growth of pedunculate oak seedlings, showed that apical bud accumulates always-high ¹⁴C-DMO than the internode [9]. For the same species, the resting period is characterised by energetic deficiency resulting from a weak capacity to synthesis adenylic and non-adenylic nucleotide [5]. The seed size and their storability had a great effect on the number of leaves and was well correlated with growth, but did not affect the final number of leaves because of the large variation between seedlings.

Leaf chlorophyll concentration may be related to leaf photosynthetic activity in plants grown in the same light environment. It was reduced as seed storage time increased and was indifferent with seed size. This fact reinforces the idea that seedling size (final shoot height and stem diameter) depends more strongly on the initial cotyledonary reserves than on the photosynthates produced after germination. Bonfil [14] studying the effect of cotyledon removal showed that the reserves remaining in the seed 1 month after germination still contributed to seedling survival. The decrease in biomass of different seedling parts from the stored smallest seeds, which contain probably few reserves, also supports this idea. Seed size also affected root biomass of *Quercus rugosa* at the age of 5 months [14].

The shoot/root ratio is another important variable that can be used to predict seedling performance in the field. It becomes even more important on dry sites where soil moisture is critical for survival [22]. It is known that soil drought is the first cause of seedling mortality just after planting [13, 28]. The seed storage affected the values of shoot/root by reducing them and no significant differences were observed between parental trees. In fact, the shoot/root value of seedlings from stored seed was about 1.5 and that from seedlings from fresh seed was about 2. The equilibrium in the biomass of seedling components could play an important role at planting time, as it reduces the water loss by evapotranspiration and increases water uptake. For Douglas fir, a good shoot/root ratio would be 1.5, whereas a poor shoot/root ratio can be as much as 3 [22].

The increase in size of the root systems of seedlings issued from stored seed was directly related to the increase of taproot biomass and, probably, to the carbohydrate reserves accumulated there. For many species, e.g. *Quercus rubra* the allocation of carbohydrate reserves could vary as a function of the phenology of shoot growth, and the species with the most determinate shoot growth patterns had the highest total mass of carbohydrate reserves [17]. If this is true, our seedlings from large seeds could accumulate more carbohydrate reserves because of their rapid growth. It has been showed [1, 19, 24, 27] that the carbohydrate reserves play an important role in lateral root emergence, and that seedling performance depends on the rapidity of emergence of lateral roots [6, 16, 37].

We conclude that producing seedlings from stored seed could have a double strategical interest in the nursery. It would enable to counter the irregular acorn production and to supply, at any time, acorns able to

germinate. It would also give the opportunity to choose the seedling age and the best time to plant. The reduction in the time of emergence, the improvement of emergence uniformity and increase of root system size as a result of seed storage, are the best objectives requested by the nursery.

Acknowledgements: We thank the Estação Florestal Nacional (EFN), which made its seed laboratory available for germination tests and the CENASEF staff for their storage room chamber availability. This work was financed by an EC project, contract FAIR5-CT97-3480.

REFERENCES

- [1] Aarrouf J., Darbelley N., Demandre C., Razafindramboana N., Perbal G., Effect of horizontal clinorotation on the root system development and on lipid breakdown in Rapeseed (*Brassica napus*) seedlings, *Plant Cell Physiol.* 40 (4) (1999) 396–405.
- [2] Actes du séminaire méditerranéen sur la régénération des forêts de chêne liège, Tabarka, 22–24 octobre 1996, *Annales de l'INRGREF*, No. spécial, 1998, 253 p.
- [3] Aissa D., Étude sur la germination des semences de chêne vert (*Quercus ilex* L.) I : Influence de l'arbre producteur et de la taille des semences, *Rev. Cytol. Bio. Végét. Bot.* 6 (1983), 5–14.
- [4] Alatou D., Barnola P., Lavarenne S., Gendraud M., Caractérisation de la croissance rythmique du chêne pédonculé, *Plant. Physiol. Biochem.* 27 (2) (1989) 275–280.
- [5] Alatou D., Recherches sur le déterminisme de la croissance rythmique du chêne : *Quercus pedunculata* Ehrh- *Quercus mirbeckii* Durieu- *Quercus suber* L. – Étude morphologique, biochimique et écophysiological, Thèse de Doctorat d'État en Sciences Naturelles, Université de Constantine (Algérie) et Blaise Pascal Clermont II (France), 1990, 109 p. et annexe.
- [6] Aussenac G., El Nour M., Évolution du potentiel hydrique et du système racinaire de jeunes plants de cèdre, pin laricio de Corse et pin noir plantés à l'automne et au printemps, *Ann. Sci. For.* 43 (1986) 1–14.
- [7] Aussenac G., El Nour M., Reprise des plants et stress hydriques, *Rev. For. Fr.* XXXVIII (3) (1986) 264–270.
- [8] Aussenac G., Guehl J.M., Kaushal P., Granier A., Grieu Ph., Critères physiologiques pour l'évaluation de la qualité des plants forestiers avant plantation, *Rev. For. Fr.* XL (No. spécial) (1988) 131–139.
- [9] Barnola P., Alatou D., Lacoïnte A., Lavarenne S., Étude biologique de la croissance rythmique du chêne pédonculé (*Quercus robur* L.). Effet de l'ablation des feuilles, *Ann. Sci. For.* 21 (1990) 619–631.

- [10] Barnola P., Alatou D., Parmentier C., Vallon C., Approche du déterminisme du rythme de croissance endogène des jeunes chênes pédonculés par modulation de l'intensité lumineuse, *Ann. Sci. For.* 50 (1993) 257–272.
- [11] Barnola P., Crochet A., Payan E., Gendraud M., Lavarenne S., Modification du métabolisme énergétique et de la perméabilité dans le bourgeon apical et l'axe sous-jacent cours de l'arrêt de croissance momentané de jeunes plants de chêne, *Physiol. Vég.* 24 (3) (1986) 307–314.
- [12] Barnola P., Lavarenne S., Gendraud M., Dormance des bourgeons apicaux de frêne (*Fraxinus excelsior* L.) : évaluation du pool des nucléosides triphosphates et éventail des températures actives sur le débourrement des bourgeons en période de dormance, *Ann. Sci. For.* 43 (3) (1986) 339–350.
- [13] Blake T.J., Sutton R.F., Variation in water relations of black spruce stock types planted in Ontario, *Tree Physiol.* 3 (1987) 331–343.
- [14] Bonfil C., The effects of seed size, cotyledon reserves, and herbivory on seedling survival and growth in *Quercus rugosa* and *Q. laurina* (Fagaceae), *Am. J. Bot.* 85 (1) (1998) 79–87.
- [15] Brookes P.C., Wigston D.L., Variation of morphological and chemical characteristics of acorns from populations of *Quercus petraea* (Matt.) Liebl., *Q. robur* L. and their hybrids, *Watsonia* 12 (1979) 315–324.
- [16] Burdett A.N., Physiological processes in plantation establishment and the development of specification for forest planting stock, *Can. J. For. Res.* 20 (1990) 415–427.
- [17] Canham C.D., Kobe R.K., Latty E.F., Chazdon R.L., Interspecific and intraspecific variation in tree seedling survival: effects of allocation to roots versus carbohydrate reserves, *Oecologia* 121 (1) (1999) 1–11.
- [18] Carvalho J.B., Morais C.J.E., Análise da florestação em Portugal 1966-1995, Reunião de Especialistas em Reabilitação de Ecossistemas Florestais Degradados, Instituto Florestal, Lisboa, 1996, Portugal.
- [19] Chenevard D., Frossard J.S., Lacoïnte A., Lipid utilization and carbohydrate partitioning during germination of English walnut (*Juglans regia*), *Ann. Sci. For.* 51 (1994) 373–379.
- [20] Costa-e-Silva F., L Carla., Fabião A., Almeida M.H., Produção de sobreiros em viveiro : Qualidade e Certificação, in *Recopilación de trabajos, Congreso sobre Forestación en las dehesas, Mérida, 20–22 mayo 1999.*
- [21] Croizeau D., Roguet M., Faculté de reprise de glands prélevés en forêt après germination, *Rev. For. Fr.* XXVIII (4) (1976) 275–279.
- [22] Duddles R.E., Landgren C.G., Selecting and buying quality seedlings, *The woodland workbook, reforestation, Oregon State University, 1993, 1–11.*
- [23] Eriksson O., Seed size variation and its effect on germination and seedling performance in the clonal herb *Convallaria majalis*, *Acta Oecologica* 20 (1) (1999) 61–66.
- [24] Ernst M., Chatterton N.J., Harrison P.A., Carbohydrate changes in chicory (*Cichorium intybus* L. var. *foliosum*) during growth and storage, *Scientia Horticulturae* 63 (1995) 251–261.
- [25] Frochot H., Picard J.F., Dreyfus Ph., La végétation herbacée obstacle aux plantations, *Rev. For. Fr.* XXXVIII (3) (1986) 271–278.
- [26] Girard S., Clément C., Boulet-Gercourt B., Guehl J.M., Effects of exposure to air on planting stress in red oak seedlings, *Ann. Sci. For.* 54 (1997) 395–401.
- [27] Girard S., Déterminants écophysologiques de la crise de transplantation de plants d'espèces forestières résineuses (*Pinus nigra* ssp. *laricio* Poir. var. *Corsicana*) et feuillue (*Quercus rubra* L.). Effet du stockage des plants, Thèse de Magister, Institut de Doctorat en Biologie Forestière, Université Henri Poincaré-Nancy I, 1996, 84 p.
- [28] Hansson A.C., Zhao A.F., Andrén O., Fine-root growth dynamics of two shrubs in semiarid rangeland in Inner Mongolia, China, *Ambio* 23 (1994) 225–228.
- [29] Hopper N.W., Overholt J.R., Martin J.R., Effect of cultivar, temperature and seed size on the germination and emergence of soya beans (*Glycine max* (L.) Merr.), *Ann. Bot.* 44 (1979) 301–308.
- [30] Johson J.D., Cline M.L., Seedling quality of southern Pine, in: Duryea M.L., Dougherty P.M. (Eds.), *Forest regeneration manuel, Kluwer Academic Press, Netherlands, 1991, pp. 143–159.*
- [31] Kolb P.F., Robberecht R., High temperature and drought stress effects on survival of *Pinus ponderosa* seedlings, *Tree Physiol.* 16 (1996) 665–672.
- [32] Kormanik P.P., Can oak seedling quality be quantified by lateral root number?, in: *Third workshop on seedling physiology and growth problems in Oak plantings, Carbondale, Illinois, 12–13 February 1986 (Abstract), US, p. 19.*
- [33] Lavarenne S., Champagnat P., Barnola P., Croissance rythmique de quelques végétaux ligneux des régions tempérées cultivés en chambres climatisées à température élevée et constante et sous diverses photopériodes, *Bull. Soc. Bot. Fr.* 118 (1971) 131–162.
- [34] Louro G., Avaliação da aplicação de programas de apoio à floresta na região do algarve, *Direcção Geral das Florestas (DGF-Lisboa), Portugal, 1999.*
- [35] Mattsson A., Predicting field performance using seedling quality assessment, *New For.* 13 (1997) 227–252.
- [36] McKay H.M., Jinks R.L., McEvoy C., The effect of desiccation and rough-handling on the survival and early growth of ash, beech, birch and oak seedling, *Ann. For. Sci.* 56 (1999) 391–402.
- [37] McKay H.M., Root Electrolyte Leakage and Root Growth Potential as Indicators of Spruce and Larch Establishment, *Sylva Fennica* 32 (3) (1998) 241–252.
- [38] Merouani H., Acherar M., Istanbuli A., Recherche de quelques contraintes biotiques et abiotiques à la régénération naturelle du chêne liège *Quercus suber* L., Séminaire Méditerranéen sur la Régénération des Forêts de Chêne Liège. Tabarka, 22–24 octobre 1996, *Annales de l'INRGREF (No. spécial)* (1998) 225–243.
- [39] Merouani H., Branco C., Almeida M.H., Pereira J.S., Comportement physiologique des glands de chêne liège

(*Quercus suber* L.) durant leur conservation et variabilité inter-individus producteurs, Ann. For. Sci. 58 (2001) 143–153.

[40] Messaoudène M., Résultats des essais de semis directs du chêne liège à Melata, Rapport interne, Institut National de Recherche Forestière (INRF-Algérie), 1984, 10 p.

[41] O'Reilly C., McCarthy N., Keane M., Harper C.P., Gardiner J.J., The physiological status of Douglas fir seedlings and the field performance of freshly lifted and cold stored stock, Ann. For. Sci. 56 (1999) 391–402.

[42] Petruzelli L., Taranto G., Wheat aging: the contribution of embryonic and non-embryonic lesions to loss seed viability, Physiol. Plant 76 (1989) 289–294.

[43] Pezet Y., Si-Mohamed C., Croissance, morphogenèse et dynamique de l'état physiologique des bourgeons de jeunes plants de Châtaignier (*Castanea sativa* Miller) en conditions naturelles et contrôlées, Ann. Sci. For. 45 (1) (1988) 17–32.

[44] Riedacker A., Production et plantation de plants à racines nues ou en conteneurs, Rev. For. Fr. XXXVIII (3) (1986) 226–236.

[45] Sondergaard P., Essais de semis du chêne liège *Quercus suber* L. dans la forêt de Bab-Azhar, une subéraie de montagne au Maroc, Ann. Rech. Forest. Maroc 25 (1991) 16–29.

[46] Susko D.J., Lovett-Doust L., Patterns of seed mass variation and their effects on seedling traits in *Alliaria petiolata* (Brassicaceae), Am. J. Bot. 87 (2000) 56–66.

[47] Tinus R.W., Root growth potential as an indicator of drought stress history, Tree Physiol. 16 (1996) 795–799.

[48] Vale R., Chambel RM., Matos A., Moura S., Pereira C., Almeida MH., Técnicas de produção de plantas de sobreiro em viveiro : efeito do contentor e do substrato, in Recopilación de trabajos, Congreso sobre Forestación en las dehesas, Mérida, 20–22 mayo 1999.