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Georgios Anagiotos, Marianthi Tsakaldimi, Petros Ganatsas*

Variation in acorn traits among natural populations of *Quercus alnifolia*, an endangered species in Cyprus

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Abstract: *Quercus alnifolia* is an endemic species in Cyprus, and it is rated as Vulnerable (VU) in the Red List of Oaks. Although *Q. alnifolia* has a great ecological importance, there are only few studies about this species. In this work we have studied the natural variability of this species by analyzing acorn dimensions, acorn mass, length of embryo, moisture content of acorns and seed germinability among the seven acorn provenances, collected in Cyprus. We also determined the seeds responses to drying. Germination trials were also carried out, and differences in seed germination among populations were examined. Rate of water loss and its effect on seed germination was also estimated by application of specific desiccation treatments. The results showed that acorn characteristics significantly differed between the populations, following a general trend to reduce their dimensions and mass with the altitude increase. Seed germination was high for all studied populations, and germination behaviour was similar for all populations. Desiccation of acorns below 35% resulted in a great reduction of seed germination capacity.

Additional key words: seed germination, desiccation, golden oak, morphology, population variability

Address: G. Anagiotos, M. Tsakaldimi, P. Ganatsas, Aristotle University of Thessaloniki, School of Forestry and Natural Environment, Laboratory of Silviculture, P.O.Box 262, GR 54124, Thessaloniki, Greece, e-mail: marian@for.auth.gr

Introduction

Quercus alnifolia Poech (golden oak) is an endemic tree species in Cyprus, and it is rated as Vulnerable (VU) in the Red List of Oaks (Oak ICRA checklist, 2010). The species is also protected by the forest law in Cyprus and by the Habitat directive 92/43/EEC. Its habitat type "Scrub and low forest vegetation of *Quercus alnifolia* (9390)" is a priority habitat of Annex I. Therefore, large forest expanses have been proposed for inclusion in the Natura 2000 network, to protect, among others, the endemic forests of golden oak. Its main threats are the recreation activities and disturbance of natural habitats (Environment Department of Cyprus 2011).

The species is an evergreen shrub or small tree that can reach up to 10 m, exceptionally reaching 14 m under optimal conditions (Meikle 1977). In exceptional cases, golden oak attains dimensions of great trees, which are regarded as nature monuments. *Q. alnifolia* flowers from April to May and the fruits ripen from November to December. Its fruit is a narrowly obovate or subcylindrical nut (acorn), with brown colour at maturity; the cupule, at the base of acorn, is closely covered with strongly recurved scales (Natural Resources and Environment, Ministry of Agriculture of Cyprus 2011). *Q. alnifolia* belongs to subgenus *Cerris* O. Schwarz (Schwarz 1936; Camus 1938). Nevertheless, according to Toumi and Lumaret (2001), this species should be classified to the same cluster of

Mediterranean oaks that corresponds to subgenus *Sclerophyllodrys* (Neophytou et al. 2007).

Q. alnifolia appears on the central and south-western part of Cyprus, where it forms extensive forests over the entire Troodos mountain range, either in pure stands, or in mixture with *Arbutus andrachne* or as an understorey of the pine stands. It grows on ultrabasic igneous rocks of the ophiolite complex at an altitude of 400 to 1800 m where it dominates locally. It occupies dry habitats in association with *Pinus brutia* or forms dense maquis in mesic habitats, characterized by deep forest soils (Meikle 1977; Barbéro and Quezel 1979). Its total area of distribution is 23,700 ha (Esser 1996). It is very common in the Paphos, Troodos, Adelphi, Limassol and Macheras forests.

Its whole habitat is characterized by a Mediterranean climate with increasing winter cold and decreasing summer aridity at the higher altitudes. At the lower altitudes it is restricted to the moister sites and it is gradually replaced by more drought tolerant species (Knopf 2008). Its upper altitudinal limit is characterized by relatively harsh winters with temperatures dropping to –15°C and snow cover lasting 3–4 months (Tsintides et al. 2002).

Within its distribution range, *Q. alnifolia* plays an important ecological role. It occurs in dry habitats, where it is associated with pines (*P. brutia* or *P. nigra*) or, less often, forms high maquis in mesic habitats characterized by deep forest soils with mull humus (Barbero and Quezel 1979). It grows in rocky, steep and gentle slopes. Moreover, it colonizes loose diabasic screes contributing to soil stabilization. *Q. alnifolia* can readily regenerate vegetatively after fire or felling through coppicing. Besides its significant role in soil protection against erosion, its fruits offer an excellent food source to the local fauna. In earlier times its hard wood has been widely used in the past for construction of tools, chairs and parquet floors (Neophytou 2010). Nowadays, it is still used for production of high quality charcoal (Knopf 2008). It has an ornamental value and can be used in roadside plantations and gardens, in the submountainous and mountainous areas (Natural Resources and Environment, Ministry of Agriculture 2011).

Although the great ecological importance of *Q. alnifolia.* there are only few studies about this species (Neophytou et al. 2007, 2011) and almost no studies concerning the seed ecology and characteristics of this species. It is worth to point out that the species *Q. alnifolia* belongs to the very few oaks that the radicles of the acorn protruding near the proximal end of the fruit unlike the other oak species (Fig. 2) (Cinar-Yilmaz and Akkemik 2007). For conservation purposes and even from a strictly economic perspective, knowledge of the variability of acorn characteristics and germination ability of this species is important.

Thus, in order to provide valuable information on seed ecology of *Q. alnifolia* this study was done to evaluate the variation in acorn morphological traits and germination ability among natural populations of the species in Cyprus. Considering that acorns are recalcitrant seeds and when they lose moisture then lose their viability, this study was also done to examine the acorns responses to drying (desiccation).

The specific goals of this study were: (i) to measure acorn dimensions, mass, moisture content among the selected populations, (ii) to estimate the seed germination capacity of these populations, (iii) to determine the seed responses to drying (moisture loss and germination capacity) and (iv) to determine any differences between the different populations in seed characteristics and germination behaviour.

Materials and methods

Data collection and analysis

Acorns were collected in autumn 2010 from the region that the species occurs in Cyprus, the wide area of Troodos mountain (Fig. 1). Seven sampling locations were selected, four from the east side of the mountain, and three from the west side, following an altitudinal gradient, and covering almost all altitudinal range the species occurs (600–1700 m asl.). The selected locations were: Lefkara village, Kapedes village, Vavatsinia village, and Machairas (Kionia) peak, from the east side of the Troodos mountain area, and Phini village, Leminthou village, and Troodos Square from the west side. Ten dominant and semi-dominant trees were selected from each location; these were randomly selected at a distance of at least 50 m (Dangasuk and Panetsos 2004; Ganatsas et al. 2008). At least 50 acorns per tree per location were collected. Acorns were transported to the laboratory for measurements. The acorns were first immersed in water for 24 hours to eliminate insect damaged or infested acorns. Acorns that appeared abnormal or obviously defective were discarded (Tilki and Alptekin 2006). Then acorns were stored moist at 3°C in polyethylene bags with a wall thickness of 0±1 mm, prior to experiments (Bonner 1996).

Acorn measurements

Ten viable acorns per tree for each sampling location were selected for morphological measurements. In total 700 acorns were marked with a number and measured. Acorns measurements accomplished in the laboratory were: length (cm), width in the maximum dimension (cm), fresh weight (g), dry weight (g), the weight of 1000 seeds (g), length of embryo (cm) and moisture content (on fresh weight basis, %).

The length and the width (at the widest point) of the acorns were measured with vernier calipers.

Fig. 1. Map showing the sampled populations of *Quercus alnifolia* in Cyprus

Acorn weight was determined as follows: each acorn was weighted in a precise balance to find its fresh weight. Then, we put each acorn in the oven at 103°C for 17 hours (Bonner and Vozzo 1987; ISTA 1993), and afterwards we re-weighted it for getting their dry weight. Based on the weight measurements, we calculated acorn moisture content percentage according to ISTA rules (Hanson 1985), as follows: M = FW – DW * 100 / FW, where: M: moisture content (%), FW: fresh weigth (g) and DW: dry weight (g). For embryo measurements, a random sample of 5 acorns per tree per sampling location was selected, and we cut longitudinally each of them, then we measured the embryo length with a rule, in accuracy of 0.01 cm.

Fig. 2. *Quercus alnifolia* germinated acorns

Germination trials

Germination capacity tests were performed on ten acorns with three replications per tree and sampling location. Acorns were cut in half and we kept the back half with the cup scar. We placed them cut side down on two sheets of filter paper moistened with deionized water in Petri dishes (Bonner and Vozzo 1987). Then Petri dishes were transferred to growth chamber with an 8-h photoperiod and day/ night temperatures of 30/20°C (Connor and Sowa 2003; Tilki and Alptekin 2006), under fluorescent lighting (approximately 20 μmol m^{-2} s^{-1}) (Bonner and Vozzo 1987). Prior to germination the acorns were dusted with fungicide in order to prevent fungal infection. Germination was recorded every two days and was considered complete when no additional seeds germinated. A seed is scored as germinated if both radicle and shoot exhibit normal growth and morphology (Bonner and Vozzo 1987).

Desiccation treatments

Seeds of *Quercus* species are recalcitrant and they loose their germination when their water content decreases (Finch-Savage et al. 1996, Sobrino-Vesperinas and Belén-Viviani 2000). However, the rate of water loss depends on species, and the environmental conditions (Bonner 1990; Connor and Sowa 2003; Tilki and Alptekin 2006). In order to found the rate of water reduction with the time, a random sample of 30 acorns with three replications were selected. Acorns were weighted for fresh weight measurement; afterwards, the sampled acorns were air-dried in room temperature conditions (around 16–20°C) for 1 day (D1), 3 (D3), 5 (D5), 7 (D7) and 9 days (D9) (Connor and Sowa 2003). After each treatment, we re-weighted the acorns in order to find the water loss. Afterwards, we put them in the oven in 103°C for 17 hours to take their dry weight. The dried seeds were transferred on the balance by a desiccator. The moisture loss percentage after desiccation was calculated as follows: Moisture loss after desiccation (%) (ML) = FW – WAD * 100 / FW, where FW: Fresh weight (g) and WAD: Weight after desiccation (g). Afterwards new 30 acorns were subjected to each desiccation treatment and they tested for germination capacity. Germination capacity tests were performed on ten acorns with three replications per desiccation treatment and the same procedure as previously described was followed.

Statistical analysis

In order to determine the differences in acorn morphological characteristics, among the populations studied, ANOVA analysis was conducted using SPSS software, and the Waller-Duncan criterion was used for comparison of the means. Distribution was tested for normality by Kolmogorov-Smirnov criterion and the homogeneity of variances was tested by Levene's test (Snedecor and Cochran 1988; Norusis 1994). Hierarchical cluster analysis was performed for grouping the studied populations based on the similarity of acorn morphological characteristics. Ward's method was used for the calculation of the shortest Euclidean distances and cluster identification, and the results displayed in a dendrogram (Valero-Galvan et al. 2011).

Results

Site characteristics of the sampling locations

The species *Q. alnifolia* in Cyprus is distributed mainly on the wide area of Troodos Massif, covering an altitude range between 600–1700 m asl. The sampled locations lay in different altitude, covering all the altitude range the species appears. The lower sampling locations (villages Lefkara P5 and Kapedes P6) lay in altitude of 600–700 m. (Table 1); three populations (Vavatsinia village P4, Lemithou village P3, and Phini village to Trooditissa P2) lay in a medium altitudinal zone (950–1300 m), and the rest two populations Machairas (Kionia) peak P1, and Troodos Square P7 lay in the higher altitudinal zone (over 1350 m). According to the data of the Meteorological Service of Cyprus (2011), the climate of all the sampled areas is dry Mediterranean, and it belongs to the type ***Csa*** of the *Koeppen* classification, even though there are some differentiations, such as lower temperature and higher precipitations in the higher altitudes compared to the areas of the lower and medium altitudes. Overall, the climate is characterized as warm temperate, mesothermal, with dry period during the summer, with very hot summer, and average temperature of the hottest month over 22°C.

According to Geological Map of Cyprus (Geological Survey Department, Cyprus, 1979), all the populations of *Q. alnifolia* grow on geological substrate (the Troodos Ophilite). However, the bedrock is greatly differentiated from Gabrro, in the west sites Phini and Lamithou villages, Diabase in Vavatsinia and Machairas (Kionia) peak, Meta basalts in Kapedes, Chalk, marly chalk and chalky marl in Lefakara, and serpentinized harzburgite in Troodos Square. The soils are generally silicate row soils on igneous rocks, usually shallow, except for the sites of Kapedes and Lefkara, where the soils are shallow brown earths. Most of the populations are distributed in generally southern faced slopes, except for the population in Kapedes, where the population grows on north faced slopes, Kionia in east faced slopes, and in Lemithou village in west faced slopes.

Acorn morphological characteristics

According to the data analysis, the morphological characteristics of the acorns significantly differed among the seven sampling locations (Table 2). The largest and heaviest acorns were found in the population of Lemithou village (1200 altitude and south faced slopes) P3, having mean values of length, width and dry weight, 3.59 cm, 1.48 cm and 3.29 g, respectively, and the population near to Kapedes village (altitude 700 m and north faced slopes) P6 having an average acorn length of 3.80, width 1.45 cm, and dry weight 2.82 g. In contrast, the smaller acorns were found in the populations grown in high altitude, in Troodos square (1600–1700 m) P7, presenting mean values of length, width and dry weight of 2.47 cm, 1.02 cm, and 0.87 g, respectively, followed by the population of Machairas (Kionia) peak P1, in an altitude of 1400 m, with mean values of length, width and dry weight, 2.84 cm, 0.99 cm, and 1.06 g, respectively. The differences observed between the high and the low-medium altitudinal populations were very high (the mean values of the last is lower than 1/3 of the former).

Similar to the acorn length, the embryo length presented significant differences, among the populations, ranging between 3.35 cm (in the lower population – Kapedes village P6) to 2.02 cm (in the higher population (Troodos Square P7). On the contrary, the moisture content of the sampled acorns slightly ranged between the populations, presented average values ranging from 42.2% to 47.4%. Statistical analysis revealed significant differences between the two group of populations, the high altitudinal (1400–1700 m asl.) and the rest populations (650–1350 m asl.) (Table 2).

Acorn dimensions and their mass showed a tendency toward reducing with the increase of altitude; especially, the populations of Troodos Square (P7) and

Table 1. Site characteristics of acorn collection locations of *Q. alnifolia* in Cyprus

Troodos Massif	Location	Population code	Altitude (m) asl.	General aspect	Climate *(Koeppen* classificatio)	Geological substrate	Soil type
West side	Lemithou village	P3	1200	West	*Csa*	Gabbro	Silicate row soils on igneous rocks
	Phini village (to Trooditisa)	P2	1300–1350	South	*Csa*	Gabbro	Silicate row soils on igneous rocks
	Troodos Square	P7	1600–1700	South	*Csa*	Serpentinized harzburgite	Silicate row soils on igneous rocks
East side	Lefkara village	P5	650	South	*Csa*	Chalk, marly chalk and chalky marl	Brown earths
	Kapedes village	P6	700	North	*Csa*	Meta basalts	Brown earths
	Vavatsinia village	P4	950	South	***Csa***	Diabase	Silicate row soils on igneous rocks
	Machairas (Kionia) peak	P1	1400	East	***Csa***	Diabase	Silicate row soils on igneous rocks

Kionia peak (P1) laying in altitude over 1350 m asl, the acorns' size was found to be greatly reduced. Those populations were found to greatly differed from the other populations based on the similarity of acorn morphological characteristics. These differences are shown in the dendrogram of the Figure 3, constructed based on hierarchical Euclidean cluster analysis according to Ward's Method. As it is shown in this figure, the performed hierarchical cluster analysis identified two main clusters; the first includes the five populations of the low and medium altitudes (650–1350 m) that did not differ distinctly (P2, P3, P4, P5, P6), and the second cluster includes the two populations (P1 and P7) of the high altitude (1400–1700 m). The high length of the horizontal branches between the two clusters indicates that there is a high degree of dissimilarity between the clusters. The above data lead to the conclusion that the main factor affected seed morphological characteristics seems to be the site altitude; stands laying in the medium (and lower) altitude produce the larger and heavier acorns compared to those of the high altitude.

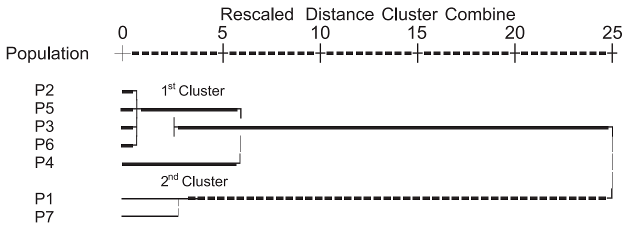

Fig. 3. Graphical representation of the distances of acorn morphological characteristics, established after Ward's clustering method analysis using squared Euclidian distance for the studied populations of *Q. alnifolia*. P1, P2,…..P7 – population codes, as they described in Table 1. The high length of the horizontal branches between the clusters indicates the high degree of their dissimilarity

Table 2. Morphological characteristics of *Q. alnifolia* for the seven populations in Cyprus (P1, P2,…..P7 – population codes, as they described in Table 1). Values are means ± standard error of mean (in parenthesis). Values in the same row followed by different letter are significantly different (P <0.05)

Populations/ acorn characteristics	P1	P2	P3	P4	P5	P6	P7	Mean value
Acorn length (cm)	2.84 (0.04)d	3.30 (0.05)c	3.59 (0.07)b	3.44 (0.06)bc	3.48 (0.06)b	3.80 (0.04)a	2.47 (0.05) e	3.19 (0.03)
Acorn width (cm)	0.99 (0.08)d	1.28 (0.20)c	1.48 (0.18)a	1.39 (0.16)b	1.32 (0.20)c	1.45 (0.12)ab	1.02 (0.20)d	1.25 (0.25)
Length to width ratio	2.87 (0.05)a	2.58 (0.04)b	2.43 (0.06)c	1.81 (0.06)d	2.64 (0.07)b	2.62 (0.05)b	2.42 (0.06)c	2.55 (0.03)
Embryo length (cm)	2.39 (0.04)	2.83 (0.05)	3.21 (0.08)	3.01(0.08)	3.10(0.08)	3.35 (0.06)	2.02(0.07)	2.75 (0.04)
Fresh weight (g)	1.97 (0.10)d	3.38 (0.24)c	5.88(0.37)a	4.55 (0.24)b	4.29(0.28)b	4.90 (0.18)b	1.65 (0.18)d	3.56 (0.13)
Dry weight (g)	1.06 (0.06)d	1.94 (0.15)c	3.29 (0.22)a	2.63 (0.16)b	2.41 (0.17)b	2.82 (0.12)b	0.87 (0.12)d	2.01 (0.09)
1000 seeds weight (g)	1973.4 (100.9)d	3380.0 (244.0)c	5885.5 (365.1)a	4550.2 (240.4)b	4288.3 (277.1)b	4904.3 (180.8)b	1654.0 (183.2)d	3563.8 (134.0)
Moisture content (%)	46.3 (0.64)a	42.6 (1.81)b	44.1 (0.91)b	42.2(1.01)b	43.8(0.80)b	42.5 (0.83)b	47.4(1.30)a	43.6 (0.50)

Acorn germination behaviour

According to the data analysis, seed germination of *Q. alnifolia* was very high for all populations, and did not significantly differ among the populations (Fig. 4). However, the highest seed germination capacity (97.5%) was observed in acorns collected from the Lemithou village of medium altitude (1200 m), and the lowest in acorns of the high altitudinal locations, Troodos Square, 1600–1700 m, and Machairas (Kionia) peak, 1400 m. Acorns of all populations have a similar pattern of germination, while acorn germination was completed between the 12th and the 14th day for all populations.

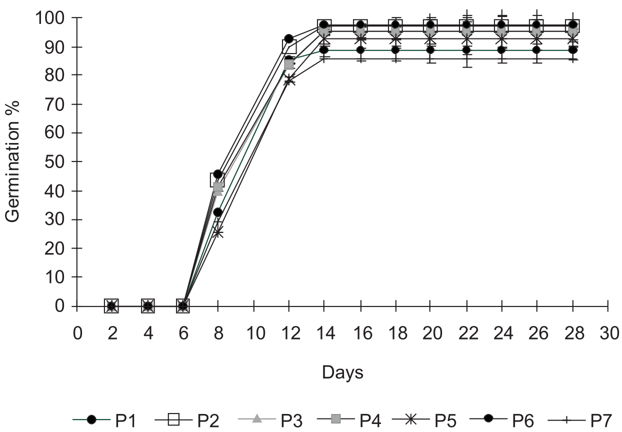

Fig. 4. Cumulative germination of *Q. alnifolia* seeds for the seven populations in Cyprus. Vertical lines represent ± Std. error values

Desiccation effect

Figure 5 shows the route of the water loss of the sampled acorns due to the desiccation effect. The moisture content decreased from the mean value of 43.54 to 25.1% after 9 days drying. The observed rate of water loss in a room temperature was 1.89%, 6.39%, 9.11%, 11.92% and 16.55% after 1, 3 5, 7, and 9 days respectively. These percentages of water loss should be considered significant for germination ability, since oak seeds are considered recalcitrant and loose the germination capacity with the water loss (Bonner and Bozzo 1987; Bonner 1996). As expected for recalcitrant seeds, germination strongly decreased with desiccation. Germination capacity was highly dependent on moisture content and started to decline significantly after 5 days of drying, when moisture content reached to 34.4% (Fig. 5), while the seed viability was nearly lost within 7 days of drying when acorn moisture content declined to 31.61%. Finally, after 9 days drying the recorded germination was zero.

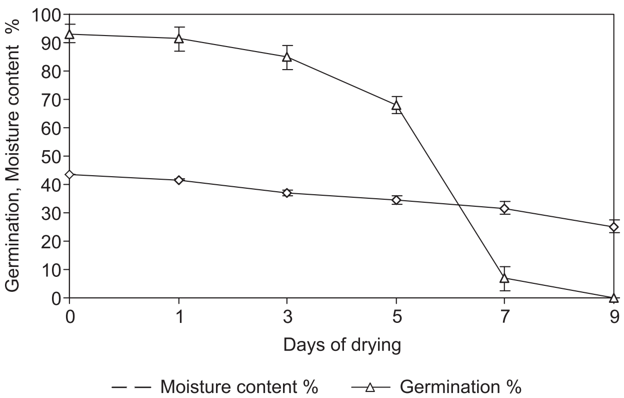

Fig. 5. Desiccation sensitivity of *Q. alnifolia* seed in different drying periods at 16–20°C. Vertical bars indicate ± s.e. of the means where larger than the symbols

Discussion

Morphological characteristics of *Q. alnifolia* acorns were influenced by altitudinal variation among the samplings locations. The general trend observed was that acorns collected from sites of medium altitudes are the largest and heaviest while their morphological characteristics decreased with the increase of altitude. Especially, acorns collected from areas with an altitude over 1350 m asl. were found to exhibit significantly lower size. This could be attributed to the climatic or ecological variations in elevation. Populations variability with respect to acorn morphometry have earlier been reported in some *Quercus* spp. Singh et al. (2010) found that acorn length and weight of *Quercus glauca* showed significant inverse correlation with elevation range of seed source. Wenying and Wanchun (2005) studying the morphological characteristics of populations of *Quercus mongolica* found that the acorn got smaller as the altitude became higher. Similarly, Valero-Galvan et al. (2011) found that *Quercus ilex* populations growing in lowest altitudes presented the longest and broadest acorns; on the contrary, populations growing highest altitudes presented the smallest and thinnest acorns.

Oaks show wide variation in seed size within a species. Seedlings emerging from seeds of varied sizes exhibit differential competitive performance due to variation in emergence time and growth rate (Khan and Shankar 2001). Seed size is assumed to play an important role in seedling development. For example, Kormanik et al. (1998) reported the dependence of *Quercus rubra* seedling development on acorn size. Plant height, root collar diameter and seedling survival were also reported to be significantly correlated with acorn mass (Nikolic and Orlovic 2002).

Germination of *Q. alnifolia* seeds was found to be very high (over 86%) for all sampling locations, and did not significantly differ among the populations. Acorns' germination for all populations followed a similar pattern; a rapid increase in germination from the 8th to 12th day and germination completion within 12–14 days. However, acorns collected from the Lemithou village P3 (medium altitude) and Kapedes village P6 (lower altitude, north faced slopes) exhibited the highest seed germination capacity, while acorns collected from the higher altitudinal locations, Troodos Square P7 and Machairas peak (Kionia) P1, presented the lower one.

Water loss of acorns due to the desiccation effect (drying) was found to follow an almost linear descending trend, reaching 25.1%, after nine days drying in room temperature. This acorns' water content was proved to be crucial for seed germination capacity for *Q. alnifolia* which completely lost their germination capacity, as for many oak species (Bonner and Bozzo 1987; Bonner 1996). Seeds of *Q. alnifolia* like the seeds of other oak species are recalcitrant, means desiccation-sensitive, and thus they loose their germination capacity when drying to the low moisture contents typical of orthodox seeds. Similar effect of drying was observed by Finch-Savage et al. (1996) for *Q. robur* seeds. Also, reduction of moisture content below mature levels (44%) severely affected germination of bur oak acorns (Schroeder and Walker 1987), while this level was found lower (40%) for Polish provenances (Suszka and Tylkowski 1980). This should be considered in forestry practice and management, since it causes practical problems for the storage of recalcitrant seeds both for continuity of supply and long-term genetic conservation (Roberts 1973; Finch-Savage et al. 1996). Comparing our results with those reported by Finch-Savage (1992) for *Q. robur* acorns, we can conclude that the moisture content for viable *Q. robur* acorns was much bigger (42–49%); this could be attributed to the fact that this species grows in humid and oceanic climates, while *Q. alnifolia* grows only in Cyprus, the hottest and driest island in the Mediterranean basin. On the contrary, Tilki and Alptekin, (2006) reported for *Quercus vulcanica* quite lower critical values for the minimum moisture content (11–16%). The critical moisture content below which recalcitrant seeds should not be dried varies from 12 to 31%, depending on the species (Roberts 1973). The critical minimum moisture content was found to be 10–12% for *Quercus nigra* seeds (Bonner 1996).

The results from this study demonstrate that there is a great diversity in acorn morphological characteristics of *Q. alnifolia* that was not associated with acorn germination behaviour. Taking into consideration, the restricted geographical distribution of the species, existing only in Cyprus, this diversity may play a critical role in species conservation in the future, and thus it should be considered in species habitat management. However, additional studies on diversity and ecology in *Q. alnifolia* would help to create efficient and effective conservation strategies for this species.

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