

Ecological significance of seed desiccation sensitivity in *Quercus ilex*

Thierry Joët^{1,*}, Jean-Marc Ourcival² and Stéphane Dussert¹

¹IRD, UMR DIADE, BP 64501, 34394 Montpellier, France and ²CNRS, CEFE, 34293 Montpellier, France

* For correspondence. E-mail thierry.joet@ird.fr

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- **Background and Aims** Several widespread tree species of temperate forests, such as species of the genus *Quercus*, produce recalcitrant (desiccation-sensitive) seeds. However, the ecological significance of seed desiccation sensitivity in temperate regions is largely unknown. Do seeds of such species suffer from drying during the period when they remain on the soil, between shedding in autumn and the return of conditions required for germination in spring?
- **Methods** To test this hypothesis, the Mediterranean holm oak (*Quercus ilex*) forest was used as a model system. The relationships between the climate in winter, the characteristics of microhabitats, acorn morphological traits, and the water status and viability of seeds after winter were then investigated in 42 woodlands sampled over the entire French distribution of the species.
- **Key Results** The percentages of germination and normal seedling development were tightly linked to the water content of seeds after the winter period, revealing that *in situ* desiccation is a major cause of mortality. The homogeneity of seed response to drying suggests that neither intraspecific genetic variation nor environmental conditions had a significant impact on the level of desiccation sensitivity of seeds. In contrast, the water and viability status of seeds at the time of collection were dramatically influenced by cumulative rainfall and maximum temperatures during winter. A significant effect of shade and of the type of soil cover was also evidenced.
- **Conclusions** The findings establish that seed desiccation sensitivity is a key functional trait which may influence the success of recruitment in temperate recalcitrant seed species. Considering that most models of climate change predict changes in rainfall and temperature in the Mediterranean basin, the present work could help foresee changes in the distribution of *Q. ilex* and other oak species, and hence plant community alterations.

Key words: *Quercus ilex*, acorn, desiccation sensitivity, holm oak, drought, ecological filtering, germination, Mediterranean climate, recalcitrance, winter rainfall.

INTRODUCTION

In most flowering plant species, seeds are in a dry quiescent state at maturity, which enables them to survive adverse environmental conditions after dispersal, such as periods of cold or drought. In these species, seed drying occurs *in planta* during development, after the acquisition of desiccation tolerance, i.e. the ability to withstand removal of intracellular water without irreversible damage and then to resume normal metabolism after rehydration (Leprince and Buitink, 2010). Such desiccation-tolerant seeds are called ‘orthodox’ due to their ability to survive storage for very long periods under conventional genebank conditions (Roberts, 1973).

However, about 8% of the world’s flowering plants (about 20 000 species; Tweddle *et al.*, 2003) produce seeds that are not orthodox, i.e. they either only withstand partial drying (intermediate seeds) or are extremely sensitive to dehydration (recalcitrant seeds) (Roberts, 1973; Ellis *et al.*, 1990). In both cases, seeds are characteristically shed from the mother plant with high moisture content and are usually not dormant. Plants in the non-orthodox categories prevail in specific habitats, such as tropical rain forests, where they represent >40% of plant species (Tweddle *et al.*, 2003). The most parsimonious explanation for the expansion of species displaying seed desiccation sensitivity in these particular biotopes relies on the convergent loss of seed desiccation tolerance, probably

due to the lack of seasonal drought as selective pressure, from several taxonomically unrelated desiccation-tolerant ancestors (Farnsworth, 2000). Indeed, factors involved in stress tolerance, such as accumulation of heat shock proteins, may have fitness costs under conditions that are not stressful (Hoffmann, 1995; Krebs and Feder, 1998).

Although non-orthodox seed species are widespread in tropical forest systems, seed desiccation sensitivity also occurs in temperate plants. Species of the genus *Quercus* (Finch-Savage, 1992), *Acer pseudoplatanus* (Dickie *et al.*, 1991), *Aesculus hippocastanum* (Tompsett and Pritchard, 1998) and *Castanea sativa* (Leprince *et al.*, 1999) are temperate trees with recalcitrant seeds. The occurrence of recalcitrant seed species in temperate regions may be puzzling at first sight considering that water stress occurs seasonally in most temperate biotopes, in particular in the Mediterranean region, which is known for its periods of severe drought. Actually, many extant taxa with desiccation-sensitive seeds originated during the warm and wet global climate of the Tertiary period (65 to 2 million years BP; Xiang *et al.*, 1998; Manos and Stanford, 2001), and thus probably lost seed desiccation tolerance for similar reasons to those mentioned above for tropical species. It has recently been suggested that such ancient species were protected from drier conditions during the Quaternary by the facilitative nurse effects of modern Quaternary species, which provided favourable

microhabitats for seed germination and seedling establishment (Valiente-Banuet *et al.*, 2006). In line with this hypothesis, one may wonder whether desiccation sensitivity is a trait which affects seed survival in temperate regions. Do seeds of recalcitrant seed species of these areas suffer from drying during the period from shedding (usually in autumn) to when the conditions required for germination return (usually in spring)? To our knowledge, no study has specifically focused on the ecological implications of seed desiccation sensitivity in temperate species, whereas various ecological aspects of this trait have been investigated in tropical biotopes (e.g. Dussert *et al.*, 2000; Pritchard *et al.*, 2004; Yu *et al.*, 2008). This also contrasts with the substantial efforts made to decipher the physiological mechanisms involved in the sensitivity to drying of recalcitrant seeds (e.g. Hendry *et al.*, 1992; Leprince *et al.*, 1999; Roach *et al.*, 2010).

Among the different temperate forests, the Mediterranean sclerophyllous forest provides a valuable system to investigate the impact of seed sensitivity to desiccation on the reproductive success of recalcitrant seed species. Indeed, one of the most emblematic tree species of this biome is an oak – *Quercus ilex*, holm oak – and is thus likely to produce recalcitrant seeds (Finch-Savage, 1992; Xia *et al.*, 2012a). Moreover, this forest still occupies very large areas in the Mediterranean basin. In addition, the Mediterranean climate is characterized by irregular and unpredictable precipitation, thus maximizing the chance to observe contrasted autumn-to-spring cumulative rainfall across locations within a single year of study. Finally, most global and regional climate models suggest that the Mediterranean region might be especially vulnerable to global change (Gibelin and Deque, 2003; Giorgi and Lionello, 2008). These models predict significant increases in temperature and decreases in precipitation in the Mediterranean basin and, in particular, a pronounced decrease in precipitation in winter in northern Mediterranean areas. These changes may have major effects on existing Mediterranean forests, particularly on the distribution of oak species (e.g. Cheaib *et al.*, 2012; Ruiz-Labourdette *et al.*, 2012). In this respect, a better understanding of the significance of seed desiccation sensitivity may improve our capacity to foresee changes in plant community structure in this region.

The holm oak is an evergreen oak which is native to the Mediterranean region. It is found from Turkey to Spain on the European side of the Mediterranean Sea, and from Morocco to Tunisia on the African side, and it has also colonized the majority of Mediterranean islands (Barbero *et al.*, 1992). Most *Q. ilex* forests are regarded as rare cases of woodlands that have undergone very little silvicultural management, and are therefore of great value for ecological observations (Lumaret *et al.*, 2008). In France, the holm oak occupies the entire Mediterranean rim and Corsica. In the present study, to test whether environmental conditions after seed dispersal affect seed viability in temperate recalcitrant seed species, the relationships between winter climate, topography, acorn morphological traits, and seed water status and viability after the winter period were investigated in 42 populations of *Q. ilex* sampled over the entire French distribution of the species.

MATERIALS AND METHODS

Study area, topography and mesoclimatic data

This study covered the entire French distribution area of *Quercus ilex* where the climate is Mediterranean (Fig. 1). The area where *Q. ilex* occurs naturally was recently updated by the French national institute of geographical information, IGN (Inventaire forestier national, 2011; <http://www.ifn.fr>). Our sampling area covered three administrative regions ('Languedoc-Roussillon', 'Rhône-Alpes' and 'Provence-Alpes-Côte d'Azur') ranging from latitude 42°27'08"N to 44°51'08"N and from longitude 02°07'24"E to 07°02'26"E. A total of 41 holm oak woodlands were sampled in continental France (Fig. 1; Supplementary Data Table S1). An additional sample was collected in Corsica (latitude 42°20'23"N; longitude 09°32'20"E). Three locations were sampled two or three times (Supplementary Data Table S1). In these cases, two of three microsites within a location differed in their topography. The topography of each microsite was characterized by two qualitative variables (shade and soil cover) and two quantitative variables (slope and exposure). Slope was calculated using numerical topographic maps (<http://www.geoportail.gouv.fr/accueil>). Exposure corresponded to the azimuth (0 to +180°) measured between north and the main gap in the canopy. Shade was assessed according to the density of trees and exposure: 0, low density woodlands and borders of groves; 1, medium density woodlands and low density woodlands with no or very little direct sunlight; and 2, understorey

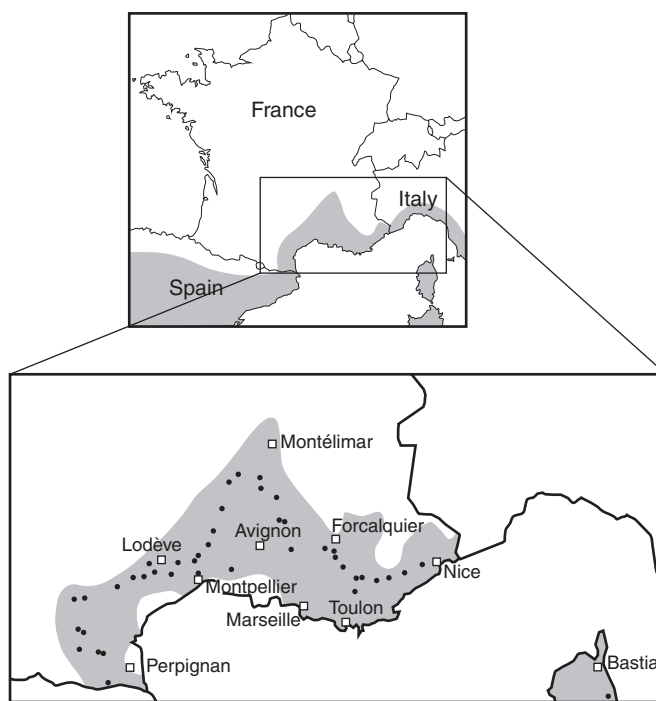


FIG. 1. Geographical distribution of *Quercus ilex* (shaded) and locations of the 42 woodlands sampled (dots). The distribution area in the upper map is roughly adapted from Lumaret *et al.* (2002), while that in the lower map was based on data provided by the French National Institute of Geographical Information, IGN (Inventaire forestier national, 2011).

of high density woodlands. The soil cover was also categorized into three types: litter, moss or grass, and bare soil. The climatic conditions (rainfall, minimum and maximum temperatures) experienced by acorns from shedding to the collection date were estimated using climatic data from 1 November 2011 to 31 March 2012 recorded by the nearest meteorological station (data were purchased from the public library of Météo France, <https://public.meteofrance.com/>). The distance between a sampling site and the meteorological station was always <5 km. Temperature data were corrected using the international standard atmosphere adiabatic lapse rate of 0.65 °C 100 m⁻¹ and the difference in altitude between each sampling site and its corresponding meteorological station (Supplementary Data Table S1).

Biological material

Acorns of *Q. ilex* are abiotically dispersed by gravity in autumn. They may also be biotically dispersed, mainly by the European jay *Garrulus glandarius* and to a lesser extent by the woodmouse *Apodemus sylvaticus* (Gómez, 2003; Gómez et al., 2008). This study focused on gravity-dispersed acorns which remained on the surface of the ground, i.e. those that had not been removed by dispersers, damaged by insects or consumed by ungulates such as the wild boar *Sus scrofa*. Acorns were collected from 26 March to 2 April 2012. In each site sampled, about 200 acorns were collected from the ground underneath at least four randomly selected *Q. ilex* trees, immediately enclosed in a hermetically sealed plastic bag, and stored in the dark at ambient temperature for a maximum of 2 d until transport to the IRD laboratory (Montpellier, France). Acorns were then directly processed and all experiments were carried out using sound acorns, which were sorted from unsound ones by visual screening after the pericarp has been removed. The moisture content, expressed on a fresh weight basis, and the dry mass of seeds and pericarps were determined gravimetrically after oven drying for 17 h at 103 °C, using ten seeds per sample.

Viability assays

Seed viability was assessed using both percentage germination and the percentage of normal seedling development. For each location sampled, six batches of six seeds were placed on 18 g of vermiculite fully moistened with 45 mL of sterile water in closed plastic Magenta[®] boxes and kept at 25 °C in the dark. Germination was recorded when the radicle had grown at least 10 mm after 1 week of culture, therefore ensuring that protrusion was not due to mechanical elongation of the hypocotyl but to true growth of the radicle. Normal seedling development was recorded using the criteria of radicle geotropic growth and opening of primary leaves after 4 weeks of culture.

Desiccation tolerance assays

Freshly shed mature acorns were collected from a single plot close to Montpellier (Pic Saint-Loup, 43°47'17"N, 03°48'22"E) in October 2011. After pericarp removal, seeds were desiccated by equilibration over various saturated salt

solutions for 20 d at 25 °C in the dark as previously described by Dussert et al. (1999). For the measurement of seed desiccation sensitivity, batches of 36 seeds were desiccated over NH₄NO₃ [62 % relative humidity (RH)], NaCl (75 % RH), KCl (85 % RH), KNO₃ (92 % RH) and K₂SO₄ (97 % RH) saturated solutions. Seed viability after desiccation was assessed using percentage of germination as described above.

Statistical analyses

All statistics were carried out using Statistica software (Statsoft, Tulsa, OK, USA). Desiccation sensitivity was quantified using the previously developed quantal response model (Dussert et al., 1999) and tested using the least square regression computed by the Quasi-Newton method. Correlations between environmental variables, seed water content and seed viability were analysed by linear regression using Pearson's correlation coefficient. The effect of two qualitative microtopography descriptors, shade and soil cover, was tested by analysis of variance (ANOVA). Post-hoc comparison of means was performed using the Newman and Keuls test. Principal component analysis (PCA) was used to analyse the respective contributions of mesoclimatic and microtopographic variables to the variation in seed water content and viability.

RESULTS

Desiccation sensitivity of fresh mature *Quercus ilex* seeds

To our knowledge, the level of tolerance to drying of holm oak seeds has never been investigated. The desiccation sensitivity of fresh mature holm oak seeds was first measured *in vitro*, using mature acorns collected in autumn from trees of a single location close to Montpellier (Pic Saint-Loup). The initial water content of these seeds was 40.7 % fresh weight (f. wt). When germination was plotted against water content, the typical S-shaped pattern of desiccation-sensitive seeds was observed (Fig. 2A, open squares). The seed water content (WC) at which 50 % of the initial viability was lost, WC₅₀, was very high (30.6 % f. wt) and corresponded to an equilibrium RH₅₀ of 93.4 %. This level of desiccation sensitivity is in full agreement with that previously observed in freshly shed mature seeds of *Q. rubra* and *Q. robur* (Pritchard, 1991; Finch-Savage, 1992; Finch-Savage and Blake, 1994). On the basis of these results, *Q. ilex* seeds can undoubtedly be categorized as recalcitrant.

In situ drying is a major cause of *Q. ilex* seed mortality during winter

Very high variability in seed viability was observed among the 42 locations sampled (Fig. 1; Supplementary Data Table S1). Complete loss of viability was observed in 18 samples, and, in the remaining samples, seed germination ranged from 2 to 100 %, and only nine samples had a germination percentage >60 %. Seed viability after the winter period was very nicely explained by the water status of seeds at the moment of collection (Fig. 2A). The proportion of variance explained by the quantal response model was very high (94 %),

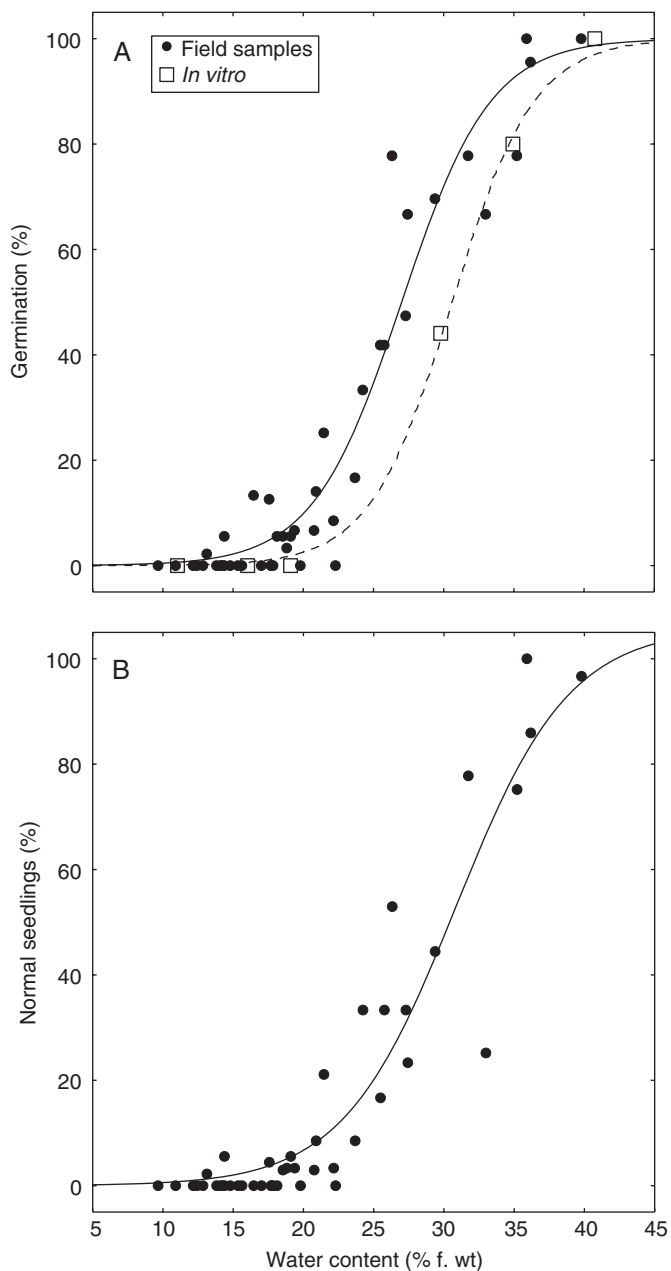


FIG. 2. Relationships between the viability of *Quercus ilex* seeds collected in 42 woodlands in the South of France in spring 2012 and their water content (circles). Viability was estimated by the percentage both of germination (A) and that of normal seedling development (B). Seed desiccation sensitivity was also quantified *in vitro* using fresh mature acorns collected near Montpellier, France (squares). Solid and dashed lines correspond to the fitted patterns using the quantal response model described in Dussert *et al.* (1999).

demonstrating a very homogeneous response of *Q. ilex* seeds to drying, independent of their origin. Germination was highly significantly ($P < 10^{-6}$) correlated with seed water content within the 20–40% f. wt hydration window. A similar trend was obtained when normal seedling development was chosen as the criterion for seed viability (Fig. 2B). The relationship between viability and water content obtained with seeds

TABLE 1. Relationships between water content and viability of *Quercus ilex* seeds collected during spring in 42 locations throughout the French distribution area of the species, and their mass, winter climatic conditions and microtopography of the microsites sampled

	Seed water content (% f. wt)		Germination (%)	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Seed dry mass (g)	-0.082	0.592	-0.096	0.531
Rainfall (mm)	0.563	0.000	0.513	0.000
T_{\min} (°C)	0.124	0.418	0.030	0.845
T_{\min} aver (°C)	0.140	0.360	0.046	0.762
T_{\max} aver (°C)	-0.455	0.002	-0.489	0.001
T_{aver} (°C)	-0.179	0.240	-0.275	0.067
$T^{\circ}\text{C}$ range (°C)	-0.386	0.009	-0.336	0.024
Days rain < 2 mm	-0.167	0.272	-0.161	0.289
Days rain < 5 mm	-0.373	0.012	-0.314	0.036
Slope (%)	0.253	0.093	0.194	0.202
Exposure (° to North)	-0.540	0.000	-0.504	0.000

T_{\min} , absolute minimum temperature; T_{\min} aver, average minimum temperatures; T_{\max} aver, average maximum temperatures; T_{aver} , average daily temperatures; $T^{\circ}\text{C}$ range, average daily temperature range; Days rain < 2 mm, number of consecutive days with < 2 mm daily rainfall; Days rain < 5 mm, number of consecutive days with < 5 mm daily rainfall.

Significant correlations are in bold.

which remained on the soil during the whole winter period was almost identical to that observed *in vitro* with fresh mature seeds dehydrated in controlled conditions (Fig. 2A). The level of desiccation tolerance of seeds collected in the spring, as quantified by WC_{50} (27.0% f. wt), was also very close to that of fresh seeds. The large number of samples displaying complete loss of viability (two-fifths of the sites sampled) clearly shows that acorns that remain on the soil until spring are subject to high environmental risks. Climatic parameters and/or microsite topography would thus be expected to be important factors in determining seed survival.

Rainfall and maximum temperatures during the winter period have a direct impact on seed water content and survival

The 42 locations sampled throughout the French distribution area of *Q. ilex* displayed very high variability in cumulative rainfall during the 2011–2012 winter, ranging from 13 to 293 mm (Supplementary Data Table 1). The average minimum and maximum temperatures also varied significantly from -3 to +8 °C and from +9 to +16 °C, respectively. Relationships between seed water content and survival and environmental factors were analysed by linear regression (Table 1, Fig. 3). Neither the average minimum temperature (Table 1, Fig. 3B) nor the absolute minimum temperature (which ranged from -18.5 to -0.8 °C, Table 1) was significantly correlated with seed water content and seed viability. The proportion of variance explained by these variables was < 2% (with $P > 0.05$), suggesting that holm oak acorns are highly tolerant to frost.

In contrast, a highly significant ($P < 10^{-6}$) positive correlation was observed between seed water content and winter

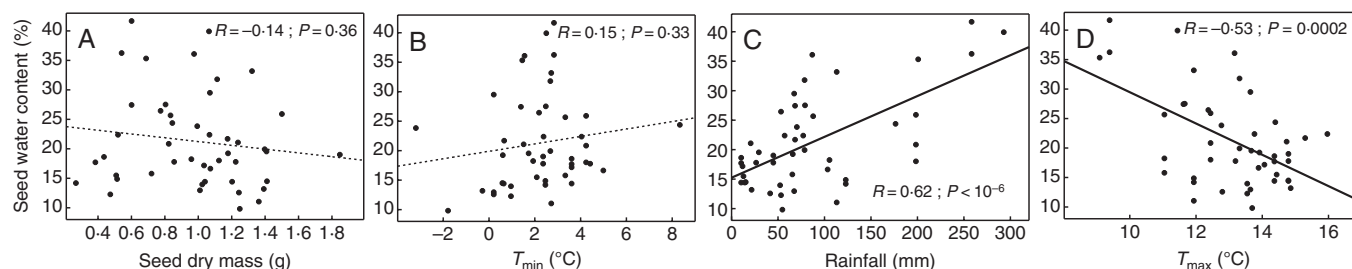


FIG. 3. Correlations between the water content of *Quercus ilex* seeds collected in 42 woodlands in the South of France in spring 2012 and their dry mass (A), and three climatic parameters used to describe the environmental conditions in winter: average minimum winter temperature (B), winter rainfall (C) and average maximum winter temperatures (D). *R* and *P* are Pearson's linear correlation coefficient and probability of significance, respectively.

TABLE 2. Effect of shade and soil cover on the water content and percentage germination of *Quercus ilex* seeds collected during spring in 42 locations throughout the French distribution area of the species

Factor	Category	<i>n</i>	Water content (% f. wt)	Germination (%)
Shade	Shade	20	24.5 ^a	38.3 ^a
	Mid-shade	15	20.8 ^a	16.7 ^{ab}
	Light	11	14.3 ^b	2.2 ^b
	<i>F</i>		9.0	5.8
	<i>P</i>		0.0006***	0.0059**
Soil cover	Leaf litter	9	26.6 ^a	47.0 ^a
	Grass and moss	17	23.1 ^a	30.0 ^a
	Bare soil	20	16.5 ^b	6.2 ^b
	<i>F</i>		9.1	7.1
	<i>P</i>		0.0005***	0.0022**

Results of one-way ANOVA: *F* and *P*.

Means followed by the same letter did not differ significantly according to the Newman and Keuls test.

n, number of samples per category.

cumulative rainfall from December to March (Table 1, Fig. 3C). This climatic variable explained a significant proportion (38 %) of the variance for seed water content. In the 12 locations which experienced a severe winter drought (<50 mm cumulative rainfall), seed water content was never higher than 21 % f. wt, and percentage germination was always <15 % (Fig. 3C). In contrast, the seed water content of acorns collected in the few locations which received >250 mm rainfall during the same period was very high (35–40 % f. wt). The local average maximum temperature also displayed highly significant ($P = 2 \times 10^{-4}$) negative correlation with seed water content and explained >28 % of variance (Table 1, Fig. 3D). The seed water content of acorns sampled in 13 locations with high winter maximum temperatures (above +14 °C) was always lower than 23 %, while acorns with a high water content (35–40 % f. wt) at the end of winter were all sampled in the few woodlands with low maximal temperatures (less than +10 °C).

Microsite topography also contributes to seed survival

The effect of two qualitative microsite topography parameters – shade and ground cover – was assessed using

ANOVA and a post-hoc Newman and Keuls test. There was a highly significant effect of both parameters on seed water content and survival, as assessed by percentage germination and the percentage of normal seedling development (Table 2). Seeds collected in sites with little or no shade had a very low water content at the end of winter and were in fact almost all dead. Among the three types of soil cover at our sample sites, leaf litter best prevented seed drying and subsequent loss of viability (Table 2). However, grass and moss were considerably less detrimental than bare soils. The influence of two other quantitative topographic parameters – exposure and slope – was tested by linear regression. While slope had no apparent effect on the status of seeds in spring, exposure had a significant impact on seed water content, and northern exposure was clearly less injurious (Table 1).

In order to examine the respective contribution of all climatic and topographic variables to the status of seeds after winter, the two topographic qualitative variables – soil cover and shade – were transformed into scores ranked according to their impact on seed viability as indicated by ANOVA. All variables were then analysed by PCA, soil cover and shade being supplementary variables. The four main principal components (PC1–PC4) explained 82 % of the variance of the whole data set (Table 3). Variance of seed viability and water content was mainly explained by PC1 and PC3 (Table 3). These two components, which accounted for about 50 % of the overall variance, were therefore chosen for the analysis of the respective contributions of climate and topography (Fig. 4). As expected from the above results, seed viability and water content vectors were collinear. Moreover, the correlation circle clearly demonstrates that the mesoclimatic variables shown to influence seed survival individually (as assessed by linear regression above) mainly contributed to PC1, while microtopographic parameters were associated with PC3. PC1 was positively correlated with seed water content ($R = 0.70$), germination ($R = 0.66$) and rainfall ($R = 0.89$), but negatively correlated with the average maximum temperature ($R = -0.74$), diurnal temperature range ($R = -0.82$) and the number of consecutive days without significant rainfall ($R = -0.70$) (Table 3). PC3 was significantly correlated with germination ($R = 0.640$), shade ($R = -0.57$) and exposure ($R = -0.78$). This key finding demonstrates that climate explained most between-site variation in seed survival, but microtopography explained most of the residual part of it.

TABLE 3. Correlation of the four first principal components resulting from principal component analysis (PCA) of the whole data set (46 *Quercus ilex* samples collected in 42 locations) with individual seed traits, climatic and microtopographic variables

Factor	PC1	PC2	PC3	PC4
Seed dry mass	-0.279	-0.041	0.146	-0.723 (52.3)
Seed water content	0.703 (49.4)	-0.251	0.606 (36.7)	-0.059
Germination	0.661 (43.7)	-0.338	0.583 (34.0)	0.021
Rainfall	0.888 (78.8)	-0.120	-0.159	-0.200
T_{\min}	0.409	0.837 (70.1)	0.087	-0.148
T_{\min} aver	0.485	0.861 (74.2)	0.027	0.051
T_{\max} aver	-0.738 (54.5)	0.407	0.178	-0.337
T_{aver}	-0.079	0.948 (89.8)	0.131	-0.172
$T^{\circ}\text{C}$ range	-0.823 (67.8)	-0.412	0.087	-0.246
Days rain < 2 mm	-0.675 (45.6)	0.075	0.561 (31.5)	0.198
Days rain < 5 mm	-0.701 (49.1)	0.260	0.349	0.464
Slope	-0.014	-0.179	0.359	-0.450
Exposure	-0.209	-0.014	-0.782 (61.2)	-0.131
Shade*	0.106	-0.063	0.566 (32.0)	-0.017
Soil cover*	-0.359	0.171	-0.317	0.008
Eigen value	4.419 (33.99)	2.975 (22.88)	1.996 (15.35)	1.269 (9.77)

All variables were active, with the exception of two qualitative variables, shade and soil cover, which were supplementary variables and are indicated by an asterisk.

Percentage contributions of variables to the principal components are indicated in parentheses for significant correlations. The Eigen value of each PC and the corresponding percentage of total variance explained (in parentheses) are also indicated in the bottom row.

T_{\min} , absolute minimum temperature; T_{\min} aver, average minimum temperatures; T_{\max} aver, average maximum temperatures; T_{aver} , average daily temperatures; $T^{\circ}\text{C}$ range, average daily temperature range; Days rain < 2 mm, number of consecutive days with < 2 mm daily rainfall; Days rain < 5 mm, number of consecutive days with < 5 mm daily rainfall.

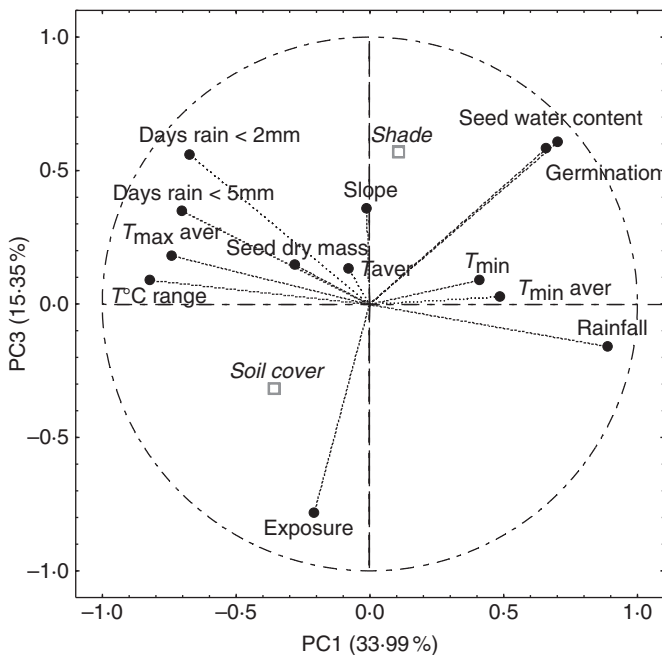


FIG. 4. Correlation circle of all seed, climatic and topography variables using the first and the third components (PC1 \times PC3) resulting from the principal component analysis (PCA) of data collected in 42 locations over the French distribution area of *Quercus ilex*. All variables were active (circles), with the exception of two qualitative variables, shade and soil cover, which were converted into scores for PCA but analysed as supplementary variables (squares). T_{aver} , average daily temperatures; T_{\max} aver, average maximum temperatures; T_{\min} aver, average minimum temperatures; T_{\min} , absolute minimum temperature (recorded in February); $T^{\circ}\text{C}$ range, average daily temperature range; Days rain < 2 mm, number of consecutive days without significant rainfall (daily rainfall not more than 2 mm).

The size and pericarp thickness of acorns did not influence their water status after winter

To test whether the variability of acorn morphology among the 42 locations sampled, possibly caused by intraspecific genetic variability or phenotypic plasticity, also contributed to the hydration and viability status of seeds after the winter period, the relationships between seed water content and germination, seed mass and pericarp thickness were tested by linear regression. The size of seeds, estimated by their dry mass, did not significantly influence their water status in spring (Fig. 3A). Similarly, the thickness of the acorn pericarp, estimated by the ratio of the pericarp to seed dry mass, was not significantly correlated with seed water content in a sub-set of 18 samples ($R = 0.26$; $P = 0.37$, data not shown).

DISCUSSION

It is now well established that environmental conditions can affect various traits of developing seeds (Donohue, 2009), including size (Daws and Jensen, 2011), desiccation tolerance (Daws et al., 2004) and longevity (Kochanek et al., 2010). For instance, latitudinal gradient was shown to cause climatic variations (sum of temperatures) that were large enough to trigger phenotypic variation in the level of desiccation sensitivity of seeds in *Acer pseudoplatanus*, probably due to variation in seed maturity at the end of the summer period (Daws et al., 2006a). Immature seeds are indeed usually shorter lived and more sensitive to desiccation than those that have reached peak maturity (e.g. Probert et al., 2007). In contrast, our results demonstrate a very homogeneous response of *Q. ilex* seeds to drying which is independent of their origin.

The relationship between germination and water content was highly consistent over the entire French distribution of the species, and very similar to that obtained *in vitro* with fresh mature acorns, suggesting that full maturity was reached in all the locations sampled (Supplementary Data Table S1). This important finding strengthens the value of the Mediterranean holm oak forest as a model to investigate the ecological filtering effect of winter environmental conditions: results are not biased by the occurrence of a geographical variation in seed tolerance to drying before winter.

Similarly, one may hypothesize that the genetic variability detected among *Q. ilex* populations in the South of France (Lumaret *et al.*, 2002) influences the pattern of seed desiccation tolerance in this region. The homogeneity of seed response to drying observed throughout the French distribution of *Q. ilex* rather suggests that this intraspecific genetic variation has no or very little impact on this trait. Neither did we find any evidence of a relationship between the size of holm oak seeds, the thickness of the pericarp and their survival. This finding may appear inconsistent with recent studies on the role played by seed size and seed coat thickness and anatomy in surviving pre-germination environmental conditions (Daws *et al.*, 2006b; Hill *et al.*, 2012; Xia *et al.*, 2012b). However, it is worth noting that up to now, all significant relationships identified between these traits resulted from studies carried out at the interspecific level and not within a single species. Besides, a recent study demonstrated that it is necessary to use descriptors more sophisticated than those used in the present work to investigate the role played by the anatomy of the pericarp in *Quercus* sp. (Xia *et al.*, 2012b).

Until now, little attention has been paid to the significance of seed desiccation sensitivity in temperate ecosystems. To our knowledge, this is the first study to investigate whether this trait can influence recruitment in recalcitrant seed species. Using *Q. ilex* as a model system, we established that temperate desiccation-sensitive seeds may suffer from drying during the period between shedding and the return of favourable conditions for germination. Within its French distribution area, the length of the period when *Q. ilex* seeds remain on the ground is very long since seed dispersal takes place in autumn and seedlings emerge at the beginning of spring. Our results demonstrate that winter mortality of desiccation-sensitive seeds can be particularly high in Mediterranean environments where the onset of the wet spring season is preceded by winter rainfall events that may be erratic in timing and amount. In addition to the major impact of winter rainfall, we showed that in Mediterranean forest ecosystems, the water content of seeds on the soil, and subsequently their viability, is dramatically influenced by winter maximum temperatures and microtopography. There is a significant amount of literature on the regeneration of oak species which focused either on mortality resulting from biotic factors (pathogenic fungi as well as insect and mammal predation) or on the death of newly emerged seedlings caused by frost or summer drought (Gomez, 2004; Garcia and Houle, 2005; Pons and Posas, 2007; Smit *et al.*, 2008; Monnier *et al.*, 2012; Perez-Ramos *et al.*, 2012). Our study establishes that seed drying during the winter period is another major cause of mortality in the transient soil seed bank. Henceforth, this issue should be taken into account in

studies dedicated to recruitment in temperate recalcitrant seed species.

Our study also revealed that the characteristics of a given microsite may account for the part of the between-location variation in seed water status and viability which is not explained by mesoclimatic variables. This finding corroborates the increasing number of studies which established the crucial importance of the microhabitat for seedling survival and growth in oak species (Monnier *et al.*, 2012; Perez-Ramos *et al.*, 2012). We observed that drying and subsequent mortality were significantly lower when acorns were dispersed in leaf litter than in grass, moss or on bare soil. Leaf litter, and even burial by dispersers, has already been suggested to protect holm oak acorns against desiccation (Kollmann and Schill, 1996; Garcia *et al.*, 2002; Gomez, 2004). Our study also suggests that shade plays a significant role in *Q. ilex* seed survival in winter. Similarly, shade provided by shrubs has been shown to facilitate seedling recruitment in many *Quercus* species, including *Q. ilex* (Callaway, 1992; Smit *et al.*, 2008). The topography parameters analysed in the present study were simply recorded as qualitative descriptors with a limited number of categories. Considering the significance of the results obtained with these two parameters, further characterization of microsites using complementary variables – such as light availability based on canopy openness, light spectral quality, litter composition and depth, soil moisture and temperature – should improve our understanding of the effects of microhabitat characteristics on the water status of seeds that remain on the soil throughout the winter period.

For long-lived species such as holm oak, sporadic winter drought events are not sufficient to compromise natural recruitment. However, an increase in the frequency of prolonged winter drought periods and a rise in winter temperatures could hamper the regeneration of non-orthodox seed species. In the context of climate change, one may thus assume that seed desiccation sensitivity is a key functional trait that could dramatically affect vegetation composition. In particular, climate projections consistently predict significant increases in temperature and decreases in precipitation in the Mediterranean basin (Gibelin and Deque, 2003; Giorgi and Lionello, 2008), suggesting possible future changes in the distribution of holm oak. For example, changes in precipitation were shown to have dramatic effects on *Quercus emoryi* recruitment in woodlands in southeastern Arizona (Weltzin and McPherson, 2000). Our results therefore illustrate the importance of considering the behaviour of seeds, particularly their sensitivity to drying, which is often overlooked when predicting the response of woody plants to impending climate change in Mediterranean regions.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of Table S1: seed mass and water content, percentages of germination and of normal seedling development in 46 batches of *Quercus ilex* acorns collected at the end of winter in 42 holm oak woodlands. Climatic data for the 4 months preceding collection and geographical data are also provided.

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