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Original article

# Effects of waterlogging on seed germination of three Mediterranean oak species: Ecological implications

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ABSTRACT

Soil water saturation during prolonged periods of time generates a negative impact on nearly all terrestrial plants. In Mediterranean woodlands, precipitation can be very abundant during the wet season, inducing temporary soil waterlogging, coinciding with the seed dispersal and germination time of many species. We investigated the effects of waterlogging on seed germination and early root growth of three coexisting oak species (*Quercus canariensis*, *Q. suber* and *Q. pyrenaica*), by completely flooding of seeds for various periods of time. The three oak species showed a certain level of tolerance to waterlogging, only being affected those seeds subjected for long periods of submersion (over 30 days). Waterlogging during prolonged periods of time decreased the probability of seed germination in the three oak species, lengthened the time to germination, and hampered root development in two of the studied species. The main differences between oak species occurred in terms of root growth (*Q. canariensis* being the less affected, and *Q. suber* the most); these differential responses could be related to a species rank of waterlogging tolerance. Thus inter-specific differences in germination responses to waterlogging could contribute to explain, at least partially, species habitat and distribution patterns across landscapes. Seed mass also played an important role on different aspects of germination, though its relative importance varied as function of species and waterlogging treatment. The tolerance to stress induced by waterlogging increased with seed mass, but only in the case of *Q. canariensis*.

## 1. Introduction

Soil water saturation over prolonged periods of time generates a negative impact on nearly all terrestrial plants, as a consequence of the slow diffusion rates of gases in water (Jackson, 1985; Armstrong, 2002), which hampers oxygen supply to roots and reduces respiration and photosynthesis rates (Pezeshki, 1994; Voesenek et al., 2006). Several studies have shown that soil waterlogging as a result of periodic-to-continuous flooding of bottomland is the major factor affecting tree regeneration in many temperate forested wetlands (e.g., Streng et al., 1989; Kevin and Brooks, 2003; Sakio, 2005; Trowbridge et al., 2005; Battaglia and Sharitz, 2006). Mediterranean ecosystems experience contrasting two-phase precipitation dynamics, receiving almost all of their yearly rainfall during the autumn–spring cold period (which can lead to pulses of over-abundant water levels), in contrast with the prolonged, warm dry summer. Water shortage during the summer has commonly been claimed to be a major limiting factor for seedling recruitment in this type of ecosystems (e.g., García, 2001; Herrera et al., 1994; Sack et al., 2003; Marañón et al., 2004; Pulido and Díaz, 2005; Gómez-Aparicio et al., 2004; Castro et al., 2005), but potential negative effects of excess water during the wet period have been overlooked. Precipitation can be very abundant in autumn–spring, with frequent events of soil waterlogging that can be more prominent and persistent in clayey soils of low permeability.

Tree species can show important differences in tolerance to waterlogging during early stages of regeneration, even within the same genus, depending on the environmental conditions of their habitats. Several authors have reported that seeds of tree species occurring in potentially flooded sites are not affected by waterlogging (Guo et al., 1998) or they are able to keep under water during long periods of time without significant loss of viability (Hosner, 1957; DuBarry, 1963). Contrarily, other studies have shown that excess water affects negatively the capacity of seed germination in species that are less adapted to flooding (Guo et al., 1998; Walls et al., 2005). Inter-specific differences in tolerance to waterlogging may determine the structure and composition of the community along a gradient of soil humidity. For example, in forested wetlands, plant community changes occur primarily as the result of variation in

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flood-tolerance among plants and the effect of flooding on growth rates (Megonigal et al., 1997). However, ecological implications of differences between species in the responses to soil waterlogging are still unknown in Mediterranean woodlands.

In a recent field study in a mixed oak woodland located in southern Spain, we demonstrated that soil waterlogging was one of the main factors limiting initial stages of seedling recruitment in the three dominant oak species (*Q. canariensis*, *Q. suber*, and *Q. pyrenaica*) (Urbieta et al., 2008a). Over-abundant water levels during the wet season reduced the probability of germination and emergence and lengthened time-to-emergence of seedlings, which in turn decreased their survivorship during summer drought. However, the mechanisms by which soil waterlogging affects initial stages of oak species recruitment are not clear.

In this study, we specifically investigated the effects of waterlogging on different aspects of seed germination in three Mediterranean oak species. We carried out a laboratory experiment, where groups of seeds of each species were completely submerged under water (with measures to prevent fungal infections) for different periods of time. We wanted to answer the following specific questions: (1) does waterlogging – and the consequent restriction of oxygen supply – decrease the probability of seed germination? (2) How long can seeds stay viable under submersion conditions? (3) What is the relative importance of seed mass on tolerance to submersion? (4) How does waterlogging affect root growth? (5) Are there differences between the three oak species in their responses to waterlogging? If so, how are such inter-specific differences related to species distribution patterns?

## 2. Materials and methods

### 2.1. Study species and area

*Quercus suber* L. (cork oak) is an evergreen tree dominating forests in sub-humid Mediterranean-type climate, on acidic soils. Its distribution range covers the western half of the Mediterranean Basin (France, Italy, Spain, Portugal, Morocco, Algeria, and Tunisia). Its seed is one of the largest in the Mediterranean forest, and its dispersal is restricted to the autumn/early winter season (between October and February).

*Quercus canariensis* Willd. is a semi-deciduous oak, locally abundant in moister sites, and usually mixed with *Q. suber*. The distribution range is restricted to the Iberian Peninsula (Spain and Portugal) and NW Africa (Morocco and Algeria). The seeds are somewhat smaller than those of *Q. suber*, and their dispersal is earlier (September–December).

*Q. pyrenaica* is a deciduous oak distributed from SW France to N Morocco. Its seeds are usually larger than those of the other two oak species, and dispersal timing is similar to *Quercus canariensis*.

The three studied species coexist in the mixed oak forests of the Aljibe Mountains, near the Strait of Gibraltar, in southern Spain (see a detailed description of the experimental site in Quilchano et al., 2008 and Pérez-Ramos et al., 2008). The dominant bedrock in the area is Oligo–Miocenic sandstone, with rugged terrain and a highest peak of 1094 m a.s.l. Climate is subhumid Mediterranean-type, with cool and wet winters, alternating with warm and dry summers. Mean annual temperature ranges from 14.6 to 18.48 °C, with a mean monthly maximum of 36.8 °C (July) and mean monthly minimum of 2.8 °C (January). Mean annual rainfall varies from 701 to 1331 mm (mean of 1056 for 15 weather stations), depending on the effects of the local orographic relief. Overstorey canopy of these forests is co-dominated by *Q. suber* and *Q. canariensis*, whereas *Q. pyrenaica* is only present in scarce populations at higher altitudes (>900 m). Most of the forested area has been protected within *Los Alcornocales* (meaning cork oak forests) Natural Park, covering about 1700 km$^2$.

### 2.2. Experimental design

To encompass intra-specific variation, more than 1000 seeds of *Q. suber* and *Q. canariensis* were collected from various trees (at least 10 of each species) in the surroundings of the study area, when seed-drops of both species overlap (late November 2005). Seeds of *Q. pyrenaica* (with scarce seed production in Aljibe mountains) were brought from Sierra Morena stands, an inland area also located at southern Spain. A sample of healthy, normal-sized seeds (i.e., discarding aborted acorns) was made, using the floating method to discard those infected by moth or beetle larvae (Gribko and Jones, 1995). The selected seeds were stored on a moist substrate at 2–4 °C until used in the experiment (with a storage timing below 15 days). Subsequently, selected seeds were buried in a moist sand (pure silica) bed on plastic trays, and completely submerged in distilled water (at 5 cm of depth) for different periods of time. We used distilled water to avoid the possible effects of nutrient content on seed germination and early root growth. Three waterlogging treatments were established as a function of the submersion time ($W_{15}$, $W_{30}$, and $W_{60}$ for groups of seeds submerged for 15, 30, and 60 days, respectively) and another treatment ($W_0$) for non-submerged seeds. The duration of $W_{15}$ and $W_{30}$ treatments is comparable to field observations of soil waterlogging timing at the study site, especially during rainy years (Fig. 1). The $W_{60}$ treatment was also selected for this experiment in order to check how long seeds of the three species are able to stay viable under submersion conditions. The trays used for seed submersion were set up within a germination chamber at constant temperature (≈20 °C).

After waterlogging treatment, four groups of 20 seeds (per treatment and species) were randomly selected and set to germinate semi-buried, separated from each other by approx. 7 cm, in a new bed of sand (pure silica), 8 cm deep covering the bottom of 48 × 33 × 10 cm plastic trays. Previously (just after submersion), the selected seeds were washed with sodium hypochlorite (2%) and then treated with a commercial fungicide based on copper oxychloride (50%) to prevent fungal infections. All selected seeds were initially weighed to the nearest 0.01 g. Mean ± SD (standard deviation) seed fresh mass (g) was 3.11 ± 0.71 for *Q. canariensis*, 3.19 ± 1.13 for *Q. suber*, and 6.32 ± 1.55 for *Q. pyrenaica*. We used acorn fresh weight as a surrogate of seed mass, according to Quero et al. (2007).

Trays for germination monitoring were set up within a germination chamber, where they stayed for 28 days at constant temperature (≈20 °C) and in darkness, which have been

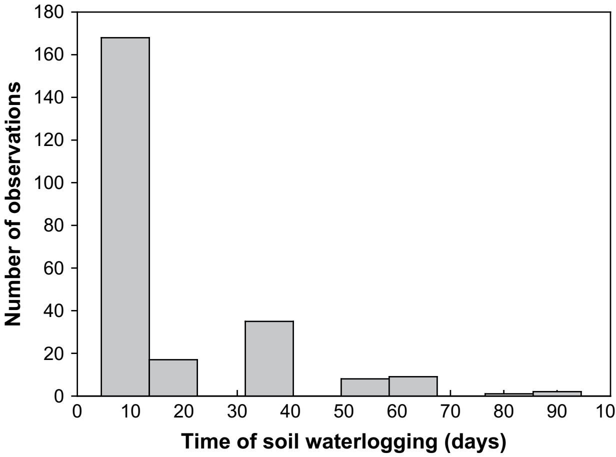

**Fig. 1.** Histogram of frequencies of soil waterlogging timing, based on field observations at the study site during the wet season (2004 year). See Urbieta et al. (2008) for methodological and analytic details.

considered as optimal conditions for seed germination in other *Quercus* species (Pritchard and Manger, 1990; Branco et al., 2002). Specifically, mean air temperature inside the chamber was 18.7 °C, while relative air humidity averaged 90%. Both air parameters were monitored using a continuous sensor linked to a data-logger (*Pro Series*, Hobo Onset, USA). To keep optimal humidity conditions for seeds, all trays were periodically watered (at intervals of 2–3 days) by adding 10 mL of distilled water around each seed.

Germination was monitored at intervals of 2–3 days, and it was noted when the radicle was higher than 2 mm. At the end of the experiment (after 28 days), the radicle of all germinated seeds was separated, and its maximum length was measured using a digital calliper. All radicles were oven-dried, and weighed using a balance with a precision of 0.0001 g.

### 2.3. Data analysis

The probability of seed germination was treated as a binomial ordinal dependent variable (1 = successful germination; 0 = non-germination), assuming a logit relationship between the dependent and explanatory variables (link function). The effects of soil waterlogging and seed mass on probability of seed germination were evaluated using generalised linear models (GLZ, McCullagh and Nelder, 1989). To test the effect of soil waterlogging on the time-to-germination and on different parameters of root growth (length, final dry weight, and growth rate), an analysis of covariance (ANCOVA) was carried out, including the initial seed mass as continuous predictor (covariate variable). Specific differences between the four waterlogging treatments, for each oak species, were determined using the *post hoc* Tukey test. Additionally, simple regressions were calibrated between seed mass and all parameters of germination and root growth for each waterlogging treatment. The criterion false discovery rate (FDR) was applied at the 5% level to control the inflation of type I error derived from repeated testing (García, 2004). Root growth rate was calculated as the ratio between final root weight and number of days between the emergence of the radicle and the end of the experiment. Before the analyses, those variables not normally distributed were log- or square-root-transformed to fulfil assumptions of normality and homoscedasticity. Normality was tested using the Kolmogorov–Smirnov test. All analyses were carried out using STATISTICA version 6.0 (StatSoft Inc., 2001).

## 3. Results

In general, waterlogging produced a negative effect on different aspects of seed germination and root growth in the three oak species, although the impact on each of them differed slightly.

### 3.1. Waterlogging and seed germination

Waterlogging decreased the probability of seed germination in the three oak species, but only after long periods of submersion (more than 30 days). In *Q. canariensis* and *Q. suber* seeds, the probability of germination was significantly lower in $W_{30}$ treatment, in comparison with $W_{15}$ and with $W_0$ (Table 1). In the case of *Q. pyrenaica*, the probability of germination in $W_{30}$ seeds was also lower than in $W_{15}$, but higher than in $W_0$ seeds (Table 1). The negative effect of waterlogging was more evident in those seeds which were kept submerged for 60 days ($W_{60}$ treatment): no case of germination was recorded for *Q. canariensis* or *Q. suber* seeds, and only three cases for *Q. pyrenaica* (Fig. 2). However, for short periods of submersion ($W_{15}$), the probability of seed germination did not decrease in comparison with $W_0$ treatment, and even significantly increased in the case of *Q. pyrenaica* (Table 1 and Fig. 2).

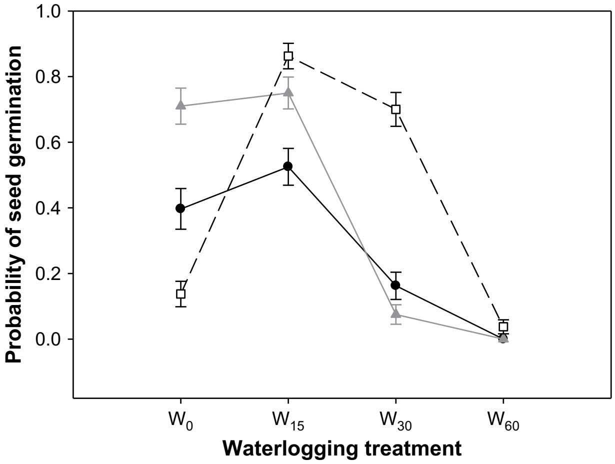

**Fig. 2.** Probability of seed germination ($n = 80$) as a function of the time of submersion ($W_{15}$ = 15 days; $W_{30}$ = 30 days; $W_{60}$ = 60 days; $W_0$ = 0 days). *Q. canariensis* is represented with circular symbols and solid black line, *Q. suber* with triangular symbols and a solid grey line, and *Q. pyrenaica* with quadrangular symbols and dashed line.

In general, waterlogging treatment also had a significant effect on the time to germinate (Table 2). A long period of submersion (30 days) lengthened the time until seed germination, in comparison with $W_{15}$, although the effect was significant only in the case of *Q. pyrenaica* ($p = 0.009$; while for *Q. canariensis* $p = 0.38$, and for *Q. suber* $p = 0.20$). In contrast, a shorter period of submersion (15 days) decreased the time to germinate, in comparison with $W_0$ treatment, in both *Q. canariensis* ($p = 0.03$) and *Q. pyrenaica* ($p < 0.001$; while for *Q. suber* $p = 0.79$) (Fig. 3a).

### 3.2. Interactions with seed mass

In general, seed mass significantly affected different parameters related to seed germination and root growth, explaining a large proportion of the total variance for many of them (Table 2). However, its relative importance varied depending on oak species and waterlogging treatment.

**Table 1**
Results of the analysis of generalised linear models for evaluating the differences among waterlogging treatments for the three studied species. The *W*-values (that correspond to values of statistic based on the asymptotic normality property of maximum likelihood estimates) and the level of significance (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) are indicated. The $W_{60}$ treatment has not been included into the analyses because no cases of germination were recorded for *Q. canariensis* or *Q. suber* seeds, and only three cases for *Q. pyrenaica*.

| | *Q. canariensis* | | | *Q. suber* | | | *Q. pyrenaica* | | |
|---|---|---|---|---|---|---|---|---|---|
| | $W_0$ | $W_{15}$ | $W_{30}$ | $W_0$ | $W_{15}$ | $W_{30}$ | $W_0$ | $W_{15}$ | $W_{30}$ |
| $W_0$ | – | 2.31 | **9.43**$^{**}$ | – | 0.15 | **46.36**$^{***}$ | – | **63.98**$^{***}$ | **43.66**$^{***}$ |
| $W_{15}$ | 2.31 | – | **21.32**$^{***}$ | 0.15 | – | **52.82**$^{***}$ | **63.98**$^{***}$ | – | **5.93**$^{*}$ |
| $W_{30}$ | **9.43**$^{**}$ | **21.32**$^{***}$ | – | **46.36**$^{***}$ | **52.82**$^{***}$ | – | **43.66**$^{***}$ | **5.93**$^{*}$ | – |

**Table 2**
Results of the analysis of covariance for some parameters related with seed germination and root growth, according to waterlogging treatments and seed mass. The proportion of the explained variance (SSx/SStotal) and the level of significance (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) for each factor and oak species are indicated. $R^2$ is the proportion of total variance absorbed by the model. The $W_{60}$ treatment has not been included into the analyses because of the scarcity or absence of seed germination in this treatment for the three species.

| Parameter | *Q. canariensis* | | | *Q. suber* | | | *Q. pyrenaica* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Treatment | Seed mass | $R^2$ | Treatment | Seed mass | $R^2$ | Treatment | Seed mass | $R^2$ |
| Time to germination | 12.08** | 5.00* | 17.08 | 5.79* | 9.26*** | 15.05 | 25.75*** | 0.21 | 25.96 |
| Root length | 14.22** | 10.01** | 24.23 | 1.35 | 4.72* | 6.07 | 27.58*** | 0.65 | 28.23 |
| Root dry weight | 4.72 | 4.61 | 9.33 | 6.97* | 12.72*** | 19.69 | 29.20*** | 1.17 | 30.37 |
| Root growth rate | 2.73 | 2.3 | 5.03 | 18.84*** | 4.37* | 23.21 | 27.11*** | 4.03** | 31.14 |

On one hand, the probability of seed germination increased with seed mass, although the effect was significant only in the case of *Q. canariensis* ($W = 9.2$; df = 1; $p = 0.02$; while for *Q. suber* $W = 0.39$; df = 1; $p = 0.53$, and for *Q. pyrenaica* $W = 2.01$; df = 1; $p = 0.16$). For this species, significant correlations between seed mass and germination probability, after controlling the false discovery rate, were only found for the $W_{30}$ treatment (Table 3). The time-to-germination was also significantly affected by seed mass, except in the case of *Q. pyrenaica* (Table 2): the higher the seed mass, the shorter the time-to-germination.

On the other hand, seed mass was positively correlated with root growth parameters, although with marked differences between oak species and waterlogging treatments (Table 2). In the case of *Q. canariensis*, correlations between seed mass and root weight/growth were stronger for long periods of submersion ($W_{30}$), and with root length for non-submerged seeds (Table 3). In contrast, for *Q. suber*, the different root growth parameters were significantly correlated with seed mass in $W_0$ and $W_{15}$ treatments, but there were no significant correlations for long periods of submersion (Table 3). Finally, seed mass of *Q. pyrenaica* was significantly correlated with root growth rate only in $W_{15}$ treatment (Table 3).

### 3.3. *Root growth*

Soil waterlogging produced a significant effect on several parameters of root growth, although with marked differences between the three oak species.

The greatest differences between treatments, in terms of root growth, occurred in *Q. pyrenaica* (Table 2). $W_{30}$ seeds produced a radicle shorter than that of $W_{15}$ seeds ($p < 0.001$), with a lower growth rate ($p < 0.001$) and, consequently, a lower final dry weight ($p < 0.001$) (Fig. 2). However, non-treated seeds ($W_0$) had the lowest root growth, in comparison with $W_{15}$ ($p = 0.0013$ for growth rate, and $p < 0.001$ for root length and final dry weight) and with $W_{30}$ ($p = 0.01$ for root length, $p = 0.03$ for final dry weight, but $p = 0.37$ for growth rate) (Fig. 3).

In the case of *Q. suber*, final dry weight was significantly ($p < 0.001$) lower in both $W_{15}$ and $W_{30}$ than in the $W_0$ treatment. Root length and growth rate were also significantly lower in $W_{15}$ ($p = 0.018$ and $p = 0.0015$, respectively), but there were no significant differences between $W_{30}$ and $W_0$ treatments ($p = 0.11$ for root length and $p = 0.19$ for root growth rate) (Fig. 3).

Submersion treatments did not affect root growth in germinated seeds of *Q. canariensis* (Figs. 3C,D). The effect of soil waterlogging

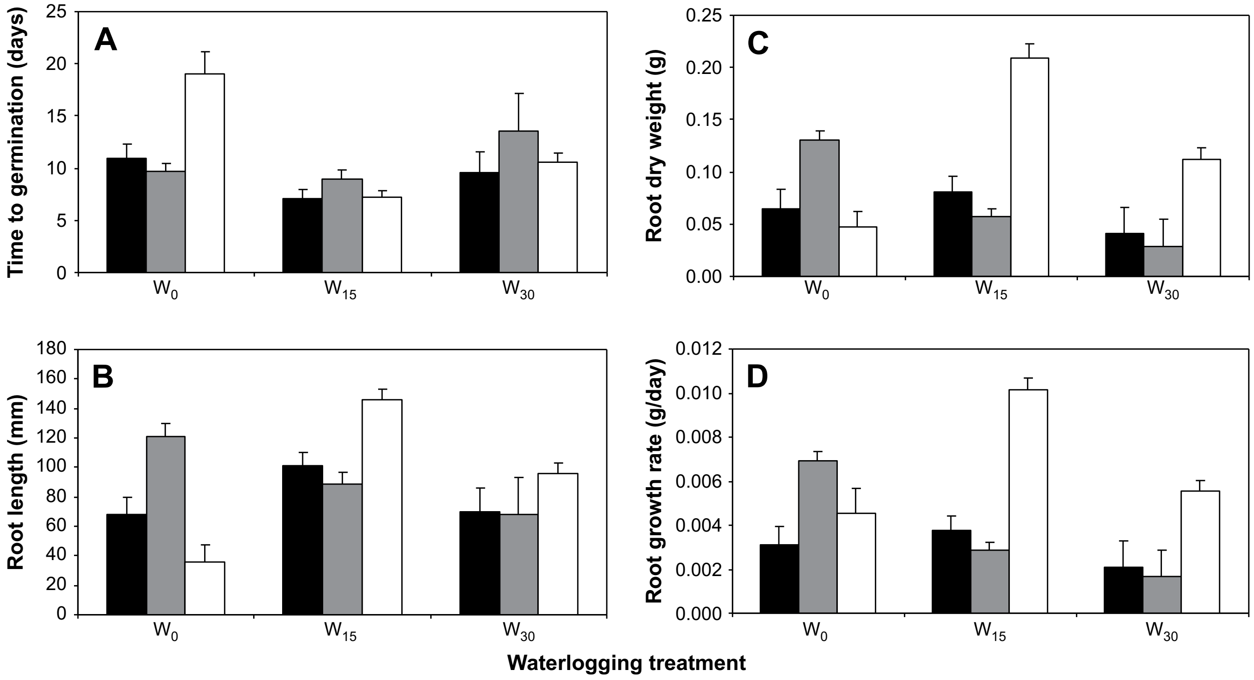

**Fig. 3.** Differences between waterlogging treatments ($W_{15} = 15$ days; $W_{30} = 30$ days; $W_0 = 0$ days) and oak species (*Q. canariensis* with black bars, *Q. suber* with grey bars and *Q. pyrenaica* with white bars) for some parameters related with seed germination and root growth: (A) time to germination (days); (B) root length (mm); (C) root dry weight (g); and (D) root growth rate (g/day). Mean and SE bars are indicated. The $W_{60}$ treatment has not been represented because of the scarcity or absence of seed germination in this treatment for the three species.

**Table 3**
Matrix of correlations between initial seed mass and some parameters related with seed germination and root growth. Results are separated according to oak species and waterlogging treatments. The level of significance is indicated as follows: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$, and those values that remained significant after controlling the false discovery rate are highlighted with bold letters. The $W_{60}$ treatment has not been included into the analyses because of the scarcity or absence of seed germination in this treatment for the three species.

| Parameter | *Q. canariensis* | | | *Q. suber* | | | *Q. pyrenaica* | | |
|---|---|---|---|---|---|---|---|---|---|
| | $W_0$ | $W_{15}$ | $W_{30}$ | $W_0$ | $W_{15}$ | $W_{30}$ | $W_0$ | $W_{15}$ | $W_{30}$ |
| Probability of germination | 0.14 | 0.23$^{*}$ | **0.32**$^{**}$ | 0.08 | −0.1 | −0.1 | −0.07 | −0.03 | −0.18 |
| Time to germination | −0.28 | 0.03 | −0.42 | **−0.42**$^{**}$ | **−0.33**$^{*}$ | 0.59 | −0.005 | −0.04 | −0.07 |
| Root length | **0.44**$^{*}$ | 0.29 | −0.005 | 0.22 | 0.23 | −0.58 | −0.30 | 0.10 | 0.10 |
| Root dry weight | 0.39 | 0.02 | **0.59**$^{*}$ | **0.45**$^{**}$ | 0.29$^{*}$ | −0.56 | −0.27 | 0.21 | 0.15 |
| Root growth rate | 0.30 | 0.02 | **0.62**$^{*}$ | **0.33**$^{*}$ | 0.14 | −0.27 | −0.30 | **0.34**$^{**}$ | 0.18 |

was significant only for final root length, with a longer radicle being recorded in $W_{15}$ seeds ($p = 0.047$) than in non-submerged ones (Table 2 and Fig. 3B).

## 4. Discussion

Under controlled conditions, waterlogging not only decreased the probability of seed germination, but also hampered root development in two of the studied species. The negative effects of soil waterlogging occurred even in absence of other possible associated factors, such as attacks of pathogenic organisms. Therefore, we can infer that seeds may have experienced low oxygen concentration (hypoxia) during submersion treatments, potentially curtailing respiration and impeding complete radicle development (e.g., Kozlowski, 1997; Schmull and Thomas, 2000). The effects of excess water on seed germination and root growth varied between oak species, and these differential responses could contribute to explain, at least partially, oak species habitat and distribution patterns (see below).

### 4.1. *Importance of soil waterlogging period*

The three studied species were not able to germinate under water but showed a certain level of tolerance to waterlogging – only those seeds subjected to long periods of submersion (over 30 days) were affected. In rainy years, the soil of woodlands where the three oak species coexist – Aljibe mountains in south Spain – can remain over-watered for long periods of time, comparable to those simulated in our laboratory experiments (see Fig. 1). Similarly, Guo et al. (1998) found that seeds of oaks, even of those species living in flooded habitats, could not germinate after long periods of late season flooding.

However, the effect of soil waterlogging was neutral (in the case of *Q. suber*) or even positive (for *Q. canariensis*, considering root growth) in those seeds subjected to short periods of submersion (15 days). The particular case of *Q. pyrenaica*, that showed a lower probability of seed germination when their seeds were not submerged, could be attributed to two possible reasons: (i) the seeds of this species likely require for germination a minimum level of water that was not furnished by the $W_0$ treatment; and/or (ii) they could suffer a problem of over-desiccation during transport.

Therefore, in spite of the relative tolerance to soil waterlogging, the successful seedling establishment of these oaks will probably be favoured during years with enough rains to moisten the soil without producing persistent waterlogging. In contrast, frequent and abundant precipitations during rainy years will generate over-abundant levels of water on the forest ground, and tree recruitment will probably be hampered, especially in those microhabitats prone to over-watering, such as ill-drained clayey patches.

### 4.2. *Interactions with seed mass*

Seed mass played an important role on the early stages of regeneration, as has been documented in other studies (e.g., Bonfil, 1998; Lloret et al., 1999; Gómez, 2004; Poorter and Rose, 2005; Quero et al., 2007). Larger seeds increased germination rates (in the case of *Q. canariensis*), accelerated germination timing, and enhanced root growth rates. Since oak seedlings receive most of energy from the seed during the first growth season (Long and Jones, 1996; Quero et al., 2007; Pérez-Ramos et al., in revision), it is not surprising to find a positive correlation between seed mass and different parameters of root growth (Tripathi and Khan, 1990; Sonesson, 1994; Bonfil, 1998). However, the relative importance of seed mass for germination performance varied depending on species and waterlogging treatment. In the case of *Q. suber*, the influence of seed mass was significant only for non-submerged seeds or for those submerged for short periods of time and, thereby, the negative effects of a longer waterlogging were not attenuated by a higher amount of reserves contained in the seed. In contrast, in the case of *Q. canariensis*, positive correlations with seed mass were maintained or even more marked in those seeds submerged for long periods of time. Interestingly, the tolerance to waterlogging for this oak species increased with seed mass, with the largest seeds being more tolerant than the smallest ones.

### 4.3. *Waterlogging-tolerance and oak species distribution*

Waterlogging had different effects on oak regeneration. On one hand, waterlogging during prolonged periods of time (over 30 days) lengthened the time-to-germination, although the differences between treatments were significant only in the case of *Q. pyrenaica*. A delayed germination induced by waterlogging – until environmental conditions turn favourable for seedling establishment – has been also reported for the common ash (*Fraxinus excelsior*) by a recent study that simulates flooding under controlled conditions (Dacasa and Dounavi, 2008).

On the other hand, waterlogging reduced the root development in two of the three studied species, with more pronounced differences in the case of *Q. suber*. Under field conditions, a less developed root system decreases the survival rate of the resultant seedling (Lloret et al., 1999) by reducing its ability to absorb water deeper during the dry season (Nicotra et al., 2002). Our laboratory results allow us to explain the delay in time-to-emergence and the lower survival of oak seedlings found in over-watered microhabitats under field conditions (Urbieta et al., 2008a). Similarly, in temperate forested wetlands, flooding increased time-to-emergence, and reduced seedling survival and total biomass (Jones et al., 1997).

The main differences between oak species occurred in terms of root growth, with a gradient of responses probably associated with waterlogging tolerance, when the two dominant oak species at the study site are compared. *Q. canariensis* might be considered the species most tolerant to excess water due to waterlogging treatments did not produce any negative effect on root growth parameters, and a longer radicle was even detected in those seeds submerged for short periods of time. On the contrary, *Q. suber* was much more sensitive to waterlogging than its co-dominant oak species. In general, submerged seeds of this species showed a lower root growth rate and, consequently, a shorter and smaller final root system in comparison with the non-treated seeds. Finally, the root development of *Q. pyrenaica* seeds was also impaired by waterlogging, but only for long periods of submersion (over 30 days). Indeed, likely as a consequence of a lack of initial hydration, the non-treated seeds delayed its germination and produced a lower root system than those that were submerged during 15 or even 30 days.

Main ecological factors such as topography, soil fertility, or human disturbances influence Mediterranean forest composition (e. g., Maltez-Mouro et al., 2005; Ajbilou et al., 2006; Coca, 2007; Urbieta et al., 2008b). However, inter-specific differences in the responses to waterlogging can also contribute to explain oak species habitat and distribution patterns. Indeed, in Mediterranean ecosystems, factors associated with water availability are commonly found to have a great influence on forest composition (Piggot and Piggot, 1993; Zavala et al., 2000). At the studied forest sites, the distribution pattern of the three oak species differs along the soil moisture gradient. *Q. canariensis* is locally abundant in the moister sites, associated with areas near streams, while *Q. suber* appears in a wider range of soil humidity and is more frequent in plots located further from the drainage network (Urbieta et al., 2008b). Even more, *Q. canariensis* is the most abundant tree in the riparian forests of the region, and contributes to build favourable habitat conditions for a set of Tertiary plant relicts (Mejías et al., 2007). Finally, the case of *Q. pyrenaica* is different, restricted to scarce populations at higher altitudes, probably associated to its higher tolerance to frost.

## 5. Conclusions

In this study, we have experimentally demonstrated that waterlogging can act as an important factor limiting germination and root growth of three Mediterranean oak species, supporting the importance of heterogeneity in soil water content for the early regeneration stages of oak species, as found in parallel field experiments (Urbieta et al., 2008a). Excess water during prolonged periods of time not only decreased the probability of seed germination in these three oak species, but also lengthened the time to germination and hampered root development in two of the studied species. The main differences between oak species occurred in terms of root growth (*Q. canariensis* being the less affected, and *Q. suber* the most); these differential responses could be related to a species rank of waterlogging tolerance. Thus inter-specific differences in germination responses to excess water could contribute to explain, at least partially, species habitat and distribution patterns across landscapes.

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