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Research paper

Increased root investment can explain the higher survival of seedlings of ‘mesic’ *Quercus suber* than ‘xeric’ *Quercus ilex* in sandy soils during a summer drought

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In Mediterranean-type ecosystems, drought is considered the main ecological filter for seedling establishment. The evergreen oaks *Quercus ilex* L. and *Quercus suber* L. are two of the most abundant tree species in the Mediterranean Basin. Despite their shared evergreen leaf habit and ability to resist low soil water potentials, traditionally it has been suggested that Q. *ilex* is better suited to resist dry conditions than Q. *suber*. In this study, we examined how seedlings of Q. *ilex* and Q. *suber* grown in sandy soils responded to different levels of water availability using natural dry conditions and supplemental watering. Specifically, we estimated survival and water status of seedlings and explored the role of acorn mass and belowground biomass in seedling performance. To our surprise, Q. *suber* was better able to survive the summer drought in our experiment than Q. *ilex*. Nearly 55% of the Q. *suber* seedlings remained alive after a 2-month period without rain or supplemental water, which represents almost 20% higher survival than Q. *ilex* over the same period. At the end of the dry period, the surviving seedlings of Q. *suber* had strikingly higher water potential, potential maximum quantum yield of photosystem II (F_v/F_m) and stomatal conductance (g_s) than those of Q. *ilex*. Acorn mass was associated with the probability of survival under dry conditions; however, it did not explain the differences in survival or water status between the species. In contrast, Q. *suber* had a higher root ratio and root:shoot ratio than Q. *ilex* and these traits were positively associated with predawn leaf water potential, F_v/F_m, g_s and survival. Taken together, our results suggest that the higher relative investment in roots by Q. *suber* when growing in a sandy acidic substrate allowed this species to maintain better physiological status and overall condition than Q. *ilex*, increasing its probability of survival in dry conditions.

Keywords: drought avoidance, drought tolerance, oaks, root depth, root ratio, seed mass, seed size.

Introduction

Drought is one of the most important selective factors in nature, affecting plant establishment, long-term survival, growth and reproduction (Malhi and Wright 2004, Sheffield and Wood 2008, Allen et al. 2010, Ramírez-Valiente et al. 2010, 2015, McDowell et al. 2011, Asner et al. 2016). Recently, severe and prolonged seasonal droughts inducing dieback events have been increasingly reported across many ecosystems globally (Malhi and Wright 2004, Sheffield and Wood 2008, Allen et al. 2010, McDowell et al. 2011, Asner et al. 2016). Seedlings are particularly vulnerable to water stress (Cavender-Bares and Bazzaz 2000, Mediavilla and Escudero 2004), and their ability to cope with water shortage is critical to the long-term sustainability of drought-prone ecosystems (Lloret et al. 1999, Padilla and Pugnaire 2007). Under a scenario of increasing drought, understanding trees' and particularly seedlings' responses to water limitation is critical for forest conservation and management (Choat et al. 2007, Engelbrecht et al. 2007, Bonan 2008, Feeley et al. 2011, Anderegg et al. 2016).

In Mediterranean-type ecosystems, drought is considered the main ecological filter for seedling establishment (Fotelli et al. 2000, Larcher 2000, Ackerly 2004, Arosa et al. 2015). The evergreen oaks *Quercus ilex* L. and *Quercus suber* L. are two of the most abundant tree species in the Mediterranean Basin (EUFORGEN database www.euforgen.org/species, Caudullo et al. 2017). They are adapted to withstand seasonal drought through their small leaf lamina, thick leaves, low shoot water potential at turgor loss point, low cuticular transpiration and high resistance to drought-induced cavitation when compared with more mesic oaks (Kerstiens 1996, Tyree and Cochard 1996, Corcuera et al. 2002, Vilagrosa et al. 2003, Quero et al. 2006, Montserrat-Martí et al. 2009, Peguero-Pina et al. 2014). These species also have deep and well-developed root systems that allow them to access groundwater (David et al. 2004, Lubczynski and Gurwin 2005, Kurz-Besson et al. 2006), and close their stomata at lower water potentials than other congeneric species from more mesic areas (Corcuera et al. 2002, Mediavilla and Escudero 2003, Vilagrosa et al. 2003). However, despite their shared evergreen leaf habit and ability to withstand low water potentials, these two species have different and contrasting distribution ranges (Figure 1). For example, in the Iberian Peninsula, where both species are present, *Q. ilex* is the dominant tree in the drier and cooler areas inland, whereas the calcifugous *Q. suber* is mainly restricted to the south-western region, characterized by a wetter and warmer climate and acidic soils (Díaz-Fernández et al. 1995, Jiménez et al. 1996). The high sensitivity of *Q. suber* to low temperatures (Aranda et al. 2005) contrasts with *Q. ilex*, which has developed an efficient leaf photo-protection strategy to cope with low temperatures in winter (García-Plazaola et al. 1999). There are some areas where both species occur and occasionally hybridize (Belahbib et al. 2001, Soto et al. 2007, Burgarella et al. 2009) (Figure 1). It is typically suggested that their geographical distributions reflect differences in the species' response to a combination of abiotic factors including water availability, temperature and soil type (Cavender-Bares et al. 2005, David et al. 2007).

Dry conditions are traditionally thought to suit *Q. ilex*, particularly *Q. ilex* spp. *rotundifolia*, better than *Q. suber* (David et al. 2007); in part, this is because of its higher leaf mass per area (LMA), leaf and mesophyll thickness (Mediavilla and Escudero 2004, Peguero-Pina et al. 2017). Likewise, *Q. ilex* is more resistant to xylem embolism than *Q. suber* (Tyree and Cochard 1996). In contrast, it has been hypothesized that *Q. suber* should displace *Q. ilex* in more mesic Mediterranean areas because of its higher carbon assimilation and growth rates (Quero et al. 2006, 2008). However, there are some inconsistencies in the results of studies comparing the two species in terms of growth potential and drought resistance. For instance, Peguero-Pina et al. (2017) reported that *Q. ilex* subsp. *rotundifolia* and *Q. suber* had similar carbon assimilation rates when water was not a limiting factor for seedlings. On the other hand,

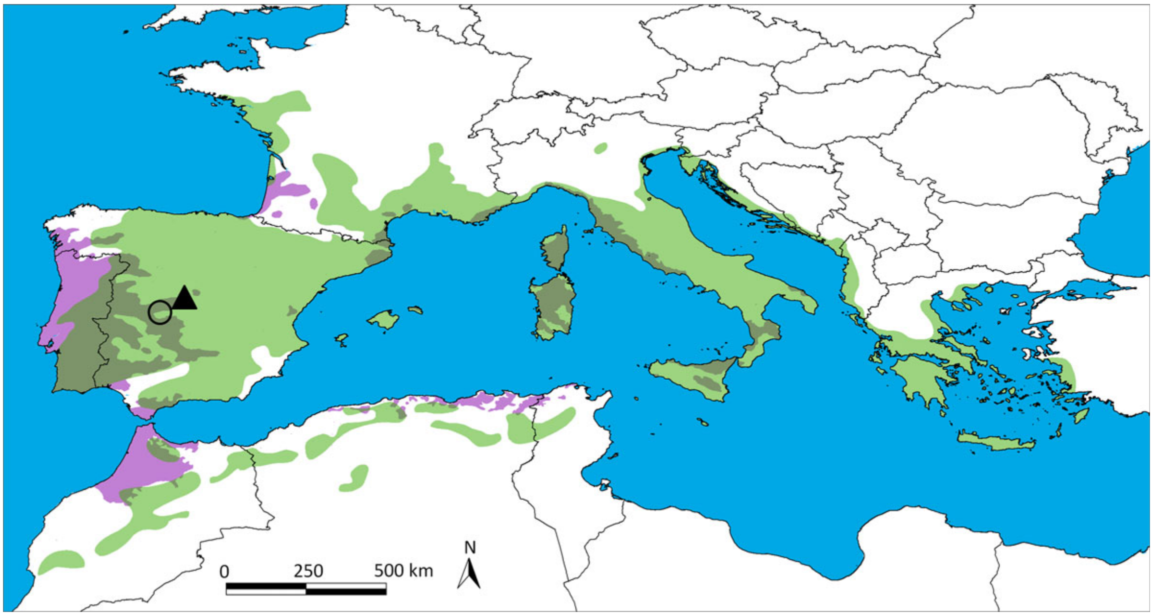

Figure 1. Distribution ranges of *Q. ilex* (green) and *Q. suber* (purple). Overlapping areas between ranges of both species are shown in brown. Modified from EUFORGEN database (www.euforgen.org/species) and Caudullo et al. (2017). The open circle indicates the area where the maternal families for the common garden were sampled. The black triangle indicates where the field experiment was established.

Corcuera et al. (2002) found that *Q. suber* and *Q. ilex* subsp. *rotundifolia* had a similar water potential at the turgor loss point and maximum bulk modulus of elasticity, which would confer on both species similar tolerance to moderate water stress. Surprisingly, none of the studies comparing *Q. suber* and *Q. ilex* subsp. *rotundifolia* have examined how drought affects fitness-related traits such as survival.

We devised a field experiment to test how seedlings of *Q. ilex* and *Q. suber* respond to different levels of water availability, using natural conditions and supplemental watering to create differential soil-moisture treatments during summer drought. In particular, this allowed us to evaluate the potential role of investment in roots for the water status, photosynthetic potential and ultimately survival of the oak seedlings. Allocation to belowground organs is considered a drought avoidance mechanism that allows plants to increase their probability of survival during dry periods (Lloret et al. 1999, Markesteijn and Poorter 2009). Several studies have found a higher root biomass ratio (i.e., the biomass invested in roots compared with the total biomass), or root–shoot ratio (i.e., the biomass invested in roots compared with the aboveground biomass), to increase survival in oaks (Villar-Salvador et al. 2004*a*, 2004*b*, 2012). Analyses of soil and leaf water potentials have demonstrated that deep soil layers can supply most of the water required by *Q. suber* during summer drought (Otieno et al. 2006). Likewise, isotope analyses have shown that mature trees of *Q. ilex* obtain most of their water from deep soil layers during dry periods (Barbeta and Peñuelas 2017). These studies suggest that root investment might be critical in allowing both *Q. ilex* and *Q. suber* to cope with water stress during prolonged droughts. In addition, seed mass is also considered a key trait determining the successful recruitment of seedlings, particularly in dry environments (Leishman and Westoby 1994, Seiwa 2000, Baraloto et al. 2005, Rodríguez-Calcerrada et al. 2011). The importance of seed mass lies in the influence that it exerts on traits such as above- and belowground growth during seedling establishment under both competitive and resource-limiting conditions (Moles and Westoby 2004). Positive associations between seed mass and survival have previously been reported in *Q. ilex* and *Q. suber* (Gómez 2004, Ramírez-Valiente et al. 2009), likewise an acorn size–growth relationship (Quero et al. 2008), but the mechanisms mediating such a pattern have not yet been elucidated in these species.

In this study, our specific research questions were: Do seedlings of *Q. ilex* and *Q. suber* differ in their response to natural vs supplemental precipitation regimes in terms of survival, growth, root ratio, photosystem II (PSII) efficiency and stomatal conductance (g_s)? Are acorn mass, root ratio, PSII efficiency and g_s associated with survival under dry conditions?

Since *Q. ilex* is usually found in more xeric areas than *Q. suber*, we expected that *Q. ilex* would have higher survival under dry conditions than *Q. suber*, whereas the latter would have higher growth rates under mesic conditions (Quero et al. 2006, David et al. 2007, Peguero-Pina et al. 2017). Previous studies reveal that *Q. ilex* has a greater capacity for osmotic adjustment and consequently tolerates low soil water potential better than *Q. suber* (drought tolerance strategy) (e.g., Salleo and Lo Gullo 1990, Tognetti et al. 1996, Villar-Salvador et al. 2004*a*, 2004*b*, Otieno et al. 2006, Castro-Díez and Navarro 2007). Less-consistent differences between these species have been reported in drought avoidance mechanisms such as allocation patterns and g_s (e.g., González-Rodríguez et al. 2011).

Materials and methods

Seed collection and experimental design

In winter 2006, we collected acorns from *Q. suber* and *Q. ilex* stands in an area located in Central Spain (40°20′N, 7°00′W), where both species coexist (Figure 1). The climate in this area is typically Mediterranean and soils are dystric and eutric cambisols (Díaz-Fernández et al. 1995, Jiménez et al. 1996). Twenty open-pollinated trees (10 per species) were sampled. Sampled trees were each separated by more than 150 m in order to avoid kinship structures (Soto et al. 2007). Seeds were stored at 4 °C. Most viable seeds germinated at this temperature before the synchronous sowing in May 2007. A common-garden experiment was established in a field site at the Centro Nacional de Recursos Genéticos Forestales (CNRGF) growth facilities (40°25′N, 3°44′W). The soils in the experimental plot and surrounding area where both species naturally coexist are dystric and eutric cambisols with a pH between 6.0 and 7.0 (Martín-Albertos et al. 1998). In the plot, the analyses revealed that the soil has sandy loam texture, 2% of organic matter in the top layer and very low content of carbonates (Table S1 available as Supplementary Data at *Tree Physiology* Online for more details). The experimental design consisted of two randomized complete blocks. Each block was divided into two subplots where watering treatments were later implemented. Between 2 and 37 seeds per family per subplot (depending on availability) were directly sown on a grid at 25 × 25 cm spacing (n = 1120). Once emerged, seedlings were kept under well-watered conditions (20–30% volumetric soil water content) and ambient temperature (average T = 19.6 °C) for 60 days after sowing. At the end of this period, two watering treatments were applied for another 60 days. In the well-watered treatment, we watered every 2 days. In the dry treatment, no water was added. Soil water content was monitored every 3–7 days over the experiment at four depths: 20, 30, 40 and 60 cm, using TDR probes (Time Domain Reflectometry, Trime®IT IMKO, Germany) (see Figure S1 available as Supplementary Data at *Tree Physiology* Online and results for details). There was no rainfall during the watering treatments.

Trait measurements

Due to the logistical limitations, we randomly selected 211 seedlings from five families per species (3–17 plants/family/species/treatment depending on availability) to monitor growth, survival, allometry and physiology traits. Stem height, basal diameter and the number of leaves were measured at the beginning and at the end of the watering treatments (i.e., 0 and 60 days after the start of the watering treatments). We used these variables to estimate initial (M_{initial} at $t = 0$ days; T_{initial}) and final biomass (M_{final} at $t = 60$ days; T_{final}) with allometric equations developed from a subsample of plants harvested at the end of the experiment ($n = 36$ plants per species). Absolute growth rate (AGR, g day^{-1}) was calculated as $(M_{\text{final}} - M_{\text{initial}})/(T_{\text{final}} - T_{\text{initial}})$. Relative growth rate (RGR, mg g^{-1} day^{-1}) was calculated as $[\log_e(M_{\text{final}}) - \log_e(M_{\text{initial}})]/(T_{\text{final}} - T_{\text{initial}})$.

Twenty days after the start of the watering treatments ($t = 20$), dark- and light-acclimated chlorophyll fluorescence measurements were performed on leaves of the 211 selected seedlings using FMS-2 (Hansatech Instruments Ltd, Norfolk, UK). Measurements were repeated on two further occasions at intervals of 15 days ($t = 35$ and $t = 50$). Dark measurements were made predawn or very early in the morning using dark-adaptation clips. Light measurements were made on leaves receiving full sun on clear sunny days at midday, when the irradiance was highest. We measured F_m and F_0, F_m' and F_s. F_m is the maximal chlorophyll fluorescence measured during a 0.8 s pulse of saturating white light, and F_0 is the minimal chlorophyll fluorescence measured during exposure to a weak modulated measuring beam. F_m' and F_s are the maximal and the steady-state chlorophyll fluorescence measured during actinic illumination, respectively. For further analysis, we calculated: $F_v/F_m = (F_m - F_0)/F_m$, which is the potential maximum quantum yield of PSII in a dark-adapted leaf, and $\Phi_{\text{PSII}} = (Fm' - F_s)/Fm'$, the effective quantum yield of PSII in an irradiated leaf, which is related to its CO_2 fixation rate and growth rates in oaks (Cavender-Bares and Bazzaz 2004).

On the same three dates ($t = 20$, $t = 35$ and $t = 50$), we measured area-based stomatal conductance (g_s) in each plant of the five selected families per species. We made two measurements per leaf (which were averaged prior to statistical analyses), and measured at two time intervals during the day, early in the morning and at midday. Stomatal conductance was measured with a leaf porometer (SC-1; Decagon Devices, Pullman, WA, USA).

We also monitored survival on the same dates as above. At $t = 50$, we measured predawn leaf water potential (Ψ_{PD}) in the surviving plants. At the end of the experiment ($t = 60$), we harvested a randomly selected subsample of plants within each treatment to estimate total biomass and investment in roots, leaves and stems ($n = 49$ plants per species). All plant parts were dried in an oven at 65 °C. Root ratio (also known as root mass fraction), leaf and stem ratios (i.e., each as a fraction of total dry biomass) were calculated. The root:shoot ratio [root biomass/(stem + leaf biomass)] was also estimated.

Statistical analyses

A linear mixed model was used for statistical analyses of growth traits, allometric traits and leaf water potential. Species and Treatment were fixed-effect factors, Block and Family nested within Species were treated as random-effect factors and acorn mass was included as a covariate. The model equation for these traits was

$$Y_{ijklp} = \mu + S_i + T_j + F(S)_{ik} + B_l + ST_{ij} + TF(S)_{ijk} + M_p + E_{ijklp} \quad (1)$$

where Y_{ijklp} is the observed value for the variable considered in the pth individual of the kth open-pollinated family from the ith species in the jth treatment into the lth block; μ is the overall mean; S_i is the fixed effect of the ith species; T_j is the fixed effect of the jth treatment; $F(S)_{ik}$ is the random effect of the kth family nested within the ith species; B_l is the random effect of the lth block; ST_{ij} is the fixed effect of the interaction between the ith species and the jth treatment, $TF(S)_{ijk}$ is the random effect of the interaction between the jth treatment and the kth family nested within the ith species; M_p is the value of acorn mass for the pth individual; and E_{ijklp} is the residual error term.

This linear mixed model was amended for F_v/F_m and Φ_{PSII} to include Date as an additional fixed-effect factor because measurements were made on three different dates, making the model a split-plot-in-time. The mixed model for g_s also included time-of-day as a fixed-effect factor because this trait was measured in the morning and at midday. The model equations for F_v/F_m, Φ_{PSII} and g_s are included in Appendix S1 available as Supplementary Data at *Tree Physiology* Online. When necessary, data were transformed (logarithm, square root) to meet normality and homoscedasticity assumptions.

A generalized linear mixed model was implemented for final survival (i.e., survival at the end of the experiment) using a binomial distribution and logit link function. The model was only applied to seedlings from the dry treatment because there was no mortality in the well-watered treatment. This model included Species as a fixed-effect factor, and Block and Family nested within Species as random-effect factors. The model equation for survival was

$$Y_{iklp} = \mu + S_i + F(S)_{ik} + B_l + M_p + E_{iklp}$$

where the description of the terms is consistent with that of Eq. (1). Analyses were performed with the package lme4 (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2017) in R 3.4.2 (R Development Core Team 2015). Degrees of Freedom (df), chi-square values (χ^2) and significance P-values were obtained from likelihood-ratio tests comparing reduced models to the full model using function 'anova'.

We also tested differences between species in acorn dry mass using a mixed model including Species as a fixed factor and Family nested within species as a random factor. To test for associations between traits within treatments, we performed linear regressions at the level of the individual and maternal family. Three linear regressions were performed for each pair of traits, one for *Q. ilex*, one for *Q. suber* and one for both species together. The trait g_s was log-transformed for some tests. Relationships between survival and traits were examined by performing linear regressions using family means. To test for associations across treatments, g_s, Ψ_{PD} and Φ_{PSII} were log-transformed (see Savage et al. 2009 for a similar approach). Regression analyses were performed in STATISTICA 10.0 (StatSoft, Inc., Tulsa, OK, USA). Sigmaplot 12.5 (Systat Software Inc., San Jose, CA, USA) was used to plot figures.

Results

In the well-watered treatment, volumetric soil water content (VWC) ranged between 20% and 30% at 20, 30 and 40 cm depth, and between 25% and 40% at 60 cm (see Figure S1 available as Supplementary Data at *Tree Physiology* Online). In the dry treatment, VWC decreased from 20–30% to 5–10% after 2 months in the top 20 cm of soil (see Figure S1 available as Supplementary Data at *Tree Physiology* Online). Reductions in VWC were also evident at 30, 40 and 60 cm soil depth but to a lesser extent than those at 20 cm depth (see Figure S1 available as Supplementary Data at *Tree Physiology* Online). The experiment finished with first rains of autumn (60 days after the start of the differential watering treatments). The average temperature during this period was $T = 22.9$ °C (maximum $T = 39.2$ °C and minimum $T = 7.8$ °C). Acorns of *Q. ilex* had a higher dry mass (0.900 ± 0.085 g) than those of *Q. suber* (0.762 ± 0.085 g) but differences were not significant ($\chi^2 = 1.32$, $P = 0.251$). The effect of acorn mass was significant for basal diameter, g_s and final survival (Tables 1–4).

There were differences between treatments in absolute and relative growth rates (AGR and RGR), during the period after the start of watering treatments, and in aboveground growth (height and number of leaves) at the end of the experiment (Table 1). However, treatment effects on aboveground growth traits were only evident for *Q. suber* not for *Q. ilex*, with the exception of RGR (Figure 2) (see also species × treatment interactions below). As expected, plants growing in the dry treatment had lower AGR, RGR, accumulated height growth and number of leaves (Figure 2). Predawn leaf water potential (Ψ_{PD}) differed between treatments in both species and plants growing in the dry environment exhibited lower values (Table 1, Figure 2). Differences between treatments in root ratio and root:shoot ratio were only evident for *Q. suber*, such that seedlings growing in the dry environment had higher root and root:shoot ratios (Table 1, Figure 3). Leaf ratio did not differ between treatments and stem ratio only differed between treatments for *Q. suber* (Table 1, Figure 3). The watering treatment affected leaf physiological traits (Tables 2 and 3): seedlings had lower F_v/F_m, Φ_{PSII} and g_s in the dry treatment compared with the well-watered treatment for both species (Figure 4). The differences between treatments in F_v/F_m and g_s increased at the end of the experiment (significant interaction watering treatment × date of measurement), as expected since the summer drought increased the difference between the treatments in VWC over time (Tables 2 and 3, Figure 4).

There were also differences between species, or species × treatment interaction, for most traits. In the well-watered treatment, *Q. suber* had higher AGR, total height, number of leaves, stem ratio and stem biomass but a lower leaf ratio than *Q. ilex*, which typically has larger thicker leaves (Table 1, Figures 2 and 3; see Figure S2 and Table S2 available as Supplementary Data at *Tree Physiology* Online), implying that the two species use different strategies in allocating resources to leaf traits. In the dry treatment, *Q. suber* had higher Ψ_{PD}, root ratio and root:shoot ratio than *Q. ilex* (Table 1, Figures 2 and 3). In the dry treatment, both species had similar growth rates (AGR, RGR), aboveground growth (height, number of leaves and diameter) and aboveground leaf, root and stem biomass (Figure 2; see Figure S2 available as

Table 1. Results of mixed models for absolute growth rate (AGR), relative growth rate (RGR), height, number of leaves (N leaves), diameter, predawn leaf water potential (Ψ_{PD}), root ratio, root:shoot ratio, leaf ratio and stem ratio. Chi-squares (χ^2) and P-values are shown. Fixed factors: Species, Treatment. Random factors: Family (Species), Block. Covariate: Seed mass.

		AGR	RGR	Height	N leaves	Diameter	Ψ_{PD}	Root ratio	Root:shoot	Leaf ratio	Stem ratio
Species	χ^2	**5.61**	3.22	**9.99**	**8.65**	0.12	**38.64**	**11.02**	**12.53**	**32.93**	0.71
	P	**0.004**	0.073	**0.002**	**0.003**	0.7241	**<0.001**	**0.001**	**<0.001**	**<0.001**	0.400
Treatment	χ^2	**38.46**	**60.67**	**9.10**	**31.01**	1.52	**172.75**	**18.51**	**10.97**	1.83	**9.92**
	P	**<0.001**	**<0.001**	**0.003**	**<0.001**	0.217	**<0.001**	**<0.001**	**0.001**	0.176	**0.002**
Species × Treatment	χ^2	**8.82**	**4.33**	**5.99**	**5.71**	0.47	**49.24**	**7.76**	**5.92**	0.32	**6.22**
	P	**0.003**	**0.038**	**0.014**	**0.017**	0.492	**<0.001**	**0.005**	**0.015**	0.571	**0.013**
Block	χ^2	0	0.00	0.01	0	0.14	0	0	0	0	0
	P	–	0.975	0.908	–	0.710	–	–	–	–	–
Family (Species)	χ^2	0	0	0.01	0.01	3.04	0	**4.01**	2.31	0.11	0
	P	–	–	0.944	0.910	0.081	–	**0.045**	0.059	0.740	–
Treatment × Family (Species)	χ^2	0	0	3.17	0.02	0.00	0	0	0	0	0
	P	–	–	0.075	0.898	0.966	–	–	–	–	–
Seed mass	F	3.44	1.37	0.18	1.32	**6.47**	1.07	1.98	1.80	1. 85	0.50
	P	0.063	0.241	0.674	0.250	**0.011**	0.302	0.159	0.180	0.173	0.478

Note: Significant values are in bold face ($P < 0.05$).

Table 2. Results of mixed models for F_v/F_m and effective quantum yield of PSII (Φ_{PSII}). Degrees of freedom (df), Chi-squares (χ^2) and *P*-values are shown. Fixed factors: Species, Treatment, Date. Random factors: Family(Species), Block. Covariate: Seed mass.

	df	χ^2	*P*-value
F_v/F_m			
Species	1	33.56	**<0.001**
Treatment	1	167.1	**<0.001**
Date	2	150.5	**<0.001**
Species × Treatment	1	26.99	**<0.001**
Species × Date	2	31.10	**0.001**
Treatment × Date	2	71.91	**<0.001**
Species × Treatment × Date	2	19.44	**<0.001**
Block	1	2.76	0.097
Family(Species)	1	0.39	0.532
Treatment × Family(Species)	1	0	–
Date × Family(Species)	1	0	–
Seed mass	1	3.70	0.054
Φ_{PSII}			
Species	1	20.87	**<0.001**
Treatment	1	466.6	**<0.001**
Date	2	30.80	**<0.001**
Species × Treatment	1	0.33	0.564
Species × Date	2	2.59	0.274
Treatment × Date	2	3.45	0.178
Species × Treatment × Date	2	0.90	0.639
Block	1	10.85	**<0.001**
Family(Species)	1	0	–
Treatment × Family(Species)	1	0	–
Date × Family(Species)	1	0	–
Seed mass	1	0.23	0.631

Note: Significant values are in bold face ($P < 0.05$).

Table 3. Results of the mixed model for area-based stomatal conductance (g_s). Degrees of freedom (df), Chi-squares (χ^2) and *P*-values are shown. Fixed factors: Species, Treatment, Date, Daytime. Random factors: Family(Species), Block. Covariate: Seed mass.

	df	χ^2	*P*-value
Species	1	22.51	**<0.001**
Treatment	1	501.3	**<0.001**
Date	2	42.27	**<0.001**
Daytime	1	78.57	**<0.001**
Species × Treatment	1	28.61	**<0.001**
Species × Date	2	41.18	**<0.001**
Species × Daytime	1	3.68	0.055
Treatment × Date	2	44.10	**<0.001**
Treatment × Daytime	1	66.26	**<0.001**
Species × Treatment × Date	2	33.86	**<0.001**
Species × Treatment × Daytime	1	1.57	0.211
Block	1	15.60	**<0.001**
Family(Species)	1	4.49	**0.034**
Treatment × Family(Species)	1	0.21	0.650
Date × Family(Species)	2	0	–
Daytime × Family(Species)	1	0	–
Seed mass	1	9.93	**0.002**

Note: Significant values are in bold face ($P < 0.05$).

Table 4. Results of the generalized linear mixed model for final survival ($t = 50$ days). Degrees of freedom (df), Chi-squares (χ^2) and *P*-values are shown. Fixed factor: Species. Random factors: Family(Species), Block. Covariate: Seed mass.

	df	χ^2	*P*-value
Species	1	**5.78**	**0.016**
Block	1	**8.32**	**0.004**
Family(Species)	1	0.00	–
Seed mass	1	**6.68**	**0.010**

Note: Significant values are in bold face ($P < 0.05$).

Supplementary Data at *Tree Physiology* Online). *Quercus suber* had higher F_v/F_m and g_s in the dry treatment in the latter half (from day 35) of the experiment (Tables 2 and 3, Figure 4). Differences between species in the well-watered treatment for these traits were small or absent (Figure 4). *Quercus suber* seedlings had greater survival than *Q. ilex* at the end of the experiment (Table 4, Figure 5).

Linear regressions did not show an association between acorn mass and survival using family means, indicating that differences in survival between species could not be explained by acorn mass (see Figure S3 available as Supplementary Data at *Tree Physiology* Online). Root biomass was positively associated with survival (see Figure S3 available as Supplementary Data at *Tree Physiology* Online). Acorn mass was not associated with root ratio but it was associated with root biomass (see Figure S3 available as Supplementary Data at *Tree Physiology* Online), which could explain the positive effect of acorn mass on survival at the individual level (Table 4).

In the dry treatment, traits related to the water status of the plants such as Ψ_{PD}, F_v/F_m and g_s were associated with root ratio and leaf ratio across species (Figure 6). Seedlings with a higher root ratio and lower leaf ratio had higher Ψ_{PD}, F_v/F_m and g_s (Figure 6; see Figure S4 available as Supplementary Data at *Tree Physiology* Online). *R*-squared values were moderate, particularly for associations of leaf traits/survival with leaf ratio/root ratio (Figure 6; see Figure S4 available as Supplementary Data at *Tree Physiology* Online). Similar associations were present between Ψ_{PD}, F_v/F_m and g_s with root:shoot ratio or root biomass (data not shown). Plants that had higher root ratio and Ψ_{PD} maintained higher g_s in the dry treatment (Figure 6; see Figure S5 available as Supplementary Data at *Tree Physiology* Online). However, when species were analysed separately, linear regressions between root ratio and Ψ_{PD}, F_v/F_m and g_s were not significant, indicating that associations were caused by species differences in these traits (Figure 6; see Figure S4 available as Supplementary Data at *Tree Physiology* Online). Higher investment in roots was also associated with greater survival after 2 months of drought (Figure 6). In the well-watered treatment, RGR was positively associated with g_s using individual values and Φ_{PSII} using family means (see Figure S6 available as Supplementary Data at *Tree Physiology* Online). Both g_s and Φ_{PSII} were associated across treatments with predawn water potential (Ψ_{PD}) in negative log-linear relationships

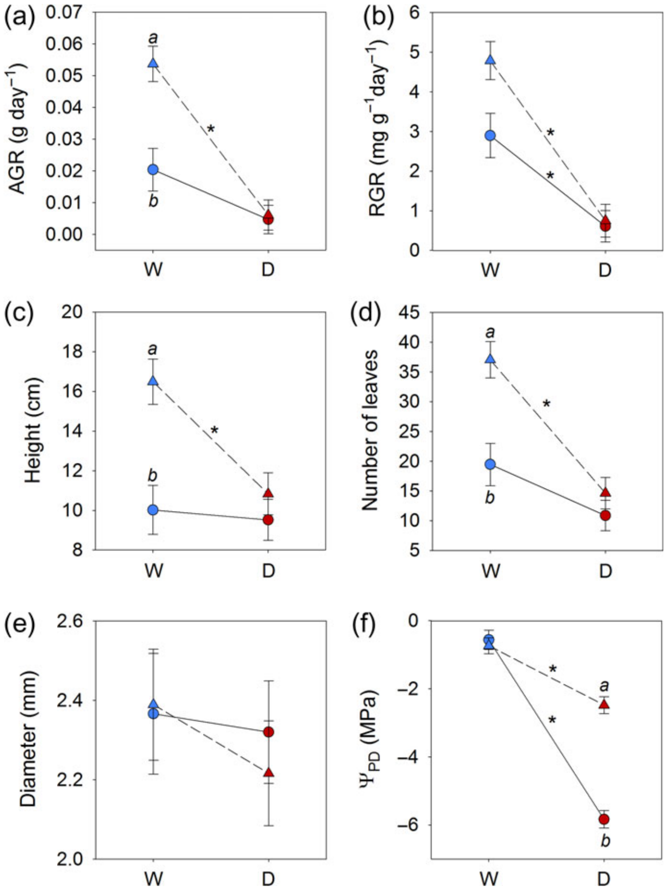

Figure 2. Least squares means (±SE) of (a) absolute growth rate (AGR), (b) relative growth rate (RGR), (c) height, (d) number of leaves, (e) basal diameter and (f) predawn water potential (Ψ_{PD}) for *Q. suber* (triangles) and *Q. ilex* (circles) under well-watered (W) and dry (D) treatments. Asterisks (*) indicate differences between treatments for each species. Letters indicate differences between species within treatments.

(see Figure S7 available as Supplementary Data at *Tree Physiology* Online).

Discussion

Contrary to our hypothesis, *Q. suber* was better able to survive the summer drought than *Q. ilex* in our experiment (Figure 5). Nearly 55% of the *Q. suber* seedlings remained alive after a 2-month period without water: this represents almost 20% higher survival than *Q. ilex* over the same period. At the end of the dry period, the surviving seedlings of *Q. suber* had strikingly higher water potential, F_v/F_m and g_s than those of *Q. ilex* (Figures 2 and 4). The most striking difference between the species was that *Q. suber* had a higher root ratio than *Q. ilex* (Figure 3), and this trait was positively associated with Ψ_{PD}, F_v/F_m, g_s and survival across species (Figure 6). Taken together, our results suggest that higher relative investment in roots by *Q. suber* allowed this species maintain better physiological status and overall condition than *Q. ilex*, increasing its probability of survival under dry conditions in a sandy acidic substrate.

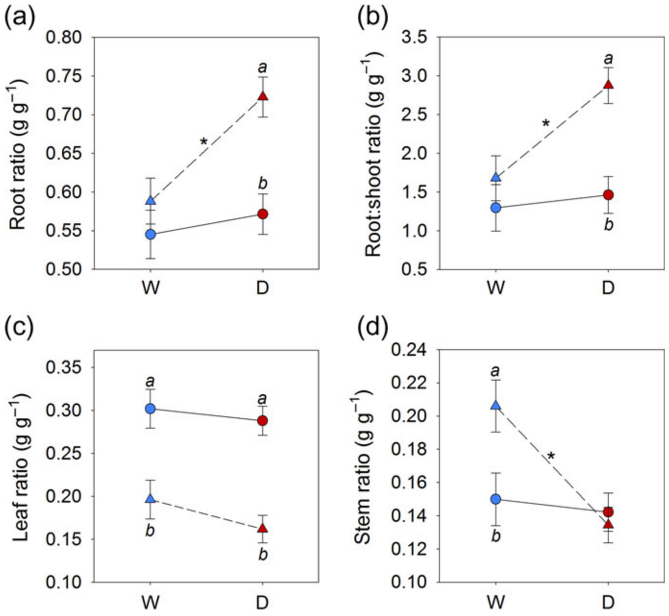

Figure 3. Least squares means (±SE) of (a) root ratio, (b) root:shoot ratio, (c) leaf ratio and (d) stem ratio for *Q. suber* (triangles) and *Q. ilex* (circles) under well-watered (W) and dry (D) treatments. Asterisks (*) indicate differences between treatments for each species. Letters indicate differences between species within treatments.

Numerous studies have reported that bigger acorns lead to increased seedling growth, which can improve seedling performance by providing the resources to grow deep and extensive root systems, giving access to water from deep within the soil profile (Stock et al. 1990, Tripathi and Khan 1990, Osunkoya et al. 1994, Gómez 2004, Ramírez-Valiente et al. 2009, Rodríguez-Calcerrada et al. 2011). In fact, strong associations have been reported between acorn mass and survival in *Q. ilex* and *Q. suber* (Gómez 2004, Ramírez-Valiente et al. 2009). In our experiment, high acorn mass was positively associated with the probability of survival under dry conditions at the individual level probably because of an increased capacity for root growth (Table 4; see Figure S3 available as Supplementary Data at *Tree Physiology* Online), but this did not explain the species differences in survival (see Figure S3 available as Supplementary Data at *Tree Physiology* Online).

On the other hand, although the total and aboveground biomass, height and number of leaves in the dry treatment were similar in the two species, *Q. ilex* had higher relative investment in leaves, i.e., leaf ratio. Higher leaf ratio might be caused by a larger leaf lamina area, thicker leaves or both. The interpretation of our results would differ depending on the main mechanism underlying the difference in leaf ratio. If leaf area is the main factor mediating differences in leaf ratio between the two species, then the lower survival and poor water status of *Q. ilex* seedlings could, at least in part, be the result of depletion of soil moisture due to their higher whole-plant transpiration (Poorter and Remkes 1990, Villar et al. 2017). We did not measure specific

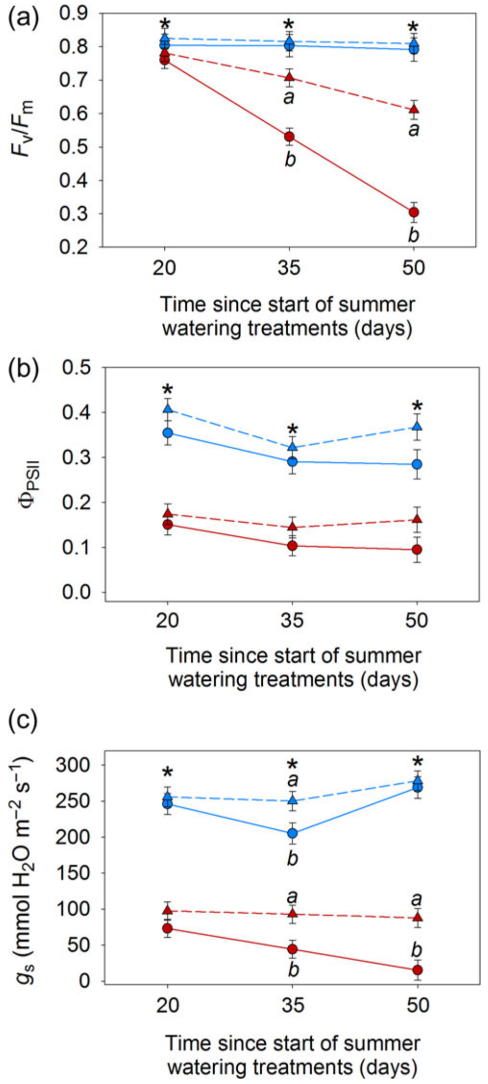

Figure 4. Least squares means (±SE) of (a) potential quantum yield of PSII in dark-adapted leaves (F_v/F_m), (b) effective quantum yield of PSII in leaves receiving full solar irradiance (Φ_{PSII}) and (c) stomatal conductance (g_s) for *Q. suber* (triangles) and *Q. ilex* (circles) under dry (red) and well-watered (blue) treatments on three occasions during the summer drought vs watering treatments ($t = 50$, 35 and 50 days). Asterisks (*) indicate differences between treatments for each species. Letters indicate differences between species within treatments.

leaf area (ratio between leaf area and leaf mass) in our study, but the facts that *Q. ilex* usually has thicker leaves than *Q. suber* (e.g., Cavender-Bares et al. 2005, Peguero-Pina et al. 2017) and that root ratio was better correlated with survival and water status of seedlings than leaf ratio (see Figure S4 available as Supplementary Data at *Tree Physiology* Online) suggest that whole-plant transpiration area have had a low impact on the differences in the performance between *Q. ilex* and *Q. suber*.

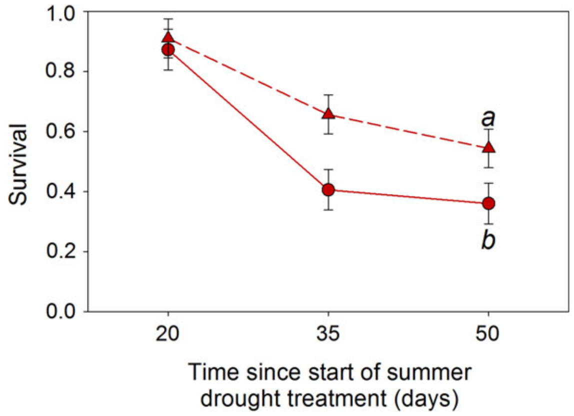

Figure 5. Least squares means (±SE) of survival measured in *Q. suber* (triangles) and *Q. ilex* (circles) on three occasions during the summer drought vs watering treatments. Only the dry (red) treatment results are shown since all seedlings survived in the well-watered treatment. Letters indicate differences between species within treatments at the end of the experiment.

Root ratio, which represents the investment in roots relative to the total biomass, seemed to underpin the different probability of survival under dry conditions between these two evergreen oak species. Mediterranean oak species have tended to favour belowground growth over aboveground growth in studies where water is limiting (Villar-Salvador et al. 2004*a*, 2004*b*, Cubera et al. 2009, Corcobado et al. 2014). In our study, *Q. suber* exhibited higher root ratio than *Q. ilex* after 2 months of drought, similar to the findings reported by Quero et al. (2008) and González-Rodríguez et al. (2011). A higher root ratio in oaks is produced by increases in both lateral and tap roots allowing a larger volume and depth of soil to be explored (Chiatante et al. 2005, Chirino et al. 2008). The size and length of the root system have also been found to predict predawn water potential and survival under seasonal drought (Bellot et al. 2002, Grossnickle 2005, Padilla and Pugnaire 2007, Chirino et al. 2008, Villar-Salvador et al. 2012). Although high relative investment in roots is a characteristic of most species from the genus *Quercus*, there can be species-specific differences such as those reported by Espelta et al. (2005) when comparing *Q. ilex* and *Quercus cerrioides* Willk.. We did not measure root length, but the fact that root ratio was positively correlated with leaf water potential, F_v/F_m and g_s across species suggests that high root biomass allocation allowed seedlings of *Q. suber* to increase water uptake, probably because their deeper roots reached soil with higher water content (Otieno et al. 2006). Consistent with these findings, 3-year-old seedlings of *Q. suber* exhibited higher survival than *Q. ilex* seedlings of the same age growing in a fluvisol (González-Rodríguez et al. 2011). In another study, adult trees of *Q. suber* had higher predawn leaf water potentials than those of *Q. ilex* under summer drought in an open woodland where both species coexist (Mediavilla and Escudero 2003). However, the opposite result has been also recorded on several occasions in both adult trees and seedlings, i.e., *Q. ilex* exhibited higher predawn leaf water potentials than *Q. suber* when growing under low water availably (Acherar and Rambal 1992, David et al. 2007, Vaz et al. 2010). In addition, seedlings of *Q. ilex* typically have a lower water potential at the turgor loss point (π_{tlp}) than those of *Q. suber*,

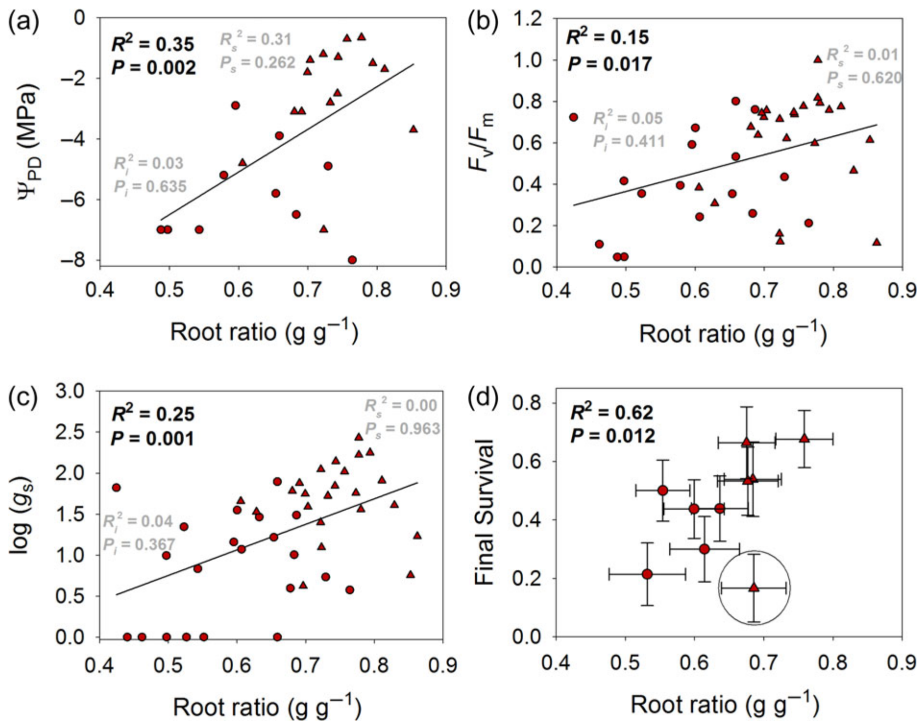

Figure 6. Relationships between root ratio and (a) predawn leaf water potential (Ψ_{PD}), (b) F_v/F_m and (c) stomatal conductance (log-transformed, g_s) in the dry treatment measured 50 days after the start of watering treatments. Results of regression analyses conducted for each species separately are shown with subscripts (*i* – *Quercus ilex*, *s* – *Quercus suber*) in linear fits (R^2) and significant levels. (d) The relationship between root ratio and survival in the dry treatment at the end of the experiment using family means (±SE). *Quercus ilex*: circles, *Quercus suber*: triangles. Linear fit (R^2) and significant level are shown when excluding one of the *Q. suber* families, which was an extreme outlier for some traits including survival and Φ_{PSII} (see also Figure S6 available as Supplementary Data at *Tree Physiology* Online).

indicating higher drought tolerance including when grown in common environmental conditions (Salleo and Lo Gullo 1990, Sala and Tenhunen 1994, Tognetti et al. 1996, Nardini et al. 1999, Villar-Salvador et al. 2004*a*, 2004*b*, Pardos et al. 2005, Otieno et al. 2006, Castro-Díez and Navarro 2007). Only Corcuera et al. (2002) reported similar values of π_{tlp} for both species.

Taken together, these previous findings suggest that *Q. ilex* seedlings better tolerate low soil water potentials than *Q. suber* seedlings probably because of their better ability to accumulate solutes and lower their osmotic potential under dry conditions (a drought-tolerance strategy). In contrast, our results suggest that *Q. suber* seedlings increased root biomass in response to low soil water potentials whereas *Q. ilex* did not (a drought avoidance strategy), which is consistent with a previous study (González-Rodríguez et al. 2011). Evolutionary trade-offs between drought tolerance and avoidance strategies have been reported for other oaks from seasonally dry ecosystems (Cavender-Bares and Ramírez-Valiente 2017, Ramírez-Valiente and Cavender-Bares 2017). The key question is; what are the conditions that favour one strategy over the other in terms of fitness? The fact that *Q. suber*, which is usually found in more mesic areas than *Q. ilex*, survived better under dry conditions opens a debate on whether rainfall and the severity and length of the dry season are the main selective forces shaping the distribution ranges of *Q. ilex* and *Q. suber*. Several studies have shown that *Q. suber* is more sensitive to cold temperatures than *Q. ilex*. For example, Cavender-Bares et al. 2005 found that *Q. suber* was less resistant to xylem embolisms than *Q. ilex* under freezing conditions. Aranda et al. (2005) found that PSII efficiency of *Q. suber* seedlings was sensitive to cold temperatures, but PSII is not usually sensitive to cold in *Q. ilex* (Nardini et al. 2000, Corcuera et al. 2005, Camarero et al. 2012). *Quercus suber* is also considered a strictly calcifugous species and is absent from basic soils, whereas *Q. ilex* tolerates a wider range of soil types (Valdecantos et al. 2006). For example, Serrasolses et al. (2009) showed that *Q. suber* seedlings, established in siliceous sandstone soils under high annual precipitation, had higher growth rates than seedlings growing in carlcareous soils and low annual precipitation regimes. In contrast, Sardans et al. (2006) did not find any effect of soil type (siliceous or calcareous) on seedling growth and biomass allocation patterns in *Q. ilex*. These differences in physiological tolerance and habitat preference highlight the need to explore the effect on the performance of *Q. ilex* and *Q. suber* of interactions among multiple abiotic factors including drought, soil chemical properties, soil granulometry and temperature to ascertain their relative roles in shaping the distributions of these evergreen oaks.

In conclusion, our results revealed that *Q. suber* seedlings survived better than *Q. ilex* during a 2-month summer drought period without any precipitation. Surviving seedlings of *Q. suber* also had strikingly higher water potential, F_v/F_m and g_s than those of *Q. ilex*. The relative investment in roots was strongly linked to survival, water potential, F_v/F_m and g_s across species, suggesting that the higher probability of survival and the better water status of seedlings of *Q. suber* was probably explained by a drought avoidance strategy that allowed them to maintain higher water content throughout the dry season in a sandy soil. Comparative studies of these two species regarding complementary drought tolerant traits, such as osmotic adjustment and xylem vulnerability to cavitation and their relationship with fitness at different ontogenetic stages, are needed to provide a definitive perspective on the drought resistance mechanisms of these two oaks and explain the main factors driving their distribution and coexistence patterns.

Supplementary Data

Supplementary Data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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