

Summer and winter can equally stress holm oak (*Quercus ilex* L.) in Mediterranean areas: A physiological view.

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Highlights

- Holm oak is an evergreen and sclerophyllous tree closely associated to Mediterranean-type climates.
- However, the origin of this species can be found in pre-Mediterranean lineages, under tropical or subtropical climates.
- To survive in Mediterranean-type climate implies to withstand dry and hot summer, cold winters or both.
- The overall response of holm oak to stress factors (drought, high or low temperatures) suggests the existence of a marked within-species variability.
- The current taxonomical consideration of holm oak as a complex of two different subspecies might go beyond morphological traits or geographical distribution.

Abstract

Q. ilex is a circum-Mediterranean species which must deal with two stressful periods throughout the year: summer and winter. However, the intensity of these abiotic stresses is very variable depending on the specific area of its distribution range. Besides, two subspecies are usually distinguished, even sometimes recognised as two different true species. Nevertheless, differences in the response to both summer and winter stresses among populations overstep the purely taxonomic question. Regarding to temperature, this species has shown a high resistance to both extremes. On the one hand, critical temperature at leaf level has been estimated in 50.2°C for a provenance in the Iberian Peninsula (Soria, Spain), a temperature which even in the hottest areas of its distribution range is hardly ever reached. On the other hand, minimal temperature for the survival of the leaves has been estimated in -19.8°C for Italian provenances and -26.6°C for one of the coldest provenances (Soria, Spain). Similarly, these temperatures are quite rarely reachable in their respective provenances. Concerning to summer-drought, this may compromise xylem stability in severe cases of scarcity of water in the soil, inducing cavitation when water soil potential is too negative. In this sense, *Q. ilex* has demonstrated to have a high resistance to cavitation, represented by quite negative values of P_{50} and P_{88} when compared to other species. Besides, Iberian provenances (i.e. the most continental ones) have shown a higher resistance to cavitation than French or Italian ones. Likewise, holm oak may suffer cavitation during winter, but in this case by

a completely different factor: freezing-thawing cycles. Winter-cavitation seems to be related to vessel size, with wider vessels being more vulnerable to cavitate than narrower ones. In spite of its paleotropical origin, *Q. ilex* has achieved to develop multiple adaptations and physiological strategies that has enabled it not only to withstand the hard climatic conditions imposed by Mediterranean climate, but to colonize and even dominate great part of the Mediterranean landscape.

Keywords: Drought; Frost; Xylem-cavitation; *Quercus ilex*; Mediterranean-type climates

1.

Introduction

As a result of its geological history and its current geographical situation between 30° and 40° latitude, the Mediterranean Basin has been recognized to have its own climate, the Mediterranean one, which is shared with only other four and less-extensive areas at similar latitudes in both hemispheres. This genuine climate is characterized by dry summers and cold or mild moist winters (Walter, 1985; Lionello et al., 2006). Consequently, the vegetative period is mainly split into two seasons: spring and autumn, since plant growth is constrained by drought during summer and low temperatures during winter (Mitrakos, 1980). This particularity has led to consider that the Mediterranean climate presents a highly adapted vegetation type. In this respect, Schimper (1903), who firstly synthesized the worldwide geobotanical information in his book “Plant-geography upon a physiological basis”, stated that “the mild temperature districts with winter rain and prolonged summer drought are the home of evergreen xerophilous woody plants, which, owing to the stiffness of their thick, leathery leaves, may be termed as sclerophyllous woody plants”. Later, Walter (1985) summarized the climates in the world and assigned to the Mediterranean region its own Zonobiome: the Zonobiome IV or “Zonobiome of Sclerophyllic Woodlands”. In this regard, Breckle (2002) claimed that the original zonal vegetation of this Zonobiome was evergreen sclerophyllous forest and woodlands with *Q. ilex*.

Holm oak is widely considered a circum-Mediterranean tree species that covers a large geographic range in the Mediterranean Basin, from the Iberian Peninsula and northwestern Africa to Greece and northern Anatolian Peninsula. Besides, there are remarkable differences in the altitude that this species occupies within the Mediterranean Basin, from coastal forests of *Q. ilex* in the Italian part of Adriatic Sea and Tyrrhenian Sea (Gratani, 1997), the Turkish Black Sea coast or the southeastern Mediterranean coast of France up to 2500-2800 m a.s.l (metres above sea level) in the Moroccan western part of High Atlas (Barbero et al., 1992). For these reasons, holm oak can be found in very different habitats with a quite ample range of rainfall regime, from semiarid to perhumid (Niinemets, 2015). However, there is no doubt that this species has its optimum in the western Mediterranean, and it begins to lose importance as we approach to Middle East, where holm oak is often substituted by *Quercus calliprinos* Webb in coastal and milder habitats (Barbero et al., 1992). In the transitional areas, towards other climatic regimes, the presence of *Q. ilex* can be seriously restricted

due to the strong competition with other species, many of them congeneric ones. Thus, when both aridity and mean annual temperature decreases (i.e. the transition to Zonobiome VI or “Zonobiome of Deciduous Forests”), *Q. ilex* would be substituted by winter deciduous submediterranean *Quercus* species such as *Quercus faginea* Lam. and *Quercus pyrenaica* Willd. in Western Mediterranean, or *Quercus cerris* L. and *Quercus frainetto* Ten. in the Eastern Mediterranean. Furthermore, when summer drought disappears as far as temperate climates are reached, forests are dominated by species like *Quercus robur* L., *Quercus petraea* (Matt.) Liebl. or *Fagus sylvatica* L. On the other hand, holm oak can also be restricted by the increase in climatic hardness. In this sense, *Quercus coccifera* L. substitutes *Q. ilex* in the transition towards Zonobiome III or “Zonobiome of Hot Deserts”, where deserts and semi-arid areas are found. And, finally, when the mean annual temperature decreases but the average rainfall keeps similar (i.e. towards the Zonobiome VII or “Zonobiome of Steppes and Cold Deserts”), it can be assumed that only one of the recognized subspecies, *Q. ilex* subsp. *ballota* (Desf.) Samp., that has been also considered a separate species (*Quercus rotundifolia* Lam. Encycl. 1: 723 (1785)) can withstand the cold conditions of the innermost part of the Iberian Peninsula. In the eastern part of its distribution, and under the same circumstances, holm oak would be substituted by *Quercus baloot* Griff. (Gil-Pelegrín et al., 2017).

Therefore, it is reasonable to identify *Q. ilex* as a paradigm species of the Mediterranean climate. However, is *Q. ilex* a truly Mediterranean species originated under Mediterranean conditions? Or, conversely, is it an older taxon that presents pre-adaptations to Mediterranean climate? Genus *Quercus* is currently divided into eight sections grouped in two subgenera: subgenus *Quercus* and subgenus *Cerris*, the latter comprising the three most ancient sections, e.g. section *Ilex*, to which *Q. ilex* belongs (Jiang et al. 2019). Section *Ilex* was originated in Asia, as suggested by the existence of macrofossils with a clear affinity to species of this group, being the oldest one dated approximately 34 Ma (early Oligocene) (Su et al., 2019a). Phylogenetic analyses supported this fact and estimated the divergence of sections *Ilex* and *Cerris* some million years before, during Eocene (ca. 48 Ma) (Jiang et al., 2019). For that epoch, ancestor of section *Ilex* species occupied tropical and subtropical forests in the Himalaya-Hengduan mountains. The clade of Mediterranean species diverged during Oligocene ca. 28 Ma ago and migrated via a humid, forested Tibet Himalaya corridor (Jiang et al., 2019).

This migration continued during Miocene, and the convulse environmental changes that Europe underwent during Neogene were likely to facilitate both speciation and colonization of Europe (Jiang et al., 2019). This fact is supported by fossil evidence of *Quercus drymeja* Unger and *Quercus mediterranea* Unger, morphotype complexes of several fossil taxa that resemble the extant Himalayan, East Asian, and Southeast Asian species of *Quercus* section *Ilex*, which may indicate subtropical, relatively humid conditions (He et al 2014, Denk et al., 2017) that were maintained up to middle-Miocene. In Himalaya-Hengduan mountains, during Pliocene until present, these humid tropical and subtropical conditions have remained, and despite that, some species exhibit xeromorphic-like traits as is evidenced by fossil records such as *Quercus yangyiensis* He, suggesting that xeromorphic traits do not necessarily imply dry or xeric conditions (He et al., 2014). Whereas in the Mediterranean region, during the middle and late-Miocene, a progressive decrease in rainfall and variation in temperature started due to tectonic movements (e.g., closure of communication between Mediterranean Sea and Indian Ocean) and glaciations (Pons et al., 1995). By 14 Ma ago, all tropical elements in the north-western Mediterranean Basin disappeared and a seasonally contrasted temperature regime was initiated (Suc, 1989). Although the dryness seems to be characteristic of Mediterranean Basin since Miocene, it was not until Pliocene (ca. 3.2 Ma ago) when temperature regimes dropped once again (Suc, 1984) and induced drier conditions, establishing the thermally contrasted seasons throughout this area. This phenomenon can be considered the origin of the current Mediterranean climate (Pons et al., 1995). These climatic changes are supported by palynological evidences: *Quercus* species became predominant from 3.2 Ma onwards and other xerophytic taxa became more frequent (*Phillyrea*, *Olea*, *Cistus*, *Pistacia*) (Suc, 1984).

Therefore, according to the evolutionary history of *Q. ilex* and the *Ilex* section it belongs to, we can finally conclude that this species was not originated under Mediterranean conditions, but in tropical and subtropical forests in Asia under humid conditions with the absence of a summer-dry season. This fact contrasts with the palaeobotanical origin of *Q. faginea*, an oak species of the *Quercus* group co-occurring with *Q. ilex* in the inland areas of the Iberian Peninsula, whose fossil records coincided with the development of the Mediterranean seasonality during the Pliocene (Roiron, 1983). Interestingly, other Himalayan-Hengduan oak species of the *Ilex* group have parallel-developed xeromorphic traits quite similar to Mediterranean species, especially in terms of sclerophylly (Alonso-Forn et al., 2020). This fact could be associated with

the existence of a period in the Himalayas during the pre-monsoon months (March to May) with scarce rainfall and increased temperatures that can potentially induce water stress in these plant species (Singh and Zobel, 1995; Shrestha et al., 2007). In fact, several sclerophyllous oak species occurring in this region showed the minimum water potential values during this period (Singh et al., 2000; Poudyal et al., 2004; Poudyal, 2013). This fact leads to think that *Ilex* section presents pre-adaptations to cope with drought that have subsequently been developed by natural selection in different parts of the world in response to similar climatic conditions, i.e. the existence of a hot-dry season.

Regardless of its palaeobotanical origin, another issue that should not go unnoticed is that holm oak currently shows a huge morphological and ecophysiological variability (Peguero-Pina et al., 2014). According to the leaf morphology, botanists usually distinguish two subspecies of holm oak, *Q. ilex* subsp. *ballota* (= *Q. rotundifolia* Lam.) and *Q. ilex* subsp. *ilex* L. On one hand, *Q. ilex* subsp. *ilex*, which presents larger and elongated leaves, is restricted to the milder, coastal and humid or subhumid areas of the northern and eastern Mediterranean region. On the other hand, *Q. ilex* subsp. *ballota*, which has smaller and thicker rounded leaves than *Q. ilex* subsp. *ilex*, occupies the driest and most continental parts of the western Mediterranean region, especially in the inner areas of the Iberian Peninsula. Thus, the analysis of the ecophysiological variability of this species is a matter of special interest that will be discussed hereunder, as both subspecies live under different climatic conditions but must cope with two potentially stressful periods throughout the year, i.e. summer drought and winter cold. Specifically, throughout this review, we will describe how holm oak is affected by both stressful periods, underscoring the mechanisms developed by this species to cope with them, mainly in terms of i/ resistance of the xylem to cavitation and ii/ tolerance to drought and extreme temperatures by photoprotection mechanisms at cell level.

2. Summer stresses: drought and heat

Summer drought is one of the main constraints for the growth and distribution of plant species subjected to Mediterranean climate conditions (Gil-Pelegrín et al., 2017). However, even though holm oak is widely considered a circum-Mediterranean tree species, there is no consensus about its resistance degree to high levels of water stress. To analyse the palaeobotanical origin of holm oak may help to shed light on this aspect.

The uncertainty on the *Q. ilex* capacity to withstand severe water stress is due to the duality between its current distribution and the evolutionary history of the section *Ilex*. Indeed, *Q. drymeja* complex is intimately related to the current Asian tropical and humid-subtropical oak species, the so-called *Heterobalanus* species (e.g. *Quercus floribunda* Lindl. ex Camus, *Quercus engleriana* Seemen, *Quercus tarokoensis* Hayata or *Quercus setulosa* Hickel & A.Camus). Conversely, *Q. mediterranea* complex is more similar to current Mediterranean sclerophyllous oak species from section *Ilex* occurring in the most arid parts of the Mediterranean Basin (Denk et al., 2017), which share a common origin with these fossil taxa (Denk and Grimm, 2010). Both floras, fossil and present, are sclerophyllous, with similar leaf morphology and some xerophytic features, but fossil-taxa occupied, migrated and evolved under humid subtropical conditions (Alonso-Forn et al., 2020). These fossil-taxa are remnants of laurophyllous vegetation, humid warm-temperate forests occupying subtropical or temperate climates, but all of them inhabiting with the absence of summer-drought (Axelrod, 1975). In short, part of the current Mediterranean flora, *Q. ilex* included, has a pre-Mediterranean origin and has not originated and evolved under Mediterranean conditions (Verdú et al., 2003). Therefore, the presence of these fossil-taxa does not imply mediterraneity, mainly because this climate did not even exist by then. It is not until the truly Mediterranean climate arises 5 Ma ago when its characteristic summer-drought periods started to appear (Suc, 1984). Nowadays, *Q. ilex* occupies some areas where drought is not severe or is even absent, especially in the northern and eastern extremes of its distribution area (Fig. 1). On the other hand, this species must definitely cope with drought in its southernmost distribution range (Fig. 1). Hence, holm oak should have developed mechanisms and strategies to survive under situations of soil water deficit and high atmospheric dryness (i.e. an elevated vapor pressure deficit, VPD) that can affect plant functioning in several ways (Fig. 2), and which will be further analysed below in this section. Thus, both dualities, the presence of xeromorphism despite of humid subtropical conditions during Tertiary and the occupation of current areas with and without summer-drought, needs to be well investigated to better clarify at what extent *Q. ilex* adapts to drought stress.

2.1. Xylem resistance to drought-induced cavitation

The long-distance transport of water through the xylem from roots to leaves is a basic function for plant growth and survival in terrestrial environments. The decrease in soil water availability is one of the main factors that can induce embolism formation (i.e. the formation of air or water vapor bubbles inside a xylem conduit), which can cause the loss of xylem functionality by cavitation. Besides, the bigger the vessels are, the more susceptible to cavitate they are. In angiosperms, an ample loss of functionality (between 80% and 90%) by cavitation of the xylem vessels seems to lead irretrievably to plant death (Urli et al., 2013). Drought-induced cavitation at moderate levels below this critical point does not provoke the death of the plant, but it will mainly affect its hydraulic function and secondarily its photosynthetic capacity, as plants close their stomata under drought conditions in order to minimize water depletion. Stomatal closure in response to soil water depletion is a widespread mechanism that has been described for holm oak by several authors (e.g. Peguero-Pina et al. 2009, 2018; Alonso-Forn et al., 2021).

Xylem resistance to drought-induced cavitation is considered a good indicator of the ability of the plants to withstand water stress. Specifically, the values of xylem tension (MPa) inducing 50% and 88% loss of hydraulic conductivity in the stems (P_{50} and P_{88} , respectively) are considered proxies of the tolerance to drought-induced embolism (Urli et al., 2013). However, how much is known about this issue in holm oak? Tyree and Cochard (1996) were among the first to elaborate vulnerability curves for some *Quercus* species, including holm oak. In the subsequent years, several studies have been published about the resistance of different provenances of holm oak to drought-induced cavitation (Table A1). Choat et al. (2012) published a meta-analysis where they compiled P_{50} and P_{88} values for many plant species, including several *Quercus* species. We took advantage of these data in order to i/ compare the resistance to drought-induced cavitation of *Q. ilex* with congeneric species, and ii/ analyse the within-species variability between both subspecies of holm oak. In a first instance, we could appreciate the high heterogeneity of genus *Quercus* in terms of cavitation resistance (Fig. 3), with species living in drier habitats showing higher values of both P_{50} and P_{88} (Fig. 3), as previously reported by Maherali et al. (2004). Regarding holm oak, we could appreciate at a glance the high degree of within-species variation in P_{50} and P_{88} , very similar to that found for a representative sample of the whole genus (Fig. 3). In spite of this, it can be concluded that holm oak was on average more resistant to cavitation than the rest of the species of the genus, especially concerning to P_{50} values.

Within *Q. ilex*, we could observe two tendencies that clearly corresponded with each subspecies (Fig. 3). *Q. ilex* subsp. *ballota* tended to present higher values for both P_{50} and P_{88} , except in the study accomplished by Pinto et al. (2012) in Portugal, where oddly low values for P_{50} were obtained (-3.21 MPa). Nevertheless, provenance does not seem to be responsible of the result, since Tyree and Cochard (1996) give a P_{50} value of -5.54 MPa for *Q. ilex* subsp. *ballota* also in Portugal (Martin-StPaul et al., 2014). Regarding *Q. ilex* subsp. *ilex*, we found lower values for both P_{50} and P_{88} when analysed French, Italian or Spanish provenances, albeit there was more heterogeneity than that showed by *Q. ilex* subsp. *ballota* (Fig. 3). The high variability in the resistance to drought-induced cavitation among provenances of *Q. ilex* subsp. *ilex* could be attributed to the high values measured by Peguero-Pina et al. (2014) for three Italian provenances measured in Zaragoza (Spain). Thus, these authors reported values of P_{50} = -5.6, -5.8 and -6.6 MPa and values of P_{88} = -8.2 and -10.1 MPa for Italian provenances, which are quite more typical of *Q. ilex* subsp. *ballota*. Notwithstanding this, the differences found in P_{50} and P_{88} between both subspecies were statistically significant ($P < 0.05$).

The fact that *Q. ilex* subsp. *ilex* provenances are able to develop a resistance to drought-induced cavitation similar to those for *Q. ilex* subsp. *ballota* provenances under climatic conditions typical of this last subspecies (Peguero-Pina et al., 2014) leads to think about the plasticity of this species in terms of xylem vulnerability. According to Cochard et al. (1997), the xylem vulnerability is not only species- or organ-specific, but can also depend on the acclimation of the plants to environmental conditions. Besides, Hacke et al. (2000) claimed that the resistance to cavitation may adjust to the amount of drought experienced in situ. In this sense, several studies have found plasticity in xylem vulnerability to embolism. For instance, Beikircher and Mayr (2009) found that forest plants of *Ligustrum vulgare* L. and *Viburnum lantana* L. were significantly less resistant to drought-induced cavitation than drought-treated garden plants. Besides, they are among the first in proposing the hypothesis that species do not develop high resistance to drought-induced embolism a priori, but are able to acclimate when extended drought periods occur. In line with this, Awad et al. (2010) found that severely water-stressed plants of *Populus x canescens* (Aiton) Sm. showed more negative P_{50} values than well-watered plants. In addition, Corcuera et al. (2011) found that *Pinus pinaster* Ait. under severe drought achieved a better resistance to xylem embolism (P_{50}). Finally, Stojnić et al. (2018) proved how marginal populations of *F. sylvatica* exhibited significant variation in xylem vulnerability to embolism. By contrast, it should be noted that some

studies have not found this plasticity. Maherali et al. (2000), for example, did not find significant changes in P_{50} when comparing populations of *Pinus ponderosa* Douglas ex C. Lawson growing in montane and desert conditions. Likewise, Cornwell et al. (2007) did not find evidence of plasticity in cavitation resistance when comparing wet and dry sites of Hawaiian *Metrosideros polymorpha* Gaudich. Concerning *Q. ilex*, no significant differences in the ability of branches to resist xylem cavitation along a climatic gradient were found either (Limousin et al., 2010; Martin-StPaul et al., 2013). Therefore, the scope of plasticity in xylem resistance to drought-induced cavitation as a main feature of plants to cope with drought is still under discussion.

The resistance to cavitation seems to be related to xylem anatomy and especially vessel size. Awad et al. (2010) postulated that the decrease in vulnerability to cavitation in plants growing under soil drought conditions was correlated with decreased vessel diameter, increased vessel wall thickness and a stronger bordered pit field. Hence, plants experiencing low (i.e. more negative) water potential values need to reinforce their vessel walls to avoid cell collapse before suffering cavitation (Hacke and Sperry, 2001; Nardini et al., 2014). Several studies have corroborated this hypothesis, showing that lower vulnerability to drought was associated with larger wood density (Hacke and Sperry, 2001; Jacobsen et al., 2007; Pratt et al., 2007). Thus, diffuse-porous species like *Q. ilex* would be more resistant to drought-induced cavitation than ring-porous species, such as *Q. robur* (Cochard et al., 1992; Bréda et al., 1993). Besides, holm oak presents an intraspecific plasticity regarding to vessel size (Campelo et al., 2010). To evaluate this within-species variation in vessel size in *Q. ilex*, we measured this trait in one-year old seedlings from three provenances of *Q. ilex* subsp. *ilex* (Veneto, Lazio and Sardinia; Italy) and four provenances of *Q. ilex* subsp. *ballota* (Cuenca, Ciudad Real, Jaen and Soria; Spain). To do this, middle segments of seedling stems were transversally sectioned with a sliding microtome (Microm HM 350S, Thermo Scientific, Walldorf, Germany). Sections with a thickness of 20 μ m were dehydrated by means of ethanol series and stained with safranin (Corcuera et al., 2004). The cross-sections were viewed with a Leica DM LB microscope (Leica Microsystems, Wetzlar, Germany) and photographed with a digital camera (Moticam 2300, Motic, Hong Kong, China). The resulting micrographs were analysed using Image-J software (<http://rsb.info.nih.gov/nih-image/>) to measure vessel number and diameter. The results obtained from these measurements suggest that *Q. ilex* subsp. *ilex* tended to have wider

vessels, having on average more vessels over 18 μm than *Q. ilex* subsp. *ballota*, which seemed to have narrower vessels (Fig. 4).

Another issue that should be considered when analyse both intraspecific and interspecific differences in resistance to cavitation may be related to safety margin. Brodribb and Hill (1999) defined the safety margin as the difference between soil water potential inducing stomatal closure (namely turgor loss point, TLP) and P_{50} . Alternatively, Meinzer et al. (2009) defined the safety margin as the difference between the minimum water potential experienced by the tree in the field during the dry season and the water potential inducing 12, 50 and 88% loss of hydraulic conductivity. In the case of holm oak, values of safety margin were quite similar regardless of the criterion used, evidencing meaningful differences between both subspecies (Fig. 5). Thus, turgor loss point and minimum water potential recorded in the field oscillated from -2.23 to -4 MPa and from -2.6 to -4.91 MPa, respectively. If we notice that *Q. ilex* subsp. *ilex* has on average a less negative P_{50} than *Q. ilex* subsp. *ballota*, we can infer that the former will have lower safety margin that the latter. Indeed, Martin-StPaul et al. (2013) reported a safety margin value of just 0.3 MPa for a population of *Q. ilex* subsp. *ilex* in northern Greece. Similarly, Savi et al. (2015) found very low (0.01 and 0.04 MPa) or even negative (-0.43 and -0.72 MPa) safety margin values for *Q. ilex* subsp. *ilex* in Croatia.

Finally, the overall plant resistance to cavitation not only depends on stem vulnerability but it is also related to the capacity of other plant organs to withstand xylem embolism (Skelton et al., 2018). In this sense, the most expendable organs (e.g. the leaves) would be more vulnerable to cavitation than the most important ones (e.g. the stem), which is known as “vulnerability segmentation” (Hochberg et al., 2017). Peguero-Pina et al. (2015) reported this phenomenon in the winter-deciduous Mediterranean oak *Quercus subpyrenaica* E.H. del Villar, which explained the premature leaf withering as a response to a severe-drought period. Similarly, Alonso-Forn et al. (2021) also demonstrated the existence of vulnerability segmentation in *Q. faginea*, another winter-deciduous Mediterranean oak. However, both studies did not find evidence of vulnerability segmentation in *Q. ilex* subsp. *ballota*, which exhibited high resistance to drought-induced cavitation in both leaves and stem. This conservative leaf strategy could be related to the longer leaf life span of holm oak, which allows this species to assimilate carbon during more than one vegetative period (Corcuera et al., 2005).

2.2. Resistance to summer drought and high temperatures at leaf cell level

The implementation of a conservative leaf strategy, such as that shown by *Q. ilex* not only relies on leaf hydraulic resistance, but also in an increased resistance to drought at cell level. Thus, when water potential becomes more negative and reaches a critical threshold, water stress can induce the collapse of the cells, which may be associated to the physical damage of the cell membrane during cell buckling and electrolyte leakage (Farrant, 2000). Therefore, the resistance of the leaf to drought at cell level can be explored through the assessment of cell membrane injury by measuring electrolyte leakage in response to decreases in leaf water potential (Ψ) (Vilagrosa et al., 2010). To assess this issue in holm oak, we compared the relationships between Ψ and relative electrical conductivity (ECr, %) for *Q. ilex* and the co-occurring winter-deciduous *Q. faginea* following the methodology described by Vilagrosa et al. (2010). We used five leaves $n = 5$ from three-year old seedlings in which we had previously measured Ψ with a Scholander pressure chamber. From each leaf, three leaf disks (0.2 cm²) were collected and incubated in 3 mL of distilled water at 10 °C for 5 h (ECi). The disks were removed from the vial, plunged into liquid nitrogen and placed in another vial to be stored for 12 h at -25 °C. We then added 3 mL of distilled water and again stored the disks in the dark at 10 °C for 5 h (ECf). Initial (ECi) and final (ECf) electrical conductivities were measured at 25 °C with a conductivity meter (Crison CM 2202, Barcelona, Spain). Relative electrical conductivity (ECr, %) of each sample was calculated as: $ECr = (ECi/ECi + ECf) \times 100$. According to our data, ECr showed better membrane stability at lower water potentials in *Q. ilex* than in *Q. faginea*. Specifically, *Q. faginea* displayed ECr values at -4 MPa that reflected a high degree of membrane destabilization (ca. 80 %), whereas ECr reached only ca. 30 % at the same Ψ in *Q. ilex* (Fig. 6). From this Ψ value, *Q. ilex* showed a strong increase in ECr, reaching ca. 80 % when Ψ was ca. -6 MPa (Fig. 6).

Besides the stability of cell membrane, other factors should also be considered when analysing the resistance to summer drought at cell level. As stated above, stomatal closure under drought conditions is a well-known mechanism that minimizes water depletion at the expense of a reduction in photosynthetic activity, which has been widely described for *Q. ilex* (Acherar and Rambal, 1992; Mediavilla and Escudero,

2003, 2004; Peguero-Pina et al., 2009, 2018; Alonso-Forn et al., 2021). Overall, these studies reported that stomatal closure occurs in this species at leaf water potential values (ca. -3 MPa) much higher than P_{50} and P_{88} (see above in this manuscript). Thus, this species is able to withstand severe drought periods with leaf water potentials lower than those inducing stomatal closure, but much higher than cavitation limit (i.e. a wide hydraulic safety margin, according to Martin-StPaul et al. 2017). Under such circumstances, since the light-harvesting complexes of both photosystems continue collecting light, excitation energy cannot be directed to the photosynthetic electron transport chain and may exceed the amount that can be used for photosynthesis (Demmig-Adams and Adams, 2006). Electrons not consumed in CO₂ fixation may react with O₂ generating reactive oxygen species and increasing the possibility of oxidative damage (Hernández et al., 2012). To cope with this situation, plants have developed different mechanisms that allow the protection of the photosynthetic apparatus. Regarding *Q. ilex*, it is widely acknowledged its great capacity for the implementation of photoprotective mechanisms. For example, the presence of trichomes in the adaxial leaf surface could contribute to a decrease in susceptibility to photodamage through the increase in leaf reflectance (Morales et al., 2002). Moreover, a significant number of studies have analysed the role of thermal dissipation of the excess energy in response to water scarcity in this species, such as the non-photochemical quenching (NPQ) of chlorophyll fluorescence and the de-epoxidation of the xanthophyll cycle pigments (García-Plazaola et al., 2017).

In this sense, Méthy et al. (1996) stated that an increase in NPQ in *Q. ilex* subsp. *ilex* when leaf predawn water potential was lower than -4 MPa implied the onset of photoprotective mechanisms associated with the dissipation of excessive energy in the photosynthetic apparatus. Moreover, these authors concluded that photoprotection is probably an important component of water-stress tolerance in *Q. ilex* as could allow the recovery of this species after an intense drought period. Corcuera et al. (2005) firstly reported the association of the down-regulation of photosynthesis during summer in *Q. ilex* subsp. *ballota* with the thermal energy dissipation and NPQ through the increase of de-epoxidated forms of the xanthophyll cycle pigments, antheraxanthin (A) and zeaxanthin (Z). In line with this, Peguero-Pina et al. (2009) also showed that *Q. ilex* subsp. *ballota* experienced a drought-mediated increase in the thermal dissipation of the energy excess (through an increase in A and Z) coupled with an increase in the amount of absorbed energy that was dissipated in the PSII antenna ($1-\Phi_{exc}$). Furthermore, these

authors evidenced that plants of *Q. ilex* subsp. *ballota* subjected to intense water stress retained overnight the de-epoxidated forms A and Z accumulated during the day, which might indicate that photoprotective mechanisms of this species could be related in some way to the persistence of a trans-thylakoid proton gradient (ΔpH). In addition, the persistence of A and Z at predawn coincided with the decrease of dark-adapted, maximum quantum efficiency of PSII (F_v/F_M), which seemed to be related to an additional photoprotective mechanism that preserved an intact photosynthetic pigment machinery. According to these authors, this mechanism may play an important role in the survival of species such as *Q. ilex* subsp. *ballota* in sites with long and intense summer drought periods. These findings were further confirmed for *Q. ilex* subsp. *ilex* by Chiatante et al. (2015), who found that drought-induced a decrease in F_v/F_M and in the actual PSII efficiency (Φ_{PSII}) that was reversible when plants recovered from water stress, which confirmed the resilience of the photosynthetic apparatus of *Q. ilex* subsp. *ilex* in response to water scarcity. More recently, Sancho-Knapik et al. (2018) showed that *Q. ilex* subsp. *ballota* displayed an early decrease in Φ_{PSII} in response to drought, downregulating the photosynthetic electron transport when CO_2 assimilation was severely limited. These authors also reported a strong increase in NPQ that was closely related to changes in the physiological reflectance index (PRI), as both parameters reflect in many cases photoprotection processes related to ΔpH and/or de-epoxidation of the xanthophyll cycle (Peguero-Pina et al., 2008a, 2013). Variations in PRI with changes in water status of holm oak have been also reported by Tsonev et al. (2014) and Zhang et al. (2017).

Therefore, drought triggers a decrease in Φ_{PSII} and a rise in NPQ related to the de-epoxidation of xanthophylls that efficiently reduce the risks of photodamage under drought conditions for both *Q. ilex* subsp. *ilex* and *Q. ilex* subsp. *ballota*. In addition, both subspecies down-regulate F_v/F_M when leaf water potential drops below the value inducing stomata to close. The evidences of an ample safety margin in *Q. ilex* subsp. *ballota* would be consonant with this ability to preserve photoprotective mechanisms since both, leaves and branches, are able to tolerate water potential levels where photosynthetic activity is impaired due to stomatal closure but are far from the cavitation threshold. This conservative leaf strategy based on the high resilience of the photosynthetic apparatus plays an important role in the rapid recovery of this species

after a long summer drought period (Vaz et al., 2010), and would contribute to maintain the leaves functional for more than one vegetative period (Corcuera et al., 2005).

Besides drought, leaves of holm oak must deal with high temperatures during summer, especially in its southernmost and westernmost distribution range (Fig. 7), where maximum temperatures during the hottest days of summer usually exceed 40°C. It should be noted that this area is occupied by *Q. ilex* subsp. *ballota*, whose leaves are much smaller than those of *Q. ilex* subsp. *ilex* (Peguero-Pina et al., 2014). The smaller the leaves are, the thinner the boundary layer is, which facilitates a sensible heat loss in summer since they tend to be better coupled to air temperature and can avoid overheating through a faster convective cooling (Peguero-Pina et al., 2020). In any case, the resistance of the leaf to high temperatures should be analysed for holm oak, especially for *Q. ilex* subsp. *ballota*, as thermal stress may jeopardize several processes occurring in the leaves at cell level, especially those related to photosynthetic activity. Thus, when leaves are exposed to extreme temperatures, they accumulate damage to photosystem II (PSII) (Arnold et al., 2021). Temperature-dependent changes in minimum chlorophyll fluorescence in the dark-adapted state (F_0) can be used to identify the critical temperature at which PSII is irreversibly damaged. In this sense, we performed an essay for this review where we calculated this critical temperature according to Schreiber and Berry (1977) for four leaves of one-year old seedlings of *Q. ilex* subsp. *ballota*. This procedure consisted on subjecting leaves to an increasing ramp of temperature and measuring F_0 with a FMS II modulated fluorometer (Hansatech Instruments, Norfolk, UK) at regular time intervals. Initially, F_0 remained constant up to a certain temperature where it suddenly increased due to thermal damage in PSII. The critical temperature for *Q. ilex* subsp. *ballota* was of 50.2 °C (Fig. 8), which was similar to those measured in other species (Ranney and Peet, 1994; Krause et al., 2013). In any case, it is far above enough from maximum temperatures usually reached in circum-Mediterranean areas where holm oak lives.

However, although high temperatures themselves do not normally compromise the survival of the plant, the combination of hot temperatures and reduced moisture in the air during summer leads to an increase in VPD (Fig. 2). A higher evaporative demand by the atmosphere implies a higher transpiration rate when stomata are opened during gas exchange (McAdam and Brodribb, 2015; Kimm et al., 2020). In this sense, the plant risks to significant water losses under a very negative VPD. For this reason, most plants respond to VPD closing stomata, either passively, i.e. induced by leaf water status (e.g.

gymnosperms) or even actively, through a local increase in abscisic acid (e.g. in angiosperms) (McAdam and Brodribb, 2015) when a certain value of VPD is reached, even though they have available water in the ground. These plants are said to follow a conservative strategy, that is, water-saver plants (Levitt, 1980). Mediavilla and Escudero (2003) showed that *Q. ilex* exhibits this conservative strategy, presenting significant reduction of stomatal conductance at approximately 2.0 kPa. Holm oak was the species with the lowest VPD threshold when compared with other three co-occurring *Quercus* species (*Quercus suber* L., *Q. faginea* and *Q. pyrenaica*), where deciduous oaks showed the highest VPD at which they begin to close stomata. This water-saver strategy used by *Q. ilex* and *Q. suber* is likely to be related to being evergreen, since they need to avoid irreversibly damage at leaf level in order to preserve leaves in the best possible condition during its life span (Mediavilla and Escudero, 2003).

Finally, the response of stomata closure is common to high VPD and scarcity of water in the soil due to drought. Thus, the additive effect of these two phenomena, which are often linked and even prolonged in time (Perez-Martin et al. 2009), means that the plant is only able to open stomata and photosynthesise for a few hours mainly at sunrise on many summer days. To the aforementioned effects of closing stomata, the fact of having a substantial reduction of photosynthesis has consequences in carbon fixation, where a lower carbon fixation is related to a slower growth rate and, consequently, a minor productivity at ecosystem level (Grossiord et al., 2020).

3. Winter cold

With reference to winter cold, less negative soil water potentials are expected. However, low temperatures (Fig. 9), commonly below zero during many hours daily, followed by a continuous freeze-thawing cycles may compromise xylem stability due to freeze-induced embolism. Additionally, low winter temperatures affect the photosynthetic activity of *Q. ilex* by reducing or even nullifying it in the coldest areas (Crescente et al., 2002). Compared to very high temperatures, low ones are worse for the same level of light, since photosynthesis levels are minimal and, therefore, the combination of temperatures below 0°C and the excess of radiation can trigger damages in the photosynthetic apparatus (Corcuera et al., 2005).

3.1. *Xylem resistance to cavitation induced by low temperatures*

Holm oak surviving throughout the cold winters is not only a matter of leaf tolerance to damages induced by frost, as above expressed. Freezing and thawing of the xylem water, in single or multiple cycles, may induce hydraulic conductivity losses and limit the survival of a tree species and its geographical distribution (Pockman and Sperry, 1996; Willson and Jackson, 2006). In the absence of refilling mechanisms during the spring, that have been reported in some tree species associated to the development of positive pressures (Cochard et al., 2001; Améglio et al., 2002), the loss of conductivity after the winter colds can be almost complete, especially in the wide vessels of ring-porous oaks (Cochard and Tyree, 1990; Corcuera et al., 2006). Cavitation induced during winter has been largely explained in different studies (Feng, 2015 and references therein). Basically, bubbles produced during the freezing of the xylem water expand when thawing, assuming a certain tension in the xylem. The wider the bubbles are the more likely will be the process of cavitation, being the bubble diameter proportional to the dimension of the xylem conduit concerned (Ameglio et al., 2002). In fact, Davis et al. (1999) proposed 44 micrometres as the conduit diameter above which the vessel will be very prone to be cavitared due to this process. Of course, the water tension in the xylem when thawing also affects the process, relating both stresses (drought and frost) in the final induction of hydraulic loss, which is especially critical in evergreen species that maintain their transpiration area during the winter (Willson and Jackson, 2006).

Does this process of frost-induced cavitation affect holm oak survival in its natural areas? Unfortunately, the number of published studies concerning this process is really limited, especially if compared to those related to drought-induced embolism. First data about frost effect on hydraulic conductivity in *Q. ilex* can be found in LoGullo and Salleo (1993), when comparing the relative importance of both factors inducing cavitation – drought and frost – in this species in Sicily (Italy). The widest vessels in one-year old stem that they measured in this population – diameter class from 40 μm to 60 μm – were highly vulnerable to freezing temperatures in terms of hydraulic conductivity losses, while narrowest – fewer than 30 μm – were not affected at any of the temperatures experienced by the plants in their experiment.

Comparison of native embolism in plants from two contrasted climates in Italy, namely at Sicily (Southern Mediterranean Basin) and Friuli Venezia Giulia (Northeastern Italy), evidenced that cavitation during the winter in this last location was

clearly higher than the measured during the summer, and also higher than that experienced in the location in Sicily (Nardini et al., 2000). The authors of this study concluded that *Q. ilex* is more limited by frost than by drought in its geographical distribution, confirming the assumption that holm oak is confined to freeze-free areas due to its overall susceptibility to frost injuries (Nardini, 1998).

However, this is not applicable to most of the populations in other territories of the distribution area of the species. Corcuera et al. (2004) studied a population of holm oak growing under a typical continental subtype of the Mediterranean-type climate, with a short freeze-free period (May to October) at Cubel (Zaragoza, Spain). The mean vessel diameter in one-year old stem in this population was below 20 μm , with a few vessels above 30 μm . No data of vulnerability to frost-induced cavitation were reported in this area, but the much narrower vessels in the studied population could indicate that the stems are probably free of risk concerning this process. In fact, the perfusion with phloxine of branches in this population revealed that functional vessels (dye-stained when perfused at low pressure) were found in three year-old growth rings (unpublished results).

3.2. Resistance to low temperatures at leaf cell level

Evergreen species living under Mediterranean-type climates such as holm oak are often subjected to freezing temperatures during winter months that impair the enzymatic processes involved in photosynthesis. Hence, under these conditions, overwintering species need mechanisms to protect the photosynthetic apparatus, which are mainly based on sustained energy dissipation and antioxidants (see García-Plazaola et al., 2017 and references therein).

Sustained thermal energy dissipation during winter is characterized by reductions in F_v/F_m together with overnight retention of the de-epoxidized forms of the VAZ cycle (A and Z). This mechanism protects the photosynthetic apparatus from excess excitation pressure when plants are exposed to high light and low temperatures during winter (Verhoeven, 2014). Several studies have described the implementation of this photoprotective mechanism in response to low temperatures in *Q. ilex*. Thus, the occurrence of chronic winter photoinhibition in *Q. ilex* subsp. *ballota* was firstly reported by Martínez-Ferri et al. (2004), who registered low predawn F_v/F_m values (ranging from 0.4 to 0.7) due to cumulative effect of low temperatures and high

excitation energy throughout winter. These authors also suggested that the association of this phenomenon with the overnight retention of Z and A might be attributed to the inhibitory effect of chilling temperatures on the enzymatic conversion of Z and A to violaxanthin (V) (Adams and Demmig-Adams, 1995; Adams et al., 1995). In line with this, Corcuera et al. (2005) demonstrated the presence of A and Z at predawn in leaves of *Q. ilex* subsp. *ballota* after cold nights due to an increase in NPQ. This study also confirmed that this species showed long-term PSII down-regulation (i.e. low predawn F_v/F_m values) during winter, which could be partly caused by changes in the chlorophyll pigment bed associated with the presence of A and Z in the PSII antenna (Gilmore and Ball, 2000). Similar results were further obtained by Baquedano and Castillo (2007), who reported chronic photoinhibition and increased de-epoxidation of xanthophyll pigments at predawn for this species during winter. However, contrary to previous studies, these authors did not find high NPQ values in winter. Regarding *Q. ilex* subsp. *ilex*, Arena et al. (2008) put in evidence the essential role of thermal dissipation as the main process to dissipate the excess absorbed light during winter. These authors concluded that the drawdown of F_v/F_m and the increased NPQ in the early morning at the end of the winter constituted an effective photoprotective mechanism to avoid photo-oxidative damage to chlorophylls, which provided remarkable stability to the photosynthetic apparatus when subjected to winter stress conditions (Gratani et al., 1998). In line with this, Sperlich et al. (2014) also stated that increased NPQ and lower F_v/F_m values in *Q. ilex* subsp. *ilex* could be related to several photoprotective mechanisms (i.e. re-organization of the thylakoid membrane, closure of reaction centres and/or reduced antennal size) that allowed this species to keep the photosynthetic apparatus intact in response to low temperatures.

Besides sustained energy dissipation, several studies have also analysed the role of antioxidants in holm oak as a mechanism to avoid the risk of oxidative damage associated with the potential generation of reactive oxygen species (ROS) under low winter temperatures. With respect to non-enzymatic antioxidants, García-Plazaola et al. (1999) registered a high synthesis of ascorbate during winter when compared to spring in *Q. ilex*. However, García-Plazaola et al. (2003) found that the most highly induced non-enzymatic antioxidant in *Q. ilex* for the coldest winter was α -tocopherol. On the other hand, regarding enzymatic antioxidants, García-Plazaola et al. (1999) reported an induction in glutathione reductase (GR) and monodehydroascorbate reductase (MDAR) activity in *Q. ilex* during winter, reducing the over-excitation of electron transport chain

and ROS production. In line with this, Corcuera et al. (2005) registered a high activity of superoxide dismutase (SOD) and GR in leaves of *Q. ilex* subsp. *ballota* during winter, which implied an increased ability to withstand oxidative stress at low temperature (Kingston-Smith and Foyer, 2000). In fact, these authors did not observe leaf photo-oxidative stress, in accordance with the absence of changes in chlorophyll concentration during winter.

In summary, holm oak has developed drastic antioxidant and photoprotective mechanisms in response to winter stress that could be of adaptive importance. Thus, this species could improve the carbon balance under favourable winter conditions, re-adjusting and protecting its photosynthetic machinery when these relatively favourable conditions changed (García-Plazaola et al., 2017). The full recovery of photosynthetic capacity and PSII efficiency when temperature increased in spring also evidences the adaptive capacity of holm oak to withstand low temperatures during winter, both for *Q. ilex* subsp. *ilex* (Gratani et al. 1998) and *Q. ilex* subsp. *ballota* (Corcuera et al. 2005). Throughout this review we have shown that photoprotection mechanisms described for *Q. ilex* in response to low temperatures are very similar to those implemented by this species to cope with summer drought (see above in this manuscript). With regard to this issue, Verhoeven et al. (2018) noted that the ability of plants to withstand dehydration stress is closely related to the tolerance to freezing temperatures, and the similarities in response at the cellular level are marked. Moreover, these authors claimed that maintenance of cellular structure is critical to tolerance of desiccation and low temperature conditions.

To examine this issue, we have taken advantage of our own unpublished data where it was examined freezing tolerance in leaves of *Q. ilex* subjected to different temperatures by measuring F_v/F_M changes as an indicator of photoinhibition induced by low temperatures, as firstly proposed by Peguero-Pina et al. (2008b). Specifically, leaves of four provenances of *Q. ilex* subsp. *ballota* from Spain (Cazorla, Cuenca, Soria and Extremadura) and four provenances of *Q. ilex* subsp. *ilex* from Spain (Gerona) and Italy (Veneto, Lazio and Sardinia) were exposed to various freezing temperatures between -5°C and -40°C and F_v/F_M was subsequently measured with a FMS II modulated fluorometer (Hansatech Instruments, Norfolk, UK) following the methodology described by Sangüesa-Barreda et al. (2018). The results obtained revealed that all studied provenances showed high resistance to freezing (Fig. 10), ranging from -19.8°C (Veneto and Sardinia) to -26.6°C (Soria provenance) (data not

shown). It should be noted that these temperatures were much lower than those typically occurring in the geographical location of each provenance (Fig. 9).

4. Conclusions

Holm oak has been considered as a genuine representative species of the Mediterranean flora by different geobotanical synthesis. The hard (sclerophyllous) and evergreen leaves developed by this species have been interpreted as an evolutive response to the stressful situations potentially imposed by the Mediterranean-type climate, i.e. hot-dry summers and cold winters. Nevertheless, the evolutionary origin of this taxon can be traced back to pre-Mediterranean lineages living in humid, tropical or subtropical climates. In fact, most of the species of the section *Ilex* inhabit now in territories under tropical, subtropical or temperate climates, while showing similar leaf morphology and phenology than *Q. ilex* itself. The existence of an extensive amount of information about the ecophysiological response of *Q. ilex* from different territories, supplemented with some unpublished results, allowed us to perform this metanalysis.

The analysis of previous published studies revealed that *Q. ilex* showed a considerable degree of within-species variability in the ability to cope with water stress in terms of xylem vulnerability to drought-induced cavitation, one of the main constraints for plant growth or survival in Mediterranean areas. As an example, P_{50} values from ca. -2 to -7 MPa have been reported, with a similar variation range when P_{88} is considered. A deeper analysis of this information allows concluding that these high P_{50} and P_{88} values are mostly found in studies concerning populations from the subspecies found in the western distribution range (*Q. ilex* subsp. *ballota*). Concerning the effect of freeze-thaw cycles on the xylem cavitation, only a few data are available. However, while *Q. ilex* is assumed to be highly vulnerable to this process, occupying coastal areas in the central and eastern locations, the populations in the western area (Iberian Peninsula and North of Africa) can occupy continental-type climates where freeze-thaw events are probable during more than three months in the winter. So, in terms of the vulnerability to cavitation in the stem, two contrasting performances can be observed within holm oak, corresponding to both different subspecies.

Contrastingly, the analysis of the response of holm oak at leaf-level to summer drought and winter cold indicated that both subspecies developed effective antioxidant and photoprotective mechanisms that minimized the risk of photodamage, preserving the leaves when photosynthetic activity is impaired. Moreover, leaves of both

subspecies showed a high resistance at cell level to extremely low and high temperatures. Overall, the analysis of these results revealed that holm oak is characterized by a conservative leaf strategy, irrespective of the subspecies and/or the climatic conditions. The maintenance of the photosynthetic machinery after a stress period is of paramount importance for improving the carbon balance in an overwintering species such as *Q. ilex*. Therefore, this species can be considered a paradigm of tolerance to extreme temperatures at leaf level, in spite of its evolutionary origin under tropical or subtropical climates.

Finally, when atmospheric dryness is considered, VPD can impose very restrictive limitations for the plant to open stomata and photosynthesise in the case of *Q. ilex* due to its water-saver strategy. Thus, especially during summer, the stressful conditions imposed by Mediterranean summers derives in a trade-off between growth and survival.

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Author contributions

R.M.-S., J.J.P.-P. and E.G.-P. conceived the review and prepared the original draft; all authors contributed to the writing and review of the final version of the manuscript.

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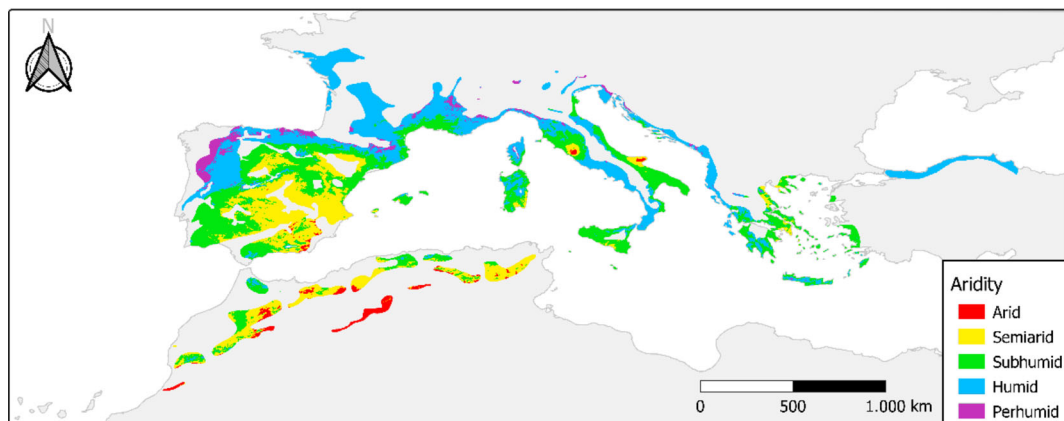


Fig. 1. Aridity index of the distribution area of *Quercus ilex* calculated according to Emberger index (Emberger, 1930). Information about distribution was taken from EUFORGEN (European Forest Genetic Resources Programme, <http://www.euforgen.org/>) and modified according to the map of provenances for *Quercus ilex* in the Iberian Peninsula (MITECO, <https://www.miteco.gob.es>) and the “Climatic Modelling of Distribution Ranges of Plant Species” Research project of the Martin-Luther-University Halle-Wittenberg (2006).

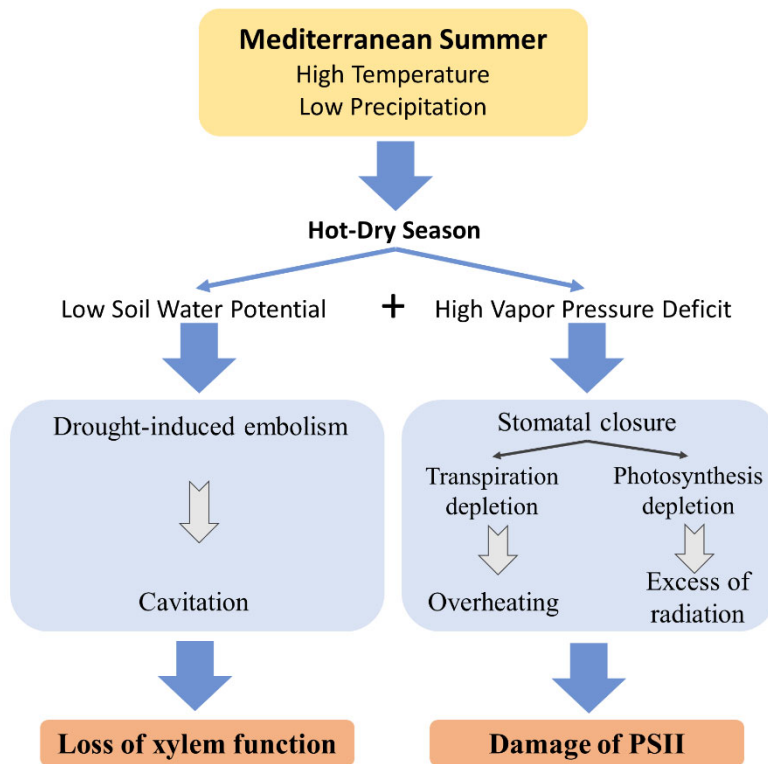


Fig. 2. Schematical representation about the effects of summer stresses on the functionality of the plant.

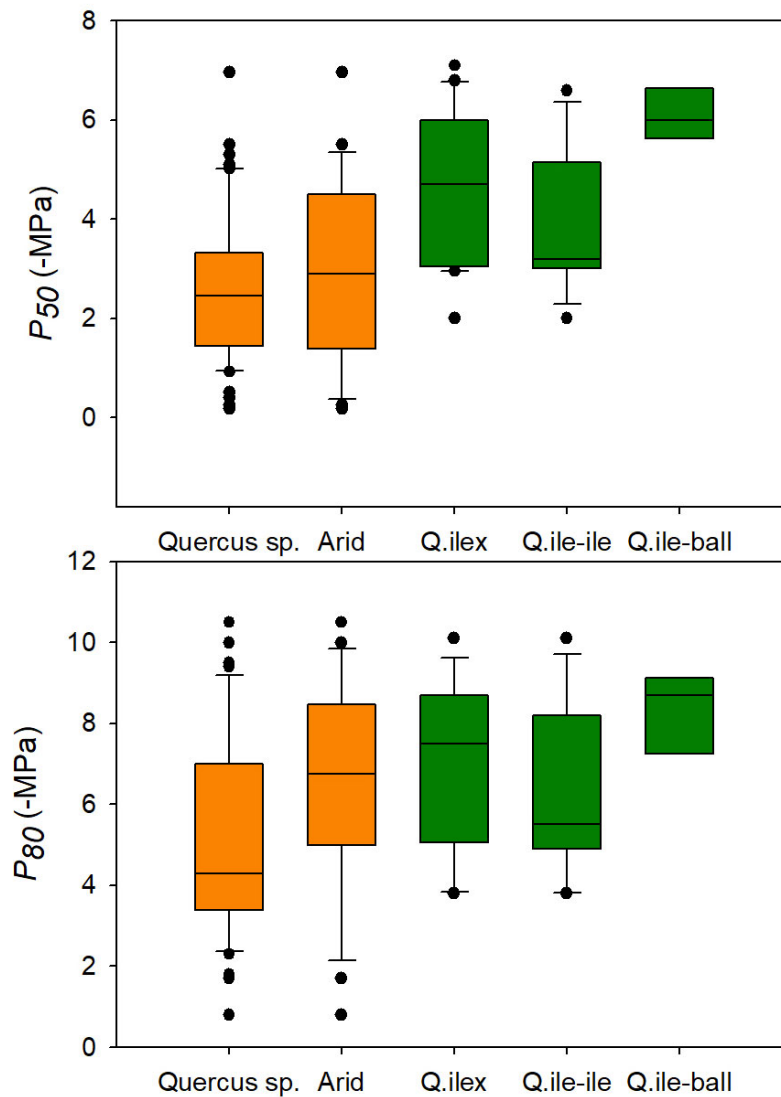


Fig. 3. Box-plots of stem water potential at 50% (P_{50}) (upper panel) and 80% (P_{80}) (lower panel) loss of conductivity for 24 *Quercus* species (*Quercus* sp., n=67, in orange), from which 13 species occupy arid areas (Arid, n=42, in orange), besides *Q. ilex* (n=19) as a single species, and distinguishing between both subspecies: *Q. ilex* subsp. *ilex* and *Q. ilex* subsp. *ballota* (in green). References and values used in the analysis for *Quercus* species are shown in Table A2, and references, values and methodology used for its calculation in the case of *Q. ilex* are shown in Table A1.

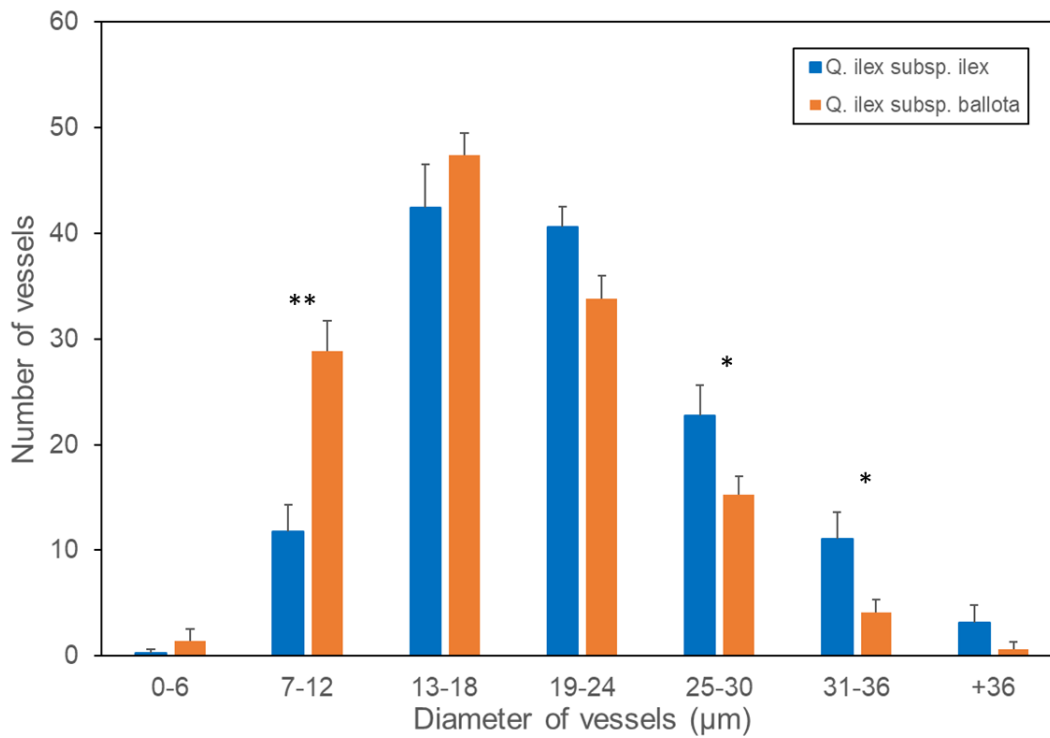


Fig. 4. Average number of vessels for each diametrical class in one year-old seedlings of *Q. ilex* subsp. *ilex* (blue bars) and *Q. ilex* subsp. *ballota* (orange bars) (unpublished results). Values were calculated from 24 seedlings corresponding to three provenances of *Q. ilex* subsp. *ilex* (Veneto, Lazio and Sardinia, Italy) and 32 seedlings corresponding to four provenances of *Q. ilex* subsp. *ballota* (Cuenca, Ciudad Real, Jaen and Soria, Spain). Data are mean \pm SE. Asterisks indicate significant differences between both subspecies for each diametrical class (Mann–Whitney U test) (*, $0.05 < P < 0.1$; **, $0.001 < P < 0.05$).

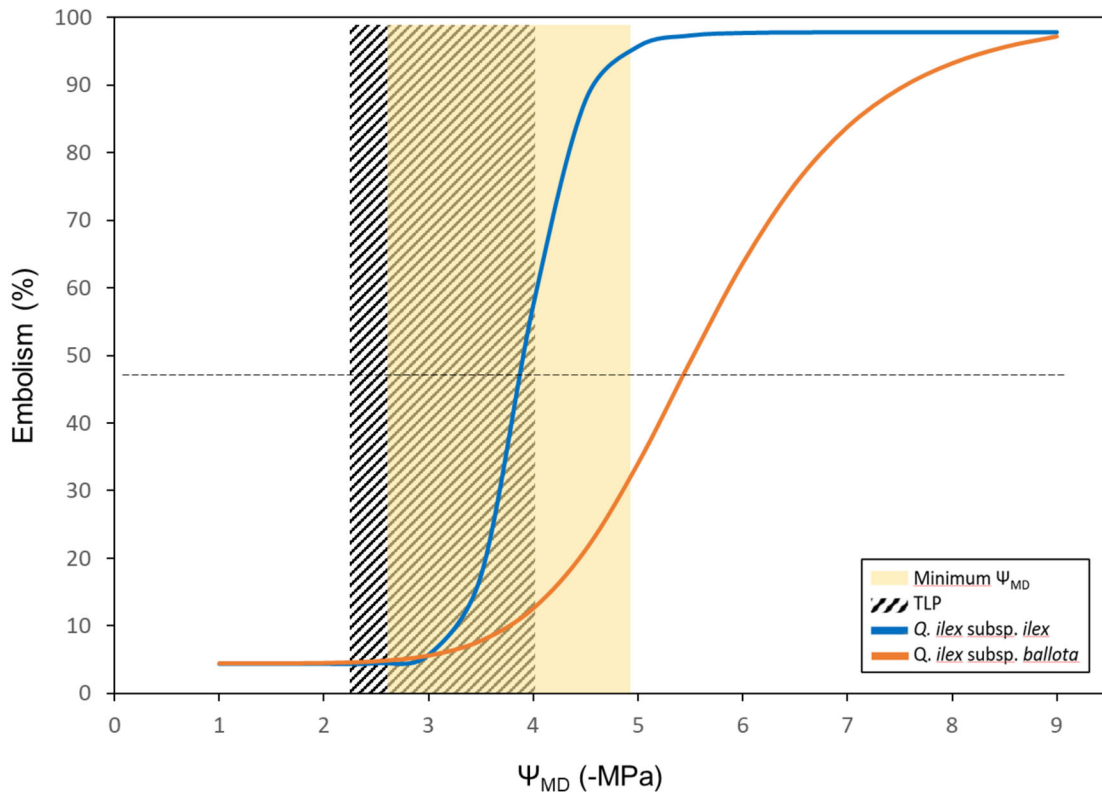


Fig. 5. Embolism type-curves of *Q. ilex* subsp. *ilex* (blue) and *Q. ilex* subsp. *ballota* (orange) and the range of water potentials values found in the literature (Table A3) for the two criteria of safety margin calculation: i) minimum water potential measured in the field at midday (Ψ_{MD} , in yellow, from -2.6 to -4.91 MPa) and ii) turgor loss point (TLP, with dashed lines, from -2.23 to -4 MPa). The horizontal dotted line represents the P_{50} values for both subspecies. Type-curves for each subspecies are extracted from Alonso-Forn et al. (2020) for *Q. ilex* subsp. *ballota* and from our own unpublished data for *Q. ilex* subsp. *ilex*.

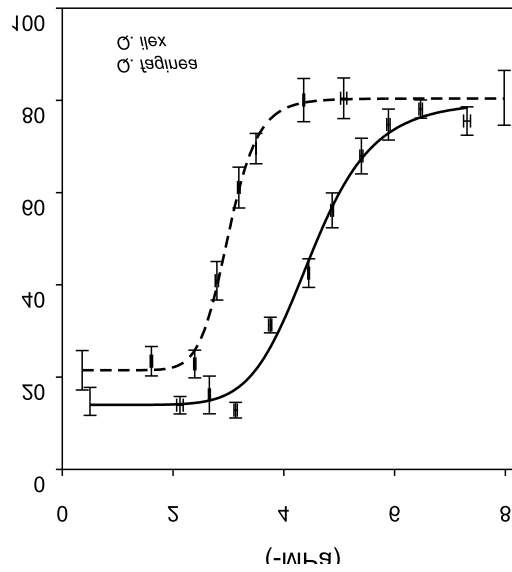


Fig. 6. Cell membrane injury (% of relative conductivity, ECr) measured by means of the electrolyte leakage technique as a function of water potential for *Q. ilex* (grey symbols) and *Q. faginea* (white symbols) (unpublished results). Data were adjusted to a four-parameter logistic curve, both for *Q. ilex* (solid line, $R^2 = 0.99$, $P < 0.0001$) and *Q. faginea* (dashed line, $R^2 = 0.99$, $P < 0.0001$). Data are mean \pm SE.

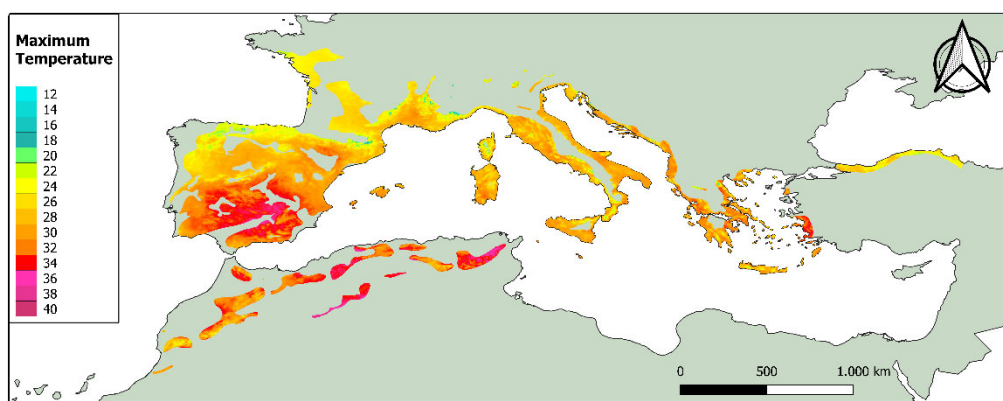


Fig. 7. Average maximum temperature of the warmest month that *Quercus ilex* endures in its natural distribution area (represented by coloured map). Information about distribution was taken from EUFORGEN (European Forest Genetic Resources Programme, <http://www.euforgen.org/>) and modified according to the map of provenances for *Quercus ilex* in the Iberian Peninsula (MITECO, <https://www.miteco.gob.es>) and the “Climatic Modelling of Distribution Ranges of Plant Species” Research project of the Martin-Luther-University Halle-Wittenberg (2006).

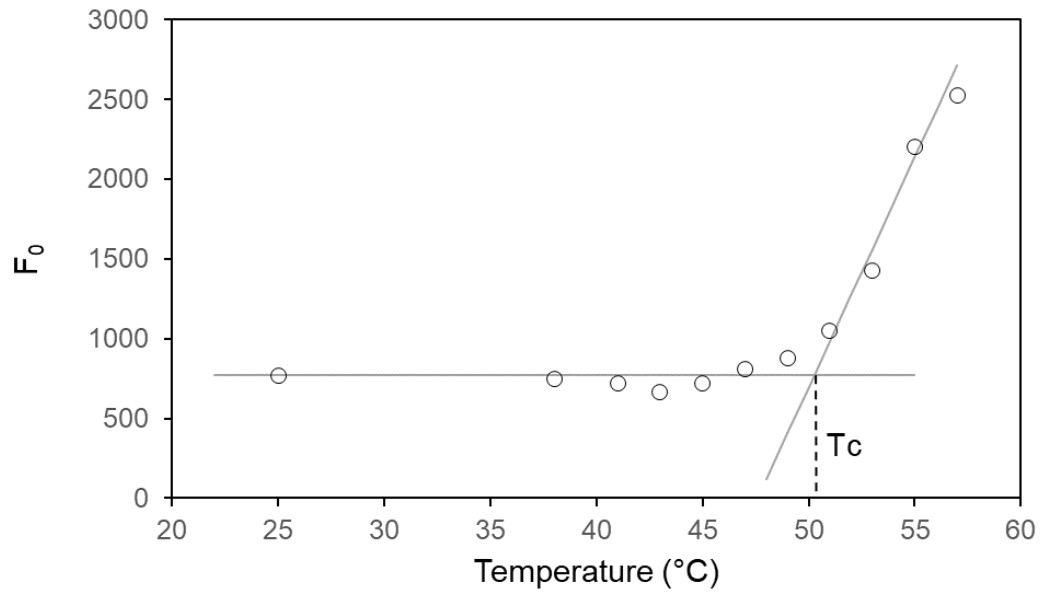


Fig. 8. Relationship between temperature and minimum chlorophyll fluorescence in the dark-adapted state (F_0) for leaves of *Q. ilex* subsp. *ballota* (n=4) (unpublished results). Critical temperature (T_c) was calculated following the procedure described by Schreiber and Berry (1977) and estimated at 50.2 °C.

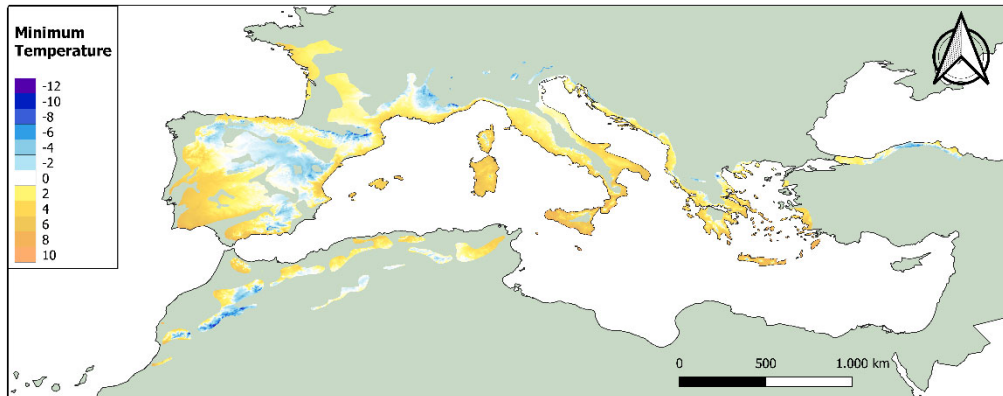


Fig. 9. Average minimum temperature of the coldest month that *Quercus ilex* endures in its natural distribution area (represented by coloured map). Information about distribution was taken from EUFORGEN (European Forest Genetic Resources Programme, <http://www.euforgen.org/>) and modified according to the map of provenances for *Quercus ilex* in the Iberian Peninsula (MITECO, <https://www.miteco.gob.es>) and the “Climatic Modelling of Distribution Ranges of Plant Species” Research project of the Martin-Luther-University Halle-Wittenberg (2006).

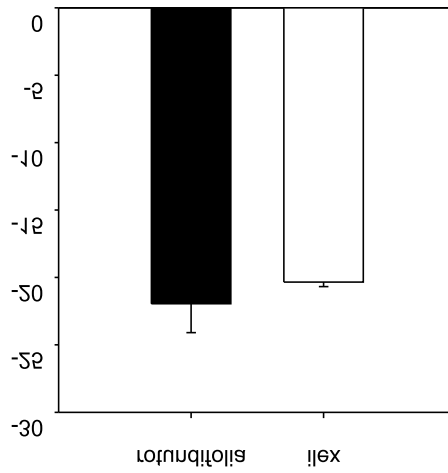


Fig. 10. Freezing tolerance values (°C) for *Q. ilex* subsp. *ballota* (black bar) and *Q. ilex* subsp. *ilex* (white bar) estimated through the changes in F_v/F_M as an indicator of photoinhibition induced by low temperatures (Peguero-Pina et al., 2008b) (unpublished results). Data are mean \pm SE of four provenances per subspecies.

Table A1. Compilation of studies that have proportionated the values of xylem tension (MPa) inducing 50% and 88% loss of hydraulic conductivity in the stems (P_{50} and P_{88} , respectively) for *Quercus ilex* or have published an embolism curve from which P_{50} and P_{88} values have been inferred (values marked with an asterisk). The kind of plant material used in the study, subspecies (when is specified) and the geographical provenance of that material are also indicated. Besides, the method used in each experiment is shown according to the terminology of Martin-StPaul et al. (2014).

Source	Plant material	Provenance	Method	P_{50} (- MPa)	P_{88} (- MPa)
Lo Gullo & Salleo (1993)	<i>Q. ilex</i> . Potted plants grown at 800 m. altitude	Sicily (Italy)	Native embolism throughout a drying cycle in summer. Dehydration <i>in planta</i>	2.95 (53%)	3.84 (85%)
Tyree & Cochard (1996)	<i>Q. ilex</i> . Branches	Non specified	Bench drying	3*	3.8*
Tyree & Cochard (1996)	<i>Q. ilex</i> . Branches	Non specified	Bench drying	5.52	6.5*
Tognetti et al (1998)	<i>Q. ilex</i> . Branches from trees in the field	Siena (Italy) 43°17' N, 11°35' E and 350 m.a.s.l.	Bench drying	3	5
Martínez-Vilalta et al (2002)	<i>Q. ilex</i> . Branches from trees in the field	Prades (Spain) 41°13'N, 0°55'E and 1000 m a.s.l.	Bench drying	2	5.5*
Gartner et al (2003)	<i>Q. ilex</i> . Seedlings grown in a greenhouse	Acorns from Nîmes (France)	Air injection (double-ended pressure chamber)	3.2	5.1
Corcuera et al (2004) a	<i>Q. ilex</i> subsp. <i>ilex</i> . Potted seedlings growing in Zaragoza (Spain) 41°50'20" N, 0°51'07"W	Mallorca (Spain)	Air injection (double-ended pressure chamber)	3	5.5*
Corcuera et al	<i>Q. ilex</i> subsp. <i>ballota</i> . Potted seedlings	Cubel (Spain) 41°00'07" N, 1°03'09"	Air injection (double-	5.6	9*

(2004) b	growing in Zaragoza (Spain) 41°50'20" N, 0°51'07" W	W, 1177 m a.s.l.	ended pressure chamber)	
Cochard et al (2005)	<i>Q. ilex</i> . Branches from trees in the field	Montpellier (France) 3°35'45" E, 43°44'29" N, 270 m a.s.l.	Centrifuge	4.13
Limousin et al (2010)	<i>Q. ilex</i> . Trees in the field	Montpellier (France) 3°35'45" E, 43°44'29" N, 270 m a.s.l.	Air injection (double-ended pressure chamber)	3.8 8*
Nardini et al (2012)	<i>Q. ilex</i> . Trees in the field	Cernizza (Italy) 45°46' 49" N, 13°35'31" E; 25 m a.s.l.	Bench drying	3.5
Pinto et al (2012)	<i>Q. ilex</i> subsp. <i>ballota</i> . Trees in the field	Evora (Portugal) 38°32' N, 8°00' E	Bench drying	3.21 8.5*
Urli et al (2013)	<i>Q. ilex</i> . Seedlings grown in a greenhouse (4–5 years old)	Non specified	Cavitron	5.52 7.08
Martin-SiPaul et al (2013)	<i>Q. ilex</i> . Trees in the field. From three forests with different level of xericity	1) Les Mages 44°13' N, 4°08' E, 360 m a.s.l. 2) Puechabon 43°44' N, 3°35' E, 270 m a.s.l. 3) Vic la Gardiole 43°29' N, 3°45' E 170 m a.s.l.	Air injection (double-ended pressure chamber)	3.8
Martin-SiPaul et al (2014)	<i>Q. ilex</i> . Current year resprouts from trees planted in 1963. CNRS campus of the CEFÉ at Montpellier (France)	Acorns from Puechabon forest 3°35'45" E, 43°44'29" N	Bench drying	4.7 6*
Peguero-Pina et al (2014)	<i>Q. ilex</i> subsp. <i>ilex</i> . Trees planted in a common garden in Zaragoza (Spain) 41°50'20" N, 0°51'07" W	Veneto (Italy) 45°44' N, 10°48' E, 617 m a.s.l.	Bench drying	5.6 8.2
Peguero-Pina et al (2014)	<i>Q. ilex</i> subsp. <i>ilex</i> . Trees planted in a common garden in Zaragoza (Spain) 41°50'20" N, 0°51'07" W	Lazio (Italy) 41°13' N, 13°03' E, 29 m a.s.l.	Bench drying	5.8 8.2
Peguero-Pina et al (2014)	<i>Q. ilex</i> subsp. <i>ilex</i> . Trees planted in a common garden in Zaragoza (Spain) 41°50'20" N, 0°51'07" W	Sardinia (Italy) 39°21' N, 08°34' E, 627 m a.s.l.	Bench drying	6.6 10.1
Peguero-Pina et al (2014)	<i>Q. ilex</i> subsp. <i>ballota</i> . Trees planted in a common garden in Zaragoza (Spain) 41°50'20" N, 0°51'07" W	Cazorla (Spain) 38°06' N, 02°33' W, 1236 m a.s.l.	Bench drying	6 9.5
Peguero-Pina et al (2014)	<i>Q. ilex</i> subsp. <i>ballota</i> . Trees planted in a common garden in Zaragoza (Spain) 41°50'20" N, 0°51'07" W	Ciudad Real (Spain) 39°27' N, 04°24' W, 724 m a.s.l.	Bench drying	7.1

Peguero-Pina et al (2014)	<i>Q. ilex</i> subsp. <i>ballota</i> . Trees planted in a common garden in Zaragoza (Spain) 41°50'20" N, 0°51'07" W	Soria (Spain) 41°46' N, 02°29' E, 1074 m a.s.l.	Bench drying	6.2	8.9
Uribe et al (2015)	<i>Q. ilex</i> . Trees in the field.	Natural Reserve of Hourtin (France) 45° 11' 14" N, 1°03' 23" W 18 m a.s.l.	Cavitron	5.52	7.08
Peguero-Pina et al (2018)	Potted plants growing (8 years old) growing in Zaragoza (Spain) 41°50'20" N, 0°51'07" W	Soria (Spain) 41°46' N, 02°29' E, 1074 m a.s.l.	Native embolism throughout a drying cycle in summer. Dehydration <i>in planta</i>	6	
Alonso-Forn et al (2021)	Potted plants growing (9 years old) growing in Zaragoza (Spain) 41°50'20" N, 0°51'07" W	"Alcarria-Serrania de Cuenca" (Spain) 40°19' N, 2°15' W, 950 m a.s.l.	Native embolism throughout a drying cycle in summer. Dehydration <i>in planta</i>	6.8	7.5
Alonso-Forn et al (unpublished data)	Potted plants growing (9 years old) growing in Zaragoza (Spain) 41°50'20" N, 0°51'07" W	Lazio (Italy) 41°13' N, 13°03' E, 29 m a.s.l.	Native embolism throughout a drying cycle in summer. Dehydration <i>in planta</i>	3.93	5.5

Table A2. Values of xylem tension (MPa) inducing 50% and 88% loss of hydraulic conductivity in the stems (P_{50} and P_{88} , respectively) for 22 *Quercus* species and references where they have been extracted.

Species	P_{50} (- MPa)	P_{88} (- MPa)	Reference
<i>Q. agrifolia</i>	2	5	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. <i>Nature</i> . 491, 752–755.
<i>Q. alba</i>	1.37	2.6	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. <i>Nature</i> . 491, 752–755.
<i>Q. berberidifolia</i>	5.1	9.5	Jacobsen, A.L., Pratt, R.B., Ewers, F.W., & Davis, S.D., 2007. Cavitation resistance among 26 chaparral species of southern California. <i>Ecol. Monogr.</i> 77(1), 99–115.
<i>Q. berberidifolia</i>	-	6.5	Venturas, M.D., MacKinnon, E.D., Dario, H.L., Jacobsen, A.L., Pratt, R.B., Davis, S.D., 2016. Chaparral Shrub Hydraulic Traits, Size, and Life History Types Relate to Species Mortality during California's Historic Drought of 2014. <i>PLoS ONE</i> . 11(7): e0159145.
<i>Q. berberidifolia</i>	1.5	-	Jacobsen, A.L., Pratt, R.B., Davis S.D., Ewers, F.W., 2007. Cavitation resistance and seasonal hydraulics differ among three arid Californian plant communities. <i>Plant Cell Envir.</i> 30: 1599–1609.
<i>Q. berberidifolia</i>	2.6	9.4	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. <i>Nature</i> . 491, 752–755.
<i>Q. coccifera</i>	6.96	10	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. <i>Nature</i> . 491, 752–755.
<i>Q. cornelius-mulleri</i>	3.17	5.5	Paddock, III, W.A., Davis, S.D., Pratt, R.B., Jacobsen, A.L., Tobin, M.F., López-Portillo, J., Ewers, F.W., 2013. Factors determining mortality of adult chaparral shrubs in an extreme drought year in California. <i>Aliso</i> . 31(1), 49–57.
<i>Q. faginea</i>	3.9	5.65	Esteso-Martínez, J., Camarero, J.J., Gil-Pelegrín, E., 2006. Competitive effects of herbs on <i>Quercus faginea</i> seedlings inferred from vulnerability curves and spatial-pattern analyses in a Mediterranean stand (Iberian System, northeastern Spain). <i>Ecoscience</i> . 13(3), 378–387.
<i>Q. falcata</i>	0.92	1.8	Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. <i>Nature</i> . 491, 752–755.

<i>Q. frainetto</i>	4.5	8.38	Choat, B., Jansen, S., Brodrribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. <i>Nature</i> . 491, 752–755.
<i>Q. gambelii</i>	0.25	0.8	Sperry, J.S., Christman, M.A., Torres-Ruiz, J.M., Tanseda, H., Smith, D.D., 2012. Vulnerability curves by centrifugation: is there an open vessel artefact, and are 'r' shaped curves necessarily invalid? <i>Plant Cell Environ.</i> 35(3), 601–610.
<i>Q. gambelii</i>	0.18	-	Choat, B., Jansen, S., Brodrribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. <i>Nature</i> . 491, 752–755.
<i>Q. gambelii</i>	1.08	-	Christman, M.A., Sperry, J.S., Smith, D.D., 2012. Rare pits, large vessels and extreme vulnerability to cavitation in a ring-porous tree species. <i>New Phytol.</i> 193, 713–720.
<i>Q. laevis</i>	1.89	3.6	Esteso-Martínez, J., Camarero, J.J., Gil-Pelegrin, E., 2006. Competitive effects of herbs on <i>Quercus faginea</i> seedlings inferred from vulnerability curves and spatial-pattern analyses in a Mediterranean stand (Iberian System, northeastern Spain). <i>Ecoscience</i> . 13(3), 378–387.
<i>Q. nigra</i>	1.31	2.7	Choat, B., Jansen, S., Brodrribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. <i>Nature</i> . 491, 752–755.
<i>Q. oleoides</i>	3.03	3.9	Choat, B., Jansen, S., Brodrribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. <i>Nature</i> . 491, 752–755.
<i>Q. petraea</i>	1.9	-	Nardini, A., Pedà, G., La Rocca, N., 2012. Trade-offs between leaf hydraulic capacity and drought vulnerability: morpho-anatomical bases, carbon costs and ecological consequences. <i>New Phytol.</i> 196, 788–798.
<i>Q. petraea</i>	3.5	4.2	Choat, B., Jansen, S., Brodrribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. <i>Nature</i> . 491, 752–755.
<i>Q. petraea</i>	3.46	4.58	Urli, M., Porté, A.J., Cochard, H., Guengant, Y., Burtlett, R., Delzon, S., 2013. Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. <i>Tree Physiol.</i> 33, 672–683.
<i>Q. petraea</i>	3.4	4.2	Tyree, M.T., Cochard, H., 1996. Summer and winter embolism in oak: impact on water relations. <i>Ann. For. Sci.</i> 53, 173–180.
<i>Q. phellos</i>	1.42	2.3	Choat, B., Jansen, S., Brodrribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. <i>Nature</i> . 491, 752–755.
<i>Q. prinus</i>	1.7	-	Choat, B., Jansen, S., Brodrribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. <i>Nature</i> . 491, 752–755.

<i>Q. pubescens</i>	2.75	-	Nardini, A., Pedà, G., La Rocca, N., 2012. Trade-offs between leaf hydraulic capacity and drought vulnerability: morpho-anatomical bases, carbon costs and ecological consequences. <i>New Phytol.</i> 196, 788–798.
<i>Q. pubescens</i>	1.7	3.2	Tognetti, R., Longobucco, A., Raschi, A., 1999. Seasonal embolism and xylem vulnerability in deciduous and evergreen Mediterranean trees influenced by proximity to a carbon dioxide spring. <i>Tree Physiol.</i> 19, 271–277.
<i>Q. pubescens</i>	3.3	5.5	Choat, B., Jansen, S., Brodrribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Haecke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. <i>Nature.</i> 491, 752–755.
<i>Q. pubescens</i>	3.5	> 4.5	Cochard, H., Bréda, N., Granier, A., Aussenac, G., 1992. Vulnerability to air embolism of three European oak species (<i>Quercus petraea</i> (Matt) Liebl, <i>Q. pubescens</i> Willd., <i>Q. robur</i> L.). <i>Ann. Sci. For.</i> 49, 225–233.
<i>Q. pubescens</i>	3	-	Tyree, M.T., Cochard, H., 1996. Summer and winter embolism in oak: impact on water relations. <i>Ann. For. Sci.</i> 53, 173–180.
<i>Q. robur</i>	2.8	3.46	Choat, B., Jansen, S., Brodrribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Haecke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. <i>Nature.</i> 491, 752–755.
<i>Q. robur</i>	2.8	3.5	Cochard, H., Bréda, N., Granier, A., Aussenac, G., 1992. Vulnerability to air embolism of three European oak species (<i>Quercus petraea</i> (Matt) Liebl, <i>Q. pubescens</i> Willd., <i>Q. robur</i> L.). <i>Ann. Sci. For.</i> 49, 225–233.
<i>Q. robur</i>	2.7	3.3	Tyree, M.T., Cochard, H., 1996. Summer and winter embolism in oak: impact on water relations. <i>Ann. For. Sci.</i> 53, 173–180.
<i>Q. robur</i>	2.83	3.41	Urli, M., Porté, A.J., Cochard, H., Guengant, Y., Burllett, R., Delzon, S., 2013. Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. <i>Tree Physiol.</i> 33, 672–683.
<i>Q. robur</i>	1.36	-	Venturas, M.D., Rodriguez-Zaccaro, F.D., Percolla, M.L., Crous, C.J., Jacobsen, A.L., Pratt, R.B., 2016. Single vessel air injection estimates of xylem resistance to cavitation are affected by vessel network characteristics and sample length. <i>Tree Physiol.</i> 36, 1247–1259.
<i>Q. robur</i>	1.67	4.3	Tobin, M.F., Pratt, R.B., Jacobsen, A.L., Guzman, M.E., 2013. Xylem vulnerability to cavitation can be accurately characterised in species with long vessels using a centrifuge method. <i>Plant Biol.</i> 15(3): 496–504.
<i>Q. robur</i>	2.26	4.3	Tobin, M.F., Pratt, R.B., Jacobsen, A.L., Guzman, M.E., 2013. Xylem vulnerability to cavitation can be accurately characterised in species with long vessels using a centrifuge method. <i>Plant Biol.</i> 15(3): 496–504.
<i>Q. rubra</i>	2.5	3.4	Cochard, H., Tyree, M.T., 1990. Xylem dysfunction in <i>Quercus</i> : vessel sizes, tyloses, cavitation and seasonal changes in embolism. <i>Tree Physiol.</i> 6, 393–407.
<i>Q. rubra</i>	1.61	3.1	Choat, B., Jansen, S., Brodrribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Haecke, U.G., Jacobsen, A.L., Lens, F., Maherali, H.,

				Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. <i>Nature</i> . 491, 752–755.
<i>Q. rubra</i>	2.4	3.7		Cochard, H., Bréda, N., Granier, A., Aussenac, G., 1992. Vulnerability to air embolism of three European oak species (<i>Quercus petraea</i> (Matt) Liebl, <i>Q. pubescens</i> Willd., <i>Q. robur</i> L.). <i>Ann. Sci. For.</i> 49, 225–233.
<i>Q. rubra</i>	2.3	4.1		Tyree, M.T., Cochard, H., 1996. Summer and winter embolism in oak: impact on water relations. <i>Ann. For. Sci.</i> 53, 173–180.
<i>Q. stellata</i>	1.44	2.5		Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Field, T.S., Gleason, S.M., Hacke, U.G., Jacobsen, A.L., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., Mencuccini, M., Mitchell, P.J., Nardini, A., Pittermann, J., Pratt, R.B., Sperry, J.S., Westoby, M., Wright, I.J., Zanne, A.E., 2012. Global convergence in the vulnerability of forests to drought. <i>Nature</i> . 491, 752–755.
<i>Q. suber</i>	5.02	7		Vaz, M., Cochard, H., Gazarini, L., Graça, J., Chaves, M.M., Pereira, J.S., 2012. Cork oak (<i>Quercus suber</i> L.) seedlings acclimate to elevated CO ₂ : Photosynthesis, Growth, Wood Anatomy and Hydraulic Conductivity. <i>Trees - Struct. Funct.</i> 26, 1145–1157.
<i>Q. suber</i>	5.3	5.9		Tyree, M.T., Cochard, H., 1996. Summer and winter embolism in oak: impact on water relations. <i>Ann. For. Sci.</i> 53, 173–180.
<i>Q. suber</i>	2.89	7.3		Pinto, C.A., David, J.S., Cochard, H., Caldeira, M.C., Henriques, M.O., Quilhó, T., Paço, T.A., Pereira, J.S., David, T.S., 2012. Drought-induced embolism in current-year shoots of two Mediterranean evergreen oaks. <i>For. Ecol. Manag.</i> 285, 1–10.
<i>Q. suber</i>	2.94	7.8		Pinto, C.A., David, J.S., Cochard, H., Caldeira, M.C., Henriques, M.O., Quilhó, T., Paço, T.A., Pereira, J.S., David, T.S., 2012. Drought-induced embolism in current-year shoots of two Mediterranean evergreen oaks. <i>For. Ecol. Manag.</i> 285, 1–10.
<i>Q. subpyrenaica</i>	5	8.7		Peguero-Pina, J.J., Sancho-Knapik, D., Martín, P., Saz, M.Á., Gea-Izquierdo, G., Cañellas, I., Gil-Pelegrín, E., 2015. Evidence of vulnerability segmentation in a deciduous Mediterranean oak (<i>Quercus subpyrenaica</i> EH del Villar). <i>Trees</i> 29, 1917–1927.
<i>Q. wislizenii</i>	1.37	5		Tobin, M.F., Pratt, R.B., Jacobsen, A.L., Guzman, M.E., 2013. Xylem vulnerability to cavitation can be accurately characterised in species with long vessels using a centrifuge method. <i>Plant Biol.</i> 15(3): 496–504.
<i>Q. wislizenii</i>	1.72	7		Tobin, M.F., Pratt, R.B., Jacobsen, A.L., Guzman, M.E., 2013. Xylem vulnerability to cavitation can be accurately characterised in species with long vessels using a centrifuge method. <i>Plant Biol.</i> 15(3): 496–504.
<i>Q. wislizenii</i>	1.39	7		Tobin, M.F., Pratt, R.B., Jacobsen, A.L., Guzman, M.E., 2013. Xylem vulnerability to cavitation can be accurately characterised in species with long vessels using a centrifuge method. <i>Plant Biol.</i> 15(3): 496–504.

Table A3. Values of Turgor Loss Point (TLP) and Minimum water potential recorded in the field at midday (Ψ_{MD}) for *Quercus ilex* and references from which data have been extracted.

Criterion	Value (Mpa)	Reference
TLP	-2.85	
	-3.2	Sala, A., Tenhunen, J.D., 1994. Site-specific water relations and stomatal response of <i>Quercus ilex</i> in a Mediterranean watershed. <i>Tree physiol.</i> 14(6), 601–617. https://doi.org/10.1093/treephys/14.6.601 .
	-3.4	
	-4	
	-2.95	Nardini, A., Salleo, S., Gullo, M.A.L., Pitt, F., 2000. Different responses to drought and freeze stress of <i>Quercus ilex</i> L. growing along a latitudinal gradient. <i>Plant ecol.</i> 148(2), 139–147. https://doi.org/10.1023/A:1009840203569 .
	-2.43	Villar-Salvador, P., Planelles, R., Oliet, J., Peñuelas-Rubira, J.L., Jacobs, D.F., González, M., 2004. Drought tolerance and transplanting performance of holm oak (<i>Quercus ilex</i>) seedlings after drought hardening in the nursery. <i>Tree physiol.</i> 24(10), 1147–1155. https://doi.org/10.1093/treephys/24.10.1147 .
Ψ_{MD}	-2.95	Gullo, M.A.L., & Salleo, S., 1993. Different vulnerabilities of <i>Quercus ilex</i> L. to freeze and summer drought induced xylem embolism: an ecological interpretation. <i>Plant, Cell Environ.</i> 16(5), 511–519. https://doi.org/10.1111/j.1365-3040.1993.tb00898.x .
	-2.23	Serrano, L., Penuelas, J., 2005. Contribution of physiological and morphological adjustments to drought resistance in two Mediterranean tree species. <i>Biol. Plant.</i> 49(4), 551–559. http://dx.doi.org/10.1007/s10535-007-0087-8 .
	-2.6	Urli, M., Lamy, J.B., Sin, F., Burrett, R., Delzon, S., Porté, A.J., 2015. The high vulnerability of <i>Quercus robur</i> to drought at its southern margin paves the way for <i>Quercus ilex</i> . <i>Plant Ecol.</i> 216(2), 177–187. https://doi.org/10.1007/s11258-014-0426-8 .
	-2.8	

- 3.5 Pinto, C.A., David, J.S., Cocharad, H., Caldeira, M.C., Henriques, M.O., Quilhó, T., Paço, T.A., Pereira, J.S., David, T.S., 2012. Drought-induced embolism in current-year shoots of two Mediterranean evergreen oaks. *For. Ecol. Manag.* 285, 1–10. <https://doi.org/10.1016/j.foreco.2012.08.005>.
- 2.6 Baquedano, F.J., Castillo, F., 2007. Drought tolerance in the Mediterranean species *Quercus coccifera*, *Quercus ilex*, *Pinus halepensis*, and *Juniperus phoenicea*. *Photosynthetica* 45, 229–238. <https://doi.org/10.1007/s11099-007-0037-x>.
- 4.2 Pesoli, P., Gratani, L., Larcher, W., 2003. Responses of *Quercus ilex* from different provenances to experimentally imposed water stress. *Biol. Plant.* 46(4), 577–581. <https://doi.org/10.1023/A:1024823830225>.
- 2.7 Bussotti, F., Bettini, D., Grossoni, P., Mansueto, S., Nibbi, R., Soda, C., Tani, C., 2002. Structural and functional traits of *Quercus ilex* in response to water availability. *Environ. Exp. Bot.* 47(1), 11–23. [https://doi.org/10.1016/S0098-8472\(01\)00111-3](https://doi.org/10.1016/S0098-8472(01)00111-3).
- 4.91 Limousin, J.M., Longepierre, D., Hue, R., Rambal, S., 2010. Change in hydraulic traits of Mediterranean *Quercus ilex* subjected to long-term throughfall exclusion. *Tree Physiol.* 30, 1026–1036. <https://doi.org/10.1093/treephys/tpq062>.
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