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## Research paper

# Differential drought resistance strategies of co-existing woodland species enduring the long rainless Eastern Mediterranean summer

Päivi J. Väänänen<sup>1,2</sup>, Yagil Osem<sup>2</sup>, Shabtai Cohen<sup>3</sup> and José M. Grunzweig<sup>1,4</sup>

<sup>1</sup>Institute of Plant Sciences and Genetics in Agriculture, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 7610001, Israel; <sup>2</sup>Department of Natural Resources, Agricultural Research Organization, Volcani Center, Bet-Dagan 50250, Israel; <sup>3</sup>Institute of Soil, Water and Environmental Sciences, Agricultural Research Organization, Volcani Center, Bet-Dagan 50250, Israel; <sup>4</sup>Corresponding author (jose.grunzweig@mail.huji.ac.il)

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**In anticipation of a drier climate and to project future changes in forest dynamics, it is imperative to understand species-specific differences in drought resistance. The objectives of this study were to form a comprehensive understanding of the drought resistance strategies adopted by Eastern Mediterranean woodland species, and to elaborate specific ecophysiological traits that can explain the observed variation in survival among these species. We examined leaf water potential ( $\Psi$ ), gas exchange and stem hydraulics during 2–3 years in mature individuals of the key woody species *Phillyrea latifolia* L., *Pistacia lentiscus* L. and *Quercus calliprinos* Webb that co-exist in a dry woodland experiencing ~6 rainless summer months. As compared with the other two similarly functioning species, *Phillyrea* displayed considerably lower  $\Psi$  (minimum  $\Psi$  of  $-8.7$  MPa in *Phillyrea* vs  $-4.2$  MPa in *Pistacia* and *Quercus*), lower  $\Psi$  at stomatal closure and lower leaf turgor loss point ( $\Psi_{\text{TLP}}$ ), but reduced hydraulic vulnerability and wider safety margins. Notably, *Phillyrea* allowed  $\Psi$  to drop below  $\Psi_{\text{TLP}}$  under severe drought, whereas the other two species maintained positive turgor. These results indicate that *Phillyrea* adopted a more anisohydric drought resistance strategy, while *Pistacia* and *Quercus* exhibited a more isohydric strategy and probably relied on deeper water reserves. Unlike the two relatively isohydric species, *Phillyrea* reached complete stomatal closure at the end of the dry summer. Despite assessing a large number of physiological traits, none of them could be directly related to tree mortality. Higher mortality was observed for *Quercus* than for the other two species, which may result from higher water consumption due to its 2.5–10 times larger crown volume. The observed patterns suggest that similar levels of drought resistance in terms of survival can be achieved via different drought resistance strategies. Conversely, similar resistance strategies in terms of isohydricity can lead to different levels of vulnerability to extreme drought.**

**Keywords:** diurnal leaf gas exchange, drought resistance strategies, dryland shrubs, hydraulic safety margins, hydraulic vulnerability, Mediterranean trees, stomatal closure, turgor loss point.

## Introduction

Recently, increased tree mortality in various forest ecosystems around the world has been related to extensive periods of extreme heat and drought (Allen et al. 2015, Hartmann et al. 2018). In the Eastern Mediterranean, woodlands have been reported to respond to declining precipitation levels with decreased growth and increased mortality (Körner et al. 2005, Sarris et al. 2007). In view of the threat to forests exposed

to a drier climate, and to enable projecting future changes in forest dynamics, there is an urgent need to understand species-specific differences in drought resistance as well as the physiological mechanisms behind them.

The behavior of plants under water deficiency has been divided into two categories of drought resistance strategies based on the plant's tendency to regulate leaf water potential ( $\Psi$ ) through stomatal conductance (Tardieu and Simonneau

1998). Isohydic plants tend to keep  $\Psi$  relatively stable under drought conditions, and thus, leaf tissues are less subjected to drought stress, whereas anisohydric species use less stomatal control allowing their  $\Psi$  to drop and their tissues to experience stress conditions. McDowell et al. (2008) developed the theory further by suggesting the following two distinct mortality pathways related to these strategies: as isohydric species tend to keep  $\Psi$  stable through early stomatal closure, they would be to risk of carbon starvation; in contrast, by postponing stomatal closure, anisohydric species are more prone to hydraulic failure. Since its publication, this classification has been widely used to assess drought resistance across species and has directed scientific research over plant function and mortality (Hochberg et al. 2018). The underlying assumptions of the classification, which may be a continuum by nature (Klein 2014), are currently under vigorous testing, and doubts have been cast on its use for explaining plant function and mortality (see Garcia-Forner et al. 2017, Martínez-Vilalta and Garcia-Forner 2017, Hochberg et al. 2018).

In Mediterranean woodlands, both isohydric and anisohydric species have been found co-existing at the same site (Lo Gullo and Salleo 1998, Nardini et al. 1999, Klein et al. 2013). Tree species exhibited different levels of resistance to drought, which led to differential mortality among species under extreme drought events (Peñuelas et al. 2000, Martínez-Vilalta and Piñol 2002, Mueller et al. 2005, Breshears et al. 2005). There is no evidence about the superiority of one drought resistance strategy over the other, despite reports of greater levels of mortality among more isohydric species under semi-arid and Mediterranean climate regimes (Peñuelas et al. 2000, Martínez-Vilalta and Piñol 2002, Breshears et al. 2005, Garcia-Forner et al. 2017; but see West et al. 2012).

The distinction between isohydric and anisohydric strategies is further reflected in other characteristics related to water use, e.g. vulnerability to embolism (Martínez-Vilalta et al. 2002, Mitchell et al. 2013), and leaf functional traits, such as turgor loss point (Bartlett et al. 2012). For example, plants that allow their water potential to drop during a period of water deficiency typically have shallow rooting (Nardini et al. 2016), low turgor loss point (Meinzer et al. 2014) and high resistance to embolism (Choat et al. 2012). Hence, drought resistance strategy can be viewed as an association of plant traits adapted by a species to enable it to survive under drought (Levitt 1980). Alternatively, individual plant traits serving as a possible proxy for drought resistance per se have also been under investigation (Greenwood et al. 2017, O'Brien et al. 2017). Among the most promising candidates currently are vulnerability to embolism (Anderegg et al. 2012, 2013, Urli et al. 2013, Brodribb and Cochard 2009, Skelton et al. 2015, Adams et al. 2017) and leaf turgor loss point (Bartlett et al. 2012, Maréchaux et al. 2015). Plant vulnerability to embolism is known to vary interspecifically and has been linked to tree

survival (Maherali et al. 2004, Brodribb and Cochard 2009, Delzon et al. 2010, Urli et al. 2013, Anderegg et al. 2016) and species distribution patterns (Engelbrecht et al. 2007, Mitchell et al. 2008, Poot and Veneklaas 2013). Hydraulic safety margins describe how close species operate to the levels of mortality-inducing hydraulic failure, and despite their limitations in predicting tree mortality (Klein et al. 2014), it seem to play an important role in describing drought resistance of trees (Adams et al. 2017, Choat et al. 2012). Turgor loss point ( $\Psi_{TLP}$ ) defines the point at which the cell turgor pressure is zero and  $\Psi$  is determined solely by cell osmotic potential (Schulte and Hinckley 1985). Species with more negative turgor loss point extend the range of  $\Psi$  at which the leaf remains turgid and maintains function (Brodribb and Holbrook 2003, Sack et al. 2003, Lenz et al. 2006), and such  $\Psi_{TLP}$  might be a key trait to characterize a plant's resistance to drought. Recent research has shown that  $\Psi_{TLP}$  (Bartlett et al. 2012, 2014, 2016, Maréchaux et al. 2015) strongly correlated with water availability within and across biomes. However, the relationship of individual plant traits to drought resistance is often observed in broad-scale meta-analyses where drought resistance is not derived from actual mortality levels under drought (Choat et al. 2012, Bartlett et al. 2012, 2014; but see Adams et al. 2017, Greenwood et al. 2017). Thus, it remains unclear whether those plant traits can predict the differences in survival and performance among individual plant species under drought (Farrell et al. 2017).

Woodlands in the Mediterranean basin are water-limited systems dominated by mostly evergreen sclerophyllous shrubs and trees. The composition of the dry-subhumid oak woodlands in the Eastern Mediterranean region shows similarities with the Western Mediterranean *Quercus ilex*-dominated forests, often consisting of dominant oaks, such as *Quercus calliprinos* Webb, accompanied by other key woody and numerous herbaceous species. However, the Eastern Mediterranean woodlands developed under even harsher climate conditions than their western counterparts. The dry, rainless summer extends to  $\sim 6$  months on average, without interruption by occasional rainfall as in the Western Mediterranean region (Ne'eman and Goubitz 2000). Despite being drought-adapted, increased tree mortality in Israeli forests has led to concerns about their future (Klein et al. 2019).

This study investigated the water status and function of mature individuals of key woody Mediterranean species to offer a comprehensive view of drought resistance of species co-existing in their natural habitat. The objectives of this study were to investigate: (i) how the co-existing Eastern Mediterranean key woodland species differ in their function under drought; (ii) how differences in function among species are related to drought resistance strategies; and (iii) whether there are specific physiological traits that can explain the variation in survival under drought among these species. By investigating a wide

range of ecophysiological traits related to drought resistance, we intended to promote a comprehensive understanding of the drought resistance strategies of these species. The study focused on individuals of *Phillyrea latifolia* L., *Pistacia lentiscus* L. and *Q. calliprinos* growing in an area that is considered marginal for growth and survival of *Q. calliprinos*. We hypothesized that, in accordance with current definitions of drought resistance strategies, the species showing lowest minimum water potential also shows the highest resistance to embolism, the lowest turgor loss point and lowest level of stomatal control. Additionally, the species with lowest minimum water potential is expected to continue to transpire even under prolonged drought stress compared with other species, and to operate close to hydraulic failure. Based on the spatially limited presence of *Q. calliprinos* in the study site and the observed mortality of this species (see below), we assumed that *Q. calliprinos* is more vulnerable to drought than *P. latifolia* and *P. lentiscus*. We tested these hypotheses by studying plant function under natural drought conditions by assessing the diurnal and seasonal cycles of water potential and gas exchange, hydraulics and leaf functional traits.

## Materials and methods

### Study site and experimental design

The study was conducted in a natural, ungrazed part of Ramat Hanadiv Nature Park in the southern part of the Carmel mountain range in Northern Israel (32° 30' N, 34° 57' E). The experimental site is located on a plateau 120 m above sea level. The parent rock is mostly composed of hard, layered limestone and dolomite, and the soil is Terra rossa (red Mediterranean soil). The climate is Mediterranean with mean annual precipitation of 530 mm (20-year mean; minimum = 282 mm, maximum = 832 mm) occurring mainly between October and March. The dominant woody species are the evergreen, sclerophyllous *P. latifolia*, *P. lentiscus* and *Q. calliprinos*, hereafter referred to by their genus names. *Phillyrea* and *Quercus* are multi-stemmed small trees or large shrubs up to 6-m high; *Pistacia* is a shrub that rarely exceeds 2 m in height. Because of lithological (karstic bedrock) and related hydrological constraints, the study site is marginal for the development of *Quercus*.

On April 2011, six circular sampling plots of 8-m radius were established within the study site. In each plot, two individuals of each of the studied species were selected as sample trees or shrubs, i.e. altogether 12 individuals of each species. The crown dimensions (height and diameter in two directions) of the sample trees were measured on November 2012. The plots were located 300–500 m from each other and shared similar topography, geology and soil type. Vegetation in the site was a dense oak shrub formation (maquis) dominated by the three studied species, with a woody understory of shrubs, dwarf shrubs and climbers, together with a mixture of annual and

perennial herbs that grow during the moist winter and spring seasons. Total woody vegetation volume at the study site was 4.15 m<sup>3</sup>/m<sup>2</sup> (±SE 0.54), assessed within the plots with line transect method (Boyd et al. 2007, Osem et al. 2011). The total cumulative contribution of *Phillyrea*, *Pistacia* and *Quercus* to the total woody vegetation volume at the study site exceeded 70%.

### Field measurements

The water status of *Phillyrea*, *Pistacia* and *Quercus* was tracked with regular measuring campaigns of  $\Psi$  for 3 years. Within this time period, several physiological variables related to, e.g. leaf gas exchange, vulnerability to embolism and leaf traits from pressure–volume curves, were assessed to characterize the drought response of the species and to establish the relationship between the above variables and plant water status. Not all physiological variables were assessed simultaneously for logistic reasons. Water potentials of small apical shoots ( $\Psi$ ) were measured during nine measurement campaigns under typical spring (May), summer (August) and winter (December–January) conditions from May 2011 to January 2014 at predawn ( $\Psi_{PD}$ ; from 1 to 3 h before sunrise) and at noon ( $\Psi_{MD}$ ; within 1 h of solar noon). Additionally,  $\Psi$  was determined concurrently with the leaf gas exchange measurements (see below). An additional campaign was conducted at the end of an extremely long and rainless summer on November 2013. To obtain diurnal dynamics of  $\Psi$  in each season, occasional measurements were also conducted in morning and evening hours. The shoots (12 individuals per species, two–three shoots per plant) were cut from the sample plants, sealed in plastic bags and placed in a cooler filled with ice. Water potential was measured within 2 h from the time samples were collected using a Scholander-type pressure bomb (PMS Instruments, Corvallis, OR, USA).

In 2–5 plants per species, leaf gas exchange was measured on April, June and September 2012 and in January 2013. Leaf stomatal conductance ( $g_s$ , mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), net assimilation ( $A_n$ ,  $\mu$ mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) and transpiration ( $E$ , mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) were measured on two sun-exposed leaves per plant in the morning, midday and afternoon of every field day using an LI-6400XT Photosynthesis System equipped with a 6400-40 Leaf Chamber Fluorometer (Li-Cor Inc., Lincoln, NE, USA). Microclimatic and light conditions inside the leaf chamber were adjusted close to ambient, and measurements were made by maintaining the natural inclination of the leaves. Water potential of shoots from the same branch was measured simultaneously with leaf gas exchange measurement. Climatic data, such as air temperature and precipitation, were available from a weather station located 300 m from the study site. Crown volume of the sample plants was calculated from crown height and diameter measurements.

In February 2017, following a decade of generally below average rainfall (Figure S1 available as Supplementary Data at

*Tree Physiology Online*), a survey was conducted in the study site to assess mortality rates among species. A transect of 600 m was established, and we counted all *Phillyrea*, *Pistacia* and *Quercus* individuals that grew within 4 m around the transect, and classified them as live or dead. A tree was classified as dead when the aboveground biomass was dried out. The vast majority of *Quercus* trees that dry out do not show any subsequent recovery and have to be declared as dead (Y.Navon, J.M.Grünzweig, unpublished results). Altogether, 211 individuals were classified.

### Pressure–volume curves

In December 2012 and in January, May and August 2013, five apical shoots with four to eight fully expanded leaves were collected from the sample trees and shrubs to generate pressure–volume curves (Tyree and Hammel 1972). Samples were transported to the laboratory in an ice chest and rehydrated for 2 h by submerging cuts end in water, according to the recommendations by Arndt et al. (2015). Subsequently, the samples were allowed to dehydrate by free transpiration while being periodically re-weighed and measured for  $\Psi$  using the pressure bomb (see above). Shoot dry mass was determined after drying at 65 °C until constant mass. Osmotic water potential at full turgor ( $\Psi_o$ ), water potential at the turgor loss point ( $\Psi_{TLP}$ ), apoplastic water fraction ( $A_f$ ) and bulk modulus of elasticity against symplastic water content ( $\varepsilon$ ) were determined as in Bartlett et al. (2012). Predawn  $\Psi$  of the sample trees was determined on additional shoots by following the protocol described above prior to collecting samples for pressure–volume curves.

### Loss of conductivity curves

The data set for vulnerability curves was obtained using the bench dehydration method described in detail by Tyree and Sperry (1989) and previously used to measure vulnerability curves in the Mediterranean oak *Q. ilex* (Martinez-Vilalta et al. 2002). Branches (~0.75–1.2 m long, up to 2.5-cm thick) were sampled in the field from at least five individuals per species during conditions of high water availability (January and April 2014). The incision was performed under water by using long and narrow water containers that were secured around the branch and filled with water. An additional sampling (five samples) was carried out during conditions of low water availability (August 2014) for *Phillyrea*, as it proved difficult to dehydrate this species under laboratory conditions to reach low levels of  $\Psi$  (below –8 MPa). Branches were transferred in plastic bags to the laboratory about an hour drive away where they were allowed to dehydrate. Branch  $\Psi$  was monitored by measuring leaves from the branches, and when the desired level of dehydration was reached, a segment of the sampled branch at least 10-cm long and with a diameter between 6–9 mm was re-cut under water. A fresh razor blade was used to shave off

the ends of the sample (Wheeler et al. 2013). The length of the segment and its basal diameter were determined. After removing the bark, branch ends were connected to a tubing system linked to a water reservoir (a burette) with a 70-cm head causing a pressure difference of ca 7 kPa. The system was filled with a filtered and degassed solution of KCl (0.02 M), and flow rate through the stem segment was recorded after steady-state was reached as an average of three consequent measurements. The initial specific hydraulic conductivity ( $K_{\text{hinit}}$ , kg s<sup>-1</sup> MPa<sup>-1</sup> m<sup>-1</sup>) was calculated as the ratio between the flow (kg s<sup>-1</sup>) and the pressure gradient along the segment (MPa m<sup>-1</sup>) normalized by stem cross sectional area (m<sup>-2</sup>). For determining the maximum hydraulic conductivity ( $K_{\text{hmax}}$ ), the segments were flushed at high pressure (ca 100 kPa) with a high-pressure flow meter for 15 min to remove all native embolisms, and then re-measured using the same methodology as described above.

Problems related to conductivity measurements in species with long vessels have been discussed in the literature (Cochard et al. 2013, Delzon and Cochard 2014, Torres-Ruiz et al. 2014, Wheeler et al. 2013). Open vessel errors occur when conductivity is measured on branch samples that are considerably shorter than the maximum vessel length, creating a significant number of vessels that are cut open from both ends. Without intervessel pits, the contribution of these open vessels to the total conductivity is high (Wheeler et al. 2005, Hacke et al. 2006). This may lead to underestimation of the embolism (The mathematical proof is presented in Supplementary Data, available at *Tree Physiology Online*, pp 7–10). Preliminary measurements of maximum vessel length for the species were made on long branches (1.5–2 m) sampled from the field after Skene and Balodis (1968), where the end of the branch was immersed in water and pressurized air (at 65 kPa) was forced through the branch from the cut base. The branch was cut shorter in 2-cm intervals until bubbles appeared in the water. *Phillyrea* had a maximum vessel length of ~32 cm, and the maximum vessel length of *Pistacia* and *Quercus* exceeded the sample length (>1.5 m). As most of the plants available for this experiment had branched stems longer than 20–30 cm, a methodology was developed to correct the open vessel error by determining the portion of conductivity caused by open vessels. The conductivity of open vessels was determined by using pressurized air that was forced through fresh wood. At low pressure, air flows through vessels cut open at both ends but does not move from one vessel to another because air flow is blocked by wet pit membranes (Skene and Balodis 1968).

The conductivity of 'closed' vessels ( $K_{\text{closed}}$ ) was defined as follows:

$$K_{\text{closed}} = K_{\text{total}} - K_{\text{open}}, \quad (1)$$

where  $K_{\text{total}}$  is the total conductivity of a stem segment, and  $K_{\text{open}}$  is the conductivity of open vessels. After determining  $K_{\text{hinit}}$

and  $K_{\text{hmax}}$  of a branch segment, both ends of the segment were trimmed with a fresh razor blade, and the branch was attached to a measuring apparatus, which is described in details in Cohen et al. (2003). Pressurized air (at 65 kPa) was forced through the branch and the outflowing air was transferred to a sealed water tank. The displaced water from the water tank was collected in a beaker that was placed on a precision balance. When steady flow was reached, the volume of the airflow could be measured as the volume of displaced water, and the volumetric flow was converted into gravimetric flow by multiplying with air density at 20 °C. The gravimetric flow rate of air through a branch was calculated as a ratio of the flow ( $\text{kg s}^{-1}$ ) and the pressure gradient along the branch segment ( $\text{MPa m}^{-1}$ ).

In order to determine  $K_{\text{open}}$  for water, the flow of air through the branch segment needed to be converted into corresponding flow of water. The Hagen–Poiseuille equation describes a laminar flow of fluid inside a cylindrical pipe:

$$\Delta P = 8\mu LQ / (\pi R^4), \quad (2)$$

where  $\Delta P$  is the pressure difference between the ends of the branch,  $L$  is the length of the branch,  $\mu$  is the dynamic viscosity of the fluid,  $Q$  is the volumetric flow rate and  $R$  is the radius of the stem. Because  $\Delta P$ ,  $L$  and  $R$  of the system remain constant, the following equations can be written:

$$8\mu_{\text{water}}LQ_{\text{water}} / (\pi R^4) = 8\mu_{\text{air}}LQ_{\text{air}} / (\pi R^4), \quad (3)$$

and further

$$Q_{\text{water}} = \mu_{\text{air}} / \mu_{\text{water}} * Q_{\text{air}}, \quad (4)$$

where  $\mu_{\text{water}}$  and  $\mu_{\text{air}}$  are the viscosity of water and air, and  $Q_{\text{water}}$  and  $Q_{\text{air}}$  are the flow of water and air, respectively. Water is about 50 times more viscous than air, and using table values of the two viscosities for lab temperatures, the ratio  $\mu_{\text{air}} / \mu_{\text{water}}$  was taken as  $1.98 \times 10^{-2}$ .  $Q_{\text{water}}$  determined in this manner was used to determine  $K_{\text{open}}$  in Eq. (1).  $K_{\text{closed}}$  is then the corrected conductivity, which was used for the vulnerability curves.

### Calculations and statistical analyses

Water potential at stomatal closure ( $\Psi_{g0}$ ) was estimated by plotting  $\Psi_{\text{MD}}$  against  $\Psi_{\text{PD}}$  and extrapolating the regression line to the 1:1 line (Martínez-Vilalta et al. 2014), but using data collected during summer only. During periods of high soil water availability when  $\Psi_{\text{PD}}$  approaches zero,  $g_s$  is mostly affected by other factors, such as irradiance (Meinzer et al. 2016). In Mediterranean climate, stomatal closure due to water deficiency may be expected mainly in summer, when also various physiological adjustments occur that affect leaf function (Bartlett et al. 2014 and the references therein). Additionally,  $\Psi_{g0}$  was estimated by using the relationship between  $\Psi$  and measured values of  $g_s$  and defining  $g_0 = 0.01 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ . Minimum water potential ( $\Psi_{\text{min}}$ ) was defined as the lowest  $\Psi$

measured in the field during the entire study period. Percent loss of conductivity (PLC) was calculated as  $K_{\text{hinit}} / K_{\text{hmax}} * 100$  using both corrected and not corrected  $K_{\text{hinit}}$  and  $K_{\text{hmax}}$  values.

Vulnerability curves were constructed by plotting PLC against corresponding  $\Psi$  values in 0.5 MPa bins, and the vulnerability curve was fitted to an exponential sigmoidal equation (Pammenter and Van der Willigen 1998). Water potentials corresponding to 50 ( $\Psi_{50}$ ) and 88% ( $\Psi_{88}$ ) loss of conductivity were determined from these curves. The PLC values for  $\Psi_{g0}$  and  $\Psi_{\text{min}}$  were also determined. Hydraulic safety margins were calculated for  $\Psi_{50}$  and  $\Psi_{88}$  as the respective distances from  $\Psi_{g0}$  and  $\Psi_{\text{min}}$ . Gas exchange variables were related to concurrent  $\Psi$  and leaf-to-air vapor pressure deficit (VPD) by an exponential regression. Stomatal sensitivity to VPD ( $m$ ) was calculated as  $-m = -d g_s / d \ln \text{VPD}$  (Oren et al. 1999). Slopes of linear curves were tested for differences among species by examining the interaction of the covariate and the species in ANCOVA. Seasonal and diurnal gas exchange were statistically analyzed by a three-way ANOVA with season, time of the day and plant species as main effects, and plotted as random factor. Two-way ANOVA was executed separately for each season using species and time of the day as main effects. Post hoc differences were analyzed by Tukey's HSD test. Predawn and midday  $\Psi$  were compared for each species by two-way ANOVA, with season and time of day as main effects. Statistical analyses were conducted with JMP Pro 14 (SAS Institute, 2018, Cary, NC, USA) and PLC-curve fitting and related analyses with fitplc-package in R (Duursma and Choat 2017).

## Results

### Climatic conditions, and tree size and mortality in the field

Weather followed the typical Eastern Mediterranean seasonal pattern, with rains occurring mainly during winter months (between October and March), and VPD generally rising from winter to summer, though peaking during hot and dry spells in spring and autumn (Figure 1). Total precipitation amounted to 416, 514 and 527 mm in the hydrological years (October–September) of 2010–11, 2011–12 and 2012–13, respectively. The duration of the dry summer period was exceptionally long in 2012 (235 days with no rain event > 6 mm) and 2013 (227 days) as compared with the dry period in 2011 (131 days). Mean daily maximum VPD during the dry period was considerably higher in 2013 (1.78 kPa) than in 2012 (1.55 kPa; data not shown). Annual rain amounts during 5 years prior to the beginning of the study were below the multiannual mean (Figure S1 available as Supplementary Data at *Tree Physiology Online*).

The mean crown volume ( $\pm \text{SE}$ ;  $n = 12$ ) of the sample individuals was  $22.2 \pm 3.0$ ,  $5.5 \pm 1.4$  and  $57.4 \pm 10.4 \text{ m}^3$  for *Phillyrea*, *Pistacia* and *Quercus*, respectively. The survey of mortality rates among the studied species showed that 27% of

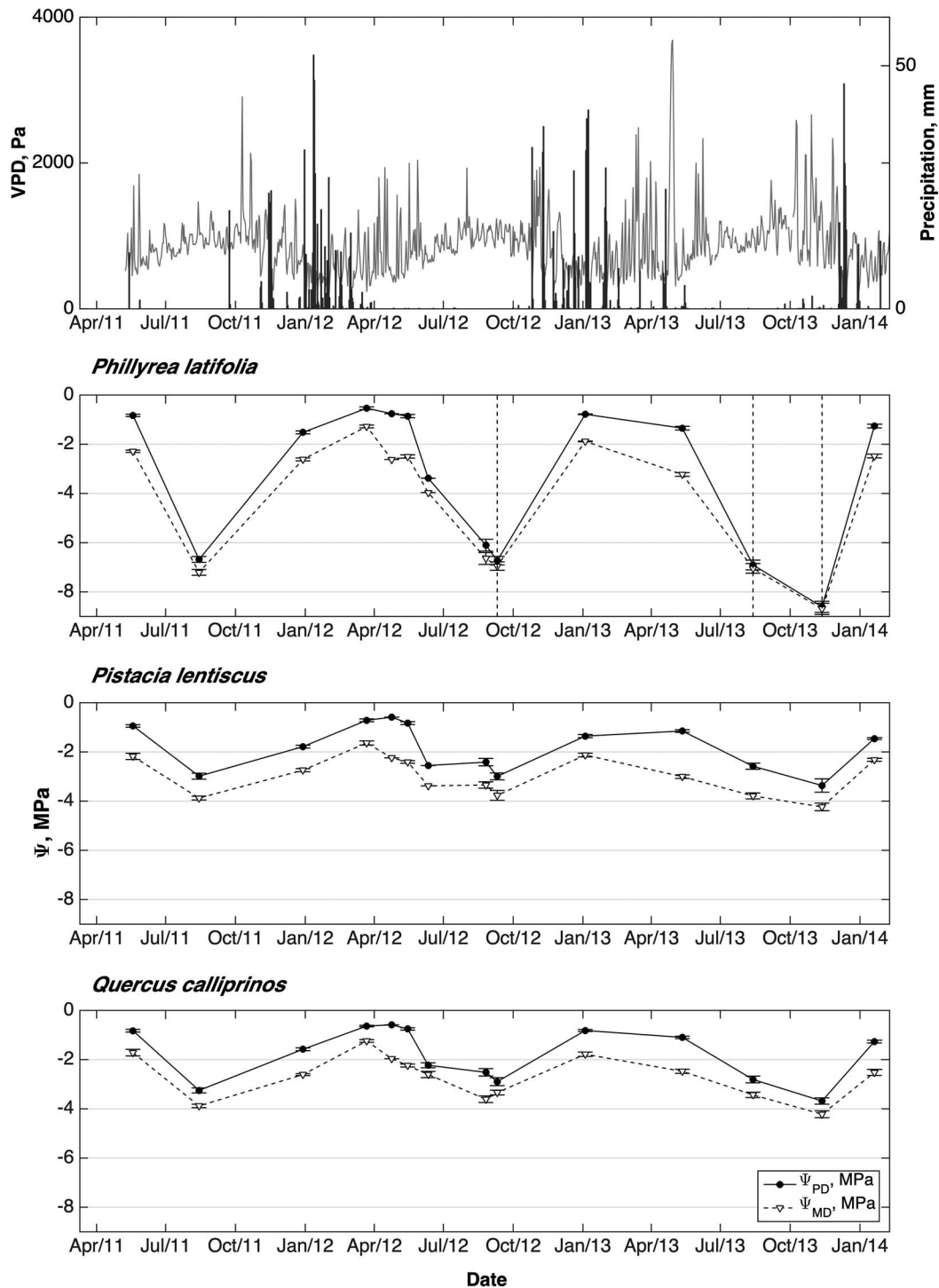


Figure 1. Daily mean VPD, precipitation and predawn ( $\Psi_{PD}$ ) and midday ( $\Psi_{MD}$ ) water potential of *Phillyrea*, *Pistacia* and *Quercus* measured from April 2011 to January 2014. Error bars indicate one standard error of the mean,  $n = 3$ –12 individuals per species. Vertical dashed lines indicate dates when there were no statistically significant differences between  $\Psi_{PD}$  and  $\Psi_{MD}$ .

the 60 monitored *Quercus* individuals were dead. In contrast, only 2% of the *Pistacia* (out of 55 individuals) and none of the *Phillyrea* individuals (out of 96 individuals) were found dead. *Quercus* mortality was not related to biological agents (L.Hadar, personal communication).

#### Leaf water potential

Predawn water potential measured throughout the year showed significant seasonal variation in all species, as follows: high  $\Psi_{PD}$  values (close to  $-1$  MPa) during the moist winter and early spring seasons (January–April), and steadily declining  $\Psi_{PD}$

values towards the summer (Figure 1). The different species exhibited similar  $\Psi_{PD}$  during times of high water availability but differed substantially during the extended dry season when *Phillyrea* showed significantly lower  $\Psi_{PD}$  than *Pistacia* and *Quercus* (mean  $\Psi_{PD} \pm SE$  of June–November being  $-6.92 \pm 0.12$ ,  $-3.22 \pm 0.11$  and  $-2.92 \pm 0.09$  MPa for *Phillyrea*, *Pistacia* and *Quercus*, respectively). Lowest values of  $\Psi_{PD}$  were reached in November 2013 ( $-8.61$ ,  $-3.37$  and  $-3.68$  MPa) following a long summer drought, which ended with the first significant rain event ( $>6$  mm) at an exceptionally late date (December 2013).

For *Pistacia* and *Quercus*,  $\Psi_{MD}$  was more negative than  $\Psi_{PD}$  on each sampling day, with  $P$ -value ranging between 0.0001 and 0.023 (Figure 1), and was close to daily minimum  $\Psi$  in all seasons of each year (Figure S2 available as Supplementary Data at *Tree Physiology Online*). Consequently,  $\Psi_{MD}$  was chosen to represent daily minimum  $\Psi$  hereafter. *Phillyrea* showed significant differences between  $\Psi_{PD}$  and  $\Psi_{MD}$  during the winter, spring and early summer ( $P = 0.0001$ – $0.0067$ ), but not during the driest parts of the seasonal drought in September 2012 and in August and November 2013 ( $P > 0.05$ ; Figure 1).

The relationship between  $\Psi_{PD}$  and  $\Psi_{MD}$  was fairly strong and linear for all three species in all of the seasons (Figure 2). The slope of the fitted line ( $\sigma$ ) for summer data (June to the first winter rain) was significantly different from  $\sigma$  in spring (March–May) for *Pistacia* and *Quercus* ( $P = 0.005$  and  $0.0135$ , respectively) and from  $\sigma$  in winter (first rain to February) for *Phillyrea* ( $P < 0.0001$ ). Consequently,  $\sigma$  and  $\Psi_{g0}$  were estimated using the data sets of summertime  $\Psi_{MD}$  vs  $\Psi_{PD}$  (Table 1), with the differences of  $\sigma$  between species not being significant ( $P = 0.3537$ , ANCOVA). The differences of  $\Psi_{g0}$  between species were assessed by 95% prediction intervals, which were overlapping for *Pistacia* and *Quercus* but not for *Phillyrea*.  $\Psi_{g0}$  estimated from the relationship of  $\Psi$  and  $g_s$  (Figure 3,  $\Psi_{g0}$  values in Table 1) yielded slightly lower values for *Phillyrea* and slightly higher values for *Pistacia* when compared with  $\Psi_{g0}$  estimated from  $\Psi_{MD}$  vs  $\Psi_{PD}$ . Calculating water potential and stomatal parameters from  $\Psi_{MD}$  vs  $\Psi_{PD}$  pooled across all seasons led to the following respective values for *Phillyrea*, *Pistacia* and *Quercus*:  $\sigma = 0.84$ ,  $0.81$  and  $0.79$  ( $P = 0.5361$ );  $\Psi_{g0} = -9.01$ ,  $-7.44$  and  $-6.28$  MPa.

### Gas exchange

Net photosynthesis,  $E$  and  $g_s$  varied seasonally, with the highest levels being measured during winter (January) and the lowest at the end of the dry season (September) in all of the species (Figure S3 and Table 1). For all variables of gas exchange, species interacted significantly with season (Table S1 available as Supplementary Data at *Tree Physiology Online*). The three species differed in  $A_n$ ,  $g_s$  and  $E$  in September ( $P = 0.0018$  for  $A_n$ , and  $P < 0.0001$  for  $g_s$  and  $E$ ), ranking *Phillyrea*  $<$  *Pistacia*  $<$  *Quercus*. Notably, time of day was not a statistically significant factor

for  $A_n$  and  $g_s$ , but seasonal effects changed with time of day for  $E$ .

Leaf gas exchange was strongly related to  $\Psi$  determined at the time of gas exchange measurements. Net assimilation,  $g_s$  and  $E$  decreased diurnally and seasonally with decreasing  $\Psi$  (Figure 3). The trend of declining  $A_n$  and  $g_s$  with  $\Psi$  below ca  $-2$  MPa was less pronounced for *Phillyrea* than for the two other species. In contrast to *Pistacia* and *Quercus*, *Phillyrea* eventually reached zero  $g_s$  at very low  $\Psi$ , as also obvious from the  $\Psi_{MD}$  and  $\Psi_{PD}$  data (points on the 1:1 line in Figure 2). Leaf gas exchange was not only related to instantaneous  $\Psi$  but also to environmental factors, mainly soil water availability represented by  $\Psi_{PD}$  and evaporative demand expressed as VPD (Figure S4 available as Supplementary Data at *Tree Physiology Online*). The relationship between daily maximum  $g_s$  ( $g_{max}$ ) and  $\Psi_{PD}$  was especially strong in *Phillyrea*, the species showing highest  $g_{max}$  throughout the range of  $\Psi_{PD}$ . Additionally,  $g_s$  was related to VPD in *Pistacia* and *Phillyrea*, and less so in *Quercus*. The stomatal sensitivity to VPD was rather similar between the species [ $0.11$ ,  $0.09$  and  $0.07$  mol m $^{-2}$  s $^{-1}$  ln(kPa) $^{-1}$  for *Phillyrea*, *Pistacia* and *Quercus*, respectively,  $P = 0.222$ ].

### Leaf turgor loss point

Water potential at turgor loss point ( $\Psi_{TLP}$ ) decreased considerably from the wet to the dry season (December vs August 2013; Figure S5 available as Supplementary Data at *Tree Physiology Online*) in *Phillyrea* and to lesser extent in the two other species. The interspecific differences in  $\Psi_{TLP}$  were most pronounced in August 2013, when the three species differed significantly from each other ( $P < 0.001$ ).

The relationship between  $\Psi_{TLP}$  and  $\Psi_o$  was positive and significant for all the species ( $P < 0.0001$ ), especially in *Phillyrea* (Figure S5 available as Supplementary Data at *Tree Physiology Online*). Elastic modulus was not directly related to the adjustment of  $\Psi_{TLP}$  of the studied species ( $R^2 = 0.01$ – $0.14$ ,  $P = 0.1125$ – $0.7110$ ), but there was a positive and significant relationship between  $A_f$  and  $\Psi_{TLP}$  in *Pistacia* and *Quercus* ( $R^2 = 0.24$ ,  $P = 0.0326$  and  $R^2 = 0.46$ ,  $P = 0.0015$ , respectively; Figure S6 available as Supplementary Data at *Tree Physiology Online*). See Figure S7 available as Supplementary Data at *Tree Physiology Online* for the pressure–volume curves of each species.

The relationship of  $\Psi_{TLP}$  to  $\Psi_{PD}$  depended on the season. During winter and early spring, when soil water availability was high,  $\Psi_{PD}$  remained above  $\Psi_{TLP}$  in all species (Figure 4). During the summer drought, however, *Phillyrea* allowed  $\Psi_{PD}$  to drop well below  $\Psi_{TLP}$ , while  $\Psi_{PD}$  of the other two species were close to and mostly above  $\Psi_{TLP}$ .

### Hydraulic vulnerability and safety margins

*Phillyrea* had significantly lower  $K_{hmax}$  than *Pistacia* and *Quercus* ( $P < 0.005$ ), which displayed similar  $K_{hmax}$  values (0.64,

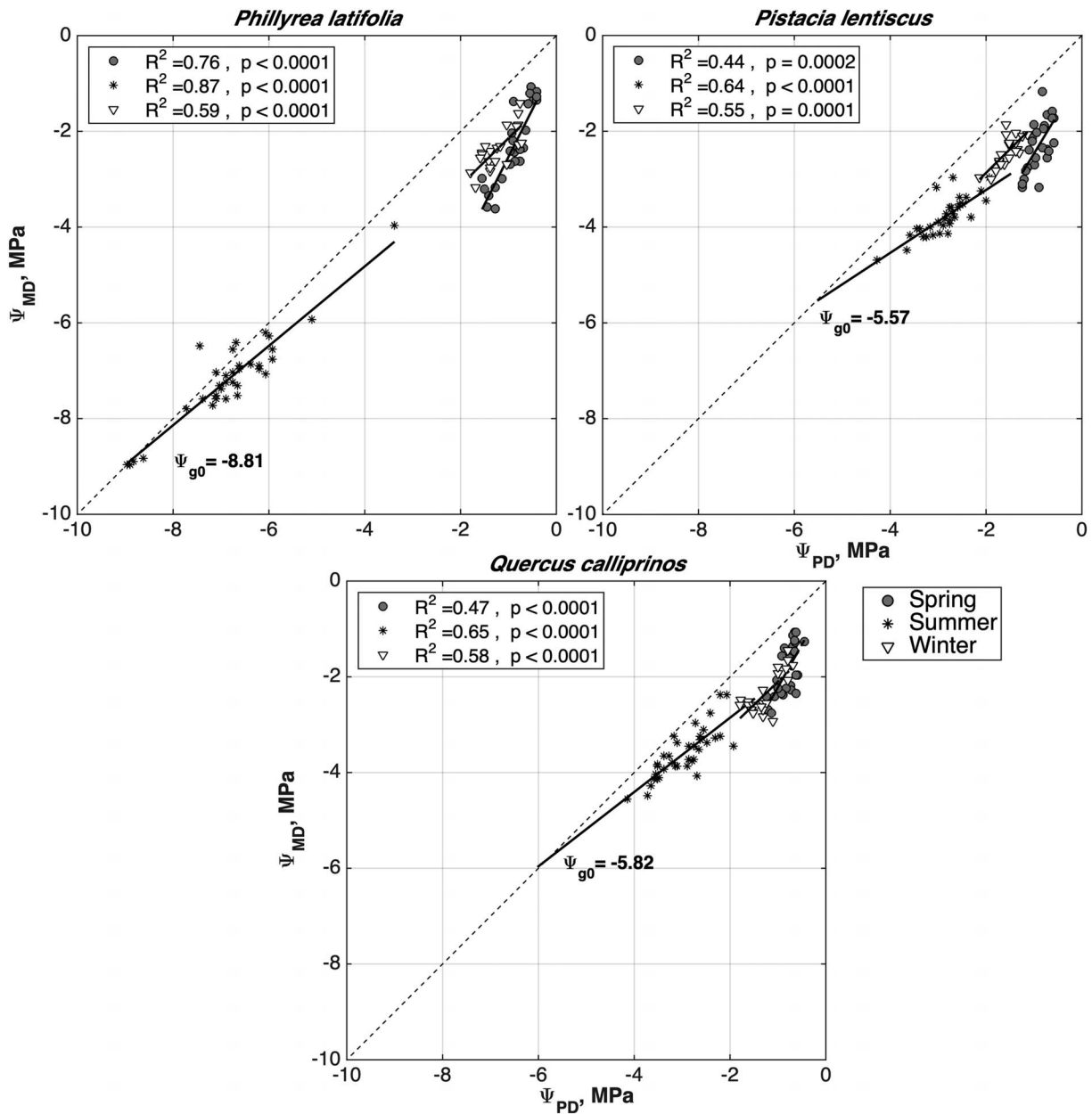


Figure 2. Midday water potential ( $\Psi_{MD}$ ) vs predawn water potential ( $\Psi_{PD}$ ) in winter, spring and summer for *Phillyrea* (equations for seasonal fits  $y = 1.99x - 0.55$ ,  $y = 0.83x - 1.50$  and  $y = 0.98x - 1.16$  for winter, spring and summer, respectively), *Pistacia* ( $y = 1.66x - 0.80$ ,  $y = 0.66x - 1.91$  and  $y = 0.99x - 0.89$ ) and *Quercus* ( $y = 1.78x - 0.43$ ,  $y = 0.78x - 1.30$ ,  $y = 0.96x - 1.14$ ). The summer data were used to define the theoretical water potential of stomatal closure  $\Psi_{g0}$  ( $\Psi_{MD} = \Psi_{PD}$ ).

1.97 and 2.12  $\text{kg s}^{-1} \text{MPa}^{-1} \text{m}^{-1}$  for *Phillyrea*, *Pistacia* and *Quercus*, respectively; Table 1. Hydraulic vulnerability followed the same pattern among the species as  $K_{hmax}$ , when the species showing higher  $K_{hmax}$  also had higher  $\Psi_{50}$  and  $\Psi_{88}$  values (Figure 5, Table 1). Regarding other hydraulic parameters,  $\Psi_{min}$  was higher than  $\Psi_{g0}$  in *Quercus* and *Pistacia*, but almost equal in *Phillyrea*. Safety margins calculated based on  $\Psi_{50}$  values were negative for all of the species, indicating that the species

regularly exceeded this value. The hydraulic safety margins based on  $\Psi_{88}$  were positive and widest for *Phillyrea*, while *Quercus* and *Pistacia* exhibited similar values Table 1.

The vulnerability curves based on PLC values with open vessel correction (Figure 5) differed from those based on non-corrected PLC values (Figure S8 and Table S2 available as Supplementary Data at *Tree Physiology Online*) in *Pistacia* and *Quercus*, but not in *Phillyrea*. The parameters derived from



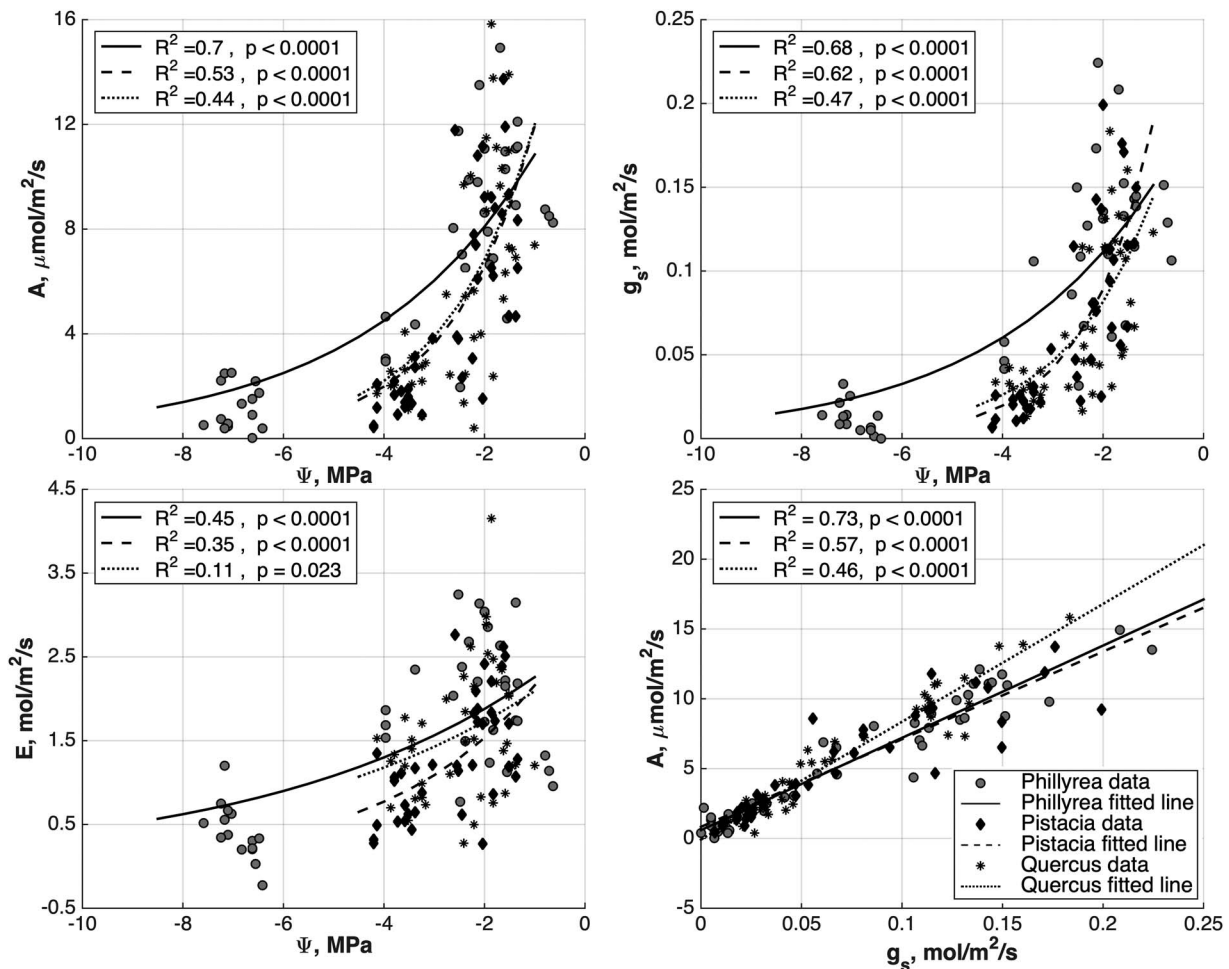


Figure 3. Relationship between leaf water potential ( $\Psi$ ) and net assimilation ( $A_n$ ), stomatal conductance ( $g_s$ ) and transpiration ( $E$ ) and the relationship between  $A_n$  and  $g_s$  of *Phillyrea* (circle, solid line), *Pistacia* (diamond, dashed line) and *Quercus* (star, dotted line). Equations for fitted lines for *Phillyrea*, *Pistacia* and *Quercus*, respectively:  $A_n$ :  $y = 14.55 \times \exp(0.29x)$ ,  $y = 21.7\text{EXP}(0.6x)$  and  $y = 21.11\text{EXP}(0.56x)$ ;  $g_s$ :  $y = 0.4\text{EXP}(0.75x)$  and  $y = 0.26\text{EXP}(0.57x)$ ;  $E$ :  $y = 2.72\text{EXP}(0.18x)$ ,  $y = 3.05\text{EXP}(0.34x)$  and  $y = 2.53\text{EXP}(0.19x)$ ; and  $A_n$  vs  $g_s$ :  $y = 66.09x + 0.59$ ,  $y = 62.77x + 0.83$  and  $y = 84.38 \times -0.08$ .

non-corrected vulnerability curves were considerably lower than when using data with open vessel correction, and accordingly, the calculated hydraulic safety margins were wider.

## Discussion

The Mediterranean woodland species *P. latifolia*, *P. lentiscus* and *Q. calliprinos* co-exist in the same habitat, yet vary significantly in their response to drought and in mortality (Figure 6). Differences between the species in  $\Psi$ ,  $g_s$  and  $\Psi_{\text{TLP}}$  were generally small during periods of high water availability but became pronounced during the long and dry summer season. At the end of the summer, *Phillyrea* lost turgor and reached stomatal closure, while *Pistacia* and *Quercus* maintained positive turgor and continued to transpire, albeit at a low rate. Despite the lower  $\Psi_{\text{min}}$ , hydraulic safety margins were wider in *Phillyrea* than in *Pistacia* and *Quercus*. Notably, *Pistacia* and *Quercus* apparently performed similarly under drought, yet a

much higher fraction of *Quercus* individuals was found dead at the site compared with *Pistacia*; no dead *Phillyrea* trees were observed.

### Drought response vs drought resistance strategy

Ecophysiological and morphological traits related to higher resistance to drought include low  $\Psi_{\text{TLP}}$  (Bartlett et al. 2012, Maréchaux et al. 2015) and high resistance to embolism (Maherali et al. 2004, Brodribb and Cochard 2009, Delzon et al. 2010, Urli et al. 2013, Anderegg et al. 2016). Without doubt, these attributes are highly significant when trying to characterize species' response to extreme drought as prevailing in the Eastern Mediterranean region. However, we were unable to relate any of these traits to higher resistance to drought across all species.

**Turgor loss point** As hypothesized, the effect of increased water loss from the leaf was counteracted by a considerably

Table 1. Summary of water potential, stomatal behavior, hydraulics and hydraulic safety margins for *Phillyrea*, *Pistacia*, and *Quercus*. Parameters for hydraulics and safety margins apply to measurements after open vessel correction

Topic	Variable	<i>n</i>	<i>Phillyrea</i> <sup>4</sup>	<i>Pistacia</i> <sup>4</sup>	<i>Quercus</i> <sup>4</sup>
Water potential <sup>1</sup>	Min $\Psi_{PD}$	5	-8.61 ( $\pm 0.23$ ) b	-3.37 ( $\pm 0.27$ ) a	-3.68 ( $\pm 0.13$ ) a
	$\Psi_{min}$	5	-8.68 ( $\pm 0.22$ ) b	-4.23 ( $\pm 0.15$ ) a	-4.22 ( $\pm 0.14$ ) a
	$\sigma$		0.83 A	0.66 A	0.78 A
Stomatal behavior <sup>2</sup>	$\Psi_{g0}$		-8.81 ( $\pm 0.76$ )	-5.57 ( $\pm 0.71$ )	-5.82 ( $\pm 0.74$ )
	<i>m</i>		0.11 A	0.09 A	0.07 A
Hydraulics <sup>3</sup>	$K_{hmax}$	12–15	0.64 ( $\pm 0.09$ ) b	1.97 ( $\pm 0.22$ ) a	2.12 ( $\pm 0.39$ ) a
	$\Psi_{50}$		-6.38 (-5.18, -10.0)	-3.56 (-2.93, -4.02)	-2.03 (-0.11, -3.04)
	$\Psi_{88}$		-12.98 (-10.41, -26.15)	-5.49 (-4.54, -7.06)	-5.51 (-3.92, -8.15)
Hydraulic safety margins <sup>3</sup>	$\Psi_{min} - \Psi_{50}$		-2.30	-0.67	-2.19
	$\Psi_{g0} - \Psi_{50}$		-2.42	-2.01	-3.79
	$\Psi_{min} - \Psi_{88}$		4.30	1.26	1.29
	$\Psi_{g0} - \Psi_{88}$		4.18	-0.08	-0.31

<sup>1</sup>Water potential: minimum water potential measured in the field at predawn (minimum  $\Psi_{PD}$ , MPa) and midday ( $\Psi_{min}$ , MPa,  $\pm$  standard error); the slope between a fitted line of  $\Psi_{PD}$  vs.  $\Psi_{MD}$  during summer time ( $\sigma$ ).

<sup>2</sup>Stomatal behavior: water potential when stomatal conductance equals 0 ( $\Psi_{g0}$ , MPa) estimated from fitted line of  $\Psi_{PD}$  vs.  $\Psi_{MD}$  during summer ( $\Psi_{g0}$ , MPa,  $\pm$  95% prediction interval); stomatal sensitivity to VPD [*m*, mol m<sup>-2</sup> s<sup>-1</sup> ln(kPa)<sup>-1</sup>].  $\Psi_{g0}$  derived from the relationship of  $\Psi$  and *g*<sub>s</sub> (when defining *g*<sub>0</sub> = 0.01 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>): -9.82, -4.92 and -5.71 MPa for *Phillyrea*, *Pistacia* and *Quercus*, respectively.

<sup>3</sup>Hydraulics: maximum hydraulic conductivity ( $K_{hmax}$ , kg s<sup>-1</sup> MPa<sup>-1</sup> m<sup>-1</sup>,  $\pm$  standard error); water potentials corresponding to 50 and 88% of loss of conductivity ( $\Psi_{50}$  and  $\Psi_{88}$ , MPa, 95% confidence intervals). Hydraulic safety margins are in MPa.

<sup>4</sup>Statistically significant differences (*p*  $\leq$  0.05) between species are marked with non-identical lower-case letters when tested with Tukey's HSD test and with non-identical upper-case letters when tested with ANCOVA.

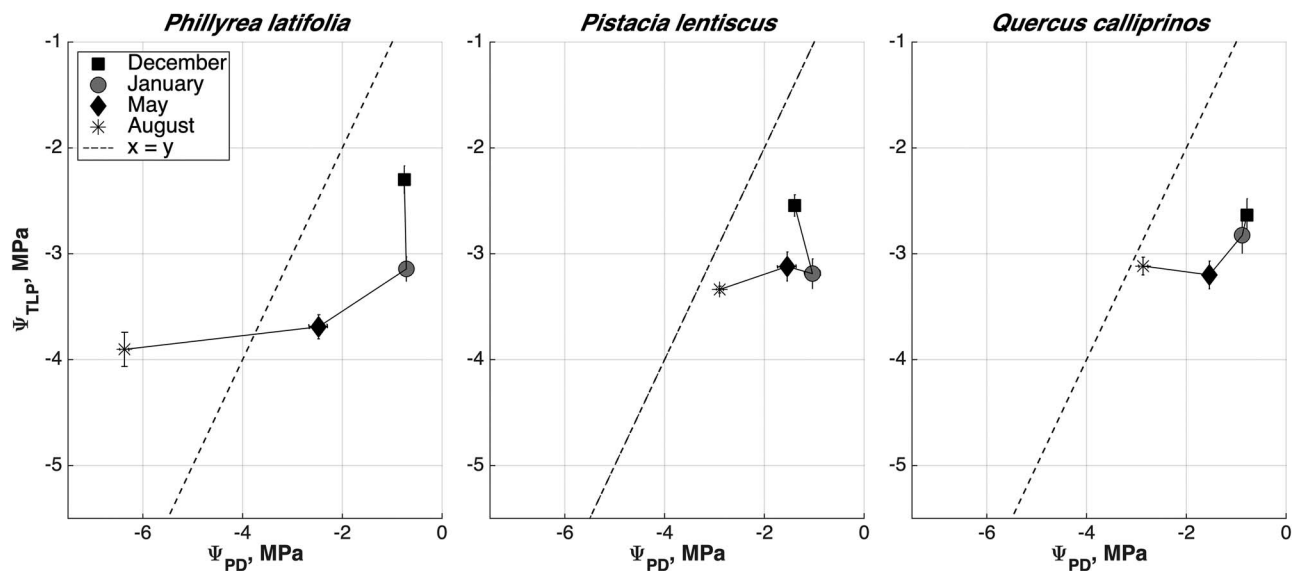


Figure 4. Relationship between predawn water potential ( $\Psi_{PD}$ ) and water potential at turgor loss point ( $\Psi_{TLP}$ ) in *Phillyrea*, *Pistacia* and *Quercus* from December 2012 to August 2013. Error bars indicate one standard error of the mean, *n* = 4–6 individuals per species and per season. The dashed lines indicate  $\Psi_{TLP} = \Psi_{PD}$ .

greater decrease in  $\Psi_{TLP}$  in *Phillyrea* than in *Quercus* and *Pistacia*. However, summer  $\Psi_{TLP}$  in *Phillyrea*, which was extremely low on a global scale (Bartlett et al. 2012), was exceeded by  $\Psi$  in August. Although  $\Psi_{TLP} < \Psi$  has been recently interpreted as a methodological artifact of the established protocol for pressure–volume analyses (Meinzer et al. 2014), this finding supports

our interpretation of an anisohydric strategy for *Phillyrea*. When summer drought ended  $\Psi$  returned to normal winter levels, showing that even passing  $\Psi_{TLP}$  did not cause permanent damage to the leaf tissue. Negative turgor pressure has been shown to form in the mesophyll of species with small cells, permitting plants to endure negative values of  $\Psi$  with relatively little water

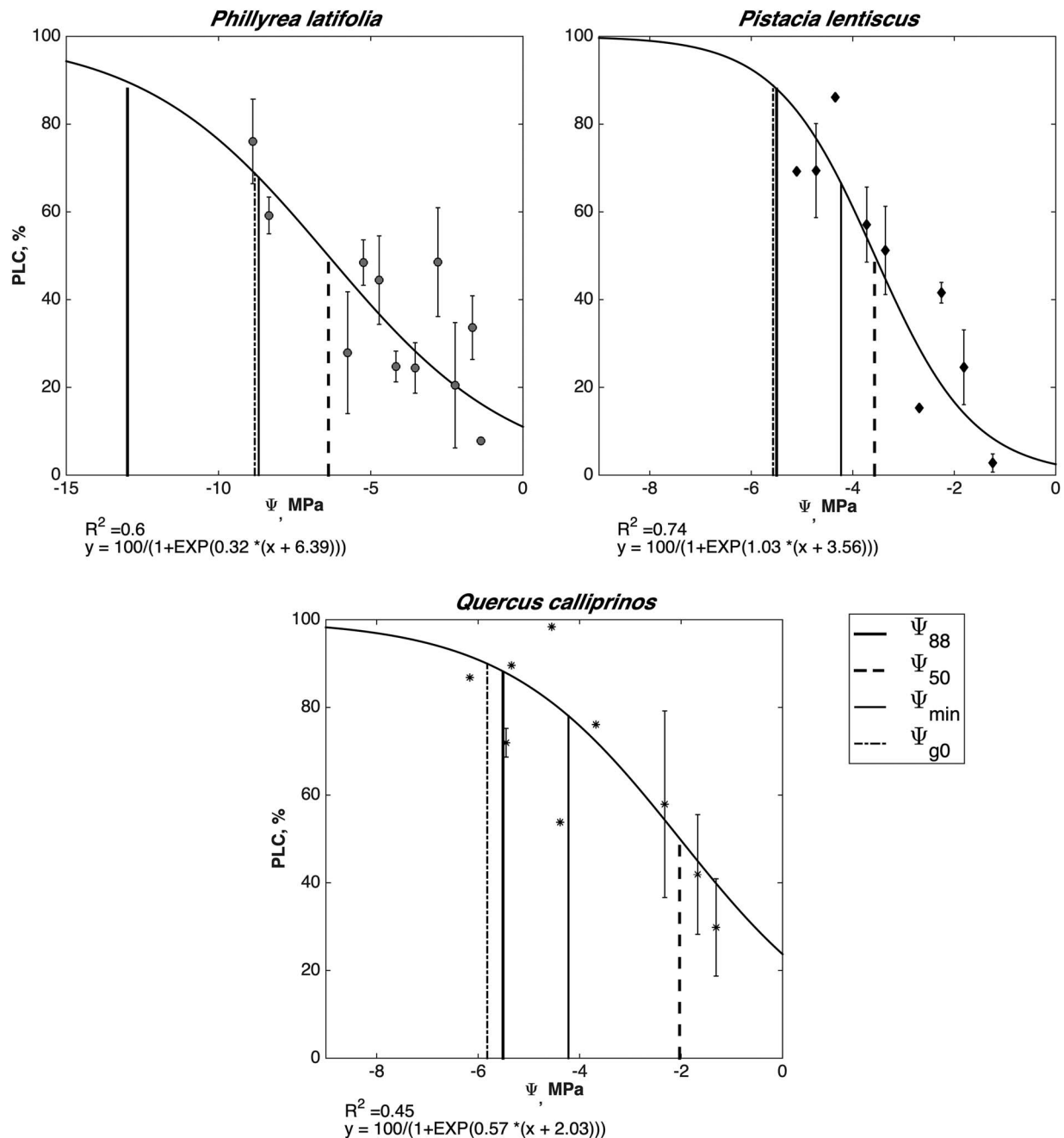


Figure 5. Relationship of water potential ( $\Psi$ ) and percent loss of hydraulic conductivity (PLC) in *Phillyrea*, *Pistacia* and *Quercus* when open vessel correction has been applied. Solid, thick line represents  $\Psi$  corresponding to 88% loss of conductivity ( $\Psi_{88}$ ); dashed, thick line is  $\Psi$  corresponding to 50% loss of conductivity ( $\Psi_{50}$ ); solid, thin line is the minimum  $\Psi$  measured in the field ( $\Psi_{\min}$ ); and dashed, thin line is  $\Psi$  at total stomatal closure ( $\Psi_{g0}$ ). Error bars indicate one standard error of the mean,  $n = 1\text{--}6$  observations per 0.5 MPa bins.

loss (Ding et al. 2014, Yang et al. 2017). This may contribute to the survival of *Phillyrea* under summer drought, but is not captured by the traditional pressure–volume curve analysis, and should be considered as a possible source of error. Maintenance of turgor pressure is important to leaf function (Blackman and Overall 2001, Roberts and Oparka 2003), and stomatal closure has been reported to correlate with  $\Psi_{\text{TLP}}$  (Hinckley et al. 1983, Brodribb and Holbrook 2003). Indeed, stomata appeared to

be sensitive to turgor loss in all three species. In *Pistacia* and *Quercus*, it was manifested by the synchronization of  $\Psi_{\text{PD}}$  and  $\Psi_{\text{TLP}}$  during drought conditions, while in *Phillyrea*,  $g_s$  decreased considerably once  $\Psi$  dropped below  $\Psi_{\text{TLP}}$ . A plant's ability to decrease  $\Psi_{\text{TLP}}$  under drought has been suggested a functional trait for determining drought resistance (Sack et al. 2003, Bartlett et al. 2012, 2014), but this was not supported by our study. *Pistacia* and *Quercus* exhibited similar values of  $\Psi_{\text{TLP}}$ , but

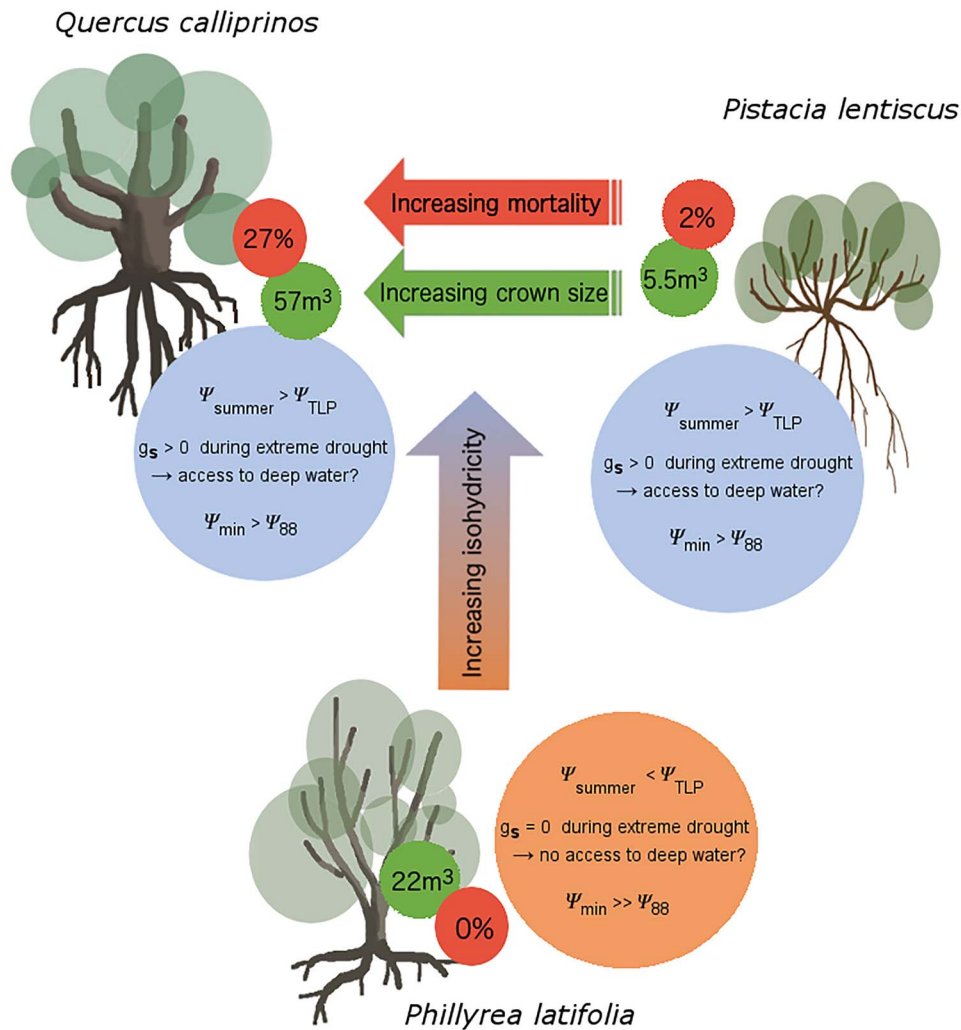


Figure 6. Synthesis of the factors affecting differential mortality in *Phillyrea*, *Pistacia* and *Quercus*.

different levels of vulnerability to drought in terms of mortality. Low  $\Psi_{TLP}$  itself may not be a measure of drought resistance of these species, but rather a manifestation of drought resistance strategy (Levitt 1980).

**Resistance to embolism** Methodological challenges related to measuring PLC in long-vesselled species are well acknowledged in the literature (Wheeler et al. 2013, Cochard et al. 2013, Torrez-Ruiz et al. 2014, David-Schwartz et al. 2016). In this study, we suggested that the bench dehydration method may be susceptible to bias caused by open vessels, and introduced a simple method for correcting the error in PLC curves caused by it. The results clearly indicate an underestimation of the level of xylem embolism, if no correction is applied to cases of significant conductivity due to open vessels in the sample. Yet, there are potential sources of error of the open vessel correction, including the possibility of air flow passing from one vessel element to another through pits, which would lead to an overestimation of  $K_{open}$  and to non-constant  $K_{open}$  for  $K_{hinit}$  and

$K_{hmax}$  (see a more thorough discussion in Supplementary Data available at *Tree Physiology Online*, pp 7–10).

The species studied here seemed to undergo high levels of loss of hydraulic conductivity on a regular basis during the summer season. The measured  $\Psi_{min}$  corresponded to 67% of loss of conductivity in *Phillyrea*, 66% in *Pistacia* and 77% in *Quercus* (as estimated from the PLC - curves). However, the high PLC values were not fatal, as  $\Psi$  had recovered to levels typical to the wet season when re-assessed after the rains started, indicating that  $\Psi_{88}$  might be a better threshold for hydraulic failure in angiosperms than  $\Psi_{50}$ , as recently suggested (Choat et al. 2012, 2018, Delzon and Cochard 2014, and Urli et al. 2013). The low  $\Psi_{88}$  values and associated large hydraulic safety margins indicate high resistance to embolism for *Phillyrea* (Martínez-Vilalta et al. 2002). Contrary to the hypothesis of McDowell et al. (2008), *Pistacia* and *Quercus*, the more isohydric species, were operating closer to hydraulic failure. Greater safety margins have been related to tree survival under drought (Plaut et al. 2012, Nardini et al. 2016), and while

this may apply to *Phillyrea*, it fails to explain the difference in mortality between *Quercus* and *Pistacia*.

### Gas exchange in relation to drought resistance strategy

Stomatal behavior in regulating  $\Psi$  has been the basis of the classification to isohydric and anisohydric strategies (Tardieu and Simonneau 1998). The high seasonal variability in  $\Psi$  confirms the more anisohydric strategy of *Phillyrea* as compared with *Pistacia* and *Quercus*. Gas exchange, and especially  $g_s$ , was very responsive to instantaneous  $\Psi$ , as shown by Abrams and Kubiske (1990) and Baquedano and Castillo (2006). The concurrent decrease in  $g_s$  and  $\Psi$  was less steep for *Phillyrea* than for *Quercus* and *Pistacia* (a decline of 75% in  $g_s$  over 3 MPa vs 1 MPa drop in  $\Psi$ ), again indicating an anisohydric strategy of *Phillyrea* (McDowell et al. 2008). However, the seasonal patterns of gas exchange and the observed complete stomatal closure under extreme drought conditions in *Phillyrea* emphasize the fact that species with different drought resistance strategies and rooting depth operate at different temporal scales of  $\Psi$  (Martínez-Vilalta and Garcia-Forner 2017, Garcia-Forner et al. 2017). This contradicts the classic view of anisohydric species being able to maintain  $g_s$ , and hence,  $A_n$ , for longer periods than isohydric species. Similar results were recently obtained by Garcia-Forner et al. (2017) for *Phillyrea* and *Q. ilex* in an experimental setup with large saplings.

### Tree mortality

Deep rooting has been related to higher survival under drought conditions (Nardini et al. 2016, Johnson et al. 2018). Even though estimating rooting depth was beyond the scope of this study, we found water-use patterns suggesting greater access of *Pistacia* and *Quercus* to deeper water reserves compared with *Phillyrea*. At the end of the dry season, when volumetric soil moisture at the site typically falls below 10% (0–20 cm; J.M.Grünzweig, unpublished results), *Phillyrea* reached stomatal closure, implying the depletion of water reserves available in its root zone (Mitchell et al. 2013). At the same time, *Pistacia* and *Quercus* still exhibited partial stomatal opening, as indicated by leaf gas exchange and lower  $\Psi_{MD}$  than  $\Psi_{PD}$ . Potentially greater rooting depth allowing enhanced access to deep water, in addition to higher  $K_{hmax}$ , may explain higher summertime  $\Psi$  enabling higher rates of  $E$  and  $g_s$  in *Pistacia* and *Quercus* as compared with *Phillyrea* (Schiller et al. 2003, Bhaskar and Ackerly 2006, David et al. 2007, Bucci et al. 2009, Pivovarov et al. 2016). Although there are reports indicating deeper rooting patterns for *Phillyrea*, *Pistacia* and *Quercus* species (Alessio et al. 2004, Mereu et al. 2009, Schiller et al. 2010, Barbata et al. 2015), the rooting depth of our co-existing species has not been directly compared so far.

Greater access to deep water reserves may contribute to tree survival under short-term drought (West et al. 2012, Barbata et al. 2015, Johnson et al. 2018). However, such a dependency

on deeper water reserves may be considered a risk under a changing climate, when precipitation amounts and patterns shift, potentially reducing summertime levels of deep water resources (Moutahir et al. 2016). Precipitation was below average during most of the decade preceding our mortality survey, which could have lowered the deep water reserves, thus affecting species with greater dependency on deep water and explaining the observed higher mortality in *Quercus* than in *Phillyrea*. Similarly, Barbata et al. (2015) concluded that extreme drought reduced the reserves of deep water in Mediterranean woodlands and resulted in enhanced defoliation in *Q. ilex*. The discrepancy in mortality between *Pistacia* and *Quercus* may be the large difference in size of the two species (Figure 6), as larger trees tended to be more vulnerable to drought than smaller ones (Bennett et al. 2015). *Quercus* individuals had mean crown volumes 10 times larger than *Pistacia* individuals, and thus, water consumption should be considerably higher in *Quercus*.

### Conclusions

The observed physiological traits of species exhibiting different levels of mortality indicated drought resistance strategies rather than actual drought stress experienced by the plants. *Phillyrea* adapted a more anisohydric drought resistance strategy, with continued transpiration until the loss of leaf turgor and subsequent stomatal closure, while *Pistacia* and *Quercus* exhibited a more isohydric strategy and probably relied on deeper water reserves. Despite assessing a large number of physiological traits, none of them could be directly related to tree mortality. Instead, we found that drought resistance strategy was not necessarily linked to tree survival, as *Quercus* displayed higher mortality than *Pistacia*, despite similar physiological trait values. Thus, similar levels of drought resistance in terms of survival can be achieved via different drought resistance strategies among co-existing species. Conversely, similar resistance strategies in terms of isohydricity can lead to different levels of vulnerability to drought.

### Supplementary Data

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

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### Conflict of interest

The authors declare no conflicts of interest.

## Authors' contributions

P.J.V., Y.O. and J.M.G. designed the study; P.J.V. collected the data; P.J.V. and Y.O. analyzed the data; S.C. designed the open vessel correction method; P.J.V. and J.M.G. wrote the first drafts of the manuscript, and all authors contributed to the final version of the manuscript.

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