Xylem anatomical traits reveal different strategies of two Mediterranean oaks to cope with drought and warming

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Performance and survival of Mediterranean trees depend on their capacity to cope with water stress. In harsh environments, xylem growth strategies are critical to ensure efficient water transport, though these are largely unknown for many species, including most Mediterranean oaks. We investigated xylem anatomical traits in tree-ring series of deciduous Quercus ithaburensis and Q. boissieri in the South-Eastern Mediterranean Basin to assess xylem response to climate variability, with special attention to dry and warm years and to temperature increase over the last decades. Abundant precipitation and low temperature during the wet season (November–April) benefited xylem formation of both species. However, intra-ring analysis revealed different patterns, as the size of early-formed vessels in Q. boissieri was related to previous-year ring, while in Q. ithaburensis it was strongly affected by current wet season conditions. During dry years, vessel size and number in Q. ithaburensis were strongly reduced, but they fully recovered in the following year (high resilience). Conversely, Q. boissieri vessels were barely reduced during dry or warm years (high resistance). Different strategies allow both species to overcome dry and warm years, but their capacity to withstand long-term warming seems to differ. Q. ithaburensis xylem traits did not evidence any trend over the last few decades, while Q. boissieri was facing a decline in some traits. Our analysis suggests that the growth strategies of Q. ithaburensis would be effective under future drier conditions, while temperature increase could induce a hydraulic deterioration and a potential decline in the less fit Q. boissieri.

\section*{1. Introduction}

As a global warming hotspot, the Mediterranean basin will likely be affected by increased temperature, reduced precipitation, and enhanced frequency of extremely dry spells (Giorgi and Lionello, 2008; IPCC, 2014). This could have dramatic consequences on terrestrial biomes and on the survival and distribution of tree species (Petit et al., 2005; Matesanz and Valladares, 2014). As migration and genetic adaptation are relatively slow processes for long-living organisms like trees, morphological and physiological adjustment would be critical to cope with a dryer climate (Sultan, 2000; Bussotti et al., 2014; Lloret et al., 2016). Given the importance of this, a large research effort has been made to elucidate Mediterranean species structural traits and adaptive strategies to withstand drought (Lo Gullo and Salles, 1988; Nardini and Pitt, 1999; Ogaya et al., 2011; Matias et al., 2012). Currently, most attention has been paid to short-term physiological and metabolic processes that determine plant capacity to overcome summer drought (David et al., 2007; McDowell et al., 2008; Quero et al., 2011; Niinemets and Keenan, 2014), especially at the leaf level. However, xylem hydraulic properties also play a role in the survival or mortality of plants during drought (Martínez-Vilalta et al., 2002; Limousin et al., 2012). Additional knowledge on xylem formation mechanisms, including strategies to deal with climate variations and extreme events, is therefore required to assess how climate change will affect Mediterranean species. Trees unable to produce safe and efficient conductive tissues under altered environmental conditions will experience hydraulic deterioration and a consequent physiological decline, which could trigger mortality (Levanic et al., 2011; Camarero et al., 2015). Indeed, hydraulic system failure has been indicated as a major cause of tree species dieback in the Mediterranean area (Nardini et al., 2014; Pellizzari et al., 2016).

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Different genera, species within a genus, and ecotypes within a species, have distinctive xylem anatomical traits adapted to prevailing environmental conditions, which influence their ecological amplitude (Baas and Carlquist, 1985; Fahn et al., 1986). The genus Quercus comprises many species with specific morphological and physiological traits, including very different xylem structure (Tessier et al., 1994; Nardini and Tyree, 1999). Some species present drought-adaptive traits to survive at the dry limit of broadleaved trees in the Mediterranean area. However, while Mediterranean oaks will likely replace less drought-tolerant species in most of Central and Southern Europe under warming climate (Hanewinkel et al., 2013), a drastic reduction in productivity and distribution in some dry Mediterranean regions (Di Filippo et al., 2010; Bussotti et al., 2015; Natalini et al., 2016) raises a doubt on Quercus species capacity to cope with warming at the dry limit of their natural distribution.

To elucidate how oaks respond to climate, an increasing number of studies have investigated secondary growth and xylem anatomy of different species in the Mediterranean region (e.g., Cherubini et al., 2003; García-Gonzalez and Fonti, 2006; Gea-Izquierdo et al., 2012; González-González et al., 2015). However, knowledge on intra-seasonal growth mechanisms, strategies to withstand drought, or current and carry-over xylem response to dry events, is still scant for most oak species (Pérez-de-Lis et al., 2016), especially in the Eastern Mediterranean.

With this study, we aimed at increasing knowledge on Quercus strategies to cope with drought in the Mediterranean area, by investigating xylem response to climatic variability, a critical aspect for assessing the genus vulnerability to climate change. We analyzed xylem anatomical traits along tree-ring series in two deciduous oak species at the south-eastern limit of genus distribution (Dufour-Dror and Ertas, 2004), Q. ithaburensis Deccne. subspecies ithaburensis and Q. boissieri Reut., to test the following hypotheses: (1) being in a water-limited area for tree growth, xylem traits of both species are related to precipitation variability, but the response magnitude and timing depend on the species and local conditions; (2) being naturally adapted to the local environment, they possess – possibly different – strategies to produce efficient vessels during and after dry and warm years; (3) despite neither species evidencing any symptoms of decline, growth rates and some xylem traits have been negatively affected over the last decades by unprecedented climate warming.

2. Material and methods

2.1. Investigated species and study area

Q. ithaburensis Deccne. (hereafter QUIT) is a deciduous oak widely distributed in the East-Mediterranean area. The subspecies ithaburensis (Tabor oak) typically grows in open stands in lowlands up to 500 m a.s.l. between Israel, Jordan and Syria (Dufour-Dror and Ertas, 2004; Schiller et al., 2007; Cooper et al., 2014). Despite being considered winter deciduous, Ne’eman (1993) reports a wide morphological and phenological variability within the species, and Kaplan and Gutman (1999) indicate that a few individuals (10–15%) keep their leaves during winter. The wood is ring- to semi-ring-porous (Fahn et al., 1986). Three study sites were surveyed, at a distance of a few kilometres apart in the Lower Galilee: Alonei Abba (32°73′N, 35°18′E, 140 m a.s.l.); Bet Zarzir (32°73′N, 35°20′E, 180 m a.s.l.); Qiryat Tiv’on (32°71′N, 35°13′E, 140 m a.s.l.). All three sites feature open woodlands with oak Trees 6–8 m tall on flat or gently slopes (< 10%).

Soils are Brown Rendzina (Rendolls and Xerorthents in the American classification, Dan et al., 1972; Singer, 2007), developed over a 0.5–2 m deep Nari layer, i.e. a hard crust developing on top the soft chalk rock during pedogenic processes. Due to the irregular topography of the Nari crust, numerous soil pockets occur up to 1.5 m deep. Through them, tree roots penetrate the rock below which serves as a water reservoir during the summer season (Herr et al., 2000, 2016). Moisture tension at the end of the summer in the Brown Rendzina soil, at 20 cm depth, is around 20–40 bar, enabling roots survival (Singer, 2007). Lower Galilee is classified as Dry subhumid, and mean aridity index in the study sites is 0.61 (UNEP, 1992). High temperature and the typical Mediterranean precipitation regime determine a long dry period between April and October (Fig. 1).

Q. boissieri Reut. (Boissier oak, hereafter QUBO) is a deciduous oak distributed between western Iran, Turkey, Lebanon, and Israel, mainly between 500 and 1200 m a.s.l. (Danin, 1992). The wood is generally ring-porous, sometimes semi-ring-porous (Fahn et al., 1986). All the samples were collected in open woodland in the Golan highlands (Merom Golan, about 60 km from Q. ithaburensis sites; 33°13′N, 35°76′E, 960 m a.s.l.), where 8–10 m tall individuals occur in mixture with other broadleaf species (mainly Q. calliprinos Webb). The land is flat or gently sloping.

![Fig. 1. Walter-Lieth ombrothermic diagrams for Lower Galilee (left), the Q. ithaburensis sample site, and Merom Golan (right), the Q. boissieri sample site. Lower Galilee weather data comes from Tavor Kadoorie climate station, 145 m a.s.l., and Allone Abba–Waldeheim rain station, 170 m a.s.l. Merom Golan data comes from Merom Golan Picman Man climate station, 942 m a.s.l.](image-url)
(< 20%), and soils are Red Mediterranean soils (Rhodoxeralfs in the American classification, Dan et al., 1972) developed over alkaline olivine basalts. Average soil depth is about 1.5 m, with roots throughout the entire profile (Singer, 2007). Although the hydraulic conductivity of the soil is lower than that of the basaltic rocks below it, the ground water availability in the region is not clear (Gvirtzman, 2002). As regards the climate, higher elevation determines slightly cooler and moister conditions compared with Lower Galilee (Fig. 1), with a mean aridity index of 1.14.

2.2. Sample collection and processing

Field surveys were conducted between autumn 2014 and spring 2015. Two 5 mm thick increment cores at 1.3 m above ground were collected from 20 adult trees for each species. After sanding the cores, ring widths were measured to the nearest 0.001 mm using a sliding micrometer stage (“TA” measurement system, Velux Inc., Bloomfield, NY, USA) and the Tellervo dendrochronological analysis package (Brewer, 2014). Cross-dating accuracy of ring-width series was checked using the COFECHA software (Holmes, 1983). For the following anatomical analysis, we selected a subsample of 12 cores (corresponding to 12 trees) for each species, avoiding samples with visible defects.

The cores were divided into 4–5 cm long pieces. Transversal micro-sections (16–18 µm thick) were obtained with a rotary microtome (Leica, Heidelberg, Germany), stained with safranin (1% in distilled water) and fixed on permanent slides with Eukitt (BiOptica, Milan, Italy). Digital images were captured with a light microscope at 20 × magnification (Nikon Eclipse 80 mounted with distortion-free oculars), and stitched together with PTGui software (New House Internet Service B.V., Rotterdam, The Netherlands). The images, 3.5–4.5 mm wide and with 0.417 pixels/µm resolution, were then processed with the image analysis software ROXAS v2.1 (von Arx and Carrer, 2014). Tree-ring borders were visually identified by vessel lumen area patterns, and by marginal parenchyma bands. The software delivered the lumen size and centroid position of each vessel within the dated annual ring. Lower limit for vessel size was set at 2500 µm², a much lower threshold respect to most previous studies on oaks, which just focus on large earlywood vessels (e.g. Garcia-Gonzalez and Fonti, 2006; Gea-Izquierdo et al., 2012; González-González et al., 2015). Vessels below the threshold were not considered, as they were hardly identifiable, and almost negligible for water transport.

To separately analyze vessels formed during different periods of the growing season, they were assigned to different ring parts, based on the relative distance from the earlywood ring border. As vessel distribution apparently differed between the species, two approaches were used: for QUIT, with vessels along the whole ring, rings were virtually divided into three equal-width sectors, named S1, S2, and S3 (Abrantes et al., 2013). For QUBO, which exhibited mostly a ring-porous vessel distribution, vessels with centroid distance from the earlywood ring border < 1.5 d, where d is the diameter of the largest vessel in the ring, were assigned to the first row, R1. Vessels with centroid distance from the border between 1.5 and 3 d were assigned to the second row, R2, while the remaining ones were assigned to a third group, R3 (similarly to González-González et al., 2014) (Fig. 2a and b).

The following xylem anatomical parameters were measured or computed for the whole ring: mean ring width (MRW, mm); number of vessels (Num); maximum vessel lumen area (Max, µm²); mean area of the three largest vessels (Max3, µm²); mean hydraulic diameter (Dh, µm), automatically calculated by ROXAS according to the Hagen-Poiseuille equation as the fourth power of the pore radius (Tyree and Zimmermann, 2013); xylem-specific potential hydraulic conductivity (Ks, kg m⁻¹ Mpa⁻¹ s⁻¹); tree-ring specific hydraulic conductivity (Kr, kg m Mpa⁻¹ s⁻¹), i.e. the sum of hydraulic potential conductivity of all the vessels in a ring (Castagneri et al., 2015; González-González et al., 2015). Moreover, we assessed the maximum vessel lumen area within each sector (S1, S2, and S3, for QUIT) and row (R1, R2, and R3, for QUBO). As Num and Ks depend on the image size under analysis, these values were standardized to a fixed frame with a tangential width of 1 mm (Castagneri et al., 2015; González-González et al., 2015). Significance of differences between the two species traits was tested by t-test, or by Mann-Whitney U test when conditions of parametric tests were not met.

2.3. Anatomical time series

During tree ontogeny, hydraulic-related xylem traits are expected to increase during juvenile phases, and to stabilize when height growth decreases or stops (Anfodillo et al., 2006; Carrer et al., 2015). Similarly, radial growth, expressed as two-dimensional basal area increment, increases during ontogenesis and stabilizes in mature trees (Biondi and Quedan, 2008). Decline of these parameters can thus be considered as an indicator of stress (Duchesne et al., 2003; Levanic et al., 2011; Pellizzari et al., 2016). We assessed potential long-term declines due to climate warming as the parameter’s trends, computed as the slope of local linear regressions fitted to each parameter (Bigler and Bugmann, 2003; Castagneri et al., 2015) over the last 25 years, i.e. the time frame when warming became evident in the region. As MRW, Num and Kr are linear measures of two-dimensional growth (radial growth occurs along the stem circumference), they were scaled from the ring to the area of the corresponding annulus (Biondi and Quedan, 2008).

To investigate inter-annual variability of xylem traits we: (1) removed the size/age trends (Carrer et al., 2015) by filtering all anatomical series with a cubic smoothing spline function with 50% frequency cut-off of 50 years; (2) calculated the ratio between the observed and fitted values for each annual ring to obtain detrended series (Cook and Kairiukstis, 1990) and (3) averaged the detrended series of 12 trees by bi-weight robust mean to build the mean anatomical chronologies, using R package dpdIR (Bunn, 2008). These were finally truncated at a minimum sample size of five series, resulting in a time span covering 1941–2013 for QUIT, and 1974–2013 for QUBO. The correlation matrix of the anatomical chronologies of each species was built to evaluate common patterns in the inter-annual variability of anatomical parameters and any potential carry-over effect on the following year parameters.

2.4. Assessing climate influence on xylem traits

The influence of inter-annual climatic variability on QUIT and QUBO xylem traits was assessed through bootstrap correlation analysis using the R package bootRes (Zang and Biondi, 2013). The 10 anatomical chronologies obtained for each species were contrasted with the corresponding time series of monthly, bi-monthly, and wet season (November–April) mean temperature and precipitation sum. To account for the joint effect of temperature and precipitation, we also tested correlation with 1-month and 6-month Standardized Precipitation Evapotranspiration Index (SPEI) (see Supplementary Table 1). As these indices did not provide any more robust or detailed information, we performed successive analyses just on temperature and precipitation.

For the climate variables showing the highest correlation with each anatomical parameter, we tested for the linearity of the relationship by fitting a second-order polynomial function (D’Arrigo et al., 2004). Nonlinear and linear models were compared based on the adjusted coefficient of determination (R² adj) and Akaike Information Criterion (AIC).
Finally, for the same climate variables we selected dry and warm years (respectively, precipitation below and temperature above one standard deviation from the mean in the study period), and assessed for each anatomical parameter: (1) the resistance, as the ratio between the anatomical parameter during and before the selected year, and (2) the resilience, i.e. the capacity to recover in the following year and return to the preceding years levels, as the ratio between the anatomical parameter after and before the selected year (Lloret et al., 2011). The antecedent condition was assessed as the prior 3-years average with respect to the selected year (Pretzsch et al., 2013), and significant deviations from this period were tested by Mann-Whitney U test. Resistance and resilience of each anatomical parameter were assessed as the mean response of 12 trees in all selected years.

3. Results

3.1. Xylem traits of Q. ithaburensis and Q. boissieri

The high-resolution images were used to quantitatively assess size and distribution within the ring of all the vessels larger than 2500 μm²; we measured a total of 29,352 vessels for QUIT and 21,341 for QUBO. QUBO trees were younger than QUIT ones, had wider rings and larger vessels (Table 1). In both the species, larger
vessels were confined to the early part of the ring. However, in QUIT we observed a smooth and monotonous shrinking of vessel size along the ring, with conduit elements present within the whole ring (semi-ring-porosity) (Fig. 2c and d). On the contrary, QUBO featured typical ring porosity, with most vessels confined to the very first part of the ring, a step-like decrease in vessel size thereafter, and few detectable vessels in the second half of the ring.

Within this general pattern, vessel size, number and distribution showed evident inter-annual variability (Fig. 2e and f). In both the species, MRW and Num were strongly correlated to each other, as well as Max, Max3 and Dh (Fig. 3). High correlation between Max, Max3, and S1 (QUIT) or R1 (QUBO), indicated that the largest vessels always occurred in the first part of the ring. S1, S2 and S3 were strongly correlated to each other, while R1, R2 and R3 were less associated. Kr was positively associated to both vessel size and Num, while Ks was strongly negatively associated to MRW and Num, particularly in QUBO. This was probably due to Ks formulation, as it decreases with increasing xylem unit area. In QUIT, a moderate negative correlation occurred only between S3 and some parameters in the following year (Fig. 3). In QUBO, a strong carry-over effect occurred for R2, Num, MRW, and mainly Kr, with respect to Max, Max3, Dh, R1, and Kr in the following year.

As regards long-term trends, just 1–2 QUIT trees showed a significant decline in some parameters over the last 25 years, whereas more than half of QUBO trees showed significant negative trends in radial growth and R2 (Figs. 4 and 5).

### 3.2. Relationships between inter-annual climate variability and xylem traits

In general, wet season (November to April) precipitation was positively related to ring width, vessel number and size in both species, while temperature showed a negative relation (Fig. 6). Climate conditions in the previous- and current-year summer were scarcely associated to xylem structure (Supplementary Table 1). Within this general pattern, the response of some xylem traits to intra-seasonal climate variability evidenced remarkable differences between the two oaks.

![Cross correlations between the anatomical parameters of the two species within the same (above) or with the following (below) year.](image)

**Fig. 3.** Cross correlations between the anatomical parameters of the two species within the same (above) or with the following (below) year. For parameter abbreviations, see Table 1. Significant correlation values are coded according to the chromatic scale in the key.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Q. ithaburensis</th>
<th>Q. boissieri</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rings</td>
<td>(n)</td>
<td>67 ± 14</td>
<td>41 ± 4</td>
</tr>
<tr>
<td>Max</td>
<td>(µm²)</td>
<td>53166 ± 7675</td>
<td>68161 ± 8386</td>
</tr>
<tr>
<td>Max3</td>
<td>(µm²)</td>
<td>46547 ± 6955</td>
<td>59701 ± 7283</td>
</tr>
<tr>
<td>Dh</td>
<td>(µm)</td>
<td>207 ± 15</td>
<td>243 ± 18</td>
</tr>
<tr>
<td>S1–R1</td>
<td>(µm²)</td>
<td>51303 ± 8015</td>
<td>68121 ± 8411</td>
</tr>
<tr>
<td>S2–R2</td>
<td>(µm²)</td>
<td>31587 ± 6315</td>
<td>35626 ± 8435</td>
</tr>
<tr>
<td>S3–R3</td>
<td>(µm²)</td>
<td>14623 ± 3360</td>
<td>18879 ± 5060</td>
</tr>
<tr>
<td>Num</td>
<td>(n)</td>
<td>11.5 ± 4</td>
<td>11.4 ± 4</td>
</tr>
<tr>
<td>MRW</td>
<td>(mm)</td>
<td>1.64 ± 0.68</td>
<td>3.28 ± 1.40</td>
</tr>
<tr>
<td>Kr</td>
<td>(kg m Mpa⁻¹ s⁻¹)</td>
<td>2.7 × 10⁻¹⁰ ± 9.1 × 10⁻¹²</td>
<td>3.8 × 10⁻¹¹ ± 9.0 × 10⁻¹²</td>
</tr>
<tr>
<td>Ks</td>
<td>(kg m⁻¹ Mpa⁻¹ s⁻¹)</td>
<td>1.9 × 10⁻⁹ ± 4.1 × 10⁻⁷</td>
<td>1.4 × 10⁻⁹ ± 5.7 × 10⁻⁷</td>
</tr>
</tbody>
</table>

**Table 1.** Mean (±standard deviation) anatomical parameters of Q. ithaburensis and Q. boissieri, and P value of the t test. Rings is the number of tree-rings per tree, Max is the maximum vessel lumen area in the ring, Max3 is the mean area of the three largest vessels. Dh is the mean hydraulic diameter. S1, S2, and S3 for QUIT, and R1, R2, and R3 for QUBO, are the maximum vessel lumen area within each ring sector (QUIT) or row (QUBO). Num is the number of vessels, MRW is ring width, Ks is the xylem-specific potential hydraulic conductivity, Kr is the tree-ring specific hydraulic conductivity. Note that Num and Ks have been standardized to a fixed frame with a tangential width of 1 mm.
during early and correlations. and P (For precipitation November precipitation there Fig. 4. Number of Q. ithaburensis and Q. boissieri trees presenting significant (at P < 0.05) increasing or declining trends in the anatomical series over the last 25 years (1989–2013). Number of trees is coded according to the chromatic scale in the key. For anatomical parameter abbreviations, see Table 1.

In QUIT, MRW and Num were strongly positively related to precipitation during the wet season, particularly during the rainiest months, November, December and January. Max, Max3, and Dh were also positively related to precipitation, especially during late winter. Along the ring, S1 was strongly related to November–April precipitation, while S2 was mainly related to precipitation between February and April, and not to autumn and early winter rain. Kr was associated to precipitation in both early and late wet season, while Ks had opposite and weaker correlations. Wet season temperature, particularly during spring-time, was negatively associated to most parameters.

In QUBO, MRW and Num were positively related to wet season precipitation, although not as strongly as in QUIT (Fig. 6). However, there was also a positive association with May and June precipitation, which occasionally occurs in the Golan region. Max, Max3, Dh and R1 were not or scarcely related to precipitation, while the size of the following vessels (R2 and R3) was related to February–March and May–June precipitation. Max, Max3, Dh and R1 were negatively associated to early winter temperature, while R2, R3, Num, MRW and Kr to February and March temperature. Similarly to QUIT, Ks showed moderate but opposite correlation patterns compared to the other parameters.

3.3. Non-linear response to climate

Comparison between first-order and second-order regression models showed that several xylem anatomical traits in QUIT did not have a linear relation with precipitation (particularly Num, MRW and Kr) or temperature (Table 2). In almost all cases, the linear and quadratic parameters had the opposite sign (Supplementary Table 2), i.e. the benefit of increasing precipitation (or decreasing temperature) tends to decrease for very high (or low) values. As an example, when years with wet season precipitation above 800 mm were removed, the performance of the model for Kr strongly increased (Fig. 7). Clearly, precipitation above 800 mm did not benefit Kr (and other parameters), which levelled off with precipitation around 700 mm. Conversely, most parameters in QUBO had a linear relationship with climate parameters, and second-order models performed slightly better than linear models only for Num-precipitation and MRW-temperature.

3.4. Resistance and resilience to drought

Low precipitation during the months that mostly influenced anatomical parameters (see Fig. 6) caused significant reduction in QUIT vessel size (Fig. 8). Num, MRW and Kr were even more affected, decreasing by about 40% during dry years. High temperatures also affected xylem traits, but to a lesser degree. Remarkably, in the following year, all these parameters recovered, returning to previous values. Conversely, in QUBO, warm and dry years caused just moderate (mostly non-significant) reduction in

Fig. 4. Number of Q. ithaburensis and Q. boissieri trees presenting significant (at P < 0.05) increasing or declining trends in the anatomical series over the last 25 years (1989–2013). Number of trees is coded according to the chromatic scale in the key. For anatomical parameter abbreviations, see Table 1.

Fig. 5. Temporal variability of vessel size in Q. ithaburensis and Q. boissieri calculated as the average of 12 trees per species (upper graphs). Dark green line represents the area of the largest vessel in the first sector (S1, Q. ithaburensis) or first row (R1, Q. boissieri), light green line the largest vessel in the second sector (S2) or second row (R2), mint green line the largest vessel in the third sector (S3) or after the second row (R3). Below, wet season (November–April) precipitation sum (blue) and mean temperature (red) in Lower Galilee (left) and in Merom Golan (right) during the common period 1974–2013. Regression line and p value are reported for significant trends during the period 1989–2013. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
most parameters, and only MRW and Ks were strongly affected by reduced precipitation. Notably, these two parameters did not fully recover in the subsequent year.

4. Discussion

4.1. Climate influence on oak’s xylem formation—similarities and differences

Among Quercus species, xylem structure presents a remarkable high variability. Deciduous QUBO and QUIT are both described as ring- to semi-ring-porous species (Fahn et al., 1986), but we observed different tree-ring structure (Fig. 2). Ring-porous xylem in QUBO can be profitable under a Mediterranean climate, as large earlywood vessels guarantee high water transport in the first part of the season, when water is usually still abundant and evaportranspiration low (Fahn et al., 1986). However, semi-ring-porous xylem of QUIT is probably safer in dry lowlands, as large earlywood vessels operate with narrow hydraulic safety margins, being more prone to hydraulic failure in case of water stress (Choat et al., 2012).

Wood anatomy provided some evidence of oak’s cambial phenology. Information retrieved from long-term wood anatomical time series has been proved to match short-term cambial activity observations in Mediterranean trees (Pacheco et al., 2016). Significant correlations between xylem traits and climate stopped in April to May for QUIT (Fig. 6), suggesting the end of the xylem growing phase before the summer dry period (Fahn, 1953). Significant correlations with May-June precipitation indicate that QUBO, being at higher elevation, experiences a longer growing season, at least in favorable years with late-spring precipitation. The absence of correlations in summer and autumn suggest the lack of cambial reactivation after the dry season rest for both species (Kaplan and Gutman, 1999).

Concerning the climate influence on xylem structure, previous-year summer temperature did not affect any xylem trait, suggesting that extremely dry summer conditions do not stress the species. Conversely, winter and spring precipitations showed a strong positive influence on vessel number and size in both species. Modifications of xylem anatomical traits according to water availability have been observed in both adult trees in natural environments (e.g., Corcuera et al., 2004; Alla and Camarero, 2012; but see Limousin et al., 2010) and saplings under controlled conditions (e.g., Fonti et al., 2013; Cao et al., 2014). Indeed, water availability can directly affect final vessel size determining the cell turgor pressure necessary to stretch the primary cell wall of the forming cells (Taiz and Zeiger, 2006).

For QUIT, low precipitation throughout the wet season led to a strong reduction in most xylem traits. However, very high precipitation (above 800 mm) did not stimulate any evident increase (Table 2, Fig. 7). This likely suggests an adaptive strategy to a dry environment: a threshold response in xylem structure to overly high water availability would prevent the production of very wide vessels, reducing the risk of embolism during late spring and summer (Cochard and Tyree, 1990). Nonetheless, we cannot exclude that surplus rain could be used for other physiological processes (e.g. root growth, fruit production, non-structural carbon accumulation, etc.).

![Fig. 6. Correlations between anatomical parameters in Q. ithaburensis and Q. boissieri with bi-monthly and seasonal (November–April) precipitation sums (above) and temperature means (below). For parameter abbreviations, see Table 1. Significant correlation values are coded according to the chromatic scale in the key.](image-url)
Intra-ring analyses of xylem traits revealed that, in a first phase, QUIT produces large vessels, i.e. the most important for water transport (Tyree et al., 1994), using groundwater accumulated during the wet season. The following vessels are not related to autumn and early winter precipitation, but to the rainfalls between February and April. When dry winters occur, and the first formed vessels are reduced, this species can take advantage of late wet season rain for building fairly large vessels that can substantially contribute to water transport.

In QUBO, xylem development processes were apparently different. In line with previous studies on ring-porous oaks, which indicate a strong dependency of earlywood vessels on the wood formed in the previous year (Barbaroux and Breda, 2002; Zweifel et al., 2006; Sass-Klaassen et al., 2011; Pérez-de-Lis et al., 2016), we observed that the first-row vessels were strongly associated to the previous-year xylem traits, in particular to the potential conductivity (Fig. 3). Wet season precipitation, the main constraint for QUIT, had no effect on them. Nonetheless, abundant rain allows QUBO to produce wider rings, increasing the number of large vessels after the first row, while May and June precipitation, when present, can promote the formation of further vessels.

The negative influence of precipitation on the potential hydraulic conductivity (Ks) cannot be interpreted as a reduced xylem efficiency during rainy years. Indeed, as ring width increases, vessel density and thus Ks decrease (Gea-Izquierdo et al., 2012; Kniesel et al., 2015). Interpretation of Ks patterns in ring-porous species should be cautious (Fahn et al., 1986), as a big part of the ring is not conductive. Indeed, reverse relationship with ring width was more evident in ring-porous QUBO than in semi-ring-porous QUIT.

The negative effect of high temperature on xylem traits of both species can be related to different but not-exclusive causes. (1)
phenology, flowering, acorn ripening (Ne’Eman 1993; Kaplan and Gutman, 1999; Limousin et al., 2012; Bussotti et al., 2015), which can reduce resource availability for xylogenesis.

4.2. Responses to drought and warm events

Tree response to unusual climate events has received increasing attention, as climate extremes, projected to increase in the future (IPCC, 2014), can have dramatic consequences on terrestrial ecosystems (Lloret et al., 2011; Frank et al., 2015). Different tree genera show different growth response to drought events (Pretzsch et al., 2013; Anderegg et al., 2015; Bussotti et al., 2015). However, the effects of extreme events on tree xylem structure are still unknown for most species (Carrer et al., 2016). Our study showed good oak xylem fitness to cope with drought (Corcuera et al., 2004; Kniesel et al., 2015), but evidenced quite different responses in the two species (Fig. 8). Xylem traits in QUBO were severely reduced under dry and, to less extent, warm conditions, but showed a complete recovery in the following year. This supports what was discussed in the previous section: xylem formation mostly depends on wet season climate conditions, with no legacies from the previous year. QUBO response was completely different, being able to build large conduits even after dry winters, while warm conditions had a moderate negative effect only on a few traits. This can be partially explained by the dependency of the first cell rows on previous-year ring. However, besides different growth mechanisms compared to QUIT, the reduced drought sensitivity of QUBO was probably related to local environmental conditions. More abundant precipitations in Golan compared to Lower Galilee prevented depletion of the groundwater reservoir, limiting water stress. Only ring width was severely affected during dry years. In this case, the lower priority of not-conductive tissues compared to vessel formation resulted in higher xylem-specific conductivity.
4.3. Long-term response to climate warming

Remarkable differences between the two species were also observed in the long-term context (Figs. 4 and 5). Despite temperature increase in the last decades, most QUIT trees did not show any evident decline. On the contrary, ring width and second-row vessel size were significantly reduced in many QUBO trees. Age-size trends cannot be completely ruled out to explain the second-row vessel decline, as vessel distribution in rings can vary with age (Evert, 2006; Schweingruber, 2007). However, given the very high sensitivity of this trait to late-winter temperature, the corresponding decline in radial growth and to a lesser extent in other xylem traits, and the timing of the decline matching the warming trend in the region (Fig. 5), we conclude that raised temperature is the most likely cause for the observed pattern. This should be taken carefully into consideration, as decline in growth rates and conduit size can predispose trees to mortality in the successive years or decades (Bigler and Bugmann, 2003; Levanic et al., 2011; Pellizzari et al., 2016).

4.4. Different strategies and prospects under future climate warming

This study provides evidences of different xylem response to drought of two deciduous Mediterranean oaks, suggesting different growth strategies. Unlike QUBO, QUIT does not take advantage of very abundant precipitation, suggesting that it would be less competitive than QUBO in relatively moist Mediterranean regions. Xylem formation in QUIT is strictly related to wet season climate conditions, a strategy typical of Mediterranean species highly adapted to dry conditions (Penuelas et al., 2001; Russotti et al., 2014). Elastic response to precipitation variability allows a full recovery in xylem structure the year after dry events. Effectiveness to cope with short-term droughts, and the absence of decline in vessel size and number in the last decades, suggest that on-going warming and increased drought frequency will not compromise future QUIT performances.

QUBO xylem development partially depends on previous-year conditions. In less stressful conditions compared to QUIT, this strategy allows the species to produce large earlywood vessels even after dry winters. However QUBO, more sensitive to high winter temperature than QUIT, is experiencing a decadal decline in radial growth and some xylem traits. Increased temperature has not yet severely affected ring potential to transport water. However, under future climate, we can expect that consecutive warm years could reduce the size of all vessels formed in the growing season. In the long term, this could affect the tree hydraulic efficiency and, ultimately, its vitality.

Author contribution

DC, LR, EB and MC conceived the analysis. DC and LR collected and prepared the samples. EB provided administrative and logistic support in Israel. DC performed laboratory analyses under MC supervision in Italy. DC analyzed the data. DC and MC wrote the manuscript, LR and EB provided intellectual contribution to it.

DC, the corresponding author, takes responsibility for the integrity of the work as a whole.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.envexpbot.2016.10.009.

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