XYLEM ADJUSTMENT IN *ERICA ARBOREA* TO TEMPERATURE AND MOISTURE AVAILABILITY IN CONTRASTING CLIMATES

G. Gea-Izquierdo1,*, G. Battipaglia1,2, H. Gärtner1 and P. Cherubini1

1Swiss Federal Research Institute WSL, 8903 Birmensdorf, Switzerland
2Second University of Naples, via Vivaldi 43, 81100 Caserta, Italy
*Corresponding author; e-mail: gea.guillermo@inia.es, guigeiz@gmail.com

ABSTRACT

Plasticity of xylem architecture can be a species specific strategy to reduce vulnerability to climate change. To study how the evergreen shrub *Erica arborea* regulates its xylem at different time scales as a response to climatic variability, we compared time series of annual xylem traits such as ring-width (growth), vessel size (hydraulic diameter and mean vessel area), vessel density, and potential conductivity (\(K_h\)) at two sites characterized by contrasting Mediterranean climates in Italy and Spain. Shrubs regulated their xylem in response to major differences in climate by modifying mostly their growth and vessel density. The different adjustment of xylem observed at the two sites was partly explained by the nonlinear nature of the relationship between the studied traits and temperature. Xylem development was mostly limited by low winter temperature at the cold and moist site, where plants produced more vessels per unit area of xylem and reduced growth in comparison with the warm and dry site. The responses of vessel size and density to climate were opposite. Vessel size and \(K_h\) were similar at the two sites and exhibited less sensitivity to climate than vessel density and growth. Humid conditions in spring increased growth and vessel size but decreased vessel density at the cold site, whereas the effect on xylem adjustment of high temperatures during the vegetation period was generally contrary to that of high moisture availability. These results likely express within species adaptations of the hydraulic function and the safety-efficiency trade-off in response to climate. A decline in growth in response to a recent decrease in precipitation at the dry site could be interpreted as a first sign of vulnerability to increasing drought severity. Similar to other species, climate change may have contrasting effects for *E. arborea* at its cold and dry distributional limits.

**Keywords:** Mediterranean, tree rings, climate change, adaptation, limiting factor, drought.

INTRODUCTION

Plants respond simultaneously to changes in environmental conditions through short-term physiological regulation and long-term anatomical acclimation (Mencuccini 2003). Plant growth performance depends partly on xylem plasticity because the water
and carbon balances are closely related to the hydraulic architecture (Tognetti et al. 2001; Breda et al. 2003; Fichot et al. 2011). Leaf water balance, carbon assimilation and stomatal conductance depend on the water supply to foliage, which is also directly linked to the xylem hydraulic architecture (Tyree & Ewers 1991; Hubbard et al. 2001; Breda et al. 2006). Plants adjust their xylem architecture to fit the trade-off between maximizing hydraulic conductivity while preserving safety (Tyree & Zimmermann 2002; Preston et al. 2006; Cai & Tyree 2010; Zanne et al. 2010). Thus xylem plasticity should partly determine plants’ capacity of acclimation to changes in the physical environment (Fonti et al. 2010; Poorter et al. 2010).

According to Liebig’s Law of the Minimum, adjustment of plants’ functional traits will be determined by the limiting factor (Fritts 1976). Ubiquitous plants are ideal to test hypotheses on adaptations of functional traits within species (Liu & Noshiro 2003) because they need to be adapted to withstand a wide range of climatic conditions and thus they need to have plastic physiological and anatomical functionality to optimize their performance under different climates (Kolb & Sperry 1999). The diffuse-porous (i.e., xylem presenting unimodal distribution of vessels [Limousin et al. 2010; Umebayashi et al. 2010]) species Erica arborea L. colonizes a wide range of climates from subtropical maritime sites in Macaronesia to high elevations in Eastern Africa and the Mediterranean Region (Désamoré et al. 2010). This large distributional area includes sites with different limiting factors, ranging from low temperatures and frost in winter to reduced moisture availability in the growing season and mild winters.

In Mediterranean areas plants need to adapt to two annual periods that limit plant development (Mitrakos 1980; Cherubini et al. 2003). Plants need to regulate to withstand embolism produced by winter freeze-thaw events and to low water potential during summer droughts (Sperry et al. 1994; Tognetti et al. 2001; Hacke & Sperry 2001; Taneda & Sperry 2008). Many shrubs in the Mediterranean maquis, including E. arborea, are pioneer species and establish after major disturbances, particularly fire events. If they want to succeed in the race for colonization, plants should exhibit fast growth while regulating their physiology to cope with abiotic stress. Erica arborea has been reported to develop extensive and deep root systems (Gratani & Varone 2004) which may be related to the species’ characteristic lower tolerance to low negative potentials inducing cavitation compared to that of co-occurring more drought-tolerant taxa (Tognetti et al. 2001; Quero et al. 2011).

In this study we analyzed within a species the acclimation of xylem to climatic variability combining tree-ring growth and xylem anatomical characteristics to better interpret the response to climate of two populations of E. arborea growing in contrasting climatic conditions at the limits of the species distribution: one coastal population under a maritime climate on a Mediterranean island and one population at high altitude on a continental Mediterranean mountain range. By using dendrochronological methods we could analyze the response of shrubs to both the short- and long-term variability of climate. Since the study was conducted on contrasting sites across the species distribution range, we aimed to assess whether the effect of climatic factors on the xylem anatomy could be generalized on a regional scale or whether, in contrast, plants modified their xylem architecture differently in response to local environmental conditions.
We hypothesize that *E. arborea* will modify its xylem features constrained by the efficiency-safety trade-off at different spatial (between sites) and temporal (among years) scales; that differences in the xylem adjustment between sites of contrasting climate will be expressed by the existence of non-linearity in the response to climate; and that shrubs will respond to mesic conditions in the studied Mediterranean ecosystems (*i.e.*, either less water stress driven by more abundant precipitation or less temperature stress driven by higher temperatures in winter) by increasing vessel size, conductivity and growth while decreasing vessel density both in the short- and the long-term.

MATERIAL AND METHODS

**Study sites**

The two-largest diameter basal stem cross sections from multi-stem individual *Erica arborea* shrubs were collected at two sites characterized by contrasting climate within the wide distribution area of the species (Table 1). One site was a relatively cold and humid mountain population at 1550 m a.s.l. in Central Spain (Sierra de Madrid, see Table 1 for details on site climatic data). The other site was a warm maritime location at 400 m a.s.l. on Monte Perrone on the Isle of Elba (Italy). In Madrid shrubs were collected from deforested patches of a surrounding forest dominated by *Pinus sylvestris* L. and *Quercus pyrenaica* Willd. currently occupied by scattered shrubland (less than 75 plants/ha of plants with height below 2 m) under full light conditions. On Elba the site was a typical dense maquis, a mixed Mediterranean thermophyllous shrubland dominated by species such as *Erica arborea*, *Arbutus unedo* L., *Quercus ilex* L. and *Phyllirea* spp. with more than 300 plants/ha of canopy height over 4 m where stand interspecific competition was much higher than in Madrid (Table 1; Battipaglia et al. 2010). Both sites had sandy soils coming from acidic siliceous bedrock.

Table 1. Data characteristics.

Ppt = annual precipitation; TminJan-Feb = minimum of the mean minimum temperatures in January and February for the studied period; db = basal diameter; RW = mean ring width; Rbr = mean RW series intercorrelation. Standard deviations are shown between parentheses.

<table>
<thead>
<tr>
<th>Site</th>
<th>Altitude (m)</th>
<th>Ppt (mm)</th>
<th>Tmean (°C)</th>
<th>TminJan-Feb (°C)</th>
<th>Shrubs</th>
<th>Radii #</th>
<th>Period</th>
<th>Length (# years)</th>
<th>Age (years)</th>
<th>db (cm)</th>
<th>RW (mm)</th>
<th>Rbr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Madrid</td>
<td>1550</td>
<td>700</td>
<td>9.4</td>
<td>1.3</td>
<td>5</td>
<td>5</td>
<td>1972–2009</td>
<td>38</td>
<td>37.6 (2.7)</td>
<td>4.5</td>
<td>0.55</td>
<td>0.407 (0.24)</td>
</tr>
<tr>
<td>Elba</td>
<td>460</td>
<td>375</td>
<td>15.3</td>
<td>7.5</td>
<td>5</td>
<td>5</td>
<td>1968–2007</td>
<td>40</td>
<td>38.0 (3.1)</td>
<td>8.5</td>
<td>1.06</td>
<td>0.424 (0.33)</td>
</tr>
</tbody>
</table>

Xylem data processing

Cross sections were polished using sand paper of different grain size and ring width (RW) measurements made with a resolution of 0.01 mm, using LINTAB measurement equipment fitted with a stereoscope and analyzed with TSAP software (Frank Rinn, Heidelberg, Germany). Crossdating was performed visually using standard methods. When macroscopic crossdating was difficult, microsections (see below) were used to
do the visual RW crossdating. Final crossdating was checked with a master *Arbutus unedo* chronology collected at the same site (Battipaglia et al. 2010) on Elba and with a *Quercus pyrenaica* chronology (Gea-Izquierdo et al.: In review) from the nearby forest to the patches in Madrid where we collected the *E. arborea* samples (Fig. 1). Regularly used statistics in dendrochronology were calculated to characterize our data (Table 1 & 2), including the mean sensitivity ($m_{sx}$), which is a measurement of the relative difference from one tree ring to the next and thus the year-to-year variation (Fritts 1976). In addition, to construct chronologies of xylem anatomical characteristics, the longest radius of each cross section was split out to cut microsections (one microsection for each annual ring) of a thickness of 20 µm using a GSL1 sledge microtome, then stained using Safranin and Astrablue, dehydrated with ethanol, immersed with xylol and mounted on slides with Canada Balsam (Gärtner et al. 2001). The microsections were studied under a light microscope, equipped with a photo-microadapter and a camera (see Fig. 2 for an example). The microphotographs taken ring by ring were digitized, calibrated and vessel measurements carried out year by year using WinCELL (Régent

![Figure 1. Mean annual growth chronologies of *Erica arborea* compared with mean growth of (a) *Quercus pyrenaica* in Madrid; (b) *Arbutus unedo* in Elba. $r = $ is the significant correlation between mean RW chronology of ERAB (*Erica arborea*) and QUPY (*Quercus pyrenaica*) or ARUN (*Arbutus unedo*) in Elba and Madrid, respectively.](image-url)
Figure 2. Microsections of two years from one individual in Madrid (upper picture) and another in Elba (bottom picture). See annual boundaries as one or several rows of thicker-walled fibres and smaller vessels. In 1991 in Elba an intra-annual density fluctuation in latewood is evident.
Instruments Inc.). Then we calculated the annual mean vessel area (MVA), the density of vessels (VD), a hydraulically weighted diameter (\(D_h = \frac{\sum n d_5}{\sum n d_4}\) for \(n\) vessels of diameter \(d\) in year \(t\), where \(d\) is calculated as the mean between vessel-lumen width and length) proportional to the Hagen-Poiseuille (HP) conductivity and thus to xylem hydraulic conductivity (Sperry et al. 1994; Fichot et al. 2009), and the theoretical conductivity (\(K_h, \text{kg} \cdot \text{m}^{-1} \cdot \text{s}^{-1} \cdot \text{MPa}^{-1}\)) of the ring in year \(t\) according to the HP equation (Tyree & Ewers 1991): \(K_h = \left(\frac{\pi \rho}{128 \eta A_{\text{image}}}\right) \cdot \sum n d_4^2\), where \(\rho\) is the density of water at 20 ºC (998.2 kg·m\(^{-3}\)), \(\eta\) the viscosity of water at 20 ºC (1.002·10\(^{-9}\) MPa), \(A_{\text{image}}\) the area of the image analyzed of the ring in year \(t\) and \(d_t\) the diameter of one of the \(n\) vessels measured in year \(t\).

Table 2. Bootstrap correlations between residual chronologies of xylem traits (GI\(_{\text{res}}\)) and mean sensitivity of chronologies in the two studied sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Variable</th>
<th>RW</th>
<th>VD</th>
<th>MVA</th>
<th>Dh</th>
<th>Kh</th>
<th>msx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elba</td>
<td>RW</td>
<td>–</td>
<td>-0.544</td>
<td>0.341</td>
<td>0.335</td>
<td>-0.157</td>
<td>0.261</td>
</tr>
<tr>
<td></td>
<td>VD</td>
<td>-0.396</td>
<td>–</td>
<td>-0.488</td>
<td>-0.479</td>
<td>0.419</td>
<td>0.141</td>
</tr>
<tr>
<td></td>
<td>MVA</td>
<td>0.281</td>
<td>-0.200</td>
<td>–</td>
<td>0.944</td>
<td>0.506</td>
<td>0.089</td>
</tr>
<tr>
<td></td>
<td>Dh</td>
<td>0.127</td>
<td>-0.130</td>
<td>0.723</td>
<td>–</td>
<td>0.535</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>Kh</td>
<td>-0.132</td>
<td>0.436</td>
<td>0.596</td>
<td>0.766</td>
<td>–</td>
<td>0.139</td>
</tr>
<tr>
<td></td>
<td>msx</td>
<td>0.218</td>
<td>0.116</td>
<td>0.089</td>
<td>0.043</td>
<td>0.232</td>
<td>–</td>
</tr>
</tbody>
</table>

Meteorological data

On Elba, monthly temperature and precipitation data were obtained from the Portoferraio meteorological station located approximately 10 km from the sites (42° 49' N, 10° 20' E, 25 m a.s.l.). In Madrid climatic data were obtained from the KNMI Climate Explorer (http://climexp.knmi.nl) and AEMET (http://www.aemet.es). On Elba annual average precipitation was 375 mm during the period 1970–2007 and mean annual temperature of 15.3 ºC and in Madrid 700 mm and 9.4 ºC, respectively. Temperatures below zero in winter were common only in Madrid (Table 1; Fig. 3, 4).

Statistical analyses

We compared the mean xylem characteristics (RW, MVA, Dh, VD and Kh) and checked whether there were significant differences between the two sites. This was done by fitting a linear mixed model including a random intercept by individual growth series and a first order autoregressive moving average (ARMA[1,1]) variance-covariance structure (Diggle et al. 2002). Both the long-term variation (including the biological trend and climate in the long-term) and the short-term response of xylem traits to climate...
Figure 3. Long-term response of *Erica arborea* hydraulic conductivity ($K_h$, black solid lines) in relation to maximum annual temperature (°C, grey polygon and thick dashed line) from Madrid (above, a) and Elba (below, b). Thick lines correspond to 25-year spline fits.

Figure 4. Long-term response of *Erica arborea* growth (black solid lines) in relation to annual precipitation (grey polygon and thick dashed line) from Madrid (above, a) and Elba (below, b). Thick lines correspond to 25-year spline fits. A vertical dashed line was plotted in Elba to show apparent start of growth and precipitation declines after 1985.
were studied. To study the long-term response, we compared raw data of xylem variables to climatic data smoothed using cubic splines. To study the short-term response we detrended all xylem traits to remove the age biological trend of xylem traits using individual cubic splines with a 50% frequency cut-off at 40 years (maximum age observed) and used residual chronologies ($G_{Ires}$) in subsequent analyses with climate (Cook & Kairiukstis 1990). Relationships of the short-term response of xylem traits with temperature and precipitation were studied using correlations calculated for the common period 1973–2008. We compared climate data for the hydrological year (from September of the previous year to August of the year of growth), winter (February-March) and the months of May, June and November. We selected these periods because on a preliminary analysis the xylem traits studied exhibited a maximum response to climatic variables during those months/periods, which also reflected differences in phenology between the two sites. Additionally, these periods or months correspond to key periods of plants’ responses to climate in Mediterranean ecosystems, including the effect of winter temperature limitation and overall availability of water in the growing period, which includes spring and fall (Gratani & Varone 2004).

RESULTS

Long-term acclimation of xylem: disentangling age- from climate-related trends

Plants modified the vessel size ($MVA$ and $D_h$) and VD in opposite direction in response to climate (Table 2). Despite higher plant densities, hence competition, and lower annual precipitation, shrubs at Elba exhibited larger RW and similar vessel size but presented xylem with much lower VD compared to Madrid (Table 3; Fig. 5). Nevertheless, as for other species analyzed along climatic gradients (Pourthahmasi et al. 2011; Gea-Izquierdo et al. 2012) shrubs at both sites exhibited similar $K_h$ (Table 3). Also, at both sites we observed an increase in temperature in the last decades simultaneous to the increase in vessel size and conductivity (Fig. 3, 5). At the maritime site (Elba) there was a decline in precipitation since the late 1980s, which seemed to trigger a simultaneous decline in growth (Fig. 4) also evident in the *Arbutus unedo* reference chronology (Fig. 1). Neither a decrease in precipitation nor a steep recent decrease in growth was observed in Madrid.

Table 3. Annual average vessel feature values with standard deviations (SD) in parentheses. $MVA$ = mean vessel area; $RW$ = ring width; $VD$ = density of vessels; $D_h$ = hydraulic diameter; $K_h$ = calculated potential conductivity. Different letters correspond to significant differences between sites at $\alpha = 0.01$ as calculated using a mixed model (see Material and Methods).

<table>
<thead>
<tr>
<th>Site</th>
<th>RW (mm)</th>
<th>VD (mm$^{-2}$)</th>
<th>MVA (10$^3$.mm$^2$)</th>
<th>$D_h$ (mm)</th>
<th>$K_h$ (kg·m$^{-1}$.s$^{-1}$.MPa$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Madrid</td>
<td>0.552 a</td>
<td>137.89 a</td>
<td>0.822 a</td>
<td>0.072 a</td>
<td>4.09 a</td>
</tr>
<tr>
<td></td>
<td>(0.305)</td>
<td>(33.95)</td>
<td>(0.173)</td>
<td>(0.007)</td>
<td>(1.40)</td>
</tr>
<tr>
<td>Elba</td>
<td>1.063 b</td>
<td>88.59 b</td>
<td>0.856 a</td>
<td>0.075 a</td>
<td>3.24 a</td>
</tr>
<tr>
<td></td>
<td>(0.486)</td>
<td>(28.54)</td>
<td>(0.215)</td>
<td>(0.009)</td>
<td>(1.92)</td>
</tr>
</tbody>
</table>
Xylem adjustment to the short-term variability of climate

Chronologies of all traits were correlated and RW had the highest sensitivity (Table 2). Although mean vessel size and conductivity were similar at the two sites, individuals exhibited some inter-annual variability within site in all traits in response to climatic variability. $D_h$ and MVA (vessel size) were very closely related and their sensitivity
to climate was lower than that of the other traits (Table 2; Fig. 6), which seems to be a normal feature also for other species (Pourtahmasi et al. 2011; Gea-Izquierdo et al. 2012). The relationship with climate was complementarily expressed by the different xylem traits (Fig. 6). RW exhibited a significant relationship mostly with precipitation at different periods whereas the vessel traits responded both to different periods of precipitation and temperature (Fig. 6). Shrubs responded to cold in winter and to low moisture availability in spring (which could be considered stressful conditions in a Mediterranean environment) by growing less and producing smaller vessels but in a higher number per sapwood area (Table 3; Fig. 6). In the short-term shrubs increased the VD and $K_h$ but decreased vessel size as a response to cold winters only at the mountain site (Fig. 6). In spring, vessel size and RW responded positively to precipitation in Madrid but negatively to temperature, whereas the VD exhibited an opposite response (Fig. 6). In Elba, the response of xylem traits to late spring climate was opposite to that of Madrid and the growth of trees at that site responded positively to fall precipitation (Fig. 6). Analyses in Figure 6 were performed separately for the two sites and focused

Figure 6. Bootstrap correlations between selected climatic covariates and residual chronologies of xylem traits. Results from Madrid are in grey whereas those from Elba are in black. Filled bars correspond to correlations with precipitation, bars with a high density of lines correspond to correlations with Tmax and bars with low density to those with Tmin. RW = ring width; VD = density of vessels; $D_h$ = hydraulic diameter; MVA = mean vessel area; $K_h$ = HP conductivity. Black horizontal dashed lines correspond to 0.05 significance levels and grey horizontal dashed lines to 0.10 significance levels.
in the linear relationship between covariates. However, when we represented the raw xylem traits of both sites together, the relationship between the xylem traits and winter temperature (particularly that of density) was non-linear, whereas a non-linear relationship with precipitation was not evident (Fig. 7).

Figure 7. Overall relationships between climate and xylem traits (data from both sites together). In (a), (c) and (e) we show winter maximum temperature (Tmax) against hydraulic diameter (Dh, in mm), mean vessel area (MVA, in mm²) and vessel density (VD, vessels·mm⁻²), respectively. In (b), (d) and (f) the same covariates but against hydrological year precipitation (Ppt). Either a best non-linear or linear fit is represented as a solid grey line with its coefficient of determination (R²) for comparison of the different relationships.

**DISCUSSION**

Xylem anatomical traits vary across genera and species along environmental gradients (Hacke et al. 2006; Preston et al. 2006; Poorter et al. 2010). The xylem plasticity exhibited helped *Erica arborea* to withstand an ample range of climatic conditions and showed that variability in xylem anatomy along spatio-temporal gradients is also expressed within species. Although main anatomical characteristics are genetically determined, the existence of certain variability in xylem architecture within a species in response to environmental factors has been reported for several other species (Kolb & Sperry 1999; Pourtahmasi et al. 2011; Gea-Izquierdo et al. 2012). However, there
are also examples of widespread species with rather constant anatomical features along their distribution (Liu & Noshiro 2003). This discrepancy among species merits further research and could be explained by species-specific physiological or anatomical traits not analyzed here (Choat et al. 2008; Lens et al. 2011). Additionally, with the present study design we could not separate the phenotypic expression on the hydraulic architecture variability from that related to the genotype, and major differences between the two sites should be a combination of both. The different adjustment to climate at the two sites was partly explained by the non-linear relationship between the studied xylem traits and temperature. The slower growth, higher vessel density and the response to temperatures observed suggested that the most limiting factor for *E. arborea* at the cold site was the low temperature in winter. Conversely, at the warm and dry maritime location the species exhibited a greater relationship with water availability than with temperature (particularly in the long-term) and an almost absent negative response of any trait to winter temperatures. This suggests that the most limiting factor for xylem development at the warm site was the moisture availability. Studies across species demonstrate that plants exhibiting higher tolerance to water-stress form denser xylem (Hacke et al. 2001), which can be directly linked to slower growth. We could not assess whether this applied to our species because it would require analyzing inter-annual variability in xylem density (Cook & Kairiukstis 1990).

**Xylem adjustment of Erica arborea to long-term changes in climate**

As is common in Mediterranean environments low water stress conditions expressed by high precipitation or low temperatures in the growing season were positive for growth of shrubs and the RW was positively correlated with vessel size but inversely to vessel density and $K_h$ (Pourthahasi et al. 2011; Gea-Izquierdo et al. 2012). Shrubs increased their vessel size and $K_h$ simultaneously with rising temperatures over the last decades at both sites and a small variation in vessel area may imply important differences in xylem functionality and vulnerability (Tyree & Zimmermann 2002; Hacke et al. 2006; Christman et al. 2012). Although the observed positive response of vessel size to maximum temperature in winter at the cold site suggests a positive effect of warming temperatures for *E. arborea*, the increase in vessel size in recent years was equally evident at both sites, which means that it was most likely driven by ontogeny rather than the changing climatic conditions (Tyree & Ewers 1991; McCulloh & Sperry 2005). Conversely, long-term relationships between growth and precipitation were only observed on Elba, which suggests that growth of shrubs declined probably in response to long-term increase in water stress resulting from reduced precipitation since the late 1980s. This growth decline in Elba was also evident in co-occurring *Arbutus unedo* and a similar growth decline in trees with increased drought has been reported elsewhere in the Mediterranean, e.g. in conifers (Linares et al. 2009; Sarris et al. 2011), in *Fagus sylvatica* (Peñuelas et al. 2008; Piovesan et al. 2008) and in different oak species (Di Filippo et al. 2010; Gea-Izquierdo et al. 2011). This suggests that even these shrub species, which are particularly adapted to dry conditions, may be threatened by climate change at certain dry sites (McDowell et al. 2011).
Different xylem traits reflect strategies to cope with climatic variability

Shrubs produced xylem with higher vessel density when water stress increased similar to results from other species such as *Populus* sp. or *Rhizophora* sp. (Verheyden *et al.* 2005; Fichot *et al.* 2009). Additionally, vessel density increased and vessel size decreased with lower temperatures in winter at the cold site. This suggests that the species produced xylem with a denser vessel network as a response to higher environmental stress (D’Arrigo *et al.* 2004; Loehle 2009; Gea-Izquierdo *et al.* 2011). Our results reflected the existing trade-off between vessel size and the density of vessels (Preston *et al.* 2006; Zanne *et al.* 2010; Pourtahmasi *et al.* 2011; Gea-Izquierdo *et al.* 2012) and the sensitivity of vessel size to low temperatures in winter at the cold site could express to some extent the balance between hydraulic efficiency and vulnerability to cavitation (Tyree & Zimmermann 2002; Preston *et al.* 2006; Cai & Tyree 2010; Zanne *et al.* 2010). The risk of cavitation as a result of freeze-thaw events is directly related to conduit size (Brodribb & Hill 1999; Hacke & Sperry 2001; Taneda & Sperry 2008) and among diffuse-porous species those growing under more limiting cold environments (higher frequency of freeze-thaw events) tend to have smaller vessels (Cordero & Nilsen 2002; Umebayashi *et al.* 2010). Greater vulnerabilities to low temperatures compared to drought stress have been proposed as one explanation for the northern distribution limit of other evergreen Mediterranean species (Corcuera *et al.* 2004), suggesting that freezing-induced cavitation would play a major role in their competitive capability (Tognetti *et al.* 2001). Nevertheless, in our results vessel density was more sensitive to climate than vessel size, which exhibited the minimum variability among the studied xylem traits. Additionally mean vessel size was similar at the two sites and shrubs responded to major differences in climate between sites by modifying other xylem traits such as vessel density and growth.

The risk of cavitation as a result of negative water potentials is explained by rather complex interrelationships between conduit characteristics related to different pit membrane features, to conduit length and the network connectivity (Hacke *et al.* 2006; Loepfe *et al.* 2007; Choat *et al.* 2008; Lens *et al.* 2011; Christman *et al.* 2012) with studies across species showing that water stress tolerance does not seem to be explained by size of the hydraulic conduits (Lens *et al.* 2011; Christman *et al.* 2012). However, within species there seems to be a relationship with vessel size and the largest vessel diameters would have a higher probability of failure by water-stress related air seeding (Kolb & Sperry 1999; Choat *et al.* 2008; Cai & Tyree 2010; Christman *et al.* 2012). Thus the observed relationships between vessel traits and climate for *E. arborea* would suggest enhanced inter-annual vulnerability to water-stress with larger vessels (Choat *et al.* 2008; Christman *et al.* 2012). The relationship of climate with vessel size and conductivity had different shapes because conductivity is related to the fourth power of conduit diameter (Tyree & Zimmermann 2002) hence a small variation in vessel size would have a high effect on conductivity compared to that of vessel number (Granier *et al.* 1994; Tyree & Zimmerman 2002). The calculated $K_h$ could slightly misestimate actual annual conductivity because potential conductivity is not totally reflecting actual conductive capacity at a certain time, because in the sapwood of diffuse-porous species several rings are able to conduct sap at a time and also because all the current year
vessels do not have to be simultaneously functional (Tyree & Ewers 1991; Choat et al. 2008; Umebayashi et al. 2010). Hydraulic efficiency depends on the conductance capacity per sapwood or leaf area and it tends to decrease when the availability of a limiting resource increases (Tyree & Ewers 1991; Mencuccini 2003), which agrees with the relationship we observed between $K_h$ and climate.

Since the two sites were on contrasting climates their phenology could differ, as expressed by correlations with climate in the short-term. For instance, VD was negatively affected by cold winters but positively by warm springs at the cold site (Fig. 6) and the response to temperatures in late spring was opposite between the two sites. Similar to other Mediterranean species (Baldocchi et al. 2010) E. arborea shows two peaks in net productivity in spring and fall (Gratani & Varone 2004). Therefore, as for other Mediterranean species (Camarero et al. 2010) the negative correlation between growth and precipitation in late fall in Elba could reflect that there is cambial activity at that time of the year leading to the formation of intra-annual density fluctuations (Copenheaver et al. 2010; Battipaglia et al. 2010; De Micco et al. 2012) which furthermore were much more common in Elba (not shown). Plants become more competitive when they maximize conductivity and growth (Russo et al. 2010). Plant traits can be interrelated and taller stems (as in Elba) would likely produce larger vessels. In addition higher plant densities should affect xylem features similarly to an increase in water stress because inter-plant competition reduces available resources for individual plants, e.g., inter-plant competition for light reduced vessel size in Rhododendron spp. (Cordero & Nilsen 2002). It could be argued that different levels of competition at the two sites could have biased the discussed relationships between the xylem traits and climate. Shrub density was higher in Elba where, hence, competition was higher. Therefore under identical climatic conditions, assuming that soil-related constraints were comparable (soils were similar at both sites) and that the effect of competition on vessel size was dominant to that of stem height, we would have expected shrubs in Elba to present smaller vessels and grow less than those in Madrid. However, growth was faster in Elba, which would support our discussion on xylem architecture and climate. We compared single stems as representative from multi-stem plants. There could be adjustments to climate at the plant level or of other hydraulic features (Lens et al. 2011) that we neglected and should be further investigated.

CONCLUSIONS

The studied species modified its stem xylem architecture as a response to climatic variability and the environmental most limiting factors, particularly moisture availability and winter temperature. The vessel density and growth of the studied species exhibited a higher variability in response to climate than the vessel size and conductivity. Plants adjusted the vessel size in opposite direction to the density of vessels. Within each site shrubs responded to mesic conditions (mild winters and wet springs) increasing growth and producing larger vessels but in a lower number per xylem area. However, the mean vessel size and potential conductivity did not differ between the two sites and shrubs responded to major differences in climate between sites by modifying the xylem vessel density and growth. The Mediterranean species studied expressed a major
effect of temperature constraining the development of its hydraulic architecture when growing in a mountain environment close to the cold limit of its distribution. At this site, shrubs produced xylem with a higher density of vessels whereas, as expressed by its faster growth, at the warm and dry maritime site the species was more competitive than at the cold site. However, in contrast to the other site studied, xylem development at the warmer location seemed to be more limited by moisture availability and shrubs exhibited a growth decline simultaneous to a decrease in precipitation over the last years, which might suggest that local populations are threatened by climate change. Differences in the response to climate at the two sites were discussed within the efficiency-safety trade-off and partly explained by the non-linear adjustment of the xylem traits to temperature. To understand how plants will cope with climate change it is crucial to characterize any non-linear responses to climate.

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