Do changes in spring phenology affect earlywood vessels? Perspective from the xylogenesis monitoring of two sympatric ring-porous oaks

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Introduction

Climate is a major driving force for tree growth, which is acclimated to variations in environmental conditions overtime. Regulation of the timings of seasonal development is a key mechanism for trees to face climatic instability, as occurs in temperate regions (Vitasse et al., 2014). Current warming-induced advancement of growth resumption is lengthening the growing season, with potentially dramatic impacts on forest trees, mainly regarding their carbon balance, health, growth performance, and wood structure (Menzel et al., 2006; Rossi et al., 2011). Integrating tree phenology and its associated responses on growth is crucial to reduce uncertainties about how trees cope with environmental changes (Cleland et al., 2007), which may shift competitive interactions among taxa, potentially modifying the relative dominance of sympatric species at a community level (Tylianakis et al., 2008).

Ring-porous trees, as other angiosperms, are assumed to be particularly sensitive to climate warming, because they operate with narrow hydraulic safety margins (Choat et al., 2012). Cavitation can result in a severe loss of water transport capacity that impairs plant productivity, and may induce mortality via hydraulic failure (McDowell, 2011). Although larger earlywood vessels are more efficient in water transport, they are also more prone to collapse by freeze–thaw cycles of xylem sap and by drought-induced air-seeding (Hacke et al., 2006). A recent study performed on pedunculate oak showed that trees dying after a severe drought event had higher hydraulic capacity than those surviving (Levanic et al., 2011). Therefore, earlywood vessel size is a critical functional aspect for hydraulic adjustment to climate variation, with a major concern if frequency and intensity of water stress increase (Bréda et al., 2006).

Xylem structure and function result from concurrent metabolic processes as a response to specific environmental signals during the growing season. In fact, long tree-ring series reveal that earlywood vessel anatomy, the main feature determining stem hydraulic conductance in ring-porous trees, is sensitive to environmental factors (García-González & Eckstein, 2003; Fonti et al., 2010). Detailed analyses on cambial phenology are therefore needed to elucidate how the environment drives the processes involved in wood formation (Rossi et al., 2012). Some studies have addressed xylogenesis in ring-porous oak species, focusing on the relationship between leaf and cambial phenology (Zasada & Zahner, 1969; Suzuki et al., 1996; Sass-Klaassen et al.,

Summary

• This study addresses relationships between leaf phenology, xylogenesis, and functional xylem anatomy in two ring-porous oak species, the temperate Quercus robur and the sub-Mediterranean Q. pyrenaica.
• Earlywood vessel (EV) formation and leaf phenology were monitored in 2012 and 2013. Ten individuals per species were sampled at each of three sites located in NW Iberian Peninsula. EV areas measured on microcore sections were used to calculate the hydraulic tree diameter ($D_h$), in order to model relationships to phenology. Thermal requirements were evaluated using growing degree days (GDD).
• A species-specific timing of growth resumption was found. The onset of EV formation and budburst were associated to a particular GDD in each species. The onset and duration of EV enlargement affected $D_h$ (and EV size) in Q. robur, but hardly in Q. pyrenaica.
• The relationship between the timings of EV formation and xylem structure appears to be stronger for the temperate oak, whose larger vessels may result from thermal-induced earlier resumption. In contrast, the sub-Mediterranean oak would maintain a more conservative hydraulic architecture under warming conditions.

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analysing carbon reserves (Michelot et al., 2012). However, the linkage between xylem development and its resulting anatomical features is still unknown for ring-porous trees. In conifers, the duration of tracheid enlargement was recently reported to be the main xylogenetic phase determining wood anatomical features in tree rings (Cuny et al., 2014).

*Quercus robur* L. is a deciduous temperate oak, widespread across Europe from the Baltic Sea to the Iberian Peninsula, wherein it coexists with sub-Mediterranean marcescent oaks, such as *Quercus pyrenaica* Willd. Temperate oaks lack multiple morphological (e.g. densely pubescent leaves and twigs) and physiological (e.g. lower leaf water potential at the turgor-loss point) traits to cope with water stress, which are present in sub-Mediterranean ones (Corcuera et al., 2002). Albeit well adapted to the summer drought, *Q. pyrenaica* woodlands inhabit mountain ranges, bearing adaptations to avoid low temperatures in late winter, such as delayed flushing and flowering (Jato et al., 2002). Earlywood formation mirrors these different strategies as shown by González-González et al. (2014), who found that vessel size chronologies of *Q. pyrenaica* were related to previous summer rainfall, whereas those of *Q. petraea* (Matt.) Liebl., another temperate oak species, were unrelated to climate.

This study concerns the influence of spring phenology on the hydraulic performance as determined by vessel size in one temperate (*Q. robur*) and one sub-Mediterranean (*Q. pyrenaica*) oak species. For this purpose, timings of secondary growth, bud-burst, and leaf unfolding were monitored in mixed stands in NW Iberian Peninsula along a north-to-south gradient. The objectives of this research are: to test whether timings of flushing and earlywood formation are similarly coupled in both species; to evaluate species-specific environmental requirements to accomplish crucial phenological events; and to determine whether the onset of wood formation affects vessel size, and therefore potential water conductivity. We hypothesize that species-specific environmental thresholds for growth resumption determine particular timings of earlywood formation, which in turn modulates the hydraulic performance.

**Materials and Methods**

**Study sites**

Three mixed stands of *Q. robur* (hereafter *Qrob*) and *Q. pyrenaica* (hereafter *Qpyr*) were selected along a transitional gradient between the Atlantic and Mediterranean biomes in the northwest Iberian Peninsula, following a north-to-south direction (Table 1; Fig. 1). *Qrob* is the dominant tree species at Bermui (BE) and Labio (LA), whereas *Qpyr* prevails at the southernmost location Moreiras (MO). Tree species as *Betula alba* L. and *Castanea*

<table>
<thead>
<tr>
<th>ID</th>
<th>Site</th>
<th>Elevation (m asl)</th>
<th>Density (trees ha⁻¹)</th>
<th><em>Quercus robur</em></th>
<th><em>Quercus pyrenaica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DBH (cm)</td>
<td>H (m)</td>
</tr>
<tr>
<td>BE</td>
<td>Bermui</td>
<td>395</td>
<td>1178</td>
<td>27.04 ± 3.83</td>
<td>13.9 ± 3.8</td>
</tr>
<tr>
<td>LA</td>
<td>Labio</td>
<td>690</td>
<td>1082</td>
<td>27.86 ± 3.82</td>
<td>13.2 ± 1.6</td>
</tr>
<tr>
<td>MO</td>
<td>Moreiras</td>
<td>450</td>
<td>531</td>
<td>26.95 ± 4.51</td>
<td>13.3 ± 2.1</td>
</tr>
</tbody>
</table>

DBH, diameter at breast height; H, tree height; asl, above sea level. Values are mean ± SE.

![Fig. 1 Study area in the northwestern Iberian Peninsula, and location of the sampling sites Bermui (BE), Labio (LA) and Moreiras (MO).](image-url)
sativa Mill., and understorey species as *Pyrus cordata* Desv., *Ilex aquifolium* L., *Daboecia cantabrica* (Huds.) K. Koch, and *Vaccinium myrtillus* L. were present at BE and LA. By contrast, MO was located within a broad river valley under Mediterranean conditions, coexisting with thermophilous plants such as *Q. suber* L., *Laurus nobilis* L., *Arbutus unedo* L., *Osyris alba* L., and *Daphne gnidium* L. Canopies of *Q. pyr* at the northernmost BE site were affected by the oak powdery mildew (*Erysiphe alphitoides* Griffon & Maubl.) in 2013.

**Meteorological data**

Air temperature was monitored at each site during 2012 and 2013 using sensor devices (iButton Hygrochron DS1923, San Jose, CA, USA). Gaps in temperature time series were interpolated by linear regressions from nearby meteorological stations belonging to the weather service Metegalicia (http://www.meteogalicia.es/), which also provided data for precipitation and photoperiod. These stations were selected within 16 km from each study site at similar latitude and elevation. Sites were located at low altitude except LA (Table 1), where the lowest temperature was recorded (Fig. 2a,b). The southernmost site MO, under Mediterranean influence, exhibited the highest temperature and the longest photoperiod (Fig. 2b,c). The shortest photoperiod was registered at the northernmost BE site (Fig. 2c), which displayed a considerable oceanic influence, and an average difference of 20–35 min in March and April to the other sites. BE showed mild temperatures and high precipitation in winter, as opposed to the more continental conditions at LA and MO (Fig. 2d). Contrasting weather conditions during late winter and spring occurred between 2012 (warm and dry) and 2013 (cold and wet) at all study sites.

**Sampling and sample processing**

We selected 10 trees per species at each site, from those exhibiting similar height and stem diameter (Table 1), and leaf development was weekly monitored from February to July, in 2012 and 2013. Dates of budburst and full expansion of leaves were recorded using binoculars (×10 magnification) at c. 10 m distance from the tree, and expressed as a particular day of year (DOY). Green-colored expanding buds with no unfolded leaves, and leaves attaining at least the 50% of their final size, constituted the criteria to respectively identify budburst and leaf full expansion. Each phase was registered when observed in branches on the uppermost part of the crown.

Two microcores of 2 mm diameter were weekly taken per tree at 1.3 m above ground from February to December in 2012 and 2013, using a Trephor device (Vitzani, Belluno, Italy), and following a helicoidal pattern around the bole (Rossi et al., 2006). After extraction, microcores were placed in microtubes with a 50% ethanol solution and stored at 5°C until processing, which included dehydration of samples by successive immersions in ethanol and xylene, and paraffin embedding using a tissue processor (Leica TP1020, Wetzlar, Germany). Cross-sections with a thickness 8–10 μm, obtained by cutting each paraffin block with a manual rotary microtome (Leica RM2125 RTS), were placed on microscope slides. After a 5-min immersion in xylene for removing the residual paraffin, samples were rehydrated and stained in two consecutive solutions of safranin and fast green.

![Fig. 2](image-url) Temporal variation of (a) daily mean temperature, (b) growing degree days (GDD), (c) photoperiod, and (d) precipitation at the study sites Bermui (BE; red), Labio (LA; blue) and Moreiras (MO; green) in 2012 and 2013.
FCF in ethanol (80%) for 10 min and 30 s, respectively (Cutler et al., 2008). Afterwards, they were newly submerged in xylene, and permanently fixed with Eukitt® resin (O. Kindler GmbH, Freiburg, Germany).

Data collection
In order to collect data relative to xylogenesis and earlywood vessel (EV) formation, microcore cross-sections were observed with a transmitted light microscope (Olympus BX40, Tokyo, Japan), using a white light polarizing filter to detect cell wall thickening (×40 magnification). The four phenophases recorded from the cross-sections were: (1) dormant cambium (Fig. 3a), (2) cambial resumption (Fig. 3b), (3) onset of EV differentiation and enlargement (Fig. 3c,d), and (4) onset of EV maturation (Fig. 3e,f). The occurrence of cell divisions was the criterion to identify cambial resumption (Frankenstein et al., 2005; Fig. 3b). The start of the period of EV enlargement was considered when the most recent ring contained only enlarging vessels. Distinctions between the first and second row were not straightforward because some samples simultaneously showed both enlarging and maturing first-row vessels (Fig. 3c,d). The onset of EV maturation was defined when both wall thickening and lignification were detected, as these processes appeared to occur simultaneously in vessels, so that we were usually not able to discern between them (Fig. 3e,f). Images of 5184 × 3456 pixels were obtained from all the samples in which EV were mature (i.e. collected in late spring, summer and autumn), using a digital camera attached to the microscope.

Data analysis and statistics
Heat units from 1 January to the dates of budburst and onset of enlargement, expressed as cumulative growing degree days (GDD), were assessed for each site from daily air temperature in 2012 and 2013 according to McMaster & Wilhelm (1997) by the equation:

\[
\text{GDD}_{\text{daily}} = \frac{(T_{\text{max}} + T_{\text{min}})}{2} - T_{\text{base}},
\]

where \(T_{\text{max}}\) and \(T_{\text{min}}\) are maximum and minimum temperature respectively, and \(T_{\text{base}}\) is an arbitrary threshold set to 5°C, according to previous studies performed on temperate species (Prislan et al., 2013). No thermal accumulation was considered when both \(T_{\text{max}}\) and \(T_{\text{min}}\) were below \(T_{\text{base}}\). GDD requirements for budburst and onset of EV enlargement were compared among sites and between species using a factorial analysis of variance and a multiple comparison analysis.

Within each tree, 50 EV located in the first and second rows of the outermost ring were measured out of the samples wherein EV were mature, setting a limit of 7500 \(\mu \text{m}^2\) because of the substantially higher contribution of large conduits to overall hydraulic conductivity (González-González et al., 2014). Measurements of vessel lumen area were semi-automatically performed using ImageJ version 1.48v (Schneider et al., 2012). Two Qpyr individuals from BE showing suppressed growth were excluded from the analysis. The diameter of each equivalent circular vessel lumen
was derived from the vessel area, and used to compute the weighted average of hydraulic diameters ($D_h$) according to the equation:

$$D_h = \frac{\sum_{n=1}^{N} D_n^5}{\sum_{n=1}^{N} D_n^4},$$

where $D_n$ is the diameter of the vessel $n$ (Sperry et al., 1994). $D_h$ is proportional to hydraulic conductivity, defined according to the Hagen-Poiseuille equation as the fourth power of the pore radius (Tyrree & Zimmermann, 2002).

The analysis of xylogenesis and leaf phenology was carried out using generalized linear mixed models (GLMM) for binary data (Bolker et al., 2009), considering ‘tree’ as a random effect, and applying multiple comparisons for parametric models to compare secondary growth with leaf phenology. Pearson’s correlations among phases of EV formation and budburst were also calculated to test for collinearity. The interaction between each tree $D_h$ and the timing of enlargement was tested by applying a generalized least squares model (GLS) selection procedure, allowing for different variances per site. The analysis was repeated for vessel areas using GLMM for gamma distributions with ‘tree’ as a random effect. The Akaike’s Information Criterion (AIC) was used to rank models, and those variables appearing in the most parsimonious model (fewest variables) showing $\Delta$AIC $< 2$ were considered as meaningful. All statistical analyses were performed in R 3.1.1 (R Core Team, 2014), using the ‘lme4’ package for GLMMs, the ‘nlme’ package for GLS model calculations, and the ‘multcomp’ package for multiple comparisons.

### Results

**Spring tree phenology**

Cambial resumption (i.e. cambial divisions) started from mid-February to late March (DOY 45–86) in $Q rob$, and from late February to early April (DOY 58–94) in $Q pyr$ throughout the study gradient (Fig. 4). The date on which cambium resumed differed between species and among sites, while differences between years were meaningless. (Table 2a). EV enlargement occurred from early March to early July (DOY 64–184) in $Q rob$, and from mid-March to early July (DOY 71–182) in $Q pyr$ (Fig. 4), significantly varying among sites and between years (Table 2b,c). The onset of EV enlargement occurred significantly earlier in $Q rob$ than in $Q pyr$, whereas differences at the end of EV enlargement were negligible between species (Table 2b,c). Consequently, $Q rob$ individuals exhibited longer periods of EV enlargement than $Q pyr$ ($F=13.53$, $P<0.001$), with mean values of 63 and 54 d, respectively. The enlargement period up to the onset of maturation phase in the first-formed vessels entailed 43.36 $\pm$ 4.84% of the whole period of EV enlargement in $Q rob$, while 59.32 $\pm$ 5.30% in $Q pyr$. EV maturation started from late March to mid-May (DOY 80–137) in $Q rob$ and from early April to mid-June (DOY 100–162) in $Q pyr$ (Fig. 4). As observed for EV enlargement, differences among species, years, and sites were also significant in EV maturation initiation (Table 2d). Similar results were revealed by the models applied on budburst (Table 2e), which started earlier in $Q rob$ (DOY 71–137) than in $Q pyr$ (DOY 100–147). Leaf expansion was completed between early April and early June (DOY 93–154) in $Q rob$, and between early

**Fig. 4** Timing of earlywood vessel formation (cambial resumption, enlargement, and maturation) and budburst for *Quercus robur* (blue) and *Quercus pyrenaica* (red), according to the day of year (DOY), at the study sites Bermui (BE), Labio (LA) and Moreiras (MO) in 2012 and 2013. Shaded areas represent the proportion (%) of the sampled trees exhibiting each phenophase. C. resumption, cambial resumption.
The best model is highlighted in bold. AIC is increment on AIC value.

Models are sorted following the Akaike’s Information criterion (AIC). Models were fitted for binomial distributions, including tree as random effect. The best model is highlighted in bold. ΔAIC is increment on AIC values respect to that of the model with lowest AIC.

df, degrees of freedom; LL, Log Likelihood.

Table 2 Comparison of generalized linear mixed models (GLMM) assessing the effect of species (Spp), site, year and their interactions on earlywood vessel (EV) formation and budburst

<table>
<thead>
<tr>
<th>Phenophase</th>
<th>df</th>
<th>LL</th>
<th>ΔAIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Cambial resumption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site + Spp</td>
<td>6</td>
<td>–118.55</td>
<td>0.00</td>
</tr>
<tr>
<td>Site + Spp + Year</td>
<td>7</td>
<td>–120.16</td>
<td>5.22</td>
</tr>
<tr>
<td>(b) Onset of EV enlargement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site + Spp + Year</td>
<td>7</td>
<td>–142.61</td>
<td>0.00</td>
</tr>
<tr>
<td>Site + Spp</td>
<td>6</td>
<td>–147.21</td>
<td>7.20</td>
</tr>
<tr>
<td>(c) End of EV enlargement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site x Year</td>
<td>8</td>
<td>–150.18</td>
<td>0.00</td>
</tr>
<tr>
<td>Site x Spp</td>
<td>8</td>
<td>–161.60</td>
<td>22.85</td>
</tr>
<tr>
<td>(d) Onset of EV maturation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site + Spp + Year x Year</td>
<td>8</td>
<td>–121.79</td>
<td>0.00</td>
</tr>
<tr>
<td>Site + Spp + Year</td>
<td>7</td>
<td>–148.00</td>
<td>50.42</td>
</tr>
<tr>
<td>(e) Budburst</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site + Spp + Year x Year</td>
<td>8</td>
<td>–92.03</td>
<td>0.00</td>
</tr>
<tr>
<td>Site + Spp</td>
<td>8</td>
<td>–108.41</td>
<td>32.76</td>
</tr>
</tbody>
</table>

Thermal drivers of phenology

The studied species showed different GDD requirements for both primary and secondary growth resumption throughout the study gradient. Qrob required lower GDD values in order to reactivate growth, particularly for budburst (GDD Qrob = 374.17 ± 12.46; GDD Qpyr = 536.35 ± 15.80; F = 158.89, P < 0.001), and EV enlargement (GDD Qrob = 273.51 ± 11.3; GDD Qpyr = 384.73 ± 13.89; F = 99.02, P < 0.001). Sensitivity to temperature forcing varied across the north-to-south gradient, as demonstrated by the higher GDD values observed at the moment of budburst (GDD BE = 572.71 ± 19.13; GDD LA = 385.01 ± 14.55; GDD MO = 408.05 ± 17.60; F = 99.02, P < 0.001), and EV enlargement (GDD BE = 435.46 ± 13.51; GDDL = 257.49 ± 11.23; GDMO = 294.41 ± 14.45; F = 94.16, P < 0.001) at the northernmost BE site. According to the multiple comparisons, trees at sites LA and MO required similar GDD, both for the onset of enlargement (t = 1.31, P = 0.391) and budburst (t = −0.17, P = 0.984). Details of GDD and their comparisons are shown in Supporting Information Tables S1 and S2.

Vessel enlargement and hydraulic diameter

Both species exhibited similar EV features, with frequent multiple rows of wide vessels, and the largest ones being mostly located in the first and second rows. Only 17 individuals developed one single row of EV (6 in Qrob and 11 in Qpyr), 10 of them were located at BE. The EV area measurements performed (3000 EV for Qrob, and 2900 for Qpyr), showed differences among sites, exhibiting Qpyr at BE and LA the lowest and highest mean values respectively (Table S3; Fig. S1). Mean lumen area was 48.711.39 ± 387.88 μm² (D = 242.55 ± 1.03 μm) for Qrob and 49.036.95 ± 411.08 μm² (D = 242.90 ± 1.09 μm) for Qpyr, whereby differences in Dₕ between species (on average 271.52 ± 4.03 μm for Qrob and 273.09 ± 4.35 in Qpyr) were non-significant (Table 4a). Best-fitted GLS models differed between the species (Table 4b). The onset of EV formation influenced Dₕ exclusively in Qrob (Fig. 5; Table 4b). In this case, the earlier the EV enlargement was, the larger the Dₕ (Table 4b). Longer periods of EV enlargement were also related to increasing Dₕ in Qrob, despite the meaningless influence of the end of this phase (Fig. 5). Conversely, Dₕ was scarcely affected by the onset and duration of EV enlargement in Qpyr, and the variability among individuals was only explained by the site factor (Table 4b). Additionally, Dₕ significantly decreased from 2012 to 2013 but

Table 3 Pearson’s correlation coefficients (r) among phenophases of earlywood vessel (EV) formation and budburst

<table>
<thead>
<tr>
<th>Phenophase</th>
<th>Cambial resumption</th>
<th>Onset of EV enlargement</th>
<th>Onset of EV maturation</th>
<th>End of EV enlargement</th>
<th>Budburst</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cambial resumption</td>
<td>0.58 (&lt; 0.001)</td>
<td>0.34 (0.009)</td>
<td>0.45 (&lt; 0.001)</td>
<td>0.61 (&lt; 0.001)</td>
<td></td>
</tr>
<tr>
<td>Onset of EV enlargement</td>
<td>0.36 (0.005)</td>
<td>0.66 (&lt; 0.001)</td>
<td>0.37 (0.004)</td>
<td>0.66 (&lt; 0.001)</td>
<td></td>
</tr>
<tr>
<td>Onset of EV maturation</td>
<td>0.38 (0.002)</td>
<td>0.45 (&lt; 0.001)</td>
<td>0.49 (&lt; 0.001)</td>
<td>0.54 (&lt; 0.001)</td>
<td></td>
</tr>
<tr>
<td>End of EV enlargement</td>
<td>0.35 (0.005)</td>
<td>0.49 (&lt; 0.001)</td>
<td>0.49 (&lt; 0.001)</td>
<td>0.38 (0.003)</td>
<td></td>
</tr>
</tbody>
</table>

P-values of the coefficients are included in brackets. Shaded cells (lower left) correspond to Quercus pyrenaica; regular ones (upper right), to Quercus robur.
only for *Qrob*, which is consistent with the delayed onset of enlargement during the second year in this species. Similar results were obtained using EV area instead of $D_h$ (Table S3; Fig. S2).

**Discussion**

**Linkage between leaf phenology and earlywood formation**

Phenological phases in the crown (budburst), and stem (cambial resumption, EV enlargement, and EV maturation) were highly correlated. This indicates that growth resumption in both apical and lateral meristems involves a chain of interconnected physiological mechanisms strongly determined by the first phenological event (Rossi *et al.*, 2012; Rossi & Bousquet, 2014). This correspondence between flushing and earlywood growth may be due to the fact that expanding leaves are major sources of auxin indole-3-acetic acid, which is considered the primary hormone among those promoting vascular differentiation. Despite the hormonal control of xylogenesis has not been fully disentangled, variations in cell sensitivity to auxin and its relative concentration along the season were reported to alternatively promote cell division and vascular differentiation (Sorce *et al.*, 2013). Conversely, increasing concentrations of gibberellin acid, which is produced in mature leaves, have been mostly associated with fiber production and latewood growth (Aloni, 2015).

Cell divisions in the cambial zone started 5–6 wk earlier than the enlargement of the first EV, attaining longer periods between cambial resumption and the onset of vessel differentiation than previous studies for other ring-porous species (Frankenstein *et al.*, 2005; Kudo *et al.*, 2014). Budburst was coupled to the beginning of wall thickening in both species, although secondary wall deposition had not been initiated at the moment of budburst for some first formed *Qrob* vessels. According to Takahashi *et al.* (2013), delayed vessel lignification with respect to the date of budburst implies that not all the first-formed vessels are available to contribute to water transport at the time of flushing. Some previous works pointed out that leaf unfolding in ring-porous species occurs when first-formed vessels are already expanding (Suzuki *et al.*, 1996; Sass-Klaassen *et al.*, 2011; Michelo *et al.*, 2012), evidencing that auxin supply from growing leaves is not required for triggering cell division and vascular differentiation (Funada *et al.*, 2002; Kudo *et al.*, 2014). Endogenous auxin levels in the dormant cambium, probably provided in winter by either dormant or swelling buds (Funada *et al.*, 2002; Aloni, 2015), may induce xylogenesis due to the higher cell sensitivity to auxin in ring-porous species (Aloni, 2015). Our results also agree to the fact that ring-porous trees yearly restore their photosynthetic apparatus and hydraulic architecture using exclusively carbon reserves from the previous season (Barbaroux & Bréda, 2002). This seems to be particularly true for *Qpyr*, whose leaves were not fully expanded until the end of earlywood formation.

**Environmental control of spring phenology**

Trees at LA and MO exhibited similar thermal requirements for leaf appearance and vessel enlargement, despite their different timing of spring phenology. This supports the conspicuous thermal driving of both leaf phenology (Vitasse *et al.*, 2009), and earlywood vessel formation (Kudo *et al.*, 2014). Enhanced sensitivity to auxin stimuli in cambial cells could occur under rising temperatures (Aloni, 2015), promoting cell division and EV differentiation. However, higher heat requirements at the northernmost site suggest that growth reactivation requires a certain threshold of photoperiod to be accomplished. Flushing in temperate trees is driven by counterbalanced relations between photoperiod and temperature, as stated by Basler & Körner (2014), who also noted a species-specific sensitivity to daylength and temperature. Indeed, phenological sensitivity to photoperiod, heat forcing, and chilling has been associated to life plant strategy (Caffarra & Donnelly, 2011), with long-lived late successional species being relatively more sensitive to daylength. Interestingly, cambial cell divisions within each site started at the same time in both years, but such synchrony was exclusively observed for this phenophase, probably suggesting a weak thermal influence as compared to the onset of vessel enlargement and budburst.

**Species-specific patterns of spring phenology**

Our results confirmed the earlier leaf phenology in *Qrob* than in *Qpyr*. The onset of cambial divisions and the subsequent phases of earlywood formation also occurred earlier in *Qrob*, leading to a longer period of earlywood vessel enlargement. Thereby, *Qrob* exhibited lower thermal requirements for break dormancy than *Qpyr*, as pointed out by Jato *et al.* (2002) after comparing

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**Table 4** Comparison of generalized least squares (GLS) models for hydraulic diameter data: (a) models evaluating the effect of species, site and year; (b) models evaluating the effect of EV enlargement, site and year for *Quercus robur* and *Quercus pyrenaica*

<table>
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<tr>
<th></th>
<th>df</th>
<th>LL</th>
<th>$\Delta$AIC</th>
</tr>
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<td>(a) Species comparison</td>
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<tr>
<td>Site + Year</td>
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<td>563.65</td>
<td>0.00</td>
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<tr>
<td>Site + Year + Spp</td>
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<td>562.11</td>
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<tr>
<td>(b) EV Enlargement</td>
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<tr>
<td>Site + Year + Onset</td>
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<td>-273.75</td>
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<td>3.16</td>
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<tr>
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<tr>
<td>Site</td>
<td>4</td>
<td>289.40</td>
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*Quercus robur*

<table>
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<td>Site + Year + End</td>
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<tr>
<td>Year</td>
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<td>284.09</td>
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</table>

Models are sorted following the Akaike’s Information criterion (AIC). Onset, Duration, and End terms are all referred to EV enlargement. The best model is highlighted in bold. $\Delta$AIC is increment on AIC values respect to that of the model with lowest AIC. df, degrees of freedom; LL, Log Likelihood.
budburst heat requirements. Vitasse et al. (2009) defined the phenological sensitivity to temperature as species-dependent. A delayed leaf appearance is assumed to be an adaptive response to avoid damage by late frost events (Vitasse et al., 2014), as could be the case of Qpyr. Remarkably unstable year-to-year climate conditions, causing either late frosts or drought stress, frequently occur at the Iberian ranges where Qpyr inhabits. On the other hand, mild oceanic climate conditions in optimal areas for Qrob would result less critical for recently formed tissues. However, our results contrast with the synchronous pattern reported by González-González et al. (2013) at one mixed stand of Qrob and Qpyr after a 1-yr monitoring. This discrepancy is consistent with the strong spatial and inter-annual variability of tree phenology, especially in Qrob, as we observed among sites, and between the 2 yr of monitoring.

Phenology-dependent hydraulic features of earlywood vessels

Models pointed out that the size of earlywood vessels, which in turn determines hydraulic diameter, was markedly dependent on the onset of cell differentiation in Qrob, affecting the potential water conductivity along the stem. Wider conduits were observed to be related to extended periods of earlywood enlargement in Qrob probably because of the longer duration of individual vessel expansion. In conifers, tracheid lumen area throughout the tree ring is determined by the duration of cell enlargement phase (Cuny et al., 2014). Similarly, the widening of tracheid diameter in conifers from the apices to the roots has been attributed to the increasing duration of cell enlargement downwards in the stem (Anfodillo et al., 2012). In contrast, the timing of enlargement barely influenced earlywood vessel conductivity in Qpyr, and probably larger vessels were associated to faster rates of cell expansion, rather than duration of the enlargement phase.

Climate signals in multi-decadal tree-ring series indicate that vessel traits in ring-porous species are mostly influenced by climatic conditions in two physiologically distinct periods, during the previous year, and at the onset of cambial activity (Fonti & García-González, 2004; Tardif & Conciatori, 2006). Noteworthy, an evident effect of temperature on vessel anatomy during dormancy, quiescence, and growth resumption periods has been reported in tree rings of Qrob (Fonti et al., 2009; Matsions & Brumelis, 2012). This is consistent with our results, suggesting that the anatomical structure and functionality of vessels in Qrob is modulated by the onset of enlargement, which is in turn driven by exogenous factors (mostly temperature and photoperiod) in winter and spring.

EV size in Qpyr may be less sensitive to thermally induced phenological modulation than Qrob. Although studies performed on Qpyr tree rings are still scarce, previous summer rainfall has been stated as the most relevant climatic factor determining vessel size, rather than temperature in winter and spring (González-González et al., 2014, 2015). In fact, moisture availability was reported to be the most limiting factor driving radial growth in Iberian Qpyr populations (Gea-Izquierdo & Cañellas, 2014). As a whole, these findings suggest that vessel size in the sub-Mediterranean Qpyr
could be more dependent on environmental signals operating at time windows that are different from those of vessel enlargement, such as precipitation during the previous growing season. Formation of narrower vessels is considered an adaptive response of trees to water stress in ring-porous species (Lovisolo & Schubert, 1998; Eilmann et al., 2009; Galle et al., 2010; G. Pérez-de-Lis benefited from a PhD. FPU-ME grant (no. AP2010-4911) funded by the Spanish Ministry of Education, and from a short-term scholarship funded by the FRQNT-Ministry of Education and Sports of Québec (no. 185330) to visit the UQAC. The authors gratefully thank L. Costa and C. Franco of the Fragas do Eume Natural Park, Forest Service of Xunta de Galicia, and M. Souto of the MVMC of Moreiras, for facilitating the field work. Two anonymous reviewers considerably contributed to improve this manuscript. The frame of the COST Action FP1106 ‘STReESS’ inspired this research.

References


Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Distribution of earlywood vessel areas for Quercus robur and Quercus pyrenaica within each study site.

Fig. S2 Relationships between mean vessel area and the onset, end, and duration of the earlywood enlargement for Quercus robur and Quercus pyrenaica.

Table S1 Growing degree days at the onset of vessel enlargement and budburst

Table S2 Effects of site, species, and their interaction on the growing degree days for earlywood vessel enlargement and budburst

Table S3 Comparison of linear mixed models for earlywood vessel area information

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