

Do ring-porous oaks prioritize earlywood vessel efficiency over safety? Clues from short and long-term responses of earlywood vessel anatomy to climate

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Highlights

- The timing of tyloses reflects tree responses to variable drought severity.
- Under drier conditions, ring-porous oaks show larger vessels and more tyloses.
- Tyloses are less frequent for the more drought tolerant *Quercus pyrenaica*.
- *Quercus robur* has narrower earlywood vessels but wider latewood increments.

Do ring-porous oaks prioritize earlywood vessel efficiency over safety? Clues from short and long-term responses of earlywood vessel anatomy to climate

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Abstract

The impact of climate on xylem structure and function has been profusely studied for a variety of species in the last decades, but the ecological role of ring porosity under increasing levels of environmental stress has been scarcely assessed. In this study, we analyze the timing of earlywood vessels occlusions by tyloses in two ring-porous species with contrasting ecological strategies (*Quercus robur* and *Q. pyrenaica*) along a seasonal drought gradient, and relate it to variations in earlywood vessel diameter and latewood width obtained from tree-ring series. The number of trees showing tyloses increased in summer as a result of more frequent cavitation events under drier conditions, and was more reduced for the more drought-tolerant *Q. pyrenaica*, although *Q. robur* had a lower hydraulic diameter (D_h) and higher latewood increments. D_h values decreased towards the wettest sites and were negatively related to warm and rainy conditions in winter. Our results showed that large earlywood vessel diameters are not necessarily accompanied by high rates of tyloses formation in summer or limited latewood growth. We hypothesized that trees in seasonal environments can take advantage from large earlywood vessels, because benefits from a more efficient hydraulic system during favourable periods are higher than the risk of xylem impairment in summer.

Key words: climate seasonality, hydraulic diameter, latewood width, tree rings, tyloses, water availability

1. Introduction

Wood anatomy influences the ability of trees to track environmental changes (Anderegg et al., 2016; Pratt and Jacobsen, 2017). Conductive cells have attracted the attention of wood anatomists because the efficiency/safety trade-off is deemed to be a prime evolutionary force (Gleason et al., 2016), which may somewhat account for the variation in vessel morphology across species and biomes (Cavender-Bares et al., 2005). The ring-porous structure represents a unique example, showing a bimodal vessel size distribution along the ring (Kitin and Funada, 2016). According to the ‘rare pit’ hypothesis, leaky intervacular pits are more probable to occur in large vessels, easing air-seeding into the vessel and leading to cavitation occurrence (Sperry et al., 2006). The more efficient earlywood vessels are thus less resistant than latewood ones to cavitation (Hernández-Santana et al., 2008; Sperry et al., 2006), being normally dysfunctional within one year (Umebayashi et al., 2010). Cavitated vessels are eventually sealed by outgrowths called tyloses that originate from adjacent parenchyma cells (De Micco et al., 2016; Kitin and Funada, 2016). Tyloses reflect tree responses to drought and freezing events, wounds, and vascular pathogens (De Micco et al., 2016), but their formation has been barely monitored so far (Cochard and Tyree, 1990).

Earlywood vessel diameter in ring-porous species is controlled by complex interactions between internal and external factors (Hacke et al., 2017). Tree size imposes physical constraints to conduits dimensions, whereby wider vessels are required at the tree base as trees grow taller and water-conducting pathways become longer (Carrer et al., 2014). Previous studies associate these allometric relationships to specific auxin production and transport, offering theoretical support to the increasing vessel dimensions with tree size and age (Carrer et al., 2014; García-González et al., 2016). Concurrently, the environment exerts a strong influence on earlywood vessel diameter by inducing changes in the timing and rate

of processes involved in cambial cell division and earlywood differentiation (Fonti et al., 2007; Hacke et al., 2017; Pérez-de-Lis et al., 2016b).

White oaks are prevalent ring-porous species occurring under a wide range of environmental conditions throughout the Northern Hemisphere. The deciduous *Quercus robur* is widespread in Europe under mild and rainy conditions, reaching the temperate-Mediterranean transition where marcescent oaks such as *Q. pyrenaica* become dominant. The latter is more tolerant than *Q. robur* to adverse abiotic conditions, as revealed by a shorter growing season and lower xylem increment (Pérez-de-Lis et al., 2017), as well as by the profuse leaf pubescence (Sánchez de Dios et al., 2009). Nevertheless, radial growth and distribution were predicted to decline in both species under future climatic scenarios (Sánchez de Dios et al., 2009; Gea-Izquierdo et al., 2013; Urli et al., 2015). Oaks bearing large vessels could be prone to experience drought-induced mortality (Levanic et al., 2011), although earlywood vessels were suggested to become narrower under water shortage conditions (Castagnieri et al., 2016; Corcuera et al., 2004; Eilmann et al., 2009; Galle et al., 2010; Gea-Izquierdo et al., 2012). Therefore, identification of key environmental factors driving vessel enlargement is decisive to accurately estimate ring-porous trees resistance to environmental stress.

In this work, we combine the study of multi-decadal tree-ring records with the timing of tyloses appearance over the course of two consecutive growing seasons (2012 and 2013) in co-occurring *Q. robur* and *Q. pyrenaica* trees. We particularly ascertain inter- and intra-specific variation in earlywood vessel diameter and latewood increment along a water-availability gradient. We hypothesize that (1) trees of both species show narrower earlywood vessels under drier conditions; (2) the temperate *Q. robur* exhibits larger and more numerous vessels than the more drought-tolerant *Q. pyrenaica*, also resulting in larger latewood increments; (3) tyloses occlusion of embolized earlywood vessels occurs earlier under drier conditions, being less recurrent in the more drought-resistant *Q. pyrenaica*.

2. Materials and methods

2.1 Study sites

The study was conducted in 2012 and 2013 at three stands covering a north-to-south gradient at the temperate-Mediterranean climatic transition in the NW Iberian Peninsula (Fig. 1A). Climate at the study region is characterized by the alternation of a mild period of abundant rainfall from autumn to spring, and a warm period of lower rain in summer (Fig. 1B). The northernmost site Bermui (hereafter ATL, Atlantic low altitude) is located at low elevation nearby the Atlantic coast (395 m a.s.l.), while the intermediate site Labio (hereafter ATH, Atlantic high altitude) lies inland at higher elevation (690 m a.s.l), showing colder winters and drier summers than the former location. Stronger Mediterranean climatic influence occurs at Moreiras (hereafter MED, Mediterranean), on the southernmost edge of the gradient at 450 m a.s.l., with a lower precipitation and frequent episodes of summer drought (Fig. 1B). Winter and spring were drier and warmer in 2012 than in 2013 (Fig. 1C), and also for the period October-November, especially at the Atlantic locations. Scarce rainfall and elevated day temperatures were registered in summer of both years.

Species composition and structure at the study sites reflect diverging climate conditions along the gradient. Although both species coexist at the three study sites, *Q. robur* (*Qrob*) prevails at ATL and ATH while *Q. pyrenaica* (*Qpyr*) is more frequent at MED. In addition, tree density at ATL and ATH was respectively 1,178 and 1,082 trees ha⁻¹, whereas 530 trees ha⁻¹ at MED. At this latter site, there was vigorous understory vegetation including *Arbutus unedo* L., *Laurus nobilis* L., and *Acacia dealbata* Link. individuals.

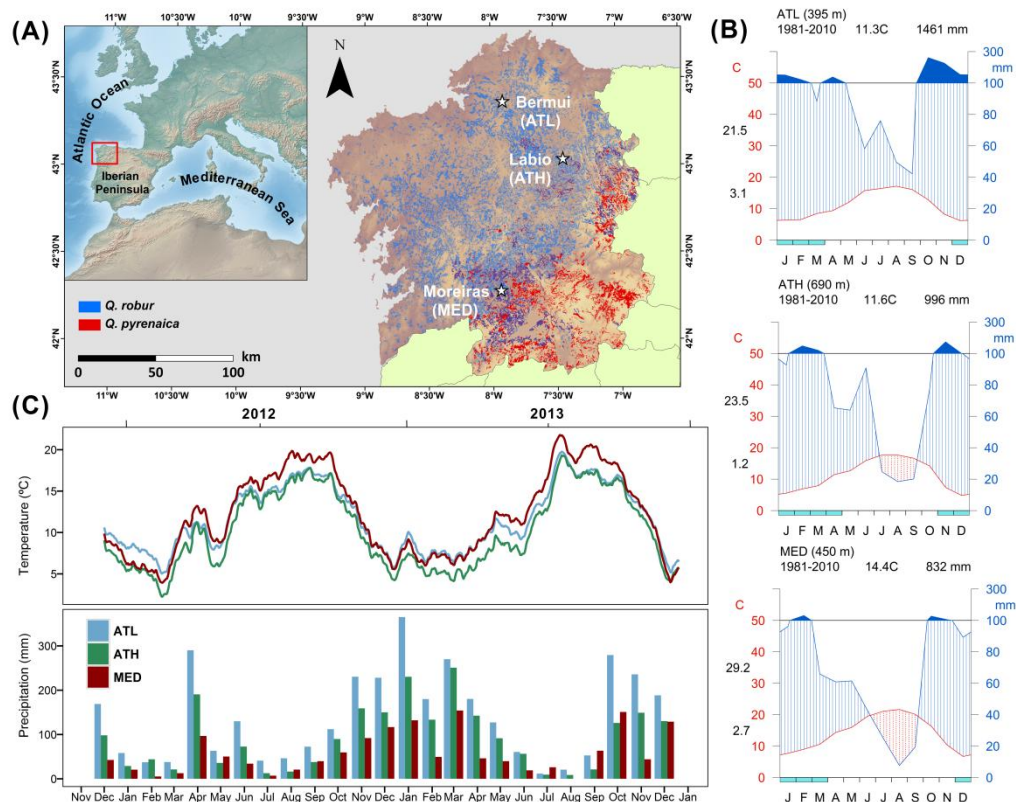


Figure 1 Location of the study sites ATL (Atlantic low altitude), ATH (Atlantic high altitude), and MED (Mediterranean) in the NW Iberian Peninsula (A), along with their respective climatic diagrams (B), and 10-day moving average of mean temperature, and monthly precipitation for the period 2012–2013 (C). The climodiagrams show mean temperature and precipitation for each month, and the period with potential frost days (horizontal bars). Sensors iButton Hygrochron DS1923 (San Jose, CA, USA) recorded temperature hourly at each site, and nearby meteorological stations the from the weather service Meteogalicia (<http://www.meteogalicia.es>) provided daily precipitation and climodiagram data.

2.2 Monitoring of tyloses formation in earlywood vessels

Tyloses formation was monitored in 10 trees per site, species and year from February 2012 to December 2013 ($N_{2012} = 60$, $N_{2013} = 60$). The selected individuals were comparable in size at the three study sites (Table 1). Two microcores per tree were weekly collected using a Trephor (Vitzani, Belluno, Italy) following a helicoidal pattern around the bole (Rossi et al., 2006), and immediately stored into a cooling box in the field. One microcore per tree was dehydrated at the laboratory with a tissue processor (Leica TP1020, Wetzlar, Germany) containing various ethanol solutions, xylene, and a final station for paraffin embedding, while the other microcore was stored at 5 °C. The cuts obtained in a rotary microtome (Leica RM2125 RTS, Wetzlar, Germany) were placed on slides, oven-dried at 60 °C for 2 hours, and

then stained in 80% ethanol solutions of safranin and fast green. More details on sample processing are provided in Pérez-de-Lis et al. (2016b). Tyloses formation was analyzed on the cross-sections by using a binocular microscope (Olympus BX40, Tokyo, Japan) at 40× magnification. For each sampling date, we quantified the number of individuals showing earlywood vessels of the outermost ring partially or totally occluded by tyloses (Fig. 2).

Table 1 Tree characteristics and wood anatomical variables for the period 1980–2013.

	<i>Q. robur</i>			<i>Q. pyrenaica</i>		
	ATL	ATH	MED	ATL	ATH	MED
D_h (μm)	246.91 ± 1.09	247.79 ± 1.20	270.76 ± 1.05	265.40 ± 1.47	261.94 ± 1.07	290.96 ± 0.99
MVA (μm ²)	38,223 ± 303	38,396 ± 366	45,326 ± 348	43,830 ± 434	42,747 ± 342	52,104 ± 350
NV	37.01 ± 0.49	48.31 ± 0.53	40.85 ± 0.71	33.14 ± 0.50	44.31 ± 0.68	35.35 ± 0.40
Stem diameter ₁ (cm)	29.36 ± 1.34	28.04 ± 0.76	33.97 ± 2.71	29.35 ± 1.51	26.26 ± 0.95	29.65 ± 1.10
Tree height ₁ (m)	17.17 ± 0.32	13.33 ± 0.36	14.89 ± 0.72	16.89 ± 0.46	13.33 ± 0.32	16.15 ± 0.57
Cambial age ₁ (yrs)	40.59 ± 0.36	36.82 ± 0.36	66.27 ± 0.68	49.86 ± 0.57	40.00 ± 0.35	68.88 ± 0.56
Number of trees ₁	13	12	18	12	12	16
EW (mm)	0.50 ± 0.01	0.62 ± 0.01	0.67 ± 0.01	0.44 ± 0.01	0.60 ± 0.01	0.60 ± 0.01
LW (mm)	1.51 ± 0.03	2.01 ± 0.03	2.15 ± 0.05	0.96 ± 0.02	1.65 ± 0.02	1.26 ± 0.03
RW (mm)	2.01 ± 0.03	2.63 ± 0.03	2.82 ± 0.05	1.40 ± 0.02	2.25 ± 0.03	1.85 ± 0.04
Stem diameter ₂	29.23 ± 0.16	28.04 ± 0.09	36.32 ± 0.38	29.19 ± 0.18	26.26 ± 0.11	30.16 ± 0.13
Tree height ₂	17.15 ± 0.04	13.32 ± 0.04	15.54 ± 0.10	16.82 ± 0.05	13.33 ± 0.04	16.50 ± 0.07
Cambial age ₂	38.45 ± 0.27	35.31 ± 0.27	50.26 ± 0.76	45.64 ± 0.36	36.80 ± 0.28	59.88 ± 0.66
Number of trees ₂	24	23	26	23	23	23

Values are expressed as mean ± standard error. D_h , hydraulic diameter; MVA, mean vessel area; NV, number of vessels; EW, earlywood width; LW, latewood width; RW, total ring width. Cambial age in 2013 is shown. The subscripts ₁ and ₂ represent the trees used to assess vessel-related and width-related variables, respectively.

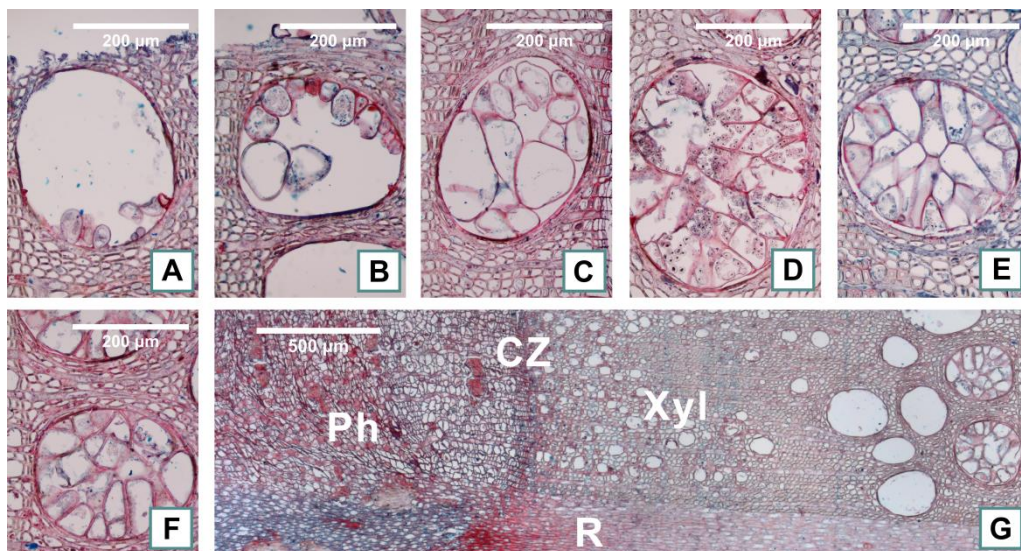


Figure 2 Earlywood vessels of *Q. robur* and *Q. pyrenaica* partially or totally occluded by tyloses. Early-stage tyloses partially blocking vessels (A-B); tyloses occupying the whole vessel section (C-F); tyloses in first-row earlywood vessels of the current growing season (G). Starch granules can be perceived inside the outgrowths (B, D, E). Xyl, xylem; Ph, phloem; CZ, cambial zone; R, ray parenchyma.

2.3 Dendrochronological survey

Trees selected for microcoring were also sampled with an increment borer in September 2013, collecting two wood cores per tree. We selected a few additional trees from those showing a larger size at each site, whereby the total number of sampled trees was 47 at ATL, 46 at ATH, and 49 at MED (Table 1). Stem diameter and tree height were measured for all selected individuals with a diameter tape and a Blume-Leiss hypsometer respectively. Cores were air-dried and mounted on wooden supports, cut with a sledge microtome (WSL, Birmensdorf, Switzerland), and sanded in order to distinguish xylem cells in the transversal section (Gärtner and Nievergelt, 2010). We measured both earlywood and latewood widths by using a sliding-stage micrometer (Velmex TA UniSlide, Velmex Inc. Bloomfield NY, USA). Tree-ring series were visually crossdated, and possible mistakes were detected by applying the COFECHA software (Holmes, 1983).

2.4 Anatomical measurements and cambial age estimation

In wood cores, we removed dust and tyloses inside earlywood vessels with high-pressure water blast. The surface was coloured with black ink, and conduits were refilled with white chalk in order to enhance contrast between vessels and the bulk tissue. We took images of 5184×3456 pixels by using a digital camera attached to a binocular microscope (Olympus SZ60). All images obtained along a radius were subsequently merged to get one image per sample. Image analysis of earlywood vessels was performed on around half of the samples collected at each site with ImageJ (Schneider et al., 2012). This procedure consisted of automatic object recognition on grey scales, which allows the estimation of the detected transversal areas. Vessel outlines were manually corrected when boundaries were not accurately identified. According to recommendations by García-González et al. (2016), both

size ($> 10,000 \mu\text{m}^2$) and circularity (> 0.5) filters were used to prevent measurements on latewood vessels and/or small artefacts in output files.

We calculated the mean vessel area and the hydraulic diameter (D_h) for each tree ring by using earlywood vessel area measurements. D_h was calculated according to the equation: $D_h = \sum_{n=1}^N d_n^5 / \sum_{n=1}^N d_n^4$, where d_n is the diameter of the n conduit within a tree-ring containing n vessels (Sperry et al., 1994). D_h is proportional to the hydraulic conductivity, and in ring-porous species it is thought to be close to the mean diameter of the conduits responsible for approximately 95 % of the total stem conductance (Corcuera et al., 2006). The number of vessels (NV) was also registered, being standardized using a tangential width of 10 mm for each core. Tree-ring width measurements were split into earlywood width and latewood width (LW). In order to assess cambial age, the number of rings was counted from bark to pith in each wood core; when pith was lacking, cambial age was estimated by adapting the graphical method described by Rozas (2003). According to this procedure, we estimated the missing radius length by graphically extending aggregate rays from the innermost ring boundary until convergence, and calculated the number of missing rings by dividing the missing radius length between mean width of the innermost four rings.

2.5 Model calculations

Observations of tyloses were transformed into binomial data and analyzed with generalized linear mixed-effects models (GLMM). Raw series of D_h , NV and LW were used to evaluate differences between species along the gradient. We used linear mixed-effects models (LMM) for Gaussian-distributed variables (D_h), while a GLMM approach was more suitable for Gamma-distributed variables (NV, LW). Effects of 'site', 'species', 'cambial age', 'stem diameter', and 'tree height' were set as the fixed part of the model, Cambial age, stem diameter, and tree height were centred by subtracting the mean. Factor 'radius nested within

tree' was included as a random factor together with factor 'year'. Since we used raw measurements to fit our models, values before 1980 were not considered in order to avoid possible juvenile trends. Variance-inflation factors below five indicated no worrying multicollinearity in our models (Appendix A, Table A.1). Calculations were performed in the package 'lme4' of R version 3.4.0 (R Core Team, 2017).

2.6 Chronology building and relationships with climatic factors

Detection of climatic factors affecting D_h required the extraction of low-frequency patterns mostly associated to non-climatic variation, such as tree age and size (Carrer et al., 2014). We averaged raw series of D_h and NV belonging to the same tree on a year-by-year basis, and a 32-year spline function with a 50% frequency response was fitted by applying algorithms from the ARSTAN software (Cook and Krusic, 2005). Pre-whitening was complementarily applied in order to remove autocorrelation. The strength of the common signal was assessed through the mean correlation between trees, the expressed population signal, and percentage of variance in the first eigenvector. Temporal variability was evaluated in the chronologies by calculating mean sensitivity and the first-order autocorrelation coefficient (Cook and Kairiukstis, 1990).

Monthly gridded time series of maximum and minimum temperatures, and precipitation were used for climate-growth relationships. Time series were taken from the CRU TS3.23 dataset, which are available in the Climate Explorer of the Royal Netherlands Meteorological Institute (<http://climexp.knmi.nl/>). The grid had a resolution of 0.5° longitude $\times$ 0.5° latitude, whereby different climatic time series were applied in each location. Pre-whitened chronologies of D_h and NV were used to calculate bootstrapped correlations for the period 1967–2013. The time window of the correlative analyses comprised from the previous to the current June. Confidence intervals of the Pearson's coefficients were assessed by computing 10,000

bootstrap iterations for each coefficient according to recommendations by Mason and Mimmack (1992). Calculations were performed using a routine written in Embarcadero Delphi 10 Seattle.

3. Results

3.1 Tyloses formation in summer

Tyloses occluding current-year earlywood vessels were firstly observed in May-June, increasing along the growing season, with maximum values in autumn (Fig. 3). Over the period June-September (days of year 170–265), more trees showed tyloses in the drier 2012 than in wetter 2013 (Table 2), as well as at MED than at the temperate locations ATL and ATH (Fig. 3). *Qrob* was more affected by tyloses than *Qpyr* (Table 2). The percentage of *Qrob* and *Qpyr* trees containing occluded vessels at MED in July 2012 was respectively 60 % and 40 %, whereas 25 % and 0 % in July 2013 (Fig. 3). Site, species and year were found to be informative when information criterion scores were calculated (Appendix A, Table A.3).

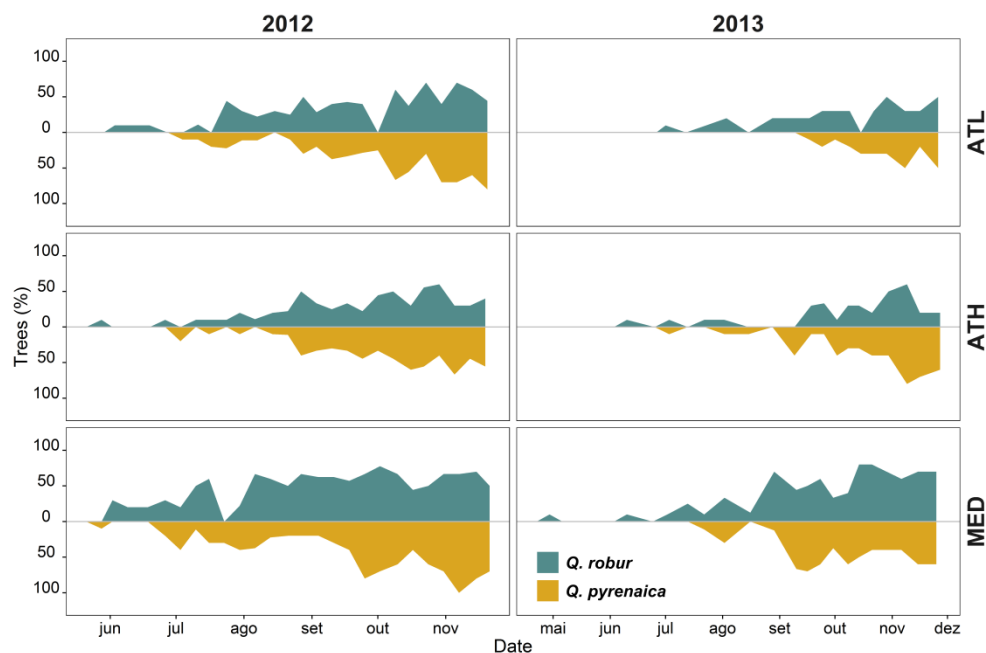


Figure 3 Frequency of trees showing tyloses in last-formed earlywood vessels for *Q. robur* (blue) and *Q. pyrenaica* (yellow) at ATL (Atlantic low altitude), ATH (Atlantic high altitude), and MED (Mediterranean) in 2012 (dry) and 2013 (wet).

Table 2 Statistics of the null and full fixed-effects models for the probability of detecting earlywood vessels blocked by tyloses in summer (days of year 170–265) for 2012 (dry) and 2013 (wet).

	Tyloses frequency	
	Binomial mixed models	
	Probit link function ($N = 1,295$)	
	Null model	Full model
Fixed Effects		
Intercept (<i>Qrob</i> : ATL : 2012)	−1.04 (0.07) ***	−4.43 (0.41) ***
Date		0.02 (0.00) ***
Spp (<i>Qpyr</i>)		−0.36 (0.12) **
Site (ATH)		−0.02 (0.16)
Site (MED)		0.73 (0.15) ***
Year (2013)		−0.55 (0.13) ***
Random Effects		
VC _[Tree]	0.296	0.162
VC _[Residuals]	0.785	0.724
VC _[Fixed effects]		0.423
PCV _[Tree]		70.15 %
PCV _[Residuals]		7.76 %
R^2 (marginal)		0.109
R^2 (conditional)		0.151
AICc	1211.989	1076.589
−log Likelihood	603.990	531.251
BIC	1222.312	1112.666

Significant fixed-effect estimates are highlighted in bold. Standard errors for fixed-effect estimates are provided in parentheses. In the full model, the intercept represents *Q. robur* at site ATL in 2012. *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$. N , number of observations; Spp, species; *Qpyr*, *Q. pyrenaica*; VC, variance components; PCV, percentage change of the variance between the null and full models; $R^2_{\text{GLMM (m)}}$, marginal coefficient of determination; $R^2_{\text{GLMM (c)}}$, conditional coefficient of determination; AICc, corrected Akaike information criterion; BIC, Sawa's Bayesian information criterion.

3.2 Spatial variation of xylem anatomical traits

The mixed-effect models applied on tree-ring series for the period 1980–2013 showed a good fit, attaining a conditional R^2 of 0.64, 0.38 and 0.56 for D_h , NV and LW, respectively (Table 3). Fixed factors explained a considerable amount of the observed variance for D_h and LW, with a substantial change in variance components and information criterion scores from null to full models, but those effects were more limited for NV. *Qpyr* had wider vessels (higher D_h) and more reduced LW than *Qrob*, while differences for NV were not significant between species (Table 3, Fig. 4). Noteworthy, the observed variance in D_h , NV and LW was mostly accounted by differences among sites. Model estimates for D_h were significantly lower at

ATL and ATH than at MED, while LW values at the former location were more reduced than at ATH and MED (Fig. 4). Trees at ATH produced a higher number of earlywood vessels however. As expected, NV and LW declined with tree age, and were higher in fast-growing trees, whereas the effect of cambial age and stem diameter on D_h was negligible (Table 3). The limited influence of tree height on D_h , NV, and LW probably reflected the scarce height variation among trees in our study.

Table 3 Statistics of the null and full fixed-effects models for the hydraulic diameter, number of vessels, and latewood width.

	Hydraulic diameter (D_h) Linear mixed model ($N = 5,269$)		Number of vessels (NV) Generalized mixed model Log link function ($N = 5,269$)		Latewood width (LW) Generalized mixed model Log link function ($N = 9,191$)	
	Null model	Full model	Null model	Full model	Null model	Full model
Fixed Effects						
Intercept (Q_{rob} : ATL)	265.22 (2.77)	247.28 (4.42)	3.63 (0.04)	3.55 (0.06)	0.32(0.06)	0.06 (0.07)
Spp (Q_{pyr})		18.57 (4.00)		−0.04 (0.05)		−0.17 (0.05)
Site (ATH)		3.83 (6.00)		0.22 (0.08)		0.47 (0.08)
Site (MED)		19.32 (5.35)		0.10 (0.07)		0.48 (0.07)
Cambial age		0.14 (0.12)		−0.01 (0.00)		−0.03 (0.00)
Stem diameter		0.54 (0.29)		0.02 (0.00)		0.03 (0.01)
Tree height		0.41 (1.09)		−0.03 (0.01)		0.01 (0.02)
Random Effects						
$VC_{[Tree : Radius]}$	800.169	547.940	0.023	0.017	0.136	0.054
$VC_{[Year]}$	82.276	72.890	0.003	0.002	0.017	0.012
$VC_{[Residuals]}$	506.117	506.118	0.083	0.082	0.295	0.265
$VC_{[Fixed effects]}$		286.706		0.030		0.275
$PCV_{[Year]}$		21.51 %		24.90 %		48.55 %
$PCV_{[Radius]}$		53.11 %		44.74 %		84.12 %
$PCV_{[Residuals]}$		−0.00 %		−0.00 %		−0.00 %
$R^2_{(marginal)}$		0.203		0.228		0.453
$R^2_{(conditional)}$		0.642		0.377		0.563
AICc	48500.126	48444.533	39276.801	39242.219	19005.782	18751.930
−log Likelihood	24246.059	24212.246	19634.397	19611.089	9498.889	9365.953
BIC	48526.397	48510.187	39303.072	39307.873	19034.282	18823.166

Significant fixed-effect estimates are highlighted in bold. Standard errors for fixed-effect estimates are provided in parentheses. In full models, the intercept represents *Q. robur* and site ATL. N , number of observations; Spp, species; Q_{pyr} , *Q. pyrenaica*; VC, variance components; PCV, percentage change of the variance between the null and full models; $R^2_{(marginal)}$, marginal coefficient of determination; $R^2_{(conditional)}$, conditional coefficient of determination; AICc, corrected Akaike information criterion; BIC, Sawa's Bayesian information criterion.

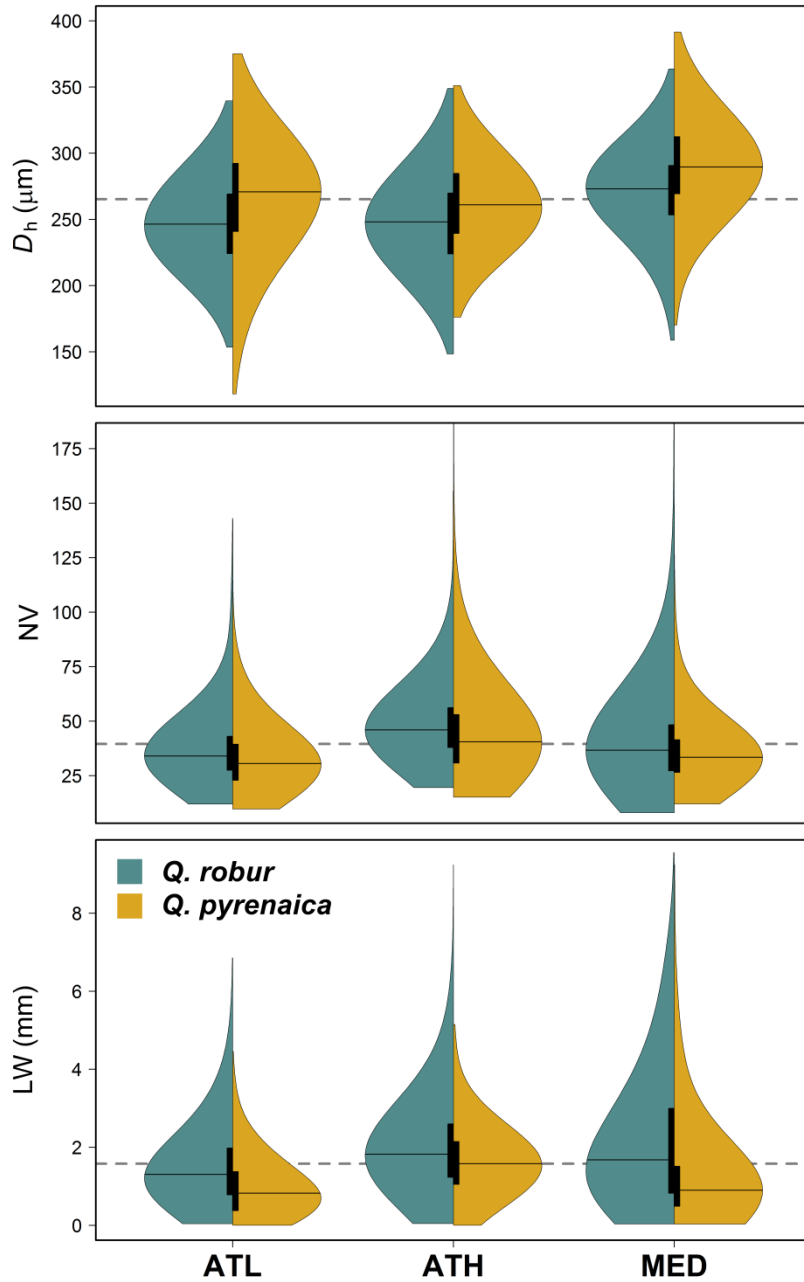
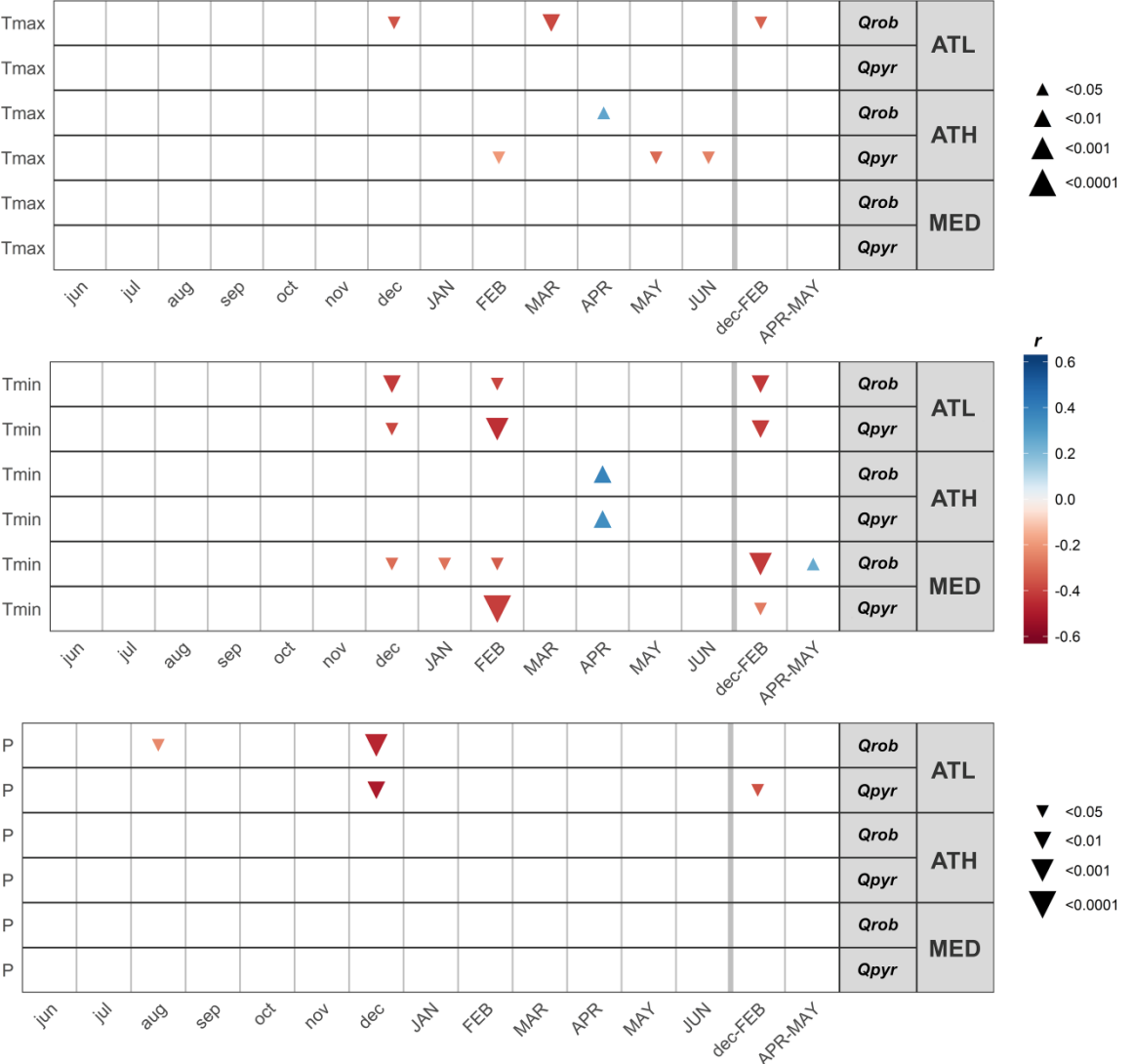


Figure 4 Values of the hydraulic diameter (D_h), number of vessels (NV) and latewood width (LW) for *Quercus robur* (blue) and *Q. pyrenaica* (yellow) in 1980–2013 at sites ATL (Atlantic low altitude), ATH (Atlantic high altitude), and MED (Mediterranean). Horizontal solid lines represent the median, and black boxplots show the extent of 25th and 75th percentiles. Dashed lines represent the mean value for each variable.

3.3 Climatic drivers of earlywood vessel diameter and number

Individual pre-whitened series and average chronologies for D_h are shown in Appendix A (Fig. A.1). Response patterns of D_h to temperature and precipitation were similar in both species, with prevalence of weather conditions during the dormant season (Fig. 5). High

275 temperature in previous December and February elicited low D_h values, namely at ATL and
 276 MED, which frequently exhibit warmer conditions in winter. In contrast, increasing
 277 temperature in April prompted vessel diameter at the colder site ATH, although significant
 278 inverse correlations were present for May and June (Fig. 5). Low D_h values were strongly
 279 related to high precipitation in December at ATL in both species. Overall, responses to
 280 previous summer conditions were negligible. Common signal of D_h chronologies was
 281 stronger at ATH and ATL than at MED (Appendix A, Table A.2).



282 **Figure 5** Summary of the significant correlations of the hydraulic diameter (D_h) with minimum
 283 (Tmin) and maximum (Tmax) temperatures, and precipitation (P) for *Q. robur* (Qrob) and *Q.*
 284 *pyrenaica* (Qpyr) at sites ATL (Atlantic low altitude), ATH (Atlantic high altitude), and MED
 285 (Mediterranean). Red (blue) symbols indicate negative (positive) correlations. Symbol size is
 286 proportional to the significance level of the coefficient. Months of the previous year are represented in
 287 lowercase letters.
 288

4. Discussion

4.1 Environmental effects on earlywood vessel diameter

According to the mixed-effects models applied on our 30-year-long series, cambial age and stem diameter affected NV and LW, but barely influenced D_h , indicating that scaling relationships between tree age and vessel dimensions failed to explain the observed variation in D_h . NV and LW decreased with tree age but increased in large trees, suggesting that tree size and age effects on wood production are not necessarily coupled. Site conditions predicted a significant portion of D_h variability, but contrary to our first expectation, earlywood vessels were wider at the seasonally dry site (MED) than at the humid Atlantic locations. The more delayed onset of xylogenesis reported by Pérez-de-Lis et al. (2016b) at the Atlantic sites as compared to MED partially supports our results because late-reactivated *Qrob* trees may form narrower vessels.

On the contrary, previous studies reported narrower earlywood vessels under dry conditions (Castagnieri et al., 2016; Corcuera et al., 2004; Eilmann et al., 2009; Galle et al., 2010; Gea-Izquierdo et al., 2012), probably as a consequence of the more limited turgor-driven enlargement during earlywood formation (Hacke et al., 2017). Nevertheless, our oaks may not regularly experience drought stress during earlywood formation, as evidenced by the strong inverse correlation between winter precipitation and D_h at ATL. González-González et al. (2015) attributed similar results to possible flood-induced anoxia, which would reduce the amount of carbohydrates available for earlywood formation through enhanced consumption in roots. In this regard, a recent study hints that low sugar content in sapwood at the onset of dormancy may reduce NV in *Qpyr* and delay budburst in both study species (Pérez-de-Lis et al., 2016a). Gričar et al. (2013), by contrast, found similar average vessel diameters in oaks experiencing two different winter flood regimes. Therefore, other factors potentially affecting

cambial activity that were not evaluated in our study, such as site-specific stand structure, local tree density, genetic diversity, and latitudinal variation in daylength, cannot be ruled out.

Warm winters appeared to limit earlywood vessels enlargement at ATL and MED, as it was commonly reported for other ring-porous species (Alla and Camarero, 2012; Castagnieri et al., 2016; Fonti et al., 2007; Gea-Izquierdo et al., 2012). The time window for cell enlargement could be shortened under warm conditions insofar as wall thickening and lignification rates increase with temperature (Cuny and Rathgeber, 2017). Yet, temperature has a dual effect on phenology; whereas chilling requirements are fulfilled by low temperature, warming hastens the onset of growth (Cooke et al., 2012). The latter would be consistent with the positive relation found in April at ATH and MED. On the other hand, Hölttä et al. (2009) reported that irreversibly embolized vessels release water, contributing to tissue hydration. Since water transport is not restored until budburst (Kitin and Funada, 2016), we hypothesize that high temperature preventing freezing-induced embolism could somehow limit water supply to buds, delaying the onset of growth. Moreover, overwintering sugar content may decline under warm conditions because of the enhanced respiratory demand of carbohydrates (Salomón et al., 2016), and the more limited starch hydrolysis (Morin et al., 2007).

Our second expectation cannot be confirmed because the more drought-sensitive *Qrob* had a safer xylem structure (lower D_h) than *Qpyr*. Similar vessel diameter across species were observed by González-González et al. (2015) in a site located near ATH, where inter-specific variation was however more limited. We also found convergent response patterns to climate factors across species. Meanwhile, *Qrob* trees exhibited larger latewood increments than *Qpyr*, with lower values at the humid site ATL than at the more seasonal MED or ATH sites. Differences between species are consistent with previous research (Rozas et al., 2009), and

support the idea that *Qpyr* has a more conservative growth strategy than *Qrob*, despite the more efficient hydraulic network. According to Hietz et al. (2017), a more limited latewood increment in *Qpyr* reduces overall xylem construction costs, while the narrower latewood vessel network is probably offset by the higher earlywood vessel efficiency.

4.2 Tyloses formation in earlywood vessels occurs earlier under drier conditions

Tyloses occluding current-year earlywood vessels appeared in early summer, in a noticeably earlier date than previously reported in oaks (Cochard and Tyree, 1990). As expected, the amount of trees showing tyloses was remarkably higher under drier conditions, namely in summer of 2012 at MED. This suggests that increasing drought levels prompted cavitation events, although larger (more vulnerable) vessels at MED could strengthen differences along the gradient. However, fewer *Qpyr* trees showing tyloses contrasted with their wider earlywood vessels. Hydraulic resistance to drought in ring-porous species probably relies on traits other than earlywood vessel diameter (Pratt and Jacobsen, 2017). Indeed, *Qpyr* exhibits multiple morphological traits limiting dehydration, such as profuse leaf and sprout pubescence, which are absent in *Qrob* (Aranda et al., 1996). In addition, vigorous *Qpyr* trees are able to access water stored in deep soil layers through their extensive root system (Hernández-Santana et al., 2008), but also sharing water with neighbouring individuals thanks to their outstanding root grafting capacity (Salomón et al., 2016).

4.3 The ring-porous structure is favoured under highly seasonal conditions

According to the positive relation between vessel diameter and transport efficiency (Hacke et al., 2017; Sperry et al., 2006), trees regularly experiencing summer drought at MED formed a more efficient earlywood vessels than those under mild oceanic conditions. Under sub-Mediterranean climate, there is a rapid progression from cold to warm weather conditions in spring (Sánchez de Dios et al., 2009). Developing shoots, catkins, and leaves in trees with

362 delayed leaf-out dates would have a lower risk of frost damage (Lenz et al., 2013), but a
363 higher exposition to drought stress (Hernández-Santana et al., 2008). Wider vessels may
364 therefore be an adaptive strategy in highly seasonal environments insofar a higher hydraulic
365 capacity enables rapid flushing. Assuming this hypothesis, wider vessels in the sub-
366 Mediterranean *Qpyr* would be also consistent with a later and faster foliage development as
367 compared to the temperate *Qrob* (Pérez-de-Lis et al., 2016b). Indeed, earlywood vessels of
368 ring-porous trees are assumed to be mostly devoted to sustain foliage development in spring
369 (Corcuera et al., 2006; Hernández-Santana et al., 2008; Taneda and Sperry, 2008), as
370 evidenced by the fact that first-formed vessels become functional around leaf-out dates (Kitin
371 and Funada, 2016; Pérez-de-Lis et al., 2016b).

372 Although increasing vessel diameter implies a higher xylem vulnerability to drought and
373 freezing (Cavender-Bares et al., 2005), LW increment in *Qrob* was maximum at MED, which
374 notably contrasted with the more frequent tyloses formed in this case. This evidence suggests
375 that partial disruption of the earlywood vessel network in summer has a limited effect on
376 subsequent latewood production. Although ring-porous oaks experience 50 % conductivity
377 loss at relatively high water potentials (Corcuera et al., 2006; Tyree and Cochard, 1996; Urli
378 et al., 2015), hydraulic failure in angiosperms only occurs when the loss of conductivity
379 reaches 88 % (Urli et al., 2013). These observations may corroborate the idea that functional
380 latewood vessels can fulfil water demand in summer (Corcuera et al., 2006; Hernández-
381 Santana et al., 2008). If so, the higher capacitance of large earlywood vessels would help to
382 avoid latewood vessels failure (Jupa et al., 2016; Pratt and Jacobsen, 2017).

383 Our results suggest that ring-porous oaks may form larger vessels under markedly seasonal
384 drought conditions unless water shortage in spring directly limit vessel enlargement
385 (Castagnieri et al., 2016; Gea-Izquierdo et al., 2012; but see Villar-Salvador et al., 1997).
386 Ring porosity is associated to other anatomical traits that are considered to be adaptations to

climate seasonality (Zanne et al., 2006). In fact, vessel dysfunction costs may be low in species that entirely rebuilt their earlywood conducting system each spring (Urli et al., 2013). However, the risk of hydraulic failure would dramatically increase under extreme drought episodes (Anderegg et al., 2016; Levanic et al., 2011), namely if latewood vessels are dysfunctional or absent. This is consistent with the aforesaid porosity decline at the intra-specific level reported under more xeric conditions (Castagnieri et al., 2016; Gea-Izquierdo et al., 2012), along with the ongoing replacement of ring-porous oaks by diffuse-porous ones reported in southern Europe (Urli et al., 2015).

4.4 Conclusions

The monitoring of tyloses formation pointed out that irreversible dysfunction of earlywood vessels rapidly progresses under drought conditions in summer, suggesting that latewood vessels are probably essential for water transport in highly seasonal environments with a markedly dry season. Wider earlywood vessels were found in the most drought-tolerant species and at the Mediterranean site, which supports that ring porosity is a strategy to cope with climate seasonality. Climate control of vessel dimensions was reflected by the high D_h sensitivity to winter-spring conditions. In this regard, we need to ascertain whether ring-porous species become more vulnerable in a context of ongoing environmental change. This understanding would be achieved by performing analyses of earlywood vessel series over wide geographical and temporal scales. Our results also convey that the progression of tyloses formation in earlywood vessels can be integrated with other procedures, such as dye injection, to assess the functionality of water-conducting pathways in ring-porous trees experiencing stressful conditions.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found in the online version at <http://www.sciencedirect.com/science/journal/01681923>.

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APPENDIX: Do ring-porous oaks prioritize earlywood vessel efficiency over safety? Clues from short and long-term responses of earlywood vessel anatomy to climate

Gonzalo Pérez-de-Lis, Vicente Rozas, Rosa Ana Vázquez-Ruiz and Ignacio García-González

Table A.1 Variance-inflation factors evaluating multicollinearity in the mixed-effects models assessed for the hydraulic diameter, number of earlywood vessels and latewood width.

	Hydraulic diameter (D_h) ($N = 5,269$)	Number of vessels (NV) ($N = 5,269$)	Latewood width (LW) ($N = 9,191$)
Spp (Q_{pyr})	1.1071	1.1035	1.1452
Site (ATH)	2.1249	2.1248	2.1337
Site (MED)	1.8514	1.8144	1.7280
Cambial age	1.8054	1.7687	1.8991
Stem diameter	2.0410	2.0418	2.1097
Tree height	2.5701	2.5809	2.9206

591 The intercept is *Q. robur* at ATL. Spp, species; Q_{pyr} , *Q. pyrenaica*.

Table A.2 Descriptive statistics characterizing the common signal of the standardized chronologies for the hydraulic diameter (D_h).

	Rbt	EPS	PC1	MS	AR1
<i>Q. robur</i>					
ATL	0.30	0.80	38.79	0.04	0.32
ATH	0.40	0.86	47.16	0.04	0.16
MED	0.17	0.75	25.75	0.03	0.14
<i>Q. pyrenaica</i>					
ATL	0.37	0.84	45.29	0.05	0.13
ATH	0.51	0.90	56.85	0.04	-0.16
MED	0.26	0.85	34.28	0.04	0.12

Rbt mean correlation between trees, EPS expressed population signal, PC1 percentage of variance in the first eigenvector, MS mean sensitivity, AR1 first-order autocorrelation coefficient.

Table A.3 Selection of the potential variables affecting the probability of detecting tyloses in summer (days of year 170–265) for 2012 (dry) and 2013 (wet).

Fixed effects	Random effects	<i>df</i>	–log-Lik	ΔAICc
Date + Site + Spp + Year	Tree	7	531.25	0.00
Date + Site $\times$ Spp + Year	Tree	9	529.70	0.94
Date + Spp + Site $\times$ Year	Tree	9	530.07	1.69
Date + Site + Spp $\times$ Year	Tree	8	531.16	1.85
Date + Site $\times$ Spp + Site $\times$ Year	Tree	11	528.40	2.42
Date + Spp $\times$ Site + Spp $\times$ Year	Tree	10	529.61	2.80
Date + Site $\times$ Year + Spp $\times$ Year	Tree	10	530.02	3.63
Date + Site $\times$ Spp + Site $\times$ Year + Spp $\times$ Year	Tree	12	528.37	4.40
Date + Site $\times$ Spp $\times$ Year	Tree	14	526.35	4.45
Date + Site + Year	Tree	6	535.52	6.52
Date + Site $\times$ Year	Tree	8	534.54	8.61
Date + Site + Spp	Tree	6	540.56	16.60
Date + Site $\times$ Spp $\times$ Year	<i>Null</i>	13	533.59	16.87
Date + Site $\times$ Spp	Tree	8	539.32	18.17

Generalized mixed models for binomial data were fitted. Spp, species; *Df*, degrees of freedom; ΔAIC , difference between each model and the best-fit one for the Akaike Information criterion score. Selected variables are those contained in the model with the lowest ΔAICc .

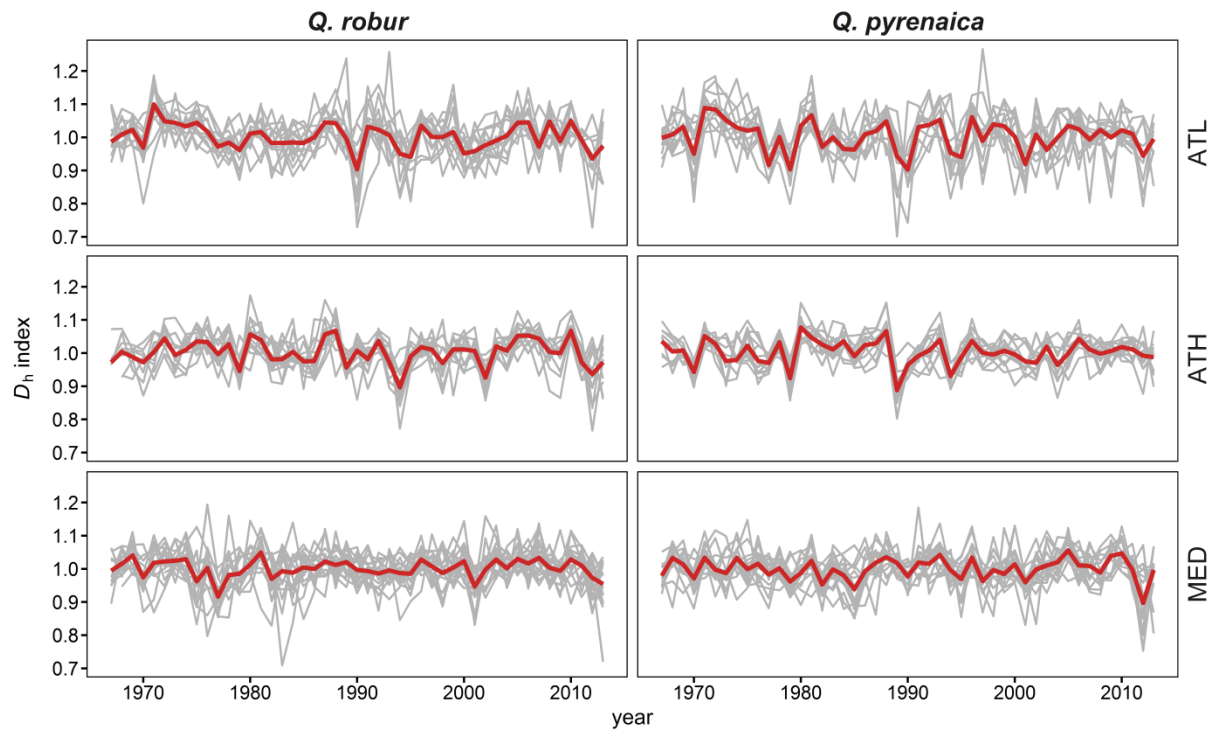


Figure A.1 Individual pre-whitened chronologies (grey lines), and average chronology (red line), of the hydraulic diameter (D_h) in the period 1967–2013 for each species at sites ATL (Atlantic low altitude), ATH (Atlantic high altitude), and MED (Mediterranean).