Response patterns of xylem and leaf phenology to temperature at the southwestern distribution boundary of *Quercus robur*: A multi-spatial study

Authors: Guillermo Guada[1], Rosa Ana Vázquez-Ruiz[1], Ignacio García-González[1]

[1]Departamento de Botánica, Universidade de Santiago de Compostela, Campus Terra, Escola Politécnica Superior de Enxeñaría, Lugo 27002, Spain.

Author for correspondence:

Guillermo Guada Prada

ORCID iD: 0000-0001-9579-543X

Escola Politécnica Superior de Enxeñaría

Universidade de Santiago de Compostela

Campus Terra, 27002 Lugo (Spain)

Tlf:+34 982822491

E-mail: guillermo.guada@gmail.com

Abstract

We investigated how temperature patterns affect cambial activity and leaf phenology of oak across a wide range of natural woodlands at its southwestern distribution boundary. Understanding the climatic control of wood formation in dominant species is very relevant to infer tree responses to ongoing environmental changes and their impact on the carbon cycle.

We selected nine sites along two elevation gradients from the coastline in northwestern Iberia, and sampled ten trees per site biweekly during 2012 and 2013. Leaf and cambial phenological phases were related to mean air temperature for 10-60 day running periods along the year to identify the most relevant time windows for cambium and leaf phenophases, and the relationships among them.

The first earlywood vessels expanded before the appearance of small leaves, and subsequently underwent maturation to meet water requirements for full leaf unfolding. The advance or delay of cambial reactivation and budburst varied among sites and years modulated by spring temperature, and were respectively maximized by maximum and minimum values.

Temperature can modify the onset of early phenophases of primary and secondary growth differently, and also the synchronicity between them. However, the maturation of the first earlywood vessels is necessary to undergo full leaf extension.

Keywords: budburst, cambium, earlywood, latewood, ring-porous wood, xylogenesis.

1.Introduction

Quercus robur L. is a nemoral tree species widely distributed under different climatic conditions throughout Europe (Gilliam, 2016), from the Baltic Sea to its southern distribution boundary in the Iberian Peninsula. Along this boundary, the transition to the Mediterranean region involves its progressive replacement by more drought-tolerant species (Sánchez de Dios et al., 2009), first nemoral oaks, and evergreen oaks under more limiting conditions.

One of these transitional areas is located in northwestern Iberian, where prevailing Atlantic conditions involve high precipitation records mainly during autumn and winter, but summer drought can often occur as a consequence of Mediterranean influence. In coastal areas, winter temperature is not limiting, but warm summer conditions facilitate the occurrence of thermophile species, or even subtropical elements where summer drought is not limiting (Izco et al., 1990). As a result, trees need to couple their patterns of phenology and wood formation to these meteorological conditions.

Activity of primary and secondary meristems follows a distinct annual pattern in crown and cambium, respectively. These processes are constraint to a well-defined time period from spring to autumn, when conditions are favorable for growth and reproduction, whereas trees enter dormancy during winter. Spatial and temporal changes in environmental conditions can modify tree phenology (Menzel et al., 2006; Vitasse et al., 2017), as species adjust the timing and length of their growth and reproduction phases to climate, with variations that depend on specific regional drivers, local adaptations, or individual plasticity (Rossi et al., 2013). Though also interacting with photoperiod (Basler and Korner, 2014), phenological events related to growth resumption are mainly driven by temperature (Begum et al., 2013; Rossi et al., 2016), and consequently changes related to the ongoing global warming have been observed (Menzel et al., 2006).

Q. robur, as a deciduous ring-porous hardwood species, needs to yearly renew its xylem vessel network, so that the cambium must build the mechanical and conductive support to fulfill water requirements from roots to leaves. The main pathways of sap flow are the networks of earlywood vessels of the current year, because those from the previous season are no more functional after a year (Chaney and Kozlowski, 1977; Ellmore and

Ewers, 1986; Umebayashi et al., 2008). The differentiation of current year's earlywood vessels has been detected to take place before the onset of bud growth (Lavrič et al., 2017; Pérez-de-Lis et al., 2016; Puchałka et al., 2017; Sass-Klaassen et al., 2011; Takahashi et al., 2015). However, the importance of their contribution for crown development is still under discussion (Kudo et al., 2018). Recent studies on the specific moment when the networks of vessel elements become functional as conduits for water movement suggest that the requirements of the early stages of leaf expansion can still be fulfilled by latewood vessels formed in previous years (Kitin and Funada, 2016; Kudo et al., 2015). Therefore, it is necessary to understand not only the mechanisms of wood formation, but also physiological aspects of the tree growth.

Variations in the meteorological conditions prior or/and at the moment of specific phenological events can affect the synchronization between primary and secondary growth in a different way. Buds, leaves, and flowers that grow from primary meristems are easily visible to the naked eye, and numerous records allow assessing variations in the timing of leaf phenology. For example, Rossi (2015) found that temperature is a predominant factor driving the ecotypic differentiation of budburst in black spruce. Recent studies on different oak provenances in England (Wilkinson et al., 2017) reported an advance in spring budburst across a range of temperatures, and Čufar et al. (2012) found that March and April temperatures were related leaf unfolding in beech (*Fagus sylvatica*) in Slovenia. During the growing season, vascular cambium cannot be directly observed to record phenological events, but in the last decades, a great effort has been made to identify stages of wood formation at a cellular level, although this monitoring of xylogenesis is a very time-consuming method.

In conifers, linear and nonlinear patterns of timings and duration of wood phenology showed local annual temperature as the main driver of cambial activity in the northern hemisphere (Rossi et al., 2016; Rossi et al., 2013). Similarly, a phenological advance in xylem phenology linked to temperature was found to modify the production of wood biomass in northeastern France (Cuny et al., 2015). However, the study of xylogenesis in hardwoods has deserved less attention. In the case of ring-porous oaks (*Q. robur* and *Q. pyrenaica*), Pérez-de-Lis et al. (2017) demonstrated that the length of the growing season was modulated by predisposing the number of dormant cambium cells, whereas the size of the first earlywood vessels was affected by the timing of earlywood enlargement (Pérez-de-Lis et al., 2016). The reactivation of cambial cells by heat

65 treatment was also proven for deciduous species (Begum et al., 2013; Kudo et al.,
66 2014); similarly, differences in the yearly onset can be explained by temperature
67 variations (Prislan et al., 2013).

68 Although there is much evidence that temperature is one of the main driving forces for
69 plant growth in terrestrial ecosystems, its influence on wood formation has been mainly
70 analyzed on conifers, often at high latitudes or elevations (Lorena et al., 2016; Rossi et
71 al., 2016), whereas studies on cambial dynamics of temperate hardwood species such as
72 oaks are limited to very few sites (Lavrič et al., 2017; Pérez-de-Lis et al., 2017;
73 Puchałka et al., 2017). On the other hand, several studies dealt with the variation of
74 phenology across gradients, but were not linked to secondary growth (Vitasse et al.,
75 2017). In order to fill this gap, we intend to evaluate the influence of the temperature
76 regime on the primary and secondary growth of native *Q. robur* forests within a region
77 that covers broad environmental gradients. For this, we compiled a data set of cambial
78 dynamics and leaf phenological phases at nine sites during 2012 and 2013, and related
79 their different phases to temperature. These sites were located towards the most
80 southwestern distribution boundary of oak, and represent Atlantic and Cantabrian
81 influences, from mild coastal areas to high continental inland, which to our knowledge
82 provides the widest range of these data currently available for oak. The aim of our study
83 is to establish the role of temperature on wood formation and crown development by i)
84 comparing the timings of cambial activity and leaf phenological phases, and ii)
85 evaluating how these relationships vary along different microclimatic gradients.

86 **2. Material and Methods**

87 **2.1 Study site and tree selection**

88 The study was conducted at nine Q. *robur* stands in northwestern Iberia (Table 1). The
89 selected sites covered the course of two rivers, Eume (E) and Sor (S), from their upper
90 watershed to their mouths into the Atlantic Ocean or the Cantabrian Sea respectively
91 (Fig. 1a). The area presents a mild Atlantic climate, with a mean annual temperature
92 ranging 9-13 ºC among sites, and a total precipitation of 900-1,500 mm; maximum
93 rainfall occurs during autumn-winter, and there is a varying degree of summer drought
94 depending on elevation or position towards the coast. Oak forests in this area are

characterized by moist and warm conditions, which even result in the occurrence of some subtropical plants (Izco et al., 1990), especially at low altitude.

Within each watershed, we followed an altitudinal gradient that covered the whole species distribution, with increasing degrees of precipitation with elevation, and continentality from the coast to the inland mountains. Nevertheless, these two rivers represent two dominant climatic influences on oak formations in northwestern Iberia. Whereas the Atlantic coast is dominated by a certain Mediterranean trend during summer (higher temperature, more intense drought), the Cantabrian coast is exposed to mild northern winds that increase humidity and attemperate maximum temperature (Martínez Cortizas and Pérez Alberti, 1999).

We monitored these forests for two consecutive years, from March to November in 2012 (1,175 trees sampled), and from February to September in 2013 (1,000 trees sampled). For this, 90 trees (9 locations × 10 trees) with a diameter of 20-40 cm were randomly selected at each sampling date within an area of ca. 2 ha per forest. The sampling interval was 12-15 days in spring, and 20 days in summer, because changes during earlywood formation or transition to latewood take place much faster than later in the season. Trees selected were dominant or codominant in the canopy, and individuals with polycormic stems, partially dead crowns, or evident damage, were avoided. The random selection of trees at each date prevented the study at the individual tree level, but provided a representative sample of the whole forest as a single population, avoiding the bias of a specific tree. In addition, this strategy increased the number of individuals that could be analyzed to relate primary and secondary growth, i.e., to associate leaf and cambium phenophases.

2.2 Site meteorological data

Air temperature was hourly monitored at each site during the sampling period using sensors (iButton DS1922L, San Jose, CA, USA) set at 2 m above the ground surface. We calculated daily mean, maximum, and minimum values, as well as the corresponding thermal amplitudes. Temperature time series were corrected to complete missing data by simple linear regression with daily records from nearby meteorological stations belonging to the weather service Meteogalicia (http://www.meteogalicia.gal/). We compared all study sites according to their monthly mean temperatures for both

study years using hierarchical cluster analysis. The Euclidean distance was selected to calculate dissimilarities among sites, whereas the average linkage was used as grouping method (Fig. 1b).

2.3 Xylem sampling and wood phenophases

At each sampling date, a minimum of two microcores (2 mm in diameter, 15 mm in length) was extracted at breast height (1.3 m) out of each selected tree by means of a Trephor tool (Rossi et al., 2006), in perpendicular direction to the slope. Samples contained mature and developing xylem of the current year, the cambial zone and adjacent phloem, and at least one previous complete tree ring. After extraction, one microcore per tree was processed by embedding in paraffin, cutting into thin sections with a rotary microtome, and staining following the same protocol as in previous works (Guada et al., 2018; Pérez-de-Lis et al., 2016).

Observation and width measurements of the wood phenophases of the 2,173 microcore cross-sections were performed on images taken with a digital camera (Canon EOS 600D, Tokyo, Japan), coupled to a transmitted light microscope (Olympus BX40, Tokyo, Japan); a white light polarizing filter allowed the detection of secondary cell wall deposition (40 × magnification). We measured the width of cell expansion and maturation zones along three radial lines per image (Fig. S1); and each phase of cambial activity was expressed as particular day of year (DOY), including: beginning of cell enlargement in the earlywood (bE), beginning of earlywood maturation (bM), beginning of latewood maturation (bLW), and cessation of cell expansion (cE). The beginning of cell enlargement was considered when the most recent ring contained at least one enlarging cell; whereas the beginning of earlywood vessel maturation was defined by the deposition of the secondary cell wall in the vessels, detected by birefringence under polarizing light; the cessation of cell expansion corresponded to the moment when no expanding cells were further detected, and the beginning of latewood maturation was defined as the moment when earlywood growth had already ceased, and maturation was detected in cells formed beyond the earlywood.

The durations between the onset and cessation of these phases were also calculated for cell expansion (dE = cE − bE), earlywood maturation (dEW = bLW − bM), latewood

maturation (dLW = cE − bLW), and cell expansion of first row of earlywood vessels (d1r = bM − bE).

2.4 Leaf phenological observations

Leaf phenology of each sampled tree was recorded in the field. Observations were performed at the upper main branches using binoculars (10 × magnification), and expressed as DOY.

We considered four different phenophases, namely budburst (BB), leaf unfolding (LU), appearance of small leaves (SL; leaves < 50% of their apparent final size), and full extension (FL; leaves > 50% of their apparent final size). Bud dormancy corresponded to the overwintering stage, while bud swelling was identified by the apparent separation of buds from the stem, together with the exposition of areas of lighter colored tissue as a result of the initial extension of cataphylls with the separation of scales. Budburst was characterized by green-colored expanded buds with no unfolded leaves, and leaf unfolding ended as soon as the leaf blade was clearly visible, but not the petiole. Appearance of small leaves was defined as the moment when at least one leaf was completely out of the bud, and current year twigs and petiole could be visually appreciated; and full extension was recorded when leaves attained at least the 50% of their apparent final size.

2.5 Statistical analyses

In order to assess the critical dates of xylem and leaf phenology, the information on the absence or presence of each phenological event per site and year was expressed as binary data (0 no active phenophase; 1 active phenophase). Afterwards, we performed logistic regressions by GLM for each phenophase using the DOY as independent variable, and considering a probability of 0.5 as the most likely DOY for the activity of a given event (Rathgeber et al., 2011). The effects of year, site, and their interaction were studied by comparing the best logistic regression fit when these factors were included isolated or in combination as classification variables. Independent variables considered in the models tested were as follows: DOY + year; DOY+ site; DOY + year + site; DOY + year × site. The model providing the lowest corrected Akaike's Information Criterion (AICc) for each event was selected as the best fit, and a multiple comparison between years and sites was performed using the Tukey test.

Relationships among the dates of wood phenological events estimated from the logistic regression and their durations were related using Pearson's correlations. The DOYs of cessation of cell expansion among sites diverged significantly from normality, and were transformed using the Y^2 formula.

We used two approaches to establish relationships between primary and secondary growth at tree scale, and specifically to look for a possible link between vessel formation and budbreak. First, we selected the trees achieving the expansion of first row of earlywood vessels, i.e., those showing enlarging vessels adjacent to the previous annual ring, but still no maturation, and classified them according to their leaf phenology in order to obtain the proportion of trees that started crown development before earlywood maturation. The second approach consisted of selecting the trees with already mature earlywood vessels for each phenological crown phase, and consequently we established the proportion of trees that had started earlywood maturation for each stage of leaf development.

In order to detect the time window that influenced primary and secondary growth, we obtained moving averages of climatic data, and compared them to the DOYs of each phenological event along the growing season. Thus, we preliminary used 30-day averages of maximum and minimum temperatures, as well as thermal amplitude, and correlated them to each phenological event every 15 days. A more detailed analysis of the most relevant variables involved running temperature means of 10 to 60 days, shifted in only one day. For these analyses, we assumed that the increase of temperature advanced along with tree growth, and performed a linear model between the temperature before the event and the timing of each event per site, including the year as class variable. For each linear model, the *p*-value of a Type I test, and the corresponding percentage of variation explained, were provided for the temperature effect and for the difference between years. We also estimated temperature thresholds from those in the DOY predicted by the GLM logistic curve per site and year. All statistical analyses were performed using R statistical software (R Core Team, 2017).

3. Results

3.1 Temperature across sites

The sites had a temperate regime, with an average variation of annual mean of 3.8 ºC across the whole area, from 10.1 ºC at S5 (continental influence) to13.8 ºC at E1 (Atlantic influence). All sites (Table 1) exhibited a mild winter with an overall range of daily mean temperatures from -0.1 ºC to 15.4 ºC, respectively corresponding to the continental sites and to the sites closest to sea level; values below 0 ºC were only one day in February at the two most continental and highest locations. The minimum absolute temperature of all sites ranged from -3.5 to -0.25 ºC, with a period below 0 ºC of only four days at the most Atlantic site (E1), and up to 30 days under continental influence (E4). The maximum absolute temperature ranged between 25 ºC in July at S3, and 41 ºC in June at E1.

The sites were grouped into three main clusters based on their monthly mean temperatures of the two study years (Fig. 1b). The first cluster included only one site (E1), namely the lowest location (100 m a.s.l.) at the Atlantic side, where the monthly mean temperature ranged between 7.8 and 21 ºC. The second cluster held the lowest Cantabrian site S1(100 m a.s.l.), and all sites at mid elevation (200-500 m a.s.l.), regardless of being at the Atlantic or Cantabrian side; the average temperature range in this group was from 5.6 to 19 ºC. The remaining locations belonged to the third cluster, and included the two highest sites (650 m a.s.l.) of both watersheds, with monthly mean temperatures ranging from 4.6 to 17.6 ºC, being the coldest and most continental sites.

As regards differences between the altitudinal gradients along both rivers, mean monthly temperatures differed more among the locations on the Atlantic side (western exposure) than those towards the Cantabrian sea (northern exposure). There were also differences between both study years, mainly an increase in March maximum temperature in 2012, yielding an increment of the thermal amplitude due to the absence of precipitations (Fig. S2).

3.2 Relationships between wood phenological timings and the duration of wood formation

Many of the dates and durations of cambium phenological events were correlated to each other (Table 2). Dates of detection of the first enlarging earlywood vessels were positively correlated with those of the beginning of maturation in both earlywood (r= 0.821; $P < 0.001$), and latewood ($r = 0.560$; $P < 0.05$). Thus, a late onset of vessel

enlargement was associated to a delayed onset of vessel maturation. In contrast, no correlation was observed between beginning of cell enlargement and its cessation of expansion in latewood.

The duration of cell enlargement was negatively correlated to its onset (r = –0.478; P < 0.05), and positively to the date of cessation of cell expansion (r = 0.791; P < 0.001), with a higher correlation for the latter. The duration of earlywood maturation was positively correlated to the beginning of latewood maturation (r = 0.665; P < 0.01), while there was no correlation to the onset of cell maturation or earlywood cell enlargement although the present definition of latewood maturation has to be bear in mind. The duration of latewood had a strong positive correlation to both duration (r = 0.808; P < 0.001) and cessation (r = 0.726; P < 0.001) of cell expansion, while it was negatively correlated to the duration of earlywood formation (r = –0.700; P < 0.01), and to the beginning of latewood maturation (r = 0.703; P < 0.01). Consequently, the duration of cell enlargement and latewood maturation were more closely associated to the cessation than to the onset of cell enlargement.

3.3 Phenological timings of xylem and leaves

The best fit of the logistic regression for all events, except for the cessation of xylem growth (cE), was achieved by the models including the interaction between year and site (Table 3). This indicates that the effect of the year on the delay or advancement of xylem and crown growth varied across sites. For the cessation of xylem growth, the best fit was provided by the model with year and site as independent main effects.

The beginning of cell enlargement started on average 10 days earlier in 2012 than in 2013, but while the differences were significant at six out of the nine sites (E1, E3, E4, S2, S4, and S5), ranging from 23 days at E4 (Z = –3.17, P < 0.01) to 11 days at S2 (Z = –2.34, P < 0.05), this was not the case for the other three sites (E2, S1, and S3), which ranged from one day at S3 (Z = 0.34, P = 0.73) to five days at S1 (Z = 1.03, P = 0.30).

The onset of cell maturation, which is linked to that of cell enlargement, also started on average six days earlier in 2012 than in 2013, although differences were only significant at three sites (E1, E3, and S5), and ranged from 15 days at S5 (Z = –2.59, P < 0.01) to 20 days at E3 (Z = –2.92, P < 0.01). At the other six sites (E2, E4, S1, S2, S3, and S4)

the differences between years ranged from one day at S4 (Z = 0.17, P = 0.86) to nine days at E4 (Z = –1.53, P = 0.13).

With regard to leaf phenology, budburst occurred on average 20 days later than the beginning of cell expansion in 2012 (P< 0.001), but synchronously in 2013 (P = 0.57). Differences in the onset between years were significant at five sites (E4, S2, S1, S3, and S4), ranging from 12 days at S4 (Z = –3.4, P< 0.001) to 29 days at S2 (Z = 6.46, P < 0.001), while they were only from one day at E3 (Z = –0.16, P = 0.87) to seven days at E2 (Z = 1.51, P = 0.13) at the other four sites (E1, E2, E3, S5).

The onset of leaf unfolding was synchronous with that of earlywood maturation (bM) in 2012 (Z = 0.03, $P \approx 1$). Differences between years were only non-significant at S1 (13 days, Z = 2.41, P = 0.01) and S2 (11 days, Z = 2.21, P = 0.02), while the appearance of small leaves was synchronous with earlywood maturation in 2013 (Z = 0.579, P = 0.98), and did differ between years (Z = –0.028, P = 0.97).

3.4 Relationship between primary and secondary growth

Although the chronology of phenological events derived from the logistic regression provided some information on the relationship between primary and secondary growth, the random selection of trees per date allowed a further insight into this relationship, with a high number of samples at tree level.

A total of 314 individuals from the whole data set showed the cambial phenophase ‘cell expansion’, but had not started earlywood vessel maturation yet; we consequently calculated the proportion of these individuals with different crown phenophases (Fig. 2a). Out of the 174 trees sampled in 2012, only one showed expanded leaves (more than 50% of their apparent final size), while there were eight trees out of 140 in 2013. The number of trees at earlier phenological phases of crown development was higher, reaching 42 at budbreak in 2012, and 61 in 2013, while even a higher number (107 in 2012; 30 in 2013) still presented close buds.

Our second approach aimed at defining which proportion of trees with mature earlywood vessels showed each of the different crown phenophases, out of a total of 750 observations (Fig. 2b). The results showed an increasing proportion of trees with mature cells during the successive phenophases of crown development. The evolution

was similar in both years, and trees with visible leaves were those that had a greater proportion of mature earlywood cells.

These analyses point to a synchronization between primary and secondary growth, as practically no expanding leaves existed at the moment earlywood cells began to enlarge. A percentage of 91% out of 314 trees enlarging the first cells did not show any phenological phases that were more advanced than leaf unfolding, whereas ca. 75% of 103 trees had begun the maturation of the first row of vessels concomitantly to the appearance of small leaves.

3.5 Temperature and tree growth relationships

The sole mean annual temperature did not explain the differences in the onset and cessation of primary and secondary growth among these nearby sites, but the adjusted R^2 of their linear regression with progressive monthly temperatures highlighted the relevance of certain periods prior to the phenological events (Fig. 3, Fig. 4). The adjusted R^2 ranged between 0.00 and 0.61; the highest values corresponded to the period between the second fortnight of February and the second fortnight of April for maximum, minimum, and mean temperatures on the beginning of cell enlargement; and from mid-March to mid-April for the effect of minimum temperature on the beginning of budburst (Fig. 4). On the contrary, the cessation of cell enlargement did not show any significant correlation. The thermal amplitude was only significant for the beginning of cell enlargement, from February to the second fortnight of April, which is the period with the greatest variations between years, as can be seen for March temperatures (Fig. S2).

In order to verify if these variations appeared to drive the start of the active period, the onset of each phenological event was related to March temperature at each site, including the effect of year as a fixed factor (Table 4). For all linear regressions, the interaction between year and temperature was not significant, suggesting that the response to temperature was similar in both years.

Budburst was not affected ($P > 0.05$) by maximum temperature from mid-March to mid-April, which only explained 15% of the total variation, while mean temperature explained 47% and 53% of total and the within-year variation (Table 4). Early-spring minimum temperature explained 56% of the variation in the onset of budburst among

sites, which averaged 107 ± 12.8 and 98 ± 9.3 DOY in 2012 and 2013, associated to mean minimum temperatures of 5.3 and 5.8 ºC respectively. Dates of budburst predicted by these temperatures were 107 ± 2.5 and 104 ± 2.5 DOY in 2012 and 2013 respectively, decreasing by 7.22 days $^{\circ}C^{-1}$ (Table 4). The beginning of the successive phenophases (leaf unfolding, leaves < 50% of their final size, and > 50% of their final size) was also explained by mid-March to mid-April temperature, because the delay in budburst affected the successive leaf phenophases (Fig. 3).

March maximum temperature explained 62% of the variation in the onset of cell enlargement, which averaged 86 ± 7.8 and 98 ± 7.7 DOY in 2012 and 2013 respectively. The predicted values were 86 ± 1.9 and 97 ± 1.9 DOY, advancing by 2.82 days $^{\circ}C^{-1}$ (Table 4), for mean maximum temperatures in March of 15.8 and 11.7 ºC in 2012 and 2013.

The beginning of latewood cell maturation was also explained by March temperature (Table 4), because the delay in cell enlargement affected the onset of cell maturation and latewood, as shown by the strong correlation between both events (Table 2 and Fig. 5). However, the interval between these two phases, which is the duration of enlargement of the first row of vessels (d1r), was neither correlated ($P > 0.05$) to the onset cell expansion nor to maturation (Table 2). Nevertheless, the linear model showed that 43% of the variation recorded in this interval (d1r) was explained by the average maximum temperature during its duration (April) with an advance of 2.22 days $^{\circ}C^{-1}$ (Table 4). The average maximum temperature in April across all sites was 11.3 ºC in 2012, and 13.3 ºC in 2013; and predicted durations of enlargement of the first vessels row were 32 ± 1.5 and 28 ± 1.5 days, with recorded averages of 33.1 ± 5.9 and 27.6 ± 2 days, respectively.

The duration of maturation of both earlywood ($P > 0.5$) and latewood could not be explained by the variation in temperature, although latewood duration tended ($P = 0.05$) to respond to October maximum temperature (Table 4), probably pointing out the existence of a late pulse of growth in autumn if weather conditions permit.

As a result, it seems that the thermal influences of late winter (March) are transferred to the successive phenological events of primary and secondary growth, because one phenological phase leads to comparable shifts in the successive phases (Fig. 5).

4. Discussion

4.1 Linear patterns between events and durations of xylogenesis

The correlations between xylem timings and durations let us verify the existence of common relationships, and also support the evidence of internal and external (mainly thermal) drivers. Wood growth dynamics seemed to be homogeneous among sites, and the sequence of dates of successive phenological timings and durations are closely interconnected.

The beginnings of the different recorded events of xylogenesis (bE, bM, bLW) were positively correlated. This was somehow expected, since they are consecutive timings where the first phenological phase (bE) leads to comparable shifts of the successive phases, as previously reported for *Q. pyrenaica* and *Q. robur* under temperate climates by Pérez-de-Lis et al. (2016), and for conifers in cold environments (Rossi et al., 2013). In contrast, the onset and cessation of cell enlargement were not directly related. To this respect, it should be noted that onset and end of xylogenesis involve different parts of the xylem, i.e., the first and the last cell produced (Rossi et al., 2013). Nevertheless, both phenological events showed significant correlations with the duration of cell enlargement. Although cessation of xylem growth explained more variation in the relation between events and durations (62%) than the beginning of cell enlargement (23%), the latter becomes important because the variation of spring phenology is more closely linked to favorable conditions for photosynthesis than variations in autumnal growth season (Chuine, 2010).

The duration of vessel expansion in the first row as associated to spring temperature (mainly maximum), but was neither correlated to the onset of cell enlargement nor to the beginning of cell maturation. These results do not support the existence of a causal relationship between overall duration of vessel expansion and the dates of their onset and cessation. However, Pérez-de-Lis et al. (2016) found that longer periods of vessel expansion were related to higher hydraulic diameters in the first row of vessels for *Q. robur*, while Souto-Herrero et al. (2017) analyzed long vessel chronologies of *Q. robur*, showing that environmental conditions in early spring were also linked to vessel diameter in this first row. Consequently, we suggest that it is spring temperature that controls the duration of vessel expansion, which in turn determines the final vessel size.

4.2 Primary and secondary growth relationships

There is an increasing interest in the relationships between primary and secondary growth in ring-porous trees (e.g., Sass-Klaassen et al. (2011)), in order to understand the influence of climate on the development of earlywood vessels, because it has been shown to be a powerful dendroclimatic proxy (Souto-Herrero et al., 2017). This linkage between crown phenology and intra-annual dynamics of xylem formation has been investigated for different ring-porous hardwood species, as summarized by Kitin and Funada (2016). According to the literature, the enlargement of the first vessel elements starts before budburst, but their maturation onset differs among studies (Kudo et al., 2015; Pérez-de-Lis et al., 2016; Puchałka et al., 2017); in fact, Puchałka et al. (2017) attributed such differences to genetic variability. But the number of individuals was very limited in previous studies; in contrast, our study based on a sample size of 750 trees randomly selected during spring in two years. We observed that only 38-46% of the individuals had started earlywood vessel maturation at the moment of leaf unfolding, and even most of the trees with small leaves (76-85%) had already undergone the maturation of the first vessel row. In addition, out of a sample of 314 trees with earlywood cell enlargement and still no secondary wall, more than the half had not achieved budburst yet (75% in 2012, 56% in 2013), and a small proportion (14% in 2012, 22% in 2013) was at the stage of budburst; later leaf phenophases were hardly detected at this stage of vessel enlargement.

In view of these findings, we hypothesize that the newly-formed vessels are not capable of supplying enough water for bud swelling and leaf unfolding in most trees yet. In this sense, Pérez-de-Lis et al. (2016) also reported that secondary wall deposition was not initiated at the moment of budburst in some *Q. robur* trees, Kudo et al. (2015) found that the first earlywood vessels were completed along the entire stem only when small leaves were visible to the naked eye in *Q. serrata*; and Guada et al. (2018) described the maturation of the first vessel row to be concomitant with the leaf phenophases having the greatest demand for water, such as leaf unfolding and extension. Kitin and Funada (2016) concluded that water requirements for the initial leaf development needed to be fulfilled by latewood vessels formed in previous years. Therefore, these latewood vessels are probably enough to provide water before the appearance of small leaves, as requirements at this stage are minimal in contrast to the amount of water needed for full leaf expansion, which should require new functional earlywood vessels for more water

conduction (Lavrič et al., 2017). Thus, Guada et al. (2018) quantified the water content of different leaf phenophases in *Q. pyrenaica*, and found that water transport of newly-formed earlywood vessels was required to maintain the turgidity of extending leaves and stems, but was not fundamental for the earliest phenological stages (bud swelling and leaf unfolding).

4.3 Phenology and temperature

The relationship between cambial activity and leaf development previously described help us understand the role of new earlywood vessels on the successful completion of crown development in ring-porous hardwoods. However, this internal relationship can be modified by external factors such as climatic conditions, which can somehow lead to advances or delays in the different phenological events.

Spring temperature is the major driving force for cambium and crown reactivation, but most investigations on its relation to xylogenesis were based on conifers at high latitudes or elevations (Rossi et al., 2016; Rossi et al., 2008). Our results based on a temperate ring-porous hardwood suggest that resumption of primary growth requires a minimum threshold temperature, which is probably related to the need of a certain thermal accumulation (Wilkinson et al., 2017). On the other hand, expansion of cambial derivatives is more dependent on concomitant warm temperatures. The fact that budburst is more linked to minimum temperatures while cell enlargement relies on maximum values causes that the process of vessel maturation can take place slightly before or after leaf unfolding depending on the conditions of each specific year. In fact, we estimate that minimal temperature from mid-March to mid-April increases budburst by 7.2 DOYs $^{o}C^{-1}$, while March maximum temperature anticipates cell enlargement by 2.8 DOYs $^{o}C^{-1}$, so that the variations between both processes are not coupled. However, the new functional earlywood vessels are required for full shoot and leaf expansion (Guada et al., 2018).

The influence of temperature on cambium reactivation has also been confirmed for other deciduous hardwoods. Thus, Kudo et al. (2014) showed that localized heating for six weeks induced earlier cambial reactivation in seedlings of *Q. serrata*, while a similar result was found for a 4-week heating in poplar (*P. sieboldii* x *P. grandidentata*) (Begum et al., 2007). In both studies, elevated temperature (20 ± 5 ^{o}C) caused earlier

xylem differentiation, suggesting that the variation of temperature modifies the onset of cell enlargement. Our study evidenced that the maximum temperature and thermal amplitude (i.e., the difference between maximum and minimum daily temperatures) anticipated cell enlargement in 2012 compared to 2013, as shown by the average of 90 trees. In contrast, the minimum temperature was not able to explain the differences in cell enlargement between years. According to Begum et al. (2010), cambial reactivation can be predicted considering the daily maximum temperature; likewise, we are the opinion that an increasing temperature (and thermal amplitude) is an important factor for cambial differentiation of the first vessels elements in the ring-porous hardwood *Q. robur*.

Although crown development has been reported to require a minimum threshold to break dormancy (Caffarra and Donnelly, 2011; Prislan et al., 2013; Wilkinson et al., 2017), budburst is considered to be also induced by photoperiod (Basler and Korner, 2014). Yet, photoperiod differences are small in our study due to the latitudinal proximity among all sites; as a result, minimum spring temperature appears to be the main factor affecting budburst of *Q. robur* in this area. This is consistent with the results reported by Wilkinson et al. (2017) for the south of England, who found a negative correlation between the date of budburst and the mean daily air temperature for *Q. robur* and *Q. petraea*, whereby spring warming had a considerably larger effect on budburst than winter chilling.

While wood formation responds to temperature increments probably by increasing its growth rate, it is the variation in the minimum and maximum temperatures that advances or delays the onset of primary and secondary growth differently across years and sites (Fig. **6a**). The broad sampling strategy involving nine sites and two study years allowed us to estimate the average threshold temperature for each event (Fig. 6b). The different timing for the beginning of cell expansion can be explained by the considerable variation in maximum temperature between years; but this is not the case for leaf unfolding, which hardly varied between 2012 and 2013, probably due to the similar values of minimum temperature.

4.4 Conclusions

Primary and secondary growth of *Q. robur* at its southwestern distribution boundary was closely linked to the moment of budburst and vessel enlargement. Practically all trees began earlywood vessel enlargement before budburst, and only when at least the first new vessels had completed maturation, did current leaves undergo full expansion. Therefore, previous year's vessels are enough to fulfill water requirements for budburst and initial leaf growth, but current year's vessels are needed to provide enough water for further leaf and shoot development.

Differences in spring (or late winter) temperature regime between years and among sites were able to explain the advancement and delay of leaf and earlywood development. However, shoot and cambium activity were not controlled by the same environmental conditions, because minimum temperature determined budburst, whereas maximum temperature was responsible for the onset of vessel formation. Consequently, understanding the environmental control of growth resumption in *Q. robur* is not straightforward, because not only the timings, but also the relationship between primary and secondary growth, can be modified by external factors.

5. Acknowledgments

This study was supported by the Spanish Ministry of Science and Innovation (Research Project BFU-2010-21451) and Xunta de Galicia (Research Project ROCLIGAL, 10MDS291009PR). G. Guada acknowledges a predoctoral fellowship from FPI program (BES-2011-050172) by the Spanish Ministry of Economy and Competitivity. I. García-González and Rosa Ana Vázquez-Ruiz are grateful to Xunta de Galicia for the recognition of Competitive Reference Group (2015/008, GI-1809-BIOAPLIC). The authors are also grateful to L. Costa and C. Franco from 'Fragas do Eume' Natural Park (Xunta de Galicia), M. Souto-Herrero for mapping, and G. Pérez-de-Lis for field assistance. The frame of the COST Action FP1106 'STReESS' inspired this research.

6. Author Contribution

I.G-G and G.G. planned and designed the research. G.G. performed experiments, conducted fieldwork, and analysed data. G.G, I.G-G., and R. V-R interpreted the results. G.G. and I.G-G. wrote the manuscript, with inputs from R.V-R.

Tables

Table 1 Description of the sites included in the analysis, with their identification codes (ID), the corresponding river catchment (Eume, Atlantic; and Sor, Cantabrian), site name, geographical location, elevation, and diameter at breast height (DBH) of the sampled trees. Annual temperature (Temp) and precipitation (Prec) values obtained from Rodríguez-Lado et al. (2016) (source: digital database at www.rgis.cesga.es).

ID	River	Latitude (N)	Longitude (E)	Elevation (m a s l)	DBH (cm)	Temp (ºC)	Prec (mm)
E1	Eume	43.416991	-8.064395	125	29.1 ± 4.5	13.2	1158
E2	Eume	43.372757	-7.991181	350	29.0 ± 5.4	12.2	1268
E3	Eume	43.469576	-7.788688	450	29.3 ± 4.4	12.0	1427
E4	Eume	43.448001	-7.634295	600	30.0 ± 4.8	9.5	1526
S1	Sor	43.676000	-7.708347	125	29.5 ± 5.2	13.0	1194
S2	Sor	43.594782	-7.721225	275	28.0 ± 5.4	12.9	1232
S3	Sor	43.556076	-7.740287	350	30.0 ± 5.8	12.4	1346
S4	Sor	43.529401	-7.736388	500	24.8 ± 3.9	12.4	1361
S5	Sor	43.495749	-7.739470	625	25.7 ± 3.6	11.0	1442

Table 2 Pearson's correlation matrix between the date of cambium phenology events (DOY) and the duration of wood maturation phases (days). The events are: beginning of cell enlargement (bE); earlywood maturation (bM); latewood maturation (bLW); and cessation of cell expansion (cE with a normal-transformation Y^2). The durations are referred to cell expansion (dE), earlywood maturation (dEW), latewood maturation (dLW), and cell expansion of first row of earlywood vessels (d1r).

	bE	**cE**	**bM**	**bLW**	**dE**	**dEW**	**dLW**	**d1r**
bE			**< 0.001**	**< 0.05**	**< 0.05**			
cE	0.170				**< 0.001**		**< 0.001**	
bM	0.821	0.358						
bLW	0.560	-0.022	0.562			**< 0.01**	**< 0.01**	
dE	-0.478	0.791	-0.197	-0.357			**< 0.001**	
dEW	-0.086	-0.349	-0.244	0.665	-0.240		**< 0.01**	
dLW	-0.270	0.726	-0.138	-0.703	0.808	-0.700		
d1r	-0.192	0.361	0.402	0.069	0.428	-0.282	0.197	

Table 3 Generalized linear models to evaluate the effect of site and year on the occurrence of the main phenological events. AIC increments (DAICc) for each model are shown as compared to those of the model with the lowest score (the best-fitted model). Selected models are highlighted in bold. Beginning of cell enlargement (bE); beginning of maturation (bM); beginning of latewood maturation (bLW); cessation of cell expansion (cE); budburst (BB); leaf unfolding (LU); <50% of final leaf size (SL); >50% of final leaf size (FL); d.f. refers to degrees of freedom.

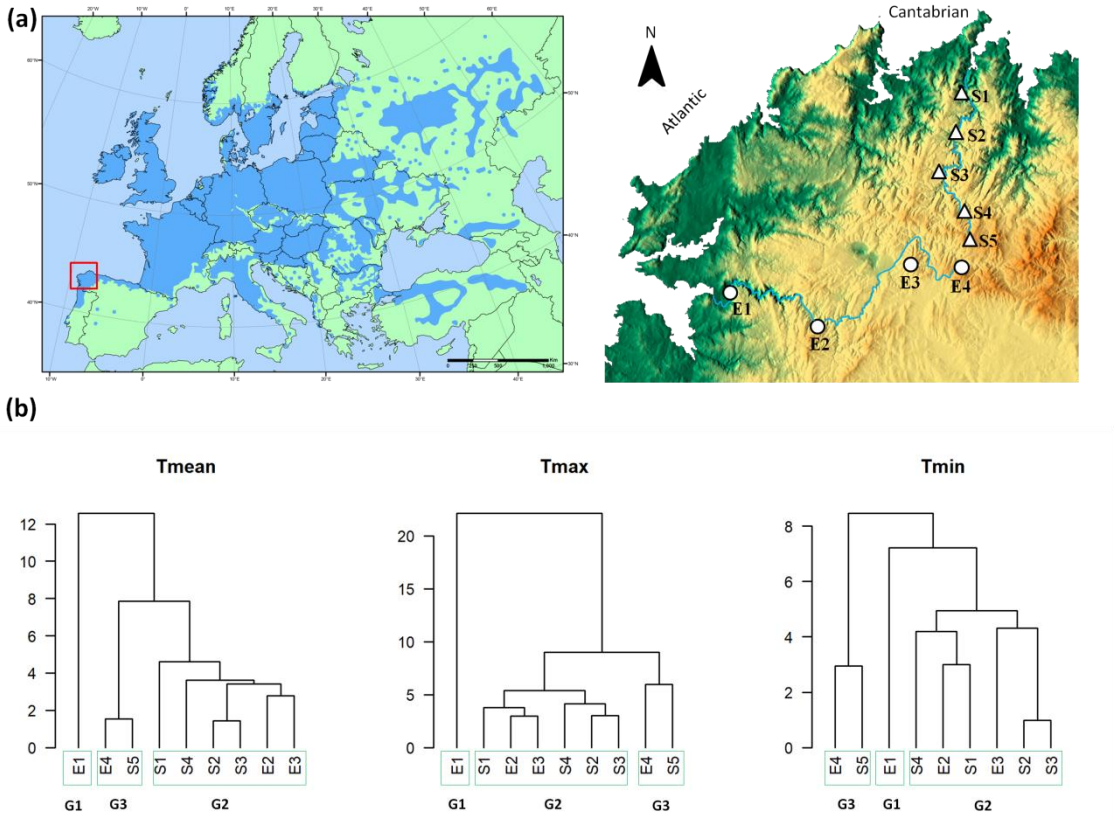
		Xylogenesis				**Leaf phenology**			
Fixed effects	**d.f.**	**bE**	**bM**	**bLW**	**cE**	**BB**	**LU**	**SL**	**FL**
DOY + Site x Year	19	**0**	**0**	**0**	3.50	**0**	**0**	**0**	**0**
DOY + Site + Year	11	30.94	11.32	6.52	**0**	84.29	4.01	21.34	13.92
DOY + Site	10	66.06	19.69	52.80	5.98	106.54	5.05	19.45	23.63
DOY + Year	3	130.39	87.88	43.53	20.97	208.96	170.68	199.68	231.26

549 **Table 4** Significant ($P < 0.05$) statistical values of the linear regression for the different
550 events, expressed as day of the year (DOY) or duration in days as dependent variable;
551 and the mean, amplitude (Ampl.) minimum (Min.), and maximum (Max.) site
552 temperature for the period of each event as independent variable; year is considered as
553 fixed factor. The values shown are: intercept (a); slope (b); residual standard error of the
554 regression (RSE); and adjusted R^2 of the model, including temperature and year effects
555 (Adj. R^2). The *P*-value of Type I test is provided for the temperature effect, as well as
556 the difference between years, expressed in days, whenever this effect was significant.
557 Variables with the highest R^2 are shown in bold.

Events	Period	Temp.	b	a	RSE	Year effect	Type I *P*	Adj. R^2
Beginning of cell enlargement (bE)	March	Mean	137.99	-5.09	6.68	ns	< 0.001	0.5219
		Max.	**130.69**	**-2.82**	**5.99**	**ns**	**< 0.001**	**0.6161**
		Ampl.	122.31	-3.90	6.24	ns	< 0.001	0.5821
Budburst (BB)	Mid-March to mid-April	Mean	165.90	-6.40	8.94	-10	< 0.01	0.472
		Min.	**145.79**	**-7.22**	**8.06**	**ns**	**< 0.001**	**0.567**
Beginning of cell maturation (bM)	March	Mean	**172.51**	**-5.54**	**6.90**	**ns**	**< 0.001**	**0.555**
		Min.	147.78	-5.37	8.21	9	< 0.05	0.3719
		Max.	190.16	-4.48	6.36	-12	< 0.001	0.6225
Duration of 1st vessels enlargement (d1r)	April	Mean	53.98	-2.72	4.79	ns	< 0.01	0.367
		Min.	46.62	-2.83	5.13	ns	< 0.05	0.271
		Max.	**57.77**	**-2.20**	**4.52**	**ns**	**< 0.01**	**0.434**
Duration of latewood (dLW)	October	Max.	-27.103	5.78	14.26	-23	0.05	0.45

558

559 **Figures**



560
561 **Figure 1**. Location of the study sites in the northwestern Iberian Peninsula, and
562 corresponding hierarchical classification following temperature regime. **(a)** *Q. robur*
563 distribution map (www.euforgen.org); and the study sites, Eume (E1-E4) and Sor (S1-
564 S5) river. **(b)** Dendrograms of monthly mean, maximum, and minimum temperatures
565 for the nine sites, clustered into three similar climatic conditions G1, G2, G3.
566

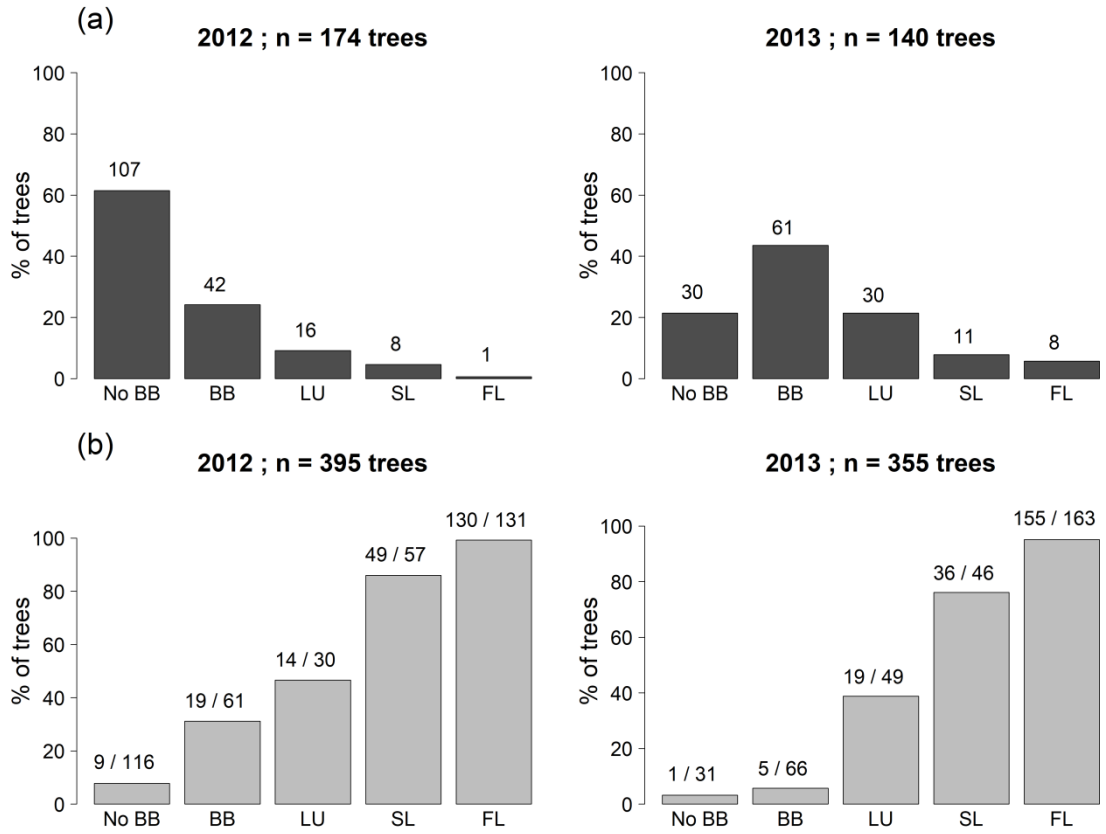


Figure 2 Relationship of primary and secondary growth at tree scale. **(a)** Percentage of different crown phenophases at the moment of beginning of cell enlargement; number of trees per each crown phenophase are indicated above the column. **(b)** Percentage of trees with vessel maturation for each crown phenophase in spring; the number of trees with maturation out of the total amount trees for each specific crown phenophase is indicated above the column. No budburst (No BB), budburst (BB), leaf unfolding (LU), small leaves (SL), full leaf expansion (FL).

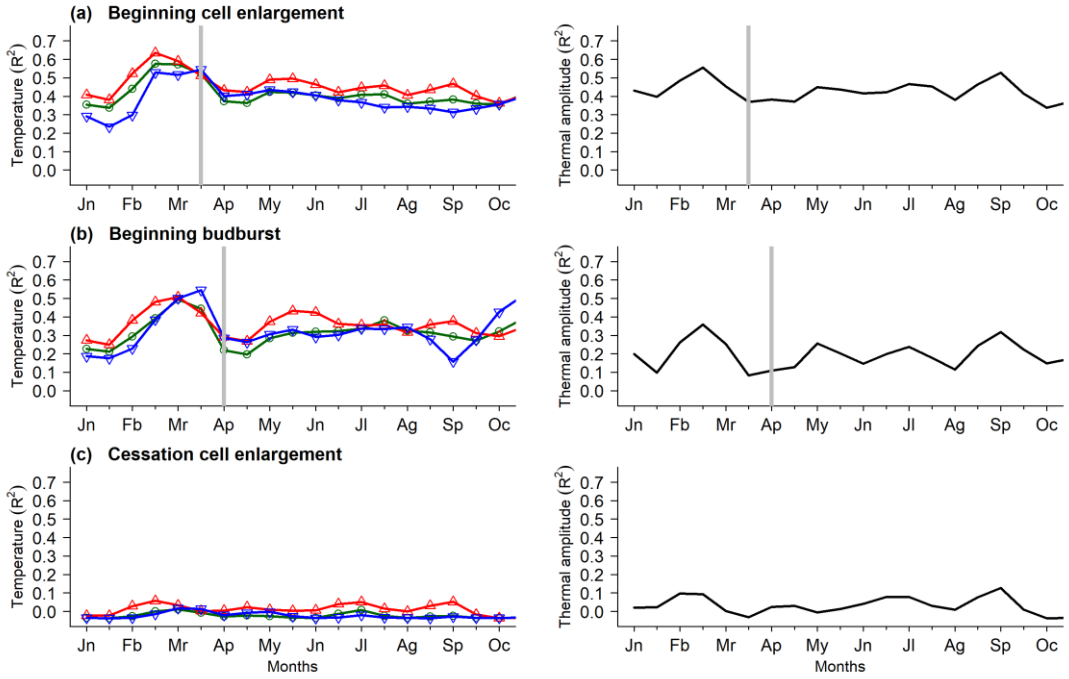
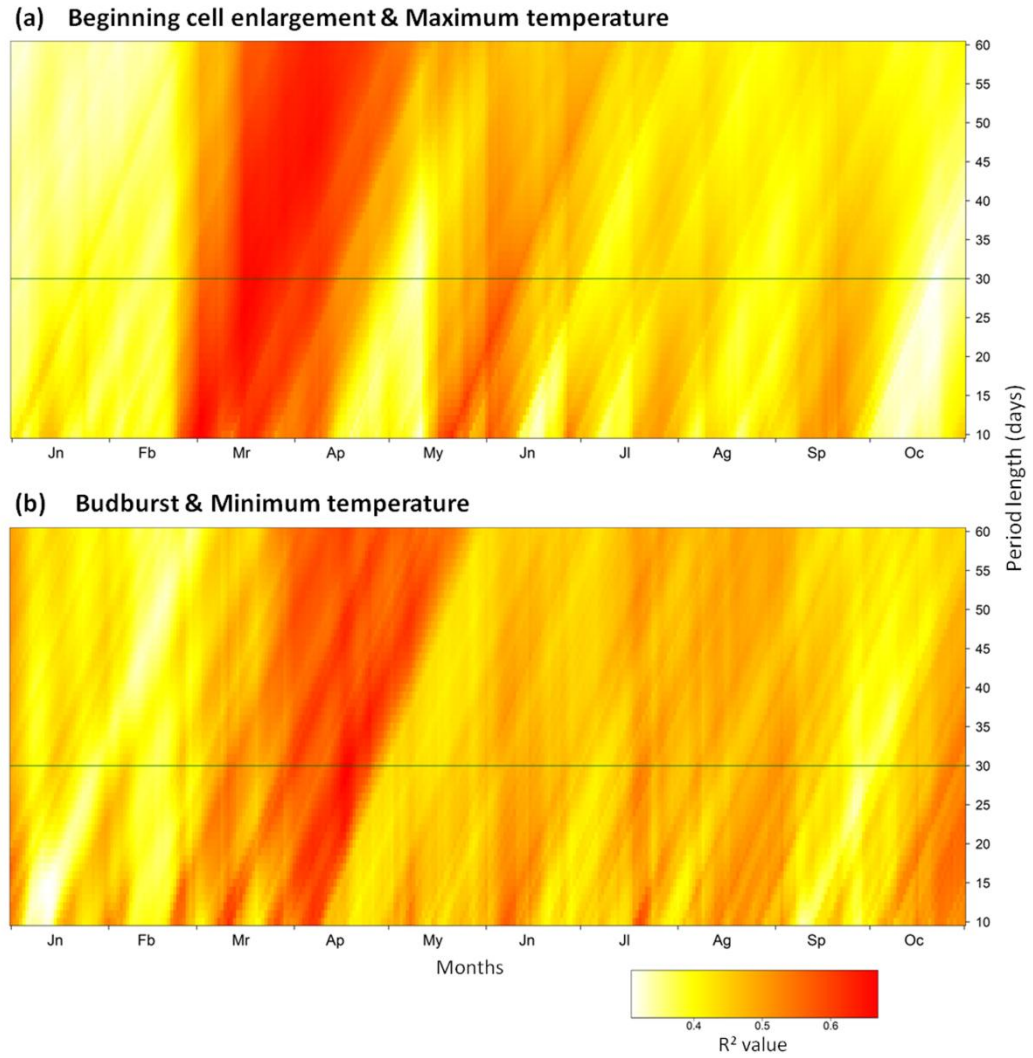


Figure 3 Adjusted R-square of the linear regression of 30-day average temperate at each site on the dates of beginning and cessation of vessel enlargement and budburst. Regressions were calculated for progressive periods separated in 15 days. *Line (without points)*: thermal amplitude; *blue line (triangle down):* minimum temperature; *green line (circle):* mean temperature; and *red line (triangle up):* maximum temperature.



583

584 **Figure 4** Adjusted R-square of linear regression relating the dates of **(a)** maximum

585 temperature on beginning of cell enlargement, and **(b)** minimum temperature on

586 budburst. Running means were calculated for periods of 10 to 60 days, shifted in one

587 day. Horizontal line indicates the average for a 30-day period.

588

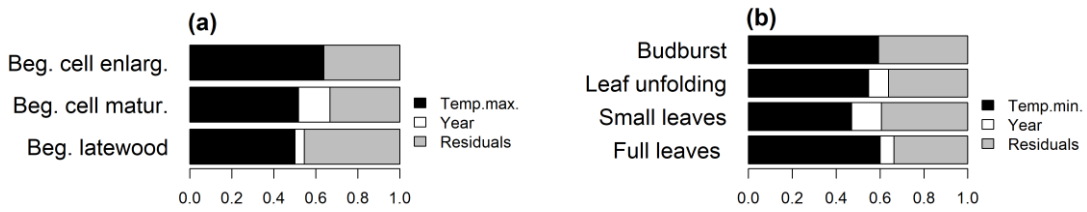
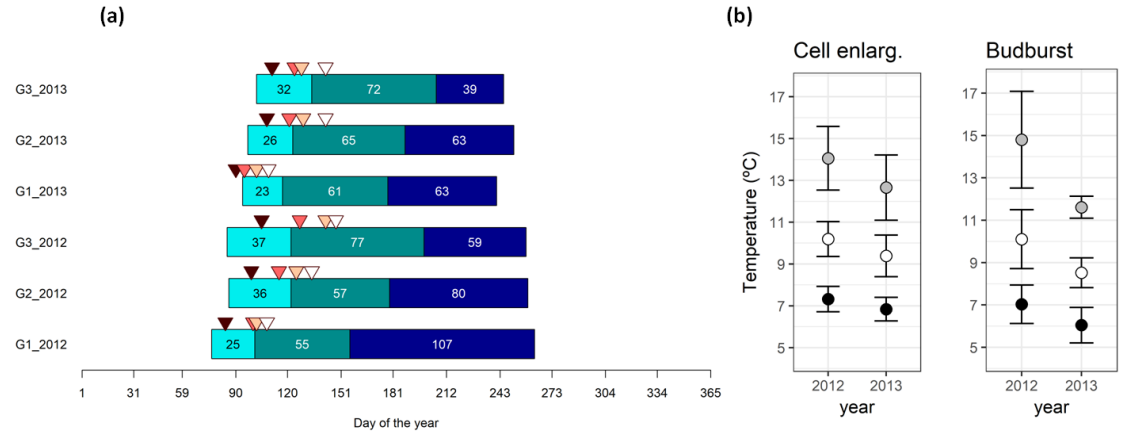


Figure 5 Variance partition of phenological events with temperature and year as independent variables. **(a)** Contribution of the March average maximum temperature to total variability of the phases of the xylem (beginning of cell enlargement, beginning cell vessel maturation, and beginning latewood maturation). **(b)** Contribution of the average minimum temperature from mid-March to mid-April to total variability of the phases of the crown (budburst, leaf unfolding, small leaves, and full leaf extension).



598

599 **Figure 6** (a) Sequence of the timings for the events of wood formation and crown
600 phenology for groups and years. G1 (Atlantic influence; N=10), G2 (intermediate;
601 N=60), G3 (continental influence; N=20) in 2012 and 2013. Wood formation events are
602 indicated by boxes in the bar diagrams (pale: cell enlargement; intermediate: earlywood
603 maturation; dark: latewood maturation; triangles refer to leaf phenology, with lighter
604 colors progressively referring to later stages (budburst, leaf unfolding, leaves with
605 <50% of its apparent final size, and leaves with >50% of its apparent final size. (b)
606 Thresholds of temperatures (minimum (black dots), mean (white dots) and maximum
607 (grey dots)), corresponding with the 0.5-probability of appreciation of cell enlargement
608 and budburst for Q. robur in 2012 and 2013. Error bars indicate the standard deviation
609 among the nine sites.

610

References

Basler, D. and Korner, C., 2014. Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species. Tree Physiology, 34(4): 377-88. http://dx.doi.org/10.1093/treephys/tpu021.

Begum, S., Nakaba, S., Oribe, Y., Kubo, T. and Funada, R., 2007. Induction of cambial reactivation by localized heating in a deciduous hardwood hybrid poplar (Populus sieboldii x P-grandidentata). Annals of botany, 100(3): 439-447. http://dx.doi.org/10.1093/aob/mcm130.

Begum, S., Nakaba, S., Oribe, Y., Kubo, T. and Funada, R., 2010. Cambial sensitivity to rising temperatures by natural condition and artificial heating from late winter to early spring in the evergreen conifer Cryptomeria japonica. Trees, 24(1): 43-52. http://dx.doi.org/10.1007/s00468-009-0377-1.

Begum, S., Nakaba, S., Yamagishi, Y., Oribe, Y. and Funada, R., 2013. Regulation of cambial activity in relation to environmental conditions: understanding the role of temperature in wood formation of trees. Physiologia Plantarum, 147(1): 46-54. http://dx.doi.org/10.1111/j.1399-3054.2012.01663.x.

Caffarra, A. and Donnelly, A., 2011. The ecological significance of phenology in four different tree species: effects of light and temperature on bud burst. International Journal of Biometeorology, 55(5): 711-721. http://dx.doi.org/10.1007/s00484-010-0386-1.

Čufar, K., De Luis, M., Saz, M.A., Črepinšek, Z. and Kajfež-Bogataj, L., 2012. Temporal shifts in leaf phenology of beech (Fagus sylvatica) depend on elevation. Trees, 26(4): 1091-1100. http://dx.doi.org/10.1007/s00468-012-0686-7.

Cuny, H.E. et al., 2015. Woody biomass production lags stem-girth increase by over one month in coniferous forests. Nature Plants, 1: 15160. http://dx.doi.org/10.1038/nplants.2015.160.

Chaney, W.R. and Kozlowski, T.T., 1977. Patterns of water movement in intact and excised stems of *Fraxinus americana* and *Acer saccharum* seedlings. Annals of Botany, 41(176): 1093-1100.

Chuine, I., 2010. Why does phenology drive species distribution? Philosophical Transactions of the Royal Society B-Biological Sciences, 365(1555): 3149-3160. http://dx.doi.org/10.1098/rstb.2010.0142.

644 Ellmore, G.S. and Ewers, F.W., 1986. Fluid-flow in the outermost xylem increment of a
645 ring-porous tree, Ulmus americana. American Journal of Botany, 73(12): 1771-
646 1774. http://dx.doi.org/10.2307/2444244.
647 Gilliam, F.S., 2016. Forest ecosystems of temperate climatic regions: from ancient use
648 to climate change. New Phytologist, 212(4): 871-887.
649 http://dx.doi.org/doi:10.1111/nph.14255.
650 Guada, G., García-González, I., Pérez-de-Lis, G., Vázquez-Ruiz, R.A. and Montserrat-
651 Martí, G., 2018. Dry matter content during extension of twigs, buds and leaves
652 reflects hydraulic status related to earlywood vessel development in Quercus
653 pyrenaica Willd. European Journal of Forest Research, 137(3): 307-319.
654 http://dx.doi.org/10.1007/s10342-018-1104-5.
655 Izco, J., Amigo, J. and Guitián, J., 1990. Los robledales galaico-septentrionales, 15.
656 Acta Botánica Malacitana.
657 Kitin, P. and Funada, R., 2016. Earlywood vessels in ring-porous trees become
658 functional for water transport after bud burst and before the maturation of the
659 current-year leaves. Iawa Journal, 37(2): 315-331.
660 http://dx.doi.org/10.1163/22941932-20160136.
661 Kudo, K. et al., 2014. The effects of localized heating and disbudding on cambial
662 reactivation and formation of earlywood vessels in seedlings of the deciduous
663 ring-porous hardwood, Quercus serrata. Annals of botany, 113(6): 1021-7.
664 http://dx.doi.org/10.1093/aob/mcu026.
665 Kudo, K. et al., 2018. Formation of new networks of earlywood vessels in seedlings of
666 the deciduous ring-porous hardwood Quercus serrata in springtime. Trees.
667 http://dx.doi.org/10.1007/s00468-018-1667-2.
668 Kudo, K., Yasue, K., Hosoo, Y. and Funada, R., 2015. Relationship between formation
669 of earlywood vessels and leaf phenology in two ring-porous hardwoods,
670 *Quercus serrata* and *Robinia pseudoacacia*, in early spring. Journal of Wood
671 Science, 61(5): 455-464. http://dx.doi.org/10.1007/s10086-015-1487-6.
672 Lavrič, M., Eler, K., Ferlan, M., Vodnik, D. and Gričar, J., 2017. Chronological
673 Sequence of Leaf Phenology, Xylem and Phloem Formation and Sap Flow of
674 Quercus pubescens from Abandoned Karst Grasslands. Frontiers in Plant
675 Science, 8(314). http://dx.doi.org/10.3389/fpls.2017.00314.

Lorena, B. et al., 2016. Compensatory mechanisms mitigate the effect of warming and drought on wood formation. Plant, Cell & Environment, 39(6): 1338-1352. http://dx.doi.org/doi:10.1111/pce.12689.

Martínez Cortizas, A. and Pérez Alberti, A., 1999. Dominios ombrotérmicos. In: A. Martínez Cortizas and A. Pérez Alberti (Eds.), Atlas climático de Galicia. Xunta de Galicia, pp. 109-111.

Menzel, A. et al., 2006. European phenological response to climate change matches the warming pattern. Global Change Biology, 12(10): 1969-1976. http://dx.doi.org/10.1111/j.1365-2486.2006.01193.x.

Pérez-de-Lis, G. et al., 2017. Environmental conditions and vascular cambium regulate carbon allocation to xylem growth in deciduous oaks. Functional Ecology, 31(3): 592-603. http://dx.doi.org/10.1111/1365-2435.12789.

Pérez-de-Lis, G., Rossi, S., Vázquez-Ruiz, R.A., Rozas, V. and García-González, I., 2016. Do changes in spring phenology affect earlywood vessels? Perspective from the xylogenesis monitoring of two sympatric ring-porous oaks. New Phytologist, 209(2): 521-530. http://dx.doi.org/10.1111/nph.13610.

Prislan, P., Gričar, J., de Luis, M., Smith, K.T. and Čufar, K., 2013. Phenological variation in xylem and phloem formation in *Fagus sylvatica* from two contrasting sites. Agricultural and Forest Meteorology, 180: 142-151. http://dx.doi.org/10.1016/j.agrformet.2013.06.001.

Puchałka, R., Koprowski, M., Gričar, J. and Przybylak, R., 2017. Does tree-ring formation follow leaf phenology in Pedunculate oak (Quercus robur L.)? European Journal of Forest Research, 136(2): 259-268. http://dx.doi.org/10.1007/s10342-017-1026-7.

Rathgeber, C.B.K., Longuetaud, F., Mothe, F., Cuny, H. and Le Moguedec, G., 2011. Phenology of wood formation: Data processing, analysis and visualisation using R (package CAVIAR). Dendrochronologia, 29(3): 139-149. http://dx.doi.org/10.1016/j.dendro.2011.01.004.

Rodríguez-Lado, L., Tapia del Río, L., Rodríguez-Pérez, M., Martínez-Cortizas, A., Macías, F., Abad, E., 2016. Atlas digital de propiedades de suelos de Galicia. Universidade de Santiago de Compostela, Santiago de Compostela (Spain). http://rgis.cesga.es.

Rossi, S., 2015. Local adaptations and climate change: converging sensitivity of bud break in black spruce provenances. International Journal of Biometeorology, 59(7): 827-835. http://dx.doi.org/10.1007/s00484-014-0900-y.

Rossi, S. et al., 2016. Pattern of xylem phenology in conifers of cold ecosystems at the northern hemisphere. Global Change Biology, 22: 3804–3813. http://dx.doi.org/10.1111/gcb.13317.

Rossi, S. et al., 2013. A meta-analysis of cambium phenology and growth: linear and non-linear patterns in conifers of the northern hemisphere. Annals of botany, 112(9): 1911-1920. http://dx.doi.org/10.1093/aob/mct243.

Rossi, S., Anfodillo, T. and Menardi, R., 2006. Trephor: A new tool for sampling microcores from tree stems. Iawa Journal, 27(1): 89-97. http://dx.doi.org/ 10.1163/22941932-90000139

Rossi, S. et al., 2008. Critical temperatures for xylogenesis in conifers of cold climates. Global Ecology and Biogeography, 17(6): 696-707. http://dx.doi.org/10.1111/j.1466-8238.2008.00417.x.

Sánchez de Dios, R., Benito-Garzón, M. and Sainz-Ollero, H., 2009. Present and future extension of the Iberian submediterranean territories as determined from the distribution of marcescent oaks. Plant Ecology, 204(2): 189-205. http://dx.doi.org/10.1007/s11258-009-9584-5.

Sass-Klaassen, U., Sabajo, C.R. and den Ouden, J., 2011. Vessel formation in relation to leaf phenology in pedunculate oak and European ash. Dendrochronologia, 29(3): 171-175. http://dx.doi.org/10.1016/j.dendro.2011.01.002.

Souto-Herrero, M., Rozas, V. and García-González, I., 2017. A 481-year chronology of oak earlywood vessels as an age-independent climatic proxy in NW Iberia. Global and Planetary Change, 155: 20-28. http://dx.doi.org/https://doi.org/10.1016/j.gloplacha.2017.06.003.

Takahashi, S., Okada, N. and Nobuchi, T., 2015. Relationship between vessel porosity and leaf emergence pattern in ring- and diffuse-porous deciduous trees in a temperate hardwood forest. Botany, 93(1): 31-39. http://dx.doi.org/10.1139/cjb-2014-0129.

Umebayashi, T. et al., 2008. Conducting Pathways in North Temperate Deciduous Broadleaved Trees. Iawa Journal, 29(3): 247-263. http://dx.doi.org/10.1163/22941932-90000184.

741 Vitasse, Y., Signarbieux, C. and Fu, Y.H., 2017. Global warming leads to more uniform
742 spring phenology across elevations. Proceedings of the National Academy of
743 Sciences, 115: 1004-1008. http://dx.doi.org/10.1073/pnas.1717342115.
744 Wilkinson, M., Eaton, E.L. and Morison, J.I.L., 2017. Variation in the date of budburst
745 in Quercus robur and Q-petraea across a range of provenances grown in
746 Southern England. European Journal of Forest Research, 136(1): 1-12.
747 http://dx.doi.org/10.1007/s10342-016-0998-z.

Supporting Information

Click here to download Supplementary Interactive Plot Data (CSV): Supporting Information_Robur_AFM.docx