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TESE DE DOUTORAMENTO

EFFECTS OF CLIMATE ON WOOD FORMATION OF *QUERCUS ROBUR* L. AND *QUERCUS PYRENAICA* WILLD. ALONG A MEDITERRANEITY GRADIENT IN GALICIA:

AN INTEGRATED ANALYSIS USING PHENOLOGY, ANATOMY, AND DENDROECOLOGY

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Memoria para optar ao grado de Doutor, baixo a dirección do **Dr. D. Ignacio García González**, profesor contratado doutor do Departamento de Botánica da Universidade de Santiago de Compostela, e do **Dr. D. Vicente Fernando Rozas Ortiz**, profesor axundante doutor da Área de Botánica da Universidad de Valladolid.

O diretor da Tese	O co-diretor da Tese
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O autor

DEPARTAMENTO DE BOTÁNICA
PROGRAMA DE MEDIO AMBIENTE E RECURSOS NATURAIS
ESCOLA POLITÉCNICA SUPERIOR

LUGO
2016

O meu pensamento na nada
a minha alma não sofre
os meus pés descansam
o caminho aguarda
com paciência ilimitada
Entre desnudos
carvalhos centenários
por onde o vento
sopra sigiloso

Manuel P. de Lis

À minha família

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interest, increasing the potential audience of my investigation. I would also thank to Annie Deslauriers, who made valuable comments on a former version of this Thesis. Finally, many thanks to Hubert Morin, Miguel, Maxence, Audrey, Lorena, Angelo, François, Germain, and Danielle for their efforts in making me feel warm in one of the coldest regions in the world. Je n'oublierai jamais mon séjour à Chicoutimi!

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Vigo, em 17 de Abril de 2016

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Summary

In woody plants, xylem tissue is involved in multiple key functions, such as long-distance water and nutrient transport, mechanical support, and storage. Tree phenology and carbon reserves are assumed to influence earlywood formation in ring-porous species, affecting hydraulic performance and wood production. However, the functional relationships between tree phenology, seasonal dynamics of growth, wood anatomy, and carbon reserves have not been previously addressed for these species. *Quercus robur* is widespread across Europe, habiting areas under oceanic climatic influence. By contrast, *Q. pyrenaica* is usually restricted to mountain areas along the temperate-Mediterranean transition, mainly in the Iberian Peninsula. *Q. pyrenaica* is therefore more tolerant to both winter frost and summer drought than *Q. robur*.

This thesis combines the analysis of leaf phenology, xylogenesis, carbon reserves, and tree-ring series in order to provide a mechanistic knowledge on the climatic regulation of leaf and cambial phenology in ring-porous oaks, and to understand their consequences on wood anatomy and carbon allocation to growth. In addition, this investigation seeks to assess whether divergent stress-tolerance strategies held by the coexisting *Q. robur* and *Q. pyrenaica* imply a contrasting adjustment of phenology, wood anatomy, and carbon reserves to shifting environmental conditions in the temperate-Mediterranean transition.

I selected three sites in Galicia (Spain) along a water-availability gradient where both study species coexist. Leaf and cambial phenology were weekly monitored for ten individuals per site in 2012 and 2013. Different anatomical parameters were also registered basing on microscopic xylem sections. Soluble sugars and starch concentrations were quantified in sapwood cores of 40 trees per species and site by using the anthrone method. Additionally, seasonal dynamics of carbohydrate reserves were monitored bimonthly in 2012. To analyze tree ring series, two cores per tree were collected out of a total of 20 trees per site and species. Yearly series of tree-ring widths and earlywood vessel areas enabled identifying those climatic drivers modulating xylem growth and anatomy.

Warm conditions in late winter advanced phenology in both species, although cambial activity resumed earlier in *Q. robur* than in *Q. pyrenaica*. Whereas a longer period of earlywood formation resulted in larger vessels for *Q. robur*, earlywood vessel diameter in *Q. pyrenaica* probably relied on the rate rather than on duration of earlywood enlargement. The number of cambial cells at dormancy fostered growth capacity in both species, allowing for a

longer active period and a wider xylem increment. Earlywood formation relied on winter reserves in study oaks, whilst current assimilates supported latewood growth concurrently with storage refilling. Feedbacks between soluble sugar content and earlywood vessels were stronger for *Q. pyrenaica*, which appeared to have a carbon saving strategy to face climatic extremes commonly occurring in the sub-Mediterranean area. High temperature during the dormant and quiescent periods played a key role in earlywood formation in study oaks, suggesting a mediation by the timing of growth resumption and soluble sugar content.

Water stress in late spring and summer was the main constrain for xylem production, although cold autumn conditions limited latewood formation at the most humid location. Rising water shortage increased the complexity of wood formation, as demonstrated by the autumn cambial resumption observed in both species at the driest location. In addition, hydraulic efficiency was prioritized over safety under increasing xeric conditions, being concurrent with an increment of the content of stored carbohydrates. Our results suggest that persistence of ring-porous oaks in the Iberian Peninsula may be compromised under future climate change scenarios insofar extreme episodes hinder earlywood and foliage development in spring. This would be also true for *Q. pyrenaica*, despite its multiple adaptations to cope with environmental stress.

CHAPTER I

General introduction

Temperate deciduous forests: a natural heritage of inestimable value

Forests play a vital role for human populations around the world, being an essential natural resource that provides multiple benefits to people. Regulation of biochemical cycles and timber production are the best known services provided by forests, but they are also a key repository of biological diversity. The impact of trees on carbon and water cycles, as well as on the earth surface reflection of sunlight, conveys the existence of feedbacks between forests and climate (Peñuelas *et al.*, 2009). Sustainable management of forest resources have consequently become of crucial importance to mitigate the rapid environmental changes. Deciduous temperate forests have been replaced by conifer plantations over the last centuries in Europe, which affected albedo, canopy roughness, and evapotranspiration from the land surface, thereby contributing to warming (Naudts *et al.*, 2016). At the same time, ecosystem management has been shown to be a primary driver of solar energy conversion into biomass in plants, potentially affecting carbon fluxes at the global scale (Campioli *et al.*, 2015). Currently, European Union forest policies encourage protection and maintenance of native deciduous forests in order to preserve their multiple ecosystem services, with a special concern for biodiversity conservation. In this context, development of mechanistic knowledge about growth responses to climate in deciduous species is crucial to provide forest management guidelines safeguarding temperate woodlands in the face of climate warming.

Linking wood structure and functionality

In woody plants, multiple functions such as long-distance transport of water and nutrients, mechanical support, and storage rely on the xylem tissue, which originates from the vascular cambium (Fig. 1.1). Evolutionary trends under changing environmental conditions at both temporal and geographic scales resulted in highly diversified types of xylem structure (Carlquist, 2012). Whilst xylem of conifers is mostly formed by tracheids, which are responsible for long-distance water transport and mechanical support, specialized vessel elements, parenchyma, and fibres are respectively committed for water transport, storage, and mechanical support in angiosperm trees (McCulloh *et al.*, 2010; Johnson *et al.*, 2012). Most of angiosperms have a diffuse-porous xylem structure, showing vessels of a similar lumen diameter throughout the ring. In contrast, a reduced group of species, most of them with a deciduous leaf habit in the Northern Hemisphere, exhibit an abrupt change in vessel diameter between earlywood and latewood (Zanne *et al.*, 2006; Boura & De Franceschi, 2007). According to the Hagen-Poiseuille law, wide vessels are more efficient conductors than narrow ones, and allow trees to maximize water transport capacity and carbon uptake under a high evaporative demand (Tyree & Zimmerman, 2002). In fact, more than 90 % of water is carried by large earlywood vessels in ring-porous species (Edmore & Ewers, 1985; Corcuera *et al.*, 2004). Nevertheless, there is a trade-off between efficiency and safety, because large vessels are more prone to dysfunction than small ones (Sperry *et al.*, 1994; Wheeler *et al.*, 2005; Hacke *et al.*, 2006; Christman *et al.*, 2009; Delzon & Cochard, 2014).

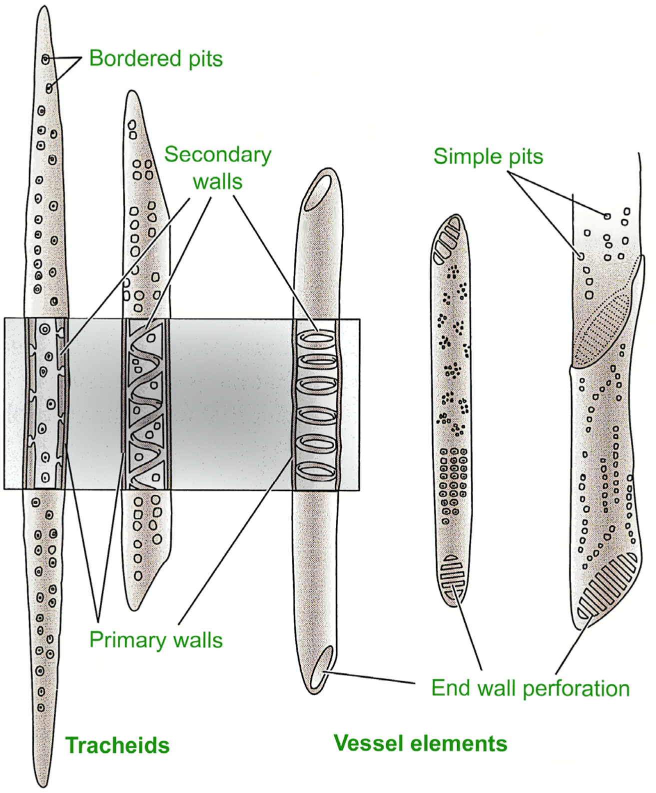


Figure 1.1 Conducting cell types in the xylem of conifers and angiosperms. Adapted from Taiz & Zeiger (2010).

Cavitation resistance is of major relevance for species adaptation to environmental conditions (Lens *et al.*, 2013; Delzon & Cochard, 2014). Choat *et al.* (2012) found that angiosperms had narrower safety margins than conifers, although the percentage of conductivity loss inducing tree mortality in angiosperms is closer to 88 %, higher than the 50 % reported for conifers (Urli *et al.*, 2013). In fact, the more resistant latewood vessels allow trees to maintain water transport after massive vessel dysfunction in the earlywood (Hernández-Santana *et al.*, 2008; Taneda & Sperry, 2008; Urli *et al.*, 2013). A more risky hydraulic architecture is probably mitigated by the abundant axial parenchyma in angiosperms, which enables embolism repair by releasing sugars into disrupted vessels (Nardini *et al.*, 2011; Johnson *et al.*, 2012; Brodersen & McElrone, 2013). Repeated cycles of vessel embolism and repair induce cavitation fatigue (Hacke *et al.*, 2001b; Anderegg *et al.*

2013). However, hydraulic deterioration is counteracted by renewing the hydraulic system (Brodribb *et al.*, 2010). As large earlywood vessels usually operate over one single growing season in ring-porous trees (Tyree & Cochard, 1996), these species restore almost the whole hydraulic network each spring. These features reflect the high adaptive capacity of ring-porous species to face the strongly changing conditions throughout the seasonal cycle, taking advantage of a more limited period for growth (Boura & De Franceschi, 2007). Therefore, large but risky earlywood vessels may enable a rapid development of new shoots and leaves insofar favourable conditions occur in spring, whilst resistant latewood vessels can avoid desiccation in summer. Zanne *et al.* (2006) found that ring-porosity is tightly associated to vascular sectoriality, which is a prevalent anatomical trait in drought-tolerant species. In this regard, rather humid conditions have been observed to lead ring-porous species to be replaced by diffuse-porous ones (Carlquist, 2012).

Tree phenology: integrating primary and secondary growth at the tree scale

Phenology in woody plants involves a concatenation of events occurring periodically during their whole life-span, which are largely controlled by abiotic factors (Vitasse *et al.*, 2009; Caffarra & Donnelly, 2011; Rossi & Bousquet, 2014; Basler & Körner, 2014), although endogenous factors, such as sugar concentrations in quiescent tissues, are assumed to play a relevant role (Cooke *et al.*, 2012). Tree growth takes place as long as environmental conditions are favourable, whilst adverse winter conditions are avoided by entering dormancy (Fig. 1.2), involving the acquisition of physiological resistance to freezing damage (Morin *et al.*, 2007; Repo *et al.*, 2008; Vitasse *et al.*, 2014). Timing and duration of the active and dormant periods are highly variable across temperature and photoperiod gradients, which results in a gradually longer winter rest towards high latitudes and elevations (Körner & Basler, 2010; Rossi & Bousquet, 2014; Vitasse *et al.*, 2014; Gill *et al.*, 2015). Temperate deciduous trees shed their leaves before dormancy, rebuilding their complete photosynthetic system when species-specific temperature and photoperiod requirements are fulfilled (Caffarra & Donnelly, 2011; Basler & Körner, 2014). Temperature has two antagonistic effects on the release of dormancy: the transition from endodormancy (intrinsic dormancy) to ecodormancy (quiescence) is mediated by chilling temperatures, whilst the break of ecodormancy is triggered by warming (Körner & Basler, 2010; Chuine, 2010; Cooke *et al.*, 2012). Interspecific differences in the complex control exerted by these factors have relevant ecological consequences. Increased photoperiod sensitivity has been identified as a mechanism to prevent frost damage in spring, when young tissues are more vulnerable

(Vitasse *et al.*, 2014; Basler & Körner, 2014). Photoperiod and temperature have been widely considered to be two important drivers on the induction of dormancy in autumn (Gill *et al.*, 2015), but soil water content or wind have concomitant effects (Panchen *et al.*, 2015). It is broadly accepted that water stress reduce photosynthesis via stomatal closure (Klein, 2014), but also cambial activity (Hsiao, 1973; Ryan, 2011), which can enter a quiescent period in summer if drought is persistent (Montserrat-Martí *et al.*, 2009; Vieira *et al.*, 2014). This is the case of the Mediterranean area, where winter frost and summer drought split the growing season into two different periods in spring and autumn (de Luis *et al.*, 2007; Linares *et al.*, 2009; Battipaglia *et al.*, 2010; Camarero *et al.*, 2010).

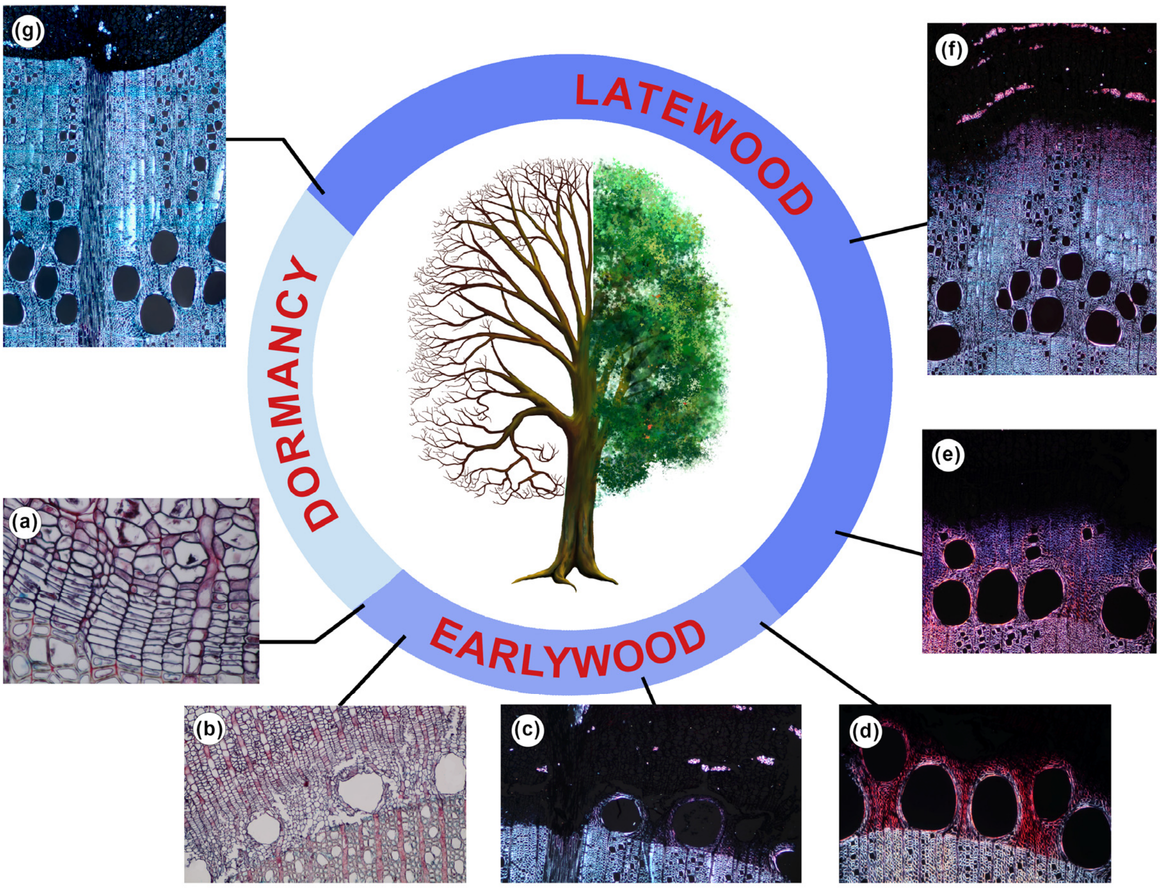


Figure 1.2 Different phases of xylem formation over the course of the growing season in deciduous ring-porous trees: (a) cambial resumption, (b) earlywood enlargement, (c) Onset of earlywood maturation, (d) End of earlywood formation, (e,f) latewood formation, (g) xylem growth cessation. (c–g) Cell maturation (wall-thickening and lignification) is distinguished using a light polarized filter.

Developmental processes occurring in different plant compartments are coordinated at the tree level (Thibeault-Martel *et al.*, 2008; Huang *et al.*, 2014). Time for leaf-out in deciduous species is coupled with the onset of growth in the xylem, while fine root production in ring-porous oaks peaks around the time of maximum xylem growth (McCormack *et al.*, 2014). As a consequence, processes involved in tree phenology appear to be finely tuned. This is somewhat connected to the strong association among different phenophases occurring over the course of the growing season (Rossi *et al.*, 2012, 2013; Rossi & Bousquet, 2014). However, such a synchronicity varies between species with contrasted xylem structure. Leaf unfolding, flowering and shoot enlargement in ring-porous species is assumed to be supported by newly formed vessels at the expense of massive dysfunction of the previous year's ones (Tyree & Cochard, 1996). Whereas diffuse-porous trees have photosyntetically active leaves when cambial activity resumes, enlargement of the first-row vessels in ring-porous trees often predates flushing (Suzuki *et al.*, 1996; Sass-Klaassen *et al.*, 2011; Takahashi *et al.*, 2013). However, only a few studies concurrently dealt with leaf phenology and xylogenesis for ring-porous species (Fig. 1.3), and consequently our knowledge on this topic is still fragmentary.

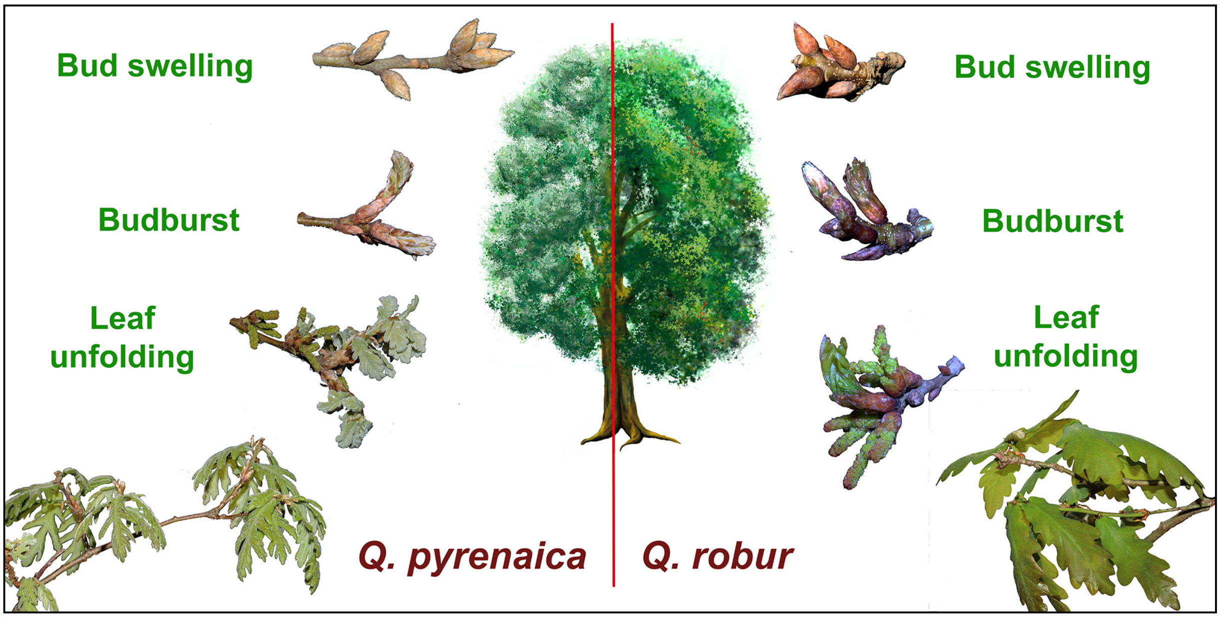


Figure 1.3 Main stages of bud and leaf development in spring.

Cambial activity: a functional approach to the processes involved and their environmental control

The cambial zone is formed by several layers of narrow thin-walled cells, which undergo multiple morphological and biochemical changes in the dormant-active transition (Lachaud *et al.*, 1999). Xylem originates from periclinal divisions in the inner face of the cambial zone, whilst phloem is formed outwards (Fig. 1.4). Xylem ontogeny during the active period involves the succession of five major steps: cambial cell division, cell enlargement, cell wall thickening and lignin deposition, programmed cell death, and heartwood formation (Plomion *et al.*, 2001; Samuels *et al.*, 2006). Dividing cells in the cambial zone give rise to xylem mother cells committed to differentiation. Primary wall loosening elicits irreversible cell expansion as a consequence of the increasing turgor pressure in the symplast (Taiz & Zeiger, 2010; Wang & Ruan, 2013). To prevent further volume changes, the protoplast undertakes secondary cell wall thickening through gradual deposition of cellulose, hemicelluloses and pectins (Samuels *et al.*, 2006). As wall thickening progresses, lignin infiltrates the polysaccharide matrix, starting in cell corners and middle lamela (Kaneda *et al.*, 2010). Lignin is a complex phenolic polymer dehydrating and conferring stiffness to cell wall, which is essential to prevent water-conducting cell implosion (Hacke *et al.*, 2001a). The final stage for fibre, vessel and tracheid maturation is programmed cell death, which involves the autolysis of their living protoplast (Courtois-Moreau *et al.*, 2009). Vessel functioning requires the loss of cytoplasm and organelles in order to reduce hydraulic resistivity, and allow sap to flow throughout the complete vessel conduit.

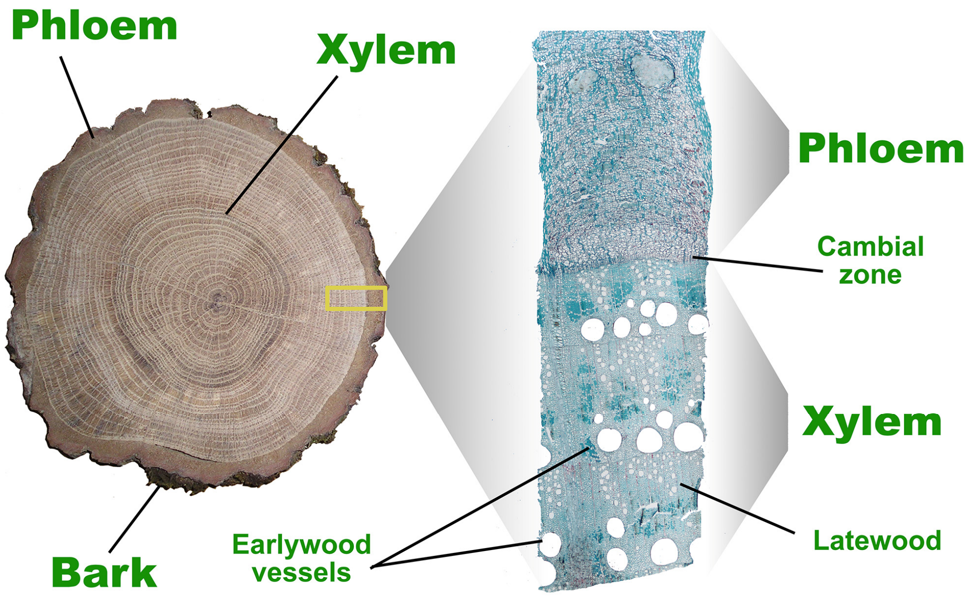


Figure 1.4 Cambial zone, xylem, and phloem in a ring-porous tree and their location in the stem.

Ontogenetic processes in different organs need to be controlled by the interaction of a wide variety of both endogenous and exogenous factors. In temperate deciduous species, earlywood formation in spring is concurrent with shoot elongation and leaf unfolding. Developing buds and shoots are a major source of auxin, which is basipetally transported through the cambial zone (Sundberg *et al.*, 1991). Auxin is considered to be the primary hormone regulating cell production and differentiation (Sorce *et al.*, 2013). The exact mechanism of regulation involves a radial gradient of auxin concentration, which provides positional information to newly formed cells (Tuominen *et al.*, 1997; Uggla *et al.*, 1998).

However, plant growth regulation involves other plant hormones and their combined effects. For instance, gibberellins synthesized in mature leaves foster fibre differentiation and woodiness (Aloni, 2015), likely operating on the transition from earlywood to latewood. There is strong evidence of that cytokinins promote radial growth (Nieminen *et al.*, 2008), while soluble sugars were recently found to act as growth signals and elicit auxin biosynthesis (Lilley *et al.*, 2012).

The timing and rate of such developmental processes are assumed to be fundamental to determine xylem structure. The most obvious example is annual xylem increment, which is positively correlated to duration of the growing season and the rate of cell division (Lupi *et al.*, 2010; Rathgeber *et al.*, 2011b). In contrast, lumen area of conductive cells may be limited by increasing rates of cell division and maturation, according to the auxin gradient hypothesis (Sorce *et al.*, 2013). Downward increasing tracheid diameters in the stem were attributed to gentle auxin gradients fostering the time for tracheid expansion (Anfodillo *et al.*, 2012). Indeed, disbudding oaks showed narrow earlywood vessels (Kudo *et al.*, 2014). Cuny *et al.* (2014) demonstrated that tracheid lumen area and wall thickness are in tune with the duration of tracheid enlargement, while carbon investment in wood formation is rather stable over the growing period. Such linkage between growth and structure represents a mechanism to adjust xylem anatomy to prevailing environmental conditions.

Temperature controls cell responsiveness to auxin (Schrader *et al.*, 2003), with the lowest influence registered during dormancy (Nilsson *et al.*, 2008). This may account for an accelerated resumption of cambial cell divisions when local heating is applied to the stem (Gricar *et al.*, 2006; Begum *et al.*, 2008). Cell maturation is also controlled by temperature (Cuny *et al.*, 2015), which agrees to the observation of unlignified latewood cells as a response to abrupt temperature reduction (Piermattei *et al.* 2015). This is related to that maximum carbon investment in wood formation is concomitant with the time of maximum temperature, ca. one month after the maximum rate of cell expansion (Cuny *et al.*, 2015). On the other hand, rates of wood increment are related to daylength, with maximum values coinciding with summer solstice (Rossi *et al.*, 2006b), whilst water shortage impair cell expansion (Hsiao, 1973; Ryan, 2011). In fact, water supply is considered a major driver of xylem production in both temperate and Mediterranean ecosystems (Olano *et al.*, 2014; Vieira *et al.*, 2014).

An increasing number of studies carried out during the last decades have worthily improved our mechanistic understanding of tracheid production and differentiation in conifers, along with their consequences on wood anatomy and functionality (Rathgeber *et al.*, 2011a; Cuny *et al.*, 2013; Rossi *et al.*, 2013). In recent years, some studies have shed light on the timing of secondary growth in temperate deciduous species (Čufar *et al.*, 2011; Sass-Klaassen *et al.*, 2011; Oladi *et al.*, 2011; González-González *et al.*, 2013; Prislan *et al.*, 2013). Nevertheless, the linkage between xylogenesis and xylem anatomy in deciduous species is largely unknown. Despite these studies performed in conifers have provided a valuable mechanistic knowledge on wood formation, this is not fully applicable to deciduous ring-porous species due to their contrasting wood structure, xylem cell types, different leaf habit and ecological strategy.

Non-structural carbohydrates: a keystone for hydraulic functioning and growth

Photosynthesis allows plants to acquire non-structural carbohydrates (NSC), which are essential for plant survival and responsible for multiple key functions (Fig. 1.5), such as osmoregulation, cryoprotection, maintenance of respiration, vessel embolism repair, protection against predators or pathogens, growth, and reproduction (Atkin & Tjoelker, 2003; Améglio *et al.*, 2004; Salleo *et al.*, 2009; Dietze *et al.*, 2014; Martínez-Vilalta, 2014). However, carbohydrate assimilation and consumption are frequently uncoupled in trees. This asynchrony is solved by accumulating carbohydrate reserves in different organs, mainly as starch (Chapin *et al.*, 1990). When carbon demand in the sinks exceeds photosynthetic acquisition in the sources, starch is catalyzed by hydrolysis, and soluble sugars are released. Yet, storage is an active carbon sink competing for carbohydrates, rather than a passive compartment for carbon surplus (Dietze *et al.*, 2014). In deciduous trees, stored carbon pools must provide enough energy and materials for respiration maintenance during the leafless period, and for development of the photosynthetic apparatus at the onset of the growing season (Barbaroux & Bréda, 2002; El Zein *et al.*, 2011). Winter carbon reserves were found to support a great proportion of earlywood formation in deciduous species (Kagawa *et al.*, 2006; Skomarkova *et al.*, 2006). Dependence on carbohydrate stores seems to be greater for ring-porous trees, because functional earlywood vessels are rebuild every spring (Tyree & Cochard, 1996), and their formation predates flushing (Suzuki *et al.*, 1996; Zweifel *et al.*, 2006; Sass-Klaassen *et al.*, 2011; Michelot *et al.*, 2012; Takahashi *et al.*, 2013). Furthermore, earlywood anatomy in ring-porous species often responds to environmental conditions in the previous year (Fonti & García-González, 2004), which has been largely attributed to a supposed limiting effect of NSCs on growth (Tardif & Conciatori, 2006; Gea-Izquierdo *et al.*, 2012; González-González *et al.*, 2014).

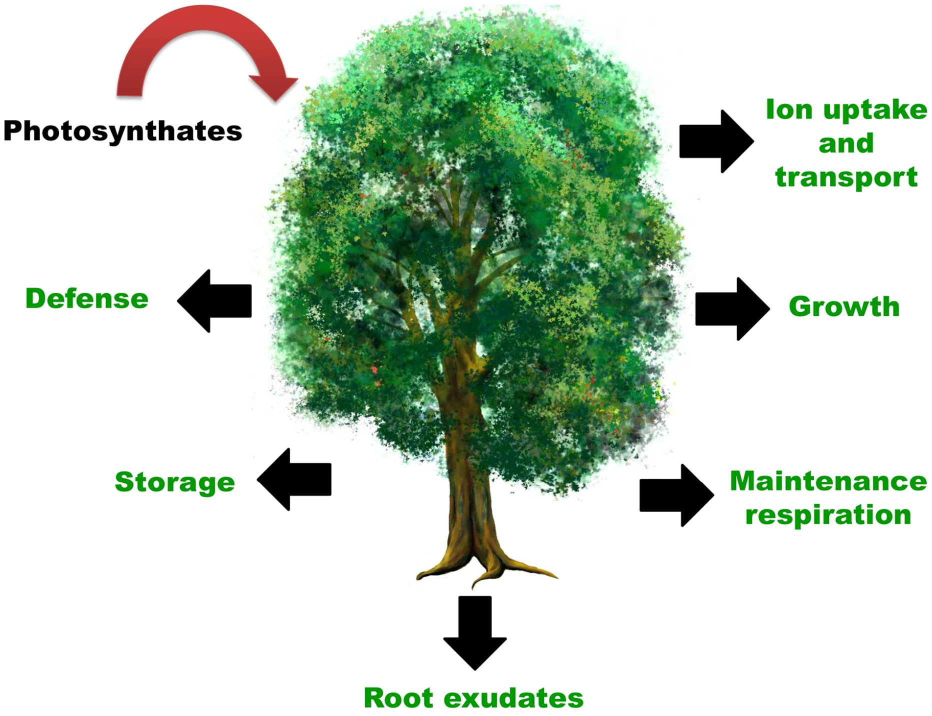


Figure 1.5 Diagram showing competing sinks of non-structural carbohydrates in trees.

Numerous studies have found that NSC content is rarely exhausted in trees, being actually enhanced as abiotic stress reduces carbon demand for growth (Palacio *et al.*, 2007; Sala & Hoch, 2009; Piper, 2011; Sala *et al.*, 2012). This is underpinned by the relatively abundant starch granules that can be observed in sapwood through the growing season (Fig. 1.6). However, feedbacks between carbon storage, osmotic regulation, and growth are notably complex (Sala *et al.* 2012; Dietze *et al.*, 2014), and recent studies argued that source limitation may be somehow concurrent with the broadly accepted sink limitation (Guillemot *et al.*, 2015; Schiestl-Aalto *et al.*, 2015). For instance, Deslauriers *et al.*, (2009) reported that the xylem of fast-growing poplar clones contained higher sucrose levels than their slow-growing counterparts. More recently, drought-induced reduction in the radial growth of black spruce was attributed to the lack of cell turgor and to mobilization of carbohydrate pools for osmotic purposes (Deslauriers *et al.*, 2014). Detrimental effects of drought stress on carbohydrate stores, as a possible mechanism of tree death, elicited the interest of a broad comunity of plant biologists in recent years (McDowell, 2011; Anderegg *et al.*, 2012). However, seasonal dynamics of carbohydrates have been scarcely studied in ring-porous species (Barbaroux & Bréda, 2002; Barbaroux *et al.*, 2003; El Zein *et al.*, 2011) and, with a few exceptions, their conection to xylem growth has been precluded (Michelot *et al.*, 2012; Salomón *et al.*, 2016).

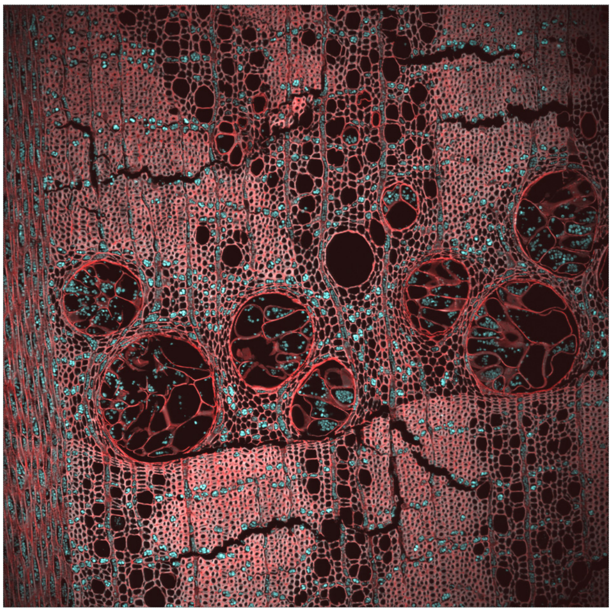


Figure 1.6 Confocal laser scanning image showing starch granules (green) in xylem parenchyma and dysfunctional vessels (tyloses) in October 23rd 2012.

Environmental control of wood formation: a dendroecological approach for ring-porous species

Predictable seasonal changes in temperature and photoperiod determine the annual course of xylem formation, and alternating periods of activity and quiescence lead numerous species to bear distinguishable rings of growth disposed in layers from the pith to the vascular cambium. Nevertheless, trees need to cope with unpredictable changes in temperature and moisture that modulate growth processes. Thereby, shifts in climatic conditions affecting multiple anatomical traits are recorded in the year of formation, giving rise to a variety of environmental proxies, among which tree-ring width has been the most used (Fritts, 1976; Hughes, 2002). For the last decades, innovative techniques have encouraged studies on additional proxies, such as wood density fluctuations, or isotopic composition, as well as on the abundance and morphology of different cell types (Skomarkova *et al.*, 2006; Olano *et al.*, 2014; Campelo *et al.*, 2015; Fernández-de-Uña *et al.*, 2016). Multiproxy approaches allow different environmental signals registered on the tree-rings to be integrated across multiple spatiotemporal scales (McCarroll *et al.*, 2003). As a result, the analysis of tree-ring series have provided valuable insights into climatology, stand dynamics, environmental hazards, and human impact on forest ecosystems (Swetnam & Anderson, 2008; Fonti *et al.*, 2010; Olano *et* *al.*, 2014; Rozas *et al.*, 2015; Campelo *et al.*, 2015). This technique has indeed become greatly relevant for the accuracy of past climate reconstructions and predictions on the global responses of forest ecosystems to the ongoing climate change.

Development of image analysis tools paved the way to build chronologies of quantitative xylem features (Fig. 1.7), mostly for water-conductive cells (Fonti *et al.*, 2010; Carrer *et al.*, 2014). A number of studies published over the last decade set the methodological basis for the analysis of earlywood vessels, but also revealed convergent climatic constraints for vessel expansion across the temperate area, such as high temperature in winter or reduced precipitation in summer (García-González & Eckstein, 2003; Fonti & García-González, 2004; García-González & Fonti, 2006; Gea-Izquierdo *et al.*, 2012; González-González *et al.*, 2014). Nevertheless, the linkage between wood anatomy and growth physiology is frequently hidden due to the lack of understanding on cambial processes, which represents a relevant shortcoming for the ecological interpretation of tree-ring series (Fonti *et al.*, 2010). A recent study linking long tree-ring series with intra-annual growth dynamics in conifers demonstrate that combining intra-annual growth dynamics and long tree-ring series may help us to identify functional growth responses to climate fluctuations (Pacheco *et al.*, 2015).

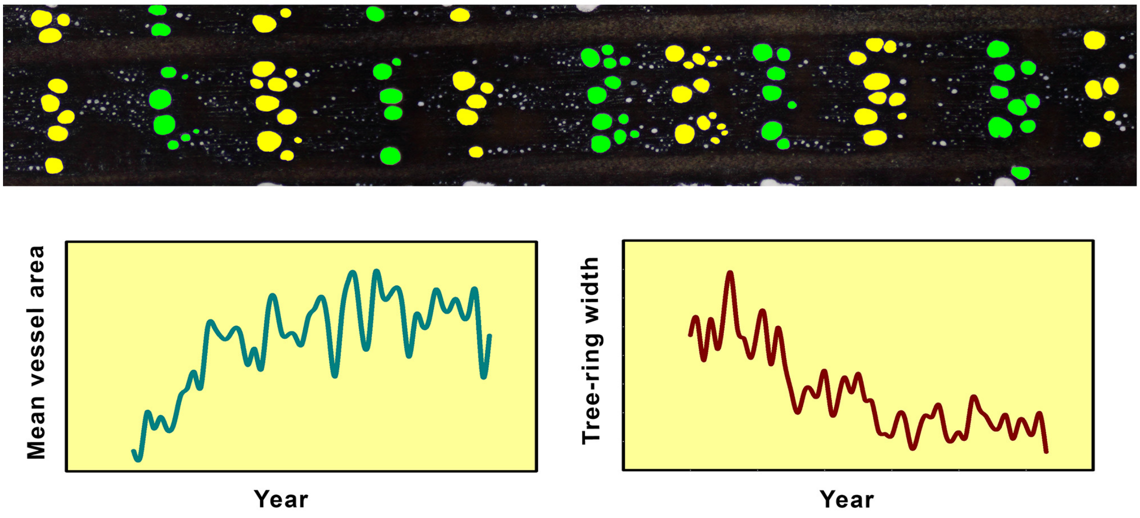


Figure 1.7 Surface of a wood core and theoretical representation of a tree-ring series for vessel area and ring width after cross-dating.

Responses of forest ecosystems to the ongoing climate change

Rapid environmental change has been suggested to overcome limits of phenotypic plasticity and genotypic evolution in numerous plant species, which may undergo distributional shifts to offset detrimental climatic conditions (Allen & Breshears, 1998; Peñuelas *et al.*, 2013; Urli *et al.*, 2015). This is in line with the widespread forest die-off related to drought stress, which has been reported across the globe for the last decade (Allen *et al.*, 2010; Anderegg *et al.*, 2012). Concurrently, environmental change can alter the relative dominance of coexisting species at the community level (Tylianakis *et al.*, 2008).

Several studies have intended to predict future distributional ranges for different tree species according to climate change scenarios (Benito Garzón *et al.*, 2009), but the understanding of current physiological responses of species to climate instability at their range margins is necessary to validate such predictions (Hampe & Petit, 2005). Although several lines of evidence support the idea that more drought-tolerant species will potentially replace their temperate counterparts in future decades (Sánchez de Dios *et al.*, 2009; Benito Garzón *et al.*, 2009; Urli *et al.*, 2015), the existence of stabilizing processes may counteract the effects of extreme events, reinforcing community resilience (Lloret *et al.*, 2012). In this regard, long-term persistence and colonizing capacity across a deeply humanized and highly fragmented landscape should be considered in order to determine the ability of long-lived ring-porous species to withstand environmental change.

Tree phenology is assumed to be a main predictor of tree species range related to the ability of trees to cope with climate constraints (Chuine & Beaubien, 2001). Climate change is altering phenological patterns of organisms and populations at a global scale (Peñuelas *et al.*, 2013). A lengthening of the growing season as a response to climate warming has been widely documented in multiple biomes (Linderholm, 2006; Menzel *et al.*, 2006; Morin *et al.*, 2010; Zhu *et al.*, 2012). In turn, phenological variation is thought to affect climate, through positive and negative feedbacks, either mitigating or aggravating the effect of climate warming in forests (Peñuelas *et al.*, 2009). Thereby, tree phenology at the rear edge of a given species may represent an appropriate indicator to analyze the effects of climate change in forests.

Study species: oak forests in the sub-Mediterranean climatic zone

From an ecological perspective, some of the most relevant ring-porous trees and shrubs belong to genus *Quercus*, which has undergone an intense speciation process from the Oligocene onwards, and currently includes 400–500 species spread across the Northern Hemisphere (Nixon, 1993; Manos *et al.*, 1999). Mild and humid conditions during the last glacial interstitial allowed deciduous oaks re-colonize Europe from their southern refugia

(Brewer *et al.*, 2002), but increasingly drier conditions caused their replacement by marcescent and evergreen Mediterranean oaks in southern Europe. The temperate oak *Quercus robur* is widespread in Europe, reaching the Anatolian Peninsula and the Caucasus (Fig. 1.8), whereas the more drought-tolerant *Q. pyrenaica* is restricted to mountain areas in the Iberian Peninsula, SW France and N Morocco (Olalde *et al.*, 2002). In the Iberian Peninsula, *Q. pyrenaica* is abundant in mountain ranges of the sub-Mediterranean area (Aranda *et al.*, 1996; Corcuera *et al.*, 2006; Sánchez de Dios *et al.*, 2009). Although both species are sympatric along their respective range boundaries (Fig. 1.8), *Q. robur* grows under mild oceanic climatic conditions, whereas *Q. pyrenaica* is more abundant under continental conditions and on acidic soils. This latter species exhibits multiple morphological leaf adaptations that confer drought resistance, such as pubescence and deep lobation (Sisó *et al.*, 2001), as well as a higher cell-wall elasticity and lower water content at the turgor loss point than *Q. robur* (Aranda *et al.*, 1996; Corcuera *et al.*, 2002). It is noteworthy that *Q. pyrenaica* is also more tolerant to winter frost than *Q. robur*, showing delayed flushing to avoid eventual freezing damage in spring (Jato *et al.*, 2002; Lenz *et al.*, 2013). In addition, it has an outstanding capacity for vegetative reproduction, which is characteristic of species adapted to high levels of abiotic stress (Garcia & Zamora, 2003).

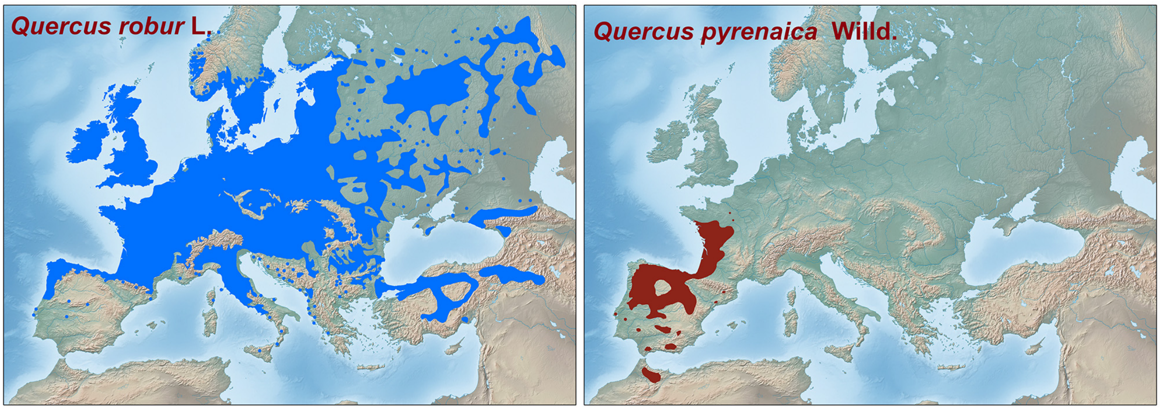


Figure 1.8 Distributional ranges of *Quercus robur* and *Quercus pyrenaica*. Base map: EUFORGEN (http://www.euforgen.com) for *Q. robur*, and the European Atlas of Forest Tree Species for *Q. pyrenaica* (San-Miguel-Ayanz *et al.*, 2016).

Most of native oak forests in Europe have been transformed into agricultural land over the last centuries due to human demographic growth. *Q. pyrenaica* stands were mainly managed as coppices to obtain firewood (Camisón *et al.*, 2015), while pollarding was a common practice in both species (Rozas, 2004). However, their populations were gradually recovered during the second half of the twentieth century as a consequence of rural flight and the replacement of firewoods for heating by fossil fuels.

Study sites: a water-availability gradient along the transition between the temperate and Mediterranean biomes.

We selected three stands of *Q. robur* (*Qrob*) and *Q. pyrenaica* (*Qpyr*) along a north-to-south gradient in Galicia, which is located in NW Spain (Fig. 1.9). Transitional climatic conditions between the temperate and Mediterranean regions induce mild temperature and abundant rainfall from autumn to spring, alternating with frequent drought in summer. Drought severity increases southwards according to the stronger Mediterranean climatic influence.

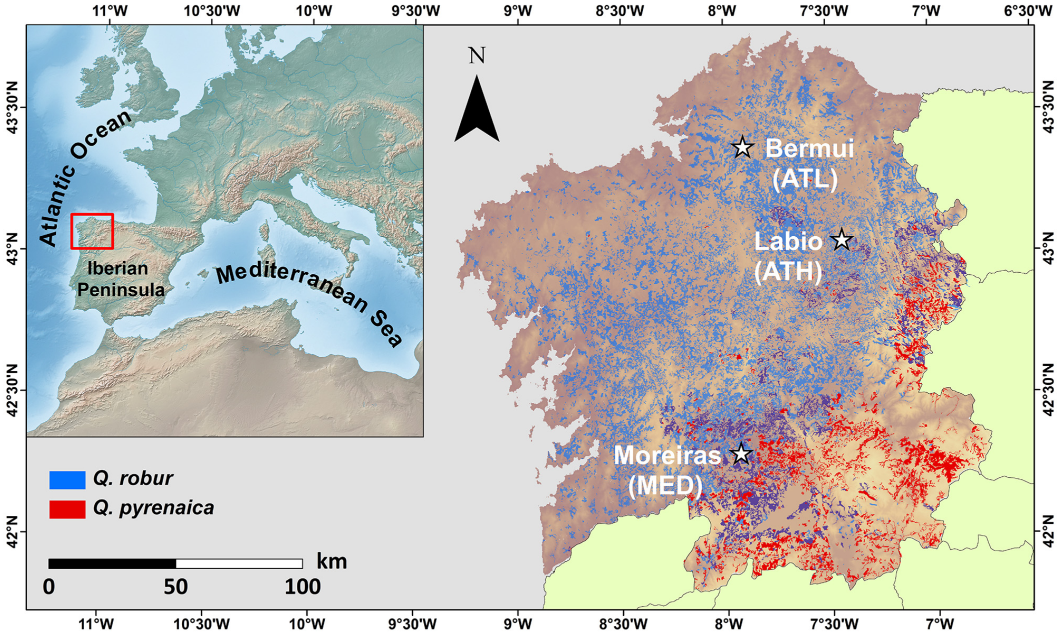


Figure 1.9 Map of Galicia (NW Spain) with current distribution of *Quercus robur* (blue) and *Quercus pyrenaica* (red), and location of the study sites: Bermui (ATL), Labio (ATH), and Moreiras (MED). Source map: Spanish Third National Forest Inventory (IFN3) conducted in 1997–2007.

The northernmost site Bermui (ATL) has Atlantic climate, with 1,461 mm of mean annual precipitation, being located at low altitude (395 m a.s.l). The intermediate site Labio (ATH) has also Atlantic climate and 996 mm of mean annual precipitation, although it shows colder winter conditions due to the higher elevation (690 m a.s.l). Moreiras, the sub-Mediterranean site (MED), lies on the southernmost edge of the gradient at 450 m a.s.l., showing lower rainfall (832 mm of mean annual precipitation), and more frequent summer drought than the previous locations (Fig. 1.10). Mean annual temperature is lower at ATL (11.3 ºC) and ATH (11.6 ºC) than at MED (14.4 ºC). Although both species are present at the three locations, *Qrob* is more abundant at the mesic sites (ATL, ATH), being accompanied by other nemoral tree species such as *Pyrus cordata* Desv., *Ilex aquifolium* L., *Betula alba* L. and *Castanea sativa* Mill., and understorey species such as *Daboecia cantabrica* (Huds.) C. Koch, and *Vaccinium myrtillus* L. Conversely, *Qpyr* is more abundant than *Qrob* at MED (Fig. 1.9), where several termophile plant species such as *Q. suber* L., *Laurus nobilis* L., *Arbutus unedo* L., *Osyris alba* L., and *Daphne gnidium* L. are frequent. Decreasing rainfall along the gradient leads stand density to drop from ATL (1,178 trees ha^{-1}) and ATH (1,082 trees ha^{-1}) to MED (530 trees ha^{-1}). Soil is loam or sandy loam derived from gneiss bedrock at ATL, and calco-alkaline granitoids at ATH and MED.

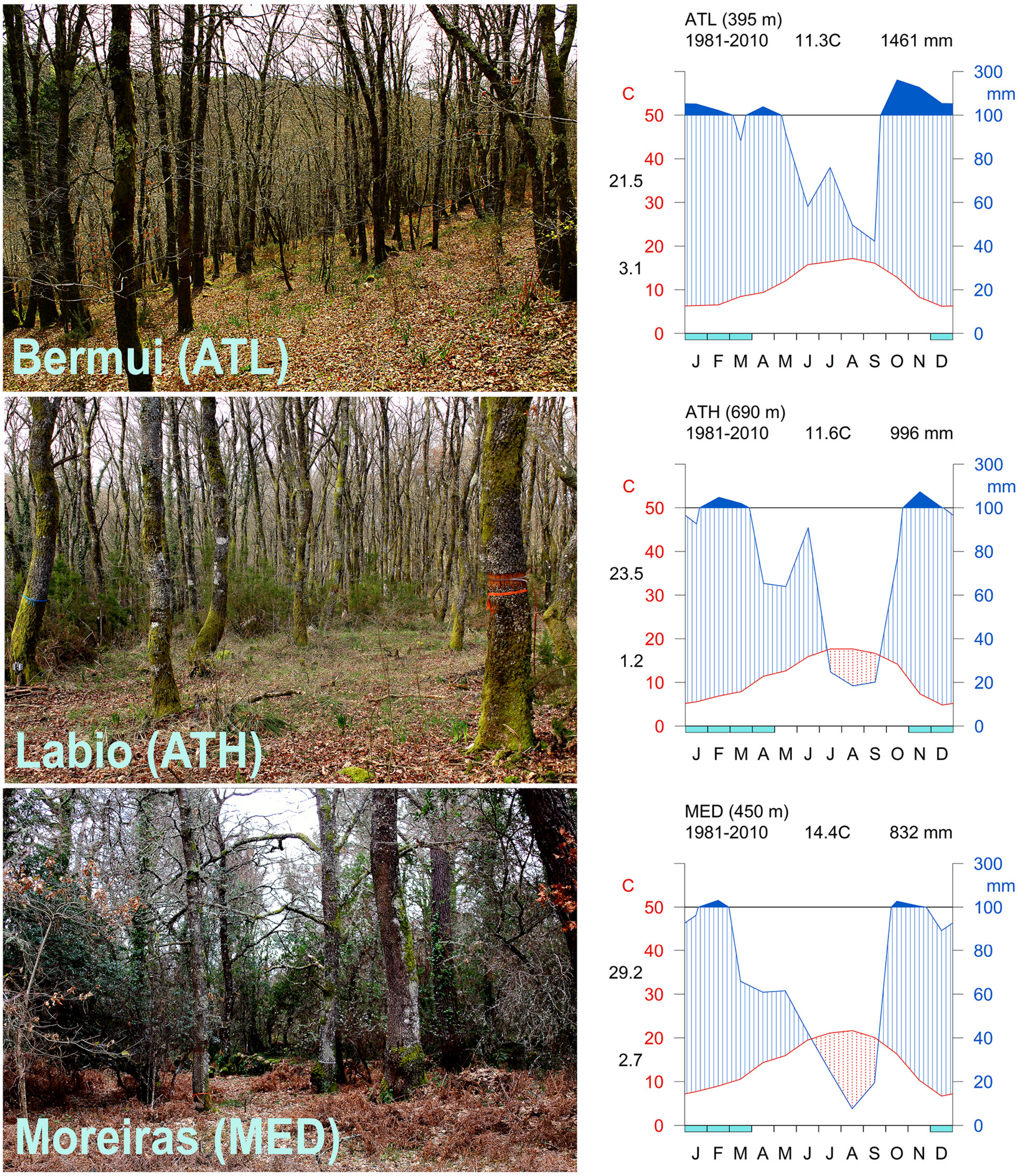


Figure 1.10 Images and climatic diagrams of the study locations that include site altitude; mean, maximum and minimum temperature; and mean annual precipitation for the period 1981-2010.

General objectives

This thesis combines the analysis of leaf phenology, xylogenesis, carbon reserves, and tree-ring series in order to provide mechanistic knowledge on the climatic regulation of leaf and cambial phenology in ring-porous oaks, and their consequences on wood anatomy and carbon allocation to growth. In addition, this work is aimed at assessing whether divergent stress-tolerance strategies held by the coexisting *Q. robur* and *Q. pyrenaica* imply a contrasting adjustment of phenology, wood anatomy, and carbon reserves to shifting environmental conditions in the temperate-Mediterranean transition.

The particular objectives of this thesis are: (i) to describe seasonal dynamics of xylem formation, leaf phenology, and sapwood NSC concentrations along the study gradient; (ii) to assess the impact of phenology on earlywood vessel expansion; (iii) to examine feedbacks between earlywood anatomy and carbohydrate stores, as well as their influence on phenology and latewood increment; and (iv) to elucidate climatic factors controlling earlywood vessel size and tree-ring width, and their connections to phenology and carbohydrate contents. I hypothesize that *Q. pyrenaica* exhibits a shorter growing period, a less vulnerable hydraulic system, and a more conservative growth strategy than *Q. robur* under increasing xeric conditions. Therefore, I expect that *Q. pyrenaica* trees in the temperate-Mediterranean transition become favoured by the more frequent climatic extremes expected in future decades as compared to coexisting *Q. robur* individuals.

Thesis Outline

This thesis includes results from three original experiments carried out for both study species at the three selected locations. (i) The first survey consists of a two-year monitoring of leaf and cambial phenology. (ii) The second experiment comprises the assessment of non-structural carbohydrate concentrations in sapwood after leaf shedding, along with a one-year bimonthly monitoring in a subsample of trees. (iii) The third research includes the collection of increment cores to build up long tree-ring chronologies for a variety of wood anatomical traits. Results provided by this research are structured in four chapters and a general discussion.

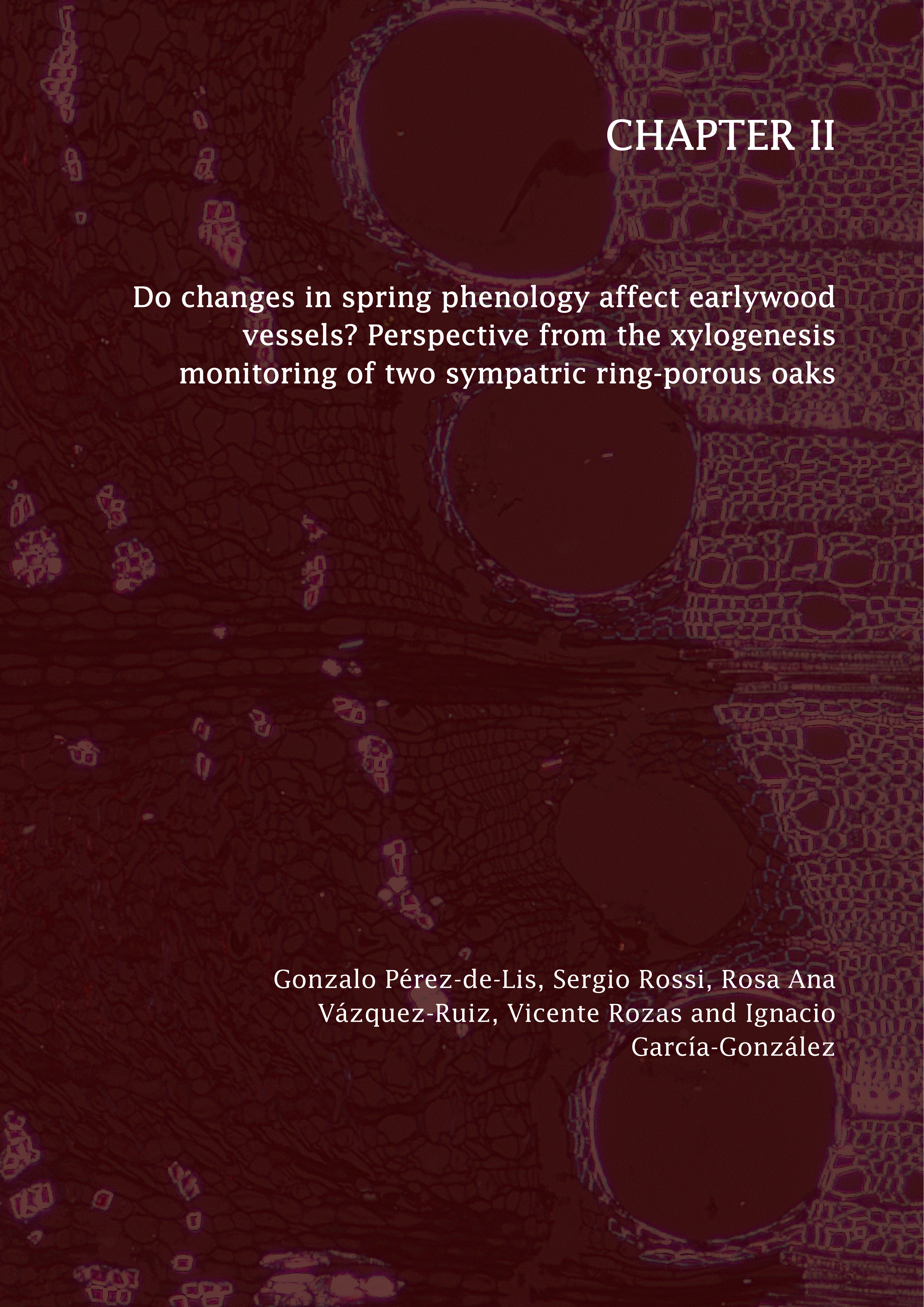
In Chapter II, I use data from phenology monitoring to analyze the timing of secondary growth in spring, and its synchronicity with budburst and leaf unfolding under contrasting climatic conditions. For this purpose, a linear mixed-effects model for binomial data is applied on phenological information. Furthermore, earlywood vessel lumen areas are measured to test whether the timing of earlywood formation affects the hydraulic performance as determined by vessel size. Differences between species are discussed as related to their contrasting stress-tolerance strategy.

Chapter III provides findings on seasonal dynamics of cambial activity over the course of two growing seasons, and their connections to tree-vigour and the mobile carbon pool. Data on non-structural carbohydrate concentrations along one growing season are detailed here. I further apply a novel analysis based on generalized additive models in order to detect if complexity of xylem growth patterns increases under Mediterranean climatic conditions. To conclude, dependencies among annual xylem production, duration of the growing season, maximum xylem growth rate, and the number of cell layers in dormant cambium are assessed by using the structural equation model approach.

In Chapter IV, data on vessel lumen diameters in the previous year, mobile carbon pools in winter, and leaf-out dates, are used to disentangle the influence of their mutual interactions on vessel production, for which I apply an innovative approach based on structural equation models. This chapter also encompasses the analysis of differences in carbon partitioning and allocation to growth between study species. Finally, I assess the effects of earlywood vessel expansion and production, foliage density, and winter carbon pool on latewood production.

Chapter V contains data of multidecadal tree-ring series, in addition to dendrometer data and timing of tyloses formation. The number and size of earlywood vessels, and latewood width are the wood anatomical traits selected to build up tree-ring chronologies. A mixed-effects modelling approach is used to analyze the adjustment of these key wood anatomical traits to increasing xeric conditions, focusing on possible divergences between study species. This is complemented with a correlative analysis on the long-term growth responses to temperature and precipitation.

Chapter VI includes the general discussion of this thesis, where major findings from the four previous chapters are integrated to draw a compelling picture of the connections between tree growth responses to climate and phenology. Besides examining particular results, I intend to go ahead on the possible effects of climate change on deciduous forests from the perspective of the mutual interactions between phenology, carbon reserves, and functional wood anatomy.



CHAPTER II

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

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

Summary

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

Introduction

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

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

Xylem structure and function result from concurrent metabolic processes as a response to specific environmental signals during the growing season. In fact, long tree-ring series reveal that earlywood vessel anatomy, the main feature determining stem hydraulic conductance in ring-porous trees, is sensitive to environmental factors (García-González & Eckstein, 2003; Fonti *et al.*, 2010). Detailed analyses on cambial phenology are therefore needed to elucidate how the environment drives the processes involved in wood formation (Rossi *et al.*, 2012). Some studies have addressed xylogenesis in ring-porous oak species, focusing on relations between leaf and cambial phenology (Zasada & Zahner, 1969; Suzuki *et al.*, 1996; Sass-Klaassen *et al.*, 2011; González-González *et al.*, 2013; Takahashi *et al.*, 2013), or analysing carbon reserves (Michelot *et al.*, 2012). However, the linkage between xylem development and its resulting anatomical features is still unknown for ring-porous trees. In conifers, the duration of tracheid enlargement was recently reported to be the main xylogenetic phase determining wood anatomical features in tree rings (Cuny *et al.*, 2014).

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

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

Materials and methods

Study sites

Three mixed stands of *Q. robur* (*Qrob*) and *Q. pyrenaica* (*Qpyr*) were selected along a transitional gradient between the Atlantic and Mediterranean biomes in the NW Iberian Peninsula, following a north-to-south direction (Table 2.1). *Qrob* is the dominant tree species at Bermui (Atlantic site at low altitude, ATL) and Labio (Atlantic site at high altitude, ATH), whereas *Qpyr* prevails at the southermost location Moreiras (Mediterranean site site, MED). Tree species as *Betula alba* L. and *Castanea sativa* Mill*.,* and understorey species as *Pyrus cordata* Desy*., Ilex aquifolium* L*., Daboecia cantabrica* (Huds.) K. Koch*,* and *Vaccinium myrtillus* L. were present at ATL and ATH. In contrast, MED was located within a broad river valley under Mediterranean conditions, coexisting with termophilous plants such as *Q. suber* L., *Laurus nobilis* L., *Arbutus unedo* L., *Osyris alba* L., and *Daphne gnidium* L. Canopies of *Qpyr* at the northernmost ATL site were affected by the oak powdery mildew (*Erysiphe alphitoides* Griffon & Maubl.) in 2013.

Meteorological data

Air temperature was monitored at each site during 2012 and 2013 using sensor devices (iButton Hygrochron DS1923, San Jose, CA, USA). Gaps in temperature time series were interpolated by linear regressions from nearby meteorological stations belonging to the weather service Meteogalicia (http://www.meteogalicia.es/), which also provided data for precipitation and photoperiod. These stations were selected within 16 km from each study site at similar latitude and elevation. Sites were located at low altitude except ATH (Table 2.1), where the lowest temperature was recorded.

Table 2.1 Characteristics of the study sites and sampled individuals.

ID	Elevation (m a.s.l.)	Density (trees ha^{-1})	*Q. robur*		*Q. pyrenaica*	
			DBH (cm)	H (m)	DBH (cm)	H (m)
ATL	395	1178	27.04±3.83	13.9±3.8	27.35±4.47	16.5±2.0
ATH	690	1082	27.86±3.82	13.2±1.6	26.29±4.90	13.6±1.4
MED	450	531	26.95±4.51	13.3±2.1	28.49±4.61	15.9±2.8

DBH, diameter at breast height. H, tree height. Values are mean ± SE.

The southernmost site MED, under Mediterranean influence, exhibited the highest temperature and the longest photoperiod (Fig. 2.1a–c). The shortest photoperiod was registered at the northernmost ATL site, which displayed a considerable oceanic influence, and an average difference of 20-35 minutes in March and April to the other sites (Fig. 2.1c). ATL and ATH showed mild temperatures and high precipitation in winter, as opposed to warmer and drier conditions at MED (Fig. 2.1a,d). Contrasting weather conditions during late winter and spring occurred between 2012 (warm and dry) and 2013 (cold and wet) at all study sites.

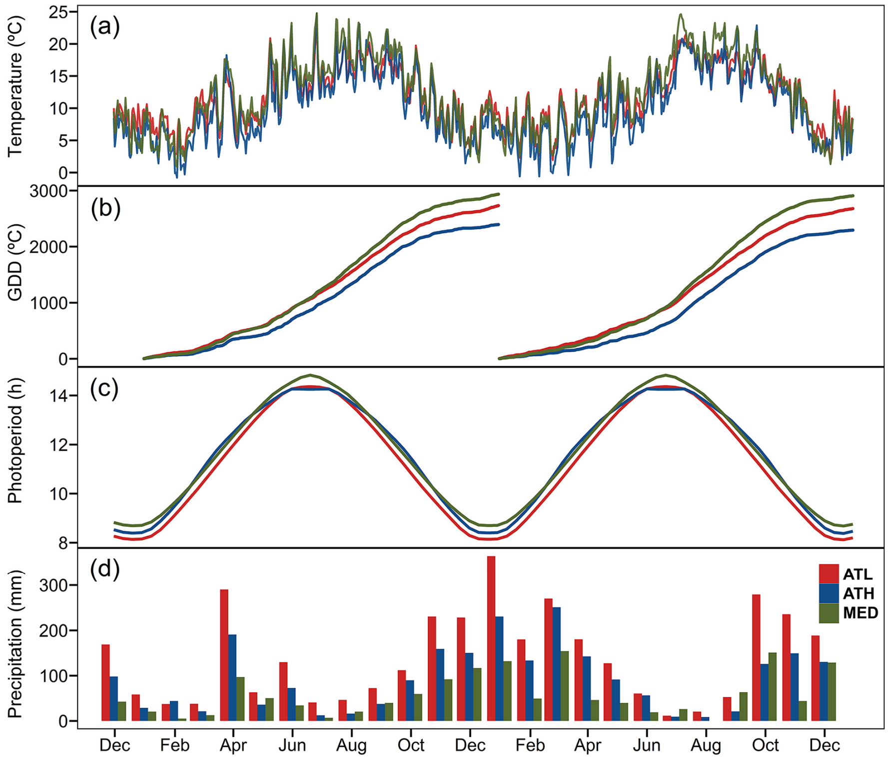


Figure 2.1 Temporal variation of (a) daily mean temperature, (b) growing degree days (GDD), (c) photoperiod, and (d) precipitation at the study sites (ATL, ATH and MED) throughout 2012 and 2013.

Sampling and sample processing

We selected 10 trees per species at each site, from those exhibiting similar height and stem diameter (Table 2.1), and leaf development was weekly monitored from February to July, in 2012 and 2013. Dates of budburst and full expansion of leaves were recorded using binoculars (10×) at ca. 10 m distance from the tree, and expressed as a particular day of year (DOY). Green-colored expanding buds with no unfolded leaves, and leaves attaining at least the 50% of their final size, constituted the criteria to respectively identify budburst and leaf full expansion. Each phase was registered when observed in branches on the uppermost part of the crown.

Two microcores of 2 mm diameter were weekly taken per tree at 1.3 m above ground from February to December in 2012 and 2013, using a Trephor device (Vitzani, Belluno, Italy), and following a helicoidal pattern around the bole (Rossi *et al.*, 2006a). After extraction, microcores were placed in microtubes with a 50% ethanol solution and stored at 5 ºC until processing, which included dehydration of samples by successive immersions in ethanol and xylene, and paraffin embedding using a tissue processor (Leica TP1020, Wetzlar, Germany). Cross-sections with a thickness 8-10 µm, obtained by cutting each paraffin block with a manual rotary microtome (Leica RM2125 RTS, Wetzlar, Germany), were placed on microscope slides. After a 5-minute immersion in xylene for removing the residual paraffin, samples were rehydrated and stained in two consecutive solutions of safranin and fast green FCF in ethanol (80%) for 10 minutes and 30 seconds respectively (Cutler *et al.*, 2008). Afterwards, they were newly submerged in xylene, and permanently fixed with Eukitt® resin (O. Kindler GmbH, Freiburg, Germany).

Data collection

In order to collect data relative to xylogenesis and earlywood formation, microcore cross-sections were observed with a transmitted light microscope (Olympus BX40, Tokyo, Japan), using a white light polarizing filter to detect cell wall thickening (40×). The four phenophases recorded from the cross-sections were: (1) dormant cambium (Fig. 2.2a), (2) cambial resumption (Fig. 2.2b), (3) onset of earlywood differentiation and enlargement (Fig. 2.2c,d), and (4) onset of earlywood maturation (Fig. 2.2e,f).

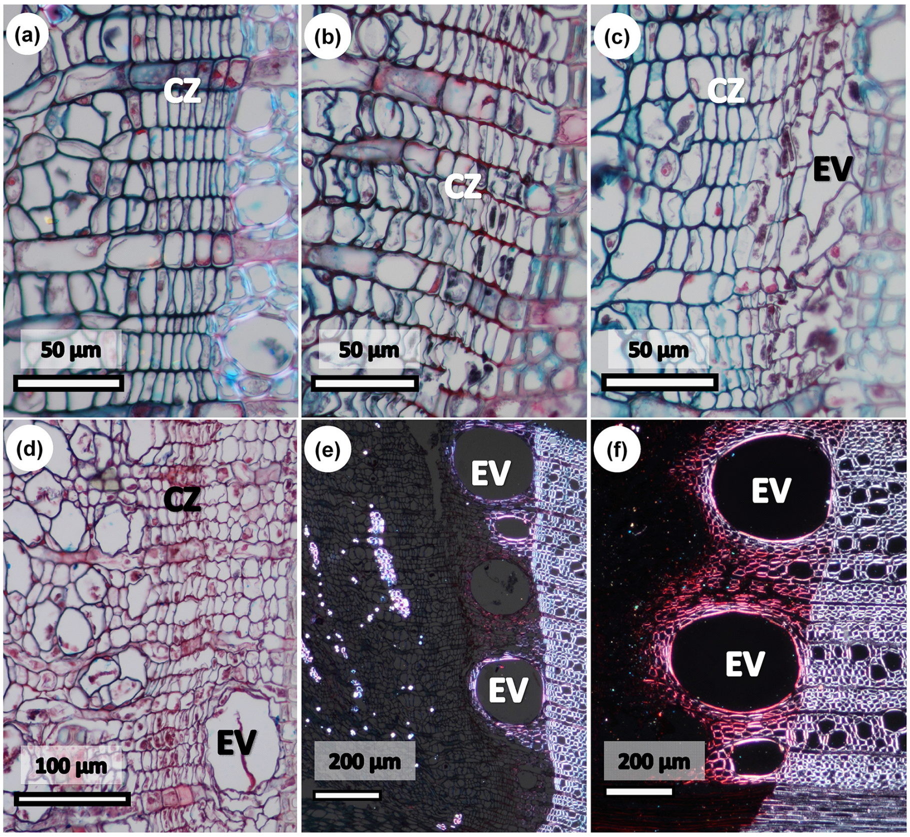


Figure 2.2 Transverse sections of cambium and new xylem showing the four recognized phenophases of earlywood formation. (a) Dormant cambium, (b) active cambium, (c, d) vessel differentiation and enlargement, (e, f) vessel maturation: wall-thickening and lignification, observed under polarized white light. CZ is cambial zone, and EV is earlywood vessel.

The occurrence of cell divisions was the criterion to identify cambial resumption (Frankenstein *et al.*, 2005) (Fig. 2.2b). The onset of earlywood enlargement was considered to occur when xylem mother cells started to differentiate. Distinctions between the first and second row were not straightforward because some samples simultaneously showed both enlarging and maturing first-row vessels (Fig. 2.2c,d). The onset of earlywood maturation was defined when both wall thickening and lignification were detected, as these processes appeared to occur simultaneously in vessels, so that we were usually not able to discern between them (Fig. 2.2e,f). Images of 5184 × 3456 pixels were obtained from all the samples in which vessels were mature (i.e. collected in late spring, summer and autumn), using a digital camera attached to the microscope.

Data analysis and statistics

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

$$GDD_{daily} = \frac{(T_{max} + T_{\min})}{2} - T_{base}$$

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

Within each tree, 50 mature vessels located in the first and second rows of the outermost ring were measured, setting a limit of 7,500 μm^2 because of the substantially higher contribution of large conduits to overall hydraulic conductivity (González-González *et al.*, 2014). Measurements of vessel lumen area were semi-automatically performed using ImageJ version 1.48v (Schneider *et al.*, 2012). Two *Qpyr* individuals from ATL showing suppressed growth were excluded from the analysis. The diameter of each equivalent circular vessel lumen was derived from the vessel area, and used to compute the weighted average of hydraulic diameters (D_h) according to the equation:

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

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

The analysis of xylogenesis and leaf phenology was carried out using generalized linear mixed models (GLMM) for binary data (Bolker *et al.*, 2009), considering ʽtree' as a random effect, and applying multiple comparisons for parametric models to compare secondary growth with leaf phenology. Pearson's correlations among phases of earlywood formation and budburst were also calculated to test for collinearity. The interaction between each tree D_h and the timing of enlargement was tested by applying a generalized least squares model (GLS) selection procedure, allowing for different variances per site. The analysis was repeated for vessel areas using GLMM for gamma distributions with ʽtree' as a random effect. The Akaike's Information Criterion (AIC) was used to rank models, and those variables appearing in the most parsimonious model (fewest variables) showing ΔAIC < 2 were considered as meaningful. All statistical analyses were performed in R 3.1.1 (R Core Team, 2014), using the ʽlme4' package for GLMMs, the ʽnlme' package for GLS model calculations, and the ʽmultcomp' package for multiple comparisons.

Results

Spring tree phenology

Cambial resumption (i.e. cambial divisions) started from mid-February to late March (DOY 45–86) in *Qrob*, and from late February to early April (DOY 58–94) in *Qpyr* throughout the study gradient (Fig. 2.3). The date on which cambium resumed differed between species and among sites, while differences between years were negligible (Table 2.2). Earlywood enlargement occurred from early March to early July (DOY 64–184) in *Qrob*, and from mid-March to early July (DOY 71–182) in *Qpyr* (Fig. 2.3), significantly varying among sites and between years (Table 2.2). The onset of this phase occurred significantly earlier in *Qrob* than in *Qpyr*, whereas differences at the end of enlargement were negligible between species (Table 2.2). Consequently, *Qrob* individuals exhibited longer periods of earlywood formation than *Qpyr* ($F = 13.53$, $P < 0.001$), with mean values of 63 and 54 days, respectively.

The enlargement period up to the onset of maturation phase in the first-formed vessels entailed 43.36 ± 4.84 % of the whole period of enlargement in *Qrob,* while 59.32 ± 5.30 % in *Qpyr.* Vessel maturation started from late March to mid-May (DOY 80–137) in *Qrob* and from early April to mid-June (DOY 100–162) in *Qpyr* (Fig. 2.3). As observed for the enlargement, differences among species, years, and sites were also significant in maturation initiation (Table 2.2). Similar results were revealed by the models applied on budburst (Table 2.2), which started earlier in *Qrob* (DOY 71–137) than in *Qpyr* (DOY 100–147). Leaf expansion was completed between early April and early June (DOY 93–154) in *Qrob,* and between early May and late June (DOY 126–177) in *Qpyr.* These phenophases started earlier at MED, the southernmost site, with a marked delay in 2013 as compared to 2012 (Fig. 2.3).

Table 2.2 Comparison of generalized linear mixed models (GLMM) assessing the effect of species, site, year and their interactions on earlywood formation and budburst.

	df	Log-Lik	ΔAIC
Cambial resumption			
Site + Species	**6**	**-118.55**	**0.00**
Site + Species + Year	7	-120.16	5.22
Onset of enlargement			
Site + Species + Year	**7**	**-142.61**	**0.00**
Site + Species	6	-147.21	7.20
End of enlargement			
Site × Year	**8**	**-150.18**	**0.00**
Site × Species	8	-161.60	22.85
Onset of maturation			
Site + Species × Year	**8**	**-121.79**	**0.00**
Site + Species + Year	7	-148.00	50.42
Budburst			
Site + Species × Year	**8**	**-92.03**	**0.00**
Site × Species	8	-108.41	32.76

Models are sorted following the Akaike's Information criterion (AIC). Models were fitted for binomial distributions, including tree as random effect. The simplest model with ΔAIC < 2 is in bold. ΔAIC is increment on AIC values with respect to best-fit model. *df*, degrees of freedom. Log-Lik, log-likelihood value.

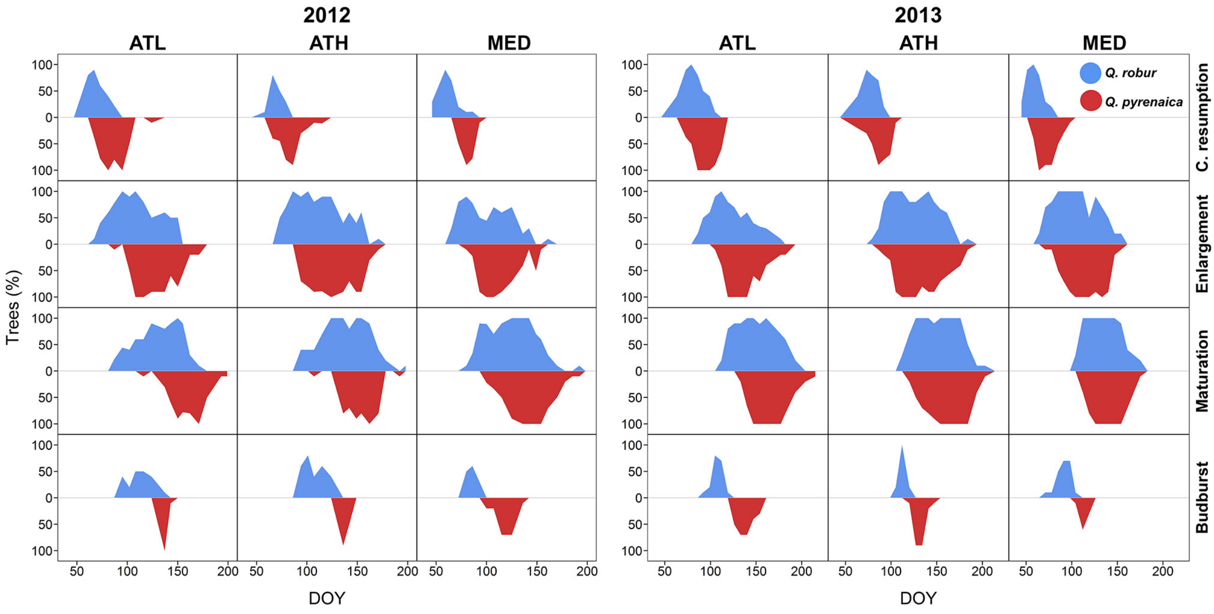


Figure 2.3 Timing of earlywood formation (cambial resumption, enlargement, and maturation) and budburst in 2012 and 2013, according to the day of year (DOY) at the study sites ATL, ATH, and MED. Shaded areas represent the proportion (%) of the sampled trees exhibiting each phenophase in both oak species. C. resumption is cambial resumption.

Dates of cambial resumption and onset of earlywood formation (enlargement and maturation) were highly correlated (Table 2.3), indicating that every phase involved in earlywood development relies on those occurring previously. Budburst dates were highly correlated to those of wood formation, particularly to the onset of maturation in *Qpyr,* and to the beginning of enlargement in *Qrob.* The onset of maturation was synchronous to budburst in both species ($Z = 2.51$ and 1.16, $P > 0.050$ in *Qrob* and *Qpyr,* respectively), although a few *Qrob* individuals exhibited earlier budburst than maturation. Full leaf expansion and the end of earlywood enlargement were simultaneous ($Z = –2.02$, $P = 0.331$) in *Qpyr,* but leaves were fully expanded on average 14 days before earlywood enlargement ceased in *Qrob* ($Z = –6.48$, $P < 0.001$). Consequently, earlywood growth and leaf unfolding showed to be coupled. Duration of leaf expansion strongly varied among sites and between years, lasting from 13 days in both species to 51 days in *Qpyr* and 55 days in *Qrob.*

Table 2.3 Pearson's correlation coefficients (*r*) among phenophases of earlywood formation and budburst. *p*-values of the coefficients are included in brackets.

	CR	OE	OM	EE	Budburst
CR		0.58 (<0.001)	0.34 (0.009)	0.45(<0.001)	0.61 (<0.001)
OE	0.58 (<0.001)		0.66 (<0.001)	0.37 (0.004)	0.66 (<0.001)
OM	0.36 (0.005)	0.66 (<0.001)		0.38 (0.003)	0.54 (<0.001)
EE	0.38 (0.002)	0.45 (<0.001)	0.49 (<0.001)		0.38 (0.003)
Budburst	0.35 (0.005)	0.49 (<0.001)	0.67 (<0.001)	0.56 (<0.001)	

Shaded cells (lower left) correspond to *Q. pyrenaica*; regular ones (upper right), to *Q. robur*. CR cambial resumption, OE onset of enlargement, OM onset of maturation, and EE end of enlargement.

Thermal drivers of phenology

The studied species showed different GDD requirements for both primary and secondary growth resumption throughout the study gradient. *Qrob* required lower GDD values in order to reactivate growth, particularly for budburst ($\mathrm{GDD}_{Qrob} = 374.17 \pm 12.46$; $\mathrm{GDD}_{Qpyr} = 536.35 \pm 15.80$; $F = 158.89$, $P < 0.001$), and earlywood enlargement ($\mathrm{GDD}_{Qrob} = 273.51 \pm 11.3$; $\mathrm{GDD}_{Qpyr} = 384.73 \pm 13.89$; $F = 99.02$, $P < 0.001$). Sensitivity to temperature forcing varied across the north-to-south gradient, as demonstrated by the higher GDD values observed at the moment of budburst ($\mathrm{GDD}_{\mathrm{BE}} = 572.71 \pm 19.13$; $\mathrm{GDD}_{\mathrm{LA}} = 385.01 \pm 14.55$; $\mathrm{GDD}_{\mathrm{MO}} = 408.05 \pm 17.60$; $F = 99.02$, $P < 0.001$), and earlywood enlargement ($\mathrm{GDD}_{\mathrm{BE}} = 435.46 \pm 13.51$; $\mathrm{GDD}_{\mathrm{LA}} = 257.49 \pm 11.23$; $\mathrm{GDD}_{\mathrm{MO}} = 294.41 \pm 14.45$; $F = 94.16$, $P < 0.001$) at the northernmost ATL site. According to the multiple comparisons, trees at sites ATH and MED required similar GDD, both for the onset of enlargement ($t = 1.31$, $P = 0.391$) and budburst ($t = –0.17$, $P = 0.984$). Details of GDD and their comparisons are shown in Supporting Information (Tables A.2.1 and A.2.2).

Vessel enlargement and hydraulic diameter

Both species exhibited similar earlywood features, with frequent multiple rows of wide vessels, and the largest vessels being mostly located in the first and second rows. Only 17 individuals developed one single row of vessels (6 in *Qrob* and 11 in *Qpyr*), 10 of them were located at ATL. The vessel area measurements performed (3,000 vessels for *Qrob,* and 2,900 for *Qpyr*), showed differences among sites, exhibiting *Qpyr* at ATL and ATH the lowest and highest mean values, respectively (Supplementary information, Table S.2.3, Fig. S.2.1). Mean lumen area was 48,711.39 ± 387.88 μm^2 (D = 242.55 ± 1.03 μm) for *Qrob* and 49,036.95 ± 411.08 μm^2 (D = 242.90 ± 1.09 μm) for *Qpyr,* whereby differences in D_h between species (on average 271.52 ± 4.03 μm for *Qrob* and 273.09 ± 4.35 in *Qpyr*) were non-significant (Table 2.4a).

Table 2.4 Comparison of generalized least squares (GLS) models assessing the effect of earlywood enlargement on the hydraulic diameter. (a) Models evaluating the effect of species, site and year. (b) Models evaluating the effect of earlywood enlargement.

	df	Log-Lik	ΔAIC
(a) Species comparison			
Site + Year	**7**	**-563.65**	**0.00**
Site + Year + Species	8	-563.59	2.18
Site × Year × Species (full model)	15	-562.11	16.61
(b) Enlargement			
Q. robur			
Site + Year + Onset	**8**	**-273.75**	**0.00**
Site + Year + Duration	8	-275.34	3.16
Site + Year	7	-277.65	5.79
Site + Year + End	8	-277.29	7.07
Year	3	-288.57	19.63
Site	4	-289.40	23.29
Q. pyrenaica			
Site	**4**	**-280.90**	**0.00**
Site + Year	5	-280.07	0.34
Site + Year + Onset	6	-279.42	1.04
Site + Year + Duration	6	-279.65	1.51
Site + Year + End	6	-280.03	2.27
Year	3	-284.09	4.38

Models are sorted following the Akaike's Information criterion (AIC). Onset, Duration, and End terms are all referred to earlywood vessel enlargement. Models were built using generalized least squares. The simplest model with ΔAIC < 2 is in bold. ΔAIC is increment on AIC values respect to that of the model with lowest AIC. *df,* degrees of freedom. Log-Lik, log-likelihood value.

Best-fit GLS models differed between the species (Table 2.4b). The onset of earlywood formation influenced D_h exclusively in *Qrob* (Fig. 2.4, Table 2.4b). In this case, the earlier the enlargement started, the larger the D_h (Table 2.4b). Longer periods of earlywood enlargement were also related to increasing D_h in *Qrob*, despite the meaningless influence of the end of this phase (Fig. 2.4). Conversely, D_h was scarcely affected by the onset and duration of earlywood enlargement in *Qpyr,* and the variability among individuals was only explained by the site factor (Table 2.4b). Additionally, D_h significantly decreased from 2012 to 2013 but only for *Qrob*, which is consistent with the delayed onset of enlargement during the second year in this species. Similar results were obtained using vessel area instead of D_h (Supplementary information, Table S.2.3, Fig. S.2.2).

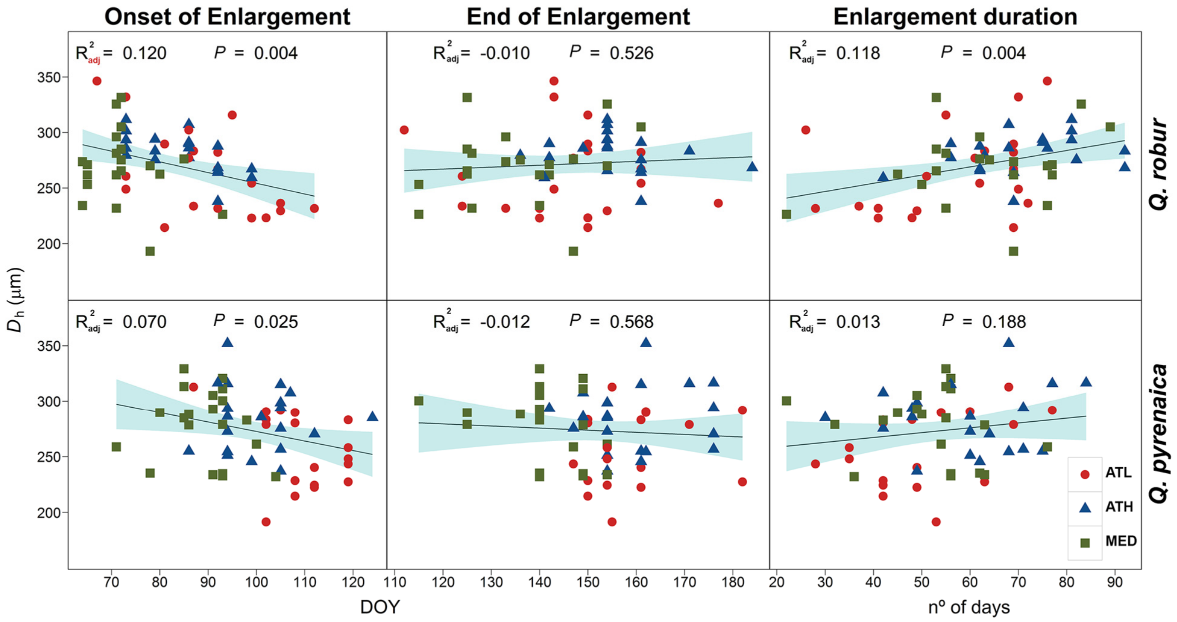


Figure 2.4 Relationships between tree hydraulic diameter (D_h) and the onset, end, and duration of the earlywood enlargement in *Q. robur* and *Q. pyrenaica*. Onset and end of vessel enlargement are expressed in day of year (DOY), and vessel enlargement duration in number of days. Dot shapes, and dot and line colors, represent the different study sites (ATL, ATH, and MED). Adjusted R^2 and *P*-value are provided for each relationship.

Discussion

Linkage between leaf phenology and earlywood formation

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

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

Environmental control of spring phenology

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

Species-specific patterns of spring phenology

Our results confirmed the earlier leaf phenology in *Qrob* than in *Qpyr*. The onset of cambial divisions and the subsequent phases of earlywood formation also occurred earlier in *Qrob,* leading to a longer period of earlywood vessel enlargement. Thereby, *Qrob* exhibited lower thermal requirements for break dormancy than *Qpyr,* as pointed out by Jato *et al.* (2002) after comparing budburst heat requirements. Vitasse *et al.* (2009) defined the phenological sensitivity to temperature as species-dependent. A delayed leaf appearance is assumed to be an adaptive response to avoid damage by late frost events (Vitasse *et al.*, 2014), as could be the case of *Qpyr*. Remarkably unstable year-to-year climate conditions, causing either late frosts or drought stress, frequently occur at the Iberian ranges where *Qpyr* inhabits. On the other hand, mild oceanic climate conditions in optimal areas for *Qrob* would result less critical for recently formed tissues. However, our results contrast with the synchronous pattern reported by González-González *et al.* (2013) at one mixed stand of *Qrob* and *Qpyr* after a one-year monitoring. This discrepancy is consistent with the strong spatial and inter-annual variability of tree phenology, especially in *Qrob*, as we observed among sites, and between the two years of monitoring.

Phenology-dependent hydraulic features of earlywood vessels

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

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

Earlywood vessel size in *Qpyr* may be less sensitive to thermally-induced phenological modulation than *Qrob*. Although studies performed on *Qpyr* tree rings are still scarce, previous summer rainfall has been stated as the most relevant climatic factor determining vessel size, rather than temperature in winter and spring (González-González *et al.*, 2014; González-González *et al.*, 2015). In fact, moisture availability was reported to be the most limiting factor driving radial growth in Iberian *Qpyr* populations (Gea-Izquierdo & Cañellas, 2014). As a whole, these findings suggest that vessel size in the sub-Mediterranean *Qpyr* could be more dependent on environmental signals operating at time windows that are different from those of vessel enlargement, such as precipitation during the previous growing season. Formation of narrower vessels is considered an adaptive response of trees to water stress in ring-porous species (Lovisolo & Schubert, 1998; Eilmann *et al.*, 2009; Gallé *et al.*, 2010; Gea-Izquierdo *et al.*, 2012). Such adjustment, also found by Tardif & Conciatori (2006) in *Q. rubra* and *Q. alba*, could be somewhat triggered by declining carbon gain after severe drought (Bréda *et al.*, 2006), due to the reliance of ring-porous oaks on the reserves of the previous year for earlywood formation and canopy development (Barbaroux & Bréda, 2002; Zweifel *et al.*, 2006).

The trade-off between hydraulic efficiency and safety suggests that larger vessels could jeopardize tree survival during severe droughts, increasing the detrimental effects of cavitation on water transport (Hacke *et al.*, 2006). Levanic *et al.*, 2011 found that *Qrob* trees dying after a severe drought were those showing a larger D_h, probably because their xylem was maladjusted to dry conditions. Hydraulic architecture in *Qrob* appears to be more sensitive to environmental conditions that influence the timing of vascular differentiation. An earlier reactivation in warm springs is expected to produce larger vessels, which are more efficient, but vulnerable to drought-induced embolisms; whereas a cold early season would delay growth resumption, resulting in smaller vessels and thus restricting hydraulic efficiency. On the contrary, *Qpyr* vessels are less affected by an early or late reactivation due to spring temperatures, and have therefore a lower vulnerability during dry or cold periods, but do not benefit under favourable conditions. Such strategy is consistent with the adaptations of *Qpyr* to continental conditions in Mediterranean mountains (Corcuera *et al.*, 2002), including delayed resumption and faster earlywood development to avoid both cold conditions in late winter-spring and drought in summer.

Conclusions

To our knowledge, this is the first research specifically addressing the effect of spring phenology on vessel size in ring-porous species. We were able to show that two coexisting oaks differentially adjust their xylem architecture to thermal drivers of plant growth resumption in spring. Hydraulic properties were affected by the timing of earlywood enlargement exclusively in the temperate *Qrob*, whereas they were more independent of phenological variability in the sub-Mediterranean *Qpyr* as an adaptation to cope with the enhanced stress of the Mediterranean region. Although more approaches at longer temporal and spatial scales are needed, our results evidenced a major role of functional wood anatomy as a response to shifting phenology, providing valuable information to understand how trees face climatic instability.

Acknowledgements

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Supplementary information

Table S.2.1 Growing degree days at the onset of vessel enlargement and budburst.

	Year 2012		Year 2013	
	Q. robur	*Q. pyrenaica*	*Q. robur*	*Q. pyrenaica*
Onset of enlargement				
ATL	356.44±26.17	495.22±10.70	382.78±16.25	507.41±11.27
ATH	228.23±13.04	364.73±8.51	197.28±6.01	239.72±9.78
MED	261.41±22.98	418.74±16.60	214.91±7.51	282.56±14.28
Budburst				
ATL	522.91±25.26	728.32±0.00	429.02±12.00	610.60±11.85
ATH	368.67±8.92	527.56±0.00	281.64±5.05	362.17±5.48
MED	360.81±19.81	548.28±20.22	281.97±10.84	441.15±11.42

Values are mean ± SE.

Table S.2.2 Effects of site, species, and their interaction on the growing degree days for earlywood vessel enlargement and budburst.

	df	MS	*F*-value	*P*
Onset of enlargement				
Site	2	352892	94.16	<0.001
Species	1	371103	99.02	<0.001
Site×Species	2	4471	1.19	0.307
Residuals	114	3748		
Budburst				
Site	2	419178	84.41	<0.001
Species	1	789041	158.89	<0.001
Site×Species	2	14543	2.93	0.057
Residuals	114	4966		

df, degrees of freedom. MS, mean square.

Table S.2.3 Comparison of linear mixed models assessing the effect of earlywood enlargement on vessel area.

	df	Log-Lik	*Δ*AIC
(a) Species comparison			
Site + Year	6	-629.72	0.00
Site	**5**	**-631.19**	**0.92**
Site +Year + Species	7	-629.72	2.00
Site × Year × Species (full model)	14	-627.57	11.75
(b) Earlywood enlargement			
Q. robur			
Site + Year + Onset	**7**	**-309.01**	**0.00**
Site + Year + Duration	7	-311.52	5.02
Site + Year	6	-314.51	9.01
Site + Year + End	7	-314.32	10.62
Year	4	-317.43	10.85
Site	5	-316.45	10.89
Q. pyrenaica			
Site	**5**	**-313.66**	**0.00**
Site + Year	6	-313.53	1.74
Site + Year + Onset	7	-312.62	1.91
Site + Year + Duration	7	-313.12	2.91
Site + Year + End	7	-313.52	3.73
Year	4	-319.68	10.04

df, degrees of freedom. Log-Lik, log-likelihood value.

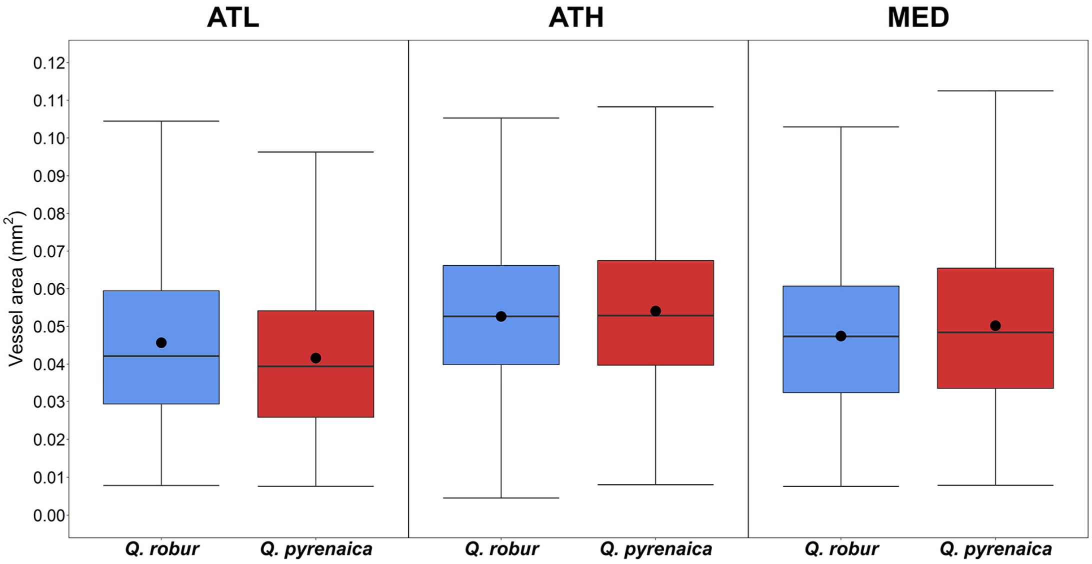

Fig. S.2.1 Distribution of earlywood vessel areas, sorted by tree species, within each study site.

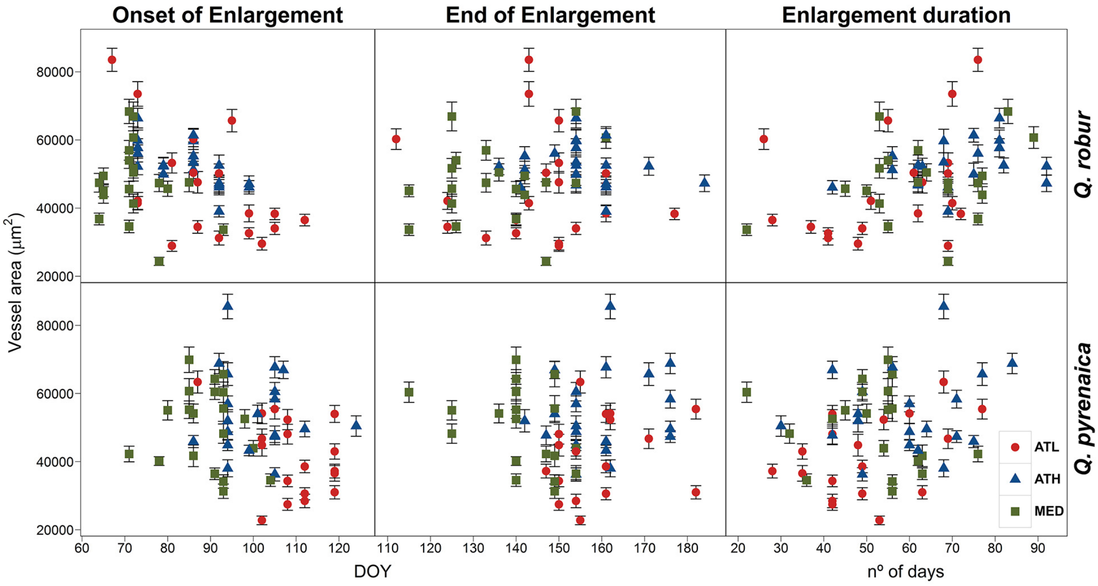

Fig. S.2.2 Relationships between mean vessel area and the onset, end, and duration of the earlywood enlargement.

CHAPTER III

Environmental conditions and vascular cambium regulate carbon allocation to xylem growth in deciduous oaks

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Summary

Combined effects of climate conditions and tree vigour are assumed to affect cambial activity, controlling allocation of non-structural carbohydrates (NSC) to growth. However, seasonal dynamics of xylem formation and NSC under changing environmental conditions, together with the influence of vascular cambium on xylem production, have rarely been integrated in the same study. We monitored xylogenesis during 2012 and 2013, and NSC in 2012 for the drought-sensitive *Quercus robur* and the drought-tolerant *Quercus pyrenaica* along a water-availability gradient in north-western Iberian Peninsula. We analyzed dependencies of xylem production and phenology on the number of cambial cells in winter. Xylem growth patterns were more complex towards the driest site, where growth resumed in autumn after a period of drought-induced quiescence. Sapwood NSC content however declined under humid conditions. *Q. pyrenaica* showed a shorter growing period and a lower productivity than *Q. robur*, but convergent NSC dynamics. Earlywood formation relied on NSC stored during the previous year, whilst current assimilates supported latewood growth concurrently with reserve refilling. Enhanced xylem production in fast-growing oaks was related to their higher number of cell layers in the dormant cambium, which also lengthened the growing season. Our study suggests that deciduous oaks respond to rising xeric conditions by increasing the complexity of wood formation dynamics, along with stored NSC, whose allocation to xylem growth is regulated by the number of cells in the dormant cambium. Therefore, dormant cambium size acts as a predisposing factor, modifying xylem growth demands during the growing season.

Introduction

Wood production is controlled by the plastic responses of trees to environmental cues, which set the pace of successive developmental processes occurring at a particular time window (Rossi *et al.*, 2012). In extratropical regions, trees enter a predictable dormancy phase to minimize detrimental effects of adverse winter conditions, such as frost damage (Morin *et al.*, 2007; Repo *et al.*, 2008). Hence, the timing of cambial phenology is tightly adjusted to decreasing temperature and incident solar radiation from the Equator to the poles, with a longer dormant period observed at higher latitudes (Gill *et al.*, 2015). At boreal and temperate biomes, growth is arrested from autumn to spring due to low temperature and daylength (Gričar *et al.*, 2006; Begum *et al.*, 2010; Cuny *et al.*, 2015). During the active period, xylem production describes a bell-shaped curve, which is defined by the onset of growth, the maximum growth rate, and the time of growth cessation (Rathgeber *et al.*, 2011b; Cuny *et al.*, 2015). In the Mediterranean region, by contrast, winter temperature and summer drought exert a double climatic control on cambial activity (Camarero *et al.*, 2010; Lempereur *et al.*, 2015). Severe drought triggers a period of quiescence (Montserrat-Martí *et al.*, 2009), which can be partially reversed if groundwater is recharged (de Luis *et al.*, 2007; Battipaglia *et al.*, 2010; Vieira *et al.*, 2015). Thus, xylogenesis of several Mediterranean conifers is frequently split into two active periods in spring and autumn (de Luis *et al.*, 2007; Linares *et al.*, 2009; Camarero *et al.*, 2010; Vieira *et al.*, 2015).

Seasonal changes in cambial activity are accompained by fluctuations of stored non-structural carbohydrates (NSC), which serve multiple key functions in plants, such as osmotic regulation, metabolic energy supply, and growth regulation (Barbaroux & Bréda, 2002; Sala *et al.*, 2012; Deslauriers *et al.*, 2014). At the end of the dormant season, NSC content is mobilized to meet growth demands, giving rise to new woody tissues and leaves (Skomarkova *et al.*, 2006; Begum *et al.*, 2010). Stored carbohydrates are thought to be recovered over the second half of the active period, particularly after growth cessation (Barbaroux & Bréda, 2002; Zweifel *et al.*, 2006; El Zein *et al.*, 2011). Seasonal fluctuations in NSC are assumed to be particularly pronounced in deciduous ring-porous oaks because the onset of radial growth occurs before budburst (Barbaroux & Bréda, 2002; Zweifel *et al.*, 2006; Pérez-de-Lis *et al.*, 2016). However, only a few studies mostly performed in moist ecosystems addressed the potential connection between xylogenesis and NSC levels in these species (Michelot *et al.*, 2012; Salomón *et al.* 2016).

The environmental control of secondary growth in a given species cannot be properly identified if we preclude the influence of individual characteristics. Large and fast-growing trees are thought to have a longer growing season and an enhanced cambial activity (Rathgeber *et al.*, 2011b; Vieira *et al.*, 2015). In the same line, tree vigour is mirrored on dormant cambium size (Vaganov *et al.*, 2006), and trees having more cambial cell layers during dormancy exhibit wider xylem rings (Gričar *et al.*, 2014). Such interplay must be necessarily mediated by the rate of cell proliferation and/or duration of growth, which are key predictors of wood production (Vaganov *et al.*, 2006; Lempereur *et al.*, 2015). In turn, the date of growth cessation is related to the amount of cells produced earlier in the season (Lupi *et al.*, 2010), whereby the dormant cambium size, along with the maximum growth rate, might affect the length of the growing season. However, our understanding of the interactions between tree-level factors and xylem formation dynamics is still fragmentary and little is known about their effects in the species-specific ability to tackle abiotic stress.

Temperate and sub-Mediterranean oaks showing divergent ecological traits due to different evolutionary pressures are sympatric at the transition between the temperate and Mediterranean biomes (Sánchez de Dios *et al.*, 2009). This is the case of the temperate *Quercus robur* L. and the sub-Mediterranean *Q. pyrenaica* Willd. The former has a broad distribution range comprising Europe and the Anatolian Peninsula, while the latter is present in western France and mountain areas of the Iberian Peninsula and northern Morocco. As compared to *Q. robur*, *Q. pyrenaica* is a late-flushing species better adapted to summer drought (Pérez-de-Lis *et al.*, 2016). Divergent ecological traits could also cause species-specific growth responses to environmental drivers, accounting for a different tolerance to the ongoing climate warming, which would bring out critical consequences on forest composition, productivity, and carbon sequestration (Rossi *et al.*, 2011).

The objective of this study is to analyze seasonal dynamics of cambial activity and their connexions with stem wood NSC content in *Q. robur* and *Q. pyrenaica* at the temperate-Mediterranean transition, a challenging boundary for the adaptation of the species to shifting climatic conditions. We specifically aim (i) to assess seasonal trends of xylogenesis and carbohydrate reserves along a water-availability gradient in 2012–2013, and (ii) to evaluate the interplay among tree size and vascular cambium features, considering the interspecific differences. We expect that increasing xeric conditions along the gradient will be associated to the occurrence of growth stagnation in summer. Stem wood NSC content is expected to show convergent fluctuation patterns in both species, with a decline at the time of earlywood formation and replenishment after growth cessation. In addition, we hypothesize that a higher number of cambial cells at dormancy predisposes trees to higher growth rates and to a longer growing season, resulting in an enhanced xylem growth.

Materials and methods

Study sites

The study was conducted at three mixed stands of *Q. robur* (*Qrob*) and *Q. pyrenaica* (*Qpyr*), located along a water availability gradient at the temperate-Mediterranean transition in the NW Iberian Peninsula. Bermui (ATL) and Labio (ATH) are the northernmost locations, where *Qrob* is the most abundant species. In contrast, the sub-Mediterranean *Qpyr* is more frequent than *Qrob* at the southernmost location Moreiras (MED). Soils are acidic, with loamy or sandy loam textures derived from gneiss bedrock at the ATL site, and calco-alkaline granitoids at ATH and MED.

Sensor devices (iButton Hygrochron DS1923, San Jose, CA, USA) were set at each site in order to register hourly variations in air temperature during 2012 and 2013. Precipitation data were collected from meteorological stations of the weather service MeteoGalicia (http://www.meteogalicia.es/), located within 16 km from each of the study sites. Summer is the driest period of the year, while maximum precipitation occurs from October to May. Site ATL, located 22 km away from the coast, has 1,461 mm of rain per year (period 1981–2010), whereas precipitation at ATH and MED reaches 996 and 832 mm per year, respectively. Site ATH, located at the highest elevation (690 m a.s.l.), shows cooler conditions in January-February than the other locations, with a mean minimum temperature of 2.5 ºC for the period 1981–2010, whereas ATL and MED respectively have 3.6 and 4.1 ºC. Site MED exhibits a higher mean maximum temperature (27.6 ºC) than ATH (22.8 ºC) and ATL (20.8 ºC) between June and September, experiencing frequent summer drought episodes. Precipitation registered at ATL, ATH, and MED in January-March 2012 was respectively 37, 26, and 13 % of the 1981-2010 average, whereas it was 225, 170, and 115 % for the same period of 2013.

Sampling and sample processing

Xylogenesis was monitored on 10 trees (20–30 cm stem diameter) per site and species from February 2012 to December 2013 (4,440 samples). Different trees were used in each year of monitoring. Wood microcores were weekly collected by pinning a Trephor (Vitzani, Belluno, Italy) in the trunk at breast height, as described by Rossi *et al.* (2006). Samples contained the vascular cambium, living phloem, and the outermost xylem rings. Microcore processing consisted of progressive sample dehydration in a tissue processor (Leica TP1020, Wetzlar, Germany), paraffin embedding, and cut of the paraffin blocks with a microtome (Leica RM2125 RTS, Wetzlar, Germany). As a result, we obtained 8–10 µm thick cross-sections, which were placed on microscope slides and dried before the staining, which included consecutive solutions of xylene, ethanol, safranin, and fast green FCF (Cutler *et al.*, 2008). Microsections were permanently fixed with Eukitt® resin (O. Kindler GmbH, Freiburg, Germany).

Non-structural carbohydrate concentrations were quantified in the sapwood of 10 additional trees per site and species. We took one 5-mm increment core per tree every two months, from mid-February 2012 to mid-February 2013 (n = 427). Cores were placed into a cool box in the field, and then stored at –20 ºC to prevent carbohydrate degradation. We oven-dried the cores at 60 ºC for 72 hours. The outermost 2-cm-long xylem fraction was selected for the analysis, which was performed after grounding the samples in a mixer mill (Retsch MM 400, Düsseldorf, Germany). Soluble sugars (SS) and starch fractions were calculated using the perchloric acid / anthrone method (Morris, 1948; Olano *et al.*, 2006). We used the anthrone reagent to spectrophotometrically determine SS concentrations, which were previously extracted from 20 mg of dry mass in 1 ml of ethanol (80 %) at 80 ºC for 30 min, and centrifuged 10 min at 4,000 rpm. Starch contained in the residue was then hydrolized with 1 ml of perchloric acid (35 %) for 1 h. Determination of starch concentration was then performed using the anthrone reagent as previously described for SS. Total NSC, SS, and starch concentrations are expressed as percentage of dry matter (DM).

Anatomical and phenological measurements

Anatomical observations on the cross-sections were performed at 40 × magnification, using a transmitted light microscope (Olympus BX40, Tokyo, Japan), equipped with a white light polarizing filter to discriminate maturing from enlarging cells. A digital camera attached to the microscope allowed us to acquire images of 5184 × 3456 pixels from all the samples. Measurements were performed in ImageJ version 1.48v (Schneider *et al.*, 2012). In a total of 6,002 images we counted the number of cell layers in the cambial zone, and measured the width of both enlarging and maturing zones on the outermost ring portion along three randomly selected radial lines within each cross-section.

The number of cambial cell layers and the width of the enlarging zone were used as proxies for the rates of cell division and xylem growth, respectively. The number of dormant cambial cell layers was obtained by averaging the values from the first and the last sampling dates of each year. We registered the dates of onset and cessation of xylem growth, along with those of maximum number of cambial cell layers (i.e. maximum rate of cell division) and widest enlarging zone (i.e. maximum xylem growth rate). All phenological events were expressed as day of year (DOY). The onset of xylem growth was assumed to occur the first DOY at which enlarging earlywood cells were observed, while growth cessation was identified the first DOY from which enlarging latewood cells were no longer detected.

Intra-annual dynamics of radial growth and carbon reserves

Data of xylem growth were transformed to binary information, according to the presence/absence of the observed events. We used generalized linear mixed models (GLMM) for binomial distribution to compare species, sites, and years. Generalized additive models (GAM) for binary data were fitted in order to analyze periods of xylem growth (Wood 2006). This model assumed that the probability of detecting cambial divisions varied over time (i.e. with DOY), for which a penalized cubic regression spline was fitted. Seasonal dynamics of carbohydrate concentrations in sapwood were analyzed in order to identify the main seasonal trends of carbon storage. SS, starch, and total NSC concentrations were quantified for each sampling date. SS levels were evaluated fitting generalized linear models (GLM) for gamma distributions. Starch and total NSC concentrations were evaluated with generalized least squares (GLS), allowing for heterogeneous variances, and a first-order autocorrelation structure. Model selection was conducted by optimizing the Akaike's information criterion (AIC) scores and the distribution of residuals (Zuur *et al.*, 2009). We also estimated the proportion of the ring formed by smoothing individual curves of maturing tissue width. Model calculations were performed in R (R Development Core Team, 2014), by using the packages 'lme4' (GLMM), 'mgcv' (GAM), and 'nlme' (GLS).

Structural equation models

We applied structural equations models (SEM) to assess the way in which the relations between tree size, the number cell layers in the dormant cambium, the maximum width of enlarging xylem, and duration of the growing season determined the annual xylem increment. This approach is suitable for testing direct and indirect effects of multiple parameters occurring in complex biological systems (Grace, 2006). We aimed to verify a proposed hypothetical structure, which relied on the evidences mentioned in the introduction (Supplementary information, Fig. S.3.1). Analyses were performed separately for each species with pooled data from the three locations. Model coefficients were estimated by maximum likelihood, using standard evaluation methods (Grace, 2006), which comprises χ^2 test, the adjusted goodness of model fit index (AGFI), and the root mean square error of approximation (RMSEA). We assumed that the model fit was acceptable if AGFI > 0.90 and RMSEA < 0.05. SEM was performed with AMOS 18.0 software (AMOS Development Corp., Mount Pleasant, South Carolina, USA).

Results

Cambial phenology and radial growth

The onset of xylem enlargement occurred from mid to late March in *Qrob* (DOY 73–89), and from late-March to mid-April in *Qpyr* (DOY 90–109), being significantly earlier for *Qrob*, and at MED (Table 3.1, Fig. 3.1). The number of cambial cells increased until the occurrence of a plateau from mid- to late-June (DOY 169–179) in *Qrob*, and from mid-June to early-July (DOY 173–183) in *Qpyr* (Fig. 3.1). The width of the enlarging zone increased at the onset of earlywood formation and declined before the onset of latewood enlargement, which took place from late-May to late-June (DOY 144–181) in *Qrob*, and from mid-June to early-July (DOY 165–188) in *Qpyr*.

Table 3.1 Generalized linear mixed models (GLMM) to evaluate the effect of species, years and sites on the occurrence of main phenological events.

	df	Log-Lik	ΔAIC
Onset of earlywood enlargement			
Year + Site + Species	7	-142.61	0.00
Site + Species	6	-147.21	7.20
Onset of latewood enlargement			
Year + Site + Species	7	-128.54	0.00
Year + Site	6	-148.46	37.83
Maximum number of cambial cell layers			
Year	4	-185.62	0.00
Year + Species	5	-185.51	1.78
Year + Species + Site	7	-190.29	15.35
Maximum width of enlarging xylem			
Year + Site + Species	7	-174.47	0.00
Year + Site	6	-176.51	2.07
Site + Species	6	-185.52	20.09
Cessation of growth			
Year	4	-216.87	0.00
Year + Site	6	-215.04	0.35
Year + Species	5	-222.51	13.28

Models were fitted for binomial distributions, including tree as a random effect. Models are sorted following the Akaike's Information criterion (AIC). Only the best-fit models are shown. ΔAIC is increment on AIC values respect to that of the model with lowest AIC. *df* is degrees of freedom. Log-Lik is the log-likelihood value.

Growth rates raised again afterwards (Fig. 3.1), and the maximum width of enlarging xylem occurred approximately 20 days later (DOY 179–199 in *Qrob* and DOY 186–203 in *Qpyr*), with considerably higher variation in mean values between years at MED than at ATL and ATH (Fig. 3.1). The rate of xylem production gradually slowed down from July until growth cessation, occurring from mid- to late-October in 2012 (*Qrob*, DOY 286; *Qpyr*, DOY 303), and in early-October in 2013 (*Qrob*, DOY 279; *Qpyr*, DOY 275), with a substantial year and site effect, but not between species (Table 3.1). At MED, the growing season of 2013 (wet) was shorter ($F = 25.90$, $P < 0.001$), whilst xylem increment doubled ($Z = –3.61$, $P < 0.001$) that of 2012 (dry). Complementarily, the mean leaf period (from budburst to leaf abscission) at the study sites comprised 239 ± 3 days in *Qrob*, from mid March-early April to November-December, whilst only 196 ± 3 days in *Qpyr*, from April-May to November.

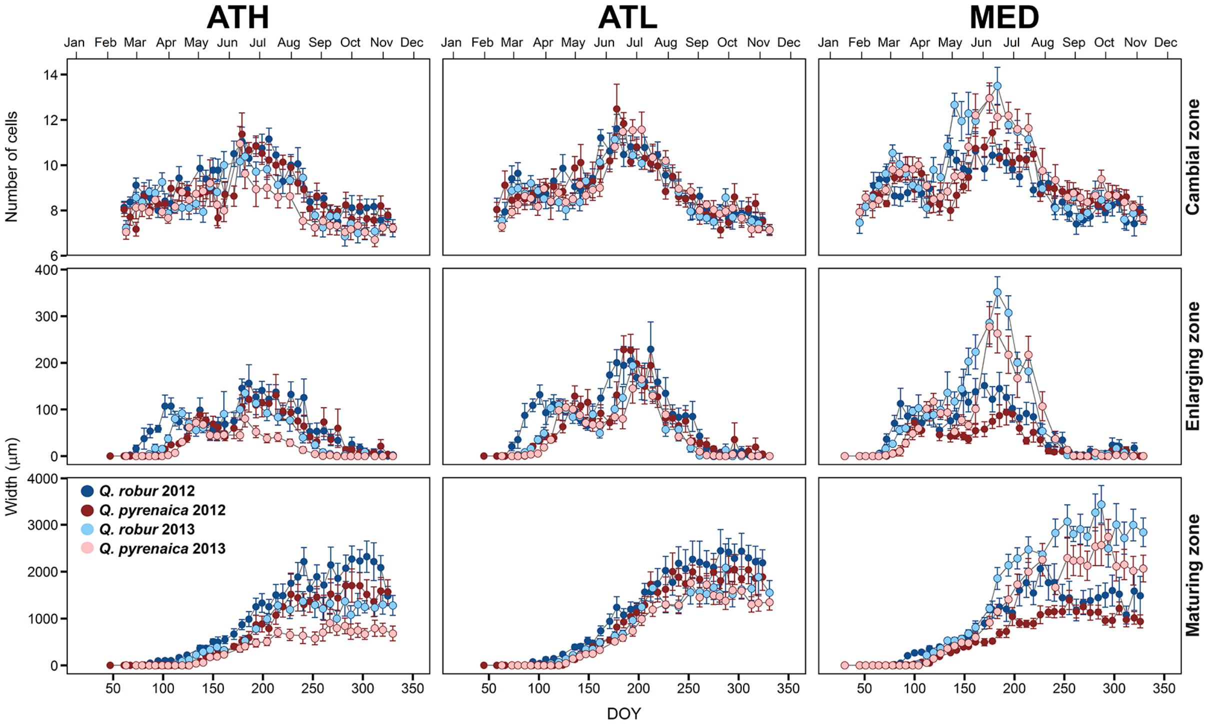

Figure 3.1 Seasonal dynamics of radial growth for *Q. robur* (blue dots) and *Q. pyrenaica* (red dots) at the three study sites (ATL, ATH and MED) in 2012 (dark dots) and 2013 (light dots). Vertical bars represent the standard error.

GAMs confirmed the high plasticity of cambial phenology. The smoothing term of the best-fit model (explained variance of 74.4 %) pointed out that seasonal patterns changed between species, sites, and years (Table 3.2). Model predictions indicated that the onset of xylem enlargement occurred earlier for *Qrob*, and at MED (Fig. 3.2). The probability of growth declined earlier at this site (Fig. 3.2), but a brief recovery period in October was observed for both species during the dry year (2012) and only for *Qrob* during the wet year

(2013). Such pattern was noted in 13 *Qrob* and 14 *Qpyr* trees at MED, whilst only in 6 (7) *Qrob* and 10 (9) *Qpyr* at site ATL (ATH). Yet, xylem formed during autumn reactivation was merely 6.91 ± 1.25 % of the annual xylem increment for *Qrob* and 5.51 ± 1.42 % for *Qpyr* (Fig. 3.1; Supplementary information, Fig. S.3.2). It is noteworthy that premature withering was perceived in September 2012 in 6 individuals for each species, all of them at the MED.

Table 3.2 Comparison of generalized additive models (GAM) evaluating the time-related effect of species, site and year on cambial activity. Models are sorted following the Akaike's Information criterion (AIC). Shading area represents those models including the smoothing term that optimized AIC scores.

		df	AIC	R_{adj}^2 (%)
Smoothing term	Linear responses			
DOY (Species × Year × Site)	Species + Site	94.739	2055.652	0.786 (74.4)
DOY (Species × Year × Site)	Species + Year	93.315	2056.993	0.786 (74.3)
DOY (Species × Year × Site)	Species	94.343	2057.293	0.786 (74.4)
DOY (Species × Year × Site)	Species + Year + Site	95.032	2057.658	0.786 (74.4)
DOY (Species × Site)	Species + Year + Site	50.958	2188.025	0.767 (71.4)
DOY (Site × Year)	Species + Year + Site	48.675	2244.531	0.756 (70.6)
DOY (Site)	Species + Year + Site	29.090	2354.739	0.742 (68.5)
DOY (Year × Species)	Species + Year + Site	36.984	2459.747	0.724 (67.3)
DOY (Species)	Species + Year + Site	21.554	2504.951	0.719 (66.2)
DOY (Year)	Species + Year + Site	22.089	2570.679	0.707 (65.4)
DOY	Species + Year + Site	14.581	2612.112	0.703 (64.6)
None (generalized linear model)	Species + Year + Site	5.000	7226.285	0.010

df, degrees of freedom. R_{adj}^2, adjusted R^2. The explained deviance is included in brackets and expressed as a percentage.

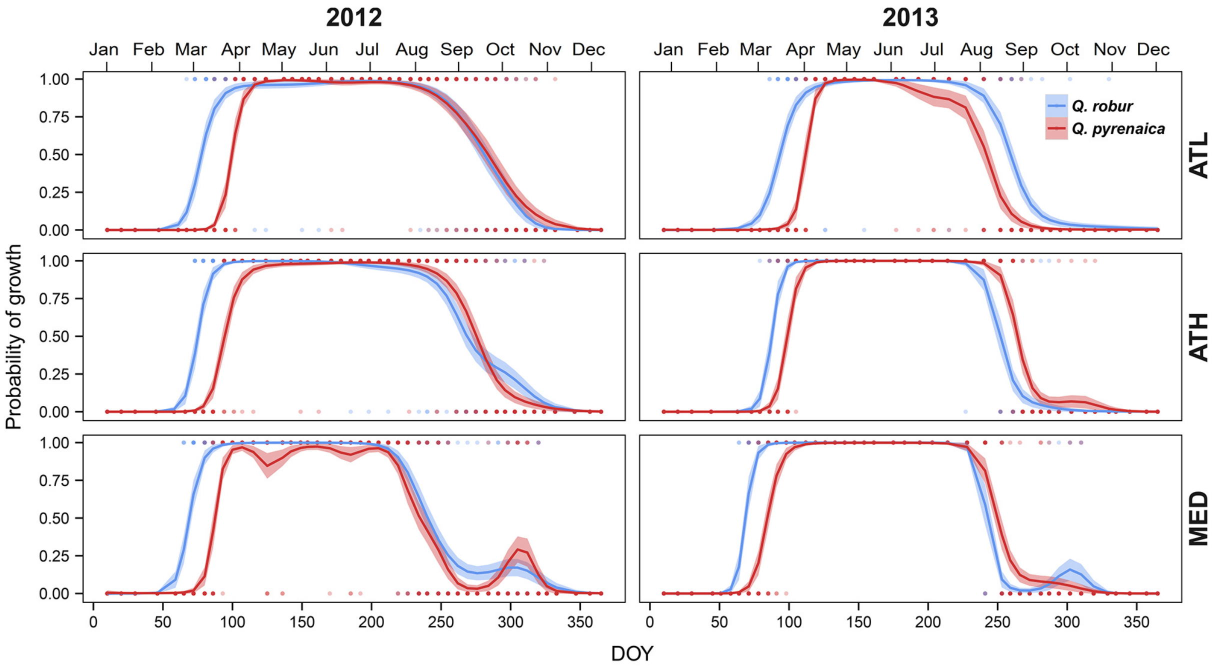

Figure 3.2 Seasonal variation of the probability of growth predicted by the GAM models for *Q. robur* (blue) and *Q. pyrenaica* (red). Dots represent the observations of each species, expressed as binary data (0, no growth; 1, growth). Shaded areas represent standard error estimates.

Seasonal dynamics of carbon reserves

Carbohydrate concentrations showed strong seasonal and spatial variability (Table 3.3), with the highest mean values at MED (SS = 4.40 ± 0.14, starch = 5.62 ± 0.17 % DM), and the lowest ones at ATL (SS = 3.83 ± 0.12, starch = 3.99 ± 0.13 % DM). Both species exhibited similar total NSC concentrations, but a different relative contribution of SS and starch fractions (Table 3.3). The mean SS content during 2012 was 3.91 ± 0.11 % DM for *Qrob* and 4.35 ± 0.12 for *Qpyr*, whereas starch represented 4.93 ± 0.13 % DM for *Qrob* and 4.33 ± 0.13 for *Qpyr* (Table 3.3).

Table 3.3 Evaluation of soluble sugars (SS), starch levels, and total non-structural carbohydrates (NSC) considering the effect of the sampling date, location, and species.

	df	Log-Lik	*Δ*AIC
NSC (GLS)			
Date + Site	17	-870.95	0.00
Date + Site + Species	18	-870.05	0.37
Date + Site × Species	20	-868.62	1.90
Date	15	-893.61	40.98
SS (GLM)			
Date + Site + Species	11	-564.56	0.00
Date + Site × Species	13	-564.07	3.28
Date + Site	10	-573.32	15.43
Starch (GLS)			
Date + Site + Species	20	-788.80	0.00
Date + Site × Species	22	-787.81	2.47
Date + Site	19	-795.27	10.74

Generalized linear models (GLM) for gamma-distributed data were fitted for SS, and generalized least squares (GLS) were used for total NSC and starch concentrations. Models are sorted following the Akaike's Information criterion (AIC). Only the best-fit models are shown. *df* is degrees of freedom. Log-Lik is the log-likelihood value. *Δ*AIC is increment on AIC values respect to that of the model with lowest AIC.

SS and starch concentrations showed a strong variation during the growing season (Table 3.3, Fig. 3.3). In *Qrob*, a lower seasonal variation in total NSC content was noted at ATL compared to MED and ATH (*F* = 4.37, *P* = 0.017). In *Qpyr*, these differences were only marginally significant (*F*= 2.82, *P* = 0.077). SS and starch levels dropped from February to June, during earlywood formation and leaf expansion (Fig. 3.3). Starch levels increased from June to August at site ATL, but only in October at ATH and MED. SS recovery initiated in June at MED, but in August at ATL and ATH (Fig. 3.3). From October onward, starch content decreased simultaneously with a pronounced increase of SS concentrations.

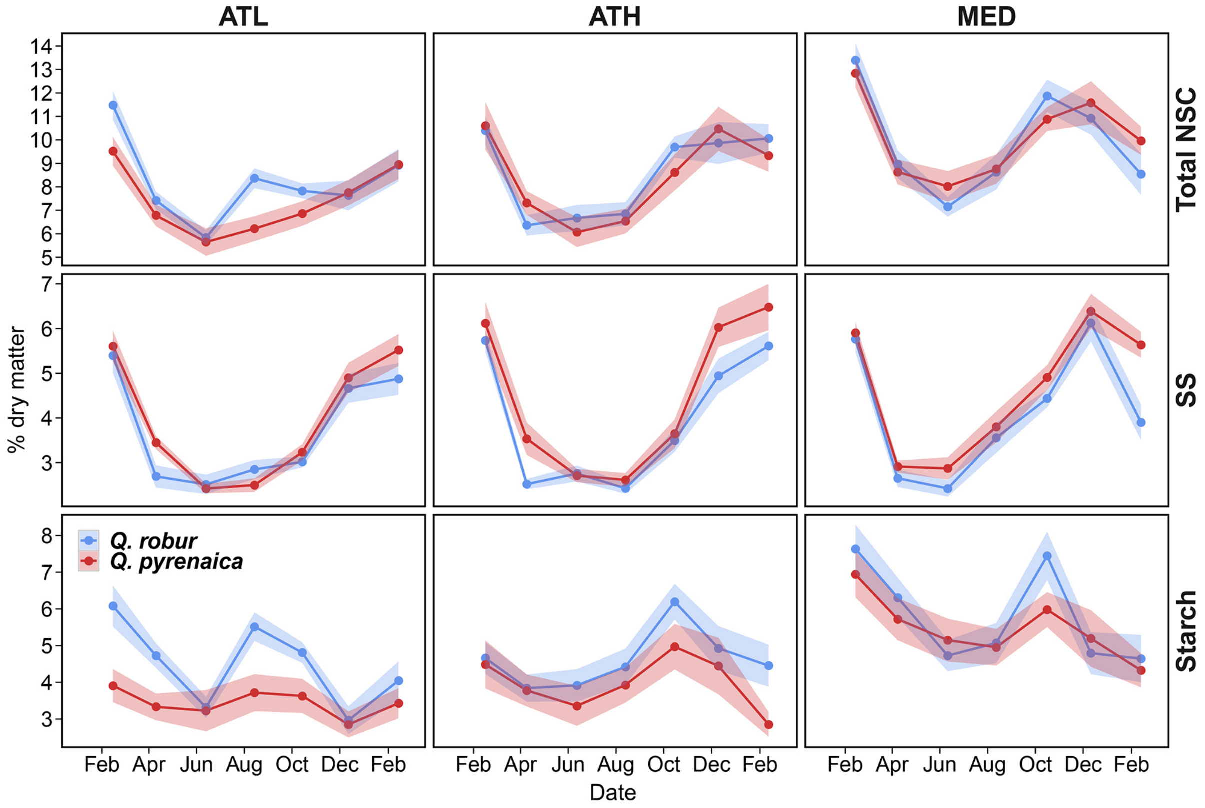

Figure 3.3 Seasonal dynamics of non-structural carbohydrate content for *Q. robur* (blue) and *Q. pyrenaica* (red) in 2012. Levels of total non-structural carbohydrates (TNSC), soluble sugars (SS), and starch are represented for the three study sites. Lines and shaded areas represent mean and standard error, respectively.

Growth modulation by tree-level factors

SEM model exhibited a good fit on the observed dataset ($P_{\chi2} > 0.050$; AGFI > 0.900; RMSEA < 0.001), revealing multiple dependencies among intrinsic tree-level factors modulating xylem formation (Fig. 3.4). A large percentage of variability in annual xylem increment could be explained by the model (64 % for *Qrob* and 74 % for *Qpyr*). The effect of stem diameter on growing season length, maximum growth rate, and annual xylem growth, was indirect and mediated by its positive effect on the dormant cambium (Fig. 3.4). Larger and fast-growing trees exhibited more cambial cell layers during dormancy than their slow-growing counterparts. Number of cell layers in the dormant cambium had a positive direct effect on growing season length. Trees having more cambial cell layers ceased growth later for both *Qpyr* ($r = 0.43$, $P < 0.001$) and *Qrob* ($r = 0.37$, $P = 0.004$), being also active at an earlier date in *Qpyr* ($r = –0.37$, $P = 0.004$). Dormant cambial cell number also had an indirect effect on growing season length through its direct effect on maximum rate of xylem enlargement. Trees with a larger maximum rate of xylem growth entered dormancy later ($r = 0.37$; $P = 0.004$ for both species). Maximum growth rate showed a strong control of annual xylem increment, with growing season length only exerting a minor effect in *Qrob* (compare Fig. 3.4a with 4b).

The number of dormant cambial cells correlated to maximum cambial cell number for *Qpyr* ($r = 0.41$, $P < 0.001$), and marginally for *Qrob* ($r = 0.23$, $P = 0.075$).

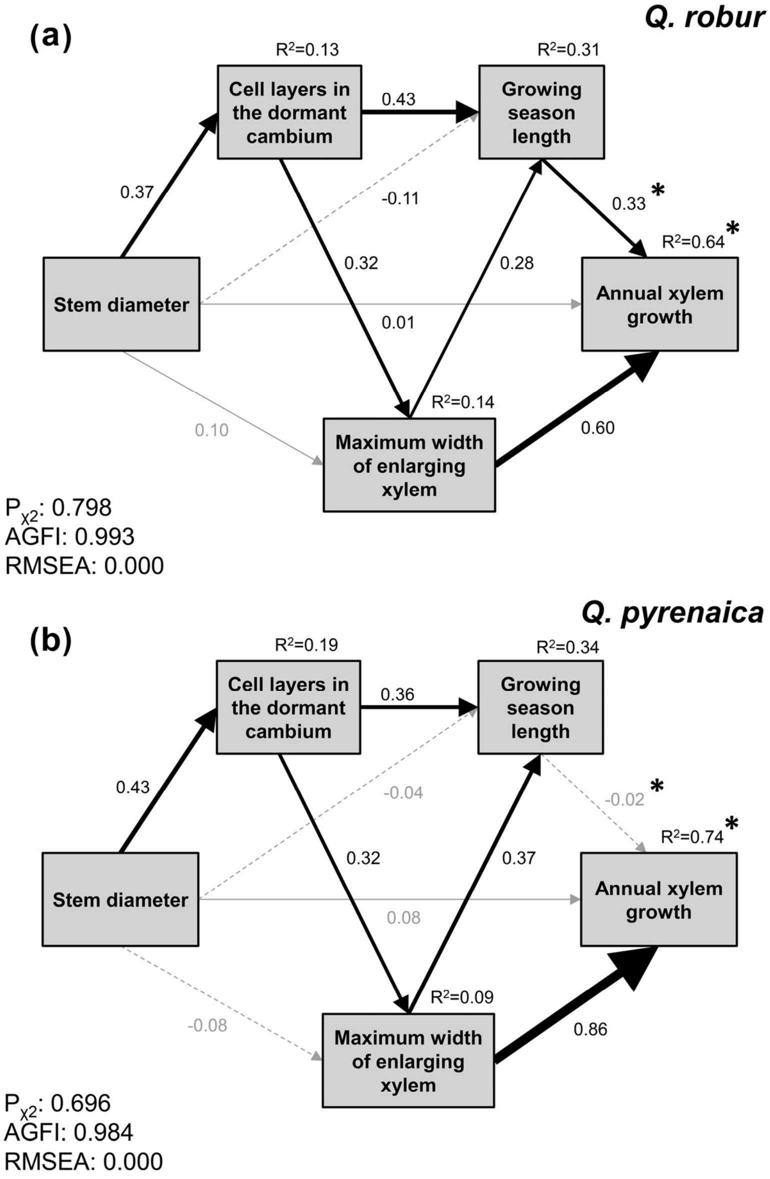

Figure 3.4 Structural equation models fitted for (a) *Q. robur* and (b) *Q. pyrenaica*. Explained variance for endogenous parameters are shown above the boxes. Black solid (positive effects) and dashed (negative effects) arrows denote significant relations, while non-significant relations are shown as grey arrows and coefficients. Asterisks denote paths or error values significantly different between the species models by multiple comparison (* $P \leq 0.01$). The x^2 test, the adjusted goodness of model fit index (AGFI), and the root mean square error of approximation (RMSEA) are shown for each model.

Qrob exhibited a higher maximum rate of xylem enlargement than *Qpyr*, which also showed a shorter growing season and smaller annual xylem increment (Table 3.4). However, the maximum radial number of cells in both dormant (7.51 ± 0.11 for *Qrob* and 7.60 ± 0.12 for *Qpyr*) and active cambium (12.42 ± 0.20 for *Qrob* and 12.61 ± 0.26 for *Qpyr*) did not differ between species. The number of cell layers in the dormant cambium did not differ between species (7.51 ± 0.11 for *Qrob* and 7.60 ± 0.12 for *Qpyr*). Differences among sites were only detected in the dormant cambium for *Qpyr*, with a higher number of cambial cell layers at MED (Table 3.4).

Table 3.4 Tree characteristics recorded for each species and site, and comparisons between *Quercus robur* (*Qrob*) and *Quercus pyrenaica* (*Qpyr*), and among sites within species.

	DBH (cm)	Cell layers in dormant cambium	Maximum cambial cell layers	Maximum width of enlarging xylem (μm)	Annual xylem growth (μm)	Growing season length (days)
Qrob	27.29±0.52	7.51±0.10	12.42±0.20	**282.48±15.54**	**1999.44±127.40**	**193.80±4.25**
ATL	27.04±0.86**a**	7.55±0.21**a**	11.93±0.29**a**	238.19±21.52**a**	1681.10±172.50**a**	191.35±6.29**a**
ATH	27.89±0.85**a**	7.35±0.16**a**	12.33±0.26**a**	282.26±27.96**ab**	1977.50±181.36**a**	193.95±7.92**a**
MED	26.95±1.01**a**	7.63±0.18**a**	13.02±0.42**a**	327.00±28.24**b**	2339.79±277.69**a**	196.10±8.09**a**
Qpyr	27.38±0.60	7.60±0.12	12.61±0.26	**220.10±14.68**	**1488.07±105.49**	**182.30±4.47**
ATL	27.35±1.00**a**	7.39±0.24**a**	12.18±0.52**a**	165.67±25.39**a**	1117.94±167.97**a**	169.65±8.78**a**
ATH	26.29±1.10**a**	7.34±0.22**a**	12.65±0.43**a**	257.81±20.97**b**	1709.81±140.48**b**	185.45±6.83**a**
MED	28.49±1.03**a**	8.07±0.12**b**	13.00±0.38**a**	236.83±26.05**ab**	1636.47±211.60**ab**	191.80±6.99**a**

Values are reported as mean ± standard error. GLM models were fitted to test differences between species (shading) and among sites (non shading), for which multiple pairwise comparisons were subsequently applied. Values in bold indicate a significant effect of the species. Within each species, values follow by the same letter are not statistically different.

Discussion

Cambial activity at the transition between temperate and Mediterranean biomes

In line with previous studies for other deciduous species (Oladi *et al.*, 2011; Prislan *et al.*, 2013), the onset of xylem enlargement showed a pronounced temporal and spatial variability. Temperature is deemed to play a key role in driving cambial resumption (Gričar *et al.*, 2006; Deslauriers *et al.*, 2008; Begum *et al.*, 2010), promoting the onset of cell differentiation (Pérez-de-Lis *et al.*, 2016), with growth resumption responding to site and year temperature (Deslauriers *et al.*, 2008; Moser *et al.*, 2009). This temporal hierarchy in xylogenesis onset was translated to the rest of the wood formation phases, reflecting the dependence of each phenophase on the previous ones (Rossi *et al.*, 2012). Initiation of earlywood-to-latewood transition matched the completion of leaf expansion, probably because gibberellins synthesized in mature leaves control signalling pathways for cell division, fibre differentiation, and woodiness (Aloni, 2015). In fact, the maximum width of enlarging xylem, a proxy of the maximum growth rate, occurred from late June to mid-July, just after the onset of latewood growth. This is convergent with the synchrony between maximum growth rate and maximum day-length previously reported for evergreen and deciduous species (Montserrat-Martí *et al.*, 2009; Oladi *et al.*, 2011; Kalliokoski *et al.* 2013; Prislan *et al.*, 2013; Cuny *et al.*, 2015). Growing season was longer than previously reported for temperate oaks, with most of trees ceasing enlargement in October (Zweifel *et al.*, 2006; Michelot *et al.*, 2012). Such an extended active period could reflect the longer photoperiod and/or warmer conditions experienced in our study sites (Bauerle *et al.*, 2012; Gill *et al.*, 2015).

Overall, growth dynamics depicted a bimodal pattern, with a first minor peak during earlywood enlargement, a declining rate during earlywood maturation, and a second major peak at the onset of latewood growth. This pattern is comparable to that found by Cuny *et al.* (2013) for temperate conifers. However, several *Qrob* and *Qpyr* trees showed a temporal suppression of cambial activity in September followed by a brief recovery period in October, thus leading to a trimodal growth pattern. According to our model, this complex behaviour was especially evident at site MED, where premature withering occurred in September, conveying drought stress (Montserrat-Martí *et al.*, 2009). In addition, differences in the maximum growth rate between years were more pronounced at this location. Soil water content modulates cambial activity in Mediterranean areas (Battipaglia *et al.*, 2010; Camarero *et al.*, 2010), but also in temperate forests (Zweifel *et al.*, 2006; Delpierre *et al.*, 2015). Nonetheless, a peak of cambial activity in autumn is characteristic of the Mediterranean region (de Luis *et al.*, 2007; Linares *et al.*, 2009; Battipaglia *et al.*, 2010; Camarero *et al.*, 2010; Montserrat-Martí *et al.*, 2009; Vieira *et al.*, 2015, Arzac *et al.*, 2016), but presumably absent in temperate oaks, even in extremely dry years (Zweifel *et al.*, 2006; van der Werf *et al.*, 2007). Discrepancies between our study and those performed under cooler climates might respond to low autumn temperatures precluding cambial reactivation as a response to soil water recharge (Arzac *et al.* 2016). Nevertheless, post-drought recovery was reported in beech trees under temperate conditions (van der Werf *et al.*, 2007), while Balducci *et al.* (2016) demonstrated the high resilience of cambial activity to drought in black spruce seedlings growing under controlled conditions. Taken together, these findings suggest that plastic responses of cambial activity to climate are widespread among taxa, but their occurrence is restricted to particular climatic conditions.

Seasonal variation of carbohydrate concentrations in relation to radial growth dynamics

According to our second expectation, study species showed convergent carbohydrate fluctuations in sapwood, being also comparable to those previously reported for *Qpyr* (Salomón *et al.*, 2016) and sessile oak (Barbaroux and Bréda, 2002; El Zein *et al.*, 2011, Michelot *et al.*, 2012). This result indicates that temporal dynamics of carbon allocation to storage and growth in study oaks reflected their identical ring-porous xylem structure and deciduous leaf habit (Barbaroux and Bréda, 2002; Zweifel *et al.*, 2006). SS and starch declined in outermost xylem from February to June, until the completion of earlywood formation. This was also concurrent with shoot growth and flushing, although rapid leaf development after budburst may have alleviated reserve mobilization (Montserrat-Martí *et al.*, 2009). In addition to aboveground growth, NSC reserves fuel root production, which was reported to occur between May and July in ring-porous oaks (McCormack *et al.*, 2014). Short-distance NSC mobilization was detailed in larch by Begum *et al.* (2010), who found that augmented sink activity during cambial resumption reduced the size and number of starch granules in the cambial zone. Likewise, Skomarkova *et al.* (2006) found that about 20–30 % of the tree ring was supported by stored NSC in deciduous beech. Such percentage could be even larger for our study oaks, given that xylem growth resumption predates leaf-out in ring-porous species (Barbaroux and Bréda, 2002; El Zein *et al.*, 2011; Sass-Klaassen *et al.*, 2011; Pérez-de-Lis *et al.*, 2016). Indeed, Zweifel *et al.* (2006) stated that early phases of radial growth in pubescent oak were entirely supported by stored carbohydrates. In contrast, the minimum NSC content in the outermost sapwood coincided with maximum growth rates in June-July, which is consistent with latewood growth as being supported by recently obtained photosynthates (Kalliokoski *et al.*, 2013).

In accordance to previous studies, NSC replenishment started in summer (Barbaroux and Bréda, 2002; El Zein *et al.*, 2011), even though carbon demand for xylem growth is assumed to peak at that time (Cuny *et al.*, 2015). Completion of shoot elongation and leafing contributes to reduce NSC demand to these competing carbon sinks. In addition, photosynthetic activity is enhanced in early summer due to the longer photoperiod (Morecroft *et al.*, 2003; Bauerle *et al.*, 2012), which presumably prompt carbon allocation to storage and xylem growth. SS accumulation might be also fostered in summer to prevent dehydration in living tissues under increasing dry conditions (Regier *et al.*, 2009; Deslauriers *et al.*, 2014). This idea is consistent with the more pronounced NSC increment observed from August onward at ATH and MED, which was related to a higher seasonal variation. Photosynthesis is assumed to be less sensitive than cambial activity to water stress (Lempereur *et al.*, 2015), whereby diminished NSC allocation to growth under moderate drought stress probably fostered storage (Delpierre *et al.*, 2015). Yet, the lower sapwood NSC concentration noted at ATL was concurrent with a more limited latewood increment, which indicates that differences in carbon allocation to storage along the gradient cannot be solely attributed to the contrasting growth demand. The shorter growing period at ATL probably reduced carbon gain (Morecroft *et al.*, 2003), whilst the excessive soil humidity in winter might exacerbate carbohydrate consumption in roots (Baley-Serres and Voesenek, 2008; Ferner *et al.*, 2012). Furthermore, a more severe powdery mildew infestation was noted at ATL, likely affecting photosynthetic performance and growth (Marçais & Desprez-Loustau, 2012).

Maximum carbohydrate content was attained at the onset of dormancy, probably due to nutrient recycling from senescent leaves (El Zein *et al.*, 2011). Carbon reserves are necessary for maintenance respiration during the leafless period (Edwards & Hanson, 1996; Atkin & Tjoelker, 2003; Deslauriers *et al.*, 2014; Salomón *et al.*, 2015), but also to provide energy and substrate for the formation of new tissues in spring (Barbaroux and Bréda, 2002; Begum *et al.*, 2010). This NSC increase was however coupled with a pronounced decline of the starch content. A high SS concentration reduces the risk of freezing damage in the symplast, whereby starch breakdown may be associated to cold hardening before dormancy (Morin *et al.*, 2007; Repo *et al.*, 2008). Low temperatures and leaf senescence are thought to promote starch breakdown (Morin *et al.*, 2007; Repo *et al.*, 2008; Vitasse *et al.*, 2014). The higher SS concentration in *Qpyr* is thus consistent with its shorter growing period and higher cold tolerance than *Qrob*. This suggests that different carbohydrate partitioning between the study species may be mostly attributed to species-specific responses of phenology to environmental cues.

We noted that *Qpyr* was less productive than *Qrob*, exhibiting a more conservative growth strategy despite the greater SS concentration. Rodríguez-Calcerrada *et al.* (2008) attributed contrasting growth responses in *Qpyr* and sessile oak to the more conservative carbon-use strategy of the former species. Several lines of evidence point out that stress-tolerant species deploy mechanisms to limit resource turnover, affecting both sources and sinks (Valladares *et al.*, 2000). This idea is reinforced by the fact that *Qrob* trees growing at the rear edge (site MED) were more productive than their *Qpyr* counterparts, even under moderate drought stress.

Vascular cambium and growth capacity

As previously reported for conifers, fast-growing oaks showed wider tree rings and longer growing season than slow-growing ones (Rathgeber *et al.*, 2011b; Vieira *et al.*, 2015). However, such relationship was mediated by the interaction between tree size and the number of dormant cambial cells, which had a direct effect on maximum growth rate. This result confirmed our second hypothesis pointing out the crucial role played by the radial number of cambial cells in the adjustment of xylem production to tree vigour. The association between tree size and number of cambial cell layers was likely mediated by the amount of auxin basipetally transported through the cambial zone, which is involved in the maintenance of meristem identity (Uggla *et al.*, 1996; Tuominen *et al.*, 1997). In this regard, Uggla *et al.*,

(1998) noted a wider radial gradient of auxin concentration and more cambial cell layers in the stem of large and vigorous trees. Since auxin concentrations in the cambial zone are similar in the active and dormant periods (Uggla *et al.*, 1996), we can assume that the association between tree vigour and the number of dormant cambial cells was maintained during dormancy, regardless of the limited auxin transport capacity (Schrader *et al.*, 2003).

Dormant cambium size implicitly affected xylem production through the maximum growth rate, which is a prominent explanatory factor of annual xylem increment (Delpierre *et al.*, 2015). Given that the number of periclinal cell divisions in the cambial zone is in concert with the amount of xylem cells produced (Gregory, 1971; Vaganov *et al.*, 2006), trees having more dividing cells at the onset of cambial resumption might be able to produce more xylem cells during the active period. Thereby, enhanced cell production may partially account for the higher maximum growth rate observed in those trees with more cambial cells in winter. In fact, the number of dormant cambial cells was correlated to the maximum number of cambial cells during the active period for *Qpyr*, though only marginally for *Qrob*.

Delayed growth cessation was reported to occur when larger amount of xylem cells were produced earlier in the season (Lupi *et al.*, 2010). This is consistent with the positive dependency of growing season length on maximum growth rate in our study oaks. However, dormant cambium size had a complex effect on cambial phenology, with both direct and indirect positive influences, as demonstrated the fact that *Qpyr* trees having numerous dormant cambial cells exhibited an earlier onset of enlargement. A substantial effect of growing season length on annual xylem increment was exclusively evidenced for *Qrob*, suggesting that fast-growing *Qpyr* trees were not able to take advantage from their longer growing season. This result implies a reduced responsiveness of wood anatomy to tree phenology in *Qpyr*, which would play a role in the physiological resistance to stress (Pérez-de-Lis *et al.*, 2016). Persistent winter frost and summer drought in the sub-Mediterranean area would have elicited a stress-tolerance mechanism through which growth is speeded up to avoid unfavourable conditions (Montserrat-Martí *et al.*, 2009; Sánchez de Dios *et al.*, 2009).

SEM model was a first attempt to disentangle the potential connection between the dormant cambium size and xylem production reported in previous studies (Gričar *et al.*, 2014). In the context of climate change, the lengthening of the growing season might offset drought-induced growth decline according to the plastic behaviour of cambial activity (Balducci *et al.*, 2016). Although shorter growing period and more conservative carbon use strategy in *Qpyr* may improve its physiological resistance to abiotic stress, *Qrob* could be able to increase carbon allocation to growth as a consequence of the longer active period. On the other hand, drought can induce premature defoliation and poor bud production (Montserrat-Martí *et al.*, 2009), which might reduce the number of cambial cells, deteriorating productive capacity in subsequent years.

Conclusions

The extensive monitoring performed during two years provided new insights on the environmental control of cambial activity in deciduous oaks. For the first time, plastic trimodal xylogenesis in response to summer drought was detected in a temperate deciduous oak, indicating that rising xeric conditions increases the complexity of growth patterns. Moderately dry conditions in summer prompted NSC storage, increasing carbohydrate availability the following spring. Moreover, dormant cambium size was in tune with wood production. Our work suggests that further investigation on the endogenous predictors of cambial activity may help to predict changes in the ability of trees to sequestrate atmospheric carbon under increasing abiotic stress. In addition, understanding how environmental cues modulate the number of cell layers in the dormant cambium should be a priority due their critical impact on wood production.

Acknowledgements

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Supplementary information

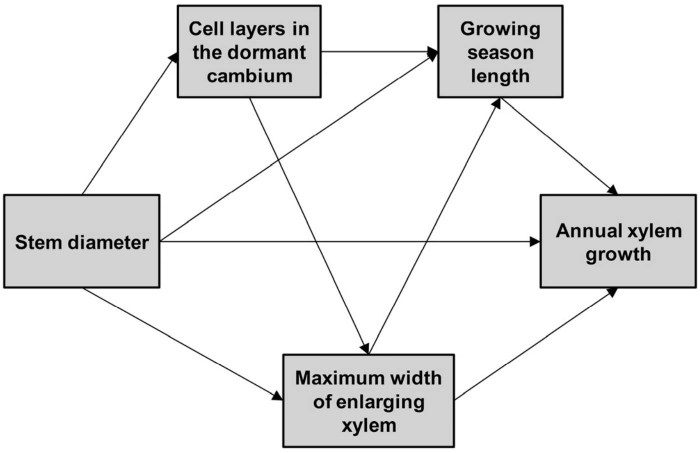


Figure S.3.1 The conceptual model structure showing the hypothesized dependencies among stem diameter, number of cell layers in the dormant cambium, maximum width of enlarging xylem, growing season length, and annual xylem growth.

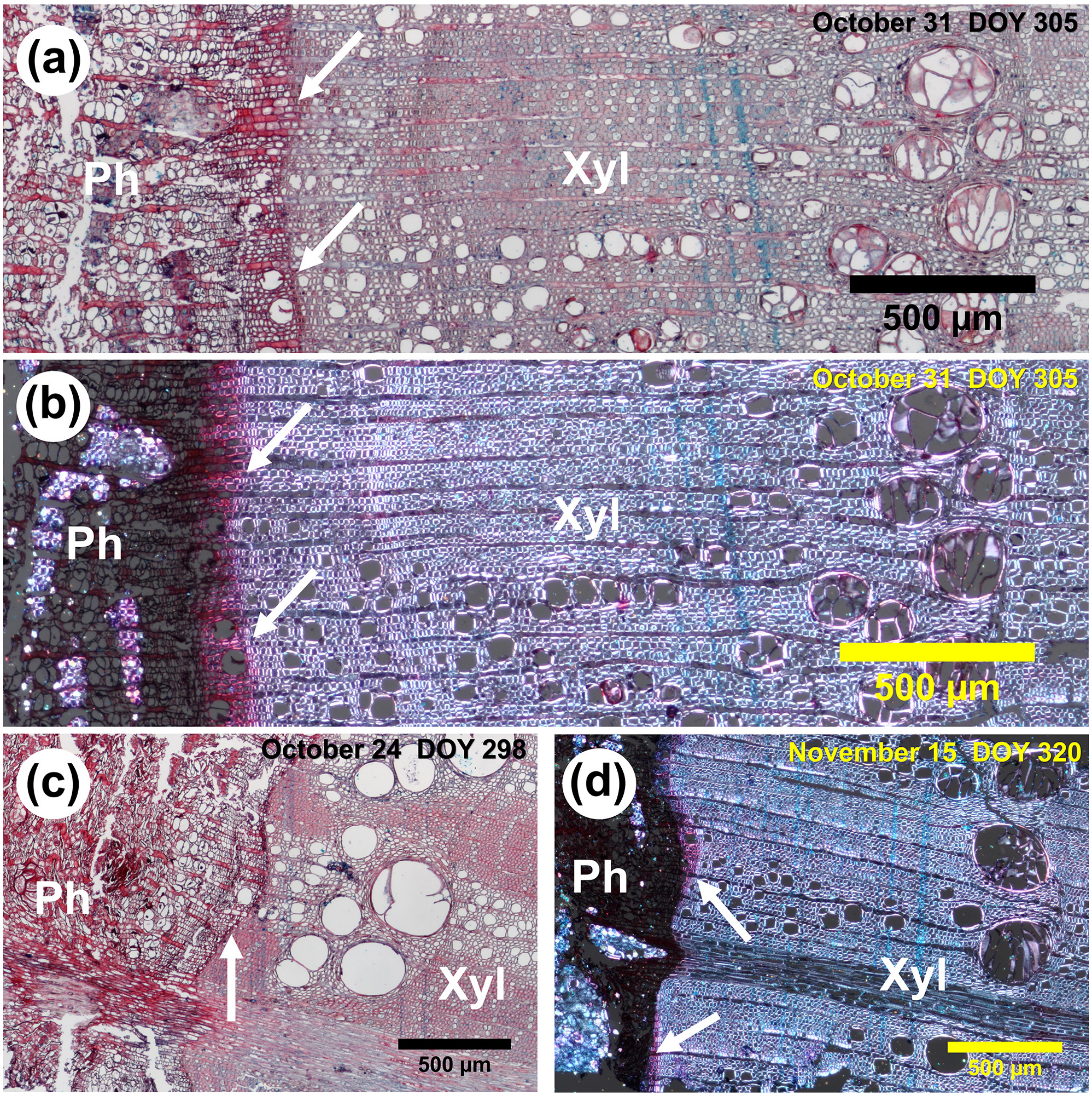
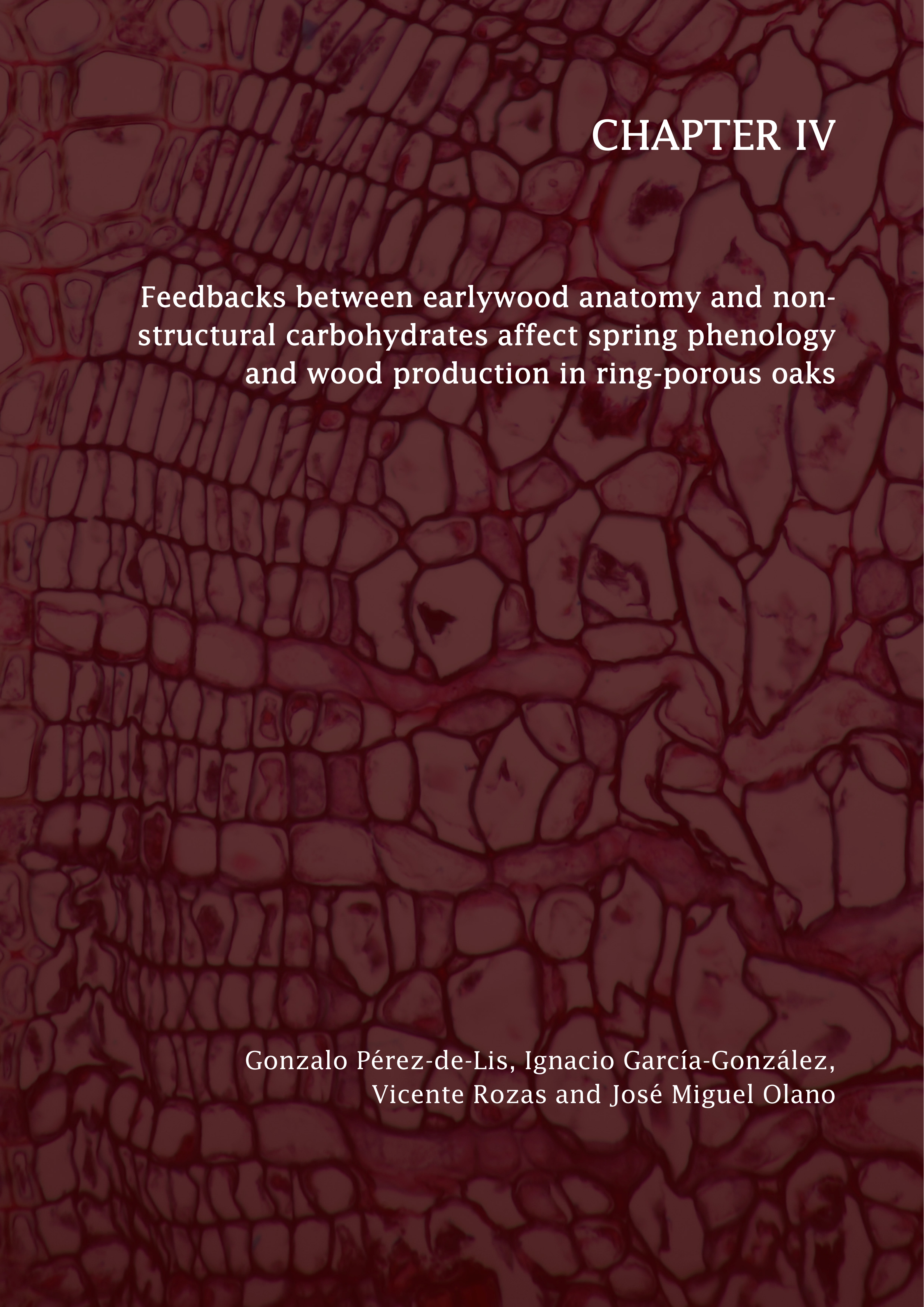


Figure S.3.2 Transverse sections of new xylem (Xyl) and phloem (Ph) showing growth recovery in autumn (white arrows). A sharp intra-annual fluctuation indicates the occurrence of a period of growth detention (A, B, C, D). Enlarging and maturing cells can be distinguished under polarized white light (B, D).



CHAPTER IV

Feedbacks between earlywood anatomy and non-structural carbohydrates affect spring phenology and wood production in ring-porous oaks

Gonzalo Pérez-de-Lis, Ignacio García-González,
Vicente Rozas and José Miguel Olano

Summary

Non-structural carbohydrates (NSC) play a central role in the construction and maintenance of the vascular system, but feedbacks between the NSC status of trees and wood formation are not fully understood.. We aimed to evaluate multiple dependencies among wood anatomy, winter NSC, and phenology for coexisting temperate (*Quercus robur*) and sub-Mediterranean (*Q. pyrenaica*) oaks along a water-availability gradient in NW Iberian Peninsula. Sapwood NSC was quantified at three sites in December 2012 ($n = 240$). Leaf phenology and wood anatomy were surveyed in 2013. Structural equation modelling was used to analyze the interplay among hydraulic diameter (D_h), winter NSC, date of budburst, and earlywood vessel production (EVP), while the effect of D_h and EVP on latewood width was assessed by using a mixed-effects model. NSC and wood production increased under drier conditions in both species. *Q. robur* showed narrower D_h and lower soluble sugar (SS) concentration (3.88–5.08 % dry matter) than *Q. pyrenaica* (4.06–5.57 % dry matter), but *Q. robur* exhibited larger EVP and wider latewood (1,403 µm) than *Q. pyrenaica* (667 µm). Large D_h enabled trees to have higher SS concentration in winter and early flushing in spring, which encouraged EVP in *Q. robur*. EVP in *Q. pyrenaica* was in concert with SS content in winter, evidencing a carbon saving strategy. Latewood production was controlled by D_h and EVP, rather than by foliage density and growing season duration. Advanced spring phenology and enhanced earlywood formation in wide-vessel bearing oaks may be associated to their large SS content. *Q. pyrenaica* exhibited a tighter control of carbohydrate allocation to wood growth than *Q. robur*, which is probably related to the acquisition of physiological resistance to stress in the sub-Mediterranean area.

Introduction

Non-structural carbohydrates (NSC) have multiple key functions in trees, such as fuel maintenance respiration, osmoregulation, cryoprotection, or growth regulation (Morin *et al.,* 2007; Wang & Ruan, 2013; Dietze *et al.,* 2014). The asynchrony between carbon assimilation and consumption is solved by accumulating non-structural carbohydrate (NSC) reserves (Chapin *et al.,* 1990; Sala *et al.,* 2012), which are mostly stored in stem, branches and coarse roots as soluble sugars (SS) and starch (Barbaroux *et al.,* 2003). Much of the NSC budget of the tree is invested in construction and maintenance of the aboveground vascular system (Salleo *et al.*, 2009; Nardini *et al.*, 2011; Brodersen & McElrone 2013; Wang & Ruan 2013), as well as in fine root production and turnover (McCormack *et al.*, 2014). The hydraulic network in ring-porous oaks is highly vulnerable to dysfunction due to cavitation of their large vessels, which operate at a narrow safety margin (Urli *et al.,* 2015). The refilling of these embolized vessels needs restoration of osmotic gradients through releasing SS into the conduits (Salleo *et al.,* 2009; Nardini *et al.*, 2011; Brodersen & McElrone 2013). Alternatively, the hydraulic function can be recovered through the formation of new conduits the following spring (Brodribb *et al.,* 2010). In ring-porous species, earlywood vessels are generally functional during only one year (Urli *et al.,* 2015), and cambial resumption precedes leaf formation (Pérez-de-Lis *et al.,* 2016). Large NSC reserves are therefore needed in order to provide energy and materials for leaf expansion and cambial activity at the onset of the growing season (Barbaroux *et al.,* 2003; El Zein *et al.,* 2011).

Scaling relationships between tree size and xylem conduits convey that bigger trees form wider vessels in order to compensate height-related hydraulic resistance in the stem (Petit *et al.*, 2008; Anfodillo *et al.*, 2012). Therefore, larger vessels are probably formed in dominant trees, which are frequently taller than neighbouring individuals (Matsushita *et al.*, 2015). On the other hand, fast-growing trees commonly exhibit larger NSC levels and faster NSC turnover than their slow-growing counterparts (Sundberg *et al.*, 1993; Sala and Hoch, 2009; Carbone *et al.*, 2013). Large earlywood vessels probably allow dominant trees to ameliorate carbon gain since stomatal conductance increases with the hydraulic capacity under high evaporative demand (Fichot *et al.*, 2009). However, little is known about how feedbacks between earlywood anatomy and NSC affect growth processes in ring-porous oaks.

Entangled relationships between tree vigour and growth are influenced by the timing of tree phenology, given that dominant trees show larger growing periods and more intense cambial activity (Rathgeber *et al.,* 2011b). Although the break of dormancy is mostly controlled by temperature and photoperiod (Basler & Körner 2014), some studies suggest that high NSC concentrations in developing buds speed up leaf-out dates (Maurel *et al.,* 2004). In winter, phloem of deciduous trees was suggested to be non-functional, whereby sapwood might be involved in carbon translocation through the plant body (Lacointe *et al.,* 2004). The influx of sucrose from xylem conduits into the buds was reported to be tightly correlated to bud swelling rates (Bonhomme *et al.,* 2010), whilst high sucrose concentrations in mutant poplars have been associated to advanced budburst (Park *et al.,* 2009). In ring-porous oaks, earlywood vessel production has been reported to be affected by winter temperature, which has been attributed to direct effects of temperature on both the timing of phenology and carbon demand for maintenance respiration (Gea-Izquierdo *et al.,* 2012; González-González *et al.,* 2014). However, the influence of sapwood NSC levels in dormancy release is frequently precluded, and the possible effect of their interplay in tree growth is poorly understood.

There is a tight association between the timing of primary and secondary growth at the whole-tree level (Huang *et al.*, 2014). In turn, ecological requirements modulating phenology are linked to functional species-specific strategies (Basler & Körner 2014). This is the case of the ring-porous oaks *Quercus robur* L. and *Q. pyrenaica* Willd., which coexist in NW Iberian Peninsula. The latter is a sub-Mediterranean species that exhibits late flushing (Pérez-de-Lis *et al.,* 2016), along with several morphological and physiological adaptations to cope with summer drought and winter frost. Such differences could impact carbon metabolism and allocation to growth (Valladares *et al.,* 2000; Regier *et al.*, 2009; Piper, 2011; Guillemot *et al.,* 2015), as well as the rate of developmental processes (Deslauriers *et al.,* 2009), affecting the adaptive capacity to track rapid climate change (Jump & Peñuelas 2005). Water shortage is deemed to influence carbon metabolism in a complex manner by constraining the activity of both source and sink organs (Sala *et al.,* 2012). Whereas some studies reported that NSC are accumulated under drought (Sala & Hoch 2009; Lempereur *et al.,* 2015), other authors found a drought-induced reduction in starch concentration, coupled with changes in the SS composition (Rosas *et al.,* 2013; Deslauriers *et al.,* 2014). Therefore, we need to understand how phenology and growth are coordinated with NSC and wood anatomy in order to better predict plant responses to climate in the context of global warming.

In this research, data from sapwood NSC concentration in winter 2012, xylem anatomical traits, and phenology in spring 2013 are used to disentangle the influence of their mutual interactions on the radial growth of ring-porous oaks growing along a water-availability gradient in NW Iberian Peninsula. We focused on the possible differences in xylem anatomy and NSC between *Q. robur* and *Q. pyrenaica* along the gradient. In this regard, we hypothesized that the more drought-tolerant *Q. pyrenaica* will have a larger NSC pool and more reduced growth than *Q. robur*. Wood production and NSC concentration are expected to vary along the gradient, with reduced growth but increasing NSC storage under drier conditions. At the species level, we aim to test the following hypotheses: (i) tree size influences earlywood vessel diameter, which in turn affects NSC content; (ii) higher SS content in winter predisposes trees to advance growth resumption in spring, thereby fostering earlywood production; and (iii) earlywood anatomical features are important predictors of latewood growth in oaks.

Materials and methods

Study sites

The study area is located in NW Iberian Peninsula, at the transition between the Atlantic and Mediterranean biogeographical regions. Three mixed stands of *Q. robur* (*Qrob*) and *Q. pyrenaica* (*Qpyr*) were selected along a north-to-south gradient of decreasing water supply. Annual rainfall decreases, from 1,461 mm at the northernmost site Bermui (Atlantic site at low elevation, ATL), to 996 mm at Labio (Atlantic site at high elevation, ATH), and to 832 mm at the southernmost site Moreiras (Mediterranean site, MED). Mean annual temperature is lower at sites ATL (11.3 ºC) and ATH (11.6 ºC) than at MED (14.4 ºC). Sampled stands are dominated by *Qrob* at ATL and ATH, whereas *Qpyr* is more frequent at MED.

Sites ATL and ATH include temperate trees and understory shrubs, such as *Betula alba* L., *Castanea sativa* Mill., *Pyrus cordata* Desy., *Ilex aquifolium* L., *Daboecia cantabrica* (Huds.) K. Koch, and *Vaccinium myrtillus* L. By contrast, thermophilic Mediterranean flora, such as *Q. suber* L., *Laurus nobilis* L., *Arbutus unedo* L., *Osyris alba* L., and *Daphne gnidium* L., dominates at MED. Stand tree densities are 1,178, 1,082, and 530 ha^{-1} at ATL, ATH, and MED, respectively.

Drought episodes can appear at the study region in summer, particularly at MED, which is the driest site within the gradient. Incident rainfall in 2012 at ATL, ATH, and MED was respectively 8, 14, and 33 % lower than the 1981–2010 average, whereas it was 35, 36, and 3 % higher than the average in 2013 (Table 4.1). In summer 2013, there were only 34 (45 mm), 11 (20 mm), and 8 (35 mm) days of precipitation at ATL, ATH and MED. Furthermore, mean maximum temperature in the same period was 22.0 ºC at ATL and ATH, but 25.3 ºC at site MED.

Table 4.1 Climatic information of the study sites in 2012 and 2013.

Site	P (mm)	Rainy days	Tm (ºC)	Tmax (ºC)	Tmin (ºC)
2012					
ATL	1346.8	210	11.7	16.4	7.8
ATH	858.3	169	10.4	14.9	7.1
MED	555.0	172	12.4	16.8	8.5
2013					
ATL	1979.0	225	11.6	15.8	8.0
ATH	1351.6	190	10.2	14.2	7.2
MED	856.3	168	12.3	16.4	8.8

P mean precipitation, *Tm* mean temperature, *Tmax* mean maximum temperature, *Tmin* mean minimum temperature.

Phenology and NSC concentration

At each study site, 40 trees per species were selected for sampling (overall n = 240). Stem diameter was measured for each tree in October 2012. Leaf phenology was weekly monitored during 2013 using binoculars (10×) at *ca.* 10 m distance from each tree. Budburst was identified as the day of year (DOY) in which the apical buds on the uppermost part of the canopy were green and expanding, but no leaves were distinguishable yet. In late autumn, leaf shedding was identified as the date in which at least 50% of the leaves were shed from the crown. In addition, foliage density was visually estimated in July 2013 by counting the proportion of gaps in the crown, being expressed as a percentage of the theoretical maximum foliage density.

In order to analyze feedbacks between NSC and xylem anatomy, we quantified the content of NSC in sapwood by sampling one 5-mm diameter wood core per tree with an increment borer at breast height. Cores were taken in mid-December 2012, soon after the completion of leaf abscission, which occurred between mid and late November for both species. After extraction, cores were immediately placed into a cool box, and subsequently stored at –20 ºC to prevent carbohydrate degradation. Before NSCs extraction, bark and traces of heartwood were removed, and the cores were oven-dried at 60 ºC for 72 hours. Sapwood was then finely grounded with a mixer mill (Retsch MM 400, Düsseldorf, Germany). We quantified NSC concentration for the whole sapwood by using the anthrone method (Olano *et al.,* 2006). SS were extracted from 20 mg of dry mass in 1 ml of ethanol (80%) at 80 ºC for 30 min. The extract was centrifuged 10 min at 4,000 rpm, and the supernatant was collected for the spectrophotometrical determination of SS concentrations, for which we used the anthrone reagent. Starch contained in the residue was hydrolized with 1 ml of perchloric acid (35%) for 1 hour, and determination was conducted by using the anthrone reagent, as previously described for SS. Total NSC, SS, and starch concentrations were expressed as percentage of dry matter.

Wood anatomical measurements

One additional core per tree was collected in October 2013 to perform wood anatomical measurements. Cores were air-dried and mounted on wooden supports to be cut using a microtome (WSL Core Microtome, Zurich, CH) and polished. Cross-sectional surfaces were photographed with a digital camera (Canon EOS 600D, Tokyo, Japan), attached to a transmitted light microscope (Olympus BX40, Tokyo, Japan). Image analysis was applied on the rings formed in 2012 and 2013 using ImageJ 1.48v (Schneider *et al.,* 2012), in order to quantify the lumen area of earlywood vessels, latewood width, and the number of earlywood vessels, which is a *proxy* of earlywood vessel production (EVP) according to findings by González-González *et al.* (2014). For each vessel, we estimated the diameter of the equivalent circle, obtaining the hydraulic diameter (D_h) at the tree level according to the equation:

$$D_\mathrm{h} = \sum_{n=1}^{N} d_n^5 \,/ \sum_{n=1}^{N} d_n^4,$$

where d_n is the diameter of the n vessel (Sperry *et al.,* 1994). According to the Hagen-Poiseuille equation, D_h is proportional to the hydraulic capacity.

Comparisons along the gradient

Differences among sites and between species for NSC, dates of budburst and leaf shedding, wood anatomical traits, and foliage density were evaluated by applying generalized linear models (GLM) for gamma-distributed variables. Multiple pairwise comparisons between factor levels were also assessed. This analysis was performed by using the packages 'lme4' and 'multcomp' for R 3.1.1 (R Core Team, 2014).

Connections among earlywood anatomy, sapwood NSC content and spring phenology

We performed structural equation models (SEM) to disentangle, at the species level, the role of winter NSC as possible regulators of budburst and EVP in 2013. Thereby, data from all the sites were pooled, and a unique model was fit for each species. SEM approach provides an adequate representation for interacting systems, in which simultaneous influences and responses including direct and indirect effects are explored (Grace 2006). The structure of a hypothetical SEM, and its calculation, requires incorporating available *a priori* knowledge.

According to the lines of evidence showed in the introduction, we hypothesized that fast-growing trees (larger stem diameter) show higher SS and starch concentrations due to their larger D_h (Supplementary information, Fig. S.4.1). In turn, high SS and starch concentrations in winter are expected to speed up tree phenology (date of budburst) and boost EVP during the subsequent year.

Standardized coefficients were estimated by the maximum likelihood method, and model evaluation was performed using a χ^2 test. A *P*-value below 0.05 indicates that discrepancy between observed and expected covariance matrices is acceptable. The adjusted goodness of model fit index (AGFI), and the root mean square error of approximation (RMSEA) were complementarily performed in order to consider the effect of sample size on the model fit evaluation. Values of AGFI and RMSEA respectively above 0.90 and below 0.05 indicate an acceptable fit of the model in relation to the degrees of freedom. A χ^2 test for multi-group invariance was applied to evaluate differences between the models fitted for each species. SEM analyses were performed with AMOS 18.0 software (AMOS Development Corp., Mount Pleasant, South Carolina, USA).

Predictors of latewood formation

We performed generalized linear mixed-effects models (GLMM) to identify which factors affected latewood production in 2013 at the species level. The effect of site was included as a random component, while winter NSCs, earlywood anatomy (D_h and EVP in 2013), growing season length, and foliage density were the explanatory variables of the model. Collinearity was surveyed by calculating the generalized variance-inflation factors for each species. GLMM models were fitted by a log-link function with a gamma distribution, being ranked according to the second-order Akaike's Information Criterion (Bolker *et al.,* 2009). We averaged the 95% confidence set of models according to the Akaike weights, and the relative importance of a given variable was calculated as the sum of the Akaike weights across all the models in which it was contained (Burnham and Anderson 2002). We used the packages 'lme4' and 'MuMIn' for R 3.1.1 (R Core Team, 2014) to assess GLMMs.

Results

Variation in NSC, wood anatomy and leaf phenology along the gradient

Mean SS concentrations at the sites ranged from 3.88 to 5.08 % dry matter in *Qrob*, and from 4.06 to 5.57 % dry matter in *Qpyr* (Fig. 4.1a), being similar to those of starch, which ranged from 4.28 to 5.11 % dry matter in *Qrob*, and from 3.47 to 5.11 % in *Qpyr* (Fig. 4.1b). *Qpyr* exhibited therefore greater SS content than *Qrob* ($F = 18.27$, $P < 0.001$), while both starch and NSC concentration did not differ between species, although were marginally significant for NSC ($F_{\text{starch}} = 2.14$, $F_{\text{NSC}} = 0.62$; $P > 0.050$). Such pattern resulted in a higher SS-to-starch ratio for *Qpyr* than for *Qrob* ($F = 18.07$, $P < 0.001$), especially at ATL and ATH (Fig. 4.1c), whereas there was no variation along the gradient ($F = 0.21$, $P = 0.814$). In contrast, NSC substantially varied among sites ($F = 22.34$, $P < 0.001$), with decreasing concentrations from MED to ATL (Fig. 4.1a,b). SS content followed a similar geographical pattern, in both *Qrob* ($F = 17.72$, $P < 0.001$) and *Qpyr* ($F = 21.89$, $P < 0.001$). Site MED exhibited a higher starch content than ATL site for *Qpyr* ($F = 8.59$, $P < 0.001$), whereas no clear geographical pattern was found for *Qrob* ($F = 2.52$, $P = 0.085$).

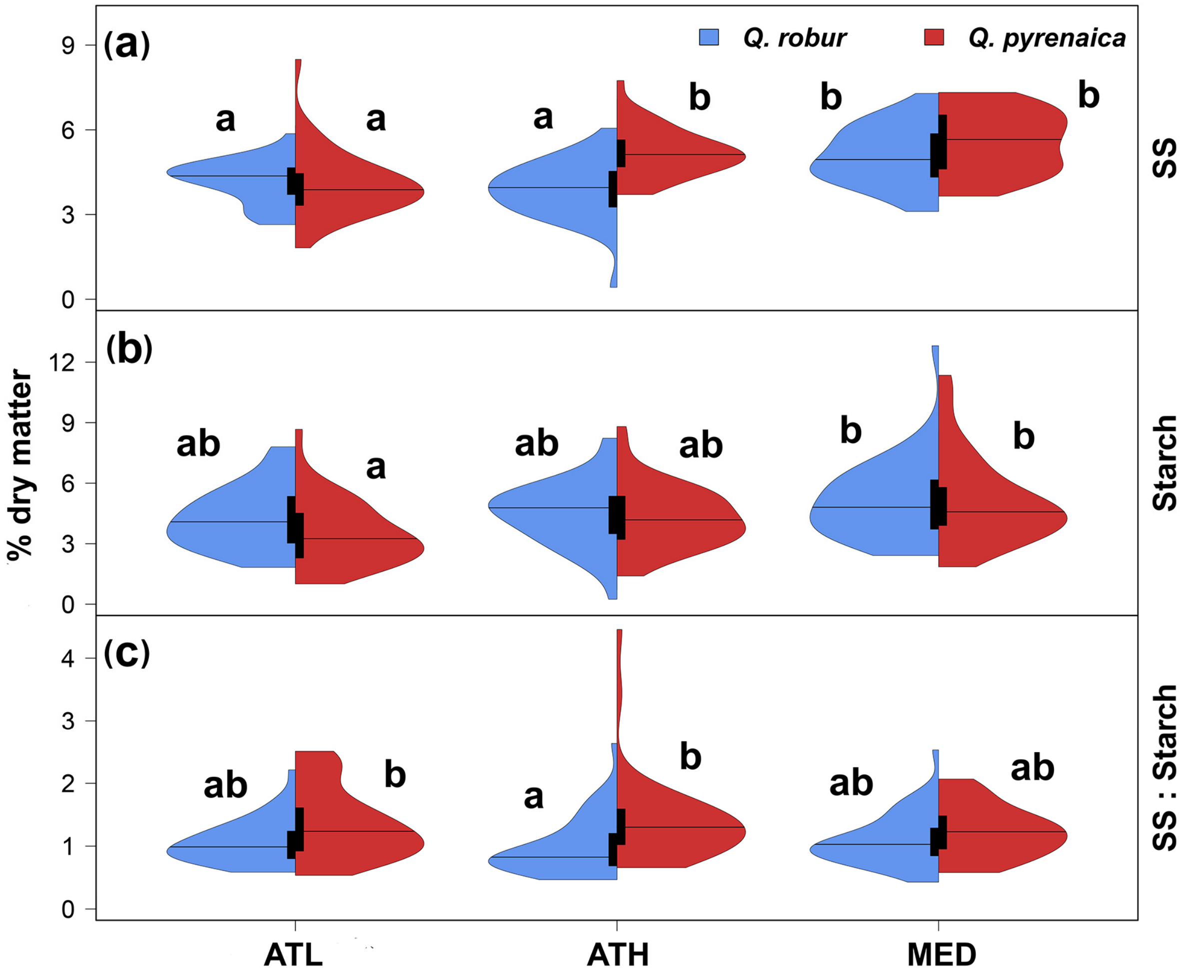


Figure 4.1 Distribution of (a) soluble sugars (SS), (b) starch concentrations, and (c) SS-to-starch ratio, for *Quercus robur* and *Q. pyrenaica* at the three study sites. Horizontal lines represent the median, and black boxplots show the extent of 25th and 75th percentiles. Lower case letters indicate statistically significant differences along the gradient according to multiple pairwise comparisons.

Overall, *Qpyr* exhibited a higher D_{h} than *Qrob* ($F_{2012} = 7.76$, $F_{2013} = 8.31$; $P < 0.010$). Yet, *Qrob* had larger EVP ($F = 30.28$, $P < 0.001$) and wider latewood ($F = 51.15$; $P < 0.001$) than *Qpyr* (Fig. 4.2a,b). D_{h} was substantially lower at ATL in 2012 ($F = 7.67$, $P < 0.001$) and 2013 ($F = 3.72$; $P = 0.027$) for *Qpyr*, whereas much less variation was found among sites for *Qrob* ($F_{2012} = 2.89$, $F_{2013} = 0.18$; $P > 0.050$). EVP and latewood width differed along the gradient ($F_{\mathrm{EVP}} = 8.45$, $F_{\mathrm{LW}} = 12.50$; $P < 0.001$), with the lowest values at ATL (Fig. 4.2c,d). Conversely, the highest values for both variables occurred at MED for *Qrob*, and at ATH for *Qpyr*.

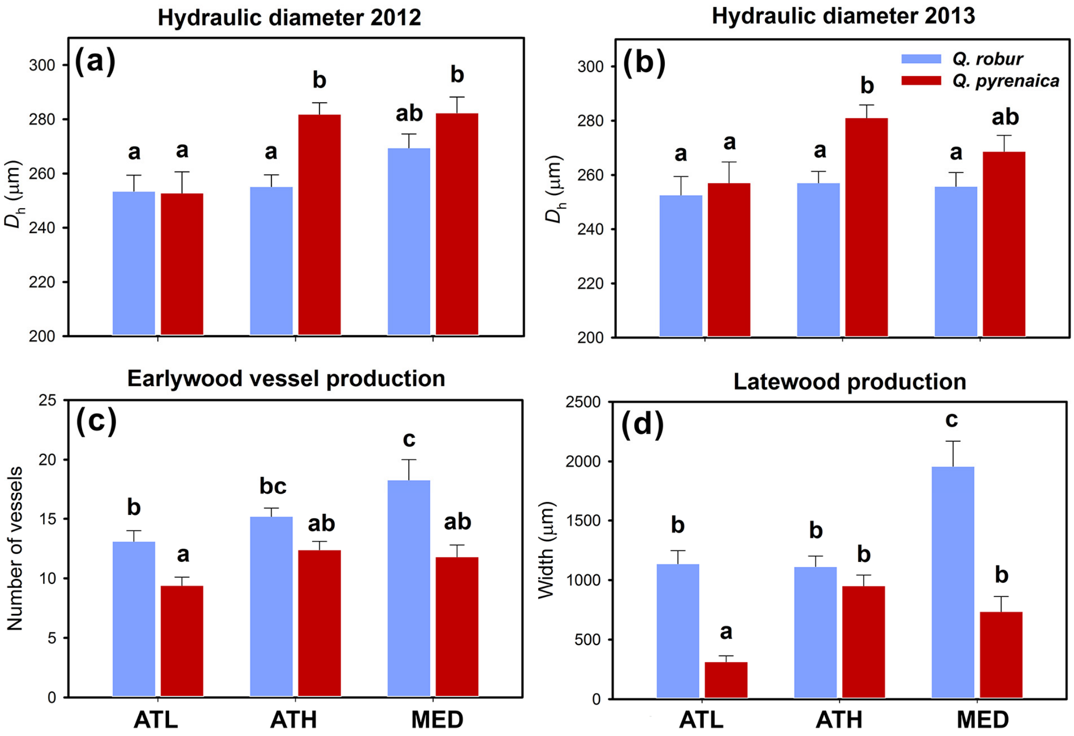


Figure 4.2 Mean values and SE of (a) hydraulic diameter in 2012, (b) hydraulic diameter in 2013, (c) earlywood vessel production, and (d) latewood production in 2013 for *Quercus robur* and *Q. pyrenaica*. Lower case letters indicate statistically significant differences along the gradient according to multiple pairwise comparisons.

Stem diameter was positively correlated with tree height in *Qrob* ($r = 0.60$, $P < 0.001$) and *Qpyr* ($r = 0.58$, $P < 0.001$). At site ATL, stem diameter was larger for *Qrob* than for *Qpyr* ($Z = 3.29$, $P = 0.012$), whereas the studied species showed similar values at ATH and MED (Fig. 4.3a). Trees at MED had a larger stem diameter than at ATH (*Qrob* $Z = 5.08$, *Qpyr* $Z = 7.58$, $P < 0.001$) and ATL (*Qrob* $Z = 2.67$, $P = 0.021$; *Qpyr* $Z = 7.13$, $P < 0.001$). *Qpyr* exhibited a later budburst than *Qrob* ($F = 527.83$, $P < 0.001$), occurring from mid to late April at MED, and from late April to late May at ATL and ATH (Fig. 4.3b). Budburst occurred synchronously at ATL and ATH (*Qrob* $t = 1.92$, *Qpyr* $t = -0.54$, $P > 0.05$), but comparatively earlier (*Qrob* $F = 128.45$, *Qpyr* $F = 79.49$, $P < 0.001$) at MED (Fig. 4.3b). Trees showing an earlier budburst had a delayed senescence (*Qrob* $r = -0.36$, *Qpyr* $r = -0.68$, $P < 0.001$). Leaf shedding was first recorded at ATL and ATH for *Qpyr* (DOY 312 on average), whereas some green leaves could be perceived until late December at MED (Fig. 4.3c; *Qrob* DOY 357, *Qpyr* DOY 354). The period from budburst to leaf shedding in 2013 was on average 42 days longer for *Qrob* than for *Qpyr* ($F = 450.90$, $P < 0.001$). Foliage density was similar along the gradient for *Qrob*, but significantly lower at ALT for *Qpyr* (Fig. 4.3d). It is also relevant to consider that numerous *Qpyr* trees had powdery mildew on their leaves in spring 2013 at this latter location.

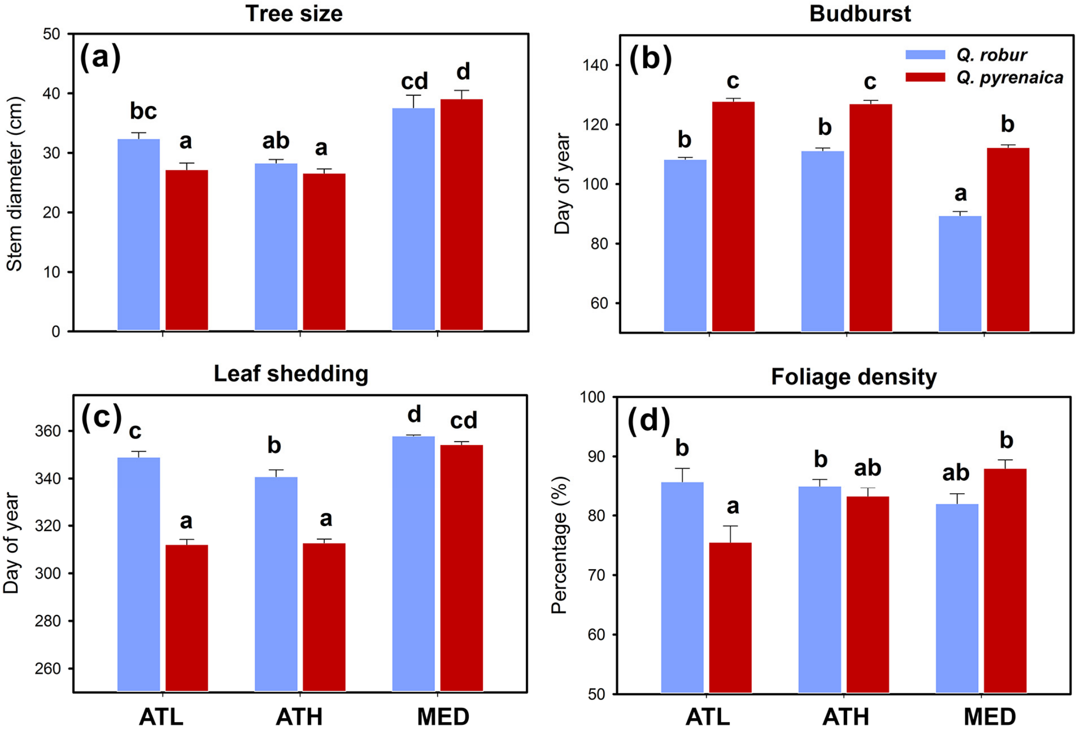


Figure 4.3 Mean values and SE of (a) stem diameter, (b) date of budburst, (c) date of leaf shedding, and (d) foliage density in 2013 for *Quercus robur* and *Q. pyrenaica*. Lower case letters indicate statistically significant differences along the gradient according to Multiple Pairwise comparisons.

Species-specific models on functional relationships affecting wood production

SEM models showed a good fit for both species ($df = 1$; *Qrob*, $\chi^2 = 0.202$, $P = 0.653$; *Qpyr*, $\chi^2 = 0.118$, $P = 0.732$), with AGFI > 0.90 and RMSEA < 0.1 (Fig. 4.4). Large trees exhibited a higher D_h in 2012, having a positive indirect effect on SS levels, irrespective of the species. SS concentration in December showed positive covariation with starch content in both species (Fig. 4.4). High SS contents were associated to advanced budburst, whereas starch concentration and date of budburst were unrelated. In *Qpyr*, SS concentrations had a positive direct effect in EVP. By contrast, this relationship was not direct, but mediated by SS effect on budburst date in *Qrob* (Fig. 4.4). The proportion of variance explained by SEM models was lower for D_h than for budburst date. Similarly, R^2 scores for EVP substantially differed between species, with values of 0.06 and 0.20 in *Qrob* and *Qpyr*, respectively (Fig. 4.4).

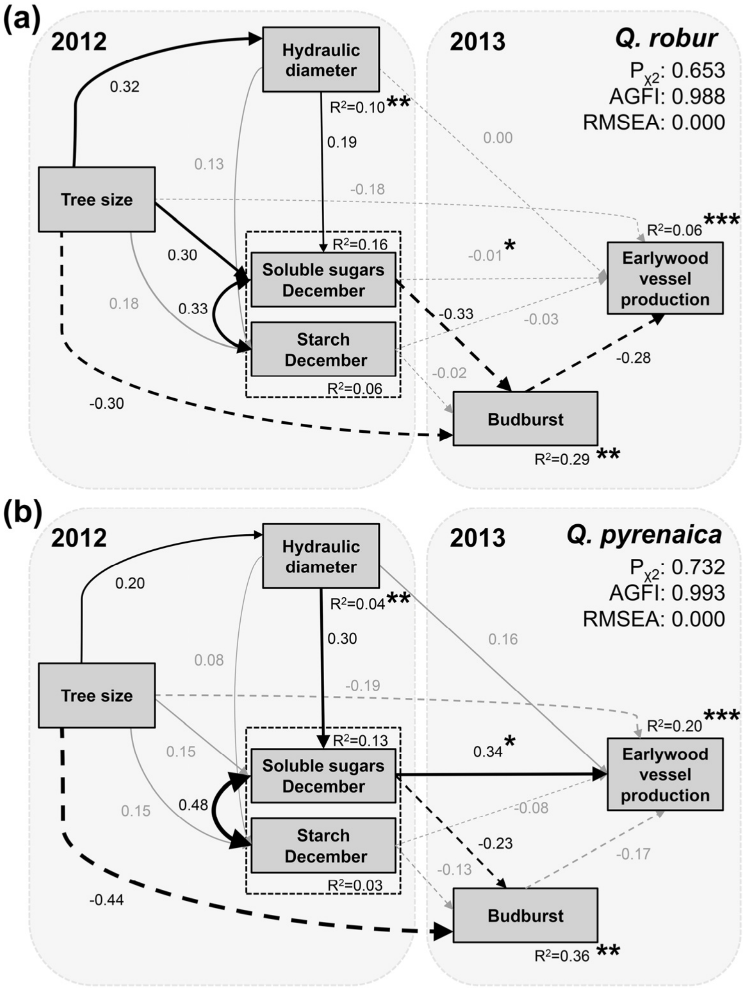


Figure 4.4 Structural equation models fitted for (a) *Quercus robur* and (b) *Quercus pyrenaica*. Variables of the conceptual model are tree size (stem diameter), hydraulic diameter in 2012, soluble sugars and starch concentrations in December 2012, date of budburst, and earlywood growth (number of vessels) in 2013. Explained deviances of endogenous variables are shown near the boxes. Black solid (positive effects) and dashed (negative effects) arrows denote significant relations, while non-significant relations are shown as grey coefficients and arrows. The Chi-square test, the adjusted goodness of model fit index (AGFI), and the root mean square error of approximation (RMSEA) are shown for each model. Asterisks indicate paths or error values significantly different between the models of both species. *** $P \leq 0.001$, ** $P \leq 0.01$, and * $P \leq 0.05$.

EVP and D_h of the current year had a strong positive influence on subsequent latewood growth in both species, attaining a relative influence above 85 % (Fig. 4.5). A second group of variables included in the models was related to tree vigour, such as foliage density, with a positive effect and a relative importance of 40–60 %; and length of the growing season, with a stronger influence for *Qrob* (40 %) than for *Qpyr* (31 %). As expected, a higher foliage density together with a longer growing season predicted a larger latewood growth. Winter NSC accounted for a marginal weight in both species, having however a negative impact on latewood growth for *Qrob*, whereas positive for *Qpyr* (Fig. 4.5). Problems of collinearity were not detected among the predictors included in the model (Supplementary information, Table S.4.1).

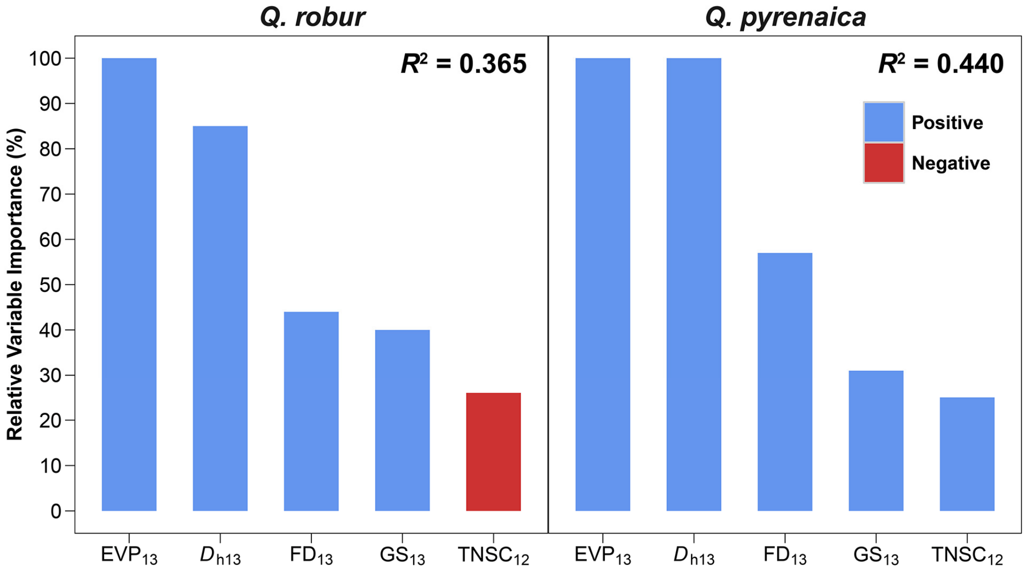


Figure 4.5 Relative importance of the variables driving latewood production in 2013, expressed as percentage. Location effect was included as random factor in the model. NSC_{12} is total non-structural carbohydrates in December 2012, D_{h13} is hydraulic diameter in 2013, EVP_{13} is earlywood vessel production in 2013, GS_{13} is length of the growing season in 2013, and FD_{13} is foliage density in 2013. Different colours of bars denote variables with either a positive or negative effect. We provide the coefficient of determination (R^2) of the full model.

Discussion

NSC allocation to xylem growth reflects contrasting stress-tolerance strategies in oaks

According to our expectations, the sub-Mediterranean *Qpyr* exhibited a higher SS-to-starch ratio at the onset of dormancy than the temperate *Qrob*. Sugars are involved in the osmotic protection against freezing damage (Améglio *et al.,* 2004), whereby cold acclimation requires the accumulation of high symplastic SS concentration in winter (Morin *et al.,* 2007; Repo *et al.*, 2008). This process is in concert with the timing of leaf shedding, which occurred earlier in *Qpyr* than in *Qrob*, reflecting the stronger cold tolerance of the former species. Additionally, SS content is maintained over a certain threshold to mitigate detrimental effects of eventual extreme events in long-lived trees (Sala *et al.,* 2012). This may be of high relevance in the Mediterranean area, where fire and defoliation episodes are frequent (Rosas *et al.,* 2013; Camarero *et al.,* 2015). Indeed, drought-tolerant species are thought to increase stored NSC at the cost of reducing growth (Valladares *et al.,* 2000; Piper, 2011; Regier *et al.*, 2009). This idea is further supported by the higher SS concentrations reported at MED, which also exhibited higher starch content for *Qpyr*. Under drought, SS contribute to prevent desicattion through osmotic regulation and cavitation repair (Salleo *et al.,* 2009; Nardini *et al.*, 2011; Brodersen & McElrone 2013; Pantin *et al.,* 2013; Deslauriers *et al.,* 2014), although the prevalence of vessel embolism and refilling is still under discussion (Delzon and Cochard

2014). In this regard, it is noteworthy that *Qpyr* exhibited larger vessels at ATH and MED, being even larger than those of *Qrob*. This is in accordance with the fact that more drought resistant species tend to form wider vessels than more sensitive ones (Zanne *et al.*, 2006). Trade-offs between efficiency and safety thus suggest that *Qpyr* prompted long-distance water transport under drier conditions, but concurrently increased the risk of vessel dysfunction (Sperry *et al.,* 1994). One posible explanation is that enhanced SS concentration might be involved in compensating hydraulic vulnerability in this species, although further research on SS fractioning may be required to confirm such hypothesis.

Wood formation declined along with NSC from site MED toward site ATL, which is opposed to our expectations. A lower tree density at MED than at the Atlantic sites, probably favoured carbon uptake and growth due to more reduced inter-tree competition (Fernández-de-Uña *et al.,* 2016). Nevertheless, strong differences between the two Atlantic sites in *Qpyr* might be attributed to contrasting moisture, instead to the similar tree density. Indeed, *Qpyr* trees exhibited sparser foliage and more severe powdery mildew infestation at ATL, which may reduce carbon uptake and growth (Améglio *et al.,* 2001; Martínez-Vilalta, 2014; Camarero *et al.,* 2015). On the other hand, soil water excess in winter could exacerbate carbon consumption associated to fermentation processes and root anaerobic stress (Baley-Serres and Voesenek, 2008; Ferner *et al.,* 2012). Furthermore, the growing season was shorter at ALT and ATH, probably restricting the carbon gain (Morecroft *et al.,* 2003), as well as xylem formation (Rathgeber *et al.,* 2011b). Interestingly, growth decline and tree dieback were recently reported in oaks suffering from both high competition levels and water excess after extremely rainy periods (Rozas & García-González 2012b). In contrast, more immediate effects of water shortage on stem growth than on photosynthesis likely favour carbohydrate accumulation in summer (Sala *et al.,* 2012; Lempereur *et al.,* 2015).

EVP and latewood width was generally lower for *Qpyr* than for *Qrob*, but such differences were striking at MED in spite of the more Mediterranean climatic conditions. This apparently contradictory result supports that *Qpyr* is more conservative than *Qrob* in allocating NSC to wood production. This is also consistent with results from Rodríguez-Calcerrada *et al.,* (2008), who suggested that temperate oaks are more competitive than sub-Mediterranean ones. Hence, the carbon saving strategy here suggested for *Qpyr* could entail a high opportunity cost in favourable environments (Chapin *et al.,* 1990), where coexisting *Qrob* probably outcompetes *Qpyr*. In this regard, Grossiord *et al.* (2014) reported that temperate sessile oaks exerted a negative effect on coexisting Turkey oaks, which experienced a reduction of their transpiration fluxes as a result of increasing water stress.

Dependencies among NSC, phenology, and earlywood vessels

The wide range of environmental conditions encompassed by our SEM model allowed us to confirm the hypothesized functional relationships among earlywood anatomy, NSC, and date of budburst at the species level. Tree size had a positive direct effect on the hydraulic capacity (i.e. larger vessels), and SS content at dormancy. Wider vessels would be formed in larger trees to counteract the increasing hydraulic resistance with height (Petit *et al.*, 2008). This was corroborated by the significant correlation between stem diameter and tree height in both studied species. Vessels of large diameter may boost carbohydrate uptake under a high evaporative demand (Steppe & Lemeur, 2007; Fichot *et al.*, 2009), which is a consequence of the enhanced water transport capacity (Meinzer *et al.,* 2005). Alternatively, since large vessels are thought to be more vulnerable to cavitation (Sperry *et al.,* 1994), higher SS concentrations may be required in the sapwood of trees bearing wider vessels to maintain long-distance water transport (Nardini *et al.*, 2011; Brodersen & McElrone 2013).

Trees having a higher SS concentration in the stem showed earlier budburst the following spring, which is consistent with previous works performed in peach (Maurel *et al.,* 2004) and poplar (Park *et al.,* 2009). This is related to the fact that carbohydrate influx from vessels may be involved in bud growth regulation (Bonhomme *et al.,* 2010). In this regard, Lacointe *et al.* (2004) suggested that carbon transport during the dormant period relies on xylem vessels. In addition, xylem sap osmolarity plays a role in the generation of stem pressure, which is needed to reverse winter embolisms in early spring (Améglio *et al.,* 2001). It is noteworthy that vessels of previous years may be responsible for carrying water over quiescence because first-formed earlywood vessels are not functional at least until budburst (Pérez-de-Lis *et al.,* 2016). Thereby, reduced ability to repair winter embolism could affect negatively the supply of water to swelling buds in those trees showing lower SS content in the xylem sap (Améglio *et al.,* 2001).

In *Qrob*, the observed positive effect of SS concentration on EVP was mediated by the timing of budburst. The observation that budburst coincides with the onset of vessel maturation in the stem suggests that early-flushing trees had an advanced onset of earlywood formation (Pérez-de-Lis *et al.,* 2016). Therefore, larger EVP in early-flushing trees could result from the longer period of earlywood formation, which is underpinned by that greater

EVP values measured at the location with earlier budburst. Yet, the impact of SS concentration on EVP was more pronounced for *Qpyr*, as demonstrated the fact that EVP was in tune with SS concentration. Presumably, high overwintering sugar levels in sapwood somehow increased energy and materials as well as water supplied to growing tissues in spring, even though starch mobilization may be initiated at that time (Améglio *et al.,* 2001). Furthermore, sugars act as elicitors of auxin biosynthesis and distribution (Lilley *et al.,* 2012; Sairanen *et al.,* 2012), and as growth promoters (Stewart *et al.,* 2011). Although relations between carbon allocation to storage and growth are complex, and mainly related to the activity of carbon sinks (Lempereur *et al.,* 2015), a growing body of literature suggests that NSC availability is involved in growth regulation (Pantin *et al.,* 2013; Dietze *et al.,* 2014; Guillemot *et al.,* 2015). This might be particularly true for earlywood given its reliance on winter NSC reserves (Skomarkova *et al.,* 2006). This idea also agrees with the positive association between tree vigour, growth rates, and the NSC pool found across taxa in multiple environments (Sundberg *et al.,* 1993; Deslauriers *et al.,* 2009; Carbone *et al.,* 2013).

Feedbacks between SS content and EVP differed between species, which may be attributed to their contrasting stress tolerance (Guillemot *et al.,* 2015). Our results therefore suggest that the more drought-resistant *Qpyr* limits construction costs under favourable conditions in spring if NSC levels decrease, which conveys its more conservative carbon use strategy. This result underpins that storage is an actively competing sink, rather than a passive compartment (Wiley & Helliker, 2012; Dietze *et al.,* 2014). Deslauriers *et al.* (2014) attributed drought-induced growth decline in black spruce to the increasing demand of NSC for osmotic purposes, together with dehydration effects on cell turgor. In the same line, Anderegg *et al.* (2013) suggested that reduced carbon uptake under drought can impair growth in subsequent years. Yet, we noted increasing NSC concentrations and higher EVP under drier conditions, whilst sapwood starch concentrations scarcely affected growth. EVP was probably affected by the starch breakdown rate at the onset of dormancy, rather than by drought-induced changes in the reserve pool the previous summer. Our results hint that earlywood vessel anatomy and winter temperature connections reported by dendrochronological studies (Gea-Izquierdo *et al.,* 2012; González-González *et al.,* 2014) could be attributed to thermal-induced changes in carbon partitioning during cold hardening (Améglio *et al.,* 2004; Morin *et al.,* 2007; Repo *et al.*, 2008). However, not all the starch that is contained in sapwood is readily accessible (Sala *et al.,* 2012). Thereby, the assessment of mean starch concentrations in sapwood probably failed to reflect actual starch availability.

Earlywood anatomy is a predictor of latewood growth

The most influential predictors driving latewood growth did not differ between *Qrob* and *Qpyr*, suggesting common underlying mechanisms for both species. Latewood width was influenced by earlywood properties within the same tree ring, and, to a lesser extent, by foliage density and phenology. This confirms the positive impact of enhanced water transport capacity on xylem growth (Fichot *et al.,* 2009), which is largely related to both conduit size (Sperry *et al.,* 1994), and total conductive area (Meinzer *et al.,* 2005). Despite the higher NSC costs, abundant vessels of distinct size could be useful to mitigate the physiological effects of drought, because still functioning small vessels would also serve as local water reservoirs for neighbouring collapsed large vessels (Brodersen & McElrone 2013), which could promote water refilling of disrupted vessels. This is consistent with the higher EVP observed in *Qrob* at MED. In addition, an efficient hydraulic network may enable carbon gain to be maximized under favourable conditions (Fichot *et al.,* 2009), protecting key processes in which sugars are involved, such as osmotic regulation of long-distance transport (Sala *et al.,* 2012; Deslauriers *et al.*, 2014), embolism repair (Salleo *et al.,* 2009; Nardini *et al.*, 2011), and turgor pressure driving cell elongation (Wang and Ruan, 2013).

Latewood growth was also higher in trees exhibiting high foliage density, which is consistent with the reduced carbon reserves and radial growth found in defoliated trees for evergreen oaks (Rosas *et al.,* 2013; Camarero *et al.,* 2015), or walnut trees (Améglio *et al.,* 2001). The lower importance of this factor on latewood growth could also be related to the high overall foliage density levels at the study sites. Trees having earlier budburst showed delayed leaf abscission and thus longer growing season, as found in previous studies on cambial activity (Deslauriers *et al.,* 2009; Rathgeber *et al.,* 2011b). The duration of the growing season had limited influence on latewood width, suggesting that cambial activity rates were more influential on xylem production than phenology (Deslauriers *et al.,* 2009; Rathgeber *et al.,* 2011b).

Conclusions

In this study, non-structural carbohydrates in sapwood, wood anatomy, and leaf phenology were comprehensively addressed in two ring-porous species during one year, along a broad geographical range in NW Iberian Peninsula. Our results reveal that feedbacks between earlywood vessels and soluble sugars affect wood production. Earlywood vessel production in *Q. pyrenaica* showed a tighter control by soluble sugar concentration than in *Q. robur*, suggesting a more conservative carbon use strategy in the former species. These lines of evidence support that non-structural carbohydrates play a role in the acquisition of resistance to cope with harsh environmental conditions in the sub-Mediterranean area. This study is a first attempt to unravel the interactions between non-structural carbohydrates, wood anatomy, and phenology in ring-porous oaks. We acknowledge the need for further research comprising a longer time span, soluble sugar fractioning, additional tree compartments such as branches and roots, and a comprehensive dataset on cambial phenology instead of isolated leaf phenophases. However, this study hints the existence of stable functional interactions between sapwood carbohydrate levels, xylem anatomy, and phenology in ring-porous oaks. In the light of these results, we suggest that *Q. pyrenaica*, and to a lesser extent *Q. robur*, might mitigate increasing hydraulic vulnerability under climate warming by prioritizing carbon accumulation over growth. Nevertheless, such mechanism would impose additional limitations for secondary growth if adverse climate episodes become more frequent in future decades.

Acknowledgements

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Supplementary information

Table S.4.1 Diagnosis of collinearity for the generalized linear mixed-effects models identifying predictors of latewood width.

	Latewood width				
	EVP_{13}	D_{h13}	FD_{13}	GS_{13}	$TNSC_{12}$
Q. robur	1.03	1.07	1.06	1.17	1.16
Q. pyrenaica	1.09	1.07	1.24	1.26	1.26

Generalized variance-inflation factors are given for each predictor of the models. *BB* is date of budburst in 2013, EVP_{13} is earlywood vessel production in 2013, D_{h13} is hydraulic diameter in 2013, *FD* is foliage density, GS_{13} is length of the growing season in 2013, and $TNSC_{12}$ is total non-structural carbohydrates in December 2012.We consider 10 as a threshold for detecting collinearity.

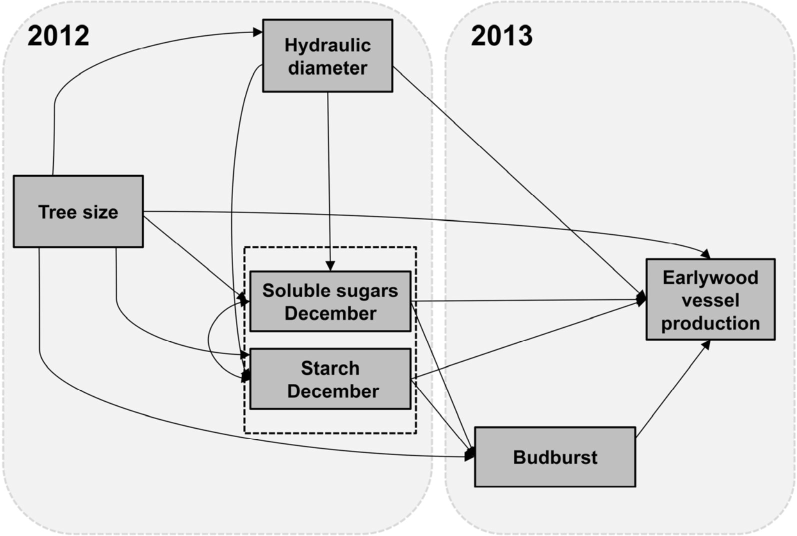
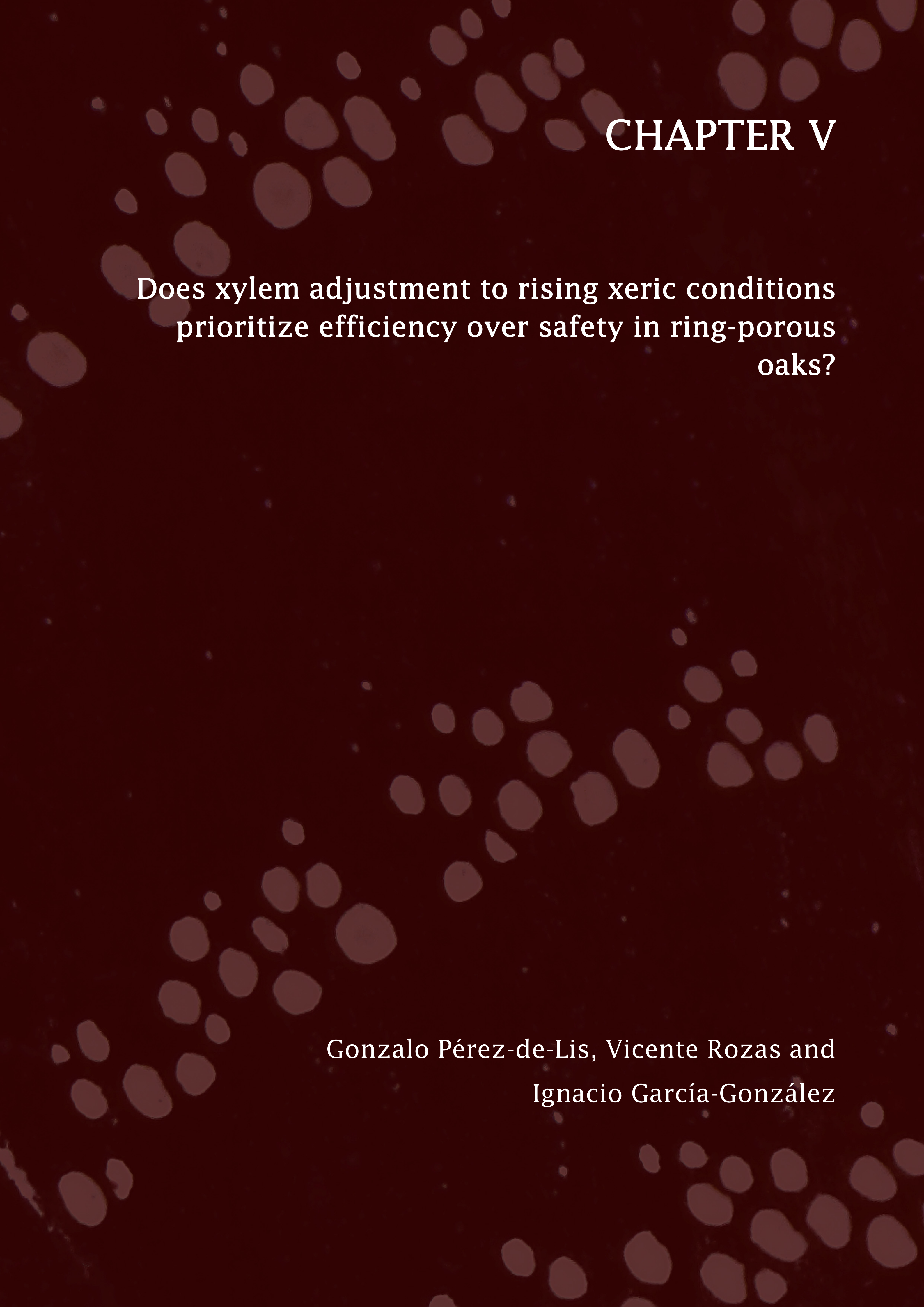


Figure S.4.1 Structure of the hypothetical conceptual model showing interactions among tree size (stem diameter), hydraulic diameter in 2012, soluble sugars and starch concentrations in December 2012, and date of budburst and earlywood vessel production in 2013.



CHAPTER V

Does xylem adjustment to rising xeric conditions prioritize efficiency over safety in ring-porous oaks?

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Ignacio García-González

Summary

Climate affects the timing and rate of radial growth, modulating xylem structure and functioning. However, we lack a complete understanding of the connection between xylogenesis and wood anatomical traits. In this study, we ascertain the effect of climate on wood formation and structure in two sympatric ring-porous species with contrasting ecological strategies, the temperate *Quercus robur* and the sub-Mediterranean *Q. pyrenaica* in NW Iberian Peninsula. Cambial phenology, stem girth increment, and tyloses appearance were surveyed in 2012 and 2013. Long-term responses of wood anatomy to environmental conditions were identified by using tree-ring chronologies of hydraulic diameter (D_h), number of earlywood vessels (NV), and latewood width (LW). Tree-ring series at the driest site exhibited larger D_h values, especially in *Q. pyrenaica*, which showed larger D_h but lower wood production than *Q. robur.* This latter species had a longer growing period and a higher stem girth increment than *Q. pyrenaica,* despite it was less resistant to cavitation, with more frequent tyloses formed under drought. Warm and rainy winters negatively affected D_h and NV, while dry conditions in late spring and early summer constrained the expansion of late-formed vessels and LW. Our results suggest that earlywood formation in these oaks is largely controlled by winter temperature, while water supply in summer controls latewood growth. Study ring-porous oaks seem to prioritize hydraulic efficiency over safety in moderately dry environments, probably to hasten foliar and vascular renewal in spring.

Introduction

Stem wood anatomy influences its hydraulic and mechanical properties, determining tree performance (Lachenbruch & McCulloh, 2014), which directly impact on the ability of long-lived trees to track rapid climate change (Jump & Peñuelas, 2005). Studies on quantitative anatomy in woody angiosperms have been mostly focused on vessels (Fonti *et al.*, 2010), which are specialized cell types responsible for water transport. Vessel morphology and distribution strongly differs across phylogenetic groups and biomes (Zanne *et al.*, 2006; Carlquist, 2012). In ring porous species, there is an abrupt change in vessel size between earlywood and latewood (Boura & De Franceschi, 2007). Wood porosity is a trait present in deciduous species that is related to the acquisition of both frost and drought tolerance (Zanne *et al.*, 2006; Boura & De Franceschi, 2007). But although large earlywood vessels carry water more efficiently through the plant body (Steppe & Lemeur, 2007), they are presumably more vulnerable to cavitation (Sperry *et al.*, 1994; Tyree & Cochard, 1996; Wheeler *et al.*, 2005; Hacke *et al.*, 2006; Christman *et al.*, 2009). Despite cavitation resistance is assumed to play a relevant role in adaptation to drought (Delzon and Cochard, 2014), angiosperm trees show narrow safety margins across different phylogenetic groups and biomes (Choat *et al.*, 2012). In this regard, earlywood vessels of ring-porous trees are assumed to become dysfunctional within one year (Sperry *et al.*, 1994; Tyree and Cochard, 1996), being then blocked by tyloses originated in adjacent parenchyma cells (Cochard & Tyree, 1990). The more resistant latewood vessels are thus crucial to maintain water transport if earlywood vessels irreversibly cavitate during the active period (Cochard & Tyree, 1990; Hernández-Santana *et al.*, 2008; Taneda & Sperry, 2008).

White oaks are one of the most prevalent groups of ring-porous species being frequent at intermediate latitudes throughout the northern Hemisphere. The temperate *Quercus robur* (*Qrob*) is widespread in Europe under mild and rainy conditions, reaching the temperate-Mediterranean transition at its southern rear edge. Marcescent sub-Mediterranean oaks are dominant in transitional areas between the temperate and the Mediterranean biomes. One of these species, *Q. pyrenaica* (*Qpyr*), is a late-flushing and stress-tolerant species adapted to cope with both winter frost and moderate summer drought (Sánchez de Dios *et al.*, 2009). In the Iberian Peninsula*, Qpyr* substitutes *Qrob* at higher altitudes under more continental conditions, as well as at xeric sites. Both species coexist along their respective distributional edges in northern Iberian Peninsula. Their performance and relative abundance could be altered as a consequence of the ongoing global warming, which is predicted to induce shifts in species distribution, even in long-lived trees less capable of tracking rapid environmental change (Jump & Peñuelas, 2005). The area currently occupied by sub-Mediterranean forests is deemed to become considerably reduced under future climatic scenarios (Sánchez de Dios *et al.*, 2009). In fact, growth decline has been observed at low elevation *Qpyr* stands in dry areas of the Iberian Peninsula (Gea-Izquierdo & Cañellas, 2014).

Much effort has been made during the last century to provide a comprehensive interpretation of the environmental control on a variety of xylem features, with a special focus on ring width (Fritts, 1976; Cook & Kairiukstis, 1990). More recent studies demonstrated the suitability of earlywood vessel traits to be used as climate proxies (García-González & Eckstein, 2003), together with earlywood and latewood widths (Mérian *et al.*, 2011; Rozas & García-González, 2012a). Recently developed image analysis tools have sped up vessel measuring and data collection (Fonti *et al.*, 2010), increasing the number of studies focused on the adjustment of vessel anatomy to environmental fluctuations (Fonti *et al.*, 2007). For instance, a number of studies revealed that warm winters are associated to narrow earlywood vessels in ring-porous species (Fonti *et al.*, 2007; Gea-Izquierdo *et al.*, 2012). However, the understanding of these relations considerably relies on the available knowledge on xylogenesis, allowing different portions of the ring to be unequivocally assigned to a particular time window of formation within the growing season (Cuny *et al.*, 2015; Pérez-de-Lis *et al.*, 2016). Dendrometer bands have been complementarily used to register short-term girth variations related to stem growth and water storage (Zweifel *et al.*, 2006). Hence, a recent study performed in pine combined long tree-ring width series with both xylogenesis and dendrometer surveys (Pacheco *et al.*, 2015), but so far, studies combining seasonal growth dynamics and tree-ring chronologies have not been performed in ring-porous species.

In this work, we combine the study of growth dynamics and the timing of tyloses appearance over the course of two consecutive growing seasons (2012 and 2013) with long-term growth responses to climate conditions in *Qrob* and *Qpyr* trees co-occurring at their respective distribution boundaries. We particularly ascertain (i) if temporal trends in radial growth and earlywood vessel anatomy reflect species-specific strategies to cope with climatic variation along a water-availability gradient, and (ii) if seasonal growth dynamics can account for responses of wood anatomy to particular climatic factors. We hypothesize that (1) *Q. robur* is more competitive, exhibiting enhanced radial growth and hydraulic capacity (larger and more numerous vessels) than *Q. pyrenaica*; (2) *Q. pyrenaica* is more sensitive to warm conditions in winter than *Q. robur*, being however more tolerant to water shortage than the latter, and (3) latewood width is expected to be directly influenced by water availability in summer and indirectly by temperature-related earlywood development.

Materials and methods

Study sites

The study was conducted at three stands covering a north-to-south gradient at the temperate-Mediterranean climatic transition in the NW Iberian Peninsula. Climate 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. Climate in the northern part of the study region is Atlantic, with abundant rainfall and mild conditions from October to May, but with warm and dry conditions in summer. The northernmost site Bermui (ATL) is located at low elevation nearby the Atlantic coast (395 m a.s.l.), while the intermediate site Labio (ATH) is located 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 (MED), which is located in the southernmost edge of the gradient at 450 m a.s.l., showing lower precipitation and more frequent summer drought episodes than the other study sites. Noteworthy, winter and spring in 2012 were considerably drier and warmer than the same period in 2013, whilst the same occurred for the period October-November, especially at the Atlantic locations. Scarce rainfall and elevated day temperatures were registered in summer for both years. Although both species coexist at the three study sites, *Qrob* prevails at ATL and ATH whereas *Qpyr* does at MED.

Intra-annual dynamics of growth

Xylogenesis and leaf phenology were weekly monitored on 10 trees per location and species from February 2012 to December 2013 (n = 120). The selected individuals within each location had approximately the same size regardless of the species (Table 5.1). Dates of budburst and leaf abscission were identified at *ca.* 10 m distance from each tree with binoculars (10×). We registered budburst when the apical buds on the uppermost part of the crown were broken, but no unfolding leaves were yet observed. Leaf abscission was registered at the end of the growing season when at least 50% of leaves were shed from the crown. We used a Trephor (Vitzani, Belluno, Italy) to collect two microcores per tree (4,440 samples) and date following a helicoidal pattern around the bole (Rossi *et al.*, 2006a), storing microcores into a cooling box. Sample processing consisted of a succession of dehydration phases programmed with a tissue processor (Leica TP1020, Wetzlar, Germany) containing ethanol and xylene, and concluding with paraffin embedding. The cuts obtained in a rotary microtome (Leica RM2125 RTS, Wetzlar, Germany) were placed in slides, oven-dried at 60 ºC for 2 hours, and then stained in 80% ethanol solutions of safranine and fast green. More details on sample processing are provided in Pérez-de-Lis *et al.* (2016).

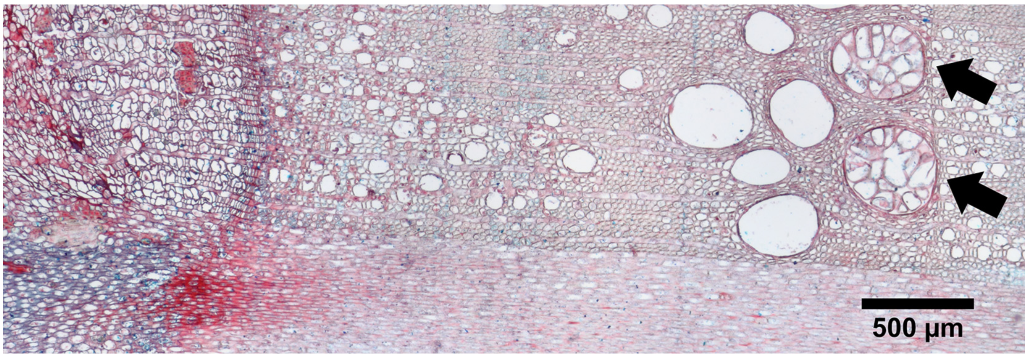

Figure 5.1 Transverse wood section of *Q. robur* showing earlywood vessels blocked by tyloses the 21 August 2012 (DOY 234).

Table 5.1 Tree characteristics and anatomical variables used to build up the chronologies for the period 1967–2013.

	Q. robur			*Q. pyrenaica*		
	ATL	ATH	MED	ATL	ATH	MED
DBH	29.36 ± 1.34	28.04 ± 0.76	33.97 ± 2.71	29.35 ± 1.51	26.26 ± 0.95	29.65 ± 1.10
H	17.17 ± 0.32	13.33 ± 0.36	14.89 ± 0.72	16.89 ± 0.46	13.33 ± 0.32	16.15 ± 0.57
D_h	244.60 ± 1.22	235.35 ± 1.71	260.15 ± 1.31	264.45 ± 1.56	248.39 ± 1.58	292.02 ± 0.98
D_{r1}	254.08 ± 1.33	246.48 ± 1.82	270.73 ± 1.36	273.33 ± 1.65	259.09 ± 1.68	302.47 ± 1.10
MVA	36346 ± 325	34506 ± 462	41131 ± 377	42076 ± 443	38346 ± 451	51107 ± 341
MVA$_{r1}$	43741 ± 446	43291 ± 613	50394 ± 491	50954 ± 584	47912 ± 617	63962 ± 512
NV	41.42 ± 0.60	51.76 ± 0.71	46.98 ± 0.95	36.61 ± 0.65	47.62 ± 0.83	36.40 ± 0.41
EW	0.53 ± 0.01	0.61 ± 0.01	0.63 ± 0.01	0.48 ± 0.01	0.59 ± 0.01	0.60 ± 0.01
LW	1.79 ± 0.03	1.98 ± 0.03	2.01 ± 0.05	1.20 ± 0.02	1.63 ± 0.02	1.19 ± 0.03
RW	2.32 ± 0.04	2.59 ± 0.03	2.64 ± 0.06	1.69 ± 0.03	2.23 ± 0.03	1.79 ± 0.04
N$_1$	13	12	18	12	12	16
N$_2$	24	23	26	23	23	23

Values are expressed as mean ± standard error. DBH is stem diameter, H is tree height, D_h (D_{r1}) hydraulic diameter (first-row), MVA (MVA$_{r1}$) mean vessel area (first-row), NV number of vessels, EW earlywood width, LW latewood width, RW total ring width, N$_1$ and N$_2$ represent the sample size respectively for vessel-related (D_h, MVA, NV) and width-related (EW, LW, RW) variables.

The timing of cambial resumption (only mitoses initiation), earlywood enlargement and maturation, and latewood formation were analyzed on the cross-sections by using a binocular microscope (Olympus BX40, Tokyo, Japan) at 40× magnification. For each sampling date, we registered the number of individuals showing earlywood vessels in the outermost ring partially or totally blocked by tyloses (Fig. 5.1). These data were transformed into absence/presence binomial information. We also installed band dendrometers (D1 UMS GmbH, München, Germany) with an accuracy of 0.01 mm on the trees used for xylogenesis monitoring (n = 120) in order to register changes in stem girth at 1,30 m. Dendrometers were weekly read from February 2012 to December 2013, which allowed us to calculate cumulative stem girth increments for each date. Data reading was performed at the same time in each location to minimize the effect of diurnal stem size variations (Drew & Downes, 2009).

Dendrochronological survey

Trees used in the phenological survey were cored in September 2013, collecting two samples from each bole by using an increment borer. We sampled in 2014 a few additional trees in order to increase the length and strength of the chronologies, whereby the total number of sampled trees was 47 at ATL, 46 at ATH, and 49 at MED. Samples were air-dried and mounted on wooden supports, then cut with a sliding microtome, and sanded in order to distinguish xylem cells in the transversal section (Gärtner & 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 (Holmes, 1983).

Anatomical measurements

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 were performed on around half of the samples collected at each site (Table 1), employing the software for image processing ImageJ (Schneider *et al.*, 2012). This procedure consisted of performing automatic object recognition on grey scales. Vessel outlines were manually corrected when boundaries were not accurately identified. Both size (> 10,000 μm^2) and circularity (> 0.5) filters were used to prevent measurements on artefacts and/or small latewood vessels in output files. First-row vessels were split from those occurring in subsequent rows in order to carry out two distinct correlation analyses. Vessels encountered alongside the ring boundary, together with those starting before the middle line of these vessels, were considered as integrating the first row.

Chronology building and models calculation

Tree-ring width measurements were split into earlywood width (EW), latewood width (LW), and total ring width, calculated as the yearly sum of EW and LW. We calculated the mean vessel area and the hydraulic diameter (D_h) for each tree ring by using earlywood vessel size 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 all the cores. These calculations were separately performed for first and non first-row vessels.

Individual raw series were used to evaluate differences between the species along the gradient. We used linear mixed-effects models for normally-distributed variables (D_h, mean vessel area), while generalized linear mixed-effects models (GLMM) were more suitable for Gamma-distributed variables (NV, EW, LW, ring width). We selected values between 1980–2013 in order to avoid juvenile trends present in raw series (Carrer *et al.*, 2014). Effects of 'site', 'species', and their interaction were set as the fixed part of the model. Factor 'sample', which was nested within factor 'tree', was included as a random factor together with factor 'year'. Data from the girth bands for the period October-December were used in a GLMM model to test differences in the annual girth increment between sites, species, and years. Observations of tyloses were converted to binomial data and analyzed with a GLMM approach. Model selection relied on Akaike's information criterion (AIC) scores (Bolker *et al.*, 2009), and *post hoc* pairwise comparisons allowed us to assess differences among levels for each explanatory variable included in the model. We also performed a Principal component analysis (PCA) in order to identify temporal associations among anatomical variables (Fonti & García-González, 2004). Model assessment and the PCA were performed in R (R Core Team, 2015), using the package 'lme4' for model calculations.

Detection of climatic factors affecting wood anatomical parameters requires the extraction of low-frequency patterns mostly associated to non-climatic variation (Carrer *et al.*, 2014). We averaged raw series 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 & Krusic, 2005). Pre-whitening was complementarily applied in order to remove first-order 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 & Kairiukstis, 1990).

Correlative analysis of growth responses to climate

Monthly gridded time series of maximum and minimum temperatures, and precipitation were used for climate-growth relationships calculations. 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 0.5º longitude × 0.5º latitude, whereby different climatic time series were applied in each location. In order to assess climate-growth relationships, pre-whitened chronologies of earlywood variables (D_h, D_r1, D_rn1, NV) and LW 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 for earlywood, and from the previous June to the current November for latewood. 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.

Results

Spatial variation of xylem anatomical traits

Study species exhibited similar trends of variation along the gradient for number and size of earlywood vessels, as well as for LW (Tables 5.1 and 5.2). *Qpyr* had larger D_h than *Qrob*, but lower earlywood vessel production than *Qrob* (Tables 5.1 and 5.2). In both species, D_h values at ATL and ATH were similar ($P = 0.992$; Fig. 5.2), but notably lower than at MED ($P < 0.001$; Fig. 5.2). A similar number of vessels was found at ATH and MED ($P = 0.512$), but trees at ATL formed fewer vessels ($P = 0.023$). *Qrob* showed larger LW and ring width than *Qpyr* (Tables 5.1 and 5.2). Trees at MED and ATH attained similar LW values ($P = 0.413$), whereas latewood was narrower at ATL ($P < 0.010$). Results for mean vessel area and ring width were comparable to those obtained for D_h and LW, respectively (Supplementary information, Table S.5.1).

Table 5.2 Evaluation of the effects of site and species for the hydraulic diameter, no. of vessels, and latewood width.

Fixed effects	Random effects	*df*	Log-Lik	*Δ*AICc
Hydraulic diameter				
Site + Species	Year + Tree × Sample	8	–24133.58	0.00
Site × Species	Year + Tree × Sample	10	–24133.40	3.65
Site	Year + Tree × Sample	7	–24139.67	10.18
Species	Year + Tree × Sample	6	–24144.10	17.02
Number of vessels				
Site + Species	Year + Sample	7	–9852.29	0.00
Species	Year + Sample	5	–9855.61	2.63
Site × Species	Year + Sample	9	–9851.74	2.93
Site	Year + Sample	6	–9855.39	4.19
Latewood width				
Site + Species	Year + Tree × Sample	8	–9086.74	0.00
Site × Species	Year + Tree × Sample	10	–9084.92	0.37
Species	Year + Tree × Sample	6	–9095.92	14.35
Site	Year + Tree × Sample	7	–9096.35	17.23

Linear mixed models were applied for the hydraulic diameter (normally distributed data). Generalized mixed models for Gamma-distributed data were fitted for the number of vessels and latewood width. *df* is degrees of freedom, Log-Lik is the log-likelihood value, and *Δ*AICc is the difference between each model and the best-fit one for the corrected Akaike Information criterion score.

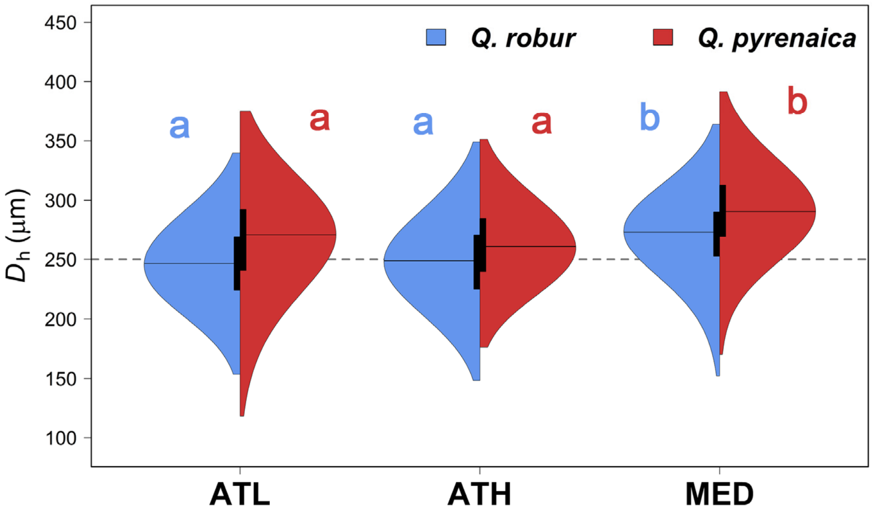

Figure 5.2 Distribution of the hydraulic diameter (D_h) in *Quercus robur* and *Q. pyrenaica* at the three study sites (ATL, ATH, MED). Horizontal solid lines represent the median, and black boxplots show the extent of 25th and 75th percentiles. Lower case letters indicate statistically significant differences along the gradient according to pairwise comparisons.

According to the PCA, variables related to vessel size (D_h, D_{r1} and D_{nr1}) were separated from LW, although distances were more reduced at MED than at ATL and ATH (Fig. 5.3). NV and EW were located close to LW for *Qrob* at ATL and *Qpyr* at MED, indicating that earlywood production was directly associated to latewood in study species at their respective range boundaries. LW had a stronger common variation than D_h and NV, with an expressed population signal of 0.85–0.91. Common signal of the chronologies was stronger at ATH and ATL than at MED for D_h and LW in both species (Supplementary information, Table S.5.2). In contrast, the statistical quality of the NV chronology at ATL was lower than at ATH and MED. Individual pre-whitened series for D_h, NV and LW are shown in the Supplementary information (Fig. S.5.1–S.5.3).

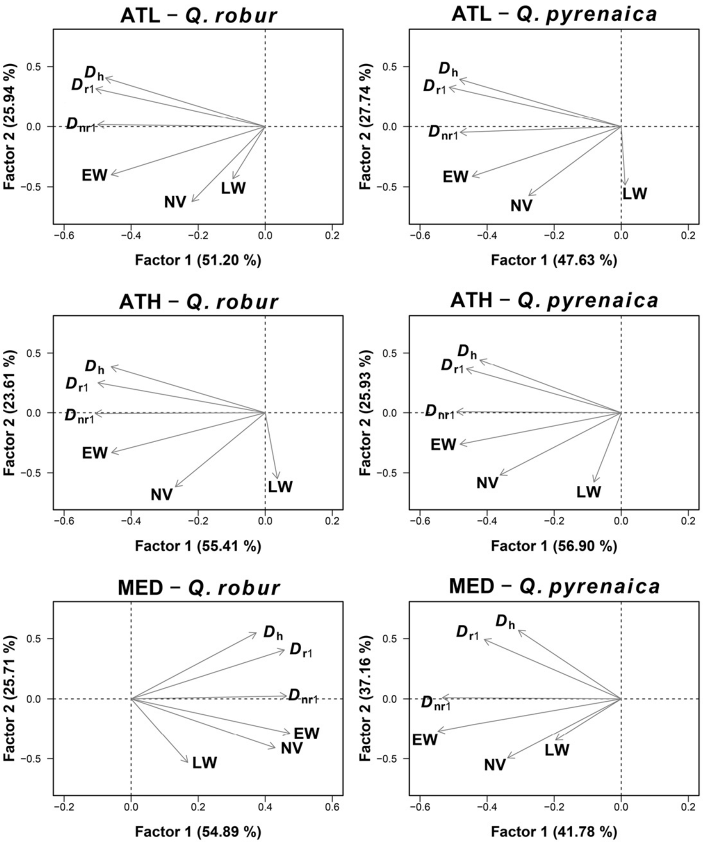

Figure 5.3 Principal component analyses (PCA) based on the correlation matrices of the pre-whitened chronologies for *Q. robur* (*Qrob*) and *Q. pyrenaica* (*Qpyr*) at ATL, ATH and MED sites. The period of analysis is 1967–2013. D_h hydraulic diameter, D_{r1} hydraulic diameter of first-row vessels, D_{nr1} hydraulic diameter of non first-row vessels, NV number of vessels, EW earlywood width, LW latewood width.

Tree phenology, intra-annual growth dynamics, and tyloses appearance

Cambial resumption lasted from early March to April at ATL and ATH regardless of the species and for *Qpyr* at MED, but from mid February for *Qrob* at MED (Fig. 5.3), with a duration of *ca.* 20 and 26 days in *Qrob* and *Qpyr*, respectively. The growing season (wood enlargement) lasted for 194 ± 4 days in *Qrob* and for 182 ± 4 days in *Qpyr*. Earlywood enlargement in *Qrob* proceeded from March to June, although this phase was concluded one month earlier at ATL and MED in 2012 (Fig. 5.4). In *Qpyr*, earlywood enlarged from late March to June at MED, but also at ATL and ATH in 2012, whereas in 2013, the end of this phase occurred in July at these latter locations. Maturation of first-row vessels was firstly observed in *Qrob*, from late March to early April in 2012, but one month later in 2013. Maturation in *Qpyr* started in April at MED and ALH, and mostly in May at ATL. Latewood growth started between mid May and late June in both species, lasting 117 ± 5 days and 111 ± 5 days in *Qrob* and *Qpyr*, respectively (Fig. 5.4). The last formed enlarging cells were observed at mid or late November in 2012 at the three sites and also in 2013 at ATH and MED, but one and two months earlier at ATL in 2013 for *Qrob* and *Qpyr*, respectively. Study species at MED exhibited a period of growth stagnation from mid September to early

October, after which cambial activity resumed again. On average, the leaf period (from budburst to leaf shedding) comprised 8 months in *Qrob*, from mid March-April to November-December, while 6.6 months in *Qpyr*, from April-May to November (Fig. 5.4). Most of the sampled trees exhibited a second flush in late June and July. It is relevant to note that the *Qpyr* canopy at ATL was notably affected by powdery mildew in 2013.

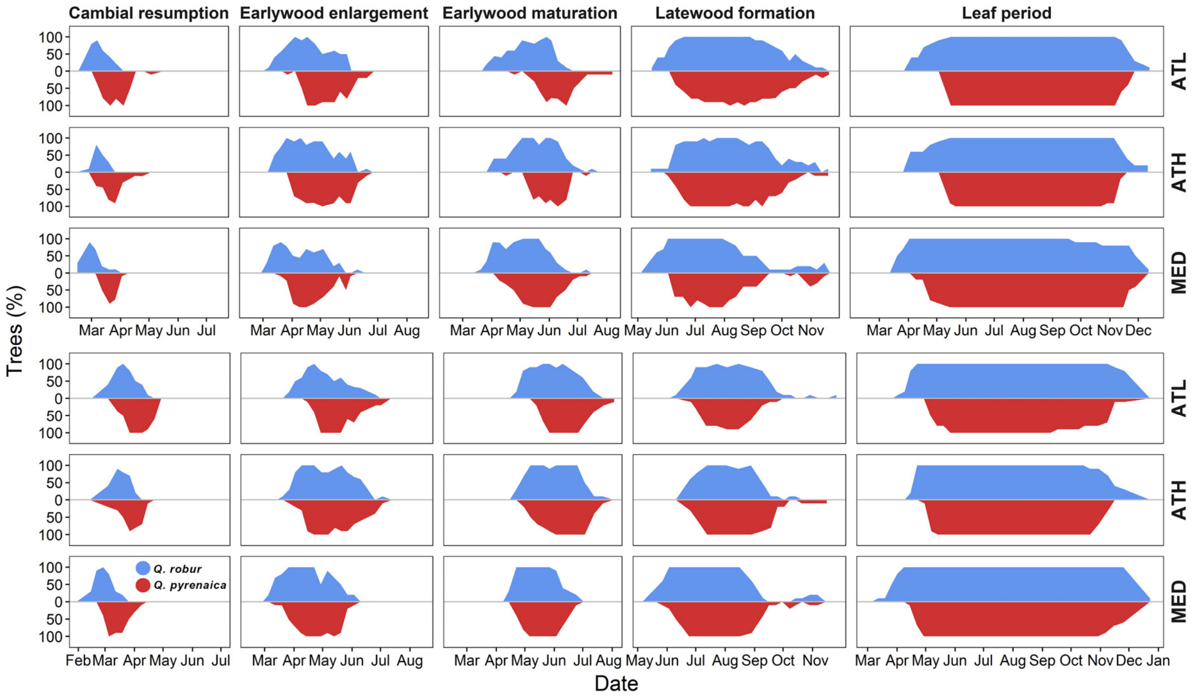

Figure 5.4 Timing of cambial resumption, earlywood enlargement and maturation, latewood formation, and duration of the leaf period for *Q. robur* (blue) and *Q. pyrenaica* (red) at the study sites (ATL, ATH, and MED) in 2012 (dry) and 2013 (wet). Shaded areas represent the proportion of sampled trees showing each phase per date.

The main pulse of stem girth increment occurred from late June to early August at MED and ATH, but up to late August and September at ATL in 2012. Growth in October-November, if any, scarcely contributed to total radial increment regardless the species (Fig. 5.5). *Qrob* showed a larger radial increment than *Qpyr* (Supplementary information, Table S.5.3) at the end of the growing season at ATL ($P = 0.002$) and MED ($P = 0.008$), but not at ATH (Fig. 5.5), where both species showed similar values ($P = 0.078$). In agreement with the overall shorter period of latewood formation observed during 2013, radial growth diminished in that year compared to 2012 (Supplementary information, Table S.5.3). *Qpyr* exhibited a lower radial increment at ATL than at ATH ($P < 0.001$) and MED ($P < 0.001$). Weaker differences were however found along the gradient for *Qrob*, with only marginal differences ($P = 0.054$) between ATL and ATH (Fig. 5.5).

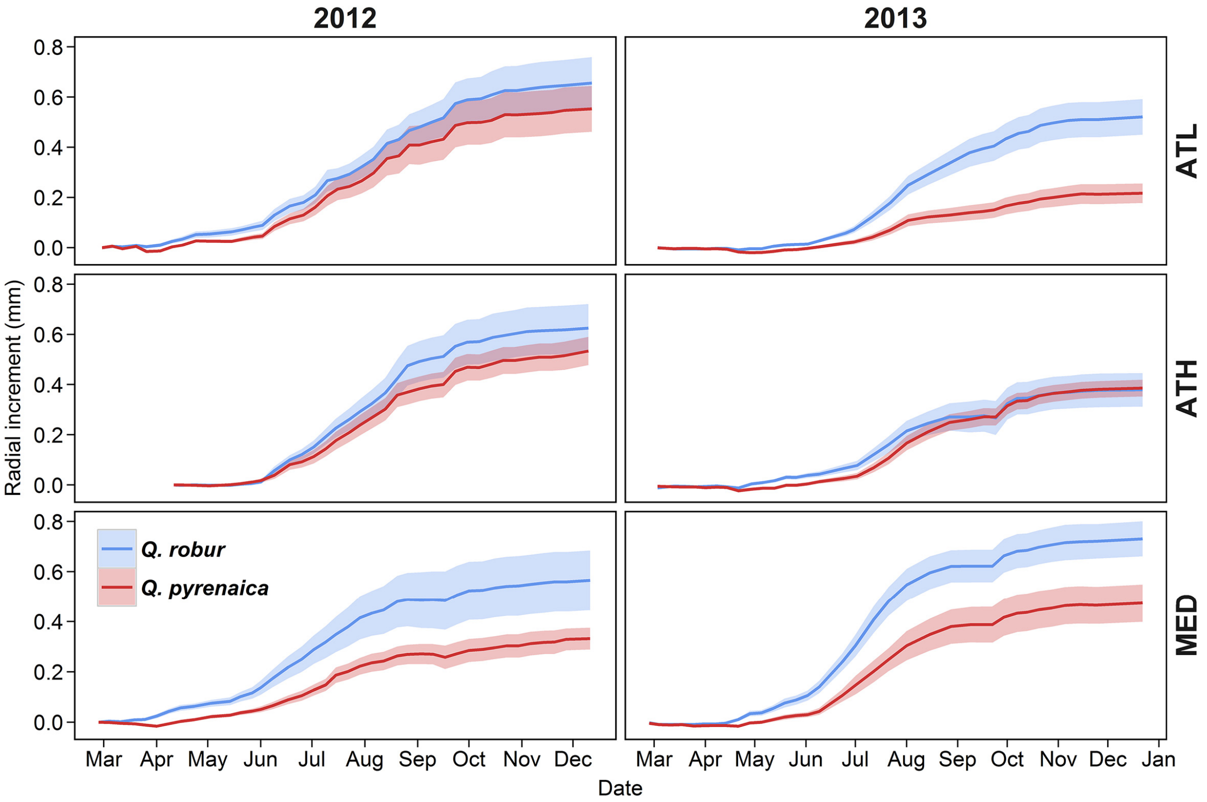

Figure 5.5 Cumulative radial increment of *Q. robur* (blue) and *Q. pyrenaica* (red) for the study sites (ATL, ATH, and MED) at 2012 (dry) and 2013 (wet). Shaded areas represent standard error.

Tyloses blocking earlywood vessels were firstly observed in May-June, increasing along the growing season ($P < 0.001$) until maximum values were attained in autumn (Fig. 5.6). According to pairwise comparisons calculated on the best-fit mixed model, tyloses were more frequently observed in the drier 2012 than in the wetter 2013 ($P < 0.001$), being more abundant at the driest site (MED) than at the Atlantic locations ATL and AHT ($P < 0.004$, Fig. 5.6). At the end of the growing season, frequency of tyloses in *Qpyr* at ATL exceed that in *Qrob* ($P < 0.001$), whilst no differences were observed at ATH ($P = 0.119$) and MED ($P = 0.051$). Over the period July-September, *Qrob* vessels showed substantially more tyloses than *Qpyr* ones at ATL ($P = 0.020$) and MED ($P = 0.010$), whereas differences between species at ATH were negligible ($P = 0.819$). The percentage of *Qrob* and *Qpyr* trees containing any blocked vessel at MED in early July 2012 was respectively 60 % and 40 %, whereas 25 % and 0 % in 2013 (Fig. 5.6). Model results are summarized in the Supplementary information (Supplementary information, Table S.5.4).

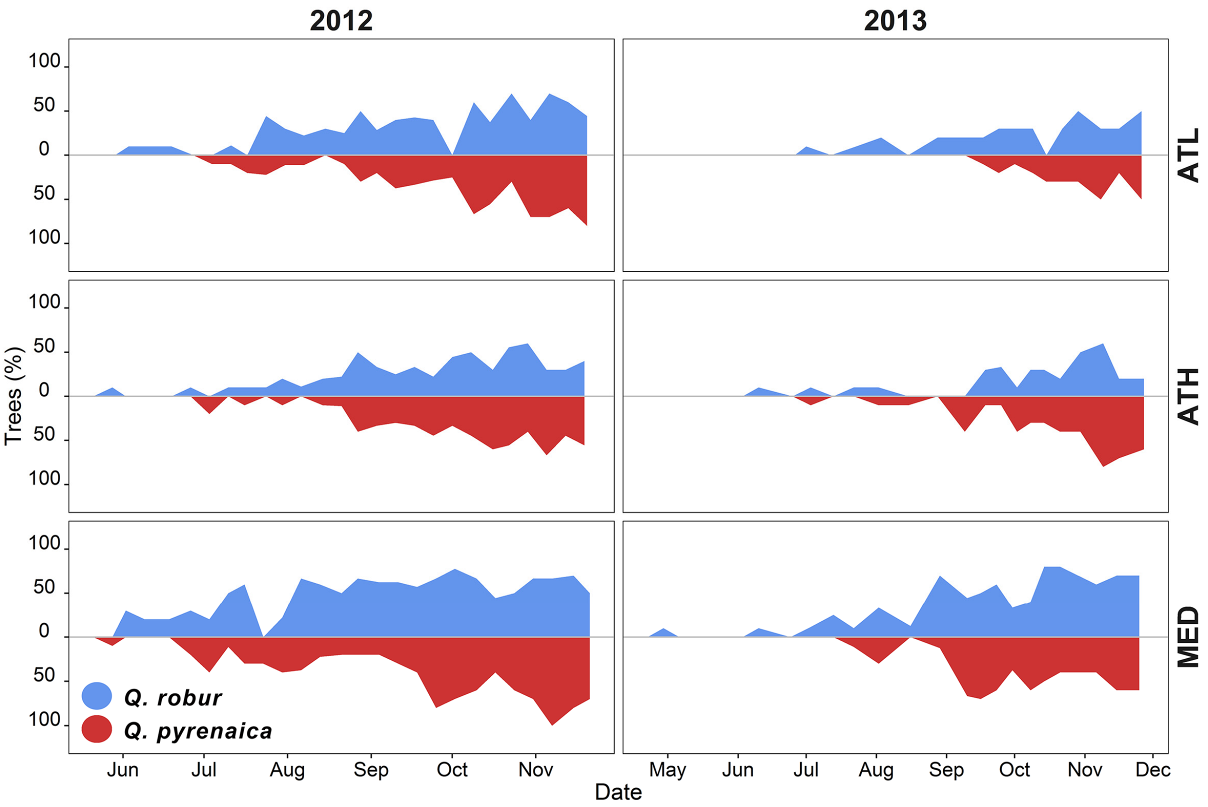

Figure 5.6 Tyloses formation in earlywood vessels of *Q. robur* (blue) and *Q. pyrenaica* (red) at the study sites (ATL, ATH, and MED) in 2012 (dry) and 2013 (wet). Shaded areas represent the proportion of sampled trees showing vessels blocked by tyloses per date.

Climatic drivers of xylem growth

Different climatic factors appeared to control vessel expansion along the gradient, with prevalence of weather conditions during the quiescent period and growth resumption (Table 5.3). At ATL, warm and rainy conditions in December reduced D_{r1} for both species and D_{nr1} for *Qrob*, whilst high temperatures in February (both species) and March (*Qrob*) were related to low D_{r1} values. These effects were weaker at MED, where high precipitation (both species) and night temperature (*Qrob*) in December, as well as warm conditions in January-February, limited vessel expansion. Yet, D_{r1} was positively influenced by high precipitation and night temperature in April-May at MED (Table 5.3). Trees at ATH showed a remarkable positive response of D_{r1} (both species) and D_{rn1} (for *Qrob*) to night temperatures in April (Table 5.3). The occurrence of warm conditions or scarce precipitation in March-April (*Qpyr*) and April-June (*Qrob*) reduced D_{nr1} at MED, while similar effects were observed in May-June at ATL and ATH (*Qpyr*). Warm January (ATL, ATH) and February-March (MED) was related to high NV in *Qrob*, while the combination of cold February and warm April fostered vessel production in *Qpyr* (ATL and ATH). Conversely, high day temperature in April was related to larger NV in this latter species (Table 5.3). Interestingly, rainfall in previous November and in

April-May boosted vessel production in *Qpyr* at MED and ATL, while rainfall in April-May induced high NV in *Qrob* at MED. Non-significant correlations were found during the previous summer. Detailed information on the correlations with maximum and minimum temperatures is shown in the Supplementary information (Table S5).

Table 5.3 Summary of the significant correlations between earlywood traits and minimum (downward triangles) and maximum (upward triangles) temperatures, and precipitation (squares) for *Q. robur* (*Qrob*) and *Q. pyrenaica* (*Qpyr*) along the gradient (ATL, ATH, and MED sites).

			dec	JAN	FEB	MAR	APR	MAY	JUN	jul-dec	dec-FEB	FEB-MAR	APR-MAY	MAY-JUN
ATL	*Qrob*	D_h	▽□		▽	△				▽□	▽	△		
		D_{r1}	▽□		▽	△				□	▽	△		
		D_{nr1}	▽ □						△■	▽□	▽□			■
		NV		▼						□				
	Qpyr	D_h	▽□		▽						▽□	▽		
		D_{r1}	▽□		▽						▽□	▽		
		D_{nr1}		▼	△	△		△■				△	△■	△■
		NV			△			■						■
ATH	*Qrob*	D_h					▼							
		D_{r1}					▼							
		D_{nr1}				△	▼				△	△		
		NV		▼						▼	▼			
	Qpyr	D_h			△		▼	△	△					△
		D_{r1}			△		▼	△	△			△		△
		D_{nr1}				△		△	▽			△		△
		NV					▼					△		
MED	*Qrob*	D_h	▽	▽	▽						▽		▼	
		D_{r1}		▽				■			▽		■	
		D_{nr1}	□										■	■
		NV			▼	▲□							■	
	Qpyr	D_h			▽						▽			
		D_{r1}	□		▽									
		D_{nr1}			▽	△						△		
		NV					■							

Open (close) symbols indicate negative (positive) correlations. Symbol size is proportional to the significance level of the coefficient ($P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$). D_h hydraulic diameter, D_{r1} hydraulic diameter of first-row vessels, D_{nr1} hydraulic diameter of non first-row vessels, NV number of vessels.

Climatic conditions limiting LW varied along the gradient, although oaks from all study sites exhibited convergent responses to water availability in the spring-summer transition, which was the most relevant climatic factor (Table 5.4). Overall, LW in *Qpyr* was more responsive to climatic conditions than *Qrob*. LW in the former species positively responded to humid conditions in May-July at the Atlantic sites, while excessive day temperature and reduced rain in May-June constraint xylem production at MED. Abundant rainfall in such period had a positive influence on LW for *Qrob* solely at ATL. Nevertheless, low temperatures and abundant rainfall in September and October were strongly related to low LW in both species at ATL, and to a lesser extent in *Qpyr* at MED (Table 5.4). Weather conditions occurring earlier than latewood formation were also relevant predictors of LW in both species, which positively responded to high day temperatures in autumn-winter and April at MED (Table 5.4). This is in connection with excessive rainfall in March-April limiting LW in both species at ATL. Although latewood formation in *Qpyr* was encouraged by high temperatures in April at ATH, warm conditions in February-March at this location induced a narrow LW in both species (Table 5.4).

Table 5.4 Summary of the significant correlations between latewood width and minimum (downward triangles) and maximum (upward triangles) temperatures, and precipitation (squares) for *Q. robur* (*Qrob*) and *Q. pyrenaica* (*Qpyr*) along the gradient (ATL, ATH, and MED sites).

		nov	dec	FEB	MAR	APR	JUN	JUL	SEP	OCT
ATL	*Qrob*								▲□	▲□
	Qpyr				□			△■	▲□	▲
ATH	*Qrob*	□								
	Qpyr		▽		▽	▲		■		
MED	*Qrob*	▼▲	▼▲■	▲		▲				
	Qpyr			▼		▼▲	▽△■	△	▲□	

		dec-FEB	MAR-APR	MAY-JUN	JUN-JUL	JUL-AGO	SEP-OCT
ATL	*Qrob*		□		■		▲□
	Qpyr		□		■		▲□
ATH	*Qrob*						
	Qpyr				■	■	
MED	*Qrob*	▼▲	▲				
	Qpyr			△■	▽△		□

Open (close) symbols indicate negative (positive) correlations. Symbol size is proportional to the significance level of the coefficient ($P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$).

Discussion

Xylem adjustment to increasing xeric conditions at the temperate-Mediterranean transition

Long-term tree ring series revealed larger D_h at the driest site (MED) than at the Atlantic locations. These results are somehow opposed to previous studies reporting narrower vessels under drier conditions (Tardif & Conciatori, 2006; Eilmann *et al.*, 2009; Gallé *et al.*, 2010; Gea-Izquierdo *et al.*, 2012), although such adjustment has not been observed for sub-Mediterranean oaks (Villar-Salvador *et al.*, 1997; Corcuera *et al.*, 2004). Large vessels are more vulnerable to cavitation, suggesting that earlywood vessels in our study oaks were misadjusted to increasing xeric conditions (Delzon & Cochard, 2014). Indeed, study species exhibit low embolism resistance, with water potentials of –2.9 MPa for *Qpyr* (Corcuera *et al.*,

2006) and between –2.7 and –2.9 MPa for *Qrob* (Tyree and Cochard, 1996; Urli *et al.*, 2015) inducing a 50 % conductivity loss. Yet, angiosperms experience irreversible hydraulic failure when conductivity loss reach 88 % rather than 50 % (Urli *et al.*, 2013), which is in part attributed to the ability of these species to repair embolism (Johnson *et al.*, 2012; Delzon & Cochard, 2014). It is relevant to note that frequency of tyloses was remarkably higher during the drier year, particularly at the most xeric location (MED), where more vulnerable vessels were formed. A high proportion of trees exhibited tyloses in mid-summer under increasing xeric conditions, which is significantly earlier than previously reported for temperate oaks (Cochard & Tyree, 1990). In fact, withering leaves were perceived at MED in late summer, pointing out that some branches experienced vascular dysfunction (Peguero-Pina *et al.*, 2015).

In our study oaks, cavitation resistance apparently played a secondary role in the environmental control of earlywood formation. One explanation could be that latewood act as an 'emergency tissue' under moderate drought, preventing hydraulic failure if earlywood become dysfunctional (Corcuera *et al.*, 2006; Hernández-Santana *et al.*, 2008; Taneda & Sperry, 2008). In addition, the cost of cavitation may be low for ring-porous species that entirely rebuild their functional earlywood vessel network each spring (Urli *et al.*, 2013). More interestingly, tyloses formation in summer was lower for *Qpyr*, which is contradictory with its larger vessels. Therefore, xylem anatomy failed to reflect cavitation resistance at the whole-tree level, which is likely more related to morphological leaf traits, such as pubescence, deep lobation, and cell-wall elasticity (Aranda *et al.*, 1996; Corcuera *et al.*, 2002; Sisó *et al.*, 2001). This would be combined with an extensive root system conferring the ability to access water in deep soil layers in summer (Gallego *et al.*, 1994; Hernández-Santana *et al.*, 2008). The outstanding capacity of *Qpyr* for root grafting may also confer resistance to drought at the population level, allowing vigorous trees to share water and carbohydrates (Salomón *et al.*, 2016). In addition, late leaf-out dates in *Qpyr* might minimize detrimental effects of drought due to the shorter period of elevated evapotranspiration and water soil consumption before summer (Fischer *et al.*, 2007).

Since wide conduits carry water more efficiently (Steppe & Lemeur, 2007), our results suggest that increasing xeric conditions favoured hydraulic efficiency. It is noteworthy that drought tolerance was found to be positively associated to vessel diameter and ring porosity (Zanne *et al.*, 2006). Earlywood vessels are devoted for sustaining growth in spring (Corcuera *et al.*, 2006; Hernández-Santana *et al.*, 2008; Taneda & Sperry, 2008), as suggests the fact that latewood formation is induced by gibberellins synthesized in mature leaves (Aloni, 2015).

This is in accordance with the fact that first-row vessel maturation is coupled with budburst (Pérez-de-Lis *et al.*, 2016), but also with the appreciable number of trees showing dysfunctional earlywood vessels in July at MED. A higher hydraulic capacity may enhance water supply to developing leaves and shoots (Steppe & Lemeur, 2007), speeding up foliage development, which is probably adaptive in areas where climatic seasonality restrict favourable conditions to a short period in spring (Zanne *et al.*, 2006). This is the case of the sub-Mediterranean area, where *Qpyr* prevails (Sánchez de Dios *et al.*, 2009). Late flushing reduces the risk of late frost damage (Lenz *et al.*, 2013), but this also implies that shoots, catkins and leaves must be promptly developed before water shortage in summer (Hernández-Santana *et al.*, 2008). This was confirmed by the later but faster flushing perceived in *Qpyr*. In fact, *Qpyr* was reported to maintain high transpiration rates under dry conditions (Gallego *et al.*, 1994; Hernández-Santana *et al.*, 2008). Nevertheless, this strategy could be detrimental for trees facing extreme drought episodes (Levanic *et al.*, 2011)

Despite their lower hydraulic capacity, *Qrob* exhibited larger latewood width than *Qpyr*, with higher radial increments over the two-year monitoring period, particularly at the warmer sites ATL and MED. Similar results were reported in a previous study (Rozas *et al.*, 2009), confirming our expectations that *Qpyr* has a more conservative growth strategy than *Qrob*. On the other hand, drier conditions at MED limited latewood growth, being the only study site where stem increment was lower during the drier year (2012). This result suggests that summer drought at the temperate-Mediterranean transition is severe enough to limit growth, even for the sub-Mediterranean *Qpyr*. It is known that water stress impairs cell production (Hsiao, 1973; Eilmann *et al.*, 2009; Gallé *et al.*, 2010; Wang & Ruan, 2013), whereby yearly changes in summer drought severity probably induced high inter-annual LW variation, which is in accordance with previous observations in ring-porous species (Fonti *et al.*, 2007; Alla & Camarero, 2012; Gea-Izquierdo *et al.*, 2012; González-González *et al.*, 2014).

Linking the climatic modulation of wood anatomy to phenological patterns

Correlative analyses revealed that vessel enlargement was mostly limited by high night temperatures at the onset of dormancy and during quiescence, before the time of cambial resumption at the sites showing warmer winter conditions (ATL and MED), but also at ATH for the more cold-tolerant *Qpyr*. Similar results have been recurrently observed for temperate (Tardif & Conciatori, 2006; Fonti *et al.*, 2007) and Mediterranean (Alla & Camarero, 2012;

Gea-Izquierdo *et al.*, 2012) ring-porous species, being attributed to (i) an insufficient chilling that may delay the break of dormancy, and to (ii) an excessive consumption of carbohydrate reserves due to enhanced respiration rates with high temperature.

The timing of growth resumption has been shown to affect earlywood vessel anatomy, although such linkage was stronger for *Qrob* than for *Qpyr* (Pérez-de-Lis *et al.*, 2016). In turn, tree phenology is controlled by the complex interplay among temperature, photoperiod, and growth regulators. Chilling temperatures reduce the dormancy state, whereas warming accelerates the break of dormancy once chilling requirements are fulfilled (Cooke *et al.*, 2012; Basler & Körner, 2014; Kudo *et al.*, 2014). Radial gradient of auxin concentration provides positional information to cells determining rates of cell division and differentiation (Ursache *et al.*, 2013), but responsiveness to this morphogen is activated by thermal forcing in spring (Schrader *et al.*, 2003). Therefore, spatial variation in auxin concentrations and thermal-induced variation in cell sensitivity may determine the rate of cell division and the time for vessel expansion. A trade-off between earlywood vessel expansion and production may explain why NV and D_h were decoupled between species and sites, although both different climatic signals and aggregation to different functional groups for these variables conveyed a limited impact of such trade-off on earlywood anatomy (Carrer *et al.*, 2014). This framework would underpin the previously discussed effect of low temperature on D_h, but also the fact that warming at the time of earlywood enlargement fostered cell division and vessel expansion at ATH. This latter response was coupled for both species, affecting first-row vessels in *Qpyr*, but also conduits located in subsequent rows for *Qrob*. Such positive influence would probably be a consequence of the altitudinal pattern of decreasing temperature affecting the timing of growth resumption in spring (Pérez-de-Lis *et al.*, 2016), being comparable to that observed at the northern edge of the *Qrob* range (Matisons *et al.*, 2012).

Besides providing materials and energy for growth and respiration, sugars play a key role in growth signalling, being also involved in the osmotic restoration of long-distance water transport in spring (Améglio *et al.*, 2004; Morin *et al.*, 2007; Repo *et al.*, 2008). However, the extent to which carbohydrate reserves limit growth is still a matter of discussion (Palacio *et al.*, 2014). The acquisition of freezing tolerance at the end of the growing season involves starch hydrolysis, which was found to be fostered at non-freezing low temperatures (Améglio *et al.*, 2004; Morin *et al.*, 2007; Repo *et al.*, 2008). Thereby, persistent high temperature in late autumn at low elevation might limit overwintering sugar concentrations, which in turn could delay the timing of growth reactivation (Cooke *et al.*, 2012), as well as affect rates of cell division in spring (Deslauriers *et al.*, 2009). Sugar consumption may be aggravated during the leafless period if warm conditions boost respiration rates (Edwards & Hanson, 1996; Deslauriers *et al.*, 2014; Salomón *et al.*, 2015). In addition, the strong negative response to the excessive winter precipitation at the most humid location ATL, which was previously found in declining oak forests (Rozas & García-González, 2012b; González-González *et al.*, 2014), could be related to a high sugar demand in roots under conditions of anoxia in the soil (Baley-Serres and Voesenek, 2008; Ferner *et al.*, 2012).

Maximum temperature and/or low precipitation in April-May (at the two Atlantic sites) and May-June (at site MED) limited vessel expansion, which is probably related to occasional episodes of spring drought in the temperate-Mediterranean transition (García-González & Eckstein, 2003). However, the effect of previous summer conditions found in previous studies was negligible for our oaks (González-González *et al.*, 2014). On the other hand, latewood formation was mostly boosted by high moisture between May and August, i.e. high precipitation at both Atlantic sites and low day temperature at MED. Similar results were observed for *Qrob* and other ring-porous species from temperate and Mediterranean forests (Rozas, 2005; Di Filippo *et al.*, 2010; Mérian *et al.*, 2011; Alla & Camarero, 2012). The highest rate of radial increment occurred between June and early August for both species, whereby climatic conditions at this period may result critical for wood production. This is in line with previous studies suggesting that wood production considerably relies on the maximum growth rate, which is attained around the time of maximum photoperiod (Cuny *et al.*, 2015). Concurrently, high precipitation in early summer may ameliorate water soil reserves that are needed to withstand late summer drought (Hernández-Santana *et al.*, 2008).

The role played by autumn phenology appeared to be relevant at ATL for both species, with cool and humid conditions in September and October limiting latewood growth, probably due to the advancement of growth cessation, as occurred in 2013 compared to 2012. In addition, excessive precipitation (ATL) and cold conditions (ATH and MED) between March and May negatively affected latewood formation, likely because this impaired leaf development. Powdery mildew infections are fostered by high winter temperature and humid conditions in spring, being especially acute when budburst is delayed (Marçais & Desprez-Loustau, 2012). This is in accordance with the strong limiting effect of high spring precipitation on LW observed at ATL, where we found a severe mildew infection in 2013.

Vulnerability of ring-porous oaks under future climatic scenarios

Stronger cavitation resistance (less frequent tyloses) and conservative growth in *Qpyr* is in line with its other multiple adaptations to minimize water loss (Corcuera *et al.*, 2002), conveying the higher ability of sub-Mediterranean oaks to withstand extreme climatic events. However, *Qpyr* showed a risky hydraulic adjustment, forming wider earlywood vessels but a less extent hydraulic network under increasing xeric conditions. Thereby, acute drought in spring could challenge long-distance water transport (Levanic *et al.*, 2011; Anderegg *et al.*, 2014). Convergent results have been observed in other sub-Mediterranean oaks, such as *Q. faginea* (Granda *et al.*, 2013) and *Q. cerris* (Di Filippo *et al.*, 2010), revealing the high vulnerability of sub-Mediterranean oaks to the ongoing environmental change (Gea-Izquierdo *et al.*, 2013; Gea-Izquierdo & Cañellas, 2014). Nevertheless, our results suggest that warm conditions in winter may cause a decline in vessel diameter. This compensatory mechanism would offset increasing drought-related cavitation risk under climate warming, although slow foliage development may increase vulnerability to extreme events (i.e. frost damage and early drought episodes). There is therefore limited evidence that *Qpyr* could displace *Qrob* in the temperate-Mediterranean transition under future climatic scenarios, attributing to evergreen diffuse-porous oaks, such as *Q. ilex* and *Q. suber*, more chances of success due to their higher tolerance to both high temperature and scarce precipitation (Urli *et al.*, 2015).

Conclusions

In this chapter, we combined long-tree ring series with seasonal dynamics of wood formation and frequency of tyloses, which allowed us to shed light on the connections between climate factors driving growth and key anatomical traits. Less frequent tyloses and conservative growth in *Qpyr* conveyed the higher ability of sub-Mediterranean oaks to withstand extreme climatic events. However, both species formed a less safe hydraulic network under increasing dry conditions. Although such configuration probably allows for a rapid foliage development, the risk of hydraulic failure would dramatically increase if extreme episodes occur in spring. Nevertheless, the inverse association between winter temperature and vessels suggests that drought-induced cavitation risk would be compensated in future decades insofar temperatures rise in winter. Our results demonstrate that intra-annual growth dynamics can improve our understanding on the environmental information contained in long tree-ring series, even though the limited duration of our monitoring is a relevant shortcoming. Thus, further studies including multi-year monitoring of xylogenesis and leaf phenology are needed to improve our understanding on the environmental control of xylem anatomy in ring-porous species under shifting climate conditions

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Supplementary information

Table S.5.1 Evaluation of the effects of site and species for mean vessel area, earlywood width, and total ring width.

Fixed effects	Random effects	*df*	Log-Lik	ΔAIC
Mean vessel area				
Site + Species	Year + Tree × Sample	8	–54346.23	0.00
Site × Species	Year + Tree × Sample	10	–54346.14	3.82
Site	Year + Tree × Sample	7	–54350.26	6.05
Species	Year + Tree × Sample	6	–54352.27	8.06
Earlywood width				
Site + Species	Year + Tree × Sample	8	2834.20	0.00
Site	Year + Tree × Sample	7	2832.64	1.12
Site × Species	Year + Tree × Sample	10	2834.56	3.28
Species	Year + Tree × Sample	6	2822.69	19.05
Ring width				
Site + Species	Year + Tree × Sample	8	–10420.04	0.00
Site × Species	Year + Tree × Sample	10	–10418.75	1.41
Site	Year + Tree × Sample	7	–10427.77	13.45
Species	Year + Tree × Sample	6	–10429.93	15.76

Generalized mixed models data were fitted for Gamma-distributed variables. *df* is degrees of freedom, Log-Lik is the log-likelihood value, and *Δ*AICc is the difference between each model and the best-fit one for the corrected Akaike Information criterion score.

Table S.5.2 Descriptive statistics characterizing the common signal of the standardized chronologies.

	Rbt			EPS			PC1			MS			AR1		
	D_h	NV	LW	D_h	NV	LW	D_h	NV	LW	D_h	NV	LW	D_h	NV	LW
Q. robur															
ATL	0.30	0.15	0.39	0.80	0.61	0.91	38.79	26.48	43.08	0.04	0.08	0.22	0.32	0.21	0.13
ATH	0.40	0.23	0.44	0.86	0.72	0.87	47.16	33.03	47.79	0.04	0.12	0.26	0.16	-0.25	0.10
MED	0.17	0.22	0.36	0.75	0.80	0.88	25.75	28.62	39.76	0.03	0.11	0.31	0.14	0.35	0.26
Q. pyrenaica															
ATL	0.37	0.19	0.35	0.84	0.68	0.91	45.29	29.91	39.58	0.05	0.08	0.26	0.13	0.43	0.05
ATH	0.51	0.19	0.45	0.90	0.67	0.90	56.85	30.62	48.93	0.04	0.08	0.26	-0.16	0.29	-0.07
MED	0.26	0.26	0.25	0.85	0.85	0.85	34.28	32.84	29.31	0.04	0.08	0.21	0.12	0.38	0.11

Rbt mean correlation between trees, EPS the expressed population signal, PC1 the percentage of variance in the first eigenvector, MS mean sensitivity, AR1 the first-order autocorrelation coefficient. D_h hydraulic diameter, NV number of vessels, LW latewood width.

Table S.5.3 Models evaluating differences among site, species and year in the annual radial growth of 2012 and 2013.

Fixed effects	Random effects	*df*	Log-Lik	ΔAIC
Site × Species + Year	Tree	9	1896.73	0.00
Site × Year	Tree	8	1893.90	3.62
Site + Species × Year	Tree	8	1892.70	6.01
Site × Species	Tree	8	1892.40	6.62
Site + Species + Year	Tree	7	1888.42	12.56
Site × Species × Year	Tree	14	1886.10	31.50
Site × Species × Year	*Null*	13	105.84	3589.62

Generalized mixed models for Gamma-distributed data were fitted. *df* is degrees of freedom, Log-Lik is the log-likelihood value, and *Δ*AIC is the difference between each model and the best-fit one for the Akaike Information criterion score.

Table S.5.4 Models evaluating probability of tyloses detection between sites, species and years.

Fixed effects	Random effects	*df*	Log-Lik	*Δ*AIC
Date + Site × Species + Site × Year	Tree	11	–1270.84	0.00
Date + Site × Species + Year	Tree	9	–1272.93	0.15
Date + Site + Species + Year	Tree	7	–1275.52	1.32
Date + Site × Year + Species	Tree	9	–1273.55	1.40
Date + Site + Year	Tree	6	–1276.73	1.73
Date + Site × Species + Site × Year + Species × Year	Tree	12	–1270.82	1.96
Date + Site × Year	Tree	8	–1274.87	2.02
Date + Site × Species + Species × Year	Tree	10	–1272.92	2.14
Date + Site + Species × Year	Tree	8	–1275.52	3.32
Date + Site × Year + Species × Year	Tree	10	–1273.54	3.38
Date + Site × Species × Year	Tree	14	–1270.34	5.04
Date + Site × Species	Tree	8	–1284.15	20.59
Date + Site × Species × Year	*Null*	13	–1291.93	46.16

Generalized mixed models for binomial data were fitted. *df* is degrees of freedom, Log-Lik is the log-likelihood value, and *Δ*AIC is the difference between each model and the best-fit one for the Akaike Information criterion score.

Table S.5.5 Summary of the significant correlations between earlywood traits and minimum (downward triangles) and maximum (upward triangles) temperatures for *Q. robur* (*Qrob*) and *Q. pyrenaica* (*Qpyr*) along the study gradient (ATL, ATH, and MED sites).

			dec	JAN	FEB	MAR	APR	MAY	JUN	dec-FEB	FEB-MAR	MAR-APR	MAY-JUN
ATL	*Qrob*	D_h	▽△		▽	△				▽△	▽△		
		D_{r1}	▽△		▽	△				▽△	▽△		
		D_{nr1}	▽△						▽△	▽△			
		NV		▼									
	Qpyr	D_h	▽		▽					▽	▽		
		D_{r1}	▽		▽					▽	▽		
		D_{nr1}		▼	△	△		△			△		△▽
		NV			△								
ATH	*Qrob*	D_h					▼▲					▼	
		D_{r1}					▼▲					▼	
		D_{nr1}				△	▼▲			△	△		
		NV		▼						▼			
	Qpyr	D_h			△		▼	△	△				△
		D_{r1}			△		▼	△	△		△		△
		D_{nr1}				△		△	▽		△		▽△
		NV					▼				△		
MED	*Qrob*	D_h	▽	▽	▽					▽			
		D_{r1}		▽						▽			
		D_{nr1}											
		NV			▼	▲						▲	
	Qpyr	D_h			▽					▽			
		D_{r1}			▽								
		D_{nr1}			▽	△					△	△	
		NV											

Open (close) symbols indicate negative (positive) correlations. Symbol size is proportional to the significance level of the coefficient ($P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$). D_h hydraulic diameter, D_{r1} hydraulic diameter of first-row vessels, D_{nr1} hydraulic diameter of non first-row vessels, NV number of vessels.

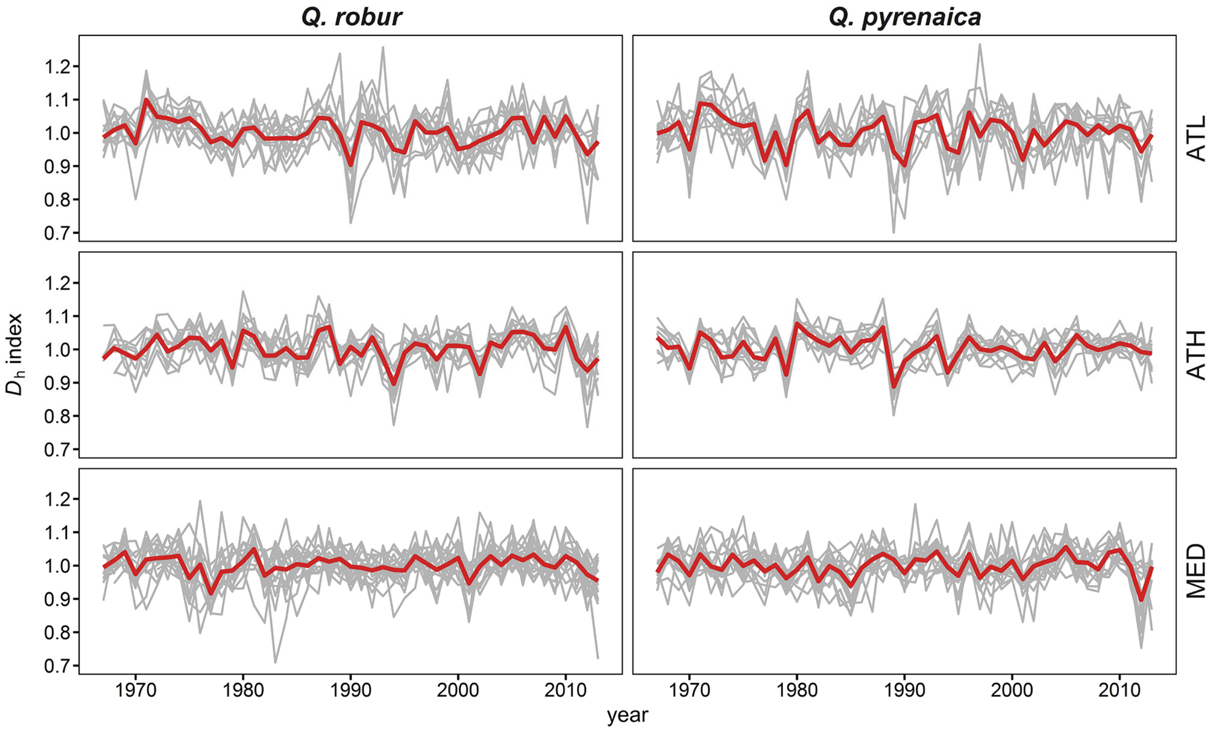

Figure A.5.6 Individual pre-whitened chronologies of the hydraulic diameter (D_h) in the period 1967–2013 for each species and site.

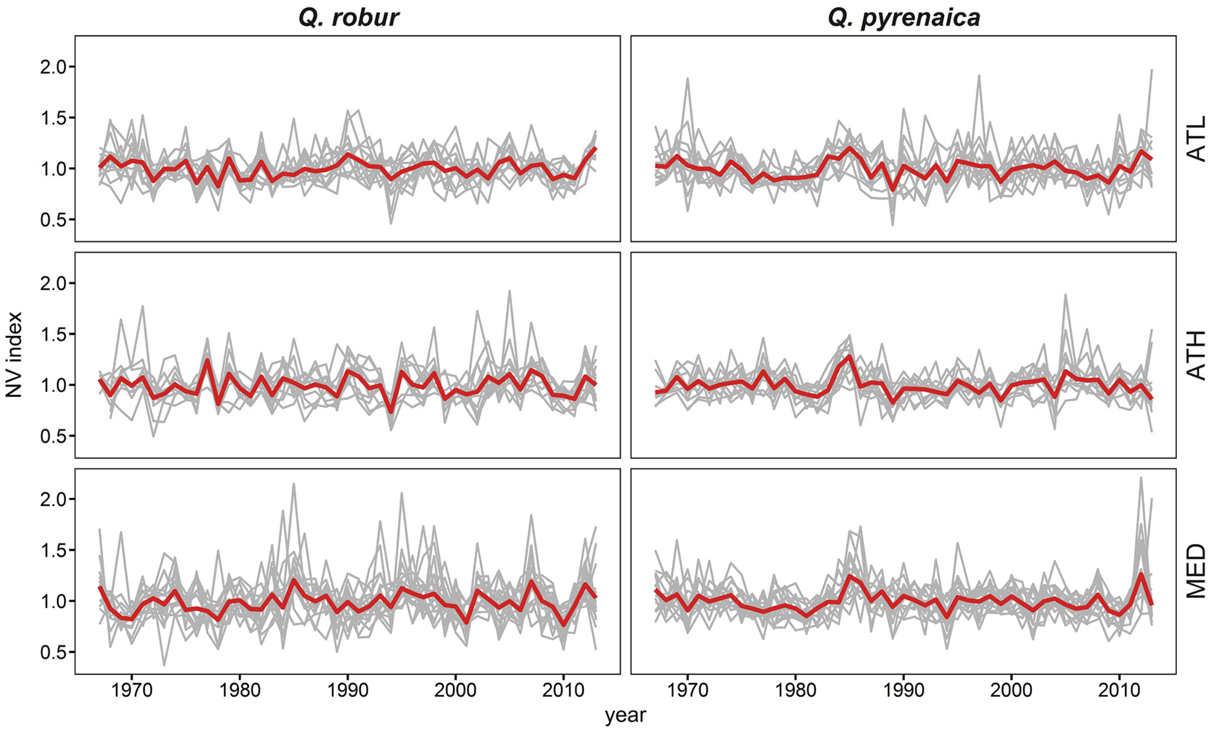

Figure A.5.7 Individual pre-whitened chronologies of the number of vessels (NV) in the period 1967–2013 for each species and site.

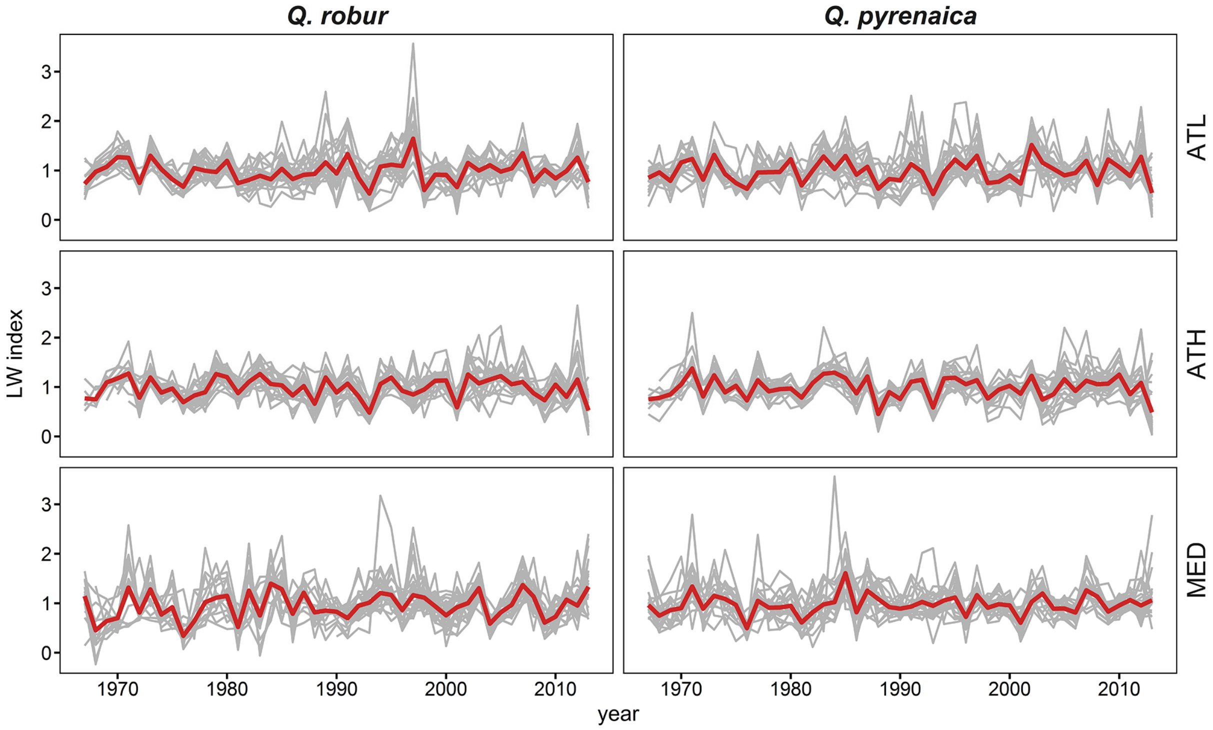

Figure A.5.8 Individual pre-whitened chronologies of latewood width (LW) in the period 1967–2013 for each species and site.

CHAPTER VI

General discussion

Summary of results

In this Thesis, stem growth was addressed in coexisting temperate (*Quercus robur*) and sub-Mediterranean (*Q. pyrenaica*) oaks through a multidisciplinary approach, which encompassed the study of leaf and cambial phenology, non-strucutural carbohydrates, and tree-ring series. The information obtained shed light on the plastic adjustment of phenology and xylem structure to varying climate conditions in ring-porous deciduous oaks. Nonetheless, full understanding of the mechanisms underlying the various functional relationships between phenology, production and structure of xylem, and sapwood NSC content presented here requires reviewing the major findings of this Thesis. Furthermore, this integrative work help us assessing the capability of ring-porous oaks to withstand increasing abiotic stress in the context of climate warming.

At each of three sites selected along a water-availability gradient in NW Iberian Peninsula, xylogenesis and leaf phenology were monitored in 2012 and 2013 in ten individuals per species. Timings of flushing and earlywood formation were evaluated in Chapter II by using generalized linear mixed-models, assessing specific thermal requirements for dormancy release. Growth plasticity in relation to drought stress was assessed in Chapter III during one growing period, for which a generalized additive modelling approach was used. Since the connection between phenology and xylem structure was a major concern in this Thesis, Chapter II examined how the timing of earlywood vessel formation affected the conductive area of the stem. Chapter III went further into this subject through the dependencies of xylem production and growing season duration on tree vigour, represented by vascular cambium traits.

Sapwood NSC concentration was monitored for 30 individuals of each species at the three selected sites along the growing season of 2012. This was complemented with the assessment of NSC concentration in December 2012 in 90 additional trees of each species. The main findings of the first experiment were detailed in Chapter III along with xylogenesis data. The focus of the second experiment was on the linkage between winter reserves in 2012 with the timing of spring phenology and earlywood vessel production in 2013. These connections were analyzed in Chapter IV by using structural equation models. In this latter Chapter, carbon allocation to wood was argued in terms of the contrasting species strategies to cope with environmental constraints.

This Thesis concluded with a retrospective analysis of the various key anatomical parameters considered throughout this dissertation. Tree-ring chronologies of the hydraulic diameter, earlywood vessel production, and latewood width were built up on trees used for phenological assessment. Changes in these variables among sites and between species were analyzed by applying mixed-effects models. Complementarily, a correlative analysis enabled the identification of main climate drivers for cell proliferation and vessel expansion in longer time-scales. These results were shown in Chapter V, which also contained a detailed portrayal of the timing of tree phenology and stem girth increment, as well as an examination of tyloses formation over the course of two growing seasons. Such an integrative analysis facilitated the identification of adaptive mechanisms for xylem adjustment to climate in ring-porous oaks.

Environmental control of tree phenology in deciduous oaks

Cambial resumption was the first event perceived after dormancy, between February and early April (Fig. 2.3; Fig. 5.4). This phase was identified as the period when cambial cell divisions but not differentiation could be perceived. The hypothesis that differentiated overwintering cells give rise to first-formed vessels (Frankenstein *et al.*, 2005) was not confirmed in this case, as also reported for *Q. serrata* in recent investigation (Kudo *et al.*, 2014). Budburst was coupled to the beginning of wall thickening of first-formed vessels, confirming previous studies in ring-porous species (Suzuki *et al.*, 1996; Sass-Klaassen *et al.*, 2011; Michelot *et al.*, 2012; Takahashi *et al.*, 2013). Furthermore, both phases were correlated with cambial resumption and onset of earlywood enlargement. These results confirmed the tight coordination among different ontogenetic processes at the whole tree level (Thibeault-Martel *et al.*, 2008; Rossi *et al.*, 2012, 2013; Rossi & Bousquet, 2014), which are probably mediated by the polar transport of auxin from expanding buds downwards through the cambial zone (Tuominen *et al.*, 1997; Schrader *et al.*, 2003). Since new-formed vessels are required to be functional for flushing, time for budburst is probably signalled by vessel maturation, which is in turn dependent on changes in auxin fluxes and responsiveness during bud swelling (Schrader *et al.*, 2003). Delayed break of dormancy at the northernmost sites within the study gradient, and over the colder year of monitoring, proved that spring leaf phenology is tightly controlled by environmental conditions, especially by temperature, but also by photoperiod. Pronounced temporal and spatial variability was previously found for other deciduous species, underpinning the plastic adjustment of phenology to unstable climatic conditions (Moser *et al.*, 2010; Oladi *et al.*, 2011; Prislan *et al.*, 2013). This is related to that temperature plays an essential role in regulating dormant state in buds (Caffarra & Donnelly, 2011; Basler &

Körner, 2014), as well as in the cambial zone (Gricar *et al.*, 2006; Begum *et al.*, 2008). Nevertheless, cambial resumption was remarkably coincident in both years, suggesting a weak thermal influence in this phase as compared to the onset of vessel enlargement and budburst.

It is noteworthy that thermal requirements for budburst and onset of enlargement were larger for *Qpyr*, which is consistent with its more delayed timing of growth resumption (Jato *et al.*, 2002). In addition, I suggest that *Qpyr* is probably more sensitive to photoperiod than *Qrob*. The lower thermal sensitivity in *Qpyr* may be related to its stronger tolerance to cold conditions because a delayed leaf-out prevents damage from late freezing episodes (Lenz *et al.*, 2013; Vitasse *et al.*, 2014). In this regard, it should be highlighted that *Qpyr* is a high-elevation species within the sub-Mediterranean area (Sánchez de Dios *et al.*, 2009).

The onset of latewood formation was however convergent in both species, coinciding with the second flush and the increment of xylem growth rates, which peaked around the time of maximum day-length. Such events, detailed in Chapter III (Fig. 3.1), occurred once leaf unfolding was completed probably because mature leaves are major sources of gibberellins, which foster cell division and fibre differentiation (Aloni, 2015). Furthermore, enhanced carbon uptake in long days could contribute to increase growth, which may account for the association between xylem increment rates and photoperiod in multiple species across biomes (Rossi *et al.*, 2006b; Montserrat-Martí *et al.*, 2009; Prislan *et al.*, 2013; Huang *et al.*, 2014; Cuny *et al.*, 2015). The last formed enlarging cells were observed in October-November, and leaf shedding was completed in November-December, occurring significantly later at the warmest location within the gradient (MED). On average, the growing and leafing periods lasted respectively for 6.5 and 8 months in *Qrob*, but 6 and 6.6 months in *Qpyr*. Such values are longer than those previously observed for temperate deciduous oaks (Zweifel *et al.*, 2006; Michelot *et al.*, 2012), which is probably due to the fact that a longer photoperiod and warmer conditions at the study sites had a lingering effect on growth cessation and leaf senescence (Bauerle *et al.*, 2012; Gill *et al.*, 2015).

Detection of tyloses on dysfunctional vessels augmented with summer drought severity (Fig. 5.6), with 40–60 % of trees at MED showing their earlywood partially blocked by tyloses in July 2012 (drier year). Tyloses in temperate oaks had not been detected by October in previous studies (Cochard & Tyree, 1990).This result may indicate that study oaks underwent a certain level of summer stress. In fact, trees at this site experienced a drought-induced quiescent period in late summer, which was released in October when water soil was recharged. Such behaviour was detected through the application of generalized additive models (Table 3.2, Fig. 3.2), pointing out that deciduous oaks exhibit a plastic trimodal xylogenesis as a response to summer drought. The negative impact of summer drought on xylem production in temperate and Mediterranean biomes is widely documented (Bréda *et al.*, 2006; Montserrat-Martí *et al.*, 2009; Pacheco *et al.*, 2015), but autumn ressumption was considered to be exclusive of Mediterranean species (de Luis *et al.*, 2007; Linares *et al.*, 2009; Battipaglia *et al.*, 2010; Camarero *et al.*, 2010; Vieira *et al.*, 2015). The absence of this pattern for temperate oaks in previous research suggests that low autumn temperatures in cooler climates could prevent cambial reactivation in less frequent dry years (Zweifel *et al.*, 2006; van der Werf *et al.*, 2007; Arzac *et al.*, 2016). Therefore, plastic responses of cambial activity to drought are present in temperate oaks, but they are probably restricted to particular climatic conditions in the temperate-Mediterranean transition.

Connection between phenology, temporal patterns of sapwood NSC content, and growth

Seasonal NSC patterns of studied species were convergent with those reported for other ring-porous oaks (Barbaroux & Bréda, 2002; El Zein *et al.*, 2011; Michelot *et al.*, 2012; Salomón *et al.*, 2016), revealing that xylem structure and leaf habit strongly influence the tree's carbon balance. In this regard, earlywood formation was proved to rely on carbon reserves in deciduous species (Kagawa *et al.*, 2006; Skomarkova *et al.*, 2006). Since the previous year's earlywood vessels are assumed to be dysfunctional at the time of flushing (Cochard & Tyree, 1990; Sperry *et al.*, 1994; Tyree & Cochard, 1996; Taneda & Sperry, 2008), dependence on carbon reserves should be particularly high in ring-porous trees at early stages of wood and leaf development (Barbaroux & Bréda, 2002; Zweifel *et al.*, 2006). This may account for NSC concentrations declining from February to June (Fig. 3.3), which would reflect carbon mobilization in different tree compartments in order to support earlywood formation, crown development, fine root turnover, and flowering.

Minimum NSC content coincided with maximum growth rates in June-July, the time at which carbon replenishment started. Previous studies consistently showed that latewood growth is mostly supported by current photosynthates (Kagawa *et al.*, 2006). Starch concentration increased in summer, probably because the longer photoperiod prompted photosynthetic activity (Bauerle *et al.*, 2012), even though carbon investment in wood peak at that time (Cuny *et al.*, 2015). Such refilling was less pronounced at the most humid site

(ATL), which indeed, lead to a more reduced NSC content in winter. Since photosynthesis is less sensitive to water stress than cambial activity is (Palacio *et al.*, 2007; Lempereur *et al.*, 2015), it could be argued that drought-induced growth stagnation reduced carbon demand in summer at MED. This is in line with studies suggesting that reduced xylem formation under moderate drought is related to physiological limitations of water shortage on cambial activity, rather than to declining carbon uptake (Palacio *et al.*, 2007; Piper, 2011; Delpierre *et al.*, 2015). However, tree-ring series evidenced that xylem production at ATL was also lower than at ATH and MED (Tables 5.1 and 5.2). One feasible explanation is that the lower tree density at the Mediterranean site affected competition levels for resources and growth (Fernández-de-Uña *et al.*, 2016). Strong differences between the two Atlantic locations however suggest that excessively humid conditions at site ATL affected carbohydrate storage. The later leaf-out at this location could be detrimental for carbohydrate assimilation (Morecroft *et al.,* 2003), while probable soil flooding in autumn and winter could exacerbate carbon consumption (Ferner *et al.*, 2012). Furthermore, leaves of *Qpyr* were considerably infested with powdery mildew at ATL, which should affect photosynthetic efficiency and growth (Marçais & Desprez-Loustau, 2012; Martínez-Vilalta, 2014).

Maximum NSC values were attained at the onset of dormancy, probably because reduced carbon demand after growth cessation favoured storage, together with nutrient recycling from senescent leaves (El Zein *et al.*, 2011). As previously mentioned, stored NSC play a key role at the onset growth, providing energy and structural material for the formation of new tissues, and are also necessary to fuel respiration in winter (Edwards & Hanson, 1996; Atkin & Tjoelker, 2003; Salomón *et al.*, 2015). SS concentration in sapwood might also affect the capability of trees to repair freeze-thaw induced embolisms (Améglio *et al.*, 2001), influencing water status before new vessels are formed in spring. In addition, NSC accumulation in autumn was synchronized with a considerable conversion of starch into SS, which is likely associated to cold hardening given that SS play a role in preventing freezing damage (Morin *et al.*, 2007; Repo *et al.*, 2008). Starch hydrolysis is thought to be fostered by low non-freezing temperatures in autumn (Améglio *et al.*, 2004; Begum *et al.*, 2010), although this process is linked the timing of leaf shedding. Thus, early senescence can allow sugar concentrations in the symplast to be higher in winter (Morin *et al.*, 2007; Vitasse *et al.*, 2014). In this regard, *Qpyr* trees showed higher SS-starch ratios than their *Qrob* counterparts, which could be attributed to its earlier senescence indicating a better cold tolerance (Fig. 4.1).

The SEM model in Chapter IV (4.4) conveyed that feedbacks between sapwood NSC concentration and earlywood vessel size affected timing of spring phenology and xylem growth. Oaks showing larger vessels had larger sugar concentrations. Presumably, enhanced hydraulic capacity in trees bearing larger vessels could enhance carbon gain under high evaporative demand (Fichot *et al.*, 2009). Complementarily, formation of larger vessels increases hydraulic vulnerability, whereby sugar concentration might have an adaptive value in terms of hydraulic safety (Johnson *et al.*, 2012). SS concentration appeared to speed up budburst, whilst starch reserves were unrelated to phenology. Xylem was suggested to be responsible for carbon transport in winter (Lacointe *et al.*, 2004), while sugars act as osmolytes generating positive stem pressures (Ewers *et al.*, 2001). Bud development relies on sugar influx from vessels to buds (Bonhomme *et al.*, 2010), whereby increasing SS intake in buds speeds up bud swelling (Maurel *et al.*, 2004). This is related to the fact that water uptake in buds depends on the regulation of osmotic potentials (Améglio *et al.*, 2001). That interaction was accompanied by a positive direct effect of SS concentration on earlywood vessel production, although this association was exclusively observed in *Qpyr*. Therefore, earlywood production appeared to be controlled by sapwood SS concentration only in the more stress-tolerant species. Although the knot of carbon-regulated growth has not been already untied (Dietze *et al.*, 2014), our results underpin that winter SS concentration somewhat reflected earlywood formation. Furthermore, differences between *Qrob* and *Qpyr* point out that feedbacks between carbohydrates and growth capacity probably vary among species adapted to different environmental conditions (Guillemot *et al.*, 2015).

Contrasting impact of phenology on wood anatomical traits

Models provided in Chapter II (Table 2.4, Fig. 2.4) pointed out that earlywood vessel size, which in turn affects the conductive area of the stem, was markedly dependent on the onset of earlywood enlargement in *Qrob*. Earlywood vessels of early-reactivated trees thus attained larger lumen diameters because they exhibited a longer enlargement phase. These results agree with those obtained in temperate conifers, proving the clear linkage between time elapsed for enlargement and conduit lumen area (Anfodillo *et al.*, 2012; Cuny *et al.*, 2014). By contrast, the timing of earlywood enlargement hardly influenced vessel size in *Qpyr.* One feasible explanation is that larger vessels are associated to faster rates of cell expansion in this species, rather than to duration of the enlargement phase, probably as a consequence of the more delayed onset of growth in *Qpyr*, which in fact, exhibited a shorter enlargement period. This mechanism may prevent flushing at the wrong time (Vitasse *et al.*, 2014; Basler &

Körner, 2014), but it further implies that earlywood growth and flushing must be accelerated to avoid detrimental effects of summer drought in the sub-Mediterranean area (Sánchez de Dios *et al.*, 2009; Montserrat-Martí *et al.*, 2009). In light of these results, *Qrob* appears to have a more plastic strategy of xylem adjustment in relation to the expected advancement in spring phenology due to global change (Menzel *et al.*, 2006; Morin *et al.*, 2010). The trade-off between hydraulic capacity and safety suggest that early-resumed trees would form a more efficient hydraulic network at the cost of increasing vulnerability to drought. Hence, effects of warming might be advantageous in humid environments, where large-vessel bearing trees can be more competitive than neighbouring ones, whereas enhanced vulnerability to severe drought could threat hydraulic functioning at drier locations (Wheeler *et al.*, 2005; Hacke *et al.* 2006; Christman *et al.*, 2009; Levanic *et al.*, 2011). On the contrary, the sub-Mediterranean oaks would maintain a more stable hydraulic architecture despite either cooling or warming in spring. One possible explanation is that expansion rates in *Qpyr* are more relevant than duration, because late resumption reduces the time for flushing before the onset of summer drought in the temperate-Mediterranean transition.

The number of cell layers in dormant cambium had a positive effect on the duration and rate of xylem production (Fig. 3.4), which is probably caused by the higher amount of cells capable of undergoing division (Uggla *et al.*, 1996; Gričar *et al.*, 2014). In turn, enhanced xylem increment early in the season delayed growth cessation, which is consistent with observations for conifers by Lupi *et al.* (2010). This latter result emphasize the strong dependence of annual xylem increment on the maximum growth rate (Rossi *et al.*, 2006b; Deslauriers *et al.*, 2009; Delpierre *et al.*, 2015), which were generally attained at the onset of latewood formation. It is relevant that growth was further prompted by growing season length in *Qrob*, whereas this association was not found for *Qpyr*. Fast-growing *Qpyr* trees did presumably not benefited from their longer growing season, which is somewhat consistent with results in Chapter II (Table 2.4, Fig. 2.4) evidencing the reduced impact of phenology on xylem anatomy. Indeed, earlywood anatomical traits, which probably affect growth capacity (Fichot *et al.*, 2009), were reported to be more influential than foliage density on latewood growth, growing season length, or winter NSC (Fig. 4.5).

Xylem adjustment under increasing xeric conditions

Long-term tree ring series revealed that trees at drier locations in the gradient exhibited larger vessel lumina, being this pattern even more pronounced for *Qpyr* (Table 5.2, Fig. 5.2). Indeed, this species showed wider vessels than *Qrob*, indicating the higher vulnerability of *Qpyr* vessels to cavitation despite the more conservative adjustment argued in Chapter II. Although some authors stated that more reduced xylem conduits are formed in oaks as a response to drought (Tardif & Conciatori, 2006; Eilmann *et al.*, 2009; Gallé *et al.*, 2010; Gea-Izquierdo *et al.*, 2012), other studies performed in the Iberian Peninsula revealed either similar or wider vessels following a dry winter (Villar-Salvador *et al.*, 1997; Corcuera *et al.*, 2004). In this regard, large vessel diameter and ring-porosity are traits strongly linked to climate seasonality in temperate areas, where trees must cope with both freezing and drought episodes (Zanne *et al.*, 2006). In the sub-Mediterranean area, formation of wide vessels might be even more relevant, since favourable conditions last for a short period in spring. As it was suggested in Chapter V, enhanced water transport capacity may allow trees to speed up foliage development in dry environments, taking advantage of the more limited favourable period. Resistance to cavitation was thus subordinated to efficiency in order to minimize drought effects on growth. Yet, larger vessels in *Qpyr* than in *Qrob* did not imply more frequent tyloses in early summer for the former species, as we noted in 2012 and 2013. In fact, *Qpyr* was less vulnerable than *Qrob* to irreversible cavitation during the growing season. Resistance to drought was probably related to multiple traits in leaves and root networks present in *Qpyr* (Aranda *et al.*, 1996; Sisó *et al.*, 2001; Corcuera *et al.*, 2002; Salomón *et al.*, 2015, 2016), rather than to vessel size in the stem. Furthermore, although larger vessels could increase the risk of hydraulic failure under extreme drought episodes (Levanic *et al.*, 2011), ring-porous trees were reported to withstand losses up to 88% of total conductivity (Urli *et al.*, 2013). This idea suggests that the latewood vessel network is probably more decisive than earlywood vessels to overcome drought in late summer (Taneda & Sperry, 2008), being the observed earlywood adjustment risky only for those trees lacking latewood. On the other hand, hydraulic costs of summer cavitation may be lower for ring-porous species than for conifers or diffuse-porous species given that the earlywood vessel network has to be entirely renewed after freezing-thaw induced dysfunction (Urli *et al.*, 2013). In addition, abundant axial parenchyma in angiosperms reflects their capability to repair vessel embolism, allowing sugars to be readily transferred into vessels (Johnson *et al.*, 2012). In this regard, a more elevated SS content could be somehow related to earlywood vessel size, given that it was higher in *Qpyr* and at the driest location along the gradient.

According to retrospective tree-ring series, *Qpyr* was less productive (lower earlywood and latewood production) than *Qrob*, despite its more efficient hydraulic network and higher SS content. This was corroborated with band dendrometer information (Fig. 5.5), as well as by the maximum xylem growth rate showed in Chapter III (Fig. 3.1). A longer active period in *Qrob* (Fig. 3.1, Fig. 5.4), together with the stronger relevance of phenology on growth observed in Chapters II (Table 2.4, Fig. 2.4) and III (Fig. 3.4), appears to enhance xylem production. These results suggest that *Qpyr* exhibit strategy which is more focused on carbon saving. It is interesting to note that plants growing under harsh environmental conditions show lower resource turnover than those in more favourable environments (Valladares *et al.*, 2000). Rodríguez-Calcerrada *et al.* (2008) met a similar conclusion after studying responses of seedlings to light, stating that temperate oaks are more competitive than their Mediterranean counterparts. The carbon saving strategy is well documented in plants adapted to harsh environments, where resource acquisition is hindered (Chapin *et al.*, 1993; Valladares *et al.*, 2000; Regier *et al.*, 2009; Piper, 2011). This strategy would be possible if we assumed that storage is an active NSC sink that competes for carbohydrates with growth (Wiley & Helliker, 2012; Dietze *et al.*, 2014). This framework could therefore account for the linkage between SS and earlywood production in *Qpyr*. As discussed in Chapter IV, this more conservative growth strategy entails a high opportunity cost at humid environments (Chapin *et al.*, 1990), which is related to the probable suppression by neighbouring *Qrob* individuals.

The correlative analysis between dendrochronological data and monthly series of temperature and precipitation for the period 1967-2013 revealed that winter conditions drive earlywood formation in ring-porous species (Fonti & García-González, 2004; Fonti *et al.*, 2007; Matisons & Brumelis, 2012; González-González *et al.*, 2014). Warm and rainy weather during dormancy and quiescence negatively affected vessel expansion and production. High winter temperature may impair chilling requirements to be fulfilled (Cooke *et al.*, 2012; Basler & Körner, 2014), being this effect more probable for *Qrob* due to the stronger association between phenology and xylem structure. But high temperature could also limit starch breakdown, affecting SS concentration in overwintering tissues (Améglio *et al.*, 2004; Morin *et al.*, 2007). In line with the interaction between SS and earlywood vessel production held for *Qpyr*, reduced starch-to-sugar conversion could represent a limiting factor for earlywood growth in this species. Indeed, Deslauriers *et al.* (2009) reported higher growth in trees showing a larger amount of sugars in the outermost sapwood segment. Starch mobilization at the onset of the growing season may assure cell division and subsequent differentiation (Améglio *et al.*, 2001; El Zein *et al.*, 2011). Yet, this idea is opposed to the scarce effect of starch levels on earlywood production observed in Chapter IV (Fig. 4.4). High overwintering sugar levels may somehow foster developmental processes, by enhancing the supply of energy, materials, or water to growing tissues. In addition, high precipitation could boost carbon consumption associated to anoxic stress (Rozas & García-González, 2012b), detracting sugars for maintenance respiration, osmotic purposes, and early season growth.

Dry conditions in late spring and early summer constrained the enlargement of late-formed vessels and latewood in both study species, even at the most humid location. Adverse conditions in spring were found to limit expansion of last-formed vessels (González-González *et al.*, 2014), while detrimental effects of summer drought on radial growth were widely documented under temperate and Mediterranean climate conditions (Corcuera *et al.*, 2004; Montserrat-Martí *et al.*, 2009; Eilmann *et al.*, 2009; Granda *et al.*, 2013; Rozas *et al.*, 2015). Similarly to other deciduous trees (Rozas *et al.*, 2015), the highest correlation scores in the study oaks were found at the time of maximum growth rate, which is consistent with the relevance of early summer growth rate in predicting annual xylem increment (Fig. 3.4). These observations hint that late summer quiescence had little impact on overall xylem production, which is consistent with the larger xylem increment recorded at the driest locations. At the most humid site ATL, a significant detrimental effect of cold weather in autumn was observed, probably related to the shortening of the growing season. In fact, this effect was stronger for *Qrob*, presumably due to the connection between growing season duration and xylem production in this species (Fig. 3.4). This is also consistent with the notably earlier growth cessation observed during the cold year at the ATL location.

Vulnerability of ring-porous oaks under future climatic scenarios

The advancement of spring phenology predicted in future decades (Linderholm, 2006; Menzel *et al.*, 2006; Rossi *et al.*, 2011) could lead *Qrob* to become more vulnerable to drought-induced embolisms through enhanced vessel expansion, especially in those stands at the rear edge of this species. This may be aggravated by the fact that earlier leaf-out could speed up water soil depletion through a longer period of elevated evapotranspiration (Fischer *et al.*, 2007). In contrast, the risk of xylem maladjustment to drought as a consequence of an earlier reactivation may be reduced for *Qpyr*. Yet, both study oaks prioritized efficiency over safety as a response to increasing xeric conditions, likely to ensure leafing and flowering before the arrival of drought stress. Larger but less numerous earlywood vesssels in *Qpyr*, which were likely a consequence of late resumption, would increase vulnerability to drought-induced cavitation (Wheeler *et al.*, 2005; Hacke *et al.*, 2006; Brodersen & McElrone, 2013; Delzon &

Cochard, 2014). But although this xylem structure could jeopardize hydraulic functioning under extreme drought (Levanic *et al.*, 2011; Anderegg *et al.*, 2014), the carbon saving strategy found in *Qpyr* may contribute to offset hydraulic dysfunction through vessel repair, or growth reiteration (Brodribb *et al.*, 2010; Johnson *et al.*, 2012). Therefore, even if low growth is likely detrimental for *Qpyr* trees coexisting with *Qrob* under favourable conditions, it could be beneficial to cope with a rapid environmental change. This physiological response, together with several leaf traits minimizing water loss in *Qpyr* (Aranda *et al.*, 1996; Sisó *et al.*, 2001; Corcuera *et al.*, 2002), conveys a higher drought tolerance to drought despite the more vulnerable hydraulic network in the stem.

Growth is widely assumed to be reduced as a consequence of global warming (Di Filippo *et al.*, 2010; Cuny *et al.*, 2015). In the face of this challenge, it is recognized that lengthening of the growing season, together with the plastic behaviour of cambial activity, is assumed to offset negative impacts of rapid environmental change on xylogenesis (Balducci *et al.*, 2016). Nevertheless, the advancement of drought could compromise the effectiveness of such compensatory mechanisms, given the outstanding influence of pre-summer climate conditions on xylem growth, water availability, and carbon uptake in sub-Mediterranean oaks (Hernández-Santana *et al.*, 2008; Granda *et al.*, 2013). It is noteworthy that increasing warm conditions in winter may constrain vessel enlargement, at least at low elevation, reducing cavitation risk in summer. However, reduced hydraulic capacity may slow down foliage development, increasing vulnerability to late-frost damage, as well as to sporadic drought in spring.

Distributional ranges of ring-porous oaks were predicted to become considerably reduced in the next decades in the Iberian Peninsula (Sánchez de Dios *et al.*, 2009; Benito Garzón *et al.*, 2009). This would be also applicable to the drought-tolerant *Qpyr*, which was reported to have declining growth at low elevation stands in Spain (Gea-Izquierdo & Cañellas, 2014). Although data gathered in this Thesis do not allow me to confirm this statement, further evidences of the vulnerability of ring-porous species to extreme events, particularly those hindering growth in spring, are now provided. Nevertheless, mutual interactions among plastic phenology, carbon storage, and wood structure reveal compensatory mechanisms in oaks, offsetting enhanced climatic stress in future decades.

CHAPTER VII

Conclusions

(i) Findings presented in this Thesis evidenced a strong thermal regulation of both budburst and the onset of xylogenesis, which are coordinated at the whole-tree level. Warm conditions in late winter hasten dormancy release in both species, although the higher cold tolerance of *Quercus pyrenaica* was reflected by its later cambial resumption. Rising xeric conditions increase the complexity of wood formation dynamics in the temperate-Mediterranean transition, as demonstrated by the autumn cambial resumption, which was registered in study species for the first time.

(ii) The study species notably differ on carbohydrate allocation to xylem growth, which are in tune with their specific stress tolerance. Yet, both species show convergent seasonal dynamics of sapwood NSC content, with winter reserves supporting earlywood formation, and current assimilates concurrently fuelling latewood growth and NSC refilling.

(iii) The interplay between phenology and wood structure is dependent on the species. Whereas a lengthening of earlywood growth implies formation of wider vessels in *Q. robur*, earlywood vessel diameter in *Q. pyrenaica* probably relies on the rate rather than on duration of earlywood enlargement. The number of cambial cells at dormancy fosters growth capacity, allowing for a longer active period, which is a relevant growth predictor in *Q. robur*.

(iv) High temperatures during the dormant and quiescent periods play a central role in earlywood vessel expansion and cell proliferation, suggesting a probable meditation by the timing of growth resumption and soluble sugar content. Low water availability in late spring and summer is the main constrain for xylem production, although mild autumn conditions probably promote latewood formation at the most humid location.

(v) The study oaks seem to prioritize hydraulic efficiency over safety under increasing xeric conditions, being concurrent with an increment of stored carbohydrates. Feedbacks between the xylem network and carbohydrate content are stronger for *Q. pyrenaica*, which appeared to have a carbon saving strategy to face eventual harsh climatic episodes, commonly occurring in the sub-Mediterranean area. A lower frequency of tyloses also indicated a higher drought resistance in *Q. pyrenaica* than in *Q. robur*.

(vi) Although ring-porous oaks are vulnerable to extreme events impairing earlywood and foliage development in spring, the effects of plastic phenology on carbon storage and wood structure may contribute to offset the increasing climatic stress expected in future decades, especially for *Q. pyrenaica*.

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Appendix

Resumen

Efectos del Clima en la formación de madera de *Quercus robur* L. y *Quercus pyrenaica* Willd. a lo largo de un gradiente de mediterraneidad en Galicia: un análisis integrado mediante fenología, anatomía y dendroecología

Las plantas leñosas tienen la capacidad de crecer a lo largo de toda su existencia. El cambium vascular es el responsable de originar el crecimiento secundario, dando lugar al floema y el xilema. Este último tejido es a través del cual tiene lugar el transporte de agua y nutrientes desde las raíces hasta las hojas, siendo también responsable de dar soporte mecánico a la copa y, junto a otros tejidos, de almacenar reservas de carbono. En la mayoría de las angiospermas, la conducción de agua y nutrientes en el xilema se produce a través de unas células especializadas llamadas vasos conductores. El fallo hidráulico es el principal mecanismo de mortalidad en árboles como consecuencia de la falta de agua. Esto ocurre como consecuencia de la cavitación de los vasos cuando los potenciales hídricos descienden en condiciones de sequía. Asimismo, los vasos pueden cavitar en invierno como consecuencia de los ciclos de hielo-deshielo en el interior de los mismos. No obstante, las plantas tienen mecanismos para contener la pérdida de capacidad conductiva como la regulación estomática. Por otro lado, las células de parénquima del xilema proporcionan azúcares necesarios para la restauración de los vasos, una vez que estos se vuelven disfuncionales, mediante la regulación de los potenciales osmóticos en el flujo de savia. Sin embargo, aún se desconoce la importancia relativa de la resistencia a la cavitación y los mecanismos de recuperación en el xilema. Por ejemplo, en algunas angiospermas los vasos formados en primavera son mucho mayores que aquellos formados en verano, lo cual estaría relacionado con el hábito caducifolio y la adecuación a la estacionalidad climática de estas especies denominadas de anillo poroso. Los vasos de gran tamaño son muy eficientes en la conducción de agua, pero al mismo tiempo son más vulnerables a la cavitación. Es por ello que un aumento en la duración y severidad de la sequía como consecuencia del cambio climático podría resultar crítico para la supervivencia de las especies de anillo poroso.

La formación de xilema tiene lugar de manera cíclica, en estrecha relación con determinados factores ambientales que dan lugar a una gran variabilidad espacial y temporal en las fases ontogenéticas. Esto es debido a la plasticidad de la fenología, lo cual permite a las

plantas enfrentar la variabilidad climática. Algunas especies reducen su exposición al daño por helada tardía, retrasando el inicio del crecimiento gracias a la imposición de ciertos umbrales en su sensibilidad a la temperatura y el fotoperíodo. De la misma manera, el crecimiento y la fotosíntesis son sensibles a la disponibilidad de agua en verano, lo que permite mantener respectivamente los niveles de reservas de carbono y la integridad hidráulica en ambientes con marcada sequía estival. En especies caducifolias de anillo poroso, los grandes vasos de madera temprana son probablemente funcionales durante una sola estación de crecimiento, mientras que la madera tardía se mantiene funcional durante varios años. Se sospecha que la formación de la copa depende notablemente de la formación previa de madera al inicio de cada estación debido a que se necesitan vasos de primavera funcionales durante la brotación. Esta característica también implica que el inicio del crecimiento radial depende en las reservas almacenadas durante la estación anterior. Sin embargo nuestro conocimiento sobre la relación entre la formación de la copa, la xilogénesis, y las reservas de carbono en especies de anillo poroso es todavía escaso. Esto resulta sorprendente si tenemos en cuenta que el análisis de las reservas de carbono y su posible efecto sobre el crecimiento en condiciones de estrés ambiental ha atraído la atención de una amplia comunidad de investigadores en los últimos años.

Los efectos del clima sobre el crecimiento secundario quedan reflejados en los anillos de los árboles, por medio de diferentes características anatómicas como la cantidad de madera formada, el área conductora, o la composición isotópica. De esta manera, el estudio de las series de anillos nos permite acceder a una gran cantidad de información ambiental que se encuentra almacenada en su estructura. En los últimos años, el estudio del área transversal de los vasos de primavera ha sido objeto de un notable avance en el ámbito de la dendrocronología gracias a la aparición de herramientas automáticas para el análisis de imagen. En el caso de las especies de anillo poroso del sur de Europa, se ha observado un efecto recurrente de las condiciones del invierno previo sobre las características de los elementos conductores de primavera, así como de las condiciones de verano sobre la producción anual de madera. Sin embargo, el control que ejerce el clima sobre la anatomía y la fisiología de los árboles es notablemente complejo, por lo que la integración de datos fisiológicos como el contenido de carbohidratos no estructurales, los patrones fenológicos en diferentes órganos de la planta y las series de crecimiento es crucial para poder interpretar de forma sistemática la respuesta de estos parámetros anatómicos al clima. La identificación de los factores climáticos de mayor importancia sobre el crecimiento sería de gran utilidad para evaluar la vulnerabilidad de los ecosistemas forestales templados y Mediterráneos al estrés ambiental.

Algunas de las especies de anillo poroso de mayor relevancia ecológica pertenecen al género *Quercus*. Algunas de ellas, como *Quercus robur*, se distribuyen por toda Europa y el Oeste de Asia, ocupando zonas de clima templado oceánico. Sin embargo, en áreas de clima Mediterráneo los robles esclerófilos de poro difuso, como la encina y el alcornoque, sustituyen a los robles templados. En la transición entre el clima oceánico y el Mediterráneo aparecen los robles marcescentes de anillo poroso, como *Q. pyrenaica*, cuyo rango de distribución se restringe al suroeste de Francia y zonas montañosas de la Península Ibérica y Marruecos. *Q. pyrenaica* es una especie más tolerante que *Q. robur* al frío en inverno y la sequía en verano, a pesar de lo cual ambas especies coexisten en zonas del norte de la Península Ibérica. No obstante, el cambio climático podría alterar la dominancia relativa de estas especies en aquellos lugares donde cohabitan, desplazando la especie más tolerante a la sequía a aquella de clima templado.

Esta Tesis de Doctorado combina el análisis de la fenología foliar, la xilogénesis, las reservas de carbono y las series de anillos de crecimiento con el objetivo de contribuir en el conocimiento de los factores ambientales que regulan la fenología en robles de anillo poroso (*Q. robur* y *Q. pyrenaica*) y las consecuencias funcionales que esta interacción tiene en la anatomía y la utilización de las reservas de carbono. Por otro lado, este trabajo pretende averiguar si la fenología, la estructura del xilema y los niveles de reservas de carbono reflejan las estrategias de tolerancia frente al estrés desarrolladas por *Q. robur* y *Q. pyrenaica* en la transición entre el ambiente templado y el Mediterráneo. En este sentido se espera que *Q. pyrenaica* exhiba un periodo de crecimiento más corto y presente un sistema hidráulico menos vulnerable a la sequía que *Q. robur*. Asimismo, se espera que *Q. pyrenaica* tenga una estrategia de uso de carbono más conservadora que *Q. robur* como respuesta al estrés ambiental. En consecuencia, *Q. robur* podría verse desplazado por *Q. pyrenaica* si los efectos del cambio climático avanzan en décadas futuras en aquellas áreas en las que ambas especies coexisten.

De acuerdo con los objetivos de esta investigación se llevaron a cabo tres líneas de estudio: la dinámica estacional de crecimiento primario y secundario y su relación con la productividad, el contenido de reservas de carbono y sus efectos sobre el crecimiento y el estudio dendrocronológico de la información climática contenida en las series de anillos del xilema. Seleccionamos tres localidades en Galicia (España) a lo largo de un gradiente ambiental con grado de mediterraneidad variable, en las cuales las dos especies de estudio estaban presentes. Se hizo un seguimiento de la fenología foliar y cambial con frecuencia semanal durante los años 2012 y 2013, para lo cual se seleccionaron diez individuos por especie, localidad y año. Las muestras de madera se obtuvieron con un dispositivo Trephor, siendo estas procesadas en laboratorio para poder obtener secciones microscópicas de madera. Con la observación de los cortes se pudieron registrar diferentes eventos, así como medir diferentes parámetros anatómicos entre los que se encuentra el área y el número de vasos de primavera, así como la anchura de madera tardía. El área de los vasos se utilizó para calcular el diámetro hidráulico en el último anillo, siendo este un parámetro que refleja la capacidad conductiva de cada individuo. El estudio del contenido invernal de reservas de carbono en el tronco se realizó en cuarenta individuos por especie y localidad. Para ello, se utilizó una barrena de pressler con la que obtener muestras de albura, a partir de las cuales se determinó la concentración de azúcares solubles e insolubles usando el método de la antrona. También se hizo un seguimiento bimensual de la dinámica estacional de las reservas de carbono en la albura entre febrero de 2012 y febrero de 2013. Para el estudio dendrocronológico se tomaron dos muestras de al menos veinte individuos por especie y localidad, utilizando una barrena de pressler. Los testigos de madera se cortaron y pulieron en sección transversal para poder distinguir los anillos de crecimiento y el contorno de los vasos en la lupa. La medición y sincronización de las series de anchura de los anillos y tamaño de los vasos permitió estudiar de manera retrospectiva los factores climáticos que modulan la formación de madera.

El inicio de la estación de crecimiento fue registrado en el momento en el que comenzaron las divisiones celulares en la zona cambial. El inicio de la formación de los vasos de primavera se adelantó cuando las condiciones fueron más cálidas, mientras que el inicio de las divisiones cambiales fue más estable en cada especie, siendo posiblemente más sensible al fotoperiodo que a la temperatura. Los resultados obtenidos indicaron que *Q. pyrenaica* tiene una reactivación del crecimiento primario y secundario más tardía que *Q. robur*, que presentó un menor requerimiento térmico para la rotura de la dormición. El retraso del inicio del crecimiento permitiría a *Q. pyrenaica* evitar los daños por heladas durante la primavera, lo que es consistente con la abundancia de esta especie en áreas montañosas de la Península Ibérica. Sin embargo, esto provocó que el período de formación de madera temprana fuese más corto en *Q. pyrenaica* que en *Q. robur*. A pesar de las diferencias en la ocurrencia de las fases fenológicas, el inicio de la brotación coincidió con la maduración de los primeros vasos en las dos especies estudiadas, evidenciando que el inicio de la formación de la madera temprana depende de las reservas de carbono acumuladas durante la estación de crecimiento anterior. El diámetro hidráulico en *Q. robur* aumentó con la duración del período de formación de madera temprana, mientras que en *Q. pyrenaica* esta relación no resultó significativa. De hecho, la fecha de inicio de formación de madera temprana influyó sobre el diámetro hidráulico únicamente en *Q. robur*. Este resultado sugiere que *Q. robur* ha desarrollado una estrategia de formación de madera temprana más plástica que *Q. pyrenaica*, lo que le permitiría formar un sistema vascular más eficiente en el caso que un hipotético aumento de la temperatura adelantase la reactivación en primavera. No obstante, una menor dependencia del tamaño de vaso en la fenología permitiría a *Q. pyrenaica* evitar incrementar excesivamente su vulnerabilidad frente a la cavitación en zonas cálidas y secas, así como garantizar un cierto umbral de eficiencia en años o lugares en los que las condiciones ambientales pasan rápidamente del frío a la sequía.

El inicio de la formación de madera tardía ocurrió alrededor del solsticio de verano, coincidiendo con una segunda brotación de pequeña entidad. Esta fase fue mucho más sincrónica entre especies y localidades que las correspondientes a la madera temprana. En los árboles que formaron leño tardío, la tasa máxima de formación de madera se alcanzó precisamente en ese momento, lo cual es coincidente con los estudios previos realizados en zonas templadas tanto para coníferas como para frondosas. Uno de los resultados más notables de esta investigación fue el hecho de que en la localidad más seca numerosos árboles retomaron la formación de madera en Octubre, después de sufrir un período de quiescencia en septiembre debido a la sequía. Esto ocurrió especialmente en 2012, el año de estudio más seco, en el que los modelos reflejaron que la probabilidad de observar tejido en crecimiento durante el otoño era mayor que al final del verano. Este resultado demuestra que la actividad cambial presenta una respuesta plástica a los cambios en la disponibilidad hídrica no solo en especies Mediterráneas, sino también en robles de áreas templadas o de transición entre el clima templado y el Mediterráneo. Por otro lado, el cese de la actividad cambial se adelantó en la localidad más húmeda, especialmente en *Q. pyrenaica*.

Las reservas de carbohidratos no estructurales contenidas en la albura siguieron una dinámica anual semejante en ambas especies, probablemente debido a la coincidencia de la estructura anatómica y el hábito foliar. El almidón y los azúcares solubles acumulados en la estación previa descendieron notablemente entre febrero y junio, incluso después de que las hojas estuviesen totalmente desarrolladas. Estos resultados confirman la dependencia de la madera temprana en las reservas de carbono. En verano se observó una acumulación de almidón concurrente con la formación de madera tardía. El contenido de almidón descendió al transformarse en azúcares solubles durante el otoño hasta la llegada de la dormición. Esta acumulación comenzó con anterioridad en la localidad más seca, lo que probablemente está relacionado con el cese del crecimiento en respuesta a la sequía estival. Una concentración alta de azúcares en la albura mejoraría la regulación osmótica y facilitaría la recuperación de vasos disfuncionales. El contenido total de carbohidratos así como el de azúcares solubles alcanzó su máximo valor al inicio de la dormición. El mantenimiento de niveles altos de azúcares solubles en invierno tiene probablemente un alto valor adaptativo en estas especies dado el rol que juegan estas substancias en la protección frente al daño por helada, como fuente de energía metabólica durante la dormición, o como fuente de materiales y energía durante la reactivación en primavera. En este sentido, nuestros resultados indicaron la existencia de una relación positiva entre la concentración de azúcares en la albura al inicio de la dormición y el adelanto de la brotación. En *Q. pyrenaica* la concentración de azúcares también ejerció un efecto directo sobre la producción de madera temprana durante la primavera. De acuerdo con estos resultados, la concentración de azúcares podría haber influido en la capacidad de recuperar la conductividad de la madera tardía después de eventos de helada, facilitando el proceso de hinchamiento de las yemas al mejorar el aporte de agua y azúcares. Probablemente, la relación más intensa entre nivel de azúcares solubles y formación de madera observado en *Q. pyrenaica* refleja la existencia de una estrategia de crecimiento más conservadora, encaminada a evitar un consumo excesivo de recursos. Este resultado se vio reflejado en el menor crecimiento radial observado en esta especie tanto en las secciones de madera, como en los datos procedentes de dendrómetros durante los años 2012 y 2013. De hecho, *Q. pyrenaica* mostró una mayor proporción de azúcares solubles frente almidón que *Q. robur*, lo que probablemente está relacionado con la mayor tolerancia de la primera a eventos de sequía en verano y bajas temperaturas en invierno.

El número de células en la zona cambial durante la dormición, que es una característica ligada al vigor del árbol, fue un factor determinante para la formación de madera y la fenología. De esta manera, los árboles con mayor número de células cambiales al inicio de la estación estaban predispuestos a generar más tejido en el momento de máximo crecimiento anual que aquellos que presentaban menos células. A su vez, este hecho contribuyó en el alargamiento de la estación de crecimiento. En *Q. robur* ambos factores, duración y tasa máxima de crecimiento, influyeron en el incremento anual de madera, mientras que en *Q. pyrenaica* únicamente la tasa máxima de crecimiento contribuyó de manera significativa. Este resultado confirma que la asociación entre fenología y anatomía es más débil en *Q. pyrenaica*, lo cual podría ser una consecuencia de su limitado período de crecimiento. En todo caso, nuestros resultados sugieren que la movilización de carbohidratos no estructurales para el crecimiento está modulada en ambas especies por parámetros estructurales como el número de células cambiales.

Las series de anillos para el período 1980-2013 indicaron que tamaño de los vasos en ambas especies aumentó en el sitio más seco con respecto a los más húmedos, siendo este patrón algo más pronunciado en *Q. pyrenaica*. Este resultado, unido a que las mayores concentraciones de carbohidratos fueron observadas precisamente en el sitio más seco, parece indicar que los robles subordinaron la resistencia a la cavitación a la eficiencia hidráulica en condiciones de mayor estrés hídrico, lo que probablemente compensaron aumentando el contenido de azúcares. Esto podría atribuirse, al menos en *Q. robur*, al hecho de que la localidad con mayor sequía estival es también la que tiene el inicio de la estación de crecimiento más adelantado. En el caso de *Q. pyrenaica*, el aumento del tamaño de vaso podría atribuirse al aumento del ritmo de expansión con la temperatura, más que a un incremento de la duración de la formación de madera temprana. En zonas de clima Mediterráneo los eventos de sequía son más precoces que en ambientes templados, por lo que el período favorable, en el cual la copa puede desarrollarse, se encontraría más limitado. Este proceso podría acelerarse mediante una mejora en el suministro de agua a las células en expansión y una mayor fijación de carbono si la eficiencia fuese aumentada con el tamaño de vaso. El notable incremento de las tilosis en los vasos de primavera entre agosto y octubre, especialmente en la localidad más seca y para *Q. robur*, sugiere que el mantenimiento integro del sistema de vasos de primavera puede no ser necesario para la supervivencia en verano. Esta idea sería compatible con la estrategia de mejora de la eficiencia en primavera.

El análisis correlativo entre las series de anillos y los datos interpolados de temperatura y precipitación para el período 1967-2013 confirmó la gran influencia de las condiciones de invierno en la formación de madera temprana en especies de anillo poroso. Temperaturas altas y precipitación abundante durante el período de dormición afectó negativamente la expansión de los vasos de primera fila, así como la producción de madera temprana. Es sabido que la existencia de altas temperaturas en inverno puede retrasar la rotura de la dormición interna. Simultáneamente, la concentración de azúcares podría verse afectada por las altas temperaturas debido a una reducida conversión de almidón en azúcares al inicio de la dormición y un aumento en el consumo asociado a la respiración. Esto podría suponer una limitación funcional para el árbol dado el papel central que desempeñan los azúcares solubles tanto a nivel hidráulico como metabólico. En este sentido, el exceso de precipitación podría asimismo afectar al balance de carbono debido al estrés generado por falta de oxígeno en los suelos encharcados. Sin embargo, el ascenso de las temperaturas durante la propia expansión de vasos, favoreció la formación de vasos más grandes. Por otra parte, la falta de agua y no el exceso es lo que limitó la expansión de vasos al final de la primavera, incluso en la localidad más húmeda. Esto indica la existencia de pulsos de sequía en primavera en la zona de estudio. La escasez de agua fue, de hecho, el principal factor limitante de la producción de madera tardía, lo que corrobora los resultados obtenidos en una gran cantidad de estudios desarrollados en zonas templadas y Mediterráneas. Las correlaciones más altas fueron registradas durante el inicio de formación de la madera tardía, lo cual es consistente con el registro de la mayor tasa anual de producción de madera justo en ese período. Además, se observó un efecto negativo del descenso de la temperatura y el aumento de la precipitación en septiembre y octubre, lo cual podría estar asociado con un adelanto del cese del crecimiento, como así se observó en esa localidad durante el 2013 (año húmedo).

En resumen, la información obtenida en esta Tesis de Doctorado permite arrojar luz sobre las relaciones funcionales entre la fenología, la producción y estructura del xilema, y el contenido de carbohidratos, así como sobre los mecanismos que subyacen a dichas relaciones. Además, esta investigación supone un avance significativo en el conocimiento de la capacidad de los robles de anillo poroso para enfrentar el aumento de estrés abiótico en el contexto del calentamiento climático. En este sentido, nuestros resultados sugieren que *Q. pyrenaica* está mejor adaptado que *Q. robur* a un eventual aumento de los eventos extremos de sequía en verano, sin embargo, ambas especies estarían limitadas en el caso que los eventos de sequía se adelantase a la primavera. En tal caso, *Q. pyrenaica* se vería especialmente afectado al tener una arquitectura hidráulica más vulnerable en la madera temprana. Además, en sitios con alta disponibilidad hídrica, la mayor plasticidad fenológica y alta productividad permitiría a *Q. robur* ser más competitivo que *Q. pyrenaica*.

Resumo

Efeitos do Clima na formação de madeira de *Quercus robur* L. e *Quercus pyrenaica* Willd. ao longo de um gradiente de mediterraneidade na Galiza: uma análise integrada mediante fenologia, anatomia e dendroecologia.

As plantas lenhosas têm a capacidade de crescer em toda a sua existência. O crescimento secundário tem a sua origem no cambium vascular, resultando em floema e xilema. O último destes tecidos é através do que tem lugar o transporte de água e nutrientes desde as raízes para as folhas, sendo também responsável de lhe proporcionar suporte mecânico ao dossel arbóreo, e de permitir o armazenamento das reservas de carbono. Na maioria das angiospermas, a água e os nutrientes são levados pelo xilema através de células especializadas chamadas vasos condutores. A falência hidráulica é o principal mecanismo de mortalidade em árvores como consequência da falta de água. Isto acontece quando os potenciais hídricos descendem em condições de seca, o que produz a cavitação dos vasos. Além disso, os vasos podem sofrer a cavitação no inverno como consequência dos ciclos de congelamento-descongelamento que têm lugar no interior deles próprios. No entanto, as plantas têm mecanismos para conterem a perda de condutividade como é a regulação estomática. Por outro lado, as células de parênquima fornecem açúcares solúveis necessários para a restauração da condutividade através da regulação dos potenciais osmóticos no fluxo de seiva depois da cavitação. Porem, ainda se desconhece a importância relativa dos mecanismos de resistência à cavitação e de recuperação no xilema. Por exemplo, em algumas angiospermas, os vasos formados na primavera são muito maiores do que os formados no verão, o que estaria relacionado com o hábito caducifólio e a adequação à sazonalidade climática de estas espécies chamadas de anel poroso. Os vasos de grande tamanho são muito eficientes na condução de água, mas também são mais vulneráveis à cavitação. É por isso que o aumento da seca como consequência da mudança climática poderia resultar critico para a sobrevivência das espécies de anel poroso .

A formação de xilema acontece ciclicamente, em relação estreita com determinados fatores ambientais que produzem uma grande variabilidade espacial e temporal na ocorrência das fases ontogenéticas. Isto é devido à plasticidade da fenologia, o que permite que a variabilidade do clima possa ser enfrentada pelas plantas. Algumas espécies reduzem a sua exposição aos danos causados pelas geadas tardias, graças a que retardam o início do

crescimento por meio da imposição de certos limites na sua sensibilidade à temperatura e o fotoperíodo. Do mesmo jeito, o crescimento e mais a fotossíntese são sensíveis à disponibilidade de água, o que permite manter, respectivamente, os níveis de carbono e a integridade hidráulica em ambientes com forte seca estival. Em espécies decíduas de anel poroso, os grandes vasos de madeira inicial são possivelmente funcionais apenas uma estação de crescimento, enquanto que a madeira de verão pode ser funcional durante vários anos. Supõe-se que a formação da copa depende da formação de certa quantidade de vasos com anterioridade devido a que os vasos de primavera começam a ser funcionais durante o brotamento. Esta característica sugere também que o início do crescimento radial depende das reservas armazenadas durante a estação anterior. Contudo, a nossa compreensão da relação entre a formação da copa, a xilogénese, e as reservas de carbono na espécie de anel poroso é ainda muito escassa. Isto causa estranheza quanto que a análise de das reservas carbono e seu possível efeito sobre o crescimento em condições de estresse ambiental tem atraído a atenção de uma ampla comunidade de investigadores nos últimos anos.

Os anéis das árvores refletem o efeito do clima sobre o crescimento secundário através de diferentes características anatômicas, como a quantidade de madeira formada, a área condutora ou a composição isotópica. Assim, o estudo das séries de anéis dá-nos acesso a uma grande quantidade de informação sobre o ambiente que se encontra armazenada na sua estrutura. Graças ao aperfeiçoamento das ferramentas de análise automatizada de imagem, tem-se observado nos últimos anos um progresso notável no estudo dos vasos de primavera no contexto da dendrocronologia. Em espécies de anel poroso do sul da Europa, recentes estudos verificaram um forte efeito das condições do inverno anterior sobre as características dos elementos condutores, mas também das condições de verão sobre a produção anual de madeira. No entanto, o controle exercido pelo clima na anatomia e fisiologia das árvores é extremamente complexo, de maneira que a integração de dados fisiológicos como os carboidratos não estruturais, os padrões fenológicos em diferentes órgãos da planta ou as séries de anéis de crescimento é crucial para interpretarmos a resposta dos parâmetros anatômicos ao clima. Ademais, a identificação dos fatores climáticos a limitar o crescimento seria útil para avaliarmos com sucesso a vulnerabilidade dos ecossistemas florestais temperados e Mediterrâneos ao estresse ambiental.

Ao gênero *Quercus* pertencem algumas das espécies de anel poroso de maior relevância ecológica. Por exemplo, *Quercus robur* encontra-se na Europa e Oeste da Ásia, ocupando áreas de clima temperado oceânico. Na área Mediterrânea espécies esclerofilas, como os azinheiros ou os sobreiros, substituem aos carvalhos de anel poroso. Porem, a transição entre o clima temperado e o Mediterrâneo é dominada por carvalhos marcescentes de anel poroso, como é o *Q. pyrenaica*, cuja área de distribuição é restrita ao sudoeste da França e áreas de montanha da Península Ibérica e Marrocos. *Q. pyrenaica* é mais tolerante ao frio no inverno e seca no verão do que *Q. robur*, embora ambas as espécies coexistam em áreas do norte da espécie da Península Ibérica. No entanto, o aquecimento global poderia produzir alterações na dominância relativa destas espécies em florestas onde elas coabitam, com um retrocesso da espécie temperada em favor da mais Mediterrânea.

Esta tese de Doutoramento combina a análise da fenologia foliar e da xilogénese, o contido de carboidratos não estruturais e as séries de anéis de crescimento, a fim de contribuir na compreensão dos fatores ambientais que regulam a fenologia nos carvalhos de anel poroso (*Q. robur* e *Q. pyrenaica*) e as consequências funcionais que esta interação tem sobre a anatomia e o uso das reservas de carbono. Além disso, este trabalho tem o objetivo de esclarecer se as estratégias de tolerância ao estresse na transição entre o ambiente temperado e o Mediterrâneo são refletidas na fenologia, na estrutura do xilema ou no estado das reservas de carboidratos de *Q. robur* e *Q. pyrenaica*. A este respeito, espera-se crescimento mais reduzido e um sistema hidráulico menos vulnerável em *Q. pyrenaica* do que *Q. robur*. Espera-se também uma estratégia de uso de carbono mais conservadora em *Q. pyrenaica* do que em *Q. robur* em resposta ao estresse ambiental. Em consequência, *Q. robur* poderia ser substituído por *Q. pyrenaica* se progredirem nas próximas décadas os efeitos do aquecimento global em aquelas áreas onde ambas as espécies coabitam.

Em conformidade com os objetivos de esta investigação executaram-se três linhas de estudo: a dinâmica sazonal de crescimento primário e secundário e a sua relação com a produtividade, o conteúdo de carboidratos não estruturais e os seus efeitos sobre o crescimento e o estudo dendrocronológico da informação climática contida nas séries de anéis do xilema. Na Galiza (Espanha), foram selecionados três sítios ao longo de um gradiente de Mediterraneidade nos que as duas espécies de estudo estavam presentes. Analisou-se a fenologia foliar e cambial com frequência semanal durante os anos 2012 e 2013, para o que dez indivíduos por espécie, localidade e ano foram selecionados. As amostras de madeira foram extraídas com um dispositivo Trephor, para depois serem processadas no laboratório até a obtenção das secções microscópicas de madeira. Com a observação dos cortes puderam-se registrar diferentes eventos e medir vários parâmetros anatômicos entre os que se encontram a área e o número de vasos de primavera, bem como o incremento da madeira de verão. A área dos vasos foi empregada para calcular o diâmetro hidráulico no ultimo anel, devido a que este é um parâmetro que reflete a capacidade condutiva de cada individuo. O estudo do conteúdo invernal de reservas de carbono no tronco foi realizado em quarenta indivíduos por espécie e sítio. Empregou-se uma verruma de pressler para extrair amostras de alburno, as concentrações de açúcares solúveis e insolúveis (amido) foram obtidas com o método da antrona. Para além disso, fez-se um seguimento bimensal da dinâmica sazonal das reservas de carbono no alburno entre Fevereiro de 2012 e Fevereiro de 2013. Duas amostras num mínimo de vinte indivíduos foram tiradas por espécie e sítio com uma verruma pressler com o objetivo de fazer o estudo dendrocronológico. As amostras de madeira foram cortadas e polidas em seção transversal para os anéis de crescimento e os contornos dos vasos serem distinguidos na lupa. A medição e sincronização das series de incrementos de anéis e de tamanho dos vasos permitiu estudar de maneira retrospectiva os fatores climáticos que modulam a formação da madeira.

O início da estação de crescimento foi registado no tempo em que as divisões celulares começaram na zona cambial. O começo da formação dos vasos de primavera adiantou-se sob as condições mais cálidas, enquanto que o começo das divisões cambiais foi mais estável, sendo se calhar mais regulado pelo fotoperíodo do que pela temperatura. Os resultados indicaram que *Q. pyrenaica* tem uma reativação do crescimento mais tardia do que *Q. robur*, que apresentou um limiar térmico mais reduzido para a quebra da dormência. Uma reativação mais tardia protege a *Q. pyrenaica* fronte os danos causados pela geada durante a primavera, o que concorda com a abundância desta espécie em áreas montanhosas da Península Ibérica. Porem, isso fez com que o período de formação do lenho inicial fosse menor do que em *Q. robur*. Apesar dos estádios fenológicos das espécies estudadas acontecerem em tempos diferentes, iniciação de brotamento coincidiu com o amadurecimento dos primeiros vasos nas duas espécies, o que demonstra que o começo da formação de madeira de primavera em carvalhos de anel poroso depende das reservas de carbono acumuladas durante o ano anterior. O diâmetro hidráulico em *Q. robur* cresceu com a duração do período de formação do lenho inicial, enquanto que em *Q. pyrenaica* essa relação não foi significativa. De fato, apenas em *Q. robur* o diâmetro hidráulico foi afetado pela data de iniciação da formação do lenho. Este resultado sugere que *Q. robur* desenvolveu uma estratégia de produção de lenho inicial mais plástica do que *Q. pyrenaica*. Em consequência, *Q. robur* pode ter um sistema vascular mais eficiente caso que um hipotético aumento na temperatura adiante a reativação em primavera.

Contudo, desligar o tamanho de vaso da duração de alargamento do lenho inicial poderia ser vantajoso para *Q. pyrenaica* ao evitar um incremento excessivo da vulnerabilidade à cavitação em áreas secas e cálidas, mas também ao garantir certo nível de eficiência hidráulica se as condições mudarem rapidamente do frio de inverno à seca de verão.

O início da formação do lenho final ocorreu por volta do solstício de verão, coincidindo com um segundo brotamento de menor entidade. Esta fase foi mais síncrona entre espécies e sítios de estudo do que a correspondente para o lenho inicial. Nas árvores que formaram lenho tardio, a taxa de incremento máximo de madeira aconteceu nessa altura, o que é consistente com estudos prévios realizados em coníferas e frondosas de clima temperado. Um resultado notável foi que no sítio mais seco muitas árvores mostraram uma recuperação do crescimento em outubro, depois de sofrer uma parada em setembro devido à seca. Isto foi evidente em 2012, o ano mais seco, no que os modelos refletiram que a probabilidade de observar tecido em crescimento no outono era maior do que ao final do verão. Este resultado revela que a resposta da atividade cambial às mudanças na disponibilidade de água é plástica não apenas em espécies Mediterrâneas, mas também em espécies de clima temperado ou de transição. Por outro lado, a cessação da atividade cambial aconteceu com anterioridade no sítio mais úmido, nomeadamente em *Q. pyrenaica*.

As reservas de carboidratos não estruturais contidas no alburno seguiram uma dinâmica sazonal semelhante em ambas as espécies, o que talvez seja devido à coincidência na sua estrutura anatômica e no seu hábito foliar. O amido e os açúcares solúveis acumulados na temporada anterior caíram significativamente entre Fevereiro e Junho, mesmo depois das folhas estarem totalmente desenvolvidas. Estes resultados confirmam a dependência que o lenho inicial teve nas reservas de carbono. A formação da madeira foi, porem, concorrente com a acumulação de amido durante o verão. Depois, o conteúdo de amido decresceu para se tornar em açúcares solúveis até a chegada da dormência. A acumulação de açucares começou mais cedo no sítio mais seco, o que talvez esteja relacionado com a cessação do crescimento em resposta à seca de verão. Se calhar, uma maior concentração de açúcares no alburno iria melhorar a regulação osmótica e a recuperação de vasos disfuncionais. O conteúdo total de hidratos de carbono e açúcares solúveis atingiu o seu pico no início da dormência. Estes resultados sugerem que a conservação de níveis altos de açúcares solúveis no inverno tem um grande valor adaptativo nestas espécies, dado o papel desempenhado por estes compostos na proteção contra os danos da geada, como fonte de energia metabólica durante a dormência, ou como fonte de materiais e energia na primavera para a reativação. A este respeito, os nossos modelos indicaram a existência de uma relação positiva entre a concentração de açúcares no alburno ao início de dormência e o avanço da quebra da dormência. A concentração de açúcares solúveis em *Q. pyrenaica* também exerceu um efeito direto sobre a produção de madeira no início da primavera. Em concordância com estes resultados, a concentração de açúcares solúveis pôde ter influenciado a capacidade para recuperar a funcionalidade dos vasos de lenho final após da geada, melhorando o fornecimento de água e açúcares as gemas em inchamento. A relação mais intensa entre o nível de açúcares solúveis e a formação de lenho inicial advertida em *Q. pyrenaica* evidencia a existência de uma estratégia de crescimento conservadora destinada a evitar o consumo excessivo de carbono. Este resultado foi verificado no menor crescimento radial medido em *Q. pyrenaica*, quer nas series de anéis de crescimento para o período 1980-2013, quer nos dados de dendrômetros em 2012 e 2013. De fato, *Q. pyrenaica* mostrou mais açúcares solúveis em relação ao conteúdo de amidodo do que *Q. robur*, fato que talvez esteja associado com a maior tolerância da primeira espécie aos eventos de seca no verão e baixas temperaturas no inverno.

O número de células na zona cambial durante a dormência, característica ligada ao vigor da árvore, foi um fator determinante para a regulação da formação da madeira e a fenologia. Assim, as árvores com maior número de células cambiais no início da época de crescimento foram predispostas a gerar mais tecido no momento de crescimento máximo do que aquelas outras que tinham menos células. Por sua vez, este fato contribuiu para a elongação da estação de crescimento. Em *Q. robur*, ambos os fatores, duração e taxa máxima de crescimento, influíram no incremento anual de madeira, enquanto que em *Q. pyrenaica* somente a taxa máxima de crescimento contribuiu de jeito significativo. Este resultado confirma que a ligação entre fenologia e anatomia é mais fraca em *Q. pyrenaica*, fato que poderia ser uma consequência do seu limitado período de crescimento. Mesmo assim, os nossos resultados sugerem que a mobilização de hidratos de carbono não estruturais para o crescimento é modulado em ambas as espécies por parâmetros estruturais, tais como o número de células do cambium.

As séries de anéis de crescimento no período de 1980-2013 revelaram que a área de vaso cresceu no sítio mais seco em relação aos dois mais chuvosos, havendo um padrão mais pronunciado em *Q. pyrenaica*. Este resultado, combinado com as concentrações mais elevadas de hidratos de carbono no sítio mais seco, sugere que em condições de baixa disponibilidade hídrica os carvalhos subordinam a resistência à cavitação fronte a eficiência hidráulica, o que talvez seja compensado através do aumento dos níveis de açúcares solúveis. Em *Q. robur*, isto poderia ser atribuído ao fato que a localidade mais seca também foi aquela que mostrou uma iniciação de crescimento mais adiantada. Em *Q. pyrenaica*, o aumento da área dos vasos poderia ser causado por um incremento do ritmo de expansão com a temperatura mais do que a um adiantamento da fenologia. Em áreas de clima Mediterrâneo, os eventos de seca são mais precoces do que em ambientes temperados, assim que o período favorável para as folhas serem desenvolvidas é mais limitado. Este processo poderia ser acelerado através da melhora no subministro de água e a fixação de carbono se a eficiência hídrica do xilema tornar aumentada. O notável aumento entre Agosto e Outubro das tiloses a bloquear os vasos do lenho inicial, nomeadamente no sítio mais seco e para *Q. robur*, sugere que a conservação da integridade dos vasos de lenho inicial pode não ser necessária para a sobrevivência no verão. Esta ideia é compatível com a estratégia de melhoramento da eficiência na primavera.

A análise de correlação entre as séries de anéis e os dados interpolados de temperatura e precipitação durante o período de 1967-2013 verificou a grande influência das condições de inverno na formação dos primeiros vasos nos carvalhos de anel poroso. Temperaturas altas e chuva abundante durante o período de dormência afetou negativamente à expansão dos vasos e a produção de lenho inicial. Sabe-se que a existência de temperaturas elevadas no inverno pode retardar a fim da dormência interna. Ao mesmo tempo, a concentração de açúcares iria ser afetada devido à descida na conversão de amido em açúcares solúveis no início de dormência, mas também a um aumento no consumo associado com a respiração. Em concordância com o papel que os açúcares solúveis desempenham a nível hidráulico e metabólico, uma redução na disponibilidade de açúcares solúveis poderia supor uma limitação funcional para a árvore. A este respeito, o excesso de chuva poderia ter afetado o balanço de carbono devido ao estresse gerado pela falta de oxigênio no solo. No entanto, temperaturas altas durante a expansão dos vasos favoreceram a formação de vasos grandes. Por sua parte, a falta de água ao final da primavera limitou a expansão dos últimos vasos do lenho inicial, mesmo no sítio de estudo mais úmido. Isto parece indicar que na área de estudo existem episódios de seca na primavera, embora o inverno seja frequentemente chuvoso. A escassez de água foi, de fato, o principal fator limitante da produção de lenho final, o que confirma os resultados de um grande número de estudos realizados em áreas temperadas e do Mediterrâneo. As correlações para o lenho final foram nomeadamente altas entre Maio e Julho, o que concorda com a maior taxa anual de produção de madeira a acontecer nesse período. Além disso, a diminuição da temperatura e o aumento da precipitação em Setembro e Outubro teve um efeito negativo na produção de madeira no sítio mais chuvoso, o que pode estar associado a uma interrupção precoce do crescimento, como assim aconteceu nesta localidade durante o 2013 (ano úmido).

Em resumo, a informação obtida nesta tese de doutoramento contribui ao esclarecimento das relações funcionais entre fenologia, produção e estrutura do xilema e o conteúdo de carboidratos não estruturais, bem como os mecanismos subjacentes a estas relações. Além do mais, esta pesquisa supõe um avanço significativo na compreensão da capacidade dos carvalhos de anel poroso para enfrentarem o aumento do estresse abiótico no contexto do aquecimento global. A este respeito, os nossos resultados sugerem que *Q. pyrenaica* seria mais resistente do que *Q. robur* a um possível aumento da seca extrema no verão, ainda que, mesmo assim, ambas as espécies seriam limitadas caso que a seca tornasse frequente na primavera. Deste jeito, *Q. pyrenaica* poderia ser afetado ao ter uma arquitetura hidráulica mais vulnerável no lenho inicial. Além disso, em solos com alta disponibilidade hídrica, a maior plasticidade fenológica e alta produtividade fariam com que *Q. robur* fosse mais competitivo do que *Q. pyrenaica*.

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Pérez-de-Lis G, Rossi S, Vázquez-Ruiz RA, Rozas V & 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**, 521–530, doi: 10.1111/nph.13610.

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Pérez-de-Lis G, Olano JM, Rozas V, Rossi S, Vázquez-Ruiz RA & García-González I. 2016. Environmental conditions and vascular cambium regulate carbon allocation to xylem growth in deciduous oaks. *Functional Ecology*, doi: 10.1111/1365-2435.12789.

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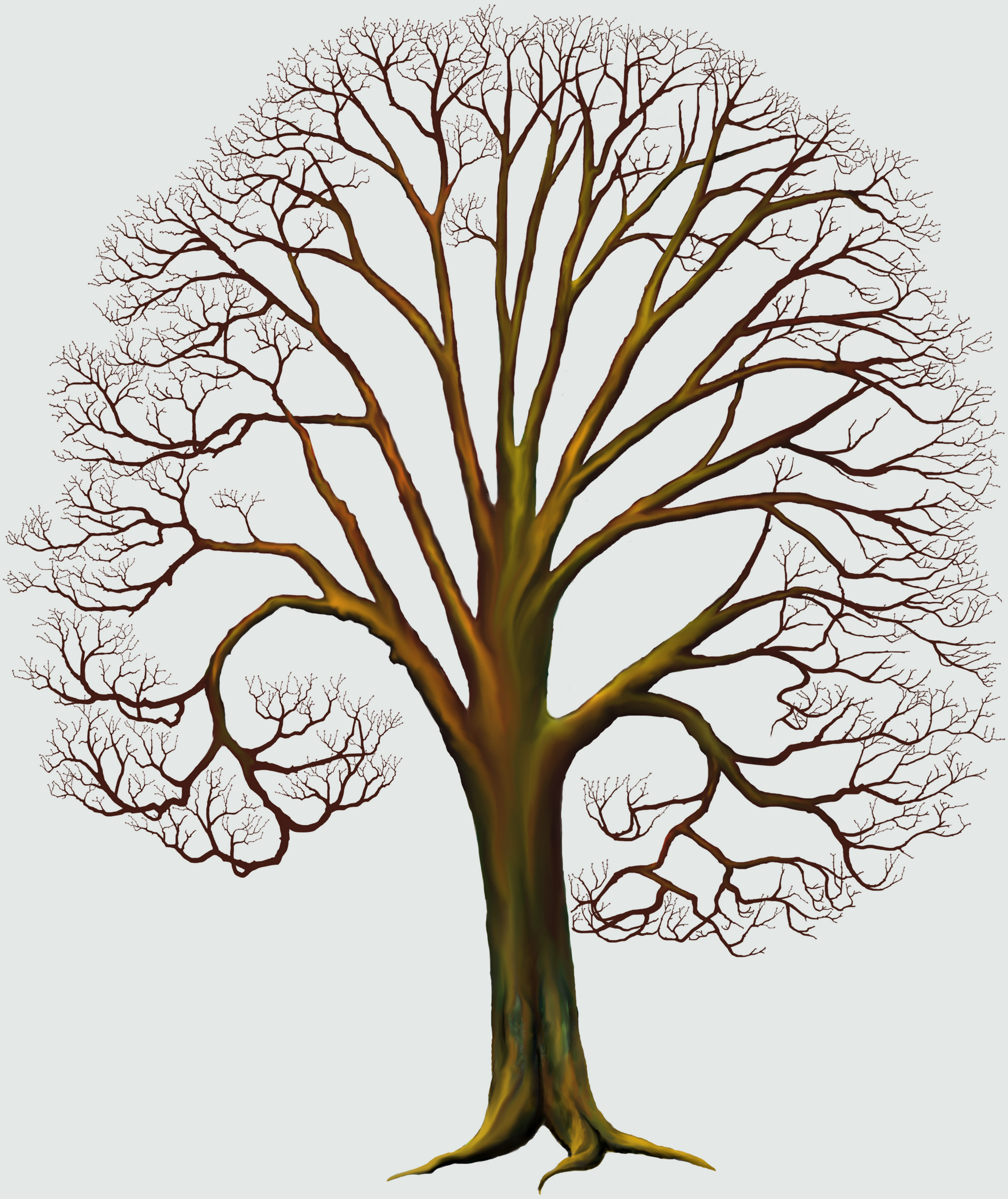

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