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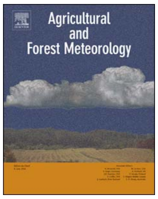

Research paper

Do ring-porous oaks prioritize earlywood vessel efficiency over safety? Environmental effects on vessel diameter and tyloses formation

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ABSTRACT

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

1. Introduction

Wood anatomy influences the ability of trees to track environmental changes (Anderegg et al., 2016; Fonti and Jansen, 2012; Pratt and Jacobsen, 2017). Conductive cells have attracted the attention of wood anatomists because the efficiency/safety trade-off is deemed to be a prime evolutionary force (Gleason et al., 2016), which may somewhat account for the variation in vessel morphology across species and biomes (Cavender-Bares et al., 2005). The ring-porous structure represents a unique example, showing a bimodal vessel size distribution along the ring (Kitin and Funada, 2016). According to the 'rare pit' hypothesis, leaky intervascular pits are more probable to occur in large vessels, easing air-seeding into the vessel and leading to cavitation occurrence (Sperry et al., 2006). The more efficient earlywood vessels are thus less resistant than latewood ones to cavitation (Hernández-Santana et al., 2008; Sperry et al., 2006), being normally dysfunctional within one year (Umebayashi et al., 2010). Cavitated vessels of numerous taxa are eventually sealed by outgrowths called tyloses that originate from adjacent parenchyma cells (De Micco et al., 2016; Kitin and Funada, 2016). Tyloses reflect tree responses to drought and freezing events, wounds, and vascular pathogens (De Micco et al., 2016), but their formation has been barely monitored so far (Cochard and Tyree, 1990).

Earlywood vessel diameter in ring-porous species is controlled by complex interactions between internal and external factors (Hacke et al., 2017). Tree size imposes physical constraints to conduits dimensions, whereby wider vessels are required at the tree base as trees grow taller and water-conducting pathways become longer (Carrer et al., 2014). Previous studies associate these allometric relationships to specific auxin production and transport, offering theoretical support to the increasing vessel dimensions with tree size and age (Carrer et al., 2014; García-González et al., 2016). Concurrently, the environment exerts a strong influence on earlywood vessel diameter by inducing changes in the timing and rate of processes involved in cambial cell division and earlywood differentiation (Fonti et al., 2007; Hacke et al., 2017; Pérez-de-Lis et al., 2016b).

White oaks are prevalent ring-porous species occurring under a wide range of environmental conditions throughout the Northern Hemisphere. The deciduous *Quercus robur* is widespread in Europe under mild and rainy conditions, reaching the temperate-

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Mediterranean transition where marcescent oaks such as *Q. pyrenaica* become dominant. The latter is more tolerant to adverse abiotic conditions than *Q. robur*, as revealed by a shorter growing season and lower xylem increment (Pérez-de-Lis et al., 2017). Under future climatic scenarios, growth performance in oaks is expected to decline (Sánchez de Dios et al., 2009; Gea-Izquierdo et al., 2013; Urli et al., 2015), while predictions for vessel dimensions are still uncertain. At the intra-specific level, narrower vessels were reported under dry conditions in Mediterranean areas (Castagneri et al., 2017; Corcuera et al., 2004; Eilmann et al., 2009; Gea-Izquierdo et al., 2012), but also under wet conditions in mesic sites (Fonti and García-González, 2008; Fonti et al., 2009). Since trees bearing large vessels could be prone to experience drought-induced mortality (Levanic et al., 2011), identification of key environmental factors driving vessel enlargement is decisive to accurately estimate ring-porous trees resistance to environmental stress.

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

2. Materials and methods

2.1. Study sites

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

Species composition and structure at the study sites reflect diverging climate conditions along the gradient. Although both species coexist at the three study sites, *Q. robur* (*Qrob*) prevails at ATL and ATH while *Q. pyrenaica* (*Qpyr*) is more frequent at MED. In addition, tree density at ATL and ATH was respectively 1178 and 1082 trees ha^{-1}, whereas 530 trees ha^{-1} at MED. At this later site, there was vigorous understory vegetation including *Arbutus unedo* L., *Laurus nobilis* L., and *Acacia dealbata* Link. individuals.

2.2. Monitoring of tyloses formation in earlywood vessels

Tyloses formation was monitored in 10 trees per site, species and year from February 2012 to December 2013 ($N_{2012} = 60$, $N_{2013} = 60$). The selected individuals were comparable in size at the three study sites (Table 1). Two microcores per tree were weekly collected using a Trephor (Vitzani, Belluno, Italy) following a helicoidal pattern around the bole (Rossi et al., 2006), and immediately stored into a cooling box in the field. One microcore per tree was dehydrated at the laboratory with a tissue processor (Leica TP1020, Wetzlar, Germany) containing various ethanol solutions, xylene, and a final station for paraffin embedding, while the other microcore was stored at 5 °C. The cuts obtained in a rotary microtome (Leica RM2125 RTS, Wetzlar, Germany) were placed on slides, oven-dried at 60 °C for 2 h, and then stained in 80% ethanol solutions of safranin and fast green. Samples exhibiting cracks in the cambial zone were occasionally lost during staining. In those cases, we processed the second sample collected for each tree and sampling date. More details on sample processing are provided in Pérez-de-Lis et al. (2016b). Tyloses formation was analysed on the cross-sections by using a binocular microscope (Olympus BX40, Tokyo, Japan) at 40 × magnification. For each sampling date, we registered those individuals showing earlywood vessels of the outermost ring partially or totally occluded by tyloses (Fig. 2).

2.3. Dendrochronological survey

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

2.4. Anatomical measurements and cambial age estimation

In wood cores, we removed dust and tyloses inside earlywood vessels with high-pressure water blast. The surface was coloured with black ink, and conduits were refilled with white chalk in order to enhance contrast between vessels and the bulk tissue. We took images of 5184 × 3456 pixels by using a digital camera attached to a binocular microscope (Olympus SZ60, Tokyo, Japan). All images obtained along a radius were subsequently merged to get one image per sample. Image analysis of earlywood vessels was performed on around half of the samples collected at each site with ImageJ (Schneider et al., 2012). This procedure consisted of automatic object recognition on grey scales, which allows the estimation of the detected transversal areas. Vessel outlines were manually corrected when boundaries were not accurately identified. According to recommendations by García-González et al. (2016), we used a size filter ($> 10{,}000\ \mu m^2$) to prevent measurements of latewood vessels or other cell types, while a circularity filter (> 0.5) allowed us to discriminate vessels from artefacts in output files.

We calculated the mean vessel area and the hydraulic diameter (D_h) for each tree ring by using earlywood vessel area measurements. D_h was calculated according to the equation: $D_h = \sum_{n=1}^{N} d_n^5 / \sum_{n=1}^{N} d_n^4$, where d_n is the diameter of the n conduit within a tree-ring containing n vessels (Sperry et al., 1994). D_h is proportional to the hydraulic conductivity, and in ring-porous species it is thought to be close to the mean diameter of the conduits responsible for approximately 95% of the total stem conductance (Corcuera et al., 2006). The number of vessels (NV) was also registered, and weighted to a tangential width of 10 mm for each core in order to minimize the influence of ring orientation on NV values. Tree-ring width measurements were split into earlywood width and latewood width (LW), the latter distinguished due to the absence of large vessels. In order to assess cambial age, the number of rings was