Original Article

Magnetic resonance imaging suggests functional role of previous year vessels and fibres in ring-porous sap flow resumption

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Stem water flow resumption in ring-porous oak

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Abstract

Reactivation of axial water flow in ring-porous species is a complex process related to stem water content and developmental stage of both earlywood-vessel and leaf formation. Yet empirical evidence with non-destructive methods on the dynamics of water flow resumption in relation to these mechanisms is lacking.

Here we combined *in vivo* Magnetic Resonance Imaging (MRI) and wood-anatomical observations to monitor the dynamic changes in stem water content and flow during spring reactivation in four-year-old pedunculate oaks (*Quercus robur* L.) saplings.

We found that previous year latewood vessels and current year developing earlywood vessels form a functional unit for water flow during growth resumption. During spring reactivation, water flow shifted from latewood towards the new earlywood, paralleling the formation of earlywood vessels and leaves. At leaves full expansion, volumetric water content of previous rings drastically decreased due to the near-absence of water in fibre tissue.

We conclude i) that in ring-porous oak, latewood vessels play an important hydraulic role for bridging the transition between old and new water-conducting vessels and ii) that fibre tissue and parenchyma cells provides a place for water storage.

Key words: Cambial activity, Earlywood vessel formation, MRI, Phenology, *Quercus robur* L., Water content, Water flow.

Introduction

The change in deciduous hardwood trees from winter dormancy to active growth is a recurrent phenomenon in nature. Within a short period of time, trees reactivate their conductive tissues and form leaves in order to resume photosynthesis. Xylem vessels, which are mostly embolised after experiencing several freeze-thaw cycles during winter, need either to be refilled or renewed (Sperry and Sullivan 1992, Ameglio et al. 2002, Tyree and Zimmermann 2002). Diffuse-porous species, such as *Acer*, *Betula* or *Juglans*, usually rely on multiple sapwood rings and in some cases they are able to refill embolised vessels with positive pressure coming from the root (Sperry et al. 1988, Hacke and Sauter 1996, Ameglio et al. 2001). However, ring-porous tree species such as *Quercus* or *Fraxinus*, which form large earlywood vessels in spring followed by small latewood vessels later on in the growing season need to renew their conductive tissues every year as the earlywood vessels become dysfunctional after frost (Cochard and Tyree 1990, Utsumi et al. 1996, Davis et al. 1999). This makes ring-porous species highly dependent on their newly formed earlywood vessels, which are formed synchronously or just prior to leaf development (Essiamah and Eschrich 1986, Cochard and Tyree 1990, Granier et al. 1994, Fonti et al. 2007, Sass-Klaassen et al. 2011, Copini et al. 2016, Pérez-de-Lis et al. 2016).

In ring-porous species the large earlywood vessels play a dominant role for axial water flow since, according to the Hagen-Poiseuille law, one large earlywood vessel with a size of 300 μm in diameter transport as much water as 10000 latewood vessels with diameters of 30 μm . However, these small latewood vessels might become of vital importance during spring resumption once the large previous year earlywood vessels have lost their transporting capacity since embolised during the cold winter (Zimmermann 1964, Braun 1970, Granier et al. 1994, Utsumi et al. 1999, Tyree and Zimmermann 2002). Although the exact mechanisms of where

water flows during leaf development and vessels formation are not known, wood-anatomical studies have revealed that earlywood vessels are connected to previous year latewood vessels. In *Fraxinus lanuginosa* the connection is achieved via common pits (Kitin et al. 2004, Kedrov 2012) while in *Quercus robur* these connections are provided by vasicentric tracheid cells surrounding the latewood vessels (Braun 1970, Sano et al. 2011). In addition, it has been suggested that previous latewood vessels may already be conductive in axial direction during buds swelling in early spring (Essiamah and Eschrich 1986) as water is needed e.g. for hydrolyses of starch and for providing the necessary turgor for cell expansion. Based on dye injection and cryo-scanning electron microscopy, Sano et al. (2011) suggested that previous year latewood tracheids in *Quercus crispula* may also conduct water during the growing season. Yet, as contemporary measurement techniques such as heat-based methods (heat balance and heat dissipation) or radioisotope methods, usually lack the required spatial resolution or are invasive (Clearwater and Clark 2003, Renninger and Schafer 2012, Vandegehuchte and Steppe 2013), and thus it was only possible to speculate about these processes.

High spatially-resolved *in vivo* observations of both water content and axial water flow in the stem of actively transpiring plants are now made possible with the application of Magnetic Resonance Imaging (MRI) (Windt et al. 2006, Van As et al. 2009, Borisjuk et al. 2012, De Schepper et al. 2012, Windt and Blümner 2015). The most important feature of MRI is the possibility to non-invasively map the presence and mobility of protons (i.e., the nucleus of the hydrogen isotope ^1H) within the plant. Over the last decades MRI has become an important technique to study cavitation, root development, anatomy, and xylem- and phloem-water flow (Windt et al. 2006, Scheenen et al. 2007, Helfter et al. 2007, Homan et al. 2007, Borisjuk et al. 2012, De Schepper et al. 2012, Van As et al. 2012, Robert et al. 2014, Peuke et al. 2015).

99 In this study, we applied Magnetic Resonance Imaging (MRI) on four-year-old ring-
100 porous pedunculate oak saplings (*Quercus robur* L.) to explorative investigate the dynamics of
101 water flow resumption in relation to leaf phenology and the processes of earlywood-vessel
102 formation during spring reactivation. We hypothesize that (i) previous year latewood vessels
103 conduct water during the period of earlywood vessel and leaf formation (Zimmermann 1964,
104 Utsumi et al. 1999, Tyree and Zimmermann 2002), whereas earlywood vessels conduct water
105 during the remainder of the growing season, (ii) that earlywood-vessel formation starts at the
106 position where latewood vessels are proximity to the tree-ring boundary (Braun 1970, Kitin et
107 al. 2004), and (iii) during leaf and earlywood-vessel formation the sapwood water distribution
108 changes as more water is needed in the outermost ring for both cambial activity and axial water
109 flow (Arend and Fromm 2003, Woodward 2004, Arend and Fromm 2007).

Material and Methods

Plant material and leaf phenology

For this study, we used 12 four-year-old pedunculate oak saplings (*Quercus robur* L.) with similar stem diameter (12.5 ± 1.5 mm at 50 cm stem height, mean $\pm$ SD) and tree height (171 ± 12 cm, mean $\pm$ SD). The trees were grown in 17 l containers (diameter 30 cm, height 24 cm) in a sand-loam mixture and placed on a 1 x 1 m grid in Wageningen, the Netherlands (51.9884°N , 5.6644°E). The trees were watered, using a semi-automatic fertigation system. During the study period, all the trees were scored according to the spring and summer leaf phenology on their upper branch at every second day from April 15th to June 15th 2009. For the classification of spring and summer phenology, we used the criteria provided by Derory et al. (2006) and used six phenophases: 0 = dormant buds; I = swollen buds; II = leave visible; III = extending internodes; IV = fully developed leaves; V = swollen buds upon the second growth flush.

MRI measurements

To monitor the dynamics of the stem water content and sap flow, we selected two trees at every phenophase. These trees were scanned in a 3T MRI scanner (Bruker, Karlsruhe, Germany) consisting of an Avance console (Bruker, Karlsruhe, Germany) and a superconducting magnet with a vertical 0.5 m-diameter bore (Magnex, Oxford, UK) generating a magnetic field of 3 T (128 MHz proton frequency) (Van As 2007). An openable radio frequency coil with an inner diameter of 4 cm, surrounded by a gradient coil with a maximum gradient strength of 1 T/m was mounted around the stem (Homan et al. 2007, Van As 2007). All measurements were conducted in the centre of the gradient coil at ca. 50-cm stem height. To stimulate transpiration, light was generated by 8 halogen projection bulbs (Philips, type 13117, 17V, 150W, GX5.3)

providing photosynthetically active radiation of ca. $350 \mu\text{mol m}^{-2}\text{s}^{-1}$ at a temperature between 25 and 27 °C within the upper part of the crown. The relative humidity varied between 30 and 60%.

The amount of water per voxel and the T_2 relaxation times were measured in a matrix of 256×256 pixels and a slice thickness of 3 mm, representing a field of view of 2.0×2.0 cm (1 voxel = $78 \times 78 \times 3000 \mu\text{m}$) (Fig. 1) using a multiple spin echo (MSE) imaging sequence (Edzes et al. 1998). Measurements were performed using a repetition time 2500 ms and 64 spin echoes with an echo time of 7.5 ms. The decay of the spin echoes was fitted to a mono-exponential curve to obtain both the amount of water and T_2 relaxation value per voxel. The amount of water per voxel or the volumetric water content - for simplicity hereafter referred to as water content – within the tree was calibrated against the signal intensity of the water in the reference tubes that were placed around the tree to provide a 100% value. It should be noted that the signal of water with a T_2 relaxation time of less than a few milliseconds, as found in the most tightly packed fibres in the wood, cannot be detected by the MSE method here employed. For this reason the assessed amount of water underestimates the true volumetric water content, but still provide evidences on the relative changes over time.

Axial water flow was measured in a matrix of 64×128 voxels representing a field of view of 2.0×2.0 cm at a slice thickness of 3 mm (1 voxel = $312.5 \times 156.25 \times 3000 \mu\text{m}$) (Fig. 1) using a pulsed field gradient – spin echo – turbo spin echo sequence (PFG-SE-TSE) (Scheenen et al. 2001, Windt et al. 2006). These measurements were performed using a turbo factor of 16, with a small delta of 4 ms, a big delta of 50 ms, and a maximum gradient of 0.4 T/m. On average seven successive sap flow measurements were conducted of which the mean and standard deviation were calculated yielding the mean water flow (mm^3/s) and the mean linear velocity (mm/s) per voxel (Scheenen et al. 2001, Windt et al. 2006).

Leaf area and stem anatomical measurements

Immediately after the MRI measurements, we assessed both the leaf area (phenophase IV – VI) and the development of the forming ring. The leaf area was determined by scanning all leaves with a resolution of 600 dpi using a flatbed scanner (Epson Expression 10,000 XL) and the measurements were performed using the software Image J (version 1.44) (Rasband 1997-2012).

The assessment of the stem anatomy has been performed on the same position immediately after the MRI measurements. To keep track of the correct orientation of the 3-mm thick stems segments, its west side was marked before cutting and stored in a 50% ethanol solution at 4 °C. Thin cross-sections of 20-25 µm thickness (Fig. 1) were then cut using a sliding microtome (Gärtner et al. 2014). All cross-sections were stained with a solution of safranin and astra blue for 5 min and gradually dehydrated in graded series of ethanol (50–95–100%), before embedding into Canada balsam. Photos of the entire cross sections were taken with a digital camera (DFC 320, Leica, Cambridge, UK) mounted on a microscope (DM2500, Leica, Cambridge, UK) using the Leica imaging software (version 3.6.0) with a resolution of 2.19 µm/pixel). Subsequently the photos from the same section were stitched together using the software PTgui (version Pro 9.1.7, New House Internet Service B.V., Rotterdam, The Netherlands).

The area of mature and unlignified earlywood vessels was measured for the entire circumference during each leaf phenophase using Image J software (version 1.44) (Rasband 1997-2012). Vessel maturation was recognizable by the reddish cell wall colour provided by safranin. We also counted the number of earlywood vessels connected to previous year latewood-vessel or to fibre tissue (Fig. 1) to test with a Chi-square tests for privileged tissues connections while taking into account the proportion of fibre or latewood-vessel tissue covering the outermost tree-ring boundary.

Water content and flow related to wood tissues

185 To monitor the dynamic changes in water content and flow within the stem, we overlaid the
186 spatial explicit MRI measurements (i.e. the tissue specific water content and flow data,
187 including the T₂ time-relaxation values) with the referenced wood anatomical images of the
188 stem thin sections using the ArcMap 10.2.1 package (ESRI Redlands, California, U.S.). Masks
189 including the different tree rings, the pith, and the bark were previously prepared to allow tissue-
190 specific analyses (Fig. 1). For the previous year tree ring, additional masks were created to also
191 differentiate the latewood vessels, vasicentric tracheid and radial parenchyma cells from the
192 fibre tissue consisting of libriform fibres and axial parenchyma (Fig. 1). The masks were used
193 to estimate the mean flow and mean amount of water in the different compartments (tree rings,
194 bark, pith).

Results

Earlywood-vessel formation along with development of leaf phenology

Results show that the timing of cambial and leaf phenologies are coordinated and that the transition of flow from latewood vessels of previous year to current year earlywood vessels occurs in synchronicity with increasing transpiration from the larger leaf area. At time of first anatomical observation, i.e. during bud swelling (phenophase I), we observed already the presence of new formed earlywood vessels irregularly distributed around the circumference (Fig. 2), but with large differences in proportion among the two analysed trees. At phenophase I, the amount of new lignified earlywood ranged from 6 to 41% of all present earlywood vessels (Table 1) and the vessel density (number of vessels per millimetre of circumference) varied from 2.3 and 3.0 vessels. At the successive phenophases, i.e. when the first leaves were visible (II), the amount of lignified vessels increased to around 50%. In one of the two-selected tree there was already second-row earlywood vessels. Successively, vessel formation progressed steadily. At phenophase IV, i.e. when the leaves were fully developed, more than 80% of all present earlywood vessels had been lignified and the vessel density reached values between 7 and 9 vessels per mm of circumference. At phenophase V - during the second flush of apical growth – the overall mean of earlywood vessel lumen area of lignified vessels ranged between 4050 and 9113 μm^2 .

Earlywood vessel position relative to previous year latewood vessels

The first new forming earlywood vessels were positioned with significantly higher probability (Chi square tests, $P = < 0.001$) close to previous year latewood-vessel (LWT) than to vessel-free fibers. At bud swelling, almost 90% of the new earlywood vessels were connected to previous year tissue rich in latewood-vessel and which was only present along 28% of the outermost tree-

ring boundary (Fig. 3A). During all phenophases significantly more vessels-elements were connected to previous year latewood-vessels (Chi square tests, $P = < 0.001$), although the privileged connections became weaker in later phenophases. At the beginning of the second growth flush about 50% of earlywood vessels were connected to previous year latewood-vessels tissue (Fig. 3A), which only occupied 25% of the circumference of the tree-ring boundary in the selected trees.

Resumption of axial water flow along with development of cambial and leaf phenology

During bud dormancy, in early March, water flow did not yet occur (Fig. 2 and 3). At time of bud swelling (I), water transport in terms of flow volume predominately took place in the latewood vessels, and, in one of the two trees, in some new lignified earlywood vessels of the outermost tree ring. Water flow was limited to the transition between previous-year latewood and new-year earlywood vessels (Fig. 2) with low values of 0.02 and 0.05 mm³/s (Fig. 3). The mean linear velocity was 0.48 mm/s. When the leaves became visible (phenophase II), water flow differed considerable between the two trees depending on amount of earlywood vessel development: whereas one tree only conducted 0.05 mm³/s at a velocity of 0.47 mm/s, the other tree, with the largest mean earlywood size, transported more water i.e. 0.27 mm³/s with a velocity of 0.84 mm/s (Table 1). Once the internodes started expanding (III) the flow was still low and ranged between 0.07 to 0.15 mm³/s, while the total leaf area was 710 and 883 cm² (Table 1). When the leaves were fully developed (IV) and the leaf area was 1760 and 2753 cm², a significant increase in water flow occurred in concomitance with the development of the earlywood vessel tissue (Fig.2) with values of 0.76 and 0.90 mm³/s and a high mean velocity of 1.2 and 1.3 mm/s (Table 1). At the onset of the second growth flush, flow was 1.2 and 1.3 mm³/s with mean flow velocities of 1.2 and 0.8 mm/s and a leaf area of 3000 and 3500 cm² respectively.

Spatial dynamics of stem water content

The mean water content in the imaged stem cross-section barely differed in relation to leaf phenology and ranged between 29 and 37% (Table 1). However, the position of the water within the stem changed considerably from the dormant trees to the trees with newly formed leaves and earlywood (Fig. 2). During dormancy, the water was distributed evenly over the stem and the highest water content values of approx. 37% were found in the outermost tree ring formed in the previous growing season (Fig. 4). Notably the earlywood vessels of the 2008 tree ring contained hardly any water (Fig. 2, arrow). In the latewood of 2008, the highest water content (approx. 42%) was found in latewood-vessel tissue and approx. 32% in the fibre and axial parenchyma tissue. At the time of bud swelling, when earlywood-vessel formation had started, most water (approx. 50%) was found in the developing tree ring (Fig. 4). Also, during the following phenophases most water was located in the outermost developing ring, whereas the water content in the previous ring decreased until on average 15% during the second growth flush in summer (Fig. 4). This water was almost exclusively located in the latewood-vessel tissue (35%); hardly any water (7%) was measured in the fibre and axial parenchyma tissue.

Discussion

Axial water flow gradually shifts from previous latewood vessels to new earlywood vessels

Our observations from *in vivo* monitoring and assessment of water content and flow resumption in young ring-porous oak trees, although based on only two individuals per phenophases, seems to support the hypothesis that previous year conducting latewood tissue and the new-year earlywood vessels operate as a functional hydraulic unit. Axial flow has been observed to only occur in connection to latewood vessels of previous annual rings and to constantly increase along with the increasing leaf (transpiration) and new conductive (earlywood vessels) area. This observation is in line with earlier studies using a combination of invasive dye injection and cryo-scanning electron microscopy, that showed the presence of water only in previous year latewood vessels of *Fraxinus mandshurica* after freeze-thaw cycles (Utsumi et al. 1999) and both in vasicentric tracheids around previous year latewood vessels and around the outermost earlywood vessels in ring-porous *Quercus crispula* and *Q. robur* (Sano et al. 2011). Our hypothesis is strongly supported by similar recent observations performed in *Quercus serrata* seedling showing the involvement of previous year's late- wood vessels in supplying water to new networks of current year's earlywood vessels (Kudo et al. 2018).

In our study, we also provide evidence that axial water flow initially occurring in the previous year latewood vessels, is progressively taken over by new lignified earlywood vessels. At the end of earlywood vessel formation, these are responsible for about 75% of the total water flow. This observation indicates that, even in young oaks with relatively narrow earlywood vessels (lumen area of 4000 - 9000 μm^2), only the previous year latewood vessels and new earlywood vessels are conductive, confirming the general view that earlywood vessels are only functional during one growing season (Zimmermann 1964, Ellmore and Ewers 1986, Cochard and Tyree 1990, Granier et al. 1994, Hacke and Sauter 1996). Our estimates of a 69 to 75% share in water

flow by the outermost ring matches results by Granier et al. (1994), who combined different sap flow-measurement techniques in pedunculate oak and estimated that 70 to 80% of the total flow occurs in the outermost tree ring. The higher values reported in Ellmore and Ewers (1986) and determined by using tracer dye in *Ulmus americana* trees might be related to species-specific properties or due to the fact that our assessment was not including the contribution of current year latewood vessels.

Water flow in our experimental setting was generally low which was most likely the consequence of the restricted light conditions of ca. 350 $\mu\text{mol m}^{-2}\text{s}^{-1}$. At bud burst few earlywood vessels were fully expanded with differentiated secondary as similarly observed on *Kalopanax septemlobus* (Kitin and Funada 2016) and in mature oak trees (Sass-Klaassen et al. 2011; Lavrič et al. 2017). Lavrič et al (2017) also showed that before budswell water flow was minimal and increased with increasing leaf area. Indeed, a significant increase in axial flow has been observed only when the leaves were fully expanded and about 80% of the earlywood vessels were lignified. This increase in flow confirms that current-year earlywood vessels becomes functional for water transport by the time when the first new leaves are mature (Kitin and Funada 2016, Kudo et al. 2015, Kudo et al. 2014). At full leaf expansion, most water is obviously needed for transpiration, which leads to increasing flow. In the preceding period, i.e. during budswell, water is also needed e.g. for the hydrolysis of starch, the reactivation of cambial activity and for cell expansion (Essiamah and Eschrich 1986, Zweifel et al. 2006) but the quantities required are considerably lower.

Earlywood-vessel formation starts near previous year latewood vessels and occurs before bud burst

The importance of previous year latewood vessel during growth resumption is also supported by the observation that the first earlywood vessels appeared at the position where previous year latewood vessels were present at the tree-ring boundary. To our knowledge the onset of earlywood-vessel formation has never been studied in relation to their position within other stem tissues and still little is known about where and how radial flow occurs. Rays, comprising ray parenchyma and/or ray tracheid cells, are assumed to play an important role in safeguarding water transport to the cambium (von Arx et al. 2017, Van Bel 1990, Fuchs et al. 2010, Spicer 2014, Pfautsch et al. 2015). However, Barnard et al. (2013) challenged the importance of radial conductance through rays and showed that tracer dye did not infiltrate ray tracheids, and hardly penetrated into ray-parenchyma cells in different conifers. In line with our finding that suggest a possible additional radial flow between latewood vessels and newly forming earlywood vessels, there are previous studies on several species that have observed the existence of pits on the tangential walls of the last latewood conduits in both conifers (Kitin et al. 2009) and angiosperms (Fujii et al. 2001, Kitin et al. 2004). According to Kitin et al. (2004) all earlywood vessels at the tree-ring boundary of *Fraxinus lanuginosa* made contact via inter-vessel pits with latewood vessels in the previous tree ring. Kitin et al. (2004) also showed that the cambium is bounded by vessels containing many bordered pits. In *Quercus* the transfer of water from vessel to vessel or from vessel to the cambium is most likely mediated by vasicentric tracheids (Braun 1970). Together with the results in axial water flow, it indicates that water needed for earlywood-vessel expansion (turgor), is provided by previous year latewood vessels. This means that previous year latewood vessels and newly formed earlywood vessels form a functional unit across the tree-ring boundary. Future research combining high resolution X-ray computed tomography (Brodersen et al. 2011) with MRI may further support our claim of a tree- ring crossing conductive vessel network.

Stem water dynamics during water flow resumption

When we compared trees at various stages of leaf and wood formation, the overall stem-water content barely differed, however the distribution of water across the stems changed significantly. During dormancy most water was located in the outermost tree ring, while during leaf- and earlywood-vessel formation most water occurred around the cambial zone, i.e. the physiological active cells such as the cambium, differentiating xylem and phloem cells and conductive sieve elements. This latter observation is in line with MRI studies on *Pinus thunbergii* (Umebayashi et al. 2011), *Populus sp.* (Windt et al. 2006) and *Quercus ssp.* (Kuroda et al. 2006, De Schepper et al. 2012) and with studies using cryo-scanning electron microscopy on *Picea jezoensis*, *Larix kaempferi* and *Abies sachalinensis* (Utsumi et al. 2003). The observed continuous decrease in water content of the previous tree ring is mainly related to a significant decrease of water within fibre tissue, i.e. libriform fibres and surrounding parenchyma. This points to the relevance of fibre tissue and axial parenchyma within the sapwood for water storage, especially during winter dormancy. This finding is at odds with the general consensus that libriform fibres have a supporting function because of their thick cell walls and narrow lumina and do not play a role in tree-water relations (Metcalf and Chalk 1983). Nevertheless, different studies report that fibres act as a source for water storage (Utsumi et al. 1999, Zimmermann and Milburn 1982). Utsumi et al. (1999) studied *Fraxinus mandshurica* trees and found that fibre lumen were normally empty but after cavitation of earlywood vessels, water was observed in fibre lumina, indicating that water might have moved from embolised vessels to fibres (Utsumi et al. 1999). Zimmermann and Milburn (1982) reported on water storage in xylem fibres, especially in *Acer* trees during leaf dormancy. De Schepper et al. (2012) found water to be present in fibre tissue in proximity of the cambium at the end of the growing season in young pedunculate oak trees. Potentially, fibre tissues may also play a role in diurnal patterns in water movement resulting from depletion and replenishments of internal stem-water reserves

caused by a delay between leaf transpiration and soil-water uptake (Zweifel et al. 2000). Integrating MRI and stem anatomy with paralleling eco-physiological measurements such as gas exchange and stem diameter fluctuations, may provide in-depth understanding of causal processes occurring during tree development or in response to expected environmental changes such as increased drought.

Conclusions

Our combination of xylem-anatomical observation with *in vivo* MRI monitoring has led to new insights into the dynamics of water content and flow resumption in a ring-porous species. Our results suggest that previous year conductive latewood tissue and new developing earlywood vessels form a functional unit that conducts water in the beginning of the growing season. This radial flow (in addition to the one provided by via symplastic transport in ray parenchyma) might occur through connections between latewood and earlywood vessels across the tree-ring boundary. The hydraulic contribution of latewood vessels during the process of reactivation in spring is gradually taken over by the new earlywood. Our observations also indicate that water stored in fibre and axial parenchyma tissue of the previous year ring might plays an important role for vessel formation and reactivation of water transport during spring. The presented approach shows great potential to further linking stem structure to function, which is crucial to understand climate change impacts on tree functioning.

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Table 1. Characteristics of the studied oak trees. Bold values for EW vessels (Mean $\pm$ SD) refers to unligified EW vessels. Variability around the mean (SD) for the MRI measurements are given from the number of MRI measures

Fig. 1. Overview of performed MRI (left) and wood anatomical (right) measurements. A: Percentage of water content. B: T₂ relaxation times for the identification of newly formed earlywood vessels, which are red since they include water with long T₂ relaxation time. C: Xylem-flow (mm³/s). D: Thin sections with relative masks to differentiate the tissue belonging to the bark, the pith and the individual tree rings (TR). The mask also included the identification of latewood tissue (LWT) formed during the previous growing season. Earlywood vessels indicated with asterisks indicate earlywood vessels that are connected to latewood tissue. The arrow indicates an unligified (blue stained) vessel. The dashed line at the tree-ring boundary represents the circumference used to calculate the earlywood-vessel density.

Fig 2. Overview of stem water content and flow in relation to the developing tree-ring and leaf phenology. 0 = dormant buds; I = swollen buds; II = leave visible; III = extending internodes; IV = fully developed leaves; V = swollen buds upon the second growth flush. The white arrow indicates that previous year earlywood vessels. Scale bars on the wood anatomical images indicate 200 μ m.

Fig. 3. Percentage of current year earlywood vessel-elements connected to previous year latewood vessel-elements (A) and mean ($\pm$ SD) flow (B) expressed as the proportion of axial flow in the outermost ring relative to the previous ring measured for two oak individuals along developing leaf phenophases; 0 = dormant buds; I = swollen buds; II = leave visible; III = extending internodes; IV = fully developed leaves; V = swollen buds upon the second growth flush.

Fig. 4. Percentage of water content in a stem upon the second growth flush, phenophase V (A). Note that in older tree rings water is mainly present in latewood tissue whereas there is hardly any water present in the intermediate fibre tissue. Percentage of water content in the different tree rings (B) and in the bark and pith (C) measured for two oak individuals along developing leaf phenophases. 0 = dormant buds; I = swollen buds; II = leave visible; III = extending internodes; IV = fully developed leaves; V = swollen buds upon the second growth flush.

Figure 1

Figure 2

Figure 3

Figure 4