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REVIEW

Is embolism resistance in plant xylem associated with quantity and characteristics of lignin?

Luciano Pereira[1] · Adilson P. Domingues-Junior[1] · Steven Jansen[2] ·
Brendan Choat[3] · Paulo Mazzafera[1]

Abstract

Key message **We suggest, based on data available for lignin content and Ψ_{50}, a limiting relationship between embolism resistance and lignin content across various groups of seed plants.**

Abstract The appearance of lignin during plant evolution was of defining importance in the colonization of the terrestrial environment. Among several evolutionary advantages, lignin deposition in the xylem increased mechanical support allowing plants to become tall, enabled more efficient water transport, and made the conduits strong enough to transport water under tension. Greater lignin content in the xylem may also improve resistance to embolism induced by drought. In this review we suggest, based on 91 species (54 angiosperms and 37 gymnosperms) data available for lignin content and Ψ_{50} (the water potential when 50% of conductivity in the xylem is lost), a limiting constraint of lignin content to embolism resistance across various groups of seed plants. For a Ψ_{50} reduction of approximately -0.3 MPa, plants allocate at least one percent of extra lignin to their wood. Species with low lignin content seem to be more vulnerable to embolism, whereas species with higher content show considerable variation in embolism resistance. Lignin content may play an indirect role in embolism resistance, since higher total lignin content is related to thicker cell walls. We also discuss the vulnerability to embolism regarding the different composition of lignin between gymnosperms and angiosperms and the performance of transgenic plants with modified lignin content and composition.

Keywords Embolism · Plant cell wall · Plant hydraulics · Water transport · Xylem · Pit membrane

Communicated by M. Buckeridge.

✉ Luciano Pereira
 lpereira@unicamp.br

1 Department of Plant Biology, Institute of Biology, State University of Campinas – UNICAMP, CP 6109, Campinas, SP 13083-970, Brazil

2 Institute of Systematic Botany and Ecology, Ulm University, Albert-Einstein-Allee 11, 89081 Ulm, Germany

3 Hawkesbury Institute for the Environment, University of Western Sydney, Richmond, NSW 2753, Australia

Introduction

Lignin is the most abundant compound in trees, after cellulose (Donaldson 2001). The appearance of lignin coincides with the colonization of the terrestrial environment by plants (Popper et al. 2011), possibly due to its fundamental roles in mechanical support and water transport (Niklas 1992; Pittermann 2010). In addition, its biosynthetic pathway may have played a role in the protection from UV radiation and microbial infection in a unicellular ancestor (Popper et al. 2011). Lignin is present in all derived land plants, except for bryophytes, but it was also found in *Calliarthron cheilosporioides,* a red algae that shared a common ancestor with land plants over 1 billion years ago (Martone et al. 2009), while lignin-like compounds occur in primitive green algae (Delwiche et al. 1989).

Lignin is a hydrophobic polymer (Laschimke 1989) and although having low resistance to tensile strength it confers rigidity to the cell wall by keeping the cellulose

microfibrils free of water (Niklas 1992). Lignin is also a bulking agent, increasing the compression resistance of cells (Niklas 1992).

During transpiration, the negative pressure generated in the xylem can reach values lower than -10 MPa. The rather thin conduit cell walls must resist these high-tension values imposed by drought-induced stress (Hacke et al. 2001). In this scenario, lignin plays a role in providing rigidity to the conduit cell wall, avoiding implosion. The strength of the conduit is proportional to the ratio between the wall thickness and lumen diameter, which determines the necessary investment needed to avoid implosion, particularly in vessels (Hacke et al. 2001; Sperry et al. 2006). In tracheids, however, the ratio of the wall thickness to lumen diameter is higher than in vessels, which might be an evolutionary consequence of the combined function of water transport and mechanical support (Hacke et al. 2001; Sperry et al. 2006; Bouche et al. 2014). The collapse of xylem conduits is very rare and unlikely to occur in the field, but the few existing observations of gymnosperms show that it was always associated with an extremely weak degree of lignification of the secondary wall and severe dehydration (Barnett 1976; Donaldson 2002). However, higher lignin content in needles from early growth stages of *Pinus pinaster* than adult trees (Mediavilla et al. 2014) seems to suggest that the difference in thickness to span ratio and not lignin concentration explains higher levels of xylem tracheid deformation in needles from seedlings than mature trees (Bouche et al. 2015).

As xylem sap is typically transported under negative pressure, gas emboli can form in conduits, which interrupt the water transport to shoots. Depending on the number of conduits embolised and the degree to which water transport is impaired, this can have lethal consequences to woody plants (Anderegg et al. 2016). Several hypotheses have been proposed to explain embolism formation. Some of them are related to the characteristics of the conduit walls, such as (1) the "air-seeding" mechanism, which means that air–water menisci will penetrate through the cellulose microfibrils of bordered pit membranes between two neighbouring vessels (Zimmermann 1983; Sperry and Tyree 1988; Brodersen et al. 2013; Lens et al. 2013; Schenk et al. 2015); (2) heterogeneous nucleation from surface bubbles that are associated with a hydrophobic conduit wall (Tyree et al. 1994; Zwieniecki and Secchi 2015); or (3) surfactant coated nanobubbles in xylem sap may become unstable under certain conditions of gas concentration, temperature, or pressure (Jansen and Schenk 2015; Schenk et al. 2015). Thus, a direct effect of lignin could be expected in avoiding or inducing embolism formation, especially if lignin would be present in intervessel pit membranes, or if the presence of lignin on inner vessel walls would affect its hydrophobic nature. However, an indirect correlation could also exist between embolism resistance and the amount of wood lignin content, since there is evidence that thicker conduit walls are associated with higher resistance to xylem embolism (Hacke et al. 2001; Cochard et al. 2008).

A strong line of evidence supporting the idea that lignin influences embolism resistance comes from work with mutants and transgenic plants. It has been shown that mutants deficient in lignin can have increased embolism vulnerability (Coleman et al. 2008; Voelker et al. 2011; Awad et al. 2012). Several mutants for reduced lignin content with a dwarf phenotype showed a deficient capacity to transport water (Anterola and Lewis 2002; Bonawitz et al. 2014). On the other hand, several plants with a modified lignin composition do not have reduced growth or other apparent functional problems (Bonawitz et al. 2014; Wilkerson et al. 2014; Wagner et al. 2015). It has been recently shown in *Arabidopsis thaliana* that the dwarf phenotype seems to be caused by the disruption of the transcription factor Mediator, which rescues the stunted growth of a lignin-deficient mutant (Bonawitz et al. 2014).

Here we assessed the literature aiming at finding possible correlations between lignin contents and composition with embolism resistance in wood.

Embolism resistance and lignin content

Lignin is fundamental for the development of an efficient water transport system in plants, conferring rigidity and impermeability to conduits. Here, we discuss the evidence that a greater lignin deposition may increase embolism resistance. Although lignin-deficient mutants show greater embolism vulnerability (Coleman et al. 2008; Voelker et al. 2011; Awad et al. 2012), the functional effects of natural variation in lignin content are not known.

Data are available on the total wood lignin contents for many species (Fengel and Grosser 1975; Pettersen 1984). This allows direct and/or simple comparison with hydraulic traits. Embolism can be estimated by vulnerability curves (VC), which is the relationship between water potential and loss of conductivity. A useful parameter to compare different species is the water potential in which 50% loss of conductivity occurs (Ψ_{50}, MPa). Thus, to assess a possible relationship between lignin content and embolism resistance, we used the Ψ_{50} values from the xylem functional traits (XFT) database (Choat et al. 2012) and the lignin content of angiosperms and conifers (Fengel and Grosser 1975; Pettersen 1984). Although these databases rely on different plant materials, we assumed that interspecific differences are greater than intraspecific variation. Although lignin content may vary among plant tissues (Donaldson 1985, 1991) lignin

content is relatively stable if samples are collected from the same plant parts, such as normal wood, with no reaction wood or knots (Uprichard and Lloyd 1980; Sykes et al. 2008; Novaes et al. 2009, 2010). The lignin data were obtained from standard samples, as characterized by a sampling procedure from Technical Association of the Pulp and Paper Industry (TAPPI Standards, see Pettersen 1984). In short, discs with a thickness less than 25 mm were cut from logs, discarding bark, knots, compression and tension wood. The discs were then cut into two or four equal portions and then they were reduced to sawdust to maintain unchanged the proportions of sapwood and heartwood (TAPPI 1992). We also carefully considered in our analyses the methodology that was used to obtain the Ψ_{50} data as well as potential differences between plant organs (e.g., roots, stems) in order to avoid any possible bias, even considering that in most methods terminal branches or roots are used to measure embolism. We also assumed that the difference in lignin content is proportional between trunks and terminal branches. Nonetheless, even with these precautions, the interpretation of a causal relation (e.g., linear regression) should be avoided and, thus, we analysed functional relations by applying a quantile regression, using the R package Quantireg (Koenker 2012), which considers an increasing number of unmeasured factors that limits the distribution of data (Cade et al. 1999; Cade and Noon 2003). In this way, we expected a limiting relationship with lignin content as the limiting factor, while other non-controlled variables could provide noise.

We considered different criteria to include and exclude Ψ_{50} values, with and without data from stems and root samples; sigmoidal versus exponential vulnerability curves (Cochard et al. 2013); the cavitron method, which may overestimate Ψ_{50} for long-vesseled angiosperms species (Pivovaroff et al. 2016); and non-flushed versus flushed samples, which may result in more negative Ψ_{50} values (Pausas et al. 2016).

Data on lignin concentration (quantified as % of total dry mass) were based on the Klason method as published by Pettersen (1984; including 695 entries of 589 species), and Fengel and Grosser (1975; including 153 species). We used species-specific average values for specimens for which various values of lignin content were available. Based on these datasets it was possible to compare Ψ_{50} and lignin content for 91 species (54 angiosperms and 37 gymnosperms). Various species had more than one Ψ_{50} value in the XFT database, resulting in a total of 294 specimens, which allowed us to compare different methods and curve types. Analyses were also carried out using Ψ_{50} mean values for each species. We considered the 95th quantile (corresponding to the 5th quantile for a negative parameter such as Ψ_{50}).

The results are shown in Fig. 1 and Table 1, and additional comparisons in the Fig. S1 and Table S1. We found a similar and significant limiting relationship between lignin content and Ψ_{50} in all analyses (Fig. 1 [$y = -0.29 \pm 0.03x + 1.75 \pm 0.72$; $P < 0.002$]). When analysed separately, gymnosperms also showed a limiting relationship ($y = -0.51x + 8.41$; $P < 0.003$), but with a greater slope, while there was no significant relation for the angiosperms. Considering all species, a low lignin content was strongly related with high Ψ_{50}, i.e., with low resistance to embolism. Species with greater lignin content, however, exhibited greater variation in Ψ_{50}, i.e., they were not constrained to high or low embolism resistance.

The greater lignin content may be evolutionarily related to other functions in addition to water transport, such as UV tolerance and protection to pests and pathogens. It is important also to consider that Ψ_{50} values used in our analyses were based on terminal branches, whereas the lignin content was extracted from mature wood, which could be more embolism resistant than terminal branches (Pivovaroff et al. 2014). This may partially explain why there are species with high lignin content but low resistance to embolism. However, a similar limiting relationship to gymnosperms was found for the relationship among lignin content from stems and Ψ_{50} from roots (Fig. S1), since the root database was mainly formed by this gymnosperm species. Despite possible differences in Ψ_{50} between roots, trunks, and terminal branches, there were few species beyond the limiting relationship in all analyses (Figs. 1, S1). Thus, the differences between databases did not alter the position of the species with low lignin content and the limiting relationship was found to be consistent.

The limiting relationship considering all species may indicate a biophysical limitation to embolism formation in relation to lignin content in the cell wall, i.e., plants must have a certain amount of lignin to avoid embolism regardless of the plant group (angiosperms or gymnosperms), because there were few species beyond this limit. The investment of 1% of lignin in wood may therefore result in a −0.3 MPa less negative Ψ_{50} value (Fig. 1).

Figure 1 also suggests that the limiting relation between lignin content and Ψ_{50} was significant for different plant groups of woody plants. Herbs and grasses present a significant relationship between Ψ_{50} and the percentage of lignified stem tissues (Lens et al. 2016). However, the lignin content in these plants ranges from 1 to 15% dry mass (Knudsen 1997; Fukushima and Hatfield 2004), which is lower than in angiosperm wood. Many herbaceous species presented Ψ_{50} values lower than −2 MPa (Lens et al. 2016), although there is no information about total lignin content for these species. Thus, life forms may be important to relate lignin content with Ψ_{50}.
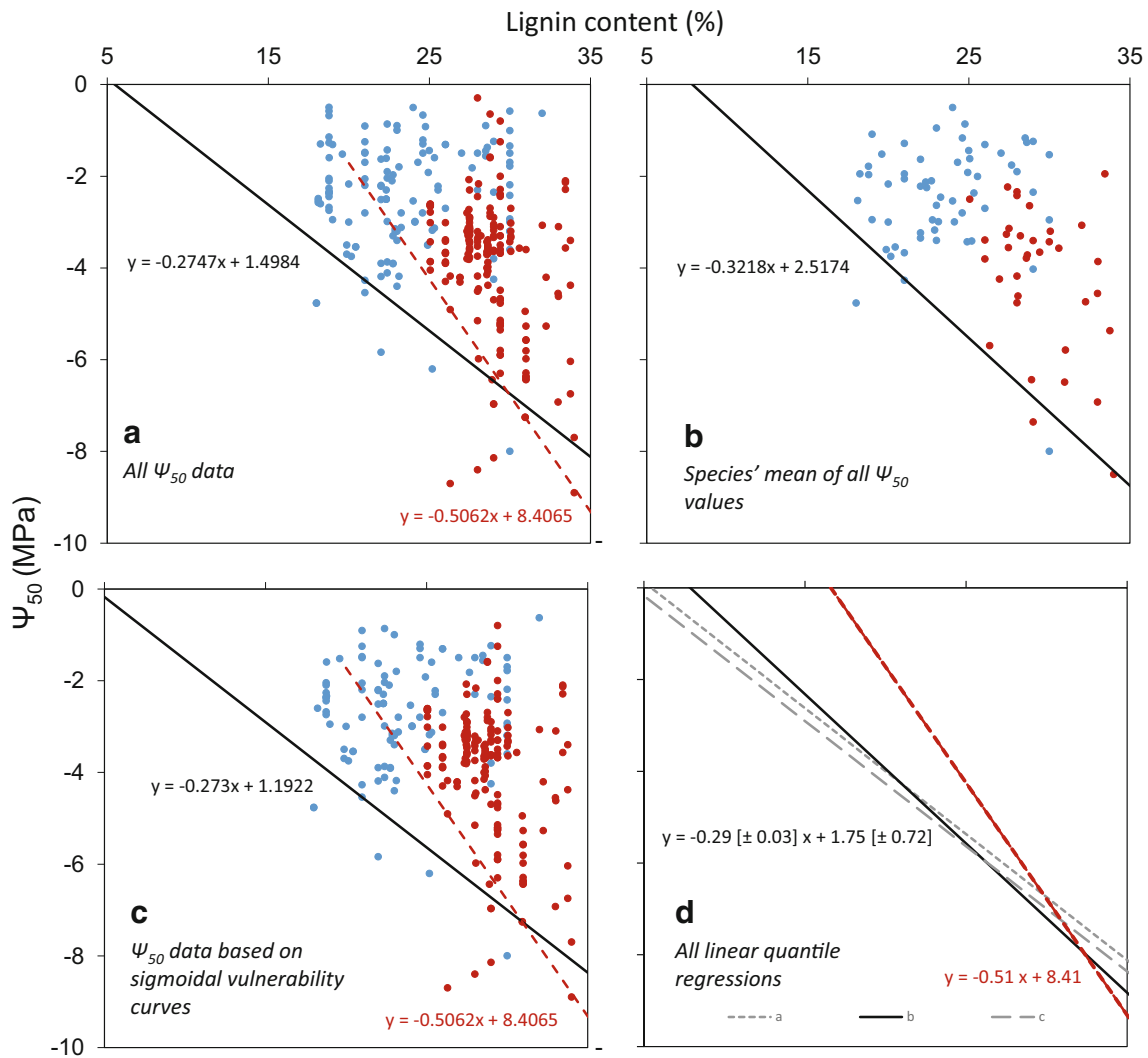
Fig. 1 Relationship between lignin content (%) and Ψ_{50} (MPa) for gymnosperms (*red* points) and angiosperms (*blue* points) while considering the different criteria listed in Table 1: **a** all Ψ_{50} values from stems (some species have more than one Ψ_{50} value); **b** mean of Ψ_{50} from stems; **c** only Ψ_{50} data from sigmoidal curves from stems; **d** all linear quantile regressions. The *solid lines* represent the limiting relationship considering all species and *red dashed line* are for gymnosperms only. There was no significant relationship for the angiosperms only. As Ψ_{50} is a negative variable, the *lines* represent 5% quantile regression ($P < 0.05$), which is equivalent to the 95% quantile for a positive response variable

Table 1 Overview of the criteria used to analyse the possible relationship between Ψ_{50} and lignin concentration in wood, with reference to the plant group, slope, standard error (Std error) and

P values from linear quantile regression, considering the 5th quantile. Ψ_{50} is the water potential at which 50% of conductivity is lost

Ψ_{50} database	Group	Slope	Std error	P value
Ψ_{50} data from stems	All	−0.27	0.05	0.001
	Gymnosperms	−0.51	0.05	0.003
Species' mean of all Ψ_{50} values from stems	All	−0.32	0.04	0.000
	Gymnosperms	NS	NS	NS
Ψ_{50} data based on sigmoidal stem vulnerability curves	All	−0.27	0.08	0.002
	Gymnosperms	−0.50	0.17	0.003

The different results found for gymnosperms and angiosperms might be attributed to a different wood anatomy and chemical composition. According to the data from Pettersen (1984), the wood of the gymnosperms has higher lignin content than angiosperms (Fig. 2; Mann–Whitney U test, $Z = -7.26$, $P = 0.000$; Gymnosperms: 29.7 ± 2.8 g g^{-1}, $N = 100$; Angiosperms: 26.1 ± 5.1 g g^{-1}, $N = 489$). Additionally, the unicellular conduits of gymnosperms (tracheids) are shorter and narrower than the multicellular vessels of angiosperms, and have the dual function of water transport and mechanical support, while vessels are specialized for conduction (Sperry et al. 2006). Such differences in terms of cell dimensions might indicate a different strategy against drought-induced embolism resistance. While most lignin in gymnosperms is present in tracheids, in angiosperms a lower content is found in vessels (see "Lignin distribution and lignin types"). Thus, most wood lignin content is not directly related to embolism resistance in this group, although it would play indirect roles (see below). That might explain why there is no significant relationship for angiosperms, although a significant limiting relationship was found considering both groups. This limit for all species possibly indicates that a biophysical limitation to construct water transport system is acting independently of embolism resistance strategies in angiosperms and gymnosperms species.

Wood density also has been considered to represent an indicator of embolism resistance (Hacke et al. 2001). To assess any significa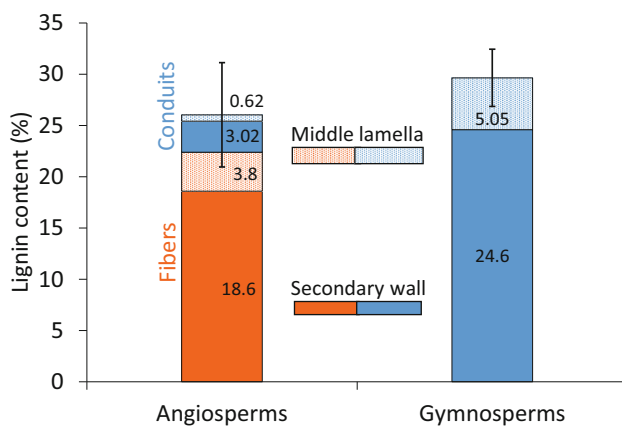nt relationship between wood density and lignin content, we used data from Pettersen (1984) for lignin content and the Global Wood Density database (Zanne et al. 2009; Chave et al. 2009). We compared data from 279 species (222 angiosperms and 57 gymnosperms) and could not find a significant relationship when analyzing all data together or separately for gymnosperms and angiosperms (Fig. S2).

Our comparisons suggest that only species with greater lignin content are more resistant to embolism, as indicated by the Ψ_{50} values. Although the number of species included in this analysis lends some credibility to the relationship found, it is important to remember that the data were taken from different sources (i.e., different plant specimens, populations, locations, subspecies, etc.). Thus, a direct relationship might be obtained by carrying out studies under controlled conditions with a significant and representative number of gymnosperms and angiosperms. Additionally, treatments inducing variation in the lignin content in a single species, including transgenic plants, might be another alternative to reveal mechanisms relating lignin and embolism resistance.

Lignin and mechanisms of embolism resistance

Understanding embolism resistance is a key factor to elucidate plant responses to drought and adaptive strategies in both dry and wet environments (Choat et al. 2012; Lens et al. 2013; Anderegg et al. 2016). A key feature that contributes to embolism resistance in angiosperms seems to be pit membrane thickness (Choat et al. 2008; Jansen et al. 2009; Li et al. 2016). The reduced diameter of the pores confers a greater capillary resistance to air entry (Zimmermann 1983; Choat et al. 2008). Although most papers suggest that lignin does not occur in intervessel pit membranes (Bamber 1961; Sano and Fukuzawa 1994; Fineran 1997; Donaldson 2001), few papers suggest that cellulose microfibrils in the pit membrane are impregnated with lignin (Fromm et al. 2003; Boyce et al. 2004; Schmitz et al. 2008; Herbette et al. 2015). Additional work using antibodies or specific staining reactions (e.g., Fineran 1997) would be needed to test the potential presence of lignin in various angiosperm species. The occurrence of lignin in the pit membrane may reduce the wettability, i.e., the contact angle between the pit membrane and an air–water meniscus, which has consequences for the bubble point pressure difference (P) needed for air-seeding. A reduced wettability would result from the Young–Laplace law ($P = k4T.\cos \alpha/D$; where k is the shape correction factor, T is the surface tension of water and D is the pore diameter) in a reduced air-seeding pressure, increasing the vulnerability to embolism. However, a controlled adjustment of the deposition of less hydrophobic lignin

Fig. 2 Percentage of wood lignin content for angiosperms ($N = 489$) and gymnosperms ($N = 100$) according to the data from Pettersen (1984), and hypothetical lignin distribution in fibers (*orange*), vessels or tracheids (*blue*) and middle lamella region or secondary wall (*dotted areas*), assuming: lignin concentration is 50% in the middle lamella region (including primary wall) and 20% in the secondary wall; middle lamella and secondary wall represent 10 and 90% of the cell wall respectively, in both angiosperms and gymnosperms; wood of angiosperms are composed by 53% of fiber walls and 8% of vessel walls (and lignin is only present in theses tissues). See details in the "Lignin distribution and lignin types". *Bars* represent standard deviation

monomers could improve the mechanical strength of the pit membrane, while maintaining a high wettability depending on other wall components, and thus maintaining high embolism resistance. The presence of lignin might increase the youngś modulus, resulting in greater flexural rigidity of the pit membrane, since air-seeding may occur by enlargement of the pores or formation of micro cracks in the pit membrane (Tixier et al. 2014; Hillabrand et al. 2016). It is also important to consider that the wettability may be affected by the type of lignin in relation to other wall components, which could be relevant for the occurrence of surface nanobubbles on hydrophobic parts of inner conduit walls.

Other anatomical traits that have been indirectly related to embolism resistance include conduit wall thickness (Hacke et al. 2001; Cochard et al. 2008) and fibre wall thickness (Jacobsen et al. 2005; Cochard et al. 2007). Thickening of the secondary walls of the conduits is directly related to implosion resistance (Hacke et al. 2001; Jacobsen et al. 2005; Sperry et al. 2006). Due to a correlation between vessel wall thickness and pit membrane thickness (Jansen et al. 2009; Li et al. 2016), the wall thickness is indirectly correlated with air-seeding via pit membranes. It is possible that reinforced walls of conduits, neighbouring fibers, or parenchyma cells surrounding vessels, avoid micro-cracks through which embolism nucleation may occur or air could be sucked in (Jacobsen et al. 2005). Another possibility is that the conduit lumen is reduced when walls are thick, with consequent low conductivity, and it is known that species resistant to embolism show low conductivity (Gleason et al. 2015).

Lignin distribution and lignin types

To understand the potential role of lignin for plant hydraulics, it is important to know the distribution of this polymer in the cell walls and in different tissues. It is also important to know how cell wall monomeric composition varies. Lignin in the cell wall forms chemical bonds with hemicellulose, which in turn adheres to cellulose microfibrils (Donaldson 2001), but the concentration of these components varies widely. According to Donaldson (2001), the middle lamella and primary wall are composed of more than 50% of lignin in both angiosperms and gymnosperms, whereas about 20% is found in the secondary wall, depending on the cell type. However, the secondary wall contains most of the total lignin content due to its greater volume. A hypothetical contribution of the middle lamella region (including the primary wall) and secondary wall to the total lignin content is shown in Fig. 2. It was considered that the middle lamella region represents 10%, and the

secondary wall 90% of the cell wall volume (Fergus et al. 1969). In this case, the contribution of the middle lamella and secondary wall to the total lignin content is about 17 and 83%, respectively.

Lignin is also heterogeneously distributed in wood because of the different cell types in angiosperm xylem. Secondary walls of vessels and fibers are composed of roughly 20% of lignin and ray cells about 44% when the latter are lignified (Donaldson 2001). Wood may be composed of up to 53% of fiber walls and up to 8% of vessel walls (Ziemińska et al. 2013). If we consider only cell walls of fibers and vessels, since parenchyma cell wall are comparatively thinner [although in some cases they are large and lignified (Plavcová and Jansen 2015)], 86 and 14% of the lignin in wood should be present in fibers and vessels, respectively (Fig. 2).

Lignin composition also varies between gymnosperms and angiosperms. Three canonical precursors produced in the phenylpropanoids pathway are involved in the biosynthesis of lignin—p-coumaryl, coniferyl and sinapyl alcohols, which after incorporation into lignin are referred to as p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units, respectively (Bonawitz and Chapple 2010; Cesarino et al. 2012). Their proportion varies among cell types, taxa and between tissues in the same plant, but other phenylpropanoids may also be part of the lignin and are incorporated at varying levels (Raes et al. 2003). The incorporation of H and G units into lignin starts during early phases of the primary cell wall formation, but are only incorporated in the actual cell wall after initiation of the secondary wall deposition (Grabber 2005). The lignin of angiosperms is mainly composed of G and S units and has traces of H units, while the lignin of gymnosperms is mostly composed of G units with small amounts of H units (Vanholme et al. 2010). Although it is generally accepted that angiosperm vessels are composed mostly of G units and minor part of S units, variation exists among species (Takabe et al. 1992; Wu et al. 1992; Watanabe and Fukazawa 1993).

Possible functions of different types of lignin

Is it possible to explain functional aspects of vessels, fibers and tracheids by their lignin composition? Although there are few detailed descriptions of lignin distribution among species (Terashima et al. 1986, 1988; Terashima and Fukushima 1988; Fukushima and Terashima 1990, 1991; Takabe et al. 1992; Wu et al. 1992; Watanabe and Fukazawa 1993; Saito et al. 2012) and even less about the natural environmental influences on lignin composition (e.g., Stackpole et al., 2011), some characteristics of lignin polymers might define their tissue functionality.

In theory, 16 linkages can be formed among H, G and S units (Baucher et al. 1998). In gymnosperms, there is a greater deposition of H units in the middle lamella and cell corners, with G units mainly deposited in the secondary wall (Fukushima and Terashima 1991). The greater lignification rate of the primary wall should increase the mechanical adhesion between adjacent cells (Niklas 1992). The higher investment in H units can be attributed to its strength, since compression wood shows large amounts of H units, both in the middle lamella and secondary wall (Fukushima and Terashima 1991), thus indicating that lignin formed by H units is more condensed (Baucher et al. 1998). This trait may increase the mechanical strength and supports the stem (Du and Yamamoto 2007). The distribution of H units can give us clues about the mechanical function of H units in gymnosperms. In addition, there is a decrease of hydraulic conductivity in compression wood (Boyce et al. 2004; Pittermann et al. 2006). On the other hand, since H units are deposited in the middle lamella of vessels (Fukushima and Terashima 1990), but in a small amount (Baucher et al. 1998), they may not play a direct role in angiosperm plant hydraulic traits.

Lignin rich in G units is more cross-linked because more biphenyl and other carbon–carbon bonds are formed, resulting in a more rigid and hydrophobic polymer than lignin rich in S units (Koehler and Telewski 2006; Bonawitz and Chapple 2010). The relatively higher abundance of G units in conduits, and possibly more hydrophobic parts of the wall, can be a paradox since it could reduce the capillary pressure and increase the nucleation of embolism (Tyree et al. 1994). However, McCully et al. (2014) recently suggested that bordered pits are generally hydrophilic, composed of hydrophilic pit chambers and hydrophobic pit borders, which may become hydrophilic or hydrophobic if the conduit is water or gas filled, respectively. Herbette et al. (2015) suggested the occurrence of non-condensed lignin (composed of S or G + S units) in pit membranes of hybrid poplar (*Populus tremula × alba*). Those polymers have free phenolic hydroxyl groups that increase the wettability and are more linear and less cross-linked by the presence of a methoxyl group than lignin rich in G units. This may result in a strong but flexible polymer (Koehler and Telewski 2006; Bonawitz and Chapple 2010). This characteristic can maximize the mechanical support function of the fibers as well as give flexibility to vessels, avoiding cell wall fractures when xylem sap is under negative pressure (Jacobsen et al. 2005; Koehler and Telewski 2006).

There is evidence from molecular studies that deposition of lignin in different xylem cell types is highly controlled (Ralph et al. 2004). Thus, the distinct cell functions of fibers and vessels in angiosperms and their different S/G ratios are good indicators of a functional control of the lignin monomers deposition. Stackpole et al. (2011) found a strongly positive relationship between latitude and S/G ratio in populations of *Eucalyptus globulus.* Although they did not identify the cause of the variation, they suggested an adaptive response to abiotic and/or biotic factors. However, as mentioned above, the tendency of increase in S units suggests a more elastic polymer, which influences the hydraulic as well as mechanical properties of the cell wall.

Transgenic plants

Several transgenic plants with altered lignin content and/or deposition show impaired vascular system development, such as collapsed vessels, which is directly related to stunted growth shown by these plants (Anterola and Lewis 2002). However, most of this information comes from transgenic model plants grown in growth chambers or greenhouse (Pilate et al. 2012). For instance, there is a clear increase in vulnerability to embolism in poplar trees with reduced lignin content (Coleman et al. 2008; Voelker et al. 2011; Awad et al. 2012). These results were obtained in plants with lignin reduced by 2% to more than 50%. Although lignin has been generally related to embolism resistance, it is possible that the increase of vulnerability in transgenic plants is due to the reduction of G units, affecting the vessels directly (Coleman et al. 2008). But, in other, the lower content of S units, possibly in the fibers, also resulted in an increase of embolism vulnerability (Voelker et al. 2011). Curiously, several transgenic plants did not show altered water transport and mechanical properties, although they had increased vulnerability to embolism (Awad et al. 2012).

Transgenic plants of *A. thaliana* have been produced, containing very high amounts of H units, from 10 to 15-fold more than normal cell walls (Sundin et al. 2014) to lignin that is almost exclusively composed of H units (Bonawitz et al. 2014). Under controlled conditions in growth chambers they showed very few collapsed vessels and although these were similar to normal cell walls, they were thinner than wild plants (Bonawitz et al. 2014).

Despite the production of mutants with modified lignin and without apparent functional problems (Bonawitz et al. 2014; Wilkerson et al. 2014), these plants have to be tested under conditions of drought stress, which is likely to result in impaired growth. Also, these plants do show some collapsed vessels even under controlled conditions (Bonawitz et al. 2014).

Perhaps a better understanding of the importance of structural lignin alteration in the embolism process might be obtained using T-DNA insertion mutants of *A. thaliana* available in seed stocks (Arabidopsis Biological Resource

Center—https://www.arabidopsis.org/abrc/index.jsp). The inflorescence stems of these plants could be an interesting model to study embolism resistance (Tixier et al. 2013).

Conclusions

The limiting constraint of lignin content to Ψ_{50} seems to suggest that plants require a minimum amount of lignin to tolerate embolism, indicating an investment in the secondary wall and an indirect relation with pit characteristics. Different proportions of lignin monomers (S/G ratio) may modulate the hydrophobicity (McCully et al. 2014; Herbette et al. 2015), and therefore the hydraulic properties of the conduits. It is possible that differential deposition of lignin occurs in response to the environment (Stackpole et al. 2011), despite the known variation in earlywood, latewood, compression and tension wood. Further studies need to be focused on the functional consequences for growth and embolism resistance in transgenic plants with modified lignin.

Author contribution statement LP, PM, APD-J analysed the data and wrote the first manuscript draft. SJ and BC contributed data, ideas and assisted with writing the final manuscript. PM and SJ assisted substantially with manuscript development.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Anderegg WRL, Klein T, Bartlett M et al (2016) Meta-analysis reveals that hydraulic traits explain cross-species patterns of drought-induced tree mortality across the globe. PNAS 113:2–7. doi:10.1073/pnas.1525678113

Anterola AM, Lewis NG (2002) Trends in lignin modification: a comprehensive analysis of the effects of genetic manipulations/mutations on lignification and vascular integrity. Phytochemistry 61:221–294

Awad H, Herbette S, Brunel N et al (2012) No trade-off between hydraulic and mechanical properties in several transgenic poplars modified for lignins metabolism. Environ Exp Bot 77:185–195. doi:10.1016/j.envexpbot.2011.11.023

Bamber RK (1961) Staining reaction of the pit membrane of wood cells. Nature 4786:409–410

Barnett JR (1976) Rings of collapsed cells in *Pinus radiata* stemwood from lysimeter-grown trees subjected to drought. N Z J For Sci 6:461–465

Baucher M, Monties B, Van Montagu M, Boerjan W (1998) Biosynthesis and genetic engineering of lignin. CRC Crit Rev Plant Sci 17:125–197. doi:10.1080/07352689891304203

Bonawitz ND, Chapple C (2010) The genetics of lignin biosynthesis: connecting genotype to phenotype. Annu Rev Genet 44:337–363. doi:10.1146/annurev-genet-102209-163508

Bonawitz ND, Kim JI, Tobimatsu Y et al (2014) Disruption of Mediator rescues the stunted growth of a lignin-deficient Arabidopsis mutant. Nature 509:376–380. doi:10.1038/nature13084

Bouche PS, Larter M, Domec JC et al (2014) A broad survey of hydraulic and mechanical safety in the xylem of conifers. J Exp Bot 65:4419–4431. doi:10.1093/jxb/eru218

Bouche PS, Delzon S, Choat B et al (2015) Are needles of Pinus pinaster more vulnerable to xylem embolism than branches? New insights from X-ray computed tomography. Plant Cell Environ. doi:10.1111/pce.12680

Boyce CK, Zwieniecki MA, Cody GD et al (2004) Evolution of xylem lignification and hydrogel transport regulation. PNAS 101:17555–17558. doi:10.1073/pnas.0408024101

Brodersen CR, McElrone AJ, Choat B et al (2013) In vivo visualizations of drought-induced embolism spread in *Vitis vinifera*. Plant Physiol 161:1820–1829. doi:10.1104/pp.112.212712

Cade BS, Noon BR (2003) A gentle introduction to quantile regression for ecologists. Front Ecol Environ 1:412–420. doi:10.1890/1540-9295(2003)001[0412:AGITQR]2.0.CO;2

Cade BS, Terrell JW, Schroeder RL et al (1999) Estimating effects of limiting factors with regression quantiles. Ecology 80:311–323

Cesarino I, Araújo P, Domingues Júnior AP, Mazzafera P (2012) An overview of lignin metabolism and its effect on biomass recalcitrance. Braz J Bot 35:303–311. doi:10.1590/S0100-84042012000400003

Chave J, Coomes D, Jansen S et al (2009) Towards a worldwide wood economics spectrum. Ecol Lett 12:351–366. doi:10.1111/j.1461-0248.2009.01285.x

Choat B, Cobb AR, Jansen S (2008) Structure and function of bordered pits: new discoveries and impacts on whole-plant hydraulic function. New Phytol 177:608–625. doi:10.1111/j.1469-8137.2007.02317.x

Choat B, Jansen S, Brodribb TJ et al (2012) Global convergence in the vulnerability of forests to drought. Nature 491:752–755. doi:10.1038/nature11688

Cochard H, Casella E, Mencuccini M (2007) Xylem vulnerability to cavitation varies among poplar and willow clones and correlates with yield. Tree Physiol 27:1761–1767

Cochard H, Barigah ST, Kleinhentz M, Eshel A (2008) Is xylem cavitation resistance a relevant criterion for screening drought resistance among Prunus species? J Plant Physiol 165:976–982. doi:10.1016/j.jplph.2007.07.020

Cochard H, Badel E, Herbette S et al (2013) Methods for measuring plant vulnerability to cavitation: a critical review. J Exp Bot 64:4779–4791. doi:10.1093/jxb/ert193

Coleman HD, Samuels AL, Guy RD, Mansfield SD (2008) Perturbed lignification impacts tree growth in hybrid poplar—a function of sink strength, vascular integrity, and photosynthetic assimilation. Plant Physiol 148:1229–1237. doi:10.1104/pp.108.125500

Delwiche CF, Graham LE, Thomson N (1989) Lignin-like compounds and Sporopollenin in Coleochaete, and algal model for land plant ancestry. Science 245:399–401. doi:10.1126/science.245.4916.399

Donaldson LA (1985) Within and between-tree variation in lignin concentration in the tracheid cell wall of *Pinus radiata*. N Z J For Sci 15:361–369

Donaldson LA (1991) Seasonal changes in lignin distribution during tracheid development in *Pinus radiata* D. Don. Wood Sci Technol 25:15–24. doi:10.1007/BF00195553

Donaldson LA (2001) Lignification and lignin topochemistry—an ultrastructural view. Phytochemistry 57:859–873

Donaldson LA (2002) Abnormal lignin distribution in wood from severely drought stressed *Pinus radiata* trees. IAWA J 23:161–178. doi:10.1163/22941932-90000295

Du S, Yamamoto F (2007) An overview of the biology of reaction wood formation. J Integr Plant Biol 49:131–143. doi:10.1111/j.1672-9072.2007.00333.x

Fengel D, Grosser D (1975) Chemische zusammensetzung yon nadel- und laubhözern. HOLZ als Roh—und Werkst 33(33):32–34

Fergus BJ, Procter AR, Scott JAN, Goring DAI (1969) The distribution of lignin in sprucewood as determined by ultraviolet microscopy. Wood Sci Technol 3:117–138. doi:10.1007/BF00639636

Fineran BA (1997) Cyto- and histochemical demonstration of lignins in plant cell walls: an evaluation of the chlorine water/ethanolamine-silver nitrate method of Coppick and Fowler. Protoplasma 198:186–201. doi:10.1007/BF01287568

Fromm J, Rockel B, Lautner S et al (2003) Lignin distribution in wood cell walls determined by TEM and backscattered SEM techniques. J Struct Biol 143:77–84. doi:10.1016/S1047-8477(03)00119-9

Fukushima RS, Hatfield RD (2004) Comparison of the acetyl bromide spectrophotometric method with other analytical lignin methods for determining lignin concentration in forage samples. J Agric Food Chem 52:3713–3720. doi:10.1021/jf035497l

Fukushima K, Terashima N (1990) Heterogeneity in formation of lignin. XIII. Formation of p-hydroxyphenyl lignin in various hardwoods visualized by microautoradiography. J Wood Chem Technol 10:413–433. doi:10.1080/02773819008050250

Fukushima K, Terashima N (1991) Heterogeneity in formation of lignin. Part XV: formation and structure of lignin in compression wood of *Pinus thunbergii* studied by microautoradiography. Wood Sci Technol 25:371–381

Gleason SM, Westoby M, Jansen S et al (2015) Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency across the world's woody plant species. New Phytol. doi:10.1111/nph.13646

Grabber JH (2005) How do lignin composition, structure, and cross-linking affect degradability? A review of cell wall model studies. Crop Sci 45:820–831. doi:10.2135/cropsci2004.0191

Hacke UG, Sperry JS, Pockman WT et al (2001) Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. Oecologia 126:457–461. doi:10.1007/s004420100628

Herbette S, Bouchet B, Brunel N et al (2015) Immunolabelling of intervessel pits for polysaccharides and lignin helps in understanding their hydraulic properties in *Populus tremula × alba*. Ann Bot 115:187–199. doi:10.1093/aob/mcu232

Hillabrand RM, Hacke UG, Lieffers VJ (2016) Drought-induced xylem pit membrane damage in aspen and balsam poplar. Plant Cell Environ. doi:10.1111/pce.12782

Jacobsen AL, Ewers FW, Pratt RB et al (2005) Do xylem fibers affect vessel cavitation resistance? Plant Physiol 139:546–556. doi:10.1104/pp.104.058404.result

Jansen S, Schenk HJ (2015) On the ascent of sap in the presence of bubbles. Am J Bot 102:1561–1563. doi:10.3732/ajb.1500305

Jansen S, Choat B, Pletsers A (2009) Morphological variation of intervessel pit membranes and implications to xylem function in angiosperms. Am J Bot 96:409–419. doi:10.3732/ajb.0800248

Knudsen KEB (1997) Carbohydrate and lignin contents of plant materials used in animal feeding. Anim Feed Sci Technol 67:319–338. doi:10.1016/S0377-8401(97)00009-6

Koehler L, Telewski FW (2006) Biomechanics and transgenic wood. Am J Bot 93:1433–1438. doi:10.3732/ajb.93.10.1433

Koenker R (2012) Quantreg. R package version 5.3. R Foundation for Statistical Computing

Laschimke R (1989) Investigation of the wetting behaviour of natural lignin—a contribution to the cohesion theory of water transport in plants. Thermochim Acta 151:35–56. doi:10.1016/0040-6031(89)85335-3

Lens F, Tixier A, Cochard HH et al (2013) Embolism resistance as a key mechanism to understand adaptive plant strategies. Curr Opin Plant Biol 16:287–292. doi:10.1016/j.pbi.2013.02.005

Lens F, Picon-Cochard C, EL Delmas C et al (2016) Herbaceous angiosperms are not more vulnerable to drought-induced embolism than angiosperm trees. Plant Physiol. doi:10.1104/pp.16.00829

Li S, Klepsch M, Jansen S et al (2016) Intervessel pit membrane thickness as a key determinant of embolism resistance in angiosperm xylem. IAWA J 37:152–171. doi:10.1163/22941932-20160128

Martone PT, Estevez JM, Lu F et al (2009) Discovery of lignin in seaweed reveals convergent evolution of cell-wall architecture. Curr Biol 19:169–175. doi:10.1016/j.cub.2008.12.031

McCully M, Canny M, Baker A, Miller C (2014) Some properties of the walls of metaxylem vessels of maize roots, including tests of the wettability of their lumenal wall surfaces. Ann Bot 113:977–989. doi:10.1093/aob/mcu020

Mediavilla S, Herranz M, Gonzalez-Zurdo P, Escudero A (2014) Ontogenetic transition in leaf traits: a new cost associated with the increase in leaf longevity. J Plant Ecol 7:567–575. doi:10.1093/jpe/rtt059

Niklas K (1992) Plant biomechanics: an engineering approach to plant form and function. University of Chicago Press, Chicago

Novaes E, Osorio L, Drost DR et al (2009) Quantitative genetic analysis of biomass and wood chemistry of Populus under different nitrogen levels. New Phytol 182:878–890. doi:10.1111/j.1469-8137.2009.02785.x

Novaes E, Kirst M, Chiang V et al (2010) Lignin and biomass: a negative correlation for wood formation and lignin content in trees. Plant Physiol 154:555–561. doi:10.1104/pp.110.161281

Pausas JG, Pratt RB, Keeley JE et al (2016) Towards understanding resprouting at the global scale. New Phytol 209:945–954. doi:10.1111/nph.13644

Pettersen RC (1984) The chemical composition of wood. In: Rowell RM (ed) The chemistry of solid wood. American Chemical Society, Washington, DC, pp 57–126

Pilate G, Dejardin A, Leple J-C (2012) Field trials with lignin-modified transgenic trees. Adv Bot Res 61:1–36

Pittermann J (2010) The evolution of water transport in plants: an integrated approach. Geobiology 8:112–139. doi:10.1111/j.1472-4669.2010.00232.x

Pittermann J, Sperry JS, Wheeler JK et al (2006) Mechanical reinforcement of tracheids compromises the hydraulic efficiency of conifer xylem. Plant Cell Environ 29:1618–1628. doi:10.1111/j.1365-3040.2006.01539.x

Pivovaroff AL, Sack L, Santiago LS (2014) Coordination of stem and leaf hydraulic conductance in southern California shrubs: a test of the hydraulic segmentation hypothesis. New Phytol 203:842–850. doi:10.1111/nph.12850

Pivovaroff AL, Burlett R, Lavigne B et al (2016) Testing the "microbubble effect" using the Cavitron technique to measure xylem water extraction curves. AoB Plants. doi:10.1093/aobpla/plw011

Plavcová L, Jansen S (2015) The role of xylem parenchyma in the storage and utilization of nonstructural carbohydrates. Springer, Cham

Popper ZA, Michel G, Hervé C et al (2011) Evolution and diversity of plant cell walls: from algae to flowering plants. Annu Rev Plant Biol 62:567–590. doi:10.1146/annurev-arplant-042110-103809

Raes J, Rohde A, Christensen JH et al (2003) Genome-wide characterization of the lignification toolbox in Arabidopsis. Plant Physiol 133:1051–1071. doi:10.1104/pp.103.026484

Ralph J, Lundquist K, Brunow G et al (2004) Lignins: natural polymers from oxidative coupling of 4-hydroxyphenyl- propanoids. Phytochem Rev 3:29–60. doi:10.1023/B:PHYT.0000047809.65444.a4

Saito K, Watanabe Y, Shirakawa M et al (2012) Direct mapping of morphological distribution of syringyl and guaiacyl lignin in the xylem of maple by time-of-flight secondary ion mass spectrometry. Plant J 69:542–552. doi:10.1111/j.1365-313X.2011.04811.x

Sano Y, Fukuzawa K (1994) Structural variations and secondary changes in pit membranes in *Fraxinus mandshurica* var. japonica. IAWA J 15:283–291

Schenk HJ, Steppe K, Jansen S (2015) Nanobubbles: a new paradigm for air-seeding in xylem. Trends Plant Sci 20:199–205. doi:10.1016/j.tplants.2015.01.008

Schmitz N, Koch G, Schmitt U et al (2008) Intervessel pit structure and histochemistry of two mangrove species as revealed by cellular UV microspectrophotometry and electron microscopy: intraspecific variation and functional significance. Microsc Microanal 14:387–397. doi:10.1017/S143192760808077X

Sperry JS, Tyree MT (1988) Mechanism of water stress-induced xylem embolism. Plant Physiol 88:581–587. doi:10.1104/pp.88.3.581

Sperry JJS, Hacke UG, Pittermann J (2006) Size and function in conifer tracheids and angiosperm vessels. Am J Bot 93:1490–1500. doi:10.3732/ajb.93.10.1490

Stackpole DJ, Vaillancourt RE, Alves A et al (2011) Genetic variation in the chemical components of *Eucalyptus globulus* wood. G3 genes. Genomes Genet 1:151–159. doi:10.1534/g3.111.000372

Sundin L, Vanholme R, Geerinck J et al (2014) Mutation of the inducible *Arabidopsis thaliana* cytochrome P450 reductase2 alters lignin composition and improves saccharification. Plant Physiol 166:1956–1971. doi:10.1104/pp.114.245548

Sykes R, Kodrzycki ÆB, Tuskan ÆG et al (2008) Within tree variability of lignin composition in Populus 42:649–661. doi:10.1007/s00226-008-0199-0

Takabe K, Miyauchi S, Tsunoda R, Fukazawa K (1992) Distribution of guaiacyl and syringyl lignins in Japanese beech (*Fagus crenata*) variation within an annual ring. IAWA J 13:105–112

TAPPI (1992) TAPPI test methods T 257 cm-85: sampling and preparing wood for analysis

Terashima N, Fukushima K (1988) Heterogeneity in formation of lignin XI: an autoradiographic study of the heterogeneous formation and structure of pine lignin. Wood Sci Technol 22:259–270

Terashima N, Fukushima K, Takabe K et al (1986) Heterogeneity in formation of lignin. VII. An autoradiographic study on the

formation of guaiacyl and syringyl lignin in poplar. J Wood Chem Technol 6:495–504. doi:10.1080/02773818608085241

Terashima N, Fukushima K, Sano Y (1988) Heterogeneity in formation of lignin X. Visualization of lignification process in differentiating xylem of Pine by microautoradiography. Holzforschung 42:347–350

Tixier A, Cochard H, Badel E et al (2013) *Arabidopsis thaliana* as a model species for xylem hydraulics: does size matter? J Exp Bot 68:2295–2305. doi:10.1093/jxb/ert087

Tixier A, Herbette S, Jansen S et al (2014) Modelling the mechanical behaviour of pit membranes in bordered pits with respect to cavitation resistance in angiosperms. Ann Bot 114:325–334. doi:10.1093/aob/mcu109

Tyree M, Davis S, Cochard H (1994) Biophysical perspectives of xylem evolution: is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction? IAWA J 15:335–360

Uprichard JM, Lloyd JA (1980) Influence of tree age on the chemical composition of radiata pine. N Z J For Sci 10:551–557

Vanholme R, Demedts B, Morreel K et al (2010) Lignin biosynthesis and structure. Plant Physiol 153:895–905. doi:10.1104/pp.110.155119

Voelker SL, Lachenbruch B, Meinzer FC et al (2011) Transgenic poplars with reduced lignin show impaired xylem conductivity, growth efficiency and survival. Plant Cell Environ 34:655–668. doi:10.1111/j.1365-3040.2010.02270.x

Wagner A, Tobimatsu Y, Phillips L et al (2015) Syringyl lignin production in conifers: proof of concept in a Pine tracheary element system. Proc Natl Acad Sci 112:6218–6223. doi:10.1073/pnas.1411926112

Watanabe Y, Fukazawa K (1993) Lignin heterogeneity of the cell walls on the genus Acer. Res Bull Coll Exp For Univ 50:349–389

Wilkerson CG, Mansfield SD, Lu F et al (2014) Monolignol ferulate transferase introduces chemically labile linkages into the lignin backbone. Science 344:90–93. doi:10.1126/science.1250161

Wu J, Fukazawa K, Ohtani J (1992) Distribution of syringyl and guaiacyl lignins in hardwoods in relation to habitat and porosity form in wood. Holzforschung 46:181–185

Zanne AE, Lopez-Gonzalez G, Coomes DA et al (2009) Data from: towards a worldwide wood economics spectrum. Ecol Lett. doi:10.5061/dryad.234

Ziemińska K, Butler DW, Gleason SM et al (2013) Fibre wall and lumen fractions drive wood density variation across 24 Australian angiosperms. AoB Plants 5:1–14. doi:10.1093/aobpla/plt046

Zimmermann MH (1983) Xylem structure and the ascent of sap. Springer, New York

Zwieniecki MA, Secchi F (2015) Threats to xylem hydraulic function of trees under "new climate normal" conditions. Plant Cell Environ 38:1713–1724. doi:10.1111/pce.12412