

Wood structure and function change with maturity: Age of the vascular cambium is associated with xylem changes in current-year growth

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Abstract

Xylem vessel structure changes as trees grow and mature. Age- and development-related changes in xylem structure are likely related to changes in hydraulic function. We examined whether hydraulic function, including hydraulic conductivity and vulnerability to water-stress-induced xylem embolism, changed over the course of cambial development in the stems of 17 tree species. We compared current-year growth of young (1–4 years), intermediate (2–7 years), and older (3–10 years) stems occurring in series along branches. Diffuse and ring porous species were examined, but nearly all species produced only diffuse porous xylem in the distal branches that were examined irrespective of their mature xylem porosity type. Vessel diameter and length increased with cambial age. Xylem became both more conductive and more cavitation resistant with cambial age. Ring porous species had longer and wider vessels and xylem that had higher conductivity and was more vulnerable to cavitation; however, these differences between porosity types were not present in young stem samples. Understanding plant hydraulic function and architecture requires the sampling of multiple-aged tissues because plants may vary considerably in their xylem structural and functional traits throughout the plant body, even over relatively short distances and closely aged tissues.

1 | INTRODUCTION

As a tree grows and matures, the structure of the xylem conduits that are produced changes. These changes in xylem structure with cambial age are evident when xylem structure is analysed across growth rings, with many trees showing a pronounced change in the structure of their inner growth rings, produced when the trees were younger, compared with their mature outer rings (Panshin & Zeeuw, 1980; Cook, Briffa, Shiyatov, & Mazepa, 1990). Such changes are also evident axially through large structural differences that occur from branch apices toward the tree base (Anfodillo, Carraro, Carrer, Fior, & Rossi, 2006; Jacobsen et al., 2018; Olson et al., 2018; Petit, Anfodillo, & Mencuccini, 2008; Rosell, Olson, & Anfodillo, 2017), with shoot diameter (Zimmermann & Potter, 1982; Ewers & Fisher, 1989; Ewers,

Fisher, & Chiu, 1990; Jacobsen, Pratt, Tobin, Hacke, & Ewers, 2012), with stem age (Cochard & Tyree, 1990; Lo Gullo, Salleo, Piaceri, & Rosso, 1995), and between juvenile and mature wood (De Micco, Aronne, & Baas, 2008; Domec et al., 2008; Olson et al., 2014; Schweingruber, Börner, & Schulze, 2007). In general, conduit diameters increase toward tree bases relative to apices and in older xylem relative to younger xylem; many other changes may also occur in wall structure, pitting, cellular arrangement, and cell type abundance as wood transitions between juvenile and mature characteristics.

Changes in xylem structure with cambial age may be particularly important for understanding whole-tree hydraulic function. Age- and development-related changes in hydraulic function have been found when comparing stem ages and sampling location within the tree (Domec & Gartner, 2001, 2002; Domec, Warren, Meinzer, &

Lachenbruch, 2009; Lo Gullo et al., 1995); however, these patterns are rarely incorporated into studies of plant hydraulics. Most xylem functional studies have focused on relatively young branch apices (e.g., see reviewed xylem functional data in Choat et al., 2012), with comparatively little research on the impact of age-related structural changes on hydraulic function.

Along the shoot hydraulic transport pathway, sap must be transported through current-year growth produced by both an older vascular cambium (toward the tree base) and xylem created by a younger vascular cambium (at branch apices) in series. Any point along this pathway may become a point that reduces whole-plant conductance or increases susceptibility to water-stress-associated hydraulic failure (Alder, Sperry, & Pockman, 1996; Pratt et al., 2007; Tsuda & Tyree, 1997). Transport through the current year's growth ring is especially important considering that this is the tissue that supplies most sap to current-year leaves (Ellmore & Ewers, 1986).

There are two hypotheses that describe potential patterns of plant hydraulic functional changes along the length of the shoot. The vulnerability segmentation hypothesis suggests that younger distal stems will be more vulnerable to water-stress-induced embolism than are older and less expendable stems that supply water to larger sections of the canopy, and the hydraulic segmentation hypothesis predicts that distal organs will have lower hydraulic conductance (Pivovarov, Sack, & Santiago, 2014; Tyree, Cochard, Cruiziat, Sinclair, & Ameglio, 1993). These nonmutually exclusive hypotheses thus predict that distal stems will have low conductivity and be vulnerable to embolism formation, a pattern that is notably divergent from tissue-level predictions for a safety–efficiency hydraulic trade-off. A contrasting prediction may be that distal stems, which experience the most negative pressures, will be more resistant to cavitation to tolerate the more negative pressures that they experience relative to more basal xylem segments. Prior studies have found varying levels of support for these hypotheses in young stems versus older shoot tissue (note that we are not including the discussion here of studies testing these hypotheses between stems and leaves, because leaf patterns are not directly relevant to the topic of the present study). For instance, in contrast to the vulnerability segmentation hypothesis, some studies have reported that younger stems were more resistant to cavitation than were older xylem (Domec & Gartner, 2001; Johnson et al., 2016; Melcher, Zwieniecki, & Holbrook, 2003), whereas others have found that smaller stems were less resistant to cavitation than were wider diameter stems (Hacke & Sauter, 1996; Tyree, Snyderman, Wilmot, & Machado, 1991). Some of these differences may relate to whether young stems were compared with older distal stems or to the bole. An additional confounding factor is that measures conducted on multiyear growth in older stems may include xylem that has experienced prior stress and exhibits cavitation fatigue, although this pattern is likely variable, because some species appear to be relatively resilient to fatigue (Hacke, Stiller, Sperry, Pittermann, & McCulloh, 2001; Sperry, Perry, & Sullivan, 1991).

Changes in xylem structure and function with cambial age may also be related to changes in growth ring porosity and the size distribution of vessels. Species that produce ring porous xylem in their

mature wood (i.e., ring porous species) often produce diffuse porous xylem in their younger stems (Cochard & Tyree, 1990; Gilbert, 1940; Lo Gullo et al., 1995). We were interested in the timing of the transition between diffuse and ring porous xylem production within ring porous species, especially as this may relate to changes in their xylem hydraulic function. Ring porous species were predicted to have very large changes in xylem structure and function with vascular cambium age, given the shift in growth ring porosity that they undergo with cambial age. We were interested in how this might differ from the cambial age changes that occur in diffuse porous species that do not experience a similar shift in porosity as they age.

We examined changes in xylem structure (vessel diameter, vessel length, and growth ring porosity) and function (hydraulic conductivity, cavitation resistance) with cambial age across 17 tree species. Samples were collected in series, such that sap would have been transported through current-year xylem of older samples, into intermediate aged samples, and to terminal young stems within a sample; across species, sampled age categories overlapped. Only the outermost growth ring was sampled to control for interannual variation, time since xylem production, and the potential for cavitation fatigue. All trees were irrigated and growing in a common garden on campus at California State University, Bakersfield, to minimize variation in response to the environment. We predicted that vessel diameter and length would both increase with increased cambial age in woody angiosperm trees and that the ratio of the diameters of earlywood and latewood vessels (E:L) would also increase with cambial age, specifically in ring porous species. Finally, we predicted that hydraulic function would change, such that xylem produced by young cambia at stem apices would have lower hydraulic conductivity (hydraulic segmentation) and be more vulnerable to cavitation (vulnerability segmentation).

2 | MATERIALS AND METHODS

We measured the xylem structure and function of 17 species of mature (>30-year-old) trees growing on campus at California State University, Bakersfield (Table 1; Figure S1). An 18th species was originally intended to be included in this study, *Ulmus parvifolia* Jacq. (Ulmaceae), but we were unable to measure the hydraulic function of this species due to copious mucilage production in cut stems. For the measured species, samples from six or more individuals were measured per species. All sampled individuals were growing within campus landscaping, were irrigated, were in full sunlight, and were healthy (Figure S2). Sampling occurred during the summers of 2013 and 2014, after spring and early-summer xylem formation had ceased.

Xylem structural and functional traits were measured following the harvesting of branches from trees using a hand saw, large loppers, or a rope saw. Very large branches (3 to 5 m) were collected from trees in the early morning and brought immediately to the lab where they were processed. Samples were selected in series so that they were a minimum of 1, 2, and 3 years old in samples that had annual increments of branch elongation of more than 0.15 m and spanned several ages and included older samples in species that had shorter

TABLE 1 Tree species included in the present study as well as their leaf habit and mature wood growth ring porosity type

Scientific name	Common name	Family	Leaf habit	Mature wood porosity
<i>Callistemon</i> sp. (cultivar)	Bottlebrush	Myrtaceae	Evergreen	Diffuse
<i>Ceratonia siliqua</i> L.	Carob tree	Fabaceae	Evergreen	Diffuse
<i>Cercis canadensis</i> L.	Redbud	Fabaceae	Deciduous	Ring to semiring
<i>Cinnamomum camphora</i> (L.) J. Presl	Camphor tree	Lauraceae	Evergreen	Diffuse to semiring
<i>Eucalyptus polyanthemos</i> Schauer	Silver dollar gum tree	Myrtaceae	Evergreen	Diffuse
<i>Fraxinus americana</i> L.	White ash	Oleaceae	Deciduous	Ring
<i>Koelreuteria paniculata</i> Laxm.	Golden rain tree	Sapindaceae	Deciduous	Ring
<i>Laurus nobilis</i> L.	Bay laurel	Lauraceae	Evergreen	Diffuse
<i>Magnolia grandiflora</i> L.	Southern magnolia	Magnoliaceae	Evergreen	Diffuse
<i>Magnolia</i> × <i>soulangiana</i> Soul.-Bod.	Saucer magnolia	Magnoliaceae	Deciduous	Diffuse
<i>Parkinsonia floridum</i> (Benth. ex Gray) S. Wats.	Palo verde	Fabaceae	Deciduous (photosynthetic bark)	Diffuse to semiring
<i>Pistacia chinensis</i> Bunge	Chinese pistachio	Anacardiaceae	Deciduous	Ring to semiring
<i>Platanus occidentalis</i> × <i>orientalis</i> = <i>Platanus</i> × <i>hispanica</i> Mill. ex Münchh.	London planetree	Platanaceae	Deciduous	Ring to semiring
<i>Pyrus calleryana</i> Decne. (cultivar)	Flowering pear	Rosaceae	Deciduous	Diffuse
<i>Quercus robur</i> L.	English oak	Fagaceae	Deciduous	Ring
<i>Schinus molle</i> L.	Peppertree	Anacardiaceae	Evergreen	Diffuse
<i>Triadica sebifera</i> (L.) Small	Chinese tallow tree	Euphorbiaceae	Deciduous	Diffuse

increments of annual elongation (Figure 1). Vascular cambium ages were classified as being young (1 to 4 years), intermediate (2 to 7 years), or older (3 to 10 years; age ranges for each age category for each species are included in Table 2). Cambial age was determined by counting terminal bud scars (when visible) and was confirmed through cross-sectional analysis of growth ring number. Only the outermost growth ring was sampled (i.e., current-year growth) for all anatomical and functional measures.

2.1 | Hydraulic conductivity and vulnerability to cavitation

Young, intermediate, and older xylem segments were identified in series on a branch, and 0.15-m or longer segments were cut from the branches underwater for each cambial age category. Segments were then further trimmed underwater using fresh razor blades to a length of 0.14 m. In intermediate and older segments, the inner growth rings were sealed using cyanoacrylate resin, which sets underwater, so that flow was restricted to the current year's growth. Prior to the outset of measurements, active xylem staining (Jacobsen et al., 2018) was used to verify that this resin blocked flow through the inner sealed rings. Samples were sealed, and dye was pulled up through the xylem under mild suction following the methods of Jacobsen et al. (2018). This was followed by sectioning to evaluate dye patterns. In these samples, dye was predominantly restricted to the outer growth ring, with only minimal dye spread into the older growth rings at the growth ring boundary consistent with dye

“bleeding” through current-year tissues and not with flow. Thus, sealing of the inner rings was effective at limiting flow through older xylem. All samples from an individual branch were processed together on a single day, so that comparisons were controlled within each branch and represented xylem that was grown in the same year, sampled at the same time, and measured under the same conditions.

Maximum xylem-specific conductivity (K_s) was determined following the flushing of samples. Samples were flushed for 1 hr at 100 kPa using degassed ultrafiltered (in-line filter Calyx Capsule Nylon 0.1 μ m, GE Water & Process Technologies, Trevose, PA) 20 mM KCl solution. Maximum hydraulic conductivity was then measured gravimetrically using a conductivity apparatus under slight positive pressure (1.5–2 kPa), and values were corrected for background values measured at 0 kPa. Conductivity values were divided by the xylem cross-sectional area of the outer growth ring as measured microscopically from a thin cross section of the distal stem end.

Vulnerability to cavitation curves were measured on the same samples using the standard centrifuge method (Alder, Pockman, Sperry, & Nuismer, 1997) with hydration reservoirs containing foam pads (Tobin, Pratt, Jacobsen, & De Guzman, 2013). Segments were subjected to increasing negative xylem pressures by being spun in a custom centrifuge rotor, and hydraulic conductivity was measured following each spin. These values were used to construct a vulnerability curve of the loss in conductivity at each imposed water potential relative to the initial flushed (maximum) value. Curves were plotted for each sample and were fit using a Weibull curve (Microsoft Excel 2010, Microsoft, Redmond, WA) to calculate the water potential at 50% loss in hydraulic conductivity (P50).

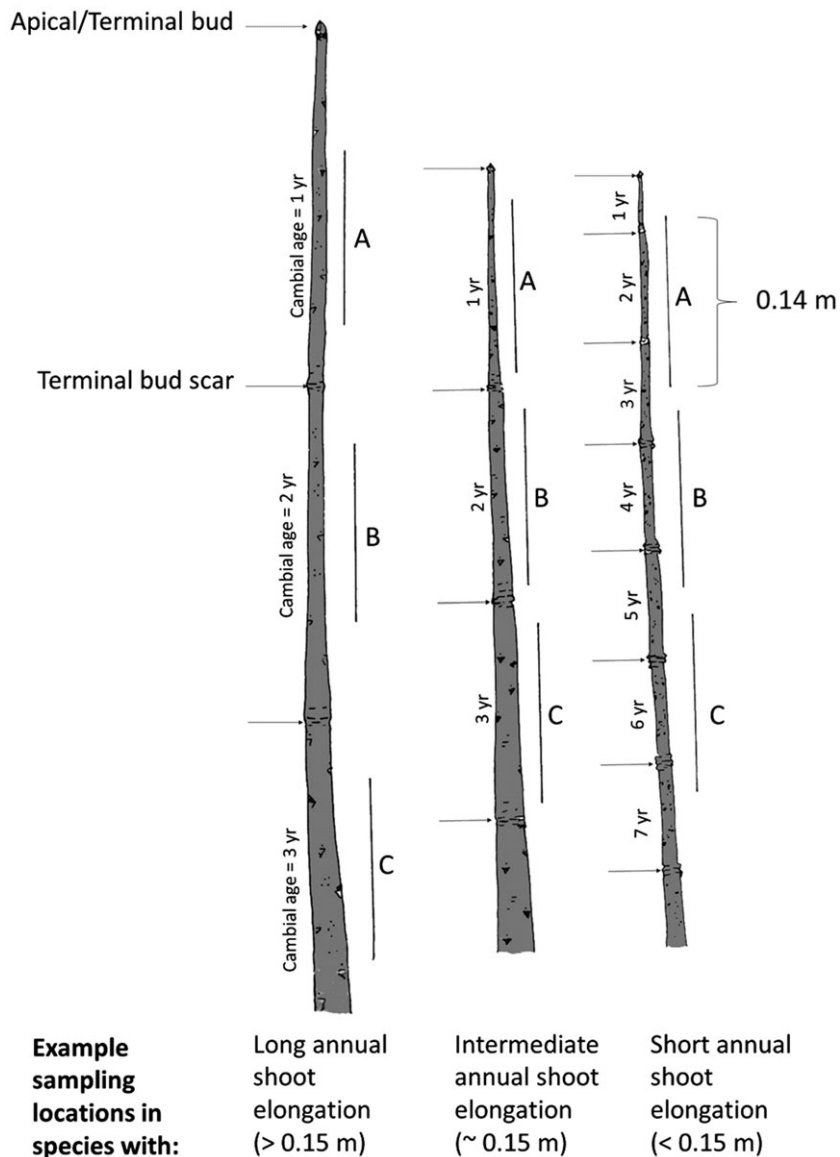


FIGURE 1 Xylem produced by vascular cambia of different ages sampled from 17 tree species. For species that produced greater than 0.15-m elongation growth each year, samples were spaced so that they represented xylem produced by 1-, 2-, and 3-year-old cambia for young, intermediate, and older aged samples (left and centre examples), with 0.15-m samples excised and then trimmed to a length of 0.14 m for functional measures. For samples with less than 0.15-m annual shoot elongation, samples were collected in series along the branch, with each stem segment containing multiple cambial ages (right). The ages or age ranges of sampled segments thus depended on shoot elongation increments for each species. The ages of sampled stem segments for each species are reported in Table 2

2.2 | Vulnerability to air-seeding using single-vessel air injection

Different methods used to generate vulnerability to cavitation curves may produce different results. The standard centrifuge technique as used in the present study, when compared with other techniques, has generally been found to produce reliable results (e.g., Sperry, Christman, Torres-Ruiz, Taneda, & Smith, 2012; Jacobsen & Pratt, 2012; Hacke et al., 2015; Venturas et al., 2016); however, some have reported potential measurement errors with similar static rotor designs (Choat et al., 2010; Torres-Ruiz et al., 2014). This has led to the recommendation that more than one method be used in order to evaluate methods within studies (Hacke et al., 2015; Sperry et al., 2012).

We selected a subset of species of special interest to examine using single-vessel air injection (SVAI) as described in Venturas et al. (2016). The first species that we selected was *Laurus nobilis*, which is a basal angiosperm that contains very short vessels (Jacobsen et al., 2012). The vulnerability of *Laurus* xylem to cavitation has been a topic

of recent interest and debate (Cochard, Delzon, & Badel, 2015; Nardini et al., 2017). The second species that we selected was *Callistemon*, which was one of the longest vesselled species included in the current study (see vessel length results below). This species was selected because some studies have suggested that vessel length may be an important factor influencing reliable vulnerability curve determination. An additional species from the present study, *Quercus robur*, has also been of recent interest (Cochard et al., 2010; Tobin et al., 2013). We examined young samples from this species; and the results of this comparison, which was conducted concurrently to the comparisons mentioned above and on the same individuals included in the present study, have already been published (Venturas et al., 2016).

Vulnerability to cavitation was measured for all three cambial age categories for our selected species using SVAI, which measures the pressure required to seed gas across the largest pit membrane pore within a vessel. This is an independent method of vulnerability to cavitation determination, because it relies on positive pressure applied to single vessels rather than the xylem tensions created by

TABLE 2 For each species, three cambial age categories were sampled, with the age range of the sampled categories dependent upon the length of annual stem expansion (Figure 1)

Species	Cambial age (category)	Cambial age (years)	Stem diameter (mm)
<i>Callistemon</i>	Young	2 to 4	3.81 ± 0.40
	Intermediate	4 to 7	4.94 ± 0.43
	Older	6 to 10	7.05 ± 0.49
<i>Ceratonia</i>	Young	1	5.31 ± 0.20
	Intermediate	2	7.89 ± 0.26
	Older	3	9.79 ± 0.21
<i>Cercis</i>	Young	1	3.55 ± 0.13
	Intermediate	2	5.25 ± 0.21
	Older	3	7.64 ± 0.21
<i>Cinnamomum</i>	Young	1 to 2	5.48 ± 0.23
	Intermediate	2 to 4	7.13 ± 0.17
	Older	3 to 5	9.38 ± 0.41
<i>Eucalyptus</i>	Young	1 to 3	4.05 ± 0.22
	Intermediate	3 to 5	5.89 ± 0.18
	Older	4 to 8	7.78 ± 0.22
<i>Fraxinus</i>	Young	1	4.68 ± 0.15
	Intermediate	2	5.93 ± 0.23
	Older	3	7.06 ± 0.51
<i>Koelreuteria</i>	Young	1	6.68 ± 0.32
	Intermediate	2	10.33 ± 0.83
	Older	3	11.45 ± 1.06
<i>Laurus</i>	Young	1 to 2	4.92 ± 0.33
	Intermediate	2 to 3	7.18 ± 0.43
	Older	3 to 5	9.46 ± 0.30
<i>Magnolia grandiflora</i>	Young	1 to 2	6.84 ± 0.33
	Intermediate	2 to 4	8.94 ± 0.23
	Older	3 to 8	10.54 ± 0.32
<i>Magnolia × soulangeana</i>	Young	1	4.02 ± 0.18
	Intermediate	3	5.71 ± 0.17
	Older	5 to 6	7.69 ± 0.33
<i>Parkinsonia</i>	Young	1 to 2	4.76 ± 0.20
	Intermediate	2 to 3	6.77 ± 0.23
	Older	3 to 4	8.58 ± 0.24
<i>Pistacia</i>	Young	1	6.50 ± 0.37
	Intermediate	2	8.35 ± 0.47
	Older	3	9.83 ± 0.54
<i>Platanus</i>	Young	1	4.16 ± 0.21
	Intermediate	2	5.68 ± 0.24
	Older	3	8.16 ± 0.48
<i>Pyrus</i>	Young	1	4.68 ± 0.22
	Intermediate	2	6.72 ± 0.16
	Older	3	9.44 ± 0.18
<i>Quercus</i>	Young	1	4.10 ± 0.42
	Intermediate	2 to 3	5.27 ± 0.18
	Older	3 to 4	7.12 ± 0.36
<i>Schinus</i>	Young	1 to 2	4.28 ± 0.16
	Intermediate	2 to 3	5.64 ± 0.20
	Older	3 to 5	7.36 ± 0.24
<i>Triadica</i>	Young	1 to 3	3.26 ± 0.21
	Intermediate	2 to 3	5.15 ± 0.37
	Older	3 to 5	7.64 ± 0.47

Note. Abbreviated names for each species are included below along with the cambial age range for each age category for each species and the stem diameters (mean ± 1 SE) from the measured samples. Full species names are included in Table 1.

centrifugation. Samples were collected and prepared as described above, and cambial age categories identified. A 55-mm segment was removed underwater from the central region of each age category sample. Prior to SVAI determination, emboli were removed from samples by submerging them in 20 mM KCl and vacuum infiltrating (−91 kPa) them for 1 hr. SVAI methods follow those described in

Venturas et al. (2016) in which each xylem segment was secured vertically under a stereo microscope (Olympus Corporation, Tokyo, Japan) with the proximal end submerged in 20 mM KCl degassed solution. A glass capillary was inserted into a single vessel within the outer growth ring, and the other end of the capillary was connected via tubing to a pressure chamber (PMS Instrument Company, Albany, OR),

which was used to slowly increase the pressure being pushed into the vessel. The proximal sample end was observed until a pressure was reached that resulted in bubbles emerging from a vessel or vessels. This indicated that gas had been seeded through a pit membrane of the injected vessel.

For each cambial age sample, the air-seeding pressure of four vessels was determined, and six samples from different individuals per species per age were measured for a total of 72 values per species. SVAI data were plotted as a cumulative distribution (%) of the pressure thresholds that seeded air through the sample. This was then used to determine the median air-seeding pressure (P_m), which represents the threshold at which 50% of vessels air seeded. The P_m values and 95% confidence intervals were calculated using the methods described in Venturas et al. (2016), with bootstrapping used to generate datasets by randomly resampling with replacement of the observed datasets.

2.3 | Vessel diameter and growth ring porosity

Diameters of the vessels within the outer growth ring were measured on the same samples as used for vulnerability curves. Stem segments were sectioned using a sledge microtome and imaged using a light microscope (Olympus BH-2, Olympus Imaging Corp., Center Valley, PA) attached to a digital camera (Spot Insight 2, v. 18.2 Color Mosaic, Diagnostic Instruments, Inc., Sterling Heights, MI). The outermost growth ring was divided visually into earlywood and latewood portions, and the area of vessel lumens was measured using an image-analysis program (ImageJ, 1.44p, National Institutes of Health, USA). For each sample, between 50 and >100 vessels were measured within the earlywood and within the latewood, and all vessels within a sector of current-year xylem were sampled. Conduit diameters were calculated from the measured lumen areas on the basis of the assumption that conduits were circular.

For each sample, multiple vessel diameter parameters were calculated. This included the mean vessel diameter and the maximum vessel diameter for the entire outer growth ring as well as the mean and maximum vessel diameter values for the earlywood and latewood separately. The porosity of the outer growth ring was quantified by taking the ratio of the earlywood mean vessel diameter to the latewood mean vessel diameter (E:L; Beery, Ifju, & McLain, 2007). For this parameter, E:L values that were not significantly different from 1 represent diffuse porous xylem, and E:L values that were significantly greater than 1 indicate ring porous xylem. Values were categorized as being greater than 1 and indicating ring porosity if the 95% confidence interval of the mean for E:L values did not include 1 within the interval.

2.4 | Vessel length

Maximum vessel length was determined using an air-injection method (Greenidge, 1952). For each tree, three different branch ages were injected at distal positions corresponding to young, intermediate, and older aged samples. Because of the need to destructively sample

branches for these measures, samples from different age categories were measured on different large branches collected from individual trees. Very large branches, even larger than those required for other measures, were required for these measures. In some cases, different trees were sampled for vessel length than those used for other measures because of the need to harvest such large branches and limitations on available material to sample. Vessel length measures were sometimes variable, and so for some species, we increased the sample size (up to as many as 15 individuals per species). In other cases, where only a limited number of individuals of a species were available, we have a reduced sample size (the lowest number of sampled individuals was four for a species). Sample sizes per species and age for vessel length measures are included in Table S1.

Stems were injected using nitrogen gas at an injection pressure of 100 kPa from branch tips toward the base. Injection points were selected to occur within each of the three cambial age categories. Branches were shortened at the basal end by 0.1-m increments when >2 m, 0.05-m increments when >1 m, 0.02-m increments when >0.5 m, and 0.01-m increments when <0.5 m. Maximum vessel length was estimated as the length of a branch at the first sight of streaming gas bubbles emerging from an open vessel plus half the length of the previously cut increment.

2.5 | Analyses

We were interested in examining the influence of cambial age and porosity on xylem vessel structure and function. For these analyses, the porosity produced by trees within their mature wood was used, regardless that their young stems produced diffuse or ring porous wood. Wood porosity classifications were based on those reported in the InsideWood database of wood traits (Wheeler, 2011). Diffuse porous mature wood included species reported as having diffuse wood (Category 5) or diffuse to semiring porous wood (Categories 4 and 5). Ring porous mature wood included species reported as having semiring to ring porous wood (Categories 3 and 4) to ring porous wood (Category 3; Wheeler, Baas, & Gasson, 1989; see Table 1 for categories for each species).

Data were analysed using a mixed-model analysis of variance with cambial age (young, intermediate, older), porosity (ring, diffuse), and age \times porosity, with individual trees as random replicates nested within species. For vessel length, there was no repeated-measures term, and the model included cambial age, porosity, and cambial age \times porosity, with species as a random treatment factor. Our sampling scheme was not designed for analysis of differences within or between species, and we focused on sampling a broader number of species rather than intensively within species. Thus, because of limited within-species sampling, we did not include any within- or between-species comparisons in our primary analyses or discussion; however, we conducted a post hoc Tukey's analysis of species \times age categories to illustrate some of the species-specific patterns in change with cambial age. For analyses, P50 was square root transformed; K_s , vessel diameter, and vessel length data were transformed using a Box-Cox

transformation; and E:L was transformed by taking the inverse for normality. Contrasts were for preplanned comparisons between age and porosity, conducted to determine statistical significance. Analyses were conducted using JMP software (JMP 13.2.1, SAS Institute Inc., North Carolina).

3 | RESULTS

3.1 | Most species produced diffuse porous xylem

Most of the samples we examined contained diffuse porous xylem (E:L not different from 1) across all three cambial age categories, including for species that later produce ring porous wood within the bole of mature trees and for samples that were up to 10 years (Figure 2). Only three species displayed ring porous wood (E:L > 1) within any of their sampled cambial age groups. *Magnolia grandiflora*, which produces diffuse porous mature wood, contained large earlywood vessels in xylem produced by young and intermediate aged cambia and then transitioned to diffuse porous wood in older samples. *Cercis* began producing ring porous wood in samples that were within the older cambial age category (3 years old for this species) but produced diffuse porous xylem in young (1 year old) and intermediate (2 years old) aged samples. *Fraxinus* produced ring porous xylem in all cambial ages examined. All other species produced diffuse porous wood across all cambial ages examined. For the analyses presented below, porosity categories were based on mature wood type. In interpreting these analyses, it is important to remember that comparisons were generally of currently diffuse porous xylem in species that may or may not later produce ring porous wood.

3.2 | Validation of vulnerability to cavitation curves

Vulnerability to cavitation was determined using the standard centrifuge method for all species and for a subset of species using a reference technique, SVAI. Hydraulic conductivity declined with decreasing xylem pressure, and these declines were used to calculate the water potential at 50% loss in hydraulic conductivity (Figure 3; P50). For our species selected for additional methods comparison, vulnerability curves obtained with the standard centrifuge and SVAI techniques were consistent. For both species and for all cambial ages, the P50 values fell within the 95% confidence limits of P_m values (Table 3, Figure 4). This was also the case for the young *Quercus* samples that were examined (results reported in Venturas et al., 2016). For *Quercus*, the standard centrifuge P50 was -1.36 MPa, and the SVAI P_m was 1.13 MPa with 95% lower and upper confidence limits of 0.61 and 1.40 MPa. Note that the SVAI pressures are positive; they measure the pressure difference required for seeding air through the largest pit membrane pore, which would be equal to the required pressure difference between the xylem (negative, $P < 0$) and atmosphere (reference, $P = 0$) for allowing air-seeding cavitation (Sperry & Tyree, 1988).

3.3 | Xylem structure and function changed with cambial age and porosity

Vessels increased in size with cambial age, including increases in both vessel length and diameter (Figure 5, Table 4). Younger stems contained narrower vessel diameters than intermediate and older stems. Vessel diameter increases were present for both diffuse and ring porous species. Mean vessel diameter was used for analyses; other vessel diameter traits showed similar patterns and were strongly correlated to mean vessel diameter (Table S2 and Figure S3). Vessel

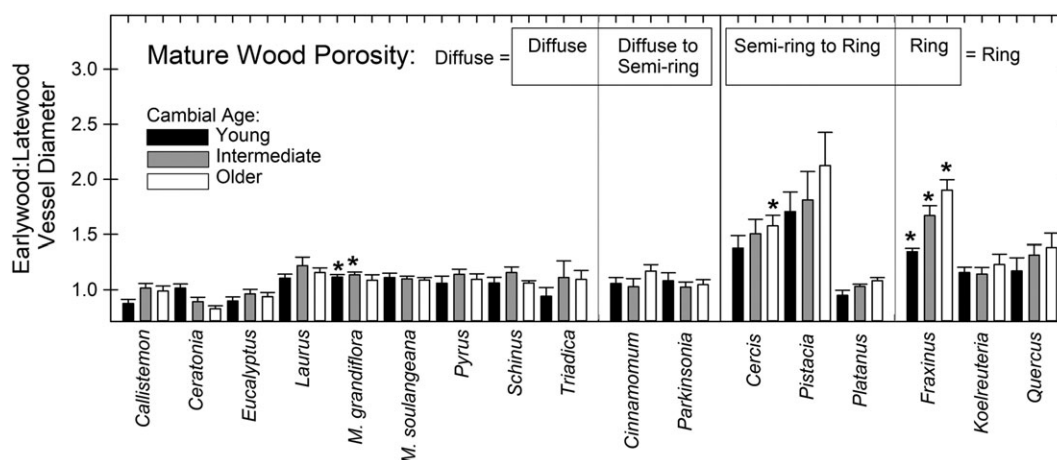


FIGURE 2 Mature wood porosity type of either diffuse (diffuse porous and diffuse to semiring porous) or ring (semiring to ring porous and ring porous) and the growth ring porosity (earlywood to latewood vessel diameter ratio) of current-year growth within measured cambial age categories. Ring porous wood has a ratio of earlywood:latewood (E:L) mean vessel diameter significantly greater than 1 (indicated by asterisks). Different cambial ages are indicated by different bar fills, and each bar represents the mean with a whisker representing 1 SE. Species are organized by mature wood porosity type, with diffuse species first (up to the first grey vertical line), then diffuse to semiring porous species (up to the black line, which separates pooled diffuse porous species), then semiring to ring porous species, and finally ring porous species. See Table 1 for full species names

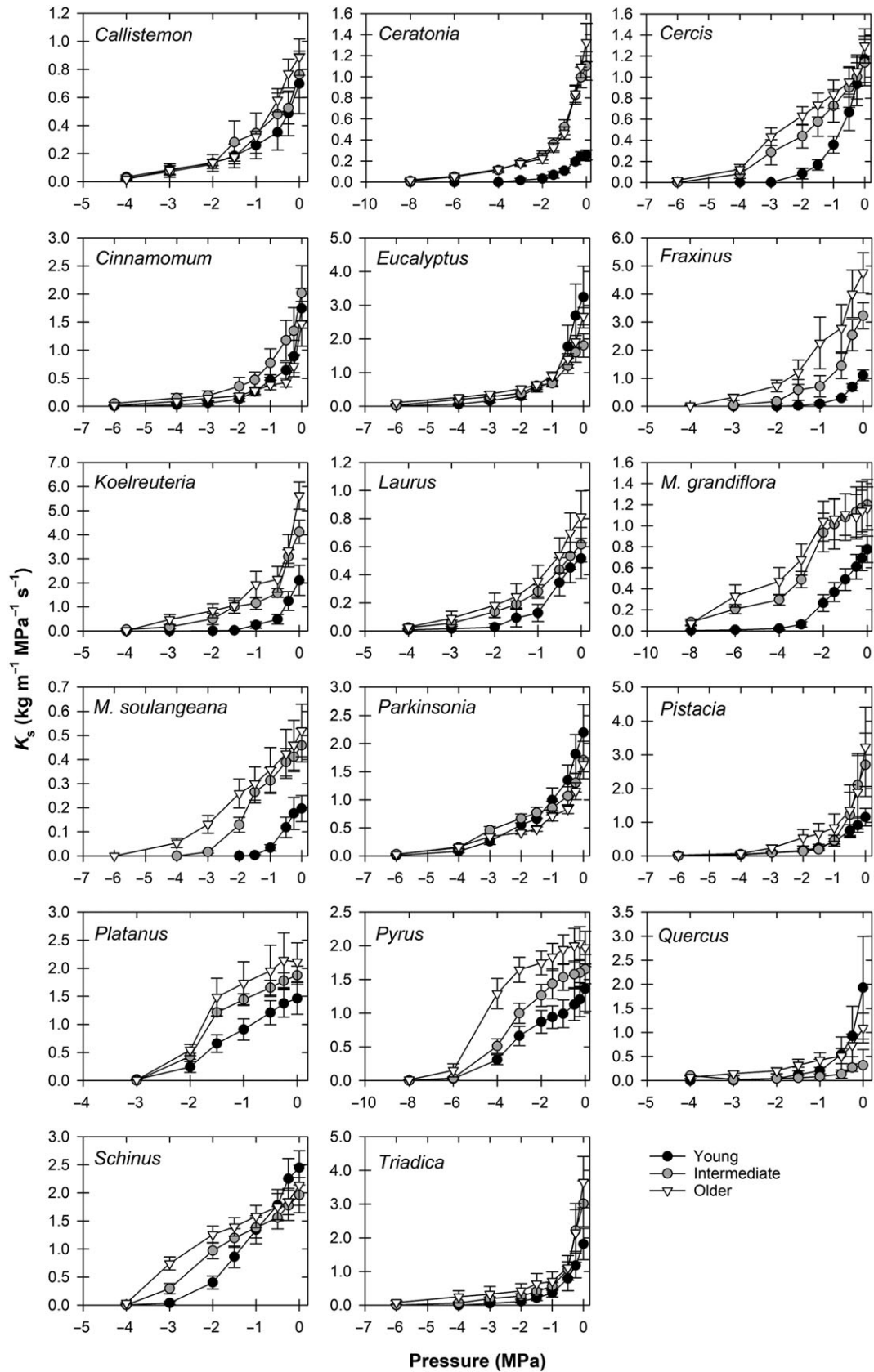


FIGURE 3 Vulnerability to cavitation curves showing the decline in xylem-specific hydraulic conductivity (K_s) with decreasing xylem pressure for 17 tree species. For each species, six stem samples were measured for each of three different cambial ages (young, intermediate, and older). Each point represents the mean $K_s \pm 1$ SE. Full species names are included in Table 1. Note that axes are on different scales for each panel

TABLE 3 The water potential corresponding to 50% loss in hydraulic conductivity (P_{50}), the median air-seeding pressure (P_m), which represents the threshold at which 50% of vessels air seeded as measured using single-vessel air injection, and the 95% confidence limits (upper and lower limits) for P_m values for two selected species and three stem cambial age categories

Species	Cambial age	P_{50} (MPa)	P_m (MPa)	P_m 95% confidence limits (MPa)
<i>Callistemon</i>	Young	-0.65 ± 0.19	0.35	0.14–0.74
	Intermediate	-0.80 ± 0.18	0.39	0.17–0.87
	Older	-0.78 ± 0.09	0.36	0.19–0.90
<i>Laurus</i>	Young	-0.70 ± 0.08	0.67	0.38–0.96
	Intermediate	-0.98 ± 0.07	0.88	0.61–1.49
	Older	-1.01 ± 0.19	1.00	0.54–1.41

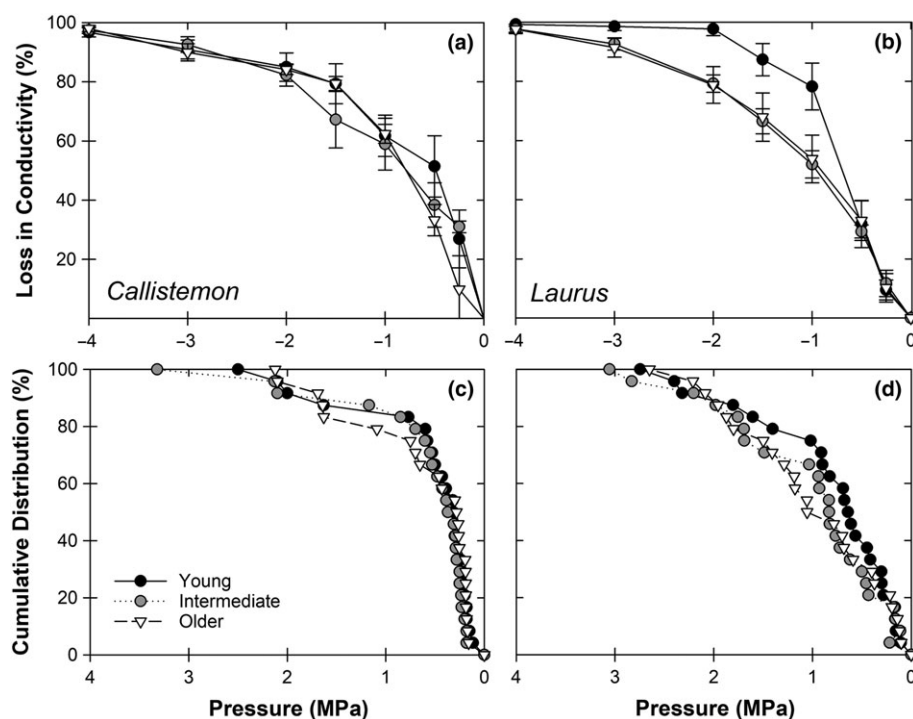


FIGURE 4 Vulnerability to cavitation curves showing the percentage decline in hydraulic conductivity with decreasing xylem pressure (a, b) and the cumulative distribution of vessel air-seeding pressures (c, d) for two selected species, *Callistemon* sp. (a, c) and *Laurus nobilis* (b, d). For hydraulic conductivity curves, six stem samples were measured for each of three different cambial ages (young, intermediate, and older), and each point represents the mean % loss in hydraulic conductivity ± 1 SE

length increased with cambial age; however, there was a significant interaction between cambial age and porosity for vessel length. Vessel length increased with cambial age for both diffuse and ring porous species, but the increase in length with age was greater for ring porous species. Growth ring porosity increased with cambial age for ring porous species, but not for diffuse porous species.

Xylem became both more conductive and more resistant to cavitation with cambial age. Xylem tissue increased in hydraulic transport efficiency (xylem-specific hydraulic conductivity; K_s) and cavitation resistance (water potential at 50% loss in hydraulic conductivity; P_{50}) with cambial age (Figure 5, Table 4; Table S3). Xylem produced by the youngest cambia had lower K_s and less negative P_{50} than had older samples. Ring porous species increased greatly in their K_s with cambial age, and diffuse porous species had more negative P_{50} with cambial age.

Vessel structure and xylem function differed between diffuse porous and ring porous species (Figure 4, Table 4). Ring porous species had longer and wider vessels and xylem that had higher conductivity and was more vulnerable to cavitation. These differences between porosity types were not present in young samples but were present among older samples.

Among the sample species, changes with cambial age varied and were often species specific. Across species and ages, vessel diameter and length were correlated (Pearson correlation, $P = 0.004$, $r = 0.402$), but the within-species patterns were variable (Figure 6; Table S4). Although limited sampling within species limited our power to detect within- and between-species differences, we note that some species diverged from the overall patterns described above. For instance, *Ceratonia* and *Pistacia* tended to be more vulnerable (had less negative P_{50}) in samples from older cambial ages (Table S3), the

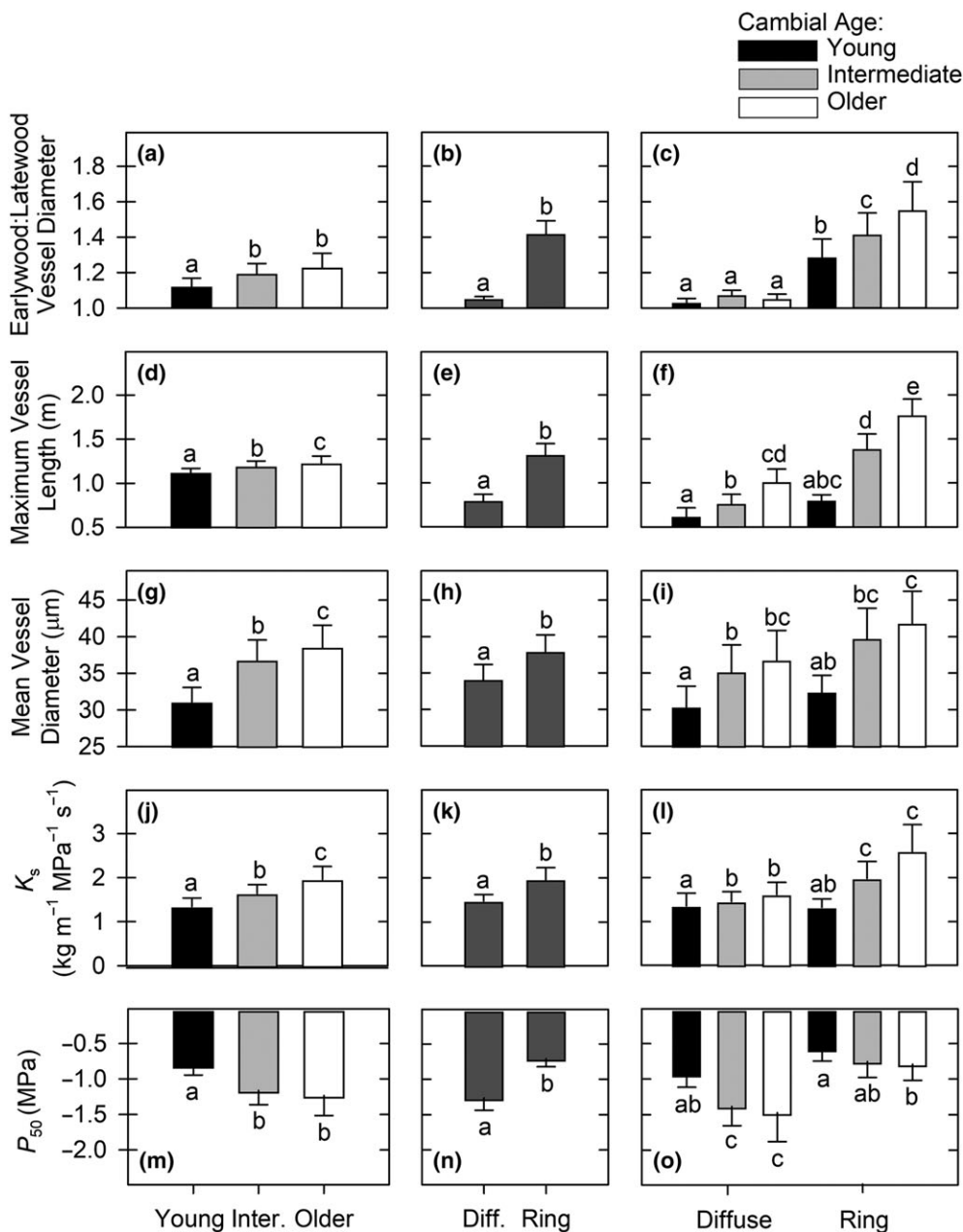


FIGURE 5 Changes in xylem structural and functional traits among 17 species with cambial age (young, intermediate, older), mature wood growth ring porosity (ring, diffuse), and the interaction between these terms for several xylem structural and functional traits including the porosity of current-year growth (ratio of earlywood to latewood vessel diameters [E:L]), maximum vessel length, mean vessel diameter, hydraulic conductivity (K_s), and cavitation resistance (P_{50}). Within each panel, different letters indicate significant differences among categories. Each bar represents the across-species mean \pm 1 SE

inverse of the overall pattern. Comparisons between each species and their different cambial age categories are shown in Table S4.

4 | DISCUSSION

4.1 | Cambial age and vessel structure in current-year growth

Across 17 angiosperm trees, xylem vessel characteristics of current-year growth changed with increasing cambial age in distal branches.

This included changes in vessel diameter, vessel length, and growth ring porosity. These changes were also associated with changes in xylem tissue hydraulic function. The vascular system of plants changed even over just a few years of cambial development, and previous studies suggest that even larger changes are likely across greater ranges of shoot cambial age (Anfodillo et al., 2006; Jacobsen et al., 2018; James et al., 2003; McCulloh et al., 2010; Olson et al., 2018; Rosell et al., 2017; Zimmermann, 1978).

Large changes in vessel structure and hydraulic function occurred between young samples and those that were intermediate and older. This pattern was especially evident in vessel diameter, vessel length

TABLE 4 Results from a mixed-model analysis of variance with cambial age (young, intermediate, older), mature wood porosity (ring, diffuse), and the interaction between these terms for several xylem structural and functional traits including the porosity of current-year growth (ratio of earlywood to latewood vessel diameters [E:L]), maximum vessel length, mean vessel diameter, hydraulic conductivity (K_s), and cavitation resistance (P50). Significant differences ($P < 0.05$) are indicated in bold text.

Trait	Cambial age	Porosity	Porosity × Cambial age
E:L	$F(2, 200) = 10.010; P < 0.001$	$F(1, 100) = 51.822; P < 0.001$	$F(2, 200) = 5.542; P = 0.005$
Vessel length	$F(2, 367) = 89.151; P < 0.001$	$F(1, 15) = 7.308; P = 0.016$	$F(2, 367) = 11.651; P < 0.001$
Vessel diameter	$F(2, 200) = 66.217; P < 0.001$	$F(1, 100) = 4.498; P = 0.036$	$F(2, 200) = 1.882; P = 0.155$
K_s	$F(2, 200) = 19.339; P < 0.001$	$F(1, 100) = 10.172; P = 0.002$	$F(2, 200) = 2.282; P = 0.105$
P50	$F(2, 200) = 11.424; P < 0.001$	$F(1, 100) = 6.859; P = 0.010$	$F(2, 200) = 0.822; P = 0.441$

of ring porous species, and cavitation resistance. The presence of primary xylem in the current year's growth of young 1-year-old stems compared with only secondary xylem tissue within older stems may partially explain this pattern. Vessels within the primary xylem tend to be both narrower and shorter than secondary xylem vessels and differ in their intermembrane pit structure (Bailey, 1944; Choat, Lahr, Melcher, Zwieniecki, & Holbrook, 2005).

4.2 | Delayed onset of ring porosity

Species that produce ring porous growth rings in their mature bole xylem generally did not produce ring porous growth rings within the distal stems examined in the present study. This is consistent with many prior studies describing the delayed onset of ring porosity (Cochar & Tyree, 1990; Gilbert, 1940; Lo Gullo et al., 1995; Rosell et al., 2017). Of six ring porous species, only two (*Cercis* and *Fraxinus*) produced ring porous xylem within the studied cambial ages. For the remaining species, the onset of ring porosity is likely delayed until cambia are older than the samples we examined. Although ring porous species were not yet producing ring porous xylem, ring porous and diffuse porous species differed in the xylem traits that we examined, with this difference driven by inclusion of older cambial samples within analyses.

Young samples of ring and diffuse porous species were not different from one another in vessel diameter, vessel length, K_s , or P50. This similarity in both structure and function of young distal stems makes it

difficult to interpret past studies that have compared the xylem of ring and diffuse porous species from young samples. For example, Sperry, Nichols, Sullivan, and Eastlack (1994) measured 1- to 3-year-old samples, Taneda and Sperry (2008) measured 2- to 3-year-old samples, and Cochar et al. (2010) measured 1-year old samples. These stem age and size ranges are typical of plant hydraulic studies (reviewed in Choat et al., 2012), and for most species, it is unlikely that these types of terminal stems displayed differences in their porosity.

Suggestions for why distal stems from ring and diffuse porous species may produce different experimental results with some methods (e.g., Cochar et al., 2013) have assumed mature wood characteristics (long and wide earlywood vessels for ring porous species) that are not always present within the young samples typically measured for xylem functional studies. In the present study, terminal current-year shoots do not differ in either xylem function or vessel structure. Comparative studies may require alternative explanations, other than vessel size, for why there may be potential differences between young stems from ring and diffuse species. Alternatively, ring and diffuse porous species comparisons should be designed to compare xylem that exhibits these different growth ring types.

4.3 | Hydraulic and vulnerability segmentation

Xylem became more efficient and more resistant to cavitation in older xylem. These changes are consistent with both the hydraulic and

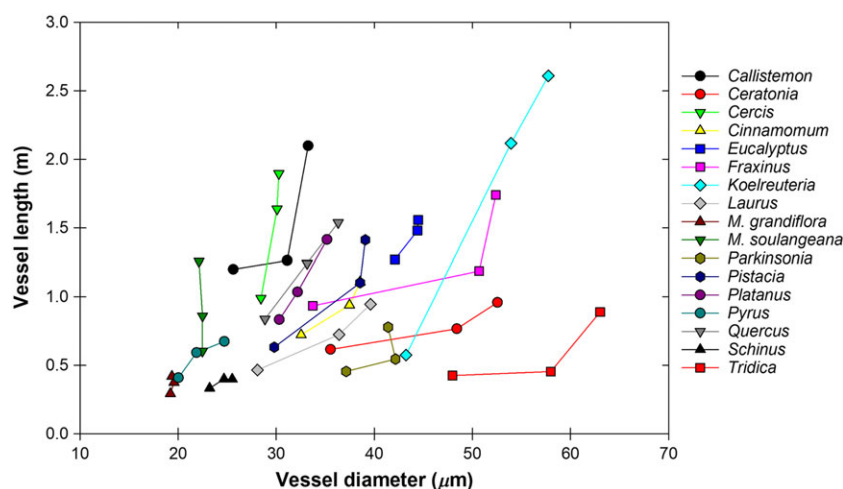


FIGURE 6 Across species and ages, vessel diameter and length were correlated (Pearson correlation, $P = 0.004$, $r = 0.402$). For each species and age category, the mean vessel diameter and maximum vessel length are shown. Within a species, lines connect the points representing young, intermediate, and older cambial samples

vulnerability segmentation hypotheses (Tyree & Ewers, 1991; Tyree & Zimmermann, 2002). Terminal young branches may represent an important hydraulic bottleneck within the whole-plant pathway, and they may also be functioning as relatively dispensable organs, given that they would experience more negative water potentials than other regions of the stem but were not more resistant.

In older samples, the combination of wider diameter and longer vessels with higher conductivity is not surprising, given the importance of vessel lumen and terminal vessel element resistances on flow through conduits (Jacobsen & Pratt, 2018; Mrad, Domec, Huang, Lens, & Katul, 2018); however, the combination of higher conductivity with decreased P50 is less intuitive (Gleason et al., 2016). This contrasts to what would be predicted on the basis of a safety–efficiency trade-off (Hacke, Sperry, Wheeler, & Castro, 2006). It seems likely that vessels produced by older cambia have different pit and pit membrane structure than have those produced in young samples. Xylem produced by older cambia could contain thicker or denser pit membranes, which would confer greater safety from cavitation. Changes in pit structure with cambial age deserves research attention, given the importance of pit traits in determining the hydraulic function of xylem tissue (Choat, Ball, Luly, & Holtum, 2003; Hargrave, Kolb, Ewers, & Davis, 1994; Lens et al., 2011; Li et al., 2016; Wheeler, Sperry, Hacke, & Hoang, 2005). In addition, it may be that younger samples have a higher proportion of fibre wall area (Jacobsen, Ewers, Pratt, Paddock, & Davis, 2005), whereas older samples may be able to partially rely on previous years' growth rings for mechanical support, allowing a greater proportion of the cross-sectional area to be vessel lumens. If all other factors were equal, this would increase efficiency (K_s) without necessarily impacting P50.

These patterns may differ across tissues produced by even older cambia than those included in the present study. The samples that we examined were relatively young and distal given the large sizes of mature trees. Although we found significant changes in both vessel structure and xylem hydraulic function, the magnitude of these changes was relatively small compared with those found between distal branches and mature wood from tree boles. In studies that have examined distal stem to bole xylem, the bole has contained very large conduits and has been more vulnerable to cavitation (Domec & Gartner, 2001; Jacobsen et al., 2018; Johnson et al., 2016; McCulloh, Johnson, Meinzer, & Woodruff, 2014; Melcher et al., 2003). Presumably, there is a point at which xylem shifts from becoming more resistant with cambial age to becoming less resistant, perhaps associated with the transition from juvenile to mature wood formation.

4.4 | Vulnerability to cavitation curves

There continues to be extensive discussion of the comparability of different vulnerability curve methods within the field of plant hydraulics. In this study, we used the standard centrifuge technique and compared those results to those of an independent reference technique, SVAI, for species of special interest. The standard centrifuge technique with the hydration reservoir modification (Tobin et al., 2013)

produced results that were similar to those obtained from SVAI for the species we examined.

The hydraulic measures presented in the current study are generally consistent with prior studies, including those that have used a broad range of methods, although comparisons are complicated by many studies not reporting the specific stem age that was examined and generally including all growth rings (not just current year's as in this study). This includes similarities between our results and those reporting the following: acoustic-emission-based curves for *Ceratonia siliqua* (Lo Gullo, 1991; Salleo, Gullo, Raimondo, & Nardini, 2001); centrifuge-based curves for *Cercis canadensis* (Maherali, Moura, Caldeira, Willson, & Jackson, 2006); acoustic emission curves for *Cinnamomum camphora* (Vander Willigen, Sherwin, & Pammenter, 2000); air-seeding thresholds for *Fraxinus americana* (Zwieniecki, Melcher, & Holbrook, 2001); acoustic emissions, hydraulics, and air injection for *L. nobilis* (Hacke & Sperry, 2003; Nardini et al., 2017; Nardini & Salleo, 2000; Salleo et al., 2001); centrifuge-based curves for *Koelreuteria paniculata* and *M. grandiflora* centrifuge (Litvak, McCarthy, & Pataki, 2012); long and short segment lengths measured with the centrifuge and SVAI for *Q. robur* (Tobin et al., 2013; Venturas et al., 2016); and centrifuge-based curves for *Triadica sebifera* (Pratt & Black, 2006). Although they agree with some prior studies, our data from our *Laurus* and *Quercus* curves differ from those of a few previous studies (e.g., Cochard et al., 2010; Cochard et al., 2015; Lamarque et al., 2018); however, these studies used either Cavitron-based methods, which are known to produce measurement artefact (Wang, Zhang, Zhang, Cai, & Tyree, 2014), or micro-computed-tomography-based estimates, which produce theoretical hydraulic conductivity estimates that may not correspond to measured conductivity because of violations of Hagen–Poiseuille flow assumptions (Jacobsen & Pratt, 2018; Mrad et al., 2018). Our results also contrast with those of studies that have suggested that longer vessels are more prone to a methodological artefact that may make samples appear more vulnerable to cavitation (Choat et al., 2010; Cochard et al., 2010; Cochard et al., 2013), because we found that older samples contained longer vessels but were more resistant to cavitation.

4.5 | Understanding whole-plant hydraulic structure and function

Vessel structure and xylem function varied with cambial age, with significant changes in vessel diameter, length, hydraulic conductivity, and cavitation resistance. Although 1-year-old stems were not different between species with different growth ring porosity types, differences between porosity types increased with cambial age. Young branches may not provide the most informative comparison of hydraulic structure and function across species nor the best indication of whole-plant hydraulic performance. Additionally, because changes in structure and function with cambial age were species specific, there does not appear to be a simple relationship that could be used to calculate the traits of older xylem from characteristics measured on young branches. Increased understanding of plant hydraulic function and architecture

likely requires the sampling of multiple-aged tissues, and plants may vary considerably in their xylem structural and functional traits throughout the plant body, even over relatively short distances or closely aged tissues. Hydraulic traits of longer lived plant body segments, such as older stems, roots, and bole xylem, may be more indicative of whole-plant water-stress tolerance than distal branches.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Vessel length measures were conducted on different stem samples than those used for other measures. In some cases, the large branches required for these measures could only be obtained from a subset of the 6 trees sampled for other measures and in other cases

we added additional trees. The number of individuals sampled, the diameter of the stem at the point of gas injection for each cambial age category (young, intermediate, and older) (mean \pm 1 SE), and the maximum vessel length of samples (mean \pm 1 SE) are included for all species and cambial age categories. Complete species names are included in Table 1.

Table S2. Vessel diameter was measured on a minimum of 50 earlywood and latewood vessels within the outer growth ring of samples of differing cambial age (young, intermediate, older). Mean (\pm 1 SE) and maximum values for several different vessel diameter traits are included below for each species and cambial age category. Complete species names are included in Table 1.

Table S3. Vulnerability to cavitation curves were generated for each of 17 tree species, with 6 samples measured for each species for each of three different cambial ages (young, intermediate, and older). Mean water potential at 50% loss in hydraulic conductivity (P50) and xylem specific hydraulic conductivity (K_s) values for each species and cambial age category are included below \pm 1 SE. Full species names are included in Table 1.

Table S4. Results from a Tukeys *post hoc* test comparing species differences for the primary traits, maximum vessel length, mean vessel diameter, hydraulic conductivity (K_s), and cavitation resistance (P50). Species differed for each of the traits ($P < 0.001$ for all), but there was a significant interaction between species and age for each of the traits ($P < 0.001$ vessel length and vessel diameter, $P = 0.024$ K_s , and $P = 0.006$ P50). For each trait, different letters indicate differences between a species*age category. Full species names are included in Table 1.

Figure S1. Representative branchlets and leaves from the 17 sampled tree species. For each species a branchlet is included as well as both

adaxial and abaxial images of leaves. All images were taken at the same time and are shown at the same scale, with a 6 cm scale bar included in the lower left corner of the image. Fruit or flowers are included if they were present in July at the time that this image was taken. Full species names are included in Table 1, and numbers correspond to each species as follows; 1: *Callistemon*, 2: *Ceratonia*, 3: *Cercis*, 4: *Cinnamomum*, 5: *Fraxinus*, 6: *Eucalyptus*, 7: *Koelreuteria*, 8: *Laurus*, 9: *M. grandiflora*, 10: *M. soulangeana*, 11: *Parkinsonia*, 12: *Pistacia*, 13: *Pyrus*, 14: *Quercus*, 15: *Schinus*, 16: *Platanus*, and 17: *Triadica*. Photo credit: ALJ.

Figure S2. Mature irrigated trees were sampled from California State University, Bakersfield, USA. Trees were > 30 years old and grown well-spaced from one another. Images show some of the sampled tree species and locations, including *Platanus* (A), *Eucalyptus* (B), and *Cinnamomum*, *Koelreuteria*, and *Pistacia* (C). Photo credit: ALJ

Figure S3. Vessel diameter traits (Table S2) were strongly correlated with one another ($P < 0.001$ for all shown correlations). Mean vessel diameter was selected for all additional analyses as a representative trait for vessel diameter traits.

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