

## INVITED SPECIAL ARTICLE

For the Special Section: Wood: Biology of a Living Tissue

# Functional lifespans of xylem vessels: Development, hydraulic function, and post-function of vessels in several species of woody plants

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**PREMISE OF THE STUDY:** Xylem vessels transition through different stages during their functional lifespan, including expansion and development of vessel elements, transition to vessel hydraulic functionality, and eventual transition to post-functionality. We used information on vessel development and function to develop a model of vessel lifespan for woody plants.

**METHODS:** We examined vessel functional lifespan using repeated anatomical sampling throughout the growing season, combined with active-xylem staining to evaluate vessel hydraulic transport functionality. These data were combined with a literature review. The transitions between vessel functional lifespans for several species are illustrated, including grapevine (*Vitis vinifera* L., Vitaceae), English oak (*Quercus robur* L., Fagaceae), American chestnut [*Castanea dentata* (Marshall) Borkh.; Fagaceae], and several arid and semi-arid shrub species.

**KEY RESULTS:** In intact woody plants, development and maturation of vessel elements may be gradual. Once hydraulically functional, vessel elements connect to form a vessel network that is responsible for bulk hydraulic flow through the xylem. Vessels become nonfunctional due to the formation of gas emboli. In some species and under some conditions, vessel functionality of embolized conduits may be restored through refilling. Blockages, such as tyloses, gels, or gums, indicate permanent losses in hydraulic functional capacity; however, there may be some interesting exceptions to permanent loss of functionality for gel-based blockages.

**CONCLUSIONS:** The gradual development and maturation of vessel elements in woody plants, variation in the onset of functionality between different populations of vessels throughout the growing season, and differences in the timing of vessel transitions to post-functionality are important aspects of plant hydraulic function.

**KEY WORDS** active-xylem staining; cavitation; chaparral; dye ascent; hydraulic conductivity; occlusions; tyloses; vessel development; vessel differentiation; wood formation.

In the secondary xylem tissue of woody angiosperms, many different cell types may occur, including fibers, vessel elements, and ray and axial parenchyma, with each of these cell types associated with specific functions of the xylem (reviewed by Pratt and Jacobsen [2017]). In mature sapwood, some of these cell types will be living, e.g., parenchyma, while others will have undergone autolysis to become functionally mature, e.g., vessel elements. Fiber types are more variable, with some

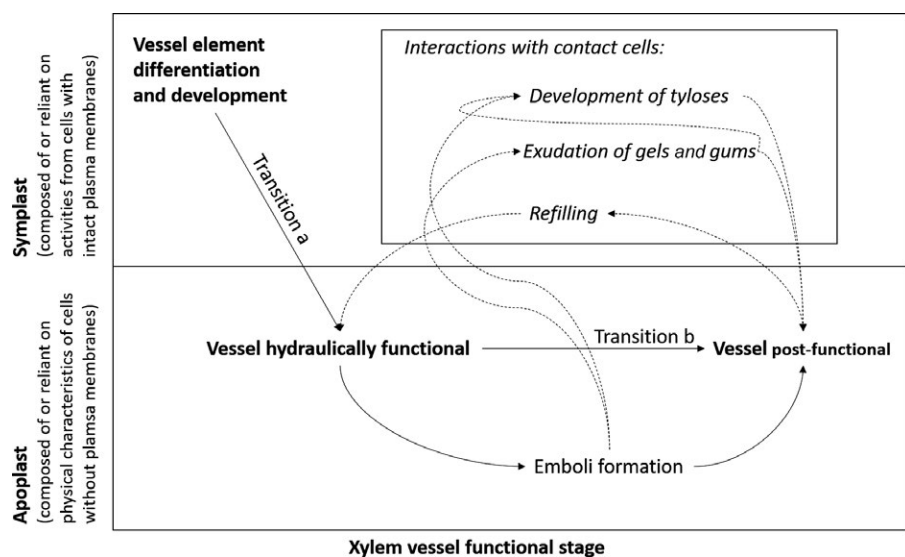
remaining alive, as in septate fibers, while others may undergo programmed cell death (Carlquist, 2013). Thus, within the secondary xylem, there are cells that maintain a cellular membrane and remain part of the plant symplast, while other cell types lose their cellular membrane and maintain only the apoplastic portion, the primary and secondary cell walls, of their cell body. These cell types interact and impact the hydraulic function of the xylem.

Bulk water transport within woody angiosperms occurs through xylem vessels. An individual vessel is composed of many vessel elements that connect axially through large openings termed perforation plates, so that many vessel elements connect to form a long continuous pipe. In the trunks of woody trees and vines, some individual vessels may extend several meters in length, but most vessels are less than a few centimeters long (Zimmermann and Jeje, 1981; Zimmermann and Potter, 1982; Ewers et al., 1990). Vessels begin and end at terminal vessel elements, which contain one end wall where a perforation plate does not form (Handley, 1936).

During the lifespan of a vessel, many changes occur (Fig. 1). Vessel elements differentiate and develop, forming connections to other vessel elements. When vessel elements lyse and their perforation plates open, they become hydraulically conductive. Vessels maintain contact with adjacent parenchyma cells. These connections occur through specialized vessel to parenchyma pits between vessel elements and neighboring contact cells, which are specialized cells of the parenchyma (Murakami et al., 1999). Eventually, vessels lose their ability to transport water after the formation of gas emboli and, in some cases, the subsequent formation of occlusions that block transport. Some species can restore vessel functionality through refilling, facilitated by living parenchyma cells within the xylem (Salleo et al., 2004). Each of these different stages within the functional lifespan of a vessel are examined and described below, with a special emphasis on the transition from vessel formation to hydraulic functionality (Transition “a” in Fig. 1) and the transition from hydraulic functionality to post-functionality (Transition “b” in Fig. 1).

## MATERIALS AND METHODS

Evaluation of the transition to hydraulic functionality requires information on vessel activity that may not be readily apparent



**FIGURE 1.** During the functional lifespan of a vessel, there are two important transitions: Transition “a” represents the transition from being a living and nonhydraulically functional vessel element to a hydraulically functional vessel, and Transition “b” represents the transition from being hydraulically functional to being non-functional. Vessels also interact with living contact cells within the xylem, particularly as related to Transition “b”. There are numerous interactions and transitions between living (symplast) elements within the xylem and cell or xylem components types that are nonliving (apoplast).

from anatomical measures alone, especially given the difficulty in fixing and sectioning samples without disrupting end walls of nearly functional elements (discussed by St. Aubin et al. [1986]). Active-xylem staining has been used for decades and is a well-supported technique to identify hydraulically active vessels or regions of xylem (e.g., Newbanks et al., 1983; Ewers et al., 1989; McManus et al., 1989; Fisher and Ewers, 1992; Hargrave et al., 1994; Kolb and Davis, 1994; Davis et al., 1999; Tibbetts and Ewers, 2000; Jaquish and Ewers, 2001; Sano et al., 2005; Jacobsen et al., 2007; Umebayashi et al., 2007; Cai and Tyree, 2010), although this technique has been used in only a limited number of studies in the context of vessel functional lifespan (Halis et al., 2012; Kudo et al., 2015; Jacobsen et al., 2015; also termed vessel “conductive lifespan”: Ellmore and Ewers, 1986; Ewers et al., 1991). With active-xylem staining, dye is often pulled up through either native or flushed samples under a mild suction or via transpiration. The use of suction recreates the negative pressure and flow direction of an intact transpiring plant, which may not be recreated with the use of positive pressure or bidirectional infiltration techniques. The dye travels through vessels that are conductive and stains their cell walls, marking them as functional in water conduction. Numerous dyes have been used for this method, such as crystal violet, safranin, and acid fuchsin, and the selection of dye depends on the specific plant material chemical and physical properties and differences in the preparation and sectioning of materials (e.g., Fisher and Ewers, 1992; Sano et al., 2005; Jacobsen et al., 2015). With all dye techniques, care is required to process and section samples to ensure limited staining of nonconductive cells due to positive pressure, capillarity, or diffusion, and many of the studies cited above have discussed how to identify and avoid these potential issues.

For the present study and review, we selected several species to serve as focal examples of different transitions during xylem vessel lifespan. These were selected because of their use in prior studies that have examined important transitions in vessel functional lifespan, including *Vitis vinifera* L. (Vitaceae) and *Quercus robur* L. (Fagaceae). We also examined *Castanea dentata* (Marshall) Borkh. (Fagaceae) and several species of arid and semi-arid shrubs, for which active-xylem staining was examined as components of prior studies. In brief, methods are described below for each of these focal examples, with references to published data when applicable.

For *V. vinifera*, active xylem data were included from Jacobsen and Pratt (2012) and Jacobsen et al. (2015). One-year-old shoots were removed from plants ( $n = 4-6$  per sample period) at the site of initial bud emergence. Sampling was repeated throughout the growing season. Shoots were collected at predawn, bagged, and transported to the laboratory where a 0.20-m stem segment was trimmed underwater from each large shoot at 1 m from the proximal end. All samples were collected at this same distance so that samples were of progressively older stems throughout the

growing season from shoots initiated at a similar time. Samples were inserted into a tubing apparatus and flushed for 1 h at 100 kPa using a degassed 20 mM KCl solution filtered to 0.1  $\mu\text{m}$  (Inline Filter, GE Water and Process Technologies, Trevose, PA, USA). A 0.10-m segment was then cut underwater from the longer flushed segments. This shorter segment was stained using a 0.1% (m/v) dye solution of crystal violet that was pulled up through the stems at approximately 2 kPa pressure for 20–30 min. Samples were flushed because the primary interest was to examine the transitions to hydraulic functionality and permanent loss in hydraulic function. Stained samples were sectioned in the central portion of the segment using a sledge microtome (Model 860, American Optical, Buffalo, NY, USA). Thin sections were mounted on slides in glycerol and photographed using a digital camera attached to a microscope (Zeiss Stereo Discover V.12 with Axiocam HRc digital camera). All vessels within the secondary xylem were counted, and each vessel was quantified as developing, hydraulically conductive, or post-functional.

The appearance and stability of gels as a permanent blockage to flow within vessels was also examined in three samples of *V. vinifera*. Each sample was scanned at high resolution (about 3  $\mu\text{m}$ ) in a high-resolution computed tomography scanner (HRCT; SkyScan 2211, Bruker, Kontich, Belgium) located at California State University, Bakersfield in the Biology 3D Imaging Center. Mildly dehydrated samples were cut in air to allow water-filled vessels to drain (i.e., gas-filled vessels become visible as black vessels rather than grey fluid-filled vessels). Relatively rapidly following cutting, gels were visible exuding from the cut sample ends as has been shown previously (Jacobsen and Pratt, 2012). Gels were visible on the surface of the scanned segments and also in the vessels immediately below the exuded gels, enabling easy confirmation of gel presence. Samples were then dried at room temperature for 24 h. During this period, the imaged cut end was open to air, but the rest of the sample was wrapped in plastic film to slow evaporation. The samples were then rescanned and the appearance and presence of the gel compared. All samples showed similar results.

Active-xylem staining was used to follow English oak (*Q. robur*) individuals for several months to examine the time from vessel element initiation to the onset of hydraulic function in young stems. Every 2 weeks from early March until the activation of most current season vessels in late May, six 1-year old stems were harvested from different individuals to record the number of new vessels forming. Samples were removed from trees while submerged underwater, then rapidly transported to the laboratory where they were trimmed underwater to 0.14 m in length. Samples were carefully flushed and their hydraulic conductivity ( $K_h$ ) measured gravimetrically.  $K_h$  was calculated by dividing the flow rate onto a balance (CP124S, Sartorius, Goettingen, Germany) by the pressure head, corrected for any non-zero background flow (Hacke et al. 2000). Samples were then stained to identify active vessels using the methods of Jacobsen et al. (2015) that are described above. Thin cross sections were prepared by hand using a razor blade (GEM single-edge stainless steel PTFE-coated blades, Electron Microscopy Sciences, Hatfield, PA, USA) and mounted on slides in glycerol for anatomical measurements. Photographs of the current year xylem and phloem were taken using a microscope (Leica DM750) with an attached camera (Leica MC120 HD). All stained vessels within each cross section were counted. Additionally, all the current-year-growth vessels were counted, and the proportion

of current-year vessels that were also hydraulically active (i.e., stained) was determined for each sample.

For American chestnut (*C. dentata*) the same methods as those described above were used for staining active xylem, although staining was conducted on native (nonflushed) samples from 1-yr-old seedlings. Active-xylem staining and anatomical measures were conducted on four individuals in mid-September to evaluate the relative proportions of developing, functional, and post-functional vessels in seedlings of this species. All samples showed similar results.

Active xylem was stained in 27 arid and semi-arid shrub species as part of the study described in Jacobsen et al. (2007). Active xylem was stained in 6–12 flushed samples from different individuals for each species during the spring and early summer. Active-xylem area was determined using a digital camera and image analysis software (Olympus SP-500UZ; Scion Image v. Beta 4.0.3, Scion Corp., Frederick, MD, USA). Stem samples were 6–8 mm in diameter and ranged from just a few years in age to more than 10 years of age in some of the desert shrub species examined.

## RESULTS AND DISCUSSION

### Differentiation and development: Pre-hydraulic function

Within the secondary xylem, vessel elements originate from meristematic cells in the vascular cambium (Esau, 1953). Cells that will become vessel elements are identifiable early in development by their wider diameter compared to other surrounding cells. The degree of cellular expansion is controlled by hormones (Doley and Leyton, 1968; Tuominen et al., 1997; Hacke et al., 2017) and potential interactions with external conditions (Doley and Leyton, 1968; Lovisolo and Schubert, 1998). Numerous studies have examined the impact of hormones and changes in expression that are associated with these early stages of vessel development and expansion (reviewed by Hacke et al., 2017).

Following early differentiation and expansion of vessel elements, secondary wall deposition begins. Secondary wall layers start to form toward the inside of developing vessel elements along the lateral walls (Esau and Charvat, 1978). Secondary wall material is not deposited along the lateral walls in the locations of forming pits (Barnett, 1982). The vessel element end walls (also termed perforation partitions; Meylan and Butterfield, 1981), which are located at the basal and apical cell ends, thicken (Esau and Hewitt, 1940; Esau and Charvat, 1978; Benayoun et al., 1981); however, these end walls later thin and dissolve (Esau, 1936). Following tonoplast rupture, organelles within the cell start to degrade and lignin deposition occurs and/or continues (reviewed by Fukuda, 1997 and Bollhöner et al., 2012).

Vessel elements may remain alive for weeks and at locations relatively distant from the vascular cambium within current-year growth. Zasada and Zaher (1969) conducted a study examining the timing vessel expansion and development in red oak (*Quercus rubra*) and at three different tree positions, including the trunk and young stems. In these trees, expansion of vessel element occurred over 5 weeks, and maturation took as long as 10 weeks in some earlywood vessels. Development was more advanced in the stems than in the bole samples, as also described by Kitin and Funada (2016). In *Populus*, Courtois-Moreau et al. (2009) found respiratory

activity of vessel elements several hundred microns distant from the cambium in *Populus*. Bollhöner et al. (2012: fig. 4D and E) showed that cell death was not triggered in vessel elements until a similar distance from the cambium.

Studies in woody plants, which show either distance from the cambium or time since differentiation prior to vessel maturation, differ from those in some nonwoody plants and cell and tissue cultures. For instance, within *Arabidopsis* hypocotyls, which undergo some secondary growth, xylem vessels undergo cell death immediately adjacent to the cambium (Bollhöner et al., 2012: fig. 4A and C), suggesting rapid maturation. In cultured cells, such as in *Zinnia* (reviewed by Fukuda, 1997), elements mature and open quickly over several days (Falconer and Seagull, 1985). However, in some nonwoody systems, vessel development is apparently a protracted process, and St. Aubin et al. (1986) described living vessels persisting as far as 0.3 m from the root tips in which they formed in maize roots.

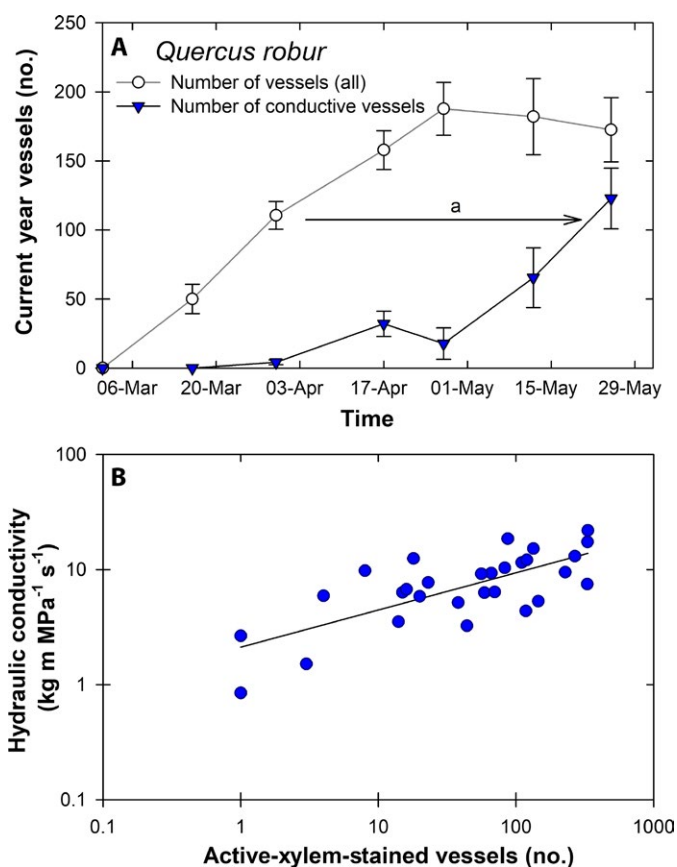
Vessel elements do not become hydraulically functional for bulk axial transport until after cell death, autolysis of cellular contents, and the dissolution and opening of the end walls to form a hollow tube. The cell membrane and remaining cellular components disintegrate at the same time or immediately following lignification of the secondary cell walls, and this may occur concurrent with end wall opening (Esau and Hewitt, 1940; Murmanis, 1978; Benayoun et al., 1981). Alternatively, the cellular contents may disintegrate without the opening of end walls, with hydraulic activity delayed. Meylan and Butterfield (1981) and Butterfield and Meylan (1982) suggested that the final removal of the perforation plate occurred due to the pull of the sap stream. This physical opening of the perforation plates may occur later and separate from autolysis of the cellular membrane. Thus, the onset of vessel hydraulic function may be delayed depending on whether end walls open at the point of cell death or if perforation plates remain intact until the transpiration stream pulls them open. In some species, the combined fragments of the perforation plates and cell membrane form a coating on the vessel walls following the opening of the vessel element. These cellular wall and membrane remnants are visible within both vessels and tracheids as a “warty” layer and as lipid fragments on the inner wall (Scott et al. 1960; Esau 1967; Parham and Baird 1974; Ohtani, 1979). Vessel elements within a vessel open in progression from the proximal to the distal end of the vessel (Esau, 1936; Eames and MacDaniels, 1947; Halis et al., 2012).

### Transition to hydraulic functionality

Using active-xylem staining, we followed English oak (*Quercus robur*) individuals for several months to examine the time from vessel element initiation to the onset of hydraulic function in young stems (Fig. 2). Vessels were initiated in early March, immediately before bud break on 16 March. At this time, some vessels from the previous year were active, but no current year vessels were active. Vessels took several weeks to fully expand, and hydraulic function for most vessels within the earlywood did not occur until 10 weeks after vessel initiation (Fig. 2A). These results are consistent with the timing of vessel element development described by Zasada and Zahner (1969) for *Q. rubra*. Leaves were flushed during this time and presumably relying on the vessels that remained active from the previous year and the active primary xylem vessels of newly elongated shoots. Consistent with this finding, Kudo

et al. (2015) also found that new vessels matured following bud break in both *Q. serrata* and *Robinia pseudoacacia*, but perforation plates may have opened more rapidly in these species than in the *Q. robur* we examined. In our samples, the number of stained vessels corresponded to the hydraulic transport capacity of the samples, including all stained vessels in the cross section, both current year growth and older. The relationship between conductivity and staining confirms that active-xylem staining was indicative of hydraulic functional status (Fig. 2B; linear regression,  $P < 0.001$ ,  $r^2 = 0.449$ ).

Grapevine has also been examined for the timing to hydraulic function of vessels in young stems. The observed amount of time from the initial differentiation of secondary xylem vessels to the appearance of hydraulically active vessels was approximately 4–8 weeks in *Vitis vinifera* (Fig. 4A; Jacobsen et al., 2015), including at least 2 weeks to differentiate and expand and another 2–4 weeks before autolysis and end-wall opening. Abundant developing and living vessels have been described for several varieties of *V. vinifera*, including ‘Glenora,’ ‘Chardonnay,’ and ‘Cabernet’ (Halis et al., 2012; Jacobsen et al., 2015).



**FIGURE 2.** English oak (*Quercus robur*) vessel development within the current growth ring of 1-yr-old stems shows a pronounced delay between the growth of new vessels and the transition of newly grown vessels to hydraulic functionality (A) (means  $\pm 1$  SE;  $n = 6$ ). This transition period is indicated with an arrow labelled with an “a”. The number of hydraulically conductive vessels (active-xylem-stained vessels) correlates with the hydraulic conductivity ( $K_p$ ) of samples (B) (each point indicates 1 sample,  $n = 36$ , linear regression shown). Bud break for these trees occurred on approximately 16 March.



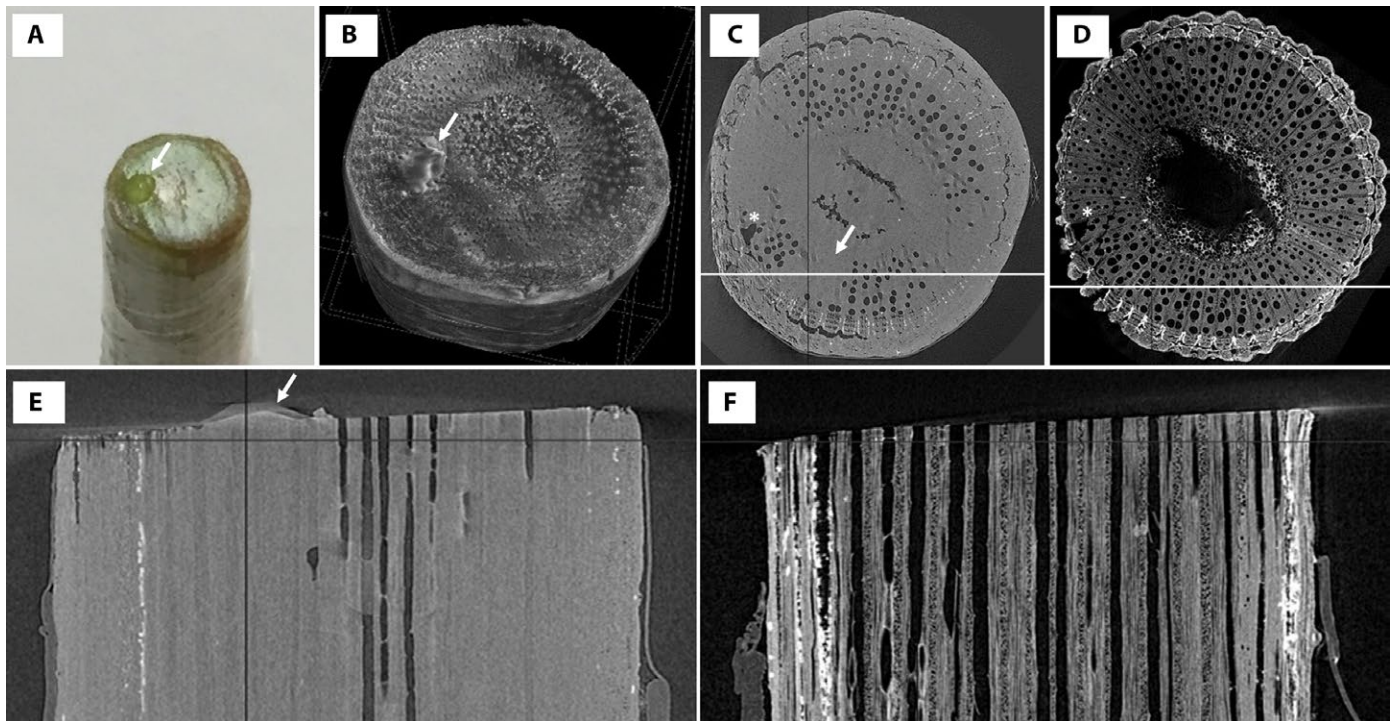
Once a vessel becomes hydraulically functional, it works as part of the vessel network to transport water from areas of higher pressure (e.g., roots in moist soil) to lower pressure (e.g., transpiring leaves) within the plant. Water and nutrients also are exchanged with living cells along the transport pathway, through contact cells. In *Populus*, contact cells form at the same time as vessel elements (Murakami et al., 1999) and exchange water with vessels as evidenced by the abundant expression of aquaporins within these cells (Almeida-Rodriguez and Hacke, 2012). There are numerous features of xylem vessels that impact the efficiency of hydraulic transport, including the diameter of vessels, vessel density, and the structure of vessel to vessel pits and pit membranes (reviewed by Venturas et al., 2017 and Pratt and Jacobsen, 2017).

### Transition to post-functionality

Once a vessel becomes functional, many factors may lead to a vessel becoming nonfunctional. Cavitation, and the resultant formation of gas emboli, is a common form of transport failure, and it occurs when water limitation results in xylem tensions that are beyond critical points of failure and gas seeds into functional conduits (Tyree and Zimmermann, 2002) and as a result of freeze-thaw stress (Sperry et al., 1994), mechanical damage (Jacobsen et al., 2005), or biotic nucleation. In some species and

under certain conditions, embolism may be reversible (Sperry et al., 1987; Hacke and Sperry, 2003; Salleo et al., 2004; Stiller et al., 2005; McCully et al., 2008). When embolism reversal occurs under negative tension, it is likely that refilling depends on connections between vessels and adjacent contact cells (Salleo et al., 2004; Nardini et al. 2011), representing another important interaction between vessels and living cells within the xylem (Fig. 1). Vessels may also become nonfunctional within the vessel network if they become isolated from other conductive vessels, either due to emboli or occlusions (Loepfe et al., 2007). While such vessels may retain the capacity to conduct water, they become nonfunctional within the xylem due to their position within the vessel network.

Other types of vessel blockages, such as gums, gels, or tyloses, often represent permanent loss of vessel hydraulic function and are produced by the living contact cells adjacent to vessels (Cochard and Tyree, 1990; Kitin et al. 2010; Jacobsen and Pratt, 2012; De Micco et al., 2016). The formation of these blockages may be triggered by dehydration and embolism (Jacobsen and Pratt, 2012), wounding (Sun et al., 2008), or infection (Vander Molen et al., 1977). Occlusions may also form as part of a controlled pattern of senescence of older vessels (Spicer, 2005). It is generally thought that occluded vessels are no longer able to transition back to hydraulic functionality, although some have suggested that gel formation may be reversible or part of the process



**FIGURE 3.** The appearance and stability during drying of gels within vessels was examined in *Vitis vinifera*. Relatively rapidly after cutting, gel was visible exuding from the cut sample end (A, white arrow). Gel was also visible on the surface of the high-resolution computed tomography (HRCT) scanned segments (B, white arrow) and in several vessels that were fully occluded with gel and visible as light grey “fluid” filled vessels in cross section (C; white line indicates the location of the longitudinal cut shown in panel e; arrow indicates location of the gel visible in A and B) and in several vessels below the exuded gel in longitudinal section (E; white arrow indicates the exuded gel and several fully occluded light grey gel-filled vessels are visible beneath the exuded gel). Gas-filled vessels appear as black vessels in HRCT images; water- and gel-filled vessels are grey. After the sample dried at room temperature for 24 h, the gel was no longer visible either at the surface of the sample nor in any of the vessels previously occluded with gel (D and F; asterisk in C and D indicate the same stem feature to assist with visual alignment of the samples). All of the panels within this figure are from the same sample.

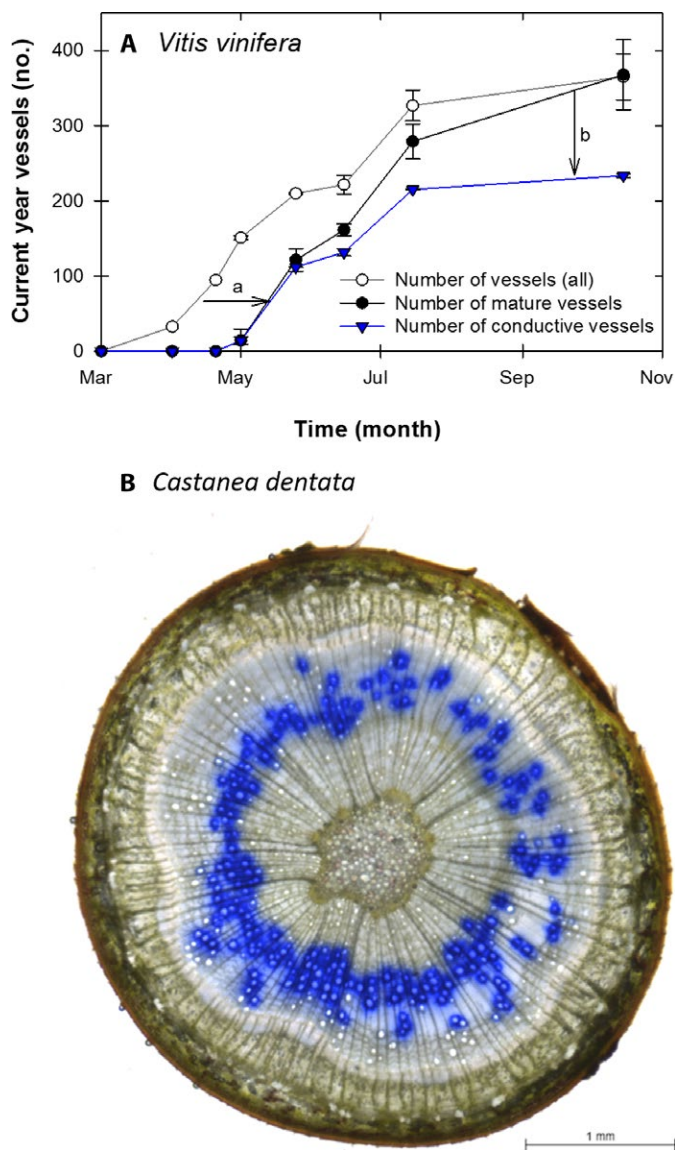
of refilling embolized conduits (Sun et al., 2008). Consistent with gels being potentially nonpermanent, we found that gels were not stable when grapevine samples were dried, even for a short time (Fig. 3).

Within woody plants, there are several examples of vessels within stems transitioning through all stages of their functional lifespan within a single growing season, especially the earlywood vessels of temperate tree species (Kozlowski and Winget, 1963; Umehayashi et al., 2010). Vessels transitioning through all stages of their lifespan within one growing season has also been reported for grapevine, where vessels within current year growth of young shoots develop, become hydraulically active (Transition “a” in Fig. 4A), and transition to post-functionality (Transition “b” in Fig. 4A) over several months (Jacobsen et al., 2015). A relatively high proportion of vessels, particularly in the earlywood, are not active by the end of the growing season, and the proportion of hydraulically functional vessels has been reported to be as low as 35% of the vessels within the secondary xylem tissue (Jacobsen et al., 2015). This same pattern of vessel functional lifespan has been observed within current year growth in American chestnut (Fig. 4B). Within stained late-summer samples, an inner ring of post-functional vessels was visible, and these vessels were predominantly filled with gums, with a few tyloses. Tyloses and gums in American chestnut have been well described, form rapidly, and are abundant (Bramble, 1938; Biggs, 1987; McManus et al., 1989; Ewers et al., 1990; McManus and Ewers, 1990). The outer ring of xylem nearer the vascular cambium contained large diameter vessels that were not yet hydraulically conductive (i.e. pre-functional), many of which were also not yet fully lignified. These changes represent loss of function within individual conduits and within sapwood, and they are separate from the changes that occur within the xylem during the sapwood to heartwood transition.

Changes in xylem hydraulic function seasonally (e.g., Sperry et al., 1987; Kolb and Sperry, 1999; Tibbetts and Ewers, 2000; Jacobsen et al., 2007) are consistent with changes in the suite of vessels active throughout the season, including changes that may occur in the specific vessels that are active, the number of vessels that are active, or the characteristics of active vessels. Vessels produced and hydraulically functional earlier in the season versus those produced later in the season may differ in hydraulic traits such as transport efficiency or resistance to water-stress-induced cavitation. Vessel lifespan impacts on hydraulic function may be most apparent in ring porous species, which have large, highly conductive vessels that are active early in the season and are more vulnerable to embolism. These vessels become nonconductive, develop tyloses in many species, and have shorter functional lifespans than narrower diameter latewood vessels (Ellmore and Ewers, 1986; Cochard and Tyree, 1990). Some species may have vessels with very short functional lifespans and rely on tracheids for water transport following periods of water stress or freezing (Carlquist, 1985; Pratt et al., 2015; Barotto et al., 2016). Although the lifespans of nonvessel conduits in angiosperms are not discussed further here, this is an interesting area of future study, especially since many economically important genera, such as *Quercus*, contain both vessels and tracheids within their xylem.

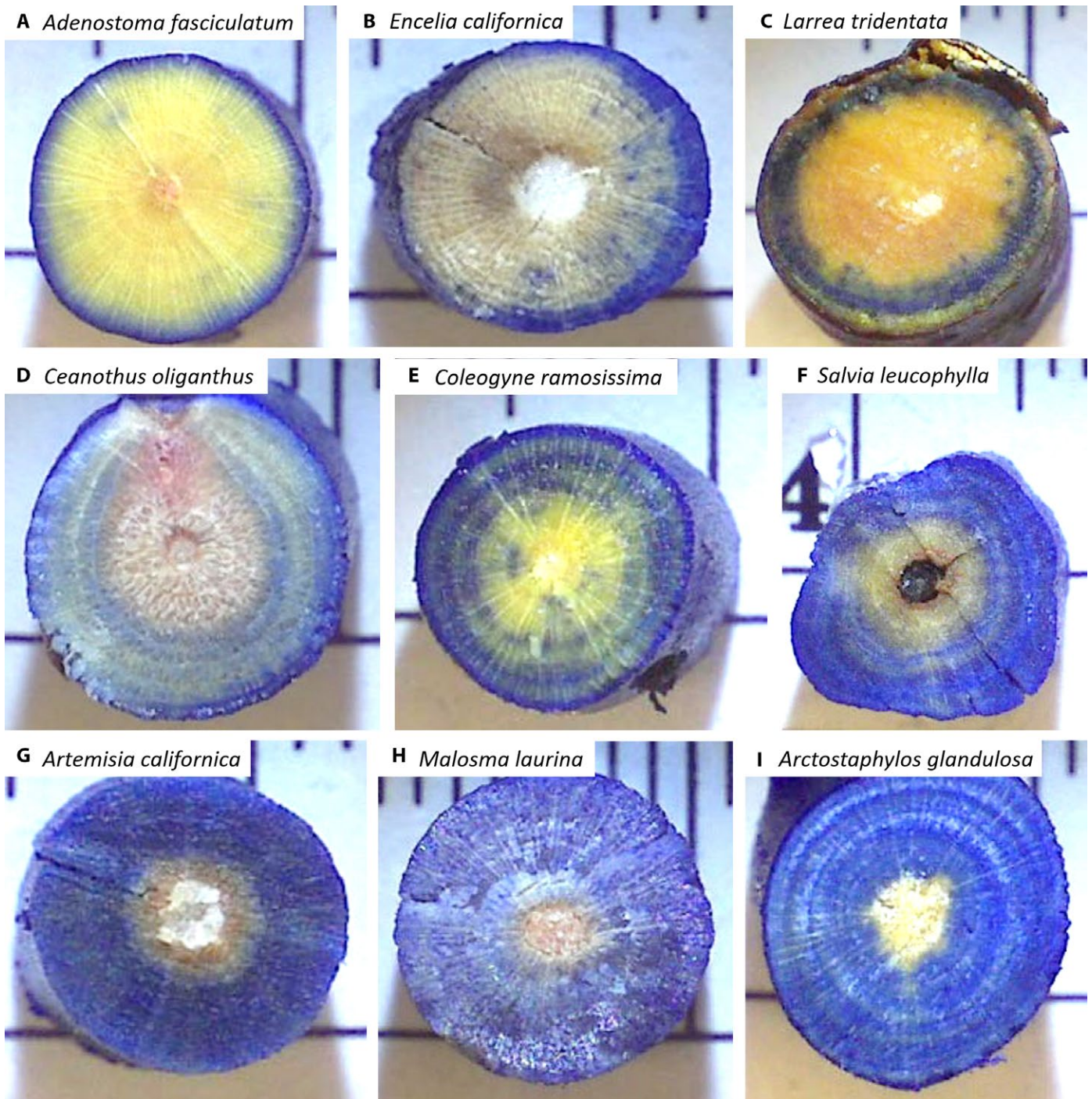
In some species, vessels may remain hydraulically functional for much longer periods within the sapwood. This divergence between species is well illustrated by the highly diverse patterns of active-xylem staining within the sapwood of different arid and semi-arid shrub species from California (Fig. 5), but has also been described for other species (Kozlowski and Winget, 1963; Gartner, 1991;

Fisher et al., 2002; Umehayashi et al., 2010). While some species rely on a relatively narrow band of active vessels near the cambium (Fig. 5A–C), others maintain several years of active xylem (Fig. 5G–I), and others are intermediate (Fig. 5D–F). These differences in



**FIGURE 4.** Grapevine (*Vitis vinifera*) vessel development within current year growth of new stems (A) showed a delay between the onset of new xylem formation and the transition of newly grown vessels to hydraulic functionality (Transition “a” marked with an arrow) and a late season transition of vessels from hydraulically functional to nonfunctional (Transition “b” marked within an arrow) (mean  $\pm$  1 SE;  $n = 4$ –6 for each point). Data from Jacobsen et al. (2015). A similar pattern is visible in late summer stems from 1-year-old American chestnut (*Castanea dentata*) seedlings (B), where there are vessels near the vascular cambium that are not yet functional, vessels that have transitioned (Transition “a”) to being hydraulically functional, and vessels in the central nonstained region of xylem near the pith that have transitioned to being hydraulically nonfunctional (Transition “b”). The central, nonfunctional region contained vessels that were occluded by gums; tyloses were visible in some samples.





**FIGURE 5.** Micrographs showing representative patterns of active-xylem staining from nine arid and semi-arid shrub species in California, arranged by smallest proportion of their xylem active (A) to the greatest (F). Species are identified for each panel. The short black ticks visible along the upper edge of each panel indicate 1 mm. All stem samples shown are several years old, but none contain heartwood, although most of these species will form a distinct heartwood region in older samples. See Jacobsen et al. (2007) for methods.

vessel functional lifespan could reflect differences in the ability of species to form permanent blockages within vessels as well as differences between species in the timing and development of heartwood (Sperry et al., 1991; Spicer, 2005). The relation of these divergent patterns of active xylem maintenance and other aspects of xylem function and hydraulic strategies could be an intriguing area of

future research and may be critical in understanding some aspects of plant physiology. To date, very few studies have examined vessel functional lifespan across different woody plant species or across different tree positions and organs, and there are abundant opportunities for study of the timing and mechanisms related to vessel functional transitions in these long-lived organisms.

## CONCLUSIONS

In some species, vessel elements within secondary xylem may take several weeks to develop, mature, and open before becoming hydraulically functional. Altering the timing of hydraulic activation and the proportion of vessels that are hydraulically active may enable plants to dynamically respond to changing conditions. Many of the transitions between different stages of vessel functional lifespans are dependent upon interactions with living elements of the xylem, particularly contact cells within xylem parenchyma. Although vessels are “dead” when hydraulically functional, they represent a component of the xylem that undergoes dynamic functional changes seasonally, annually, and inter-annually. The ability of plants to alter the cohort of hydraulically functional vessels through the maturation of new vessels and the occlusion of vessels is an underappreciated mechanism for xylem, a living tissue, to respond to changing conditions and hydraulic requirements.

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