

# Evaluating Carlquist's Law from a physiological perspective

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Accepted for publication: 21 July 2023; published online: 15 August 2023

**Summary** – “Carlquist's Law” is a striking pattern of association between anatomical features in the wood of vessel-bearing plants. It derives from Sherwin Carlquist's observation that xylem vessels tend to be solitary when embedded in a matrix of imperforate tracheary elements that appear to be conductive, whereas xylem vessels tend to be grouped when surrounded by seemingly non-conductive cells. Vessel-vessel contacts (vessel grouping) allow water to travel between conduits, but also provide pathways for air to propagate from embolized (air-filled) vessels into functional vessels. If the background matrix is conductive, it is conceivable that water could bypass embolized vessels, providing an alternative transport route in species with conductive backgrounds and solitary vessels. Much remains to be tested in this hypothesis, including the topology of the vessel networks in species with solitary versus grouped vessels and how conductive the different imperforate tracheary element types are. Exploring Carlquist's Law promises to provide key insight into the causes of embolism in plant conduits, the modes of embolism passage between conduits, and how vessels and the cells in which they are imbedded may interact to govern the pathways of water flow through plants.

**Keywords** – libriform fibers, tracheids, vasicentric and vascular tracheids, xylem embolism, xylem vessels.

*“Sherwin Carlquist describes one afternoon when the realization struck him: species with solitary vessels always have true tracheids. Was this correct? He wondered. He spent the rest of the day, until three in the morning, at the microscope, reviewing every species he could find to look at. Exhausted but elated, he found that the evidence bore out his intuition”.* (Olson, pers. commun.)

## What is Carlquist's Law?

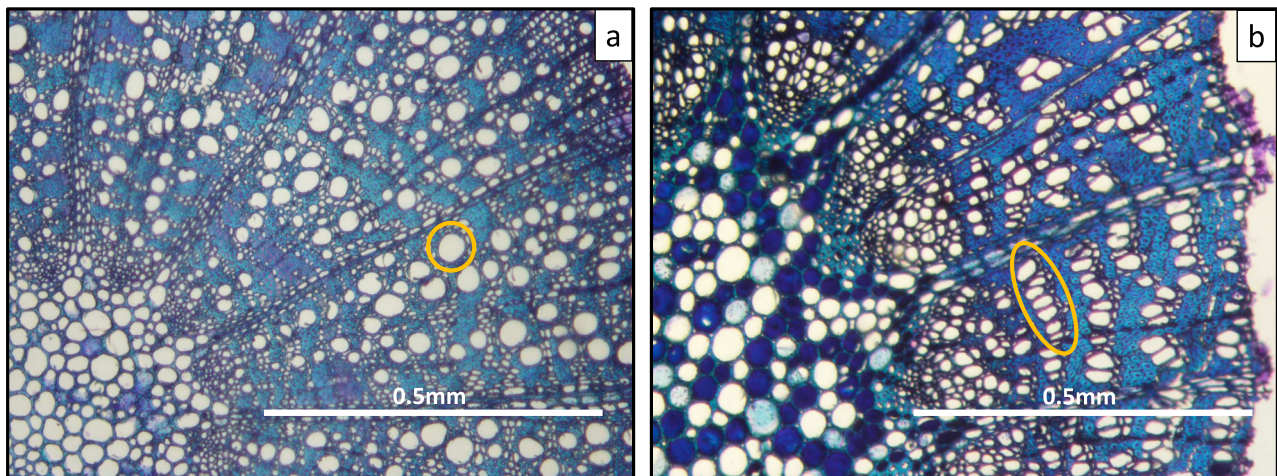
In 1984, from examination of the diverse stem-anatomy of a wide variety of woody plants from across more than 200 families, Sherwin Carlquist made the astute observation that the degree of vessel grouping in angiosperm stems is related to the nature of the cellular matrix in which they are embedded (Carlquist 1984). “Carlquist's Law” describes this observed association between vessel grouping and imperforate tracheary element type in woody, vessel-bearing plants (Olson 2020). When the background imperforate tracheary elements (those which do not have open ends and therefore do not form continuous pipes) appear to be non-conductive, vessels are grouped, but when these

background elements are conductive, vessels are almost invariably solitary (Carlquist 1984, 1987, 2001; Rosell *et al.* 2007; Lens *et al.* 2009).

The name “Carlquist’s Law” is a nod to the tradition of the late 19th and early 20th centuries of naming strong trait-trait or trait-environment patterns “laws” and honors Carlquist’s significant contribution to the field of comparative anatomy. This is not a law in the sense of being inexorable, but a striking pattern that requires the attention of comparative anatomists and xylem physiologists. To understand the precise nature of this pattern and its functional implications for the plant water transport system, there are a number of aspects which must be investigated and quantified. Here, we 1) discuss Carlquist’s functional explanation for this pattern in the context of understanding how anatomy relates to function in plants, 2) outline the key elements we must understand to evaluate Carlquist’s observation and its implications, and 3) discuss the potential implications of Carlquist’s Law.

### Vessel connectivity

When transverse sections are examined using light microscopy, ‘grouped’ vessels are defined as those which are connected to one or more adjacent vessels via pits, while ‘solitary’ vessels have no shared walls (Carlquist 2001; Fig. 1). This can be quantified using a ‘vessel grouping index’ as described by Carlquist (2001) whereby the total number of vessels is divided by the total number of vessel groupings (the number of solitary vessels plus the number of vessels in groups). A resulting vessel grouping index ( $V_G$ ) of 1 indicates that all vessels are solitary while a  $V_G > 1$  indicates increasing vessel grouping. It must be acknowledged, however, that light microscope images (microns thick) and MicroCT analysis (mm thick 3D scans obtained using powerful X-rays; McElrone 2013; Nolf *et al.* 2017) provide connectivity assessments based on only a short longitudinal stretch of a water transport system. Individual xylem vessels can, in some species, be multiple meters long (Pan *et al.* 2015), while the entire vascular system can extend over 100 meters in some trees (Koch *et al.* 2004).



**Fig. 1.** Transverse light microscope sections of small stems demonstrating contrasting vessel grouping patterns. In panel (a) *Allocasuarina verticillata* (Casuarinaceae) displays largely solitary vessels that are surrounded by abundant vasicentric tracheids which are postulated to be conductive, while *Banksia marginata* (Proteaceae; panel b) has highly grouped vessels that are surrounded by fibres which are thought to be non-conductive. Orange ellipses highlight an example of the typical vessel arrangement in the wood of both species, the pith is located in the bottom left of each image, and the white scale bars are 0.5 mm.

Vessel length and the number of pits in vessel walls (which provide pathways for water flow) are key components of vessel network connectivity that cannot be determined from microscope sections. Various methods have been used to determine the distribution of vessel length in woody tissue, many of which are laborious and some producing varied results (Ewers 1989; Link *et al.* 2018). Determining the number and distribution of pits is more straightforward and can be achieved through light microscopy but more detailed analysis of their size and features requires higher resolution approaches such as scanning electron microscopy or atomic force microscopy. Nevertheless, to truly understand vessel network connectivity, these features must be considered. With the complexities of quantifying vessel connectivity in mind, we turn to Carlquist's hypothesis for the patterns he observed.

Carlquist postulated a double-edged sword for vessel grouping in relation to the spread of air through the plant water transport system. Cavitation is the process by which air rapidly enters a xylem conduit (Tyree & Sperry 1988) resulting in the formation of an air bubble (embolism) and rendering the conduit non-functional. Cavitation often occurs due to declining water availability in drought conditions and has been shown to lead to plant damage and death (Tyree & Sperry 1988; Choat *et al.* 2012). Carlquist proposed that vessel-grouping is favored in species with non-conductive background cells because it creates redundancy in the plant water transport system, providing pathways for routing water around embolized vessels. However, vessel grouping also permits embolisms to be drawn into functional vessels. This is because vessel-vessel connections serve to increase xylem connectivity for radial water flow, but they also provide pathways for the propagation of air embolism through the vessel network. This is supported by research utilizing MicroCT to elucidate the drought-induced spread of xylem embolism in 3D. In conduit networks with high connectivity (such as tracheid-bearing Californian redwood and vessel-bearing grapevine), cavitation occurs largely in sectors, with multiple conduits embolizing together (Brodersen *et al.* 2013; Choat *et al.* 2015). Similarly, research using the Optical Vulnerability Technique (OVT; the use of optical light and cameras; Brodrribb *et al.* 2016) revealed the simultaneous cavitation of large sectors of tracheids in conifer species, *Callitris rhomboidea* (Johnson *et al.* 2021).

Therefore, it seems that water transport systems with solitary vessels would minimize the passage of gas from embolized vessels to functional ones, an idea also discussed by Zimmermann in 1983. This is supported by research using both MicroCT and the OVT which found that embolism propagates largely by single conduit events in the stems of three species with low xylem network connectivity (Johnson *et al.* 2020). Carlquist postulated that, in these species with mostly solitary vessels, the conductive background matrix provides an alternative pathway for water to move between functional vessels and around those rendered non-functional due to embolism.

### The cellular matrix: what does it mean to be 'conductive'?

The conductivity of a cellular matrix exists along a spectrum as all cells are permeable to water to some degree. Vessels have open end-walls allowing the movement of a continuous stream of water when stacked end-to-end. Living cells, on the low conductivity end of the spectrum, are permeable to water with their permeability driven largely by the density of aquaporins (membrane channels) in their walls (Hill *et al.* 2004). In cells with secondary walls, permeability is dominated by pits that mediate the flow of water between tracheary elements and provide resistance to air penetration.

Carlquist's Law defines cells as conductive or non-conductive. This classification is based on cell-anatomical features known to be associated with high conductivity or low conductivity in the context of the plant water transport system. This raises an important question, of whether the presence of these anatomical features always directly translates to the functional roles of these cells. Factors such as the placement of cells in a matrix in relation to other cell types and water sources (i.e., veins) would determine whether a cell has the capacity or opportunity to facilitate water movement. This is acknowledged by Carlquist, as he notes that cells he defines as 'conductive' are often arranged so that they provide lateral pathways between vessels (Carlquist 1984). However, to determine whether structure

relates to function in this instance, the conductivity of the cells surrounding the xylem, which are postulated to be conductive, should be tested.

## Conductive vs non-conductive imperforate tracheary elements

Carlquist's system for classifying imperforate tracheary element types is made up of five cell types, three "ground tissue" tracheary element types, and two "subsidiary" tracheary element types. "Ground tissue" imperforate tracheary elements constitute the predominant cell type of the matrix in which all of the other cell types are embedded, whereas "subsidiary conductive" tracheary elements occur only in specific locations. In Fig. 2 we illustrate and briefly summarize the five imperforate tracheary element types in Carlquist's classification system, and comment on the degree to which they are associated with solitary or grouped vessels.

### LIBRIFORM FIBERS

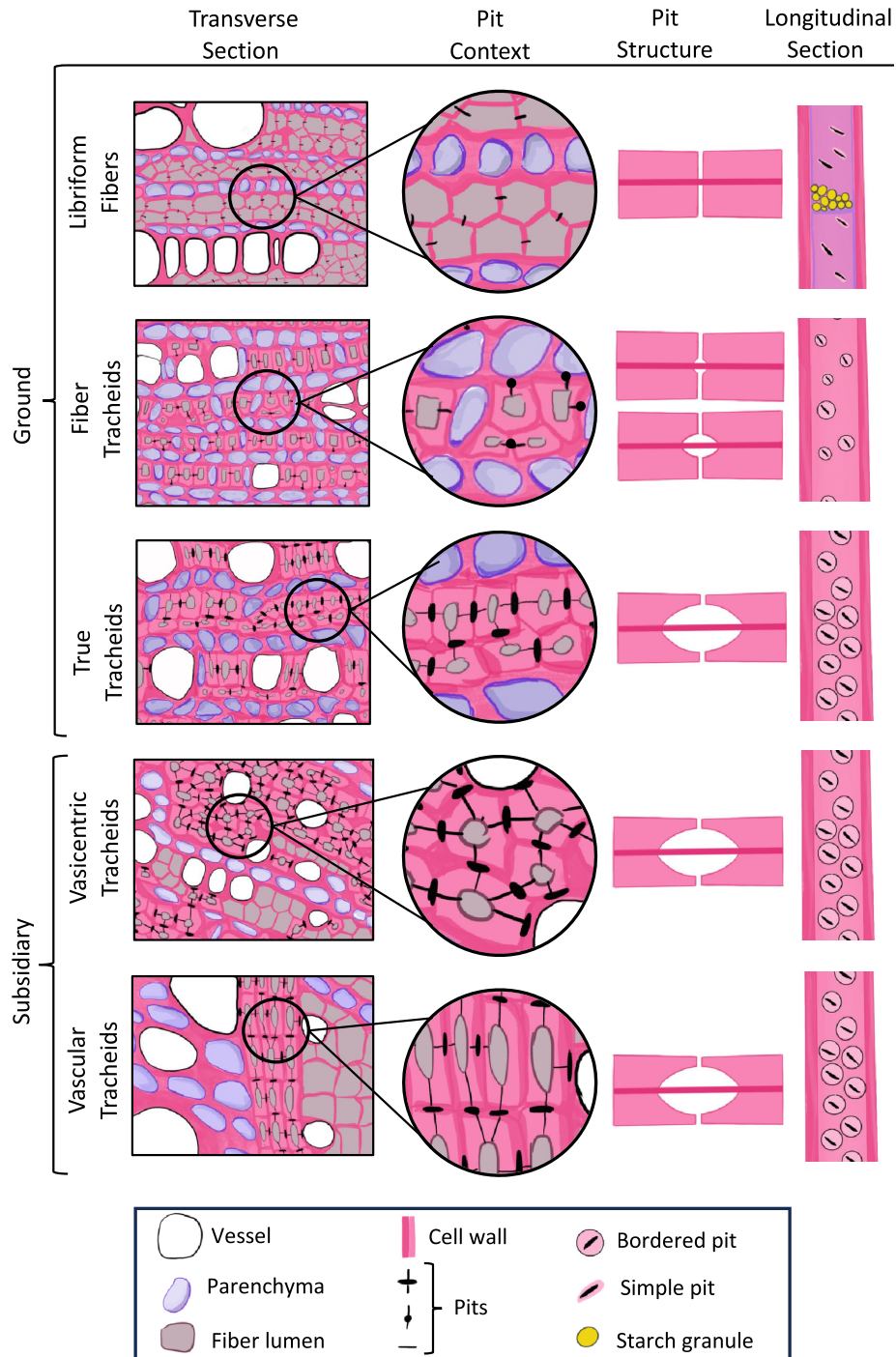
Libriform fibers are one of Carlquist's three ground tissue tracheary element types. Libriform fibers are thought to be non-conductive, and vessels are almost always grouped in their presence. Libriform fibers are the most common type of imperforate tracheary element globally (Echeverría *et al.* 2022). In Carlquist's classification, libriform fibers are regarded as having simple pits: there is no enlarged chamber associated with the pit aperture (though where the functionally relevant threshold between "simple" and "bordered" pits remains to be understood, see Baas 1986; Olson this issue). The pits tend to be sparse and are often slit-like, aligned with the cellulose microfibrils making up the cell wall. Libriform fibers are often living, as diagnosed by features such as primary wall septa traversing the lumen, storage of starch or other metabolites (Fig. 2), or the presence of nuclei, all of which would seem to preclude conductive status (Van Dam & Gorshkova 2003; Carlquist 2014; Plavcová *et al.* 2023).

### FIBER-TRACHEIDS

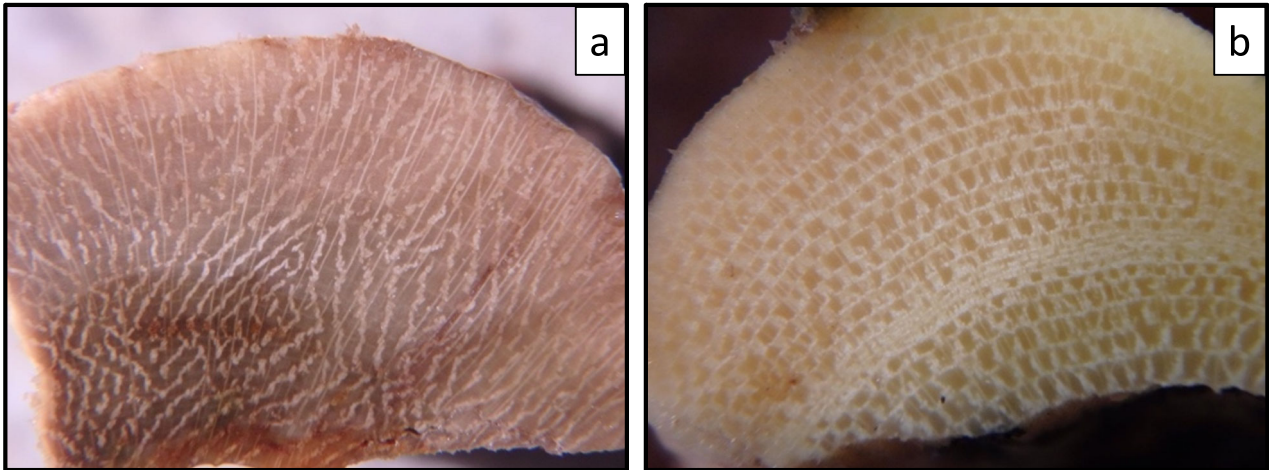
Fiber-tracheids constitute the second of Carlquist's three ground tissue tracheary element types. Like libriform fibers, fiber-tracheids are thought to be non-conductive, can be living, and vessels are almost always grouped in their presence. They are the least common of the ground tissue imperforate tracheary element types. Fiber-tracheids have very narrow bordered pits which are often sparse (Fig. 2; Van Dam & Gorshkova 2003; Carlquist 2014). Where the exact border lies between non-conductive fiber-tracheids and (conductive) true tracheids remains to be determined (Sano *et al.* 2011; Echeverría *et al.* 2022).

### TRUE TRACHEIDS

True tracheids are the only *conductive* ground tissue imperforate tracheary element type. In species with true tracheids, this is the only imperforate tracheary element present, and therefore the entire background is conductive. The "solitary vessel" end of the Carlquist's Law pattern is most dependably observed in species with true tracheids. True tracheids are the second most common imperforate tracheary element type, after libriform fibers. They have wide pit chambers relative to the pit aperture (often referred to by the term "distinctly bordered pits"; Fig. 2) and tend to be long and to have densely packed distinctly bordered pits on their lateral walls (Carlquist 1984). True tracheids are dead at maturity and never have nuclei, septa, starch, or other elements of a living cytoplasm (Sano *et al.* 2011). They also tend to have intact pit membranes (Sano *et al.* 2011). In species with vessels that are solitary throughout wood transections (i.e., not varying in solitariness throughout a growth ring), the background elements are almost always true tracheids.



**Fig. 2.** Illustrations of the imperforate tracheary element types highlighting differences in pit morphology and tracheary element type distribution. Libriform fibers have simple pits. Fiber tracheids have few or narrow bordered pits. True tracheids, vascentric tracheids and vascular tracheids have dense distributions of distinctly bordered pits, with vascentric tracheids located next to vessels and vascular tracheids in the last layers of latewood. Libriform fibers are the ground tissue in the vascentric and vascular tracheid illustrations.



**Fig. 3.** Striking patterns of vessel groupings associated with vascentric tracheids. (a) The vascentric tracheids and vessels of *Ozothamnus hookeri* (Asteraceae) create “dendritic” patterns that form jagged pale lines that contrast with the darker libriform fibers that make up the background imperforate tracheary elements. Note also the pale rays which traverse the entire section radially. (b) In closely related *Ozothamnus rodwayi*, the libriform fiber strands produce a checkerboard pattern outlined by vascentric tracheid and vessel groupings.

#### VASICENTRIC TRACHEIDS

Vascentric tracheids are “subsidiary” (or secondary) conductive imperforate tracheary elements present in species that have non-conductive libriform fibers or fiber-tracheids as their main ground tissue type. Vascentric tracheids have distinctly bordered pits that are densely packed on all faces of the cell (Fig. 2). They can be about as long as the vessel elements or, less commonly, during maturation they can elongate beyond the lengths of the vessel elements, as most imperforate tracheary elements do. The name “vascentric” describes their location, always next to vessels. Species with vascentric tracheids tend to have distinctive clustering of conductive cells with distinctly bordered pits (vessels plus vascentric tracheids) surrounded by non-conductive imperforate tracheary elements (Pratt *et al.* 2020). In many species, this vessels + vascentric tracheids tissue forms conspicuous diagonal or dendritic patterns (Fig. 3; Carlquist 1987). It can actually be difficult to distinguish vessel elements and vascentric tracheids from each other under light microscopy; they can have overlapping diameter distributions and the lateral walls of vessels and vascentric tracheids are usually anatomically indistinguishable in transections. Additionally, vascentric tracheids can vary greatly in abundance across species, ranging from just a few vascentric tracheids for dozens of vessels (Rosell *et al.* 2007; Brodersen *et al.* 2013) to highly abundant. When vascentric tracheids are rare, vessels tend to be grouped, and when they are abundant, vessels tend to be solitary.

#### VASCULAR TRACHEIDS

Like vascentric tracheids, vascular tracheids are “subsidiary” conductive imperforate tracheary elements present in species that otherwise have non-conductive ground tissue elements. Vascular tracheids are morphologically similar to vascentric tracheids (with densely packed distinctly bordered pits; Fig. 2), but histologically very different. Unlike vascentric tracheids, vascular tracheids are not intermixed with vessels. Instead, vascular tracheids are found in the last layers of latewood, often in bands just one or two cells in radial thickness. When vascular tracheids are very abundant, the vessels *adjacent* to them can be solitary, both in the last-formed latewood vessels and in the first-formed earlywood ones of the next ring. Variation in abundance of vascular tracheids affects vessel grouping has not been studied quantitatively.

Carlquist observed that conductive background imperforate tracheary elements (true tracheids, vasicentric tracheids and vascular tracheids) are associated with reduced numbers of vessels per group while non-conductive background tracheary elements (libriform fibers and fiber-tracheids) are associated with grouped vessels. However, questions surrounding the conductivity of each imperforate tracheary element type and the connectivity between vessels and these imperforate tracheary elements remain. These outstanding questions highlight the idea that Carlquist's classification of imperforate tracheary elements was designed to facilitate the generation and testing of predictions regarding xylem structure-function relationships.

### What measurements do we need to evaluate Carlquist's law?

To assess Carlquist's Law, some important factors require quantification. To begin with, the extent of vessel grouping needs to be determined for a diverse range of woody species. Vessel grouping could be calculated from microscope cross-sections, but as mentioned above, this only accounts for grouping within a thin slice of a long three-dimensional network. Vessel grouping data must therefore be accompanied by quantification of vessel length and pit number, size, and frequency and expressed in the context of these measurements. The advent of techniques such as MicroCT provides an opportunity to quantify connectivity in small 3D segments. Recent work by Wason *et al.* (2021) in grapevine has shown that this MicroCT data can be combined with pit and vessel characteristics to model connectivity and subsequently predict drought-induced air spread through the vessel network. 3D imaging could also be used to better understand the connectivity between vessels and presumed 'conductive' imperforate tracheary elements. For example, this could help to evaluate the hydraulic connectivity between vasicentric tracheids, conventional multi-celled vessels, and very short (2–3 element) vessels, which are probably abundant in some species with vasicentric tracheids (Carlquist 2012; Brodersen *et al.* 2013; Pratt & Jacobsen 2018; Olson this issue).

It is also important to determine the true conductivity of the background imperforate tracheary elements. This could be achieved by examining how water flows from the vessel network into surrounding tissues (i.e., using dye) or by blocking the vessel-network to measure water flow through the background cells using hydraulic techniques such as a low-pressure flow meter as described by Melcher *et al.* (2012), Pan *et al.* (2015) and Pan & Tyree (2019). Measurements of conductivity will be key to assessing both the potential and realized role of imperforate tracheary elements in providing an alternative route for water transport.

### Implications of Carlquist's law

Carlquist's Law may have implications for the way we understand the spread of drought-induced embolism through stems. 'Air seeding', the most widely accepted hypothesis used to explain drought-induced embolism, depicts air spreading from an embolized to a non-embolized conduit (Zimmerman 1983; Lewis 1988). However, this explanation does not hold for all incidences of embolism spread as it does not account for (1) The origin of the first embolized conduits, or (2) What happens when there are few connections between conduits, and therefore little opportunity for air-embolism spread. While the initial air-source may be external in some cases, e.g. originating from mechanical damage to the plant (Johnson *et al.* 2022), this still does not explain how embolisms propagate beyond this first event in stems with low radial xylem connectivity. Carlquist's Law may offer an explanation for how embolism propagates in these systems.

The observation that largely solitary vessels embolize one-at-a-time (Johnson *et al.* 2020), suggests that in stems where radial xylem network connectivity is low, individual conduit resistances to air-entry determine conduit vulnerabilities, rather than proximity to an already embolized conduit. This concept has also been suggested and demonstrated in other recent studies (Christman *et al.* 2012; Venturas *et al.* 2016; Avila *et al.* 2022). Air exogenous to

the xylem may therefore be the predominant source for cavitation events in these stems. This air likely originates from cells connected to the xylem, possibly the imperforate tracheary elements.

Optical vulnerability data often detects rapid changes in pixel intensity (indicative of cavitations) in short, thin ‘fibers’, but these smaller events are filtered out during image-processing, as the vulnerability of the vessel network is the focus of xylem vulnerability analysis (Brodribb *et al.* 2016; Johnson *et al.* 2020). In fact, in Johnson *et al.* 2020, the higher number of OVT detected cavitations compared to MicroCT detected events in *Eucalyptus globulus* (33%) was attributed to cavitation in smaller vascentric tracheids (Wheeler 2011), which were likely only detectable with the OVT due to the limited MicroCT resolution in this study. The high proportion of these ‘small’ events highlights the potentially significant role of imperforate tracheary elements and provides evidence that they do cavitate. Carlquist’s Law and recent research suggesting that air in the cells surrounding the xylem may be the source of cavitation in low-connectivity stems highlights the importance of understanding cavitation in these smaller conductive cells, which is possible with techniques like the OVT.

We are yet to understand the role of connectivity in the vessel network on embolism spread in angiosperm stems; including conductive imperforate tracheary elements in our assessments might give us a more complete picture of hydraulic connectivity. Some research suggests that increased clustering of vessels increases redundancy in the hydraulic pathway reducing embolism spread (Carlquist 1966, 1984; Schenk *et al.* 2008; Zhang *et al.* 2016; Mrad *et al.* 2020), while others suggest the opposite, that increased connectivity increases embolism spread (Loepfe *et al.* 2007). Conductive imperforate tracheary elements may be a source of air for embolism as discussed above, or they may provide an alternative route for water transport, perhaps by facilitating water flow around embolized conduits to those that remain functional (Carlquist 1984; Pratt *et al.* 2015; Barotto *et al.* 2016). A role for imperforate tracheary elements in water transport is supported by evidence that fibers can store and remobilize water (Copini 2019) and that true tracheids do appear to move water between vessels (Cai *et al.* 2014). If they do serve one, some, or all, of these roles the presence of conductive imperforate tracheary elements would likely influence the connectivity of the hydraulic network and the pattern of embolism spread.

The associations between anatomical traits observed by Carlquist provide a rich source of evidence for xylem structure–function hypotheses and suggest that understanding the role of imperforate tracheary elements is important for future research on the topics discussed above. Both recognizing and testing the potential physiological contributions of imperforate tracheary elements and using this to inform interpretations of their functional anatomy is critical. Combining this with techniques that allow embolism to be monitored in real-time will provide us with valuable insights into the role of these abundant and possibly highly conductive cells in stems with low vessel connectivity.

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*Edited by Marcelo R. Pace*