Comparative anatomy vs mechanistic understanding: how to interpret the diameter-vulnerability link

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Summary – Results from comparative and ecological wood anatomy combined with a number of experimental studies on plant hydraulics have led to a pervasive and longstanding assumption that wider-diameter vessels are more vulnerable to drought-induced embolism than narrower vessels. Although we agree that wider vessels tend to be more vulnerable than narrower vessels within stems and within roots across most species, our current understanding of the diameter-vulnerability link does not offer a mechanistic explanation for why increased vessel diameter should consistently lead to greater vulnerability or vice versa. Causes of drought-induced embolism formation and spread likely operate at the nano-level, especially at gas-liquid-surfactant interfaces inside intervessel pit membranes. We evaluate here new perspectives on drought-induced embolism and its key anatomical and physico-chemical drivers, of which vessel diameter is one of the parameters involved, although its linkage to embolism vulnerability is likely indirect. As such, the diameter-vulnerability link does not imply that species with on average wider vessels are consistently more susceptible to drought-induced embolism compared to species with narrower vessels. Scientific priorities for future progress should focus on more accurate predictions of how water transport in plants is affected by drought, which requires a better mechanistic understanding of xylem network topology and biophysical processes at the nano-scale level in individual vessels that determine embolism formation and spread.

Keywords – drought, embolism, pit membrane thickness, plant hydraulics, vessel diameter, wood anatomy, xylem networks, xylem sap.

Introduction

One of the most frequently measured xylem traits in angiosperms is vessel diameter, which ranges from 10–700 µm both across and within species (Hacke *et al.* 2017; Olson 2020). This trait is commonly measured for multiple reasons, most notably because vessel diameter is easily measured from cross sections of xylem, and has proven to act as an important functional trait regulating hydraulic conductivity (Sperry *et al.* 2006; Lachenbruch & McCulloh 2014) and freeze-thaw-induced embolism (Sperry & Sullivan 1992; Davis *et al.* 1999; Sevanto *et al.* 2012; Lintunen *et al.* 2013,

2020; Charrier *et al.* 2014), among other plant functional traits. Broad accessibility to this trait with relatively simple methods has also provided numerous opportunities for comparative anatomy studies that allowed for insights into plant adaptation across various terrestrial habitats, many of which were initiated by Sherwin Carlquist and followed by others (Carlquist 1966, 2001; Baas *et al.* 1983; Lens *et al.* 2004; Olson *et al.* 2023). These comparative studies show that species from dry environments tend to have a narrower mean vessel diameter than closely related species from wetter habitats, which provides evidence for the idea that species with wider vessels are more susceptible to drought-induced embolism than species with narrower vessels (Olson *et al.* 2018; Stovall *et al.* 2019). To further highlight the role of vessel diameter in a plant's drought response, Carlquist (1977) calculated indices for vulnerability (*V*; vessel diameter divided by vessel density) and mesomorphy (*M* = *V* multiplied by vessel element length) for species native to habitats with contrasting levels of aridity, and found that drought-adapted species showed *V*- and *M*-indices markedly below 1.0 and below 30, respectively (summarised in this special issue by Ewers *et al.* 2023). Yet, despite several decades of work on establishing a link between conduit diameter and vulnerability to drought-induced embolism, the current state of the underlying mechanistic understanding remains largely uncertain (Lens *et al.* 2022; Hacke *et al.* 2023).

In addition to the comparative studies of Carlquist (1966, 2001) and pioneers before him (see Olson 2020 for a historical overview), part of the theoretical background regarding the functional role of conduit diameter finds its root in the seminal work by Zimmermann (1983), which illustrates the effects of conduit diameter on idealised Hagen-Poiseuille flow in cylindrical pipes. This theoretical framework was highly influential and set the stage for many subsequent investigations to explicitly test the hypotheses presented in Zimmermann's book. Emerging over the following decades was the idea that some inherent trade off must exist between optimising the xylem for hydraulic efficiency, structural support, and hydraulic safety. That hypothetical trade off 'triangle' (see Baas *et al.* 2004; Brodersen 2015) was then critically evaluated based on large-scale data analyses, confirming (often weak) correlations between efficiency and safety (measured as P_{50}: xylem sap water potential leading to a 50% loss of conductivity; Gleason *et al.* 2016), between support and safety (Hacke *et al.* 2001), and between conduit diameter and safety (Fig. 1 in Lens *et al.* 2022; Hacke *et al.* 2023). This latter diameter-vulnerability link was also confirmed in a number of smaller-scale experimental studies (Hargrave *et al.* 1994; Cai & Tyree 2010; Nardini *et al.* 2017; Jacobsen *et al.* 2019; Isasa *et al.* 2023). However, many other smaller-scale experimental publications did not find a diameter-P_{50} link in either stems or petioles (Lens *et al.* 2011, 2016; Brodersen *et al.* 2013; Choat *et al.* 2016; Dória *et al.* 2018, 2019; Emilio *et al.* 2019; Thonglim *et al.* 2020, 2022). This link was also not retrieved in some studies on conifers (Bouche *et al.* 2014; Johnson & Brodribb 2023). Two conclusions can be drawn from these inconsistent experimental results: (1) even if a correlation between vessel diameter and P_{50} exists, this correlation is of little use without critically interpreting what it actually means in relation to total-plant drought resilience, and (2) reporting a correlation between these traits does little to inform our understanding of the mechanisms underlying drought-induced embolism formation and spread, which is currently a priority for the field (Lens *et al.* 2022).

In this opinion paper, based on the ideas already published in detail in Lens *et al.* (2022) and inspired by earlier comparative wood anatomy work by Carlquist and others, we further explore the idea of a potential mechanistic relationship between vessel diameter and vulnerability to drought-induced embolism, and discuss how this impacts the interpretation of the diameter-vulnerability link. A critical assessment of this link involves two research questions: (1) Are wider vessels always more prone to drought-induced embolism than narrower vessels (and vice versa), and (2) Are species with a higher mean vessel diameter consistently more vulnerable to embolism — and by extension more vulnerable to drought — than species with narrower vessels? These two questions are often not separated, and we believe disentangling them will lead to a more balanced and useful understanding of the role of vessel diameter in drought-induced embolism.

Even when assuming the diameter-vulnerability link to be valid across a broad range of vascular plant species, we echo the conclusion in our previous paper stating that (1) wider vessels are often (but not always) more vulnerable to drought-induced embolism compared to narrower vessels when the same organs are taken into account (see also

Fig. 2 in Hacke *et al.* 2023), and (2) that species with on average wider vessels are not necessarily more vulnerable to drought-induced embolism than species with narrower vessels. The main reasons for these two statements are that the mechanisms leading to drought-induced embolism involve a complex interaction among multiple anatomical (including vessel diameter) and physico-chemical drivers operating at different scales (Lens *et al.* 2022). Unfortunately, this complexity and the fact that we do not understand exactly how plants are able to transport water under negative pressure without constant or even frequent embolism formation, makes it difficult to generalise findings related to drought-induced embolism.

To reconcile this discrepancy, there is an urgent need to keep on critically assessing the methods used to generate anatomical and experimental data, and to dive deeper into the mechanisms behind drought-induced embolism. In addition, the vulnerability discussion concerning wide vessels contributes to debates about the frequency of drought-induced embolism events *in planta*: (1) Does embolism formation occur in the vulnerable wide vessels on a daily basis in the field also under normal conditions of precipitation, which indirectly may suggest frequent embolism repair events (Salleo *et al.* 2004; Jacobsen *et al.* 2007; Hacke *et al.* 2009; Nardini *et al.* 2011; Jacobsen & Pratt 2012), or (2) Is widespread embolism uncommon over relatively long periods in the field and limited to periods of severe drought in narrow- as well as wide-vesseled species (Cochard & Delzon 2013; Lens *et al.* 2016; Wason *et al.* 2018; Guan *et al.* 2022)? Answering these questions is crucial because embolism events could result in the loss of hydraulic conductance, tissue desiccation, and/or dieback (Davis *et al.* 2002; Venturas *et al.* 2016; Pratt *et al.* 2020; Brodribb *et al.* 2021; Mantova *et al.* 2022) and can therefore affect plant function and survival (McDowel *et al.* 2008; Allen *et al.* 2015; Anderegg *et al.* 2016; Adams *et al.* 2017; Choat *et al.* 2018; Brodribb *et al.* 2020).

Why are wider vessels not necessarily more vulnerable to drought-induced embolism than narrower vessels?

A frequently cited argument from ecological studies on wood anatomy supporting the diameter-vulnerability link is that earlywood vessels, which are often formed during the spring in temperate regions or during the wet season in seasonally dry regions, are always wider than latewood vessels formed later in the season, often under drier conditions (Carlquist 2001; Olson *et al.* 2023). We agree that earlywood will be on average more vulnerable than latewood across most of these species. However, this does not necessarily indicate that all earlywood and latewood vessels differ in vulnerability to drought-induced embolism, and it is even plausible that some of the latewood vessels are considerably more vulnerable than most earlywood vessels. With respect to the tracheid-bearing conifer species *Pseudotsuga menziesii*, Dalla-Salda *et al.* (2014) indeed found that the latewood is *more* vulnerable to drought-induced embolism compared to earlywood, probably due to inflexible interconduit pit membranes in the latewood tracheids that facilitate embolism spread. Therefore, linking conduit diameter with vulnerability to embolism can be misleading when not considering other traits that may play an equal or more important role in drought-induced embolism formation than conduit diameter. In other words, all else being equal, wider vessels are likely more vulnerable than narrower vessels within stems and roots, agreeing with the large body of literature (Lens *et al.* 2022; Hacke *et al.* 2023; Olson *et al.* 2023). However, all else is typically not equal in nature, implying that wider vessels can also be considerably more resistant to drought-induced embolism than narrower vessels.

Also, from the point of view of xylem physiology, there are three main explanations for why the impact of vessel diameter on drought-induced embolism vulnerability should be treated in a broader perspective. First, the mechanisms behind drought-induced embolisms are complex, poorly understood, and occur at much smaller scales than whole-conduit dimensions. For example, gas bubble formation (and stabilisation), and bubble expansion may arise from nano-scale interactions occurring among the multiple interfaces between liquids, gases, and solids inside conduits. These interactions are further complicated by the presence of solutes and insoluble xylem sap compounds that change the surface tension, aka surface-active agents or surfactants, which are hypothesised to

stabilise very small gas bubbles ("nanobubbles") and may result in xylem sap that is far more resilient to drought-induced embolism than traditionally thought (Schenk *et al.* 2017, 2020; Yang *et al.* 2020; Jansen *et al.* 2022; Ingram *et al.* 2021). However, the precise combination of surfaces (cell wall, pit border, pit membrane), xylem sap, and gas properties/conditions that result in embolism formation and spread remain poorly understood but are likely proximally related to embolism vulnerability. For example, it is possible that embolisms result from pre-existing gas bubbles, including tiny nanobubbles or surface bubbles associated with hydrophobic vessel surfaces, which expand in the xylem sap when these bubbles exceed a critical size threshold for a given pressure (Tyree *et al.* 1994; Hölttä *et al.* 2002; Lohse & Zhang 2015; Schenk *et al.* 2017), although experimental evidence for this idea is poor. How nanobubbles and the likelihood of embolism formation relate to vessel diameter (and length) remains to be studied. Either vessels with a larger volume contain a higher total number of nanobubbles that may increase embolism risk, or these wider and longer vessels could be more resistant if large neighbouring vessels during their initial stage of embolisation would extract gas from their functional neighbours in order to reach atmospheric pressure at the final stages of embolisation (Wang *et al.* 2015).

Second, correlation does not invoke causation, which obviously also applies to the diameter-vulnerability link. There is no known direct, mechanistic link between conduit diameter and drought-induced embolism resistance either at the whole-plant level or at the ultrastructural pit level. If such a mechanism were to exist, we should expect it to affect the relative vulnerabilities of xylem in leaves (narrower conduits) and stems (wider conduits) similarly within and across individuals and species, but it clearly has not. For example, although several authors have reported leaf xylem to be more resistant to embolism than branch or stem xylem (Zhu *et al.* 2016; Klepsch *et al.* 2018; Levionnois *et al.* 2020b; Guan *et al.* 2022), this result is far from consistent across studies, despite leaves having consistently narrower conduits than branches or stems. Likewise, several authors have reported that leaves are more vulnerable than perennial shoots (Pivovaroff *et al.* 2014; Charrier *et al.* 2016; Johnson *et al.* 2016; Creek *et al.* 2018; Skelton *et al.* 2019), or else have found no meaningful difference in xylem vulnerability between leaves, stems and/or roots of the same individuals (Skelton *et al.* 2017; Creek *et al.* 2018; Wason *et al.* 2018; Losso *et al.* 2019; Levionnois *et al.* 2020b; Smith-Martin *et al.* 2020; Wu *et al.* 2020, Lübbe *et al.* 2022). Thus, if a causal mechanism between conduit diameter and vulnerability was identified, it would need to account for the inconsistent vulnerability-diameter relationships within and across individuals. Bearing this in mind, the recent study of Isasa *et al.* (2023) showed that a negative vessel diameter-P_{50} relationship exists at the interspecific scale, but not at the intraspecific scale. In conclusion, a view that considers the entire body of research on xylem vulnerability, rather than studies that only support a vulnerability-diameter link within a single organ, does not support a mechanism operating at the scale of the whole plant.

At the pit level, researchers often cite the pit area hypothesis (Wheeler *et al.* 2005; Hacke *et al.* 2006, 2009; Christman *et al.* 2009, 2012) to mechanistically explain the diameter-vulnerability link. This hypothesis assumes that permeability to gas and embolism propagation among vessels increases with increasing intervessel pit area of a given vessel with average dimensions (Ap). While the pit area hypothesis correctly emphasises the functional importance of intervessel connectivity (Loepfe *et al.* 2007; Wason *et al.* 2021; Bouda *et al.* 2022), large pit membrane pores are thought to be exceptionally rare and, if observed, are most likely the result of artefacts (Jansen *et al.* 2008, 2009; Choat *et al.* 2008). Furthermore, the idea that a rare, single-layered pit membrane would be highly leaky due to a single, unusually large pore, leading to embolism propagation, is not compatible with recent theoretical insights on the three-dimensional, multi-layered nature of pit membranes with multiple pore constrictions that represent the bottlenecks for water and gas transport (Kaack *et al.* 2019, 2021; Yang *et al.* 2020; Zhang *et al.* 2020). These insights, based on 3D-modelling and hydraulic measurements, suggest that P_{50} has a stronger relationship with intervessel pit membrane thickness than Ap (Kaack *et al.* 2021), although Isasa *et al.* (2023) showed that intervessel membrane thickness alone cannot explain the P_{50} interspecific variability based on 12 temperate tree species. What emerges from these studies is that the pit area hypothesis should be further tested with respect to both length-diameter scaling of conduits and connectivity among both vessels and adjacent tracheids (Carlquist 1984; Sperry *et al.* 2006) before it can be used as a solid mechanistic explanation for a possible correlation between P_{50} and vessel diameter. Embolism

spread is the result of a combination of different traits that may be related (e.g., vessel connectivity or Ap) or may not be related (e.g., sap composition, vessel wall chemistry, intervessel pit membrane thickness) to vessel diameter (Lens *et al.* 2022). There is an urgent need to validate the theoretical pit membrane models with ultrastructural observations of intervessel pit membranes.

Third, several different methods have been used to quantify both mean vessel diameter and P_{50}, thereby adding noise to the diameter-vulnerability link (Brodersen *et al.* 2013; Cochard & Delzon 2013; Choat *et al.* 2016; Martin-StPaul *et al.* 2017; Creek *et al.* 2018; Emilio *et al.* 2019; Smith-Martin *et al.* 2020; Barigah *et al.* 2021). With respect to vessel diameter, there are multiple ways for a plant to arrive at the same mean vessel diameter (i.e., different vessel diameter frequency distributions), and "mean" vessel diameter may not be the most meaningful quantification of vessel width, as it relates to embolism vulnerability. In addition, mean vessel diameter is calculated in different ways (e.g., tangential diameter, equivalent circle diameter), and is often hydraulically weighted based on either all vessels in a cross-section or from a subset of these vessels (usually >100) (Scholz *et al.* 2013). Also, the pressure gradient driving water transport through the xylem may not be uniform across all vessels in a cross-section (Bouda *et al.* 2019), which would likely result in errors in estimated vulnerability across vessels of different diameters within the same cross-section. To further complicate matters, branch age, growth rate, number of growth rings, and seasonality may affect the vulnerability curve (Charrier *et al.* 2018; Olson *et al.* 2018; Pratt *et al.* 2020; Sorek *et al.* 2020; Weithmann *et al.* 2022). In summary, the interpretation of the diameter-vulnerability link will become clearer if we know the vulnerability of individual vessels, and the determinants of this vulnerability, as well as the aggregate vulnerability across many vessels.

The unexplained variation in the vulnerability-diameter correlation could also be explained by differences in P_{50} methods that may impact vulnerability curves (Cochard *et al.* 2013; Venturas *et al.* 2019; Gauthey *et al.* 2020; Hacke *et al.* 2023). The open vessel artefact is noteworthy in this regard, especially in studies focusing on stems of long-vesseled genera such as *Quercus* (Sperry *et al.* 2012; Martin-StPaul *et al.* 2014; Skelton *et al.* 2018, 2021; Percolla *et al.* 2021) and *Vitis* (Choat *et al.* 2010; Jacobsen & Pratt 2012; McElrone *et al.* 2012; Charrier *et al.* 2018). Many studies showed that this artefact occurs when embolism is artificially induced with the centrifuge method in stem (or root) segments that are considerably shorter than the maximum vessel length, thus resulting in vessels being cut at both ends ("open vessels"). These longer (and often wider) cut-open vessels embolize quickly when they are subjected to mild negative pressure, thus resulting in a rapid (exponential or "r"-shaped) decline in hydraulic conductivity with increasing xylem tension, and hence an overestimation of vulnerability (Alder *et al.* 1996; Cochard 2002; Choat *et al.* 2010; Cochard *et al.* 2010, 2013; McElrone *et al.* 2012; Martin-StPaul *et al.* 2014; Torres-Ruiz *et al.* 2014; although see Tobin *et al.* 2012, Jacobsen & Pratt 2012, Sperry *et al.* 2012, Hacke *et al.* 2014, and Jacobsen & Pratt 2023 for a different point of view). Other factors that are known to affect the shape of a vulnerability curve are temporal variation in "native" embolism (daily or seasonal) and the flushing of stems with water prior to taking an initial hydraulic measurement (Domec *et al.* 2006; Jacobsen *et al.* 2007; Hacke *et al.* 2014, 2023). Considering all these potential sources of error in embolism vulnerability measurements, a critical examination of methods and models used to estimate xylem vulnerability remains a research priority (Cochard *et al.* 2013).

Why are species with wider vessels not necessarily more vulnerable to drought-induced embolism than those with narrower vessels?

The mechanisms leading to drought-induced embolism involve several anatomical and physico-chemical drivers of drought-induced embolism. This implies that natural selection may act on combinations of traits that affect the susceptibility of xylem to drought-induced embolism at different scales, with vessel diameter only being one of these traits that may or may not have a significant impact on the drought resistance of the entire individual. The potential

xylem drivers of drought-induced embolism are discussed more in-depth in Lens *et al.* (2022) and are listed here in alphabetical order (see also Box 1 in Lens *et al.* 2022).

(1) Macro-level (vessel) traits: 3D-conduit network (Loepfe *et al.* 2007; Brodersen 2013; Mrad *et al.* 2018, 2021; Wason *et al.* 2021; Bouda *et al.* 2022), Ap (Wheeler *et al.* 2005; Kaack *et al.* 2021), and conduit diameter (this paper; Isasa *et al.* 2023).

(2) Nano-level xylem traits: intervessel pit membrane thickness (Jansen *et al.* 2009; Lens *et al.* 2011, 2013; Li *et al.* 2016; Dória *et al.* 2018; Levionnois *et al.* 2020a; Thonglim *et al.* 2020, 2022; Kaack *et al.* 2021), cell wall chemistry and thickness (Lens *et al.* 2016; Greenwood *et al.* 2017; Pereira *et al.* 2018; Dória *et al.* 2018, 2019; Thonglim *et al.* 2020, 2022; Frankiewicz *et al.* 2021; Liang *et al.* 2021; Zizka *et al.* 2022), wettability of the inner conduit wall (Lohse & Zhang 2015; Zwieniecki & Holbrook 2000; McCully *et al.* 2014; Schenk *et al.* 2017, 2018, 2020; Brodersen *et al.* 2018), dynamic surface tension of xylem sap and surfactant-coated nanobubbles (Schenk *et al.* 2015, 2017, 2020; Yang *et al.* 2020; Ingram *et al.* 2021; Jansen *et al.* 2022), and gas movement and xylem sap oversaturation (Schenk *et al.* 2016; Pereira *et al.* 2020; Guan *et al.* 2021).

Concluding remarks

From the above, it is clear from both ecological wood anatomy and experimental studies that a weak but significant diameter-vulnerability link does exist when comparing all available data (mainly from stems; Fig. 1 in Lens *et al.* 2022). This means that there is a large body of evidence that wider vessels tend to be more vulnerable than narrower vessels within stems and roots, confirming Carlquist's ideas (1966, 2001). Consequently, we agree that vessel diameter likely plays some role in drought-induced embolism formation in many clades (Lens *et al.* 2022; Olson *et al.* 2023), but most likely not within all clades (Lens *et al.* 2011, 2016; Brodersen *et al.* 2013; Choat *et al.* 2016; Dória *et al.* 2018, 2019; Emilio *et al.* 2019; Thonglim *et al.* 2020, 2022), and definitely not at the whole-plant level (Skelton *et al.* 2017; Creek *et al.* 2018; Wason *et al.* 2018; Losso *et al.* 2019; Levionnois *et al.* 2020b; Smith-Martin *et al.* 2020; Wu *et al.* 2020, Lübbe *et al.* 2022). Even if we generally accept that there is a diameter-vulnerability link, not knowing the mechanisms involved in drought-induced embolism can lead to subtle but important differences in the interpretation of what the link actually means, i.e., how and why it has arisen and its consequences on plant function. Although we agree with Olson *et al.* (2023) that the salient vessel diameter patterns in nature are valuable in directing our research questions to solve how plants respond to environmental stress, we feel that this approach overemphasises the role of vessel diameter in the drought stress debate and simplifies the relationship between vessel diameter and embolism vulnerability to the point of being misleading. The diameter-vulnerability link does not imply that (1) wider vessels are necessarily more susceptible to drought-induced embolism (and vice versa) or (2) that vessels with the same width (and length) are equally vulnerable (see also Fig. 2 in Hacke *et al.* 2023), and (3) it definitely does not mean that species with on average wider vessels are more vulnerable to drought-induced embolism or by extension are more drought-sensitive. As we all know, plants are famous for developing multiple solutions when it comes to drought response strategies (Pivovaroff *et al.* 2015; McDowell *et al.* 2022). Placing a scientific premium on the study of vessel diameter as an important and meaningful correlate of embolism vulnerability may not be the best way to prioritise future research, nor the best way to inspire research that could potentially lead to a better understanding of drought-induced embolism (Lens *et al.* 2022). Although pointing out that correlation does not imply causation may appear as a platitude, it is true that scientists still commonly cite vessel diameter in discussions about drought responses without addressing why this correlation represents a convincing argument, or not.

We suggest that a better understanding of the diameter-vulnerability link could be obtained by focusing our research efforts on three broad objectives: (1) improve our understanding of the vulnerability of individual vessels, rather than an aggregate measure of vulnerability across many vessels (although this is technically challenging, e.g., via microCT observations that span an axial segment length beyond the length of the longest vessels), and connect

this information to other drought-associated traits in the xylem and beyond, (2) develop new and more powerful non-invasive techniques to identify macro and nano-scale mechanistic driver(s) of embolism initiation and spread across different environmental conditions and different plant organs, and (3) evaluate the relative importance and timing of xylem embolism among the sequence of physiological failures that occur during drought, i.e. loss of turgor, cessation of cell division and growth, plasma membrane instability, plant death (Mantova *et al.* 2022). Once a large body of drought-associated xylem traits are investigated in different organs of carefully selected model species that grow in contrasting environments, we can further test and fine-tune the mechanisms involved in embolism formation and spread through the development of a new family of integrative, process-based, xylem models linking nano- (Li *et al.* 2020; Zhang *et al.* 2020; Kaack *et al.* 2021; Weishaupt *et al.* 2022) to macro-scale processes (Loepfe *et al.* 2007; Mrad *et al.* 2018, 2021; Wason *et al.* 2021). Integrating nano- and macro-scale xylem processes would allow the evaluation of pit membrane traits, vessel network traits (e.g., connectivity among vessels and adjacent tracheids, vessel widening, segmentation or sectoriality; Carlquist 1984; Olson *et al.* 2020; McElrone *et al.* 2021) and their interactions on whole-plant transpiration, construction costs, and carbon income. This knowledge will be important to reconcile our current state-of-the-art knowledge of embolism formation and spread with observations made from comparative anatomical studies, such as the empirical correlation between embolism vulnerability and conduit diameter. Moreover, it will help us to understand how plants will respond to a warming climate, and how to manage natural and agricultural systems under these novel environmental conditions (Choat *et al.* 2018; Brodribb *et al.* 2020).

Acknowledgements

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