

Functional Status of Xylem Through Time

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Abstract

Water transport in vascular plants represents a critical component of terrestrial water cycles and supplies the water needed for the exchange of CO₂ in the atmosphere for photosynthesis. Yet, many fundamental principles of water transport are difficult to assess given the scale and location of plant xylem. Here we review the mechanistic principles that underpin long-distance water transport in vascular plants, with a focus on woody species. We also discuss the recent development of noninvasive tools to study the functional status of xylem networks in planta. Limitations of current methods to detect drought-induced xylem blockages (e.g., embolisms) and quantify corresponding declines in sap flow, and the coordination of hydraulic dysfunction with other physiological processes are assessed. Future avenues of research focused on cross-validation of plant hydraulics methods are discussed, as well as a proposed fundamental shift in the theory and methodology used to characterize and measure plant water use.

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INTRODUCTION

The transition to terrestrial habitats approximately 470 mya (7, 38, 111) exposed early land plants to a desiccating environment, forcing plants to develop novel strategies for limiting water loss while regulating the influx of CO₂ into the plant body. The concurrent development of increasingly more efficient and complex vascular systems allowed for greater photosynthetic gains but also led to increased stature and the competition for resources in more diverse environments. The form and function of extant vascular plants have been fundamentally shaped by the biomechanical demands of maintaining an upright growth form while balancing the risk of transporting great quantities of water under tension with the reward of high carbon gain.

Vascular plants leverage the cohesive properties of water molecules to transport water with little energetic cost after the initial investment in xylem development, which must both withstand the negative liquid pressure (i.e., tension) that develops and remain functional for significant periods of time. The negative pressure in the xylem sap arises from the evaporation of liquid water inside the leaf, the diffusion of water vapor out of the plant through the stomata (i.e., transpiration) (121), and the resistance to the flow of water through the xylem. If the evaporation of water from the leaf outpaces the supply of water from the roots and soil, or the stored water within the plant body, a pressure gradient is established, with water flow in the direction of the tissues experiencing a lower relative pressure. Simple bulk flow of this type can be successfully characterized using Darcy's law (115) or modifications of Ohm's law (109), the latter of which allows water transport through different plant tissues (e.g., roots, stems, petioles, and leaves) to be described in terms of their relative resistances to flow. This characterization and study of the different components of the soil–plant–atmosphere continuum (SPAC) have enabled extensive insight into the vascular systems of plants.

One fundamental component of the cohesion–tension theory (143) that guides our understanding of plant water transport is that when water is in a negative pressure environment, it is metastable (37). As such, liquid water is susceptible to cavitation, the rapid phase change to water vapor, which forms gas bubbles that block xylem conduits and can occur when the negative pressure exceeds the tensile strength of the sap (30, 112). The negative pressure environment also allows bubbles above a critical radius to expand and block flow through the xylem (37, 128, 132), or for the aspiration gas across the membrane separating two adjacent conduits. These conditions typically arise during periods of high evaporative demand from the atmosphere, low soil moisture availability, or both (i.e., characteristics often associated with drought). Thus, while plants have

Xylem: the assemblage of cells responsible for long-distance water transport in vascular plants, structural support, carbon storage, and pathogen resistance

evolved an elegant mechanism for transporting water over long distances, the physical properties of the xylem network and the negative pressure environment it depends on for moving water are susceptible to dysfunction when plants need water most. Maintaining a functional xylem network is so critical to survival that plants prioritize water loss prevention over carbon gain through active and passive stomatal closure well before the point of cavitation (17, 58, 67). Systemic spread of gas bubbles in the xylem network ultimately disrupts water transport to the foliage, and if water delivery to the meristematic tissues such as the vascular cambium and apical meristems is disrupted, the long-term survival of a perennial plant is severely compromised.

Recent evidence strongly implicates hydraulic dysfunction in drought-induced tree mortality (24), from the legacy effects of either low-level drought events over many years or less frequent high-intensity droughts coupled with elevated temperatures. The combination of increasing ambient temperatures and decreased precipitation frequency has led to multiple large-scale tree mortality events in recent years, often resulting in subsequent catastrophic, stand-replacing fires. Furthermore, it is now clear that many plant species within their current habitat distributions are experiencing environmental conditions that are pushing them ever closer to their physiological tipping points (27). As a consequence, the xylem and its functional status during and after drought are now under intense research focus, both from a desire to understand how individual plants and tissues respond to, and recover from, hydraulic dysfunction and as a means of predicting how communities and ecosystems contribute to landscape-level water fluxes after these events. The fate of the xylem postdrought and its capacity to deliver water to the canopy are of great interest to the plant biology community, those working in agricultural systems (145), and those wishing to incorporate xylem function as it relates to landscape and ecosystem terrestrial water flux into evapotranspiration models.

Progress over the last 25 years in the field of plant hydraulics now allows for a much more sophisticated understanding of inter- and intraspecific variation in water transport and of the underlying interaction and feedbacks between the environment and the physiological control of water moving through the SPAC. Here, we characterize a general understanding of water transport within the xylem network and highlight the major technological advances that have allowed for a more careful examination of the underlying principles of the cohesion–tension theory and the relationships between plant structure and function in terms of adaptation to drought. One of the main difficulties for the study of plant vascular function is the disproportionate scaling of xylem conduit diameter and length, which are typically measured in micrometers and centimeters to meters, respectively. Furthermore, the long, narrow conduits used to transport water in vascular plants are also embedded within an opaque tissue, making detailed observations of the xylem difficult. Recent advances in noninvasive imaging have established a new set of tools for probing the fundamentals of plant–water relations.

THE ASCENT OF SAP

Water transport within the vascular tissue of plants occurs through a network of interconnected pipe-like cells that are dead upon maturity and embedded within a matrix of living and nonliving support tissue (152). Yet, at both ends of the hydraulic pathway, movement of water takes place outside the highly efficient xylem. Water absorbed by the roots can travel through several nonexclusive pathways (apoplastic, symplastic, transcellular) between the root epidermis and the endodermis (143), collectively characterized by their high resistance relative to the axial pathway inside the xylem conduits (45, 46, 77). Similarly, water leaving the leaf vasculature must also travel through a tortuous path between and through the mesophyll cells to evaporate into the

Conductivity:

measured flow rate as a function of pressure drop across the segment

Parenchyma: living cells embedded in the xylem that play numerous roles, including storage, production of tyloses, and wound response

Tracheid: xylem cell type used for water transport and structural support; most common in gymnosperms, but frequently occurs in angiosperm xylem

Vessel: series of axially aligned vessel elements with open end walls that allow efficient long-distance water transport, most commonly in angiosperms

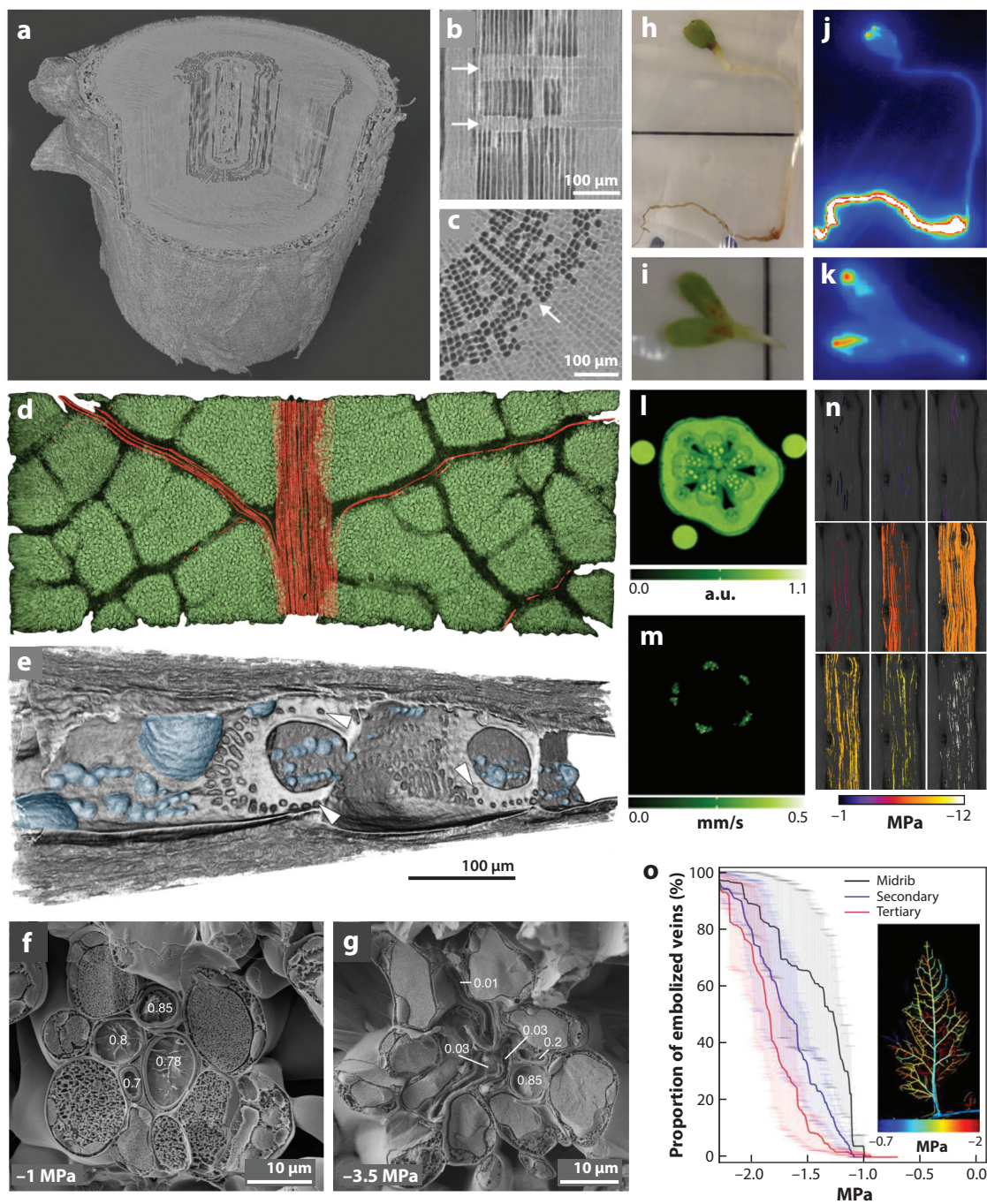
Fiber: structural cell and storage compartment for water with thickened cell walls; can be living at maturity but is usually dead

Conductance: measured flow rate as a function of the pressure driving flow, commonly normalized by leaf area or sapwood area

intercellular airspace prior to exiting the stomata. These terminal points of the hydraulic pathway represent the majority of the total resistance under normal conditions and also appear to be more vulnerable to the effects of desiccation than the xylem, leading to steep declines in outside-xylem conductivity that protect the xylem from dysfunction (35, 130, 146). Therefore, when water is not limiting and the xylem is free of blockages, the pathway through the xylem is generally not limiting in terms of hydraulic resistance relative to the outside-xylem pathways in the roots and leaves. These outside-xylem pathways are dynamic, given that they are largely composed of living cells and sensitive to the ever-changing environment. The formation of large voids within the roots between the epidermis and endodermis under mild drought conditions (35) and the reversible collapse of semirigid xylem conduits in leaves (165, 166) (**Figure 1**) strongly suggest that various adaptations have evolved at these terminal points of the hydraulic pathway to buffer the xylem from excessively negative xylem sap pressure.

From an anatomical perspective, gymnosperms and angiosperms are often described separately, given the fundamental differences between the number of xylem conduits and their packing density, and the diversity of the number, shape, and distribution of the parenchyma cells that serve in various roles. Gymnosperm xylem is generally less complex than angiosperm xylem. Most woody gymnosperm wood, and in particular, conifer wood, consists primarily of tracheids, ray parenchyma, and in some groups, resin canals. Tracheids are the low-resistance xylem conduits in gymnosperms that are relatively short (0.5–4 mm in length) and narrow (8–80 μm in diameter) and function in both structural support and xylem sap transport (108, 111). In contrast, angiosperms move most water axially in vessels that are longer (occasionally >2 m) and greater in diameter (up to approximately 500 μm) than tracheids and specialized for sap transport (152). Instead of the unicellular tracheids found in gymnosperms, vessels are a multicellular assemblage of vertically aligned vessel elements, allowing for long, low-resistance conduits. While individual transverse sections through a stem might suggest a general vertical ascent through the stem, vessels of many species take a more tortuous, complex, and winding axial path (2, 9, 168, 169). Surrounding the vessels are narrow, short, thick-walled fibers specialized for structural support and water storage. Tracheids are not exclusive to the gymnosperms and are common in many plant families (e.g., Fagaceae), but because of their reduced diameter, individual tracheids play a less significant role in axial water transport compared to vessels because of their more frequent endings and higher resistance. A few unusual examples exist of vesselless angiosperms (53, 139), but the general evolutionary trend has been toward an increasing diameter and lengthening of the xylem conduits (4). Presumably, this is to maximize the potential conductance of water to the foliage due to the fundamental power relationship with flow as described by the Hagen–Poiseuille equation, where resistance to flow is largely driven by the conduit radius (80). There also appears to be a nearly universal trend of conduit diameter tapering from the roots to the shoot apex, which likely plays a role in minimizing resistance to flow in the basal regions and establishing a sufficient pressure drop across the whole plant to support axial flow (106).

Where two neighboring conduits make contact, the conduit wall is perforated with a series of bordered pits that contain a semipermeable membrane (25). The pit membrane allows xylem sap to pass between conduits yet prevents large gas bubbles from spreading. Once water enters the xylem conduits (vessels, tracheids, and fibers, in order of increasing resistance), it travels through this low-resistance pathway through the trunk, branches, and petioles (if present) into the increasingly ramified vascular tissue of the leaf (121). Recent work strongly suggests that the living tissue embedded in the xylem, i.e., living parenchyma and fiber cells, which require considerable long-term carbon and water investments, plays a significant role in xylem maintenance and its functional status (99, 100, 102, 131).



(Caption appears on following page)

Figure 1 (Figure appears on preceding page)

Representative examples from recent advances in imaging techniques used to study the functional status of the xylem network and sap flux in planta. Microcomputed tomography (microCT) imaging has been useful in studying embolism formation and spread in the complex vascular system of intact plants via three-dimensional visualizations of (*a–c*) stems (e.g., *Sequoia sempervirens*; adapted with permission from Reference 23), (*d*) leaves (e.g., *Magnolia grandiflora*; adapted with permission from Reference 130), and roots. White arrows in panels *b* and *c* point to the ray parenchyma oriented perpendicular to the long axis of the stem. Panel *b* exposes the radial plane, and panel *c* shows a similar region but in the transverse plane. Air-filled tracheids can be seen as black regions and water-filled tissue is light gray in panels *b* and *c*. MicroCT provides good contrast between water- and air-filled tissues without the need for sample preparation or stains/dyes to reveal the presence of embolism in the xylem network embedded in otherwise opaque tissues. (*e*) MicroCT has also been used to study embolism removal in model species like *Vitis vinifera*, where droplets emerging from the vessel walls (shaded in *blue*) from the surrounding living tissue contribute to the restoration of hydraulic conductivity. Adapted with permission from Reference 11. The high magnification of these images shows minute surface features such as the small perforations in the angled perforation plates indicated by the white arrowheads. (*f,g*) Cryo-scanning electron microscopy was used to study the phenomenon of leaf xylem conduit buckling in response to excessive negative pressures in the xylem sap resulting from drought. The deformation is apparently reversible in *Quercus*, allowing leaf sap flow to recover quickly and avoid xylem cavitation: (*f*) fully hydrated versus (*g*) xylem conduit buckling (adapted with permission from Reference 165). Numbers in lower-left corner indicate the leaf water potential, and inset numbers within the xylem conduits are a measure of circularity. The novel combination of microCT with positron emission tomography (PET) shows promise for tracking the movement of radiolabeled copper nanoparticles that accumulate in the roots and are transported to the cotyledons: (*b–k*) lettuce seedling roots exposed to nanoparticles and imaged with PET scanning (adapted with permission from Reference 36). The pixel intensity color map indicates the nanoparticle concentration. Nuclear magnetic resonance imaging has yielded novel information about (*l*) the functional status of the xylem, as well as (*m*) the velocity of sap flow and its directionality (adapted with permission from Reference 161). Here, cross sections through the stem show water content and velocity relative to stationary reference samples (*green circles*). The optical vulnerability method developed by Brodribb et al. (adapted with permission from Reference 16) has now been used on both (*n*) stems (16) and (*o*) leaves (136), highlighting the effectiveness of this low-cost, open-source technique for monitoring cavitation events in planta. In panel *n* and the inset of panel *o*, the false colored pixels show the location of embolism events, with color cross-referenced with the water potential at which the event occurred.

DEVELOPMENT OF FUNCTIONAL XYLEM

Most xylem development proceeds in a regular, highly predictable pattern based on cellular divisions within the bifacial cambium that exists in most vascular plants (142). Within the cambial zone, production of successive xylem initials from additive divisions leads to orderly rows of radially aligned xylem cells (43, 129). Primary xylem serves as the principal water-conducting pathway for herbaceous species and woody plants during the initial growth phase, which is then succeeded by secondary growth in the vascular cambium of woody plants. It is worth noting that the woody plant form is believed to have been the ancestral condition for extant herbaceous plants (40); that is, woodiness has been repeatedly lost, but the syndrome of secondary woodiness is possible, often occurring in island habitats (39, 79).

While cellular development within the cambial zone occurs in regular increments, the end product (i.e., the xylem network) of these divisions is highly species-specific and sensitive to environmental conditions. Environmental cues and hormonal gradients lead to high anatomical diversity, particularly within the angiosperms (22). Furthermore, the developmental time frame and final dimensions of each of the different cellular types within the xylem can vary considerably among species and over the course of a growing season. The ratio of xylem to phloem production in terms of cross-sectional area can be nearly 1:1 or up to 10:1 (129, 164), likely reflecting both the relative quantities of xylem and phloem sap transported, and their structural roles. Because the phloem is not reinforced like the xylem, with the exception of fibers embedded within or at the periphery, phloem cells eventually collapse once they are nonfunctional, whereas the xylem persists due to its lignified cell walls and role in structural support.

Upon completion of the elongation and enlargement phase of xylem conduit development, the cellular contents are disassembled, allowing unobstructed flow of xylem sap except for the crossing of pit membranes into neighboring conduits. Whereas tracheids develop from individual xylem

initials, vessels arise from the programmed cell death of many axially arranged vessel elements and are functionally linked together by the dissolution of the end walls between them (139, 142). In temperate trees, cellular activity in apical regions leads to a cascading gradient of auxin that triggers cellular division in the vascular cambium within the trunk. Because of this delay, much of the water needed during leaf expansion in the spring is supplied by internal water storage or the xylem of previous annual rings until the current year's xylem is fully developed (74, 75). Quantifying sap flow through these conduits can be accomplished using a variety of complementary methods.

METHODS FOR MEASURING SAP FLOW

Much of our understanding of xylem transport in intact trees was obtained from using a variety of well-established, thermodynamically based methods for measuring sap flow on large trees. The instruments use a heat pulse as a tracer to quantify the rate at which water passes through the xylem and have existed since the 1930s (60, 61). As the xylem sap ascends in a plant, the heat tracer is carried convectively in the sap, moving past temperature sensors located at known distances from the heating element. Thus, if the distance the heat pulse travels during a period of time is known, a sap flow rate for a given volume of wood can be calculated. Variations in heat-based sap flow methods have been developed to more effectively measure the full range (i.e., low, high, and reverse) of sap flow velocities that can occur in plant organs (44, 86).

Most sap flow methods are semi-invasive, with the heating element and thermocouple probes inserted into holes drilled into the plant tissue at different depths. Whereas this method provides information on radial flow profiles across annual growth rings, there are important artifacts that need to be accounted for. These methods require calibration because the presence of the probes in the xylem alters the flow characteristics of the xylem network, and the spatial distribution of water in vessels, tracheids, fibers, and parenchyma varies considerably between species (**Figure 2**). Calibration has been done both empirically (via measurements of gravimetric water loss by potted plants or those grown in weighing lysimeters) and theoretically with modeling to account for wounding effects and other properties of the wood. The extent of such effects can vary with time, across species, and with abiotic and biotic stress, but Vandegehuchte & Steppe (153) have developed a nonempirical heat pulse-based method to accurately determine the water content during low flow conditions, thus enabling corrections without destructive core measurements.

Although sap flow sensors have allowed researchers to make significant advancements in studying water transport, many important yet largely untested assumptions are made, particularly the total tissue volume that is represented in a given measurement and the role of the surrounding tissue types (e.g., fibers, parenchyma) (73). A recent modeling effort based on noninvasive X-ray microcomputed tomography (microCT) scans showed that variability in the water content of the nonconductive fibers that surround xylem conduits can significantly alter the derived sap flow velocities from these sensors (88). MicroCT has also been used to evaluate the wounding effects associated with sap flow probes installed at different points of the growing season in diffuse- and ring-porous trees (85) and revealed that wounds were larger in the radial plane, more extensive in one species, and damage varied with time since insertion. Other heat-based sap flow sensors are noninvasive and deliver and detect heat dissipation externally, but these sensors provide limited information on radial flow profiles and likely capture flow only in the outermost xylem. Collectively, these methods have provided a major advancement in our understanding of both diurnal and seasonal patterns in sap flow in a wide variety of species.

Until recently, sap flow sensors were used primarily in trunks and larger-diameter stems and roots because of the physical constraints of the heater and thermocouple assembly. Methodological advancements and miniaturization have allowed for the development of sensors that can be used to resolve flows into and through much smaller organs, such as leaves, flowers, and small-diameter

**X-ray
microcomputed
tomography
(microCT):**
X-ray-based
noninvasive method
with high resolution
and magnification
capabilities

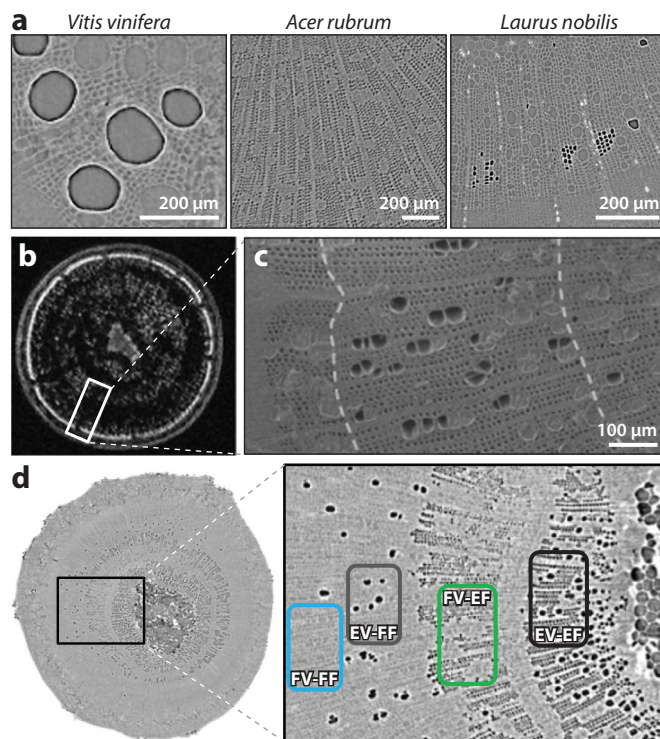


Figure 2

In vivo examples of the variability that can exist in water content status of different xylem cell types in intact plant stems. (a) Transverse microcomputed tomography (microCT) images for three species: (left to right) Fibers in grapevine (*Vitis vinifera*) are living at maturity and remain water filled, in red maple (*Acer rubrum*) are emptied under mild drought stress and prior to embolism formation, and in bay laurel (*Laurus nobilis*) exhibit patterns where the vessels and fibers can empty simultaneously. Similar patterns have been documented in *Betula platyphylla* var. *Japonica* saplings with (b) nuclear magnetic resonance imaging and (c) cryo-scanning electron microscopy. Annual growth boundaries are shown by dashed lines. (d) Variability within a single stem is illustrated in *L. nobilis*, which exhibited different combinations of water-filled vessels (FV), water-filled fibers (FF), empty vessels (EV), and empty fibers (EF) at different radial positions. Image captured with synchrotron-based X-ray microCT at the Advanced Light Source on an intact potted plant. Interpretation and calibration of sap flow sensors would benefit from this type of data to improve sensor placement, sensor output, and estimates of active cross-sectional area. Images in panels a and d adapted with permission from Reference 88; images in panels b and c adapted with permission from Reference 48.

stems and roots (117, 118, 135, 157). For example, an external heat pulse sensor was developed by Clearwater et al. (29) that was used successfully to measure low and reverse flow rates in tomato peduncles in this and other studies (148). Similarly, Hanssens et al. (54) developed a noninvasive, miniaturized version of a heat field deformation sensor that has been useful in tracking flow dynamics into and out of fruit and providing information on the vascular connectivity of fruits as they mature. Recent advances in three-dimensional (3D) printing and low-cost microcontrollers can be used to build heat pulse sensors for measuring sap flow, thereby extending their use to herbaceous species and soft tissues (96, 135). Helfter et al. (55) developed a noninvasive sap flow technique in which heating is achieved by delivering a laser pulse to a small area on the outside of small stems and an infrared camera is used as a noncontact thermometric probe to quantify sap flows; this method also showed promise in resolving the lower flow rates of the phloem.

One of the most promising new avenues for describing both the functional status of the xylem network and sap flow is the use of noninvasive imaging instruments, many of which were originally designed for medical purposes and have been adapted for use in plant sciences. While much has been learned using these methods independently, significant advancements in our understanding of plant water transport and the underlying assumptions of standard plant hydraulics methods have yet to be fully explored. Cross-validation of empirical measurements with noninvasive imaging of intact plants will be a critical step moving forward.

Nuclear magnetic resonance (NMR) imaging has been used in plant sciences for almost 20 years, and recent developments show great promise for new insights into sap flow under laboratory and field conditions (**Figure 1**). These NMR instruments have been modified for use with plants and utilize the same technology as medical NMR systems but with increased image resolution. NMR allows visualization of the functional status of the xylem network in planta, without having to cut into the xylem to detect the presence of air or water in the vessel lumen (59). Custom applications are capable of detecting the flow velocity and its directionality. Scheenen et al. (124–126) have developed instruments and NMR experimentation to partition flows to different vessel groups within a stem cross section and demonstrate the practical use of NMR to study water transport in plants. Other researchers have developed small-scale, portable NMR systems to measure sap flow directionality in model species such as *Phaseolus vulgaris* and *Populus* under field conditions (69, 101, 161), and novel flow methods are being developed and improved (20). Similarly, neutron radiography (NR) has also been utilized to visualize the functional status of the xylem in intact plants and can allow visualization of larger segments (several centimeters in length) with good temporal resolution (150). NR is also being applied to study water uptake and transport processes in the rhizosphere (98, 150, 163), allowing the user to observe how root networks acquire resources from the soil profile (**Figure 1**).

The primary limitation to several noninvasive imaging tools such as NMR, positron emission tomography (PET), and NR imaging is the resolution of the final images; the pixel dimensions are often approximately the same diameter or larger than the diameter of xylem conduits (**Table 1**). Because of this fundamental constraint, interpretation of the dynamics of the functional status of individual conduits can be reliably determined only in species with large-diameter vessels (59), whereas pixel information in scans of most species will be representative of several neighboring vessels or vessel groups. Yet the benefit of these methods is the ability to measure sap flow or the movement of tracers through the xylem. For example, radiolabeled nanoparticles (NPs) are commonly used in medical imaging applications and have been extended recently to track and quantify uptake and long-distance transport processes in plants (144, 167). Davis et al. (36) used autoradiography and PET-microCT to noninvasively track the movement of radiolabeled copper oxide NPs in lettuce seedlings, where they were rapidly absorbed across intact roots and transported to young leaves. Optically tagged NPs also have been used to investigate long-distance transport in intact plants (57, 103), but these methods can be difficult to implement due to background luminescence from the plant tissues being studied. Pairing technologies, for example, NMR with cryo-scanning electron microscopy, can help resolve spatial distributions of functional conduits (**Figure 2**).

Nuclear magnetic resonance (NMR): noninvasive imaging method that can detect water content and sap flow velocity

Neutron radiography (NR): nondestructive imaging technique and alternative to microCT because of differences in attenuation of neutrons and X-rays

Positron emission tomography (PET): imaging method often coupled with microCT to detect the movement of tracers through the xylem

THE RISE AND DECLINE IN SAP FLOW

Water flow through the xylem will decline from its maximum for a variety of reasons, although much of the literature focuses on hydraulic dysfunction or decreases in flow due to plant responses to low water availability, high vapor pressure deficit, or seasonal abiotic environmental conditions. Xylem-feeding insects, xylem-dwelling pathogens, mechanical damage, and phenological processes can all render the xylem nonfunctional and contribute to the overall decline in flow.

Table 1 Advantages and disadvantages of available and emerging technologies for monitoring the functional status of xylem networks and their spatial and temporal resolution

Technology	Spatial resolution	Temporal resolution	Advantages	Disadvantages
MicroCT	~325 nm–10 μ m	Minutes to hours	Nondestructive, cryo-compatible, 3D, can be laboratory-based and combined with NPs or other tracers	Cost, computation power, potential for X-ray damage, limited sample dimensions
NMR	50–400 μ m	Minutes to hours	Nondestructive, velocity flow imaging, measurement of flow direction	Nonmagnetic materials only, often horizontal bore, limited sample dimensions
NR	~100 μ m	Minutes to hours	Nondestructive, depletion zones at soil–root interface, uptake with deuterated water, 3D (tomography)	Low resolution, thin chambers required for root studies, neutron beam radiation exposure
SEM	<5 nm	Minutes	Exceptional magnification and depth of field, cryo-compatible, minimal sample preparation with ESEM	Limited to exposed face and small sample dimensions
OV	5–10 μ m	Seconds to minutes	Noninvasive (unless mounted to stems), inexpensive, simple construction	Limited depth of field, embolism prior to installation is unknown
PET/ autoradiography	~400 μ m	Minutes to hours	Noninvasive, can be combined with NPs as a tracer	Low resolution, positron radiation exposure

Abbreviations: 3D, three-dimensional; ESEM, environmental scanning electron microscopy; microCT, microcomputed tomography; NMR, nuclear magnetic resonance; NP, nanoparticle; NR, neutron radiography; OV, optical vulnerability; PET, positron emission tomography; SEM, scanning electron microscopy.

Losses of conductivity resulting from embolisms can be represented with vulnerability curves that plot the percent loss of conductivity (PLC) as a function of declining water potential. PLC is calculated as the native stem conductivity divided by the maximum stem conductivity obtained after removing existing embolisms (138). Much attention has been focused on the relationship between the minimum water potential experienced in a species in the field (P_{\min}) within the context of its vulnerability curve (27) and the difference between P_{\min} and the water potential that induces a 50% loss of conductivity (P_{50}), also known as the safety margin. Safety margins are useful in terms of comparing the relative resistance of species within their natural environment, but ultimately, declines in flow will occur when the water potential drops below a threshold at which stomatal conductance declines to zero (**Figure 3d**). Thus, a major point of uncertainty is related to how frequently and for how long plants experience water potentials close to but not exceeding P_{50} , which typically falls on the steepest part of the vulnerability curve. Recovery of hydraulic conductivity resulting from declines in water potential up to or beyond P_{50} can take weeks to months, typically requiring the growth of new xylem (24, 71), but water potentials inducing some degree of stomatal closure for long periods of time will significantly detract from the total amount of carbon a plant can extract from the atmosphere.

During the functional life span of a given population of xylem conduits, their overall conductance will vary depending on their location within the xylem network, their age, their distal and basal connectivity (i.e., their connection to sources and sinks of water), anatomical properties that determine the overall resistance of the conduit (e.g., length, diameter), and the pressure gradient driving the flow. The general characterization of bulk sap flow in woody plants during the growing

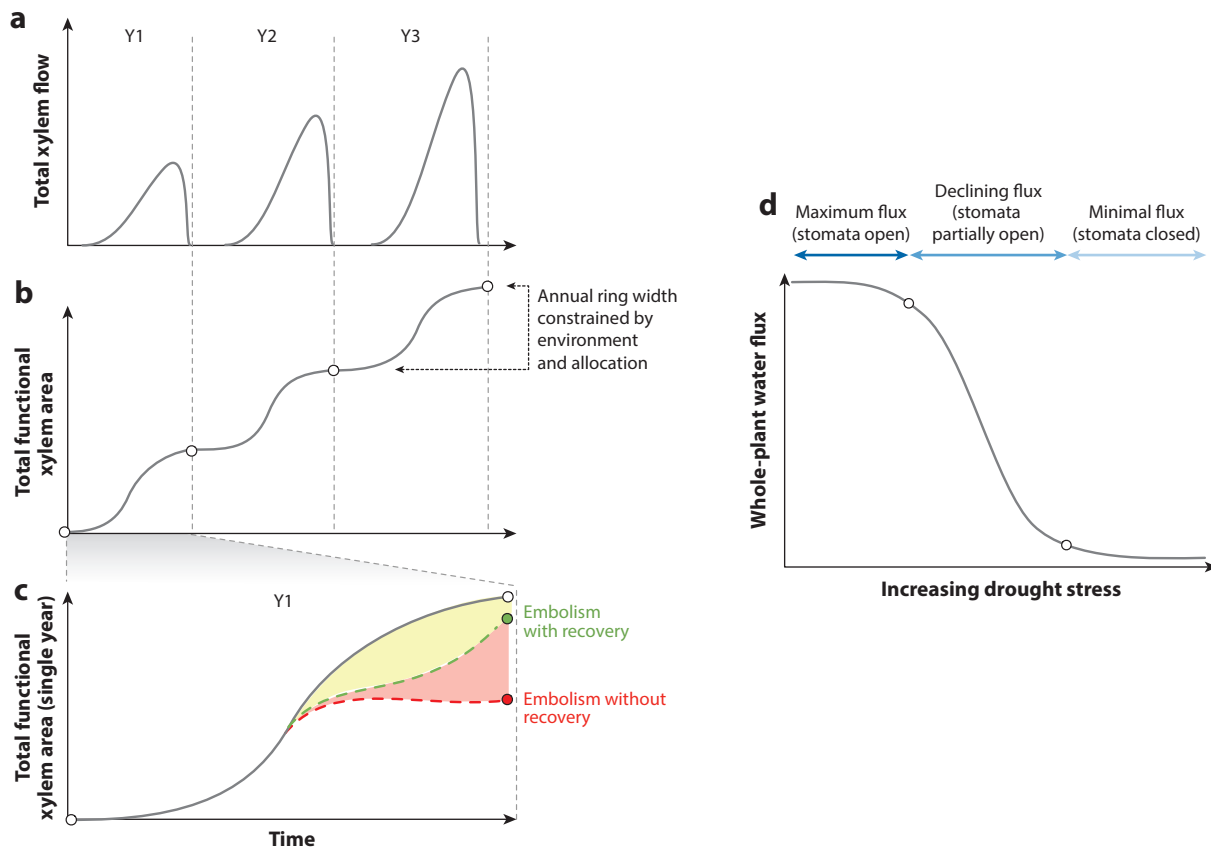


Figure 3

Conceptual changes in cumulative water transport and functional xylem area over the course of three consecutive growing seasons in a temperate, perennial woody plant. (a) In the first year (Y1), xylem sap flux lags slightly behind xylogenesis due to the developmental delay between cell differentiation and functionality. Flux increases until seasonal leaf senescence occurs, at which point the plant remains dormant and sap flow ceases due to lack of foliage (e.g., in deciduous trees) or frozen xylem sap. (b) In subsequent years (Y2 and Y3), total flux increases above the Y1 maximum because of the addition of xylem and the contribution of Y1 xylem to leaves produced in Y2. Annual ring width will be a function of environmental conditions and allocation of carbon, water, and nutrients to secondary growth that will be needed to support the canopy. (c) In a given year, realized maximum sap flux can deviate from the potential maximum because of drought-induced cavitation or damage to the vascular system. In most species, losses in hydraulic conductivity due to cavitation are irreversible during the growing season, but some species can apparently restore functionality to some proportion of their xylem network, although an opportunity cost is incurred on the total sap flow to the leaves during the recovery phase. (d) A major point of uncertainty is the amount of time that plants spend within the three domains shown, where increasing water stress leads to stomatal closure and therefore declines in whole-plant water flux.

season shows a strong diurnal pattern responding to stomatal aperture, which is in turn sensitive to light availability, exposure of various parts of a canopy, vapor pressure deficit, and water availability. Sap flow can show a lag time of several minutes after stomatal opening as tension builds in the xylem sap from water evaporation and draws on water storage and root water uptake via complex xylem pathways (50, 92). X-ray microCT imaging, a noninvasive diagnostic imaging tool, has provided new and unexpected insights into tissue-specific depletion of water from storage compartments (73, 155) that help to identify these transient adjustments between sap flow and water loss from the foliage.

Estimates of leaf area-specific hydraulic conductance of the SPAC range from less than 1 mmol H₂O m⁻² leaf area s⁻¹ MPa⁻¹ to almost 20 mmol H₂O m⁻² s⁻¹ MPa⁻¹ (95). Whole-plant hydraulic conductance varies among major plant groups, with angiosperms having both higher maximum leaf-specific conductance and a larger range than gymnosperms (5, 51). Within species, maximum leaf-specific hydraulic conductance varies both diurnally (119) and seasonally (104). Surprisingly, the highest values of leaf-specific hydraulic conductance occur among desert shrubs (95), although trees from the humid tropics, particularly pioneer species, tend to have higher conductance than trees from temperate biomes (5, 91, 162). Furthermore, Brenes-Arguedas et al. (8) showed that among tropical seedlings, dry forest species had higher leaf-specific conductance than wet-forest species. Collectively, these data suggest high rates of water transport to take advantage of precipitation when water is otherwise limited, or when rapid growth is a strong component of a specific life-history strategy. Although the majority of plant fresh mass is water, this high hydraulic conductance means that atmospheric vapor pressure deficit can drive sufficient rates of transpiration to turn over and replace leaf and plant water rapidly (63). Previous reviews estimated whole-plant water use ranged from 10 kg per day in a temperate plantation to 1,180 kg per day in an Amazonian overstory tree (162). For leaves, turnover times of water can be as fast as 9 minutes for a detached and transpiring tobacco leaf (134) but are slower, ranging from 1 to 3 hours, for intact *Calycanthus* leaves growing under natural conditions (119). In tropical trees, studies using deuterated water have revealed that at the whole-tree scale, water residence times can range from 4 to 25 days (65, 92), while in temperate conifers, the residence times range from 34 to 79 days (90). Collectively, plants exhibit an extraordinary range of hydraulic capabilities to adapt to a wide range of habitats and growth forms.

Once the secondary xylem reaches a certain age or depth behind the cambium, it becomes nonfunctional. In many species, heartwood is produced in secondary xylem, and/or the xylem is occluded with tyloses, gums, or other substances that prevent the axial conductance of water. In other cases, the radial resistance to flow across annual growth rings likely limits the contribution of older wood to axial flow. In addition to the decline in the contribution to axial transport, deposition of chemical compounds (e.g., phenolics) limits radial and lateral movement of water because of their hydrophobic properties, thereby constraining the functional role of the heartwood in water storage for capacitance or long-term storage of nonstructural carbohydrates. At this point, xylem within the heartwood largely serves a structural role (99, 133). Complicating modeling efforts are the complex interactions between xylem-dwelling bacterial and fungal pathogens, as well as hemiparasitic angiosperms (e.g., mistletoes, Santalales) that lead to varying degrees of complete or partial decline in water transport (114). In many cases, successful colonization or parasitism is dependent at least in part on continued flow. As a consequence, partitioning the influence of such organisms from the host-specific declines in sap flow due to phenology is difficult.

VISUALIZATION AND MEASUREMENT

Hydraulics Methods to Quantify Resistance to Embolism Spread in Stems

A major challenge to characterizing plant resistance to embolism formation and spread and accurately monitoring xylem functional status over time is the location and scale of the xylem within the plant. Cutting into the xylem to study it necessarily disrupts the negative pressure environment established through transpiration, sometimes leading to artifacts that overestimate the total amount of gas in the xylem. Although there are methods to artificially induce embolisms into living plants and monitor the physiological responses (140), many studies characterize stem resistance to embolism spread using excised stem segments to measure declines in hydraulic conductivity as the stem is exposed to increasingly negative xylem pressures to simulate drought (138).

Several laboratory-based methods of inducing negative pressures that simulate declining stem water potential have been developed, including benchtop dehydration, centrifugation, and air injection. Benchtop dehydration involves drying branches to a range of water potentials and measuring PLC (138, 141). Recent work has identified a number of potential artifacts with these methods along with solutions to improve accuracy (149, 159). The centrifuge and air injection techniques allow for quick generation of vulnerability curves using multiple measurements on the same stem (e.g., six stem samples per day). The centrifuge method uses centrifugal force to induce progressively more negative tensions on excised stem sections between measurements of conductivity (1, 112), and the Cavitron modification allows for simultaneous conductance measurements as the stem is spinning (32), thereby significantly increasing sample throughput. Centrifugation methods are not recommended for species with long vessels that can embolize easily in short excised stem segments using the standard 14.5-cm-diameter rotor (87). The air injection technique functions by applying positive pressure to stems and assuming that the positive pressure simulates the negative pressure of equal value but opposite sign experienced during drought (31). There has been much debate regarding the accuracy of different methods (26, 33, 64, 147), and new imaging techniques such as microCT and optical vulnerability (OV; described below) have allowed for validation and improvement (26, 72, 87).

Conductance Versus Conductivity

While measures of conductivity are often used to describe the maximum amount of water that can flow through a stem, measures of conductance more accurately quantify the functional dynamics of a plant's actual water use. Conductance takes into account differences in the functional status of vessels at a given point in time, is frequently scaled to leaf area, and is representative of the entire hydraulic architecture of the measured sample, where significant portions of the xylem (i.e., older annual rings) may not have the same capacity or direct connection to the foliage (**Figure 4**; see the sidebar titled Conductance Versus Conductivity).

Conductivity can be conceptualized as a material property because it is scaled to stem segment length and is commonly used to construct stem xylem vulnerability curves (27). Hydraulic conductivity is generally limited to measurements on nonbranching segments and necessarily avoids the complexities of xylem in branch networks, leaf insertions, petioles, and leaves that can make up most of the total resistance in a plant (152). Indeed, measurements of maximum stem conductivity are performed by artificially removing all xylem blockages by either vacuum infiltration or flushing the sample with pressurized solutions (93), thereby restoring hydraulic pathways in the xylem that may not have been functional in planta, leading to overestimates of maximum conductivity.

Meanwhile, conductance incorporates the anatomical and morphological complexities of the shoot and the actual flux of water moving through the plant to the foliage or flowers. Hydraulic conductance can be measured on stems with any branching structure using a variety of methods (74, 122) and can include intact shoots with or without leaves, leading to a much closer approximation of whole-plant hydraulic water use (41). Measuring conductance therefore allows for much more direct comparison of the hydraulic efficiency of different organs such as leaves and flowers (116). Hydraulic conductance is useful in modeling plant metabolism (123) and is commonly scaled by leaf area or dry mass, both estimates of size and resource allocation. Thus, leaf area-specific or sapwood area-specific hydraulic conductance yields a return on investment, which is important when considering the costs and benefits of different structures in terms of water, carbon, and nutrients. Most importantly, measurements of shoot hydraulic conductance explicitly include the points of highest hydraulic resistance in the shoot, namely, the branch tips and leaves, where conduits narrow (3, 105).

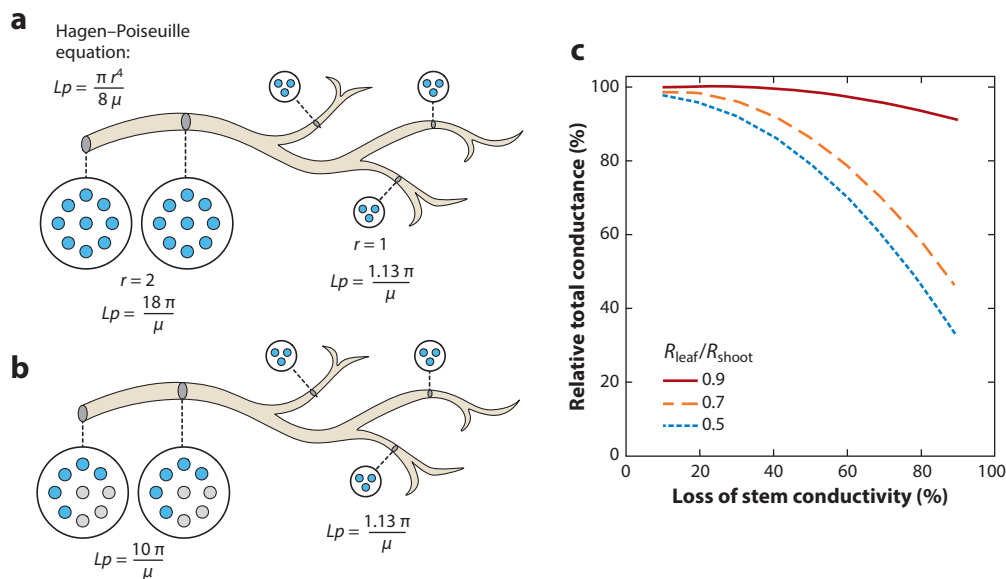


Figure 4

Fluid flow through a pipe can be modeled according to the Hagen–Poiseuille equation, in which the hydraulic conductance (L_p) is proportional to the pipe’s inner radius (r) divided by the viscosity (μ) of the fluid. Thus, the conductivity of a pipe scales with the fourth power of its radius. The axial taper in xylem conduits means that conduits in distal branches are narrower than those in basipetal stems, and the number of conduits in stems versus distal branches can vary across individuals and life stages. While gas embolism in the xylem would decrease the conductivity of a basipetal stem segment (see the sidebar titled *Conductance Versus Conductivity*), its influence on the conductance of the entire shoot and on water flux to the leaves would depend on the relative conductances of the distal branches compared to the basipetal segment. (*a, b*) A branch with conduit taper from $r = 2$ (arbitrary units) in the basipetal segment to $r = 1$ in the distal branches. Conduits colored blue are hydrated, while gray conduits are filled with gas embolism. (*a*) With no embolism, L_p of the basipetal segment (on which vulnerability measurements are typically measured) is approximately 15 times higher than L_p of the distal branch segments, whose conductances are additive in parallel. (*b*) Even with 40% embolism in the basipetal stem, this segment still has L_p approximately 8 times higher than the total L_p of the distal branches. Because terminal branches likely represent the greatest resistance in the shoot, significant levels of embolism can occur in the basipetal stem without influencing the total water flux to the leaves. (*c*) The effect of embolism-induced declines in stem conductivity on the total conductance depends on the partitioning of hydraulic resistance throughout the hydraulic pathway. When the resistance of the leaves is higher than the resistance of the shoot, there can be substantial loss of conductivity without impacting conductance and the flux of water to the leaves. As the hydraulic resistance of the shoot increases relative to the resistance of the leaves, loss of conductivity of a basipetal segment has a greater impact on shoot hydraulic conductance and supply of water to the leaves. Figure adapted with permission from Reference 89.

Given these strong differences between the commonly measured hydraulic conductivity of stem segments and more realistic estimates of water flux through whole-stem conductance, it is perhaps not surprising that hypothesized trade-offs between safety and efficiency of xylem based on measurements of stem conductivity have found little support (10, 49). Rather, such a trade-off may exist in measurements of whole-shoot hydraulic conductance, which incorporate the high resistance of the terminal branches. Because the conductance of a single conduit scales to the fourth power of the radius, changes in conduit diameter have a disproportionate effect on hydraulic conductance. Typically, measurements of PLC are specifically designed to assess the impact of embolism formation and spread on the maximum conductivity of an excised stem; however, such measurements should take into account the number of annual rings being measured and the leaf area being supplied. The assumption that the measured PLC from a uniform pressure gradient would lead to an equivalent loss in conductance is likely inaccurate (92, 154) (see the sidebar titled *Conductance Versus Conductivity*). For example, in a simplified branching shoot (**Figure 4**), the

CONDUCTANCE VERSUS CONDUCTIVITY

All measurements of hydraulics start from a common measurement of flow per unit time, frequently measured in terms of mass (kilogram) or molar equivalents (millimole) per second. For measurements of hydraulic conductance, the flow rate is divided by the pressure gradient (MPa) driving the flow. To calculate leaf- or sapwood-specific hydraulic conductance, the hydraulic conductance is divided by the leaf or sapwood area, respectively, to account for either the efficiency of water delivery to the transpiring leaf area or the return on investment in stem biomass (in both cases units are $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$). In contrast, hydraulic conductivity is calculated by multiplying the hydraulic conductance by the length of the stem segment, assuming that the pressure head driving flow is homogeneously dissipated through the length of the stem segment, which is equivalent to dividing the flow per time by the pressure drop per length (i.e., dividing mmol s^{-1} by MPa m^{-1}). Thus, conductivity is a bulk average property of a heterogeneous material (reported in units of $\text{mmol m s}^{-1} \text{MPa}^{-1}$) that can be measured only in linear systems with resistances aligned in series. However, in terms of the efficiency of the entire branching network, hydraulic conductance more closely quantifies the efficiency of water flow through the shoot network (reported in units of $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) (**Figure 4**). Because the conductance of an individual conduit scales to the fourth power of its radius, even assuming a constant number of conduits throughout a shoot reveals that an approximately 50% loss in stem conductivity can occur before measurable declines in shoot hydraulic conductance are observed (**Figure 4**). The simple model presented in **Figure 4** assumes that half of the resistance is contributed by the roots, and the effects of loss in stem conductivity on shoot conductance depend on the ratio of resistance partitioned across leaves and shoots (92).

The distinction between conductivity and conductance has important implications for our understanding of plant hydraulic architecture because the two metrics reveal different characteristics. It has long been predicted that there should be a trade-off between safety (i.e., low vulnerability to embolism) and hydraulic efficiency, though there is little evidence for the existence of this trade-off (49). One reason could be that the hydraulic conductance of the shoot can vary independently of the conductivity of a stem segment (**Figure 4**). As a result, questions of shoot hydraulic efficiency should consider the hydraulic conductance of the shoot network that supplies the leaves. Hydraulic conductance has some additional benefits:

- Hydraulic conductance can be measured on branching networks and allows comparison of different structures (e.g., whole shoots or leaves) using the same units. Although positive correlations have been shown between leaf hydraulic conductance and stem hydraulic conductivity, such correlations are not necessarily expected.
- Stems are not the major source of resistance in the hydraulic pathway. Due to conduit taper, the highest hydraulic resistance in shoots occurs in the branch termini. How much loss of conductivity decreases hydraulic conductance and water use depends on the partitioning of resistances in the hydraulic pathway, and even as much as a 50% loss of stem conductivity can have a relatively minor effect on total plant hydraulic conductance (**Figure 4**).
- Hydraulic conductance is directly linked to plant water use and can be dynamically estimated based on measurements of sap flow and water potential.
- Measuring entire branching shoots with multiple rings implicitly incorporates the anatomical complexity of the flow path and the morphological complexity of shoots that influence plant water use.
- Measuring vulnerability of hydraulic conductance would more directly link embolism formation to changes in flux, thus providing a more direct assessment of the hydraulic safety–efficiency trade-off and better facilitating incorporation of hydraulic properties in ecosystem water models.

hydraulic resistance due to conduit taper largely mitigates the loss of hydraulic conductivity in the main stem. In other words, loss of conductivity is not equal to loss in conductance, and the two should be reconciled in more than just theoretical frameworks (34, 83, 84).

One emerging consensus from models and data is that tree boles and even branches are hydraulically overbuilt for their gas exchange capacity. Because embolism refilling rarely, if ever, occurs, building redundancy into the system can maintain water transport despite accumulation of embolisms, without the need to build new xylem to maintain maximum gas-exchange capacity during a single growing season (**Figure 3**). Exactly how hydraulically redundant the stem is, however, is unclear, as the majority of water supplied to current-year leaves flows through current-year xylem (94). Resolving the apparent contradiction between sap flow studies that measure flow through older wood and hydraulic and dye-staining studies that suggest a minimal role for older wood will be important in understanding the resilience of trees to drought.

While microCT and other noninvasive methods show great promise for allowing scientists to monitor the functional status of the xylem network, factors such as accessibility, cost, logistics, and computational limitations still restrict their widespread use. To overcome some of these limitations, Brodribb et al. (16, 18) developed a simple, low-cost imaging system to detect cavitation events in leaves that has now been applied to various plant tissues, including roots and stems (120). Known as the OV method, this technique takes advantage of changes in the refractive index of xylem conduits during cavitation, when liquid water is replaced with water vapor. By taking time-lapse images of a leaf with a flatbed scanner or digital camera, the successive images can be processed to pinpoint the spatial and temporal origins of embolism spread through the xylem network. This method is particularly exciting because OV systems can be made inexpensively (<\$300/unit); they are small and lightweight; and all of the construction and programming details are open source (<http://www.opensourceov.org>). Recent work has shown good agreement with microCT imaging (136), an important step in cross-validation. Weatherproofing the housing and supplying OV systems with battery power should allow them to be used extensively in the field to corroborate results from laboratory and greenhouse experiments with mature trees in a natural environment.

Ultimately, one of the primary goals is to determine a species-specific drought index, whereby the recent environmental conditions (e.g., temperature and precipitation) can be used to predict the length of time that individual plants or whole forests can tolerate drought before succumbing to hydraulic failure and ultimately death, as well as the opportunity costs of stomatal closure during drought (6, 97). Although some work in herbaceous plants suggests that leaf vein embolism is reversible (151), a growing body of work suggests that cavitation resulting from drought severely limits the ability of a species to recover from drought, as persistent embolism blocks the transport of water needed for transpiration (15, 110, 137, 156).

Xylem Network Analysis

The spatial complexity of xylem networks makes them inherently difficult to study (152). Flow through xylem networks is dependent on numerous network parameters, including the number and frequency of network connections, the anatomical characteristics of the xylem conduits and their spatial distribution within the network locally (e.g., within an internode), and the up- and downstream connections to water sources and sinks. These hydraulic pathways also function as potential routes for the spread of embolism and pathogens. These connections therefore underpin the performance of a xylem network under drought stress because of the physical pathway for embolism movement from conduit to conduit through pits. Air can enter through physical damage

(e.g., herbivory), fire, or freeze–thaw embolism, and gas trapped within other tissues (e.g., fibers) can also enter the xylem, which introduces additional complexity. Yet, once present, movement of gas through the xylem network should be largely predictable, and therefore the comprehensive analysis of 3D xylem networks in the future will allow for additional insight into strategies that have arisen to adapt to different environmental conditions. For example, even within a tissue, xylem organization can vary significantly in both the size of the conduits and the degree of connectivity (e.g., shallow versus deep roots) (66). Imaging tools such as microCT are now allowing for a much more comprehensive understanding of the xylem network and the functional implications of where and how frequently conduits connect (12).

The porosity of interconduit pit membranes is a major factor determining whether air embolisms will spread between two xylem conduits. Therefore, the connectivity of xylem networks has a strong influence on how sap and air embolisms move through xylem. Conifer xylem represents extremely high connectivity whereby almost every tracheid is connected to several other tracheids along most of the length of their radial walls via bordered pits (25), although connections on tangential walls and across annual growth rings appear to be more limited (23). Whereas conifer xylem can be placed at one end of a connectivity spectrum, angiosperm xylem can express a wide range of connectivity, from highly connected networks found as an adaptation to mesic habitats, where the risk of cavitation and embolism spread is low, to highly isolated vessels and segmented stems prevalent in arid habitats, thereby functionally splitting the trunk into discrete, modular units (127).

High network connectivity provides many alternative pathways for water transport in the event of hydraulic dysfunction resulting from drought or freeze–thaw embolism. Connectivity alone, however, is not a good predictor of embolism resistance. Northern hemisphere conifers, for example, have highly integrated xylem networks, but the small size of their conduits and other drought adaptations make them highly resistant to cavitation. In contrast, angiosperms with integrated networks are often restricted to mesic habitats, presumably because of the risk of significant losses in hydraulic conductivity in a network with comparatively fewer large-diameter vessels as embolism spreads. Because vessels are specialized for axial transport, the supporting matrix of fibers and parenchyma becomes increasingly important for water storage in arid habitats. Initial analyses of the 3D organization of the xylem network suggest that vessels are not connected along their entire axial length (2, 12, 78, 158, 160), and instead, vessel connections can be short and infrequent.

Theoretical models treat the xylem network in a variety of ways based on the complexity of the network and the included tissues. For example, because of its relative simplicity and high connectivity, conifer xylem can be successfully modeled as a porous medium, similar to soils or synthetic foams with pores of regular, isomorphic dimensional properties (28). This model appears to be valid based on high lateral connectivity but low radial connectivity; for example, in vivo imaging of drought spread in *Sequoia sempervirens* appears to be limited to large events that lead to massive losses of tracheids within an annual ring, but the discontinuity with adjacent annual rings prevents radial spread (23).

Given the structural heterogeneity of angiosperm xylem, vessel networks with more and longer connections provide alternative pathways for water transport if an embolism forms (81). Extreme cases of this redundancy can even result in reverse flow in stems to bypass embolized vessels (78). However, high connectivity also allows for easier embolism spread to connected vessels, suggesting that there may be a trade-off between the added redundancy and safety (81). Indeed, connectivity in angiosperms has been shown to vary across species (42) and along environmental gradients (127), suggesting that this may be an important and plastic trait for plant responses to the environment.

Early attempts to quantify xylem network connectivity relied on two-dimensional (2D) cross sections. Indeed, we have learned much from these relatively easily measured 2D metrics, for example, the vessel grouping index (21, 22). However, there was early recognition that 3D representations of xylem vessel networks are needed to understand how xylem sap and air embolisms spread in xylem networks. Serial sectioning (62, 70, 152, 168, 169), insertion of fine wires into vessels (68), and resin casting (2, 47, 107) all provide important insights but are time consuming and difficult to analyze. Other techniques such as the classic double saw cut experiments (52, 82) and newer sap flow measurements (19), where lateral movement via redistribution between roots on opposite sides of large trees necessarily requires a certain degree of connectivity and hydraulic integration within the stem, demonstrate how network connectivity allows for lateral movement of sap around embolized vessels but does not directly characterize the xylem network structures making that possible.

Recently, microCT has emerged as a useful tool for understanding the complex 3D nature of xylem vessel networks and anatomy (8, 12, 14, 158). Similar to noninvasive imaging performed with NMR (59, 125), microCT images allow the user to determine the functional status of the xylem conduits. Whereas the resolution of NMR is low and the size of individual pixels is typically larger than the diameter of most xylem conduits, microCT works on a more traditional microscope optics platform and can be used with different objectives to magnify the sample, yielding pixel resolutions of 325 nm or less, although the field of view necessarily becomes much smaller at higher magnifications (**Table 1**). MicroCT has seen a significant increase in use for studying the functional status of xylem networks since some of the original work with these systems was performed (13, 23, 35, 72, 73).

3D xylem networks extracted from microCT scans of grapevines and processed using custom software have rapidly improved our understanding of 3D vessel arrangement, connectivity, and implications for flow paths of xylem sap and the spread of air embolisms (12, 78). Although these techniques require specialized equipment and extensive computing power, the greater availability of laboratory-based microCT systems and affordable high-speed computers is quickly making these techniques much more accessible. Often, a wide range of tools is necessary to address specific questions, and the rapid development of new instruments and techniques has allowed for a major surge in our understanding of plant hydraulics.

SCALING FROM LEAVES TO ECOSYSTEMS

To understand the role of vascular function at the landscape and ecosystem scales, the methods described here rely on intense measurements often of only a few individual plants. Challenges exist in scaling such measurements (56). New approaches described above to better model xylem networks, deal with the methodological constraints of sap flow techniques, and apply portable noninvasive systems can help to address uncertainty associated with upscaling from sensors to trees and to stands (56, 113). Extensively studied agricultural monocultures that contain weighing lysimeters, flux towers, and a range of other sensors (76) could also provide needed infrastructure to validate data across scales. New remote sensing tools and products provide the best opportunities for measuring and modeling ecosystem-scale water fluxes, but accurate partitioning of fluxes and energy balance components still relies heavily on field validation with sensors and physiological measurements. Understanding the role that plants play in regulating these fluxes and physical interactions with the atmospheric boundary layer still requires insight from the cell to the whole plant. Commercial application of inexpensive sensor networks (e.g., surface renewal estimates of evapotranspiration at large scale) provides a unique opportunity to better explore the role of plant

vasculature in moderating stand/ecosystem fluxes and enabling improved remote quantification of both evapotranspiration and plant water stress.

SUMMARY POINTS

1. The evolution and adaptation of the plant vascular system have allowed plants to colonize nearly every terrestrial habitat on Earth, and over 30 years of research on the topic have significantly advanced our understanding of the fundamental principles of plant water use and transport.
2. One of the major obstacles to studying the functional status of the xylem network has been the disproportionate scaling between xylem conduit diameter and length, along with the positioning of the xylem conduits within an opaque tissue and the surrounding matrix of living and nonliving cells. Recent advancements in noninvasive imaging such as nuclear magnetic resonance, microcomputed tomography, and the optical vulnerability method have provided new avenues for research into the effects of drought-induced hydraulic failure and the link to plant mortality.
3. Measurement of losses in hydraulic conductivity of stem segments exposed to simulated drought stress have been informative and work well for comparative studies of drought resistance. Conductivity, however, does not fully incorporate the spatial and temporal dynamics of downstream tissues (e.g., leaves, flowers, fruit) that represent major points of resistance in the hydraulic pathway. Complementary measurements of conductance should therefore be considered as standard measurements that will allow greater comparative power across studies.
4. Extending the work done at the leaf and whole-plant levels to the forest canopy and ecosystem is now a major focus of research, in which physiological processes such as variable stomatal conductance and xylem functional status over time can be assessed remotely and integrated into landscape flux models.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Alder N, Pockman W, Sperry J, Nuismer S. 1997. Use of centrifugal force in the study of xylem cavitation. *J. Exp. Bot.* 48:665–74
2. André J-P. 2005. *Vascular Organisation of Angiosperms: A New Vision*. Enfield, NH: Sci. Publ.

18. Demonstrated a low-cost, open-source option for observing embolism in leaves, with subsequent applications to stems and roots.

3. Anfodillo T, Petit G, Crivellaro A. 2013. Axial conduit widening in woody species: a still neglected anatomical pattern. *IAWA J.* 34:352–64
4. Bailey IW, Tupper WW. 1918. Size variation in tracheary cells: I. A comparison between the secondary xylems of vascular cryptogams, gymnosperms and angiosperms. *Proc. Am. Acad. Arts Sci.* 54:149–204
5. Becker P, Tyree MT, Tsuda M. 1999. Hydraulic conductances of angiosperms versus conifers: similar transport sufficiency at the whole-plant level. *Tree Physiol.* 19:445–52
6. Blackman CJ, Brodribb TJ, Jordan GJ. 2010. Leaf hydraulic vulnerability is related to conduit dimensions and drought resistance across a diverse range of woody angiosperms. *New Phytol.* 188:1113–23
7. Boyce CK. 2010. The evolution of plant development in a paleontological context. *Curr. Opin. Plant Biol.* 13:102–7
8. Brenes-Arguedas T, Roddy AB, Kursar TA. 2013. Plant traits in relation to the performance and distribution of woody species in wet and dry tropical forest types in Panama. *Funct. Ecol.* 27:392–402
9. Brodersen CR. 2013. Visualizing wood anatomy in three dimensions with high-resolution X-ray microtomography (μ CT)—a review. *IAWA J.* 34:408–24
10. Brodersen CR. 2016. Finding support for theoretical tradeoffs in xylem structure and function. *New Phytol.* 209:8–10
11. Brodersen CR, Knipfer T, McElrone AJ. 2018. In vivo visualization of the final stages of xylem vessel refilling in grapevine (*Vitis vinifera*) stems. *New Phytol.* 217:117–26
12. Brodersen CR, Lee EF, Choat B, Jansen S, Phillips RJ, et al. 2011. Automated analysis of three-dimensional xylem networks using high-resolution computed tomography. *New Phytol.* 191:1168–79
13. Brodersen CR, McElrone AJ, Choat B, Matthews MA, Shackel KA. 2010. The dynamics of embolism repair in xylem: in vivo visualizations using high resolution computed tomography. *Plant Physiol.* 154:1088–95
14. Brodersen CR, Roddy AB. 2016. New frontiers in the three-dimensional visualization of plant structure and function. *Am. J. Bot.* 103:184–88
15. Brodribb TJ, Bowman DJ, Nichols S, Delzon S, Burlett R. 2010. Xylem function and growth rate interact to determine recovery rates after exposure to extreme water deficit. *New Phytol.* 188:533–42
16. Brodribb TJ, Carriqui M, Delzon S, Lucani C. 2017. Optical measurement of stem xylem vulnerability. *Plant Physiol.* 174:2054–61
17. Brodribb TJ, Holbrook NM. 2003. Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiol.* 132:2166–73
18. Brodribb TJ, Skelton RP, McAdam SA, Bienaimé D, Lucani CJ, Marmottant P. 2016. Visual quantification of embolism reveals leaf vulnerability to hydraulic failure. *New Phytol.* 209:1403–9
19. Burgess S, Bleby T. 2006. Redistribution of soil water by lateral roots mediated by stem tissues. *J. Exp. Bot.* 57:3283–91
20. Buy S, Le Floch S, Tang N, Sidiboulouar R, Zanca M, et al. 2018. Flip-flop method: a new T1-weighted flow-MRI for plants studies. *PLOS ONE* 13:e0194845
21. Carlquist S. 1984. Vessel grouping in dicotyledon wood: significance and relationship to imperforate tracheary elements. *Aliso* 10:505–25
22. Carlquist S. 2013. *Comparative Wood Anatomy: Systematic, Ecological, and Evolutionary Aspects of Dicotyledon Wood*. Berlin: Springer-Verlag
23. Choat B, Brodersen CR, McElrone AJ. 2015. Synchrotron X-ray microtomography of xylem embolism in *Sequoia sempervirens* saplings during cycles of drought and recovery. *New Phytol.* 205:1095–1105
24. Choat B, Brodribb TJ, Brodersen CR, Duursma RA, López R, Medlyn BE. 2018. Triggers of tree mortality under drought. *Nature* 558:531
25. Choat B, Cobb AR, Jansen S. 2008. Structure and function of bordered pits: new discoveries and impacts on whole-plant hydraulic function. *New Phytol.* 177:608–26
26. Choat B, Drayton WM, Brodersen C, Matthews MA, Shackel KA, et al. 2010. Measurement of vulnerability to water stress-induced cavitation in grapevine: a comparison of four techniques applied to a long-vesselled species. *Plant, Cell Environ.* 33:1502–12

27. Choat B, Jansen S, Brodribb TJ, Cochard H, Delzon S, et al. 2012. Global convergence in the vulnerability of forests to drought. *Nature* 491:752–55
28. Chuang Y-L, Oren R, Bertozzi AL, Phillips N, Katul GG. 2006. The porous media model for the hydraulic system of a conifer tree: linking sap flux data to transpiration rate. *Ecol. Model.* 191:447–68
29. Clearwater MJ, Luo Z, Mazzeo M, Dichio B. 2009. An external heat pulse method for measurement of sap flow through fruit pedicels, leaf petioles and other small-diameter stems. *Plant, Cell Environ.* 32:1652–63
30. Cochard H, Barigah T, Herbert E, Caupin F. 2007. Cavitation in plants at low temperature: Is sap transport limited by the tensile strength of water as expected from Briggs' Z-tube experiment? *New Phytol.* 173:571–75
31. Cochard H, Cruiziat P, Tyree MT. 1992. Use of positive pressures to establish vulnerability curves: further support for the air-seeding hypothesis and implications for pressure-volume analysis. *Plant Physiol.* 100:205–9
32. Cochard H, Damour G, Bodet C, Tharwat I, Poirier M, Améglio T. 2005. Evaluation of a new centrifuge technique for rapid generation of xylem vulnerability curves. *Physiol. Plant.* 124:410–18
33. Cochard H, Herbette S, Barigah T, Badel E, Ennajeh M, Vilagrosa A. 2010. Does sample length influence the shape of xylem embolism vulnerability curves? A test with the Cavitron spinning technique. *Plant, Cell Environ.* 33:1543–52
34. Couvreur V, Ledder G, Manzoni S, Way DA, Muller EB, Russo SE. 2018. Water transport through tall trees: a vertically-explicit, analytical model of xylem hydraulic conductance in stems. *Plant, Cell Environ.* 41:1821–39
35. Cuneo I, Knipfer T, Brodersen C, McElrone AJ. 2016. Mechanical failure of fine root cortical cells initiates plant hydraulic decline during drought. *Plant Physiol.* 172:1669–78
36. Davis RA, Rippner DA, Hausner SH, Parikh SJ, McElrone AJ, Sutcliffe JL. 2017. In vivo tracking of copper-64 radiolabeled nanoparticles in *Lactuca sativa*. *Environ. Sci. Technol.* 51:12537–46
37. Debenedetti PG. 1996. *Metastable Liquids: Concepts and Principles*. Princeton, N.J.: Princeton Univ. Press
38. Donoghue MJ. 2005. Key innovations, convergence, and success: macroevolutionary lessons from plant phylogeny. *Paleobiology* 31:77–93
39. Dória LC, Podadera DS, del Arco M, Chauvin T, Smets E, et al. 2018. Insular woody daisies (*Argyranthemum*, Asteraceae) are more resistant to drought-induced hydraulic failure than their herbaceous relatives. *Funct. Ecol.* 32:1467–78
40. Doyle JA. 2012. Molecular and fossil evidence on the origin of angiosperms. *Annu. Rev. Earth Planet. Sci.* 40:301–26
41. Drake PL, Price CA, Poot P, Veneklaas EJ. 2015. Isometric partitioning of hydraulic conductance between leaves and stems: balancing safety and efficiency in different growth forms and habitats. *Plant, Cell Environ.* 38:1628–36
42. Ellmore GS, Zanne AE, Orians CM. 2006. Comparative sectoriality in temperate hardwoods: hydraulics and xylem anatomy. *Bot. J. Linnean Soc.* 150:61–71
43. Evert RF. 2006. *Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development*. Hoboken, NJ: Wiley
44. Fernández E, Čermák J, Cohen Y, Ferreira I, Nadezhkina N, et al. 2017. *Methods to estimate sap flow*. Rep., ISHS Work. Group Sap Flow, Korbeek-Lo, Belg. https://www.ishs.org/sites/default/files/documents/methods_0.pdf
45. Frensch J, Hsiao TC. 1993. Hydraulic propagation of pressure along immature and mature xylem vessels of roots of *Zea mays* measured by pressure-probe techniques. *Planta* 190:263–70
46. Frensch J, Steudle E. 1989. Axial and radial hydraulic resistance to roots of maize (*Zea mays* L.). *Plant Physiol.* 91:719–26
47. Fujii T, Hatano Y. 2000. The LDPE resin-casting method applied to vessel characterisation. *IAWA J.* 21:347–59
48. Fukuda K, Kawaguchi D, Aihara T, Ogasa MY, Miki NH, et al. 2015. Vulnerability to cavitation differs between current-year and older xylem: non-destructive observation with a compact magnetic resonance imaging system of two deciduous diffuse-porous species. *Plant, Cell Environ.* 38:2508–18

27. Identifies the xylem safety margin as a useful metric for interspecific comparison of drought tolerance.

29. Example of noninvasive sap flow measurements on tissues otherwise difficult to measure, e.g., stems, pedicels.

49. Well-executed challenge to the paradigm of xylem safety–efficiency trade-off in conduit dimensions and anatomy.

59. First noninvasive imaging of embolism repair in grapevine using NMR, subsequently confirmed by Brodersen et al. (11).

60. Seminal work showing the possibility of measuring sap flow in plants.

49. Gleason SM, Westoby M, Jansen S, Choat B, Hacke UG, et al. 2016. Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency across the world's woody plant species. *New Phytol.* 209:123–36
50. Goldstein G, Andrade J, Meinzer F, Holbrook N, Cavelier J, et al. 1998. Stem water storage and diurnal patterns of water use in tropical forest canopy trees. *Plant, Cell Environ.* 21:397–406
51. Granier A, Claustres J. 1989. Relations hydriques dans un épicéa (*Picea abies* L.) en conditions naturelles: variations spatiales. *Acta Oecol.* 10:295–310
52. Greenidge K. 1955. Studies in the physiology of forest trees. III. The effect of drastic interruption of conducting tissues on moisture movement. *Am. J. Bot.* 42:582–87
53. Hacke U, Sperry J, Feild T, Sano Y, Sikkema E, Pittermann J. 2007. Water transport in vesselless angiosperms: conducting efficiency and cavitation safety. *Int. J. Plant Sci.* 168:1113–26
54. Hanssens J, De Swaef T, Nadezhdina N, Steppe K. 2013. Measurement of sap flow dynamics through the tomato peduncle using a non-invasive sensor based on the heat field deformation method. *Proc. IX Int. Workshop Sap Flow* 991:409–16
55. Helfter C, Shephard JD, Martínez-Vilalta J, Mencuccini M, Hand DP. 2007. A noninvasive optical system for the measurement of xylem and phloem sap flow in woody plants of small stem size. *Tree Physiol.* 27:169–79
56. Hernandez-Santana V, Hernandez-Hernandez A, Vadeboncoeur MA, Asbjornsen H. 2015. Scaling from single-point sap velocity measurements to stand transpiration in a multispecies deciduous forest: uncertainty sources, stand structure effect, and future scenarios. *Can. J. For. Res.* 45:1489–97
57. Hirschmüller A, Nordmann J, Ptacek P, Mummenhoff K, Haase M. 2009. In-vivo imaging of the uptake of upconversion nanoparticles by plant roots. *J. Biomed. Nanotechnol.* 5:278–84
58. Hochberg U, Windt CW, Ponomarenko A, Zhang Y-J, Gersony J, et al. 2017. Stomatal closure, basal leaf embolism and shedding protect the hydraulic integrity of grape stems. *Plant Physiol.* 174:764–75
59. Holbrook NM, Ahrens ET, Burns MJ, Zwieniecki MA. 2001. In vivo observation of cavitation and embolism repair using magnetic resonance imaging. *Plant Physiol.* 126:27–31
60. Huber B. 1932. Observation and measurement of vegetable sap flow. *Rep. Ger. Bot. Soc.* 50:89–109
61. Huber B, Schmidt U. 1937. A compensation method for thermoelectric measurement of slow sap flow. *Ber. Deutsch. Bot. Ges.* 55:514–29
62. Huggett B, Tomlinson PB. 2010. Aspects of vessel dimensions in the aerial roots of epiphytic Araceae. *Int. J. Plant Sci.* 171:362–69
63. Hunt ER, Nobel PS. 1987. Non-steady-state water flow for three desert perennials with different capacitances. *Funct. Plant Biol.* 14:363–75
64. Jacobsen AL, Pratt RB. 2012. No evidence for an open vessel effect in centrifuge-based vulnerability curves of a long-vesselled liana (*Vitis vinifera*). *New Phytol.* 194:982–90
65. James SA, Meinzer FC, Goldstein G, Woodruff D, Jones T, et al. 2003. Axial and radial water transport and internal water storage in tropical forest canopy trees. *Oecologia* 134:37–45
66. Johnson DM, Brodersen CR, Reed M, Domec J-C, Jackson RB. 2013. Contrasting hydraulic architecture and function in deep and shallow roots of tree species from a semi-arid habitat. *Ann. Bot.* 113:617–27
67. Jones H, Sutherland R. 1991. Stomatal control of xylem embolism. *Plant, Cell Environ.* 14:607–12
68. Kanai Y, Fujita M, Takabe K. 1996. Vessel network tracing by wire insertion and pigment injection. *Bull. Kyoto Univ. For.* 68:127–36
69. Kimura T, Geya Y, Terada Y, Kose K, Haishi T, et al. 2011. Development of a mobile magnetic resonance imaging system for outdoor tree measurements. *Rev. Sci. Instrum.* 82:053704
70. Kitin PB, Fujii T, Abe H, Funada R. 2004. Anatomy of the vessel network within and between tree rings of *Fraxinus lanuginosa* (Oleaceae). *Am. J. Bot.* 91:779–88
71. Klein T, Zeppel MJ, Anderegg WR, Bloemen J, De Kauwe MG, et al. 2018. Xylem embolism refilling and resilience against drought-induced mortality in woody plants: processes and trade-offs. *Ecol. Res.* 33:839–55

72. Knipfer T, Brodersen CR, Zedan A, Kluepfel DA, McElrone AJ. 2015. Patterns of drought-induced embolism formation and spread in living walnut saplings visualized using X-ray microtomography. *Tree Physiol.* 35:744–55
73. Knipfer T, Cuneo I, Earles JM, Reyes C, Brodersen C, McElrone AJ. 2017. Storage compartments for capillary water rarely refill in an intact woody plant. *Plant Physiol.* 175:1649–60
74. Kolb K, Sperry J, Lamont B. 1996. A method for measuring xylem hydraulic conductance and embolism in entire root and shoot systems. *J. Exp. Bot.* 47:1805–10
75. Kudo K, Utsumi Y, Kuroda K, Yamagishi Y, Nabeshima E, et al. 2018. Formation of new networks of earlywood vessels in seedlings of the deciduous ring-porous hardwood *Quercus serrata* in springtime. *Trees* 113:1021–27
76. Kustas WP, Anderson MC, Alfieri JG, Knipper K, Torres-Rua A, et al. 2018. The grape remote sensing atmospheric profile and evapotranspiration experiment. *Bull. Am. Meteorol. Soc.* 99:1791–1812
77. Landsberg J, Fowkes N. 1978. Water movement through plant roots. *Ann. Bot.* 42:493–508
78. Lee EF, Matthews MA, McElrone AJ, Phillips RJ, Shackel KA, Brodersen CR. 2013. Analysis of HRCT-derived xylem network reveals reverse flow in some vessels. *J. Theor. Biol.* 333:146–55
79. Lens F, Davin N, Smets E, del Arco M. 2013. Insular woodiness on the Canary Islands: a remarkable case of convergent evolution. *Int. J. Plant Sci.* 174:992–1013
80. Lewis AM, Boose ER. 1995. Estimating volume flow rates through xylem conduits. *Am. J. Bot.* 82:1112–16
81. Loepfe L, Martinez-Vilalta J, Pinol J, Mencuccini M. 2007. The relevance of xylem network structure for plant hydraulic efficiency and safety. *J. Theor. Biol.* 247:788–803
82. Mackay J, Weatherley P. 1973. The effects of transverse cuts through the stems of transpiring woody plants on water transport and stress in the leaves. *J. Exp. Bot.* 24:15–28
83. Manzoni S, Vico G, Katul G, Palmroth S, Jackson RB, Porporato A. 2013. Hydraulic limits on maximum plant transpiration and the emergence of the safety–efficiency trade-off. *New Phytol.* 198:169–78
84. Manzoni S, Vico G, Thompson S, Beyer F, Weih M. 2015. Contrasting leaf phenological strategies optimize carbon gain under droughts of different duration. *Adv. Water Resour.* 84:37–51
85. Marañón-Jiménez S, Van den Bulcke J, Piayda A, Van Acker J, Cuntz M, et al. 2017. X-ray computed microtomography characterizes the wound effect that causes sap flow underestimation by thermal dissipation sensors. *Tree Physiol.* 38:287–301
86. McElrone AJ, Bleby T. 2011. *Measuring sap flow in plants*. PrometheusWiki, updated April 29, 2011. <http://prometheuswiki.org/tiki-pagehistory.php?page=Sap%20flow&preview=13>
87. McElrone AJ, Brodersen CR, Alsina MM, Drayton WM, Matthews MA, et al. 2012. Centrifuge technique consistently overestimates vulnerability to water stress-induced cavitation in grapevines as confirmed with high-resolution computed tomography. *New Phytol.* 196:661–65
88. McElrone AJ, Earles J, Knipfer T, Albuquerque C, Brodersen C, Cuneo I. 2018. Changes in xylem conducting capacity and water storage across species: How can variable air content of xylem cells affect sap flow? *Acta Hortic.* 1222:5–12
89. Meinzer FC. 2002. Co-ordination of vapour and liquid phase water transport properties in plants. *Plant, Cell Environ.* 25:265–74
90. Meinzer FC, Brooks J, Domec JC, Gartner B, Warren J, et al. 2006. Dynamics of water transport and storage in conifers studied with deuterium and heat tracing techniques. *Plant, Cell Environ.* 29:105–14
91. Meinzer FC, Goldstein G, Jackson P, Holbrook N, Gutierrez M, Cavelier J. 1995. Environmental and physiological regulation of transpiration in tropical forest gap species: the influence of boundary layer and hydraulic properties. *Oecologia* 101:514–22
92. Meinzer FC, James SA, Goldstein G, Woodruff D. 2003. Whole-tree water transport scales with sapwood capacitance in tropical forest canopy trees. *Plant, Cell Environ.* 26:1147–55
93. Melcher PJ, Holbrook NM, Burns MJ, Zwieniecki MA, Cobb AR, et al. 2012. Measurements of stem xylem hydraulic conductivity in the laboratory and field. *Methods Ecol. Evol.* 3:685–94
94. Melcher PJ, Zwieniecki MA, Holbrook NM. 2003. Vulnerability of xylem vessels to cavitation in sugar maple. Scaling from individual vessels to whole branches. *Plant Physiol.* 131:1775–80

74. Development of the apparatus now used to study hydraulic conductance in stems and other plant tissues.

81. Synthetic xylem network modeling exercise that established proof of concept for studying the implications of vessel connectivity.

95. Mencuccini M. 2003. The ecological significance of long-distance water transport: short-term regulation, long-term acclimation and the hydraulic costs of stature across plant life forms. *Plant, Cell Environ.* 26:163–82
96. Miner GL, Ham JM, Kluitenberg GJ. 2017. A heat-pulse method for measuring sap flow in corn and sunflower using 3D-printed sensor bodies and low-cost electronics. *Agric. For. Meteorol.* 246:86–97
97. Mitchell PJ, O'Grady AP, Pinkard EA, Brodribb TJ, Arndt SK, et al. 2016. An ecoclimatic framework for evaluating the resilience of vegetation to water deficit. *Glob. Change Biol.* 22:1677–89
98. Moradi AB, Conesa HM, Robinson B, Lehmann E, Kuehne G, et al. 2009. Neutron radiography as a tool for revealing root development in soil: capabilities and limitations. *Plant Soil* 318:243–55
99. Morris H, Brodersen C, Schwarze FW, Jansen S. 2016. The parenchyma of secondary xylem and its critical role in tree defense against fungal decay in relation to the CODIT model. *Front. Plant Sci.* 7:1665
100. Morris H, Plavcová L, Gorai M, Klepsch MM, Kotowska M, et al. 2018. Vessel-associated cells in angiosperm xylem: highly specialized living cells at the symplast–apoplast boundary. *Am. J. Bot.* 105:151–60
101. Nagata A, Kose K, Terada Y. 2016. Development of an outdoor MRI system for measuring flow in a living tree. *J. Magn. Reson.* 265:129–38
102. Nardini A, Gullo MAL, Salleo S. 2011. Refilling embolized xylem conduits: Is it a matter of phloem unloading? *Plant Sci.* 180:604–11
103. Nordmann J, Buczka S, Voss B, Haase M, Mummenhoff K. 2015. In vivo analysis of the size- and time-dependent uptake of NaYF₄:Yb,Er upconversion nanocrystals by pumpkin seedlings. *J. Mater. Chem. B* 3:144–50
104. O'Grady A, Cook P, Eamus D, Duguid A, Wischusen J, et al. 2009. Convergence of tree water use within an arid-zone woodland. *Oecologia* 160:643–55
105. Olson ME, Anfodillo T, Rosell JA, Petit G, Crivellaro A, et al. 2014. Universal hydraulics of the flowering plants: Vessel diameter scales with stem length across angiosperm lineages, habits and climates. *Ecol. Lett.* 17:988–97
106. Olson ME, Soriano D, Rosell JA, Anfodillo T, Donoghue MJ, et al. 2018. Plant height and hydraulic vulnerability to drought and cold. *PNAS* 115:7551–56
107. Oskolski AA, Jansen S. 2009. Distribution of scalariform and simple perforation plates within the vessel network in secondary xylem of Araliaceae and its implications for wood evolution. *Plant Syst. Evol.* 278:43–51
108. Panshin AJ, de Zeeuw C. 1970. *Textbook of Wood Technology*, Vol. I: *Structure, Identification, Uses, and Properties of the Commercial Woods of the United States and Canada*. New York: McGraw-Hill. 3rd ed.
109. Passioura J. 1984. Hydraulic resistance of plants. I. Constant or variable? *Funct. Plant Biol.* 11:333–39
110. Peguero-Pina J, Mendoza-Herrer O, Gil-Pelegrín E, Sancho-Knapik D. 2018. Cavitation limits the recovery of gas exchange after severe drought stress in holm oak (*Quercus ilex* L.). *Forests* 9:443
111. Pittermann J. 2010. The evolution of water transport in plants: an integrated approach. *Geobiology* 8:112–39
112. Pockman WT, Sperry JS, O'Leary JW. 1995. Sustained and significant negative water pressure in xylem. *Nature* 378:715–16
113. Poyatos R, Granda V, Molowny-Horas R, Mencuccini M, Steppe K, Martínez-Vilalta J. 2016. SAPFLUXNET: towards a global database of sap flow measurements. *Tree Physiol.* 36:1449–55
114. Press MC, Whittaker JB. 1993. Exploitation of the xylem stream by parasitic organisms. *Philos. Trans. R. Soc. B* 341:101–11
115. Reid DE, Silins U, Mendoza C, Lieffers VJ. 2005. A unified nomenclature for quantification and description of water conducting properties of sapwood xylem based on Darcy's law. *Tree Physiol.* 25:993–1000
116. Roddy AB, Brodersen CR, Dawson TE. 2016. Hydraulic conductance and the maintenance of water balance in flowers. *Plant, Cell Environ.* 39:2123–32
117. Roddy AB, Dawson T. 2012. Determining the water dynamics of flowering using miniature sap flow sensors. *Acta Hort.* 951:47–54
118. Roddy AB, Dawson T. 2013. Novel patterns of hysteresis in the response of leaf-level sap flow to vapor pressure deficit. *Acta Hort.* 991:261–67

119. Roddy AB, Simonin KA, McCulloh KA, Brodersen CR, Dawson TE. 2018. Water relations of Calycanthus flowers: hydraulic conductance, capacitance, and embolism resistance. *Plant, Cell Environ.* 41:2250–62
120. Rodríguez-Domínguez CM, Carins Murphy MR, Lucani C, Brodribb TJ. 2018. Mapping xylem failure in disparate organs of whole plants reveals extreme resistance in olive roots. *New Phytol.* 218:1025–35
121. Sack L, Holbrook NM. 2006. Leaf hydraulics. *Annu. Rev. Plant Biol.* 57:361–81
122. Sack L, Melcher PJ, Zwieniecki MA, Holbrook NM. 2002. The hydraulic conductance of the angiosperm leaf lamina: a comparison of three measurement methods. *J. Exp. Bot.* 53:2177–84
123. Savage VM, Bentley LP, Enquist BJ, Sperry JS, Smith D, et al. 2010. Hydraulic trade-offs and space filling enable better predictions of vascular structure and function in plants. *PNAS* 107:22722–27
124. Scheenen T, Heemskerk A, De Jager A, Vergeldt F, Van As H. 2002. Functional imaging of plants: a nuclear magnetic resonance study of a cucumber plant. *Biophys. J.* 82:481–92
125. **Scheenen T, van Dusschoten D, De Jager P, Van As H. 2000. Quantification of water transport in plants with NMR imaging. *J. Exp. Bot.* 51:1751–59**
126. Scheenen T, Vergeldt F, Heemskerk A, Van As H. 2007. Intact plant magnetic resonance imaging to study dynamics in long-distance sap flow and flow-conducting surface area. *Plant Physiol.* 144:1157–65
127. Schenk HJ, Espino S, Goedhart CM, Nordenstahl M, Cabrera HIM, Jones CS. 2008. Hydraulic integration and shrub growth form linked across continental aridity gradients. *PNAS* 105:11248–53
128. Schenk HJ, Steppe K, Jansen S. 2015. Nanobubbles: a new paradigm for air-seeding in xylem. *Trends Plant Sci.* 20:199–205
129. Schweingruber FH, Börner A. 2018. *The Plant Stem: A Microscopic Aspect*. Cham, Switz.: Springer
130. Scoffoni C, Albuquerque C, Brodersen C, Townes SV, John GP, et al. 2017. Outside-xylem vulnerability, not xylem embolism, controls leaf hydraulic decline during dehydration. *Plant Physiol.* 173:1197–210
131. Secchi F, Pagliarani C, Zwieniecki MA. 2017. The functional role of xylem parenchyma cells and aquaporins during recovery from severe water stress. *Plant, Cell Environ.* 40:858–71
132. Sevanto S, Holbrook NM, Ball M. 2012. Freeze/thaw-induced embolism: probability of critical bubble formation depends on speed of ice formation. *Front. Plant Sci.* 3:107
133. Shigo AL, Marx HG. 1977. *Compartmentalization of decay in trees*. Agric. Inf. Bull. 405. Washington, DC: US Dep. Agric.
134. Simonin KA, Roddy AB, Link P, Apodaca R, Tu KP, et al. 2013. Isotopic composition of transpiration and rates of change in leaf water isotopologue storage in response to environmental variables. *Plant, Cell Environ.* 36:2190–206
135. Skelton RP. 2013. Miniature external sapflow gauges and the heat ratio method for quantifying plant water loss. *Bio-Protocol* 7:e2121
136. Skelton RP, Brodribb TJ, Choat B. 2017. Casting light on xylem vulnerability in an herbaceous species reveals a lack of segmentation. *New Phytol.* 214:561–69
137. **Sperry JS, Alder N, Eastlack S. 1993. The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. *J. Exp. Bot.* 44:1075–82**
138. Sperry JS, Donnelly J, Tyree M. 1988. A method for measuring hydraulic conductivity and embolism in xylem. *Plant, Cell Environ.* 11:35–40
139. Sperry JS, Hacke U, Feild T, Sano Y, Sikkema E. 2007. Hydraulic consequences of vessel evolution in angiosperms. *Int. J. Plant Sci.* 168:1127–39
140. Sperry JS, Pockman W. 1993. Limitation of transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*. *Plant, Cell Environ.* 16:279–87
141. Sperry JS, Tyree MT. 1988. Mechanism of water stress-induced xylem embolism. *Plant Physiol.* 88:581–87
142. Spicer R, Groover A. 2010. Evolution of development of vascular cambia and secondary growth. *New Phytol.* 186:577–92
143. Steudle E. 2001. The cohesion-tension mechanism and the acquisition of water by plant roots. *Annu. Rev. Plant Biol.* 52:847–75
144. Stockhofe K, Postema JM, Schieferstein H, Ross TL. 2014. Radiolabeling of nanoparticles and polymers for PET imaging. *Pharmaceuticals* 7:392–418

125. Development of NMR as a tool to study the functional status of the xylem using noninvasive methods.

137. Holistic approach to studying the up- and downstream effects of changing hydraulic conductivity between tissues.

145. Tardieu F, Simonneau T, Muller B. 2018. The physiological basis of drought tolerance in crop plants: a scenario-dependent probabilistic approach. *Annu. Rev. Plant Biol.* 69:733–59
146. Thomas NB, Grace PJ, Scoffoni C, Sack L. 2015. How does leaf anatomy influence water transport outside the xylem? *Plant Physiol.* 168:1616–35
147. Tobin MF, Pratt RB, Jacobsen AL, De Guzman ME. 2013. Xylem vulnerability to cavitation can be accurately characterised in species with long vessels using a centrifuge method. *Plant Biol.* 15:496–504
148. Tonetto de Freitas S, McElrone AJ, Shackel KA, Mitcham EJ. 2013. Calcium partitioning and allocation and blossom-end rot development in tomato plants in response to whole-plant and fruit-specific abscisic acid treatments. *J. Exp. Bot.* 65:235–47
149. Torres-Ruiz JM, Jansen S, Choat B, McElrone AJ, Cochard H, et al. 2015. Direct X-ray microtomography observation confirms the induction of embolism upon xylem cutting under tension. *Plant Physiol.* 167:40–43
150. Tötze C, Miranda T, Konrad W, Gout J, Kardjilov N, et al. 2013. Visualization of embolism formation in the xylem of liana stems using neutron radiography. *Ann. Bot.* 111:723–30
151. Trifilò P, Gascó A, Raimondo F, Nardini A, Salleo S. 2003. Kinetics of recovery of leaf hydraulic conductance and vein functionality from cavitation-induced embolism in sunflower. *J. Exp. Bot.* 54:2323–30
152. Tyree MT, Zimmermann MH. 2002. Hydraulic architecture of whole plants and plant performance. In *Xylem Structure and the Ascent of Sap*, pp. 175–214. New York: Springer. 2nd ed.
153. Vandegehuchte MW, Steppe K. 2012. Sapflow+: a four-needle heat-pulse sap flow sensor enabling nonempirical sap flux density and water content measurements. *New Phytol.* 196:306–17
154. Venturas MD, Sperry JS, Hacke UG. 2017. Plant xylem hydraulics: what we understand, current research, and future challenges. *J. Integr. Plant Biol.* 59:356–89
155. Vergeynst LL, Dierick M, Bogaerts JA, Cnudde V, Steppe K. 2014. Cavitation: a blessing in disguise? New method to establish vulnerability curves and assess hydraulic capacitance of woody tissues. *Tree Physiol.* 35:400–9
156. Vilagrosa A, Bellot J, Vallejo V, Gil-Pelegrín E. 2003. Cavitation, stomatal conductance, and leaf dieback in seedlings of two co-occurring Mediterranean shrubs during an intense drought. *J. Exp. Bot.* 54:2015–24
157. Wang S, Fan J, Ge J, Wang Q, Yong C, You W. 2018. New design of external heat-ratio method for measuring low and reverse rates of sap flow in thin stems. *For. Ecol. Manag.* 419–420:10–16
158. Wason JW, Huggett BA, Brodersen CR. 2017. MicroCT imaging as a tool to study vessel endings in situ. *Am. J. Bot.* 104:1424–30
159. Wheeler JK, Huggett BA, Tofte AN, Rockwell FE, Holbrook NM. 2013. Cutting xylem under tension or supersaturated with gas can generate PLC and the appearance of rapid recovery from embolism. *Plant, Cell Environ.* 36:1938–49
160. Wheeler JK, Sperry JS, Hacke UG, Hoang N. 2005. Inter-vessel pitting and cavitation in woody Rosaceae and other vesselless plants: a basis for a safety versus efficiency trade-off in xylem transport. *Plant, Cell Environ.* 28:800–12
161. Windt CW, Blümmer P. 2013. Using portable NMR to measure sap flow and water in the intact plant. *Proc. IX Int. Workshop Sap Flow* 991:115–22
162. Wullschlegel SD, Meinzer F, Vertessy R. 1998. A review of whole-plant water use studies in tree. *Tree Physiol.* 18:499–512
163. Zarebanadkouki M, Kim YX, Carminati A. 2013. Where do roots take up water? Neutron radiography of water flow into the roots of transpiring plants growing in soil. *New Phytol.* 199:1034–44
164. Zhang L, Copini P, Weemstra M, Sterck F. 2016. Functional ratios among leaf, xylem and phloem areas in branches change with shade tolerance, but not with local light conditions, across temperate tree species. *New Phytol.* 209:1566–75
165. Zhang Y-J, Rockwell FE, Graham AC, Alexander T, Holbrook NM. 2016. Reversible leaf xylem collapse: a potential ‘circuit breaker’ against cavitation. *Plant Physiol.* 172:2261–74
166. Zhang Y-J, Rockwell FE, Wheeler JK, Holbrook NM. 2014. Reversible deformation of transfusion tracheids in *Taxus baccata* is associated with a reversible decrease in leaf hydraulic conductance. *Plant Physiol.* 165:1557–65

167. Zhang Z, He X, Zhang H, Ma Y, Zhang P, et al. 2011. Uptake and distribution of ceria nanoparticles in cucumber plants. *Metallomics* 3:816–22
168. Zimmermann MH, Tomlinson PB. 1966. Analysis of complex vascular systems in plants: optical shuttle method. *Science* 152:72–73
169. Zimmermann MH, Tomlinson PB. 1968. Vascular construction and development in the aerial stem of *Prionium* (Juncaceae). *Am. J. Bot.* 55:1100–9

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Errata

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