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Tansley review

Positive pressure in xylem and its role in hydraulic function

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Summary

Although transpiration-driven transport of xylem sap is well known to operate under absolute negative pressure, many terrestrial, vascular plants show positive xylem pressure above atmospheric pressure on a seasonal or daily basis, or during early developmental stages. The actual location and mechanisms behind positive xylem pressure remain largely unknown, both in plants that show seasonal xylem pressure before leaf flushing, and those that show a diurnal periodicity of bleeding and guttation. Available evidence shows that positive xylem pressure can be driven based on purely physical forces, osmotic exudation into xylem conduits, or hydraulic pressure in parenchyma cells associated with conduits. The latter two mechanisms may not be mutually exclusive and can be understood based on a similar modelling scenario. Given the renewed interest in positive xylem pressure, this review aims to provide a constructive way forward by discussing similarities and differences of mechanistic models, evaluating available evidence for hydraulic functions, such as rehydration of tissues, refilling of water stores, and embolism repair under positive pressure, and providing recommendations for future research, including methods that avoid or minimize cutting artifacts.

I. Introduction

Positive pressure in plant xylem is a very common phenomenon. Theophrastus (300 B.C.) first described bleeding from cuts in tree stems, as did Willughby and Ray (1669), followed by Munting's (1672) description of guttation from leaves, and the first direct measurements of xylem pressure by Hales (1727). Despite this long history, and rather incredibly, the mechanisms that cause the pressure still remain largely unknown (Singh, 2016b). Positive pressure in xylem, which we define as

above atmospheric pressure, can potentially occur in roots, stems, rhizomes, and even in leaves, but in the literature, it is generally referred to as "root pressure" even if the source of the pressure is undetermined. For a long time, positive pressure in xylem has been viewed as relative unimportant compared to transpiration-driven water transport under negative pressure via the cohesion-tension mechanism (Askenasy, 1895; Dixon & Joly, 1895), but there is a resurgence of interest in the topic (Knipfer *et al.*, 2015; Yang *et al.*, 2015; Gleason *et al.*, 2017; Hölttä *et al.*, 2018). While general reviews of the subject are available (Kramer & Boyer, 1995; Singh, 2016b), the purpose of this review is to discuss positive xylem pressure with a critical assessment of what is known about underlying mechanisms and explore by model calculations what conditions are required for root pressure to occur. Other goals include assessing the role of positive pressure in hydraulic function, including rehydration of tissues, refilling of water stores in living cells and the apoplast, embolism repair, and the different methods used for studying positive xylem pressure.

Functionally there is a clear difference between root pressure, which moves soil water from roots into plant stems and leaves, and stem pressure, which moves water out of stem and bark storage into xylem conduits (Fig. **1**). Unfortunately, few studies have been designed to distinguish between root and stem pressure, or between pressure in fine roots at the source of water uptake and woody roots, where water could also come out of storage. Methods used for studying positive pressure include measurements of "bleeding", which is exudation from wounded tissues (Fig. **1**), or guttation, which is exudation from intact leaves (Fig. **1**), normally through specialized openings in the leaf epidermis called hydathodes. Research on positive xylem pressure has often been based on measuring exudation rates, rather than pressure, from cut-off stems or roots, i.e., bleeding (Fig. **2a,b**), or, more rarely, on exudation from guttation. Cut and bleeding stems or roots have also been most commonly used for pressure measurements. Less destructive and continuous methods included pressure probes designed for short-term measurements of individual cells or vessels (Steudle & Jeschke, 1983; Balling & Zimmermann, 1990; Tomos & Leigh, 1999) or probes inserted into xylem of intact plants (Cochard *et al.*, 1994; Saha *et al.*, 2009; Hölttä *et al.*, 2018), including very small probes that minimally disturb the system (Clearwater *et al.*, 2007; Charrier *et al.*, 2017) (Fig.

2c). An indirect non-destructive method involves combined dendrometer and sap flow measurements combined with modelling (De Swaef *et al.*, 2013). These methods and their pros and cons are summarized in Table 1, which provides examples for the different approaches.

Positive xylem pressure occurs in five different scenarios that may – or may not – involve different mechanisms, and three of these scenarios are addressed in this review. (1) Seasonal pressure before leaf-out in the spring for winter-deciduous perennial plants or after rainfall for drought-deciduous plants (Fig. **1**; Table 2) is discussed in section II. (2) Diurnal pressure fluctuations that can go from positive to negative pressure almost every day are found in many plants, especially herbs (Table 2), often associated with guttation from leaves overnight and during early mornings. This is discussed in section III. (3) Positive xylem pressure in response to a water supply after drought stress is discussed in section IV. (4) Developmental positive pressure is used in some seedlings to stretch newly developing cells and tissues. This subject is outside the scope of this review and has been reviewed elsewhere (Cosgrove, 1986; McIntyre, 1987). (5) Aquatic vascular plants cannot move sap and nutrients via transpiration and therefore require positive pressure for any sap movement (Pedersen, 1993; Pedersen *et al.*, 1997). This last scenario is also outside the scope of this review.

The generation of positive xylem pressure must be related to the underlying anatomy (Figs. **3** and **4**). Pressure involving the gas phase, such as in sugar maple (Sauter, 1974; O'Malley & Milburn, 1983; Milburn & O'Malley, 1984), requires presence of fiber lumina and intercellular spaces, and pressure created by active solute transport in living xylem cells requires these cells to be located next to conduits and hydraulically connected to a water source. In either case, there must be anatomical features to contain the pressure. Unfortunately, there currently is no unequivocal evidence that points to any particular anatomical traits as being associated with positive xylem pressure. Most commonly, xylem pressure is assumed to be generated in fine roots with an intact cortex and endodermis, (Priestley, 1920; Steudle *et al.*, 1993; Knipfer & Fricke, 2010), but such assumptions are often based on a simplistic view of root anatomy and fail to account for traits, such as the endodermis containing plasmodesmata, leaks, and passage cells (Fig. **3**), all of which allow for uncontrolled fluxes of solutes and water, even more so in roots with secondary growth (Fig. **4**).

To cut through the complexity of functions, timing, mechanisms, and structure-function relationships, this review focusses on two phenomena: Seasonal root and stem pressure before leaf-out in deciduous woody plants (part II), and diurnal xylem pressure cycles that include positive pressure (part III). Part IV of this review addresses what is known about the role of positive xylem pressure in rehydration and refilling of embolized xylem. The review concludes with recommendations for future research on the mechanisms and functions of positive pressure generation in xylem.

II. Seasonal root and stem pressure before leaf-out in deciduous woody plants

1. Occurrence in trees and vines

Seasonal root and stem pressure before leaf-out has been reported for many winter-deciduous genera of woody plants in temperate and boreal environments (Table 2) and before leaf-out in drought-deciduous plants after rainfall (Borchert, 1994; Kolb & Davis, 1994; Scholz *et al.*, 2016; Van Camp *et al.*, 2017). Seasonal pressure usually disappears with leafing out and is sometimes observed again after leaves are dropped. In leafless plants, there is minimal movement of sap during the pressure development, except when stems or roots are cut for sap collection or pressure measurements. Xylem pressure before leaf-out has a strong diurnal component, most likely in response to temperature (Kramer, 1940; Hölttä *et al.*, 2018). Xylem sap before leaf-out tends to be high in solutes, including sugars (Larochelle *et al.*, 1998; Jeong *et al.*, 2012; Ozolinčius *et al.*, 2016). Hartig (1858) provided a list of tree species that bleed in the spring, including most of the genera for which positive xylem pressure has been documented (Table 2). There is no evidence for positive pressure in mature conifer trees, but root pressure has been observed in conifer seedlings and excised roots (O'Leary & Kramer, 1964; O'Leary, 1966; Lopushinsky, 1980).

2. Mechanisms causing positive xylem pressure before leaf-out

Mechanisms causing positive pressure before leaf-out may differ between species and organs, and even between different developmental stages. For sugar maple, *Acer saccharum*, there is strong support for the hypothesis that its positive sap pressure originates in stems rather than roots (Kozlowski & Pallardy, 1996) and is explained in large part by purely physical factors, including a combination of thermal and freezing/thawing effects (Sauter, 1974; O'Malley & Milburn, 1983; Milburn & O'Malley, 1984). According to the Milburn-O'Malley model (see Fig. 3.16 in Tyree & Zimmermann, 2002), warming temperatures cause both melting of frozen sap and thermal gas expansion in fibers, forcing sap into vessels and creating positive pressure. Tyree and coworkers argued that this model was incomplete and that sap sugar content and osmotic forces must be considered to explain sap pressure in maple (Tyree, 1983; Johnson & Tyree, 1992; Cirelli *et al.*, 2008). The Milburn-O'Malley hypothesis was later turned into a mathematical model (Graf *et al.*, 2015) that identified root water uptake from thawing soils or from unfrozen soil under snow cover as a key-factor for stem pressure in maple and also showed that freezing point depression due to sugar content in the sap allows ice to exist in fibers while sap in adjacent vessels remains liquid. Therefore, it seems that positive xylem pressure in sugar maple can be successfully reproduced in a model based purely on physical factors, which is not to say that metabolic processes are not involved in the living trees.

A similar physical explanation may be valid for birch. The pressures in roots and stems of birch occur at the same time, with root pressure exceeding stem pressure (Kozlowski & Pallardy, 1996), suggesting that the pressure may originate in roots or perhaps in both organs. Springtime stem pressure in *Betula pendula* is most strongly correlated with temperature, peaking at mid-day. Xylem sap osmotic pressure in one study was small in comparison to total xylem pressure and showed little diurnal variation (Hölttä *et al.*, 2018), but vertical gradients of sugar concentrations were found in birch sap during leaf emergence (Westhoff *et al.*, 2008), leaving it open whether osmotic forces play a role for positive pressure in birch. In walnut, xylem pressure was closely correlated with soil temperature and xylem sap osmolarity, and the pressure appeared to originate mainly in roots

(Améglio *et al.*, 2001). Springtime xylem pressure in walnut appeared to be related to starch-sugar conversion and active transport of sugar into vessels (Améglio *et al.*, 2004). However, the purely physical model based on freeze-thaw cycles developed for maple was also capable of explaining sap pressure in walnut without references to osmotic forces (Graf *et al.*, 2015). Loading of sugar into sap clearly provides carbon for leaf-out but may not be primarily responsible for creating positive pressure.

Positive xylem pressure in spring is also observed in some woody vines. Hales (1727) measured 93 kPa of stem pressure in a grapevine at 13.5 m away from the base of the plant and argued that "this force is not from the root only, but must also proceed from some power, in the stem and branches (p. 110)." Direct measurements of grapevine rootstocks during the growing season clearly show pressure originating from roots (Barrios-Masias *et al.*, 2015), but that observation did not pinpoint an origin in fine or woody roots and did not exclude the possibility of concurrent stem pressure. Measurements of xylem sap osmotic potentials could account generally for the observed xylem pressure in the monocot vine *Smilax rotundifolia* (Cobb *et al.*, 2007) and in *Actinidia* rootstocks (Clearwater *et al.*, 2007), but in *Actinidia* there was no correlation between drought-induced changes in root pressure and xylem sap osmolality. Xylem sap osmolality was very low in bleeding *Vitis rotundifolia* (Andersen & Brodbeck, 1989).

None of the research summarized above, except for maple, pinpoints the mechanism or actual location where positive xylem pressure is generated before leaf-out. Moreover, on balance, the literature cited above does not support a strictly osmotic origin of positive xylem pressure before leaf-out. On the other hand, because sap pressure in spring is temporary, an osmotic mechanism could work well for providing an initial supply of nutrients to emerging leaves and new stems.

III. Diurnal positive pressure in xylem

1. Occurrence in plants

While seasonal xylem pressure before leaf out typically shows a strong diurnal response to temperature, diurnal fluctuations in pressure from positive to negative in leafy (as opposed to leafless) plants during the active growing season are a different phenomenon and almost certainly not caused by purely physical forces. The positive pressure spike at night does not appear to be a constitutive phenomenon that becomes apparent only when transpiration ceases, because measurements and models show its disappearance during the day (De Swaef *et al.*, 2013). Diurnal positive xylem pressure has been observed in many, if not most, herbaceous species (Singh, 2016b) and in pseudo-woody monocots, such as bamboo (Wang *et al.*, 2011) and palms (Davis, 1961) (Table 2), but not for tall trees with true secondary growth, most likely because it takes more than 10 kPa per meter of height to push up water to the top of a tall tree. Diurnal leaf guttation has been found in a few small trees (up to 6 m tall), including several tropical (Molisch, 1902; Fisher *et al.*, 1997), and temperate, such as *Hammamelis virginiana* (Spanjer, 1898). There are also anecdotal reports of guttation from leaves of taller temperate trees in the genera *Ulmus*, *Fraxinus*, and *Salix* (Patch, 2004) and *Pterocarya* (Spanjer, 1898), but guttation in these cases may have been restricted to lower branches.

Direct, non-destructive measurements of xylem pressure in intact herbs are rare, because it is much easier to measure pressure in wood than in herbaceous tissue, as manometers and pressure gauges can be connected to small holes drilled into wood, while herbaceous stems and roots usually must be cut for pressure measurements. Maximum xylem pressures in 22 herbaceous plant species cut just above the ground ranged from 54—163 kPa (Eckerson, 1908), but is unknown if these pressures are representative for intact herbs. Diurnal root pressure of up to 155 kPa in tomato plants was estimated via modeling parameterized with sap flow and dendrometer data, with generally good agreement between estimates and destructive measurements with a manometer on de-topped plants (De Swaef *et al.*, 2013). Diurnal pressure fluctuations with positive pressure at night and in early mornings have also been measured directly in cut stems of woody and pseudo-woody species (Table 2).

2. Guttation

Guttation was first described by Munting (1672) for taro plants (*Colocasia esculenta*), and studied systematically by Hartig (1855; 1862) and Unger (1858), who both confirmed that guttation was favored by high humidity and darkness. Guttation implies the existence of at least some nocturnal sap flow (van der Velde *et al.*, 2009). Guttation from leaves occurs naturally through hydathodes, openings on leaf margins. Strong metabolic activity was found in the epithem (a thin-walled parenchymatous tissue with numerous intercellular spaces) under all hydathode pores studied (Häusermann & Frey-Wyssling, 1963), possibly involving reabsorption of some nutrients before exudation of water (Nagai *et al.*, 2013). In the presence of positive xylem pressure, guttation from hydathodes was found to prevent flooding of the mesophyll (Feild *et al.*, 2005), so guttation appears to be a necessary process in species that create positive xylem pressure diurnally. Guttation may be limited to young, developing leaves, as hydathodes can get plugged over time (Takeda *et al.*, 1991). Because positive pressure knows no direction, it is not surprising that droplets have also been observed on young root surfaces and within interstices of the rhizosheaths of guttating maize, oats, barley and crabgrass plants, particularly in the early morning (McCully, 1995). Guttation has also been observed in some tropical trees and bamboos (Molisch, 1902).

Guttation has been documented for a very large number of herbaceous species during early mornings, especially after cool nights (Volkens, 1883; Frey-Wyssling, 1941; Singh, 2016a). This is almost exactly the opposite of the exudation patterns observed for bleeding from cut plants (see below), suggesting that guttation provides a better way to determine diurnal patterns of hydraulic pressure periodicity in plants (Speidel, 1939). The periodicity of guttation and water uptake in grass seedlings appears to be driven by the periodicity of seedling growth, with growth and water uptake positively correlated with each other and guttation occurring when the other two processes cease (Engel & Heimann, 1949). Guttation in *Kalanchoë* has a normal maximum at night, but diurnal patterns could be shifted by changing light- and dark-periods and ceased after 2-3 days of placing the plant in CO_2-free air (Heimann, 1950).

3. Methodological artifacts in measurements of bleeding

Guttation and bleeding are related but different processes, as many species show both phenomena, but some species show one but not the other (Hartig, 1862). Guttation and bleeding in *Zea mays* and other cereals do not always occur in the same plants, and are not strongly correlated with each other when they do occur in the same plants (Heimann, 1950; Höhn, 1951). Research on the periodicity of bleeding from decapitated root systems has consistently confirmed findings first reported by Hofmeister (1862) that exudation rates show diurnal cycles that peak at mid-day and tend to be lowest during the night, even at constant temperature (Baranetzky, 1877; Wieler, 1893; Grossenbacher, 1938; White, 1938). These cycles were correlated with aquaporin expressions in roots (Henzler *et al.*, 1999). Most importantly, decapitation of the entire aboveground plant was found to cause a rapid decrease in root aquaporin expression and a concurrent decline in root hydraulic conductivity by about 50% over an hour (Meng *et al.*, 2016). Cutting directly affects turgor pressure in cells near the cut surface, permeability of plasmodesmata (Hernández-Hernández *et al.*, 2020), rapid expression of apoplastic peroxidases, and a burst of reactive oxygen species (ROS) (Minibayeva *et al.*, 2015). Decapitation therefore obviously creates major artifacts. Importantly, the timing of diurnal patterns in exudation from cut plants was related to the timing of decapitation, with predictable maxima in exudation occurring about 12 and 36 hours after cutting (Speidel, 1939). Clearly, decapitation is a highly problematic experimental approach (Meng *et al.*, 2016), although artifacts could potentially be reduced by removing only a small part of the shoot.

Bleeding exudate may include not only sap from xylem conduits, but also liquid from living parenchyma cells (Rowan *et al.*, 2000) and phloem (Zhang *et al.*, 2012), however, P-proteins and callose quickly terminate phloem transport after wounding. Bleeding also serves a natural function in response to wounding by herbivores or wind damage, delivering defense compounds to wounded tissues and even providing sugar in some cases that attracts ants, other predators, and parasitoids, which can defend the plant against herbivores (Heil, 2016). For all these reasons, it is clearly problematic to use bleeding plant parts for collecting either xylem or phloem sap (Schurr, 1998; Zhang *et al.*, 2012) or to assume that rates of bleeding are indicative of xylem pressure conditions in

intact plants. That said, differences in bleeding rates between plants may be useful indicators of functional traits, such as the ability to recover from drought stress after watering (Gleason *et al.*, 2017).

4. Possible mechanisms that create positive xylem pressures diurnally

Paul Kramer (1932; 1945) reviewed many early theories to explain positive xylem pressure. All explanations proposed since that time followed Kramer in assuming that diurnal pressure is generated in roots and invoked some combination of osmotic and turgor pressure components to explain active transport of water into vessels. Textbooks published within the last few decades mostly state it as fact that positive xylem pressure is caused by an osmotic process following active movement of solutes into vessels (e.g., Evert & Eichhorn, 2013; Taiz *et al.*, 2014; Nobel, 2020) and do not distinguish between seasonal and diurnal pressure fluctuations.

There is abundant evidence for a role of metabolic energy in diurnal xylem pressure generation, which excludes a purely physical explanation. Deprivation of oxygen caused a reduction in positive xylem pressure in several studies (Wieler, 1893; Grossenbacher, 1938; Speidel, 1939; Gessner, 1941; Gibbs *et al.*, 1998), although the effect was mostly not immediate and sometimes did not reduce the pressure to atmospheric (Grossenbacher, 1938; Gessner, 1941). Use of oxygen microelectrodes to determine oxygen concentrations within and outside the root stele of maize roots resulted in findings that decreasing the external O_2 concentration by about 80% decreased root pressure by about 45% to a new steady state over a period of 4-6 hours (Gibbs *et al.*, 1998). Moreover, sap exudation correlated strongly with reduction of reserve carbohydrates, such as starch in roots (Speidel, 1939), and a role of chemical energy in the form of GTP was found for exudation from maize root xylem (Zholkevich *et al.*, 2007).

 i. Mechanisms of active transport

Active water transport between cells requires active transport of solutes across a plasma membrane, almost certainly involving H^+-ATPases (proton pumps), which generally facilitate active plant membrane transport (Palmgren, 2001). Plasma membrane H^+-ATPases transport protons exclusively

from the cytoplasm into extracellular space and are abundantly expressed in vessel-associated cells (VACs) (Fink, 1982; Fromard *et al.*, 1995; Alves *et al.*, 2001; Decourteix *et al.*, 2006; Alves *et al.*, 2007), in xylem parenchyma cells and the pericycle between the endodermis and metaxylem, and especially in the root epidermis and in root hairs (Samuels *et al.*, 1992; Jahn *et al.*, 1998). H^+-ATPase activity at the boundary between VACs and vessels would be accompanied by xylem sap acidification (Box Fig. **1b**). Active transport requires a membrane, so ultimately the active transport step in any positive pressure generation is from the apoplast into the symplast (Box Fig. **1a**), most likely at an endodermis, exodermis (Lehmann *et al.*, 2000), or periderm (O'Leary, 1965), at the epidermis and/or in root hairs.

ii.   Two model scenarios for pressure generation in roots

Proposed mechanisms for pressure generation in roots can be broadly grouped into osmotic exudation and hydraulic pressure scenarios. Box 1 presents a comparison of two models that represent these two scenarios. As with textbook authors, most authors of recent papers assume that positive pressure is generated in fine roots via an **osmotic exudation scenario**, where osmotic agents – either inorganic ions, sugars, or larger organic molecules – are actively transported from the root cortex through the endodermis into the stele and then through VACs into vessels (Figs. **3** and **4**, Box Fig. **1a**). There are two problems associated with this scenario: (1) Why do solutes not move upwards toward the leaves once they enter the vessels (Overton, 1921)? (2) Solutes exuded through the plasmalemma of VACs would not move directly into vessel sap, but accumulate in the thick (200 nm to > 1 µm) apoplastic layer of the pit membrane (Figs. **3d,e** and **4c-e**) and in secondary xylem also an amorphous layer between plasmalemma and pit membrane (Fig. **4c, d**), which consists of polysaccharides and arabinogalactan-proteins (Czaninski, 1977; van Bel & van der Schoot, 1988; Morris *et al.*, 2018; Schenk *et al.*, 2018).

Hydraulic pressure scenarios explain pressurized water flow into vessels by osmotic pressure and increased turgor pressure in VACs (Box Fig. **1b**, Box Table 2) (Sachs, 1865; Priestley, 1920; Blackman, 1921; Canny, 1995; Canny, 1998; Enns *et al.*, 2000; Pickard, 2003a). These scenarios also include osmotic water flow, but strictly into living cells, not away from them. The different hydraulic

pressure scenarios all share the idea that water moves into vessels mainly due to hydraulic pressure, and that solute movement into vessels is incidental and not the main driving force for water flow, an idea first proposed by Sachs (1865) (Fig. **5**). The most detailed hydraulic pressure model (Pickard, 2003a), consisting of three-compartments and two membranes, has been referred to as the "membrane asymmetry" scenario (Clearwater & Goldstein, 2005). The model proposed passive co-transport of solutes and water from VACs into the apoplast surrounding the cells, followed by immediate active transport of solutes back into VACs (Box Fig. **1b**). Later publications proposed this same idea without acknowledging that it was the same as Pickard's earlier model (Wegner, 2014; Fricke, 2015; Wegner, 2015). In addition to aquaporins, this scenario also requires larger channels of >2 nm diameter for pressure-driven water flow, most likely consisting of the pit membrane pores between vessels and VACs (Figs. **3d,e** and **4c-e**).

The main challenge for hydraulic pressure scenarios is to explain why water would move from VACs into vessels rather than indiscriminately into the surrounding tissue. Hofmeister (1862) and Sachs (1865) first proposed a path-of-least-resistance theory, suggesting that living cells may have plasma membranes with increasing permeability to water, the closer they are to vessels, thereby favoring water flow towards vessels (Fig. **5**). At the cellular level, VACs may show differential aquaporin expressions in plasma membranes that face the half-bordered vessel-VAC pits (Figs. **3e** and **4c-e**, Box Fig. **1**), and possible gating of aquaporins on other sides of the cells. Moreover, in secondary xylem the cell walls and pits between neighboring VACs are typically lignified (Czaninski, 1977), allowing water flow only through plasmodesmata, which can be closed in response to high pressure differences between cells (Oparka & Prior, 1992).

   iii.   The problem of leaks in the pressurized system

Kramer and Boyer (1995) questioned the assumption that root pressure required an intact endodermis to contain the pressure in the stele, as experimental damage to an endodermis does not completely inhibit root pressure (Steudle *et al.*, 1993). Moreover, there are many natural leaks in the endodermis, such as plasmodesmata connecting the endodermis cells to the pericycle (Fig. **3a**), the leaks where branch roots break through (Fig. **3a**), or passage cells without suberized cell walls

(Peterson & Enstone, 1996; Waduwara *et al.*, 2008) (Fig. **3f**). Most likely, the function of an endodermis in root pressure generation is not containing the pressure but to function as a main site for active solute transport.

Plasmodesmata connect all roots cells, except for vessels and tracheids (Figs. **3c,d** and **4e**), and even connect endodermis cells both to cortex and pericycle cells (Fig. **3c**). Plasmodesmata are especially abundant in passage cells located in the endodermis (Fig. **3f**), which lack cell wall suberization and are typically located just opposite the xylem poles (Fig. **3f**). If active transport from the apoplast into the symplast occurred at the endodermis or anywhere else in roots, then plasmodesmata would have to be closed to prevent backflow of solutes and water (Box Fig. **1a**, Box Table 1), either in direct response to hydraulic pressure differences between cells (Oparka & Prior, 1992) or possibly due to callose deposition (Rinne *et al.*, 2001).

Aquaporins are required by all positive pressure scenarios, especially in plasma membranes of VACs to move water at a high-enough rate into vessels (Henzler *et al.*, 1999; Sakr *et al.*, 2003; Dustmamatov & Zholkevich, 2008). Aquaporins are abundantly expressed in xylem parenchyma, both in plasma membranes (Hachez *et al.*, 2006) and tonoplasts (Barrieu *et al.*, 1998). Aquaporin expression has been found to respond to hydraulic pressure changes (Meng *et al.*, 2016), and some aquaporins have been found to be gated by hydraulic pressure stimuli (Wan *et al.*, 2004; Chaumont & Tyerman, 2014). Aquaporin expression and/or gating in response to pressure differences could play a role in controlling water release from VACs in any positive pressure scenario.

iv.   A possible role of phloem

Of the proposed positive pressure scenarios, the "tissue pressure" model of Canny (1995; 1998) was the only one to directly invoke a role of water flow from phloem, which is typically pressurized in the order of 1 MPa. Canny attempted to explain all plant water transport with this model and was proven wrong in doing so (Comstock, 1999; Stiller & Sperry, 1999; Tyree, 1999), but the fact remains that phloem in roots and vascular bundles borders directly on xylem, and would be the most immediate source of water for xylem at sites of phloem unloading, such as sugar sinks in roots or

storage tissues (Münch, 1930; Woodhouse, 1933; Hölttä *et al.*, 2006; Nardini *et al.*, 2011; Sevanto *et al.*, 2011; Wegner, 2014). Water from phloem therefore could contribute to positive xylem pressure, including in stems and rhizomes.

v.   Empirical evidence

Only few studies have reported solute concentrations in xylem sap high enough to support an osmotic exudation scenario (Lundegårdh, 1940; Miller, 1985; Meinzer *et al.*, 1991), with one study even showing osmotic pressure values that exceeded the measured xylem pressures by a factor of 20-30 (Wang *et al.*, 2011). Lacking controls for contamination of xylem exudate with living cell content and phloem sap, these data must be interpreted with caution.

In contrast, Hofmeister (1862) found that solute concentrations in bleeding xylem sap were too low to explain sap pressure, and this has been confirmed in numerous studies since then (Heyl, 1933; Grossenbacher, 1938; Gessner, 1941; Van Overbeck, 1942; Arisz *et al.*, 1951; Zholkevich, 1981; Enns *et al.*, 1998; Enns *et al.*, 2000; Pickard, 2003b). It has been proposed that solutes might be retrieved somehow from sap just above the exudation point (Klepper, 1967), but there is no direct evidence to support this and some that directly contradicts it (Enns *et al.*, 1998). Moreover, it would be almost impossible for living cells to retrieve solutes from moving sap in vessels through pit membranes and amorphous layers, except in leaves and their hydathodes, far above where the pressure is generated.

The observation of extremely high concentrations of solutes in maize root exudate by Miller (1985) was effectively contradicted by cryo-scanning energy dispersive X-ray microanalysis (McCully *et al.*, 2010), showing that solute concentrations (mostly K and Ca) in maize root vessels were very low and that there was no vertical gradient in vessels that would have indicated local exudation (Enns *et al.*, 1998; Enns *et al.*, 2000) or retrieval of solutes from sap. Cation concentrations in vacuoles of vessel-associated xylem parenchyma exceeded the concentrations in metaxylem vessels by a factor of about 8×, and calculations of turgor pressure in xylem parenchyma suggested values of 200-300 kPa or higher, providing strong support for a hydraulic pressure scenario. McCully *et al.* (2010) also

argued that Miller (1985) had used immature maize roots without late metaxylem, causing contamination from living cells (Enns *et al.*, 2000). Whatever the active mechanism that pressurizes xylem conduits, it is clearly sensitive to osmotic potential outside the system, because changing the nature and concentration of solutes in the solution outside roots often has temporary effects on water movement into root vessels, with different solutes causing different responses (Pickard, 2003b).

In summary, abundant evidence from 150 years of research on diurnal cycles that create positive xylem pressure does not support a straight osmotic exudation scenario, but does support a hydraulic pressure or combined osmotic exudation/hydraulic pressure model (see Box 1). More research is needed on the expression of membrane transporters during positive pressure creation in xylem, including aquaporins, H^+-ATPase, and various symports and antiports for cations, sugars, and organic acids, together with detailed anatomical studies and molecular techniques to visualize the locations of these proteins.

vi.   Non-osmotic models

A proposed, non-osmotic model was based on intriguing observations of rhythmic oscillations of exudation and water uptake by sunflower roots with a periodicity of 1 to 3 minutes (Zholkevich *et al.*, 2005), causing the authors to hypothesize the existence of active cell contractions, possibly involving the cytoskeleton. However, there is no direct evidence for such cellular pumps anywhere in plants. Rhythmic patterns are common in fluid systems under pressure and by themselves are not evidence for biological actions.

Alternatively, a role of CO_2 pressure in stems could be explored, as CO_2 concentrations in xylem can exceed 20% (Teskey *et al.*, 2008) and show large diurnal fluctuations, typically rising at night (Saveyn *et al.*, 2008). Hales (1727), Rominger (1843), and Knipfer *et al*. (2015) reported small gas bubbles emerging from grapevine vessels during exudation, and Coulomb (1799) even reported pressurized gas audibly escaping from deep cuts or holes drilled into poplar tree stems, especially on warm, sunny days but not during the night or on cold, wet days. Sauter (1974) suggested that gas (CO_2)

expansion, contraction, and solubility in response to temperature could partly explain springtime sap pressure in maple. CO_2 could potentially pressurize gas-filled fibers and intercellular spaces in xylem and thereby pressurize living cells to move water from storage compartments into vessels, with pits in fibers and tracheids enabling gas diffusion.

These ideas are wildly speculative, but it is important to keep in mind that the evidence after 300 years of research on positive xylem pressure still has not produced a convincing explanation for xylem pressure fluctuations that vary from negative to positive diurnally. This suggests that some crucial factors have been overlooked.

IV. Role of positive xylem pressure in recovery from drought and embolism

Obvious functions for positive xylem pressure include the rehydration of tissues and refilling of water stores in living cells and the apoplast, either after a long dormant season, in response to rain or watering, or diurnally. Positive pressure can also move nutrients and sugars towards the leaves. While seasonal, diurnal, or watering-induced rehydration of stem and leaf tissues is well documented, there are not many measurements that show the involvement of positive pressure in this process (e.g., Yang *et al.*, 2012). The role of positive xylem pressure in embolism repair is of particular interest, because it has become clear in recent years that absolute positive pressure (atmospheric or above) is required to refill embolized (gas-filled) xylem conduits, except for very narrow conduits where capillary forces are sufficient (Rolland *et al.*, 2015). Earlier reports of embolism repair under tension (reviewed by Clearwater & Goldstein, 2005; Brodersen & McElrone, 2013) are likely to have been due to methodological artifacts caused by cutting stems (Wheeler *et al.*, 2013). Therefore, here we treat observations of xylem embolism repair as indirect evidence for positive xylem pressure, with the caveat that very few studies have actually documented a direct link between embolism repair and positive xylem pressure (e.g., Fig. 6). That said, it is clear that many species do not use positive pressure to refill their hydraulic systems and instead either grow new xylem, prevent embolism in the first place, or use another mechanism, such as water uptake via

leaves or bark tissue (Berry *et al.*, 2019; Mayr *et al.*, 2020). Here we focus on the evidence for rehydration and embolism repair under positive pressure in spring, in response to watering or rain, or diurnally.

1. Functions of positive pressure before and during leaf-out in spring

Increasing water content in wood and bark in spring is well documented for winter-deciduous angiosperm trees (Gibbs, 1935; Hinckley *et al.*, 1978), but, due to a shortage of continuous xylem pressure measurements in deciduous trees (except for Hao *et al.*, 2013; Hölttä *et al.*, 2018), there is limited evidence for a function of positive pressure in seasonal rehydration and refilling of water stores.

There is abundant evidence for the development of winter embolism in many deciduous angiosperm trees and conifers of the temperate and boreal zones, most likely due to freeze-thaw dynamics in xylem (Sperry & Sullivan, 1992; Sperry *et al.*, 1994). In some diffuse-porous trees, winter embolism can encompass 80-100% of the xylem followed by almost complete refilling before leaf-out (Utsumi *et al.*, 1998; Améglio *et al.*, 2002). Embolism reversal due to positive xylem pressure before leaf-out has been suggested for the diffuse-porous tree genera *Fagus, Acer, Betula, Salix, Alnus, Juglans* (semi-ring-porous), and some species of *Populus* (Sperry *et al.*, 1994; Hacke & Sauter, 1996; Cochard *et al.*, 2001; Améglio *et al.*, 2002; Strati *et al.*, 2003; Hao *et al.*, 2013), but not for ring-porous trees in the genera *Fraxinus* and *Quercus* (Sperry *et al.*, 1994; Hacke & Sauter, 1996). These reports were based on destructive hydraulic conductance measurements, which are prone to artifacts (Wheeler *et al.*, 2013; De Baerdemaeker *et al.*, 2019), but both the magnitude of frost-induced loss of conductivity over the winter and the recovery during spring was so strong in most of these studies that it seems likely that hydraulic recovery included vessel refilling associated with positive xylem pressure. That conclusion is supported by cryo-SEM observations of winter embolism and springtime refilling before leaf-out in a *Salix* and *Betula* species (Utsumi *et al.*, 1998). The capability for developing positive xylem pressure has been found to correlate with other hydraulic traits. Trees that generate both root and stem pressure were found to have lower specific hydraulic conductivity than trees that generate only root pressure or no positive pressure at all, and trees that generate

only root pressure had higher vulnerability to embolism than trees with no positive pressure or both root and stem pressure (Yin *et al.*, 2018).

Embolism reversal before leaf-out due to positive xylem pressure has also been reported for vines in the genera *Smilax* (Cobb *et al.*, 2007) and *Vitis* (Sperry *et al.*, 1987; Tibbetts & Ewers, 2000). Both have very large vessels, showing that vessel diameter is not an impediment to pressure generation, and suggesting that the inability of ring-porous trees to generate positive pressure may be due to the very large combined vessel volume that must be filled.

2. Functions of positive pressure in response to rain or watering

Rehydration of plant tissues in response to rain or watering is familiar to almost everyone, but the role of positive xylem pressure in this process is poorly documented. Positive pressure often spikes in response to irrigation after a drought treatment (Barrios-Masias *et al.*, 2015; Knipfer *et al.*, 2015; Gleason *et al.*, 2017), and it is reasonable to infer that the pressure aids in rehydrating tissues, but direct evidence for this is very limited.

Could positive xylem pressure after rewatering repair embolism that has formed during a drought period? According to modeling studies, if an embolus is compressed to slightly above atmospheric pressure, it should be resorbed in seconds to minutes (Pickard, 1989; Yang & Tyree, 1992), and even atmospheric pressure can be sufficient for embolism repair, including via capillary forces (Rolland *et al.*, 2015; Knipfer *et al.*, 2016; Cuneo *et al.*, 2018). In contrast, stems with many emboli require many hours to refill (Tyree & Yang, 1992), and refilling may not be possible after severe drought stress that causes embolism in leaf veins as well as damage to the photosynthetic machinery, as observed in wheat (Johnson *et al.*, 2018).

The evidence for embolism repair after drought is very limited, and research that failed to find embolism repair (including our unpublished data) is often not published because it is difficult to publish negative results. That said, root pressure-induced refilling of vessels and recovery of hydraulic stem conductivity has been found for a few species, including maize (Gleason *et al.*, 2017). MicroCT imaging revealed that maize plants developed xylem embolism after drought treatment,

but then experienced refilling and increasing hydraulic conductance facilitated by the generation of root pressure and low rates of nighttime transpiration (Gleason *et al.*, 2017) (Fig. **6c,d**). In rice, positive xylem pressure after a six-day drought was stimulated seven-fold compared with well-watered plants (Stiller *et al.*, 2003), and other research has also confirmed such effects in rice (Singh *et al.*, 2009b). Positive xylem pressure measured in *Vitis* species after rewatering was positively correlated with drought stress severity for *V. riparia* and *V. arizonica*, both of which showed embolism repair (Knipfer *et al.*, 2015) (Fig. **6a,b**). Drought stress increased xylem pressure after rewatering in hybrid rootstocks of *Vitis* compared to well-watered treatments (Barrios-Masias *et al.*, 2015), and xylem sap osmolality was too low in these experiments to explain the measured pressures. Imaging via microCT showed that rehydration of excised grapevine stems was enough to refill vessels in the absence of root pressure, with vessels being too wide and long to explain refilling by capillary forces (Knipfer *et al.*, 2016), suggesting the pressure was generated in the stem. The development of some positive xylem pressure near the base of *Vitis* plants after watering was associated with concurrent refilling of embolism in that same location (Charrier *et al.*, 2016). Positive xylem pressure in hybrid poplar seedlings and differential expression of aquaporins along the stem also coincided with a recovery of hydraulic conductance after re-watering (Leng *et al.*, 2013).

Because of methodological difficulties there is hardly any research on xylem refilling in roots, which in some species appear to be more vulnerable to embolism than stems (Sperry & Saliendra, 1994; McElrone *et al.*, 2004; Bucci *et al.*, 2013; Losso *et al.*, 2019), but can be equally or even more resistant to embolism in others species (McElrone *et al.*, 2004; Rodriguez-Dominguez *et al.*, 2018; Wu *et al.*, 2020). It is unknown if species that are prone to root embolism are more likely to exhibit root pressure than less vulnerable species.

3. Functions of nocturnal positive xylem pressure

Nocturnal rehydration of tissues and refilling of water stores in living cells and the apoplast is well documented (De Schepper *et al.*, 2012; Zhou *et al.*, 2015), but due to a shortage of continuous pressure measurements, direct evidence for links between positive nocturnal pressure and rehydration is limited. The strongest evidence for this comes from measurements of stem-diameter

variations and sap flow combined with modeling of water storage and pressure (De Swaef *et al.*, 2013).

Evidence is mixed for diurnal cycles of embolism formation during the day followed by refilling under positive xylem pressure at night. This is unlikely to occur on top of tall trees, because of the very high pressure required to push sap there. Diurnal xylem refilling has been reported for several herbaceous species (1883; Milburn & McLaughlin, 1974; Tyree *et al.*, 1986), but observations are mostly indirect and implied from pressure measurements or from acoustic emissions thought to be associated with embolism formation. Diurnal cycles of severe embolism – i.e., severe daytime drought – followed by refilling, requiring a nocturnal water source, are unlikely, because such conditions are not common in nature (Lens *et al.*, 2016). Most herbs are resistant to embolism under normal growing conditions, and it does not appear to be a common hydraulic strategy for herbs to develop embolisms during the day and refill at night (Lens *et al.*, 2016; Ahmad *et al.*, 2018; Volaire *et al.*, 2018).

Bamboos provide the most convincing evidence for diurnal cycles of xylem pressure and nocturnal refilling of xylem. In general, embolism repair under positive pressure might be more common in monocots than dicots, because monocots have no secondary growth to replace embolized vessels and hence may need mechanisms for refilling. All >60 bamboo species investigated show at least some positive xylem pressure during the night and early morning (Cochard *et al.*, 1994; Ewers *et al.*, 1997; Saha *et al.*, 2009; Wang *et al.*, 2011; Cao *et al.*, 2012; Yang *et al.*, 2012; Yang *et al.*, 2015; Mei *et al.*, 2016). In some species, nocturnal xylem pressure is high enough to cause guttation from leaves at predawn, a phenomenon referred to as bamboo rain (Molisch, 1902; Wang *et al.*, 2011), and there is clear evidence for a strong correlation between xylem pressure and height of bamboos, sufficient to push sap all the way to leaves (Ewers *et al.*, 1997; Cao *et al.*, 2012). That said, only one study actually documented bamboo leaves recovering hydraulic conductance after experiencing positive xylem pressure overnight (Yang *et al.*, 2012).

The consistent findings of diurnal cycles in xylem pressure, with positive pressures during the night and negative ones during the day, could be unique to the subfamily Bambusoideae of the grass

family, which is characterized by a rather unique pseudo-woody anatomy (André, 1998; Liese, 1998). It may well be that similar hydraulic strategies will be found in other monocots, such as perhaps in palm trees, but so far bamboos stand out as the only group of plants that appear to share a hydraulic strategy of such diurnal pressure cycles.

V. Conclusions and research outlook

After a long history of being underappreciated, positive xylem pressure is experiencing new interest as a potentially important process for rehydrating tissues, keeping plant hydraulic systems functional and interconnected, and aiding in cell and tissue growth. While positive xylem pressure is clearly restricted to certain groups of plants, it appears to be a common hydraulic strategy for rehydrating tissues and filling water stores in living cells and the apoplast. More continuous measurements of xylem pressure and water content using minimally invasive probes are required to determine the importance of positive pressure for plant water status. Positive pressure is important for seasonal embolism repair in some trees and vines, also in some plants, such as maize and grapevine, for refilling xylem after mild drought stress, and for diurnal xylem rehydration of tissues, especially in bamboos and some herbs. Many more plant species need to be surveyed for positive xylem pressure, including where in roots and/or stems the pressure is generated.

Positive xylem pressure has often been mischaracterized as being driven by a purely osmotic mechanism, and this is clearly contradicted by the evidence. On the other hand, apparent contradictions between osmotic exudation and hydraulic pressure scenarios to explain positive xylem pressure turn out to be minor, making it possible that the actual mechanisms involve a combination of – or switching between — the two mechanisms. There is an urgent need for research into the metabolic processes and membrane transporters involved in pressure generation, especially in vessel-associated cells. Possible roles of water flow from phloem, and potentially a role for CO_2 for non-osmotic pressure generation should be investigated.

Research on decapitated and bleeding plants should be avoided due to the serious artifacts involved, but more research on the magnitude of such artifacts and the strange discrepancies between diurnal patterns of guttation and bleeding could be instructive.

Because of their reliable patterns of positive xylem pressures, preferred study systems for exploring underlying physiological and physical mechanisms include bamboos for diurnal patterns and grapevines for seasonal patterns and for their responses to watering after drought. Maize is a promising model system for studies of gene expression associated with positive pressure generation. Among trees, mechanisms for seasonal positive xylem pressure in walnut and birch require more research. Lastly, positive xylem pressure in tropical plants other than bamboos requires more attention, especially in understory plants and cloud forests.

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References

Ahmad HB, Lens F, Capdeville G, Burlett R, Lamarque LJ, Delzon S. 2018. Intraspecific variation in embolism resistance and stem anatomy across four sunflower (*Helianthus annuus* L.) accessions. *Physiologia Plantarum* **163**(1): 59-72.

Alves G, Decourteix M, Fleurat-Lessard P, Sakr S, Bonhomme M, Ameglio T, Lacointe A, Julien JL, Petel G, Guilliot A. 2007. Spatial activity and expression of plasma membrane H$^+$-ATPase in stem xylem of walnut during dormancy and growth resumption. *Tree Physiology* **27**(10): 1471–1480.

Alves G, Sauter JJ, Julien JL, Fleurat-Lessard P, Ameglio T, Guillot A, Petel G, Lacointe A. 2001. Plasma membrane H$^+$-ATPase, succinate and isocitrate dehydrogenases activities of vessel-associated cells in walnut trees. *Journal of Plant Physiology* **158**(10): 1263-1271.

Améglio T, Bodet C, Lacointe A, Cochard H. 2002. Winter embolism, mechanism of xylem hydraulic conductivity recovery and springtime growth patterns in walnut and peach trees. *Tree Physiology* **22**: 1211-1220.

Améglio T, Decourteix M, Alves G, Valentin V, Sakr S, Julien JL, Petel G, Guilliot A, Lacointe A. 2004. Temperature effects on xylem sap osmolarity in walnut trees: evidence for a vitalistic model of winter embolism repair. *Tree Physiology* **24**(7): 785-793.

Améglio T, Lacointe A, Cochard H, Alves G, Bodet C, Vandame M, Valentin V, Saint-Joanis B, Ploquin S, Cruiziat P, et al. 2001. Water relations in walnut during winter. *Acta Horticulturae* **544**: 239-246.

Andersen PC, Brodbeck BV. 1989. Diurnal and temporal changes in the chemical profile of xylem exudate from *Vitis rotundifolia*. *Physiologia Plantarum* **75**(1): 63-70.

André JP. 1998. A study of the vascular organization of bamboos (Poaceae Bambuseae) using a microcasting method. *IAWA Journal* **19**: 265-278.

Arisz WH, Helder RJ, Van Nie R. 1951. Analysis of the exudation process in tomato plants. *Journal of Experimental Botany* **2**(3): 257-297.

Askenasy E. 1895. Ueber das Saftsteigen. *Verhandlungen des Naturhistorisch-medizinischen Vereins zu Heidelberg, N.F.* **5**: 325-345.

Balling A, Zimmermann U. 1990. Comparative measurements of the xylem pressure of *Nicotiana* plants by means of the pressure bomb and pressure probe. *Planta* **182**: 325-338.

Baranetzky J. 1877. Untersuchungen über die Periodicität des Blutens der krautigen Pflanzen und deren Ursachen. *Abhandlungen der Naturforschenden Gesellschaft zu Halle* **13**: 1-63.

Barrieu F, Chaumont F, Chrispeels MJ. 1998. High expression of the tonoplast aquaporin *ZmTIP1* in epidermal and conducting tissues of maize. *Plant Physiology* **117**(4): 1153-1163.

Barrios-Masias FH, Knipfer T, McElrone AJ. 2015. Differential responses of grapevine rootstocks to water stress are associated with adjustments in fine root hydraulic physiology and suberization. *Journal of Experimental Botany* **66**(19): 6069-6078.

Berry ZC, Emery NC, Gotsch SG, Goldsmith GR. 2019. Foliar water uptake: processes, pathways, and integration into plant water budgets. *Plant, Cell & Environment* **42**(2): 410-423.

Blackman VH. 1921. Osmotic pressure, root pressure, and exudation. *New Phytologist* **20**(3): 106-115.

Borchert R. 1994. Induction of rehydration and bud break by irrigation or rain in decidous trees of a tropical dry forest in Costa Rica. *Trees* **8**(4): 198-204.

Brodersen CR, McElrone AJ. 2013. Maintenance of xylem network transport capacity: a review of embolism repair capacity in vascular plants. *Frontiers in Plant Science* **4**: 108.

Bucci SJ, Scholz FG, Peschiutta ML, Arias NS, Meinzer FC, Goldstein G. 2013. The stem xylem of Patagonian shrubs operates far from the point of catastrophic dysfunction and is additionally protected from drought- induced embolism by leaves and roots. *Plant, Cell & Environment* **36**(12): 2163-2174.

Canny MJ. 1995. A new theory for the ascent of sap -- cohesion supported by tissue pressure. *Annals of Botany* **75**(4): 343-357.

Canny MJ. 1998. Applications of the compensating pressure theory of water transport. *American Journal of Botany* **85**(7): 897-909.

Cao KF, Yang SJ, Zhang YJ, Brodribb TJ. 2012. The maximum height of grasses is determined by roots. *Ecology Letters* **15**(7): 666-672.

Charrier G, Burlett R, Gambetta GA, Delzon S, Domec JC, Beaujard F. 2017. Monitoring xylem hydraulic pressure in woody plants. *Bio-protocol* **7**(20): e2580.

Charrier G, Torres-Ruiz JM, Badel E, Burlett R, Choat B, Cochard H, Delmas CE, Domec J-C, Jansen S, King A, et al. 2016. Evidence for hydraulic vulnerability segmentation and lack of xylem refilling under tension. *Plant Physiology* **172**: 1657–1668.

Chaumont F, Tyerman SD. 2014. Aquaporins: Highly regulated channels controlling plant water relations. *Plant Physiology* **164**(4): 1600-1618.

Cirelli D, Jagels R, Tyree MT. 2008. Toward an improved model of maple sap exudation: the location and role of osmotic barriers in sugar maple, butternut and white birch. *Tree Physiology* **28**(8): 1145-1155.

Clearwater MJ, Blattmann P, Luo Z, Lowe RG. 2007. Control of scion vigour by kiwifruit rootstocks is correlated with spring root pressure phenology. *Journal of Experimental Botany* **58**(7): 1741-1751.

Clearwater MJ, Goldstein G 2005. Embolism repair and long distance water transport. In: Holbrook NM, Zwieniecki MA eds. *Vascular transport in plants*. Amsterdam: Elsevier Academic Press, 375-399.

Cobb AR, Choat B, Holbrook NM. 2007. Dynamics of freeze-thaw embolism in *Smilax rotundifolia* (Smilacaceae). *American Journal of Botany* **94**(4): 640-649.

Cochard H, Ewers FW, Tyree MT. 1994. Water relations of a tropical vine-like bamboo (*Rhipidocladum racemiflorum*): root pressures, vulnerability to cavitation and seasonal changes in embolism. *Journal of Experimental Botany* **45**(8): 1085-1089.

Cochard H, Lemoine D, Améglio T, Granier A. 2001. Mechanisms of xylem recovery from winter embolism in *Fagus sylvatica*. *Tree Physiology* **21**(1): 27-33.

Comstock JP. 1999. Why Canny's theory doesn't hold water. *American Journal of Botany* **86**(8): 1077-1081.

Cosgrove D. 1986. Biophysical control of plant cell growth. *Annual Review of Plant Physiology* **37**(1): 377-405.

Coulomb CA. 1799. Expériences relatives à la circulation de la sève dans les arbres. *Mémoires de l'Institut des Sciences et Arts* **II**: 246-248.

Cuneo IF, Knipfer T, Mandal P, Brodersen CR, McElrone AJ. 2018. Water uptake can occur through woody portions of roots and facilitates localized embolism repair in grapevine. *New Phytologist* **218**(2): 506-516.

Czaninski Y. 1977. Vessel-associated cells. *IAWA Bulletin* **1977**(3): 51-55.

Davis TA. 1961. High root pressures in palms. *Nature* **192**: 227-228.

De Baerdemaeker N, Arachchige KNR, Zinkernagel J, Van den Bulcke J, Van Acker J, Schenk HJ, Steppe K. 2019. The stability enigma of hydraulic vulnerability curves: addressing the link between hydraulic conductivity and drought-induced embolism. *Tree Physiology* **39**(10): 1646-1664.

De Schepper V, van Dusschoten D, Copini P, Jahnke S, Steppe K. 2012. MRI links stem water content to stem diameter variations in transpiring trees. *Journal of Experimental Botany* **63**(7): 2645-2653.

De Swaef T, Hanssens J, Cornelis A, Steppe K. 2013. Non-destructive estimation of root pressure using sap flow, stem diameter measurements and mechanistic modelling. *Annals of Botany* **111**(2): 271-282.

Decourteix M, Alves G, Brunel N, Améglio T, Guilliot A, Lemoine R, Petel G, Sakr S. 2006. JrSUT1, a putative xylem sucrose transporter, could mediate sucrose influx into xylem parenchyma cells and be up-

regulated by freeze-thaw cycles over the autumn-winter period in walnut tree (*Juglans regia* L.). *Plant, Cell and Environment* **29**(1): 36-47.

Dixon HH, Joly J. 1895. On the ascent of sap. *Philosophical Transactions of the Royal Society of London, Series B* **186**: 563-576.

Dustmamatov AG, Zholkevich VN. 2008. Effects of $HgCl_2$ on principal parameters of exudation from maize detached root system. *Russian Journal of Plant Physiology* **55**(6): 814-820.

Eckerson S. 1908. The physiological constants of plants commonly used in American botanical laboratories. *Botanical Gazette* **45**(1): 50-54.

Engel H, Heimann M. 1949. Weitere Untersuchungen über periodische Guttation. *Planta* **37**(3): 437-450.

Enns LC, Canny MJ, McCully ME. 2000. An investigation of the role of solutes in the xylem sap and in the xylem parenchyma as the source of root pressure. *Protoplasma* **211**(3): 183-197.

Enns LC, McCully ME, Canny MJ. 1998. Solute concentrations in xylem sap along vessels of maize primary roots at high root pressure. *Journal of Experimental Botany* **49**(326): 1539-1544.

Evert RF, Eichhorn SE. 2013. *Raven biology of plants*. New York: W. H. Freeman and Company.

Ewers FW, Ameglio T, Cochard H, Beaujard F, Martignac M, Vandame M, Bodet C, Cruiziat P. 2001. Seasonal variation in xylem pressure of walnut trees: root and stem pressures. *Tree Physiology* **21**(15): 1123-1132.

Ewers FW, Cochard H, Tyree MT. 1997. A survey of root pressures in vines of a tropical lowland forest. *Oecologia* **110**(2): 191-196.

Feild TS, Sage TL, Czerniak C, Iles WJD. 2005. Hydathodal leaf teeth of *Chloranthus japonicus* (Chloranthaceae) prevent guttation-induced flooding of the mesophyll. *Plant Cell and Environment* **28**(9): 1179-1190.

Fink S. 1982. Histochemische Untersuchungen über Stärkeverteilung und Phosphataseaktivität im Holz einiger tropischer Baumarten. (Histochemical investigations on starch distribution and activity of acid phosphatases in the xylem of some tropical tree species. In German with English summary). *Holzforschung* **36**(6): 295-302.

Fisher JB, Angeles G, Ewers FW, Lopez-Portillo J. 1997. Survey of root pressure in tropical vines and woody species. *International Journal of Plant Sciences* **158**(1): 44-50.

Frey-Wyssling A. 1941. Die Guttation als allgemeine Erscheinung. *Berichte Der Schweizerischen Botanischen Gesellschaft* **51**: 321-325.

Fricke W. 2015. The significance of water co-transport for sustaining transpirational water flow in plants: a quantitative approach. *Journal of Experimental Botany* **66**(3): 731-739.

Fromard L, Babin V, Fleurat-Lessard P, Fromont JC, Serrano R, Bonnemain JL. 1995. Control of vascular sap pH by the vessel-associated cells in woody species (physiological and immunological studies). *Plant Physiology* **108**(3): 913-918.

Gessner F. 1941. Untersuchungen über die Beziehung zwischen Wurzeldruck und Atmung. *Berichte der Deutschen Botanischen Gesellschaft* **59**(5): 169–173.

Gibbs J, Turner DW, Armstrong W, Darwent MJ, Greenway H. 1998. Solute transport and water relations of oxygen deficient maize roots. I. Development of O_2-deficiency in the stele reduces radial solute transport to the xylem. *Australian Journal of Plant Physiology* **25**: 745-758.

Gibbs RD. 1935. Studies of wood. II. On the water content of certain Canadian trees and on changes in the water-gas system during seasoning and flotation. *Canadian Journal of Research* **12**: 727-760.

Gleason SM, Wiggans DR, Bliss CA, Young JS, Cooper M, Willi KR, Comas LH. 2017. Embolized stems recover overnight in *Zea mays*: The role of soil water, root pressure, and nighttime transpiration. *Frontiers in Plant Science* **8**: 662.

Goatley JL, Lewis RW. 1966. Composition of guttation fluid from rye, wheat, and barley seedlings. *Plant Physiology* **41**(3): 373-375.

Graf I, Ceseri M, Stockie JM. 2015. Multiscale model of a freeze-thaw process for tree sap exudation. *Journal of the Royal Society Interface* **12**(111): 20150665.

Grossenbacher KA. 1938. Diurnal fluctuation in root pressure. *Plant Physiology* **13**(4): 669-676.

Hachez C, Moshelion M, Zelazny E, Cavez D, Chaumont F. 2006. Localization and quantification of plasma membrane aquaporin expression in maize primary root: A clue to understanding their role as cellular plumbers. *Plant Molecular Biology* **62**(1): 305-323.

Hacke UG, Sauter JJ. 1996. Xylem dysfunction during winter and recovery of hydraulic conductivity in diffuse-porous and ring-porous trees. *Oecologia* **105**: 435-439.

Hales S. 1727. *Vegetable staticks: or, an account of some statical experiments on the sap in vegetables*. London, UK: W. & J. Innys and T. Woodward.

Hao G-Y, Wheeler JK, Holbrook NM, Goldstein G. 2013. Investigating xylem embolism formation, refilling and water storage in tree trunks using frequency domain reflectometry. *Journal of Experimental Botany* **64**(8): 2321-2332.

Hartig T. 1855. Ueber wässrige Ausscheidungen durch die Pflanzenblätter. *Botanische Zeitung* **13**: 911-913.

Hartig T. 1858. Ueber die Bewegung des Saftes in den Holzpflanzen. *Botanische Zeitung* **16**: 329-342.

Hartig T. 1862. Ueber die Bewegung des Saftes in den Holzpflanzen. *Botanische Zeitung* **20**: 73-109.

Häusermann E, Frey-Wyssling A. 1963. Phosphatase-Aktivität in Hydathoden 1. *Protoplasma* **57**(1): 371-380.

Heil M. 2016. Nightshade wound secretion: The world's simplest extrafloral nectar? *Trends in Plant Science* **21**(8): 637-638.

Heimann M. 1950. Einfluss periodischer Beleuchtung auf die Guttationsrhythmik. *Planta* **38**(2): 157-195.

Henzler T, Waterhouse RN, Smyth AJ, Carvajal M, Cooke DT, Schäffner AR, Steudle E, Clarkson DT. 1999. Diurnal variations in hydraulic conductivity and root pressure can be correlated with the expression of putative aquaporins in the roots of *Lotus japonicus*. *Planta* **210**(1): 50-60.

Hernández-Hernández V, Benítez M, Boudaoud A. 2020. Interplay between turgor pressure and plasmodesmata during plant development. *Journal of Experimental Botany* **71**(3): 768-777.

Heyl JG. 1933. Der Einfluss von Aussenfaktoren auf das Bluten der Pflanzen. *Planta* **20**(2): 294-353.

Hinckley TM, Lassoie JP, Running SW. 1978. Temporal and spatial variations in the water status of forest trees. *Forest Science* **24**(suppl_1): a0001-z0001.

Hofmeister W. 1862. Ueber Spannung, Ausflussmenge und Ausflussgeschwindigkeit von Säften lebender Pflanzen. *Flora (Jena)* **45**: 97-108, 113-120, 138-144, 145-152, 170-175, Appendix I-XXXIV.

Höhn K. 1951. Beziehungen zwischen Blutung und Guttation bei *Zea mays*. *Planta* **39**(1): 65-74.

Hölttä T, Dominguez Carrasco MDR, Salmon Y, Aalto J, Vanhatalo A, Bäck J, Lintunen A. 2018. Water relations in silver birch during springtime: How is sap pressurised? *Plant Biology* **20**(5): 834-847.

Hölttä T, Vesala T, Perämäki M, Nikinmaa E. 2006. Refilling of embolised conduits as a consequence of 'Münch water' circulation. *Functional Plant Biology* **33**(10): 949-959.

Jahn T, Baluska F, Michalke W, Harper JF, Volkmann D. 1998. Plasma membrane H$^+$-ATPase in the root apex: Evidence for strong expression in xylem parenchyma and asymmetric localization within cortical and epidermal cells. *Physiologia Plantarum* **104**(3): 311-316.

Jeong S-J, Lee C-H, Kim H-Y, Lee S-H, Hwang I-G, Shin C-S, Lee J-S, Jeong H-S. 2012. Quality characteristics of the white birch sap with varying collection periods. *Journal of the Korean Society of Food Science and Nutrition* **41**(1): 143-148.

Johnson KM, Jordan GJ, Brodribb TJ. 2018. Wheat leaves embolised by water stress do not recover function upon rewatering. *Plant, Cell & Environment* **41**(11): 2704-2714.

Johnson RW, Tyree MT. 1992. Effect of stem water-content on sap flow from dormant maple and butternut stems - induction of sap flow in butternut. *Plant Physiology* **100**(2): 853-858.

Klepper B. 1967. Effects of osmotic pressure on exudation from corn roots. *Australian Journal of Biological Sciences* **20**(4): 723-735.

Knipfer T, Cuneo IF, Brodersen CR, McElrone AJ. 2016. *In situ* visualization of the dynamics in xylem embolism formation and removal in the absence of root pressure: a study on excised grapevine stems. *Plant Physiology* **171**(2): 1024-1036.

Knipfer T, Eustis A, Brodersen C, Walker AM, McElrone AJ. 2015. Grapevine species from varied native habitats exhibit differences in embolism formation/repair associated with leaf gas exchange and root pressure. *Plant, Cell & Environment* **38**(8): 1503-1513.

Knipfer T, Fricke W. 2010. Root pressure and a solute reflection coefficient close to unity exclude a purely apoplastic pathway of radial water transport in barley (*Hordeum vulgare*). *New Phytologist* **187**(1): 159-170.

Kolb KJ, Davis SD. 1994. Drought tolerance and xylem embolism in co-occurring species of coastal sage and chaparral. *Ecology* **75**(3): 648-659.

Kozlowski TT, Pallardy SG. 1996. *Physiology of woody plants*. San Diego: Academic Press.

Kramer PJ. 1932. The absorption of water by root systems of plants. *American Journal of Botany* **19**(2): 148-164.

Kramer PJ. 1940. Sap pressure and exudation. *American Journal of Botany* **27**(10): 929-931.

Kramer PJ. 1945. Absorption of water by plants. *The Botanical Review* **11**(6): 310-355.

Kramer PJ, Boyer JS. 1995. *Water relations of plants and soils*. San Diego: Academic Press.

Larochelle F, Forget É, Rainville A, Bousquet J. 1998. Sources of temporal variation in sap sugar content in a mature sugar maple (*Acer saccharum*) plantation. *Forest Ecology and Management* **106**(2): 307-313.

Lehmann H, Stelzer R, Holzamer S, Kunz U, Gierth M. 2000. Analytical electron microscopical investigations on the apoplastic pathways of lanthanum transport in barley roots. *Planta* **211**(6): 816-822.

Leng HN, Lu MZ, Wan XC. 2013. Variation in embolism occurrence and repair along the stem in drought-stressed and re-watered seedlings of a poplar clone. *Physiologia Plantarum* **147**(3): 329-339.

Lens F, Picon-Cochard C, Delmas CE, Signarbieux C, Buttler A, Cochard H, Jansen S, Chauvin T, Chacon Doria L, del Arco M, et al. 2016. Herbaceous angiosperms are not more vulnerable to drought-induced embolism than angiosperm trees. *Plant Physiology* **172**: 661-667.

Liese W. 1998. *The anatomy of bamboo culms*. Beijing, China: International Network for Bamboo and Rattan.

Lopushinsky W. 1980. Occurrence of root pressure exudation in Pacific Northwest conifer seedlings. *Forest Science* **26**(2): 275-279.

Losso A, Bär A, Dämon B, Dullin C, Ganthaler A, Petruzzellis F, Savi T, Tromba G, Nardini A, Mayr S, et al. 2019. Insights from in vivo micro-CT analysis: testing the hydraulic vulnerability segmentation in *Acer pseudoplatanus* and *Fagus sylvatica* seedlings. *New Phytologist* **221**(4): 1831-1842.

Lundegårdh H. 1940. Anionenatmung und Bluten. *Planta* **31**(2): 184-191.

Mayr S, Schmid P, Beikircher B, Feng F, Badel E. 2020. Die hard: timberline conifers survive annual winter embolism. *New Phytologist* **226**(1): 13-20.

McCully ME. 1995. Water efflux from the surface of field-grown grass roots. Observations by cryo-scanning electron microscopy. *Physiologia Plantarum* **95**(2): 217-224.

McCully ME, Canny MJ, Huang CX, Miller C, Brink F. 2010. Cryo-scanning electron microscopy (CSEM) in the advancement of functional plant biology: energy dispersive X-ray microanalysis (CEDX) applications. *Functional Plant Biology* **37**(11): 1011-1040.

McElrone AJ, Pockman WT, Martinez-Vilalta J, Jackson RB. 2004. Variation in xylem structure and function in stems and roots of trees to 20 m depth. *New Phytologist* **163**(3): 507-517.

McIntyre GI. 1987. The role of water in the regulation of plant development. *Canadian Journal of Botany* **65**(7): 1287-1298.

Mei T, Fang D, Röll A, Niu F, Yanto H, Hölscher D. 2016. Water use patterns of four tropical bamboo species assessed with sap flux measurements. *Frontiers in Plant Science* **6**: 1202.

Meinzer F, Grantz D, Smit B. 1991. Root signals mediate coordination of stomatal and hydraulic conductance in growing sugarcane. *Australian Journal of Plant Physiology* **18**(4): 329-338.

Meng DL, Walsh M, Fricke W. 2016. Rapid changes in root hydraulic conductivity and aquaporin expression in rice (*Oryza sativa* L.) in response to shoot removal - xylem tension as a possible signal. *Annals of Botany* **118**(4): 809-819.

Milburn JA, McLaughlin ME. 1974. Studies of cavitation in isolated vascular bundles and whole leaves of *Plantago major* L. *New Phytologist* **73**: 861-871.

Milburn JA, O'Malley PER. 1984. Freeze-induced sap absorption in *Acer pseudoplatanus*: a possible mechanism. *Canadian Journal of Botany* **62**: 2101-2106.

Miller DM. 1985. Studies of root function in *Zea mays*: III. Xylem sap composition at maximum root pressure provides evidence of active transport into the xylem and a measurement of the reflection coefficient of the root. *Plant Physiology* **77**(1): 162-167.

Minibayeva F, Beckett RP, Kranner I. 2015. Roles of apoplastic peroxidases in plant response to wounding. *Phytochemistry* **112**(0): 122-129.

Molisch H. 1902. Ueber localen Blutungsdruck und seine Ursachen. *Botanische Zeitung* **60**: 45-63.

Morris H, Plavcová L, Gorai M, Klepsch M, Kotowska M, Schenk HJ, Jansen S. 2018. Vessel-associated cells in angiosperm xylem: Highly specialized living cells at the symplast-apoplast boundary. *American Journal of Botany* **105**(2): 153-162.

Münch E. 1930. *Die Stoffbewegungen in der Pflanze*. Jena: Fischer.

Munting A. 1672. *Waare oeffening der planten*. Amsterdam: Jan Rieuwertsz.

Nagai M, Ohnishi M, Uehara T, Yamagami M, Miura E, Kamakura M, Kitamura A, Sakaguchi SI, Sakamoto W, Shimmen T, et al. 2013. Ion gradients in xylem exudate and guttation fluid related to tissue ion levels along primary leaves of barley. *Plant Cell and Environment* **36**(10): 1826-1837.

Nardini A, Lo Gullo MA, Salleo S. 2011. Refilling embolized xylem conduits: Is it a matter of phloem unloading? *Plant Science* **180**(4): 604-611.

Nobel PS. 2020. *Physiochemical and environmental plant physiology*. London: Academic Press.

O'Leary JW. 1965. Root-pressure exudation in woody plants. *Botanical Gazette* **126**(2): 108-115.

O'Leary JW. 1966. Root pressure exudation from apical root segments. *Nature* **212**(5057): 96-97.

O'Leary JW, Kramer PJ. 1964. Root pressure in conifers. *Science* **145**(3629): 284-285.

O'Malley PER, Milburn JA. 1983. Freeze-induced fluctuations in xylem sap pressure in *Acer pseudoplatanus*. *Canadian Journal of Botany* **61**: 3100-3106.

Oparka KJ, Prior DAM. 1992. Direct evidence for pressure-generated closure of plasmodesmata. *Plant Journal* **2**(5): 741-750.

Overton JB. 1921. The mechanism of root pressure and its relation to sap flow. *American Journal of Botany* **8**(7): 369-374.

Ozolinčius R, Bareika V, Rubinskienė M, Viškelis P, Mažeika R, Staugaitis G. 2016. Chemical composition of silver birch (*Betula pendula* Roth.) and downy birch (*Betula pubescens* Ehrh.) sap. *Baltic Forestry* **22**(2): 222-229.

Palmgren MG. 2001. Plant plasma membrane H⁺-ATPases: Powerhouses for nutrient uptake. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**(1): 817-845.

Patch D. 2004. Trees bleeding. *Arboricultural Practice Notes* **8**: 1-8.

Pedersen O. 1993. Long-distance water transport in aquatic plants. *Plant Physiology* **103**(4): 1369-1375.

Pedersen O, Jørgensen LB, Sand-Jensen K. 1997. Through-flow of water in leaves of a submerged plant is influenced by the apical opening. *Planta* **202**(1): 43-50.

Peterson CA, Enstone DE. 1996. Functions of passage cells in the endodermis and exodermis of roots. *Physiologia Plantarum* **97**(3): 592-598.

Pickard WF. 1989. How might a tracheary element which is embolized by day be healed by night? *Journal of Theoretical Biology* **141**(2): 259-279.

Pickard WF. 2003a. The riddle of root pressure. I. Putting Maxwell's demon to rest. *Functional Plant Biology* **30**(2): 121-134.

Pickard WF. 2003b. The riddle of root pressure. II. Root exudation at extreme osmolalities. *Functional Plant Biology* **30**(2): 135-141.

Priestley JH. 1920. The mechanism of root pressure. *The New Phytologist* **19**(7/8): 189-200.

Rinne PLH, Kaikuranta PM, Van Der Schoot C. 2001. The shoot apical meristem restores its symplasmic organization during chilling-induced release from dormancy. *The Plant Journal* **26**(3): 249-264.

Rodriguez-Dominguez 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 Phytologist* **218**(3): 1025-1035.

Rolland V, Bergstrom D, Michelle, Lenné T, Bryant G, Chen H, Wolfe J, Holbrook NM, Stanton D, Eric, Ball M, C. 2015. Easy come, easy go: capillary forces enable rapid refilling of embolized primary xylem vessels. *Plant Physiology* **168**(4): 1636-1647.

Rominger CL. 1843. Versuche über die Saftführung der Gefässe. *Botanische Zeitung* **1**: 177-185.

Rowan A, McCully ME, Canny MJ. 2000. The origin of the exudate from cut maize roots. *Plant Physiology and Biochemistry* **38**(12): 957-967.

Sachs J. 1865. *Handbuch der Experimental-Physiologie der Pflanzen*. Leipzig: W. Engelmann.

Saha S, Holbrook NM, Montti L, Goldstein G, Cardinot GK. 2009. Water relations of *Chusquea ramosissima* and *Merostachys claussenii* in Iguazu National Park, Argentina. *Plant Physiology* **149**(4): 1992-1999.

Sakr S, Alves G, Morillon RL, Maurel K, Decourteix M, Guilliot A, Fleurat-Lessard P, Julien JL, Chrispeels MJ. 2003. Plasma membrane aquaporins are involved in winter embolism recovery in walnut tree. *Plant Physiology* **133**(2): 630-641.

Samuels AL, Fernando M, Glass ADM. 1992. Immunofluorescent localization of plasma membrane H$^+$-ATPase in barley roots and effects of K nutrition. *Plant Physiology* **99**(4): 1509-1514.

Sauter JJ 1974. Maple. *McGraw-Hill Yearbook Science & Technology*. New York: McGraw-Hill, 280-282.

Saveyn A, Steppe K, McGuire MA, Lemeur R, Teskey RO. 2008. Stem respiration and carbon dioxide efflux of young *Populus deltoides* trees in relation to temperature and xylem carbon dioxide concentration. *Oecologia* **154**(4): 637-649.

Schenk HJ, Espino S, Rich-Cavazos SM, Jansen S. 2018. From the sap's perspective: the nature of vessel surfaces in angiosperm xylem. *American Journal of Botany* **105**(2): 174-187.

Scholander PF, Love WE, Kanwisher JW. 1955. The rise of sap in tall grapevines. *Plant Physiology* **30**(2): 93-104.

Scholz FG, Bucci SJ, Meinzer FC, Goldstein G 2016. Maintenance of root function in tropical woody species during droughts: Hydraulic redistribution, refilling of embolized vessels, and facilitation between plants. In: Goldstein G, Santiago LS eds. *Tropical Tree Physiology: Adaptations and Responses in a Changing Environment*. Cham: Springer International Publishing, 227-241.

Schurr U. 1998. Xylem sap sampling – new approaches to an old topic. *Trends in Plant Science* **3**(8): 293-298.

Sevanto S, Höltta T, Holbrook NM. 2011. Effects of the hydraulic coupling between xylem and phloem on diurnal phloem diameter variation. *Plant Cell and Environment* **34**(4): 690-703.

Shapira O, Israeli Y, Shani U, Schwartz A. 2013. Salt stress aggravates boron toxicity symptoms in banana leaves by impairing guttation. *Plant, Cell & Environment* **36**(2): 275-287.

Singh S. 2016a. Guttation: Mechanism, momentum and modulation. *Botanical Review* **82**(2): 149-182.

Singh S. 2016b. Root pressure: Getting to the root of pressure. *Progress in Botany* **77**: 105-150.

Singh S, Singh TN, Chauhan JS. 2009a. Guttation in rice: Occurrence, regulation, and significance in varietal improvement. *Journal of Crop Improvement* **23**(4): 351-365.

Singh S, Singh TN, Chauhan JS. 2009b. Water transport in crop plants with special reference to rice: Key to crop production under global water crisis. *Journal of Crop Improvement* **23**(2): 194-212.

Spanjer O. 1898. Untersuchungen über die Wasserapparate der Gefässpflanzen. *Botanische Zeitung* **56**: 35-81.

Speidel B. 1939. Untersuchungen zur Physiologie des Blutens bei höheren Pflanzen. *Planta* **33**(1): 67–112.

Sperry JS, Holbrook NM, Zimmermann MH, Tyree MT. 1987. Spring filling of xylem vessels in wild grapevine. *Plant Physiology* **83**(2): 414-417.

Sperry JS, Nichols KL, Sullivan JEM, Eastlack SE. 1994. Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of northern Utah and interior Alaska. *Ecology* **75**(6): 1736-1752.

Sperry JS, Saliendra NZ. 1994. Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. *Plant, Cell and Environment* **17**: 1233-1241.

Sperry JS, Sullivan JEM. 1992. Xylem embolism in response to freeze-thaw cycles and water stress in ring-porous, diffuse-porous, and conifer species. *Plant Physiology* **100**(2): 605-613.

Steudle E, Jeschke WD. 1983. Water transport in barley roots. *Planta* **158**(3): 237-248.

Steudle E, Murrmann M, Peterson CA. 1993. Transport of water and solutes across Maize roots modified by puncturing the endodermis - Further evidence for the composite transport model of the root. *Plant Physiology* **103**(2): 335-349.

Stiller V, Lafitte HR, Sperry JS. 2003. Hydraulic properties of rice and the response of gas exchange to water stress. *Plant Physiology* **132**(3): 1698-1706.

Stiller V, Sperry JS. 1999. Canny's compensating pressure theory fails a test. *American Journal of Botany* **86**(8): 1082-1086.

Strati S, Patiño S, Slidders C, Cundall EP, Mencuccini M. 2003. Development and recovery from winter embolism in silver birch: seasonal patterns and relationships with the phenological cycle in oceanic Scotland. *Tree Physiology* **23**(10): 663-673.

Taiz L, Zeiger E, Møller IM, Murphy A. 2014. *Plant physiology and development*. Sunderland, MA: Sinauer Associates.

Takeda F, Wisniewski ME, Glenn DM. 1991. Occlusion of water pores prevents guttation in older strawberry leaves. *Journal of the American Society for Horticultural Science* **116**(6): 1122-1125.

Teskey RO, Saveyn A, Steppe K, McGuire MA. 2008. Origin, fate and significance of CO_2 in tree stems. *New Phytologist* **177**(1): 17-32.

Theophrastus. 300 B.C. *Enquiry into plants and minor works on odours and weather signs. 2 vols. (Περι φυτων ιστοριας. In Greek, with English translation, published 1916)*. Cambridge, Mass.: Harvard University Press.

Tibbetts TJ, Ewers FW. 2000. Root pressure and specific conductivity in temperate lianas: exotic *Celastrus orbiculatus* (Celastraceae) vs. native *Vitis riparia* (Vitaceae). *American Journal of Botany* **87**: 1272-1278.

Tomos AD, Leigh RA. 1999. The pressure probe: A versatile tool in plant cell physiology. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**(1): 447-472.

Tyree MT. 1983. Maple sap uptake, exudation, and pressure changes correlated with freezing exotherms and thawing endotherms. *Plant Physiology* **73**(2): 277-285.

Tyree MT. 1999. The forgotten component of plant water potential: a reply. Tissue pressures are not additive in the way M.J. Canny suggests. *Plant Biology* **1**: 598–601.

Tyree MT, Fiscus EL, Wullschleger SD, Dixon MA. 1986. Detection of xylem cavitation in corn (*Zea mays*) under field conditions. *Plant Physiology* **82**: 597-599.

Tyree MT, Yang S. 1992. Hydraulic conductivity recovery versus water pressure in xylem of *Acer saccharum*. *Plant Physiology* **100**(2): 669-676.

Tyree MT, Zimmermann MH. 2002. *Xylem structure and the ascent of sap*. Berlin: Springer-Verlag.

Unger F. 1858. Beiträge zur Physiologie der Pflanzen. VII. Ueber die Allgemeinheit wässriger Ausscheidungen und deren Bedeutung für das Leben der Pflanzen. *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften Wien* **28**: 111-134.

Utsumi Y, Sano Y, Fujikawa S, Funada R, Ohtani J. 1998. Visualization of cavitated vessels in winter and refilled vessels in spring in diffuse-porous trees by cryo-scanning electron microscopy. *Plant Physiology* **117**(4): 1463-1471.

van Bel AJE, van der Schoot C. 1988. Primary function of the protective layer in contact cells: Buffer against oscillations in the hydrostatic pressure in the vessels? *IAWA Bulletin n.s.* **9**(1-4): 285-288.

Van Camp J, Hubeau M, Van den Bulcke J, Van Acker J, Steppe K. 2017. Cambial pinning relates wood anatomy to ecophysiology in the African tropical tree *Maesopsis eminii*. *Tree Physiology* **38**(2): 232-242.

van der Velde M, Green SR, Clothier BE. 2009. Nocturnal sap flow measurements in squash using the T-max method: Transpiration or rehydration? *Acta Horticulturae* **846**: 113-120.

Van Overbeck J. 1942. Water uptake by excised root systems of the tomato due to non-osmotic forces. *American Journal of Botany* **29**(8): 677-683.

Volaire F, Lens F, Cochard H, Xu H, Chacon-Doria L, Bristiel P, Balachowski J, Rowe N, Violle C, Picon-Cochard C. 2018. Embolism and mechanical resistances play a key role in dehydration tolerance of a perennial grass *Dactylis glomerata* L. *Annals of Botany* **122**(2): 325-336.

Volkens G. 1883. Ueber Wasserauscheidung in liquider Form an den Blättern höherer Pflanzen. *Jahrbücher des Königlich Botanischen Gartens und des Botanischen Museums zu Berlin* **2**: 166-209.

Waduwara CI, Walcott SE, Peterson CA. 2008. Suberin lamellae of the onion root endodermis: their pattern of development and continuity. *Botany* **86**: 623-632.

Wan X, Steudle E, Hartung W. 2004. Gating of water channels (aquaporins) in cortical cells of young corn roots by mechanical stimuli (pressure pulses): effects of ABA and of HgCl2. *Journal of Experimental Botany* **55**(396): 411-422.

Wang FS, Tian XL, Ding YL, Wan XC, Tyree MT. 2011. A survey of root pressure in 53 Asian species of bamboo. *Annals of Forest Science* **68**(4): 783-791.

Wegner LH. 2014. Root pressure and beyond: energetically uphill water transport into xylem vessels? *Journal of Experimental Botany* **65**(2): 381-393.

Wegner LH. 2015. A thermodynamic analysis of the feasibility of water secretion into xylem vessels against a water potential gradient. *Functional Plant Biology* **42**(9): 828-835.

Westhoff M, Schneider H, Zimmermann D, Mimietz S, Stinzing A, Wegner LH, Kaiser W, Krohne G, Shirley S, Jakob P, et al. 2008. The mechanisms of refilling of xylem conduits and bleeding of tall birch during spring. *Plant Biology* **10**(5): 604-623.

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 & Environment* **36**(11): 1938-1949.

White PR. 1938. "Root-pressure" - an unappreciated force in sap movement. *American Journal of Botany* **25**(3): 223-227.

Wieler A. 1893. Das Bluten der Pflanzen. *Beiträge zur Biologie der Pflanzen* **6**(1): 1-211.

Willughby F, Ray J. 1669. Experiments on the motion of sap in trees. *Philosophical Transactions of the Royal Society* **4**(48): 963-965.

Woodhouse ED. 1933. Sap hydraulics. *Plant Physiology* **8**: 177-202.

Wu M, Zhang Y, Oya T, Marcati CR, Pereira L, Jansen S. 2020. Root xylem in three woody angiosperm species is not more vulnerable to embolism than stem xylem. *Plant and Soil* **450**(1): 479-495.

Yang S-J, Zhang Y-J, Goldstein G, Sun M, Ma R-Y, Cao K-F. 2015. Determinants of water circulation in a woody bamboo species: afternoon use and night-time recharge of culm water storage. *Tree Physiology* **35**(9): 964-974.

Yang S-J, Zhang Y-J, Sun M, Goldstein G, Cao K-F. 2012. Recovery of diurnal depression of leaf hydraulic conductance in a subtropical woody bamboo species: embolism refilling by nocturnal root pressure. *Tree Physiology* **32**(4): 414-422.

Yang S, Tyree MT. 1992. A theoretical-model of hydraulic conductivity recovery from embolism with comparison to experimental data on *Acer saccharum*. *Plant, Cell & Environment* **15**: 633-643.

Yin XH, Sterck F, Hao GY. 2018. Divergent hydraulic strategies to cope with freezing in co-occurring temperate tree species with special reference to root and stem pressure generation. *New Phytologist* **219**(2): 530-541.

Zhang C, Yu X, Ayre BG, Turgeon R. 2012. The origin and composition of cucurbit "phloem" exudate. *Plant Physiology* **158**(4): 1873-1882.

Zholkevich VN 1981. On the nature of root pressure. In: Brouwer R, Gašparíková O, Kolek J, Loughman BC eds. *Structure and Function of Plant Roots: Proceedings of the 2nd International Symposium, held in Bratislava, Czechoslovakia, September 1–5, 1980*. Dordrecht, Netherlands: Springer, 157-158.

Zholkevich VN, Emel'yanova IB, Sushchenko SV. 2005. Self-oscillations of water transport in the plant root. *Doklady Biological Sciences* **403**(1): 269-271.

Zholkevich VN, Popova MS, Zhukovskaya NV. 2007. Stimulatory effects of adrenalin and noradrenalin on root water-pumping activity and the involvement of G-proteins. *Russian Journal of Plant Physiology* **54**(6): 790-796.

Zhou H, Sun Y, Tyree MT, Sheng W, Cheng Q, Xue X, Schumann H, Schulze Lammers P. 2015. An improved sensor for precision detection of in situ stem water content using a frequency domain fringing capacitor. *New Phytologist* **206**(1): 471-481.

Supporting information

Notes S1 Supporting information for the model scenarios in Box 1.

Table 1 Techniques to measure positive xylem pressure and their pros and cons.

What is observed?	Measurement	Pros and Cons	Sources
Guttation	Exudation rate Chemical composition	Pros: - Non-destructive - Uncontaminated composition - Natural temporal patterns Cons: - Pressure measurement impossible - Composition may be affected by active reabsorption in hydathodes	(Goatley & Lewis, 1966) (Singh *et al.*, 2009a) (Shapira *et al.*, 2013) (Nagai *et al.*, 2013)
Bleeding	Exudation rate Chemical composition	Pros: Easiest method Measurements of both rate and pressure possible Cons: - Temporal patterns and rates affected by conditions and time of cutting - Composition affected by cut, living cells and wounding responses	(Van Overbeck, 1942) (Arisz *et al.*, 1951) (O'Leary, 1965) (Pickard, 2003b) (Zholkevich *et al.*, 2005) (Gleason *et al.*, 2017)
	Pressure; manometer or pressure sensor mounted on decapitated stems or roots	Pros: - Can measure pressure in entire root systems Cons: - Destructive: Cutting can affect the magnitude and timing of pressure - Pressure declines over time after cutting	(Hales, 1727) (Sachs, 1865) (Fisher *et al.*, 1997) (Saha *et al.*, 2009) (Wang *et al.*, 2011) (Yang *et al.*, 2015) (Knipfer *et al.*, 2015) (Gleason *et al.*, 2017)
Xylem pressure	Pressure; pressure sensor inserted into	Pros: - Minimally destructive - Continuous measurements possible	(Scholander *et al.*, 1955) (Tyree, 1983) (Cochard *et al.*, 2001)

	intact xylem	in the field	(Clearwater *et al.*, 2007)
		- Natural temporal patterns	(Charrier *et al.*, 2017)
		Cons:	(Hölttä *et al.*, 2018)
		- Unable to measure sap composition	
Vessel pressure	Pressure; pressure probe inserted into individual vessel	Pros: - Same as for xylem pressure Cons: - Technically challenging, high failure rate - Not for use in the field	(Steudle & Jeschke, 1983) (Steudle *et al.*, 1993) (Knipfer & Fricke, 2010)

The sources listed are examples for studies that have used the different approaches.

Table 2 Maximum positive xylem pressures recorded in a variety of studies.

Type of pressure	Species	Maximum pressure (kPa)	Location of measurement	Source
Seasonal	*Acer saccharum*	200	trunk*	(Tyree, 1983)
	Acer pseudoplatanus	60	trunk*	(O'Malley & Milburn, 1983)
	Actinidia macrosperma	150	rootstock*	(Clearwater *et al.*, 2007)
	Alnus crispa	22	basal branch	(Sperry *et al.*, 1994)
	Betula papyrifera	55	basal branch	(Sperry *et al.*, 1994)
	Betula pendula	250	trunk*	(Hölttä *et al.*, 2018)
	Fagus sylvatica	45	trunk*	(Cochard *et al.*, 2001)
	Juglans regia	160	lateral branch	(Ewers *et al.*, 2001)
	Smilax rotundifolia	100	branch	(Cobb *et al.*, 2007)
	Vitis labrusca	391	basal stem*	(Scholander *et al.*, 1955)
	Vitis vinifera	129	root stump	(Hales, 1727)
	Vitis vinifera	93	upper branch	(Hales, 1727)
	Vitis vinifera	107	root stump	(Hofmeister, 1862)
Diurnal				
	Bambusa multiplex	67.5	basal stem	(Wang *et al.*, 2011)
	Cissus gossypiifolia	89	basal stem	(Fisher *et al.*, 1997
	Cissus sicyoides	148	basal stem	(Fisher *et al.*, 1997
	Cocos nucifera	125	advent. root	(Davis, 1961)
	Dendrocalamus latiflorus	195	basal stem	(Cao *et al.*, 2012)
	Digitalis media	61	root stump	(Hofmeister, 1862)
	Doliocarpus major	64	basal branch	(Ewers *et al.*, 1997)
	Fuchsia speciosa	163	root stump	(Eckerson, 1908)
	Helianthus annuus	136	root stump	(Eckerson, 1908)
	Lycopsersicum esculentum	137	root stump	(Eckerson, 1908)
	Lycopsersicum esculentum	155	stem	(De Swaef *et al.*, 2013)
	Lygodium venustrum	66	lateral branch	(Ewers *et al.*, 1997)
	Monstera acuminata	137	aerial root	(Fisher *et al.*, 1997

	Myriocarpa longipes	121	basal stem	(Fisher *et al.*, 1997
	Rhipidocladum racemiflorum	120	basal stem	(Cochard *et al.*, 1994; Ewers *et al.*, 1997)
	Salvia involucrata	159	root stump	(Eckerson, 1908)
	Vitis labrusca	38	basal stem*	(Scholander *et al.*, 1955)
	Zea mays	420	root stump	(Miller, 1985)
Response to rewatering after drought	*Oryza sativa*	62	root stump	(Stiller *et al.*, 2003)
	Vitis arizonica	230	root stump	(Knipfer *et al.*, 2015)
	Vitis champinii	190	root stump	(Knipfer *et al.*, 2015)
	Vitis riparia	180	root stump	(Knipfer *et al.*, 2015)
	Zea mays	140	root stump	(Gleason *et al.*, 2017)

All measurements were done on cut and bleeding stems or roots, except where noted.

* Pressure sensor inserted into xylem

Figure captions

Fig. 1 Locations and timing of positive xylem pressure in plants. It may occur before leaf-out in deciduous species or in leaf-bearing plants. Positive pressure is most commonly observed as guttation from leaves or bleeding from cut stems. Root pressure may occur in fine roots, using soil water as the source, or in woody roots and stems, using water stored in living cells, fibers, cell walls, and intercellular spaces as the source.

Fig. 2 Methods used to measure positive xylem pressure, showing remarkably similar techniques between the earliest measurements (a) by Hales (1727) with manometers mounted on grapevine branches, (b) Sachs (1865), with a manometer mounted on a root stump, and recent measurements with small pressure probes inserted into bamboo stems (c) for continuous logging with minimal disturbance of the system. Both techniques measure relative pressure, which avoids having to account for atmospheric pressure effects. Photograph (c) taken by Joseph Michaud.

Fig. 3 Anatomical images of root cross-sections of monocots that exhibit root pressure: Ruscus bamboo (*Shibataea kumasaca*, a, c, d) and perennial teosinte (*Zea diploperennis*, b, e, f).The light microscopy images (a and b) show the overall anatomy from the rhizodermis (R) to the vascular tissues in the center (Ex = exodermis, C = cortex, En = endodermis, X = xylem, LC = lysigenous cavity), with a side root on the right in a. The stele in a and b is recognizable as being surrounded by the endodermis. Transmission electron microscopy images show details of a thick endodermis (En) traversed by two long pit canals with plasmodesmata (Pd) in c, and plasmodesmata between a vessel-associated parenchyma cell (VAC) and neighboring parenchyma cell in d. Figure e includes a VAC with cell membrane (arrow), pit membrane (PM), pit border (PB), and a primary and secondary wall (PW and SW). Folded endodermal cells (En) and passage cell (PC) opposite three tracheary xylem elements (X) are shown in f.

Fig. 4 Anatomical images of roots of woody dicots that exhibit root pressure: Silver birch (*Betula pendula*; a, c, d) and grapevine (*Vitis vinifera*, b, e), including root cross-sections imaged using light microscopy (LM, a, b), and transmission electron microscopy (c –e). The LM images show a root with secondary growth (a), with xylem tissue (X), vessels (V), and phloem tissue (P), and early developmental stages of secondary growth (b), before the endodermis (En) and cortex (C) disappear. Details of vessel-associated parenchyma cells (VAC) show an amorphous layer (AL) between cell wall and plasma membrane, pit membrane (PM), pit border (PB), secondary and primary cell wall (SW and PW), starch (S), lipids (L), and plasmodesmata (arrows) for symplastic connectivity between VACs and other parenchyma cells.

Fig. 5 Image illustrating the first explicit hydraulic pressure model taken from Sachs (1865). The diagram shows a vessel *B* surrounded by three layers of solute-containing living cells *A''*, *A'*, and *A*, separated from each other with plasma membranes *a''*, *a'*, and *a*, with cell layer *A''* separated from the vessel by plasma membrane *b*. The membranes' permeability to water, but not to solutes, is assumed to be *b* > *a''* > *a'* > *a*, causing water to flow from *A* to vessel *B* without moving solutes into *B*.

Fig. 6 Examples for refilling of vessels in response to positive xylem pressure. (a) MicroCT images of cross-sections through grapevine stems (*Vitis riparia* and *V. arizonica*) showing embolism in drought-stressed (Ψ_{stem} < −1.5 MPa) and re-watered plants (Ψ_{stem} > −0.5 MPa). Embolized vessels appear dark gray and water-filled vessels as light gray. The variably hydrated pith is visible in the center of each cross-section. (b) Relationship of stem water potential before re-watering and steady-state root pressure after re-watering for *Vitis riparia* and *V. arizonica*. Each symbol represents an individual plant. (c) Vessel refilling in *Zea mays* in response to re-watering after drought treatment. Graph shows percent water-filled (functioning) vessels measured via microCT in three watering treatments

after an overnight recovery period (10 h). Purple and orange bars represent two inbred lines, B73 and CML103. Error bars represent 1 SD. (d) Relationship between root pressure and the recovery of stem-specific conductivity measured in internodes of *Zea mays* B73. Data points represent individual plants and were fit with logistic models. Graphs reproduced with permission and slightly modified from Knipfer et al. (2015) (a and b) and Gleason et al. (2017) (c and d).
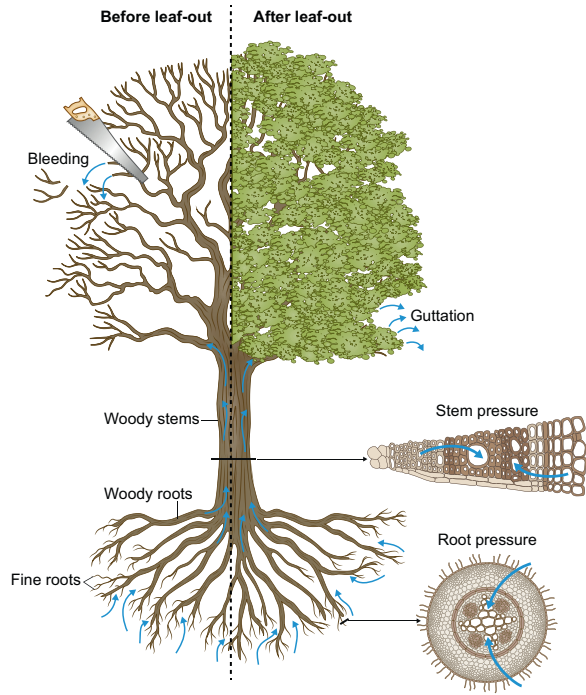
Before leaf-out | After leaf-out
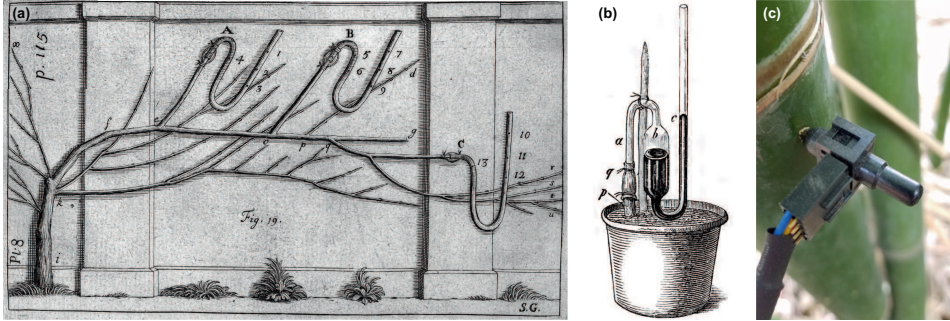
Bleeding

Guttation

Woody stems

Stem pressure

Woody roots

Root pressure

Fine roots

Figure 1

Figure 2
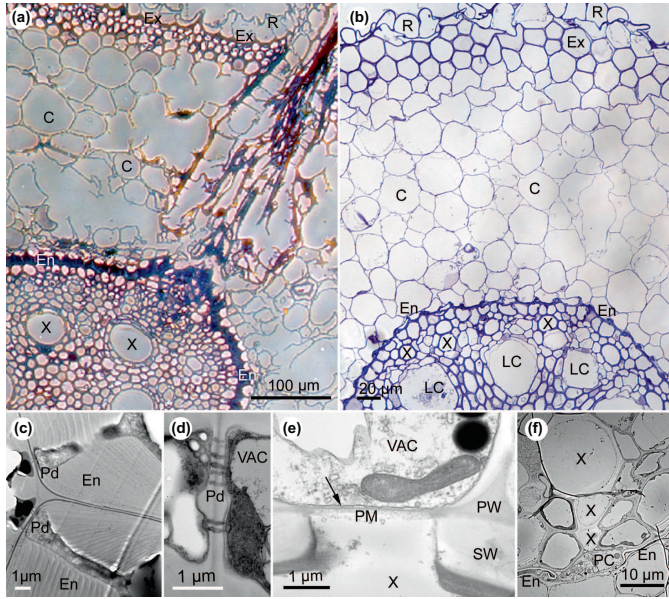
Figure 3

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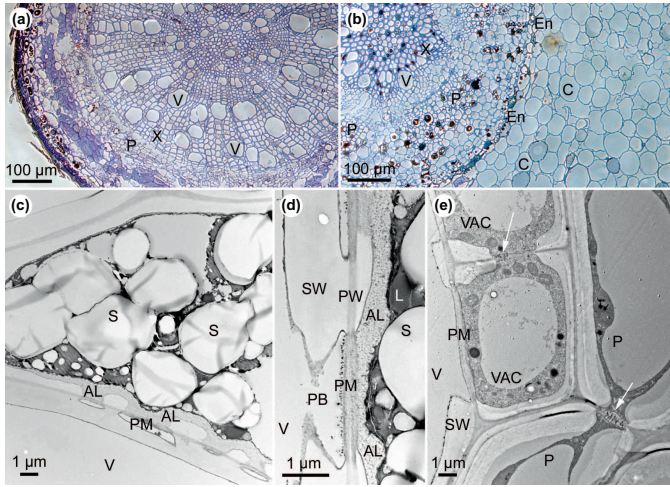
Figure 4

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Figure 5

(a)
Drought-stressed
Re-watered
Vitis riparia   *Vitis arizonica*
1 mm

(b)
Vitis arizonica
R^2 = 0.58, $P < 0.01$
Vitis riparia
R^2 = 0.55, $P < 0.01$
Steady-state root pressure (MPa)
Stem water potential (MPa)

(c)
B73
CML1031
microCT fully functional vessels (%)
No water   Partially watered   Fully watered

(d)
Pseudo R^2 = 0.63; MSE = 0.22
Stem conductivity (kg m^{-1} s^{-1} MPa^{-1})
Fully watered
Partially watered
No water
Pre-dawn root pressure (MPa)
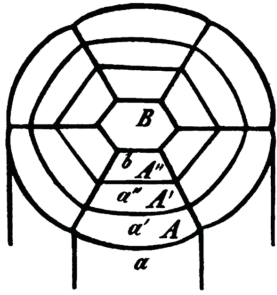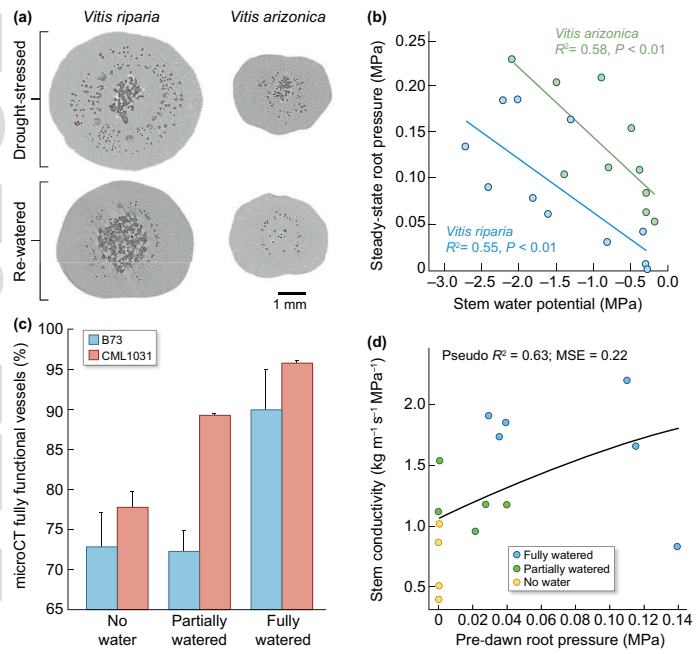
Figure 6

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Box 1. Mechanistic root pressure scenarios

Box Fig. 1 Xylem pressure generation via an (a) osmotic exudation and (b) hydraulic pressure scenario Pickard (2003). The structure shown here is for secondary xylem in a root with an amorphous layer between the pit membrane and the plasma membrane of the vessel-associated cell (VAC). Exudation from the VAC moves solutes into this layer and from there via diffusion into the vessel. A primary cell wall and pit membrane in primary xylem could play the same functional role as the amorphous layer. Note that increased expression of aquaporins (dark blue arrows) in membranes facing vessels would decrease hydraulic membrane resistance on that side of the cell and flow towards the vessel side would then be at a higher rate.

Model scenarios to explain root pressure fall into two broad categories, here referred to as osmotic exudation (A) and hydraulic pressure (B) scenarios. Both could be applicable to stems after some modification, but here we focus on models that have been proposed for fine roots. To illustrate the differences and commonalities between the two approaches, we parameterized the widely accepted osmotic exudation scenario and a hydraulic pressure scenario proposed by Pickard (2003a) for a model with five compartments: Soil (assumed to be in equilibrium with the root cortex apoplast), root symplast, vessel-associated cell (VAC), amorphous layer (or primary cell wall) around VAC, and vessel (Box Fig. **1**). The exact location where water moves from apoplast into symplast is not defined and could be at an endodermis, exodermis, or anywhere within the cortex.

The driving force (*F*, as force per area, MPa) for water transport across boundaries, such as cell membranes or a pit membrane, is calculated according to the following equation (Nobel, 2020):

$$F_{12} = (P_1 + M_1) - (P_2 + M_2) + \sigma(\pi_1 - \pi_2) \qquad \text{Eqn. (1)}$$

where *P* is pressure potential, *M* is matric potential, π is osmotic potential (all in MPa), σ is the dimensionless reflection coefficient, and the subscripts 1 and 2 refer to the compartments separated by a boundary. The reflection coefficient σ obtains a value between zero and one, where $\sigma = 0$ means that the boundary/membrane between the two compartments is equally permeable to water and the solute in question, and $\sigma = 1$ refers to perfect semipermeability, i.e.,

water can cross the boundary/membrane, but the solute in question cannot. For biological membranes, reflection coefficients lower than one are caused largely by expression of solute channels. Osmotic potential differences contribute to the driving force for water flow to the same extent as pressure differences only if $\sigma = 1$.

(a) Osmotic exudation scenario

In this scenario, osmotic substances are actively transported from the soil and cortex apoplast into the symplast, causing water to follow via osmosis. The substances are then passively secreted into the amorphous layer from VACs by solute channels. This would draw water into the vessel apoplast osmotically from the VACs and other parts of the symplast, in turn drawing more water in from the soil according to the water potential difference. Box Table 1 shows an example of osmotically generated root pressure with a driving force difference (F) of 0.1 MPa between adjacent compartments. The positive root pressure generated corresponds to 0.1 MPa, sufficient for pushing water up to a height of 10 m.

Box Table 1. Model scenario used as an example to show water potentials and driving forces across cell layers in a root for the osmotic exudation model shown in Box Fig. 1a. All data are in MPa.

Ψ	Vessel	PM	AL/CW	MB	VAC	MB	Sym	MB	Apo/Soil
P	0.10	$\sigma=0$	0.30	$\sigma=0.5$	0.30	$\sigma=1$	0.30	$\sigma=1$	0
M	0		-0.10		0		0		-0.20
π	-0.75		-0.75		-0.75		-0.65		-0.05
Ψ_{total}	-0.65		-0.55		-0.45		-0.35		-0.25
F		0.1		0.1		0.1		0.1	

Abbreviations: PM = pit membrane, MB = plasma membrane, AL/CW = amorphous layer or primary cell wall, MB = plasma membrane, VAC = vessel-associated cell, Sym = symplast, Apo/Soil = apoplast or soil, P = pressure potential, M = matric potential, π = osmotic potential, Ψ_{total} = total water potential, F = driving force for water flow, σ = reflection coefficient.

(b) Hydraulic pressure scenario

In this scenario (Pickard, 2003a), solutes moving from the symplast to the apoplast – including the amorphous layer or primary cell wall, pit membrane, and the vessel sap – are actively "retrieved" back into the cytoplasm of VACs, most likely driven by H^+-ATPases. This would cause acidification of the xylem sap by proton fluxes (Box Fig. 1 b). Box Table 2 demonstrates an example where root pressure would be generated with a driving force difference (F) of 0.1 MPa between the adjacent compartments. Note that the driving force F moves water against an apparent water potential gradient Ψ_{total} (Pickard, 2003a).

Box Table 2. Model scenario used as an example to show water potentials and driving forces across cell layers in a root for the hydraulic pressure model shown in Box Fig. **1b**. All data are in MPa.

Ψ	Vessel	PM	AL/CW	MB	VAC	MB	Sym	MB	Apo/Soil
P	0.10	$\sigma=0$	0.95	$\sigma=0.5$	0.95	$\sigma=1$	0.95	$\sigma=1$	0
M	0		-0.75		0		0		-0.20
π	-0.10		-0.10		-1.40		-1.30		-0.05
Ψ_{total}	0		0.10		-0.45		-0.35		-0.25
F		0.1		0.1		0.1		0.1	

Abbreviations: PM = pit membrane, MB = plasma membrane, AL/CW = amorphous layer or primary cell wall, VAC = vessel-associated cell, Sym = symplast, Apo/Soil = apoplast or soil, P = pressure potential, M = matric potential, π = osmotic potential, Ψ_{total} = total water potential, F = driving force for water flow, σ = reflection coefficient.

Root pressure in this modeling framework can be accomplished with a wide variety of different parameterizations. Using eqn. (1), and simplifying the structural scenario in Box Fig. **1** further to

include just three compartments (soil, symplast, including VACs, and vessel apoplast), the value of pressure in the vessel apoplast can be predicted to be (see Notes S1 for the derivation of the equation and example calculations conducted with the equation):

$$P_{APO} = \Psi_{SOIL} - \pi_{SYMP} - 2F + \sigma(\pi_{SYMP} - \pi_{APO}) \qquad \text{Eqn. (2)}$$

Where π_{sym} and π_{apo} are the osmotic potentials of the symplast and vessel apoplast, respectively, Ψ_{soil} is soil water potential, and P_{apo} is pressure in the vessel apoplast (all in MPa). The equation gives the pressure in the vessel apoplast to be either positive, signifying root pressure, or negative (no root pressure). According to the equation, the predicted pressure is higher when (1) $\Psi soil$ is higher, (2) the driving force F for water movement is lower, (3) π_{APO} is lower, (4) π_{SYMP} is lower, and/or (5) the reflection coefficient σ is lower.

Note that the same model framework (and the same Eqn. 2) describe both the osmotic exudation and hydraulic pressure scenarios. Within the framework, the difference between the two scenarios simply lies in the active transport of solutes back into VACs (Pickard, 2003a; Wegner, 2014) and the resulting difference in xylem apoplast osmotic potential, requiring expenditure of more metabolic energy than for the osmotic exudation scenario.

Rate of water movement

The rate of water movement from soil to symplast and from symplast to vessel apoplast is the driving force F multiplied by the hydraulic conductance from soil to vessels. The flux from soil to symplast, and from symplast to vessel apoplast must be the same. If flux from soil to symplast is larger than flux from symplast to vessel apoplast, then the water amount and the turgor pressure in the symplast both increase. This will decrease the flow rate from soil to symplast and accelerate the flow rate from symplast to vessel apoplast until these fluxes will be equal.

Are the two root pressure scenarios exclusive and can they work for stems?

As shown above, osmotic exudation and hydraulic pressure scenarios are not mutually exclusive. By expressing or removing solute channels and active transporters (H$^+$-ATPase) in the plasma membrane between symplast/VAC and the vessel apoplast, a root could switch between the two scenarios or run a combined mode of both, depending on the availability of

solutes in the soil medium. Both scenarios can potentially work in stems in a modified form, where the water source could be phloem and storage compartments in bark, xylem, or pith, and solutes could come out of cell storage as well.