Methods in Ecology and Evolution

British Ecological Society

Methods in Ecology and Evolution 2012, **3**, 685–694 doi: 10.1111/j.2041-210X.2012.00204.x

REVIEW

Measurements of stem xylem hydraulic conductivity in the laboratory and field

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Summary

1. Xylem hydraulic properties play an essential role in supporting growth and photosynthesis and influence sensitivity to environmental conditions such as drought and freezing. Consequently, stem hydraulic conductance can be used as a comparative measure of overall hydraulic adaptation across species and to assess the impact of environmental variation, especially drought, on water transport.

2. We summarize the main methods currently in use for measurements of stem xylem hydraulic properties. Measurements can be accomplished in a number of ways, including using a pipette, an analytic balance or a 'pressure-drop' flow meter. We provide new details on the design of a relatively inexpensive and easily field-deployable flow meter that is flexible for a variety of applications. The biological challenges associated with these measurements arise from the difficulties of working with diverse living tissues of variable geometry.

3. We provide a review of best practices and provide technical guidance, emphasizing measurements on detached samples using portable equipment.

Key-words: cavitation, embolism, flow meter, hydraulic conductivity, per cent loss conductivity, tracheid, vessel, xylem

Introduction

Measurements of stem xylem hydraulic properties provide fundamental information of a plant's capacity to supply water to photosynthetic and growing tissues (Tyree & Zimmermann 2002; Holbrook & Zwieniecki 2005; Brodribb 2009), and sensitivity to environmental stresses such as drought or freezing, affecting species distributions (e.g. Sperry, Alder & Eastlack 1993; Lipp & Nilsen 1997; Pockman & Sperry 1997; Brodribb & Hill 1999; Cavender-Bares & Holbrook 2001; Choat, Sack & Holbrook 2007; Stuart *et al.* 2007; Markesteijn *et al.* 2011). We review methods for determining stem xylem hydraulic conductivity, with an emphasis on methods using easily portable equipment. We build upon an earlier discussion of this topic (Sperry, Donnelly & Tyree 1988); interested readers are encouraged to consult that work for more information. Additionally, we note the existence of commercial instruments with excellent accompanying manuals (Bronkhorst 2008; Dynamax 2009). We provide a concise current guide, including issues associated with selection and sampling of appropriate material, and the methods used to characterize hydraulic properties. We restricted our discussion to measurements made on stems, branches and mature portions of roots. For information on techniques for determining the hydraulic properties of the xylem associated with regions of uptake, exchange and reproduction (e.g. roots, leaves, flowers and fruit), papers should be sought that deal specifically with these topics (e.g. Zwieniecki & Boersma 1997; Nardini, Tyree & Salleo 2001; Sack *et al.* 2002; Choat *et al.* 2009; Feild, Chatelet & Brodribb 2009).

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Water flow through the roughly tubular xylem conduits is nonturbulent, resulting in a ratio of volumetric flow rate to applied pressure gradient proportional to the 4th power of conduit radius as given by the Hagen–Poiseuille equation (Lewis & Boose 1995; Zwieniecki, Melcher & Holbrook 2001a). However, because the internal anatomy of xylem conduits and their interconnections is more complex than a series of parallel straight-walled tubes, values of xylem hydraulic conductance calculated from measurements of conduit radii typically overestimate measured conductivity (Tyree & Zimmermann 2002, p. 19; Sperry, Hacke & Wheeler 2005; Choat, Cobb & Jansen 2008). The hydraulic conductivity of the xylem is highly variable across species (e.g. Brodribb & Feild 2000; Nardini & Salleo 2000; Maherali, Pockman & Jackson 2004) and, for a given species, among growth environments (e.g. Schultz & Matthews 1993; Barigah *et al.* 2006; Choat, Sack & Holbrook 2007; Cornwell *et al.* 2007). For a given individual, conductivity can be dynamic, with changes resulting from embolism formation (Sperry & Sullivan 1992; Zwieniecki & Holbrook 1998; Domec & Gartner 2002; Taneda & Sperry 2008), embolism repair (Salleo *et al.* 1996; Holbrook & Zwieniecki 1999; Tyree *et al.* 1999; Holbrook *et al.* 2001; Kaufmann *et al.* 2009; Brodersen *et al.* 2010), sap ion concentration (Zwieniecki, Melcher & Holbrook 2001b; Cochard *et al.* 2010b; Nardini, Salleo & Jansen 2011) and growth (Cochard *et al.* 1997; Melcher, Zwieniecki & Holbrook 2003). Xylem conduits are dead at maturity, yet the tissues in which they function contain living cells and measurements must be taken relatively quickly to prevent wounding reactions or clogging because of fungi and bacterial growth from affecting the measured flow rates (Sperry, Donnelly & Tyree 1988; Melcher & Warchocki 2010). Hydrated material can often be transported from the field to the laboratory. However, when this cannot be done within a few days, rapid and portable techniques need to be deployed in the field or in a nearby laboratory which may lack major instruments and supplies.

Measurements of xylem hydraulic properties fall into two main categories. The first involves measuring the hydraulic conductivity, which is the hydraulic conductance, that is, the flux for a given driving force ($Q/\Delta P$), normalized by the length of the segment and referenced either to the cross-sectional area of the xylem (K_s: xylem specific conductivity) or to the leaf area supported by the xylem (K_L: leaf specific conductivity):

$$K_s = QL/(\Delta P\, A_{sw}) \qquad \text{eqn 1}$$

$$K_L = QL/(\Delta P\, A_L) \qquad \text{eqn 2}$$

where Q is the recorded flux (gravimetric or volumetric flow rate), L is the length of the measured segment, ΔP is the pressure drop across the segment, A_{sw} is the cross-sectional area of the conducting sapwood, and A_L is the total leaf area supplied by the measured segment. These two conductivities are interrelated by the ratio of A_{sw}/A_L, a parameter known as the 'Huber value' (H_v), as $K_L = H_v K_s$.

The second major measurement category addresses the vulnerability of the xylem to drought- or freeze-induced cavitation. Here, the relevant parameter is the extent to which the maximum hydraulic capacity has been reduced by cavitation:

$$\text{Per cent loss conductivity (PLC)} = 100\left(1 - K'_{\text{initial}}/K'_{\text{max}}\right) \qquad \text{eqn 3}$$

where K'_{initial} is the initial conductivity and K'_{max} is the maximum conductivity measured after any gas trapped within the conduits has been removed, using a high-pressure flush with partially degassed water or holding the measured segment in solution under a partial vacuum (Sperry, Donnelly & Tyree 1988; Tyree & Yang 1992; Hietz *et al.* 2008). Vulnerability curves relate per cent loss conductivity (PLC) to xylem 'tensions' (subambient xylem pressures). The points on the curve are typically generated by measuring conductivity after letting the plant (or cut branch) dehydrate, or after air injection at different pressures, or during or after generating xylem tensions by centrifugation (Cochard, Cruiziat & Tyree 1992; Salleo *et al.* 1992; Choat *et al.* 2010; Cochard *et al.* 2010a). Vulnerability curves provide useful information of xylem sensitivity to drought. Vulnerability to cavitation is often summarized by the tension that results in a 50% loss in hydraulic conductivity (P_{50}); a large tension is often correlated with highly negative minimum leaf water potentials and species drought tolerance (Hacke & Sperry 2001; Maherali, Pockman & Jackson 2004; Jacobsen *et al.* 2007). For the measurement of vulnerability curves, readers should additionally consider papers that focus on many important methodological issues (e.g. Beikircher *et al.* 2010; Cai & Tyree 2010; Choat *et al.* 2010; Cochard *et al.* 2010a; Domec 2011; Ennajeh *et al.* 2011). Here, we focus on the conductivity measurements from which they are derived.

Methods and instrumentation

FLOW RATE

The measurement of fluid flow can be taken in a number of ways. Owing to the low compressibility and thermal coefficient of expansion of liquid water, mass and volumetric flow rates are proportional. The simplest method for determining the flow rates through a plant sample is to connect the segment of interest to a water reservoir on one side and to a pipette on the other (Fig. 1a). Nonxylem pathways through which water could flow (e.g. in the pith) need to be blocked (e.g. with plasticine; Santiago *et al.* 2004). The change in meniscus position as a function of time gives the volume flow rate of water, while the height difference (Δh) between the water level in the reservoir and the meniscus in the horizontal pipette gives the driving gradient ($\Delta P = \rho g\, \Delta h$, where ρ is the density of water and g is the acceleration due to gravity). This somewhat labour-intensive but simple approach can be used to make excellent measurements (e.g. Tyree *et al.* 1983; Markesteijn *et al.* 2011). The conductance of a stem or branch segment, K', is defined as $Q/\Delta P$, where ΔP is the pressure and Q is the mass flow rate. In terms that are actually measured, $\Delta P = \rho gh$, and $Q = \Delta m/\Delta t$, where Δm is the volume that flowed during a

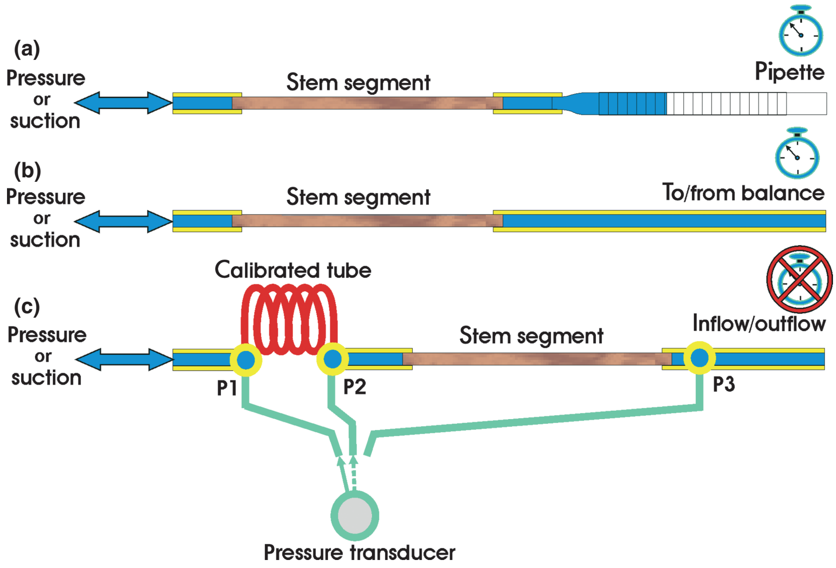

Fig. 1. Diagram of three methods for measuring flow rates through the xylem. For simplicity, the plant sample shown is an unbranched, straight segment. In (a), flow is determined by the change in the position of the meniscus in the calibrated pipette, as a function of time. In (b), flow is determined by the change in water mass of a reservoir located on an analytic balance, as a function of time. In (c), flow rate is determined by measuring the pressure drop across a tube of known conductance.

given time interval Δt. Thus in terms of the measured quantities, $K' = \Delta m/(\rho g h \Delta t)$.

Alternatively, one may replace the pipette with an analytical balance (Fig. 1b), to register the change in mass with time with a computer interface, such that the flow rate can be displayed at regular intervals on a monitor and logged into a data file. When using this approach, it is important to take into account changes in the driving gradient resulting from the net movement of fluid from one reservoir to the other. The effect of evaporation from the reservoir on the balance can be minimized by placing wet paper towels inside the analytical balance and/or by placing a layer of oil on the surface of this reservoir. We advise against using too narrow a container, lest the interactions between the water and the container wall influence the overall pressure gradient. An assumption of these measurements is that the system is at steady state (i.e. constant flow rate for a stable driving gradient). One advantage of the balance over the pipette is an ability to record the instantaneous flow rate and thus ascertain when it has stabilized (e.g. as indicated by a low coefficient of variation for the flow rate; Nardini, Tyree & Salleo 2001).

We note that the definition of conductivity in eqns 1 and 2 implies that it is possible to determine K' accurately from a single flow rate at a given pressure gradient. However, such a single point measurement of K' can only be made assuming that (i) the flow vs. pressure gradient relationship is linear and (ii) there is zero flow when there is zero applied pressure gradient (flow intercept $Q_0 = 0$). While there is usually good support for the first assumption, often Q_0 is not zero, reflecting passive uptake by the sample. Fortunately, this background flow can be accounted for easily, by subtracting Q_0 measured as the uptake into the sample from a container of water using a balance or flow meters on both sides of the sample. Alternatively, one may account for Q_0 by measuring flow rates at a series of pressure gradients and determining K' from the slope of the relationship between the flow rate and the pressure gradient (Kolb & Sperry 1999; Sperry, Hacke & Wheeler 2005).

A major limitation of using an analytical balance for field studies is its lack of portability. A low-cost portable alternative is the pressure-drop flow meter. Flow measurements based on determining the drop in pressure across a calibrated orifice or tube have long been used in engineering, and this principle was first introduced to plant physiology by Tyree *et al.* (1993, 2002). We note this instrument is a hydraulic analog to one of the most basic circuits in electronics, the voltage divider (Horowitz & Hill 1989). One places the stem or branch segment in series with a resistance tube with a hydraulic conductance known to high precision (Fig. 1c; see below). Once a steady-state flow has been achieved (and thus the flow rates through the calibrated resistance tube and the unknown sample are equal), K'_{sample} can be calculated, assuming isothermal conditions, by determining the pressure differences across the calibrated resistance tube (ΔP_{tube}) and the plant sample (ΔP_{sample}):

$$K'_{\text{sample}} = Q/\Delta P_{\text{sample}} = \Delta P_{\text{tube}} K'_{\text{tube}}/\Delta P_{\text{sample}} \qquad \text{eqn 4}$$

Pressures across a calibrated resistance tube can be measured using pressure transducers interfaced to a datalogging system to provide continuous measures of Q. One can assure the accuracy of the pressure sensor by calibrating the pressure reading against a series of pressures created using a column of water. To account for nonzero Q_0, it is preferable to make multiple measurements of Q at different values of ΔP_{sample}, by altering the pressure head, and thus to determine K'_{sample} as the slope of the regression of $Q_1 = \Delta P_{\text{tube1}} K'_{\text{tube}}$, $Q_2 = \Delta P_{\text{tube2}} K'_{\text{tube}}$, $Q_3 = \Delta P_{\text{tube3}} K'_{\text{tube}}$, etc. against $\Delta P_{\text{sample1}}$, $\Delta P_{\text{sample2}}$, $\Delta P_{\text{sample3}}$, etc.

A detailed protocol for the construction of a hydraulics flow meter of this type is available at http://prometheuswiki.publish.csiro.au/tiki-index.php?page = Constructing + and + operating + a + hydraulics + flow + meter. (Sack *et al.* 2011). The system design is very similar to that of the high-pressure flow meter (HPFM) and ultralow flow meter (ULFM) of Tyree *et al.* 1993 and 2002, which were constructed to allow hydraulics measurements driven by respectively compressed air delivering up to several bars of pressure and a vacuum pump creating levels of subatmospheric pressures. The HPFM is commercially available, as is the Xyl'em, an analogous system that contains a different type of flow meter (Bronkhorst, Ruurlo, The Netherlands), which also can be purchased separately; these systems have the advantage of off-the-shelf high performance. There are four major advantages of the self-built flow meter described here:

1 Low cost: the system can be built relatively inexpensively (< \$200 without the datalogger or computer) and is thus readily available to a broader community of plant water relation scientists across the globe.

2 Field portability: while field-portable versions of commercial instruments are available, the system we describe is small and lightweight, requiring no pressurized gas source, captive air tank, vacuum pump or other heavy gear, and can be powered by a chargeable battery and can log its output to a laptop.

3 Design flexibility: the luer-lock components in the system can be reconfigured to match a variety of hydraulic research goals, including different driving forces (e.g. vacuum-driven flow or positive pressure-driven flow), or to allow multiple stems to be connected simultaneously such that flow occurs through each and the pressure drop across each stem can be measured consecutively.

4 Full details of construction: the protocol describes how to construct the flow meter in detail, that is, from basic assembly through datalogging, also enabling field reparability of the instrument in case of malfunction.

Using a low-pressure flow meter, the ability to resolve flow rates is maximized by matching the magnitude of the reference resistance tubing with that of the plant sample. At that point, the pressure drop across the resistance tubing will equal that across the sample, and the relative measurement error will be lowest, that is, the signal-to-noise ratio is highest (Fig. 2). The ideal flow meter allows the user to switch between different reference tubes. By using a variety of PEEK tubes cut to various lengths and with various internal diameters, the conductance of the reference tube can be 'tuned' to that of the sample (Sack *et al.* 2011); notably, analysis of the sources of error shows that the flow meter can provide similar or better accuracy to the analytical balance (Beers 1957; see Appendix S1).

DRIVING FORCE

The easiest way to provide a hydraulic driving force is to create a hydraulic head between the up- and downstream reservoirs (measured as the vertical distance between the two surfaces). It is also possible to use a regulated pressurized gas source to generate additional pressure on the upstream end. To prevent gas from being forced into the perfusing solution while applying gas pressure to the water reservoir, one should use a gas-impermeable barrier (e.g. a captive air tank) or a tall column of water pressurized with a field-portable cylinder of a compressed gas with low solubility in water such as N_2.

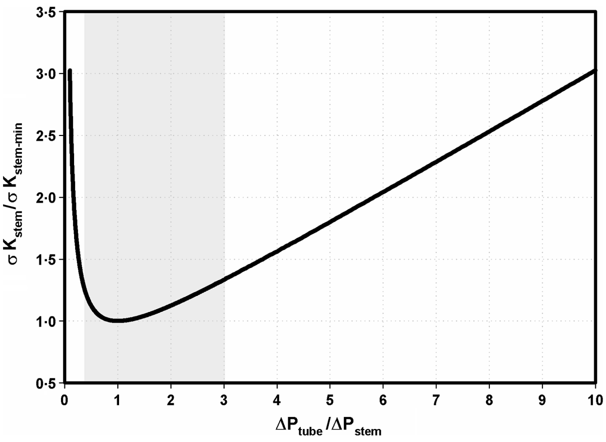

Fig. 2. Sensitivity analysis of the relative measurement error ($\partial K'_{\mathrm{stem}}/\partial K'_{\mathrm{stem-min}}$) as a function of the relative pressure differences across the calibrated tube (ΔP_{tube}) and the sample (ΔP_{stem}). The measurement error is minimized, and thus signal-to-noise ratio is maximized when $\Delta P_{\mathrm{tube}} = \Delta P_{\mathrm{stem}}$. See Appendix S1 for derivation.

In principle, the flow measuring device can be located at either the up- or downstream end of the measured sample. When measuring flow through material with more than one outflow (i.e. a branched sample), the flow measuring device is most easily situated at the upstream end. It is important that the downstream end(s) be immersed in water to eliminate back pressure because of curvature of the water menisci that will otherwise form at each outflow point. Submerging the entire sample ensures that the same driving gradient is applied to all pathways through the branch. Although not easily portable, a vacuum pump provides a useful tool for working with branched material. If the sample can be placed within a vacuum chamber, then the applied driving force will be the same at each outflow point (Kolb *et al.* 1996).

How much pressure should be used in measuring hydraulic conductivity? A higher pressure will maximize the signal-to-noise ratio for both pressure and flow rate. On the other hand, a high pressure may accidentally flush out gas emboli within conduits, a problem if one is aiming to measure droughted plants. The maximum pressure that can be used without displacing emboli can be estimated as $P = 2\ T/r_c$, where T is surface tension of water, 0·0728 N m^{-1} at 20°C, and r_c is the radius of the widest conduit in the sample (Van Ieperen *et al.* 2001). When attaching tubing to short segments, one should ensure that the other end of the tube is open, lest the act of pushing the stem into a liquid filled closed tube creates a pressure sufficient to displace gas trapped within the xylem.

PERFUSING SOLUTION

Rigorous measurements of hydraulic properties require the control of the composition and temperature of the perfusing solution. The liquid should be free of any particulate matter; notably, particles as small as 0·02 μm in diameter are excluded from pit membranes (Choat *et al*. 2004) and could in principle contribute to slow clogging. Therefore, perfusing solutions should be based on ultra-pure water, preferably refiltered (0·2 μm) immediately prior to filling the system of tubing. In addition, containers should be autoclaved when possible, and all tubing and fittings should be cleaned regularly with 10% bleach for at least 20 min to prevent the micro-organism growth.

It is essential to avoid using a flow solution supersaturated with gas, which can result in air bubble formation and blockage of the xylem (Booker & Kininmonth 1978; Espino & Schenk 2011). A number of studies describe partial de-gassing of solution, either by boiling, by placing the container in a vacuum chamber or using a membrane contactor that is designed for degassing (Espino & Schenk 2011) and storing the solution in an air-free container to minimize air contact. A simple method for degassing a small volume of solution in the field is to partially fill a 60-mL syringe, seal its outlet (e.g. with a stopcock) and pull back on the plunger. By tapping on the side of the syringe for about a minute, one can speed the movement of gas out of solution, then expel the gas from the syringe; the entire process can be repeated several times.

It has long been known that the ion composition and pH of the perfusing solution influence the measured flow rates through plant tissues (Zimmermann 1978; van Ieperen, van Meeteren & van Gelder 2000; Zwieniecki, Melcher & Holbrook 2001b; Cochard *et al*. 2010b). This phenomenon is thought to be mediated by the shrinkage and swelling of hydrogels located in intervessel pit membranes (Zimmermann 1978; Zwieniecki, Melcher & Holbrook 2001b). Changes in the ion concentration of the perfusing solution can alter the measured flow rate by *c*. 20–100% depending on species (van Ieperen, van Meeteren & van Gelder 2000; Zwieniecki, Melcher & Holbrook 2001b; Nardini, Salleo & Jansen 2011), influenced by the number of pit membranes the solution must traverse. A good choice would be to use the same ion concentration and pH as in the intact plant but this is frequently unknown. Alternatively, one can use a consistent solution for comparative purposes or use a low pH and/or high ion concentration to saturate the ion response or quantify the xylem hydraulic properties at a range of concentrations.

Studies of xylem hydraulic properties should be carried out at a standard temperature. The density of water is a function of temperature, although the change is only *c*. 0·8% across the range of biologically relevant temperatures (0–40°C). However, over this same temperature range, the viscosity of water decreases by *c*. 64% (Tyree & Zimmermann 2002), and laminar flow through capillaries is inversely proportional to viscosity. It is common practice to normalize conductivities to a standard temperature of 20°C (Yang & Tyree 1993). Ideally, the perfusing solution will be isothermal throughout the system, which can be accomplished by submerging the flow meter with branch in the same water bath. When that is not convenient, differences in temperature along the flow pathway can be measured and accounted for using thermocouple junctions at the inflow and outlet ends of both the plant sample and (in the case of the pressure-drop flow meter) the calibrated resistance tube. It should be noted that the pressure-drop flow meter measures the conductance of the sample relative to the conductance of the resistance tube at the temperature at which the tube was calibrated (Sack *et al*. 2011).

Plant material

The choice of material to measure depends on one's question and may be constrained by the growth form of the species considered. The hydraulic conductance of an intact plant can be calculated from estimates of xylem water flow rates using sap flow sensors or a gas exchange system, divided by a driving gradient estimated as the difference between mid-day leaf water potential and soil water potential (or predawn leaf water potential, which is often assumed to have equilibrated with the water potential of the soil in contact with the roots, e.g. Andrade *et al*. 1998). Such methods can achieve sufficient resolution only when stomata are open and transpiration is substantial. Further, an important assumption of this whole-plant approach is a steady state, that is, no net movement of water into or out of storage capacitance along the path. Such estimates of hydraulic properties from studies of intact plants are valuable. However, because the path lengths in the network are not well defined and the leaf area is frequently not well known, it can be difficult to normalize the calculated conductance to allow comparison between individuals of different sizes or among species. Further, it can be difficult to assess the soil-to-leaf water potential gradient. The relevant soil water potential may be obscure, as the uptake zone may be deep in large plants, and may vary throughout the day. The predawn leaf water potential may not accurately reflect soil water potential for numerous plant species that conduct nocturnal transpiration or accumulate solutes in the leaf apoplast (Donovan, Richards & Linton 2003).

Measurements of large stems have been taken by subsampling. In conifers, one may determine the hydraulic properties of very large stems from measurements of small 'dowels' excised from the main trunk, because flow takes place exclusively through tracheids of small size relative to the dimensions of the sample (Spicer & Gartner 2001). An alternative method that, in principle, could be scaled up to large stems is the microcapillary method for the determination of the hydraulic properties of individual xylem vessels (Zwieniecki, Melcher & Holbrook 2001a; Christman & Sperry 2010).

A more common approach is to examine xylem hydraulic properties by measuring flow through short stem segments. Typically, measurements are taken on small diameter branches convenient to collect. Indeed, because the specific conductivity of the xylem decreases distally along the flow path (Tyree & Zimmermann 2002), smaller branches are likely to be disproportionately important in limiting flow through the stem portion. Thus, studies have demonstrated a good correlation between the leaf specific conductivity of the small terminal branches and estimates of leaf-area-normalized whole-tree hydraulic conductance, and leaf gas exchange rates (Brodribb & Feild 2000; Nardini & Salleo 2000; Bucci *et al.* 2004; Santiago *et al.* 2004).

SAMPLE COLLECTION

The water in the xylem typically exists at pressures below atmospheric, and thus special precaution must be taken during sampling. Typically, this is carried out by cutting the sample off under water to prevent air from being sucked into the xylem, but that may only be possible if one is working with a vine or a plant with long, flexible branches. Even when cutting under water, care should be taken to prevent the uptake into the xylem of microscopic bubbles that may adhere to the cutting tool. An alternative option is to make the first cut far away from the place one wants to actually sample, after which one may excise the portion of interest under water. The cut end should be kept wet until measurement, and the downstream foliage kept covered. To prevent any air from entering into the target region, the most conservative approach is to cut sample lengths exceeding the maximum vessel length; notably, the longest vessels are extremely rare (Sperry, Hacke & Wheeler 2005), and therefore, cutting lengths somewhat shorter than this should not greatly affect the conductivity (Van Ieperen *et al.* 2001). The shortest sample length can be found by making conductivity measurements for segments of progressively longer lengths until conductivity is stable with increasing length.

A relatively simple, rapid way to estimate maximum vessel lengths is to perfuse a cut length of branch or stem with pressurized air of, for example, 0·1 MPa gauge pressure. One may use a 60-cc syringe filled with air, connected to the stem via tubing and cable ties, and place the syringe within a large caulking gun to press the plunger. The air can be pressurized by reducing the volume of the gas within the syringe, and the pressure then calculated from the ideal gas law equation $PV = nRT$. For example, compressing the air volume by half (i.e. from 60 to 30 cc) will double the applied air pressure and thus deliver 0·1 MPa pressure above atmospheric to the excised stem. The distal excised ends of the segment can be placed into a well-lit water reservoir, and a hand lens will aid the detection of fine, steady streaming bubbles that emanate from individual conduits when they are open. As air is forced in the proximal end, the underwater end may be progressively cut back by, for example, 0·5 cm, and when streaming bubbles are observed, the length of the sample is a rough estimate of maximum vessel length. The applied pressure should be sufficiently low (0·1 MPa) such that it would not pass across the air–water interface at an intact vessel–vessel pit membrane pore (assuming a pore of $<<3$ μm diameter, by the surface tension equation in which the pressure P resisting displacement of the meniscus $= -2\,T/r$, where T is surface tension of water, 0·0728 N m^{-1} at 20°C, and r is the radius of the pore; Choat *et al.* 2003). For long branches that contain small vessel diameters, we recommend waiting 1–2 min before each shortening of the segment.

This method gives the maximum vessel length, and there exist a number of other, finer scale methods for determining vessel length distributions more comprehensively in the laboratory (e.g. Handley 1936; Zimmermann & Jeje 1981; Tyree 1993; Sperry, Hacke & Wheeler 2005). Potential problems with this simple method can arise if nonxylem regions of the stem (e.g. the pith) provide an open pathway for air. Problems can also arise in material with high levels of native embolism where gas may be able to pass easily between embolized vessels if the pit membranes are not fully hydrated, making two or more conduits in series appear as a single long one. This problem might also arise if branches are cut off the plant in air while the xylem is under strong tension.

SEGMENT LENGTH AND DIAMETER

Notably, the sample length requirements for determining K_s or K_L differ from those for PLC. Hydraulic conductivity is an absolute measure of the xylem's ability to transport water, while PLC is an internally normalized ratio of the degree that the xylem hydraulic capacity is reduced because of the presence of emboli (Sperry, Donnelly & Tyree 1988). Thus, measurements of hydraulic conductivity require a true measure of the segment's conductance, while determinations of PLC only require that any difference in pre- and postflushing conductance be due to the removal of gas trapped within the vessels. In sampling for hydraulic conductivity, it is important that the measured segment be long relative to the length of a typical vessel (Fig. 3). Vessels that are open at both ends provide a high conductance path, which may lead to significant overesti-

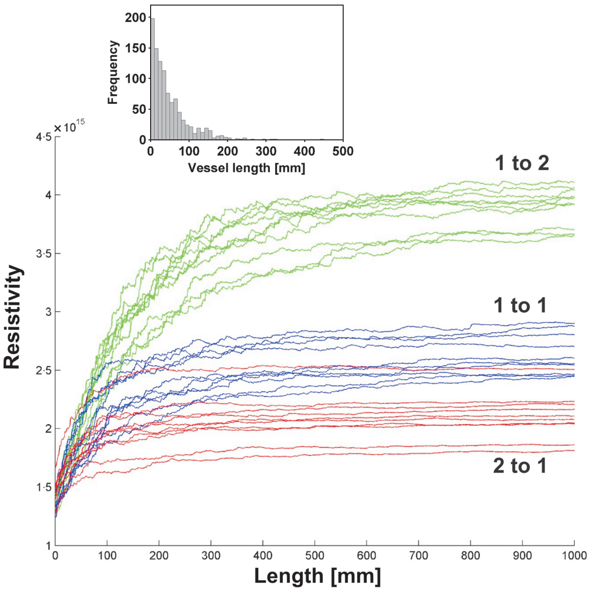

Fig. 3. Simulation model of measured resistivity [resistance/length = $(\text{conductance} \times \text{length})^{-1}$] as a function of segment length. Each simulated stem has 200 vessels in parallel, with diameter ranging from 25 to 75 μm and average diameter being 50 μm. Vessel lengths are assigned at random, with the average vessel length being 50 mm. A typical vessel length distribution for one of the simulated stems is shown in the upper left. The resistance because of vessel lumen relative to that of vessel end walls is 1 : 2 (green), 1 : 1 (blue) and 2 : 1 (red). The simulation demonstrates that when the relative resistance of the vessel ending is large, the resistivity does not achieve a stable value until the measured length is substantially longer than that of the longest vessel.

mates of hydraulic conductivity. When no information is available for the vessel length distribution of a species, the safest approach is to use stem segments that are longer than the longest vessel, which can be determined as described in the previous section. That rule is conservative, as a study of seven species reported that resistivity (i.e. 1/conductivity) was stable even for segment lengths well below that of the longest vessel because the longest vessels were rare (Sperry, Hacke & Wheeler 2005).

In measuring PLC, it is critical to avoid the removal of any gas (emboli) trapped within the xylem during the initial measurement. In this case, all measured segments should be either shorter than the median vessel length or longer than the longest vessels, that is, containing virtually all open vessels or all closed vessels. Short segments are easy to flush, for example, using a syringe full of filtered, de-gassed perfusing solution, allowing rapid measurement of K_{max}. However, short segments must be handled with extreme care so as not to inadvertently remove emboli prior to determining K_{initial}. Longer segments require extended periods of flushing (>40 min), which increases the possibility that the segment's conductance will be altered by clogging or wounding (see below). For many species, holding stem segments in the perfusion solution under partial vacuum may be a more suitable method for restoring maximum conductivity than subjecting the segments to a high-pressure flush (Espino & Schenk 2011). In particular, for conifers, the use of a high-pressure flush may result in torus displacement against pit apertures (Hietz *et al.* 2008).

The manner in which plants grow will influence how one samples and interprets measurements of xylem hydraulic properties. The diameter of measured stems should be considered carefully. Indeed, for given species, K_s can increase strongly with stem diameter (Yang & Tyree 1994; Mencuccini & Grace 1996). Interpretation of comparisons within and across species may be ambiguous if the impact of variation in diameter is not taken into account either by sampling a narrow range of stem diameters or by explicitly determining K_s as a function of stem diameter. Further, finding an unbranched segment (i.e. with a single outflow) of sufficient length frequently requires using older branches. Because leaves are only produced on the current year's extension growth, the xylem hydraulic pathways that are functional *in vivo* for shoots more than a year old include both the axial component and the lateral pathway between xylem produced in different years (Melcher *et al.* 2001). Thus, in species in which the xylem remains functional over multiple growing seasons, measurements of segments that do not extend into the current year's growth may overestimate the xylem conductivity to transpiring leaves (Melcher, Zwieniecki & Holbrook 2003). On the other hand, working with branched segments is complicated by the problem of no single path length. It may be tempting to estimate the hydraulic properties of terminal segments of a branched sample by measuring the difference in resistance calculated before and after their removal. However, such an estimate would only be exact if (i) the hydraulic path from the base of the branched segment to each point of excision is independent and of equal conductance and (ii) the conductance of each excised segment is equal (the 'independent model' in Taneda & Tateno 2007).

PROBLEMS AND PRECAUTIONS

When preparing samples for measurement, it is important to make clean cuts such that an additional resistance is not introduced by damage, distortion or blockage of conduits at the ends of the segment. We recommend using razor clippers to excise the stem ends under solution and then shave the ends with a fresh razor blade under solution prior to attachment to the hydraulic apparatus. Repeated trimming of both ends of the sample is also recommended if samples are to be measured over a long period or if mucilage is present. Similarly, it is important that the segment not be clamped tightly (Sperry, Hacke & Wheeler 2005).

Two major problems encountered in hydraulic measurements are leaks and gas bubbles. Leaks from the tubing can occur between fittings, or where the sample is attached to the tubing. While the sample should not be clamped tightly, wrapping the sample with parafilm and sealing into soft silicone tubing often create a good seal. Leaks can be tested for by gluing the end of the sample after the measurement and ensuring zero flow of pressurized solution through the sample. Air bubbles in the tubing or at the edge of the samples must be avoided; the use of partially degassed water and maintaining constant temperature during measurement will reduce this problem.

Measured flow rates may decline with time for a number of reasons (Espino & Schenk 2011). First, if the material is not fully hydrated, then significant amounts of water may be diverted from the xylem as the adjacent tissues rehydrate initially (Yang & Tyree 1993). This is particularly a problem when working with large branched segments. Second, time may be needed to stabilize the ionic/pH response to the composition of the perfusing solution. Measurements taken with deionized water (pH 7) may take a long time to stabilize, presumably related to the slow equilibration of solution composition as ions dissociate from within the pectin matrix of pit membranes. Third, bubble entry into the sample or bubble formation in the sample may cause a decline in flow. Fourth, for species that exude abundant mucilage, resins or latex from cut surfaces, the clogging of xylem conduits may cause an apparent decrease in the measured conductance. When mucilage or resin canals occur predominantly in the phloem or pith, removal of the bark and plugging the pith will help. However, for species that have secretory tissues located within the xylem, it can be difficult to obtain a good measurement. Finally, a wounding response may occur near the cut ends to an extent that appears to vary strongly across species. We ascribe a decrease in hydraulic conductivity to a wounding response or to bubbles at the sample surface if we can restore the initial flow rate by shaving off the segment ends. In *Acer rubrum*, after stems were perfused with solution for 30 min, cutting 1 cm off each end of the sample resulted in a dramatically enhanced flow rate close to the initial value, which remained relatively stable for hours (Melcher & Warchocki 2010).

Conclusions and future directions in measurement methods

The stem xylem provides a critical supply line necessary for the photosynthetic function of leaves and requires significant investment of biomass. Measurements of stem xylem hydraulic conductivity and vulnerability to cavitation can reveal ecological differences among species, point to the effects of environmental conditions on xylem transport and indicate the degree to which productivity is limited by xylem properties. Because the xylem is a living tissue, measurements should be taken promptly on material sampled appropriately to address the research question, and using best practices. Significant unresolved issues in making xylem hydraulic measurements include the lack of an easy-to-use field method for obtaining the distribution of vessel lengths, the fact that artificially driven flow rates may decline because of secretory tissues (mucilage/latex) or wounding, and the choice of perfusing solution. Achieving standard methods to cope with these issues will be hastened if published measurements of xylem hydraulic properties are accompanied by information on the age of the material, time and date of collection, temperature, perfusing solution composition and pH and segment length relative to the distribution of vessel lengths.

Acknowledgements

This work was supported by the National Science Foundation, the USDA, The Andrew W. Mellon Foundation, The Arnold Arboretum of Harvard University and the Harvard Forest.

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Received 25 September 2011; accepted 6 March 2012
Handling Editor: Robert Freckleton

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Analysis of error for flow meter.

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