Plant, Cell and Environment (2002) **25,** 1445–1450

Hydraulic architecture of leaf venation in *Laurus nobilis* L.

M. A. ZWIENIECKI, P. J. MELCHER, C. K. BOYCE, L. SACK & N. M. HOLBROOK

Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA

ABSTRACT

Veins are the main irrigation system of the leaf lamina and an understanding of the hydraulic architecture of the vein networks is essential for understanding leaf function. However, determination of leaf hydraulic parameters is challenging, because for most leaves the vein system is reticulate, contains a hierarchy of different vein sizes, and consists of leaky conduits. We present a new approach that allows for measurements of pressure differences between the petiole and any vein within the leaf. Measurements of *Laurus nobilis* leaves indicate that first- and second-order veins have high axial conductance and relatively small radial permeability, thus allowing water to reach distal areas of the leaf with only a small loss of water potential. Higher order veins tend to be more hydraulically resistant and permit greater radial leakage. This design allows for a relatively equitable distribution of water potential and thus reflects the capacity of the venation to provide a relatively homogeneous water supply across the leaf lamina, with only the leaf margins being hydraulically disadvantaged relative to the rest of the leaf.

Key-words: *Laurus nobilis* L.; leaf hydraulic; transpiration; veins architecture.

INTRODUCTION

Leaf veins have two main functions. In many leaves, the veins provide physical support for the lamina, allowing the leaf to maintain its three-dimensional structure and orientation in space. However, in all leaves the veins provide a transport system for the movement of water and carbohydrates within the leaf (Roth-Nebelsick *et al*. 2001). Water lost during transpiration from any portion of the leaf blade has to be replaced by flow from the roots via the stem and petiole. In most plants the water capacity of the leaf is small in comparison with the volume of water lost during active transpiration over the course of a day. Thus, the ability to supply and distribute water to the mesophyll is an important component of leaf design that might influence leaf size, shape and performance. Recent efforts to understand the hydraulic properties of leaves have focused on measurements of entire leaves (Nardini, Tyree & Salleo 2001; Siso, Camarero & Gil-Pelegrin 2001) with some attempts to resolve hydraulic resistance of veins in leaves with parallel venation (Martre, Durand & Cochard 2000). Although the whole-leaf approach is useful for understanding the relative contribution of leaves to the total resistance to water flow from soil to atmosphere (Yang & Tyree 1994), a more detailed approach is necessary to understand how water is supplied equitably to all parts of the lamina.

The venation patterns in the earliest laminate leaves of both ferns and seed plants consisted of a single order of open, dichotomous veins, but multiple vein orders and/or reticulate veins evolved in a variety of lineages over subsequent evolutionary history (Boyce & Knoll 2002), suggesting such vein patterns may confer functional advantages. Previous models of leaf venation suggest that water transport is maximized by straight parallel veins radiating from the point of lamina attachment, and that deviations from this architecture represent tradeoffs that increase structural support at the expense of water transport efficiency. This suggests that multiple vein orders may have evolved to maximize the efficiency of structural support (Niklas 1999). In addition, reticulation provides redundancy of transport pathways that will minimize the deleterious effect of insects and other sources of damage (i.e. cavitation) on transport pathways, while at the same time providing a physical barrier limiting the spread of such damage (Salleo, Nardini & Lo Gullo 1997). However, these innovations may play an additional role in ensuring a more even distribution of water potential throughout the leaf. In this paper we consider the hydraulic architecture of leaf venation as a mechanism to overcome the hydraulic limitations associated with increasing distance from the water source – which, in this case, is the point where the petiole joins the leaf. Within the constraints of providing an even supply of water across the lamina, we argue that leaves should minimize water potential losses and gradients so as to facilitate water delivery to the mesophyll, thus allowing the stomata to remain open for CO_2 uptake.

From a hydraulic point of view, leaf veins must be considered as a system of 'leaky' pipes (Landsberg & Fowkes 1978; Canny 1995). This contrasts with the vascular tissues of stems and major roots, which function primarily to deliver water to terminal, as opposed to distributed, sinks. Because the leaf vasculature is leaky, the design principles associated with ensuring an even supply of water to all portions of the leaf must take into account that pressure dissipation will be due to both viscous interactions (Poisieulle flow) and the exit of material from the flow path

Correspondence: Maciej A. Zwieniecki, Biological Laboratories, 16 Divinity Ave., Cambridge MA 02 138, USA. E-mail: mzwienie@oeb.harvard.edu

(leaks). Thus, the hydraulic properties of individual portions of the leaf venation cannot be characterized by a single resistance, but rather as the ratio of the radial-to-axial flow resistance (Landsberg & Fowkes 1978). A supply network that provides water at relatively equal pressures along its length requires that the ratio of radial to axial resistance be large. Large radial resistances, and the corresponding large pressure drops, are a well-recognized feature of human-engineered hydraulic networks, from the simplest pressure manifold, to extensive irrigation systems (Cuenca 1989). Thus we predict that the major veins provide little water directly to the mesophyll and that the resistance to flow from the major veins to higher order ones is relatively large. This latter expectation reflects the fact that, from the point of view of the major veins, connections with higher order veins represent lateral leaks. In addition to distributing water throughout the leaf, the leaf venation is responsible for actually delivering water to the mesophyll. Thus we anticipate that the higher order veins are themselves relatively leaky (i.e. have a low radial:axial resistance), yet function in distributing water at a local scale due to their relatively short lengths.

In this study a new approach that allows us to measure directly the pressure dissipation within the leaf vasculature was used. We are aware of only one related previous study, which estimated pressure drops within first and secondary leaf veins of *Populus balsamifera* indirectly by measuring dye flow rates and xylem conduit dimensions (Jeje 1985). The aim of the present study was to investigate the hydraulic architecture of the leaf venation of dicotyledonous plants though an intensive study of the hydraulic design of *Laurus nobilis* leaves, based on direct measurement of pressure drops across the leaf lamina, in all orders of veins, and at different flow rates into the petiole.

MATERIALS AND METHODS

Leaves examined in the study were collected from 6-year-old greenhouse-grown *Laurus nobilis* L. trees. Measurements were made during January and February and only leaves produced during the previous summer were used. A total of 42 leaves were examined; leaves were selected based on uniformity in size and venation pattern. One to three pressure measurements were made on each leaf for a total of 101 pressure measurements. Measurements were made on all four orders of veins; vein 'order' was defined as the order of branching, with the midrib the first-order vein, and the fine vein network the fourth-order veins (Nelson & Dengler 1997). All measurements were pooled to create a mean hydraulic pressure contour map (Fig. 1).

The measurement procedure consisted of supplying water at a known delivery pressure and flow rate to the petiole of an excised leaf and measuring the pressure drop to specific points in the leaf vasculature. Petioles of cut leaves were immediately attached to the outflow of a laboratory-built flow meter (described below) using a compression fitting (Zwieniecki *et al.* 2000). Water was then supplied to the petiole at a low pressure (~0·02 MPa), allowing a small amount of water to flow into the leaf. The leaf was then viewed with a dissecting microscope and the desired vein microsurgically severed (Fig. 2a). A freshly pulled, water-filled microcapillary attached to a pressure probe (described below) was placed over the cut end of the vein (Fig. 2b). The microcapillary tip and all cut surfaces were then sealed with cyanoacrylate glue (Fig. 2c). Special attention was paid to cover all cut leaf surfaces and small veins to avoid any pressure drop due to leaks caused by injury. During pressure measurements the leaf was inspected for leakage under the microscope and if any leak was observed the measurement was discarded. The pressure in the microcapillary was recorded after the 30–60 min needed for flow into the leaf to stabilize. This time was

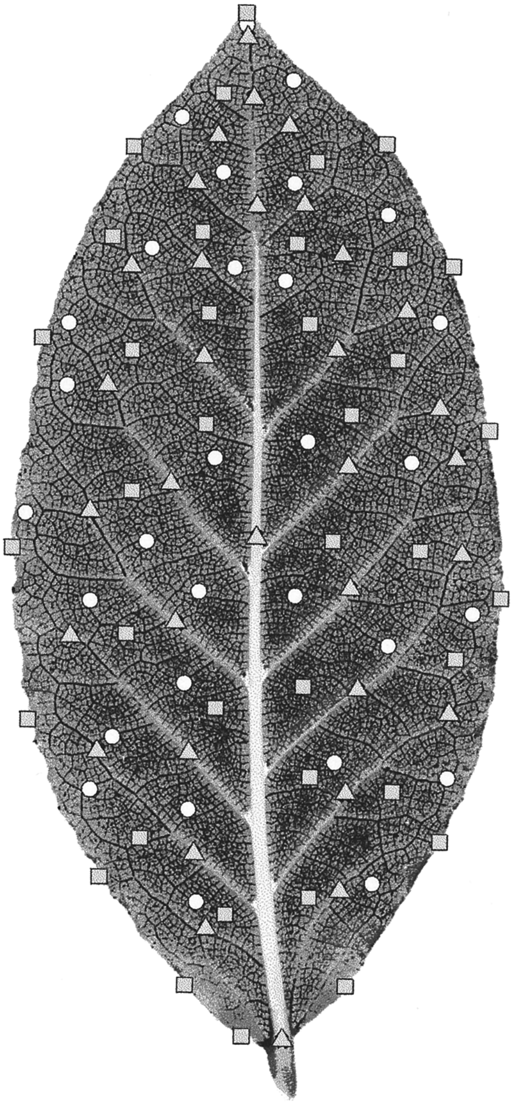

Figure 1. Distribution of the pressure sampling points across the leaf surface. Triangles show sampling points for the first and the second vein orders, circles show sampling points for the third-order veins, and squares show sampling points for the fourth-order veins.

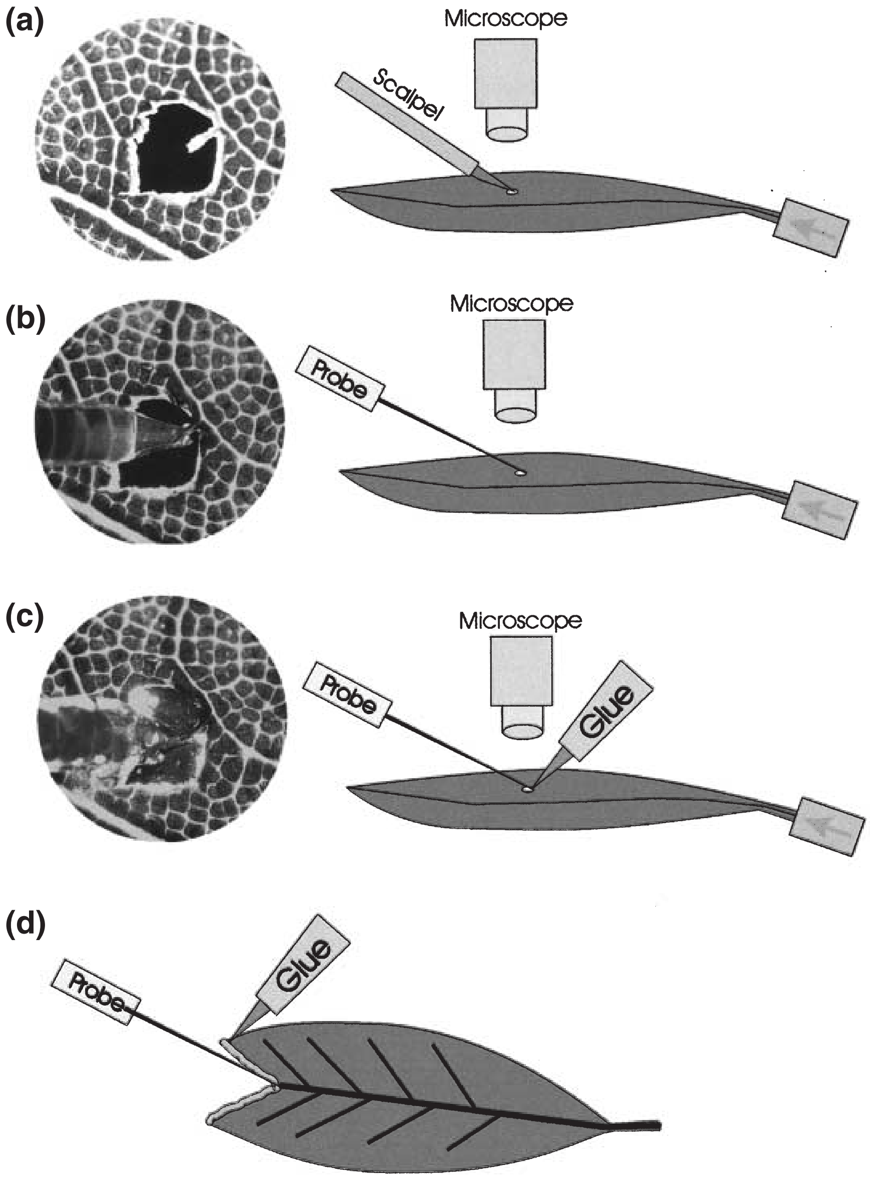

Figure 2. Schematic of experimental procedure used to measure hydrostatic pressure drop within veins of mature leaves: (a) preparation of vein including vein separation from the leaf lamina; (b) insertion of the vein in the microcapillary tube attached to the pressure probe; (c) final sealing of the vein to the pressure probe and sealing of cut leaf tissues; (d) schematic of pressure probe attachment for first- and second-order veins.

necessary for the leaf to adapt to conditions in the experimental set-up (i.e. leaf was removed from the plant, placed in a completely new environment, supplied with water, etc.). Upon completion of the experiment the petiole was cut close to the flow meter compression fitting and its cut surface plugged with glue to test for leaks around the seal. In no case was a leak detected.

The basic approach for determining flow rates into the leaf followed the methods described previously (Zwieniecki *et al.* 2000). Solution (10 mM KCl) was delivered to the leaf from a plastic pressurized reservoir through a calibrated polyetheretherketone (PEEK) capillary tube with a hydraulic conductance of $2{\cdot}512 \times 10^{-4}$ g s^{-1} MPa^{-1}. The 10 mM KCl solution was chosen based on published xylem sap ion concentration (Marschner 1995). The pressure drop across the capillary tube was measured by two pressure transducers (Omega PX236-100 Series, Omega Engineering Inc., Stamford, CT, USA). Measurement of the pressure drop across the tube allows one to calculate the flow rate into the leaf. The pressure of the second (downstream) transducer shows the delivery pressure into the leaf. Delivery pressures were always positive and ranged from 0·04 to 0·27 MPa. The pressure probe attached to the vein via the microcapillary followed a standard cell pressure probe design (Steudle & Tyerman 1983) except that for this application it was filled with water. A pressure transducer (Omega PX120–200 GV; Omega Engineering Inc.) was fitted to the pressure probe, allowing simultaneous determination of the pressure in the leaf vein and the delivery pressure. Vein pressures ranged from –0·023 to 0·26 MPa. The outputs from the three pressure transducers were recorded using a data logger and displayed on a computer. Pressure transducers were calibrated such that their outputs were within ± 0·0003 MPa of each other in the range of 0–0·3 MPa.

During measurements the leaves were illuminated and allowed to transpire freely, although the evaporative conditions surrounding the leaf were not actively controlled. Intercellular spaces did not become water-filled (based on leaf surface scan using a stereo-microscope), however, because water was forced into the leaf under positive pressure the possibility that some infiltration did occur cannot be eliminated. A subset of measurements was made with different delivery pressures to test the linearity of the pressure in leaf veins to changes in flow rate into the leaf. When measurements were made on first- and second-order veins portions of the leaf blade that were supplied by these veins were removed and the cut edges sealed to remove bypass flow across higher order veins (Fig. 2d). When higher order veins were measured, the excision of surrounding tissue was limited to the minimum that would allow for pressure probe attachment (Fig. 2a–c).

Two approaches were used to examine the structure of the xylem in the veins. The first involved measuring air permeability through the leaf venation to determine the extent of continuous conduits. Air was forced into the cut end of the petiole at an initial pressure of 0·05 MPa, with the leaf lamina under water so that bubbles could be easily observed. Cuts were then made in fourth-order veins, followed by third-, second- and first-order veins and the first occurrence of bubbles noted. The second approach involved anatomical examination of each vein order. Leaf sections containing only one of the four vein orders were surgically excised and macerated in a 10% H_2O_2 1 M HCL solution for 96 h. Each macerate was microscopically examined for the presence of vessel elements versus tracheids.

RESULTS

We found a linear relation between flow rate into the leaf and pressure drop across the leaf (ΔP_{leaf}), where ΔP_{leaf} is the difference between the pressure measured at the petiole (delivery pressure) and the pressure measured at the vein connected to the pressure probe (Fig. 3). This relation, which holds for both flows into the leaf under positive pressure or natural suction generated by evaporation from leaf surfaces, allowed us to calculate the pressure drop within the veins across the leaf blade for a specified flow rate. Maximum transpiration rates reported for *L. nobilis* growing in its native environment are ~ 3 mmol m^{-2} s^{-1} (Nardini & Salleo 2000). Flow rates into the leaf during pressure measurements ranged between 0·2 and 2·0 mmol m^{-2} s^{-1} (typically ~ 1 mmol m^{-2} s^{-1}). All ΔP_{leaf} values reported hereon were calculated for a flow rate of 1·5 mmol m^{-2} s^{-1} assuming a linear relation between pressure gradient and flow rate (Fig. 3). We then used the data collected on the ensemble of leaves to construct a pressure contour map of the entire leaf blade (Fig. 4).

Pressure differences (ΔP_{leaf}) from the petiole to the first- (mid-rib) and second-order veins were relatively small, typically 0·0–0·2 MPa (Fig. 4a). Only in the distal portion of the leaf were there larger pressure drops in the first- and second-order veins. There was a substantial drop in pressure between second- and third-order veins throughout the entire leaf blade, resulting in a fairly uniform distribution of pressures in third-order veins (Fig. 4b). The value of ΔP_{leaf} in third-order veins ranged between 0·3 and 0·5 MPa over most of the leaf. Once again, the tip of the leaf experienced a greater pressure drop in comparison with the rest of the lamina. An additional pressure drop occurred between the third- and the fourth-order veins (Fig. 4c). In *L. nobilis* it is the fourth-order veins that subdivide the leaf mesophyll into areoles. Thus, fourth-order veins form the major region for water delivery to the mesophyll and the ΔP_{leaf} of these veins represents the total pressure dissipation within the leaf vasculature. The spatial distribution of ΔP_{leaf} in fourth-order veins was less uniform than that observed in third-order veins. The value of ΔP_{leaf} in the central portion of the lamina ranged between 0·4 and 0·6 MPa. However, along the leaf margins and especially at the leaf tip ΔP_{leaf} could be 0·7 MPa or higher.

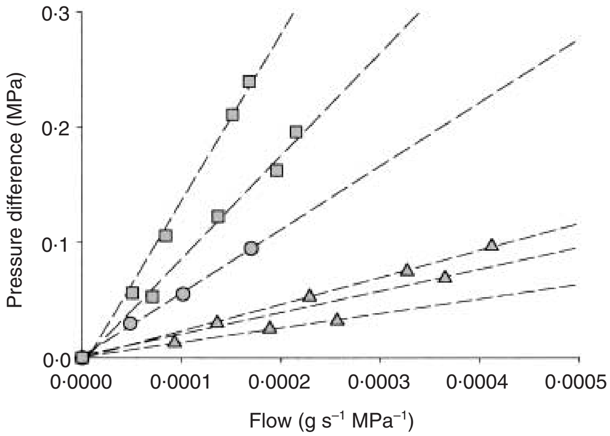

Figure 3. Regressions of ΔP_{leaf} (pressure drop across the leaf) against flow rate of solution into the petiole. Different shapes represent pressure drops at different vein orders: triangles, first and second order; circle, third order; squares, fourth order.

Air perfusion through the leaf veins showed that first- and second-order veins contain xylem conduits that are continuous with the petiole (i.e. at least one vessel). However, no bubbles were observed even at 1·0 MPa pressure when only third- and fourth-order veins were cut. This finding was consistent with the observation that macerate from first- and second-order veins contained vessel elements and tracheids, whereas macerate from third- and fourth-order veins contained only tracheids (Esau 1965).

DISCUSSION

The vasculature of *L. nobilis* leaves consists of four orders of veins, with the third-order veins interlinking the first- and second-order veins to form a reticulated network. Fourth-order veins divide the mesophyll into small regions called areoles. Vessel elements were only observed in first- and second-order veins, whereas tracheids were observed in all

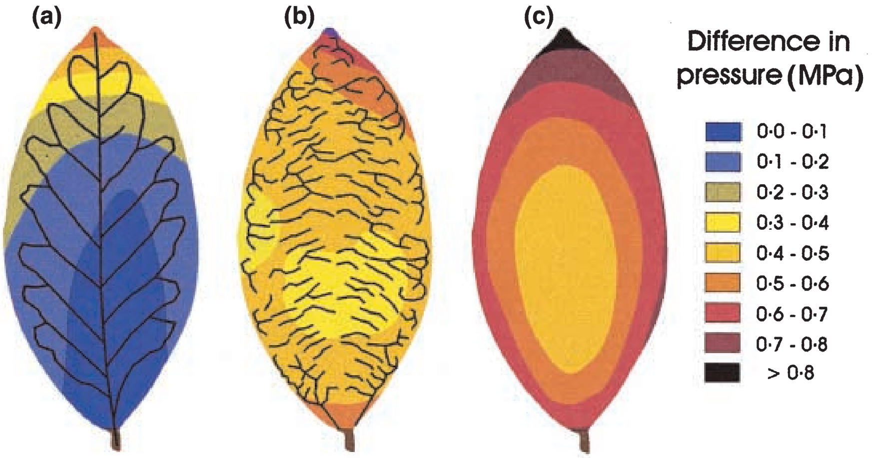

Figure 4. Spatial pattern of ΔP_{leaf} as a function of vein order for a flow rate of 0·028 g s^{-1} m^{-2} (equivalent to 1·5 mmol m^{-2} s^{-1}): (a) first- and second-order veins (black lines); (b) third-order veins (black lines); (c) fourth-order veins.

four orders. This qualitative difference in xylem structure coincides with the presence of continuous vascular conduits between first- and second-order veins, but not between second- and third-order veins (Esau 1965). This is also consistent with the existence of a major resistance between second- and third-order veins – as indicated by the relatively large pressure drop (0·2–0·4 MPa) between these two vein orders (Fig. 4).

Pressure dissipation within leaky pipes results from both viscous interactions with the walls (Poisieulle flow) and the exit of material from the conduit (i.e. lateral ‘leaks’). The extent to which one mechanism dominates the observed pressure dissipation depends upon the relative resistances of the axial and radial pathways (Landsberg & Fowkes 1978; Cuenca 1989). The design constraint of providing a distribution system that can allow similar transpiration rates and water potential to all regions of the leaf argues for a relatively high radial resistance, thus allowing the vascular system to function like a high pressure-manifold. However, an increase in radial resistance could actually reduce the water supply to the mesophyll if the resulting pressure drops are sufficient to restrict stomatal opening. We propose that the hydraulic design of leaf vasculature integrates these two design constraints through a hierarchy of resistances associated with vein order. According to this design, the ratio of radial to axial resistance is high in first- and second-order veins and low in third- and especially fourth-order veins. In this way, water can be supplied relatively evenly throughout the leaf by the major veins and then delivered to the mesophyll via a leaky system of higher order paths of limited length. Central to this view of leaf irrigation is the existence of a substantial resistance in the xylem between the first two orders of veins and the higher order veins. This view is consistent with our measurements of pressure dissipation in *L. nobilis* venation, in which the major pressure drops occur in the higher order veins, despite their much shorter lengths.

The existence of substantial pressure dissipation within the vasculature appears to leave little room for an additional pressure drop within the mesophyll. Based on the measurements of pressure dissipation in *L. nobilis* leaves, the decrease in pressure between petiole and fourth-order veins was calculated to be as much as 1·4–1·8 MPa for the maximum transpiration rates (3·0 mmol m^{-2} s^{-1}) reported in the literature (Nardini & Salleo 2000). Stomata of *L. nobilis* are reported to shut around –2·0 MPa (Nardini & Salleo 2000). Thus in the leaves of well-watered *L. nobilis* trees at maximum transpiration rate, stomata at the leaf edge would be predicted to remain open only if the extra-vascular pressure drop (between the fourth-order veins and the sites of water evaporation) is less than a few tenths of MPa. In other words, our measurements suggest that the pressure drop within the leaf vasculature could account for as much as 80% of the total pressure drop across the leaf. Further study is needed to determine the exact contribution of the vasculature to the total leaf resistance, and how pressure drops in the leaf are distributed among the leaf vasculature, bundle sheath, mesophyll, and, ultimately, the stomatal guard cells (Franks, Cowan & Farquhar 1998).

The linear relation between flow rate into the leaf and pressure dissipation in the leaf demonstrates that leaves might become hydraulically limited due to pressure drops within the vascular network. In other words, there is a limit on how much water can flow through the veins before the pressure dissipation is sufficiently large as to lead to stomatal closure. The hydraulic properties of the leaf vascular system may therefore play an important role in the response of the stomata to transpiration rate (Aasamaa, Sober & Rahi 2001). The large pressure dissipation within the vasculature helps to explain the fact that leaf size is often linked to water availability (Specht & Specht 1989). These results suggests that in *L. nobilis* leaves, the leaf margins have pressures almost 0·2 MPa lower (at maximum transpiration rate) than the middle of the leaf. Further expansion of the leaf without substantial alteration of the architecture of the leaf venation will lead to larger drops in pressure that may result in desiccation or permanent stomatal closure at leaf margins.

In the future, our technique can be used to compare the hydraulic function of the different venation types found among the angiosperms (Becker, Tyree & Tsuda 1999; Roth-Nebelsick *et al*. 2001), as well the simpler patterns found in many fern and gymnosperm lineages. These venation patterns may differ considerably in terms of their efficiency and equitability of water transport. Alternatively, the diversity of venation patterns may reflect selective pressures unrelated to water transport or simply the absence of strong selection upon a system that is so evolutionarily variable. Our model of the lower order veins providing a low-axial resistance/high-radial resistance conduit allowing equitable distribution to the leaky, higher order veins might need to be modified for taxa such as *Gnetum* or some ferns that have independently evolved a hierarchical, reticulate leaf vasculature.

ACKNOWLEDGMENTS

We thank M. Burns for helpful suggestions and A. Cobb for critical review of the manuscript. This study was supported by the Andrew W. Mellon Foundation.

REFERENCES

Aasamaa K., Sober A. & Rahi M. (2001) Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water status in temperate deciduous trees. *Australian Journal of Plant Physiology* **28**, 765–774.

Becker P., Tyree M.T. & Tsuda M. (1999) Hydraulic conductances of angiosperms versus conifers: similar transport efficiency at the whole-plant level. *Tree Physiology* **19**, 445–452.

Boyce C.K. & Knoll A.H. (2002) Evolution of developmental potential and the multiple independent origins of leaves in Paleozoic vascular plants. *Paleobiology* **28**, 69–99.

Canny M. (1995) Apoplastic water and solute movement – new rules for an old space. *Annual Review of Plant Physiology and Plant Molecular Biology* **46**, 215–236.

Cuenca R.H. (1989) *Irrigation System Design: an Engineering Approach*. Prentice Hall, Englewood Cliffs, NJ, USA.

Esau K. (1965) *Plant Anatomy*. John Wiley & Sons, Inc, New York, USA.

Franks P.J., Cowan I.R. & Farquhar G.D. (1998) A study of stomatal mechanics using the cell pressure probe. *Plant, Cell and Environment* **21**, 91–100.

Jeje A.A. (1985) The flow and dispersion of water in the vascular network of dicotylendonous leaves. *Biorheology* **22**, 285–302.

Landsberg J.J. & Fowkes N.D. (1978) Water movement through plant roots. *Annals of Botany* **42**, 493–508.

Marschner H. (1995) *Mineral Nutrition of Higher Plants*. Academic Press, San Diego, CA, USA.

Martre P., Durand J. & Cochard H. (2000) Changes in axial hydraulic conductivity along elongating leaf blades in relation to xylem maturation in tall fescue. *New Phytologist* **146**, 235–247.

Nardini A. & Salleo S. (2000) Limitation of stomatal conductance by hydraulic traits: sensing or preventing xylem cavitation? *Trees* **15**, 14–24.

Nardini A., Tyree M.T. & Salleo S. (2001) Xylem cavitation in the leaf of *Prunus laurocerasus* L. and its impact on leaf hydraulics. *Plant Physiology* **125**, 1700–1709.

Nelson T. & Dengler N. (1997) Leaf vascular pattern formation. *Plant Cell* **9**, 1121–1135.

Niklas K. (1999) A mechanical perspective on foliage leaf form and function. *New Phytologist* **143**, 19–31.

Roth-Nebelsick A., Uhl D., Mosbrugger V. & Kerp H. (2001) Evolution and function of leaf architecture: a review. *Annals of Botany* **87**, 533–566.

Salleo S., Nardini A. & Lo Gullo M. (1997) Is sclerophylly of Mediterranean evergreens an adaptation to drought? *New Phytologist* **135**, 603–312.

Siso S., Camarero J.J. & Gil-Pelegrin E. (2001) Relationship between hydraulic resistance and leaf morphology in broadleaf *Quercus* species: a new interpretation of leaf lobation. *Trees Structure and Function* **15**, 341–345.

Specht R.L. & Specht A. (1989) Canopy structure in *Eucalyptus*-dominated communities in Australia along climatic gradients. *Acta Oecologica* **10**, 191–213.

Steudle E. & Tyerman S.D. (1983) Determination of permeability coefficients, reflection coefficients, and hydraulic conductivity of *Chara corralina* using the pressure probe: effects of solute concentration. *Journal of Membrane Biology* **75**, 85–96.

Yang S. & Tyree M.T. (1994) Hydraulic architecture of *Acer saccharum* and *A. rubrum*: comparison of branches to whole trees and the contribution of leaves to hydraulic resistance. *Journal of Experimental Botany* **45**, 179–186.

Zwieniecki M.A., Hutyra L., Thompson M.V. & Holbrook N.M. (2000) Dynamic changes in petiole specific conductivity in red maple (*Acer rubrum* L.), tulip tree (*Liriodendron tulipifera* L.) and northern fox grape (*Vitis labrusca* L.). *Plant, Cell and Environment* **23**, 407–414.

Received 16 January 2002; received in revised form 30 May 2002; accepted for publication 31 May 2002