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Hydraulic architecture of leaf blades: where is the main resistance?

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ABSTRACT

The hydraulic architecture of *Laurus nobilis* L. and *Juglans regia* L. leaves was studied using three different approaches: (1) hydraulic measurements of both intact leaves and of leaves subjected to treatments aimed at removing the extra-vascular resistance; (2) direct measurements of the vascular pressure with a pressure probe; and (3) modelling the hydraulic architecture of leaf venation system on the basis of measurements of vein densities and conductivities. The hydraulic resistance of leaves (R_{leaf}) either cut, boiled or frozen–thawed was reduced by about 60 and 85% with respect to control leaves for laurel and walnut, respectively. Direct pressure drop measurements suggested that 88% of the resistance resided outside the vascular system in walnut. Model simulations were in agreement with these results provided vein hydraulic conductance was 0.12–0.28 that of the conductance predicted by Poiseuille's law. The results suggest that R_{leaf} is dominated by substantial extra-vascular resistances and therefore contrast with the conclusions of recent studies dealing with the hydraulic architecture of the leaf. The present study confirms the 'classical' view of the hydraulic architecture of leaves as composed by a low-resistance component (the venation) and a high-resistance component (the mesophyll).

Key-words: *Laurus nobilis* L.; *Juglans regia* L.; leaf hydraulic architecture; leaf venation; modelling.

INTRODUCTION

Leaves represent the terminal part of the soil–plant–atmosphere continuum and make up a significant portion of the whole plant hydraulic resistance (Yang & Tyree 1994; Nardini & Tyree 1999). Leaves are also the least understood component of the water pathway through the plant. It is generally agreed that water flows through the leaf venation and escapes xylem at the minor veins level (Canny 1990). Here, water would cross the bundle sheath cells and flow to the air spaces through or around living cells. Recent studies show that this picture is correct only in leaves with low radial water permeability of the major veins. In some species, however, major veins are largely leaky in the radial direction so that water can flow from these veins to living cells, directly (Salleo *et al*. 2003). In any case, the relative contribution of vascular and extra-vascular water pathways to the total leaf hydraulic resistance is still a matter of debate. A detailed understanding of the partitioning of the hydraulic resistances within the leaf is important both from a theoretical and practical point of view. In fact, leaf hydraulics is a major determinant of gas exchange rates (Tyree & Zimmermann 2002) and, hence, it is likely to have a significant influence on whole-plant productivity.

Early studies suggested that the major part of the leaf hydraulic resistance resided in the extra-vascular water pathway (Tyree & Cheung 1977). More recently (Tyree, Nardini & Salleo 2001; Salleo *et al*. 2003), freezing treatments aimed at disrupting cell membranes have revealed that at least 50–80% of the leaf hydraulic resistance is located outside the venous system. This finding would be in agreement with the impact of vein cavitation on leaf hydraulics which, in many cases, has been found to be less than expected on the basis of countings of ultrasound acoustic emissions and visual observations of the functionality of leaf veins (Nardini, Tyree & Salleo 2001; Nardini, Salleo & Raimondo 2003; Salleo *et al*. 2003; Trifilò *et al*. 2003). In fact, the large hydraulic resistance located outside the vascular system would buffer any change of vein hydraulic conductance as induced by embolism, damage or wall collapse (Meinzer 2002; Nardini & Salleo 2003; Sack, Cowan & Holbrook 2003; Cochard *et al*. 2004).

This 'classical' view of the hydraulic architecture of the leaf has been recently questioned by Zwieniecki *et al*. (2002) on the basis of measurements of pressure dissipation through the venation system of *Laurus nobilis* L. leaves and by Sack, Streeter & Holbrook (2004) on the basis of several cuts made through leaf blades of sugar maple and red oak. Experiments by Zwieniecki and coworkers revealed that a large pressure dissipation occurred between the petiole and minor veins, leading the authors to conclude that at least 80% of the leaf hydraulic resistance was located within the vascular system. Similar conclusions were reached by Sack *et al*. (2004) who suggested that extra-vascular water pathway accounted for only 30–40% of total leaf hydraulic resistance. Because the two hypotheses lead to different interpretations of the hydraulic architecture of the leaf and

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of the impact of water stress on it, the present study was addressed to investigate the hydraulic architecture of *L. nobilis* and *Juglans regia* L. leaves using three independent approaches: (1) hydraulic measurements of both intact leaves and of leaves subjected to treatments aimed at removing the extra-vascular resistance; (2) direct measurements of the vascular pressure with a pressure probe; and (3) modelling the hydraulic architecture of leaf venation system on the basis of measurements of vein densities and conductivities.

MATERIALS AND METHODS

Hydraulic measurements

Experiments for *L. nobilis* (laurel) were conducted on 1-year-old leaves sampled from an adult individual growing in the Botanical Garden of the University of Trieste, Italy. For *J. regia* (walnut) current year terminal leaflets were sampled on adult trees in the INRA Crouelle orchard, Clermont-Ferrand, France. Average surface area was 70.9 ± 10.5 cm^2 for laurel leaves and 98.9 ± 18.1 cm^2 for walnut leaflets. Hydraulic experiments were carried out in July 2000 and November 2002 for walnut and laurel, respectively. Leaf hydraulic resistance on a surface area basis (R_{leaf}) was measured using a high pressure flow meter (HPFM, Tyree *et al*. 1995). Briefly, leaves were excised and immediately connected to the HPFM using a compression fitting. Distilled water filtered at 0.1 μm was delivered at a pressure of 0.3 MPa. R_{leaf} was measured every 60 s and was shown to increase during the first 20–30 min. After that time, R_{leaf} reached stable values (i.e. SD of the last 10 readings was less than 5% of the mean). R_{leaf} was measured of both control (intact) leaves and of experimentally treated leaves (see below). In particular, R_{leaf} of control laurel leaves was measured both under normal laboratory irradiance (PAR < 6 μmol m^{-2} s^{-1}) and under high irradiance (PAR > 1000 μmol m^{-2} s^{-1}) provided by a fibre optic light source (KL 1500; Schott Corporation, New York, USA). This procedure had the aim of checking any possible 'light effect' on HPFM measurements of R_{leaf} as recently reported by Sack *et al*. (2002) for *Quercus rubra* L. In some cases, leaves were experimentally manipulated in the attempt to remove the hydraulic resistance of the extra-vascular water pathway. Some leaves were cut through between the second-order veins ('cut a', walnut and laurel) or along the leaf margins ('cut b', laurel only) with the aim of cutting open the minor veins, thus allowing water to bypass the extra-vascular water pathway. Cuts were made with fresh razor blades. Although 'cut a' involved damage of some second-order veins, in the case of 'cut b', particular care was taken to avoid cutting second-order veins, i.e. only veins of the third or higher order were cut open. In some other cases, physical treatments aimed at disrupting cell membranes were applied. In particular, laurel leaves were either frozen for 10 min at $T = -35$ °C and then thawed for 10 min at $T = +22$ °C, or they were immersed in boiling water for 2 min. Each experimental treatment was replicated on seven leaves.

In order to calculate the pressure drop across the venation system relative to total pressure drop across the leaf blade, field measurements of transpiration rate (E), leaf water potential (Ψ_{leaf}) and stem xylem water potential (Ψ_{stem}) were performed between 1200 and 1400 hours of a clear sunny day at the end of July 2003, and the results were compared with model simulations (see below). Water potential measurements were performed using a portable pressure chamber (Model 3005; Soilmoisture Equipment Corp., Santa Barbara, CA, USA). Transpiration rate was measured using a steady-state porometer (LI-1600; LiCor Inc., Lincoln, NE, USA). Seven leaves were bagged with plastic film and aluminium foil the evening before the experiments. This procedure has been shown to lead to equilibration of water potential of bagged leaves (Ψ_{bagged}) with Ψ_{stem} so that $\Psi_{bagged} = \Psi_{stem}$. The values of E and Ψ_{leaf} of leaves adjacent to bagged leaves were measured first and Ψ_{bagged} was measured immediately after. Under steady-state conditions, E should approximately equal liquid water flow through the leaf, while $\Psi_{bagged} - \Psi_{leaf}$ should equal the total pressure drop across the leaf. The measured value of E was used to calculate the pressure drop across the vascular system on the basis of the hydraulic model. For walnut, values of E, Ψ_{stem} and Ψ_{leaf} from previous studies (Cochard *et al*. 2000, 2002) were used.

Modelling the hydraulic architecture of leaf vasculature

A model was developed to compute the pressure drop across the venation system of dicot leaves. The model was parameterized for walnut and laurel. An experimental evaluation of the model was performed for walnut. The model is based on an explicit and exhaustive description of the vascular system.

Leaf vasculature

A representative leaf was selected for each species and cleared following the technique of Berlyn & Miksche 1976) (Fig. 1a & g). For laurel, five orders of veins were determined (V1 to V5), V1 corresponding to the midrib and V5 to the terminal veinlets. The density of V5 was determined by measuring the mean areole size. The areole is the smallest subdivision of the mesophyll delimitated by the vein network. Areole size was measured on a microscope equipped with a digitizing table for different positions on the leaf. Areoles were modelled as squares having 407 ± 8 μm ($n = 33$) width, the leaf being constituted of 29 442 areoles. The densities of V1 to V4 were adjusted in order to give a realistic pattern. For walnut, the analysis was performed on a terminal leaflet (walnut has compound leaves). We determined six orders of veins (V1 to V6), V1 being the midrib and V6 the terminal free-ending veinlet. Veins density (m m^{-2}) was determined on digital images with a standard image analysis software. For V1 and V2 we divided the total vein length by the total leaflet area. For V3 to V6 we measured vein length

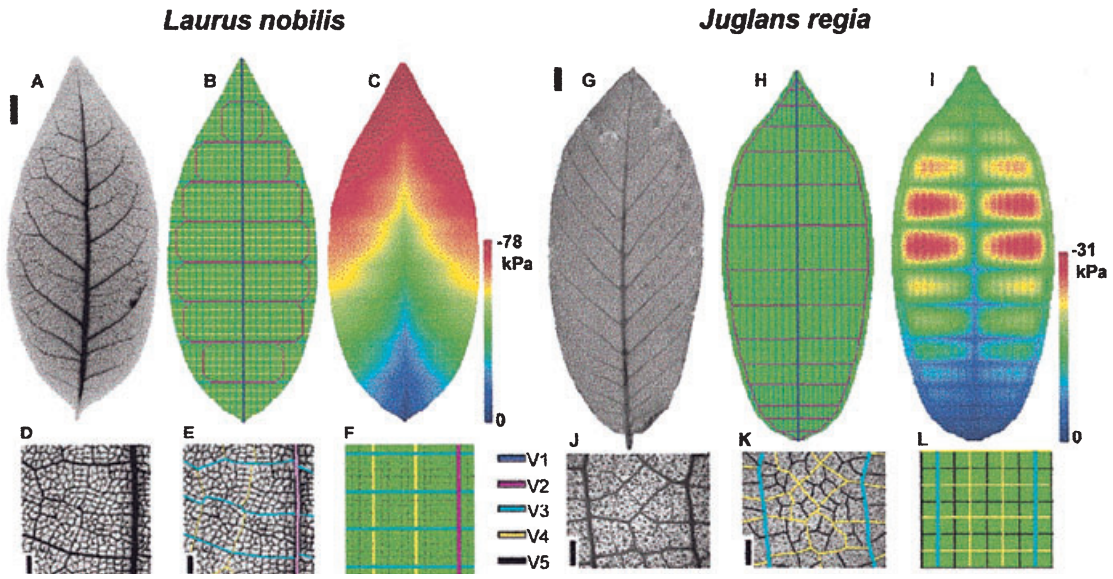

Figure 1. Model of leaf venation for *Laurus nobilis* (left) *and Juglans regia* (right). (a), (g), a typical cleared leaf used to parameterize the model. Details of the vascular system is shown in (d) and (j); (b), (f) and (h), (l): schematic representations of the leaf vasculature used in the model. Vein density of the different vein orders was adjusted in order to produce a realistic pattern. (e), (f), (k) and (l) compare the model with the actual vascular system (V6 are not shown in L). (c) and (I), Model prediction of the xylem pressure in the fifth vein order, with the xylem pressure at leaf entrance set to 0 MPa and a constant evaporation rate. Pressure drop varied between 0 (dark blue colour) at the leaf base to a miminum value (dark red) and near leaf apex that depends on the xylem efficiency. The values are given for an efficiency of 0.3. Bar scale represent 1 cm for (a), (b), (c0, (g), (h) and (i) and 1 mm for (d), (e), (f), (j), (k) and (l).

on 1 or 2 mm^2 squares from different positions on the blade.

Vein hydraulic conductivity

Theoretical hydraulic conductivities were derived from the number and dimensions of conduits in the xylem. Cross sections were obtained for the different vein orders and observed with an epifluorescent microscope. This facilitated the localization of the xylem conduits. V1 and V2 segments were sampled from different positions to account for the tapering of these veins. For each sample we measured the minimum (a) and maximum (b) lumen diameters of all the conduits in the vein. Following Lewis & Boose (1995), the theoretical vein hydraulic conductivity (K_t, mmol m s^{-1} MPa^{-1}) was computed as:

$$K_t = \sum \frac{\Pi a^3 b^3}{64\eta(a^2 + b^2)}$$

K_t usually overestimates the actual xylem hydraulic conductivity (K_x) because xylem conduits are not perfect pipes. We define the xylem hydraulic efficiency (e) as $e = K_x/K_t$ (Tyree & Zimmermann 2002). Simulations were done with e-values between 0.05 and 1. The hydraulic conductance (k) of each individual vein segment was obtained by dividing K_x by segment length. A total of 22 and 13 vein cross-sections were measured for walnut and laurel, respectively. The vein hydraulic characteristics are shown in Table 1 for the two species.

Model leaf

An exact representation of leaf vasculature is clearly out of reach. Therefore, we constructed a simplified model leaf with characteristics that were as representative as possible. The vascular system was considered as closed and reticulate; that is, as a regular mesh made by the interconnected veins. Areoles are thus represented by squares delimited by four veins (V1 to V5). For walnut, the terminal veins V6 end freely in the areoles. As V6 were lacking in laurel, they were present in the model but assigned an infinite conductance. The leaf mesophyll conductance (k_m) is in series with V6. We assumed that water was leaving the vascular system to enter the mesophyll only at the apex of V6 and that V6 were connected only to V5 (Canny 1990). However, connecting V6 to all the veins in the vasculature did not produce very different results. A total of 26 231 areoles and 157 386 veins were necessary to form the walnut leaflet blade. Laurel leaf was composed of 29 442 areoles but only 118 000 vein segments because V6 were lacking. Veins were distributed in the mesh in order to obtain densities as close as possible to the actual values. The data for the model leaves are shown in Table 1. The real and model leaves can be compared in Fig. 1.

Model computation

The details of the model calculations are given in the Appendix 1. In short, an analogy is made between the hydraulic resistances in a leaf blade and an electrical cir-

Table 1. Vein density, mean number of conduits, mean conduits' diameter and theoretical hydraulic conductivity as measured and calculated for different vein orders of *Juglans regia* L. (Walnut) and *Laurus nobilis* L. (Laurel) leaves

		Vein order					
		V1	V2	V3	V4	V5	V6
Walnut	Density (m m^{-2})	20.2	141.8	319	1462	1884	3650
	No. of conduits	268	52	18	10	5	1
	Conduit diameter (μm)	20.5	13.7	6.9	4.6	3.2	2.3
	Conductivity (mmol s^{-1} m MPa^{-1})	1.27 E–01	4.45 E–03	4.61 E–05	3.86 E–06	6.76 E–07	1.80 E–07
Laurel	Density (m m^{-2})	26.8	99.9	380	287	4217	
	No. of conduits	112	21	12	10	6	
	Conduit diameter (μm)	15.5	11.3	7.5	7.2	4.34	
	Conductivity (mmol s^{-1} m MPa^{-1})	1.19 E–02	4.72 E–04	6.11 E–05	4.07 E–05	4.5 E–06	

cuit. Two series of equations are considered. First, the water flow between two consecutive nodes is proportional to the hydraulic conductance of the vein connecting the nodes and pressure difference between the nodes (Ohm's law). Second, the water flow entering a node equals the flow leaving the node (Kirchhoff's law). The equations are solved using the Gauss–Siedel algorithm. The parameters for the model are: (1) the description of the entire vein network; (2) the theoretical hydraulic conductance for the different vein orders; (3) the xylem hydraulic efficiency; (4) the hydraulic conductance of the mesophyll; and (5) the xylem pressure at leaf entrance. If leaf evaporation rate is specified, then the model computes the pressure in the mesophyll and in all the vasculature. If the pressure in the mesophyll is specified, the pressure in the vasculature and the leaf evaporation rate are computed. Because the xylem hydraulic efficiency was unknown, the model was run for efficiency values between 0.05 and 1. When the efficiency was changed, the mesophyll resistances were adjusted to maintain whole leaf resistances at their measured values.

Model evaluations

Two experiments were designed to evaluate the predictions of the model. The principle of the evaluation was to simulate the conditions of experimentally treated leaves. The first evaluation aimed at simulating walnut leaves cut through between the second-order veins ('cut a' above) and exposed to a positive pressure at the base (P_{base}). The pressure in the mesophyll and in the cut veins were set to zero. Whole leaf resistance was computed as the ratio between the pressure drop across the blade ($P_{base} - 0$) and the flow through the leaf and was compared to the resistance of an intact leaf. Simulations were done with xylem efficiencies between 0.05 and 1. The second evaluation consisted in measuring and modelling the pressure in the vascular system of intact walnut leaves exposed to positive pressure at the base (P_{base}). The experimental set-up was in essence similar to that described by Zwieniecki *et al.* (2002) but the veins were probed in a different way (see below). The rachis of terminal leaflets was connected to a XYL'EM apparatus (Instructec, Montigny les Cormeilles, France) set to the high pressure mode. The XYL'EM measured the flow (F, mmol s^{-1}) and the pressure (P_{base}, MPa) at leaf entrance. The leaflet was exposed to ambient laboratory light and immersed in water to limit water losses by transpiration. The pressure in the vascular system (P_{veins}, MPa) was measured by two similar procedures. First, a small hole (5 mm in diameter) was made in the central part of the leaf blade between two V2. Veins V3 to V5 were thus severed. Rubber corks were pressed against both leaf surfaces in order to manage a waterproof seal around the hole. A small needle passed through one of the corks and was connected to a miniature pressure transducer. The pressure transducer measured the water pressure in the leaf hole which equalled, upon equilibration, the average pressure of the severed veins. Leaves were first perfused at 300 kPa until re-saturated (which usually took about 1 h). Then, the pressure at the leaf base was adjusted between 40 and 140 kPa and P_{veins} was determined. First results demonstrated that the equilibration time for P_{veins} was very long (several hours), probably because of the relatively large dead volume of our system. To obtain more rapid equilibration of P_{veins}, P_{base} was first set to a value between 50 and 150 kPa and then progressively decreased until P_{veins} stabilized. We then noticed that increasing slightly P_{base} increased P_{veins} and decreasing P_{base} decreased P_{veins}, thus suggesting that equilibrium conditions were obtained. The term $P_{base} - P_{veins}$ represented the pressure drop between leaf base and veins connected to the hole in the blade. On one occasion we mounted the system described above on an intact leaf with P_{base} set to 0.4 MPa. No detectable pressure variation was then noticed on the pressure transducer clamped on the leaf. The second procedure was very similar, except that a cell pressure probe (Steudle 1993) was employed to measure P_{vein}. This enabled us to reduce the hole in the blade to about 0.5 mm in diameter and thus to severe specifically V3, V4 or V5. P_{base} was adjusted between 20 and 200 kPa. The pressure in the pressure probe was adjusted by moving its internal rod to obtain a steady value. The equilibrium P_{vein} value was confirmed by slightly increasing or decreasing P_{base}. Pressure relaxation curves were also performed to test the hydraulic coupling between the probe and the vas-

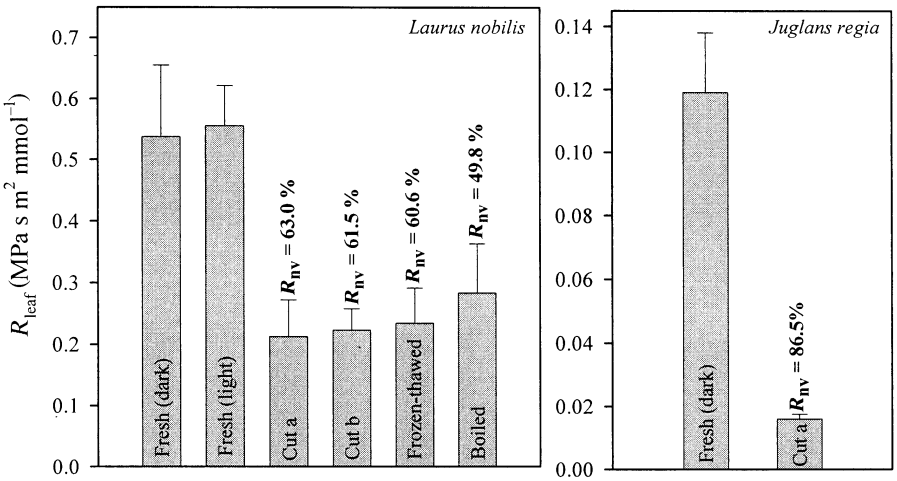

Figure 2. Leaf hydraulic resistance (R_{leaf}) of untreated (fresh) leaves measured both in light and dark conditions, and of leaves manipulated (cutting, boiling, freezing) in order to bypass or remove the extra-vascular hydraulic resistance. Error bars represent one SD ($n = 7$).

cular system. The results of typical experiments are shown in Fig. 4 for holes in V3 and V5. A total of 12 leaves were used for these measurements.

The experiment was simulated with the model described above. Virtual holes filled with water were obtained by assigning an infinite conductance to all the veins in the hole. P_{base} was assigned a value between 20 and 200 kPa and the pressure at the outlet of the mesophyl was set to 0 kPa. The whole leaf specific hydraulic conductivity ($k_l = 8.85$ mmol s^{-1} m^{-2} MPa^{-1}, SE = 0.85, $n = 8$) was derived from the relationships between P_{base} and F described above. The hydraulic conductance of the leaf mesophyll was adjusted when the xylem hydraulic efficiency was changed so that the k_l value for the model leaf equalled the actual value. P_{veins} values for virtual and experimental leaves were then compared.

RESULTS

Leaf hydraulic resistance

The R_{leaf} of control leaves of *L. nobilis* was found to be about 0.53 MPa m^2 s mmol^{-1} (Fig. 2) which was in the same range as previously reported for the same species by Nardini (2001) and Nardini & Salleo (2000). For walnut, R_{leaf} measured with the HPFM technique equalled 0.12 MPa m^2 s mmol^{-1}, not significantly different from the value measured with the XYL'EM apparatus (0.11 MPa m^2 s mmol^{-1}). No statistically significant difference was found between laurel leaves measured for R_{leaf} under low or high irradiance conditions (Fig. 2). The hydraulic resistance of leaves either cut, frozen–thawed or boiled was significantly lower with respect to values recorded in control leaves. In particular, R_{leaf} of manipulated laurel leaves was about 0.21 MPa m^2 s mmol^{-1}, with no statistically significant difference between different treatments. In all cases the hydraulic resistance of the treated laurel leaves was reduced by 49.8–63% compared to control leaves. Cutting through walnut blades between V2 removed 84.5% of leaf resistance (Fig. 2). Salleo *et al.* (2003) have reported 88% resistance reduction on frozen–thawed walnut leaves.

Cutting walnut leaf blades between V2 was simulated with the model (Fig. 3). The hydraulic resistance of the cut leaf was inversely related to the xylem hydraulic efficiency. An agreement between the model and the measurements was found for an efficiency of about 0.12.

Pressure drop in leaf vasculature

Direct pressure drop in the vasculature of walnut leaves exposed to a positive pressure water supply were measured with pressure transducers. The data reported in Fig. 4 show examples of pressure recordings with a pressure probe in contact with V3 (upper panel) and V5 (lower panel). The pressure in the probe was first adjusted to obtain steady values. When the pressure at leaf base was changed, the

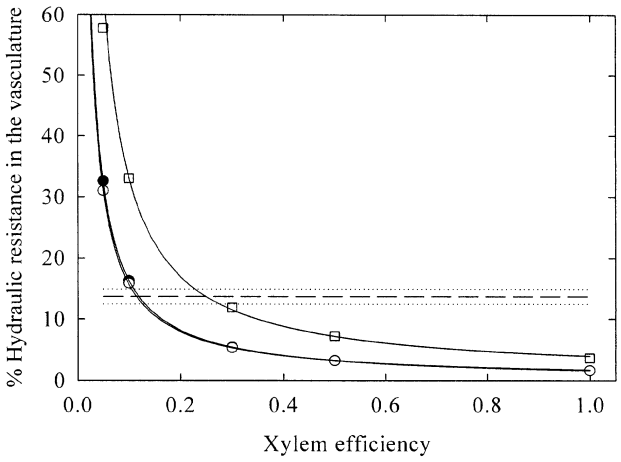

Figure 3. Measured and predicted percentages of hydraulic resistance in the vasculature of a walnut leaflet. The model predictions are given for different xylem hydraulic efficiencies. Closed circles represent the theoretical resistance of the vasculature (i.e. the resistance obtained when the mesophyll resistance was set to 0). Open circles represent the predicted resistance when leaf blades were incised through V2. Open squares represent predicted resistance when 175 small cuts were made in the blades. Curves are hyperbolic fits. Horizontal dashed and dotted lines and mean ± SE percentage resistance of leaflet having blades cut between V2.

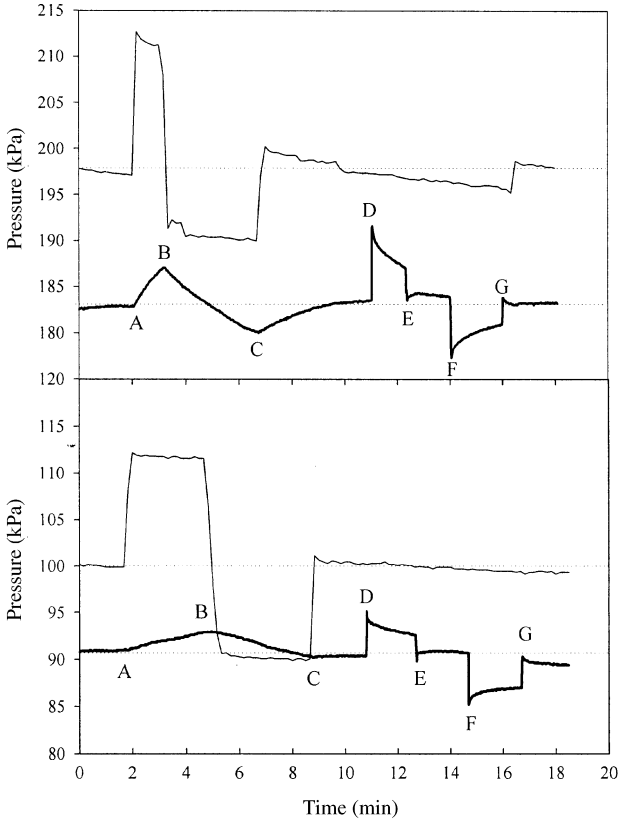

Figure 4. Typical results of pressure drop measurements in the vascular system of a walnut leaflet exposed to a positive water pressure at its base. Third and fifth vein orders were probed in the upper and lower panels, respectively. The thin line represents the pressure at leaf base (P_{base}) and the thicker line the pressure in the vein (P_{vein}). P_{vein} was in equilibrium state (P_{vein}^0) with P_{base}^0 at time 0. In A, P_{base} was increased above P_{base}^0. In B, P_{base} was reduced below P_{base}^0. In C, P_{base} was returned to P_{base}^0. In D, P_{vein} was increased above P_{vein}^0. In E, P_{vein} was returned to P_{vein}^0. In F, P_{vein} was decreased below P_{vein}^0. In G, P_{vein} was returned to P_{vein}^0.

pressure in the probe responded immediately. The response was much slower when V5 were probed. When the pressure in the probe was suddenly increased, typical relaxation curves were obtained. Taken together, these data demonstrate that the pressure probe was hydraulically connected to the vascular system and that the pressure at the onset of the experiment was equilibrated with the pressure in the sampled veins.

The relationship between the supply pressure (P_{base}) and the pressure in the different vein orders (P_{vein}) upon equilibration is shown in Fig. 5. The two techniques we used yielded similar results and are pooled in the figure. P_{vein} varied linearly with P_{base}. The difference in P_{vein} values between different vein orders was small but tended to be lower in higher vein orders. In all cases the two values were very close to each other with a maximum difference of 50 kPa when P_{base} equalled 200 kPa. The relative pressure drop for V5 [i.e. $(P_{base} - P_{vein})/P_{base}$] was 13.7%.

Simulations of pressure probe measurements were performed with the model. The pressure drop in the vasculature was inversely related to the xylem efficiency (Fig. 6). An agreement between the model and the pressure measurements in V5 was found for a xylem efficiency of 0.28.

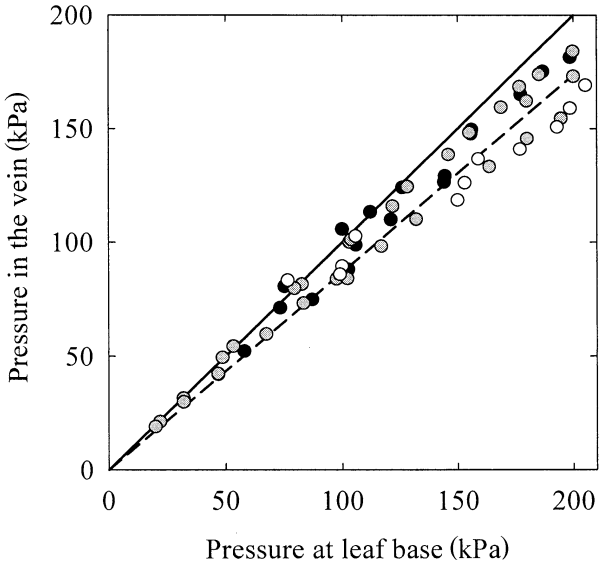

Figure 5. Measured and predicted xylem pressure in the veins (P_{veins}) of walnut leaflets perfused with a positive water pressure (P_{base}). P_{base} was applied at the leaf base and P_{vein} was measured in the middle of the blade by cutting different types of veins (different symbols). Model predictions (different dashed lines) are shown for a xylem hydraulic efficiencies of 0.3.

Xylem pressure drop *in planta*

At the end of July 2003, the value of E for *L. nobilis* leaves was 1.47 ± 0.28 mmol m^{-2} s^{-1}. Leaf (Ψ_{leaf}) and stem (Ψ_{stem}) water potential were -1.42 ± 0.09 and -1.14 ± 0.04 MPa,

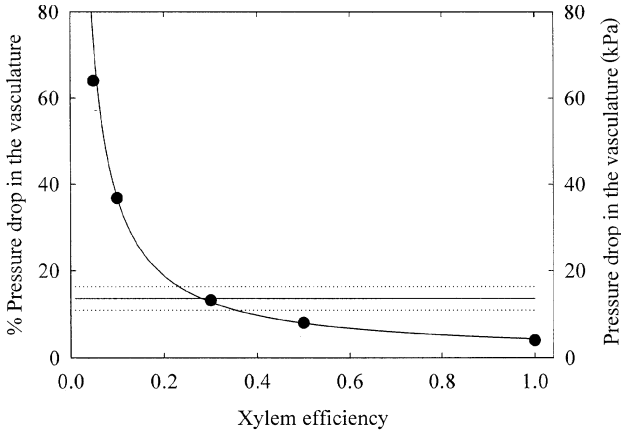

Figure 6. Measured and predicted pressure drop in the fifth vein order of walnut leaves exposed to a positive pressure water supply at their base. The results are expressed as a percentage of total pressure drop across the blade (left y axis) or in absolute value with a total pressure drop of 100 kPa. Horizontal dashed and dotted lines and mean ±SE percentage pressure drops measures in V5 with the pressure probe.

respectively, so that the total pressure drop across the leaf blade at the measured transpiration rate turned out to be 0.27 ± 0.08 MPa. Data for walnut from previous studies (see References above) indicated that E equalled 2.0 mmol m^{-2} s^{-1}, Ψ_{stem} was about –0.7 MPa and Ψ_{leaf} was about –0.9 MPa. When the hydraulic model was run using the E value measured in the field, the mean pressure in the fifth vein order was inversely related to the xylem hydraulic efficiency (Fig. 7). For laurel, when the xylem efficiency was 0.15 the mean pressure drop equalled –0.105 MPa, corresponding to 61% pressure drop in the leaf mesophyll. The variations in xylem pressure across the venation system are shown in Fig. 1(c & i) for the entire model leaves with the measured E values and a xylem efficiency of 0.3. The patterns of pressure variation along leaf blades were very different for the two species, reflecting differences in V1 and V2 hydraulic conductance.

Figure 8 shows the variation in pressure in the vasculature and the mesophyll of transpiring walnut leaves during a typical sunny day. The pressure at leaf entrance was set equal to the pressure in the stem (–0.7 MPa). We considered four extreme conditions: (1) a xylem efficiency consistent with the previous data ($e = 0.3$); (2) a very low xylem efficiency ($e = 0.05$); (3) a constant transpiration rate; and (4) a constant leaf mesophyll pressure. When the xylem efficiency was high, the predicted variations in pressure or transpiration rates were very small (upper graphs). By contrast, a low xylem efficiency resulted in very heterogeneous pressure and transpiration rates in the blade (lower graphs).

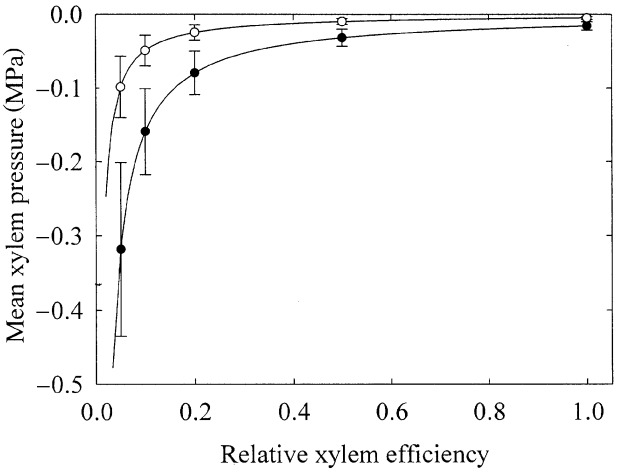

Figure 7. Mean pressure drop in transpiring leaves of walnut (○) and laurel (●) versus xylem hydraulic efficiency. Leaf evaporation rate was set to 2 and 1.47 mmol s^{-1} m^{-2} for walnut and laurel, respectively.

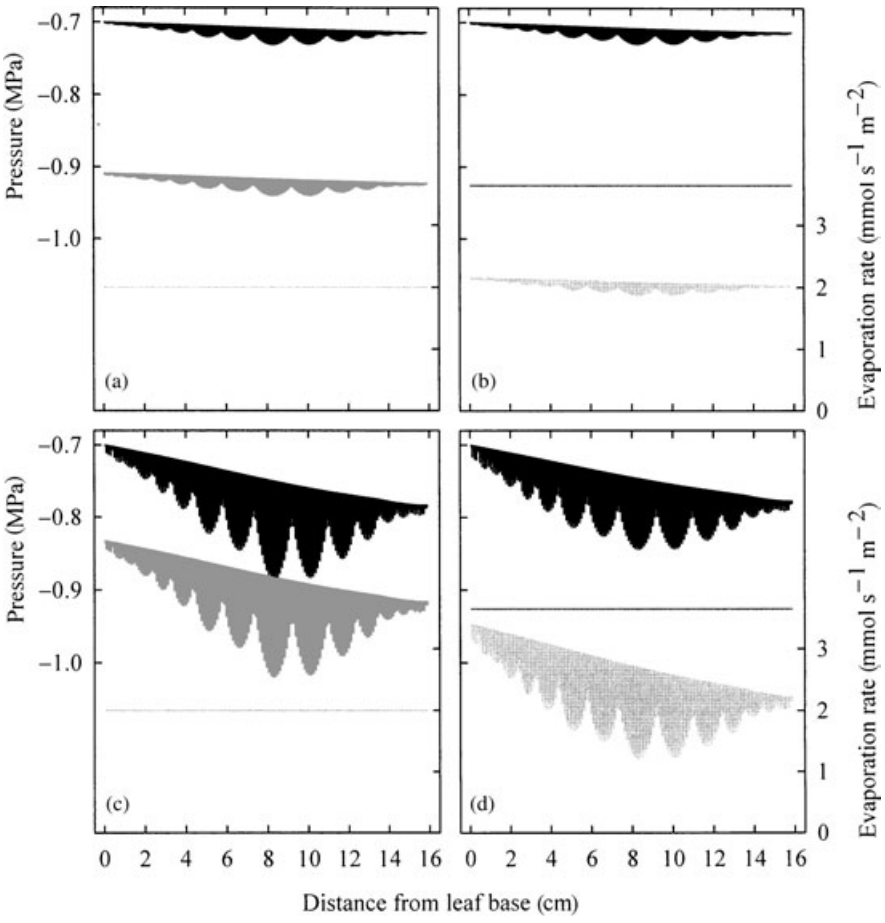

Figure 8. Water relations of a transpiring walnut leaf *in situ*. The data show, for each areole in the blade, the pressure in V5 (black lines), the pressure in the leaf mesophyll (dark grey lines) and the evaporation rate (lower light grey line, right y axis). For the simulations, the xylem pressure at the entrance of the leaf was set to –0.7 MPa. In (a) and (c) the leaf evaporation rate was constant and set to 2 mmol s^{-1} m^{-2}. In (b) and (c) the pressure in the leaf mesophyll was constant and set to –0.93 MPa. The upper (a, b) and lower graphs (c, d) show the predictions of the model with a xylem hydraulic efficiency (e) set to 0.3 and 0.05, respectively.

In Fig. 9 we show the effect of doubling or dividing by two the hydraulic conductance of each vein order on the mean pressure drop for V6 in walnut. This sensitivity analysis revealed that changing drastically the hydraulic conductivity of minor veins had little effect on the overall pressure drop across the leaf vasculature.

DISCUSSION

The hydraulic resistance of intact laurel and walnut leaves was quite high, in agreement with previous reports for these and many other species (Tyree *et al*. 1999; Nardini 2001). However, when cuts were made in the blades or when leaves were boiled or frozen, leaf hydraulic resistance was considerably decreased (by about 60 and 85% in laurel and walnut, respectively), suggesting that most of the R_{leaf} was either by-passed or removed in treated leaves. Direct pressure measurements in the vascular system of walnut leaves suggested a very small pressure drop in the vascular system. More than 86% of the pressure drop, and hence of the hydraulic resistance, was located downstream of the minor veins. Although the experimental treatments applied to leaves in the present study are not entirely novel, this is the first time that different treatments are applied to the same species and their substantial agreement is demonstrated. These experimental results are consistent with the prediction of our leaf architecture model. To our knowledge this is the first model that attempts to predict pressure drops and flows in the vasculature of a dicot leaf, which is based on a realistic and exhaustive description of the vascular system. Vasculature in previous models (e.g. Tyvand 1982; Roth *et al*. 1995; Sack *et al*. 2004) was either much simplified or based on a porous medium analysis. Prediction of xylem pressure drops, and hence vascular hydraulic resistance, were a function of the hydraulic efficiency of the xylem conduits. Any value between 100 and 1.6% vascular hydraulic resistance was predictable for walnut when the xylem efficiency was adjusted between 0 and 1 (Fig. 3). However, very low xylem efficiencies are, in our opinion, very unlikely. First of all, the values reported so far in the literature vary only between 0.26 and 1 (see Tyree & Zimmermann 2002 for a review). The minimum values were reported for very small conduits, like tracheids in conifers. Conduits in laurel and walnut leaves were found to exhibit diameters that were even smaller than tracheids in conifers so that efficiencies below 0.26 might be possible. However, a comparison of model predictions of xylem pressure drop (Fig. 6) and resistance of cut leaves (Fig. 3) with actual measurements suggests xylem efficiency values between 0.12 and 0.28.

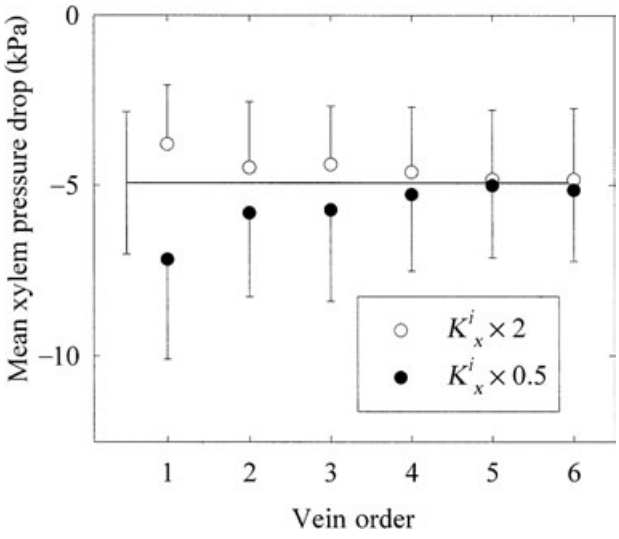

Figure 9. Effects of doubling (○) or dividing by two (●) the hydraulic conductance of one vein category (x axis) on the mean xylem pressure drop for V6. The horizontal line is the reference value for a walnut leaflet with an efficiency set to 1. The pressure at leaflet entrance was set to 0 MPa and the leaf transpiration rate equalled 2 mmol s^{-1} m^{-2}. Vertical bars represent one SD.

Our experimental results and the predictions of our model provide support to the hypothesis of a large extravascular resistance in the leaf blades of laurel and walnut. This conclusion would be in agreement with previous reports by Tyree & Cheung (1977), Tyree *et al*. (2001), Trifilò *et al*. (2003) and Nardini *et al*. (2003) for different species. However, direct pressure measurements in the vasculature of laurel by Zwieniecki *et al*. (2002), and cutting treatments of dicot leaves by Sack *et al*. (2004) have led these authors to the opposite conclusion; namely that dominant vascular resistance is present in the leaf blade. As the present study produced results that are inconsistent with those obtained by these authors, we have to consider possible reasons that could explain the discrepancies between the different studies. The experimental procedure adopted by Zwieniecki and coworkers consisted of connecting the leaf to a pressurized water source via the petiole. Then, veins of different orders were cut using a scalpel and they were inserted into a microcapillary tube attached to a pressure probe. Final sealing of the vein to the pressure probe was obtained using cyanoacrylate glue. In our study, the leaf was also pressurized with water via the petiole but the pressure probe was in contact to a water-proof clamp around a hole in the blade. We do not see any reason why the two procedures should yield different results. However, in the study by Zwieniecki and coworkers, 'the pressure in the microcapillary was recorded after the 30–60 min needed for flow into the leaf to stabilize'. In our experiments we indeed verified that the flow entering walnut leaves was constant after such a time. However, the pressure in the pressure probe was not yet stable and kept increasing. This is why we had to modify our experimental set up in order to obtain steady-state pressures more rapidly. This is also why we elaborated tests to demonstrate true steady states (Fig. 4). The problem might have been exacerbated in the study by Zwieniecky and coworkers because the minor veins were cut open in air, thus possibly increasing embolism level in the surrounding vasculature. Although leaves were perfused at 0.02 MPa during cutting and minor vein isolation, it is possible that such pressures

were too low to displace emboli, so that most minor veins surrounding the 'isolated' vein might have been non-functional during the pressure drop measurements. If this was the case, then the locally large hydraulic resistance would have considerably increased the equilibration time. On the other hand, our cutting experiments might be affected by some other problems as well. Our experimental procedure involved cutting some major veins (at least some third-order veins). According to Zwieniecki *et al*. (2002), low hydraulic resistance is associated with these veins and this might lead to underestimation of the contribution of the venation system to total leaf hydraulic resistance. However, this experimental procedure produced results that were consistent with freezing and boiling experiments, in which the integrity of the venation system was unchanged and only extra-vascular resistance associated with cell membranes was removed. Nonetheless, R_{leaf} was reduced by the same amount as in cutting experiments. It has to be pointed out that boiling and freezing leaves might increase the radial permeability of major veins by disrupting bundle sheath cells, thus short-circuiting the water flow through the venation system and leading to underestimation of the contribution of the vein network to leaf hydraulic resistance. Although caution is needed in interpreting the results of experimental treatments applied in the present study, we note that different approaches all lead to the same conclusion and this, in our opinion, provides evidence for a major role of the extra-vascular water pathway in determining the overall R_{leaf}. This view is reinforced by the hydraulic model presented in this study. The model is based on actual values of vein densities and theoretical conductivities based on xylem anatomy and, hence, it is quite well grounded. A striking agreement was found between the predictions of 'cut a' treatment and the computation of the xylem resistance (Fig. 3). This would suggest that this treatment achieved R values close to the true vascular resistance. However the agreement may just have been incidental and should be confirmed with other species.

The conclusions of Sack *et al*. (2004) of a high xylem resistance are based on cutting treatments. These authors have incrementally severed minor veins until 120–150 cuts were made. The treatment removed only 8 to 63% of total leaf resistance. We have simulated this experiment with our model by operating up to 175 1.5–2 mm long cuts through V4 and V5 randomly located on a walnut blade. Leaf resistance indeed decreased with the number of cuts but 175 cuts were not enough to remove all the extra-vascular resistance (see Fig. 3). Less than 2% of V4 and V5 were actually severed in the blade with this treatment. Our opinion is that the procedure of Sack *et al*. (2004) did not achieve the true vascular resistance but this point would deserve further investigations.

Our view of the leaf hydraulic architecture (i.e. low hydraulic resistance in the vein network) and the opposite view defended by Zwieniecki *et al*. (2002) and Sack *et al*. (2004) (i.e. high hydraulic resistance in the vein network) would result in greatly contrasting leaf functioning. Zwieniecki and coworkers have suggested that the hydraulic design of laurel leaves, as reported in their study, was optimized in order to supply water relatively evenly throughout the leaf. In fact, if the hydraulic resistance of the venation system were dominant, then water would not be distributed evenly throughout the leaf blade especially under conditions of patchy or disomogeneous transpiration rates over the leaf surface (Mott & Buckley 2000; Prytz, Futsaether & Johnsson 2003), because the high hydraulic resistance at the vein level would prevent water to be re-distributed to different regions of the leaf, rapidly. On the contrary, the hydraulic design envisioned by Zwieniecki *et al*. (2002) would induce important water potential disequilibrium between different regions of the leaf. We feel that the hydraulic design suggested by the result of our study; namely relatively minor hydraulic resistance at the vein level, is more suited for homogeneous water transport and distribution across the leaf blade to occur. The model of leaf hydraulic architecture clearly demonstrates this point (Fig. 8). The pressures in the blade and the evaporation rates were much more homogeneous when the resistance in the vasculature was small. This partitioning of hydraulic resistances within the leaf blade would result in a much more efficient leaf functioning and would also considerably buffer the consequences of embolism formation in the vasculature (Salleo *et al*. 2001).

It was not the purpose of this paper to explore in detail the predictions of our leaf architecture model. The simulations shown in Fig. 9 illustrate how the model might help understanding the cost versus benefit of different vascular constructions. Here we show that minor vein conductances have little weight on the overall conductance of the whole leaf vasculature. Minor veins have very low conductivities but the fact that they are very numerous, short, and highly interconnected minimize their hydraulic impact. However this point would deserve a more detailed analysis.

In conclusion, the experimental and the modelling approaches developed in this study both converge to a low hydraulic resistance in the leaf vasculature for walnut and laurel. The highest share of leaf resistance is most probably located in the extra-vascular water pathways in the mesophyll. The opposite situation proposed in the recent literature would only be explained by a very low xylem hydraulic efficiency in the venation system. Our model predicts that our view of leaf construction would be more efficient in terms of leaf water relations and gas exchange rates.

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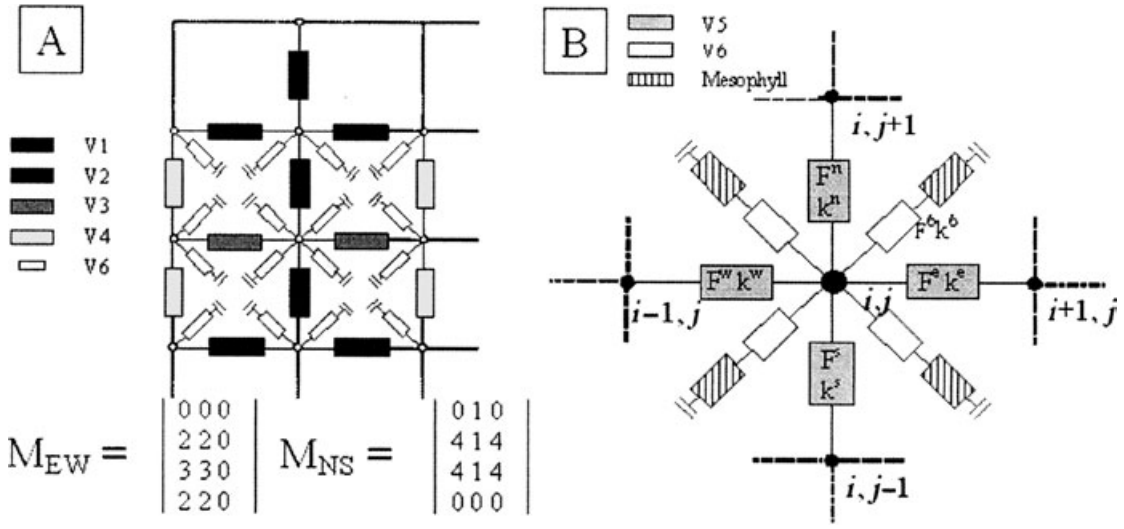
APPENDIX 1: DETAILS OF THE LEAF ARCHITECTURE MODEL

Two (I,J) matrices were used to describe the model leaf vasculature, I and J being the number of areoles along the width and the length of the leaf. Each coordinate in the matrices represent one node of the mesh. The first matrix (M_{EW}) contains the order of the vein between node (i,j) and node ($i + 1$,j); that is in the East–West direction. The second matrix (M_{NS}) contains the order of the vein between node (i,j) and node (i,$j + 1$); that is in the North–South direction. Nodes outside of the leaf were assigned null values. An example of notations is given in Fig. A. More precisely, the numbers in the matrices are the indices of arrays pointing to the conductance values for the different veins. This was necessary to better describe the hydraulics of V1 and V2 that taper along the blade.

Model computation

The flow ($F6$; mmol s^{-1}) through V6 equals the transpiration rate (E, mmol s^{-1} m^{-2}) time the leaf area attached to each V6 (usually one-quarter of the areole area A). As V6 ends



freely in the areoles, the pressure ($P6$, MPa) at the apex of V6 can be easily derived from Ohm's law:

$$P6_{i,j} = P_{i,j} - F6/k6_x \quad (1)$$

where $P_{i,j}$ is the pressure of the node the vein V6 is connected to and $k6_x$ the hydraulic conductance of V6 (see Fig. B for notations). For laurel, as $k6_x$ is infinite, $P6_{i,j} = P_{i,j}$. Similarly, the pressure in the mesophyll is computed as:

$$P^{m}_{i,j} = P6_{i,j} - F6/k_m \quad (2)$$

where k_m is the mesophyll conductance. Therefore, the model needs only to solve flow and pressures for veins V1 to V5. The electric analogy was used to solve the problem: the flow between two consecutive nodes (F^n, F^s, F^e, F^w) follows the Ohm's law:

$$F^n = [P_{i,j+1} - P_{i,j}]\, k^n_x$$

$$F^s = [P_{i,j-1} - P_{i,j}]\, k^s_x$$

$$F^e = [P_{i+1,j} - P_{i,j}]\, k^e_x$$

$$F^w = [P_{i-1,j} - P_{i,j}]\, k^w_x \quad (3)$$

and there is a conservation of mass at each node (Kirchhoff's law):

$$F^n + F^s + F^e + F^w = EA \quad (4)$$

Therefore, the xylem pressure at the node (i,j) can be derived from the pressure at the neighbouring node and the conductance of the veins connected to the node:

$$P_{i,j} = [k^n P_{i,j+1} + k^e P_{i+1,j} + k^w P_{i-1,j} + k^s P_{i,j-1}]/ [k^n + k^s + k^e + k^w] - EA \quad (5)$$

The classical iterative Gauss–Siedel method was used to solve the equations for all the nodes. In short, the equations are considered sequentially columns by columns and rows by rows, and the previously computed results are used as soon as they are available. To accelerate the rate of convergence of the iterates to the solution an extrapolation factor of 1.52 was introduced (successive over-relaxation method). This had no effect on the results. The model was first run with a zero xylem pressure at all the veins. After 10^5 iterations (about 10 min for a CPU clock at 1.4 GHz) the model converged to a stable value. The results of this simulation were used as initial conditions for other simulations. The computations were stopped when the maximum change in pressure between two iterates was less than 10^{-8} kPa. The model was used in two different ways. The first calculations aimed at simulating a transpiring leaf. The pressure at the petiole base was assigned a constant value (zero or a negative pressure) and the flow through the leaf mesophyll caused by evaporation was imposed. The model then computed the flow and the pressure for each veins and the pressure in the mesophyl. The second computations aimed at simulating a non-transpiring leaf perfused with water as described in the experiment below. The pressure at the leaf base was assigned a positive value and the pressure at the mesophyll outlet set to zero. The model then computed the flow and the pressure for each vein and the flow through the mesophyll. The model was written in C language and executed on PC or Unix computers. A PostScript file generator was developed to create two- or three-dimensional colour maps. H.C. will send the program and the code to anyone upon request.

Tests of internal model consistency

Several tests were performed to evaluate the correctness of the model calculations. For simple networks (such as in Fig. A above) for which analytical solutions can be found, we verified that the model yield the correct flow and pressure values. For the much more complex model leaves, we verified that the model was behaving like an electrical circuit having one resistance equivalent to all the resistances in serial and in parallel; for example, the pressure drop varied linearly with the flow and the resistances, lowering the pressure at the leaf entrance lowers all the pressures in the leaf by the same amount, etc.