



# The hydraulic conductance of the angiosperm leaf lamina: a comparison of three measurement methods

Lawren Sack<sup>1</sup>, Peter J. Melcher, Maciej A. Zwieniecki and N. Michele Holbrook

Harvard University, Biological Laboratories, 16 Divinity Avenue, Cambridge, MA 02138, USA

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## Abstract

**A comparison was made of three methods for measuring the leaf lamina hydraulic conductance ( $K_{\text{lamina}}$ ) for detached mature leaves of six woody temperate angiosperm species. The high-pressure method, the evaporative flux method and the vacuum pump method involve, respectively, pushing, evaporating and pulling water out of the lamina while determining the flow rate into the petiole and the water potential drop across the leaf. Tests were made of whether the high-pressure method and vacuum pump method measurements of  $K_{\text{lamina}}$  on single leaves were affected by irradiance. In *Quercus rubra*, the high pressure method was sensitive to irradiance;  $K_{\text{lamina}}$  measured under high irradiance ( $>1200 \mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetically active radiation) was 4.6–8.8 times larger than under ambient laboratory lighting ( $\sim 6 \mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetically active radiation). By contrast, the vacuum pump method was theoretically expected to be insensitive to irradiance, and this expectation was confirmed in experiments on *Hedera helix*. When used in the ways recommended here, the three methods produced measurements that agreed typically within 10%. There were significant differences in species'  $K_{\text{lamina}}$ ; values ranged from  $1.24 \times 10^{-4} \text{ kg s}^{-1} \text{ m}^{-2} \text{ MPa}^{-1}$  for *Acer saccharum* to  $2.89 \times 10^{-4} \text{ kg s}^{-1} \text{ m}^{-2} \text{ MPa}^{-1}$  for *Vitis labrusca*. Accurate, rapid determination of  $K_{\text{lamina}}$  will allow testing of the links between  $K_{\text{lamina}}$ , water-use, drought tolerance, and the enormous diversity of leaf form, structure and composition.**

Key words: Angiosperms, evaporative flux method, high-pressure method, hydraulic conductance, leaves, vacuum pump method.

## Introduction

As liquid moves through the leaf lamina, from entry at the petiole–lamina junction to the sites of evaporation, its flow rate at a given water potential difference depends on the lamina hydraulic conductance ( $K_{\text{lamina}}$ ). Several studies have suggested that  $K_{\text{lamina}}$  is small relative to conductivities of other components of the whole-plant system, therefore constraining whole plant sap flow, and, ultimately, maximum stomatal conductance (Grubb, 1984; Becker *et al.*, 1999; Nardini and Salleo, 2000; Tsuda and Tyree, 2000; Aasamaa *et al.*, 2001; Matzner and Comstock, 2001). In addition, a number of authors have hypothesized that  $K_{\text{lamina}}$  might, to some degree, explain the huge diversity in leaf characteristics such as venation architecture, stomatal dimensions and distribution, leaf size, and leaf shape (Roth-Nebelsick *et al.*, 2001; Siso *et al.*, 2001; Aasamaa *et al.*, 2001). Approaching these questions depends on accurate techniques for measuring  $K_{\text{lamina}}$ . This study tests the agreement among single-leaf versions of the three most often used techniques.

Recent studies of leaf hydraulic properties have used the high-pressure method (HPM; Yang and Tyree, 1994; Becker *et al.*, 1999; Nardini and Salleo, 2000; Tyree *et al.*, 1999; Tsuda and Tyree, 2000), the evaporative flux method (EFM; Tyree *et al.*, 1999; Nardini *et al.*, 2001; Tsuda and Tyree, 2000), and the vacuum pump method (VPM; Martre *et al.*, 2001; Nardini *et al.*, 2001). These methods drive flow through the leaf by, respectively, pushing, evaporating or pulling water out of the lamina at a determined pressure gradient across the leaf. The leaf hydraulic conductance is calculated as flow rate/pressure gradient, allowing  $K_{\text{lamina}}$  to be calculated once the petiole hydraulic conductance is known. The three methods rely on different driving forces for water movement, raising the question of whether the methods measure the conductance of the same hydraulic path through the lamina (Tyree *et al.*, 1999). In

<sup>1</sup> To whom correspondence should be addressed. Fax: +1 617 496 5854. E-mail: LSACK@oeb.harvard.edu

addition, the majority of previous studies have estimated  $K_{\text{lamina}}$  indirectly from measurements on branches, an approach that depends upon the untested assumption that the water supply to each leaf is independent. A few recent papers have reported  $K_{\text{lamina}}$  measurement using the HPM or VPM on individual leaves (Wei *et al.*, 1999; Martre *et al.*, 2001; Nardini *et al.*, 2001). This study compares the HPM, EFM and VPM, for single leaves of six woody temperate angiosperm species.

The HPM has been the most frequently used approach in the study of leaf hydraulics (see above references). However, HPM studies report values for a given species that sometimes vary by as much as 6-fold (Tyree *et al.*, 1993; Nardini and Tyree, 1999; Asamaa *et al.*, 2001; Siso *et al.*, 2001). Further, one study reported that the HPM did not produce stable values, with measured  $K_{\text{lamina}}$  decreasing continuously for hours (Melcher *et al.*, 2001). During the HPM measurement the leaf airspaces become infiltrated, and water drips from the stomata. Strong stomatal constriction could bias downward the measured  $K_{\text{lamina}}$  from its value *in vivo*, since *in vivo* the stomata contribute only to the vapour-phase resistance. Therefore, tests were carried out to discover whether changes to leaf irradiance affected the HPM measurement of  $K_{\text{lamina}}$ . The VPM method, on the other hand, drives flows at rates too slow to fill the intercellular spaces with water. However, flow rates increase with higher illumination, motivating us to test whether  $K_{\text{lamina}}$  measured with the VPM was affected by irradiance.

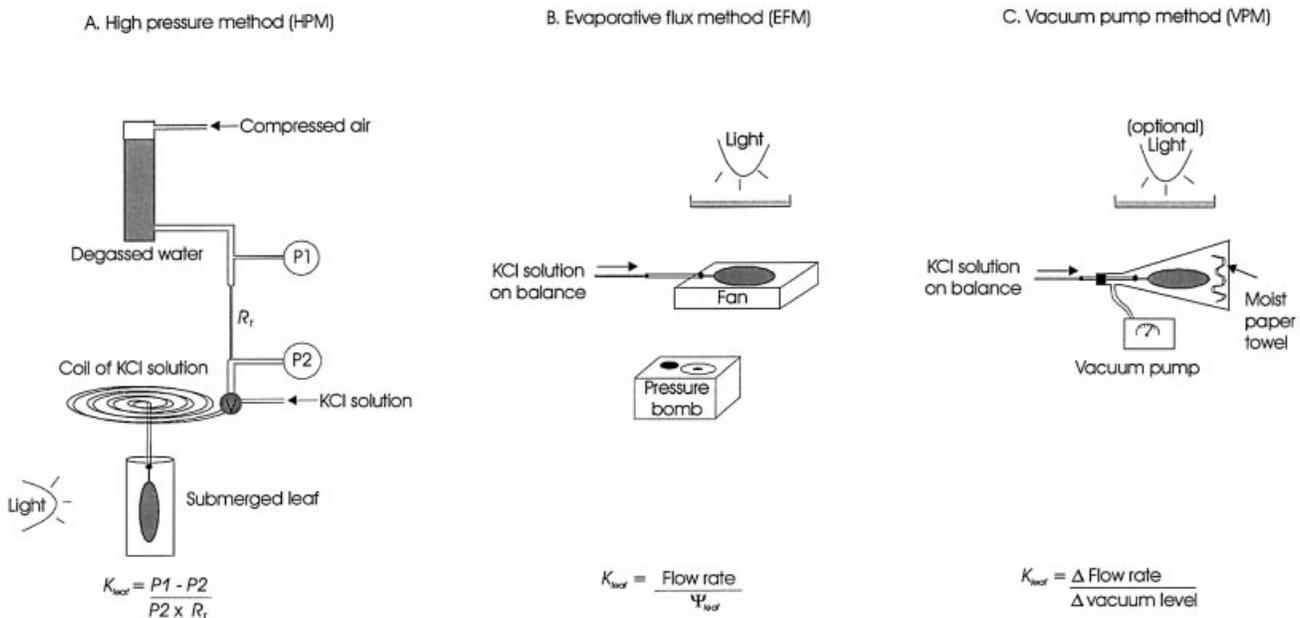
## Materials and methods

### Plant material

Six species were sampled which had leaves that differed strongly in thickness, texture, and apparent desiccation tolerance. From June to August 2001 mature trees of *Acer rubrum*, *Acer saccharum*, *Betula papyrifera*, and *Quercus rubra* were sampled at Harvard Forest in Petersham, MA (42°54' N, 72°18' W). Branches were sampled from the exposed part of the crown (5–8 m above the ground) of five trees per species, growing at exposed sites or along roads and trails within the forest. Diameters at breast height ranged between 16–39 cm (*A. rubrum*), 56–91 cm (*A. saccharum*), 7–10 cm (*B. papyrifera*), and 20–51 cm (*Q. rubra*). Shoots of *Vitis labrusca* and *Hedera helix* were collected from one or several individuals that covered more than 30 m of fence at the Harvard Forest (*V. labrusca*) or from large vines growing along a fence on the Harvard University campus (*H. helix*, sampled in October). Measurements were made using a dilute electrolyte solution, degassed 10 mM aqueous KCl, that was re-filtered to 0.2  $\mu\text{m}$  immediately prior to each use. Material collected in the field was re-cut under water and allowed to hydrate overnight by placing the cut ends of the shoots in the solution and covering the leaves with plastic. The next morning, the petioles of fully expanded, mature leaves were re-cut under the solution and attached to the measurement apparatus using a compression fitting (Omnifit A2227 bore adaptor; Omnifit, Cambridge, UK).

### High-pressure method (HPM)

The approach to measuring hydraulic conductance by pushing water into a plant part at a known flow rate and delivery pressure was first developed by MT Tyree (Tyree *et al.*, 1999). Early studies measured conductance by the 'transient' method: applying flow solution at different pressures and flow rates and calculating the conductance as the slope of flow versus pressure. However, these measurements proved difficult to interpret and to use; later studies have emphasized steady-state measurements (Yang and Tyree, 1994). The HPM



**Fig. 1.** The three methods. (A) High-pressure method, with  $P1$  and  $P2$  the pressure readings from transducers,  $R_T$  a segment of high-resistance tubing of known hydraulic resistance, and  $V$  a three-way valve connecting the reservoir coil of solution either to the system, or to a refill tube. (B) Evaporative flux method. (C) Vacuum pump method.

measurement system works by analogy to a voltage divider (Horowitz and Hill, 1989). In this study's system (Fig. 1A), pressurized (0.5–0.6 MPa), degassed, filtered water was forced through a system of tubing including a high-resistance segment ( $R_T$  in Fig. 1A, a 145 cm segment of 'red' PEEK tubing, 0.125 mm internal diameter, Upchurch Scientific, Oak Harbor, WA, USA). The hydraulic resistance of tubing  $R_T$  was determined by pushing water through it at various pressures, and calculating the slope of pressure versus flow rate to a graduated cylinder on a balance ( $\pm 0.1$  mg; Mettler AG104, Mettler-Toledo GmbH, Greifensee, Switzerland). In this system (Fig. 1A), the water pushed through the tubing  $R_T$  next passed into a 350 cm coil of low-resistance tubing (Bev-A-Line IV tubing, 3.2 mm internal diameter, Cole-Parmer, Vernon Hills, IL, USA) containing fresh degassed KCl solution. The solution in turn flowed into the leaf petiole, attached to the tubing by compression fitting, eventually leaking from the stomata. A three-way valve placed before the Bev-A-Line tubing of KCl solution allowed this reservoir to be replenished with fresh degassed KCl between measurements (Fig. 1A). Transducers (Omega PX-180; Omega Engineering, Stamford, CT, USA) measure the pressure before  $R_T$  ( $P_1$ ), and after ( $P_2$ ), i.e. before entry to the leaf. The leaf hydraulic conductance can be calculated from  $P_1$  and  $P_2$ , since  $R_T$  is known, using the voltage divider equation (Fig. 1A; see Horowitz and Hill, 1989, page 8). For each leaf  $P_1$  and  $P_2$  were recorded at 2 min intervals. Due to the capacitance of both the solution-filled tubing and the leaf,  $P_2$  increases initially as the system pressurizes, before reaching a stable value. This 'pressure-charging' appears as a decrease in the calculated 'apparent conductance' (see curves in Fig. 3). The actual leaf conductance was calculated once  $P_2$  stabilized with a coefficient of variation  $<5\%$  for 10 min, which occurred after 20–60 min. The lamina was then severed at the petiole, and within 10 min  $P_2$  was again stable, and  $P_1$  and  $P_2$  were recorded to allow calculation of petiole conductance.

Leaves for HPM were exposed to high irradiance of  $>1200 \mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetically active radiation, as provided by a fibre-optic light source (Fiber-Lite Illuminator series 180; Dolan-Jenner, St Lawrence, MA, USA; measured by Li-Cor LI-250 Light Meter, Li-Cor, Lincoln, NE, USA), except when the effect of irradiance was tested. For *Q. rubra* HPM measurements were also made at ambient

irradiance,  $\sim 6 \mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetically active radiation. For four leaves, a measurement was made under ambient lighting until stable, and then the high irradiance source was switched on.

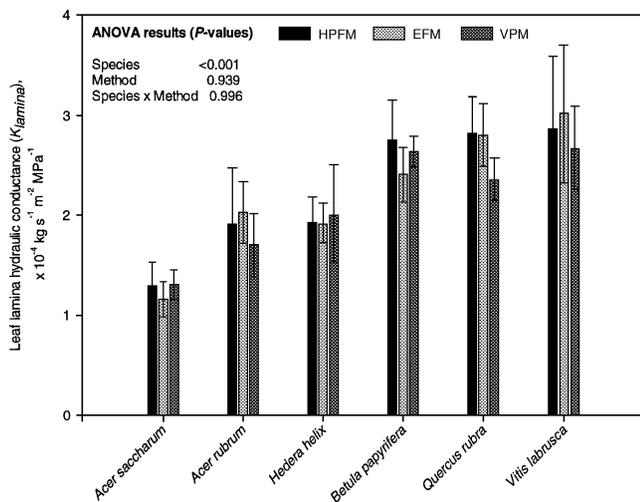
For a set of initial *Q. rubra* leaves tested, leaves were kept in a plastic bag with moist paper towel during the period in which their  $K_{\text{lamina}}$  was measured. Despite the use of a water bath to shield the leaf from the high irradiance source, the leaves heated to up to  $6^\circ\text{C}$  above ambient temperature. All subsequent leaves for HPM were submerged in a glass jar filled with water with temperature maintained within  $2^\circ\text{C}$  of ambient.

#### Evaporative flux method (EFM)

The EFM involves placing the leaf in an environment favourable to transpiration, with water uptake measured from a balance; leaf water potential ( $\psi_{\text{leaf}}$ ), representing the driving force, is determined subsequently using the pressure bomb (Boyer, 1977). In this system the leaf petiole was attached by a compression fitting to low-resistance Bev-A-Line tubing (as described above) containing solution, running to a graduated cylinder on a balance ( $\pm 0.01$  mg; Sartorius 12 MP8, Sartorius AG, Goettingen, Germany;  $\pm 0.1$  mg; Mettler AG104, Mettler-Toledo GmbH, Greifensee, Switzerland), which logged data every 60 s to a computer for the calculation of flow rate into the petiole (Fig. 1B). Leaves were supported (abaxial surface down) using a wood frame strung with fishing line, which held the leaf horizontal and immobile above a large box fan. A light source was suspended above a plexiglass container full of water above the leaf, containing waxed paper as a diffuser, producing  $>1200 \mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetically active radiation at the leaf surface while maintaining leaf temperature within  $2^\circ\text{C}$  of ambient. When the fan was turned on, water flow into the leaf increased typically for 10–30 min before stabilizing with a coefficient of variation  $<5\%$  for 10 min. For all five species, this period of 10 min was more than five times the time constant for water exchange between the xylem and the mesophyll (as determined using methods in Nobel and Jordan, 1983); the measured flow was therefore assumed to represent a steady-state flow through the lamina. Measurements were stopped if the flow suddenly began to decline, presumably due to blockage in the petiole by particles or air bubbles. The flow rate was recorded, and within 5 s the leaf was covered with a plastic bag and removed from the tubing. Before bagging the leaf, moisture was exhaled into the bag, to minimize desiccation of the bagged leaf. The leaf balancing pressure was measured with a pressure chamber (PMS Instrument Co., Corvallis, Oregon, USA). Leaf conductance was calculated as flow rate/balancing pressure.

#### Vacuum pump method (VPM)

The VPM involves pulling water through the leaf using a vacuum pump, while measuring flow rates of solution into the petiole from a balance (Fig. 1C). By using a series of partial vacuums, one can calculate the leaf conductance from the slope of the least-squares regression of flow rate into the leaf versus pressure (Kolb *et al.*, 1996; Nardini *et al.*, 2001). The apparatus is like that of the EFM method, but the leaf is sealed inside a vacuum flask with moist paper towel, with the tube of solution attached to the petiole running out through the centre of a rubber stopper to the balance. Typically five levels of partial vacuum (henceforth 'vacuum levels') were used for each leaf, between 0.017 and 0.083 MPa, in steps of  $\sim 0.017$  MPa. Generally, the maximum vacuum level was applied first, and then decreasing levels were applied; however, applying vacuum levels in different orders generally does not affect the measurement (Kolb *et al.*, 1996; Nardini *et al.*, 2001). When the first vacuum level was applied the flow typically increased for 10–30 min before stabilizing; after each subsequent change of vacuum level, values stabilized typically after 5–10 min. Readings were made after 5–10 min with a



**Fig. 2.** Means and standard errors for the  $K_{\text{lamina}}$  values determined with the high-pressure method (HPM), evaporative flux method (EFM), and vacuum pump method (VPM), all under high irradiance. For the six species, the  $n$ -values for the three methods were (5, 8, 5), (5, 5, 6), (5, 5, 6), (5, 6, 5), (11, 7, 6), and (5, 8, 5).

**Table 1.** Mean values and standard errors for lamina area, flow rate using the three methods, and driving gradient across entire leaves (petiole plus lamina) for the HPM and EFM, and leaf-area specific petiole conductivity ( $K_{\text{petiole}}$ ) for leaves of mean lamina area; for the VPM the given flow rates are for the maximum partial vacuum

Species	LA (cm <sup>2</sup> )	Flow rates (10 <sup>-5</sup> kg s <sup>-1</sup> m <sup>-2</sup> )			Driving gradient (MPa)		$K_{\text{petiole}}$ ( $\times 10^{-3}$ kg s <sup>-1</sup> m <sup>-1</sup> MPa <sup>-1</sup> )
		HPM	EFM	VPM (maximum)	HPM	EFM	
<i>Acer rubrum</i>	57±3.1	7.7±1.4	1.4±0.21	1.5±0.22	0.51±0.04	0.11±0.003	5.47±1.52
<i>Acer saccharum</i>	57±3.2	5.0±0.8	1.3±0.21	1.7±0.21	0.51±0.01	0.15±0.004	3.12±0.33
<i>Betula papyrifera</i>	33±2.4	19.0±5.2	1.3±0.53	2.0±0.12	0.52±0.01	0.05±0.002	7.93±1.15
<i>Hedera helix</i>	60±3.6	8.1±1.2	4.9±0.56	1.7±0.32	0.50±0.01	0.46±0.089	4.59±1.61
<i>Quercus rubra</i>	81±4.8	11.9±1.1	1.5±0.33	2.7±0.35	0.46±0.01	0.04±0.001	28.6±3.49
<i>Vitis labrusca</i>	190±9.2	8.2±0.9	5.2±0.43	2.2±0.44	0.34±0.03	0.31±0.010	10.0±6.08

coefficient of variation <5%. The temperature within the flask at the highest vacuum level applied was within 1 °C of ambient.

All VPM readings were made with the vacuum flask under the same light source and water bath described for the EFM, except during tests for the effect of this irradiance itself. For five *H. helix* leaves, after the series of vacuum levels under high irradiance used to calculate the leaf conductance, the flask was returned to the highest vacuum level, and the leaf was allowed to re-stabilize. Next, the high irradiance source was switched off, and when the leaf had re-established a stable flow rate, the series of descending vacuum levels were repeated, and flow rates recorded for a low irradiance leaf conductance.

#### Calculation of $K_{\text{lamina}}$

The absolute conductance of the lamina was calculated as the inverse of (the inverse of the whole leaf conductance minus the inverse of the petiole conductance).  $K_{\text{lamina}}$  was determined by dividing absolute conductance of the lamina by lamina area (determined by Li-Cor leaf area meter, Li-Cor, Lincoln, NE, USA). The study species are hypostomatous, so the area of only one lamina surface was used. Note that this conductance per leaf area (in units kg s<sup>-1</sup> m<sup>-2</sup> MPa<sup>-1</sup>) differs from the 'leaf-specific conductivity' often used for stem segments (in units such as kg s<sup>-1</sup> m<sup>-1</sup> MPa<sup>-1</sup>) because within leaves water flows through a network, making it impossible to normalize by the path length. All measurements were made at ambient temperatures (23±2 °C). Where leaves were heated above ambient temperature, as occurred for the HPM and EFM, as described above, the  $K_{\text{lamina}}$  values were reduced by 2% per °C above ambient, to normalize for the effects of temperature on viscosity (Weast, 1974; Yang and Tyree, 1993). The  $K_{\text{lamina}}$  values for the different species as determined by the three methods were analysed by two-way ANOVA (Minitab, Release 13.31) after log-transformation to increase homoscedasticity (Sokal and Rohlf, 1995).

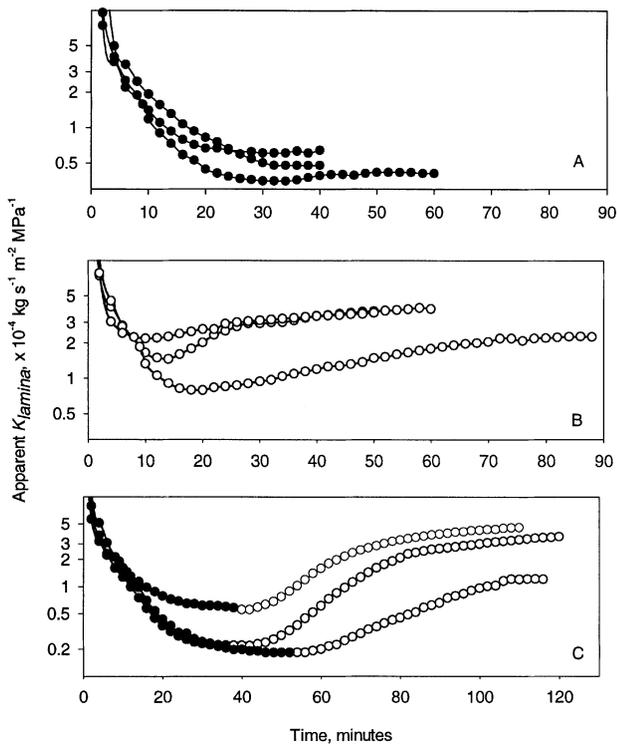
## Results

The petiole conductances for the leaves measured using the VPM and EFM were estimated from the HPM leaf petioles. For the HPM data, the regressions of petiole conductivity (petiole conductance/petiole length, in units kg s<sup>-1</sup> m) versus leaf area were significant for all species ( $n$  ranged 6 to 21;  $R^2$  ranged 0.61 to 0.90;  $P < 0.05$ ); petiole conductances could thus be estimated from lamina areas. Across species, the leaf-specific petiole conductivity ( $K_{\text{petiole}}$ ; petiole conductivity/leaf area) for leaves of

mean area (Table 1) was rank-correlated with  $K_{\text{lamina}}$  (values in Fig. 2; Spearman coefficient 0.80;  $P < 0.02$ ).

The three methods induced different magnitudes of flow rates and driving gradients (Table 1). However, the  $K_{\text{lamina}}$  values from the different methods for each given species were similar (Fig. 2), differing by 1% to 20%, but commonly by 10% or less. The differences between methods were not consistent across species, or significant (Fig. 2). The  $K_{\text{lamina}}$  values for different species varied significantly ( $P < 0.001$ ), ranging from  $1.24 \times 10^{-4}$  kg s<sup>-1</sup> m<sup>-2</sup> MPa<sup>-1</sup> for *A. saccharum* to  $2.89 \times 10^{-4}$  kg s<sup>-1</sup> m<sup>-2</sup> MPa<sup>-1</sup> for *V. labrusca*.

The HPM measurement of  $K_{\text{lamina}}$  in *Q. rubra* was sensitive to irradiance. For leaves kept in plastic bags with moist paper towel during the measurement, the  $K_{\text{lamina}}$  values for leaves under ambient irradiance were 4.6 times lower than for leaves exposed to high irradiance; the mean values ±SE were  $0.478 \pm 0.199$  and  $2.18 \pm 0.41 \times 10^{-4}$  kg s<sup>-1</sup> m<sup>-2</sup> MPa<sup>-1</sup>, respectively ( $n=4$  each;  $P < 0.01$ ; unpaired  $t$ -test). All  $K_{\text{lamina}}$  values are normalized to ambient temperature to remove the small increase of  $K_{\text{lamina}}$  that arises from viscosity changes in leaves that increased several °C above ambient (see Materials and methods). The same pattern was found for *Q. rubra* leaves kept submerged in water to control temperature variation during the measurement; leaves under ambient irradiance showed much lower  $K_{\text{lamina}}$  values than leaves exposed to high irradiance; the mean values ±SE were  $0.353 \pm 0.069$  and  $2.72 \pm 0.46 \times 10^{-4}$  kg s<sup>-1</sup> m<sup>-2</sup> MPa<sup>-1</sup>, respectively ( $n=6$  and  $7$ ;  $P < 0.001$ ; unpaired  $t$ -test; Fig. 3A, B). As further validation, leaves were measured first under ambient irradiance and then the high irradiance source was switched on. Under ambient irradiance the leaves stabilized at a mean ±SE  $K_{\text{lamina}}$  value of  $0.345 \pm 0.064 \times 10^{-4}$  kg s<sup>-1</sup> m<sup>-2</sup> MPa<sup>-1</sup>. When the high irradiance was switched on, the apparent conductance increased within 15 min, stabilizing at significantly higher values, mean ±SE  $3.02 \pm 0.669 \times 10^{-4}$  kg s<sup>-1</sup> m<sup>-2</sup> MPa<sup>-1</sup> (Fig. 3C;  $P < 0.01$ ; paired  $t$ -test). The  $K_{\text{lamina}}$  values for *Q. rubra* determined by HPM under high irradiance were similar to those obtained using the other measurement methods (Fig. 2).

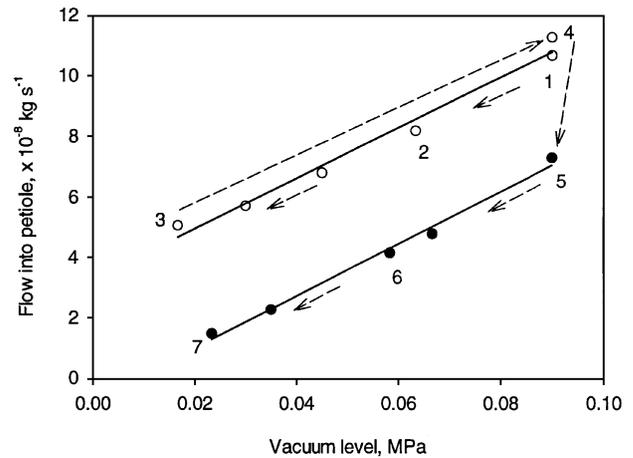


**Fig. 3.** Typical measurements of  $K_{\text{lamina}}$  using the high-pressure method for *Quercus rubra* (A) under ambient laboratory lighting ( $\sim 6 \mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetically active radiation), (B) with high irradiance ( $>1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) incident on the leaf, and (C) for leaves first under ambient lighting (filled dots), and then with high irradiance switched on (open dots). The y-axis reads 'apparent  $K_{\text{lamina}}$ ' because the measurements change through time; actual  $K_{\text{lamina}}$  is determined only when the value stabilizes.

By contrast to the HPM measurement, the VPM measurement did not fill the lamina airspaces with solution, or cause solution to drip visibly from the stomata. For *H. helix* under ambient irradiance the measured flow rates in the VPM were considerably slower than under high irradiance (Fig. 4). However, the  $K_{\text{lamina}}$  measurement was not affected.  $K_{\text{lamina}}$  determined for leaves under high and then under low irradiance was, respectively,  $1.57 \pm 0.34$  and  $1.69 \pm 0.55 \times 10^{-4} \text{ kg s}^{-1} \text{ m}^{-2} \text{ MPa}^{-1}$ , i.e. statistically identical ( $P=0.62$ ; paired *t*-test).

## Discussion

The three methods produced similar  $K_{\text{lamina}}$  values for each species, with values differing 1–20%, but commonly less than 10%. This was true despite the three methods forcing different flow rates, and thus different pressure drops across the leaf. Significant species differences were resolved, with values ranging from  $1.24 \times 10^{-4} \text{ kg s}^{-1} \text{ m}^{-2} \text{ MPa}^{-1}$  for *Acer saccharum* to  $2.89 \times 10^{-4} \text{ kg s}^{-1} \text{ m}^{-2} \text{ MPa}^{-1}$  in *Vitis labrusca*. The majority of previous studies indirectly estimate  $K_{\text{lamina}}$  from conductance measurements made on whole branches, with and without their



**Fig. 4.** Regression of flow rate versus vacuum level, determined using the vacuum pump method for measurement of  $K_{\text{lamina}}$ . A typical pattern is shown for the effect of irradiance for *Hedera helix*: steps 1–3, flow rates determined at decreasing vacuum levels under high irradiance ( $>1200 \mu\text{mol m}^{-2} \text{s}^{-1}$  incident on the leaf); step 4, the vacuum level was restored to high; step 5, the irradiance source switched off, and the flow rate restabilized; steps 5–7, flow rates again determined at decreasing vacuum levels. The high irradiance slope was  $83.2$  ( $R^2=0.98$ ;  $P < 0.001$ ); the low irradiance slope was  $86.5$  ( $R^2=0.99$ ;  $P < 0.001$ ).

leaves, using an estimation that assumes the water pathway to each leaf is parallel, independent, and equal. Such an assumption may not hold for all species (Tyree and Ewers, 1991). For example, in *A. rubrum* and *A. saccharum* the leaf-specific stem conductivity is lower for larger branches than small branches (Yang and Tyree, 1994), suggesting that the leaves are not, in fact, hydraulically autonomous. If the hydraulic architecture of the shoot is complex, with resistances in series and parallel, then estimates of  $K_{\text{lamina}}$  from whole shoot measurements may lead to values that are biased downward. The  $K_{\text{lamina}}$  values for *A. rubrum*, *A. saccharum* and *Q. rubra* in this study are, respectively, 2, 2.7 and 5.5 times higher than those found by HPM studies using branches (Tyree *et al.*, 1993; Yang and Tyree, 1994).

Low irradiance during single-leaf HPM measurement can further reduce  $K_{\text{lamina}}$ , as found here for *Q. rubra* (Fig. 3). The reduction of  $K_{\text{lamina}}$  in low irradiance might arise from the added resistance of closed stomata. The hydraulic resistance that stomatal constrictions add in series with the mesophyll can be modelled as a round plate with round pores of uniform dimensions  $8\eta d_p / \pi r_p^4 \times 1 / (LA \times SD) \times 1/\rho$  where  $d_p$  and  $r_p$  are the depth and radius of a round open stomate,  $LA$  is the lamina area,  $SD$  is the stomatal density, and  $\eta$  and  $\rho$  are the viscosity and the density of the solution (Thompson and Holbrook, 2002). Typical values for these parameters are, respectively,  $10 \mu\text{m}$  and  $5 \mu\text{m}$ ,  $80 \text{ cm}^2$ , and  $300 \text{ mm}^{-2}$  for temperate tree species, and  $1.0 \times 10^{-3} \text{ Pa s}$ , and  $1000 \text{ kg m}^{-3}$  (Jones, 1992; Kramer and Boyer, 1995). The very low  $K_{\text{lamina}}$  observed under low irradiance for *Q. rubra* may be explained by

stomatal closure under low irradiance to ~3% of maximum aperture width (or to 0.7%, if the stomata are modelled as ellipses with constant length  $l$  and variable width  $w$ ;  $64\eta d_p / \pi \times (l^2 + w^2) / l^3 w^3 \times 1 / (LA \times SD)$ ; Lewis and Boose, 1995). Such strong stomatal closure is documented in studies of detached epidermes for several species (Jarvis and Mansfield, 1980; Franks *et al.*, 1998). The HPM itself might aggravate stomatal closure in detached leaves, since pressurizing the xylem can build epidermal pavement cell pressure, which reduces stomatal aperture (Franks *et al.*, 1998; Mencuccini *et al.*, 2000). Performing the measurement on submerged leaves can also aggravate closure (Fricker *et al.*, 1991), although the low  $K_{\text{lamina}}$  under low irradiance was equally observed for leaves measured in moist air, in plastic bags. Closed stomata could explain the reported decline of apparent  $K_{\text{lamina}}$  even to extremely low values during HPM measurement on mangrove leaves under low irradiance (Melcher *et al.*, 2001). For *Q. rubra* leaves exposed to high irradiance  $K_{\text{lamina}}$  increased, and thus apparent stomatal opening occurred, even when submerged under water, as previously found for detached epidermes of *Commelina communis* (Fricker *et al.*, 1991).

The EFM is the method that most closely approximates the transpiration flow of a leaf *in vivo*. This method is the most rapid of the three, often taking 20 min or less. The greatest problem in applying this method is the need to stimulate a high enough transpiration rate to resolve the driving gradient with the pressure bomb. In expanding leaves, flow rate and  $\psi_{\text{leaf}}$  show a non-linear relation at  $\psi_{\text{leaf}}$  values as low as those in this study (Table 1): growing cells can generate strong pressure drops even as they absorb small amounts of water (Boyer, 1977). For growing leaves, therefore,  $K_{\text{lamina}}$  should be checked at a range of  $\psi_{\text{leaf}}$  values. During *in vivo* transpiration, flow rates can be much higher than those achieved for detached leaves in the laboratory, and the associated pressure drops far larger. For five leaves of *Q. rubra* sampled at midday in late August 2001, mean transpiration rate estimated by diffusion porometer  $\pm$ SE was  $1.18 \pm 0.062 \times 10^{-4} \text{ kg s}^{-1} \text{ m}^{-2}$ , and mean driving gradient, determined as the difference in pressure bomb water potential between bagged and transpiring leaves, was  $0.52 \pm 0.080 \text{ MPa}$ . The leaf conductance calculated from the transpiration rate divided by  $\Delta\psi_{\text{leaf}}$  was  $2.97 \pm 1.0 \times 10^{-4} \text{ kg s}^{-1} \text{ m}^{-2} \text{ MPa}^{-1}$  (corrected for effects of viscosity, to 25 °C). This value for the leaf conductance is similar to the  $K_{\text{lamina}}$  values found for detached leaves using the three methods (Fig. 4), although slightly higher, perhaps due to the fact that porometers often overestimate transpiration (Yang and Tyree, 1994).

The VPM measurement was expected to be insensitive to irradiance, because, for all species tested, solution did not visibly fill the lamina airspaces, or visibly drip from the stomata. The  $K_{\text{lamina}}$  measurement would therefore not be significantly affected by the stomatal constriction. The insensitivity to irradiance of the VPM measurement of

$K_{\text{lamina}}$  was confirmed for *H. helix*, despite the fact that stomata apparently closed strongly under ambient irradiance, and opened under high irradiance (Fig. 4). In the VPM, water flow through the lamina is driven both by the suction produced by the pump and the transpiration from the leaf inside the flask. The latter can be minimized by increasing the humidity in the flask with wet tissue paper (Kolb *et al.*, 1996). Nonetheless, transpiration into the flask creates a consistent driving force at different vacuum levels, i.e. it affects only the  $y$ -intercept, and not the slope of the flow versus vacuum level relationship (Fig. 4), because transpiration rate is a function of the mole fraction driving force and stomatal conductance, neither of which is affected by ambient pressure (Nobel, 1999). Irradiance effects on stomatal aperture, therefore, also affect only the  $y$ -intercept. The mole fraction of vapour in the flask apparently reaches at least an approximate steady-state, with the evaporation from the leaf and paper towel countered by the exchange of air between the flask and the outside due to the action of the pump itself.

Do the values produced by the three methods actually reflect  $K_{\text{lamina}}$  *in vivo*? The question of the pathway of liquid flow through the leaf remains complex and controversial (Boyer, 1985; Kramer and Boyer, 1995). The most obvious concern regarding the methods in this study is the degree to which the HPM and VPM, which drive flow through the leaf at higher pressures than ambient, cause the water to flow through the leaf differently than during transpiration. However, the EFM drives flow in the same way as during *in vivo* transpiration, and the  $K_{\text{lamina}}$  values found by the EFM agreed with those found by the HPM and VPM. The infiltration of airspaces in the HPM (and perhaps also to some degree, in the VPM) provides alternative pathways for water movement to those utilized during transpiration. The agreement between the three methods indicates that the mesophyll pathways that are circumvented in leaves during the HPM (and perhaps VPM) measurement would have to be of negligible resistance.

A final concern is with the EFM itself, because of its measurement of  $\psi_{\text{leaf}}$  using the pressure bomb balancing pressure. The balancing pressure corresponds to a volume-weighted average water potential, and thus is dominated by the water potential of the mesophyll and epidermis. It follows that the balancing pressure accurately represents the driving force for flow through the leaf only if there are very small water potential gradients within the leaf, that is, only if water is supplied evenly throughout the leaf, and if water is evaporating evenly from exposed cell walls. If, however, the vascular system supplies leaf regions differently, or if water preferentially evaporates from the cell walls of a relatively small population of cells, say near the stomata ('peristomatal transpiration'; Meidner, 1975), the driving gradient for flow through the lamina would arise from particular cells' very negative water potentials. This

true driving gradient would be difficult to determine, since leaf excision results in a rapid collapse of the water potential gradients throughout the lamina (Yang and Tyree, 1994). However, it has been shown that the vascular system can supply water relatively equitably through the leaf (Zwieniecki *et al.*, 2002) and, further, there are many indications that water evaporates fairly evenly through the mesophyll, both because of the large evaporative area in the leaf interior, and because the cell walls near the stomata are often cutinized (reviewed in Davies, 1986; Kramer and Boyer, 1995). Further research is needed to understand the degree to which water potential varies throughout the leaf lamina during transpiration. Generally, it is suggested that the HPM, VPM and EFM apparently measure  $K_{\text{lamina}}$  for the same pathway as transpiration, with several important associated issues remaining to be resolved.

$K_{\text{lamina}}$  (Fig. 2) is an integrated measure for all the transpiration flow paths, from the petiole–leaf junction, to the sites of evaporation, through apoplasm and symplasm. That transpired water crosses at least one layer of cells in the leaf is well supported by dye experiments showing transpired water exiting the vascular xylem into bundle sheath cells (Canny, 1990, 1995). However, some have suggested that once past the bundle sheath the transpired water might flow in and out of cells through the mesophyll (Tyree and Cheung, 1977). Membrane conductivities of plant cells reportedly range from  $1 \times 10^{-5}$  to  $2 \times 10^{-3}$   $\text{kg s}^{-1} \text{m}^{-2} \text{MPa}^{-1}$  (Maurel, 1997). Thus, the  $K_{\text{lamina}}$  values found in this study imply that the transpiration stream crosses only membranes that have conductivities at the high end of this range and/or at that crossing, the total membrane surface area crossed is large. However, an alternative view of the transpiration stream is that beyond the bundle sheath it moves through cell walls, with only growing or rehydrating tissues absorbing water into cells (Weatherley, 1963; Boyer, 1977, 1985; Kramer and Boyer, 1995). In addition, there is qualitative evidence that, in species with bundle sheath extensions, some of the water exiting the veins moves through this tissue, apoplastically or symplastically, to the epidermis (Wylie, 1943; Sheriff and Meidner, 1974). The significant species variation in  $K_{\text{lamina}}$  shown here could arise from differences in conductance of the vasculature, or from differences in the conductivities or transfer areas of the bundle sheath cell membranes, other flow path membranes, and/or the cell wall network.

There are trade-offs to consider in deciding which method to use in a given situation. The HPM requires high irradiance, if a response such as that found for *Q. rubra* is suspected. The VPM requires the longest measurement period (often >1 h), and the EFM the shortest (often <20 min), but for the EFM, attention needs to be paid to achieving high resolution of the pressure bomb water potential.

The measurement of  $K_{\text{lamina}}$  has many applications. The  $K_{\text{lamina}}$  may predict drought responses, allowing estimation of the minimum  $\Delta\psi_{\text{leaf}}$  incurred by given transpiration rates (Matzner and Comstock, 2001). If growth stops and the stomata begin to close at given  $\psi_{\text{leaf}}$  values, then for plants of similar sensitivity, those with higher  $K_{\text{lamina}}$  will maintain function under stronger evaporative demand or reduced water supply (Tsuda and Tyree, 2000). It is noted that the  $K_{\text{lamina}}$  values in this study are maximal ones for leaves detached from plants in moist soil, and saturated overnight; the  $K_{\text{lamina}}$  might decrease diurnally or due to drying soil (Kikuta *et al.*, 1997; Nardini *et al.*, 2001), as do stem and whole-plant conductivities (Passioura and Munns, 1984; Tsuda and Tyree, 2000).

$K_{\text{lamina}}$  may also be a fundamental metric for predicting optimal water use, since it seems to correlate across species with maximum stomatal conductance (unpublished data).  $K_{\text{lamina}}$  may be correlated across species with stomatal dimensions (as suggested by the data of Aasamaa *et al.*, 2001), venation architecture, and leaf shape (Siso *et al.*, 2001), as well as with petiole hydraulics, as shown here. The correlation of petiole conductivity with leaf area for a given species, described in this study, is consistent with the classic correlation of petiole xylem cross-sectional area with leaf area (Alexandrov and Djaparidze, 1934). The rank-correlation across species of  $K_{\text{lamina}}$  and  $K_{\text{petiole}}$  is a case of matching of hydraulic capacity within the leaf.

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