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Relationship between hydraulic resistance and leaf morphology in broadleaf *Quercus* species: a new interpretation of leaf lobation

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Abstract We investigated the relationship between leaf shape and leaf hydraulic resistance in a set of broadleaf *Quercus* tree species (*Q. cerris*, *Q. frainetto*, *Q. petraea*, *Q. pyrenaica*, *Q. robur*, *Q. rubra*, *Q. velutina*). Seedlings of all the studied species were grown under uniform environmental conditions. A new high-pressure flowmeter was designed to measure leaf-blade hydraulic resistance. Leaf shape was characterised by the complexity of leaf outline which was regarded as an estimate of leaf lobation. This was done using the box-counting fractal dimension of the leaf silhouette. Leaf hydraulic resistance was negatively related to leaf lobation. It is suggested that the lower hydraulic resistance in deeply lobed leaves may constitute a mechanism for improving water balance under dry atmospheric conditions.

Keywords *Quercus* · Broadleaf trees · High-pressure flowmeter · Leaf hydraulic resistance · Fractal dimension

Introduction

Diversity in leaf shape and size among broadleaf trees is surprisingly high. This is clearly shown by leaf lobation in the genus *Quercus* (Fagaceae), which includes tree species dominant in many temperate forests in the Northern Hemisphere (Krüssmann 1986). Moreover, leaf form is related to environmental variability, mainly to climatic conditions (Brenner 1902; MacArthur 1972; Givnish 1979; Hamerlynck and Knapp 1994). However, as Vogel (1970) noted, “direct evidence” of environment/leaf shape relationship is “scanty”.

Some authors have suggested complementary concepts in order to explain leaf morphology as an adaptive trait. Firstly, lobed leaves (e.g. “sun leaves”) are more effective convection-heat dissipaters than straight-edged ones (e.g. “shade leaves”) (Vogel 1968, 1970). Secondly, leaf lobes and teeth are areas of active early-season photosynthesis in immature leaves of temperate trees (Baker-Brosh and Peet 1997). Thirdly, leaf lobation influences the interception of direct solar radiation (Niklas 1989).

Additional factors contributing to leaf shape variation can be found in the influence of hydraulic architecture on water relationships in woody plants (Yang and Tyree 1993; Tyree et al. 1999). When analysing the whole-shoot hydraulic resistance in deciduous *Quercus* species, Tyree et al. (1993) concluded that leaves were responsible for 80–90% of the resistance. This high contribution of the leaves to the whole shoot hydraulic resistance led us to explore the influence of leaf shape on the hydraulic pattern of the shoot.

In fact, a previous study about the whole hydraulic resistance in shoots of *Quercus* species (Sisó et al. 2001) suggested an influence of leaf shape on leaf hydraulic resistance. Our main objective is to study this relationship in depth for a wide spectrum of *Quercus* species.

We hypothesise that a deeper lobation should be associated with a lower leaf hydraulic resistance, which directly affects the plant’s response to water stress. A lower hydraulic resistance would allow a better water supply to mesophyll cells in spite of high water-potential differences between the leaf and the atmosphere.

In order to test this hypothesis, we studied the relationship between leaf shape, specifically leaf lobation, and shoot hydraulic resistance, for a set of broadleaf deciduous *Quercus* tree species. The selected species were chosen to represent a wide spectrum of leaf form (e.g. lobed vs straight edged), but showing at the same time a high degree of phylogenetic affinity.

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Materials and methods

Plant material

In order to study a wide range of leaf morphologies, from deeply lobed to nearly straight ones, various *Quercus* species were selected, namely: *Q. cerris*, *Q. frainetto*, *Q. petraea*, *Q. pyrenaica*, *Q. robur*, *Q. rubra* and *Q. velutina*. This selection was based on our previous experience and supported by the descriptions given in Krüssman (1986). Seeds of the species found in the Iberian Peninsula (*Q. petraea*, *Q. pyrenaica*) were provided by the El Serranillo Forest Breeding Station (Ministry of Environment, Guadalajara, Spain), while the remainder were supplied by Sandeman Seeds (The Croft, Sutton, UK). Seedlings of each species were grown in cylindrical pots in the shadehouse of the Forest Research Unit, Agriculture Research Service (Zaragoza, Spain). Climatic conditions in the shadehouse were monitored with a thermohydrograph (JRI MINIDISQUE 165-00, Jules Richard, Argenteuil, France). The mean maximum and minimum temperatures during the growing season (March–August) were 24.9°C and 10.4°C, while the relative air humidity varied between 92% and 58%.

The shadehouse was covered by a shade frame to limit light intensity to a maximum of 40% of the total external radiation. The light regime was quite regular throughout the growing period because only about 5% of the days were cloudy. Plants were watered twice a day (early morning and evening) during the entire growing period, by using micro-sprinklers fixed 1.5 m above the plants.

The cylindrical pots were filled with nutrient-free sand. Nutrient supply was ensured by using a slow-liberation fertiliser (Osmocote Plus, 5–6 months liberation at 21°C, Sierra Chemical, Milpitas, Calif.) as described by Naidu and DeLucia (1997). The fertiliser (3 g l⁻¹ substrate) was added at the start of the experiment to the top 10 cm of sand. At the end of the second growth cycle (July), 6–7 seedlings per species were randomly harvested for hydraulic and leaf morphology measurements.

In all species, the second cycle of leaf growth was similar to the first one except for *Q. cerris*. The second growth cycle was selected for all leaf measurements except for *Q. cerris*, because of its great variability of leaf lobation between the first and second growth cycles. Therefore, *Q. cerris* seedlings were grouped according to their ontogenic development: the first (*Q. cerris* with even straight-edged leaves – *Q. cerris* entire leaves) and the second growth cycle (*Q. cerris* with even more lobed leaves – *Q. cerris* lobed leaves).

Leaf morphology

Throughout this study, lobation is considered to be a measure of the degree of complexity of leaf outline (McLellan and Endler 1998). In order to measure this parameter, the fractal dimension was used as a dimensionless index for a definite scale range (Mandelbrot 1982; Vlcek and Cheung 1986; Borkowski 1999). For instance, the mean leaf area (\pm SE) was 21.0 \pm 1.0 cm². In order to determine the fractal dimension, we chose the metric approach based on the Hausdorff dimension (Edgar 1990). Leaf outlines are curves whose dimension must be a number between 1 and 2. Among the several definitions of fractal dimension, the one based on box-counting (FDb) was chosen. The length of leaf outline (u) depends on the size of the box (s , scale factor) used to measure it. Therefore, the FDb can be calculated from the slope (d) in log-log plot of the outline (u) versus precision ($1/s$):

$$\text{FDb} = 1 + d \quad (1)$$

where the slope (d) of linear regression [$\log u = d \log (1/s) + b$] is estimated by the least-squares method. The best fit was obtained by removing the smallest box sizes (s , 2s; see Alados et al. 1999). The range of the box sizes was determined by the accuracy of outline digitisation (minimum s) and figure size (maximum s).

All leaves were digitised, immediately after sampling, using a page scanner (Agfa Snapscan 1236u, Agfa-Gevaert N.V.). The images were converted to black and white and stored as Macintosh PICT

files with a constant size (512 \times 512 pixels). The FDb was computed using Fractal Dimension Calculator software (Bourke 1993).

The new high-pressure flowmeter

The leaf hydraulic resistance was estimated according to the methodology of Tyree et al. (1993, 1995), which is based on the perfusion of water at a given pressure into the base of a whole shoot while, simultaneously, recording the flow in a quasi-steady state mode (Nardini and Tyree 1999). However, the system we used for recording the flow differed from that described by these authors.

The apparatus shown in Fig. 1 is similar to a syringe pump (see also Sisó et al. 2001). In this kind of device, the measurement of plunger displacement (X) and the syringe cross-section (S) allows the dispensed volume to be calculated ($V = X S$). If the time elapsed (t) is recorded simultaneously, a flow rate (F) can be calculated ($F = V t^{-1}$). When the resistance to flow is high enough, an almost constant pressure (P) within the syringe is reached as a result of the compressive force (E) exerted by the plunger in the syringe.

In this device, the syringe was made of a borosilicate glass tube (0.125 m long, 14 mm internal diameter, 19 mm external diameter; GT in Fig. 1) housed in a clear metacrilate block (MB). Its inner plunger (IP), of stainless steel and equipped with a silicone gasket, was connected to the piston rod of a pneumatic microcylinder (Model 1200-Pneumax, Lurano, Italy). The force exerted by the pneumatic microcylinder (PM) was adjusted by using a pressure regulator connected to an air tank. A three-way stopcock (TS) was incorporated in the circuit of the device to control the gas inflow. The plunger displacement was recorded by using a built-in electronic calliper with a resolution up to 10 μ m in a dynamic mode (Mitutoyo, Digimatic SD-M 572). This calliper (EC) was connected to a computer by an interface (Mitutoyo, Digimatic DMX-2). Specific software was designed to convert displacement data into flow values and show them along with time (flow rates every 5 s). A linear regression was fitted for flow values before and after leaf removal. As the slope was not significantly different from zero in all cases ($P < 0.05$), the flow was considered to be measured in a quasi-steady state mode. The flow value was estimated as the mean value before and after leaf removal (see Fig. 2). A compression fitting (CF) provided a tight connection of the shoot to the device. Water pressure was controlled using a manometer (MA) installed in the device-shoot junction. The use of transparent materials al-

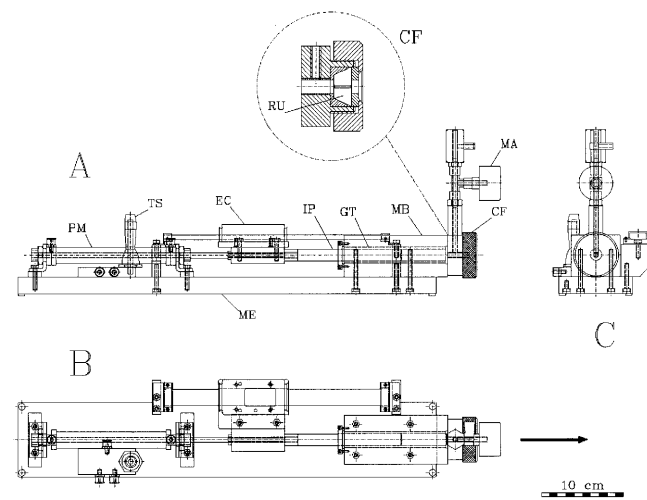


Fig. 1A–C Schematic diagram of the high-pressure flowmeter. Abbreviations of the main components of the apparatus are given in Materials and methods (The new high-pressure flowmeter). The arrow indicates the direction of water flow to the shoot. Side (A), top (B), and front (C) views are shown. The compression fitting (CF) is displayed in detail ($\times 2$) with its rubber stopper (RU)

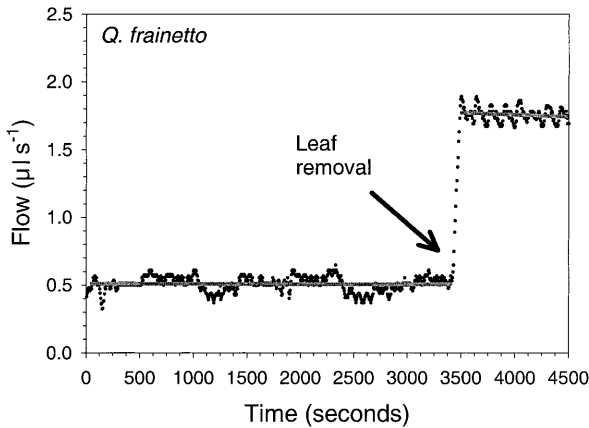


Fig. 2 Typical measurement of flow in a quasi-steady state mode of a *Quercus frainetto* shoot using the high-pressure flowmeter described in Fig. 1. The flow values for each shoot were estimated as the mean values before and after leaf removal (horizontal lines). The measurement of flow rates started when the initial flow reached stable values before leaf removal (time=0)

lowed the visual detection of any bubbles in the water, which were carefully vented prior to perfusion. Perfusion water was filtered (mesh size=0.11 µm), slightly acidified and degassed. This was done in order to prevent microorganism growth and the presence of solid particles. Non-elastic materials were used in the apparatus. The device was mounted on a metacrilate base (ME).

The flowmeter was calibrated by directing water through a rigid peak HPLC capillary tube (0.5 mm internal diameter). Flow values were kept within the next range 10^{-6} – 10^{-8} kg s⁻¹ by varying the applied pressure and the length of the capillary tube. This interval was great enough to include all flow measurements. One end of the capillary tube was connected to the flowmeter while the other end was introduced into a water container placed on a balance. Water loss due to evaporation was prevented by covering the water in the container with liquid paraffin. The flow rate was measured by observing the plunger displacement as well as the weight of water on the balance at different water pressures. The fitted functions between both flow measurements were linear and highly significant ($r^2=0.97$ – 0.99 , $P<0.001$, $n=30$).

Measurement of hydraulic resistance in *Quercus* shoots

Hydraulic resistances were measured by connecting the flowmeter to the shoot base at 0.4 MPa for 2–3 h (see Tyree et al. 1993, 1995). The initial flow rate was very high in order to infiltrate all mesophyll air spaces (usually water dripped from some stomata after this stage), and gradually decreased until reaching a stable value. Shoot normalised resistance (R_s) was calculated by applying the formula (Tyree et al. 1993):

$$R_s = P A / F \quad (2)$$

where P is water pressure (0.4 MPa), A is total leaf area per seedling measured with a leaf area meter (Delta-T Devices, Cambridge, UK), and F is stable flow.

Hydraulic resistance was determined in different seedling parts (shoot without leaves and leaf blades) by measuring resistance after removal of each part. Leaf-blade resistance (R_l) was equal to the resistance of the whole shoot (R_s) minus resistance of the shoot without leaves (R_{s-1}):

$$R_l = R_s - R_{s-1} \quad (3)$$

Resistance and leaf shape variables were compared using the non-parametric Spearman's coefficient (r_s). Mean values of several variables were compared using Mann-Whitney U test (Sokal and Rohlf 1995).

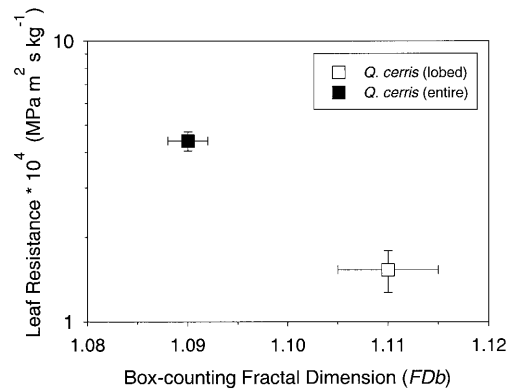


Fig. 3 Scatter plot of the mean box-counting fractal dimension and mean leaf hydraulic resistance (log scale) in *Q. cerris* according to growth cycle (first – *Q. cerris* entire; filled square – and second cycle – *Q. cerris* lobed, empty square). The error bars indicate the standard error

Results and discussion

Both inter- and intraspecific differences were found when comparing mean fractal dimension values. The species at the extremes of the fractal dimension spectrum were *Q. rubra* (straight-edged leaf) and *Q. pyrenaica* (deeply lobed leaf). In *Q. cerris*, the mean values of leaf-outline fractal dimension differed significantly ($P<0.05$) between the two growth cycles (Figs. 3, 4, 5). These results confirm the efficiency of the fractal dimension in estimating leaf outline complexity (Borkowski 1999).

The scatter of hydraulic-resistance values formed a continuous distribution, but several groups could be distinguished (Figs. 4, 5). Species with the highest leaf resistance (*Q. cerris* entire, *Q. rubra*) showed mean values almost 6 times higher than those with the lowest one (*Q. pyrenaica*, *Q. frainetto*). Intraspecific differences in hydraulic resistance were found in *Q. cerris* when its two growth cycles were taken into account (Fig. 3).

The box-counting fractal dimension of leaf outline (FDb) was significant and negatively related to leaf hydraulic resistance ($r_s = -0.75$, $r^2 = 0.56$, $n = 45$, $P < 0.001$; Fig. 4). Two opposite trends can be seen in Fig. 4. Leaves with complex outlines occupied a wide range of fractal dimension (FDb=1.11–1.16), and showed little variation in hydraulic resistance (0–1.75 10^4 MPa m² s kg⁻¹); however, leaves with less lobed margins showed greater resistance values and occupied a greater resistance range (2–5 10^4 MPa m² s kg⁻¹).

Paired interspecific comparisons of the mean fractal dimension of leaf outlines yielded three significantly different groups (Mann-Whitney U test, $P<0.05$; Fig. 5). Overall, there was a group with low fractal dimension of leaf outline and high resistance (*Q. rubra*, *Q. velutina*, *Q. cerris* entire), an intermediate group (*Q. robur*, *Q. petraea*, *Q. cerris* lobed), and a group with very lobed leaves and low hydraulic resistance (*Q. frainetto*, *Q. pyrenaica*).

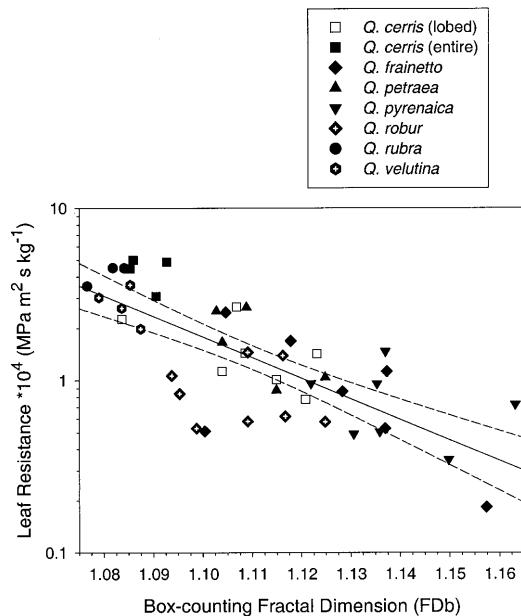


Fig. 4 Scatter plot of the relationship between the mean box-counting fractal dimension of leaf outlines (FD_b) and the hydraulic resistance (log scale) of all leaves per shoot. The inverse relationship is highly significant ($r^2=0.56$, $P<0.001$, $n=45$)

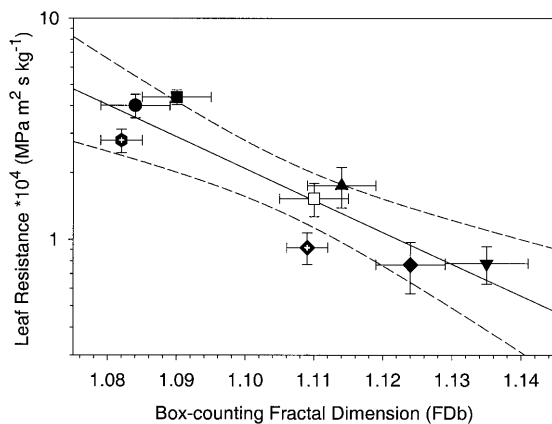


Fig. 5 Scatter plot of the relationship between the mean box-counting fractal dimension of leaf outlines (FD_b) and the hydraulic resistance (log scale) using mean values per species. The error bars are standard errors. Symbols are as in Fig. 4. The inverse relationship is significant ($r^2=0.82$, $P<0.01$, $n=8$)

The hydraulic architecture of trees is related to drought adaptation; low hydraulic resistance in the xylem increases leaf water potential, reducing water stress (Tyree and Ewers 1991). Different studies have showed that drought-adapted plants have lower hydraulic conductance with respect to water-demanding ones (e.g. Engelbrecht et al. 2000). For instance, the hydraulic architecture of deciduous broadleaf *Quercus* species has been found to vary significantly in relation to drought adaptation (Tyree et al. 1993; Nardini and Tyree 1999). Furthermore, there have been studies reporting large dif-

ferences in leaf resistance to water flow, which may greatly modify plant hydraulic resistance (Tyree et al. 1999). It is therefore expected that leaf morphology affects hydraulic resistance.

Leaf lobation can be considered as a way of reducing the mesophyll area by increasing leaf outline complexity. In leaves of similar area, such as those described in this study, this would mean a decrease in the leaf hydraulic resistance. It has been shown that leaves which are more lobed have lower hydraulic-resistance values, in agreement with our hypothesis (Figs. 4, 5).

The relationship between lobation and resistance can be interpreted as an adaptive trait. In environments where water supply can limit tree growth, leaf lobation can be a means to control water stress. It has been proven that leaf lobation is not functionally advantageous to light interception (Niklas 1989). The reduction of leaf hydraulic resistance can be interpreted as an advantageous trait under conditions that induce considerable water losses due to high transpiration flux.

From an ecological point of view, species with more lobed leaves can maintain an adequate water potential in their leaves. The case of *Q. pyrenaica*, a tree commonly found in the Iberian Peninsula, may be a good example (Aranda et al. 1996). This species grows in the nemoro-mediterranean climate, where total annual rainfall is similar to that recorded in temperate regions, but a moderate water deficit appears in the summer (Walter and Lieth 1960; Allué-Andrade 1990). During the relatively dry summer, atmospheric conditions can cause very high water potential gradients between the leaf and air. In such an environment, a water spender, sensu Levitt (1980), could prevent the development of critical water deficits by transporting water efficiently. A further decrease in leaf hydraulic resistance can enhance water supply to the transpiring leaves. The study of Gallego et al. (1994) about the ecophysiology of *Q. pyrenaica* confirms our idea, as long as these authors found a non-conservative pattern of water use in this species.

It is a common observation that lobation in oak leaves follows the pinnate pattern of the primary and secondary veins. Deeply lobed leaves experience a high reduction of the leaf blade. Therefore, a higher lobation should imply a lower leaf hydraulic resistance. The question that arises is to what extent the reduction of the leaf blade would specially affect those areas poorly supplied by water.

Anatomical and physiological studies should check this idea in the future. As an example, the relative contribution of the vascular versus the non-vascular pathway of water in the leaf should be quantified, as long as it has been found that the main hydraulic restriction in the leaf lies in the non-vascular part of the path (Tyree and Cheung 1977; Nardini and Tyree 1999).

The present study has focused on seedlings. Results for adult trees might differ from those presented here. It would also be interesting to investigate the ontogenic changes in leaf lobation and hydraulic resistance in relation to microenvironmental changes within the crown. Fu-

ture studies should also consider the relationship between leaf shape and leaf hydraulic resistance in a larger set of broadleaf temperate tree species (e.g. *Acer* or *Fagus*).

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