Effects of the experimental blockage of the major veins on hydraulics and gas exchange of *Prunus laurocerasus* L. leaves

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Abstract

The impact of leaf vein blockage on leaf hydraulic conductance (\(K_L\)), gas exchange (\(g_L\)) and water potential (\(\Psi_L\)) was studied in *Prunus laurocerasus* L., a broad-leaved evergreen. For this purpose, leaves were measured for the three variables above, either with an intact leaf blade (controls) or with the midrib cut a third of the way up (cut a), or with the midrib cut at three different points and the first-order veins cut through near their insertion to the midrib (cut b), or with the midrib cut at 2 mm from the leaf base (cut c). All the cut surfaces were sealed with cyanoacrylate. A serial decrease of \(K_L\) was recorded from cut a to cut c with respect to that measured for the controls, i.e. a \(K_L\) loss of about 37% (cut a), 57% (cut b) and 87% (cut c). A positive linear relationship appeared to exist between \(g_L\) and \(K_L\) with a high correlation coefficient (\(r^2=0.99\)) and a high statistical significance (\(P<0.01\)). Even under a severe drop in \(K_L\) (as that induced by cut c), leaf water potential remained approximately constant and not statistically different from \(\Psi_L\) measured for the controls. In fact, \(\Psi_L\) ranged between \(-0.83\) and \(-0.98\) MPa, i.e. within the cavitation threshold of leaves in terms of the critical \(\Psi_L\) inducing a significant production of ultrasound acoustic emissions which was \(-0.94 \pm 0.09\) MPa. The conclusion was that stomata were very sensitive to changes in \(K_L\) and that stomatal closure led to the homeostatic maintenance of \(\Psi_L\) and cavitation avoidance.

Key words: Cavitation, gas exchange, hydraulic conductance, leaves, major veins, *Prunus laurocerasus* L., stomatal response, water potential.

Introduction

The water status of plants is generally agreed to be well balanced when the water flow through the plant is co-ordinated with the evaporative flow from the leaves. This implies that under steady-state water flow through the plant, leaf conductance to water vapour (\(g_L\), which is a function of stomatal aperture) is co-ordinated with the hydraulic conductance of the soil-to-leaf pathway (\(K_{S-L}\), Sperry, 2000; Meinzer, 2002) or, according to Darcy’s law

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E = g_L \times VPD = K_{S-L} \times \Delta \Psi_{S-L}
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where \(E\) is the transpiration rate, \(VPD\) is the vapour pressure difference between the leaf and the external air and \(\Delta \Psi_{S-L}\) is the water potential difference between leaf and soil, i.e. the driving force for water transport through the plant. At a given \(VPD\), therefore, maximum \(g_L\) can be increased by increasing \(K_{S-L}\) or by tolerating larger \(\Delta \Psi_{S-L}\). The former variable (\(K_{S-L}\)) is a function of the conductance of three main compartments, namely the soil, the xylem (i.e. the vertical water pathway in a plant) and the extravascular water pathway (i.e. the radial water pathway) in roots and leaves (Tyree and Ewers, 1991). Recent studies have revealed that various possible mechanisms exist for short-term regulation of plant hydraulic conductance like temperature-mediated changes in water viscosity (Cochard et al., 2000; Matzner and Comstock, 2001), changes in xylem sap composition (van Ieperen et al., 2000; Zwieniecki et al., 2001) and aquaporins’ expression (Henzler et al., 1999; Morillon and Chrispeels, 2001; Javot and Maurel, 2002). However, maximum diurnal \(K_{S-L}\) is generally considered to remain relatively stable over a period of hours or a few days (Sperry, 2000; Meinzer, 2002). In turn, \(\Delta \Psi_{S-L}\) cannot be increased indefinitely, because \(K_{S-L}\) itself is a function of \(\Psi\) (Tyree and Sperry, 1989; Sperry, 2000). In fact, when xylem water

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potential drops below a critical species-specific threshold, xylem cavitation is triggered causing xylem embolism and the consequent impairment of water transport from soil to leaf (Tyree and Sperry, 1989). As a consequence, leaf gas exchange can be expected to be limited by the hydraulic conductance of the soil-to-leaf pathway and xylem vulnerability to cavitation (Bond and Kavanagh, 1999; Nardini and Salleo, 2000; Salleo et al., 2000; Sperry, 2000; Meinzer, 2002). Experimental evidence for the limitation of leaf gas exchange by plant hydraulic traits is now available for many woody and herbaceous species (Reich and Hinckley, 1989; Meinzer and Grantz, 1990; Meinzer et al., 1992; Nardini and Salleo, 2000) and experimental manipulations of root (Briggs and Wiebe, 1982) or stem hydraulic conductance (Sperry et al., 1993; Salleo et al., 2000; Matzner and Comstock, 2001) have been reported to induce parallel and rapid changes in $g_L$. However, the impact of changes in the leaf blade hydraulic conductance ($K_L$) on $g_L$ has received much less attention (Söber, 1997), despite the fact that leaves represent a significant ‘hydraulic bottleneck’ in a shoot (Yang and Tyree, 1994; Nardini and Tyree, 1999; Nardini and Salleo, 2000) and should, therefore, represent a serious limitation to leaf gas exchange.

In a previous study (Nardini et al., 2001), the hydraulic conductance of leaves of *Prunus laurocerasus* L. was measured at different leaf water potentials inducing vein cavitation as detected in terms of ultrasonic acoustic emissions (UAE) produced by the leaf blade during dehydration. The overall $K_L$ was found to remain remarkably constant at decreasing $\Psi_L$ despite the fact that the midrib had suffered up to a 70% loss of conductance. This surprising result was interpreted on the basis of a simple hydraulic model based on the hydraulic redundancy of the vein system (Nardini et al., 2001; Tyree et al., 2001). In fact, in a finely reticulate vein network like that of the leaf venous system, the blockage of one or more vein paths (Salleo et al., 2001) due to embolism or mechanical damage can be expected to have a limited impact on the overall leaf hydraulic conductance because water can bypass the damaged site easily (Roth-Nebelsick et al., 2001). This view is in accordance with Plymale and Wylie (1944) who tested the ‘wound tolerance’ of leaves of several species previously subjected to severe interruptions of their venous system. These authors found that in many cases the leaf blade was still supplied with water even in the areas downstream of the cut veins as revealed by the high survival rates of damaged leaves. These experiments, however, were not addressed to investigate the specific effects of vein blockage on leaf hydraulics or gas exchange. In the present study, measurements are reported of hydraulic conductance, stomatal conductance and water potential of *P. laurocerasus* leaves with major veins being blocked to different extents, in order to check (1) whether and to which extent severe interruptions of the major vein system induced loss of $K_L$; (2) whether and how leaf conductance to water vapour ($g_L$) was correlated to $K_L$; (3) whether the co-ordination between $g_L$ and $K_L$ resulted in some regulation of leaf water potential.

**Materials and methods**

All experiments were conducted between June and July 2002 on 1-year-old leaves of *P. laurocerasus* (a broad-leaved sclerophyllous evergreen). This species was preferred because leaves had shown large flows (Nardini et al., 2001) that made $K_L$ measurements easier. Leaves were collected from the SW-exposed part of the crown of a 30-year-old *P. laurocerasus* tree growing in the Botanical Garden of the University of Trieste (north-eastern Italy). The tree was approximately 7 m tall with a trunk 0.15 m in diameter at 1 m above the ground. The climate in the area is temperate with about 240 mm rainfall between April and June and the tree under study received no additional water supply.

**Measuring leaf blade hydraulic conductance**

One-year-old shoots bearing four to six leaves each were collected the evening before the experiments. Shoots were cut off under distilled water filtered to 0.2 μm and immediately transported to the laboratory. Shoots remained in contact with water overnight. Under these conditions, previous experiments (not reported in the present study) had shown that intact leaves reached full turgor ($\Psi_L \sim -0.02$ MPa). Moreover, preliminary experiments (not reported here) had shown that leaf blade hydraulic conductance as measured after overnight rehydration with distilled water was not significantly different from that measured immediately after leaves were harvested in the field. After shoots were put in contact with water, at least two leaves per shoot were either left intact (controls, Fig. 1) or subjected to different vein cuttings. In particular, three study cases were investigated: (a) leaves with the midrib cut a third of the way up (Fig. 1, cut a); (b) leaves with the midrib cut at three points along its length and with all the veins of the first order cut at about 4 mm from their insertion to the midrib (Fig. 1, cut b); (c) leaves with the midrib cut 2 mm from the leaf blade base (Fig. 1, cut c). Cut c had the aim of interrupting what we expected to be the main water path in a leaf. Cut b was to estimate the hydraulic conductance of a leaf where water was prevented from flowing through all the major water paths and any easy bypass of interrupted veins was impeded. Cut c had the aim of inducing an extreme limitation of water inlet into the leaf. All the cut surfaces were immediately sealed with cyanoacrylate to prevent flow. Leaves were measured for hydraulic conductance ($K_L$) 12–16 h after cutting and clogging their major veins. This procedure ensured the complete sealing of the cut surfaces.

The hydraulic conductance of leaves ($K$) was measured using the high pressure flowmeter (HPFM, Tyree et al., 1995). This instrument has been widely used for measuring the $K_L$ of different species and organs (Tyree et al., 1999; Nardini and Tyree, 1999; Nardini, 2001) and has proved to yield $K_L$ values similar to those obtained using independent methods such as the vacuum chamber technique and the evaporative flux method (Tsuda and Tyree, 2000; Nardini et al., 2001; Tyree et al., 2001; Sack et al., 2002). Briefly, leaves were connected to the HPFM using compression fittings and water was forced into the leaves at a pressure ($P$) of 0.3 MPa while recording the flow ($F$). Leaves showing leaks from the cut veins were immediately discarded. Flow readings were taken every 20 s until flow became stable, which required 20–30 min. The hydraulic conductance of the leaf blade was then calculated from $K=F/P$. At the end of experiments, leaf surface area ($A_L$) was measured using a Leaf Area Meter (LI3000A, Li-Cor Inc.) and $K$ was scaled by $A_L$. The hydraulic conductance of the soil-to-leaf pathway ($K_{SL}$) was calculated from $K_{SL} = K_{L}/g_L$. When these conditions were met, the hydraulic conductance to water vapour ($g_L$) was calculated from $g_L = F/(P \cdot A_L)$.
thus obtaining the leaf hydraulic conductance per unit leaf surface area \((K_L)\). At least seven leaves per treatment were measured.

Field measurements of leaf conductance to water vapour \((g_L)\) and water potential \((\Psi_L)\)

In order to assess the effect of the blockage of the major veins on \(g_L\) and \(\Psi_L\), leaves still attached to the tree were subjected to vein cutting as described above (Fig. 1) during the evening preceding field measurements. Leaves were measured for maximum \(g_L\) and minimum \(\Psi_L\) the next day between 12.00 h and 14.00 h (i.e. 15–18 h after cutting), when foliage was in full sunlight (photosynthetically active radiation, \(PAR\), was about 1200 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) as measured using a Quantum sensor LI190S1, Li-Cor Inc.). Leaf conductance to water vapour was measured using a steady-state porometer (LI1600, Li-Cor Inc.) equipped with a 200 mm\(^2\) leaf chamber. Measurements were taken at the distal third of the leaf lamina. Immediately after \(g_L\) had been measured, leaves were detached from the plant, wrapped in plastic film to prevent water loss and immediately measured for \(\Psi_L\) using a portable pressure chamber (3005 Plant Water Status Console, Soilmoisture). At least seven leaves per study case were measured. Intact leaves selected the evening before the experiments served as controls to check the values of \(g_L\) and \(\Psi_L\) in untreated leaves.

Measuring leaf vulnerability to cavitation

Vulnerability to cavitation of leaves was estimated on the basis of UAE production from the leaf blade during dehydration (Tyree and Dixon, 1983; Kikuta et al., 1997). Three-year-old branches bearing 80–100 leaves were cut off under distilled filtered water during the evening preceding the experiments, and immediately transported to the laboratory. They were covered with plastic bags and maintained in contact with water overnight to allow full rehydration. While branches were still in the dark, a UAE transducer (RI15I, Physical Acoustic Corp., Princeton, NJ, USA) was clamped to the proximal third of the midrib on its adaxial side and connected to a UAE counter (4615 Drought Stress Monitor, Physical Acoustic Corp.). Signals were amplified by 72 dB (52 dB by the main amplifier and 20 dB by the built-in transducer’s amplifier). While still in the dark, five to six leaves were measured for \(\Psi_L\) in order to get the base value of this variable and the absence of UAE was checked for at least 30 min. Water was then removed and branches were air dehydrated at a temperature of 22±1 °C, relative humidity of 45±5% and in the light provided by lamps (HQI-T 1000 W/D, Osram, Danvers, MA, USA) with a \(PAR\) of 350±50 \(\mu\)mol m\(^{-2}\) s\(^{-1}\). During branch dehydration, UAE were counted over periods of 60 s while \(\Psi_L\) was measured every 2 min on one leaf per time interval until \(\Psi_L\) reached about −1.5±0.1 MPa, corresponding to the minimum \(\Psi_L\) recorded in this species in summer (Nardini et al., 1996). Experiments were replicated seven times and each experiment lasted 60 to 75 min.

Results

The hydraulic conductance of leaves with an intact blade (controls, Fig. 2) turned out to be about 9.3×10\(^{-5}\) kg s\(^{-1}\) m\(^{-2}\) MPa\(^{-1}\), i.e. about the same value reported for leaves of \(P.\) laurocerasus measured using the vacuum chamber technique (Nardini et al., 2001). Leaves with the midrib cut at one-third distance from the leaf base (cut a) showed \(K_L\) values of the order of 5.9×10\(^{-5}\) kg s\(^{-1}\) m\(^{-2}\) MPa\(^{-1}\), i.e. about 36.5% less than \(K_L\) of controls. When the midrib was blocked at three different points and the first-order veins were blocked as well, \(K_L\) was further reduced to 4.0×10\(^{-5}\) kg s\(^{-1}\) m\(^{-2}\) MPa\(^{-1}\). In other words, the complete clogging of the entire major vein system resulted in about 57% loss of leaf hydraulic conductance with respect to intact leaves. As
expected, cut c (Fig. 1) caused $K_L$ to drop dramatically to only $1.2 \times 10^{-3}$ kg s$^{-1}$ m$^{-2}$ MPa$^{-1}$ with a $K_L$ loss of 87%.

Leaf conductance to water vapour measured in the field on leaves previously subjected to a blockage of veins to different extents showed a distinct and significant decrease of this variable (Fig. 3) from controls where $g_L$ was of the order of 36.3 mmol m$^{-2}$ s$^{-1}$ to 22.2 (cut a, Fig. 1), 15.7 (cut b, Fig. 1) and 8.3 (cut c, Fig. 1) mmol m$^{-2}$ s$^{-1}$. When $g_L$ values were plotted versus the corresponding $K_L$s (Fig. 3), a linear relationship appeared to exist between these two variables with a close correlation between the two ($r^2=0.987$, $P<0.01$, Pearson Product Moment Correlation). In other words, experimental reductions of $K_L$ induced proportional reductions in $g_L$.

Leaves of *P. laurocerasus* produced up to 250 UAE during air dehydration to $\Psi_L$ corresponding to minimum field values recorded in the summer. UAE counted in leaves dehydrated to different $\Psi_L$ are reported in Fig. 4 in terms of a percentage of this maximum. Taking an arbitrary number of UAE corresponding to 10% of the maximum (Salleo et al., 2000), the cavitation threshold could be estimated in terms of the critical $\Psi_L$ ($\Psi_{CAV}$) causing a consistent increase in UAE. This, turned out to be about $-0.94 \pm 0.09$ MPa (Fig. 4).

The minimum diurnal $\Psi_L$ measured in the field on leaves of the four study cases (Fig. 1) varied between $-0.83$ and $-0.98$ MPa with no statistically significant difference between controls and leaves with their major vein system blocked to different extents (Fig. 5A, one-way ANOVA). Moreover, no statistical difference was found to exist between the minimum field-recorded $\Psi_L$ and the leaf cavitation threshold ($\Psi_{CAV}$, horizontal dashed range in Fig. 5A). Despite the lack of significant differences between $\Psi_L$ measured in control and cut leaves, a general trend toward more negative values in leaves whose major veins had been blocked was observed (Fig. 5A). When $g_L$ values measured in control and cut leaves were plotted...
versus the corresponding $\Psi_L$s, a linear and statistically significant correlation was found to exist between the two variables (Fig. 5B).

**Discussion**

The impairment of the flow in the midrib and in the veins of the first order laterals caused hydraulic conductance to decrease in *P. laurocerasus* leaves. It has to be noted, however, that the interruption of the midrib at its basal third caused only about 37% loss of $K_L$ (Fig. 2), i.e. the majority of $K_L$ was conserved. This suggests that water could bypass the blocked midrib and flow within the leaf blade efficiently enough to maintain about two-thirds of the $K_L$ recorded for an intact leaf. Surprisingly, cut b implying the blockage of all the major veins (Fig. 1) resulted in a $K_L$ loss of 57% instead of the dramatically large drop expected on the basis of a leaf hydraulic architecture based on a hierarchical, serial venous system as hypothesized previously (Tyree et al., 2001). In fact, if the hydraulic architecture of leaf veins consisted of a finely branched hierarchical system where water flowed from the midrib to veins of increasing orders serially, the interruption of the midrib and that of the first-order veins should have resulted in the almost complete loss of $K_L$. Because this was not the case, alternative models of leaf hydraulic architecture have to be considered. One of these has been proposed for *P. laurocerasus* leaves by Nardini et al. (2001) and similar models have been proposed by Roth-Nebelsick et al. (2001), both based upon the high degree of redundancy of the vein system that would allow water to bypass almost any blocked vein easily, thus maintaining water flow in the leaf even in the presence of high levels of vein blockage. A second possibility might be that veins are leaky in the radial direction (Zwieniecki et al., 2002) or, in other words, that radial flow from the major veins to the living cells would be large enough to allow water to flow from the midrib into the extravascular compartment, directly. This interpretation is in accordance with the effects of cut c (Fig. 1) where the midrib was cut 2 mm from the leaf base. Here, about 13% of $K_L$ was still conserved and this suggests that some water could flow from the blocked midrib into the extravascular compartment or from the midrib into minor veins, directly. Of course, leaves are probably the most polymorphic organs among species and even in a plant (Hickey, 1979; Trivett and Pigg, 1996) so that these data cannot be generalized. Instead, experiments addressed at the evaluation of the hydraulic architecture of leaves on a taxonomic basis appear to be of great interest. A third explanation considers that leaf veins play at least two other important roles in plants, i.e. mechanical stabilization of the leaf blade (Givinish, 1979; Kull and Herbig, 1995) and transport of nutrients (Niklas, 1992; Roth-Nebelsick et al., 2001), for example, sucrose is loaded into the minor veins (Taiz and Zeiger, 1998). In this view, water transport in the leaf veins might be a collateral and not the principal (or the unique) water path in a leaf.

The capacity of *P. laurocerasus* leaves to retain over 40% of their $K_L$ in spite of the blockage of all the major veins is in accordance with the relative constancy of the hydraulic conductance of cavitated leaves of the same species (Nardini et al., 2001). In fact, the experimental

![Fig. 5. (A) Leaf water potentials ($\Psi_L$) measured in intact leaves (controls) and in leaves subjected to different cutting of the major veins (cut a to cut c, see Fig. 1) while still attached to the plant. $\Psi_L$ measurements were performed about 15 h after cutting. Vertical bars are SD of the mean ($n=7$). Note the absence of statistically significant differences among $\Psi_L$ values (n.s., one-way ANOVA). The dashed horizontal range corresponds to the leaf cavitation threshold ($\Psi_{CAV}$, mean ± SD). (B) Plot of leaf conductance to water vapour ($g_L$) versus leaf water potential ($\Psi_L$), both measured in the field, in intact and cut leaves.](https://academic.oup.com/jxb/article-abstract/54/385/1213/592769)
protocol of cutting and sealing the major veins to cause their complete and irreversible blockage, does not occur during cavitation because (1) vein cavitation has been reported not to cause complete embolization of the major veins (as recorded using leaf infiltration with fluorescein, Salleo et al., 2001) and (2) vein embolization is likely to be reversed at low transpiration (Salleo et al., 1996). Therefore, it can be assumed that if the complete blockage of all the major veins induced a $K_L$ loss of 57%, a much lower $K_L$ loss would have been recorded in the presence of the major vein system still largely functioning. Again, most of the above-cited experiments have been performed on different species with potentially different hydraulic architectures so that all the above data cannot be considered as directly comparable.

Leaf conductance to water vapour (and, hence, stomatal aperture) was apparently limited by leaf hydraulic conductance (Fig. 3). As an example, a $K_L$ loss of 37%, such as that measured for leaves with the midrib interrupted at the proximal third (cut a, Fig. 1), induced a reduction of $g_L$ of about 39% (i.e. $g_L$ decreased from 36.5 to 22.2 mmol m$^{-2}$ s$^{-1}$) and a $K_L$ loss of 57% (Fig. 3) caused an equal loss of $g_L$. This implies that stomata were quite sensitive to changes in leaf hydraulic conductance as reported by Söber (1997). On the basis of the above, the leaf (besides the root and the stem) has to be regarded as a potential site of hydraulic limitation of leaf gas exchange. Even if a leaf is able to survive extensive damage to the vein system as shown by Plymale and Wylie (1944), any $K_L$ reduction induced by mechanical damage (and perhaps by xylem embolism as well) is likely to limit CO$_2$ fixation and, hence, potential plant productivity. This conclusion has serious implications for crop species where mechanical damage to leaves is a common occurrence as a consequence of climatic events like wind and hail as well as of attacks by chewing and mining insects.

It is of interest to note that the field-recorded minimum water potential of leaves with cut veins was: (1) nearly the same as that of intact leaves and (2) in every case it remained within the $\Psi_{CAV}$ range. In other words, the decrease in $K_L$ caused a proportional decrease in $g_L$ and this, in turn, allowed the homeostasis of $\Psi_L$ even when $K_L$ was reduced by 87%. In a previous study (Salleo et al., 2000), the hypothesis was advanced that xylem cavitation might act as a hydraulic signal modulating stomatal aperture. A negative feedback was proposed to exist between stomatal conductance and $\Psi_{CAV}$ rather than between $g_L$ and bulk $\Psi_L$ per se. In fact, most stomatal responses described in the literature show poor correlation with bulk leaf water potential (Whitehead et al., 1981; Sperry and Saliendra, 1994; Meinzer et al., 1995; Meinzer, 2002). These data (Figs 4, 5) are in accordance with data reported by Salleo et al. (2000, 2001), where the water potential of leaves of Laurus nobilis L. and Ceratonia siliqua L. was found to be buffered within the $\Psi_{CAV}$ range, thus confirming the existence of a feedback linking $g_L$ to $\Psi_{CAV}$. A possible mechanistic explanation might be that stomatal aperture and transpiration rate increase in response to internal and external signals until $\Psi_L$ reaches the cavitation threshold eventually leading to some cavitation events. The loss of some xylem conduits (maybe the widest ones) would induce local $K_L$ and $\Psi_L$ drops. Turgor changes and/or $\Psi$-dependent release of chemical signals would induce partial and/or patchy stomatal closure without significant changes in the leaf bulk $\Psi_L$. In fact, the correlation between $g_L$ and $\Psi_L$, reported in Fig. 5B strongly suggests, in the authors’ opinion, that minor and/or local changes of leaf water potential which do not significantly affect bulk $\Psi_L$, might translate into a fine regulation of leaf gas exchange. In this sense, it is of interest to note that P. laurocerasus leaves underwent a 40% drop of cell turgor as a consequence of a 5% loss of symplasmic water, while bulk $\Psi_L$ was reduced by only 20% (Nardini et al., 1996). This would make leaves very sensitive to even small perturbations of water content. According to equation (1), the critical $\Psi_{CAV}$ leading to the regulation of stomatal aperture would be reached at decreasing transpiration rates consequent upon decreasing plant hydraulic conductances. While this interpretation might provide an explanation for the link between $g_L$ and $K_L$, it would also reconcile the opposing view of a $g_L$ regulation through $\Psi_L$ while bulk $\Psi_L$ remains constant.

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References


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