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ORIGINAL ARTICLE

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Changes in leaf hydraulic conductance correlate with leaf vein embolism in *Cercis siliquastrum* L.

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Abstract The impact of xylem cavitation and embolism on leaf (K_{leaf}) and stem (K_{stem}) hydraulic conductance was measured in current-year shoots of *Cercis siliquastrum* L. (Judas tree) using the vacuum chamber technique. K_{stem} decreased at leaf water potentials (Ψ_L) lower than −1.0 MPa, while K_{leaf} started to decrease only at Ψ_L <−1.5 MPa. Leaf infiltration under vacuum with Phloxine B revealed that minor veins underwent extensive embolism and became non-functional at Ψ_L <−1.5 MPa, thus indicating that leaf vein embolism was closely related to K_{leaf} changes. Field measurements of leaf conductance to water vapour (g_L) and Ψ_L showed that stomata closed when Ψ_L decreased below the Ψ_L threshold inducing loss of hydraulic conductance in the leaf. The partitioning of hydraulic resistances within shoots and leaves was measured using the high-pressure flow meter method. The ratio of leaf to shoot hydraulic resistance was about 0.8, suggesting that stem cavitation had a limited impact on whole shoot hydraulic conductance. We suggest that stomatal aperture may be regulated by the cavitation-induced reduction of hydraulic conductance of the soil-to-leaf water pathway which, in turn, strongly depends on the hydraulic architecture of the plant and, in particular, on leaf hydraulics.

Keywords Hydraulic architecture · Cavitation · Leaf · Hydraulic conductance · Stomatal conductance

Introduction

The view of xylem as a “vulnerable pipeline” (Milburn 1979) has been increasingly accepted during the last two decades (e.g. Tyree and Sperry 1989; Zwieniecki and Holbrook 1998) on the basis of several studies showing that stems (Hacke and Sauter 1995; Sparks and Black 1999), leaves (Kikuta et al. 1997; Salleo et al. 2001; Cochard 2002) and roots (Sperry and Ikeda 1997; Kavanagh et al. 1999) of both herbs and woody plants undergo xylem conduit cavitation followed by embolism and consequent blockage of conduits. Because conduit embolism impairs water transport from soil to leaf mesophyll, the vulnerability to cavitation is believed to adversely affect plant fitness and survival, although some species have convincingly proved to repair embolized conduits under low transpiration (e.g. Salleo et al. 1996; Tyree et al. 1999; Holbrook et al. 2001). Xylem cavitation has also been reported to down-regulate leaf gas exchange through stomatal closure (Sperry et al. 1993; Sperry 2000) which would help plants preventing xylem water potential to drop to increasingly negative values causing runaway embolism and catastrophic xylem dysfunction (Tyree and Sperry 1988). This “stomatal control of embolism” (Jones and Sutherland 1991) has been reported to occur in several woody species (Cochard et al. 1996; Nardini and Salleo 2000) and the hypothesis was advanced that stomatal response and xylem cavitation may be linked by a sort of negative feed back mechanism (Salleo et al. 2000). If xylem cavitation is an effective signal for short-term regulation of leaf gas exchange, stomata can be expected to respond promptly to cavitation of leaf veins (Salleo et al. 2001), because minor veins are the part of the plant vascular system nearest to stomata. Unfortunately, only a few studies have addressed the problem of the impact of vein cavitation on leaf hydraulics and gas exchange (e.g. Nardini et al. 2001; Salleo et al. 2001; Cochard 2002). Kikuta et al. (1997) surveyed the cavitation threshold of leaves of nine woody species using the acoustic technique (Tyree and Dixon 1983) and found that leaf cavitation was triggered at leaf water potentials (Ψ_L) commonly recorded in the field. However, the acoustic method like other methods proposed for measuring vein cavitation (e.g. fluorescein infiltration, Salleo et al. 2001, or cryo-scanning electron microscopy, Canny 2001) does not provide any information about the impact of vein embolism on leaf hydraulics. In other words, the above-mentioned techniques are intrinsically

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qualitative and need to be supplemented by hydraulic measurements (i.e. by measurements of changes in hydraulic conductance) in order to relate stomatal response to changes in the hydraulic properties of plant organs and/or of whole plants.

The present study reports hydraulic measurements and vulnerability to cavitation of stem and leaves of a deciduous tree, *Cercis siliquastrum* L., with the aim of checking: (1) whether leaf veins undergo cavitation at Ψ_L values commonly recorded in the field; (2) the eventual impact of leaf vein cavitation on leaf hydraulics; (3) how changes in stem and/or leaf hydraulics are related to stomatal behaviour. Because *C. siliquastrum* (Judas tree) is a Leguminous tree growing in Italy and in the Eastern Mediterranean Basin area (Davis et al. 2002) and is adapted to semi-arid climatic conditions, the vulnerability to cavitation of this species was expected to be low and stomatal response, effective.

Materials and methods

All measurements were performed between July and August 2001 on three individuals of *C. siliquastrum* over 20 years of age, growing in the Botanical Garden of the University of Trieste (north-eastern Italy), at an altitude of about 120 m. The trees were approximately 4 m tall and had a trunk diameter of about 0.4 m at 0.1 m above the ground. Between April and August, 301 mm rainfall was recorded with only 70 mm being recorded in July and August (data from the Oceanography and Meteorology Section of the Department of Earth Sciences, University of Trieste). By the time measurements started, *C. siliquastrum* plants had completed its annual growth so that all the leaves were fully expanded and anatomically mature (as revealed by serial leaf cross-sections, not shown here). All measurements were performed on branches and sun leaves collected from the SW-exposed part of the crown.

Measuring hydraulic conductance of stems and leaves

Current-year stems and whole leaves were measured for hydraulic conductance (K) using the vacuum chamber technique (Kolb et al. 1996). A detailed description of the experimental set-up can be found in Nardini et al. (2001) and further validation of the vacuum chamber technique as a tool for measuring leaf blade hydraulic conductance has been reported by Sack et al. (2002). A vacuum pump was used to reduce the pressure in the vacuum flask in 20 kPa increments. Flow readings were made at 60 s intervals at a temperature of 22±1 °C and at different pressure levels, ranging from atmospheric to 20 kPa (above vacuum) in five steps i.e. 100 kPa (= atmospheric), 80, 60, 40 and 20 kPa. Measurements started from the highest depression applied (20 kPa above vacuum) because previous work had shown that this procedure allowed the flow to become stable more promptly (Nardini et al. 2001). Volume flow rates were recorded until the flow became stable (i.e. the SD of the mean of the last ten readings was $\leq 5\%$ of the mean). The recorded flows were plotted versus the absolute pressures applied (P) and K was computed from the slope of the F to P relationship. The resulting leaf and stem hydraulic conductances were scaled by the leaf surface area (A_L, in the case of leaves) and by the A_L of all the leaves distal to the stem segment (ΣA_L, in the case of stems), thus obtaining the leaf (K_{leaf}) and stem (K_{stem}) hydraulic conductance per unit leaf surface area (Nardini et al. 2001). All A_L measurements were made using a Leaf Area Meter (LI-3000A, LiCor, Lincoln, Neb., USA).

Measurements of K_{stem} and K_{leaf} were repeated at increasing sample dehydrations to check the resulting loss of conductance. Dehydration of samples was estimated on the basis of sequential pressure bomb Ψ_L measurements made of leaves harvested from air dehydrating 3-year-old branches which had been collected in the field the evening preceding the experiments and rehydrated to near full turgor overnight. Starting from branches at near full turgor, K_{stem} and K_{leaf} were measured at Ψ_L of –0.5, −1.0, −1.5, −2.0 and −2.5 MPa.

Estimating the cavitation-induced embolism of leaf veins

The vulnerability to cavitation of the leaf minor veins was estimated in 20 leaves first dehydrated to different Ψ_L as reported above and then infiltrated with a 2% aqueous solution of Phloxine B (Sigma Aldrich). Leaf infiltration was obtained using the vacuum chamber set at a pressure P=20 kPa (above vacuum) for 15 min. Preliminary experiments had shown that this infiltration time was sufficient to obtain maximum staining of the vein network of both control (i.e. at full turgor) and water-stressed leaves. Stained veins appeared dark red. After dye perfusion, leaf images were acquired with a scanner connected to a computer. Three leaf regions with an A_L of 40–50 mm^2 each were selected from the middle part of the leaf blade and the density of the functional (red) veins was recorded in terms of the total length of stained veins divided by the surface area of the leaf sample, thus obtaining the total functional vein length per unit leaf area (mm mm^{-2}). Images were processed using SigmaScan Pro 5.0 (SPSS Science, Chicago, Ill., USA). Only third order and higher veins were measured. This is because lower order veins are likely to contain many xylem vessels; not all vessels embolize under water stress conditions and even a few functioning vessels would result in the major veins being stained thus leading to an underestimate of vein embolism.

Estimating the partitioning of hydraulic resistances within leafy shoots

The partitioning of hydraulic resistances (R) within 3-year-old shoots was estimated using the high-pressure flow meter technique (HPFM, Tyree et al. 1995). Five 3-year-old branches were collected in the field the evening preceding the experiments and rehydrated to near full turgor as described above. Branches were connected to the HPFM and perfused at a pressure of 0.3 MPa with distilled water filtered to 0.1 μm. Flow rates were recorded every 16 s until values became stable (i.e. the SD of the flows recorded during 5 min was <5% of the mean) which usually took 20–30 min. Whole shoot hydraulic resistance was calculated as the ratio of the pressure applied to the corresponding flow rate. Leaf blades were then removed and the new resistance was measured. Sequential R measurements were performed after detaching the petioles, the stems of the current-year growth and, finally 1-year-old stems. Assuming that all the above shoot compartments were arranged in series with each other (i.e. leaf blades, petioles, stems of increasing age), their R values could be calculated by difference (see Nardini and Tyree 1999). All resistances were multiplied by the ΣA_L to obtain R per unit leaf surface area.

Measuring leaf gas exchange and water potential

In order to get reference levels of leaf gas exchange and water status under natural conditions, diurnal changes of leaf conductance to water vapour (g_L) and Ψ_L were measured between July 23 and 30 for 5 clear sunny days. In particular, g_L and Ψ_L were measured every 30 min between 0630 and 1930 hours (solar time). g_L was measured on at least 15 different leaves from different plants per day time using a steady-state porometer (LI-1600, LiCor), equipped with a 200 mm^2 leaf chamber. Each measurement was completed within about 30 s while keeping the relative humidity of the air inside the chamber near to ambient. Ψ_L was measured of at least six leaves per daytime using a portable pressure chamber (Soilmoisture mod. 3005, Soilmoisture Equipment Corp., Santa Barbara, Calif., USA) with a sheet of wet filter paper inside the chamber to minimize water loss during measurement.

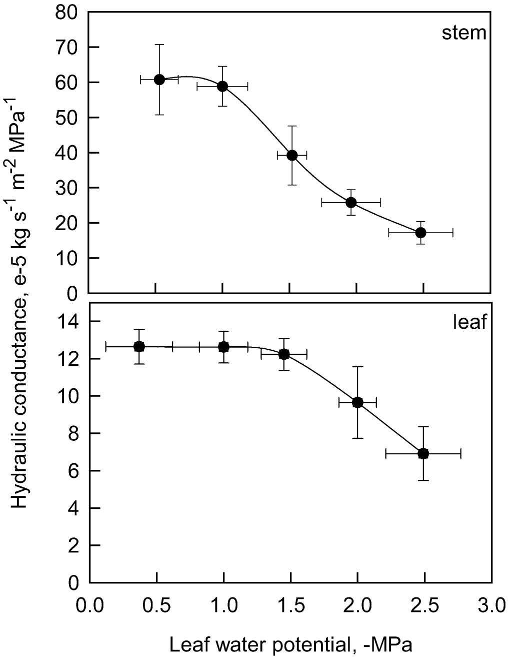

Fig. 1 Hydraulic conductance of 1-year-old stems and leaves as a function of leaf water potential. *Vertical and horizontal bars* are SD of the mean (n=5)

Results

Stem and leaf cavitation and embolism

The impact of air dehydration on the hydraulic conductance of stems and leaves is reported in Fig. 1. As dehydration proceeded (and Ψ_L decreased) both K_{stem} and K_{leaf} decreased substantially, but with different patterns and to different extents in the two organs. In particular, K_{stem} started to decrease at Ψ_L <–1.2 MPa and a 70% loss of conductance was recorded at Ψ_L =–2.5 MPa (from about 60 to about 18 e-5 kg s^{-1} m^{-2} MPa^{-1}). Meanwhile, K_{leaf} was near constant until Ψ_L dropped below –1.6 MPa and decreased from 12.7 to about 7.5 e-5 kg s^{-1} m^{-2} MPa^{-1} at Ψ_L =–2.5 MPa, i.e. leaves suffered a 44% reduction in their hydraulic conductance. Leaves infiltrated with Phloxine B (Fig. 2A) revealed that all leaf veins were still functional in leaves dehydrated up to Ψ_L =–1.5 MPa (functional vein density, FVD was 3.25 mm mm^{-2}, i.e. not different from that recorded in leaves at full turgor as well in those at Ψ_L of –0.5 and –1.0 MPa, Fig. 3). Leaves dehydrated at Ψ_L =–2.0 and –2.5 MPa, in contrast, showed a marked decrease of FVD (Fig. 2B) that dropped to 2.21 and 0.96 mm mm^{-2}, respectively (corresponding to 32% and 70% loss of FVD).

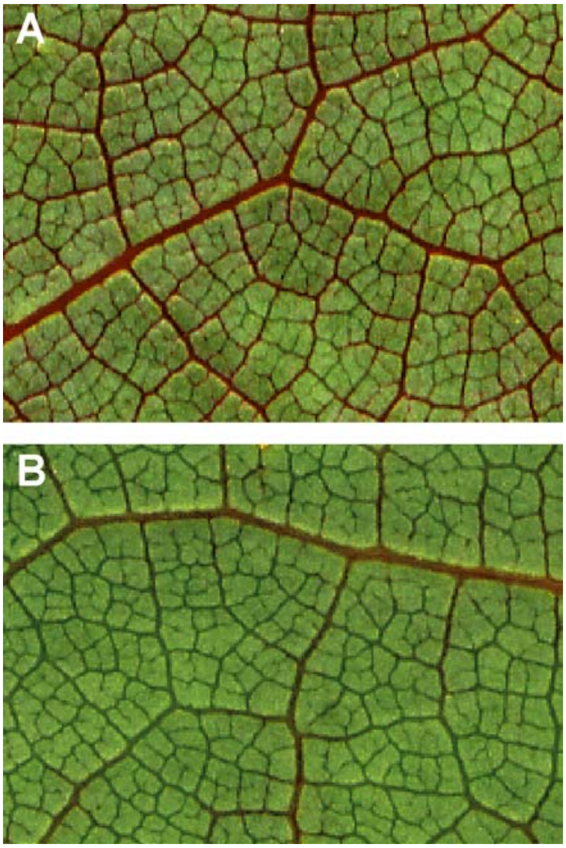

Fig. 2A, B Leaf blades infiltrated with a 2% solution of Phloxine B. **A** Leaf at Ψ_L =–0.2 MPa. Note the vein network stained with the dye. **B** Leaf at Ψ_L =–2.5 MPa. Note the numerous veins not functional. Only a few veins appear infiltrated with Phloxine B

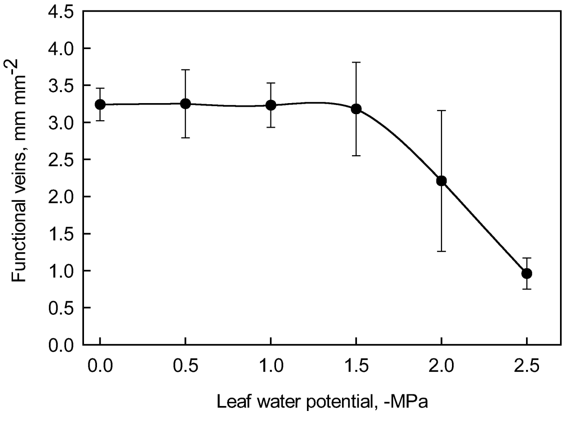

Fig. 3 Density of functional leaf veins as a function of leaf water potential, as obtained infiltrating leaves with Phloxine B. *Vertical bars* are SD of the mean (n=4)

Partitioning of hydraulic resistances within leafy shoots

The partitioning of hydraulic resistances (R) in 3-year-old shoots as measured using the HPFM, is reported in Fig. 4. Here, it can be noted that the largest fraction of the whole shoot hydraulic resistance resided in the leaf blades which, in fact, represented about 80% of the total, while both petioles and stems represented less than 10% of the total. It is worth noting that the leaf hydraulic resistance as measured using the HPFM technique was not signif-

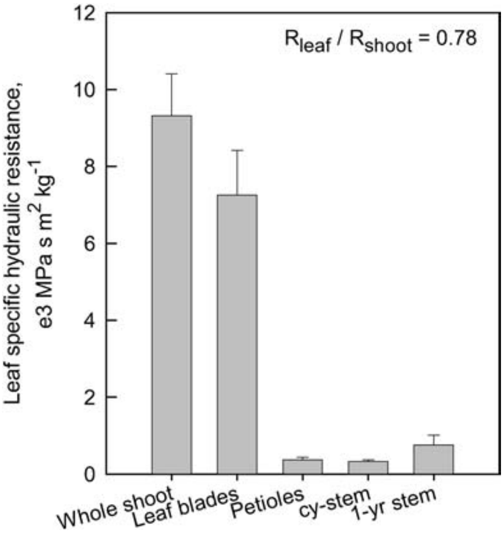

Fig. 4 Leaf hydraulic resistance scaled by leaf surface area of whole shoots and of different shoot parts including stems of the current year (*cy-stem*) and 1-year-old stems (*1-yr stem*) measured using the high pressure flow meter. Vertical bars are SD of the mean (n=5)

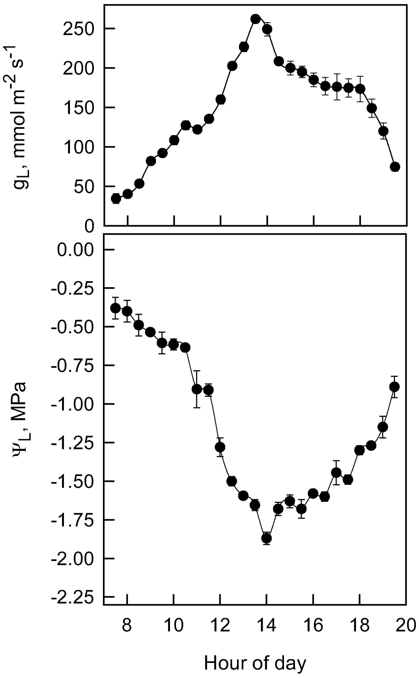

Fig. 5 Diurnal time course of leaf conductance to water vapour (g_L) and water potential (Ψ_L) in *Cercis siliquastrum* L. *Vertical bars* are SD of the mean (n=15)

icantly different from that measured using the vacuum chamber (P =0.101, one-way ANOVA). In particular, K_{leaf} was 13.77±0.86 e-5 kg s^{-1} m^{-2} MPa^{-1} (HPFM) and 12.64±0.93 e-5 kg s^{-1} m^{-2} MPa^{-1} (vacuum chamber).

Diurnal changes of g_L and Ψ_L

The diurnal time courses of g_L and Ψ_L are reported in Fig. 5. Diurnal g_L changed between 40 and 250 mmol m^{-2} s^{-1} with a peak recorded at 1400 h. Accordingly, Ψ_L decreased from about –0.35 MPa as recorded at 0630 h to about –1.89 MPa as recorded at 1400 h. This minimum diurnal Ψ_L corresponded to the onset of stomatal closure, although photosynthetically active radiation (PAR) did not decrease significantly at that time (PAR was 1000±50 μmol m^{-2} s^{-1} between 1300 and 1600 h).

Discussion

One of the objectives of the present study was to check whether stems and leaves of *C. siliquastrum* suffered cavitation and embolism at Ψ_L values that are commonly field-recorded between late spring and early summer. This was found to be the case in that field-recorded Ψ_L dropped to well below the cavitation threshold measured for stems and slightly below that for leaves (Fig. 1), i.e. to –1.89 MPa during the warmest hours of the day (Fig. 5). In particular, at the minimum diurnal Ψ_L recorded in the field, the potential loss of hydraulic conductance measured of detached stems and leaves was of the order of 50% in the stems and only 20% in the leaves. Over the entire Ψ_L range tested (up to Ψ_L =–2.5 MPa), xylem cavitation resulted in noticeable reductions of hydraulic conductance of stems and leaves, but, again, with different patterns in the two organs. In particular, stems appeared to be more vulnerable to cavitation with respect to leaves in that: (1) the Ψ_L threshold at which the loss of conductance in leaves and stems became evident was higher (Ψ_L more negative) in leaves than in stems and (2) the loss of conductance recorded at Ψ_L =–2.5 MPa was about 70% in stems and about 44% in leaves (Fig. 1). Infiltration experiments revealed that during leaf dehydration, up to 70% of leaf minor veins became non-functional at Ψ_L =–2.5 MPa (Figs. 2, 3). Similar changes in the functionality of leaf veins have been already reported by Salleo et al. (2001) for another leguminous tree (*Ceratonia siliqua* L.), but this is the first experimental demonstration that vein embolism induce changes in leaf hydraulic conductance. In fact, K_{leaf} loss and vein blockage were initiated at the same Ψ_L threshold (−1.5 MPa) and proceeded simultaneously in air-dehydrating leaves (Figs. 1, 3). The higher resistance to cavitation of leaves with respect to stems might be interpreted as the consequence of the average narrower conduit diameter in the leaf veins with respect to the stem as well as of the high degree of redundancy of the vein network in the leaf (Nardini et al. 2001). Narrow conduits, in fact, have been reported to be less vulnerable to xylem cavitation than wide ones, at least within one species (Lo Gullo et al. 1995). In turn, the presence of numerous anastomoses in the leaf vein system can be expected to allow water to bypass blocked conduits easily (Roth-Nebelsick et al. 2001), so that the embolism of even large numbers of leaf veins might have limited impact on the overall water transport in a leaf blade.

Leaves near full turgor had similar K_{leaf} values both when measured using the vacuum chamber technique and when measured using the HPFM. Sack et al. (2002) have recently shown that HPFM measurements of leaf hydraulic conductance yielded similar results when independent methods (vacuum chamber and evaporative flux) were used only when leaves were strongly illuminated (PAR =1200 μmol m^{-2} s^{-1}) in order to prevent stomatal closure. However, the authors tested this possible light effect on only one species (*Quercus rubra* L.). In our case, both HPFM and vacuum chamber measurements were performed in the laboratory under low irradiance (PAR <10 μmol m^{-2} s^{-1}); nonetheless, the two methods gave comparable K_{leaf} values, in accordance with similar results obtained on other deciduous and evergreen species (*C. siliqua*, Lo Gullo et al. 2003; *Prunus laurocerasus* L. and *Juglans regia* L.; Salleo et al., unpublished data). Therefore, light-dependence of HPFM measurements is probably not a general phenomenon, although it might be important in some species. Moreover, stomatal opening might be only one among different possible mechanisms explaining the light effect reported by Sack et al. (2002). As an example, light-mediated activation of aquaporins might be responsible for increased K_{leaf} under high irradiance (Tyree, personal communication). Clearly, our experiments do not eliminate the possibility that such a light effect would be operating in *C. siliquastrum* and we feel that more studies are needed to unravel the sources of variation in K_{leaf} measurements when different species and methods are compared (Aasamaa et al. 2001; Nardini 2001).

The diurnal time course of g_{L} and Ψ_{L} recorded in *C. siliquastrum* (Fig. 5) showed typical kinetics reported for these two variables in several mesophytes. It is well known that changes of g_{L} are partly determined by changes in incident radiation. However, the upper limit to transpiration is generally agreed to be set by the hydraulic efficiency of the water pathway between roots and leaves (Sperry 2000; Meinzer 2002). When the hydraulic conductance of this pathway was reduced experimentally or by xylem embolism, g_{L} turned out to be down-regulated, thus buffering xylem water potential above critical values leading to catastrophic xylem failure (Tyree and Sperry 1988; Sperry et al. 1993; Salleo et al. 2000). In the case of *C. siliquastrum*, however, Ψ_{L} dropped well below the cavitation threshold for stems (Fig. 1). This suggests that stomata responded to cavitation-induced changes in the hydraulic efficiency of the soil-to-leaf water pathway but were not immediately responding to stem cavitation because this did not induce any significant loss of conductance at the whole shoot level.

On the basis of HPFM measurements the ratio of leaf-to-shoot resistance was computed to be of the order of 0.8 in *C. siliquastrum*. It has to be taken into account that measurements of partitioning of resistances within the shoot by the HPFM technique involves important untested assumptions like equal and independent water supply to every leaf. Therefore, $R_{\mathrm{leaf}}/R_{\mathrm{shoot}}$ as reported in the present study has to be considered only as an estimate of the contribution of leaves to total shoot hydraulic resistance. According to Meinzer (2002), such a resistance partitioning ($R_{\mathrm{leaf}}/R_{\mathrm{shoot}}$ =0.8) would result in minor changes to the hydraulic conductance of the shoot as a whole even if in presence of stem extensive cavitation. On the basis of the simple hydraulic model reported by Meinzer (2002), we calculated that only when PLC of stem exceeded 40% could the PLC of the whole shoot be expected to increase significantly (i.e. above 10 %). In the case reported in the present study, at Ψ_{L} =−1.5 MPa, stems suffered about 30% loss of conductance (Fig. 1) which would result in less than 8% in terms of whole-shoot PLC. Such a minute loss of hydraulic conductance was apparently insufficient to induce down-regulation of stomatal conductance. In fact, g_{L} continued to increase until Ψ_{L} dropped to about −1.8 MPa, i.e. when a large number of leaf veins became non-functional and K_{leaf} started to decrease (Figs. 1, 3). On the basis of calculations as well as of measurements reported in the present study and in accordance with Meinzer (2002), we conclude that the impact of stem cavitation on plant hydraulic conductance might be of much less importance than commonly assumed at least in species with the largest fraction of shoot hydraulic resistance residing in the leaf blade. In other words, changes in leaf hydraulic conductance might have a much larger impact on plant water relations than changes in stem conductance. In turn, leaf hydraulic conductance might be a function of several biotic factors which are known to affect whole-plant hydraulic conductance like aquaporins (Kjellbom et al. 1999), abscisic acid (Morillon and Chrispeels 2001) as well as of possible cavitation fatigue (Stiller and Sperry 2002) that would influence the possible reversibility of cycles of conduit embolism and refilling. We feel, therefore, that all the recent studies on the impact of xylem embolism on leaf gas exchange cannot be the basis for generalization and should be validated by comparing laboratory measurements with the field-recorded behaviour of plants.

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