

Stomatal conductance and leaf water potential responses to hydraulic conductance variation in *Pinus pinaster* seedlings

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Received: 10 March 2006 / Revised: 23 June 2006 / Accepted: 16 January 2007
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Abstract In this study, tree hydraulic conductance (K_{tree}) was experimentally manipulated to study effects on short-term regulation of stomatal conductance (g_s), net photosynthesis (A) and bulk leaf water potential (Ψ_{leaf}) in well watered 5–6 years old and 1.2 m tall maritime pine seedlings (*Pinus pinaster* Ait.). K_{tree} was decreased by notching the stem and increased by progressively excising the root system and stem. Gas exchange was measured in a chamber at constant irradiance, vapour pressure deficit, leaf temperature and ambient CO_2 concentration. As expected, we found a strong and positive relationship between g_s and K_{tree} ($r = 0.92$, $P = 0.0001$) and between A and K_{tree} ($r = 0.9$, $P = 0.0001$). In contrast, however, we found that the response of Ψ_{leaf} to K_{tree} depended on the direction of change in K_{tree} : increases in K_{tree} caused Ψ_{leaf} to decrease from around -1.0 to -0.6 MPa, but reductions in K_{tree} were accompanied by homeostasis in Ψ_{leaf} (at -1 MPa). Both of these observations could be explained by an adaptative feedback loop between g_s and Ψ_{leaf} , with Ψ_{leaf} prevented from declining below the cavitation threshold by stomatal closure. Our

results are consistent with the hypothesis that the observed stomatal responses were mediated by leaf water status, but they also suggest that the stomatal sensitivity to water status increased dramatically as Ψ_{leaf} approached -1 MPa.

Keywords Gas exchange · Hydraulic conductance manipulation · Isohydic behavior · Leaf water status · Stomatal regulation

Introduction

Perturbations that limit water flow from roots to leaves through the xylem conduits can decrease tree hydraulic conductance (K_{tree}) and reduce the availability of water for canopy transpiration. Hydraulic limitations have been found to reduce gas exchange (Jones 1998), affect height growth (Delzon et al. 2004; Ryan et al. 2006), reduce productivity with stand age (Magnani et al. 2000), and cause partial or total foliar dieback in response to drought (Sperry et al. 2002). The analysis of hydraulic constraints can improve our understanding of how changes in K_{tree} can affect stomatal conductance and in turn limit water use and carbon gain.

In recent years, several studies have investigated the effects of directly manipulating root-to-leaf hydraulic conductance on gas exchange and bulk leaf water potential (Ψ_{leaf}), using different approaches: root pruning (Teskey et al. 1983), defoliation (Meinzer and Grantz 1990; Pataki et al. 1998), cutting transverse notches in stem (Sperry et al. 1993; Saliendra et al. 1995), root pressurization (Saliendra et al. 1995), shading lower canopy (Whitehead et al. 1996), air-injection technique (Salleo et al. 1992; Hubbard et al. 2001), and root

Communicated by H. Cochard.

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chilling (Bloom et al. 2004). These studies have suggested that the reduction in K_{tree} results in stomatal closure, while increasing K_{tree} increases stomatal conductance (g_s). Stomata should respond to changes of K_{tree} in this fashion to maintain a balance between evaporative demand and water supply, and thereby prevent water potential from declining far enough to cause runaway embolism (Tyree and Sperry 1988; Nardini and Salleo 2000). Nevertheless, the mechanism behind such a functional response of g_s to K_{tree} remains uncertain. According to Whitehead (1998), rapid and reversible short-term changes in g_s following a perturbation to the water potential gradient in the flow pathway suggest that stomata respond directly to hydrostatic signals. Indeed, a pressure change anywhere in the transpiration stream would be transmitted rapidly, at the speed of sound in water, throughout the plant (Malone 1993). Other authors (Saliendra et al. 1995; Sperry et al. 1993; Hubbard et al. 2001) have also suggested that stomata should not respond directly to changes in hydraulic conductivity, but to the consequent change in water status within the leaf. Small localized changes in leaf water status, often not detected by pressure bomb measurements, could cause a stomatal response without necessarily causing observable changes in bulk leaf water status (Sperry et al. 1993; Salleo et al. 2000; Hubbard et al. 2001). Stomata should be capable to detect these small leaf water status changes and function like a pressure regulator that controls the flow rate and maintaining Ψ_{leaf} within a limited and safe range (Sperry et al. 2002). This mechanism of functional homeostasis is typical of isohydric species where Ψ_{leaf} is kept constant irrespective of any changes in K_{tree} and soil or air humidity (Loustau et al. 1995; Tardieu and Simonneau 1998). On the other hand, some authors claimed that leaf water status plays no role in stomatal regulation, by showing that stomata can be made to re-open under various conditions regardless of leaf water status (Fuchs and Livingston 1996; Comstock and Mencuccini 1998).

The present research focuses on how gas exchange and Ψ_{leaf} of 5–6 year old *Pinus pinaster* seedlings respond to hydraulic conductance variations induced by experimental manipulation under steady-state conditions. Although several other studies have examined the effect of decreasing K_{tree} on g_s , few compare the effects of both increasing and decreasing K_{tree} . This kind of information is useful in furthering our understanding of how hydraulic architecture and stomata are interconnected. In particular, the following questions are addressed: (1) Is stomatal sensitivity high enough to prevent extreme plant water potentials in response

to reduction in K_{tree} ? (2) Does *P. pinaster* display isohydric behavior (homeostatic maintenance of constant or nearly constant bulk leaf water potential) in response to decreased K_{tree} ?

Methods

Plant material and growing conditions

The experiments were carried out in a nursery at the University of Basilicata (Southern Italy). Seventy five *P. pinaster* seedlings of French provenance were selected with similar branching patterns and leaf area and planted in three large square pots “mini-stands” (220 cm each side), according to a square spacing design (44 × 44 cm). The seedlings ranged between 1.10 and 1.30 m tall and 5–6 years old at the time of plantation (March 2003), while the experiment was carried out during summer of the same year. Plants were watered twice a day to field capacity and exposed to ambient sunlight throughout the day with temperature and relative humidity ranging between 20–30°C and 40–60%, respectively. Nutrients were supplied once a week as ammonium nitrate. The nutrient solution contained also essential nutrients, adjusted with respect to nitrogen amounts (Ingestad 1979).

Gas exchange measurements

Stomatal conductance (g_s), transpiration (E) and net photosynthesis (A) were measured using a portable infrared gas analyzer (CIRAS 1, PP Systems, Hitchin, UK). Measurements were made on four fascicles (two needles per fascicle) on the upper crown, enclosed in a broadleaf type chamber (PLC-broad, PP System). Environmental parameters inside the chamber were kept almost constant over the experiment (CO_2 concentration = 370 $\mu\text{mol mol}^{-1}$, vapour pressure difference (D) set at 25 mbar, leaf temperature = 30°C and Photosynthetic Photon Flux (PPF) = 1,200 $\mu\text{mol m}^{-2} \text{s}^{-1}$). These parameters have been chosen in order to keep environmental conditions in the chamber similar to those in the rest of the canopy. Therefore, all measurements were performed between 11:00 a.m. and 16:00 p.m., when environmental conditions were stable (i.e., avoiding windy or cloudy day etc.).

All photosynthetic variables were expressed relative to projected needle area, which was determined using a digital camera (Casio EX-23, Casio Computer Company, LTD, Japan). Needle images were analyzed using a Corel Draw program to determine leaf area.

Leaf water potential and hydraulic conductance

Leaf water potential (Ψ_{leaf}) was measured on needles excised adjacent to fascicles enclosed in the chamber, using a Scholander pressure chamber (PMS Inc., Corvallis, OR, USA). Measurements were made at regular time intervals during the experiments (every 30 min in experiment 1, and every 10 min in experiment 2). Predawn water potential was measured before dawn on ten seedlings of each experiment (1, 2). At least three/four needles of each plant were measured. Moreover, as plants were watered twice a day and kept constantly at field capacity, it was enough to measure it only three times during each experiment (lasted approximately 25 days).

Hydraulic conductance ($\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) was estimated indirectly as:

$$K_{\text{tree}} = \frac{E}{\Psi_{\text{predawn}} - \Psi_{\text{leaf}}} \quad (1)$$

where E is leaf transpiration rate (average values per treatments), ψ_{predawn} is predawn water potential (as a proxy for soil water potential) and ψ_{leaf} is the average value of leaf water potential during the experiment. In the cases where the root system or stem was removed (cuts “-1” and “-2”), the osmotic potential of the new water source (HCl and distilled water solution at pH 2) has been taken as the reference value.

Treatments

A preliminary test on ten control plants allowed us to establish whether fascicles enclosed in the chamber over the same duration of the experiment (4–5 h) showed any systematic changes in leaf gas exchange independent of the imposed changes in hydraulic conductance. Before beginning treatments, we left fascicles to acclimate in the cuvette for at least 1.5–2.0 h or until g_s , E and A were stable. The use of long term equilibration times for determining gas exchange parameters were also chosen to minimize boundary layer conductance effects and to promote steady-state flow conditions. After gas exchange had reached equilibrium, we increased or decreased stem conductance by experimental manipulations. Hydraulic conductance was decreased by two different methods: (a) in the first experiment two successive pairs of notches in the stem (indicated in the figures as cuts 1 and 2) were made on alternating sides of the stem under each internode, starting from the root collar on eight replicates; (b) in the second experiment two successive cuts of 50 and 75% of the stem radius (indicated in the

figures as for experiment 1) were made all around the stem circumference on seven replicates.

Hydraulic conductance was increased by excising the root system (cutting the stem at the collar) and then the whole stem (cutting the shoot) in succession and immediately re-cut under water (these cuts are indicated in the figures as cuts “-1” and “-2,” respectively). During the measurements, the cut ends were kept in a solution at pH 2 of HCl and distilled filtered water. In experiment 1 the same 12 plants were subject to different treatments, while in experiment 2 pairwise measurements were made on seven control and seven treated plants (where excisions were applied). After the first excision, gas exchange variables were again allowed to stabilize for at least 1.5 h to promote a new steady-state in order to proceed to the second excision.

Results

The effect of hydraulic conductance (K_{tree}) variation was studied in order to evaluate the response of both stomatal conductance (g_s) and bulk leaf water potential (Ψ_{leaf}). A preliminary test on ten control plants was carried out to assess any possible effect of cuvette on enclosed needles and consequently on g_s over the 5 h duration of the experiment (Fig. 1). After an initial decrease, g_s stabilized within 2–2.5 h. Similar trends in g_s were also found in pairwise measurements on control plants in experiment 2 (Fig. 2). This 30% decline of g_s could be probably due to slow acclimatation of fascicles in the cuvette. Figure 1 shows also the pattern of bulk leaf water potential (Ψ_{leaf}) measured in the control experiment. In agreement with previous studies

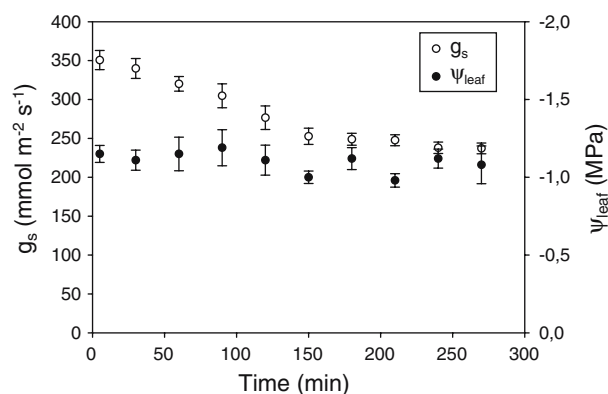


Fig. 1 Time change in stomatal conductance (g_s) and bulk needle water potential (Ψ_{leaf}) in ten control plants. Symbols are the mean values and vertical bars give the standard error on mean

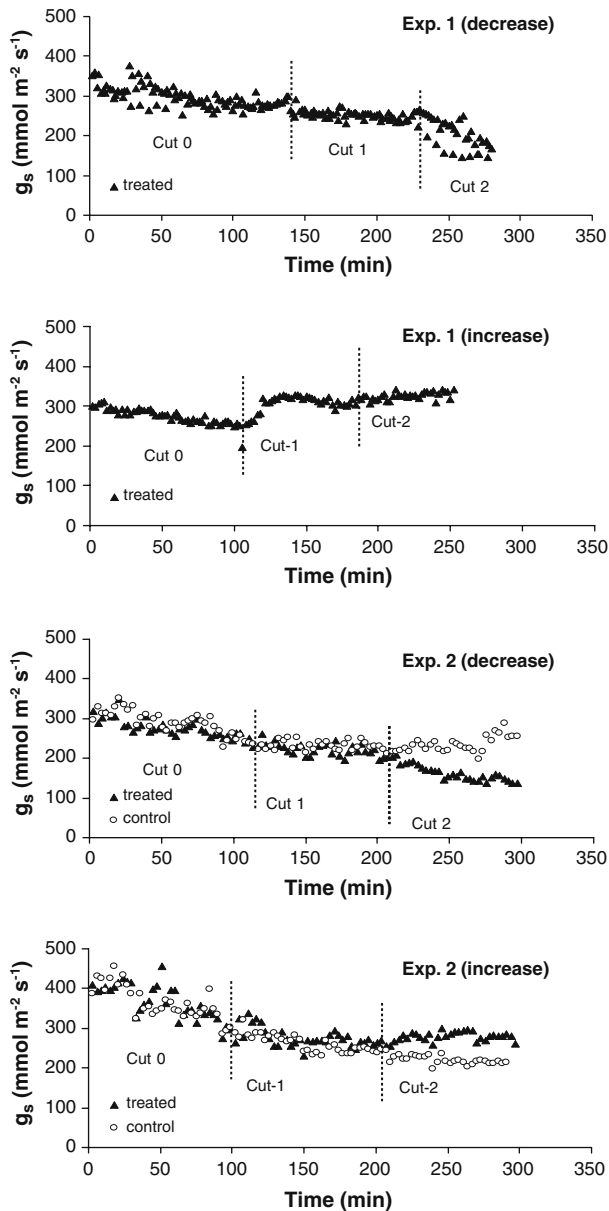


Fig. 2 Experiment 1 shows the behavior of g_s in function of the time as consequence of treatments. Each symbol gives the average value of 8 and 12 replicates for decrease and increase K_{tree} , respectively. Legend of treatments: no treatment = cut 0, first two transverse cuts in the stem = cut 1, second two transverse cuts in the stem = cut 2, roots removed = cut -1, stem removed = cut -2. Experiment 2 shows the behavior of g_s in function of the time in treated and control plants using pairwise measurements. Each symbol gives the average value of seven replicates for control, increase and decrease K_{tree} , respectively. Legend of treatments: no treatment = cut 0, 50% of stem radius all around the stem circumference = cut 1, 75% of stem radius all around the stem circumference = cut 2, roots removed = cut -1, stem removed = cut -2. Time zero represents the opening phase at the time when fascicles were enclosed in the chamber and measurements let start

(Tardieu and Simonneau 1998), *P. pinaster* displayed approximately isohydric behavior, with Ψ_{leaf} ranging only between -1.0 and -1.1 MPa.

Figure 2 shows the dynamics of g_s in response to different treatments. In both experiments we found similar trend after cuts 1 and 2: g_s decreased slightly after cut 1 and sharply after cut 2 in response to reduction in K_{tree} . In contrast, we found a different behavior of g_s in response to K_{tree} increase between the two experiments: an increase of g_s , sharp after cut -1 and slight after cut -2 in experiment 1 and a slight decrease of g_s after cut -1 and constant after cut -2 in experiment 2. Figure 3 shows the combined response of both g_s and Ψ_{leaf} when different treatments were applied. In particular, we found clear and significant (ANOVA test) decrease in g_s when treatments 1 and 2 (decrease of K_{tree}) were applied in both experiments.

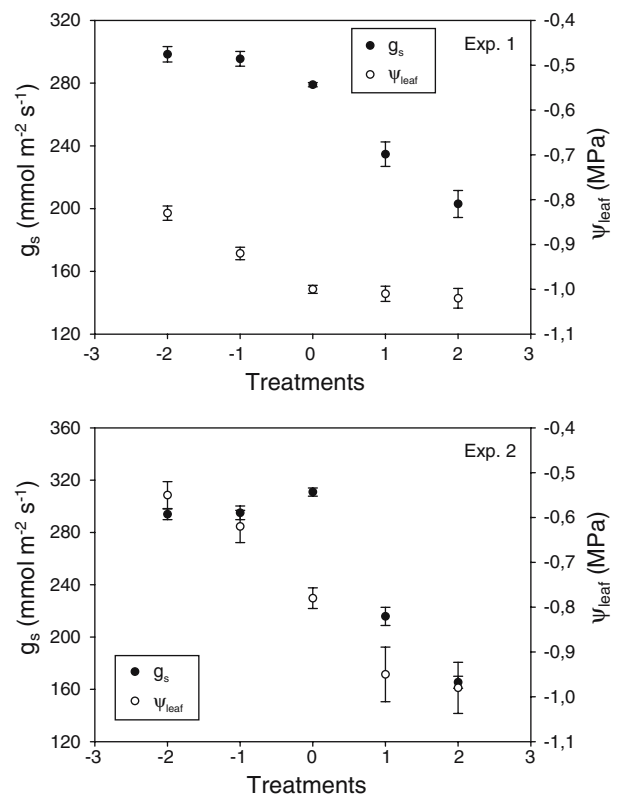


Fig. 3 Stomatal conductance (g_s) and bulk leaf water potential (Ψ_{leaf}) as a function of applied treatments: g_s , filled circles and Ψ_{leaf} , open circles. Data are means \pm S.E. ($n = 8$ for experiment 1 and $n = 7$ for experiment 2 in treatments 1 and 2; $n = 12$ for experiment 1 and $n = 7$ for experiment 2 in treatments -1 and -2). In both experiments, all values recorded in the last 50 min prior the subsequent cut were averaged for g_s and at least six values for Ψ_{leaf}

In contrast, Ψ_{leaf} was kept constant in both experiment, except at treatment 1, where Ψ_{leaf} decreased significantly (more negative value). After treatment -1 we observed a different behavior of g_s between the two experiments, with a significant increase in experiment 1 and slight decrease in experiment 2. No significant changes in g_s were observed after treatment -2 in both experiment. Ψ_{leaf} followed the same pattern in both experiment, showing a significant increase (less negative value), except at treatment -2 in experiment 2 where the increase was not significant. Stomatal conductance and net assimilation rate were both significantly and positively correlated with K_{tree} among all treatments in both experiments ($r = 0.92$, $P = 0.0001$ for g_s and $r = 0.9$, $P = 0.0004$ for A), (Fig. 4b, c). Similarly, Ψ_{leaf} increased significantly when K_{tree} was increased ($r = 0.74$, $P = 0.09$), (Fig. 4a).

Discussion and conclusions

Our results showed that *P. pinaster* displayed isohydric behavior [near homeostasis in bulk leaf water potential (Ψ_{leaf})] in response to progressively decreased tree hydraulic conductance (K_{tree}), but anisohydric behavior in response to rapidly increased K_{tree} . Moreover, our results confirmed that both stomatal conductance (g_s) and photosynthesis (A) were highly sensitive to any increase or decrease of the resistance of water transfer that is the main cause of variation in K_{tree} (Fig. 4b, c). Although g_s and K_{tree} might be auto-correlated, as both g_s and Ψ_{leaf} were used to calculate K_{tree} , A that was measured independently showed a similar strong relationship with K_{tree} . A similar high sensitivity of g_s to K_{tree} decrease has been noted previously in other species, including *Abies amabilis* (Teskey et al. 1983), woody edgerow plants (Küppers 1984), *Betula occidentalis* (Saliendra et al. 1995), sugarcane (Meinzer and Grantz 1990), *Quercus petraea* (Cochard et al. 1996), *Pinus sylvestris* (Irvine et al. 1998), Mediterranean sclerophylls and deciduous trees (Nardini and Salleo 2000), and *Pinus ponderosa* (Hubbard et al. 2001). The high sensitivity of g_s to decreased K_{tree} suggests that *P. pinaster* operates above the threshold for xylem embolism under normal conditions, and that stomatal conductance is tightly regulated to prevent reductions in Ψ_{leaf} that could induce runaway embolism.

Our results are generally consistent with the model of Buckley et al. (2003), which is based on such a feedback loop and predicts a direct relationship

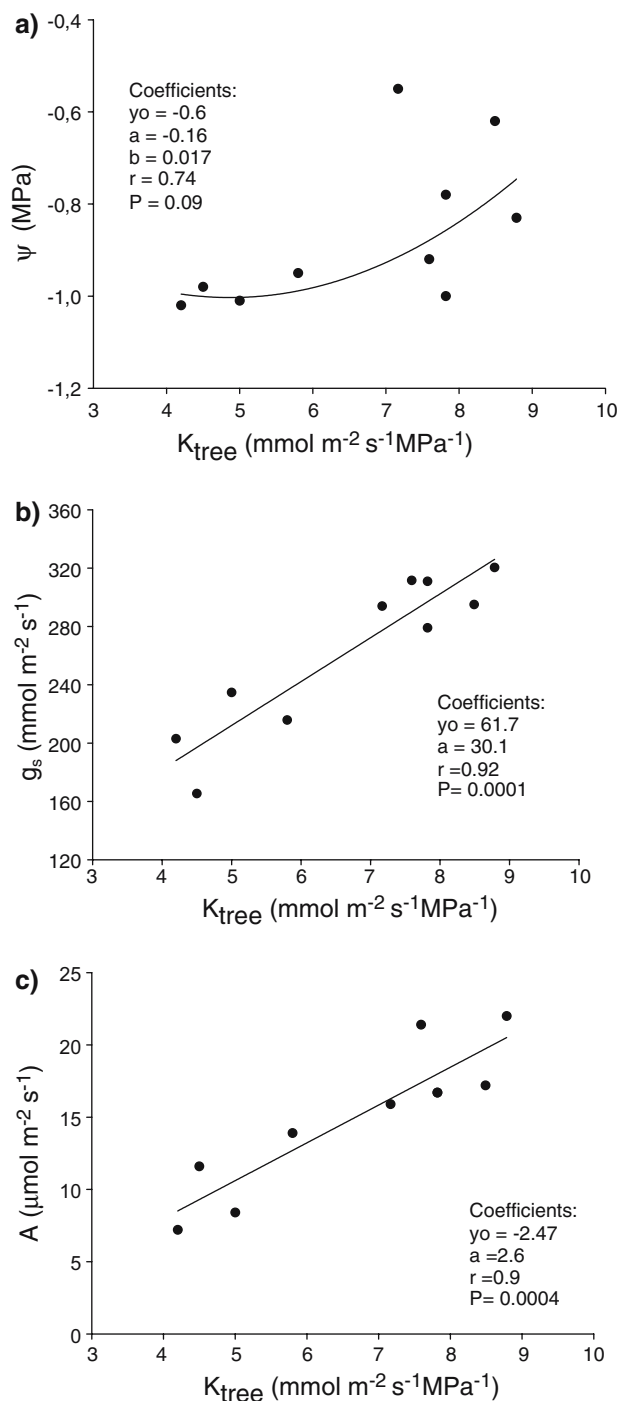


Fig. 4 Summary of results from experiments 1 and 2: **a** bulk leaf water potential (Ψ_{leaf}), **b** stomatal conductance (g_s) and **c** photosynthesis (A) as a function of hydraulic conductance (K_{tree}). Each point represents the mean: $n = 8$ for experiment 1 and $n = 7$ for experiment 2 in K_{tree} decrease; $n = 12$ for experiment 1 and $n = 7$ for experiment 2 in increase K_{tree} . In both experiments, all values recorded in the last 50 min prior the subsequent cut were averaged for g_s and A , while at least six values were averaged for Ψ_{leaf}

between g_s and K_{tree} in the steady state, similar to our observations. However, in one case (following cut -1 in experiment 2, Figs. 2, 3) g_s declined slightly in response to sudden decreased resistance of water transfer. This wrong initial response of g_s may be due to the functional link existing between floem and xylem, mediated by changes in the ionic content of the cell sap, found by some authors (Zwieniecki et al. 2001, 2004). Indeed, a decrease in xylem sap concentration following the cut -1 (phloem girdling by cut of the stem), may have induced a ion redistribution from the phloem that could be responsible of reduced increased of K_{tree} . This result may have been also due to a transient ‘wrong-way’ response, which is often observed in the minutes immediately after a change in humidity or resistance (Comstock and Mencuccini 1998), and has been attributed to sudden changes in the turgor relations between guard and epidermal cells (e.g., Cowan 1977). Following the transient, a response in the expected direction occurred after cut -2. It is also worth noting that *P. pinaster* responded within few minutes of the manipulation, in particular after the extreme shock induced by cut -1 in experiment 1 and cut 2 in both experiments (Fig. 2). In this latter, the local restriction of xylem transport caused by notching the stem induced stomata to closure in response to decreased K_{tree} . This result contrasts with the finding of Sperry et al. (1993) for *Betula occidentalis*, where decrease in g_s caused by making a transverse cut was not instantaneous, but instead occurred over a period of 20 min. According to these authors, this delay in response time to decreased K_{tree} should depend on capacitance of the leaf tissue, which would make shoots notched at mid-day more vulnerable to embolism than shoots notched in the morning, when leaf transpiration rate (E) is at a minimum. However, although in our experiment all plants were notched between 12.00 and 4.00 h, none of them showed this delay, providing further support for the idea that stomatal conductance in *P. pinaster* is very sensitively regulated to avoid embolism (Cochard et al. 1996).

Approximately isohydric behavior, with values of Ψ_{leaf} not decreasing below -1.1 MPa, could result from a sensitive and adaptative negative feedback loop between g_s and Ψ_{leaf} , that keeps values of Ψ_{leaf} above the threshold for xylem embolism and lower than reported in literature for *P. pinaster* to avoid cavitation risk. Similar findings have been previously reported on *Betula occidentalis* (Saliendra et al. 1995) where the authors under similar experimental conditions (successive cuts in the stem) have found Ψ_{leaf} constant between -1.2 and -1.3 MPa, and above 1.55 MPa corresponding to the highest Ψ predicted to induce cavitation.

Perfectly isohydric behavior, that is to say, truly constant Ψ_{leaf} , is consistent with a feedback loop between g_s and Ψ_{leaf} . Saliendra et al. (1995) noted that a decrease in Ψ_{leaf} in response to decreased K_{tree} may not be observed if the resulting stomatal response is sufficiently sensitive; pressure bomb measurements of bulk Ψ_{leaf} could mask the complex gradient of Ψ_{leaf} within the leaf to which stomata would respond. Pressure probe measurements may be able to detect such changes (Franks et al. 1998; Mott and Franks 2001). However, the precise mechanism underlying the putative feedback loop between g_s and Ψ_{leaf} is still not well understood. Some studies suggest a role for chemical signals acting from roots to leaves in the feedback loop between g_s and leaf water status (Davies and Zhang 1991; Tardieu et al. 1996). Some authors have found that the effect of root chemical signals on stomatal closure was maintained under low water soil potential (Ψ_{soil}) and high Ψ_{leaf} induced by pressurising the roots (Gollan et al. 1992; Kramer 1988; Shurr et al. 1992). Root signals can be ruled out in our experiment because the rooting system was constantly kept at field capacity in order to avoid any other induced stress. Moreover, as the response of gas exchange variables to decrease of K_{tree} occurred within few minutes, it seems unlikely that root signals have been played a role in the functional homeostasis because of long transport time from root to the stomata, that would make root-signalling ineffective for short-term stomatal regulation (Pierce and Raschke 1980; Saliendra et al. 1995). Whereas, in the case of induced K_{tree} increase, the root system was completely removed.

Other explanations could seem more likely implying an active role of leaf chemical signals alternative to root signals (Pierce and Raschke 1980; Sperry et al. 1993; Cutler and Krochko 1999; Dewar 2002; Buckley et al. 2003).

According to the hydromechanical and biochemical model proposed by Buckley et al. (2003), positive feedback between g_s and K_{tree} is provided by active regulation of guard cell osmotic pressure in response to epidermal turgor pressure or some other measure of local water status. Other authors suggest that stomata should respond to water potential gradient from epidermal to guard cells induced immediately after perturbations in humidity (Dewar 2002); however, that mechanism does not predict a response to changes in K_{tree} . Dewar proposed that stomata respond to K_{tree} by way of its effects on epidermal water potential, as in the model of Buckley et al. (2003), which alters the sensitivity of guard cells to ABA (Tardieu et al. 1993; Tardieu and Simonneau 1998; Salleo et al. 2000). Notably, both of these models attribute the response of

g_s to K_{tree} to a biochemically-mediated response to epidermal water status. Although several experimental evidence have been reporting strong relationship between g_s and ABA, we are still far from a complete understanding of the mechanism that regulate stomata in response to ABA concentration.

In synthesis, our results show that gas exchange in *P. pinaster* is strongly affected by changes in K_{tree} , and that high sensitivity of stomatal conductance to K_{tree} maintains near-homeostasis in Ψ_{leaf} , possibly indicating a sensitive embolism-avoidance strategy. We confirm the hypothesis raised by some other authors that g_s responds mainly to changes in leaf water status induced by manipulation of K_{tree} . However, the conclusions drawn here are largely preliminary. Further studies and more defined work are needed to test the hypothesis that two parallel modes of stomatal regulation could be required to explain rapid responses of g_s to changes in hydraulic conductance: one involving leaf water status, and another involving ABA. We envisage stomatal responses to ABA, perhaps already resident in leaves, further modulates hydraulic effects by ‘tuning’ stomatal sensitivity at the changes in leaf water status.

Acknowledgments This research was granted by the COFIN-2003 project (Drought and Mediterranean forests: stomatal mechanisms in the regulation of plant gas exchanges) funded by the Italian M.I.U.R. The technical assistance in field work of A. Lapolla is gratefully acknowledged. We thank Prof. Paul Kriedman and Dr. Tom Buckley for helpful comments and valuable criticism of the manuscript. We would like also to thank the Regione Campania (Vivaio Pino Amato—Baia e Latina—Caserta) for the gift of *Pinus pinaster* plants utilized in this research.

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