

# Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine

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## Abstract

Recent work has shown that stomatal conductance ( $g_s$ ) and assimilation ( $A$ ) are responsive to changes in the hydraulic conductance of the soil to leaf pathway ( $K_L$ ), but no study has quantitatively described this relationship under controlled conditions where steady-state flow is promoted. Under steady-state conditions, the relationship between  $g_s$ , water potential ( $\Psi$ ) and  $K_L$  can be assumed to follow the Ohm's law analogy for fluid flow. When boundary layer conductance is large relative to  $g_s$ , the Ohm's law analogy leads to  $g_s = K_L (\Psi_{\text{soil}} - \Psi_{\text{leaf}})/D$ , where  $D$  is the vapour pressure deficit. Consequently, if stomata regulate  $\Psi_{\text{leaf}}$  and limit  $A$ , a reduction in  $K_L$  will cause  $g_s$  and  $A$  to decline. We evaluated the regulation of  $\Psi_{\text{leaf}}$  and  $A$  in response to changes in  $K_L$  in well-watered ponderosa pine seedlings (*Pinus ponderosa*). To vary  $K_L$ , we systematically reduced stem hydraulic conductivity ( $k$ ) using an air injection technique to induce cavitation while simultaneously measuring  $\Psi_{\text{leaf}}$  and canopy gas exchange in the laboratory under constant light and  $D$ . Short-statured seedlings (< 1 m tall) and hour-long equilibration times promoted steady-state flow conditions. We found that  $\Psi_{\text{leaf}}$  remained constant near  $-1.5$  MPa except at the extreme 99% reduction of  $k$  when  $\Psi_{\text{leaf}}$  fell to  $-2.1$  MPa. Transpiration,  $g_s$ ,  $A$  and  $K_L$  all declined with decreasing  $k$  ( $P < 0.001$ ). As a result of the near homeostasis in bulk  $\Psi_{\text{leaf}}$ ,  $g_s$  and  $A$  were directly proportional to  $K_L$  ( $R^2 > 0.90$ ), indicating that changes in  $K_L$  may affect plant carbon gain.

**Key-words:** hydraulic conductivity; leaf specific hydraulic conductance; leaf water potential; stomatal control; stomatal regulation.

## INTRODUCTION

A large body of work has shown convincing, yet sometimes conflicting evidence that stomata respond to a variety of water relations parameters under light-saturating conditions. Response mechanisms have been investigated for humidity, transpiration and soil moisture. Evidence sug-

gests that the humidity response is actually a transpiration response (Mott & Parkhurst 1991) mediated by complex negative feedback via the water status of cells associated with the stomatal apparatus (Cowan 1995; Franks, Cowan & Farquhar 1997; Mott & Buckley 1998; see also Dewar 1995; Monteith 1995). Studies have shown that this response can occur without a change in bulk leaf water status, and that it is responsive to perturbations of leaf cell water potential whether caused by changes in transpiration rate or root pressurizing (Saliendra, Sperry & Comstock 1995; Willmer & Fricker 1996; Comstock & Mencuccini 1998). The soil moisture response is thought to involve chemical signals originating in the root system in response to soil drying, that are carried to the foliage in the transpiration stream where they induce stomatal closure (Zhang & Davies 1989; Loewenstein & Pallardy 1998; Tardieu & Simonneau 1998). However, root pressurizing experiments suggest that the soil moisture response may in some cases be similar to the humidity response, mediated by negative feedback in a population of sensing cells in the leaf that cause a stomatal response without necessarily causing observable changes in bulk leaf water status (Saliendra *et al.* 1995; Fuchs & Livingston 1996).

Less work has been done on stomatal responses to hydraulic conductance. Natural variation in whole-plant leaf specific hydraulic conductance ( $K_L$ ) with development (Meinzer & Grantz 1990; Saliendra *et al.* 1995; Mencuccini & Grace 1996; Hubbard, Bond & Ryan 1999), through partial defoliation by storm events (Oren *et al.* 2000), and between species (Meinzer *et al.* 1995) has shown  $K_L$  to be positively related to stomatal conductance. Stomata have also been found to respond to experimental manipulations of hydraulic conductance involving induction of xylem cavitation (Sperry & Pockman 1993), root pruning (Teskey, Hinckley & Grier 1983; Meinzer & Grantz 1990), notching of stem xylem (Sperry, Alder & Eastlack 1993), freeze-thawing of stems (Hammel 1967), and defoliation (Pataki, Oren & Phillips 1998; Hubbard *et al.* 1999). When it has been measured, the response occurs within minutes of the manipulation, and bulk leaf water status can remain nearly constant during the experiment (Teskey *et al.* 1983; Sperry *et al.* 1993; Saliendra *et al.* 1995).

The link between stomatal conductance ( $g_s$ ) and  $K_L$  arises from the fact that under photosynthetic conditions,

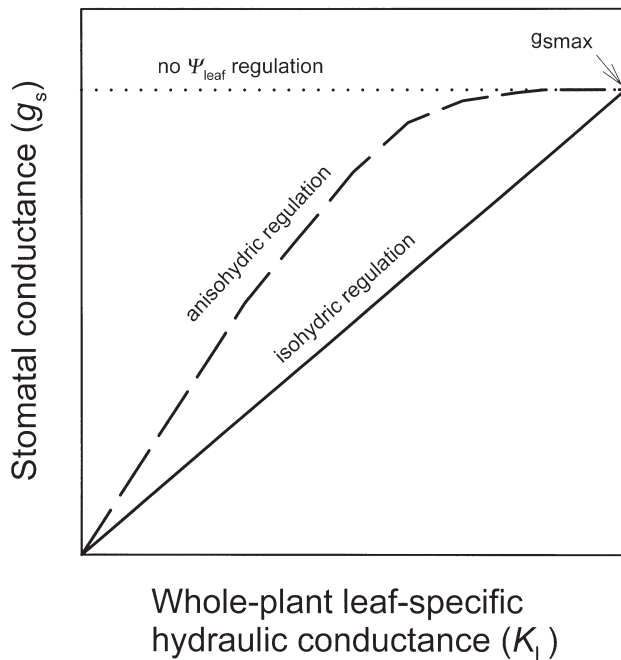
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stomata operate to enhance photosynthesis on the one hand while avoiding dehydration induced damage on the other. Such damage includes excessive cavitation and disturbance to cellular water relations and biochemistry. Consequently, stomata respond to  $K_L$  because changes in  $K_L$  influence plant water status – particularly leaf water status at the downstream end of the flow path.

The relationship between  $\Psi$ ,  $g_s$  and  $K_L$  under steady-state conditions is described by the Ohm's law analogy for fluid flow (Tyree & Ewers 1991). If boundary layer conductance is large relative to  $g_s$ , the Ohm's law analogy leads to:

$$g_s = K_L C \quad (1)$$

where  $C = \Delta\Psi/D$ . The  $\Delta\Psi$  is the water potential difference between bulk soil and leaf ( $\Psi_{\text{soil}} - \Psi_{\text{leaf}}$ ) driving the flow (assuming a negligible effect of gravity on  $\Delta\Psi$ ), and  $D$  is the vapour pressure deficit. Several possible relationships between  $K_L$  and  $g_s$  at steady-state are consistent with Eqn 1, depending on how stomata regulate  $\Psi_{\text{leaf}}$ . These are illustrated in Fig. 1 assuming constant  $\Psi_{\text{soil}}$  and  $D$  for simplicity. If stomata do not regulate  $\Psi_{\text{leaf}}$ , then there would be no



**Figure 1.** Possible steady-state responses of stomatal conductance ( $g_s$ ) to changes in whole-plant leaf-specific hydraulic conductance ( $K_L$ ) assuming (a) Ohm's law analogy for fluid flow through the soil-plant hydraulic continuum; (b) constant soil water potential; (c) constant leaf-air vapour pressure deficit; and (d) negligible influence of leaf boundary layer on leaf conductance to water vapour. Dotted line, no stomatal response associated with no regulation of bulk leaf water potential ( $\Psi_{\text{leaf}}$ ); solid line, directly proportional stomatal response for  $g_s$  below a physiological maximum ( $g_{\text{max}}$  arrow, y-axis) associated with perfect regulation of bulk  $\Psi_{\text{leaf}}$  (isohydric regulation); dashed line, curvilinear response associated with progressively stronger regulation of  $\Psi_{\text{leaf}}$  as it becomes increasingly negative with reduced  $K_L$  (anisohydric regulation).

expected response to  $K_L$  (Fig. 1, dotted line). In contrast, if they regulate  $\Psi_{\text{leaf}}$  at a constant value (isohydric regulation),  $g_s$  and  $K_L$  would be directly proportional with a slope equal to  $C$  (Fig. 1, solid line). If regulation was intermediate, gradually initiating as  $\Psi_{\text{leaf}}$  became more negative with decreasing  $K_L$  (anisohydric regulation), an intermediate curvilinear response would be expected (Fig. 1, dashed line). Previous studies have shown that  $g_s$  changes proportionally to single step changes in  $K_L$  (e.g. (Saliendra *et al.* 1995). However, no study has varied  $K_L$  systematically under controlled, steady-state flow conditions to characterize the full relationship between  $K_L$  and  $g_s$  that follows from Eqn 1 and the mode of  $\Psi_{\text{leaf}}$  regulation.

The relationship between liquid and vapour phase conductance may be part of the reason for the decline in forest productivity with age (Ryan, Binkley & Fownes 1997; Ryan & Yoder 1997). Older forests are taller forests, and  $K_L$  and  $g_s$  have been shown to decrease with increasing size and age in many species (Yoder *et al.* 1991; Saliendra *et al.* 1995; Mencuccini & Grace 1996; Hubbard *et al.* 1999; Schäfer, Oren & Tenhunen 2000). If leaf water potential is constrained from dropping with increasing tree height,  $g_s$  should decline and increasingly limit photosynthetic assimilation ( $A$ ). The fraction of  $\Delta\Psi$  required to hold water against gravity would also become significant in taller trees, reducing  $g_s$  beyond that expected from Eqn 1. Reduced  $g_s$  and  $A$  have been documented with height (Hubbard *et al.* 1999; Schäfer *et al.* 2000) yet no study has directly evaluated the response in  $A$  to manipulations of  $K_L$  under controlled conditions.

This study focuses on how stomata of ponderosa pine (*Pinus ponderosa*) seedlings respond to a systematic decrease in  $K_L$  achieved by induction of cavitation in stem xylem under controlled conditions (i.e. light,  $\Psi_{\text{soil}}$  and  $D$  held constant). Our objective was to determine which of the alternative relationships between gas exchange,  $\Psi_{\text{leaf}}$ , and  $K_L$  shown in Fig. 1 occurred in these plants. In addition, we measured the corresponding relationship between  $A$  and  $K_L$  to assess the importance of hydraulic considerations for plant carbon gain. In accomplishing these objectives, we also determined the direct relationship between gas exchange and leaf water status and the changes in stem conductivity caused by air injection. The use of conifer seedlings and long equilibration times for determining gas exchange parameters were chosen to minimize boundary layer conductance effects and to promote the steady-state flow conditions to which both Eqn 1 and Fig. 1 apply.

## METHODS

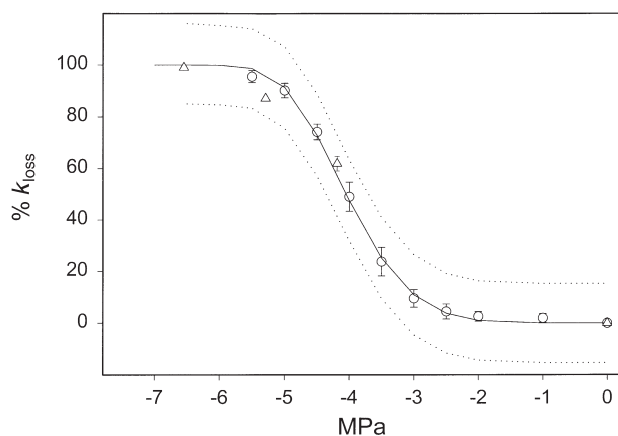
### Plant material

We selected 20 ponderosa pine seedlings with similar branching patterns and leaf area from local nurseries. The seedlings were between 0.75 and 1.0 m tall and were 4 to 5 years old. The seedlings were held in the University of Utah greenhouse and watered twice daily to avoid water stress. Plants were exposed to ambient sunlight throughout the

day with temperature and relative humidity ranging between 20 and 28 °C and 40–60%, respectively.

### Vulnerability curve

To manipulate the cavitation level in stems of intact seedlings (see Treatments) we needed to know the vulnerability curve for the stem xylem. A vulnerability curve defines the relationship between hydraulic conductivity ( $k$ ) and xylem pressure. As xylem pressure becomes more negative, cavitation events increase, causing a decrease in  $k$ . We used the centrifugal force method (Alder *et al.* 1997) to measure the vulnerability of stem xylem in six seedlings. Each seedling was cut at the root collar under water and a 142 mm segment was removed from the stem between the root collar and the first whorl of branches. The  $k$  ( $\text{kg m s}^{-1} \text{MPa}^{-1}$ ) was determined as the quotient of the mass flow rate of water through the segment ( $\text{kg s}^{-1}$ ) and the pressure gradient ( $\text{MPa m}^{-1}$ ) associated with the hydrostatic head causing flow through the segment. Conductivity differs from conductance in being a length-specific measure. Initial  $k$  ( $k_i$ ) was determined for each segment. The segment was then centred on a centrifuge rotor and spun for 3 min along its long axis. Spin rates corresponded to xylem pressures of  $-1$  and  $-2$  MPa and were then decreased by  $0.5$  MPa increments to  $-6.0$  MPa (Fig. 2). After spinning,  $k$  was re-measured for each segment and the percentage loss of  $k$  ( $k_{\text{loss}}$ ) determined as the percent decline from  $k_i$ .



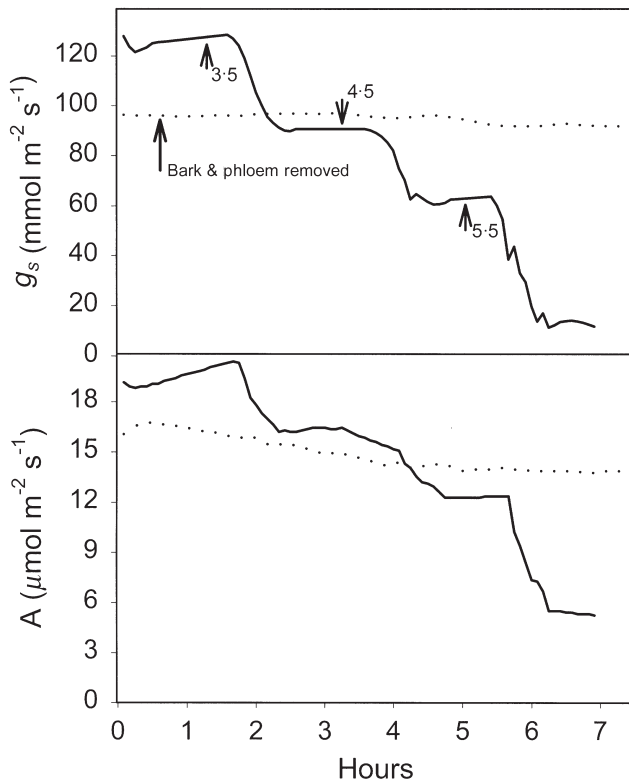
**Figure 2.** Vulnerability curve for stem xylem of ponderosa pine seedlings showing mean percentage loss of stem hydraulic conductivity ( $\%k_{\text{loss}}$ ) ( $\circ$ ,  $n = 6$ ) versus the xylem pressure ( $\Psi_{\text{xylem}}$ ). The best fit to the vulnerability curve (solid line) was a Weibull function:  $\%k_{\text{loss}} = 100 - 100 \times \exp(-(-P_{\text{total}}/b)^c)$  (see text for parameter values). Open triangles ( $\Delta$ ) represent mean percentage loss of stem hydraulic conductivity measured after air injection ( $n = 2$ ) at the actual pressure difference across the pit membrane ( $\Psi_{\text{xylem}}$  is air injection pressure). This pressure difference is equivalent to the  $\Psi_{\text{xylem}}$  axis of the vulnerability curve which assumes ambient air pressure. Dotted lines represent 95% prediction limits for the Weibull fit.

### Treatments

Each seedling was brought into the laboratory the night before treatment. The canopy of each plant was sealed into a Teflon-lined Plexiglas cuvette and fine wire-type T thermocouples were attached to five needles distributed evenly throughout the canopy. Photon flux density averaged  $1500 \text{ mmol m}^{-2} \text{ s}^{-1}$  and was supplied with two 1000 W Na-vapour HID lamps. Two fans circulated air inside the cuvette and temperature was controlled with a circulating water bath through a heat exchanger in the cuvette. We monitored  $\text{CO}_2$  and water vapour concentrations using an open gas exchange system (Field, Ball & Berry 1989). Reference and sample  $\text{CO}_2$  and water vapour concentrations were determined using two LICOR 6252 (Licor Inc., Lincoln, NE, USA) infrared gas analyzers. The  $\text{CO}_2$  concentration within the cuvette was kept near 350 p.p.m. and air saturation deficit was held at about 2.8 kPa (leaf-air mole fraction difference of approximately  $0.032 \text{ mol mol}^{-1}$ ). Boundary layer conductance of birch leaves under cuvette wind speed was previously determined to be  $1000 \text{ mmol s}^{-1} \text{ m}^{-2}$  (Saliendra *et al.* 1995). This would be even greater for needle-leaved conifers, so boundary layer conductance was assumed to have a negligible influence on total leaf conductance to water vapour. Canopy level stomatal conductance ( $g_s$ ) was determined from transpiration ( $E$ ) measurements, and canopy assimilation rate ( $A$ ) was derived from  $\text{CO}_2$  consumption. Calculations for the gas exchange parameters and a more detailed description of the gas exchange system are given in Saliendra *et al.* (1995).

An air-injection method was used (Cochard, Cruziat & Tyree 1992; Salleo *et al.* 1992; Sperry & Saliendra 1994) to induce cavitation in the stem and reduce the stem and plant hydraulic conductance. Normally, cavitation occurs when the xylem pressure drops low enough to pull air into the functional xylem conduits (the air seeding mechanism, Zimmermann 1983). In the air-injection technique, cavitation is induced by raising the air pressure around the stem high enough to push air into the conduit network. To inject the air, we removed a 10 cm section of bark and phloem to restrict air pressure to the xylem tissue and bolted a steel split chamber with a compressible gasket around the stem. The gasket provided a nearly air-tight seal. A pressure regulator held injection pressures of 3.5, 4.5 and 5.5 MPa constant despite occasional small leaks from the chamber. These injection pressures were chosen because the vulnerability curve indicated they would cause significant cavitation in stem xylem.

The experimental protocol is summarized in Fig. 3 (solid line). Before treatments began, we estimated  $\Psi_{\text{soil}}$  from pre-dawn  $\Psi_{\text{leaf}}$  measurements on three fascicles using a pressure chamber (Scholander *et al.* 1965). To begin treatments, we turned on the gas exchange system and allowed the seedling to acclimate for at least 1 h or until  $g_s$ ,  $E$  and  $A$  were stable, and steady-state flow conditions were likely to be established in these short plants. After these parameters were stable, we measured  $\Psi_{\text{leaf}}$  on three fascicles and calculated the initial  $K_L$  from Eqn 1. We then pressurized



**Figure 3.** Solid line, typical pattern of stomatal conductance ( $g_s$ ) and assimilation ( $A$ ) in response to each injection pressure (3.5, 4.5 and 5.5 MPa, duration indicated on upper  $x$ -axis) (solid line). Dotted line, typical control plant ( $n = 3$ ) showing the response of  $g_s$  and  $A$  to bark and phloem removal plus injection chamber installation (but no injection).

the stem injection chamber to 3.5 MPa for 30 min. The injection chamber was depressurized and  $g_s$ ,  $E$  and  $A$  were again allowed to stabilize for at least 1 h to promote a new steady-state equilibrium (Fig. 3). Afterwards, we re-measured  $\Psi_{\text{leaf}}$  and calculated the change in  $K_L$  caused by the injection. We repeated this sequence for injection pressures of 4.5 and 5.5 MPa. Soil moisture for the entire treatment cycle was kept at field capacity. We used three control plants to determine whether bark and phloem removal and the attachment of the air-injection chamber affected leaf gas exchange independent of any induction of cavitation (Fig. 3, dotted line). For each control plant, we measured  $g_s$ ,  $E$ ,  $A$  and  $\Psi_{\text{leaf}}$  under the same environmental conditions as our treatment plants until these parameters were stable (at least 1 h). Then we removed the bark and phloem and attached the air injection chamber. Afterwards, we monitored leaf gas exchange and  $\Psi_{\text{leaf}}$  for the duration of a typical treatment cycle (approximately 7 h).

Gas exchange parameters ( $g_s$ ,  $E$ ,  $A$ ) are reported as leaf-specific rates. We determined leaf area for each seedling by removing and weighing all of the needles from the canopy after treatment. Projected leaf area ( $\text{m}^2$ ) was determined from a leaf area : weight regression developed from similar seedlings. Fifty fascicles were removed from each of six

seedlings and the area determined by measuring the length and width of each needle with a digital micrometer.

Our methods of measuring  $g_s$  and  $K_L$  were not completely independent, because both  $g_s$  and  $K_L$  were determined in part from measurements of transpiration in the gas exchange cuvette. For this reason, we also evaluated the gas exchange response to independent estimates of the loss of stem conductivity caused by air injection ( $k_{\text{loss}}$ ). Measuring  $k_{\text{loss}}$  directly was a destructive measurement that could only be made at the end of the 5.5 MPa injection when the experiment was finished. Consequently, we estimated  $k_{\text{loss}}$  for the stem segment within the injection chamber at all injection pressures using the seedling vulnerability curve. To make this estimation, it was necessary to know both the injection pressure and the xylem pressure at the time of injection. The difference between these pressures represented the total pressure difference driving air into the vascular system. The  $k_{\text{loss}}$  was then predicted using this total pressure difference as the equivalent of the xylem pressure axis of the vulnerability curve (which was conducted at ambient air pressure). The value of  $\Psi_{\text{xylem}}$  was estimated during injection as the mid-point between  $\Psi_{\text{soil}}$  and pre-treatment  $\Psi_{\text{leaf}}$  (Linton, Sperry & Williams 1998) calculated as

$$\Psi_{\text{xylem}} = (\Psi_{\text{leaf}} + \Psi_{\text{leaf}})/2 \quad (2)$$

This (negative) value was subtracted from the injection pressure (0, 3.5, 4.5 or 5.5 MPa) to estimate the total pressure difference causing cavitation. A Weibull function was fitted to our vulnerability curve data (see *Data Analysis*) and this function was used to predict  $k_{\text{loss}}$  from the total pressure difference.

To test the estimates of  $k_{\text{loss}}$  from  $\Psi_{\text{leaf}}$ ,  $\Psi_{\text{soil}}$  and the vulnerability curve, we directly measured  $k_{\text{loss}}$  in six separate plants (two each at 3.5, 4.5 and 5.5 MPa). After subjecting the plants to the same environmental conditions as our treatment plants, we measured  $k_{\text{loss}}$  after air injection at the specific pressure and compared it with  $k_i$  based on a branch diameter versus  $k_i$  relationship developed on 12 separate ponderosa pine seedlings.

## Data analysis

A Weibull function was fitted to our vulnerability curve data to describe the relationship between  $k_{\text{loss}}$  and  $\Psi_{\text{xylem}}$ . The specific function is given as:

$$\%k_{\text{loss}} = 100 - 100 \times \exp(-(-P_{\text{total}}/b)^c) \quad (3)$$

where  $b$  and  $c$  are constants generated by the curve fitting procedure, and  $P_{\text{total}}$  is the pressure difference causing cavitation. The function was fitted and 95% prediction intervals generated using non-linear regression [Proc Nlin (SAS 1997)]. We tested for normality and homogeneity of variance and found that our vulnerability curve data did not violate either of these assumptions.

We analysed the response of leaf water potential and gas exchange parameters to changes in  $K_L$  using a general linear model [Proc Mixed (SAS 1997)]. The seedling was

the sampling unit. However, because we measured leaf gas exchange with changes in  $K_L$  at four different applied pressures (0, 3.5, 4.5 and 5.5 MPa, respectively) on the same seedling, we used a repeated measures analysis. This analysis assumes a constant correlation among measurements on the same seedling (a standard compound symmetric covariance model). We found no violation of the assumptions of normality and homogeneous variance for this analysis. The response of leaf gas exchange and  $K_L$  to changes in  $k_{\text{loss}}$  were analyzed using analysis of variance in the same repeated measures framework as described above.

## RESULTS

The Weibull function that best described our vulnerability curve for stem xylem was:

$$\%k_{\text{loss}} = 100 - 100 \times \exp(-(-P_{\text{total}}/4.3))^{5.4} \quad (4)$$

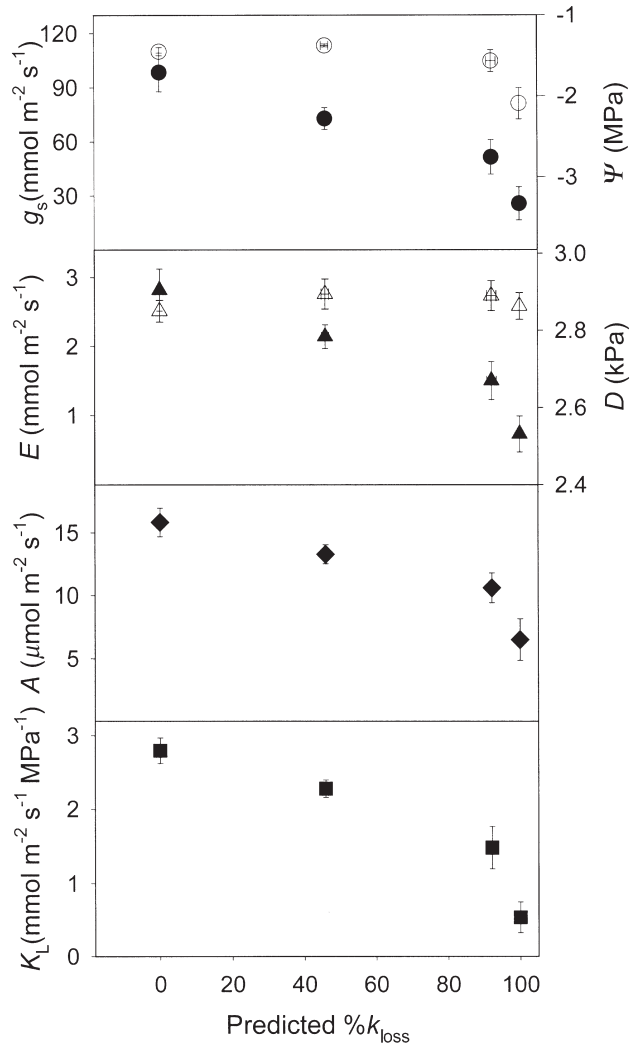
The  $R^2$  for this function (Fig. 2) was 0.92. The curve indicated that cavitation in stems of these seedlings occurred at xylem pressures between -3 and -6 MPa (at ambient air pressure).

Using the vulnerability curve (Eqn 4) and estimates of  $\Psi_{\text{xylem}}$  during injection (Eqn 2), we predicted that  $k_{\text{loss}}$  from air injections of 3.5, 4.5 and 5.5 MPa was 46, 92 and 99%, respectively (Fig. 4). When we tested these predictions by destructively sampling two plants for each pressure we found actual  $k_{\text{loss}}$  to be 62, 87 and 99%, respectively. Measured values fell within the 95% prediction intervals for the Weibull fit (Fig. 2).

The three control seedlings showed no effect of bark and phloem removal or attachment of the injection chamber on  $g_s$  or  $A$  either immediately after the treatment ( $P = 0.52$ ) or throughout the 7 h duration of the experiment (Fig. 3, dotted line). In contrast, the injected seedlings showed an abrupt decline in  $g_s$  and  $A$  within minutes after each 30 min injection (Fig. 3, solid line). Interestingly, there was no response during the injection. These declines stabilized at lower values of  $g_s$  and  $A$  within half an hour after the injection treatment.

During the gas exchange measurements, leaf temperature and  $\text{CO}_2$  concentration within the cuvette remained constant at  $26.0 \pm 0.3$  °C and  $350 \pm 5$  p.p.m., respectively. Air saturation deficit did not vary significantly ( $P = 0.72$ ) during treatment of each seedling and remained nearly constant at 2.8 kPa (Fig. 4; second panel).

Stable values of  $g_s$ ,  $E$  and  $A$  after injection showed a significant decline ( $P < 0.01$ ) with independent estimates of  $k_{\text{loss}}$  caused by injection (Fig. 4; top three panels). The decline was essentially linear until the 99%  $k_{\text{loss}}$  extreme (caused by the 5.5 MPa injection) when values decreased even more abruptly. The decline in gas exchange parameters was associated with the maintenance of a constant mean  $\Psi_{\text{leaf}}$  ( $P = 0.24$ ) of approximately -1.45 MPa up until the 99%  $k_{\text{loss}}$  extreme when  $\Psi_{\text{leaf}}$  dropped abruptly to -2.1 MPa (Fig. 4, top panel). Pre-dawn  $\Psi_{\text{leaf}}$  averaged -0.4 MPa and was not different between seedlings ( $P > 0.05$ ).

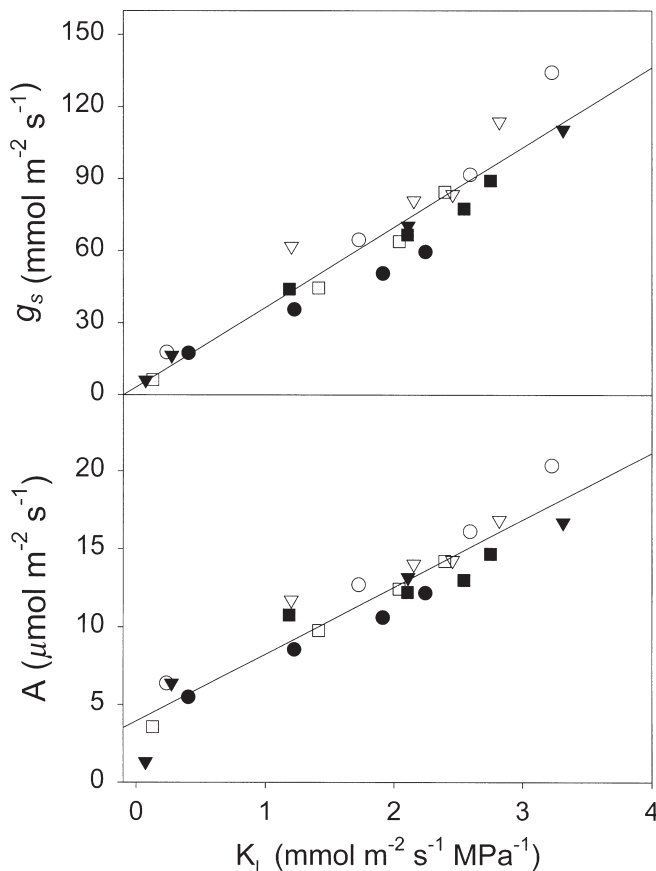


**Figure 4.** Mean leaf water potential ( $\Psi_{\text{leaf}}$ , ○), stomatal conductance ( $g_s$ , ●), transpiration ( $E$ , ▲), air saturation deficit ( $D$ , △) assimilation ( $A$ , ◆), and leaf specific hydraulic conductance ( $K_L$ , ■) versus predicted percentage loss of stem hydraulic conductivity ( $n = 6$ ). Error bars are  $\pm 1$  SE.

The bottom panel of Fig. 4 shows the decline in  $K_L$  calculated from Eqn 1 ( $K_L = E/\Delta\Psi$ ) that was associated with the  $k_{\text{loss}}$  in the stem from the air injection. The decline in  $K_L$  paralleled the decline in gas exchange (Fig. 4, upper three panels), and was approximately linear until the 99%  $k_{\text{loss}}$  point at which  $K_L$  dropped more abruptly. The non-linearity of the overall relationship indicated that to alter the conductance of the entire flow path by a given fraction, the stem segment conductivity had to be reduced disproportionately. For example, a 50% reduction in  $K_L$  required a 90% reduction in stem conductivity. This non-linearity is expected if the loss of conductivity caused by the injection did not spread equally throughout the vascular system. In support of this, air was seen to escape from the stem outside of the chamber suggesting that the high air pressures required to cause the  $k_{\text{loss}}$  dissipated within a short distance

of the chamber. Another factor contributing to the non-linearity is the fact that the initial conductivity per unit leaf area is typically at least an order of magnitude greater in the stem than in the lateral branches and leaves (Zimmermann 1978; Tyree & Ewers 1991). Consequently, stem conductivity must be lowered substantially before it can become the limiting conductance in the plant and significantly influence  $K_L$ . Finally, diurnally varying conductances in roots and shoots (Tsuda & Tyree, 2000) occurring independently of (or even in response to) the  $k_{\text{loss}}$  caused by air injection could also influence the relationship between  $k_{\text{loss}}$  and  $K_L$ .

As a result of the near-isohydric regulation of  $\Psi_{\text{leaf}}$  (Fig. 4, top panel), and the estimation of  $K_L$  from Ohm's law (Eqn 1), the summary relationship between  $g_s$  and  $K_L$  was linear ( $R^2 = 0.91$ ), with an intercept not different from zero ( $P > 0.05$ ) and a slope not different from  $\Delta\Psi/D$



**Figure 5.** Stomatal conductance ( $g_s$ ) and assimilation ( $A$ ) versus whole plant leaf specific conductance ( $K_L$ ) for each seedling ( $n = 6$ ) at each injection pressure. Different symbols represent each separate seedling. The regression equations are  $g_s = 4.5 + 496.86 \times K_L$ , and  $A = 4.06 + 64.79 \times K_L$ .  $R^2$  values for  $g_s$  and  $A$  versus  $K_L$  are 0.91 and 0.90, respectively. The intercept was not different from zero ( $P > 0.05$ ) and the slope did not differ from  $(\Psi_{\text{soil}} - \Psi_{\text{leaf}}) / \text{leaf}$  to air mole fraction difference ( $^{\circ} 0.032$ ) ( $P > 0.05$ ).

( $P > 0.05$ ). Assimilation ( $A$ ) showed a similarly strong linear relationship to  $K_L$  (Fig. 5, lower panel;  $R^2 = 0.90$ ).

## DISCUSSION

Our results showed that ponderosa pine seedlings maintained constant bulk  $\Psi_{\text{leaf}}$  as  $k$  and  $K_L$  were systematically decreased by the induction of cavitation to all but the lowest values (Figs 4 & 5). According to the Ohm's law analogy for steady-state flow through the continuum, this result requires a directly proportional response of  $g_s$  to  $K_L$  when  $\Psi_{\text{soil}}$  and  $D$  are constant, as in our experiments (Figs 1 & 5). Although it would have been desirable to measure  $K_L$  independently of  $g_s$  as has been done previously for step changes in  $K_L$  (Sperry *et al.* 1993; Saliendra *et al.* 1995), the destructive nature of these measurements made it impossible to measure the complete response on single plants. In addition, these destructive measurements of leaf and soil-root system conductances may not reflect *in situ* conductance because of changes in flow path, sap composition, and reversal of embolism caused by measurement conditions (Tyree *et al.* 1994b; Saliendra *et al.* 1995; Ieperen *et al.* 2000).

The lack of independence between the  $K_L$  and  $g_s$  measurement does not apply to corollary results. Unlike  $g_s$ ,  $A$  was measured independently from  $K_L$ , yet was also proportional (Fig. 5). Because of the strong relationship between  $g_s$  and  $A$  (Wong, Cowan & Farquhar 1985) it is unlikely that the proportionality of  $g_s$  versus  $K_L$  was an artifact of autocorrelation between the two parameters. The decline in gas exchange was also strongly related to independent measures of stem conductivity (Fig. 4). Finally, the fact that  $\Psi_{\text{leaf}}$  remained constant despite the reduction in stem conductivity (Fig. 4, top panel) requires that  $g_s$  and  $K_L$  be directly proportional under steady-state conditions (Eqn 1), which were likely to prevail in our study given the short stature of the seedlings and the 1 h equilibration periods before measurement (Fig. 2).

Other studies have shown similar stomatal response to a single step reduction in  $K_L$ . Sperry *et al.* (1993) and Sperry & Pockman (1993) found that reducing  $K_L$  in *Betula occidentalis* by notching or air injection caused lower  $g_s$  and  $E$  relative to control plants while  $\Psi_{\text{leaf}}$  remained constant. Similarly, Teskey *et al.* (1983) found that reducing the flow path of water in *Abies amabilis* via root pruning caused almost immediate stomatal closure with no change in  $\Psi_{\text{leaf}}$ . Similar conclusions were reached by Meinzer & Grantz (1990) in sugarcane. Other studies have shown that stomata close in response to lower conductance of the soil to leaf pathway via soil drying to prevent xylem cavitation (Tyree *et al.* 1994a; Sparks & Black 1999). By evaluating  $\Psi_{\text{leaf}}$  regulation in response to successive reductions in  $K_L$  in individual plants of ponderosa pine we show that the stomatal regulation is isohydric over a broad range of  $K_L$ , and that this response is also associated with a linear relationship between  $A$  and  $K_L$ . Anisohydric species would be expected to show less of a response of  $A$  to successive reductions in  $K_L$  (e.g. Fig. 1).

The simplest explanation for the rapid response of  $g_s$  to  $K_L$  (Fig. 2) is that stomata are not responding directly to  $K_L$ , but to the consequent change in water status within the leaf (Saliendra *et al.* 1995). According to this explanation, a reduction in  $K_L$  causes a momentary reduction in water status of at least a portion of the leaf tissue. Through pressure–volume changes in sensing cells or even transient cavitation within leaf veins (Salleo *et al.* 2000), the change in water status ‘signals’ a reduction in stomatal conductance (perhaps via abscisic acid (ABA) signalling within the leaf). The reduction in stomatal conductance returns leaf water status to its original level. At the bulk tissue level, these small fluctuations in leaf water status in time and space are masked, so that bulk  $\Psi_{\text{leaf}}$  remains approximately constant. This small-scale feedback loop is analogous in principle to what has been proposed for stomatal regulation in response to changes in atmospheric moisture (e.g. Cowan 1995). Evidence for some form of leaf-level feedback in the  $K_L$  response is indicated by experiments showing that if leaf water status is experimentally increased (by root pressurizing), the stomatal closure to reduced  $K_L$  can be reversed (Saliendra *et al.* 1995). The fact that leaf water potential eventually did drop in response to the most extreme reduction in  $K_L$  (99%  $k_{\text{loss}}$ , and > 75%  $K_L$  loss; Fig. 4, bottom) suggests there is a limit to the regulatory response of stomata when near-complete closure was approached.

A large body of evidence exists for root signalling in connection with stomatal regulation (Tardieu & Davies 1993; Zhang & Davies 1989) and abscisic acid has been shown to affect stomatal behaviour in conifer saplings (Jackson *et al.* 1995). However, any root signal involvement in response to changes in  $K_L$  (e.g. Meinzer, Grantz & Smit 1991) seems unlikely in our study for two reasons. First, the seedlings were well-watered throughout the experiment and second, the changes in  $K_L$  were induced *downstream* from the roots making it very unlikely they could perceive the change via alteration of water status and respond in kind.

It was interesting that we observed no decline in gas exchange until after the 30 min injection treatment was terminated (Fig. 3), whereas it is quite likely that the reduction in stem conductivity was significant within the first 10 min based on results from isolated stem segments (Sperry & Saliendra 1994). Although this may reflect the lag time in the stomatal response to changes in  $K_L$ , it seems unlikely because in stem notching experiments, the stomatal response occurred within minutes of the reduction in conductance (Sperry *et al.* 1993). It is possible that the continual release of water caused by the progressive cavitation in the stem xylem was negating the effect of reduced stem conductivity on leaf water status. Only when the injection ceased would the full effect of reduced conductivity be felt in the leaf as an incipient reduction in water status. More detailed measurements of leaf water status during the injection process would be necessary to link small scale (in time and space) changes in leaf cell water potentials to these kinds of experimental manipulations.

Our study did not directly address the adaptive significance of regulating  $\Psi_{\text{leaf}}$  at approximately  $-1.5$  MPa in

these seedlings. *A priori* we might expect it to relate to the avoidance of some form of damage from exposure to more negative values. In some previous studies, this damage was linked to extensive cavitation that would occur if pressures dropped below the regulated value (e.g. Meinzer *et al.* 1992; Saliendra *et al.* 1995). However, if the vulnerability curve for stem xylem represents that for leaf xylem, at  $\Psi_{\text{leaf}}$  of  $-1.5$  MPa, these seedlings would have a  $1.5$  MPa cushion from incipient cavitation which was initiated at  $-3$  MPa (Fig. 2). More often than not, however, there is substantial variation in cavitation resistance along the xylem (e.g. Hacke & Sauter 1996). In *Pseudotsuga menziesii*, for example, smaller distal branches were more vulnerable than thicker proximal ones and root xylem is particularly vulnerable (Sperry & Ikeda 1997). In addition to the avoidance of cavitation, there are other possible negative consequences of reduced  $\Psi_{\text{leaf}}$  including pressure–volume relations of leaf cells and organelles. However, we did not address the likelihood of such damage in this study.

Recently, Ryan & Yoder (1997) have proposed the hydraulic limitation hypothesis for reduced productivity observed in ageing forest stands. This hypothesis suggests that as trees grow older and taller,  $K_L$  declines causing stomates to close earlier in the day (relative to younger, shorter trees) to preserve  $\Psi_{\text{leaf}}$  regulation. They propose that this may explain the well-documented decline in stand NPP after canopy closure and why all trees eventually reach a maximum height. Although several studies have provided support for the hypothesis (Hubbard *et al.* 1999; Ryan *et al.* 2000), a critical test of the hydraulic limitation hypothesis requires that imposing a decrease in  $K_L$  should result in decreased carbon gain. As  $K_L$  declined after air injection in this study,  $A$  declined linearly suggesting a direct link between  $K_L$  and  $A$ , in support of Ryan and Yoder’s hypothesis. Although our results were short-term responses to experimental reductions in  $K_L$ , they are consistent with apparent responses to longer term developmental changes in  $K_L$ . Hubbard *et al.* (1999) found that old versus young ponderosa pine trees had 50% lower  $K_L$  with 25% lower  $A$  during mid-day. This is quite similar to our short-term observations in ponderosa pine seedlings where a 50% lower  $K_L$  resulted in 33% lower assimilation rates (Fig. 5). These results suggest there is little acclimation in  $A$  versus  $g_s$  relationships or in  $\Psi_{\text{leaf}}$  regulation in response to changes in  $K_L$  in this species. Understanding how changes in hydraulic conductance change with stand and tree age will be important for developing better models of forest growth (e.g. Williams *et al.* 1997) and predictions of forest response to climate change.

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