

# Impacts of tree height on leaf hydraulic architecture and stomatal control in Douglas-fir

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## ABSTRACT

This study investigated the mechanisms involved in the regulation of stomatal closure in Douglas-fir and evaluated the potential impact of compensatory adjustments in response to increasing tree height upon these mechanisms. In the laboratory, we measured leaf hydraulic conductance ( $K_{\text{leaf}}$ ) as leaf water potential ( $\Psi_l$ ) declined for comparison with *in situ* diurnal patterns of stomatal conductance ( $g_s$ ) and  $\Psi_l$  in Douglas-fir across a height gradient, allowing us to infer linkages between diurnal changes in  $K_{\text{leaf}}$  and  $g_s$ . A recently developed timed rehydration technique was used in conjunction with data from pressure–volume curves to develop hydraulic vulnerability curves for needles attached to small twigs. Laboratory-measured  $K_{\text{leaf}}$  declined with increasing leaf water stress and was substantially reduced at  $\Psi_l$  values of  $-1.34$ ,  $-1.45$ ,  $-1.56$  and  $-1.92$  MPa for foliage sampled at mean heights of approximately 20, 35, 44 and 55 m, respectively. *In situ*  $g_s$  measurements showed that stomatal closure was initiated at  $\Psi_l$  values of  $-1.21$ ,  $-1.36$ ,  $-1.74$  and  $-1.86$  MPa along the height gradient, which was highly correlated with  $\Psi_l$  values at loss of  $K_{\text{leaf}}$ . Cryogenic scanning electron microscopy (SEM) images showed that relative abundances of embolized tracheids in the central vein increased with increasing leaf water stress. Leaf embolism appeared to be coupled to changes in  $g_s$  and might perform a vital function in stomatal regulation of plant water status and water transport in conifers. The observed trends in  $g_s$  and  $K_{\text{leaf}}$  in response to changes in  $\Psi_l$  along a height gradient suggest that the foliage at the tops of tall trees is capable of maintaining stomatal conductance at more negative  $\Psi_l$ . This adaptation may allow taller trees to continue to photosynthesize during periods of greater water stress.

**Key-words:** *Pseudotsuga menziesii*; growth limitation; leaf hydraulic conductance; stomatal conductance; water stress.

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## INTRODUCTION

Stomata play a critical role in regulation of plant water status by balancing transpiration with dynamic changes in the capacity of the soil–plant hydraulic system to supply water to the leaves. Failure of stomata to sense and adequately respond to changes in the balance between water supply and evaporative demand would rapidly trigger a cascade of deleterious consequences including lethal dehydration of leaves and massive xylem embolism upstream from the leaves leading to total hydraulic failure. Despite decades of intensive research on the physiology of stomata, the specific mechanisms that permit coordination of stomatal conductance ( $g_s$ ) with plant water balance and hydraulic properties remain elusive (Meinzer 2002; Franks 2004; Buckley 2005). Regardless of the mechanism, there does seem to be general agreement that stomata sense the water potential ( $\Psi$ ) somewhere within the leaf (Comstock & Mencuccini 1998; Cochard *et al.* 2002). Nevertheless, there is ample evidence that stomatal responses to leaf water status are often modulated by root-derived chemical signals arriving in the transpiration stream (Davies & Zhang 1991; Tardieu & Davies 1993). Plant capacitance and its ability to confer elasticity on the relationship between changes in transpiration and leaf water potential (Waring & Running 1978; Goldstein *et al.* 1998; Meinzer, James & Goldstein 2004) add further complexity to interpretation of the mechanisms that govern stomatal behavior.

Leaf hydraulic architecture and its relationship to stomatal control of gas exchange have recently been investigated by a number of workers. Consistent with previous work documenting coordination of  $g_s$  with overall plant hydraulic conductance (Meinzer & Grantz 1990; Meinzer *et al.* 1995; Hubbard *et al.* 2001), recent work has shown that maximum  $g_s$  and leaf hydraulic conductance ( $K_{\text{leaf}}$ ) are also tightly coordinated within and across species (Brodribb & Holbrook 2004a, 2005a). The xylem of leaves appears to be more vulnerable to embolism than that of the stems to which they are attached even though leaves comprise the terminal portion of the liquid water transport pathway causing their xylem to be subjected to greater tension than in stems (Brodribb & Holbrook 2003; Brodribb *et al.* 2003; Bucci *et al.* 2003). The comparatively

high vulnerability to embolism in relation to tension experienced in leaf xylem results in substantial loss of  $K_{\text{leaf}}$  on a regular basis, even under non-extreme environmental conditions (Bucci *et al.* 2003; Brodribb & Holbrook 2004b; Meinzer *et al.* 2004). To compensate for daily loss of  $K_{\text{leaf}}$ , leaves apparently have vigorous embolism repair mechanisms that restore xylem conductivity over periods of minutes (Trifilo *et al.* 2003) to hours (Zwieniecki *et al.* 2000; Bucci *et al.* 2003), which suggests that embolism repair occurs even while substantial tension is present in conducting xylem adjacent to the non-conducting xylem elements. In addition,  $g_s$  appears to be tightly coupled to shifts in the dynamic balance between leaf embolism formation and repair throughout the day (Brodribb & Holbrook 2004b; Meinzer *et al.* 2004). Taken together, these observations imply that daily cycles of embolism in leaves may be an inherent component of the stomatal regulatory system rather than a phenomenon to be avoided at all costs.

In tall trees, the impact of height on physiological processes regulated by water availability must be taken into account. In the absence of transpiration, the gravitational component of water potential results in a xylem tension gradient of about 0.01 MPa m<sup>-1</sup> increase in height. When transpiration is occurring, frictional resistances come into play, increasing the vertical tension gradient as a function of the flow rate, path length and xylem characteristics. Compensatory adjustments in tree hydraulic architecture with increasing height can diminish the frictional component of the tension gradient (McDowell *et al.* 2002a,b; Phillips *et al.* 2003; McCulloh & Sperry 2005), but the hydrostatic component is independent of tree hydraulics and therefore unavoidable. In tall conifers, reduced shoot expansion with increasing height appears to be largely a consequence of reduced turgor resulting from insufficient osmotic adjustment to fully compensate for the vertical increase in tension (Koch *et al.* 2004; Woodruff, Bond & Meinzer 2004). Steadily declining  $\Psi_l$  along a tree height gradient also has implications for stomatal control of leaf gas exchange. Mean  $g_s$  and therefore photosynthesis are likely to be lower in taller than in shorter individuals of the same species growing under similar conditions, unless compensatory adjustments in leaf hydraulic architecture and water relations occur.

Here we use Douglas-fir as a model species to study the impact of increasing tree height on leaf hydraulic architecture and its relationship to stomatal control of transpiration. Douglas-fir is well suited for this type of study because it can attain heights greater than 100 m, and the crowns of old-growth specimens can span a vertical distance of over 30 m. Moreover, if  $K_{\text{leaf}}$  is found to fluctuate diurnally as a result of embolism and reversal, it can be unambiguously concluded that tracheid refilling occurs in the presence of substantial xylem tension because of the heights involved. We know of no other studies on dynamic relationships between leaf hydraulics and stomatal behavior in temperate conifers, nor of how these characteristics are affected by tree height.

## MATERIALS AND METHODS

### Field sites and sampling heights

The two study sites were located within 5 km of each other in the Wind River Basin of southwestern Washington, USA. The site with the tallest trees was a 450-year-old Douglas-fir/western hemlock [*Pseudotsuga menziesii* (Mirb.) Franco/*Tsuga heterophylla* (Raf.) Sarg.] forest located at the Wind River Canopy Crane Research Facility (WRCCRF) within the T.T. Munger Research Natural Area in the Gifford Pinchot National Forest (Shaw *et al.* 2004). The stand had a mean density of about 437 trees ha<sup>-1</sup> with a basal area of 82.9 m<sup>2</sup> ha<sup>-2</sup>. The WRCCRF contains a 75-m-tall construction tower crane with an 85 m jib that provided access to the crowns of the study trees via a suspended gondola. The second site, located in the Wind River Experimental Forest, was clear-cut in 1976 and was planted with Douglas-fir seedlings in 1978 (Phillips *et al.* 2002). During the study period, the mean density was about 1529 trees ha<sup>-1</sup> with a mean height of approximately 23 m. A 25-m-tall scaffold tower provided access to the crowns of the study trees. The elevations of the younger and older stands were 561 and 371 m, respectively.

The Pacific maritime climate of the region is characterized by wet winters and dry summers. Mean annual precipitation in the region is about 2.2 m, much of which falls as snow, with a dry season from June through September. Very low precipitation between June and September (~119 mm) typically leads to drought conditions in the upper portion of the soil profile, but readily available soil water remains accessible to Douglas-fir roots at depths greater than about 1 m throughout the summer dry period (Warren *et al.* 2005; Meinzer, Warren & Brooks, in press). The mean annual temperature is 8.7 °C with means of 0 °C in January and 17.5 °C in July. The soils are well drained and of volcanic origin.

Branch measurement and sampling locations were established near the tops of three 22- to 25-m-tall Douglas-fir trees in the younger stand, and at bottom-, mid- and upper-canopy positions in three 57- to 59-m-tall Douglas-fir trees in the old-growth stand. Mean height for the sampling locations was 20.0 m near the tops of the young trees, and 33.6, 44.0 and 55.4 m in the old trees. Sampling locations were deliberately located within trees of different height classes (old growth and young) in order to rule out the possibility of height-related trends in leaf hydraulic architecture and stomatal behaviour being driven by confounding factors such as light transmittance through the canopy.

### Leaf water status and stomatal conductance

Measurements of  $\Psi_l$  were conducted with a pressure chamber (PMS Instrument Company, Corvallis, OR, USA), and stomatal conductance ( $g_s$ ) with a steady-state porometer (LI-1600; Li-Cor, Inc., Lincoln, NE, USA). Diurnal measurements of  $\Psi_l$  and  $g_s$  were conducted concurrently at least every 30 min on 2–3 separate trees per site. Measurements of  $g_s$  were conducted repeatedly on the same

foliage over the entire diurnal period, and destructive measurements of  $\Psi_l$  were conducted on nearby adjacent foliage. Measurements commenced prior to dawn and continued until after 1600 standard time at the young site and until wind conditions created safety concerns in the crane gondola at the old site, typically between 1400 and 1500 standard time. Measurements were conducted on 20 July and 1 September 2005 at the old site, and on 23 July and 23 September 2002 at the young site.

### Measurement of $K_{\text{leaf}}$

The methods used to determine leaf hydraulic conductance were adapted from Brodribb & Holbrook (2003), and they involve the use of the following equation derived from the relationship between rehydrating a leaf and recharging a capacitor:

$$K_{\text{leaf}} = C \ln(\psi_o/\psi_t)/t, \quad (1)$$

where  $K_{\text{leaf}}$  = leaf hydraulic conductance,  $C$  = capacitance,  $\Psi_o$  = leaf water potential prior to rehydration,  $\Psi_t$  = leaf water potential after rehydration and  $t$  = duration of rehydration.

Branches approximately 30–40 cm long were collected from trees early in the morning prior to significant transpirational water loss and were placed in plastic bags with moist paper towels and stored in a refrigerator. Over the next 3 d, measurements of leaf water potential were conducted on excised twigs (~10–15 cm long) initially ( $\Psi_o$ ) and after a period of rehydration of  $t$  seconds ( $\Psi_t$ ).  $K_{\text{leaf}}$  measurement samples were collected from the three separate trees on 22 and 28 July and on 24 August 2004. Deionized water used for rehydration of  $K_{\text{leaf}}$  samples was de-gassed by vacuuming for a minimum of 2 h. Water temperature was consistently between 21 and 23 °C. The influence of light level during rehydration was assessed by conducting a series of  $K_{\text{leaf}}$  measurements at varying levels of light intensity and was found to be negligible. Some  $K_{\text{leaf}}$  samples underwent periods of hydration prior to initial measurements of  $\Psi_l$  in order to obtain a wider range of initial values of  $\Psi_l$ . Periods of rehydration following initial  $\Psi_l$  were variable and were not recorded.

$C$  was determined on an individual tree basis from the slope of the relationship between relative water content and  $\Psi_l$  obtained from pressure–volume curves (Table 1). Pressure–volume analyses (Scholander *et al.* 1965; Tyree

& Hammel 1972) were conducted on branchlets approximately 10 cm long during August 2001. These samples were excised early in the morning prior to significant transpirational water loss, sealed in plastic bags with moist paper to prevent desiccation and then stored in a refrigerator within 1–4 h of excision. Pressure–volume curves were initiated by first determining the fresh weight of the twig, and then measuring  $\Psi_l$  with a pressure chamber (PMS Instrument Company). Alternate determinations of fresh weight and  $\Psi_l$  were repeated during slow dehydration of the twig on the laboratory bench until values of  $\Psi_l$  exceeded the measuring range of the pressure chamber (–4.0 MPa). The inverse of water potential was plotted against relative water content to create a pressure–volume curve. For normalizing  $C$  on a leaf area basis, leaf areas of the branchlets were obtained with a scanner and ImageJ version 1.27 image analysis software (Abramoff, Magelhaes & Ram 2004).

### Cryo-scanning electron microscopy (SEM)

To visually determine if observed declines in  $K_{\text{leaf}}$  were associated with increasing frequency of embolized tracheids, cryo-SEM was performed on needle samples of varying levels of dehydration. Branches approximately 30–40 cm long were collected from old growth tree 3013 on 25 January 2006, placed in plastic bags with moist paper towels and stored in a refrigerator. Over the next 3 d, branchlets (~10–15 cm long) were removed from the refrigerator, placed in dark drawers and allowed to dehydrate for varying amounts of time. Branches were in the light for as short a time as possible before sampling. This ensured that all needles were equilibrated to the same water potential and prevented tension gradients from developing within the branches, which had been shown to create embolisms as samples were frozen in liquid nitrogen (Cochard *et al.* 2000). For branches over a range of water potentials (well hydrated to water stressed), two to three needles were removed with a razor blade, inserted with forceps into a fracture rivet filled with Tissue-Tek (Electron Microscopy Sciences, Hatfield, PA, USA) and plunged into liquid nitrogen. Once the sample reached liquid nitrogen temperatures (i.e. when the boiling stopped), it was inserted quickly into a pre-cooled vial and returned to the liquid nitrogen. While the needles were being frozen, the water potential of the branch was determined with a pressure chamber (PMS Instrument Company).

**Table 1.**  $\Psi_l$  at turgor loss point, leaf water capacitance ( $C$ ) prior to and following turgor loss point and osmotic potential at full turgor for foliage at different heights determined by pressure–volume analysis

| Height class | Height (m)  | $\Psi_l$ at turgor loss (MPa) | $C$ pre-turgor loss (mol m <sup>-2</sup> MPa <sup>-1</sup> ) | $C$ post-turgor loss (mol m <sup>-2</sup> MPa <sup>-1</sup> ) | Osmotic potential at full turgor (MPa) |
|--------------|-------------|-------------------------------|--------------------------------------------------------------|---------------------------------------------------------------|----------------------------------------|
| Old top      | 55.4 ± 1.37 | 3.52 ± 0.02                   | 0.42 ± 0.01                                                  | 1.24 ± 0.13                                                   | –2.77 ± 0.03                           |
| Old mid      | 44.0 ± 0.15 | 3.34 ± 0.12                   | 0.45 ± 0.03                                                  | 1.22 ± 0.05                                                   | –2.64 ± 0.06                           |
| Old low      | 33.6 ± 1.18 | 3.13 ± 0.07                   | 0.37 ± 0.02                                                  | 1.07 ± 0.12                                                   | –2.48 ± 0.09                           |
| Young top    | 15.5 ± 0.87 | 2.85 ± 0.03                   | 0.45 ± 0.03                                                  | 2.37 ± 0.22                                                   | –2.42 ± 0.04                           |

The frozen leaf samples were shipped at liquid nitrogen temperatures to the cryo-SEM facilities at the University of British Columbia. There the fracture rivets containing the needles were mounted in a cryo-prep unit (K1270; Emitech USA, Houston, USA), and the needles were fractured by touching them with a metal prong. They were then sublimed for about 10 min while being viewed in the cryo-SEM (S4700 FESEM; Hitachi High-Technologies Corp., Berkshire, UK). Various images of each needle were made at magnifications ranging from 90–5000 $\times$ . Empty tracheids within leaf vascular bundles were counted for each cryo-SEM image in order to obtain an estimate of the relative amount of leaf tracheid cavitation associated with varying levels of  $\Psi_l$ .

### Sap flow and crown conductance

Variable length heat dissipation sap flow probes with a heated and reference sensor measuring length of 10 mm at the probe tip (James *et al.* 2002) were used to estimate sap flux at a stem height of approximately 2.5 m in a 1.4-m-diameter, 58.0-m-tall Douglas-fir tree. For probe installation, two 38-gauge (2.58-mm-diameter) holes separated axially by 10 cm were drilled into the sapwood to a depth of 1.5 cm. The sensors were coated with thermally conductive silicone heat sink compound prior to insertion. All probes were protected from potential sun flecks by reflective insulation. Signals from the sap flow probes were scanned every minute, and 10 min means were recorded by a data logger (CR10X; Campbell Scientific Corp., Logan, UT, USA) equipped with a 32-channel multiplexer (AM416, Campbell Scientific). Concurrent differential voltage measurements across the copper-constantan thermocouple leads were converted to a temperature difference between the heated and reference sensor ( $\Delta T$ ), which was converted to sap flux ( $v$ ,  $\text{g m}^{-2} \text{s}^{-1}$ ) using the empirical calibration of Granier (1985):

$$v = 119k^{1.231},$$

where

$$k = (\Delta T_m - \Delta T) / \Delta T,$$

and where  $\Delta T_m$  is the temperature difference when sap flux is assumed to be zero. Data were obtained on the predominantly clear days of 31 July and 21 and 28 August 2002.

In large trees, hydraulic capacitance typically results in substantial and variable time lags in the response of sap flux near the base of the stem to changes in transpiration in the crown (Goldstein *et al.* 1998; Phillips *et al.* 2003). Data collected with a new set of sap flow probes installed in August 2005 in three upper branches at a mean height of 54 m and near the base of the trunk provided a means of estimating crown transpiration from the earlier measurements of sap flux near the base of the same tree by using crown sap flux as a proxy for transpiration. Mean daily courses of sap flux for the upper branches and trunk base were determined for a period of five clear days (20–24 August). The mean time courses were then normalized with respect to the

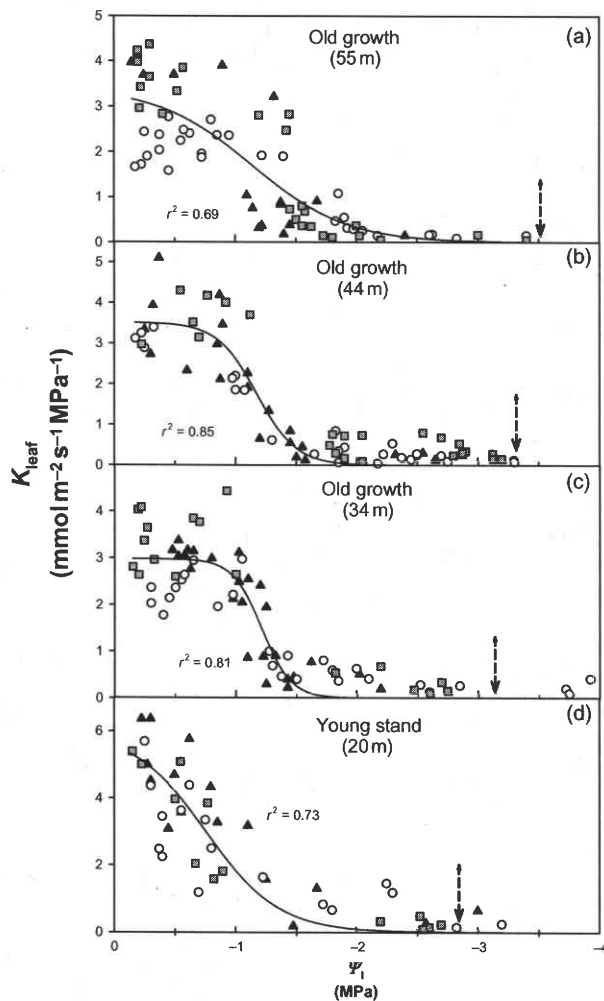
corresponding maximum sap flux at each location, and the ratio of normalized crown and basal sap flux was plotted in relation to time of day between 0700 and 1430 h, which corresponded to the time interval over which concurrent measurements of basal sap flux and  $\Psi_l$  were obtained earlier. The ratio of normalized crown and basal sap flux followed an exponential decay trajectory ( $R^2 = 0.94$ ), approaching unity by 1200 h as capacitive discharge to the transpiration stream diminished. This variable ratio constituted a time-dependent multiplier that was used to calculate crown transpiration on a unit sapwood area basis from basal sap flux at the times  $\Psi_l$  was measured. Crown conductance ( $g_c$ ) on a unit sapwood area basis ( $\text{mol m}^{-2} \text{s}^{-1}$ ) was calculated by dividing the resulting values of crown transpiration by the corresponding values of vapour pressure deficit measured by a meteorological station at a height of 60 m on the canopy crane tower.

### RESULTS

Leaf hydraulic conductance followed a sigmoidal trajectory with declining  $\Psi_l$  (Fig. 1). A three-parameter sigmoid function [ $y = a / (1 + e^{-(x-x_0/b)})$ ] yielded  $r^2$  values ranging from 0.69 to 0.85 for the dependence of  $K_{\text{leaf}}$  on  $\Psi_l$ . In the interest of identifying a functionally relevant connection between changes in  $K_{\text{leaf}}$ ,  $\Psi_l$  and control of  $g_s$ , an objective means was employed for determining a critical value of  $K_{\text{leaf}}$  in relation to  $\Psi_l$ . We used an analysis similar to that proposed for description of vulnerability curves by Domec & Gartner (2001). The value of  $K_{\text{leaf}}$  representing the midpoint between the  $\Psi_l$ -intercept of the sigmoid function and 0 ( $K_{\text{leaf}}$  midpoint, Fig. 2, dotted line) was selected for estimating the slope of the portion of the sigmoid curve containing the relatively rapid and nearly linear decline in  $K_{\text{leaf}}$ . After taking the derivative of the sigmoid function to calculate its instantaneous slope at the  $K_{\text{leaf}}$  midpoint, the  $x$ -intercept of the resulting tangent was used as an objective estimate for the value of  $\Psi_l$  at which  $K_{\text{leaf}}$  had declined to its initial minimum value (Fig. 2, dashed line). This point, which corresponds to the transition from an abrupt, nearly linear decline in  $K_{\text{leaf}}$  to a more gradual asymptotic decline, is hereafter referred to as  $\Psi_l$  at minimum  $K_{\text{leaf}}$ .

Diurnal measurements of  $g_s$  and  $\Psi_l$  showed a typical pattern of  $g_s$  increasing in the early morning once sunlight reached the foliage and then declining sharply later in the morning (Fig. 3a). There was no consistent relationship between  $g_s$  and  $\Psi_l$  because  $\Psi_l$  declined continuously as  $g_s$  rose to its maximum, then declined. However, a threshold value of  $\Psi_l$  corresponding to the point at which  $g_s$  began to decline was estimated using diurnal data for each of the four sampling heights (Fig. 3b). This point is hereafter referred to as  $\Psi_l$  at  $g_s$  decline.

When values of  $\Psi_l$  at minimum  $K_{\text{leaf}}$  and  $g_s$  decline were plotted as functions of sampling height, clear height-dependent linear relationships were apparent (Fig. 4). The value of  $\Psi_l$  at minimum  $K_{\text{leaf}}$  decreased from  $-1.34$  MPa at 20 m near the tops of young trees to  $-1.92$  MPa at 55 m near the tops of old-growth trees. Similarly, *in situ* values of  $\Psi_l$  at



**Figure 1.** Leaf hydraulic conductance ( $K_{\text{leaf}}$ ) in relation to leaf water potential ( $\Psi_l$ ) for foliage samples obtained at three mean heights in the crowns of old-growth trees, and from the upper crowns of young trees growing in a nearby stand. A three-parameter sigmoid function [ $y = a/(1 + e^{-(x-x_0/b)})$ ] was fit to  $K_{\text{leaf}}$  in relation to  $\Psi_l$ . Arrows indicate  $\Psi_l$  at loss of turgor. Different symbols indicate distinct individual trees within each height class.

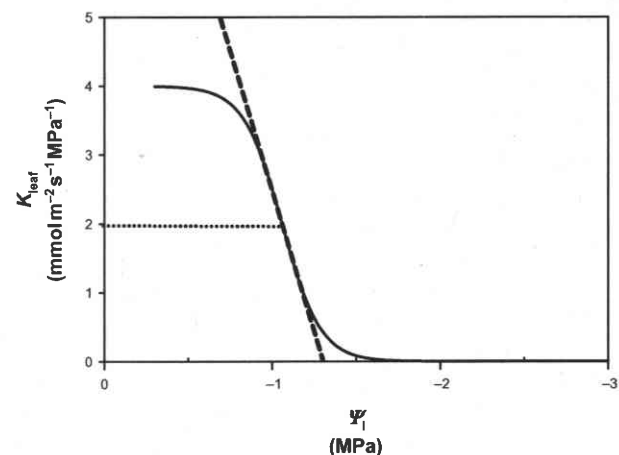
$g_s$  decline ranged from  $-1.21$  MPa at 20 m to  $-1.86$  MPa at 55 m. Values of  $\Psi_l$  at minimum  $K_{\text{leaf}}$  and  $g_s$  decline thus spanned a similar range across the height gradient such that when plotted against each other, they did not significantly depart from a 1:1 relationship (Fig. 4, inset).

Simultaneous measurements of sap flow in upper branches and near the base of the trunk of a 1.4-m-diameter, 58.0-m-tall tree provided a means of estimating crown transpiration and vapour phase conductance from earlier measurements of basal sap flow in the same tree (see Materials and Methods). A plot of  $g_c$  against concurrent values of  $\Psi_l$  measured on upper branches revealed biphasic relationship in which  $g_c$  initially increased to a maximum value as  $\Psi_l$  declined, then decreased with further reductions in  $\Psi_l$ . The threshold value of  $\Psi_l$  at which  $g_c$  began to decline was about  $-1.68$  MPa, which was similar to the

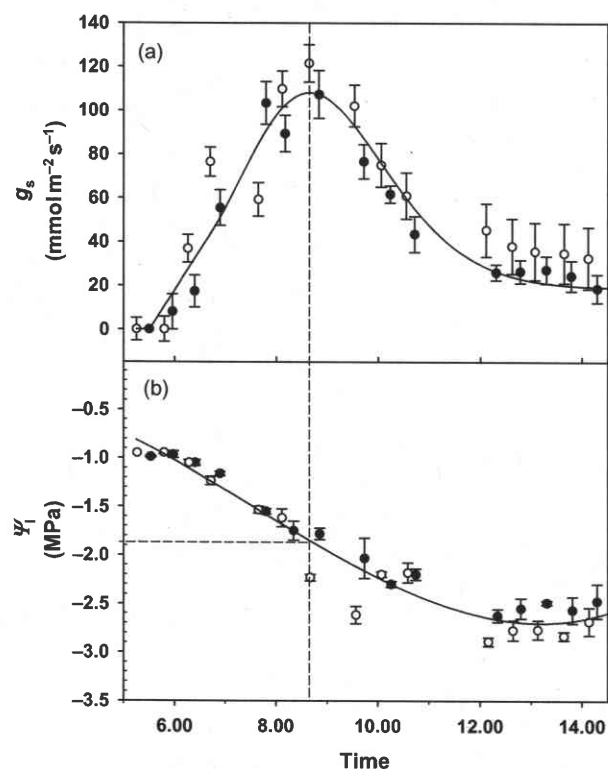
independently measured mean value of  $-1.65$  MPa for  $\Psi_l$  at decline in  $g_s$  for the three old-growth crown heights shown in Fig. 4b. Note that crown conductance is expressed on a sapwood rather than leaf area basis in Fig. 5, because the total crown leaf area was not estimated. However, using a leaf area-to-sapwood area ratio of about  $0.4 \text{ m}^2 \text{ cm}^{-2}$  as estimated by McDowell *et al.* (2002b) for Douglas-fir trees at the same study site, the maximum values of  $g_c$  in Fig. 5 are about 80 to 90  $\text{mmol m}^{-2} \text{ s}^{-1}$  on a leaf area basis, which compare favourably with the independent porometric measurements of  $g_s$  (Fig. 3a).

Cryo-SEM images of needles snap-frozen in liquid nitrogen indicated that the relative abundance of water-filled versus embolized tracheids in the central vein varied as a function of needle water status. In a sample frozen at  $\Psi_l = -0.12$  MPa, nearly all of the non-transfusion tracheids were water filled (Fig. 6a). The prevalence of water-filled and presumably functional tracheids in Fig. 6a was consistent with near maximal values of  $K_{\text{leaf}}$  at  $\Psi_l = -0.12$  MPa (Fig. 1). In contrast, an image of a sample frozen when  $\Psi_l$  was  $-2.10$  MPa revealed an abundance of embolized tracheids (Fig. 6b). Corresponding values of  $K_{\text{leaf}}$  were near 0 when  $\Psi_l$  was  $-2.10$  MPa (Fig. 1).

Well-hydrated needles frozen at  $\Psi_l$  between 0 and  $-0.5$  MPa had a mean number of empty tracheids of  $3.4 \pm 1.1$  SE (Fig. 7). Needles frozen at  $\Psi_l$  lower than  $-2.0$  MPa had a substantially larger mean number of empty tracheids of  $23.6 \pm 8.5$  SE ( $P = 0.056$ ,  $n = 16$ ). The vascular bundles of needles frozen at intermediate  $\Psi_l$  between  $-0.5$  and  $-1.25$  MPa, and between  $-1.25$  and  $-2.0$  MPa had mean numbers of empty tracheids of  $16.4 \pm 6.6$  SE and  $26.6 \pm 7.6$  SE, respectively. The nature of the relatively high error associated with empty tracheid numbers at low  $\Psi_l$  can be



**Figure 2.** Example of a typical relationship between leaf hydraulic conductance ( $K_{\text{leaf}}$ ) and leaf water potential ( $\Psi_l$ ) illustrating the method used to estimate the  $\Psi_l$  at which  $K_{\text{leaf}}$  declines to its initial minimum value. The horizontal dotted line represents the midpoint of the y-range of the sigmoid curve. The instantaneous slope at this point yielded a tangent (dashed line) whose intercept with the x-axis was considered to be the value of  $\Psi_l$  at which  $K_{\text{leaf}}$  had reached its initial minimum value.



**Figure 3.** Time courses of stomatal conductance ( $g_s$ ) (a) and leaf water potential ( $\Psi_l$ ) (b) from two trees at a mean measurement height of  $\sim 55$  m on 1 September 2005. The  $\Psi_l$  value at which  $g_s$  begins to decline is indicated by the intersection of the dashed lines. A four-parameter log-normal function was fit to diurnal courses of  $g_s$ , and a cubic polynomial function was fit to diurnal courses of  $\Psi_l$  (b).  $\Psi_l$  values at the point where  $g_s$  declined were assessed for all four sampling heights from diurnal measurements of  $\Psi_l$  and  $g_s$ . Each data point represents a mean of three measurements, and error bars are SEs.

attributed to the fact that needles frozen at  $\Psi_l$  between  $-1.25$  and  $-2.0$ , and below  $-2.0$  MPa, fell into two distinct groups; one with relatively high numbers of empty tracheids, and one with relatively low numbers of empty tracheids (Fig. 7).

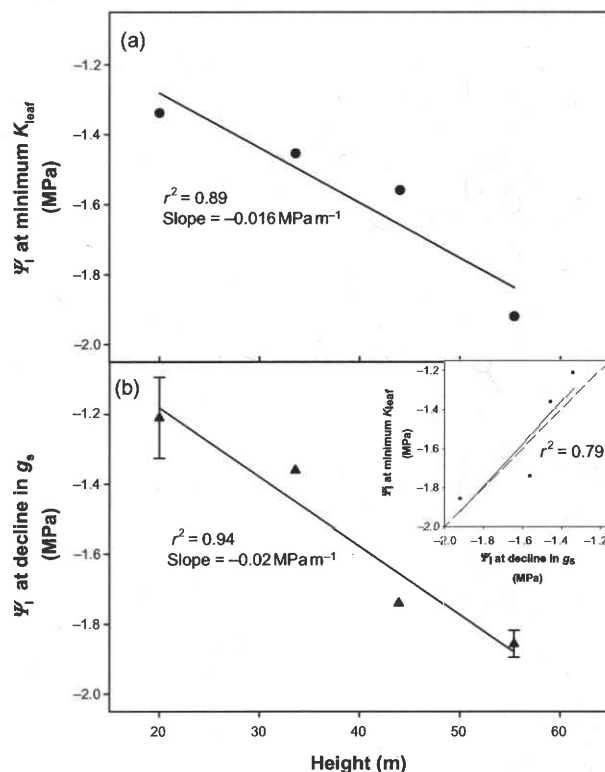
## DISCUSSION

Our results are consistent with the idea that increasing height above the ground imposes multiple constraints on the physiological functioning of leaves and other organs. In particular, features of hydraulic architecture and stomatal regulation of transpiration in Douglas-fir trees were finely tuned to vertical gradients in xylem tension. The slopes of vertical changes in the  $\Psi_l$  at which critical minimum values of  $K_{leaf}$  were attained and at which mid-morning stomatal closure began to occur were indistinguishable from each other and from the vertical gradient of  $\Psi_l$  previously measured in the same stand of trees (Bauerle *et al.* 1999; Woodruff *et al.* 2004). Furthermore, our data provide evidence for the mechanistic significance of height-dependent

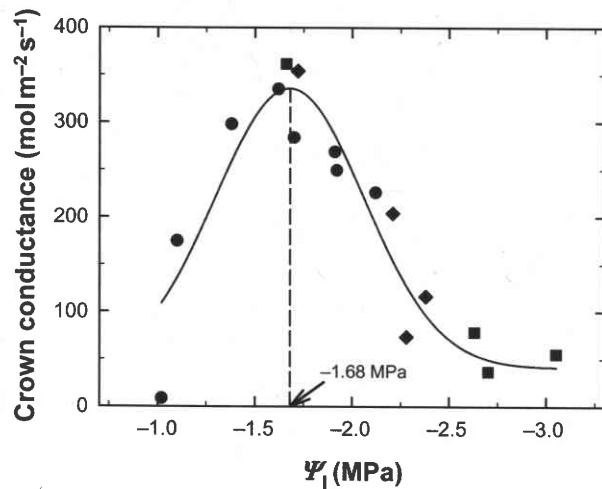
threshold values of  $\Psi_l$  associated with partial stomatal closure to limit transpiration, thereby dampening the diurnal decline in  $\Psi_l$ . Finally, cryo-SEM images suggest that tracheids in Douglas-fir needles are likely to embolize and refill on a daily basis during the growing season even under non-extreme environmental conditions.

## Compensatory adjustments to height

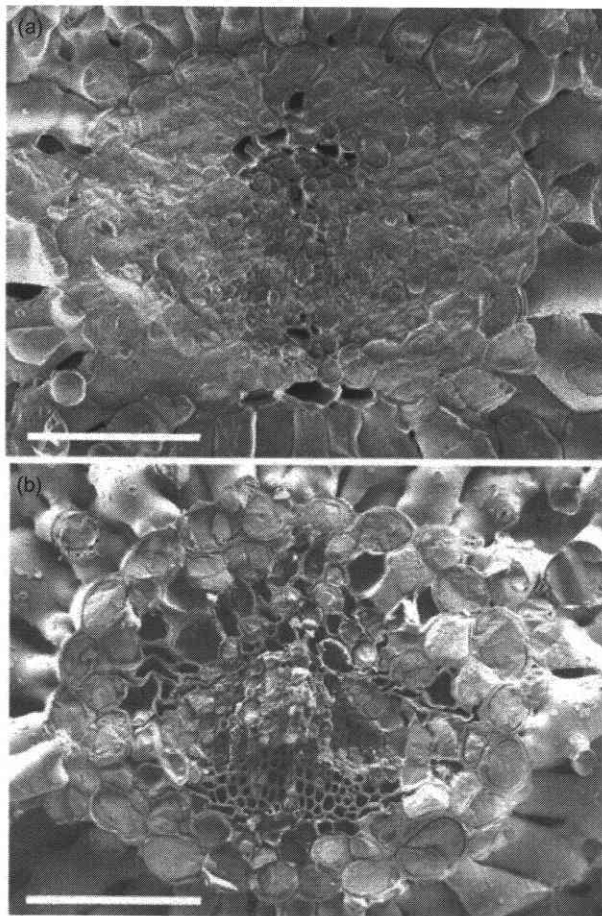
The vertical gradient in  $\Psi_l$  consists of a gravitational or hydrostatic component ( $-0.01$  MPa  $m^{-1}$ ) and a dynamic or frictional component influenced by a combination of xylem specific conductivity ( $K_s$ ), sap velocity and path length. The hydrostatic component is unalterable and persists in the absence of transpiration, but a number of compensatory mechanisms can potentially mitigate the frictional component. These include increased  $K_s$  (Domec & Gartner 2002, 2003), reductions in leaf area relative to sapwood area



**Figure 4.** Height dependence of critical values of leaf water potential ( $\Psi_l$ ). (a)  $\Psi_l$  at which leaf hydraulic conductance ( $K_{leaf}$ ) has declined to its initial minimum value. (b)  $\Psi_l$  at which stomatal conductance ( $g_s$ ) begins to decline from its daily maximum value. Measurements were conducted on 20 July 2005 (35, 44 and 55 m heights), 1 September 2005 (55 m height only) and on 23 July and 23 September 2002 (20 m height only). Error bars are from mean values of two separate diurnal sessions at the 20 and 55 m locations. Only a single sampling session was conducted at the 35 and 44 m height locations. Inset:  $\Psi_l$  at which  $K_{leaf}$  has declined to its initial minimum value in relation to  $\Psi_l$  at which  $g_s$  declines from its daily maximum for the heights indicated in Fig. 4. The dashed line represents a 1:1 relationship.



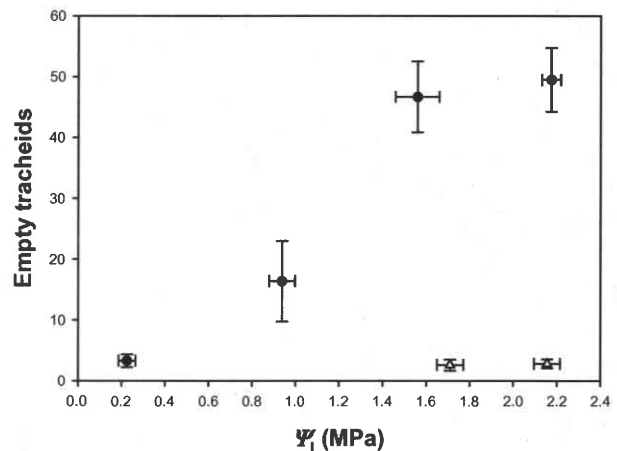
**Figure 5.** Crown vapour phase conductance in relation to leaf water potential ( $\Psi_l$ ) in a 1.4-m-diameter, 58.0-m-tall Douglas-fir tree. Data were obtained on 31 July ( $\blacklozenge$ ), 21 August ( $\bullet$ ) and 28 August ( $\blacksquare$ ) 2002.



**Figure 6.** Images observed with the cryo-scanning electron microscopy (SEM) technique of vascular bundles in Douglas-fir needles at  $\Psi_l = -0.125$  MPa (a) and  $-2.1$  MPa (b). The white bars represent 100  $\mu\text{m}$ .

(McDowell *et al.* 2002b) and increased reliance on stored water (capacitance) with increasing tree size and height (Phillips *et al.* 2003). Nevertheless, trade-offs exist that constrain compensatory adjustments that could reduce the vertical gradient in xylem tension. For example,  $K_s$  of sapwood near the base of the trunk increases with tree size in Douglas-fir (Domec & Gartner 2002), but  $K_s$  of branch wood decreases with increasing height largely because of increases in tracheid pit membrane resistance associated with decreasing membrane pore size necessary to diminish the risk of air seeding in the presence of larger tensions in terminal branches near the top of the tree (Domec, Lachenbruch & Meinzer 2006).

During the growing season, the vertical gradient in midday  $\Psi_l$  of old-growth Douglas-fir trees at the study site is about  $-0.017$  to  $-0.020$  MPa  $\text{m}^{-1}$  (Bauerle *et al.* 1999; Woodruff *et al.* 2004), which corresponds with vertical gradients in critical values of  $\Psi_l$  associated with leaf hydraulic and stomatal function in the current study ( $-0.016$  to  $-0.02$  MPa  $\text{m}^{-1}$ , Fig. 4).  $\Psi_l$  at minimum  $K_{\text{leaf}}$  at an additional sampling height of 5 m was  $-1.05$  MPa (data not shown as no concurrent data of  $g_s$  and  $\Psi_l$  were collected for this height). The inclusion of this point in the relationship between height and  $\Psi_l$  at minimum  $K_{\text{leaf}}$  left the slope of the relationship in Fig. 4a unchanged at  $-0.016$  MPa  $\text{m}^{-1}$  ( $r^2 = 0.95$ ). This convergence of vertical trends implies that there are functional links among these features that contribute to homeostasis in the operation of stomatal regulatory systems as tree height increases. The mechanisms responsible for increasing resistance to water stress-induced loss of  $K_{\text{leaf}}$  with increasing height were not investigated, but might include changes in tracheid anatomical



**Figure 7.** Number of empty tracheids per needle counted from images observed with the cryo-SEM in relation to leaf water potential ( $\Psi_l$ ) for foliage samples obtained from tree 3013. Data are grouped into four classes of  $\Psi_l$ :  $> -0.5$  MPa,  $-0.5$  to  $-1.25$  MPa,  $-1.25$  to  $-2.0$  MPa and  $< -2.0$  MPa. Data for the two most negative classes of  $\Psi_l$  ( $-1.25$  to  $-2.0$  MPa and  $< -2.0$  MPa) are divided into two categories: needles with relatively high numbers of empty tracheids ( $\bullet$ ) and needles with relatively low numbers of empty tracheids ( $\Delta$ ).

properties that could affect the tendency for tracheids to bend or collapse, as well as tracheid or pit anatomical properties that could confer greater resistance to air seeding.

### $\Psi_l$ threshold for stomatal closure

Despite considerable empirical evidence that stomatal control of transpiration is mediated by some measure of plant water status, the specific locations at which the signals are sensed, the exact nature of the signals and the means by which they are sensed remain somewhat elusive. The lack of a clear monotonic relationship between  $g_s$  and  $\Psi_l$  under field conditions (e.g. Fig. 3) has contributed to the problem. Recent efforts to explain the dynamic behavior of stomata have focused on models that include the interaction between fluctuating plant hydraulic conductance and water status, or gradients in water status within leaves, mediated by metabolic responses of guard cells (e.g. Buckley 2005; Franks 2005; Franks, Drake & Froend 2007).

It is well documented that  $g_s$  and gas exchange are tightly coordinated with overall plant hydraulic conductance within and across species (Meinzer & Grantz 1990; Meinzer *et al.* 1995; Hubbard *et al.* 2001; Meinzer 2002; Mencuccini 2003). Recent work focused on leaves points to similar coordination between  $g_s$  and  $K_{leaf}$  (Sack *et al.* 2003; Brodribb & Holbrook 2004a, 2005a) that includes a dynamic component as  $K_{leaf}$  varies diurnally (Brodribb & Holbrook 2004b; Meinzer *et al.* 2004; Lo Gullo *et al.* 2005).

Although dynamic variations in  $g_s$  and  $K_{leaf}$  appear to be broadly coordinated on a diurnal basis, it has been difficult to link dynamic stomatal behavior with consistent values of  $\Psi_l$  clearly associated with specific features of the dependence of  $K_{leaf}$  on  $\Psi_l$  (Brodribb & Holbrook 2003, 2004b). In the present study, the onset of diurnal stomatal closure was consistently associated with height-dependent threshold values of  $\Psi_l$  that coincided with  $K_{leaf}$  attaining an initial minimum value. Furthermore, independent measurements in a 58-m-tall old-growth tree indicate that the threshold value of  $\Psi_l$  at which whole-crown vapour phase conductance began to decline ( $-1.68$  MPa, Fig. 5) is consistent with mean  $\Psi_l$  thresholds for diurnal stomatal closure ( $-1.65$  MPa) and for  $K_{leaf}$  approaching an initial minimum value ( $-1.64$  MPa) in the crowns of the old-growth trees (Fig. 4). Interestingly, both  $g_s$  and  $\Psi_l$  continued to decline even though further changes in  $K_{leaf}$  were apparently small (cf. Figs 1 & 3). Measurements of whole-tree sap flux suggest that this pattern of stomatal behavior causes transpiration to remain nearly constant once threshold values of  $\Psi_l$  have been attained (data not shown). However, the continued decline in  $\Psi_l$  once sap flux reached its maximum value implies that either tree hydraulic conductance undergoes a significant decline or that internal water storage reservoirs are being exhausted resulting in an apparent decline in hydraulic conductance (Andrade *et al.* 1998; Philips *et al.* 2003). The pattern of coordination between  $g_s$  and  $K_{leaf}$  observed in Douglas-fir suggests that the dynamic

gradient in  $\Psi$  within the leaf may play a role in coordination of stomatal and leaf hydraulic properties.

### Nature of changes in $K_{leaf}$

Maximum values of  $K_{leaf}$  measured in Douglas-fir were similar to those reported for other coniferous species (Brodribb & Holbrook 2005a,b). Vulnerability of Douglas-fir needles to water stress-induced loss of conductance in this study is similar to that found by others in a number of tropical angiosperm species (Brodribb & Holbrook 2003; Bucci *et al.* 2003), but substantially greater than that observed in leaves of the tropical conifer *Podocarpus grayii* (Brodribb & Holbrook 2005b) and four temperate pine species (Cochard *et al.* 2004).

Cryo-SEM images of needles suggest that increasing xylem embolism, rather than changes in the hydraulic properties of the extra-xylary pathway, is the primary cause of the water stress-induced decline in  $K_{leaf}$ . Nevertheless, concurrent water stress-dependent changes in the conductance of other structures such as the endodermal sheath surrounding the central vein cannot be ruled out. Other studies have shown close relationships between  $K_{leaf}$  and the fraction of embolized or collapsed xylem elements using cryo-SEM (Cochard *et al.* 2004; Brodribb & Holbrook 2005b) and other techniques (Bucci *et al.* 2003). Our results imply that xylem in Douglas-fir needles undergoes daily cycles of embolism and refilling. We did not assess the timing of  $K_{leaf}$  recovery, but studies carried out on tropical species suggest that may occur rapidly during the afternoon (Bucci *et al.* 2003; Brodribb & Holbrook 2004b).

It is apparent that tracheid refilling in Douglas-fir needles must occur when substantial tension is present in non-embolized tracheid and capillary spaces because  $\Psi_l$  never rises above  $-0.3$  to  $-0.6$  MPa in the crowns of the old-growth trees studied. Bucci *et al.* (2003) proposed that afternoon refilling of xylem in leaf petioles of two tropical savanna tree species is facilitated by transient pressure imbalances that drive water radially from living cells into embolized conduits. They found that the mechanical integrity of the petiole cortex was a requirement for embolism reversal, implying that it served as a pressure-confining barrier. In Douglas-fir needles, the endodermal sheath surrounding the central vein may serve as a pressure-confining barrier that allows transient pressure imbalances to be generated between the tracheids and surrounding tissues. Daily osmotic changes in living cells may drive the water movement necessary to generate localized pressure imbalances (Bucci *et al.* 2003; Canny 1997, 1998).

In the present study, cryo-SEM images of needles at  $\Psi_l$  levels below those associated with decline in  $K_{leaf}$  fall into two distinct categories: one with relatively high numbers of empty tracheids and one with numbers of empty tracheids comparable to needles at  $\Psi_l$  levels linked to maintenance of high  $K_{leaf}$ . Non-embolized needles attached to shoot segments at low  $\Psi_l$  and the well-defined dichotomy between high and low numbers of embolized tracheids can be explained by the re-filling of embolized tracheids following

a period of cavitation. Because re-filling must occur under conditions of tension within the water-conducting pathway, it is reasonable to consider the existence of a mechanism that functions to dissociate different compartments of the overall water-conducting pathway. The refilling of tracheids at the most distal portion of the water conducting pathway (the leaves) prior to the re-establishment of continuity within the overall pathway is likely to result in a condition of non-embolized leaf tracheids on shoot segments at low  $\Psi_l$ . As more proximal cells continue to be refilled, the overall pathway becomes hydraulically reconnected and shoot water potential increases.

### Environmental effects on $K_{\text{leaf}}$

In addition to xylem tension, environmental variables such as light and temperature may influence  $K_{\text{leaf}}$ . As expected,  $K_{\text{leaf}}$  is greater in sun than in shade leaves (Sack *et al.* 2003) and in sun- versus shade-adapted species (Brodribb & Holbrook 2004a). Temperature responses of  $K_{\text{leaf}}$  exceed those expected solely from changes in viscosity, consistent with a regulatory role for symplastic components of the leaf hydraulic network (Sack, Streeter & Holbrook 2004). Diel fluctuations of  $K_{\text{leaf}}$  are likely to be superimposed on dynamic responses to environmental variables (Nardini, Salleo & Andri 2005). In the present study, the influence of xylem tension on  $K_{\text{leaf}}$  was characterized in excised shoots allowed to dehydrate slowly. Absolute values of  $K_{\text{leaf}}$  in intact Douglas-fir leaves experiencing similar magnitudes of xylem tension may differ depending on environmental conditions and the time of day. Nevertheless, daily courses of stomatal conductance and sap flow of intact Douglas-fir trees were tightly coordinated with critical values of  $\Psi_l$  identified on leaf hydraulic vulnerability curves. Vertical trends in these values of  $\Psi_l$  corresponded to combined hydrostatic and frictional tension gradients observed in intact trees, indicating that these relationships were robust and highly conserved. Although seasonal variability is to be expected in leaf pressure-volume characteristics, inter-annual variability in these characteristics is likely to be low because of the consistent seasonal drought during the growing seasons in Pacific Northwest forests (Waring & Franklin 1979), as well as to the relatively low inter-annual variability in soil moisture conditions, particularly below depths of 1 m (Warren *et al.* 2005; Meinzer *et al.*, in press).

### Implications

Douglas-fir leaves lost 50% of their hydraulic conductance at values of  $\Psi_l$  between  $-0.75$  and  $-1.25$  MPa, depending on their height above the ground. In contrast, terminal branches to which the leaves are attached do not lose 50% of their conductivity until stem  $\Psi$  falls below  $-4$  to  $-6$  MPa, depending on tree age, size and site (Kavanagh *et al.* 1999; Domec & Gartner 2002). Daily minimum values of  $\Psi_l$  during the growing season typically range between  $-2$  and  $-3$  MPa in mature trees. Therefore, leaf xylem should experience daily cycles of embolism and refilling, whereas little

or no embolism is expected to occur in stem xylem. Rapidly reversible embolism in leaves may constitute part of an essential hydraulic signal that enables stomata to maintain stem and leaf  $\Psi$  at set points that insure the integrity of the water transport system upstream. Failure of stomata to quickly respond to rapid increases in transpiration can result in sharp increases in stem xylem tension and loss of conductivity, especially if water from sapwood storage has already been exhausted. Moreover, reliance on temporary leaf cavitation to regulate the water status and degree of embolism in stems can be advantageous if embolism repair processes in woody stems are not as vigorous as those observed in leaves.

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