

# Stomatal sensitivity to vapor pressure deficit and its relationship to hydraulic conductance in *Pinus palustris*

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**Summary** We studied the response of stomatal conductance at leaf ( $g_s$ ) and canopy ( $G_s$ ) scales to increasing vapor pressure deficit ( $D$ ) in mature *Pinus palustris* Mill. (longleaf pine) growing in a sandhill habitat in the coastal plain of the southeastern USA. Specifically, we determined if variation in the stomatal response to  $D$  was related to variation in hydraulic conductance along the soil-to-leaf pathway ( $K_L$ ) over the course of a growing season. Reductions in  $K_L$  were associated with a severe growing season drought that significantly reduced soil water content ( $\theta$ ) in the upper 90-cm soil profile. Although  $K_L$  recovered partially following the drought, it never reached pre-drought values. Stomatal sensitivity to  $D$  was well correlated with maximum  $g_s$  at low  $D$  at both leaf and canopy scales, and  $K_L$  appeared to influence this response by controlling maximum  $g_s$ . Our results are consistent with the hypothesis that stomatal response to  $D$  occurs to regulate minimum leaf water potential, and that the sensitivity of this response is related to changes in whole-plant hydraulics.

**Keywords:** drought, longleaf pine, soil water, stomatal conductance, water potential.

## Introduction

Understanding environmental controls on stomatal conductance ( $g_s$ ) has been a central focus of plant physiological research for decades because of the link between  $g_s$  and carbon gain, and hence plant growth. One of the most important environmental variables to which stomata respond is the vapor pressure deficit between leaf and air ( $D$ ). Stomata generally close as  $D$  increases and the response is often depicted as a nonlinear decline in  $g_s$  with increasing  $D$ . The magnitude of the reduction, or the slope of the  $g_s$  to  $D$  relationship, reflects the sensitivity of the response (Oren et al. 1999). The sensitivity of the response is related to maximum  $g_s$  and species or individuals that have high  $g_s$  at low  $D$  also tend to show a more sensitive stomatal closure response to increasing  $D$  (Johnson

and Ferrell 1983, Yong et al. 1997, Oren et al. 1999). Although the mechanism is unknown, some studies suggest that stomatal closure with increasing  $D$  occurs as a feedback response to some aspect of transpiration (bulk leaf or epidermal) and water loss from the leaf, rather than as a direct response to humidity (Meinzer and Grantz 1991, Mott and Parkhurst 1991, Monteith 1995, Meinzer et al. 1997).

The relationship between stomatal response to  $D$  and plant water loss suggests that the response to  $D$  is related to plant hydraulics and that it may play a role in regulating leaf water potential ( $\Psi_L$ ) (Bond and Kavanagh 1999, Oren et al. 2001). These dependencies are often expressed mathematically as:

$$g_s = K_L(\Psi_s - \Psi_L - h\rho g)/D \quad (1)$$

where  $K_L$  is leaf-specific hydraulic conductance,  $\Psi_s - \Psi_L$  represents the water potential gradient from soil to leaf, and  $h\rho g$  accounts for the gravitational effects on a water column of height  $h$  and density  $\rho$  (Whitehead 1998). According to Equation 1, changes in  $g_s$  are directly proportional to changes in  $K_L$  and inversely related to changes in  $D$ . Under steady-state conditions, reductions in  $g_s$  as  $K_L$  decreases or  $D$  increases should occur so that  $\Psi_L$  is maintained above a critical threshold, below which xylem cavitation and hydraulic failure may occur (Tyree and Sperry 1988). Several authors report strong relationships between  $g_s$  and  $K_L$  and between  $g_s$  and  $D$  that are consistent with this scenario. For example, Hubbard et al. (2001) showed that experimental reductions in  $K_L$  by an air-injection technique caused rapid reductions in  $g_s$  without changing bulk  $\Psi_L$ . Similar to Saliendra et al. (1995), they concluded that the relationship observed between  $K_L$  and  $g_s$  most likely does not represent any direct effect of  $K_L$  on  $g_s$ , but rather some sort of stomatal response to changes in water status within the leaf induced by  $K_L$ . The response of  $g_s$  to  $D$  may come about in much the same way. Oren et al. (1999) found that the  $g_s$  response to  $D$ , both within and between species, was consistent

with theoretical predictions from a model that assumed regulation of  $\Psi_L$ .

Equation 1 indicates that  $K_L$  and  $D$  interact to control  $g_s$ , and therefore,  $K_L$  should be considered when interpreting the response of  $g_s$  to  $D$ . Few studies, however, have explicitly evaluated the link between  $K_L$  and the response of  $g_s$  to  $D$ , particularly for mature trees in the field. One approach for investigating this relationship is to exploit the natural variation in  $K_L$  and then determine how that variation influences the  $g_s$  to  $D$  relationship. An important factor influencing  $K_L$  is soil drought. Reductions in  $K_L$  during drought may arise as a result of loss of root hydraulic conductivity in response to xylem cavitation (Linton and Nobel 1999), tissue suberization (Stasovsky and Peterson 1991) or inhibition of aquaporins (Martre et al. 2001), all of which may occur to prevent water loss from the roots to the soil. Drought-induced loss of  $K_L$  should likewise reduce  $g_s$ . Furthermore, because the sensitivity of the  $g_s$  response to  $D$  is related to maximum  $g_s$  at low  $D$ , reductions in  $K_L$  should result in a proportional reduction in stomatal sensitivity to  $D$ .

We investigated the response of  $g_s$  to increasing  $D$  and evaluated the influence of  $K_L$  on that response in mature *Pinus palustris* Mill. (longleaf pine) during a growing season. The study year was characterized by a severe growing season drought that significantly reduced soil water content ( $\theta$ ) in the upper soil profile. Stomatal conductance was measured at both leaf and canopy scales throughout the season, and the natural variation in  $D$  was used to evaluate the sensitivity of the stomatal closure response to increasing  $D$ . Sap-flux scaled estimates of transpiration were combined with measures of the soil-leaf water potential gradient to calculate  $K_L$  and to evaluate changes in  $K_L$  associated with drought. We predicted that  $g_s$  and  $K_L$  (measured by independent methods) would be well correlated to one another and that seasonal variation in the response of  $g_s$  to  $D$  would likewise be related to changes in  $K_L$  caused by the drought.

## Materials and methods

### Study site

The study was conducted in a mixed longleaf pine-oak habitat at the Joseph W. Jones Ecological Research Center in southwest Georgia, USA (31° N, 84° W). The region's climate is described as humid-subtropical with mean daily temperatures ranging from 21–34 °C in summer to 5–17 °C in winter, and precipitation is distributed evenly throughout the year with an annual mean of 1310 mm (Goebel et al. 2001). The study site was representative of a typical sandhill habitat, in terms of woody plant community composition and structure (Monk 1968). *Pinus palustris* dominated the overstory, and several smaller stature *Quercus* spp. including *Q. laevis* (Walt.), *Q. incana* (Bartr.), *Q. margaretta* (Ashe) and *Q. virginiana* (Mill.) were present in the mid-story. The density of *P. palustris* on the site was low (54 trees ha<sup>-1</sup>), resulting in an open canopy. The soil on the site is classified as Typic Quartzipsamment with relatively low water-holding capacity

(18 cm water per m soil) and no significant accumulation of clay within the upper 3 m profile. Mean age of *P. palustris* occupying the site was 57 years (based on a survey of 71 trees by Mitchell et al. 1999). Prior to the study, scaffolding was constructed to allow canopy access to three *P. palustris* trees (three trees for all physiological measurements described below).

### Environmental measurements

The study period extended from February to October 2000. Precipitation was measured daily with a tipping bucket rain gauge (Model TE525, Campbell Scientific, Logan, UT) at a weather station located 4.94 km from the study site. Air temperature ( $T_A$ ) and relative humidity (RH) were recorded every 2 min on the site at two-thirds canopy height with a  $T_A$ -RH sensor (Model H8, HOBO Computer, Bourne, MA). Saturation vapor pressure was calculated from  $T_A$  based on the Tetens formula (cf. Murray 1967) and substituting  $T_A$  for leaf temperature ( $T_L$ ). This substitution assumes close coupling between the canopy and the atmosphere and is often made for open-canopy coniferous systems because high atmospheric turbulence maintains  $T_L$  close to  $T_A$  (Monteith and Unsworth 1990). Leaf-to-air vapor pressure deficit ( $D$ ) was calculated from saturation vapor pressure and RH. To evaluate trends in  $D$  during the measurement period, a daytime-averaged  $D$  ( $D_D$ ) was determined by averaging  $D$  during daylight hours. Volumetric soil water content ( $\theta$ ) in the upper 90 cm of soil was measured every 2 weeks (weekly during the drought period) by time domain reflectometry (TDR; Topp et al. 1980). Pairs of stainless steel rods were inserted vertically into the soil at 10 locations and  $\theta$  was measured with a cable tester (Model 1502B, Tektronix, Beaverton, OR).

### Leaf water potential and leaf gas exchange

About every 2 weeks throughout the study period, leaf water potential ( $\Psi_L$ ) was measured in the upper and lower crown thirds of each tree at predawn (~0500 h) and midday (~1300 h) with a pressure chamber (Model 1002, PMS Instruments, Corvallis, OR). One fascicle per crown position per tree was sampled on each measurement occasion, and fascicles were sampled from the same branches across measurement dates.

Leaf-level stomatal conductance ( $g_s$ ) and net photosynthesis ( $A_{net}$ ) were measured every 4–5 weeks throughout the study period with a portable photosynthesis system equipped with an artificial light source (Model LI-6400, Li-Cor, Lincoln, NE). Each measurement period spanned 2 days, over which gas exchange was measured twice in the morning and twice in the afternoon to capture the natural variation in  $D$ . Measurements were made on one fascicle in the upper and lower crown thirds for each tree, and on the same branches sampled for  $\Psi_L$ . Measurements were made on both previous- and current-year needles once new needles reached at least 50% of their anticipated final length. The first measurement date to incorporate current-year needles was August 11–12, 2000. To avoid potentially confounding effects of light on the  $g_s$  response to  $D$  (despite the open nature of the canopy), all measurements were made at light saturation (photon flux density > 1000  $\mu\text{mol m}^{-2}$

s<sup>-1</sup>, based on light response curves). Atmospheric CO<sub>2</sub> concentration was maintained at a constant 350 μmol m<sup>-2</sup> s<sup>-1</sup>. Needle temperature was kept within 1 °C of ambient air temperature, and relative humidity inside the chamber was also maintained near ambient. Needles were allowed to equilibrate inside the chamber, and data were then recorded every 15 s for 3 min. Following measurement, needles were stored on ice and transported to the laboratory for determination of leaf area. Needle radius to the nearest 0.01 mm was measured for each needle with digital calipers and from this, all-sided leaf area inside the chamber was calculated, assuming a cylindrical needle shape (Svenson and Davies 1992).

#### Canopy stomatal conductance and whole-tree hydraulic conductance

Canopy stomatal conductance ( $G_S$ ) was calculated from estimates of transpiration ( $E_L$ ; per unit leaf area) with the following equation derived from Whitehead and Jarvis (1981):

$$G_S = (G_v T_A \rho E_L) / D \quad (2)$$

where  $G_v$  is the universal gas constant adjusted for water vapor (0.462 m<sup>3</sup> kPa K<sup>-1</sup> kg<sup>-1</sup>),  $T_A$  is air temperature in degrees K,  $\rho$  is density of water (998 kg m<sup>-3</sup>) and  $D$  is vapor pressure deficit (kPa). We estimated  $E_L$  from sap flux density ( $J_S$ ) and sapwood to leaf area ratio ( $A_S:A_L$ ) for each tree. Thermal dissipation probes (Model TDP-30, Dynamax, Houston, TX) were used to measure  $J_S$ . Each probe consisted of a heated (constant energy input) and reference (non-heated) needle. Needles were inserted radially into hydroactive xylem, with the heated needle positioned ~5 cm above the reference needle. The temperature difference ( $dt$ ) between the heated and reference needle was used to calculate  $J_S$  according to an empirically derived equation provided by Granier (1987):

$$J_S = 119 \left( \frac{dt_m - dt}{dt} \right)^{1.23} \quad (3)$$

where  $dt_m$  is maximum  $dt$ , which occurs under conditions of zero flux. Sap flux data were recorded every minute with a data logger (Model CR10, Campbell Scientific, Logan, UT) and averaged over a 30-min interval. Probes were installed on the north sides of trees in the outer 30 mm of xylem at a stem height of 1.3 m and were insulated from solar radiation by reflective shielding. To account for circumferential variation in  $J_S$ , one of the study trees was equipped with a probe on its south-facing side. To account for the radial trend in  $J_S$  (Phillips et al. 1996), an additional sensor was installed on this same tree at 30–60 mm from the cambium. From these data, ratios representing south:north and inner:outer variation in  $J_S$  were developed for each measurement date. These ratios were then used to scale  $J_S$  to the entire sapwood area (Oren et al. 1998).

Sapwood area ( $A_S$ ) was determined for each tree from four increment cores extracted at the end of the measurement period after removal of the thermal dissipation probes. We did not core the trees at the beginning of the measurement period

to avoid damage to the conducting tissue. Sapwood length was determined by visual inspection of the core and converted to  $A_S$  based on the area of a circle, subtracting the areas represented by the heartwood and bark. In all cases, sapwood length was greater than the depth at which TDP probes were installed, and when scaling  $J_S$  to the entire sapwood, the flux at 30–60 mm was used to represent flux beyond 60 mm.

Tree leaf area was determined from allometric equations developed for the site both before ( $n = 15$  trees) and after ( $n = 5$  trees) the study period. Trees were randomly located along transects adjacent to the site and each selected tree was climbed. The diameter of every branch on each tree was measured, and six branches per tree were then randomly selected and cut. From harvested branches, log–log relationships between branch diameter and branch leaf area were developed to predict entire tree  $A_L$  by branch summation. Log–log relationships between DBH and  $A_L$  were developed to predict  $A_L$  of the tower-accessible trees. Seasonal change in  $A_L$  was determined from regular measurements (about every 2 weeks) of needle elongation and needle senescence in the tower-accessible trees that were made by Sheffield et al. (2003) according to the equation:

$$A_L t_x = A_L t_0 + A_L t_0 (\text{Net}_x) - A_L t_0 (\text{Nst}_x) \quad (4)$$

where  $A_L t_x$  is leaf area at time  $x$ ,  $A_L t_0$  is leaf area at the beginning of the growing season, and  $\text{Net}_x$  and  $\text{Nst}_x$  are percent needle elongation and percent needle senescence, respectively, at time  $x$ .

Soil-to-leaf hydraulic conductance ( $K_L$ ) was calculated from  $E_L$  and the water potential gradient from soil to leaf:

$$K_L = E_L / (\Psi_S - \Psi_L - h \rho g) \quad (5)$$

where  $\Psi_L$  is leaf water potential at midday and  $\Psi_S$  is soil water potential. For *P. palustris*, predawn  $\Psi_L$  provides a good estimate of  $\Psi_S$ , based on experiments with saplings (Donovan et al. 2001) and observations in this study of predawn  $\Psi_L$  near zero for mature trees. Midday transpiration estimates were used for  $E_L$ , and it was assumed that the relationship between  $E_L$  and  $\Psi_L$  at this time of day was unaffected by stored water.

#### Data analyses

Sensitivity of the stomatal response to  $D$  was determined by fitting the data to the functional form:

$$g_S = b - m \ln D \quad (6)$$

where the slope of the line ( $-dg_S/d \ln D$ ) represents the sensitivity of the response and the y-intercept represents  $g_S$  at 1 kPa  $D$ , also known as  $g_{S \text{ref}}$  (Oren et al. 1999). Stomatal sensitivity to  $D$  was determined for both leaf- and canopy-level conductance from light-saturated  $g_S$  and  $G_S$  for days that soil water content and  $K_L$  were measured. There were 11 days of available data (where data were collected over a sufficient range in  $D$ ) during the measurement period for constructing relationships between  $G_S$  and  $\ln D$ , and there were 5 days for evaluat-

ing the relationship between  $g_s$  and  $\ln D$ . The number of points in the relationships between  $G_s$  and  $\ln D$  for each tree varied according to measurement date (because the range of  $D$  varied according to measurement date), but was always between eight and 15. At the leaf level, there were four points available for each tree on each measurement date, representing the two morning and two afternoon measurements conducted on each date as described above. These relationships were analyzed by linear regression in SigmaPlot software (SigmaPlot v5.0; SPSS, Chicago, IL).

The relationship between stomatal and hydraulic conductance was constructed based on  $g_s$  and  $K_L$ , rather than  $G_s$  and  $K_L$ , because the latter are both calculated from the same estimates of transpiration and are therefore not independent. There were 6 days when measurement of  $g_s$  and  $K_L$  overlapped, and likewise measurement of  $g_s$  and  $G_s$ . Relationships between these variables were analyzed by linear regression. Variations in  $\theta$ , predawn and midday  $\Psi_L$ ,  $g_s$ ,  $A_{net}$ ,  $G_s$  and  $K_L$  over the measurement period were analyzed by repeated measures analysis of variance. Crown position (upper versus lower) was included in the models for  $\Psi_L$ ,  $g_s$ ,  $A_{net}$  and needle

age (current- versus previous-year) was included for  $g_s$  and  $A_{net}$ . These analyses were carried out with SAS software (SAS v8.1; SAS Institute, Cary, NC).

## Results

Total precipitation for the measurement period was 89% of normal, based on a 42-year mean for the area, with 69% occurring between July and October (Figure 1A). Drought occurred from the beginning of the measurement period through July, and during this time, precipitation was 52% of normal. Soil water content in the upper 90 cm varied significantly over the measurement period ( $P < 0.001$ ; Figure 1B). During the drought,  $\theta$  in the upper 90 cm of soil was reduced to 34% of maximum. Daytime-averaged vapor pressure deficit ( $D_D$ ) showed expected seasonal variation (Figure 1C). Mean monthly  $D_D$  for February, June and October corresponding to the beginning, middle and end of the season was 0.91, 1.57 and 1.16 kPa, respectively.

Both predawn and midday  $\Psi_L$  varied significantly during the measurement period ( $P < 0.001$  for both cases). Crown po-

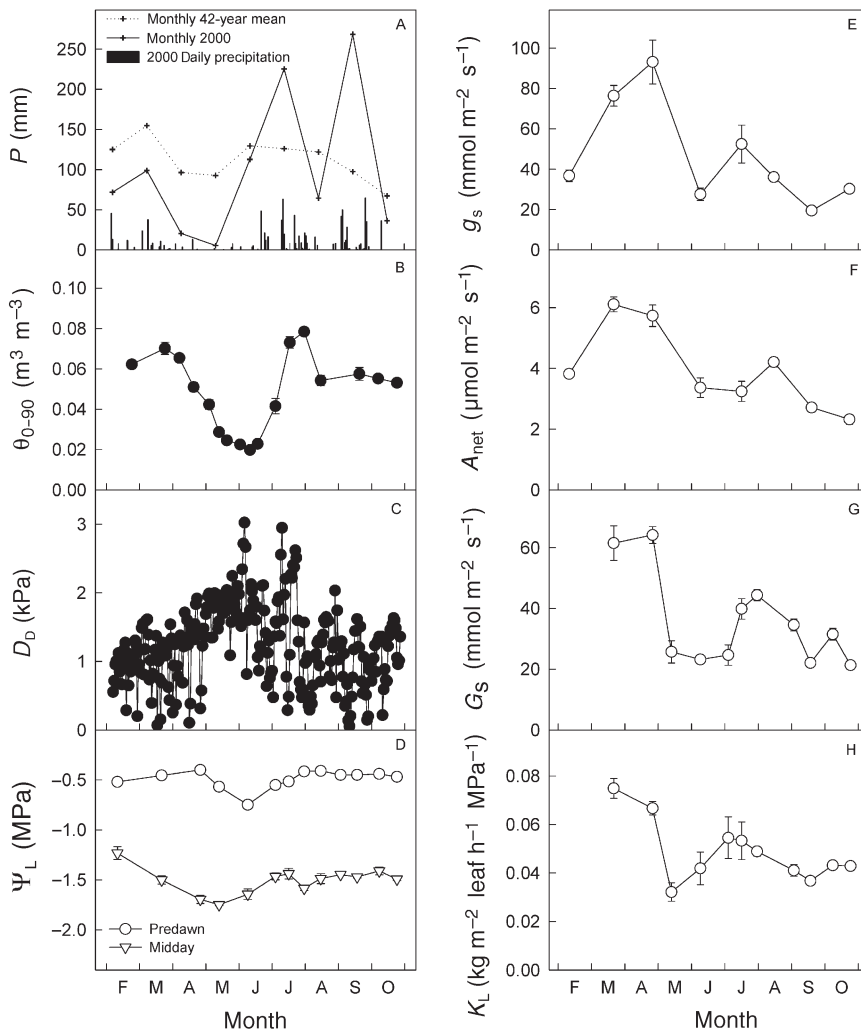


Figure 1. Observations made from February through October 2000 in a mature *Pinus palustris* stand in a sandhill habitat at the Joseph W. Jones Ecological Research Center in southwest Georgia, USA. (A) Precipitation ( $P$ ), (B) soil volumetric water content ( $\theta$ ), in the upper 90 cm of soil (means  $\pm$  SE,  $n = 10$  stations), (C) daytime averaged vapor pressure deficit ( $D_D$ ), (D) leaf water potential ( $\Psi_L$ ) before dawn and midday, (E) midday leaf stomatal conductance ( $g_s$ ), (F) midday photosynthesis ( $A_{net}$ ), (G) midday canopy stomatal conductance ( $G_s$ ), and (H) midday soil-to-leaf hydraulic conductance ( $K_L$ ). All physiological measurements (Figures D–H) are means  $\pm$  1 SE for three trees.

sition (upper versus lower) had no significant effect on  $\Psi_L$  ( $P = 0.097$ ) and there was no significant interaction between measurement date and crown position ( $P = 0.115$ ). Means presented in Figure 1D, therefore, represent means for the three trees, averaged first across crown position for each tree. Mean predawn  $\Psi_L$  excluding the drought period was  $-0.46$  MPa. During the drought, predawn  $\Psi_L$  value was reduced to  $-0.75$  MPa. Midday  $\Psi_L$  varied from  $-1.23$  MPa in February to  $-1.74$  MPa in May.

Variation in  $g_s$ ,  $A_{\text{net}}$ ,  $G_s$  and  $K_L$  over the measurement period was, in all cases, highly significant ( $P < 0.001$ ). There was no significant effect of crown position on either  $g_s$  or  $A_{\text{net}}$  ( $P = 0.734$  and  $P = 0.755$  for  $g_s$  and  $A_{\text{net}}$ , respectively), and interactions between crown position and measurement date were not present for either variable ( $P = 0.849$  and  $P = 0.581$  for  $g_s$  or  $A_{\text{net}}$ , respectively). There was also no significant effect of needle age on  $g_s$  or  $A_{\text{net}}$  ( $P = 0.164$  and  $P = 0.381$ ); interaction terms with measurement date were not significant either (minimum  $P = 0.229$ ). Means presented in Figures 1E and 1F are therefore means for the three trees, after averaging across crown position and needle age for each tree. Maximum  $g_s$ ,  $A_{\text{net}}$ ,  $G_s$  and  $K_L$  occurred during March–April and reductions in each of these parameters were recorded during the drought (Figures 1E–H). For example, measurements of  $g_s$  made in June at the peak of the drought were an average 53% of  $g_s$  measured in March. Reductions in  $A_{\text{net}}$  in response to the drought closely tracked the reductions in  $g_s$ , and overall there was a strong relationship between  $g_s$  and  $A_{\text{net}}$  ( $r^2 = 0.82$ ,  $P < 0.001$ ; data not shown). Mean  $K_L$  was reduced from  $0.0749$   $\text{kg m}^{-2} \text{ leaf h}^{-1} \text{ MPa}^{-1}$  in March to  $0.0419$  in June, and the decline in  $K_L$  over this period related well to the decline in  $\theta$  (Figure 2, Point 1 to Point 2). Some recovery of  $K_L$  as  $\theta$  continued to decline was evident (Point 2 to Point 3). As soil water content increased with the onset of precipitation following the drought,  $K_L$  likewise increased, but failed to reach pre-drought values,

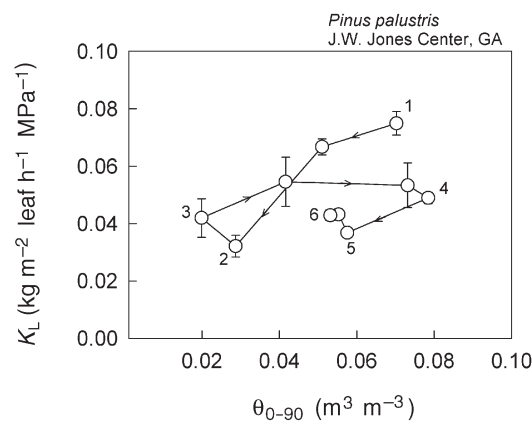


Figure 2. Relationship between soil water content ( $\theta$ ) in the upper 90 cm of soil and midday soil-to-leaf hydraulic conductance ( $K_L$ ) in three *Pinus palustris* trees (means  $\pm 1$  SE). Arrows represent the directional changes in  $K_L$  with changing  $\theta$ , and the numbers indicate measurement dates (1 = March, 2 = May, 3 = June, 4 = August, 5 = September and 6 = October).

indicating some apparently irreversible effect of drought on  $K_L$  (Point 3 to Point 4). A less severe drought that occurred later in the season caused further reductions in both  $\theta$  and  $K_L$  (Point 4 to Point 5). A slight recovery was again observed even though  $\theta$  continued to decline (Point 5 to Point 6).

Leaf- and canopy-level stomatal conductances were linearly related ( $r^2 = 0.96$ ,  $P < 0.001$ ; Figure 3), although mean  $g_s$  was 28% higher than  $G_s$  and the difference between  $g_s$  and  $G_s$  tended to be higher at greater conductances. The variation in  $g_s$  and  $G_s$  that occurred throughout the season was consistent across scales. To determine if this variation was related to hydraulic conductance along the soil–leaf pathway, we evaluated the relationship between  $K_L$  and stomatal conductance based on  $g_s$  rather than  $G_s$ . Throughout the measurement period, variation in  $g_s$  was strongly related to variation in  $K_L$  ( $r^2 = 0.88$ ,  $P < 0.01$ ; Figure 4).

Both  $g_s$  and  $G_s$  declined in response to increasing  $D$ . Fig-

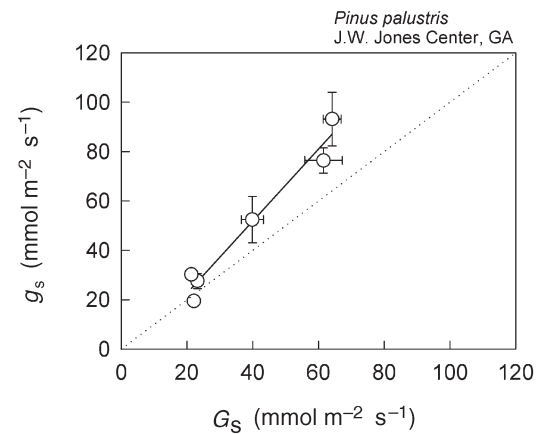


Figure 3. Relationship between leaf- and canopy-level stomatal conductance ( $g_s$  and  $G_s$ , respectively) at midday in three *Pinus palustris* trees (means  $\pm 1$  SE). The dotted line represents the 1:1 ratio.

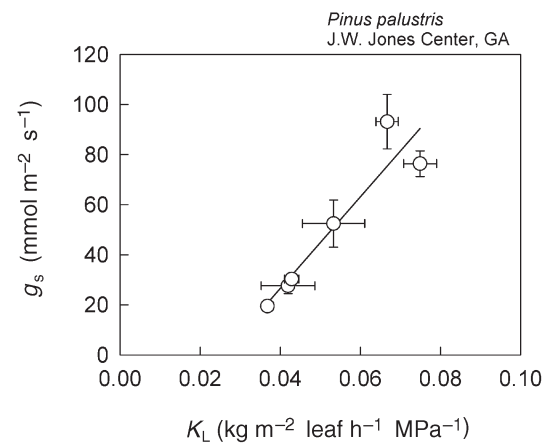


Figure 4. Relationship between midday soil-to-leaf hydraulic conductance ( $K_L$ ) and midday leaf stomatal conductance ( $g_s$ ), measured independently of one another in three *Pinus palustris* trees (means  $\pm 1$  SE).

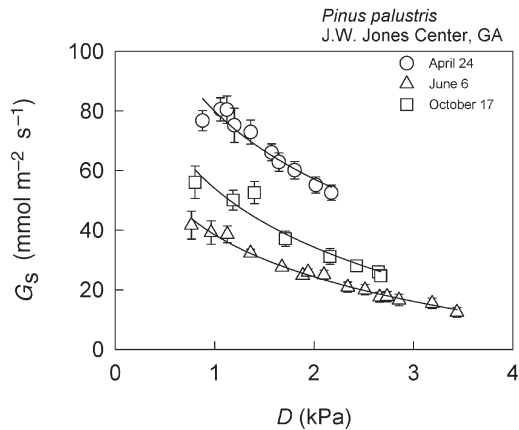


Figure 5. Decline in canopy stomatal conductance ( $G_S$ ) with increasing vapor pressure deficit ( $D$ ) for three measurement dates representing pre-drought, drought, and post-drought conditions. Symbols are means  $\pm$  1 SE in three *Pinus palustris* trees.

Figure 5 shows the relationship between  $G_S$  and  $D$  for three measurement dates representing pre-drought, drought and post-drought conditions. At a given  $D$ ,  $G_S$  was highest before the drought, lowest during the drought, and intermediate following the drought. Relationships between  $g_s$  and  $\ln D$  and between  $G_S$  and  $\ln D$  for each tree for each measurement date were generally strong (minimum  $r^2 = 0.66$  and  $0.75$  for  $g_s$  versus  $\ln D$  and  $G_S$  versus  $\ln D$ , respectively). Sensitivity of the stomatal response at both scales ( $-dg_s/d\ln D$  and  $-dG_S/d\ln D$  for leaf and canopy, respectively) was linearly related to stomatal conductance at  $D = 1$  kPa ( $g_{Sref}$  and  $G_{Sref}$ ;  $r^2 = 0.97$ ,  $P < 0.001$  and  $r^2 = 0.63$ ,  $P < 0.01$  for leaf and canopy, respectively; Figure 6). The dashed line in Figure 6 (slope = 0.6) rep-

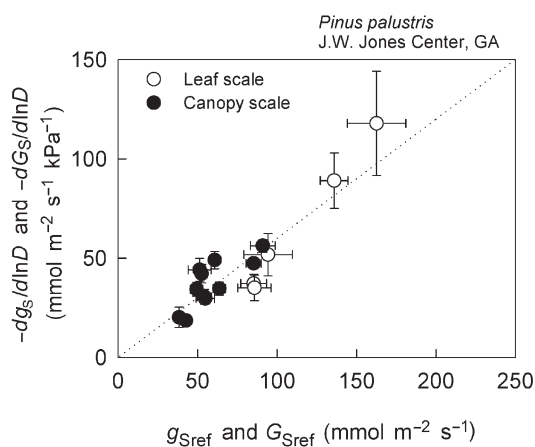


Figure 6. Relationship between the sensitivity of the stomatal response to increasing vapor pressure deficit ( $D$ ) at both leaf and canopy scales ( $-dg_s/d\ln D$  and  $-dG_S/d\ln D$  respectively) and stomatal conductance at  $D = 1$  kPa ( $g_{Sref}$  and  $G_{Sref}$  for leaf and canopy, respectively). The dotted line represents a slope of 0.6, theoretically expected if the response of  $g_s$  to  $D$  is consistent with stomatal regulation of minimum  $\Psi_L$  (Oren et al. 1999). Symbols are means  $\pm$  1 SE in three *Pinus palustris* trees.

resents the theoretical slope between stomatal conductance at 1 kPa  $D$  and stomatal sensitivity to  $D$  that is consistent with the role of stomata in regulating minimum  $\Psi_L$  (Oren et al. 1999). The data at both scales appear to cluster around this theoretical slope.

## Discussion

Given the relationship between  $K_L$  and  $g_s$  frequently reported in the literature (Sperry et al. 1993, Andrade et al. 1998, Meinzer et al. 1999, Hubbard et al. 2001), and provided that the sensitivity of the  $g_s$  response to  $D$  is related to maximum  $g_s$  at low  $D$  (Oren et al. 1999), it follows that the stomatal response to  $D$  should be related to  $K_L$  and changes therein. Our study provides strong correlative evidence for this link for mature trees in the field. Stomatal conductance at both leaf and canopy scales declined in response to increasing  $D$ , and the magnitude of the reduction varied over the measurement period. We found that  $K_L$  was the only variable measured that closely tracked the variation in  $g_s$  and  $g_s$  sensitivity to  $D$  throughout the entire measurement period. The most likely explanation for these correlations is that  $K_L$  controls maximum  $g_s$ , which in turn influences the sensitivity of the response to increasing  $D$ .

Although the study was not designed to determine how the relationships between  $K_L$  and  $g_s$  and the  $g_s$  sensitivity to  $D$  arose, the data are consistent with a scenario in which the stomatal response to  $D$  represents a feedback response of  $g_s$  to changes in leaf water status induced by  $K_L$ . The relationship observed between  $K_L$  and  $g_s$ , therefore, may not represent a direct effect of  $K_L$  on  $g_s$ , but may instead occur indirectly through the effect of  $K_L$  on leaf water status, followed by the effect of leaf water status on  $g_s$ . Stomata regulate minimum  $\Psi_L$  in such a way that tension on the water column created by declining  $\Psi_L$  does not cause excessive xylem cavitation (Tyree and Sperry 1988, Jones and Sutherland 1991). Because declines in  $\Psi_L$  occur as  $D$  increases, the stomatal response to increasing  $D$  observed in this study likely occurred to prevent  $\Psi_L$  from becoming too negative. These effects can be independent of bulk leaf water status because, as proposed by Saliendra et al. (1995), measurement of bulk leaf water potential may mask the subtle water potential gradients within the leaf that are important in controlling stomatal response. This may explain why there was no obvious relationship between  $g_s$  sensitivity to  $D$  and midday  $\Psi_L$ . It could also be argued that stomata regulate bulk  $\Psi_L$  homeostatically, i.e., that  $\Psi_L$  is maintained above some minimum value, and thus there should be no change in bulk  $\Psi_L$  as  $g_s$  declines in response to  $D$ .

Because  $g_s$  was measured independently of  $K_L$ , the relationship between stomatal conductance and hydraulic conductance represents a non-autocorrelated relationship between the variables. Numerous other studies have likewise reported this relationship based on both the natural variation in  $K_L$  and by experimentally manipulating  $K_L$ . For example,  $K_L$  has been shown to vary naturally with plant life stage (Hubbard et al. 1999), between species (Reich and Hinckley 1989), with time

of day (Andrade et al. 1998, Meinzer et al. 1999), and with declining soil water content in response to drought (Reich and Hinckley 1989, Cochard et al. 1996, Irvine et al. 1998), and in all cases, stomatal conductance was positively correlated with  $K_L$ . Experimental manipulations of  $K_L$  such as stem notching (Sperry et al. 1993), root pruning (Teskey et al. 1983), and partial defoliation (Pataki et al. 1998, Hubbard et al. 1999) further confirm this relationship and show that changes in stomatal conductance may occur within minutes of  $K_L$  manipulations.

We observed significant natural variation in  $K_L$  during the measurement period, most of which appeared to be related to the drought and its effect on soil water content. Patterns presented in Figure 2 illustrate the complexity of the relationship between  $\theta$  and  $K_L$ . An initial decline in  $K_L$  with declining  $\theta$  was present (Point 1 to Point 2), likely because of a reduction in root conductivity that could have occurred in response to xylem cavitation (Linton and Nobel 1999), or to other mechanisms designed to limit water loss from roots to soil, including formation of suberized tissue (Stasovsky and Peterson 1991) and aquaporin inhibition (Martre et al. 2001). An increase in  $K_L$  as  $\theta$  continued to decline (Points 2 to 3) may represent production of new roots, thereby increasing total root surface area and improving  $K_L$  (see Sperry et al. 2002). Roots produced during a drought, however, may have lower maximum hydraulic conductivity (Ewers et al. 2000), which may explain why  $K_L$  following the drought failed to recover fully to pre-drought values (Points 3 to 4). Maximum  $K_L$  recorded after the drought was ~70% of the maximum  $K_L$  recorded before the drought, at a similar  $\theta$  (~0.07). Additionally, this reduction in maximum  $K_L$  may be related to the failure of larger, more vulnerable conduits to refill and become active again. This pattern indicates that the effect of  $\theta$  on  $K_L$  is only partially reversible. Production of new roots and xylem may be required for complete recovery of  $K_L$ . Figure 2 illustrates that a subsequent decline in  $\theta$  that occurred later in the season likewise brought about a reduction in  $K_L$  (Points 4 to 5). When compared over a similar range of  $\theta$  (~0.06–0.08), the decline in  $K_L$  with decreasing  $\theta$  appears slightly steeper during the second drought relative to the first. One explanation for this difference in the response of  $K_L$  to  $\theta$  between the drought periods is cavitation fatigue (Hacke et al. 2001), i.e., following the first drought, xylem may be more susceptible to cavitation because of weakening of bordered pit membranes. In this case, even moderate drought may be enough to cause extensive xylem cavitation. As during the first drought, recovery of  $K_L$  as  $\theta$  continued to decline during the second drought (Point 5 to Point 6), which may again represent production of new roots.

Although we cite soil drought as the dominant factor influencing  $K_L$  in this study, other factors such as leaf area expansion and an increase in the proportion of latewood to earlywood in the xylem may explain some of the observed variation in  $K_L$ . Given the relationship between leaf area and  $K_L$  frequently reported (Pataki et al. 1998, Hubbard et al. 1999, Oren et al. 2001),  $K_L$  should decline from early to late season, corresponding to the expansion of leaf area. In *Pinus* spp., maximum leaf area occurs late in the season (Vose et al. 1994),

and, in our study stand, maximum leaf area occurred in September. Some of the reduction observed in  $K_L$  between the beginning and end of the measurement period may therefore be related to an increase in leaf area. Additionally, a decline in  $K_L$  should be expected over the season as the proportion of latewood to earlywood in the xylem increases. Mean hydraulic diameter of xylem conduits built later in the season is smaller than that of conduits constructed early in the season (Panshin et al. 1964), and the specific conductivity of these latewood cells is lower than that of earlywood (Domec and Gartner 2002). Although both of these factors (leaf area expansion and an increase in the proportion of latewood to earlywood xylem) may confound the relationship we describe between  $\theta$  and  $K_L$ , they are unlikely to exert their influence sufficiently fast to undermine our interpretations of observed patterns between consecutive measurement dates. However, they might have some effect on the interpretation of the differences in  $K_L$  at high  $\theta$  observed before and after the drought (Point 1 versus Point 4). Even if the effect were significant, it would not affect our interpretation of the relationship between  $K_L$  and stomatal conductance and stomatal sensitivity to  $D$ .

Although we have focused on demonstrating that the field data are consistent with hydraulic control, we acknowledge that chemical signalling (i.e., root- or leaf-derived abscisic acid) may also play some role in the stomatal response to changes in soil water content and  $D$  (summarized in Sauter et al. (2001) and Wilkinson and Davies (2002)). It is likely that chemical and hydraulic signals interact to control stomatal behavior (Tardieu and Davies 1993, Comstock 2002, Perks et al. 2002), and more work in this area is needed to fully understand these interactions.

However, the relationships we report between  $K_L$  and stomatal conductance and the stomatal response to  $D$  indicate some link between plant hydraulics and stomatal behavior as has been suggested by other studies (Bond and Kavanagh 1999, Oren et al. 2001). Leaf-specific hydraulic conductance itself can vary significantly over a given growing season, depending on soil water conditions and, most likely, changes in leaf area and other aspects of plant hydraulic architecture. Maximum  $g_s$  is an important predictor of stomatal response to  $D$ , and  $K_L$  appears to influence this relationship indirectly by controlling maximum  $g_s$ . To maintain adequate carbon gain yet avoid desiccation, stomata must balance the response to both  $K_L$  and  $D$ . Stomatal sensitivity to  $D$  is linearly related to plant water-use efficiency, or the ratio of carbon gain to water loss (Franks and Farquhar 1999), suggesting that the stomatal response to  $D$  is likewise coordinated with photosynthesis in some way. Understanding the nature of the relationships between  $K_L$  and  $g_s$ , and  $g_s$  response to  $D$  is therefore important for understanding the factors controlling carbon gain and plant growth, given the close relationship generally found between  $g_s$  and  $A_{net}$ .

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