

Decoupling the influence of leaf and root hydraulic conductances on stomatal conductance and its sensitivity to vapour pressure deficit as soil dries in a drained loblolly pine plantation

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ABSTRACT

The study examined the relationships between whole tree hydraulic conductance (K_{tree}) and the conductance in roots (K_{root}) and leaves (K_{leaf}) in loblolly pine trees. In addition, the role of seasonal variations in K_{root} and K_{leaf} in mediating stomatal control of transpiration and its response to vapour pressure deficit (D) as soil-dried was studied. Compared to trunk and branches, roots and leaves had the highest loss of conductivity and contributed to more than 75% of the total tree hydraulic resistance. Drought altered the partitioning of the resistance between roots and leaves. As soil moisture dropped below 50%, relative extractable water (REW), K_{root} declined faster than K_{leaf} . Although K_{tree} depended on soil moisture, its dynamics was tempered by the elongation of current-year needles that significantly increased K_{leaf} when REW was below 50%. After accounting for the effect of D on g_s , the seasonal decline in K_{tree} caused a 35% decrease in g_s and in its sensitivity to D , responses that were mainly driven by K_{leaf} under high REW and by K_{root} under low REW. We conclude that not only water stress but also leaf phenology affects the coordination between K_{tree} and g_s and the acclimation of trees to changing environmental conditions.

Key-words: *Pinus taeda*; coastal plain; conductivity; embolism; LAI; leaf phenology; soil moisture; water potential.

INTRODUCTION

Understanding what controls the diffusive conductance of stomata to water vapour (g_s) has been a critical area of research in plant physiology because of the close relationship between g_s and net carbon assimilation (Franks & Farquhar 1999). The most important environmental variable to which stomata respond is the vapour pressure deficit between leaf and air (D). Some studies suggest that stomatal closure occurs with increasing D as a feedback

response to leaf and whole plant water status in order to limit transpiration and water use, rather than as a direct response to humidity (Meinzer *et al.* 1995; Monteith 1995; Mott & Buckley 1998). In the gas phase and under steady-state conditions, when water storage is zero such as during midday (Phillips *et al.* 2002), transpiration on a leaf area basis (E in $\text{mmol m}^{-2} \text{s}^{-1}$) can be expressed mathematically using the Ohm's law analogy:

$$E = g_s D \quad (1)$$

and in the liquid phase as:

$$E = K_{\text{tree}} (\Psi_{\text{soil}} - \Psi_{\text{leaf}} - 0.01h) \quad (2)$$

where K_{tree} ($\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) is the leaf-specific hydraulic conductance of soil tree atmosphere continuum, $(\Psi_{\text{soil}} - \Psi_{\text{leaf}} - 0.01h)$ represents the driving force for water movement between the soil and the leaf over a tree height h corrected for the hydrostatic gradient of 0.01MPa m^{-1} (Whitehead 1998). Equations 1 and 2 indicate that K_{tree} and D interact to control g_s , and therefore, an understanding of K_{tree} is essential to interpret the response of g_s to D , and to quantify patterns and controls of the soil-to-root and canopy-to-atmosphere interactions (Oren *et al.* 1999; Sperry *et al.* 2002; Ewers *et al.* 2005). However, the interaction between K_{tree} and D makes it difficult to partition their individual effects on g_s . Oren *et al.* (1999) showed that stomatal sensitivity to D is proportional to the magnitude of g_s at low D ($<1 \text{kPa}$), and therefore, when D is fixed, here at 1kPa , $g_s = g_{s\text{-ref}}$ and becomes proportional to E ,

$$g_{s\text{-ref}} \approx E \quad (3)$$

from equations 2 and 3, it is clear that any change in K_{tree} will induce changes in E and $g_{s\text{-ref}}$, and any reductions in K_{tree} should result in a proportional reduction in stomatal sensitivity to D . We propose to use this framework to investigate the nature of the relationship between K_{tree} and $g_{s\text{-ref}}$, and how this relationship affects the sensitivity of g_s to D . We acknowledge that the Ohm's law analogy is a simplification

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of processes governing the long-distance water transport in plants (Katul, Leuning & Oren 2003; Chuang *et al.* 2006). However, it provides a simple and useful way to interpret the correlation between the liquid and the gas phase, as well as to study the inter-relationships among g_s and K_{tree} under steady-state conditions (see review by Meinzer 2002).

Experimental studies show a fast stomatal response to artificial manipulations in K_{tree} (Sperry & Pockman 1993; Cochard *et al.* 2002). However, while many studies have examined the relationship between K_{tree} and g_s , K_{tree} remains a somewhat mysterious factor because it is mainly dependent on K_{root} and K_{leaf} . These two components can account for more than 70% of the whole tree hydraulic resistance (Cruziat, Cochard & Ameglio 2002; Sack *et al.* 2003; Sack & Holbrook 2006), and yet remains largely unstudied in many species and ecosystems (Brodribb & Holbrook 2003; Schenk & Jackson 2005; Collins & Bras 2007). Therefore, understanding the partitioning of these two conductivities and their respective contributions to K_{tree} under various environmental conditions is crucial to understand how it influences g_s and its sensitivity to D . A mechanistic depiction of the effect of variation in K_{tree} , $g_{s\text{-ref}}$, and the sensitivity of g_s to D requires isolating the location of the main resistances to water flow and their seasonal dynamics, which is rarely done. Without knowing how these resistances change with soil drying and plant water status, we cannot understand or predict the response of plant water use to current and future environmental conditions (Maseda & Fernández 2006; McDowell, White & Pockman 2008).

Maintaining a sufficient water supply to leaves is challenging because the mechanism driving water flux (cohesion–tension) during transpiration places xylem under tension, making it vulnerable to cavitation-induced embolism. Evidence that stomata are able to sense and respond rapidly to changes in hydraulic conductance associated with variation in embolism (Salleo *et al.* 2000; Brodribb *et al.* 2003) implies that embolism in certain plant organs may play a rather different role than originally believed (Cochard *et al.* 2002; Domec *et al.* 2004). Reports of daily cycles of embolism formation, especially in leaves and roots, suggest that embolism is not necessarily avoided and that xylem hydraulic properties are dynamic, reflecting the balance between the two distinct processes of embolism and its reversal (Zwieniecki *et al.* 2000; Bucci *et al.* 2003; Domec *et al.* 2006).

The conversion of wetlands to intensively managed forest lands in eastern North Carolina was historically widespread, and yet the consequences on plant hydraulic properties and in turn on water balances have not been well studied. This study compares the hydraulic functioning of root, trunk and leaves, and quantifies their contribution to the integrated functioning of plants through the link between K_{tree} and the response of g_s to D in loblolly pine (*Pinus taeda* L.) trees. In this species, water availability is a major factor in determining leaf photosynthesis and leaf transpiration (Ewers, Oren & Sperry 2000). In 2007, a long summer drought was used to observe how the hydraulic system functioned under prolonged low soil moisture. Our

global hypothesis was that reversible changes in K_{tree} caused by short-term changes in K_{root} and K_{leaf} would act as valves responding to soil water availability and constitute hydraulic regulator that govern leaf gas exchanges. Using *in situ* field measurements, we tested the following specific hypotheses: (1) The partitioning of K_{tree} is mostly affected by K_{root} and K_{leaf} ; (2) The reduction in $g_{s\text{-ref}}$ and in g_s sensitivity to D are direct responses to decreased K_{tree} and is influenced by soil moisture; (3) Without water limitation, K_{leaf} limits K_{tree} , but as soil dries, K_{root} declines faster than K_{leaf} and regulates K_{tree} , and therefore, $g_{s\text{-ref}}$ and g_s sensitivity to D .

MATERIAL AND METHODS

Site description

The study site was a 16-year-old loblolly pine plantation with a canopy height of 14.1 m, and located at 35°48'N, 76°40'W, on the lower coastal plain of North Carolina, USA (Noormets *et al.* 2009). The area is <5 m above sea level on deep Belhaven series histosol soil (http://www.epa.gov/wed/pages/ecoregions/ncsc_eco.htm) that belongs to the outer coastal plain mixed forest province. The mean (1971–2000) annual precipitation is 1320 mm, mean temperature in July is 26.6 °C, and in January 6.4 °C. Leaf area index (LAI, projected leaf area above a unit of ground) was measured with a LAI-2000 Plant Canopy Analyzer (Licor Inc., Lincoln, NE, USA). All LAI-2000 measurements were corrected for clumping of needles and for woody surface area (Thérézien *et al.* 2007; Iiames *et al.* 2008). To confirm that most of the seasonal variations in LAI were related to loblolly pine trees, we also measured the seasonal change in leaf needle loss from 30 litter traps [0.18 m² laundry baskets suspended on polyvinyl chloride (PVC) tubes] from which pine and hardwood leaves were sorted by hand. The litter was collected every 60 d during spring and summer, and every 2 weeks during fall and early winter. The samples were separated into needles, leaves and twigs, and then oven-dried at 65 °C for 48 h. The total leaf mass collected during the year, divided by the area of the litter baskets, provided an estimate of annual leaf litter mass production per square meter. This needle mass was converted to LAI using an averaged value of leaf mass per area determined from needles harvested from the bottom, middle and top part of the canopy.

Microclimate, soil moisture content and sap flow measurements

The following micrometeorological parameters were measured above canopy: relative humidity and air temperature (HMP45AC, Vaisala, Finland), photosynthetically active radiation (LI-190, Licor) and precipitation (TE-525, Campbell Scientific, Logan, UT, USA). Soil volumetric water content (θ) was measured using a multisensor frequency domain capacitance technique (Domec *et al.* 2004; Warren *et al.* 2005). Probes consist of multiple annular capacitance sensors (Sentek PTY LTD, Adelaide, Australia) separated by 10 cm or more and placed in weatherproof PVC access

tubes. These probes are relatively insensitive to fluctuations in soil temperature (Warren *et al.* 2005). The plot had two probes installed to a depth of 1.2 m with nine independent sensors per probe. However, to compare across sites and to erase the influence of soil texture on θ , drought intensity is best quantified in the form of relative extractable soil water [relative extractable water (REW) dimensionless], and θ was converted to REW as defined by Granier, Loustau & Bréda (2000).

Stem sap flux measurements were made on six trees at 1.4 m above the ground and at 4–5 radial positions using 1 cm heat dissipation probes (James *et al.* 2002), modified after Granier (1987). Preliminary results showed that there was no significant difference in azimuthal J_s within trees ($P = 0.39$, students paired t -test, $n = 5$), probably because of the homogeneity of pine trunks, so we inserted all probes on the north–north-west side of the trees. Thirty-minute averages of temperature difference data were computed and stored in data loggers (CR10, Campbell Scientific Inc.). The sensor signal was converted to sap flux density (J_s , $\text{g m}^{-2} \text{s}^{-1}$) according to Granier (1987), which assumes that natural temperature gradients between sensors are small and accounted for the effects of non-zero night-time fluxes on the signal baseline (Oishi *et al.* 2008). Using tree sapwood area, stand LAI (converted to all-sided LAI using a conversion factor of 2.36; Rundel & Yoder 1998) and stand tree density (645 trees per hectare), J_s was scaled and converted to a tree-scale average transpiration per unit leaf area (E , in $\text{mmol m}^{-2} \text{s}^{-1}$). The total sapwood area of the trees equipped with sapflow probes was estimated from the relationship between sapwood area (from increment cores) and diameter at breast height (DBH) determined on 22 other trees and the DBH of the measured trees. Sapwood depth was determined by visual inspection of the core and converted to sapwood area based on the area of a circle, subtracting the areas represented by the heartwood and bark. In all cases, sapwood depth (ca. 75–90 mm) was greater than the depth at which the probes were installed, and when scaling J_s to the entire sapwood, the flux at 6–7 cm was used to represent the flux beyond 7 cm. To determine the seasonal increase in sapwood area as a function of the existing sapwood area, 5 trees were also cored once a month from May to December. All values of E used to calculate hydraulic conductances accounted for the seasonal dynamics of sapwood and leaf area.

Canopy conductance and analysis of its response to vapour pressure deficit

Canopy conductance derived from sapflow measurements comprises the total water vapour transfer conductance from the ‘average’ stomata of the tree canopy to the measurement height of vapour pressure deficit (D), which includes both surface boundary-layer and stomatal components. Canopy stomatal conductance (G_s) was calculated from E and D , using the simplification of the inversion of Penman–Monteith model:

$$G_s = (RT_a \rho E) / D \quad (4)$$

where R is the universal gas constant adjusted for water vapour ($0.46 \text{ m}^3 \text{ kPa K}^{-1} \text{ kg}^{-1}$), T_a is air temperature in degrees K and ρ is density of water (998 kg m^3). This simplified calculation was sufficient because in all treatments D was close to the leaf-to-air vapour pressure deficit due to high boundary-layer conductance. Indeed, given that >90% of the daytime mean wind velocity was $>0.7 \text{ m s}^{-1}$, and that leaf thickness never exceeded 1.8 mm, we estimated that the mean daytime boundary-layer conductance averaged 60 times G_s (Jones 1992). Basal sap flow can be used as a measure for diurnal crown transpiration if the measurements are corrected for the time lag with transpiration in order to more closely approximate the relationship between D and transpiration (Phillips *et al.* 1997; Ewers & Oren 2000). A commonly used method to account for the time-lag is to artificially shift the sap flow time series to maximize correlation with D or radiation (Oren *et al.* 1998; Granier *et al.* 2000; Chuang *et al.* 2006). In our system, we used a half-hour lag time that was derived from the observed time-lag of J_s with respect to D .

For isohydric species and under saturated light, Oren *et al.* (1999) showed that the decrease in g_s with increasing D is proportional to g_s at low D . Therefore, the sensitivity of the stomatal response to D when PAR was $>800 \text{ mmol m}^{-2} \text{ s}^{-1}$ (light-saturated G_s) was determined by fitting the data to the functional form:

$$G_s = b - m \times \ln(D) \quad (5)$$

where b is g_s at $D = 1 \text{ kPa}$ (hereafter designated as reference canopy stomatal conductance, $G_{s\text{-ref}}$), and m is the rate of stomatal closure and reflects the sensitivity of G_s to D [$-dG_s/d\ln D$, in $\text{mmol m}^{-2} \text{ s}^{-1} \ln(\text{kPa})^{-1}$]. The slope, $-dG_s/d\ln D$ is proportional to $G_{s\text{-ref}}$ with the proportionality averaging 0.60 across a wide range of species, and varying predictably depending on the range of D used in the analysis (Oren *et al.* 1999; Ward *et al.* 2008).

Although, it has been shown that the stomatal sensitivity to D does not vary whether g_s or G_s is used (Addington *et al.* 2004; Kim, Oren & Hinckley 2008), leaf-based g_s were used to provide an independent assessment of stomatal control of transpiration to compare with values of G_s derived from sapflow data. Measurements of g_s were conducted every 60–90 min with a LI-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA) on detached fascicles sampled from the upper crown section (height of 13 m). Our tests within this study was conformed with the report by Maier, Palmroth & Ward (2008) that the gas exchange of detached fascicles remained unchanged compared to that of attached needles for at least 20 min after excision. Diurnal measurements commenced prior to dawn and continued until after 1700 h once a month from 22 May to 3 October 2007.

Leaf water potential, K_{leaf} , K_{tree} and K_{root}

Field K_{leaf} was calculated as $K_{\text{leaf}} = E / (\Psi_{\text{branch}} - \Psi_{\text{leaf}})$, where Ψ_{leaf} is the leaf water potential of transpiring leaves and

Ψ_{branch} is the stem water potential estimated from non-transpiring covered shoots (Meinzer 2002). Under these conditions, leaf water potential is generally agreed to equilibrate to that of the adjacent xylem (Richter 1997). We measured Ψ_{leaf} and Ψ_{branch} on the same three trees used for g_s . From each of these trees, three terminal branches (<2 cm in diameter) located in the upper crown section were sampled (height of 13 m). Late morning (1000–1130 h) values of Ψ_{leaf} , Ψ_{branch} and E were used because at this time light is saturating and leaves are likely to have transpired all stored water so that steady-state water flow conditions are likely to exist. To analyse the effect of K_{leaf} on whole tree hydraulic conductance (K_{tree}), K_{tree} was calculated from the slope of the relationship between E and Ψ_{leaf} (Loustau, Domec & Bosc 1998). The regression method was used rather than the single point method [$K_{\text{tree}} = E/(\Psi_{\text{pre-dawn}} - \Psi_{\text{leaf}})$] because the slope of the regression line represents the whole tree hydraulic resistance ($1/K_{\text{tree}}$) and the potential at zero flow represents the soil water potential in contact with the roots (Cohen, Moreshet & Fuchs 1987). This approach was possible because the hysteresis effects resulting from plant capacitance were small, as previously documented for young loblolly pine trees (Ewers & Oren 2000). The cumulative daily water storage determined from the hysteresis between E and Ψ_{leaf} (Loustau *et al.* 1998) never exceeded more than 9, 7 and 5% of the daily transpiration in May, August and September, respectively. Using the ohm (electrical resistance) analogy applied to a hydraulic circuit, root to branch hydraulic conductance ($K_{\text{root-branch}}$) was calculated as: $K_{\text{root-branch}} = (K_{\text{tree}} \times K_{\text{leaf}})/(K_{\text{leaf}} - K_{\text{tree}})$. Measurements of Ψ_{leaf} and Ψ_{branch} were conducted every 2 weeks from May to September, and then once a month through mid December with a pressure chamber (Model 1000; PMS, Albany, OR, USA). Additionally, we were able to separate the effect of trunk and branch hydraulic conductance [$K_{\text{trunk-branch}} = E/(\Psi_{\text{trunk}} - \Psi_{\text{branch}})$] from $K_{\text{root-branch}}$ by measuring water potential (Ψ_{trunk}) at the trunk base. Measurements of Ψ_{trunk} were done once every 4 weeks by bagging one entire branch per tree on five adjacent trees bearing low branches (1.7 meters in height). Root hydraulic conductance (K_{root}) was computed using the electrical analogy as $K_{\text{root}} = (K_{\text{root-branch}} \times K_{\text{trunk-branch}})/(K_{\text{trunk-branch}} - K_{\text{root-branch}})$ (Nardini *et al.* 2003).

Loblolly pine trees carry 2 years of foliage during the growing season. To better understand the effect of each leaf cohort on K_{leaf} , we also determined the K_{leaf} of previous- and current-year needles ($K_{\text{leaf-current}}$ and $K_{\text{leaf-previous}}$, respectively). $K_{\text{leaf-current}}$ and $K_{\text{leaf-previous}}$ were calculated using the porometry-based transpiration and Ψ_{leaf} measured on either current- or previous-year needles. Further, to separate the contribution of current-year versus previous-year needle to the mean K_{leaf} determined using sapflow-based transpiration, we calculated an integrated K_{leaf} ($K_{\text{leaf-mean}}$) from $K_{\text{leaf-current}}$ and $K_{\text{leaf-previous}}$ weighted by the proportion of the total leaf area represented by current and previous needles. The leaf area of each cohort of needles was determined by comparing the fractional change in litter-fall (representing previous-year needle fall) to the total LAI (representing both current-

and previous-year needles). The progression of LAI of current-year needle through the year was calculated by subtracting LAI of falling needles from the total LAI. To further confirm that most of the seasonal variations in LAI were related to loblolly pine trees, the seasonal current-year needle production was documented by measuring needle elongation every 2 weeks on 18 needles per tree from five trees.

Monitoring seasonal occurrence of embolism in root, trunk and branches

To compare with the seasonal variations in K_{root} , $K_{\text{trunk-branch}}$ and K_{leaf} , specific hydraulic conductivity (k_s) and embolism were measured in roots and branches collected at the end of July, at the beginning of September and in the middle of October, 2007. Sampling started when REW was above 50% and ended when it was below 20%. At midday of each sampling date, lateral medium roots 2–4 mm in diameter and greater than 25 cm in length were excised near the base of five different trees at about 25 cm depth in the soil. Two terminal branches per tree were collected from the upper canopy level of three trees. Roots and branches were wrapped in aluminium foil and placed in a sealed plastic bag with a moist towel and immediately transported to the laboratory in a cooler, to be processed within 3 h of sampling (Domec *et al.* 2004). In the laboratory, a 10–15 cm long section of each root and branch was cut, and the proximal end was attached to a tubing system and perfused at a pressure of 5 kPa with filtered (0.22 μm) water. The hydraulic pressure head was adjusted to avoid any refilling of embolized tracheids. The rate of efflux was measured in a 1 ml graduated pipette. Specific conductivity was calculated as the mass flow rate of the perfusion solution divided by the pressure gradient across the segment, normalized by the xylem cross-sectional area. Native embolism or percent loss of specific conductivity of root and branch segments ($\text{PL}k_{s\text{-root}}$ and $\text{PL}k_{s\text{-branch}}$) was determined by comparing the initial (or native) conductivity to the maximum conductivity after removal of air emboli by soaking the samples in perfusion solution under vacuum for 48 h.

On the same days and on the same trees from which roots were collected, trunk xylem percent loss of conductivity ($\text{PL}k_{s\text{-trunk}}$) was estimated from the change in relative water content (RWC) of trunk increment cores using published relationships relating $\text{PL}k_{s\text{-trunk}}$ to RWC. Indeed, across several conifer tree species it has been shown that $\text{PL}k_{s\text{-trunk}}$ is nearly equal as twice the relative trunk water deficit (100-RWC) (Domec & Gartner 2002; Domec *et al.* 2006; Rosner *et al.* 2006; Domec & Pruyn 2008). Cores were collected at midday and wrapped in plastic film. Back at the laboratory, we determined M_f (fresh mass), V_f (fresh volume) by water displacement and M_d (dry mass) for the cores. Assuming a cell-wall material density of 1.53 g cm⁻³ (Siau 1984), RWC was calculated as $\text{RWC} = (M_f - M_d)/(V_f - M_d/1.53)$.

Error estimates and statistical analysis

Variance estimates of E and G_s were computed according to Oren *et al.* (1998) to include the variances of J_s and LAI and their mutual dependency. Similarly, variance estimates of K_{root} , K_{leaf} and K_{tree} were also computed using the variance estimate of E , and mean variance of Ψ (Phillips *et al.* 2002). Analysis of variance (ANOVA) was performed to test for differences between K_{root} , K_{leaf} and K_{tree} . To assess the difference in native embolism or percent loss of hydraulic conductivity at each date, we used a two-way ANOVA with one repeated measure factor. Variations in θ , REW and hydraulic conductivities over the measurements period were analysed by repeated measures ANOVA. The relationships among E , G_s and D were investigated, and between-REW differences in regression curves were tested by using F -test statistics for extra sum of squares (Motulsky & Christopoulos 2004). Relative errors in G_s , caused by instrument limitations were kept to <10%, by limiting G_s calculations to $D > 0.6$ kPa (Ewers & Oren 2000). Statistical analyses were performed using SAS (Version 9.1, Cary, NC, USA) and curve fits were performed using Sigmaplot (version 9.0, SPSS Inc., San Rafael, CA, USA).

RESULTS

The 2007 growing season experienced an extreme drought, with soil water content (θ) reaching its minimum in late August, and lasting until major rain events occurred in late October (Fig. 1a). Total precipitation for the measurement period was 510 mm, which was 53% of normal based on a 30-year mean for the area. In the upper 60 cm, θ varied

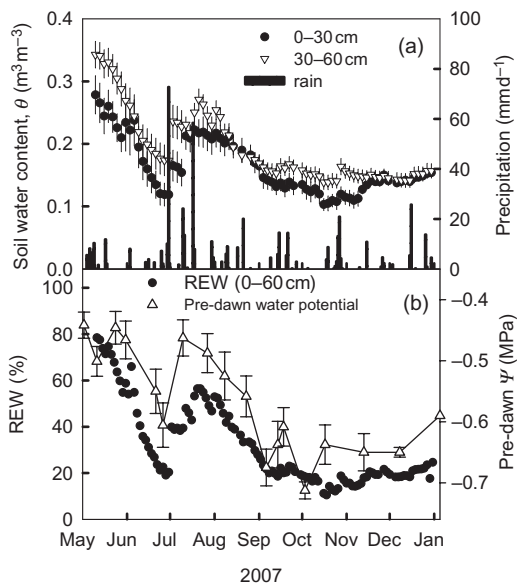


Figure 1. Seasonal variations from May to December 2007 in (a) soil volumetric water content (θ) and precipitation, and in (b) relative extractable water averaged over 60 cm of soil (REW) and pre-dawn leaf water potential (pre-dawn Ψ) corrected for the gravitational component. All measurements are means \pm SE.

significantly over the measurement period ($P < 0.001$; Fig. 1a). At the beginning of the study in May the soil was close to field capacity and mean θ of the 0–60 cm layer ranged from 0.36 to 0.28 $\text{m}^3 \text{m}^{-3}$ (Fig. 1a). In June and between August and November, gradual soil drying occurred, with θ falling to ca. 0.08 $\text{m}^3 \text{m}^{-3}$. About half of the seasonal decline in θ had occurred by the end of August. In late June, θ had already fallen to ca. 0.11 $\text{m}^3 \text{m}^{-3}$, when light rain events caused a transient partial recovery of θ . During the drought, θ in the upper 60 cm of soil was reduced to 27% of maximum, which corresponded to a decrease in REW of 80%. The seasonal decline in pre-dawn Ψ corrected for the gravitational component was very responsive to changes in soil moisture and as the soil dried out, pre-dawn Ψ declined in parallel (Fig. 1b). Pre-dawn Ψ was close to -0.4 MPa at the beginning of the study period and showed little change after late August when θ dropped below 50% REW (0.2 $\text{m}^3 \text{m}^{-3}$, Fig. 1b). The water potential at zero flow derived from the regression between Ψ and E represented the pre-dawn Ψ in contact with the roots. This value was not significantly different than measured pre-dawn Ψ ($P = 0.74$). The minimum midday Ψ_{leaf} averaged -2.2 MPa from May to October, and varied significantly throughout the growing season ($P < 0.01$), but never fell below -2.4 MPa (Data not shown).

We observed natural variation in K_{tree} during the measurement period (Fig. 2a). Mean K_{tree} was decreased from 0.82 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ in May to 0.60 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ in October. Variation in K_{tree} , K_{root} and K_{leaf} over the measurement period was, in all cases, highly significant ($P < 0.001$). From May to mid July, K_{leaf} decreased by 23% ($P = 0.02$), whereas K_{root} increased by 12% ($P = 0.04$) (Fig. 2b) during the same period. As soon as new leaves became fully expanded in early August, K_{leaf} increased back to spring values. As θ declined from September to November, K_{leaf} was reduced by 18% and K_{root} by 40%, respectively. This decline in K_{leaf} was mainly due to the sharp decrease in K_{leaf} from the previous year foliage ($K_{\text{leaf-previous}}$), which lost 50% of conductivity between July and September. In contrast, K_{leaf} from current-year foliage ($K_{\text{leaf-current}}$) almost increased by about 47% (Table 1) during the same period. The sap-flow based K_{leaf} compared well with the porometry-based K_{leaf} ($K_{\text{leaf-mean}}$ in Table 1), which was calculated using $K_{\text{leaf-previous}}$ and $K_{\text{leaf-current}}$ and the leaf area represented by previous- and current-year needles.

The root resistance (i.e. inverse of conductance) declined from 61% to 46% of the total root-to-leaf resistance between May and August, and then increased back to 58% for the rest of the year (Fig. 2c). Averaged over the growing season, $1/K_{\text{leaf}}$ and $1/K_{\text{trunk-branch}}$ represented 33% and 11% of $1/K_{\text{tree}}$, respectively. For the whole measuring period, $1/K_{\text{leaf}}$ never exceeded more than 39% of $1/K_{\text{tree}}$. Note that measurements of midday Ψ_{trunk} carried out simultaneously on basal and on upper branches (13 m above ground level) showed an average midday Ψ_{trunk} difference ranging between 0.02 and 0.05 MPa after correcting for the gravitational component. Thus, the Ψ_{trunk} difference between the base and the top of the trunk was negligible compare to

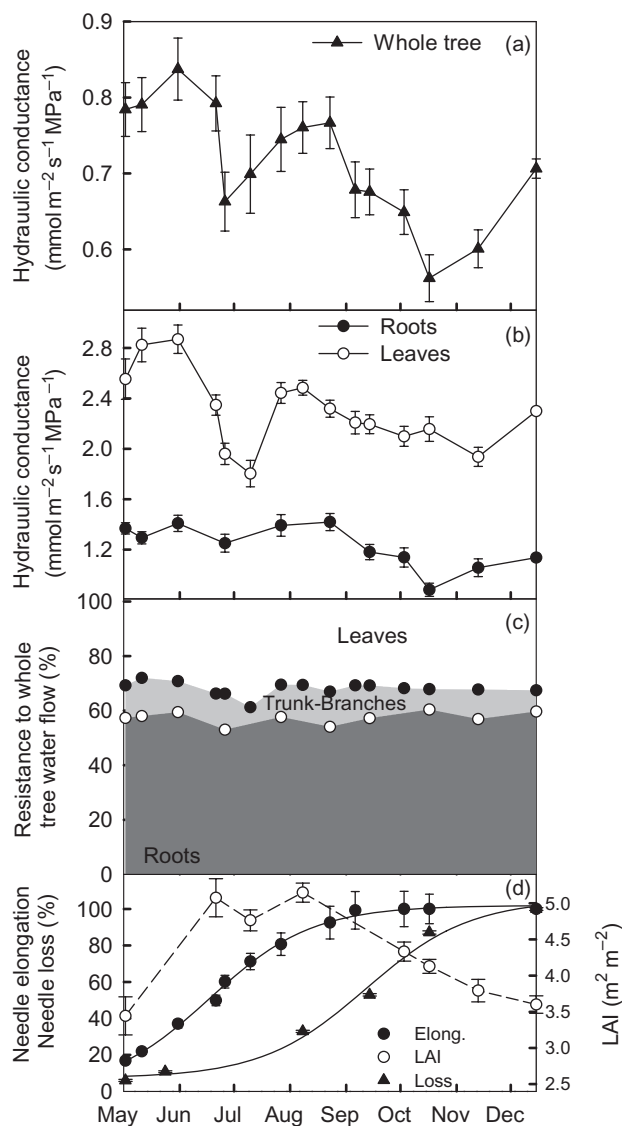


Figure 2. Seasonal variations in (a) soil-to-leaf hydraulic conductance (K_{tree}), in (b) root (K_{root}) and leaf (K_{leaf}) hydraulic conductances, in (c) the proportion of leaf, trunk to branches (Trunk-Branches) and root hydraulic resistances to the overall tree hydraulic resistance ($1/K_{tree}$), and in (d) the patterns in needle elongation (Elong.), needle loss and one-sided flat leaf area index. All measurements are means \pm SE.

Ψ_{branch} , and so most of the resistance in the trunk-branch compartment was due to branch wood. The percent loss of conductance of roots (PLK_{root}) increased significantly ($P = 0.02$) from July to October, whereas the percent loss of conductance of the trunk-branch pathway ($PLK_{trunk-branch}$) did not show any significant changes ($P = 0.54$). These results were confirmed by the measurements of native embolism of individual root, branch and trunk samples (PLK_{s-root} , $PLK_{s-trunk}$, $PLK_{s-branch}$). The average loss of specific conductivity of each different organ $PLK_{s-root-trunk-branch}$ at each sampling date was close to $PLK_{root-trunk-branch}$ (Table 1). The partitioning of native embolism indicated that the decrease in $K_{root-trunk-branch}$ was due to a reduction in the root

conductivity rather than branch or trunk conductivity and so the seasonal change in the contribution of root to K_{tree} was linked to increase root embolism (Table 1).

Stand LAI determined optically varied by about 35% over a year with a maximum of $5.1 \pm 0.2 \text{ m}^2 \text{ m}^{-2}$ in August, to a minimum of $3.5 \pm 0.3 \text{ m}^2 \text{ m}^{-2}$ in December (Fig. 2d) in November. This minimum value compared well with the LAI of $3.6 \pm 0.3 \text{ m}^2 \text{ m}^{-2}$ determined from litter fall baskets over a 3-year period, and which corresponds to the mean LAI of a single needle cohort carried by the trees in winter. LAI increased because of new foliage production during the spring, reaching a peak in July and August. The developing foliage of the first growth flush of the spring had almost attained maximum length by September (Fig. 2d) and the previous year foliage was retained at 50% until September, and was almost completely lost by November. As a consequence, previous and current needles had the same LAI in late July, but by October, total LAI was dominated at 80% by current needles (Table 1).

The variation in pre-dawn Ψ , K_{root} and K_{tree} were related to soil moisture (Fig. 3). K_{tree} decreased by 25% of its maximum value as soil water potentials approached -0.6 MPa and soil water content dropped below 40–50% REW (Fig. 3). K_{root} was similarly responsive to soil drying once the 40–50% REW threshold was reached with K_{root} decreasing by 36–38% of its maximum values between May and November.

Stomatal and canopy conductances were linearly related ($P < 0.001$; Fig. 4a), although mean g_s was 13% higher than G_s . This good agreement allowed us to use G_s as an accurate predictor of leaf level gas exchange. Diurnal variations in the two independent parameters, g_s and K_{leaf} also suggest a strong correlation between the gas and liquid phase conductances (Fig. 4b). The daily response of g_s to K_{leaf} was influenced by soil moisture such that maximum g_s decreased by 25% when REW dropped below 50% ($P = 0.02$). However, the response of g_s to midday Ψ_{leaf} was not affected by REW ($P = 0.11$) (Fig. 4c).

Daytime-averaged D showed significant seasonal variation ($P < 0.01$) from 1.4 kPa in May to 1.8 kPa and 0.91 kPa in August and November, respectively. Stomatal conductance and tree transpiration showed marked declines in response to increases in D (Fig. 5). The response of G_s and tree transpiration to increasing D was influenced by soil moisture (Fig. 5). At $D = 1 \text{ kPa}$, the decrease in reference G_s (G_{s-ref}) and E for days with REW below 50% were, respectively, 27 and 23% lower ($P < 0.03$) than for days when REW was above 50% (Fig. 5).

The shape of the relationship between G_s and D could adequately be described using Equation 5. Relationships between monthly averaged G_s and $\ln D$ were generally strong (minimum $r^2 = 0.75$). The sensitivity of the G_s to D ($-dG_s/d \ln D$) was linearly related to stomatal conductance at $D = 1 \text{ kPa}$ (G_{s-ref} ; $P < 0.001$; Fig. 6). Stomatal conductance declined in response to increasing D , and the magnitude of the reduction varied over the measurement period as shown by the decline in G_{s-ref} . The slope of the relationship between G_s and the sensitivity of the G_s to D (-0.57 ± 0.5)

	July (56%; -0.56 MPa)	September (24%; -0.74 MPa)	October (18%; -0.81 MPa)
K_{leaf} ($\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$)	$2.4 \pm 0.2\text{a}$	$2.2 \pm 0.3\text{ab}$	$2.0 \pm 0.2\text{b}$
LAI _{current}	2.3	3.1	3.2
LAI _{previous}	2.6	1.2	0.8
$K_{\text{leaf-current}}$	$1.9 \pm 0.2\text{a}$	$2.8 \pm 0.1\text{b}$	$2.4 \pm 0.3\text{b}$
$K_{\text{leaf-previous}}$	$2.6 \pm 0.3\text{a}$	$1.3 \pm 0.3\text{b}$	$0.6 \pm 0.1\text{c}$
$K_{\text{leaf-mean}}$	$2.3 \pm 0.2\text{a}$	$2.4 \pm 0.3\text{a}$	$2.1 \pm 0.3\text{a}$
PLK _{root-trunk-branch}	$13 \pm 1\text{a}$	$20 \pm 3\text{b}$	$34 \pm 4\text{c}$
PLK _{trunk-branch}	$19 \pm 3\text{a}$	$20 \pm 3\text{a}$	$23 \pm 4\text{a}$
PLK _{root}	$9 \pm 2\text{a}$	$18 \pm 3\text{b}$	$38 \pm 5\text{c}$
PLK _{s-root-trunk-branch}	$15 \pm 4\text{a}$	$24 \pm 4\text{b}$	$30 \pm 5\text{c}$
PLK _{s-branch}	$12 \pm 2\text{a}$	$22 \pm 4\text{b}$	$26 \pm 3\text{b}$
PLK _{s-trunk}	$10 \pm 5\text{a}$	$16 \pm 4\text{a}$	$20 \pm 5\text{a}$
PLK _{s-root}	$22 \pm 5\text{a}$	$35 \pm 6\text{b}$	$43 \pm 9\text{c}$

Table 1. Leaf hydraulic conductance and percent loss of hydraulic conductance and specific conductivity in the different organs sampled

One-sided flat leaf area index of previous and current needles (LAI, $\text{m}^2 \text{m}^{-2}$), relative extractable water and pre-dawn leaf water potentials at the time of sampling are also indicated. Leaf hydraulic conductance (K_{leaf}) is compared to current- and previous-year needle hydraulic conductances ($K_{\text{leaf-current}}$ and $K_{\text{leaf-previous}}$, respectively) and to the average between $K_{\text{leaf-current}}$ and $K_{\text{leaf-previous}}$, weighted by the proportion of the total leaf area represented by previous and current needles ($K_{\text{leaf-mean}}$). The total percent loss of hydraulic conductance of the root-to-branch pathway (PLK_{root-trunk-branch}) was split into PLK_{trunk-branch} and PLK_{root}. Those values of loss of hydraulic conductances were calculated using the average conductances measured in May as the reference conductances. For comparison, the percent loss of hydraulic specific conductivity of individual root, trunk and branch samples (PLK_{s-root}, PLK_{s-trunk}, PLK_{s-branch}) and their average (PLK_{s-root-trunk-branch}) are also given. Values with different letters within a row are significantly different ($P < 0.05$).

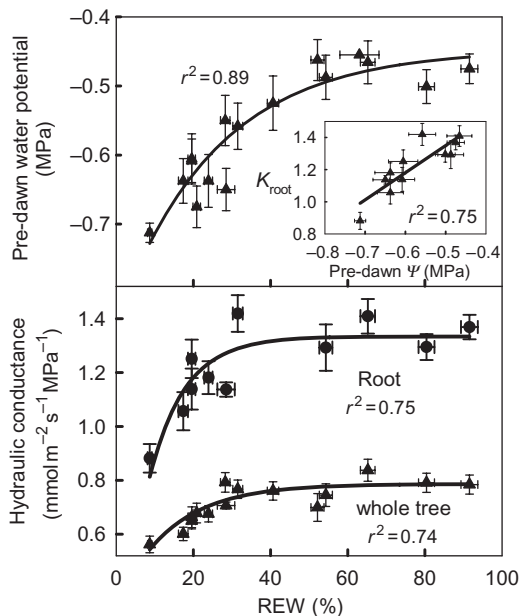


Figure 3. Pre-dawn leaf water potential (pre-dawn Ψ) corrected for the gravitational component and tree and root hydraulic conductances (K_{tree} , K_{root}) as a function of relative extractable water (REW). Lines are exponential saturation curves of the form $y = a(1 - e^{-bx})$. The inset represents the linear relationship between pre-dawn Ψ and K_{root} . All measurements are means \pm SE.

was not significantly different ($P = 0.17$) than the previously reported generic value of -0.6 based on a hydraulic model that assumes tight stomatal regulation of leaf water potential (Oren *et al.* 1999). The sensitivity of the G_s to D was also positively correlated ($P = 0.01$) with the sensitivity of $G_{s\text{-ref}}$ to K_{tree} , having a slope close to 1 (Fig. 6).

After taking into account the effect of D on G_s , soil moisture still had a major influence on G_s at field conditions, with $G_{s\text{-ref}}$ and G_s sensitivity to D decreasing to 30% of their maximum values when REW dropped from 80% to 10% (Fig. 7). Throughout the measurement period, $G_{s\text{-ref}}$ and the sensitivity of G_s to D were also responsive to K_{root} , K_{leaf} and K_{tree} . As values of K_{tree} declined by 25% from maximum, $G_{s\text{-ref}}$ declined by 35% (Fig. 7). The effect of K_{leaf} on $G_{s\text{-ref}}$ became significant ($G_{s\text{-ref}}$ declined by 7%, $P = 0.03$) when K_{leaf} decreased by 25% of its maximum value (at REW \approx 40–50%). Below a K_{leaf} threshold value of $2.3 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$, $G_{s\text{-ref}}$ kept declining without further major changes in K_{leaf} . The effect of K_{root} on $G_{s\text{-ref}}$ was such that $G_{s\text{-ref}}$ declined as soon as K_{root} decreased below $1.4 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$ (Fig. 7).

DISCUSSION

Main determinants of K_{tree}

Our study revealed that under field conditions, K_{tree} and, therefore, xylem-conducting capacity is highly dynamic, reflecting a balance between cavitation and embolism removal in roots, trunk and leaves. This relationship is consistent with reports of short-term changes in hydraulic

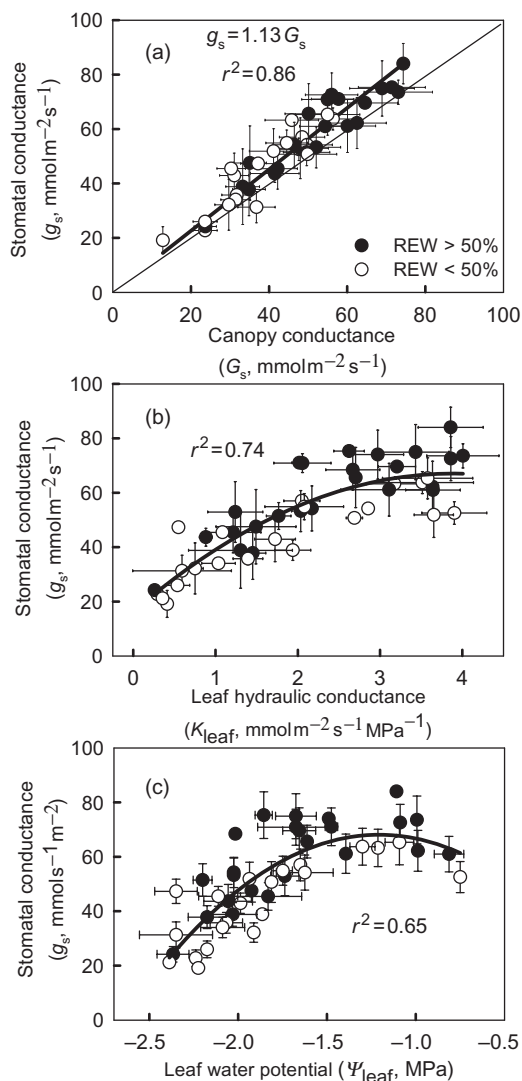


Figure 4. (a) Relationship between leaf- and canopy-level stomatal conductances (g_s and G_s , respectively), (b) between g_s and leaf hydraulic conductance (K_{leaf}) and (c) between g_s and leaf water potential for two relative extractable water ranges. In (b) and (c), quadratic curves were used to fit the data. All measurements are means \pm SE.

conductivity in different plant parts (Zwieniecki *et al.* 2000; Brodribb *et al.* 2003; Bucci *et al.* 2003; Domec *et al.* 2004). As previously reported for several tree species (Magnani & Borghetti 1995; Irvine *et al.* 1998; Addington *et al.* 2004), the seasonal decrease in K_{tree} was associated with variation in soil water availability. However, and as hypothesized, the 35% decline in K_{tree} throughout the season was highly influenced by the variation of K_{leaf} and K_{root} . This respective effect of K_{leaf} and K_{root} on K_{tree} have seldom been explicitly reported. Interestingly, at the beginning of the season, K_{tree} decreased because of the sharp decline in K_{leaf} . Later in the season, once REW dropped below 40–50%, the noticeable decrease in K_{tree} was mostly dominated by K_{root} , in accordance with several studies attributing a major role to roots

in regulating K_{tree} as soil dries (Steudle 2000; Domec *et al.* 2004).

We postulate that seasonal changes in K_{root} and $K_{\text{root-branch}}$ were mainly attributable to changes in the embolism of roots rather than to modifications in hydraulic properties at the soil-root interface or in the trunk and branch parts (Table 1). In conifers, the components K_{branch} and K_{trunk} have been shown not to vary seasonally (Domec, Prunyn & Gartner 2005; Rosner *et al.* 2008). In the current study, the seasonal drop in soil moisture and pre-dawn Ψ were related to K_{root} . It has been shown that loblolly pine roots lose 25–45% of conductivity at water potentials ranging from -0.5 to -0.75 MPa (Ewers *et al.* 2000; Hacke *et al.* 2000), which corresponded to the decline in pre-dawn Ψ when REW was below 50%, and to our maximum field loss of root specific conductivity ($k_{s-\text{root}}$, Table 1). Seasonal changes in K_{root} indicated that embolized tracheids were partially refilled by late fall before the soil had been fully recharged by precipitation, consistent with previous studies showing that a relatively small amount of water can markedly increase root conductivity (Domec *et al.* 2006). It is unlikely that the conductance recovery in the late fall reflected the production of new roots because it has been shown in

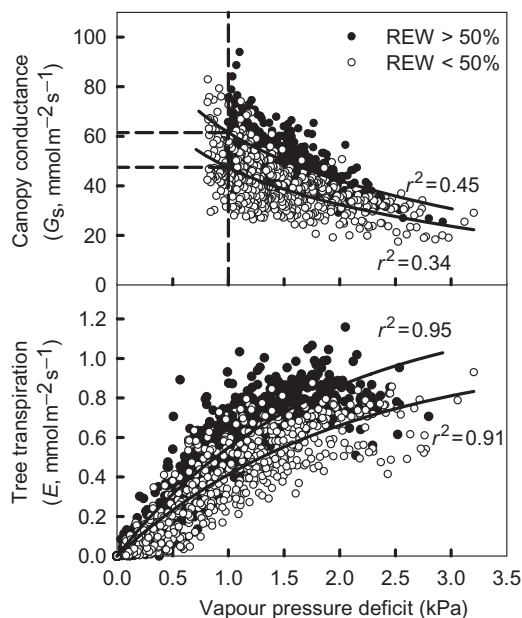


Figure 5. Responses of light-saturated canopy stomatal conductance (G_s) and transpiration (E) to water vapour pressure deficit (D) as a function of the relative extractable water (REW). Equation 5 was used to fit the relationships between G_s and D . The vertical dashed-line at $D = 1$ kPa intersects the boundary line at reference G_s ($G_{s-\text{ref}}$), which was $61 \text{ mmol m}^{-2} \text{ s}^{-1}$ for REW $> 50\%$ and $46 \text{ mmol m}^{-2} \text{ s}^{-1}$ for REW $< 50\%$. The slope of the response of G_s to $\ln D$ was 27 and $22 \text{ mmol m}^{-2} \text{ s}^{-1} \ln(\text{kPa})^{-1}$ for REW $> 50\%$ and for REW $< 50\%$, respectively (parameter m in Equation 5). The exponential saturation curves of the form $y = a(1 - e^{-bx})$ were used to fit the relationships between E and D . The maximum transpiration rate (parameter a of the fitted curve) was $1.06 \text{ mmol m}^{-2} \text{ s}^{-1}$ for REW $> 50\%$ and $0.82 \text{ mmol m}^{-2} \text{ s}^{-1}$ for REW $< 50\%$.

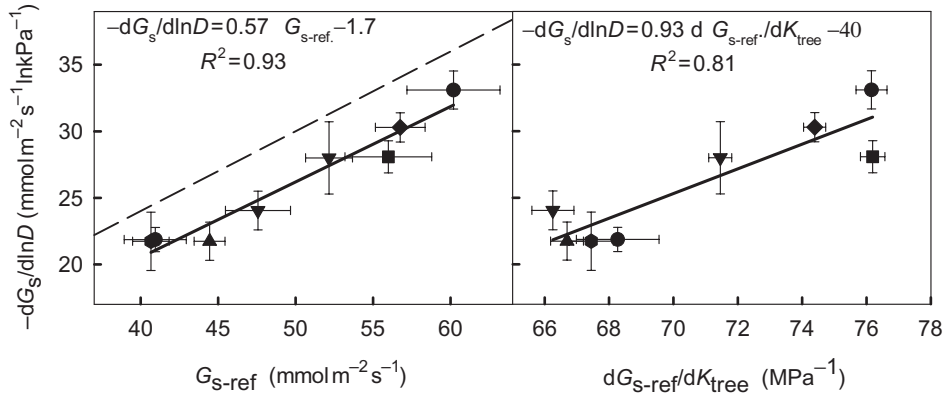


Figure 6. Linear relationship between the sensitivity of canopy conductance to vapour pressure deficit ($-dG_s/d\ln D$) and G_s at $D = 1$ kPa (G_{s-ref}) and to the stomatal sensitivity to tree hydraulic conductance (dG_{s-ref}/dK_{tree}). The dashed line (slope = 0.6) represents the theoretical slope between stomatal conductance at $D = 1$ kPa and stomatal sensitivity to D that is consistent with the role of stomata in regulating minimum Ψ_{leaf} (Oren *et al.* 1999).

several conifer seedlings that root regeneration is inhibited by more than 50% when pre-dawn Ψ drops below -0.5 MPa (Zou, Sands & Sun 2000).

It is possible that the progressive reduction of soil water content could have decreased the conductance at the soil to root interface, and consequently decreased the apparent K_{root} (Passioura 1988). However, because the Ψ at zero transpiration was not different than measured pre-dawn Ψ , soil Ψ was probably in equilibrium with tree Ψ and K_{root} represented the soil to root hydraulic conductance (Cohen *et al.* 1987).

Above-ground partitioning of hydraulic resistance

Needles constituted the major part of the above ground hydraulic resistance to water flow. Leaf resistances represented more than 35% of the total hydraulic resistances, and more than 75% of the above ground resistance, although leaves constituted less than 3% of the total hydraulic pathway. Therefore, on a distance basis, leaf conductance is the major determinant of K_{tree} , which may put some limits on maximum needle length (Zwieniecki *et al.*

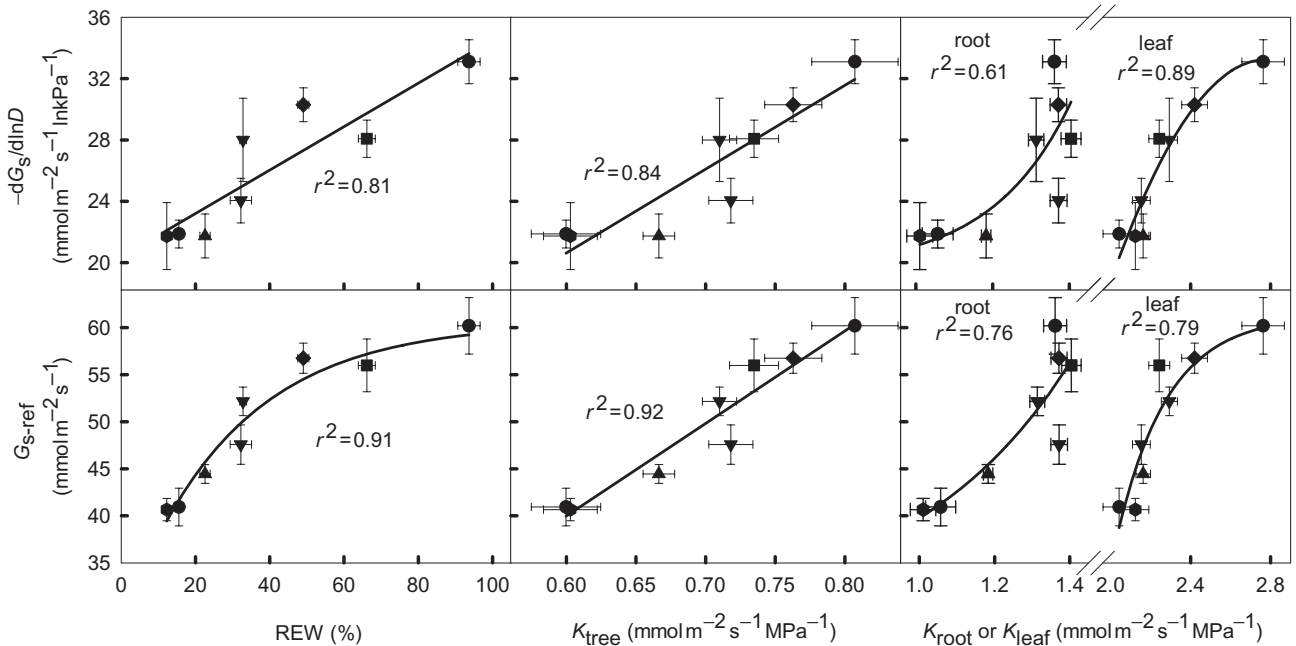


Figure 7. Relationships between the sensitivity of canopy conductance to vapour pressure deficit ($-dG_s/d\ln D$) and between G_s at $D = 1$ kPa (G_{s-ref}) to the relative extractable water (REW) and to tree hydraulic conductance (K_{tree}) and its two main components K_{root} and K_{leaf} . The function $y = a(1 - e^{-bx})$ was used to fit the relationships between G_{s-ref} and REW, as well as between G_{s-ref} and K_{leaf} and $-dG_s/d\ln D$ and K_{leaf} . The function $y = ae^{bx}$ was used to fit the relationships between G_{s-ref} and K_{root} and between $-dG_s/d\ln D$ and K_{root} . All other fitted curves were linear.

2006) and maximum tree height (Woodruff *et al.* 2007). Some of the variations observed in K_{tree} between the beginning and the end of the measurement period were related to a decrease in K_{leaf} as needles senesced, and to an increase as new foliage became hydroactive (Neumann 1987). The decline in K_{leaf} at the peak of the growing season from May to July was not induced by decreasing Ψ_{leaf} , but rather by the expression of previous-year leaf senescence and a decline in $K_{leaf-previous}$. Decrease in K_{leaf} because of leaf senescence has been reported in angiosperm species but not in conifers (Salleo *et al.* 2000; Brodribb & Holbrook 2003). Because of leaf expansion from May to September, the increase in K_{leaf} was the consequence of current-year needle development that increased hydraulic efficiency (Zwieniecki *et al.* 2006). The concurrent increase in current-year LAI and in $K_{leaf-current}$ increased the overall leaf hydraulic efficiency in mid-summer, which limited the effect of the decrease in K_{root} on K_{tree} . Many studies assume a constant LAI or constant leaf area to sapwood area ratio in the calculation of K_{tree} . Omitting such variation would affect the interpretation of the relationship between K_{tree} and stomatal conductance.

Stomatal regulation in response to soil water availability

Confirming our second hypothesis, the onset of the reduction in G_{s-ref} was tied to the decrease in soil water content below 50% REW. As pre-dawn water potential declined in response to decreasing REW, G_{s-ref} declined in order to reduce transpiration at a time of declining water availability. The threshold of 40–50% for REW, beyond which G_s was reduced, was previously reported in a large number of tree species and soil types (Bréda *et al.* 2006; Granier *et al.* 2007). The seasonal decline in G_{s-ref} was strongly related to the seasonal decline in K_{tree} (a 25% reduction in K_{tree} induced a nearly 30% reduction in G_{s-ref}) indicative of a hydraulic effect on stomata regulation. K_{tree} acted in concert with stomata to limit water loss by maintaining pre-dawn and midday Ψ_{leaf} above -0.8 MPa and above -2.2 MPa, respectively. For loblolly pine, those Ψ_{leaf} represent critical values that would induce 80% loss of K_{root} and loss of leaf turgor (Hacke *et al.* 2000). Furthermore, the response of G_s to D was closely correlated with the response of G_{s-ref} to K_{tree} (Fig. 6), which is indicative of a strong coordination between the liquid- and gas-phase (Sellin & Kopper 2005), and consistent with the view that the stomatal response to D represents a feedback response of G_s to changes in K_{tree} (Sperry & Pockman 1993; Meinzer 2002).

Our results also show that the sensitivity of G_s to D was mostly attributable to the variation in G_{s-ref} , which is again consistent with the isohydric regulation of water potential (Oren *et al.* 1999). The effect of K_{tree} on the sensitivity of G_s to D was rather surprising (Fig. 7). Although G_{s-ref} decreased sharply with decreasing K_{tree} , the decrease in the sensitivity of G_s to D became asymptotic as K_{tree} continued to decrease. The very low sensitivity of G_s to D under severe water stress

(REW < 20%) was probably because stomata were nearly fully closed and transpiration was mostly cuticular.

Co-ordination between G_{s-ref} and K_{leaf} , and between G_{s-ref} and K_{root} as soil dried

Confirming our last hypothesis, G_{s-ref} was driven by change in K_{leaf} once soil moisture was above 50%, and by K_{root} when REW was kept below 50%. This soil moisture threshold for stomatal closure was reached when K_{root} started to drop and thus when the root resistance had become an effective competitor for water with the above-ground portion of the tree (Domec *et al.* 2006). The strong effect of declining K_{leaf} on G_{s-ref} may be explained by the coupled effect of the diurnal dynamics of g_s , K_{leaf} and Ψ_{leaf} (Fig. 4; Brodribb *et al.* 2003; Woodruff *et al.* 2007), to the production of new and very hydraulically efficient needles. Many models have been proposed to describe water movement within trees and to predict tree transpiration with varying degrees of success (Katul *et al.* 2003). As suggested by Bohrer *et al.* (2005) and Chuang *et al.* (2006), a stomatal model can be coupled with a model of the hydraulic system of a tree to provide independent time series of transpiration and advance our understanding of stomatal functioning under various soil moisture and atmospheric demand. Our study provides empirical data that can be used in such theoretical hydrodynamic models to track midday water potentials in the different parts of the tree system and see which of these water potentials is more effective in affecting g_s . Such models should be able to predict whether the magnitude of seasonal water depletion and refilling of root and leaf xylem constituted a signal involved in stomatal regulation to prevent Ψ_{leaf} from declining to values that could provoke embolism in stems where embolism reversal may not be as efficient as in roots (Salleo *et al.* 2000; Domec *et al.* 2004).

In conclusion, we feel that a more complete view of tree adaptation to the environment can emerge from a description of hydraulic architecture of plant organs. The linkages between stomatal behaviour and K_{tree} shown in this study force us to reconsider the ecological implications of species-specific strategies with regard to embolism and stomatal opening. In a future with higher temperatures and evapotranspiration, trees might face hydraulic limitations in regions of increased frequency and intensity of drought (Luo *et al.* 2001; Granier *et al.* 2007). The extreme drought event that occurred in the southern United States during 2007 highlighted the need to understand the key processes that may allow trees and stands to overcome such severe water shortages (McDowell *et al.* 2008). We conclude that a given increase in D and a decrease in soil moisture in response to such climate change will reduce K_{tree} , which in turn may impose a series of constraints on the water and carbon economy of the plant (Irvine *et al.* 2004; Noormets *et al.* 2009). Finally, more work needs to be done to determine the main drivers of K_{tree} in the root to leaf continuum, and how seasonal variation in LAI and leaf phenology of different needle cohorts affect G_{s-ref} and stomatal sensitivity to D .

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