Dynamic variation in sapwood specific conductivity in six woody species

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Summary Our goals were to quantify how non-embolism-inducing pressure gradients influence trunk sapwood specific conductivity ($k_s$) and to compare the impacts of constant and varying pressure gradients on $k_s$ with KCl and H$_2$O as the perfusion solutions. We studied six woody species (three conifers and three angiosperms) which varied in pit membrane structure, pit size and frequency of axial water transport across pits (long versus short conduits). Both stepwise (“steady”) and nonlinear continuous (“non-steady”) decreases in the pressure gradient led to decreased $k_s$ in all species but white oak (Quercus garryana Dougl. ex Hook), a ring-porous and long-vesseled angiosperm. In one diffuse-porous angiosperm (red alder, Alnus rubra Bong.) and two conifers (western red cedar, Thuja plicata Donn. ex D. Don, and Douglas-fir, Pseudotsuga menziesii (Mirb.) Franco), $k_s$ was 10–30% higher under steady pressure gradients than under non-steady pressure gradients, and a decrease in the pressure gradient from 0.15 to 0.01 MPa m$^{-1}$ caused a 20–42% decrease in $k_s$. In another diffuse-porous angiosperm (maple, Acer macrophyllum Pursh) and in a third coniferous species (western hemlock, Tsuga heterophylla (Raf.) Sarg), there was no difference between $k_s$ measured under steady and non-steady pressure gradients. With the exception of western red cedar, a conifer with simple pit membranes, the differences in $k_s$ between low and high pressure gradients tended to be lower in the conifers than in the diffuse-porous angiosperms. In Douglas-fir, western red cedar and the diffuse-porous angiosperms, $k_s$ was higher when measured with KCl than with H$_2$O. In white oak, there were no differences in $k_s$ whether measured under steady or non-steady pressure gradients, or when xylem was perfused with KCl or H$_2$O. The species differences in the behavior of $k_s$ suggest that elasticity of the pit membrane was the main factor causing $k_s$ to be disproportionate to the pressure gradient and to the different pressure regimes. The results imply that, if nonlinearities in pressure–flux relationships are ignored when modeling tree water relations in vivo, large errors will result in the predictions of tree water status and its impact on stomatal control of transpiration and photosynthesis.

Introduction

The specific conductivity ($k_s$) of wood to liquid, a transport property that is necessary for accurate understanding and modeling of long distance water flow in plants, should be measured under conditions that recreate the transient behavior of water movement in vivo. Steady-state techniques have traditionally been employed for determining $k_s$, mainly because of their simplicity in theory and calculations. Steady state exists when the volumetric flow rate ($Q$) through a porous medium is constant. Although several studies have been undertaken on the mechanisms affecting steady-state flow of liquids in wood (Bolton and Petty 1978, Bolton 1988), we found only one study on non-steady-state flow of liquids through wood (Lin and Lancaster 1973). Flow through porous media can be described by a combination of the Darcy equation (Darcy 1856) and the continuity equation (conservation of mass when a fluid is in motion; Fetter 2001), with $k_s$ regarded as a function of wood structure. This empirically determined equation, which has since been derived from simplified Navier-Stokes equations (Carmen 1956, Hager and Gisonni 2002), relates the laminar flow rate of a liquid to the pressure gradient (change in hydraulic head over a distance) and $k_s$ under steady-state conditions:

$$Q(l,t) = \frac{k_s A \partial P(l,t)}{\eta l}$$

(1)

where $A$ is the area across which the flow occurs, $P$ is the liquid phase pressure, $\eta$ is the liquid dynamic viscosity and $l$ is the length of the flow path.

Following Darcy's original work, much experimental and analytical work has been devoted to the application of Darcy’s law to the description of the flow of liquids through wood.
opposite response, a decrease in flow rate with increasing ap-
high pressure gradients not likely to be found in intact trees, an
results (see Figures 12a and 12b in Sperry and Tyree 1990). At
perus virginiana
within one conduit than in the adjacent one, the fibers holding
application of force (Figure 1a). If the pressure is higher
would occupy a medial position in the pit chamber before the
pressure is that pit membranes become distorted as they are
closures, or both, rather than air blockages. We hypothesize
cluded that the decrease in flow resulted from turbulence or pit
and Tyree (1990) also reported an increase in conductivity at
pit aspiration, or plugging of pit membranes, or both. Sperry
sure (Bailey and Preston 1970). The effects were explained by
flow rarely occurs in trees because any or all of the following
can change with time: A (Tognetti et al. 1998, Eckmüllner and
Domec et al. 2005). If the resulting values of \( k \) are pressure
s in wood found decreasing flow rates at constant
pressure and disproportionate increases with increasing pres-
ure (Bailey and Preston 1970). The effects were explained by
are pressure gradient dependent, they may not explain the behavior of
water transport in trees. One study investigating the pressure de-
pendence of \( k \), in wood found decreasing flow rates at constant
and Juniperus virginiana L., but provided no explanation for such re-
results (see Figures 12a and 12b in Sperry and Tyree 1990). At
high pressure gradients not likely to be found in intact trees, an
opposite response, a decrease in flow rate with increasing ap-
plied pressure, was observed by Sucoff et al. (1965). Sperry
and Tyree (1990) and Lu and Avramidis (1999). They con-
cluded that the decrease in flow resulted from turbulence or pit
losures, or both, rather than air blockages. We hypothesize
that the main cause for the flow rate to be disproportionate to
pressure is that pit membranes become distorted as they are
distended elastically. In an un-aspirated pit, the membrane
would occupy a medial position in the pit chamber before the
application of force (Figure 1a). If the pressure is higher
within one conduit than in the adjacent one, the fibers holding
the membrane would behave like uniformly loaded beams
fixed at both ends. As the membrane begins to distend (Figure
1b), the pores become larger so the flow path will become less
restricted. As the membrane distends even further (Figure 1c),
the downstream channel between the membrane and the pit
borders will restrict flow (Figure 1c). Therefore, at low pres-
sure differentials we could expect an increase in \( k \), because of
increased bordered pit conductance, but if the pressure con-
tinued to increase, \( k \) would decrease.

The third violation of Darcy’s law occurs when \( k \) is mea-
sured with deionized water because xylem sap ionic composi-
tion and pH can affect xylem conductivity (Zimmermann
KCl concentration in the perfusion solution during measure-
ments of hydraulic conductivity caused a more than 2.5-fold
increase in \( k \), in some angiosperm species, but had a minimal
effect in conifer species. Because the response was localized in
pit membranes, Zwieniecki et al. (2001) suggested that per-
fusing solutions caused pectins in the membranes to swell or
shrink, thereby altering the size of the membrane micro-
channels.

Because of the complexity of the observed pressure–flow
relationships, studies using methods that reproduce the rele-
vant in vivo conditions on the excised wood segments studied
in the laboratory are needed to gain a better understanding of
the physics of fluid in the xylem. The goals of our study were
to assess the impact of dynamic variation in pressure gradients
on flow through xylem varying in structure, and to determine
the effects of xylem sap varying in structure, and to determine
the effect in conifer species. Because the response was localized in
pit membranes, Zwieniecki et al. (2001) suggested that per-
fusing solutions caused pectins in the membranes to swell or
shrink, thereby altering the size of the membrane micro-
channels.

The second violation of Darcy’s law when applied to living
trees is that \( k \), is often determined in vitro at pressure gradients
(0.03 to 0.10 MPa m\(^{-1}\)) exceeding those that exist in trees
(< 0.03 MPa m\(^{-1}\), Bauerle et al. 1999, Woodruff et al. 2004,
Domec et al. 2005). If the resulting values of \( k \) are pressure
gradient dependent, they may not explain the behavior of
water transport in trees. One study investigating the pressure de-
pendence of \( k \), in wood found decreasing flow rates at constant

Figure 1. Proposed model to explain the variation in flow rate and spec-
cific conductivity as the pressure gradient between two adjacent con-
ducts increases. The diagram shows a section through a bordered pit
pair, with the midline being the compound middle lamella of two ad-
jacent conduits and the pit membrane. The relative volume of water
flow through the small pores of the bordered pit membrane as pressure
increases from \( P_1 \) to \( P_2 \) to \( P_3 \) is represented by the size of the arrow-
heads. Although only a coniferous bordered pit with a central thicken-
ing (torus) in its membrane is shown, the model would be the same for
coniferous or angiosperm bordered pits with a simple membrane.
would depend on whether the pressure gradient was steady or non-steady; and (3) the magnitude of differences in $k_s$ resulting from changes in pressure regime would depend on the ionic strength of the perfusion solution. We compared results in three coniferous and three angiosperm species from the Pacific Northwest with disparate conduit and pit characteristics to further generalize conclusions about the role of pit border and membrane types.

**Material and methods**

**Wood material and sample preparation**

We studied three angiosperms and three gymnosperms with contrasting wood anatomy (Table 1) that were available locally in the McDonald-Dunn Forest near the Oregon State University campus. Among the angiosperms, Oregon white oak (*Quercus garryana* Dougl. ex Hook) is ring-porous, and as such, has long vessels and a large range of vessel diameters (Table 1). Bigleaf maple (*Acer macrophyllum* Pursh) and red alder (*Alnus rubra* Bong.) are both diffuse-porous. It is likely that pit pore sizes decrease from bigleaf maple (the most water-requiring species) to red alder, then white oak. Pit morphology varies markedly among the gymnosperms studied. Bordered pits in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) have a larger diameter, thicker torus and more margo strands than those in western hemlock (*Tsuga heterophylla* (Raf.) Sarg) (Bauch et al. 1972). Western red cedar (*Thuja plicata* Donn. ex D. Don) is one of the few North American conifers with simple pit membranes lacking torus and margo; it consists of numerous, closely packed strands (Kramer and Côté 1963).

Healthy mature trees, free of broken tops and stem deformities, were selected based on cambial age estimated from increment cores. Between July and September 2004, we felled four individuals of each species. We sampled two locations within the outer sapwood of each tree to limit variability. Immediately after felling, we cut a 25-cm thick disk from the base of each tree. The disks were transported in wet polyethylene bags to the laboratory, and stored at 3 °C until blocks were prepared within three hours of felling. Both ends of each disk were covered with wet paper towels to limit evaporation from the xylem. From each disk, four 180–200-mm-long blocks were cut from the outer part of the sapwood at regular intervals around the circumference. Following the procedures outlined by Spicer and Gartner (1998) and Domec and Gartner (2001), the blocks were split along the grain first with a maul and wedge, and then smoothed with a chisel to create axially oriented beams, 1.4 × 1.4 cm in the tangential and radial directions, and 15 cm in the tree’s axial direction. The current year (2004) was excluded in order to have samples with complete annual growth rings (5 to 12 depending on the species). The entire cross-sectional area of each sample block was assumed to be functional sapwood and was calculated from the mean of the cross-sectional areas of the two ends.

Before being tested, the samples were soaked in water and put under a vacuum for at least 48 hours to remove the existing emboli in order to determine $k_s$ values at full saturation. To test for complete refilling, the relative water content of each sample (the proportion of non-cell-wall space that is occupied by water) was calculated after the final measurement and ranged from a minimum value of 96 ± 4% in red alder to a maximum value of 99 ± 2% in western hemlock. Samples were recut at both ends and the cut surfaces smoothed with razor blades to a final length of 10.1–10.3 cm. Measurements of $k_s$ were made with a membrane-lined pressure sleeve that held a latex sleeve against the exposed axial edges of the sample to ensure that fluid did not leak from the sides of samples (Spicer and Gartner 1998). The pressure sleeve was filled with a 10% solution of agar in 0.01 M NaCl. The perfusion fluid did not leak from the sides of samples (Spicer and Gartner 1998).

### Table 1. Morphological characteristics of sampled trees and saturated specific conductivities ($k_s$) at low (0.013 MPa m$^{-1}$) and high (0.160 MPa m$^{-1}$) steady pressure gradients with deionized water as the perfusion fluid and corrected to 20 °C (mean ± SE, n = 4). Values of $k_s$ with different letters within a column or row are significantly different ($P < 0.05$).

<table>
<thead>
<tr>
<th>Tree age (years)</th>
<th>White oak (<em>Quercus garryana</em>)</th>
<th>Bigleaf maple (<em>Acer macrophyllum</em>)</th>
<th>Red alder (<em>Alnus rubra</em>)</th>
<th>Western hemlock (<em>Tsuga heterophylla</em>)</th>
<th>Douglas-fir (<em>Pseudotsuga menziesii</em>)</th>
<th>Western red cedar (<em>Thuja plicata</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>108 ± 9</td>
<td>58 ± 5</td>
<td>43 ± 1</td>
<td>51 ± 3</td>
<td>46 ± 3</td>
<td>49 ± 4</td>
<td></td>
</tr>
<tr>
<td>22.3 ± 2.2</td>
<td>29.6 ± 3.4</td>
<td>29.5 ± 0.9</td>
<td>30.2 ± 1.1</td>
<td>39.8 ± 0.8</td>
<td>24.1 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>19.4 ± 1.1</td>
<td>26.5 ± 0.7</td>
<td>28.1 ± 0.6</td>
<td>34.0 ± 2.2</td>
<td>33.5 ± 0.9</td>
<td>16.3 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>500–2500µm</td>
<td>40–250</td>
<td>30–150</td>
<td>2.2–2.9</td>
<td>2.6–3.1</td>
<td>2.1–2.7</td>
<td></td>
</tr>
<tr>
<td>$k_s$ high (kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$)</td>
<td>11.1 ± 1.1 a</td>
<td>4.3 ± 0.2 b</td>
<td>3.5 ± 0.2 c</td>
<td>3.1 ± 0.3 cd</td>
<td>4.1 ± 0.2 b</td>
<td></td>
</tr>
</tbody>
</table>
| $k_s$ low (kg m$^{-1}$ s$^{-1}$ MPa$^{-1}$) | 10.8 ± 1.1 a                     | 3.5 ± 0.3 c                       | 2.9 ± 0.1 de             | 2.6 ± 0.3 e                          | 3.8 ± 0.3 c                        | 1.4 ± 0.1 g

1 Lei et al. (1996).
2 Watson et al. (1988).
3 Gartner et al. (1997).
4 Panshin and deZeeuw (1980).
5 Domec et al. (2006b).
6 Authors’ unpublished observations.
7 Deal and Harrington (2006).
8 Siripatanadilok and Leney (1985).
ner 1998). Although the latex membrane was pressurized to 0.05 MPa, this pressure does not affect the flow rate through the samples (Spicer and Gartner 1998, authors’ unpublished observations). Hereafter, we refer to this apparatus as “the holder.” For all experiments, one end of the holder was attached to a reservoir filled with deionized and filtered (0.22 μm) water or 25 mmol KCl made with deionized and filtered water. The flow of solution through the segments was in the same direction as in the intact plant. Flow rate was determined by an electronic balance (± 0.1 mg) connected to a computer (Sperry et al. 1988).

The temperature of the solution was recorded before and after each \( k \), measurement and varied by no more than 0.5 °C during any one experiment. Fluid viscosity was corrected to 20 °C for all reported \( k \), values. Before and after each measurement, we determined the flow rate in the absence of an applied pressure head. This zero-pressure “background” flow has been shown to be zero (Cavender-Bares and Holbrook 2001) or small and negative because water can enter the samples in the absence of a pressure head (Kolb and Sperry 1999). In our study, the background flow was zero because the height of the water reservoir was adjusted to the height of the sample, and probably also because the samples were enclosed in the holder, which prevented them from losing water during the experiment.

**Experiment 1: non-uniformity of specific conductivity under steady pressure gradients**

The magnitude of the pressure gradient was controlled by adjusting the height of the fluid reservoir. The sample was positioned at the same height as the downstream height. Pressure on the upstream end of the stem segment was estimated as atmospheric pressure plus the hydraulic head produced by a water column over the vertical distance between the supply reservoir and the sample, and pressure on the downstream end was taken as atmospheric pressure. On the first subsample per tree, measurements were made starting from a maximum pressure head of 1.63 m (16 kPa), reducing stepwise to a value of 0.13 m, and then increasing stepwise to the initial pressure head. There were a total of six pressure steps between the maximum pressure head of 1.63 m (16 kPa), reducing stepwise to a value of 0.13 m, and then increasing stepwise to the initial pressure head. As soon as liquid began to flow through the sample, the pressure in the chamber decreased, creating a non-steady pressure gradient. The relationship between time and the change in water pressure (measured by the pressure transducer) was used to estimate \( k \) under non-steady pressure gradient conditions (see Equation 4 below). Flow through the sample created a feedback between the pressure and flow because the volume of water that passed through the sample was equivalent to the increase in the volume of air in the air chamber. Because samples were at full saturation, we assumed that the xylem water content remained constant across all applied pressures (no capacitive discharge or recharge) and that changes in \( k \) were the only cause of the changes in flow rate at a given transient pressure. We assumed that air in the upper chamber behaves as an ideal gas, then \( PV = nRT \), where \( n \) is the quantity of gas (moles) and \( R \) is the gas constant. The water temperature was assumed equal to that of the air in the adjacent chamber. This assumption is based on the premise that expansion occurred slowly and that the heat capacity of the chamber was sufficient to buffer temperature changes due to changes in gas pressure. Given that \( V = C/P \), we have that \( dv = -C/P^2 dP \) and Equation 2 can be rewritten as:

\[
\frac{-dP}{P^2(P - P_o)} = \frac{k_A}{C \eta l} dt
\]

Calling \( P_i \) the initial pressure at \( t = 0 \), we can integrate from \( t = 0 \) to \( t \) and from \( P_i \) to \( P \) (during these experiments, we used a \( P_i \) of 0.02 MPa):
DYNAMIC VARIATION IN CONDUCTIVITY

\[
\frac{1}{P_0^2} \ln \frac{P}{P - P_0} - \frac{1}{P_0^2} = \frac{k_s A}{C \eta} t + \left( \frac{1}{P_0^2} \ln \frac{P}{P - P_0} - \frac{1}{P_0^2} \right) \quad (4)
\]

We calculated \(k_s\) from the slope (\(S\)) of the plot of

\[
\frac{1}{P_0^2} \ln \frac{P}{P - P_0} - \frac{1}{P_0^2} = \frac{k_s A}{C \eta} t
\]

against time, given that \(A, C, \eta\) and \(l\) are known, \(k_s\) was determined as:

\[
k_s = \frac{\eta CS}{A} \quad (5)
\]

The plot of

\[
\frac{1}{P_0^2} \ln \frac{P}{P - P_0} - \frac{1}{P_0^2}
\]

versus time was often nonlinear with respect to pressure change because of non-constant \(k_s\) with applied pressure, but followed instead a quadratic polynomial relationship. The slope in Equation 5 at any applied pressure was then determined using the derivative of this quadratic polynomial curve. Specifically, \(S\) was calculated as \(t\), the time elapsed from \(P\) to \(P_0\), times the derivative at \(t\) of this quadratic polynomial curve. Therefore, by developing a simple relationship, it was possible to estimate conductivity coefficients under non-steady pressure gradients without having to employ the more complex and time-consuming numerical methods analysis.

In addition, for methodological and comparative purposes, we collected water efflux on a balance to determine \(k_s\) with Equation 1 when using the pressurized chamber. These values, referred to as \(k_s\) under “quasi-steady” pressure gradients, were computed based on the mean pressure difference and the amount of water collected during time intervals ranging from 10 s at maximum pressure to 1 min at low pressure. The values of \(k_s\) measured under quasi-steady pressure gradients were then compared with \(k_s\) determined under non-steady pressure gradients.

Statistical analysis

Because all measurements were made on the same samples, paired \(t\)-tests were performed to assess differences between the \(k_s\) values resulting from different pressure gradient manipulations, and to determine if there was an effect on \(k_s\) of the perfusion fluid used. We used the analyses to compare \(k_s\) measured under non-steady, quasi-steady and steady pressure gradients. Differences between species (Table 1) were determined by a one-way analysis of variance (ANOVA).

Results

During the steady pressure gradient measurements, \(k_s\) always declined within the first hour of measurements, whether we used deionized water or KCl as the perfusion fluid. The example for deionized water in Figure 3 shows that maximum values of \(k_s\) during the first pressure cycle (between 0 and 150 min) were always greater than maximum values during subsequent pressure cycles. This pattern was observed for every species studied but it was more pronounced in conifers.

No significant hysteresis (\(P > 0.45\)) was detected in the response of \(k_s\) to increasing or decreasing steady pressure gradients (Figure 4), indicating that the increase in \(k_s\) did not result from conduit refilling associated with increasing pressure. Pressure-dependent variation in \(k_s\) measured under quasi-steady pressure gradients followed the trends of \(k_s\) measured under non-steady pressure gradients regardless of whether we used KCl or H2O (Figure 5).

In the ring-porous species, white oak, the difference in \(k_s\) between low and high pressure gradients was not significant (\(P > 0.67\), Table 1). In this species, there was also no significant difference (\(P > 0.19\)) in \(k_s\) measured either under steady or non-steady pressure gradients, or with KCl or H2O as the perfusion fluid (Figure 6).

In both of the diffuse-porous species, bigleaf maple and red alder, there was an asymptotic increase in \(k_s\) with increasing pressure gradients under both flow regimes (Figure 6). At high pressure gradients, \(k_s\) was significantly higher than at low pressure gradients for both kinds of perfusion fluid (\(P < 0.03\)) and for both kinds of flow regime (\(P < 0.04\)). In bigleaf maple and red alder, \(k_s\) measured at the highest pressure gradient (0.160 MPa m\(^{-1}\)) was significantly higher than any \(k_s\) measured at a pressure gradient lower than 0.063 MPa m\(^{-1}\). Similarly, \(k_s\) measured at the lowest pressure gradient (0.013 MPa m\(^{-1}\)) was significantly lower than any \(k_s\) measured at a pressure gradient higher than 0.046 MPa m\(^{-1}\). In addition, for these two species, \(k_s\) was significantly higher when perfused with KCl than when perfused with H2O (\(P < 0.05\)). In red alder, \(k_s\) was significantly higher (\(P < 0.03\)) when measured under steady pressure gradients than under non-steady pressure gradients for both kinds of perfusion fluid. For this species, the increase in \(k_s\) between low and high pressure gradients under
Non-steady pressure gradient was significantly lower ($P < 0.01$) when using H$_2$O than when using KCl and ranged from 42% with H$_2$O to 74% when using KCl. Under a steady pressure gradient, the increase in $k_s$ between low and high pressure gradients in red alder was of the same order of magnitude regardless of whether we used KCl or H$_2$O and ranged from 12 to 17% (Table 1). In bigleaf maple, there was no significant difference ($P > 0.10$) in $k_s$ measured under either steady or non-steady pressure gradients (Figure 6). In bigleaf maple, the difference in $k_s$ at low and high pressure gradients was of the same order of magnitude regardless of whether we used KCl or H$_2$O, and ranged from 22 to 25% under a steady pressure gradient (Table 1) and from 39 to 43% under a non-steady pressure gradient.

In the three conifers, there was an asymptotic relationship between $k_s$ and the pressure gradient (Figure 7), with values of $k_s$ being significantly greater at high pressure gradients than at low pressure gradients for both pressure regimes ($P < 0.04$) and both perfusion fluids ($P < 0.02$). For example, in western hemlock and Douglas-fir, $k_s$ measured at the highest pressure gradient was significantly higher than any $k_s$ measured at a pressure gradient lower than 0.046 MPa m$^{-1}$, and $k_s$ measured at the lowest pressure gradient was significantly lower than any $k_s$ measured at a pressure gradient higher than 0.063 MPa m$^{-1}$. In western red cedar, $k_s$ measured at the highest pressure gradient was significantly higher than any $k_s$ measured at a pressure gradient lower than 0.063 MPa m$^{-1}$ and $k_s$ measured at the lowest pressure gradient was significantly lower than any $k_s$ measured at a pressure gradient higher than 0.046 MPa m$^{-1}$. In western hemlock, there was no difference ($P > 0.35$) between $k_s$ measured under steady or non-steady pressure gradients, and the difference between the minimum and maximum $k_s$ ranged between 14 and 20% and was smaller than in the other two conifer species. In Douglas-fir and western red cedar, $k_s$ measured with either KCl or H$_2$O under steady pressure gradients was significantly higher ($P < 0.04$) than $k_s$ measured under non-steady pressure gradients (Figure 7) with the largest differences occurring at the lowest pressure gradients (less than 0.02 MPa m$^{-1}$). In Douglas-fir, there was an 8% increase in $k_s$ from the lowest to highest pressure gradient under steady pressure gradients (Table 1) and a 50% increase under non-steady pressure gradients and the relative increase in $k_s$ between low and high pressure gradients was of the same order of magnitude regardless of whether we used KCl or H$_2$O. Moreover in Douglas-fir, under non-steady state pressure gradients, $k_s$ measured with KCl was not significantly different from $k_s$ measured with H$_2$O ($P > 0.14$). In western red cedar, $k_s$ increased by 28% under steady pressure gradients and by 40% under non-steady pressure gradients. In addition, for this species, the increase in $k_s$ between low and high pressure gradients measured either under steady or non-steady pressure gradients was significantly lower ($P < 0.02$) when using KCl than when using H$_2$O and ranged from 10 to 39% when using KCl and from 28 to 65% when using H$_2$O.

Discussion

Differences in $k_s$ measured at a given pressure gradient

Under both steady and non-steady pressure regimes, the spe-
specific conductivity ($k_s$) of fully hydrated wood increased with increasing pressure gradient; i.e., liquid flow rates increased more than expected from Darcy’s law as applied pressure increased. This observation is consistent with prior evidence that, in some cases, xylem water flux is not a linear function of the applied pressure (Peel 1965, Sucoff et al. 1965, Bailey and Preston 1970, Giordano et al. 1978). The mechanisms responsible for this nonlinearity are not yet clearly understood, but various mechanisms have been proposed, including air blockages and pit closure, as well as non-laminar (turbulent) flow. Irreversible pit aspiration and closure occurring at high pressure gradients may explain why conductivities decline within the first hour of measurements and become constant for a given pressure gradient thereafter (Figure 3). When the flow is turbulent, the flow contains unsteady vortices that interact with each other and $k_s$ declines (Rott 1990). Because our results showed that with increasing pressure gradients, $k_s$ was greater rather than lower, it is unlikely that turbulent flow occurred at high pressure gradients.

Instead, we propose that the main cause for the nonlinear relationship between flow rate and pressure gradient is that pit membranes become distorted and their conductivity changes as they distend elastically (Figure 1). If the strain of the stretched membrane is in the elastic range, then a total recovery will occur when the force is removed (Sperry and Tyree 1990), which explains the non-hysteretic reversibility of changes in $k_s$ as pressure gradients are either lowered or raised (Figure 4). The increase in $k_s$ could be caused by expansion of the membrane pores when the membrane distends, thus reducing the bordered pit resistance to flow. The incremental deflection of pit membranes decreases with increasing increments of applied pressure (Sperry and Hacke 2004), which could explain the asymptotic increase in $k_s$ with pressure gradient. At pressure gradients higher than 0.1 MPa m$^{-1}$, we hypothesize that, although increasing pit membrane pore size results in increased $k_s$, deflection of the entire membrane reduces the channeled water flow between the membrane and the edge (borders) of the pit (Figure 1c).

An inverse relationship between the pressure gradient and $k_s$ has been reported in some gymnosperms (Sperry and Tyree 1990) and one angiosperm (Sherwin et al. 1998) and was ascribed in the gymnosperms to the increased pressure forcing the torus against the pit border. However, the pressure gradients used in earlier experiments were an order of magnitude higher than the ones we used, and were imposed on branches, which necessarily have lower flow rates and lower $k_s$ than most stem sections because of their much smaller tracheids (Domec and Gartner 2002, Dunham et al. 2007). A slight decrease in $k_s$ at high pressure gradients was also occasionally seen in our study when comparing pressure gradients above 0.1 MPa m$^{-1}$. 

Figure 6. Specific conductivities of angiosperm stem xylem in relation to steady and non-steady pressure gradients using either deionized water (H$_2$O) or 25 mmol KCl as the perfusion fluid (mean ± SE, $n = 4$).
Differences in $k_s$ between steady-state and non-steady-state pressure regimes and consequences for water transport in living trees

As far as we are aware, this is the first report of interspecific comparisons of $k_s$ measured under steady and non-steady pressure regimes. We hypothesize that most of the deviations from Darcy’s law between steady and non-steady pressure regimes can be accounted for by changes in pit membrane function under different pressures and flow rates. The material that makes up pit membranes is similar to primary cell wall material and thus should exhibit rapid and appreciable deflections under low loadings. Lin et al. (1973) attributed the difference in behavior under steady and non-steady pressure regimes to pit membrane flexibility and to the delayed response (hysteresis) between the time the pressure is applied and the time the membrane starts moving. Thus, in this view, under a non-steady pressure gradient, the membranes can occupy a continuum of positions (Fengel 1972), resulting in hysteresis, which would lead to lower $k_s$ values compared with steady pressure gradients because the pores would not stretch instantaneously with the increase in pressure gradient. Because bigleaf maple and western hemlock showed no differences between steady and non-steady pressure regimes, we predict that the pit membranes of these species are flexible and respond quickly to pressure changes.

The effect of steady versus non-steady pressure gradients on $k_s$ differed in conifers and angiosperms, and is also probably related to differences in their bordered pit structure. Because the mean diameter of the pit membrane is much smaller than that of the conduit lumens, $k_s$ is largely dependent on the size and structure of the bordered pits (Pittermann et al. 2005). In both angiosperms and conifers the bordered pits can represent at least 50% of the total xylem resistance (Sperry et al. 2005, Domec et al. 2006a). In angiosperms, calculations based on morphological features of bordered pits have shown that a significant fraction of the limiting flow resistance of bordered pit membranes is located in the pit membrane pores rather than the aperture (Schulte and Gibson 1988, Sperry and Hacke 2004, Hacke et al. 2006). In contrast, in conifers, the pit aperture has been shown to represent more than two thirds of the total pit resistance (Lancashire and Ennos 2002, Hacke et al. 2004, Domec et al. 2006a). These results may explain why, with the exception of western red cedar, the differences in $k_s$ between low and high pressure gradients tended to be lower in the conifers than in the diffuse-porous angiosperm species. In other words, the majority of pit resistivity in conifers is associated with the pit border, and therefore, the membrane distension at different pressure

Figure 7. Specific conductivities of conifer stem xylem in relation to steady and non-steady pressure gradients during perfusion with either deionized water (H$_2$O) or 25 mmol KCl as the perfusion fluid (mean ± SE, n = 4).
gradients will have only a minimal impact on pit resistance. In addition, because they lack a torus and margo (Jansen et al. 2004), simple pit membranes found in most angiosperms and a few conifers like western red cedar are easier to deflect than bordered pit membranes (Sperry and Hacke 2004, Hacke et al. 2004). Thus, in contrast to the case in the typical conifer, the diffuse-porous species and western red cedar resistivity would be much more dependent on distension of the pit membrane, which we have hypothesized to be nonlinear with changes in the pressure gradient (Figure 1).

White oak, a ring-porous species with long vessels, provides a test for our hypothesis that pit characteristics determine the response of $k_s$ to variations in the pressure gradient and steady-versus non-steady-state regimes. In contrast to the diffuse-porous species, the samples of oak were shorter than the minimum vessel length (Greenidge 1952, Zimmermann 1983, Sano 2005), so very few pit membranes had to be crossed by the liquid. Therefore, the necessity of crossing more pit membranes per meter of sap ascent in the diffuse porous species would be expected to increase the dependence of xylem $k_s$ on the pressure gradient and on the type of pressure regime (Figure 6).

A recent study of Douglas-fir showed that pores within the pit membrane represent 30% of the pit resistance and around 25% of the total resistance (Domec et al. 2006b). To obtain a mean increase in $k_s$ of around 8% from low to high steady pressure gradients (Table 1), margo pore resistivity would have to decrease by 32% upon stretching. The membrane resistivity scales with the diameter of the pores to the third power, and therefore an increase in pore diameter due to membrane stretching of only 10% would decrease margo resistivity by 32%. A difference of 0.14 MPa m$^{-1}$ in pressure gradient (intermediate between the extremes of the gradients measured in this study) would give a difference across a single tracheid of 0.4 kPa. In Douglas-fir, this pressure drop would correspond to a membrane displacement of around 15% of its maximum displacement (from normal to aspirated position), which may be sufficient to increase pore diameter and increase overall $k_s$ by 10% (Domec et al. 2006a). These values are in agreement with those reported by Bolton and Petty (1978), who found that a membrane displacement of 10% occurs at a pressure difference across a single tracheid of 0.1 kPa. Previous estimates of pressure differences to initiate deflection in conifer membranes range from 7.5 to 15 kPa (Sperry and Tyree 1990), but these values represented the threshold pressures at which $k_s$ started to decrease. Such pressure differences would represent a membrane displacement of 40–50% and we think that, in such a position, the increase in pore size would be counteracted by the reduction in flow between the membrane and the border of the pit (Figure 1c).

In western hemlock, $k_s$ increased 15–20% between low and high pressure gradients, which would require a larger increase in pore diameter with increasing pressure gradient than in Douglas-fir. Although this species has a distinct torus with margo strands, the diameter of the torus is usually smaller with a denser margo and smaller pores compared with that of Douglas-fir. In addition, incrustations in the membranes are often present (Krahmer and Côté 1963, Bauch et al. 1972, authors’ unpublished observations), which are likely to increase the influence of margo pores on the overall bordered pit resistivity, and the differences in $k_s$ between low and high pressure gradients.

The maximum pressure gradient tested in the experiments was more than would occur in a transpiring hydrated plant, so that the actual $k_s$ under these conditions may be more than the maximum occurring in situ. The main changes in $k_s$ occurred at low pressure gradients, which are of the same order of magnitude as those occurring in living trees (Bauerle et al. 1999, Domec and Gartner 2002). An actively transpiring tree with trunk or foliage water potentials in the range of –1 to –2 MPa experiences pressure gradients of 0.01 to 0.02 MPa m$^{-1}$ in conifers (Woodruff et al. 2004, Domec et al. 2006b) and 0.04 MPa m$^{-1}$ in maple and alder (Fallas-Cedeño 2005). Thus it is likely that an intact transpiring tree will have a lower $k_s$ than that measured in the laboratory, which is often measured at gradients between 0.05 and 0.1 MPa m$^{-1}$. Because the steady-state method is the simplest option to determine $k_s$, we suggest that scientists standardize the measurement of $k_s$ by generating steady-state conductivity at pressure gradients encountered in the field by the plant organ studied. This approach would limit the potential error when evaluating and interpreting water transport in intact trees based on $k_s$ measurements in excised xylem. Numerous studies have generated steady-state conductivity measurements to calculate leaf specific conductivity or tree hydraulic conductance in an attempt to model water transport and to estimate whole-tree water use in vivo (e.g., Tyree 1988, Sperry et al. 2002, Tyree 2003, Sobrado 2003, Domec et al. 2005, Maseda and Fernández 2006). The differences in $k_s$ between steady and non-steady pressure gradients as well as between low and high pressure gradients observed in the present study imply that some earlier studies may have significantly overestimated xylem capacity to supply water to transpiring leaves and consequently whole-tree water use.

Discrepancies in $k_s$ values measured under steady-state and non-steady-state conditions may also be problematic for the interpretation of xylem vulnerability from embolism curves. As tension in the water column increases, vessels and tracheids become embolized (air-filled), and $k_s$ declines (Zimmermann 1983). The vulnerability curve is a plot of percent loss of $k_s$ as a function of tension in the water column. Thus, the steady-state values of $k_s$ traditionally measured during vulnerability curve determination may not be representative of the consequences of embolism for water transport under the dynamic conditions that prevail in intact plants.

**Perfusion liquid and difference in steady and non-steady $k_s$**

A limited number of studies on the influence of fluid ionic composition on $k_s$ have shown that the presence of cations in the permeating solution increases $k_s$ by reducing the resistance to flow at the pit membrane via shrinkage of pectins imbedded in the membrane (Van Ieperen et al. 2000, Zwieniecki et al. 2001). It is possible that the influence of pressure gradients on
membrane pore size may differ according to whether the pectins are swollen or shrunken. Because the effect of fluid ionic composition is localized to pit membrane pores, its influence on $k$, has been shown to be more important in angiosperms than in conifers (Zwieniecki et al. 2001), which explains why large differences were found in both of the diffuse porous species and also why white oak showed no differences (few membranes were crossed). In many conifer species, pit membranes in the sapwood contain pectins but no phenolic substances (Bauch and Berndt 1973), which may explain why western hemlock did not respond to a change in fluid composition whereas Douglas-fir did. Finally, the response of western red cedar to KCl or deionized H$_2$O was comparable with the response of both diffuse-porous species. We hypothesize that this similarity was due to the lack of a torus in the bordered pits of western red cedar, and the existence of a membrane anatomically comparable with that found in angiosperms, with the probable presence of pectic substances.

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