Responses of gas exchange to reversible changes in whole-plant transpiration rate in two conifer species

C. R. WARREN,1,2 N. J. LIVINGSTON1 and D. H. TURPIN3

1 Centre for Forest Biology, Department of Biology, University of Victoria, P.O. Box 3020 Stn CSC, Victoria, BC, V8N 3N5, Canada
2 Author to whom correspondence should be addressed (crwarren@unimelb.edu.au)
3 Department of Biology, University of Victoria, P.O. Box 3020 Stn CSC, Victoria, BC, V8N 3N5, Canada

Received November 18, 2002; accepted February 9, 2003; published online July 15, 2003

Summary  This study examined the autonomy of branches with respect to the control of transpiration (E) in Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) and western red cedar (Thuja plicata Donn) seedlings. Experiments were conducted on whole seedlings in a gas exchange system with a dual-cuvette that permitted independent manipulation and measurement of E in the upper and lower cuvettes. The value of E in one cuvette was manipulated by varying vapor pressure deficit (D) between 2.2 and 0.2 kPa, whereas D in the other cuvette was held at 2.2 kPa. Reducing D, while increasing stomatal conductance (g.), resulted in an overall decrease in E. In western red cedar, this decrease was almost threefold, and in Douglas-fir, approximately fourfold. In well-watered western red cedar, this decrease was almost threefold, and in Douglas-fir, approximately fourfold. In well-watered western red cedar, a reduction of whole-plant E by 46% (brought about by reducing D in the upper cuvette) resulted in a 12% increase in g., a 12% increase in E and a 7% increase in net assimilation (A) of untreated foliage in the lower cuvette. Responses of g., E and A of untreated foliage were similar irrespective of whether foliage was at the top or bottom of the seedling. When D in the treatment cuvette was restored to 2.2 kPa, g., E and A of foliage in the untreated cuvette returned to pretreatment values. In contrast, in well-watered Douglas-fir, there was almost no change in g., E or A of untreated foliage in one cuvette when D in the other cuvette was reduced, causing a 52% reduction in whole-plant E. However, similar manipulations on drought-stressed Douglas-fir led to 7–19% increases in g., E and A of untreated foliage. In well-watered western red cedar, daytime leaf water potential (Ψl) was maintained near –0.9 MPa over a wide range of D, whereas Ψl of Douglas-fir decreased from –1.2 to –1.5 MPa as D increased. The tighter (isohydric) regulation of Ψl in western red cedar may partly explain its greater stomatal response to D and variation in whole-plant E compared with Douglas-fir. In response to a reduction in E, measured increases in Ψl and g. of unmanipulated foliage were less than predicted by a model assuming complete hydraulic connectivity of foliage. Our results suggest the foliage of both species is partially autonomous with respect to water.

Keywords: drought, hydraulic limitation, photosynthesis, Pseudotsuga menziesii, stomatal conductance, Thuja plicata, vapor pressure deficit.

Introduction

According to the cohesion-tension theory, the flow of water from soil to leaf is seen as a “tug-of-war” on a hydraulic rope (Sperry et al. 1998). The hydraulic rope of xylem sap is under considerable negative pressure (Ψ) and is susceptible to breakage, i.e., xylem cavitation events (Pickard 1981) that render xylem elements nonconductive. The risk of xylem cavitation is a function of the rate of water loss (transpiration rate, E) and Ψ in the xylem sap, both regulated by stomatal conductance (Tyree and Sperry 1988, Jones and Sutherland 1991). Tyree and Sperry (1988) suggested that stomatal conductance is regulated such that woody plants routinely operate close to the point of xylem cavitation, a proposition supported by several studies (e.g., Cochard et al. 2002). Assuming capacitance is negligible, stomatal conductance (g. t) to water vapor can be expressed as:

\[ g. = \frac{K_{int}(\Psi - \Psi_l)}{A_l D} \quad (1) \]

where \( K_{int} \) is total conductance of the soil-to-atmosphere pathway, \( A_l \) is leaf area, \( \Psi \) is water potential of the soil, \( \Psi_l \) is water potential of the leaf and D is vapor pressure deficit between foliage and air. If stomata regulate E to prevent \( \Psi_l \) falling below a threshold or critical value (\( \Psi_{crit} \)), then as D increases, \( g. \) must decrease (e.g., Lange et al. 1971). Similarly, decreasing \( \Psi_l \) will reduce \( g. \) (e.g., Schulze 1986). The linkages between \( g. \), \( \Psi_l \), and \( D \) are directly related to \( K_{int} / A_l \) and are normally referred to as leaf-specific conductance (K). Reducing \( A_l \) will increase \( K_{int} \) and Equation 1 predicts that if \( g. \) is unchanged, \( \Psi_l \) of the remaining foliage will increase. However, a number of experiments have shown that, following a reduction in transpiring \( A_l \), \( g. \) increases whereas \( \Psi_l \) is unchanged (e.g., Meinzer and Grantz 1990, Whitehead et al. 1996). In sugarcane (Saccharum spp. hybrid), partial defoliation or shading increased \( K_{int} \) and \( g. \) (Meinzer and Grantz 1990). Similarly, \( g. \) declined rapidly when xylem hydraulic conductance was reduced by partial excision of the roots. Increases in \( K_{int} \) and \( g. \) through partial defoliation or shading have been observed in a range of species including sugarcane (Meinzer and
Materials and methods

Plant material and growth conditions

Cold-stored, clonal 2-year-old seedlings of coastal Douglas-fir were obtained from a commercial nursery in May 2001. Seedlings were planted in 3-dm³ pots filled with sand and grown outdoors at the field facility of the University of Victoria. From May to September, seedlings were watered every second day and received a nutrient solution every 2 weeks. In October 2001, 1-year-old container-grown seedlings of western red cedar were obtained from a commercial nursery and transplanted to 3-dm³ pots filled with sand and grown at the University’s field facility. These seedlings were also watered every second day and received a nutrient solution every 2 weeks.

Gas exchange measurements

Whole-seedling rates of net photosynthesis and E were measured continuously with a dual-cuvette closed gas exchange system that was a modified version of a system described previously (Livingston et al. 1994, Pepin and Livingston 1997, Pepin et al. 2002). Chamber and were measured with an infrared gas analyzer (Li-6262, Li-Cor, Lincoln, NE) and photon flux density (Q) with a quantum sensor (Li-190, Li-Cor). Net photosynthetic rates were determined by integrating the output (recorded as 2-min running averages) from a mass flow controller (Tylan, Carson, CA) used to inject CO₂ into the chamber to balance that taken up through photosynthesis. Vapor pressure was controlled by circulating air through a column of CaSO₄ when exceeded a defined set-point. The desiccant column was supported on a balance with 1 mg resolution. Transpiration rate was calculated as \( \Delta M/(At) \), where \( \Delta M \) is the change in desiccant mass over time \( t \) (typically 2 min) and \( A_t \) is projected foliage area determined with a leaf area meter (Li-3100, Li-Cor). Stomatal conductance to water vapor was calculated as \( E/D \). Whole-seedling \( E \) was determined as the sum of \( E \) (in g H₂O h⁻¹) from the upper and lower cuvettes. Intercellular \( CO₂ \) concentration \( (C_i) \) was calculated as described by Field et al. (1991). The flow rate through the chamber was approximately 0.025 m³ s⁻¹, giving rise to a high boundary layer conductance (> 2 mol m⁻² s⁻¹) for seedlings with \( A_t = 0.06 \) m²; Livingston et al. 1994) relative to \( g_s \) (typically 0.01 to 0.2 mol m⁻² s⁻¹). In separate experiments, leaf temperatures were within 0.1 °C of air temperature, and for this study, leaf and air temperatures were assumed to be the same.

The entire shoot of a seedling was sealed inside a polycarbonate chamber comprising two cuvettes. The upper and lower cuvettes were separated by a removable split polycarbonate plate. The polycarbonate plate was fixed at approximately half the seedling height, and due to this methodological limitation, there was not always an equal distribution of \( A_t \) between upper and lower cuvettes. Plasticine, which was used to seal the edges of the plate to the chamber wall, was also used to seal a hole in the center of the plate to accommodate the seedling stem. Two independent control systems were used to control \( C_i, e_a \) and temperature in each cuvette.

Soil water content and soil water potential measurements

Single-diode time domain reflectometry (TDR) probes (Hook et al. 1992) were used to determine soil water content in the
pots. Measurements of time delay were converted to volumetric water content (θ; m⁻³ m⁻³) in accordance with the relationship derived by Hook and Livingston (1996). The relationship between θ and Ψᵣ was derived from data in Pepin (1998):

\[ \ln \theta = -0.2089 \ln \Psiᵣ - 3.0541, \quad r^2 = 0.94 \]  \hspace{1cm} (2)

Variation within and between days in Ψᵣ (as indicated by predawn water potential) was small under well-watered conditions. At field capacity, pots contained ~450 ml of water and over 24 h, soil θ decreased by a maximum of 60 ml, which would have led to a decrease in Ψᵣ of < 0.1 MPa.

**Leaf water potential and hydraulic conductance measurements**

Leaf water potential was measured with a pressure chamber (Soil Moisture Equipment, Santa Barbara, CA) to determine the leaf water potential at which stomata close (Ψₘₑₜ) and Kₛ. Measurements required the partial dismantling of the upper cuvette and excision of foliage. The time from the dismantling of the upper cuvette (which caused abrupt changes in Cₕ and eₛ) to the shoot being enclosed in the pressure chamber was less than 30 s. Leaf-specific hydraulic conductance was estimated by:

\[ Kₛ = \frac{E}{\Psiₘₑₜ - \Psiᵣ} \]  \hspace{1cm} (3)

Predawn water potential (Ψₚₖ) was used as a surrogate for Ψᵣ. Under drought stress, we have only single measurements of Kₛ because in the sand used, small changes in θ below 0.07 m⁻³ have a large effect on Ψₚₖ, and thus it was difficult to obtain replicate estimates at the same Ψₚₖ. We have ignored capacitance in our calculations because all measurements were made at least 4 h after lights were turned on, at which time the water content of seedlings was assumed to be constant.

**Experimental protocol**

Seedlings were in a state of quiescence between October and April, and this partial dormancy was broken before they were placed in the gas exchange system. Seedlings were brought indoors and placed in a growth cabinet for at least 2 weeks at air temperature (Tₐₐ) = 19–22 °C, Cₛ = 360–400 µmol mol⁻¹ and eₛ = 0.7–0.9 kPa. Photoperiod inside the growth cabinet was 14 h (0700 to 2100 h) and Q at the top of the seedlings was 1000 µmol m⁻² s⁻¹, decreasing to Q > 420 µmol m⁻² s⁻¹ in the lowest foliage. During warmer months, seedlings were brought indoors and placed in the gas exchange system without this step.

Seedlings were placed in the gas exchange system and, before treatments were imposed, acclimatized for at least 1 week or until A and E were stable from day to day. Unless stated otherwise, Tₐₐ, eₛ and Cₛ inside the cuvettes were maintained at 25 °C, 2 kPa and 360–370 µmol mol⁻¹, respectively. Seedlings were exposed to a 14-h photoperiod at constant Q. Photon flux density was 1000 µmol m⁻² s⁻¹ at the top of the seedling, decreasing to 420 µmol m⁻² s⁻¹ at the bottom of the lower cuvette. In separate experiments, net photosynthetic rates and stomatal conductance in Douglas-fir and western red cedar were light-saturated at Q > 400 µmol m⁻² s⁻¹ (see also Tan et al. 1977, Pepin et al. 2002). Pots were watered to field capacity every day, unless stated otherwise.

**Leak tests**

Once a seedling was sealed in the gas exchange system, and before experiments commenced, the system was tested for leaks, including leaks between the cuvettes. In one test, which was carried out in darkness, a high concentration of CO₂ was injected into one cuvette to ensure that any changes in Cₛ in the other cuvette were consistent with respiration rates. A similar test was carried out after experiments were completed on seedlings that had died after being subjected to extreme drought stress. The validity of estimates of E from the gas exchange measurements was assessed by comparing estimates of cumulative E with the change in volumetric θ (determined by TDR). This was usually done over a period of 5 or more days. The methods always agreed to within 5%.

**Vapor pressure response of whole seedlings**

Vapor pressure response of whole plants was measured on five Douglas-fir and two western red cedar seedlings. Response curves were measured between two and four times on each seedling and averaged. Rate of net photosynthesis, E and gₛ, were measured at an eₛ of 2 kPa (D = 1.2 kPa) for at least 1 h, or until they were stable. After this, eₛ increased to 3.0 kPa and a response curve was generated by decreasing eₛ stepwise to 0.7 kPa. At each eₛ, gas exchange was allowed to stabilize for at least 1 h. During these experiments, there were no differences between eₛ in the upper and lower cuvettes.

**Response of foliage to independent manipulation of D in upper and lower cuvettes**

Experiments were carried out on five Douglas-fir and two western red cedar seedlings to determine whether A, E and gₛ of foliage in one cuvette were affected by D (and thus E) in the other cuvette. Manipulations were repeated between two and four times on each seedling. Treatments were imposed at least 1 h after lights were turned on, by which time A, E and gₛ were relatively stable. Vapor pressure in one cuvette was increased (to 2.2 kPa) or decreased (to 0.2 kPa) while D in the other cuvette (untreated foliage) was held constant at 2.2 kPa. After 24–48 h, the manipulation was reversed.

Changes in A, E and gₛ were determined by comparing pre-treatment to posttreatment values with paired t-tests (Underwood 1997) and are presented as the percentage change in absolute A, E and gₛ in that cuvette. Daytime (lights on) values of A, E and gₛ were generally averaged for periods of 12 to 14 h pre- and posttreatment. In the treatment cuvette, we did not begin integrating values until D reached a steady state, otherwise errors might have arisen because of the change in chamber eₛ and adsorption/desorption of water to the cuvette and seedling.
Response of foliage to soil drought

Soil drought was imposed on three seedlings of Douglas-fir and manipulations of $D$ were repeated as in wet soil. Water was withheld until $E$ reached a minimum and $\Psi_{pd}$ was approximately $-1.5$ MPa ($\theta = 0.035$ to $0.045$ m$^3$ m$^{-3}$); normally, this took at least 10 days. At this point, we carried out two replicate manipulations of $D$ per seedling. We attempted to maintain $\Psi_{pd}$ near $-1.5$ MPa on replicate days by, in the evening, providing the same amount of water transpired that day.

Modeling of seedling response to $D$

We used Equation 1 and measured $K_s$, $\Psi_{crit}$, $\Psi_{pd}$ and maximum $g_s (g_{s,max})$ to predict the relationship between $g_s$ (or $E$) and $D$, as described previously (Bond and Kavanagh 1999, Oren et al. 1999). The model assumes that $g_s$ is regulated such that $\Psi_l$ is never less (more negative) than $\Psi_{crit}$ and is restricted to cases where $\Psi_{pd} > \Psi_{crit}$. We assumed an infinite boundary layer conductance and that $K_s$ was invariant. Stomatal conductance was modeled as follows. A preliminary value of $\Psi_l$ was estimated using the measured $g_{s,max}$ in a rearranged version of Equation 1:

$$\Psi_l = \Psi_{pd} - (g_{s,max}D/K_s)$$

(4)

If $\Psi_l > \Psi_{crit}$, then $g_s = g_{s,max}$. If $\Psi_l < \Psi_{crit}$, then $\Psi_l = \Psi_{crit}$ and $g_s$ was calculated from Equation 1.

Predicting the responses of $g_s$ and $\Psi_l$ of untreated foliage to manipulation of $E$

We predicted the responses of $g_s$ and $\Psi_l$ in untreated foliage to imposed changes in $E$ in treated foliage based on our own and published data for branch-level manipulations on Douglas-fir (Brooks et al. 2002) and whole-tree manipulations on P. radiata (Whitehead et al. 1996). To predict changes in $g_s$ and $\Psi_l$ of untreated foliage following a reduction in $E$ of treated foliage, we considered two scenarios: (a) isohydric regulation of $\Psi_l$ (i.e., $\Psi_l$ constant, $g_s$ increases), and (b) anisohydric regulation of $\Psi_l$ (i.e., $\Psi_l$ increases, $g_s$ constant). Under control conditions, Equation 1 was solved for $K_{sat}/A$ using measured $g_{s,max}$, $\Psi_{pd}$, $\Psi_l$ and $D$. After imposing a treatment, the equation was solved again with the change in whole-plant $E$ as a surrogate for the change in $A_t$. Leaf water potential was not measured in the lower cuvette because of methodological limitations, but was assumed to be the same as $\Psi_l$ in the upper cuvette. This assumption is supported by measured $\Psi_l$ in the upper cuvette, which was the same when $D$ was 2.2 kPa in the top and 0.2 kPa in the bottom as when the differences in $E$ were reversed.

Data presentation and analysis

Throughout the experiment, all comparisons of seedlings were based on the mean of 2–4 replicate measurements for each seedling. Most analyses were based on absolute, not area-based, values of $A$, $E$ and $g_s$ (e.g., g H$^2$O h$^{-1}$ cuvette$^{-1}$). In theory, responses of untreated foliage to $E$ of treated foliage are a function of absolute rates of $E$ (Pepin et al. 2002).

The use of absolute values accounts for differences in $A_t$ distribution between the cuvettes and the change in $A_t$ over time. Leaf area was measured only when seedlings were removed from the chamber, and in spite of the species’ slow rate of growth, there may have been significant change in $A_t$ over the 2–3 week measurement period that would have confounded results.

There was significant between-seedling variation in area-based values of $A$, $E$ and $g_s$ which precluded data presentation or analysis of time-course data based on means of area-based values. To overcome this problem, statistical analysis was carried out on the change in absolute $A$, $E$ and $g_s$ in each cuvette, and to illustrate treatment effects, time courses of area-based $A$, $E$ and $g_s$ for single seedlings are presented.

Results

Response of foliage to manipulation of $D$ in upper and lower cuvettes—well-watered seedlings

At a $D$ of 2.2 kPa, $E$ ranged from 1.5 to 2.0 mmol m$^{-2}$ s$^{-1}$ in western red cedar and from 2.0 to 2.5 mmol m$^{-2}$ s$^{-1}$ in Douglas-fir (Figures 1 and 2). Decreasing $D$ from 2.2 to 0.2 kPa (by increasing $e_o$) typically took 1 h and was solely dependent on seedling $E$. The transient reduction in $g_s$ as $D$ decreases is an artifact of measurements under non-steady state conditions. The increase in chamber $e_o$ was brought about by seedling $E$ and this gave rise to erroneously low estimates of $E$ and $g_s$. When a steady state was reached, $g_s$ was greater than when $D$ was low, yet there was an overall decrease in $E$. In western red cedar, the decrease was almost threefold, and in Douglas-fir, approximately fourfold. The reduction in whole-plant $E$ (when $D$ in one cuvette was reduced) (Table 1) was a function of differences in $E$ and the distribution of $A_t$ between the upper and lower cuvettes.

Foliage in the cuvette that was held at 2.2 kPa (untreated foliage) responded to a reduction of $D$ in the other cuvette (Figure 1, Table 1). In western red cedar, reducing whole-plant $E$ by 42–46% caused $E$ of untreated foliage to increase by 9–12%, $g_s$ by 10–12% and $A$ by 5–7%. The size of these responses was the same in either cuvette when $D$ was manipulated in the treatment cuvette, i.e., in upper and lower foliage. Leaf water potential in untreated foliage of the upper cuvette increased from $-0.9$ to $-0.84$ MPa (Tables 1 and 2) when $D$ in the lower cuvette was decreased.

In Douglas-fir, reducing $D$ in one cuvette led to 24–52% reductions in whole-plant $E$. In untreated foliage, the increases in $E$, $g_s$ and $A$ of between 1 and 3% were not significant (Table 1, Figure 2). Leaf water potential in untreated foliage of the upper cuvette increased by an average of 12%, or from $-1.50$ to $-1.32$ MPa, when $D$ in the lower cuvette was decreased (Tables 1 and 2).

Increasing $D$ from 0.2 to 2.2 kPa took approximately 30 min (cf. decreasing $D$) and during this period, estimates of $E$ were erroneously large because a portion of the apparent $E$ was due to the reduction in chamber $e_o$. When $D$ was restored to 2.2 kPa
and reached a steady state, $E$, $g$, and $A$ quickly reverted to within 5% of their pretreatment values (Figures 1 and 2). This was accompanied, in untreated foliage, by slower decreases in $E$, $g$, and $A$ to within 5% of their previous values. Changes in $E$, $g$, and $A$ in untreated foliage were more rapid than when $D$ was decreased. The rapidity of these “responses” was to a large extent determined by the rate at which $D$ in the treatment cuvette changed. Standard errors are rather large owing to the differences in leaf area distribution between replicate plants. Nevertheless, the within-plant repeatability of manipulations was high. For example, when $D$ in the lower cuvette of one western red cedar seedling was varied between 2.2 and 0.2 kPa five times over a 10-day period, the change in $E$ (in untreated foliage of the upper cuvette) was $12.1 \pm 1.2\%$ (mean ± SE).
Response of foliage to manipulation of $D$ in upper and lower cuvettes—drought-stressed seedlings

In drought-stressed Douglas-fir, reducing $D$ in one cuvette led to 7–19% increases in $E$, $g_s$, and $A$ of untreated foliage. These responses were slower than those of well-watered seedlings (compare Figures 2 and 3), and were, to some extent, masked by the greater relative variability of $A$, $E$, and $g_s$ of drought-stressed compared with well-watered Douglas-fir.

Hydraulic and stomatal characteristics of seedlings

In Douglas-fir and western red cedar, $g_s$ was negatively correlated to $D$ (Figure 4). In Douglas-fir, this relationship was linear, whereas in western red cedar, it was logarithmic over the range of $D$ examined. When $D = 0.25$ kPa, $g_s$ of Douglas-fir ($0.193 \pm 0.004$ mol m$^{-2}$ s$^{-1}$; mean $\pm$ SE, $n = 5$) was significantly less than in western red cedar ($0.23 \pm 0.01$ mol m$^{-2}$ s$^{-1}$; $n = 2$) (see also Table 3). Stomatal conductance was less sensi-

Figure 2. Time courses of (a, e) vapor pressure deficit ($D$), (b, f) transpiration rate ($E$), (c, g) stomatal conductance ($g_s$), and (d, h) net photosynthetic rate ($A$) in a well-watered seedling of Douglas-fir. Projected leaf area was 85 cm$^2$ in the upper cuvette (solid lines) and 74 cm$^2$ in the lower cuvette (dashed lines). Data are for two consecutive days and are means of three replicate manipulations on the same seedling. Standard error bars are shown every 2 h for untreated foliage in the upper cuvette and were omitted for treated foliage in the lower cuvette for clarity.
Table 1. Responses of seedlings of well-watered western red cedar (WRC), well-watered Douglas-fir (D-fir) and drought-stressed Douglas-fir (D-fir drought) to manipulation of vapor pressure deficit (D). Vapor pressure deficit in the treatment cuvette was decreased from 2.2 to 0.2 kPa while D in the untreated cuvette was held at 2.2 kPa. Changes in net assimilation (A), transpiration rate (E) and stomatal conductance (gs) were determined by comparing pre- to posttreatment values. Daylight values of A, E and gs were averaged for periods of 12 to 14 h pre- and posttreatment. Data collected from the treatment cuvette were not integrated while D was changing. The change in whole-plant transpiration (AE) was determined from the sum of transpiration (g H2O h–1) in the upper and lower cuvettes. Data are means (SE) and the number of replicates (n) is in parenthesis. Each replicate is the mean of two and four manipulations on the same seedling. Statistical significance of changes in A, gs and E were compared with paired one-tailed t-tests (NS = not significant; * = P < 0.10; ** = P < 0.05; *** = P < 0.01) (Underwood 1997). For technical reasons, leaf water potential (Ψl) was measured only in the upper chamber, and thus only the response of upper foliage to manipulation of lower foliage is reported. Abbreviation: ND = not determined.

<table>
<thead>
<tr>
<th>Species</th>
<th>Percentage of foliage in cuvette</th>
<th>ΔE(%)</th>
<th>Change in untreated cuvette (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WRC (n = 2)</td>
<td>Top 57 (19)</td>
<td>−46 (16) *</td>
<td>ND</td>
</tr>
<tr>
<td>D-fir (n = 5)</td>
<td>Top 38 (5)</td>
<td>−24 (9) ***</td>
<td>+7</td>
</tr>
<tr>
<td>D-fir drought (n = 3)</td>
<td>Top 41 (8)</td>
<td>−35 (10) *</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Bottom 59 (8)</td>
<td>−50 (12) *</td>
<td>ND</td>
</tr>
</tbody>
</table>

1 Ψ1 was measured in three Douglas-fir and one western red cedar.

tive to changes in D in Douglas-fir than in western red cedar, and thus when D = 2.65 kPa, gs of Douglas-fir (0.114 ± 0.001 mol m–2 s–1) was significantly greater than that in western red cedar (0.068 ± 0.004 mol m–2 s–1) (Figure 4). When well-watered (Ψpd = −0.3 MPa), Ψ1 of western red cedar was −0.9 ± 0.1 MPa for any D between 0.5 and 2.6 kPa (Table 2). This conservative Ψ1 is denoted Ψ1-crit. In Douglas-fir, Ψ1 was not maintained within as narrow a range. Instead, Ψ1 decreased from −1.2 to −1.5 MPa as D increased from 0.5 to 2.6 kPa. Therefore, in Douglas-fir, the threshold or critical Ψ1 was more like a range than a discrete point. Nonetheless, we chose −1.5 MPa as Ψ1-crit since this was the most negative water potential until Ψ1 fell below −0.8 MPa. Leaf-specific hydraulic conductance was significantly lower in Douglas-fir (2.0 ± 0.1 mmol MPa–1 m–2 s–1) than in western red cedar (3.0 ± 0.2 mmol MPa–1 m–2 s–1) (Table 3). Under drought stress, as Ψpd decreased below −1.0 MPa, Ψ1 was no longer conserved and decreased below Ψ1-crit. When Ψpd was −1.4 to −1.7 MPa, Ψ1 (at D = 2.35 kPa) was 0.7 mmol MPa–1 m–2 s–1 in Douglas-fir. Under drought stress, we have only single measurements of Ψ1 because, in the sand used, small changes in θ below 0.07 m3 m–3 have a large effect on Ψ1, and thus, it was difficult to obtain replicate estimates at the same Ψpd.

Modeled responses of gs to D

In western red cedar, modeled responses of gs and E to D (Figure 5) were similar to the measured responses (Figure 4). The model predicted that gs of Douglas-fir would not be reduced until D was greater than −1.2 kPa, whereas measured gs decreased linearly between 0.25 and 2.65 kPa. In Douglas-fir, we contrasted the measured linear decrease in Ψ1 from −1.2 to −1.5 MPa (as D increased) with isohydric regulation of Ψ1 at −1.5 MPa. Allowing Ψ1 to decrease from −1.2 and −1.5 MPa resulted in gs being somewhat less than 1 to 2.5 kPa compared with the situation with perfect isohydric regulation.

Table 2. Measured and modeled changes in stomatal conductance (gs; mol m–2 s–1) and leaf water potential (Ψ; MPa) in response to reversible reductions in whole-plant or whole-branch transpiration rate (ΔE). To predict changes in gs and Ψ1 of untreated foliage following a reduction in whole-plant E, we considered two scenarios: (a) isohydric regulation of Ψ1 (i.e., Ψ1 constant, gs increases) and (b) anisohydric regulation of Ψ1 (i.e., Ψ1 increases, gs constant). Under control conditions, Equation 1 was solved for Kωd/Aω using measured gs, predawn water potential (Ψpd), Ψ1 and vapor pressure deficit (D). After imposing a treatment, the equation was re-solved with the change in whole-plant or whole-branch E used as a surrogate for the change in leaf area (Aω). Abbreviations: WRC = western red cedar and D-fir = Douglas-fir.

<table>
<thead>
<tr>
<th>Species</th>
<th>Pretreatment</th>
<th>Posttreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>g0</td>
<td>Ψ1</td>
<td>ΔE(%)</td>
</tr>
<tr>
<td>WRC (this study)1</td>
<td>0.085</td>
<td>−0.90</td>
</tr>
<tr>
<td>D-fir (this study)2</td>
<td>0.124</td>
<td>−1.50</td>
</tr>
<tr>
<td>D-fir (Brooks et al. 2002)3</td>
<td>0.200</td>
<td>−2.00</td>
</tr>
<tr>
<td>Pinus radiata (Whitehead et al. 1996)3</td>
<td>0.045</td>
<td>−1.50</td>
</tr>
</tbody>
</table>

1 Data are for the response of upper foliage to decreasing D in lower foliage.
2 Data are for 20-year-old trees. Ψpd = −0.85 MPa. ΔE estimated from sap flux data concurrent with gs measurements.
3 Data are for D = 12–16 mmol m–3. Ψpd was assumed to be −0.3 MPa.
Predicted responses of \( g_s \) and \( \Psi_l \) in untreated foliage to manipulation of \( E \)

Changes in \( \Psi_l \) and \( g_s \) were consistently less than predicted for a model that assumes complete hydraulic coupling (Table 2). In western red cedar, for example, if there was no stomatal response to a reduction in \( E \), we would predict that \( \Psi_l \) of untreated foliage would increase from \(-0.90\) to \(-0.64\) MPa. The small measured increase in \( \Psi_l \) (up to \(-0.84\) MPa) cannot be attributed solely to the measured increase in \( g_s \), because even with this increase, \( \Psi_l \) should have increased to \(-0.68\) MPa. Measured increases in \( \Psi_l \) and \( g_s \) were also significantly less than those predicted for Douglas-fir seedlings (this study), and
Pinus radiata trees and Douglas-fir branches (in response to partial shading).

**Discussion**

In western red cedar, when whole-plant $E$ was decreased by ~45% (by reducing $D$ in one cuvette), $A$, $E$ and $g_s$ of untreated foliage increased by 5–12% (Table 1). These responses are quantitatively consistent with those determined in the same species where varying proportions of the foliage were shaded and whole-plant $E$ was reduced (Pepin et al. 2002). Shading and decreases in $D$ both reduce $E$; however, shading decreases $A$ and $g_s$ (Pepin et al. 2002), whereas decreasing $D$ increases $A$ and $g_s$ (e.g., Figure 1). The striking similarity of responses between studies (despite the aforementioned contrasting effects on $g_s$ and $A$) is consistent with a hydraulic response of untreated foliage to whole-plant $E$. One notable difference is that responses in our study were considerably slower than those of shading treatments because of differences in the rate at which treatments were imposed. With our closed gas exchange system, a decrease in $D$ was brought about by $E$ increasing chamber $e_a$, hence $D$ changed slowly compared with the nearly instantaneous changes in $E$ induced by shading.

Table 3. Hydraulic and stomatal characteristics of well-watered seedlings of western red cedar (WRC) and Douglas-fir (D-fir). Abbreviations: $g_{smax}$ = maximum stomatal conductance measured at vapor pressure deficit ($D$) = 0.25 kPa (see also Figure 4); $g_{smin}$ = minimum stomatal conductance (cuticular conductance) measured at nighttime; $\Psi_{crit}$ = leaf water potential at which stomata close; and $K_l$ = leaf-specific hydraulic conductance measured in wet soil (predawn water potential ($\Psi_{pd}$) = –0.3 MPa) with $D = 2.35$ kPa. Data are means (SE) of two replicate seedlings of western red cedar and five Douglas-fir.

<table>
<thead>
<tr>
<th>Species</th>
<th>$g_{smax}$ (mol m$^{-2}$ s$^{-1}$)</th>
<th>$g_{smin}$ (mol m$^{-2}$ s$^{-1}$)</th>
<th>$\Psi_{crit}$ (MPa)</th>
<th>$K_l$ (mmol MPa$^{-1}$ m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WRC</td>
<td>0.226 (11)</td>
<td>0.012 (3)</td>
<td>–0.9 (0.1)</td>
<td>3.0 (0.2)</td>
</tr>
<tr>
<td>D-fir</td>
<td>0.193 (4)</td>
<td>0.019 (2)</td>
<td>–1.5 (0.1)</td>
<td>2.0 (0.1)</td>
</tr>
</tbody>
</table>

Figure 4. Responses of (a) stomatal conductance ($g_s$) and (b) transpiration rate ($E$) to vapor pressure deficit ($D$) in well-watered seedlings of western red cedar (□) and Douglas-fir (●). In Douglas-fir, $g_s = 198.4 – 33.6D$, $r = 0.98$; and $E = 1.77 + 2.33\log D$, $r = 0.98$. In western red cedar, $g_s = 137.3 – 151.6\log D$, $r = 0.99$; and $E = 1.29 + 1.12\log D$, $r = 0.97$. Data are means (± SD) of two replicate seedlings of western red cedar and five Douglas-fir. Each replicate is the mean of two to four response curves measured on each seedling.

Figure 5. Modeled responses of (a) stomatal conductance ($g_s$) and (b) transpiration rate ($E$) to vapor pressure deficit ($D$) in well-watered seedlings (soil water potential ($\Psi_s$) = –0.3 MPa) of western red cedar (□, dashed line), Douglas-fir with isohydric regulation (●, solid line) and Douglas-fir with anisohydric regulation (○, dashed line). We used Equation 1 and measured leaf specific conductance ($K_l$), critical leaf water potential ($\Psi_{crit}$) and maximum $g_s$ ($g_{smax}$) (Table 2) to predict the relationship between $g_s$ (or $E$) and $D$, as described previously (Bond and Kavanagh 1999, Oren et al. 1999). In Douglas-fir, we considered two scenarios: (1) isohydric regulation where $\Psi_{crit} = –1.5$ MPa; and (2) anisohydric regulation in which $\Psi_{crit}$ decreases linearly from –1.2 to –1.5 MPa as $D$ increases.
(Pepin et al. 2002) rather than a fundamental difference in hydraulic functioning of upper and lower portions of the canopy.

Although whole-plant responses of \( g_s \) to \( D \) (compare Figures 4 and 5) were consistent with simple hydraulic models, these models failed to predict responses of untreated foliage to changes in \( E \) in other parts of the seedling (Table 3). That is, measured changes in \( \Psi_l \) and \( g_s \) of untreated foliage were consistently less than those predicted for Douglas-fir and western red cedar seedlings (Table 2). Reexamination of published data for similar manipulations on \( Pinus radiata \) trees (Whitehead et al. 1996) and Douglas-fir branches (Brooks et al. 2002) also revealed that changes in \( \Psi_l \) and \( g_s \) of untreated foliage were significantly smaller than expected. Given the generality of model failure, one might question the application of hydraulic models assuming complete hydraulic connectivity amongst foliage.

The insensitivity of Douglas-fir foliage to changes in \( E \) is consistent with previous reports (Brooks et al. 2002) (Figure 2, Table 1) and may have one of several explanations. The absence of a response in Douglas-fir may indicate poorer hydraulic connectivity compared with western red cedar, as argued previously (Brooks et al. 2002). How \( K_l \) is distributed through the soil-to-atmosphere continuum may also affect responsiveness. For example, if leaf conductance of Douglas-fir was low relative to stem, root and rhizosphere conductances, the responses of shoot \( g_s \) would be smaller than if \( K_l \) were distributed evenly throughout the soil-to-atmosphere continuum (Becker et al. 2000). Limited feedback between \( \Psi_l \) and \( g_s \) in Douglas-fir may be another explanation. In contrast to the near isohydric regulation of \( \Psi_l \) in western red cedar, \( \Psi_l \) of Douglas-fir decreased from –1.2 to –1.5 MPa as \( D \) increased and \( \Psi_l \) of untreated foliage decreased from –1.5 to –1.32 MPa when \( E \) of treatment foliage decreased (Table 2). This anisohydric regulation of \( \Psi_l \) decreases the sensitivity of \( g_s \) to \( D \) (Figure 5, see also Oren et al. 1999), but the effect is rather minor and too small to explain the less sensitive \( g_s \)–\( D \) response of Douglas-fir compared with western red cedar (Figures 4 and 5). Furthermore, to explain the absence of a stomatal response in untreated foliage to manipulation of \( E \), \( \Psi_l \) of untreated foliage would have to increase from –1.5 to –0.88 MPa, whereas it only increased to –1.32 MPa. These findings indicate that the insensitivity of untreated foliage to manipulation of \( E \) is probably not due to less strict regulation of \( \Psi_l \) in Douglas-fir, and that other factors such as hydraulic connectivity and the distribution of \( K_l \) are likely more important.

Under moderate soil drought (\( \Psi_{soil} = –1.7 \) to –1.4 MPa), the relative responses of untreated foliage of Douglas-fir to a change in whole-plant \( E \) were greater than in wet soil (compare Figures 2 and 3). Drought decreases \( \Psi_l \) (and \( \Psi_{soil} \)), and this most likely resulted in the increased sensitivity of foliage to \( E \) elsewhere in the seedling. This is supported by Equation 1, which predicts that, as \( \Psi_l \) decreases, stomata become more sensitive to \( D \) and whole-plant \( E \) (see also Bond and Kavanagh 1999). On the other hand, the response would be reduced if soil drought decreased the hydraulic conductance of the soil-to-atmosphere continuum and caused parts of the seedling to become poorly connected hydraulically (sensu Brooks et al. 2002). That responses of Douglas-fir were greater under soil drought suggests that the change in sensitivity of \( g_s \) to \( D \) and \( E \) overrides any effects due to decreases in \( K_l \). Consideration of vulnerability curves for Douglas-fir supports our assertion that the loss of conductivity is small, and in the coarse sand used in this study it is likely that the majority of this decrease occurred in the soil, rhizosphere and small roots (Sperry and Ikeda 1997, Sperry et al. 1998, Kavanagh et al. 1999).

That changes in \( g_s \) (and \( \Psi_l \)) were smaller than expected might be explained alternatively by the regulation of \( g_s \) by chemical signals or a combination of chemical and hydraulic signals (Tardieu and Davies 1993). For example, Meinzer and Grantz (1990) argued that, following partial defoliation or partial shading, metabolic promoters (hormonal or nutritional) of stomatal opening carried in the xylem sap may reestablish the balance between water loss and water transport capacity by rapidly increasing \( g_s \). Soil pressurization experiments with seedlings of Douglas-fir (Fuchs and Livingston 1996) do not support the implication of parameters associated with soil or root water stress (such as \( \Psi_l \) or abscisic acid production by roots (Tardieu et al. 1992)) and are consistent with stomatal response to changes in \( \Psi_l \). Nevertheless, these data are consistent with suggestions that hydraulic changes could trigger the rapid generation of chemical signals in the foliage (Tardieu and Davies 1993).

Modeling \( E \) of trees is complicated if foliage in one part of a crown is affected by \( E \) elsewhere in the same crown. Most models assume complete autonomy of foliage (e.g., Leuning et al. 1995). However, in western red cedar and \( Pinus radiata \), models assuming autonomy would underestimate \( g_s \), \( A \), and \( E \) of sunlit foliage. This error would increase in direct proportion to the area of shaded foliage. An alternative model assumes complete hydraulic connectivity among all foliage within a crown. However, as our results show, such a model consistently overestimates \( g_s \), (and by inference \( A \)) of sunlit (or high \( D \)) foliage in seedlings, branches or trees with a proportion of shaded (or low \( D \)) foliage (Table 3). Improved predictions of \( g_s \), \( A \) and \( E \) in tree canopies may result if complete hydraulic maps describing hydraulic connectivity (\( K_l \)) among and between all foliage were incorporated into models.

Conclusions

Our results demonstrate that tree species differ markedly in the extent to which foliage is affected by changes in the \( E \) of foliage elsewhere in the plant. Under well-watered conditions, western red cedar responded to such manipulations, whereas Douglas-fir did not. However, Douglas-fir seedlings were affected when drought-stressed. The greater sensitivity of western red cedar to changes in \( D \) and to the manipulation of whole-seeding \( E \) is consistent with the occurrence of this species in generally wetter habitats than Douglas-fir. In response to a reduction in whole-plant \( E \), changes in \( \Psi_l \) and \( g_s \) of untreated foliage were smaller than those predicted for a simple model that assumed complete hydraulic connectivity within a seedling. Consequently, foliage on a seedling in which whole-
plant $E$ was reduced by ~50% accrued only a small advantage in terms of water status compared to foliage on a seedling where $E$ was maximum. Benefits in terms of water status may be greater where the reduction in whole-plant $E$ is larger.

Acknowledgments

This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada. CellFor Inc. and the Cairnpark Nursery are thanked for supplying Douglas-fir and western red cedar, respectively. We gratefully acknowledge Steeve Pepin and David Whitehead for useful comments on a previous version of this manuscript.

References


