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Stomatal Control of Whole-Plant Photosynthesis and Transpiration in Conifer Seedlings

by

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B.Sc., University Laval, 1989
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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Biology

We accept this dissertation as conforming to the required standard

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ABSTRACT

Because the exchange of carbon dioxide and water vapor between plants and the atmosphere is regulated by changes in stomatal conductance ($g_s$), the responses of stomata to fluctuations in environmental variables have major effects on leaf physiological processes such as carbon assimilation. This dissertation focuses on the stomatal control of whole-plant photosynthesis and transpiration in conifer seedlings. Experiments were conducted on well watered one-year-old Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and western redcedar (*Thuja plicata* Donn) seedlings to determine the effects of temperature on whole-plant photosynthetic and stomatal responses to short-term fluctuations in irradiance ($Q$). Following a step change in $Q$, time constants ($\tau$, the period over which 63% of the total change occurs) for $g_s$ and assimilation rate ($A$) decreased linearly with increasing air temperature ($T_{air}$). For example, in western redcedar $\tau_A$ decreased from $30 \pm 4$ minutes at $5 \degree C$ to $10 \pm 1$ minutes at $25 \degree C$. In all cases, $\tau_A$ was within 10–15% of $\tau_{g_s}$. There was considerable variation in $\tau$ among individuals within a given species. Differences between species became more pronounced with decreasing temperature. Multiplicative models that included functions for $\tau$ accounted for 99% of the diurnal variability in $A$ and $g_s$ for seedlings exposed to varying $T_{air}$, $Q$ and vapor pressure deficit. Estimates of daily $A$ were within 2% of those measured. Intermittent cloud cover and understory shading were approximated by exposing seedlings to 3–4 episodes ($\geq 1$ h) of shade (200 or 500 $\mu$mol m$^{-2}$ s$^{-1}$) or complete darkness during the day. In such cases, daily $A$ was overestimated by up to 4 and 21%, respectively, if a function for $\tau$ was excluded from the models. The results suggest that there is scope for selecting seedling stock for increased carbon assimilation on the basis of reduced time constants. For example, in western redcedar, a 40% reduction in $\tau$ could
lead to increases in daily carbon gains of almost 5% depending on the frequency and degree of shading. If these daily gains were translated into increased dry matter production and compounded, seasonal gains would be even larger.

Experiments were also conducted on one-year-old western redcedar seedlings to determine the response of illuminated foliage to transient and reversible changes in total photosynthesizing foliage area ($L_A$). Reductions in $L_A$ were brought about by either shading a portion of the foliage or by reducing the ambient CO$_2$ concentration ($c_a$) of the air surrounding the lower part of the seedling. In the latter case, the vapor pressure was also changed so that transpiration rates ($E$) could be manipulated independently of photosynthesis rates. It was hypothesized that following such treatments, there would be rapid short-term compensatory changes in $g_s$ and $A$ of the remaining foliage. These would be in response to hydraulic signals generated by changes in the water potential gradient rather than changes in the distribution of sources and sinks of carbon within the seedling. When a portion of the foliage was shaded, there was an immediate reduction in whole-seedling $E$ and a concomitant increase in $g_s$, $A$ and $E$ in the remaining illuminated foliage. However, the intercellular CO$_2$ concentration did not change. These compensatory effects were fully reversed after the shade was removed. When the lower foliage $A$ was reduced to $< 0 \mu$mol m$^{-2}$ s$^{-1}$, by shading or lowering $c_a$, but $E$ was either unchanged or increased, there was not a significant increase in $g_s$ and $A$ in the remaining foliage. I conclude that short-term compensatory responses in illuminated foliage occur only when reductions in $L_A$ are accompanied by a reduction in whole-plant $E$. The relation between the reduction in whole-seedling $E$ and the increase in $g_s$ or $A$ is highly linear ($R^2 = 0.69$) and confirms the hypothesis of the strong regulation of $g_s$ by hydraulic signals generated within the seedling. I suggest that the mechanism of the compensatory effects is a combination of both increased CO$_2$ supply, resulting from increased $g_s$ and a response of the rate of carboxylation, possibly related to the activity of Rubisco.
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\( A \)  
net photosynthesis rate; net assimilation rate (\( \mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \))

\( A_r \)  
ratio of whole-plant net photosynthesis rate of a partially shaded seedling to that of a fully illuminated seedling (dimensionless)

\( A_t \)  
whole-plant net photosynthesis rate (\( \mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \))

\( A_{\text{max}} \)  
steady-state assimilation rate measured over one hour, 2–3 h after the lights were switched on (\( \mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \))

\( c \)  
speed of light (m s\(^{-1}\))

\( c_a \)  
ambient \( \text{CO}_2 \) concentration (\( \mu \text{mol mol}^{-1} \))

\( c_{a, \text{lower}} \)  
ambient \( \text{CO}_2 \) concentration in the lower cuvette (\( \mu \text{mol mol}^{-1} \))

\( c_i \)  
intercellular \( \text{CO}_2 \) concentration (\( \mu \text{mol mol}^{-1} \))

\( D \)  
vapor pressure deficit (kPa)

\( D_{0.5} \)  
vapor pressure deficit when \( A \) and \( g_s \) are half the maximum (kPa)

\( D_{\text{lower}} \)  
vapor pressure deficit in the lower cuvette (kPa)

\( \text{D.C.} \)  
direct current (V)

\( e_a \)  
ambient water vapor pressure (kPa)

\( e_s \)  
water vapor pressure at saturation (kPa)

\( E \)  
transpiration rate (mmol H\(_2\)O m\(^{-2}\) s\(^{-1}\))

\( E_t \)  
whole-plant transpiration rate (mmol H\(_2\)O m\(^{-2}\) s\(^{-1}\))

\( g_s \)  
stomatal conductance to water vapor (mmol H\(_2\)O m\(^{-2}\) s\(^{-1}\))

\( g_{s, \text{max}} \)  
steady-state stomatal conductance measured over one hour, 2–3 h after the lights were switched on (mmol H\(_2\)O m\(^{-2}\) s\(^{-1}\))

\( \text{ID} \)  
internal diameter (m)

\( K' \)  
real part of the dielectric constant (dimensionless)

\( K'' \)  
imaginary part of the dielectric constant; dielectric loss (dimensionless)

\( K_a \)  
apparent dielectric constant (dimensionless)

\( K_{\text{air}} \)  
dielectric constant of air (dimensionless)

\( K_c \)  
composite dielectric constant (dimensionless)

\( K_{\text{solids}} \)  
dielectric constant of solids (dimensionless)

\( K_{\text{water}} \)  
dielectric constant of water (dimensionless)

\( l \)  
 length of a probe or transmission line (m)

\( L_a \)  
photosynthesizing foliage area (m\(^2\))

\( L_r \)  
 ratio of shaded to total foliage area (dimensionless)

\( L_t \)  
whole-plant one-sided foliage area (m\(^2\))
ns nanosecond ($10^{-9}$ s)  
OD outer diameter (m)  
$P$ probability of a statistical test  
ps picosecond ($10^{-12}$ s)  
PVC polyvinyl chloride  
$Q$ photon flux density for photosynthetically active radiation ($\mu$mol photons m$^{-2}$ s$^{-1}$)  
$Q'$ light compensation point ($\mu$mol photons m$^{-2}$ s$^{-1}$)  
$R^2$ coefficient of determination  
Rubisco ribulose 1,5-bisphosphate carboxylase/oxygenase  
t time (s or h)  
t propagation time of an electromagnetic pulse; time delay (s)  
t$_{air}$ time delay in air (s)  
t$_{dry\,soil}$ time delay in a dry soil (s)  
t$_{solids}$ time delay due to the solid fraction of a soil (s)  
t$_{water}$ time delay in water (s)  
$T$ temperature (°C)  
$T_{air}$ air temperature (°C)  
$T_{leaf}$ leaf temperature (°C)  
$T_{max}$ temperature at which $A$ and $g_s$ are at a maximum (°C)  
$T_{water}$ temperature of water (°C)  
TDR time domain reflectometry  
V velocity (m s$^{-1}$)  
WUE water use efficiency ($\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$ mmol H$_2$O m$^{-2}$ s$^{-1}$)  
$\alpha$ alpha, the level of significance of a test; probability of a Type 1 error  
$\theta_g$ gravimetric water content of a soil (Mg m$^{-3}$)  
$\theta_v$ volumetric water content of a soil (m$^3$ m$^{-3}$)  
$\varphi$ total porosity of a soil (dimensionless)  
$\rho_b$ bulk density of a soil (Mg m$^{-3}$)  
$\rho_w$ density of water (Mg m$^{-3}$)  
$\tau$ time constant (minute)  
$\tau_{g_s}$ time constant for $g_s$ (minute)  
$\tau_A$ time constant for $A$ (minute)
ACKNOWLEDGMENTS

I am truly indebted to Dr. Nigel J. Livingston. He is a great supervisor and a good friend. His generosity, understanding, patience and his numerous advices have allowed me to pursue and complete this program. I would also like to thank him for providing me with financial support through his operating grant from Natural Sciences and Engineering Research Council of Canada. I am very grateful to the members of my graduate committee, Drs. Barbara J. Hawkins, Louis A. Hobson, K. Olaf Niemann, and Robert D. Guy for offering advice during this project, for reviewing this dissertation and for providing helpful criticism and comments. I wish to thank Greg Filek and Hugh Hinskins for introducing me to the world of electronics and instrumentation, William R. Hook for sharing with me his interest and knowledge of time domain reflectometry and Brad M. Binges for his assistance in the maintenance of plant growth facilities. Thanks are also due to my lab mates: Erin, Robin, Wendy, Edgar, Sun, Sonu, Dale, Rob, Gilbert and Tracy, and to colleagues of the Centre for Forest Biology for creating such a dynamic and friendly research environment. And most importantly, special thanks to Suzanne for her support and understanding.
General introduction

Approximately one million hectares of forest are harvested annually in Canada (Anonymous 1996). A significant proportion of the harvested areas is left to regenerate naturally and the remaining areas are restocked by planting (≈45%), direct seeding (≈3%) or scarification (≈1.5%). The reforestation of ‘understocked’ forest areas and recently harvested sites requires more than 600 million seedlings each year.

Reforestation success has increased significantly over the past decades, mainly because of improvements in stock quality, planting techniques and site preparation (Anonymous 1991). Nevertheless, seedling establishment and stand productivity are often limited by a range of environmental stresses. A better understanding of the physiological processes that determine seedling responses to such stresses is central to the successful development of breeding programs for the production of high performance planting stock. Such information is also crucial for the development of mechanistic models of plant performance.

Stomatal regulation provides the mechanism by which plants control fluxes of carbon and water between their leaves and the atmosphere. A good knowledge of stomatal behavior and its influence on leaf gas-exchange is needed to predict carbon assimilation and water use by whole trees, or to model transfer processes in forest canopies (Herbst 1995). It is, therefore, hardly surprising that considerable research has been focused on characterizing the behavior and functioning of stomata. Studies on the dynamics of stomatal responses to individual environmental factors have explored several interesting approaches, ranging from experiments conducted in controlled laboratory conditions, to measurements performed in natural systems. Empirical models, rather than mechanistic models, have also been used to examine the interactions between seedlings and the aerial environment (Jones 1992). These models are generally successful in describing and predicting the overall variation in stomatal conductance (Jarvis 1976;
Livingston & Black 1987; Jones 1992). However, they have a limited scope for generalized application, as they lack a good understanding of the underlying response mechanisms. In many cases, empirical models eventually become 'semi-mechanistic' as more physiological knowledge is incorporated into them. Models that couple stomatal conductance to the rate of photosynthesis at the leaf scale provide a good example of this (Ball, Woodrow & Berry 1987; Lloyd 1991; Leuning 1995).

This dissertation investigates some of the factors that control whole-plant gas-exchange in conifer seedlings. A quantitative approach is proposed to further improve our understanding of the processes regulating stomatal conductance \( g_s \) and photosynthesis rate \( A \). In particular, the photosynthetic and stomatal responses of whole seedlings to fluctuations in photon flux density are quantified in terms of time constant (a measure of response time). Additionally, the short-term responses of whole-plant transpiration and photosynthesis to transient and reversible changes in illuminated foliage area are examined in detail. The results of these studies are presented in the two chapters of this dissertation, which has been written in a journal publication format.

In Chapter 1, the effects of temperature and varying the length of dark periods on the time constant for net photosynthesis rate and stomatal conductance \( \tau_A \) and \( \tau_{g_s} \), the period over which 63% of the total change in \( A \) and \( g_s \), respectively, occurs) of Douglas fir, western hemlock and western redcedar seedlings are described. A phenomenological gas-exchange model, based on measured photosynthetic and stomatal responses of western redcedar to three environmental variables (photon flux density, air temperature and vapor pressure deficit), is developed. The model is then used to: (i) determine if the inclusion of functions that account for photosynthetic and stomatal dynamics would improve our estimates of daily carbon gain; and (ii) determine whether the selection of individuals with lower time constant would result in increased carbon assimilation in a range of environments.
In Chapter 2, the short-term responses of illuminated foliage to transient and reversible reductions in photosynthesizing foliage area, brought about by either shading a portion of a seedling or by reducing the ambient CO\textsubscript{2} concentration of the air surrounding part of a seedling, are described. Responses to changes in vapor pressure to manipulate whole-seedling transpiration rate ($E$), independently of photosynthesis, are reported. The changes in whole-seedling $E$ and in the distribution of sources and sinks of carbon within a seedling associated with a reduction in photosynthesizing foliage area are discussed in relation to homeostatic adjustment in plant water potential and photosynthesis. Finally, the mechanisms underlying these compensatory responses are discussed.

In Appendix A, the water retention curve for the sand used in the present study, and the responses of net photosynthesis rate and stomatal conductance to changes in photon flux density, air temperature and vapor pressure deficit for western redcedar seedlings are presented.

Appendix B describes the time domain reflectometry (TDR) technique used in this study to measure soil water content. In particular, the effects of temperature on the soil apparent dielectric constant ($K_a$) and the measurement errors in $K_a$ associated with variations in soil temperature are reported. The measured changes in $K_a$ with temperature are compared with those predicted by a composite dielectric mixing model. Finally, the temperature dependence of the dielectric constant of water is discussed in relation to the effects of soil matrix on $K_a$.

Appendix C is a previously published paper that describes the whole-plant cuvette system (originally developed by Dr. Livingston) used in this study. The effects of increased temperature and vapor pressure deficit on net photosynthesis rate and stomatal conductance of western redcedar and white spruce [Picea glauca (Moench) Voss] seedlings are presented.
Appendix D is a recently published paper that describes experiments conducted in New Zealand, in collaboration with Drs. David Whitehead, Frank Kelliher and Kevin Hogan, to determine whether the compensatory mechanisms that had been observed in our laboratory experiments would also occur in trees under natural conditions. Changes in transpiration flux density, tree conductance, stomatal conductance and photosynthesis of a *Pinus radiata* D. Don tree in response to transient reductions in illuminated foliage area (by shading) are presented. The physiological processes involved in these compensatory responses are also discussed.

REFERENCES


Chapter 1

Rates of stomatal opening in conifer seedlings in relation to air temperature and daily carbon gain

INTRODUCTION

Assimilation rates (A) of photo-induced leaves adjust almost instantaneously to fluctuations in photon flux density (Q) whereas changes in stomatal conductance (gs) occur more slowly. Stomatal responsiveness to varying Q differs among species (Knapp & Smith 1989) and is influenced by a number of factors that include plant water status (Davies & Kozlowski 1975; Knapp & Smith 1989), plant nutrition (Davies & Kozlowski 1974), air temperature (Ng & Jarvis 1980; Whitehead & Teskey 1995) and light conditions (Woods & Turner 1971; Tinoco-Ojangeren & Pearcy 1993a,b).

In broad-leafed tree species, the response time of stomata (that is, the period over which stomata are in transition from one steady-state level to another) to rapid changes in irradiance typically ranges from 3–45 min (Woods & Turner 1971; Davies & Kozlowski 1975). In conifers, stomatal responses to fluctuating irradiance are generally slower. Knapp & Smith (1989) reported that in two alpine conifers (Pinus flexilis James and Pinus contorta Dougl.) gs was relatively unresponsive to alternating periods of full sunlight (Q > 1800 μmol m⁻² s⁻¹ for 8 min) and shade (Q = 400–450 μmol m⁻² s⁻¹ for 5 min). Livingston (1994) reported that in western redcedar (Thuja plicata Donn.) seedlings, once stomata were fully open, there were no significant changes in whole-plant transpiration rate (E) and gs in response to rapid and continuous fluctuations in Q (200 to 1100 μmol m⁻² s⁻¹ over 3–5 min).

In some circumstances, complete stomatal adjustment to illumination (after a period in the dark) can take over 4 h (Watts & Neilson 1978; Ng & Jarvis 1980). However, responses are usually faster. For example, the stomatal time constant (τgs, the period over
which 63% of the total change in \(g_s\) occurs), for foliage in a *Pinus radiata* D. Don tree that had been shaded for 36 h, was only 30–35 min (Whitehead et al. 1996). Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings achieved two-thirds of their stomatal response to illumination in 40 min (Watts & Neilson 1978).

Generally, stomata take longer to open than to close in response to a change in illumination (Watts & Neilson 1978; Whitehead & Teskey 1995). Further, the rate of opening is slower after overnight darkness than after a shorter period of darkness imposed during the day (Whitehead & Teskey 1995).

It has been suggested that the distribution of species among contrasting habitats might be related to the optimization of stomatal behavior in order to limit water expenditure for a given carbon gain (DeLucia 1987; Fites & Teskey 1988; Tinoco-Ojanguren & Pearcy 1993a,b). For example, it might be advantageous for trees growing in dry environments (i.e. interior provenances) to open their stomata rapidly in the morning to maximize their productivity and water use efficiency (*WUE*) by confining the bulk of their carbon assimilation to periods of relatively low \(T_{air}\) and vapor pressure deficit (*D*). It is not uncommon for seedlings growing in an understory to experience long periods of very low \(Q\) interceded by episodes (typically ranging from a few minutes to more than 1 h) of intense sunlight (Young & Smith 1979; Knapp & Smith 1988; Pfitsch & Pearcy 1989; Chen & Klinka 1997). Seedlings that rapidly open their stomata, in response to increased \(Q\), would likely minimize stomatal limitation to photosynthesis. Additionally, in the early morning, they could take full advantage of high \(CO_2\) concentrations before turbulent eddies introduce air from above the canopy at lower \(CO_2\) concentrations (Jarvis, James & Landsberg 1976). In contrast, rapid stomatal opening should be less important for carbon gain in wet (i.e. coastal provenances) and unshaded environments.

The purpose of this study was to quantify, in terms of time constants, the stomatal and photosynthetic dynamics of conifer seedlings represented by three species [Douglas-
fir (Pseudotsuga menziesii (Mirb.) Franco), western hemlock (Tsuga heterophylla (Raf.) Sarg.) and western redcedar (Thuja plicata Donn.) that have a broad distribution within British Columbia. Specific objectives were: (i) to establish the relation between air temperature and $T$ for both $A$ and $g_s$; (ii) to determine the effects of varying the length of dark periods on $\tau$; (iii) to determine whether the incorporation, within phenomenological gas exchange models, of functions that account for the photosynthetic and stomatal responses to changing irradiance would improve our ability to predict daily carbon gain; and (iv) to use such models to determine whether the selection of individuals with lower $\tau$ would result in increased carbon uptake in a range of environments.

**Materials and methods**

**Plant material and growing conditions**

One-year-old Douglas-fir of interior and coastal provenances, western redcedar from dry and wet habitats, and western hemlock seedlings were used in this study. Nursery raised seedlings were transplanted to 7 dm$^3$ plastic containers filled with fine sand and kept outdoors from mid-April to mid-November in field facilities at the University of Victoria. Seedlings were watered once or twice a week. A commercial (20:20:20) N:P:K fertilizer was applied bi-weekly.

**Measurements**

*Gas exchange*

Whole-seedling transpiration and assimilation rates were monitored continuously with a closed gas exchange system as described by Livingston *et al.* (1994). In this system, measurements of chamber CO$_2$ concentration ($c_a$) and water vapor pressure ($e_a$) are made using a dual detector infrared gas analyzer (LI-6262, Li-Cor Inc., Lincoln, NE, USA). Net assimilation rates are determined by integrating the output (recorded as 1 min run-
ning averages) of a mass flow controller (Tylan Corp., Carson, CA, USA) used to inject CO₂ into the chamber to balance that taken up by the seedling. Vapor pressure is controlled by circulating chamber air through a column of CaSO₄ when eₐ exceeds a given set point. The desiccant column is supported on a balance with 1 mg resolution. Transpiration rate is determined as the change in desiccant mass over time (typically 60 s). Instantaneous WUE is calculated as A/E. Conductance to water vapor is calculated as E/(L x D) where L is the seedling projected leaf area measured using a leaf area meter (LI-3100, Li-Cor Inc.). Intercellular CO₂ concentration (cᵢ) is calculated as described by Field, Ball & Berry (1991). Because values of E, gₛ and cᵢ are derived from measurements made at fixed intervals, they are plotted as discrete points. The flow rate in the chamber is approximately 0.025 m³ s⁻¹, giving rise to very high boundary layer conductances (≥ 2 mol m⁻² s⁻¹ for seedlings with L ≤ 0.06 m²; Livingston et al. 1994) relative to stomatal conductances (typically 0–0.2 mol m⁻² s⁻¹). In separate experiments, it was determined that leaf temperatures (Tₑₐ₉) were within 0.1 °C of air temperatures (both measured with fine wire thermocouples) and for the present study, it was assumed that Tₑₐ₉ = Tₐir.

Air temperature in the chamber is controlled to better than ± 0.1 °C over 0 to 35 °C. There are slight (< 0.25 °C) temperature excursions from the set point when the light source is switched on or off to provide an abrupt change in Q but these are corrected within 20 min of the change (Livingston et al. 1994).

*Photon flux density*

The light control system described by Livingston (1994) was used to provide constant Q (typically 1000 ± 5 μmol m⁻² s⁻¹) at seedling height over a 10–12 h photoperiod. Rapid (=10 μmol m⁻² s⁻²) or slower changes in Q are brought about by varying the amount of dyed liquid in a tank placed between the light source and the cuvette. This system was also used to accurately simulate diurnal changes in Q.
**Soil water content**

Soil water content ($\theta$) was measured using time domain reflectometry and probes with remotely shorted diodes as described by Hook et al. (1992). Transpired water was replaced every 2 or 3 days and soil water, unless stated otherwise, was generally maintained between 0.06–0.07 m$^3$ m$^{-3}$ (see Appendix A, Fig. A.1).

**Experimental protocol**

*Effects of air temperature on stomatal opening*

Prior to treatment, seedlings were acclimatized in the cuvette system for three to four days, and $A$ and $E$ measured continuously. During this period, $T_{\text{air}}$, $D$ and $c_a$ were maintained at $20 \pm 0.05 \, ^\circ\text{C}$, $1 \pm 0.02 \, \text{kPa}$ and $350 \pm 2 \, \mu\text{mol mol}^{-1}$, respectively.

Responses of whole-seedling $A$ and $g_s$ to a step change in $Q$ (from overnight darkness to 1000 $\mu\text{mol m}^{-2} \, \text{s}^{-1}$) were described as a function of time ($t$) using first-order exponential equations:

\[
A = A_{\text{max}} \left[ 1 - e^{\left(-\frac{t}{\tau_A}\right)} \right] \quad (1.1)
\]

\[
g_s = g_{s\text{ max}} \left[ 1 - e^{\left(-\frac{t}{\tau_{g_s}}\right)} \right] \quad (1.2)
\]

where $A_{\text{max}}$ and $g_{s\text{ max}}$ are the steady-state assimilation rate and stomatal conductance, respectively, measured over one hour, 2–3 h after the lights were switched on; $\tau_A$ and $\tau_{g_s}$ are the time constant for photosynthetic and stomatal responses, respectively. Estimates of $\tau$ were obtained with a non-linear least squares routine using the Levenberg-Marquardt algorithm (KaleidaGraph, Abelbeck Software, Reading, PA, USA). The time taken for a 90% change is calculated as $2.3 \, \tau$. 
Experiments were carried out on three western redcedar seedlings to determine measurement repeatability. Each seedling was held at a given air temperature for at least 20 h for the determination of $\tau_a$ and $\tau_{gs}$. Time constants were determined over a 5–25 °C range imposed at random. Any changes in $T_{air}$ were imposed at a maximum rate of 0.33 °C min$^{-1}$ and were completed at least 8–12 h before lights were switched on. At each temperature, measurements were repeated (once per day) three to nine times per seedling. For $T_{air} \geq 10$ °C, $D$ was held at 1.0 kPa and for $T_{air} < 10$ °C, $D$ was 0.6 kPa.

A second set of experiments was carried out to determine intra- and inter-species variation in $\tau_a$ and $\tau_{gs}$. Measurements were made on at least three seedlings per species and provenance. Time constants were measured at $T_{air}$ of 5, 15 and 25 °C selected at random. In all cases, $D$ was 0.6 kPa.

**Effects of length of dark periods on stomatal opening**

Experiments were conducted on western redcedar seedlings to establish the relation between the length of the dark period and $\tau_a$ and $\tau_{gs}$ in response to illumination. Time constants were determined after overnight darkness (12–14 h) and after 1–2 h periods of darkness imposed at different times during the day. Measurements were carried out at $T_{air}$ of 10, 15, and 20 °C (imposed at random) with $D$ at 1.0 kPa. At each temperature, measurements were repeated at least three times per seedling.

**Phenomenological model**

Simple multiplicative models (Jarvis 1976; Livingston & Black 1987; Jones 1992) were used to predict $A$ and $g_s$ of western redcedar seedlings as a function of $Q$, $T_{air}$, $D$ and $\tau$, whereby:

$$A = A_{\max} f(Q) g(T_{air}) h(D) j(\tau_a)$$  \hspace{1cm} (1.3)
where \( f, g \) and \( h \) are functions that describe the relation between steady-state \( A \) and \( g_s \) and \( Q, T_{\text{air}} \) and \( D \), respectively; and \( j \) describes the relation between \( A \) and \( g_s \) and time during the transition dynamics (Eqns 1.1 and 1.2). For simplicity, all variables were assumed to act independently but multiplicatively. When determining the individual functions \( f, g \) and \( h \) in Eqns 1.3 and 1.4, attempts were made to use general forms of relationships already established in the literature. Daily carbon assimilation and transpiration were calculated by integrating \( A \) and \( E \) over the corresponding photoperiod.

Estimation of the parameters for the model

Functions \( f, g \) and \( h \) used in Eqns 1.3 and 1.4 were determined for three seedlings. For each seedling, the photosynthetic and stomatal responses to the following sequence of treatments were determined over three successive days: (i) \( Q \) was progressively decreased from 1200 to 0 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) over 10 h while \( T_{\text{air}} \) was held at 25 °C and \( D = 0.75 \) kPa; (ii) \( T_{\text{air}} \) was reduced from 25 to 5 °C by 5 °C every 2 h, while \( Q \) and \( D \) were held at 1200 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) and 0.75 kPa, respectively; (iii) \( D \) was increased by 0.5 kPa every 2 h from 0.5 to 3.0 kPa with \( Q = 1200 \mu \text{mol m}^{-2} \text{s}^{-1} \) and \( T_{\text{air}} = 25 \) °C. All of the above treatments were imposed 1–2 h after the lights were switched on, when \( A \) and \( g_s \) had reached steady-state. In further experiments, seedlings were subjected to overnight changes in air temperature to determine the relation between \( T_{\text{air}} \) and respiration in the dark.

Comparison between model and measurements for dynamic changes in \( Q \)

The seedlings used to derive the coefficients in Eqns 1.3 and 1.4 were subjected to diurnal fluctuations of \( Q (\mu \text{mol m}^{-2} \text{s}^{-1}) \) and \( T_{\text{air}} \) described by the following:

\[
Q = 1200 \times [\sin 0.2618 (t - 7)] \quad \text{for} \ 7 > t < 19
\]
\[ T_{\text{air}} = 15 + [10 \times \sin 0.2618 (t - 10)] \]  

where \( t \) is the time of day in h. These conditions approximate those found in coastal southern British Columbia in mid-spring. In separate experiments, the vapor pressure deficit was either held at 0.75 kPa throughout the day or was varied between 0.4 and 2.7 kPa as a function of temperature. In both cases, the 12 h days were assumed to be cloudless. In additional experiments, seedlings were exposed to variations in \( Q \) that simulated cloudy conditions and under-canopy shading. Intermittent cloud cover and understory shading were approximated by exposing seedlings to 3–4 episodes (≥ 1 h) of shade (200 or 500 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) or complete darkness during the day.

Measurements of \( A \) and \( g_s \) were compared to estimates obtained using Eqns 1.3 and 1.4, respectively, with and without (Model 1 and Model 2, respectively) time constant functions.

**Results**

**Response of stomatal conductance and assimilation rate to changes in \( Q \)**

To facilitate comparison of the photosynthetic and stomatal responses of different conifer seedlings to a step change in \( Q \) at given air temperatures, \( A \) and \( g_s \) were normalized with respect to \( A_{\text{max}} \) and \( g_{s \text{max}} \), and time constants (\( \tau_A \) and \( \tau_{g_s} \)) were determined using Eqns 1.1 and 1.2, respectively. Coefficients of determination (\( R^2 \)) of the fits to Eqns 1.1 and 1.2 were typically > 0.90 and never less than 0.80. Standard errors of the estimates (SEE) of \( \tau_A \) and \( \tau_{g_s} \) were generally < 5%.

Between 5 and 25 °C, \( A \) and \( g_s \) generally increased with increasing temperature (Fig. 1.1). Typically, differences in \( A \) and \( g_s \) among species were largest at the lowest \( T_{\text{air}} \).

Time constants for \( A \) and \( g_s \), following a step change in \( Q \), increased linearly with decreasing air temperature (Fig. 1.2). In western redcedar seedlings, \( \tau_A \) increased three fold
(from 10 min) when $T_{air}$ was decreased from 25 to 5 °C. In general, values for $\tau_{gs}$ were slightly larger (10–15%) than those for $\tau_A$. Within a given species, there was considerable variability in $\tau$ between individual seedlings, particularly at low temperatures. For example at $T_{air}$ of 5 °C, $\tau_A$ in western hemlock seedlings ($n = 4$) ranged from 35 to 61 min. A similar variation was found in loblolly pine [$\tau_{gs}$ ranged from 36 to 73 min ($n = 6$); D. Whitehead (Landcare Research, Lincoln, NZ), personal communication]. Coefficients of variation for all species varied between 6 and 28%. However, for a given individual, variation in $\tau$ was generally small. For example, repeated measurements (one per day over 7 d) made on a western redcedar seedling at 10 °C yielded a mean $\tau_A$ of 28.7 min with a standard deviation of 2.0 min.

Differences in $\tau_A$ among species were least pronounced at 25 °C but increased significantly with decreasing $T_{air}$ (Fig. 1.2). Below 25 °C, western hemlock had the largest time constants followed by western redcedar and Douglas-fir. There was not a statistically significant difference in $\tau$ between coastal and interior provenances of Douglas-fir. While there were some individuals from wet habitats (e.g. redcedar from Jervis inlet; Fig. 1.2b) that had larger $\tau$ than those from dry habitats (Duncan), in general, for a given species, there was not a consistent relationship between habitat type and time constant. For example, $\tau_A$ for western redcedar seedlings from the Sunshine Coast (Fig. 1.2a), a relatively wet habitat, was similar to that of seedlings from the relatively dry Duncan habitat (Fig. 1.2b).

Time constants for increases in $A$, after periods of darkness that ranged from 1–2 h and were imposed at different times during a 12 h photoperiod, were generally lower than those measured after overnight darkness (Table 1.1). Even though these differences were not statistically significant ($F$-test: $P > 0.44$), the lower time constants were used to model photosynthetic and stomatal responses to short periods of darkness imposed during the day.
Figure 1.1 Time course of (a) whole-seedling assimilation rate (A) and (b) stomatal conductance to water vapor (g\textsubscript{s}) in western hemlock in response to a step change (at time = 0) in photon flux density from 0 to 1000 \(\mu\text{mol m}^{-2}\text{s}^{-1}\). Measurements were conducted over three successive days at 25, 5 and 15 °C.
Figure 1.2 Relation between time constant ($\tau_A$) for increases in assimilation rate ($A$), following a step change in photon flux density from 0 to 1000 $\mu$mol m$^{-2}$ s$^{-1}$, and air temperature ($T_{air}$, °C) for (a) three conifer species: each data point represents the average value (± SD) of at least three seedlings. Douglas-fir ($n = 6$), $\tau_A = 21.4 - 0.45T_{air}$, $R^2 = 0.99$; Western redcedar ($n = 3$), $\tau_A = 34.2 - 0.967T_{air}$, $R^2 = 0.99$; Western hemlock ($n = 4$), $\tau_A = 52.5 - 1.697T_{air}$, $R^2 = 0.97$; (b) three redcedar seedlings: one from Jervis inlet (relatively wet habitat) and two from Duncan (relatively dry habitat). Each data point represents the mean value (± SD) of three to nine measurements at each $T_{air}$ for each seedling. Soil water contents during measurements were 0.026–0.053 m$^3$ m$^{-3}$ for the Jervis inlet provenance and 0.064–0.105 m$^3$ m$^{-3}$ for the Duncan provenance. Jervis inlet: $\tau_A = 90.8 - 2.81T_{air}$, $R^2 = 0.81$; Duncan: $\tau_A = 35.8 - 1.01T_{air}$, $R^2 = 0.96$. 
Table 1.1 Time constants (mean ± SD) for increases in assimilation rates ($A$) of western redcedar seedlings following a step change in photon flux density (0 to 1000 μmol m$^{-2}$ s$^{-1}$) at three different air temperatures following (i) overnight darkness and (ii) 1–2 h dark periods imposed during the day. Numbers of measurements are shown in parentheses.

<table>
<thead>
<tr>
<th>Length of the dark period (h)</th>
<th>10 °C</th>
<th>15 °C</th>
<th>20 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–2</td>
<td>20.3 ± 4.3 (17)</td>
<td>14.9 ± 1.5 (7)</td>
<td>10.1 ± 1.7 (16)</td>
</tr>
<tr>
<td>12–14</td>
<td>24.5 ± 4.5 (13)</td>
<td>18.7 ± 3.2 (6)</td>
<td>13.4 ± 2.0 (11)</td>
</tr>
</tbody>
</table>
Phenomenological model and daily carbon gain

Following Livingston & Black (1987) and Jones (1992), photosynthetic and stomatal responses to changes in $Q$, $T_{\text{air}}$, and $D$ were best described by the following functions:

\[ f(Q) = 1 - e^{-a(Q - Q')} \]  
\[ g(T_{\text{air}}) = 1 - b(T_{\text{air}} - T_{\text{max}})^2 \]  
\[ h(D) = \frac{1}{1 + (D/D_{0.5})^c} \]

where $Q'$ is the light compensation point; $T_{\text{max}}$ is the temperature at which $A$ and $g_s$ are at a maximum; $D_{0.5}$ is the vapor pressure deficit when $A$ and $g_s$ are half the maximum, and $a$, $b$ and $c$ are constants (see Appendix A, Figs A2–A4). Values of the coefficients $a$, $b$, $c$, $Q'$, $T_{\text{max}}$ and $D_{0.5}$ used in Eqns 1.7–1.9 are given in Table 1.2. Coefficients of determination for the relationship between measured and modelled $A$ or $g_s$ using a function of any single variable ranged from 0.86 to 0.99 (Table 1.2).

Time courses of $A$, $E$, $g_s$ and WUE in response to diurnal changes (assuming a cloudless day) in $Q$, $T_{\text{air}}$ (Eqns 1.5 & 1.6, respectively), $e_a$ and $D$ (Figs 1.3a–b) were very well described by the phenomenological models regardless of whether or not a function that accounted for $T$ was included (Figs 1.3c–f). For example, there was an overall difference of 2.2% ($R^2 = 0.99$, $\chi^2 = 17$, Table 1.3) and 1.4% ($R^2 = 0.99$, $\chi^2 = 10$) between modelled and measured daily $A$ for Model 1 ($T$ function included) and Model 2 ($T$ function excluded), respectively (Fig. 1.3c). Generally, $g_s$ and $E$ were slightly overestimated for the first 2–3 h after lights were switched on, and at the end of the photoperiod (Figs 1.3d–e). Typically, there was about a 2% difference between modelled (including $T$) and measured daily $E$ when $D$ was held constant at 0.75 kPa (data not shown) and a 5% difference when $D$ was allowed to vary with $T_{\text{air}}$ (Fig. 1.3d).
Table 1.2 Values of the coefficients (± SEE) in Eqns 1.7–1.9 used to predict whole-plant assimilation rate ($A$, $\mu$mol m$^{-2}$ s$^{-1}$) and stomatal conductance ($g_s$, mmol m$^{-2}$ s$^{-1}$) of western redcedar seedlings. $Q$ is the photon flux density ($\mu$mol m$^{-2}$ s$^{-1}$); $T_{\text{air}}$ is the air temperature (°C); $D$ is the vapor pressure deficit (kPa); $Q'$ is the light compensation point ($\mu$mol m$^{-2}$ s$^{-1}$), $T_{\text{max}}$ is the temperature (°C) at which $A$ and $g_s$ are at a maximum; and $D_{0.5}$ is the vapor pressure deficit (kPa) when $A$ and $g_s$ are half the maximum. Also shown are the coefficients of determination ($R^2$) of the fits to Eqns 1.7–1.9.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Function</th>
<th>Coefficients</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$</td>
<td>$f(Q)$</td>
<td>$a = 0.0056$ (± 0.0003) $Q' = 19.2$ (± 3.1)</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$b = 0.0023$ (± 0.0003) $T_{\text{max}} = 21.2$ (± 0.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$c = 2.2$ (± 0.4) $D_{0.5} = 3.6$ (± 0.3)</td>
<td>0.89</td>
</tr>
<tr>
<td>$g_s$</td>
<td>$f(Q)$</td>
<td>$a = 0.0043$ (± 0.0003) $Q' = 100$</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$b = 0.0012$ (± 0.0004) $T_{\text{max}} = 25.3$ (± 3.3)</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$c = 2.4$ (± 0.4) $D_{0.5} = 1.5$ (± 0.1)</td>
<td>0.91</td>
</tr>
</tbody>
</table>
Figure 1.3 (a–c) Time course of (a) air temperature ($T_{\text{air}}$) and photon flux density ($Q$), (b) vapor pressure ($e_a$) and vapor pressure deficit ($D$). (c) Typical daily course of measured and modelled assimilation rate ($A$) of a western redcedar seedling. Model 1 (Eqn 1.3) includes a time constant function ($\tau_a$; Eqn 1.1) that is omitted from Model 2. The differences between measured and modelled values are shown in (c).
Figure 1.3 (d–f) Typical daily course of measured and modelled (d) transpiration rate ($E$), (e) stomatal conductance ($g_s$) and (f) water use efficiency ($WUE$) of a western redcedar seedling. Model 1 (Eqn 1.3) includes a time constant function ($\tau_A$; Eqn 1.1) that is omitted from Model 2. The differences between measured and modelled values are shown in (e).
When periods of relatively high $Q$ (1000 $\mu$mol m$^{-2}$ s$^{-1}$) were interdispersed with episodes of complete darkness, and $T_{air}$ and $D$ kept constant, Model 1 predicted $A$ successfully (Fig 1.4). There was a 0.4% difference between modelled and measured daily $A$ when $T_{air} = 10 \, ^{\circ}C$ ($R^2 = 0.99$, $\chi^2 = 42$, Table 1.3) and only a 0.2% difference when $T_{air} = 20 \, ^{\circ}C$ ($R^2 = 0.99$, $\chi^2 = 51$). In contrast, Model 2 overestimated changes in $A$ with $Q$, leading to a difference of 21.4% and 13.0% between modelled and measured daily $A$ for $T_{air}$ of 10 °C and 20 °C, respectively (Table 1.3). In cases where periods of high $Q$ (1000–1500 $\mu$mol m$^{-2}$ s$^{-1}$) alternated with periods of shade (200–500 $\mu$mol m$^{-2}$ s$^{-1}$), and $T_{air}$ and $D$ were kept constant, fluctuations in $A$ with $Q$ were well described by both models (Fig. 1.5). However, the inclusion of a function for $\tau$ led to slightly better estimates of daily $A$ (Table 1.3). The difference between modelled (including $\tau$) and measured daily $A$ was 2.3% when $T_{air} = 15 \, ^{\circ}C$ (Fig. 1.5a) and 0.6% when $T_{air} = 25 \, ^{\circ}C$ (Fig. 1.5b; Table 1.3).

In all cases where there was one or more periods of shade or total darkness during the day, the inclusion of a time constant function in Eqn 1.3 led to higher $R^2$ and lower $\chi^2$ values. This was particularly significant at low $T_{air}$.

**Discussion**

Step changes in $Q$, following prolonged periods of darkness, bring about increases in $A$ that result from changes in both stomatal conductance and photosynthetic induction state (Kirschbaum & Pearcy 1988a,b; Tinoco-Ojanguren & Pearcy 1993b; Pearcy et al. 1994). There is evidence that upon illumination with relatively high $Q$, enzyme activation (including that of Rubisco) and replenishment of metabolite pools are usually complete within 10 min (Pearcy et al. 1994). Further, studies of the regulation of Rubisco activity by light indicate that biochemical limitations of $A$ during induction do not differ among species (Seemann et al. 1988; Woodrow & Mott 1989; Tinoco-Ojanguren & Pearcy 1993b). In this study, I did not specifically examine the relative contributions of stomatal
Figure 1.4 Daily course of measured and modelled assimilation rate ($A$) of a western redcedar seedling subjected to step changes in photon flux density ($Q$) between 0 and 1000 $\mu$mol m$^{-2}$ s$^{-1}$ and held at an air temperature of (a) 10 °C and (b) 20 °C. The vapor pressure deficit was 1.0 kPa. Model 1 (Eqn 1.3) includes a time constant function ($\tau_A$; Eqn 1.1) that is omitted from Model 2. The solid bars indicate the periods during which darkness was imposed. The arrows indicate when the lights were switched on in the morning and off at night.
**Table 1.3** Values of coefficients (± SEE) in the linear regression Eqn: $A_{\text{model}} = a + b A_{\text{meas}}$ for western redcedar seedlings exposed to periods of high photon flux density ($Q$) interrupted by periods of darkness or shade, or exposed to diurnal changes in $Q$ without interruption. Air temperatures ($T_{\text{air}}$) were held at 10, 15, 20, and 25 °C, or varied diurnally. The vapor pressure deficit was 1.0 kPa when $T_{\text{air}}$ was held constant, or varied diurnally as a function of $T_{\text{air}}$. Model 1 (Eqn 1.3) includes a time constant function ($\tau_A$; Eqn 1.1) that is omitted from Model 2. Also shown are the coefficients of determination ($R^2$) and the chi-square ($\chi^2$) values of the regressions, and the differences (%) between daily $A_{\text{model}}$ and daily $A_{\text{meas}}$.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Model</th>
<th>$a$</th>
<th>$b$</th>
<th>$R^2$</th>
<th>$\chi^2$</th>
<th>*Diff (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diurnal changes in $Q$ and $T_{\text{air}}$</td>
<td>1 ($\tau_A$)</td>
<td>$-0.12$ (± 0.04)</td>
<td>$1.02$ (± 0.01)</td>
<td>0.99</td>
<td>17</td>
<td>-2.2</td>
</tr>
<tr>
<td></td>
<td>2 (no $\tau_A$)</td>
<td>$-0.08$ (± 0.03)</td>
<td>$1.02$ (± 0.01)</td>
<td>0.99</td>
<td>10</td>
<td>-1.4</td>
</tr>
<tr>
<td>Dark periods ($T_{\text{air}} = 10$ °C)</td>
<td>1</td>
<td>$0.03$ (± 0.02)</td>
<td>$0.99$ (± 0.01)</td>
<td>0.99</td>
<td>42</td>
<td>+0.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$0.49$ (± 0.08)</td>
<td>$1.03$ (± 0.02)</td>
<td>0.87</td>
<td>735</td>
<td>+21.4</td>
</tr>
<tr>
<td>Dark periods ($T_{\text{air}} = 20$ °C)</td>
<td>1</td>
<td>$-0.05$ (± 0.02)</td>
<td>$1.02$ (± 0.01)</td>
<td>0.99</td>
<td>51</td>
<td>-0.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$0.19$ (± 0.06)</td>
<td>$1.05$ (± 0.01)</td>
<td>0.93</td>
<td>462</td>
<td>+13.0</td>
</tr>
<tr>
<td>Shade periods ($T_{\text{air}} = 15$ °C)</td>
<td>1</td>
<td>$-0.01$ (± 0.02)</td>
<td>$0.98$ (± 0.01)</td>
<td>0.99</td>
<td>45</td>
<td>-2.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$0.16$ (± 0.06)</td>
<td>$1.00$ (± 0.01)</td>
<td>0.93</td>
<td>295</td>
<td>+3.6</td>
</tr>
<tr>
<td>Shade periods ($T_{\text{air}} = 25$ °C)</td>
<td>1</td>
<td>$0.16$ (± 0.03)</td>
<td>$0.98$ (± 0.01)</td>
<td>0.99</td>
<td>37</td>
<td>+0.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$0.25$ (± 0.04)</td>
<td>$0.98$ (± 0.01)</td>
<td>0.98</td>
<td>111</td>
<td>+2.3</td>
</tr>
</tbody>
</table>

$\chi^2$ approaches 0 when deviations from fit are small.

* Diff (%) = [(Model – Measured)/Measured] × 100
Figure 1.5 Daily course of measured and modelled assimilation rate ($A$) of a western redcedar seedling subjected to alternating periods of high photon flux density ($Q = 1000$ or $1500 \, \mu$mol m$^{-2}$ s$^{-1}$) and shade ($200$ or $500 \, \mu$mol m$^{-2}$ s$^{-1}$) and held at an air temperature of (a) $15 \, ^{\circ}$C and (b) $25 \, ^{\circ}$C. The vapor pressure deficit was $1.0 \, $kPa. Model 1 (Eqn 1.3) includes a time constant function ($\tau_A$; Eqn 1.1) that is omitted from Model 2. The solid and hatched bars indicate the periods during which darkness and shade, respectively, were imposed. The numbers represent $Q$ for each period of illumination. The arrows indicate when the lights were switched on in the morning and off at night.
and biochemical limitations during the various phases of induction. However, the results suggest that any differences in photosynthetic response among species and individuals that persisted for at least 10 min after a step change in $Q$, must have been related primarily to differences in their stomatal response.

The linear increases in $\tau_A$ and $\tau_{gs}$ with decreasing $T_{air}$ were almost certainly directly related to the effects of temperature on enzyme activity. Typically, the rates of reactions catalyzed by enzymes increase exponentially with increasing temperature until denaturation rapidly reduces the activity of the enzymes (Taiz & Zeiger 1991). However, for the range of $T_{air}$ used in this study, increases in reaction rates are generally linear.

There is some evidence that in woody angiosperms, stomata open more rapidly in shade-tolerant than in shade-intolerant species (Woods & Turner 1971; Davies & Kozlowski 1974). However, Pereira & Kozlowski (1977) did not find a consistent relation between $\tau$ and shade tolerance. Western redcedar and western hemlock are both considered to be very tolerant to shade, whereas Douglas-fir is regarded as having intermediate shade tolerance (Kramer & Kozlowski 1979). Based on this tolerance rating, the results suggest that shade-tolerant seedlings have relatively large $\tau$ for morning stomatal opening. However, the values of $\tau_{gs}$ for western hemlock seedlings are similar to those reported for loblolly pine seedlings (Whitehead & Teskey 1995), a species considered to be shade intolerant (Kramer & Kozlowski 1979).

Time constants for $A$ upon illumination following dark periods of 1–2 h were about 25% lower than those measured after overnight darkness, at any given temperature. This is consistent with results obtained for loblolly pine (Whitehead & Teskey 1995), that revealed that as the length of shade periods was increased from 5 to 60 min, there was a corresponding and linear increase in $\tau_{gs}$ upon illumination. However, the loblolly pine data show that beyond 60 min ($\tau_{gs} = 46 \pm 3$ min), there were only small differences in $\tau_{gs}$ following shade or overnight darkness ($\tau_{gs} = 50 \pm 6$ min). These results suggest that in
conifer seedlings, complete stomatal adjustment and enzyme deactivation are generally attained within 1 h of shade or darkness.

In experiments (data not shown) during which conifer seedlings were exposed to sinusoidal variations in temperature but \( Q \) and \( D \) kept constant, late afternoon \( A \) was consistently higher than that measured at the same temperature in the early morning. These differences in \( A \) generally disappeared when sinusoidal changes in \( T_{\text{air}} \) were imposed 1–2 h after, rather than before illumination, that is after \( A \) and \( g_s \) had both reached steady-state values. This suggests that following illumination after prolonged darkness, stomata can limit \( \text{CO}_2 \) assimilation for extensive periods. However, the extent of stomatal limitation of \( A \) does vary among coniferous species (Teskey et al. 1986; Meinzer 1982a). These results are consistent with the observations that after a step change in \( Q \), \( \tau_x \) and \( \tau_{g_s} \) were very similar, giving rise to concomitant increases in \( A \) and \( g_s \).

In other experiments when sudden shade was imposed, there was a corresponding decrease in \( \text{CO}_2 \) assimilation, followed by a slower decrease as \( A \) was limited by the slower change in \( g_s \). This type of response has also been reported by Whitehead & Teskey (1995).

Further evidence of stomatal limitation of \( A \), after a rapid increase in \( Q \), was provided, using the analysis of Kirschbaum & Pearcy (1988a) and Barradas & Jones (1996), by plots of \( A \) as a function of intercellular \( \text{CO}_2 \) concentration \( (c_i) \). Initially, \( dA/dc_i \) was negative and points were below the steady-state \( A \) vs. \( c_i \) curve (Fig. 1.6). However, over the next 15 to 20 min, changes in \( c_i \) were small as \( dA/dc_i \) approached the steady-state, and thereafter \( dA/dc_i \) was positive and constant indicating that increases in \( A \) resulted from higher \( c_i \) brought about by increases in \( g_s \).

Gas exchange models that include functions that account for photosynthetic and stomatal dynamics (e.g. Kirschbaum, Gross & Pearcy 1988; Knapp 1992, 1993; Barradas & Jones 1996; Pearcy, Gross & He 1997) are often used to assess the carbon gain and
Figure 1.6 Assimilation rate ($A$) as a function of intercellular CO$_2$ concentration ($c_i$) following a step change in photon flux density from 0 to 1000 μmol m$^{-2}$ s$^{-1}$ (open symbols). The air temperature and vapor pressure deficit were 15 °C and 1 kPa, respectively. The numbers indicate the time (min) after illumination. Also shown is a steady-state $A/c_i$ curve derived from measurements made on two western redcedar seedlings (closed symbols, $R^2 = 0.98$).
water loss associated with responses to changes in irradiance. My analysis has shown that failure to account for dynamic responses of $A$ can lead to significant errors in estimates of daily $A$, particularly when periods of relatively high $Q$ are interdispersed with periods of darkness (Table 1.3). Also, further benefits are accrued by the inclusion of a time constant function determined specifically for transitions from shade to high $Q$ rather than from darkness to high $Q$. This only applies, however, when the dark period is long enough to deactivate the several enzymes involved in carboxylation and thereby influence $g_s$. There is evidence that the loss of induction and down-regulation of Rubisco have time constants of 15–20 min (Seemann et al. 1988; Woodrow & Mott 1989).

Under conditions when changes in $Q$ are slow, as in unshaded canopies on cloud free days, time constant functions need not be incorporated into gas-exchange models. Additionally, when periods of high $Q$ alternate with periods of shade ($\geq 200 \, \mu$mol m$^{-2}$ s$^{-1}$), the inclusion of a time constant function only slightly improves the estimates of daily $A$ (Table 1.3). This is because $A$ of photo-induced needles responds more rapidly to fluctuations in $Q$ than dark-adapted needles. Furthermore, the limitation of $A$ by stomata during subsequent illuminations is smaller in shaded than in dark-adapted needles.

Whitehead & Teskey (1995) reported that modelled daily $A$ and $E$ exceeded measured values by 5% and 7%, respectively, when the dynamic responses of stomata were not taken into account (i.e. they assumed instantaneous changes in $A$ and $g_s$ for a step change in $Q$). However, they concluded that, given the added complexity, it was not worthwhile to incorporate short-term stomatal dynamics into their models. Their measurements of gas exchange were performed at $T_{air}$ between 20 and 25 °C. My results suggest that differences between modelled and measured daily $A$ increase substantially at lower $T_{air}$ and, while dependent on the frequency and duration of the fluctuations in $Q$, warrant the use of time constant functions. This, of course, would also require more intensive measurements of $Q$. 
The very large differences in time constants (but high repeatability of measurements) for $A$ and $g_s$ among individuals within a given species suggest that dynamic responses to changing irradiance are under genetic control. To determine whether there is scope for a selection program for increased carbon assimilation based on differences in $\tau$, Eqn 1.3 was used to predict the photosynthetic responses, over 14 h days, of seedlings growing under three light regimes: sinusoidal changes in $Q$ (Eqn 1.5) were interrupted by (i) four 1 h episodes of complete darkness; (ii) four 1 h episodes of shade; or (iii) two 1 h episodes of darkness and two of shade (Fig. 1.7a). Figure 1.7b shows the effects of reduced $\tau$ on carbon gain (calculated over 24 h) for western redcedar seedlings. On days with four 1 h periods of shade, those individuals that had $\tau$ 40% lower than the mean would have 2% higher carbon gain. On days with four 1 h periods of complete darkness, the same seedlings would have 4.7% greater carbon gain. Assuming that these gains are translated into increased productivity and are compounded, over a growing season or the life of a tree (if differences in $\tau$ are maintained), the selection of individuals with low $\tau$ could translate into very large increases in yield. This would be particularly true for a shade-tolerant species grown beneath a canopy.
Figure 1.7 (a) Simulated time course of air temperature ($T_{air}$) and photon flux density ($Q$) for conditions when sinusoidal changes in $Q$ are interrupted by two 1 h episodes of total darkness and two 1 h episodes of shade (200 $\mu$mol m$^{-2}$ s$^{-1}$). The solid and hatched bars indicate the periods during which darkness and shade, respectively, were imposed. The vapor pressure deficit was varied as a function of $T_{air}$. (b) Increases in daily carbon gain predicted by Eqn 1.3 vs. the ratio of the time constant for assimilation rate ($\tau_a$) to the maximum time constant ($\tau_{a,\max} = 30.5 - 1.02T_{air}$; Table 1.1). Also shown are the predicted increases in carbon gains when sinusoidal changes in $Q$ are interrupted by four 1 h episodes of total darkness or shade.
REFERENCES


Chapter 2

Short-term responses of transpiration and photosynthesis to transient and reversible changes in photosynthesizing foliage area in western redcedar (*Thuja plicata* Donn.) seedlings

**Introduction**

Under natural conditions, the relative proportions of illuminated and shaded foliage in a tree or seedling vary throughout the day and season. As transpiration rates ($E$) of different parts of the crown respond to changes in illumination, changes in water potential occur and hydraulic signals are rapidly transmitted in the xylem throughout the plant (Malone 1993). It has been proposed that $g_s$ of illuminated foliage increases in response to hydraulic perturbations brought about by partial shading (Squire & Black 1981).

Meinzer & Grantz (1990) demonstrated that in sugarcane (*Saccharum* spp. hybrid) increases in leaf specific hydraulic conductance, brought about by partial defoliation or shading, resulted in rapid increases in $g_s$ in the remaining foliage. Similarly, $g_s$ declined rapidly when the hydraulic conductance of the xylem was reduced by partial excision of the roots. They proposed that stomatal conductance is regulated by the ratio of total hydraulic conductance to total transpiring foliage area to maintain a constant water potential. Such homeostatic adjustment over a broad range of plant size, age and environmental conditions may be mediated by the rate of delivery of root metabolites to the guard cells.

In preliminary experiments, carried out on well watered western redcedar seedlings enclosed in a whole-plant cuvette, I showed that when a portion of a seedling’s foliage was shaded (by interposing an opaque screen between an overhead light and the cuvette), the reductions in whole-plant photosynthesis ($A_t$) and transpiration rate ($E_t$) were proportionally less than the changes in the area of illuminated foliage (cf. Pepin & Livingston 1994). The effects were fully and rapidly reversible. Elucidation of the mechanism of the response was not possible from the results of this preliminary study. This is because the
effects could have resulted from: (i) rapid compensatory increases in \( g_s \) and photosynthesis rates \( (A) \) of the illuminated foliage that partially offset any reduction in \( g_s \), and thus \( A \) and \( E \), in the shaded foliage; (ii) the lack of complete stomatal closure in shaded foliage so that \( A \) was still positive; or (iii) a combination of (i) and (ii).

In related field experiments, short-term responses of \( A \) and \( E \), to transient changes in illuminated foliage area were investigated in a 7-year-old *Pinus radiata* D. Don tree (Whitehead *et al.* 1996). When the lower 78% of the crown was shaded, \( g_s \) and \( A \) in the upper (illuminated) foliage increased by up to 59 and 24%, respectively. When the shade was removed, these effects were reversed within 1 min. Shading induced only small changes in bulk needle water potential but because of the rapid nature of the responses, it was concluded that changes in \( g_s \) and \( A \) must have been triggered by hydraulic signals propagated along the hydraulic pathway. Compensatory responses of lower crown foliage to shading of the upper crown were much less pronounced. Measurements of maximum \( g_s \) and \( A \), leaf nitrogen concentration and stable carbon isotope abundance revealed that the physiological capacity of the foliage decreased with depth in the crown (Livingston *et al.* 1998). This suggests that the magnitude of compensatory responses, following partial shading, might be related to the physiological capacity of the illuminated foliage as well as to the changes in whole-tree transpiration.

Another consideration, when interpreting the short-term responses to shading, is that while such treatments change whole-plant \( E \), they also have a pronounced effect on total carbon fixation and the distribution of carbon sources and sinks within the plant. There are numerous reports of homeostatic adjustment in photosynthesis following a change in sink strength (Sasek, DeLucia & Strain 1985; Thomas & Strain 1991; Layne & Flore 1993). However, at least one day is required for these responses to develop (von Caemmerer & Farquhar 1984; Geiger & Servaites 1991).
I hypothesize that short-term compensatory changes in $g_s$ and $A$ in fully illuminated parts of a tree crown resulting from changes in the fraction of illuminated foliage area occur in response to hydraulic signals generated by a change in the water potential gradient. These signals might act on stomatal guard cells, causing a rapid change in $g_s$. However, a further explanation could be that the effects are, at least in part, attributable to changes in the distribution of sources and sinks of carbon within the tree. In this paper, I describe laboratory experiments with western redcedar seedlings to distinguish between these two possible explanations. Initially experiments were undertaken to establish the effects on $g_s$, and $A$ of shade applied to part of the seedling. Subsequently, using a dual-cuvette, it was possible to change independently the environmental conditions for two parts of a seedling, while continuing to measure $g_s$ and $A$ for both parts. In contrast to shading, reductions in photosynthesizing foliage area ($L_A$) were also achieved by reducing the ambient CO$_2$ concentration ($c_a$) of the air surrounding part of the seedling. Further, it was also possible to manipulate the vapor pressure ($e_a$) in each part so that $E$ and $A$ could be manipulated independently. Consistent with the hypothesis, I anticipated that short-term changes in $g_s$ and $A$ for untreated, fully-illuminated foliage should occur only when changes in $L_A$ are accompanied by concomitant changes in $E$. A reduction in photosynthesizing foliage area with $E$ for the whole seedling remaining constant, and thus no change in the water potential gradient, should not result in a change in $g_s$ and $A$ for the untreated part of the seedling.

**Materials and methods**

**Plant material and growing conditions**

One-year-old western redcedar seedlings were used in all experiments. Nursery raised seedlings were transplanted to 3 dm$^3$ containers filled with sand and grown outdoors from mid-April to mid-November in the field facilities at the University of Victoria.
During the winter, seedlings were kept in a growth chamber (Conviron, Winnipeg, MB, Canada) at a photon flux density \( (Q) \) of approximately 350 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) at seedling height, with a 12 h photoperiod and 18/12 °C day/night air temperature \( (T_{\text{air}}) \). A commercial (20:20:20) N:P:K fertilizer was applied bi-weekly.

**Measurements**

**Gas exchange**

Whole-seedling net photosynthesis and transpiration rates were measured continuously with a closed gas exchange system, described by Livingston *et al.* (1994) and Pepin & Livingston (1997), that allows precise and independent control of chamber air temperature (± 0.05 °C), vapor pressure (± 0.02 kPa) and \( c_a \) (± 2 \( \mu \text{mol mol}^{-1} \)). Chamber \( c_a \) and \( e_a \) are monitored using a dual detector infrared gas analyser (LI-6262, Li-Cor Inc., Lincoln, NE, USA). Net photosynthesis rates are determined by integrating the output (recorded as 1 min running averages) from a mass flow controller (Tylan Corp., Carson, CA, USA) used to inject \( \text{CO}_2 \) into the chamber to balance that taken up by the seedling. Vapor pressure is controlled by circulating chamber air through a column of \( \text{CaSO}_4 \) when \( e_a \) exceeds a given set point. The desiccant column is supported on a balance with 1 mg resolution. Transpiration rate is determined as the change in desiccant mass over time (typically 60 s). Stomatal conductance to water vapor is calculated as \( E_t/(L_t \times D) \) where \( L_t \) is the whole-plant one-sided foliage area measured using a leaf area meter (LI-3100, Li-Cor Inc.) and \( D \) is the vapor pressure deficit in the chamber. Intercellular \( \text{CO}_2 \) concentration \( (c_i) \) is calculated as described by Field, Ball & Berry (1991). Because values of \( E_t \), \( g_s \) and \( c_i \) are derived from measurements made at fixed intervals, they are plotted as discrete points. The flow rate in the chamber is \( \approx 0.025 \text{ m}^3 \text{s}^{-1} \), giving rise to very high boundary layer conductances \(( \geq 2 \text{ mol m}^{-2} \text{s}^{-1} \) for seedlings with \( L_t \leq 0.06 \text{ m}^2 \); Livingston *et al.* 1994) relative to stomatal conductances (typically 0–0.2 mol m\(^{-2}\) s\(^{-1}\)).
In separate experiments, it was determined that leaf temperatures \((T_{\text{leaf}})\) are within 0.1 °C of air temperatures (both measured with fine wire thermocouples) and for the present study, it was assumed that \(T_{\text{leaf}} = T_{\text{air}}\).

In order to make independent and concurrent measurements of \(A\) and \(E\) of a seedling's lower and upper foliage, an additional cylindrical cuvette (0.15 m diameter and 0.15 m long), dual detector infrared gas analyser, two mass flow controllers and balance were incorporated into the gas exchange system (Fig. 2.1). The second cuvette was placed inside the original cuvette (0.2 m diameter and 0.3 m long) to enclose the lower foliage. Both cuvettes have removable top and bottom polycarbonate plates. Gas sampling and injection tubing, as well as cables for sensors inside the cuvettes are connected through the cylinder walls (Fig. 2.1). In the inner cuvette, air is circulated through a volume of 0.0027 m\(^3\) at \(0.006 \text{ m}^3 \text{ s}^{-1}\) by means of a 12 V D.C. fan (V369L, Micronel, Vista, California, USA).

When \(Q\) is \(\geq 1000 \mu\text{mol m}^{-2} \text{ s}^{-1}\), \(T_{\text{air}}\) (measured with a shielded fine wire thermocouple) in the inner cuvette is about 3–4 °C higher than that in the outer cuvette (cooled with an air to water heat exchange system). However, when the lights are off or the inner cuvette is shaded, this temperature difference is only 1–1.5 °C. The vapor pressure in the inner cuvette can be adjusted to compensate for these differences in \(T_{\text{air}}\) so that, when required, there are no differences in \(D\) between the cuvettes.

**Photon flux density**

The light control system described by Livingston (1994) was used to provide a constant photon flux density (typically 1000 ± 5 \(\mu\text{mol m}^{-2} \text{ s}^{-1}\) measured with a quantum sensor at seedling height) over a 10–12 h photoperiod. Changes in \(Q\) are brought about by varying the amount of dyed liquid in a tank placed between the light source (high-pressure...
Figure 2.1 Schematic diagram of the dual-cuvette gas exchange system (not to scale) where M and S denote mass flow controllers and normally closed solenoid valves, respectively.
sodium lamp) and the chamber. In the dual-cuvette configuration, a second quantum sensor was placed on the bottom plate of the inner cuvette.

Soil water content
Soil water content was measured using time domain reflectometry and probes with remotely shorted diodes as described by Hook et al. (1992). Measurements of time delay were converted to volumetric soil water content ($\theta_v$) using the relations derived by Hook & Livingston (1996). Transpired water was replaced every 3–4 days and $\theta_v$, unless stated otherwise, was generally maintained between 0.04–0.07 m$^3$ m$^{-3}$ (cf. Appendix A, Fig. A.1).

Experimental protocol
In all experiments, seedlings were acclimatized in the single cuvette system for at least three days before treatments were imposed, and $A_t$ and $E_t$ measured continuously. Unless stated otherwise, $T_{air}$, $D$ and $c_a$ were maintained at 20 °C, 1 kPa and 350 μmol mol$^{-1}$, respectively. For experiments that used the dual-cuvette system, the inner cuvette was usually installed at mid-day. Measurements were made during the rest of the day to ensure that there were no leakages of air from each half of the dual-cuvette. This was determined by summing the rates of photosynthesis from the two halves to confirm that the total was equal to the rate from the whole seedling made on the previous day. A further test carried out in darkness at night was to either inject or scrub CO$_2$ in one half of the cuvette and ensure that $c_a$ in the other half remained constant.

Whole-seedling responses to partial shading using the single cuvette
These experiments were conducted using the single cuvette system to determine the short-term responses of whole-seedling $A$ and $g_s$ to partial shading. Measurements were
carried out on four seedlings held at (i) \( Q = 500 \) and \( 1000 \, \mu \text{mol m}^{-2} \text{s}^{-1} \), and \( c_a = 350 \, \mu \text{mol mol}^{-1} \); and (ii) \( Q = 1000 \, \mu \text{mol m}^{-2} \text{s}^{-1} \), and \( c_a = 150 \) and \( 600 \, \mu \text{mol mol}^{-1} \). Reversible changes in the fraction of illuminated foliage area were brought about by interposing opaque screens (covering 25 to 75% of the cuvette’s top plate surface area) between the light source and the cuvette for 0.75–3 h periods. During these experiments, the outside walls of the cuvette were lined with a non-reflective opaque screen. At the end of the experiment, the one-sided foliage area within each quadrant of three 0.1 m layers (running from the top to the bottom of the cuvette) was determined with a leaf area meter. In addition, intensive measurements were made with four quantum sensors to confirm that, directly beneath the opaque screens, \( Q \) was 0 \( \mu \text{mol m}^{-2} \text{s}^{-1} \).

**Response in upper foliage to shading the lower foliage using the dual-cuvette**

All experiments were performed using the dual-cuvette system. Shading of the lower foliage was imposed at least 4 h after the lights were switched on and was implemented, in less than 15 min, by removing the top plate of the outer cuvette and covering the outer walls of the inner cuvette with non-reflective opaque acetate sheeting. This treatment could be reversed in slightly less time.

**(a) \( D \) in both cuvettes held constant**

These experiment were conducted to determine the response of the upper foliage \( A \) and \( g_s \) to transient shading of the lower foliage when \( D \) in both cuvettes was kept constant (1 kPa). In the first set of experiments, conducted on seven seedlings, shading was imposed for 5–24 h. For a subset of these seedlings \( (n = 4) \), treatments were repeated (once per day) three to seven times per seedling over a two week period to determine measurement repeatability. Experiments were also conducted on three additional seedlings to determine whether the effects of shading would become more pronounced over time. In
this case, shading was imposed for 3–4 d. Seedlings were monitored for at least 2 d, following shade removal.

(b) \( D \) in inner cuvette increased

These experiments were carried out, on another three seedlings, to determine whether the response of the upper foliage (to shading of the lower foliage) would be influenced by the transpiration rate of the lower foliage. To achieve this end, \( e_a \) in the inner cuvette was lowered (and thereby \( D \) increased) to partially compensate for any reduction in \( g_s \) following shading. In addition, \( c_a \) was lowered to promote stomatal opening. Further experiments were carried out to determine: (i) whether increasing lower (unshaded) foliage \( E \), by raising \( D \) in the inner cuvette, would bring about a decrease in upper foliage \( A \) and \( E \); and (ii) whether any responses of the upper foliage to this treatment would be offset or reversed by shading the lower foliage.

Response of upper foliage to exposure of lower foliage to low \( c_a \)

These experiments, using the dual-cuvette system, were carried out on ten seedlings to determine whether the upper foliage \( A \) and \( g_s \) would respond to a reduction in lower foliage \( L_a \) brought about by lowering \( c_a \) rather than by shading. Approximately 4 h after lights were switched on, \( c_a \) in the inner cuvette was reduced from 350 to 50 \( \mu \text{mol mol}^{-1} \) (in \( \approx \)10 min) for periods that ranged from 5–8 h (short-term) and 3–4 d (long-term).

(a) \( D \) held constant in both cuvettes

The vapor pressure deficit in both cuvettes was maintained at pre-treatment values following a reduction in \( c_a \) in the inner cuvette. In this case, any increase in lower foliage \( g_s \), in response to the decrease in \( c_a \), would result in increased \( E \).
(b) $E$ held constant in both cuvettes

The vapor pressure deficit and $c_a$ in the inner cuvette were reduced. In this way, any increase in $E$ resulting from an increase in $g_s$ would be offset by the decrease in $D$.

**RESULTS**

**Whole-seedling responses to partial shading**

Typical short-term responses of seedlings to partial shading are shown in Fig. 2.2. Following shading, the proportional decreases in whole-seedling $A$ and $g_s$ (and $E$, since $D$ was held constant) were always less than the change in illuminated foliage area (expressed as the ratio of illuminated to total foliage area). For example, there was only a 24% decrease in whole-seedling $A$ when 36% of the foliage area was shaded (Fig. 2.2b). Changes in $A_t$ occurred almost instantaneously. When the shade was removed, $A_t$ returned to pre-treatment values within $\approx 15$ min. In all cases, the decrease in $A_t$, after shade was imposed, was slightly faster (typically 5–7 min) than the increase in $A_t$ when the shade was removed. Stomatal conductance also responded rapidly to the imposition or removal of shade but the rate of stomatal closing was always lower than the rate of opening. There were some cases when the rate of the responses of $g_s$ approached those of $A_t$.

When a given fraction of the foliage was shaded, the overall declines in $A$ or $g_s$ were lower in seedlings held at the highest $Q$ (Fig. 2.3a). Indeed, for seedlings held at $Q = 500 \mu$mol m$^{-2}$ s$^{-1}$, the proportional decline in whole-seedling $A$, in response to shading, was only slightly smaller than the corresponding reduction in illuminated foliage area (Fig. 2.3a). The average differences ($\pm$ SD) between the ratio of the shaded to fully illuminated whole-plant $A$, and the ratio of shaded to total foliage area ($L_r$) were $2.7 \pm 2.9\%$ and $9.7 \pm 2.5\%$ for $Q = 500$ and 1000 $\mu$mol m$^{-2}$ s$^{-1}$, respectively. For seedlings held at $c_a = 150 \mu$mol mol$^{-1}$, the proportional decline in whole-seedling $A$ almost exactly corresponded to the reduction in illuminated foliage area brought about by shad-
Figure 2.2 Time course of whole-plant net photosynthesis rate ($A, \cdash\cdash$) and stomatal conductance to water vapor ($g_s, \circ$) of seedlings in response to partial shading imposed by interposing opaque screens between the light source and the cuvette. Photon flux density at seedling height was $1000 \, \mu\text{mol m}^{-2} \, \text{s}^{-1}$ while the vapor pressure deficit was 1 kPa. The solid bars indicate the periods during which shade was imposed and the numbers beneath represent the proportion of foliage that was shaded. Whole-plant one-sided foliage area ($L_t$) is: (a) 0.0383 m²; (b) 0.0244 m².
Figure 2.3 The ratio of the whole-plant net photosynthesis rate of a partially shaded seedling to that of a fully illuminated seedling ($A_r$) vs. the ratio of shaded to total foliage area ($L_r$). Values of $A_r$ are averages measured over 0.75–3 h. Measurements were made on four seedlings at: (a) photon flux densities ($Q$) of 500 and 1000 $\mu$mol m$^{-2}$ s$^{-1}$ and CO$_2$ concentration ($c_a$) = 350 $\mu$mol mol$^{-1}$; and (b) $Q$ = 1000 $\mu$mol m$^{-2}$ s$^{-1}$ and $c_a$ = 150 and 600 $\mu$mol mol$^{-1}$.
ing (Fig. 2.3b). At higher $c_a$, for a given reduction in illuminated foliage area, there was a smaller proportional reduction in whole-seedling $A$. However, there was no difference in response between those seedlings held at 350 and 600 $\mu$mol mol$^{-1}$ (data not shown). The average differences ($\pm$ SD) between $A_t$ and $L_r$ were $3.2 \pm 4.9\%$ and $10.4 \pm 3.0\%$ for $c_a = 150$ and 600 $\mu$mol mol$^{-1}$, respectively.

**Response of upper foliage to shading the lower foliage**

In all cases, when the lower foliage of fully illuminated seedlings was shaded, $A$, $g_s$ and $E$ increased in the upper foliage. In 23 out of the 26 cases, these responses were detected within minutes and upper foliage $A$, $g_s$ and $E$ reached new steady-states within 1–2 h of the imposition of shade. This usually coincided with almost complete stomatal closure in the lower foliage. In the three remaining cases, responses were much slower and were only apparent after 1–2 d.

For a given seedling and shade treatment, measurement repeatability was high. For example, when the same shade treatment (63% of the lower foliage shaded) was imposed seven times on the same seedling over a 10 d period, the mean increase ($\pm$ SD) in the upper foliage $A$ was $10.9 \pm 2.9\%$. Again, the variability in the response of different seedlings to the same treatment was low. For example, for three seedlings that had approximately the same area of lower foliage shaded (63 ± 2%), the mean increase in upper foliage $A$ was $11.4 \pm 2.3\%$.

A typical response to the imposition of shade is given in Figure 2.4. In this example, lower foliage $g_s$ and $E$ decreased by 75–80%, and $A$ declined to less than 0 $\mu$mol m$^{-2}$ s$^{-1}$ after shade was imposed. However, $A$, $g_s$ and $E$ of the upper (illuminated) foliage increased by $\approx 15\%$ (Figs 2.4a–c) but $c_i$ did not change (Fig. 2.4d). Thus, a 65% reduction in whole-seedling photosynthesizing foliage area led to 47%, 38% and 40% reductions in whole-seedling $A$ and $E$, and $g_s$ respectively.
Figure 2.4 Time course of (a) net photosynthesis rate ($A$), (b) transpiration rate ($E$), (c) stomatal conductance to water vapor ($g_s$), and (d) intercellular CO$_2$ concentration ($c_i$) when the lower 65% of a seedling's foliage (total foliage area = 0.0263 m$^2$) was shaded for 6 h. The open and solid symbols represent measurements made on the lower and upper foliage, respectively. The arrows indicate when shade was imposed. The average $A$ in the lower foliage was $-0.69$ $\mu$mol m$^{-2}$ s$^{-1}$ following shade imposition. The vapor pressure deficit was maintained at 1 kPa in both cuvettes.
In all cases, the response of upper foliage to the shading treatments became more pronounced over time. Typically, the largest increases in $A$ and $g_s$ took place within 1–2 d of the treatment, thereafter, increases in these variables were relatively small (Fig. 2.5). The increases in $A$ were similar to those in $g_s$ and consequently there was no significant change in $c_i$, even after 4 d of shading.

Lower foliage $A$, $g_s$ and $E$ increased rapidly when shade was removed and returned to pre-shade values within 1 h (Figs 2.6a–c), regardless of the length of the shade period. This was accompanied by a slower decline in $A$, $g_s$ and $E$ in the upper foliage. Because the reduction in $g_s$ in the upper foliage was slightly more pronounced than that in $A$, this led to a small decrease in $c_i$ (Fig. 2.6d).

**Response of upper foliage to changes in $D$ and shading of the lower foliage**

In treatments where the reduction in lower foliage $E$, brought about by shading, was partially offset by increasing $D$ and reducing $c_a$ in the inner cuvette, the response of the upper foliage was less pronounced than that when the lower foliage was shaded but $D$ remained constant. Figure 2.7 shows a typical example of the effects of manipulating $D$. When $D$ was increased from 1 to 2 kPa in the (unshaded) inner cuvette (enclosing 55% of the seedling’s foliage), the lower foliage $E$ increased by ≈30% within 10 min, despite a 30–35% decline in $g_s$. There was a concomitant 10–12% reduction in $g_s$, $E$ and $A$ in the upper foliage (Figs 2.7a–c). However, when the inner cuvette was shaded later in the day, and $g_s$ and $E$ of the lower foliage were reduced by 80%, $g_s$, $E$ and $A$ of the upper foliage increased by 18–20%. Intercellular CO$_2$ concentration of the upper foliage did not change significantly during these treatments (Fig. 2.7d).
Figure 2.5 Changes in net photosynthesis rate ($A$, ---) and intercellular CO$_2$ concentration ($c_i$, ...) of three seedlings when a portion of the lower foliage was shaded for 3–4 consecutive days. $L_r$ represents the ratio of shaded to total foliage area. Shade was imposed on day 0.
Figure 2.6 Time course of (a) net photosynthesis rate ($A$), (b) transpiration rate ($E$), (c) stomatal conductance to water vapor ($g_s$), and (d) intercellular CO$_2$ concentration ($c_i$) when the lower 65% of a seedling's foliage (total foliage area = 0.0263 m$^2$) was illuminated after having been shaded for 24 h. The open and solid symbols represent measurements made on the lower and upper foliage, respectively. Before shade removal, $A$ in lower foliage was $-0.87$ μmol m$^{-2}$ s$^{-1}$. The arrows indicate when shade was removed. The vapor pressure deficit was maintained at 1 kPa in both cuvettes.
Figure 2.7 Time course of (a) net photosynthesis rate \( A \), (b) transpiration rate \( E \), (c) stomatal conductance to water vapor \( g_s \), and (d) intercellular \( CO_2 \) concentration \( c_i \) when the lower 55% of a seedling’s foliage (total foliage area = 0.0174 m\(^2\)) was first subjected to an increase in vapor pressure deficit \( D_{lower} \) from 1 to 2 kPa and then shaded later in the day. The open and solid symbols represent measurements made on the lower and upper foliage, respectively. The arrows indicate when \( D_{lower} \) was increased and shade was imposed.
Response of upper foliage to changes in $c_a$ around the lower foliage with no shade applied

In all cases, $g_s$ and $E$ of the lower (illuminated) foliage increased rapidly when $c_a$ in the inner cuvette was reduced and this resulted in a concomitant reduction in $g_s$, $E$ and $A$ in the upper foliage ($c_a$ unchanged) (Figs 2.8a–c). In the example shown, $D$ in the inner cuvette was reduced to partially offset the increase in $g_s$, so $E$ increased by only 20%. Nonetheless, there was a gradual decline (30–35%) in $g_s$ and $E$ in the upper foliage over the day. However, $A$ did not change significantly so there was a pronounced decline in $c_i$ (Fig. 2.8d).

In all cases when $c_a$ was reduced in the inner cuvette and $D$ adjusted so that total $E$ for the seedling remained constant, there were no significant changes in $g_s$, $E$ and $A$ in the upper foliage (Figs 2.9a–c). When $c_a$ in the inner cuvette was increased to 350 µmol mol$^{-1}$ later in the day, lower foliage $g_s$ returned to pre-treatment values within 0.75 h. However, values of $A$ and $c_i$ were slightly lower than those before the treatment (Fig. 2.9). In some cases (data not shown), this was accompanied by a small reduction (always < 10%) in upper foliage $g_s$ and $A$.

Discussion

The results from the dual-cuvette experiments clearly demonstrate that when varying proportions of the photosynthesizing foliage area of a well illuminated seedling are shaded, and there are concomitant decreases in whole-plant $E$, then $g_s$ and $A$ rapidly increase in the remaining illuminated foliage. These compensatory responses are fully reversible. In all cases, $c_i$ of the upper (illuminated) foliage did not change when the lower foliage in the inner cuvette was shaded. This was also the case when $A$ decreased in the upper foliage resulting from an increase in $E$ in the lower foliage (Fig. 2.7). Although it was not possible to construct $A/c_i$ curves for the responses before and after the treatments were applied, a conceptual analysis of such curves in relation to CO$_2$ supply, leads
Figure 2.8 Time course of (a) net photosynthesis rate ($A$), (b) transpiration rate ($E$), (c) stomatal conductance to water vapor ($g_s$), and (d) intercellular CO$_2$ concentration ($c_i$) when the lower 63% of a seedling's foliage (total foliage area = 0.0204 m$^2$) was exposed to a reduction in ambient CO$_2$ concentration ($c_{a\text{lower}}$) from 350 to 50 µmol mol$^{-1}$. The vapor pressure deficit in the inner cuvette was lowered to partially offset the increase in $g_s$ and $E$. The open and solid symbols represent measurements made on the lower and upper foliage, respectively. The arrows indicate when $c_{a\text{lower}}$ was reduced.
Chapter 2 – Short-term responses to changes in photosynthesizing foliage area

Figure 2.9 Time course of (a) net photosynthesis rate ($A$), (b) transpiration rate ($E$), (c) stomatal conductance to water vapor ($g_s$), and (d) intercellular CO$_2$ concentration ($c_i$) when the lower 65% of a seedling's foliage (total foliage area = 0.0263 m$^2$) was exposed to changes in ambient CO$_2$ concentration ($c_{a_{lower}}$). The vapor pressure deficit in the inner cuvette was lowered so that $E$ did not change. The open and solid symbols represent measurements made on the lower and upper foliage, respectively. The arrows indicate when $c_{a_{lower}}$ was decreased from 350 to 50 μmol mol$^{-1}$ and then returned to 350 μmol mol$^{-1}$. 
to the conclusion that, for an increase in \( g_s \) and \( A \) to occur at constant \( c_i \), there must have been an increase in Rubisco activity (Sage 1994). The exception shown in Figure 2.8 also supports this interpretation since a decrease in \( g_s \) and \( c_i \) with no change in \( A \) requires an increase in Rubisco activity. This, therefore, suggests that the mechanism of the compensatory effects is a combination of both changes in CO\(_2\) supply, resulting from changes in \( g_s \) and a response of the rate of carboxylation, possibly related to the activity of Rubisco.

The results of the dual-cuvette experiments allowed me to fully interpret the results of the single cuvette shading experiments. In these experiments, reductions in whole-seedling \( A \) and \( g_s \) were proportionally less than the reduction in illuminated foliage area (Fig. 2.3). I believe that was because of compensatory increases in \( g_s \) and \( A \) in the remaining, fully illuminated foliage. Values of \( A \) and \( E \) for the shaded foliage were very low (Figs 2.4 & 2.6) and could not have accounted for the lack of proportionality between the change in \( A \) and illuminated foliage area. Following this interpretation, I calculated the change in \( A \) (\( A_c \)), for a given reduction in illuminated foliage area, as:

\[
A_c = [(A_r/(1 - L_r)) - 1
\]

where \( L_r \) is the ratio of shaded to total foliage area, and \( A_r \) is the ratio of the shaded to fully illuminated whole-plant \( A \). I assumed that: (i) for illuminated foliage, \( A \) was the same throughout the seedling (this assumption is supported by the dual-cuvette measurements which showed that in well illuminated seedlings, photosynthesis rates per unit area for the upper and lower foliage were almost the same; cf. Figs 2.4, 2.6–2.9) and; (ii) for shaded foliage, \( A \) was 0 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). Thus, for a well watered seedling, there was a highly linear increase in \( A_c \) of the non-shaded, well illuminated foliage, as the proportion of shaded foliage increased (Fig. 2.10a). Equivalent calculations for \( g_s \) were not made because it would be incorrect to assume that water loss from shaded foliage was negligible.
Figure 2.10 Changes in (a) net photosynthesis rate ($A$) and (b) stomatal conductance to water vapor ($g_s$) of photosynthesizing foliage vs. the ratio of non-photosynthesizing to total foliage area ($L_T$). Reductions in photosynthesizing foliage area were brought about by either shading ($\Diamond$ and $\bigcirc$) or lowering the ambient CO$_2$ concentration ($c_a$) below the compensation point ($\bigtriangleup$). In the latter case, the vapor pressure deficit was reduced so that $E$ did not change. Each point represents the average change in $A$ or $g_s$ measured over 1–7 h after shade was imposed or $c_a$ lowered. Solid symbols represent the overall increase in $A$ measured when treatments were imposed continuously for 3–4 d. Error bars represent the largest SD of measurements repeated (once per day) 3–7 times per seedling. In all cases, the intercept of the regression line was forced through zero. In (a): Single cuvette, $A_c = 46.6$ (± 2.0) $L_T$ ($R^2 = 0.88$), Dual-cuv. ($E$ reduced), $A_c = 19.7$ (± 0.9) $L_T$ ($R^2 = 0.68$), Dual-cuv. ($E$ constant), $A_c = -2.9$ (± 2.0) $L_T$ ($R^2 = 0.01$); in (b): Dual-cuv. ($E$ reduced), $g_{sc} = 20.8$ (± 1.6) $L_T$ ($R^2 = 0.49$), Dual-cuv. ($E$ constant), $g_{sc} = -5.0$ (± 2.7) $L_T$ ($R^2 = 0.00$).
Calculated $A_c$ (i.e. those derived from measurements in the single chamber and Eqn 2.1) were higher than those directly measured in equivalent (short-term) experiments carried out using the dual-cuvette system (Fig. 2.10a). However, in the latter case, there was also a linear relation between $A_c$ and $L_t$. It is possible that in the single cuvette system: (i) I consistently overestimated the area of shaded foliage, or (ii) penumbral effects resulted in some of the 'shaded' foliage being partially illuminated. Thus, in the latter case, $A$ might have been significantly reduced but would have still been positive. Both sources of errors would have led to an over-estimate of $A_c$. The very close correspondence between the proportional reduction in whole-plant $A$ and the proportion of foliage shaded, when seedlings were subjected to low $Q$ or $c_a$, that is, when any compensatory responses would have been limited (Fig. 2.3), suggests, however, that the penumbral effects and errors in my estimate of shaded foliage area must have been small.

Figure 2.10 summarizes the results of all experiments undertaken with the single and dual-cuvette chambers. In those experiments where, lower foliage $A$ was reduced to $< 0 \mu\text{mol m}^{-2}\text{s}^{-1}$, either by shading or by lowering $c_a$ to below the CO$_2$ compensation point, and $E$ was either unchanged or increased, $A$ or $g_s$ of the remaining foliage did not increase. Thus, when $c_a$ and $Q$ are not limiting, it is clear that compensatory responses in illuminated foliage occur only when reductions in whole-plant photosynthesizing foliage area are accompanied by a reduction in $E_t$. Further, the relation between the increase in $g_s$ or $A$ and reduction in $E_t$ is highly linear (Fig. 2.11). The slope of the regression line predicts that $A$ of illuminated foliage would increase by almost 1% following a 4% reduction in $E_t$. This confirms my initial hypothesis of the strong regulation of $g_s$ by hydraulic signals in trees.

The results are consistent with those reported by Pepin & Livingston (1994) and Whitehead et al. (1996). It is clear that $g_s$ is regulated by $E$, which accommodates the atmospheric demand for water with the supply of water from the soil (Monteith 1995),
Figure 2.11 Changes in net photosynthesis rate \( (A) \) in the upper (untreated) foliage vs. changes in whole-seedling transpiration rate \( (E_t) \) brought about by either shading the lower foliage or exposing the lower foliage to CO\(_2\) concentrations below the compensation point. Each data point represents a single measurement. \( A_c = 0.74 - 0.26E_{1c} \), \( (R^2 = 0.69) \).
however, the results suggest that there might be an additional mechanism that could account for the very rapid responses to transient perturbations in the hydraulic pathway. In the single cuvette experiments, the imposition of shade would have brought an instantaneous reduction in $A$ and a slower reduction in $g_s$, in the shaded foliage. Figure 2.2 clearly shows that whole-seedling $A$ also responded immediately to the shade treatment with a new steady-state being established within minutes. There was not a slower, secondary response (i.e. an increase in $A$) associated with the stomatal closure, and concomitant reduction in $E$, in the shaded foliage. However, when the shade was removed, whole-seedling $A$ responded less rapidly, presumably because the increase in $A$ of the previously shaded foliage was limited by the rate of stomatal opening. The time constants for such responses in western redcedar seedlings are well documented (Pepin & Livingston 1997).

There are numerous reports of almost instantaneous responses to hydraulic shocks in a range of woody species (Teskey, Hinckley & Grier 1983; Fuchs & Livingston 1996; Saliendra, Sperry & Comstock 1995). Sperry, Alder & Eastlack (1993) speculated that such hydraulic signals, transmitted through the xylem at the speed of sound, could trigger the release of chemical messengers in the apoplast which then act on the guard cells. Whitehead et al. (1996) reported that compensatory responses in $A$ and $g_s$ to transient shading were not associated with changes in bulk needle water potential (measured with a pressure chamber) but argued that any changes in guard cell turgor would likely have been masked (Sperry et al. 1993).

My results do not rule out the possibility that $g_s$ is regulated by an interaction between chemical and hydraulic signals, as, for example, proposed by Tardieu & Davies (1993). The longer-term experiments in which shade was imposed over 3–4 d, while not a primary focus of my study, do indicate that there are responses to such treatments that develop over days, in addition to those that become apparent within minutes. It has been suggested that following partial defoliation or partial shading, metabolic promoters
(hormonal or nutritional) of stomatal opening carried in the xylem sap may re-establish
the balance between water loss and water transport capacity by rapidly increasing $g_s$

It is possible that the longer-term responses were associated with homeostatic
adjustments in photosynthesis following a change in sink strength as has been reported in
other species (Sasek et al. 1985; Thomas & Strain 1991; Layne & Flore 1993). However,
this is an unlikely mechanism to explain the rapidity and reversibility of the responses I
measured. Typically, rates of photosynthesis remain unchanged for one or more days
following the onset of a source-sink imbalance (von Caemmerer & Farquhar 1984;
Geiger & Servaites 1991). Osmond, Oja & Laisk (1988) also reported that the biochemi-
cal capacity for photosynthesis responds to changes in the environment with a time
constant of one to several days.

To conclude, my results demonstrate a clear lack of branch autonomy within a
seedling and strongly support the hypothesis put forward by Pepin & Livingston (1994)
and Whitehead et al. (1996) that transpiration and photosynthesis rates in portions of a
tree’s or seedling’s foliage may, in part, be regulated by rapidly transmitted hydraulic
signals that result from transient shading of the other foliage. In turn, these signals are
likely to increase the biochemical activity of photosynthesis. In the short-term, these
responses are linearly related to the change in whole-tree transpiration rate and are not
associated with changes in the distribution of carbon sources and sinks within the tree.
REFERENCES


Appendix A

Soil water retention curve, and photosynthetic and stomatal responses to environmental factors

Soil water retention curve
The soil water retention curve for the sand used in the present study is presented in Figure A.1. Soil samples were subjected to varying water potentials using a vacuum apparatus and pressure plates, and the volumetric water content of the samples was determined after equilibrium had been reached (Soilcon Laboratories Ltd., Richmond, B.C., Canada).

Photosynthetic and stomatal responses to photon flux density
The photosynthetic and stomatal light response curves for western redcedar seedlings are presented in Figure A.2. The responses of whole-plant net photosynthesis rate (A) and stomatal conductance (gs) to changes in photon flux density (Q) were determined by progressively decreasing Q from 1200 to 0 μmol m⁻² s⁻¹ over 10 h. The treatment was imposed 1–2 h after the lights were switched on, when A and gs had reached steady-state.

Photosynthetic and stomatal responses to air temperature
The photosynthetic and stomatal temperature response curves for western redcedar seedlings are presented in Figure A.3. The responses of whole-plant A and gs to changes in air temperature (T_air) were determined by reducing T_air from 25 to 5 °C by 5 °C every 2 h. The treatment was imposed 1–2 h after the lights were switched on, when A and gs had reached steady-state.

Photosynthetic and stomatal responses to vapor pressure deficit
The photosynthetic and stomatal humidity response curves for western redcedar seedlings are presented in Figure A.4. The responses of whole-plant A and gs to changes in vapor pressure deficit (D) were determined by increasing D by 0.5 kPa every 2 h from 0.5 to 3.0 kPa. The treatment was imposed 1–2 h after the lights were switched on, when A and gs had reached steady-state.
Figure A.1 Relationship between soil water content and soil water potential for the sand used in the present study. Each data point is the mean (± SD) of three soil samples. Average bulk density, particle density and total porosity of the samples were 1.587 Mg m⁻³, 2.639 Mg m⁻³ and 39.8%, respectively (Modified from Sun 1995).
Figure A.2 Photon flux density ($Q$) vs. whole-plant (a) net photosynthesis rate ($A$) and (b) stomatal conductance ($g_s$) normalized to the maximum ($A_{\text{max}}$ and $g_{s\text{,max}}$, respectively) measured over the day. The symbols represent three western redcedar seedlings (grown outdoors in field facilities at the University of Victoria). Measurements at $Q = 300$, $500$ and $1200$ $\mu$mol m$^{-2}$ s$^{-1}$ were performed on two seedlings. The air temperature, vapor pressure deficit and ambient CO$_2$ concentration were $25^\circ$C, $0.75$ kPa and $350$ $\mu$mol mol$^{-1}$, respectively. The lines were fitted using the function $f(Q) = 1 - e^{-a(Q - Q')}$ (Eqn 1.7) and a non-linear least-squares procedure. Values of the coefficients $a$ and $Q'$ ($\mu$mol m$^{-2}$ s$^{-1}$) are (a) $0.0056$ and $19.2$, and (b) $0.0043$ and $0$ (the intercept of the regression line was forced through zero for $g_s$), respectively (cf. Table 1.2).
Figure A.3 Air temperature ($T_{\text{air}}$) vs. whole-plant (a) net photosynthesis rate ($A$) and (b) stomatal conductance ($g_s$) normalized to the maximum ($A_{\text{max}}$ and $g_{s\text{ max}}$, respectively) measured over the day. The symbols represent three western redcedar seedlings (grown outdoors in field facilities at the University of Victoria). The photon flux density, vapor pressure deficit and ambient CO$_2$ concentration were 1200 $\mu$mol m$^{-2}$ s$^{-1}$, 0.75 kPa and 350 $\mu$mol mol$^{-1}$, respectively. The lines were fitted using the function $g(T_{\text{air}}) = 1 - b(T_{\text{air}} - T_{\text{max}})^2$ (Eqn 1.8) and a non-linear least-squares procedure. The values of the coefficients $b$ and $T_{\text{max}}$ (°C) are (a) 0.0023 and 21.2, and (b) 0.0012 and 25.3, respectively (cf. Table 1.2).
Figure A.4 Vapor pressure deficit ($D$) vs. whole-plant (a) net photosynthesis rate ($A$) and (b) stomatal conductance ($g_s$) normalized to the maximum ($A_{max}$ and $g_{s\ max}$, respectively) measured over the day. The symbols represent three western redcedar seedlings (grown outdoors in field facilities at the University of Victoria). Additional measurements were carried out at $D = 0.75$ kPa. Measurements at $D = 3.0$ kPa were performed on one seedling. The photon flux density, air temperature and ambient CO$_2$ concentration were 1200 $\mu$mol m$^{-2}$ s$^{-1}$, 25 °C and 350 $\mu$mol mol$^{-1}$, respectively. The lines were fitted using the function $h(D) = 1/[1 + (D/D_{0.5})^c]$ (Eqn 1.9) and a non-linear least-squares procedure. The values of the coefficients $c$ and $D_{0.5}$ (kPa) are (a) 2.2 and 3.6, and (b) 2.4 and 1.5, respectively (cf. Table 1.2).
REFERENCES
Appendix B

Temperature-dependent measurement errors in time domain reflectometry determinations of soil water

Abstract
With the recent development of improved TDR probe design, measurement systems and calibration procedures, it is now possible to detect and quantify the effect of temperature on the soil apparent dielectric constant ($K_a$). I investigated measurement errors in $K_a$ associated with soil temperature variations and compared measured changes in $K_a$ with those predicted by a dielectric mixing model. After confirming the accuracy and resolution of my measurement system with a series of measurements on distilled water, I measured changes in $K_a$ with temperature for a range of soil types, including sand, loam and peat, at soil water contents ($\theta_v$) ranging from 0.09 to 0.81 m$^3$ m$^{-3}$. The measured variation with temperature in the dielectric constant of distilled water (0.322 °C$^{-1}$) was very close to that reported in the literature (0.356 °C$^{-1}$). In soils, changes in $K_a$ with temperature were highest at high water contents. For soils near saturation, the overall changes observed in $K_a$ with temperature were lower than those predicted by the dielectric mixing model by 17% for sand, 24% for loam and 39% for peat. These results suggest that the temperature dependence of the dielectric constant of water in a soil matrix is lower than that of bulk water. Absolute water content errors increased linearly with the size of the water fraction, ranging from $8.75 \times 10^{-5}$ m$^3$ m$^{-3}$ °C$^{-1}$ at 0.05 m$^3$ m$^{-3}$ soil water content to $1.40 \times 10^{-3}$ m$^3$ m$^{-3}$ °C$^{-1}$ at 0.80 m$^3$ m$^{-3}$ soil water content. To obtain the highest measurement accuracy, particularly at higher $\theta_v$, I suggest that a temperature correction of $0.00175\theta_v$ °C$^{-1}$ be employed.
INTRODUCTION

Electromagnetic measurements are widely used to determine the volumetric water content (\( \theta_v, \text{m}^3\text{m}^{-3} \)) of soils. Because the dielectric constant of water (\( K_{\text{water}} \approx 80 \) at 20 °C) is larger than that of other soil constituents (\( K_{\text{air}} = 1; K_{\text{solids}} = 2-5 \)), any change in the dielectric constant of a mixture of solids, air and water predominantly reflects a change in water content. The dielectric properties of a medium can be determined by measuring the propagation velocity of electromagnetic waves along a transmission line placed in that medium. The relative complex dielectric constant (\( K \)) of a material characterizes its capacity to store electric potential energy (electric polarization or dipole moment) in the presence of an electric field (Lorrain & Corson 1970) and has a real (dielectric constant \( K' \)) and an imaginary part (dielectric loss \( K'' \)) which both depend on the measurement frequency. Time domain reflectometry (TDR) operates in the frequency range of 1 MHz to 1 GHz, below the relaxation frequency of water. Hoekstra & Delaney (1974) and Davis & Annan (1977) reported little frequency-dependence of \( K' \) over this range, though the electrical conductivity contributes to dielectric loss if the soil solution contains ions (De Loor 1968). Topp, Davis & Annan (1980) indicated that \( K'' \) was small and non-significant in non-saline homogenous soils. The apparent dielectric constant (\( K_a \)) is usually used in the TDR literature to denote the measured value of \( K' \).

Reported variation in the dielectric constant of pure free water with temperature is small but significant (approximately \(-0.356 ^\circ\text{C}^{-1} \) from 5 °C to 50 °C at 1 GHz, Handbook of Physics and Chemistry 1986). Since the dielectric properties of solids and air are much less sensitive than water to changes in temperature (Roth et al. 1990), one would expect that the temperature dependence of the dielectric constant of a mixture of solids, air and water would be similar to that of free water.

There have been numerous studies using TDR technique that have investigated the temperature effect on the dielectric constants of soil (Davis & Annan 1977; Topp et al. [1977]).
These studies covered a broad range of conditions, with soil water contents varying between 0 and 0.35 m$^3$ m$^{-3}$ and temperatures ranging from 0 to 50 °C. Topp et al. (1980) suggested that no relaxation mechanisms exist which impart strong temperature dependence on $K_a$. Furthermore, they could not detect any significant effect of temperature on the soil dielectric constant from 10 to 36 °C. Zagoskii et al. (1982) also reported that $K_a$ did not change with temperature above 5 °C. However, they found an abrupt decrease in $K_a$ when clay soils were cooled to below 5 °C which they argue was due to a decrease in the volume of water, its density being maximal at 4 °C. Ledieu et al. (1986) used a correction factor of approximately 1 part in 400 °C$^{-1}$ for the temperature dependence of the dielectric constant of water, based on tabulated values. The composite dielectric models presented by Alharthi & Lange (1987) and Roth et al. (1990) also used a dielectric constant of water based on soil temperature. Roth et al. (1990) determined by sensitivity analysis that the temperature dependence of the TDR signal may have to be corrected to obtain optimum accuracy in soil water estimates. However, I have not found any published experimental data upon which temperature correction factors could be based.

The use of remote shorting diodes and calibrated reference air lines can, in many cases, considerably improve the accuracy of TDR measurements (Hook et al. 1992). The signal to noise ratio of the reflected signals can be increased by using remotely switched diodes which combined with a waveform subtraction procedure provide reliable identification of the two reflections that define $K_a$. Also, by using a time delay standard such as an external calibrated air line, time base (counting circuitry of the instrument) errors can be reduced from 66 ps to 30 ps (Hook et al. 1992; Hook & Livingston 1995). This high resolution system has the advantage of detecting very small changes in soil water content, typically, assuming no calibration errors, with an absolute accuracy of 0.012 m$^3$ m$^{-3}$ and a precision of 0.0019 m$^3$ m$^{-3}$ for a 0.3 m probe (Hook et al. 1992; Hook & Livingston...
1995). With these recent improvements in TDR probe designs, measurement systems and calibration techniques, it is possible to better quantify the effect of soil temperature on dielectric measurements and estimates of soil water. The objectives of this study were, therefore: (i) to quantify the effects of temperature variations on the apparent dielectric constant of soils with different water contents; (ii) to compare my experimental data with theoretical estimates derived from a dielectric mixing model; and (iii) to quantify the errors in estimates of water content that result from changes in soil temperature.

MATERIALS AND METHODS

TDR measurement system

The velocity ($V_o$) of an electromagnetic pulse sent through a transmission line buried in soil is calculated as $V = 2l/\hat{t}$ where $l$ is the length of the probe and $\hat{t}$ is the propagation time. In this paper, I have normalized the time of propagation in the soil, measured by the TDR instrument, to the time delay in air ($t_{air}$). This ratio is directly proportional to soil water content and is related to the apparent dielectric constant:

$$\frac{t}{t_{air}} = \frac{c}{V} = (K_a)^{1/2}$$

(B.1)

where $c$ is the speed of light. The value of $t_{air}$ need not be measured and is simply calculated as $2l/c$. Measurements were carried out using a cable tester (Tektronix, model 1502B, Redmond, OR, US.) and three-rod probes (stainless steel, 0.0032 m diameter and 0.012 m apart) combined with a remote shorting diode, similar to the transmission lines described by Hook et al. (1992). For cases where there was large signal attenuation, a three-diode single segment probe was employed. A calibrated reference air line (5.5 m long three-rod probe with a shorting slider) was used as a time delay standard (Hook et al. 1992; Hook & Livingston 1995) and the TDR unit employed only as a transfer stand-
ard. In this procedure, the apparent arrival times of the two reflections ($t_1$ and $t_2$) that define the propagation time for a probe in soil are first recorded with the TDR unit. The input to the unit is then switched from the probe to the reference air line. The dielectric slider is then moved to the positions $X_1$ and $X_2$ that give reflections that correspond to those at $t_1$ and $t_2$. The distance ($l$) between $X_1$ and $X_2$, which can be measured to an accuracy of better than 0.001 m, is related to the propagation time as: $t_2 - t_1 = 2l/c$ (Hook & Livingston 1995).

**Dielectric model**

The definition and application of composite dielectric models for the calibration of TDR in soils have been described in detail by Alharthi & Lange (1987) and Roth *et al.* (1990). The composite dielectric constant ($K_c$) of a wet soil is related to the dielectric properties ($K$) and the volume fraction of each constituent (solids, air and water) following Alharthi & Lange (1987):

$$ (K_c)^{1/2} = (1 - \varphi) (K_{\text{solids}})^{1/2} + (\varphi - \theta_v) (K_{\text{air}})^{1/2} + \theta_v (K_{\text{water}})^{1/2} $$  \hspace{1cm} (B.2)

where $\varphi$ is the total porosity of the soil, $1 - \varphi$ is the solid fraction and $\varphi - \theta_v$ is the remaining pore volume filled with air. $K_{\text{water}}$ is a function of temperature and can be calculated as:

$$ K_{\text{water}} = 78.54 \left[ (1 - 4.579 \times 10^{-3} a) + (1.19 \times 10^{-5} a^2) - (2.8 \times 10^{-8} a^3) \right] $$  \hspace{1cm} (B.3)

where $T_{\text{water}}$ is the temperature (°C) of the water ($K_{\text{water}} \pm 0.03\%$, Handbook of Physics and Chemistry 1986) and $a = (T_{\text{water}} - 25)$. The dielectric model can also be expressed in terms of time delay by combining Eqns B.1 and B.2:

$$ t/t_{\text{air}} = (1 - \varphi) (t_{\text{solids}}/t_{\text{air}}) + \varphi - \theta_v + \theta_v (t_{\text{water}}/t_{\text{air}}) $$  \hspace{1cm} (B.4)
where \( t_{\text{solids}} \) and \( t_{\text{water}} \) are the time delays due to the solid and water fractions, respectively. If \( t = t_{\text{dry\ soil}} \) for \( \theta_v = 0 \) (Table B.1), then

\[
\frac{t_{\text{dry\ soil}}}{t_{\text{air}}} = (1 - \varphi) \left( \frac{t_{\text{solids}}}{t_{\text{air}}} \right) + \varphi
\]  

(B.5)

By combining Eqns B.4 and B.5:

\[
\frac{t}{t_{\text{air}}} = \left( \frac{t_{\text{dry\ soil}}}{t_{\text{air}}} \right) + \theta_v \left[ \left( \frac{t_{\text{water}}}{t_{\text{air}}} \right) - 1 \right]
\]  

(B.6)

In this study, Eqns B.3 and B.6 were used to predict changes in \( t/t_{\text{air}} \) with temperature for different soils. Absolute values of \( \theta_v \) were determined using the time delays measured at room temperature (20–22 °C) and Eqn B.6 (Table B.1). Changes in \( \theta_v \) with temperature were calculated with reference to the room temperature value. The effects of temperature on water density and, consequently, on the volume of water (\( \theta_v \)) were also included in this analysis.

**Measurements in water**

Measurements were made in water to confirm the accuracy and resolution of the experimental and measurement system, and specifically to determine whether I could detect predicted changes in \( K_{\text{water}} \) with temperature (Eqn B.3). A covered Plexiglas container (0.13 m wide by 0.63 m long 0.12 m high) filled with distilled water was cooled to 4 °C in a refrigerator. The container was then removed from the refrigerator and allowed to warm to room temperature over 8–10 h. A 0.58 m probe with a single diode sealed in epoxy glue was immersed in the container and TDR readings taken when ever the water temperature increased by 3–5 °C. Alternatively, the container was heated to 45–50 °C in an oven, removed, the water cooled to room temperature and time delays measured during the cooling cycle. Water temperature was measured with three thermocouples.
placed 0.2 m apart, 0.01 m beneath the probe. A fourth thermocouple was placed in the middle of container 0.02 m above the probe. The outputs of the thermocouples were recorded by a Campbell CR7 data logger (Campbell Scientific, Logan, UT). To minimize temperature gradients within the container, the water was stirred frequently. Typically, differences between the thermocouples did not exceed 0.1 °C.

Measurements in soil

Precise temperature control

Experiments were conducted on near saturated sand, sand with a low θv, and on a saturated peat sample. In all cases soil temperature was precisely controlled to minimize movement of water under non-isothermal conditions. For the near saturated sand, oven-dry sand was packed into a 0.35 m long PVC cylinder (0.065 m ID) and a known amount of distilled water was slowly added to bring the soil to near saturation. For the low θv sand, known amounts of oven-dry sand and distilled water were mixed and placed in a sealed container. To ensure a uniform water distribution, the mixture was stirred frequently, allowed to stand overnight and then packed into a PVC cylinder. Both ends of the cylinder were sealed to prevent water loss. For the saturated peat experiments, an organic sample was collected at 0.30 m depth in a peatland, well below the water table. Details of the sampling procedure and the physical and chemical characteristics of the organic substrate are described elsewhere (Pepin, Plamondon & Stein 1992). The peat sample was inserted in a PVC cylinder that had dimensions similar to that used for the sand. Compaction during this manipulation probably slightly reduced the water content at saturation of the peat sample. The bulk density (ρb) of the peat sample (0.229 Mg m⁻³, Table B.1) was 25% higher than the value reported by Pepin et al. (1992).

In each cylinder, soil temperatures were monitored using five thermocouples. Four thermocouples were placed 0.06 m apart, two each at 0.01 m and 0.02 m from the cylin-
### Table B.1

$t/t_{\text{air}}$ measured at $\theta_v = 0$, bulk density, probe length and soil water content determined gravimetrically and by time domain reflectometry for sand, loam and peat soils.

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Probe length (m)</th>
<th>$(t_{\text{dry soil}}/t_{\text{air}})^\dagger$ (at $\theta_v = 0$)</th>
<th>Bulk density ($\rho_v$, Mg m$^{-3}$)</th>
<th>Soil water content ($\theta_v$, m$^{-3}$ m$^{-3}$)</th>
<th>Gravimetric$^\ddagger$</th>
<th>TDR$^\S$</th>
</tr>
</thead>
<tbody>
<tr>
<td>coarse sand</td>
<td>0.58</td>
<td>1.78</td>
<td>1.76</td>
<td>0.294</td>
<td>0.322</td>
<td></td>
</tr>
<tr>
<td>Puntledge loam</td>
<td>0.58</td>
<td>1.64</td>
<td>1.19</td>
<td>0.487</td>
<td>0.487</td>
<td></td>
</tr>
<tr>
<td>coarse sand</td>
<td>0.33</td>
<td>1.78</td>
<td>1.50</td>
<td>0.090</td>
<td>0.053 (± 0.001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>1.78</td>
<td>1.74</td>
<td>0.119</td>
<td>0.097 (± 0.003)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>1.78</td>
<td>1.85</td>
<td>0.293</td>
<td>0.309 (± 0.001)</td>
<td></td>
</tr>
<tr>
<td>mesic peat</td>
<td>0.30</td>
<td>1.14$^\dagger$</td>
<td>0.23</td>
<td>0.810</td>
<td>0.788 (± 0.004)</td>
<td></td>
</tr>
</tbody>
</table>

$^\dagger$Measured composite time delay of oven-dried soil.

$^\ddagger$Calculated from bulk density and gravimetric water content using Eqn B.7.

$^\S$Estimated from time delays measured at room temperature ($20$–$22$ °C) and Eqn B.6. Each datum is the mean value (± SD) of two to nine TDR readings for measurements with a 0.30 to 0.33 m probe and the value of one reading for measurements with the 0.58 m long probe.

$^\ddagger$Measured with a bulk density of 0.356 Mg m$^{-3}$. 

Appendix B - Temperature-dependent measurement errors in TDR

A fifth thermocouple was installed in the middle of the soil sample and used as the reference sensor for the determination of temperature gradients within the cylinders. A TDR probe, ≈0.30 m long, was inserted in the centre of each soil sample. Cylinders were immersed in a water bath (Haake D8, Haake Mess-Technik, Karlsruhe 41, Germany) that provided temperature control better than ±0.02 °C. Both heating and cooling cycles (driven by the data logger) were imposed over a 5 to 50 °C temperature range. Samples were rotated daily to ensure even distribution of water within the cylinder. Time delay measurements were taken once a day during the 3 d when the samples were maintained at constant temperature. After each 3-d measurement period, the bath temperature was increased by 5 or 10 °C at a rate of 0.21 °C h⁻¹ and held at the new temperature for three sampling days. Temperature gradients within the cylinders were always < 5 °C m⁻¹ at the time of the TDR measurements.

High resolution TDR measurements

In certain cases, time delay measurement errors can be halved by doubling the length of the probe buried in soil (Hook & Livingston 1995). I conducted a second set of experiments with soil in which I employed 0.58 m probes to maximize the resolution of the time delay measurements. This meant some sacrifice in temperature control because the water bath could not accommodate cylinders and probes longer than 0.4 m. Soil temperatures were therefore manipulated employing similar methods to those used for the distilled water experiments. A coarse sand and a loam were oven-dried and each packed in plexiglas containers to bulk densities of 1.76 and 1.19 Mg m⁻³, respectively (Table B.1). Distilled water was then slowly added to each soil until it was almost saturated. Three thermocouples were installed 0.2 m apart in the soil and 0.01 m from the probe. During warming and cooling cycles temperature differences between the thermocouples seldom exceeded 1 °C.
Gravimetric determination of soil water content

Soil samples were weighed before and after each experiment to determine if there was any water loss. Final weights were all within ± 0.15% of those measured at the start of the experiment (corresponding to a maximum change of 0.004 m³ m⁻³ in \( \theta_v \)). At the end of each experiment, samples were oven-dried to 105 °C for 48 h and soil water content determined gravimetrically (Table B.1). Gravimetric water contents (\( \theta_g \)) were converted to volumetric water contents using (Warrick 1990):

\[
\theta_v = \left( \frac{\rho_o}{\rho_w} \right) \theta_g \tag{B.7}
\]

where \( \rho_w \) is the density of water. For each dry mineral soil, the time delay (\( t_{\text{dry soil}} \)) was measured with the 0.58 m probe. The apparent dielectric constant of the dry peat was measured with a 0.30 m probe at a bulk density of 0.356 Mg m⁻³.

Data analysis

Regression analysis was used to establish the relation between \( T/T_{\text{air}} \) and soil temperature. Comparisons of the regression functions (predicted vs. experimental data) were undertaken with SAS (SAS Institute 1985) using a dummy-variable model and a level of significance of \( \alpha = 0.05 \) (Neter, Wasserman & Kutner 1985). Measurement accuracy and resolution were determined according to Hook & Livingston (1995) and are represented in Figs B.1–B.5 by large and small error bars, respectively. The measurement resolution was used as a source of variation for the data points of the predicted regression line (Eqn B.6) in this analysis. Regression parameters (slopes and intercepts) were tested with Student’s \( t \)-tests.
RESULTS AND DISCUSSION

Distilled water

Measured changes in $t/t_{air}$ in distilled water with changing temperature were very close to those predicted by Eqn B.3 (Fig. B.1) with less than a 9% difference (but significant at $P \leq 0.0001$) in slopes. Virtually all (99.6%) of the variance in time delay could be explained by temperature. The maximum difference between predicted and measured $t/t_{air}$ was at 5 °C and was only −0.66%. These data show that the TDR system provides excellent measurement accuracy and can resolve small changes in $t/t_{air}$.

Soil

Predicted vs. measured relation between $t/t_{air}$ and temperature

The empirical relationships between $t/t_{air}$ and temperature were statistically significant ($P \leq 0.0001$) for all soils except for the sand at low water contents ($\theta_v = 0.053$ and $0.097$ m$^3$ m$^{-3}$) where there were, as expected, very small changes in $t/t_{air}$ with temperature ($P = 0.539$; Figs B.2–B.5). The absolute influence of temperature on $K_c$ (or time delay) clearly increases with increasing water content (Eqn B.2). Consequently, changes in $K_c$ with temperature are small in relatively dry soils and difficult to detect, being at the limit of the resolution of the measurement system. The relationship between $t/t_{air}$ and temperature did not differ whether samples were heated or cooled and therefore these data were pooled. With the exception of the dry sand, coefficients of determination ($R^2$) were high with temperature always explaining > 90% of the total variation in $t/t_{air}$ (Figs B.2, B.4 & B.5). Coefficients of variation were not significantly higher for data collected with long (0.58 m) probes than for those obtained with shorter probes.

The slopes of plots of measured $t/t_{air}$ or $K_a$ vs. temperature were, with the exception of dry sand, all significantly lower ($P \leq 0.0104$) than those predicted by Eqns B.3 & B.6. Mean differences between measured and predicted slopes were 17% for the near
Figure B.1 Predicted and measured $t/t_{air}$ and $K_a$ vs. temperature ($T$) for distilled water using a 0.58 m long probe. The large and small error bars denote the absolute measurement accuracy and resolution, respectively. Predicted $t/t_{air} = 9.37 - 0.020T$; Measured $t/t_{air} = 9.30 - 0.018T$, $R^2 = 0.996$. 
Figure B.2 Predicted and measured $t/\tau_{\text{air}}$ and $K_a$ vs. temperature ($T$) for a wet coarse sand using (a) 0.33 m (water content, $\theta_v = 0.293 \text{ m}^3 \text{ m}^{-3}$) and (b) 0.58 m ($\theta_v = 0.294 \text{ m}^3 \text{ m}^{-3}$) long probes. Each data point is the mean value ($\pm$ SE) of two to nine TDR readings in (a) and the value of one TDR reading in (b). Standard errors are smaller than the symbol. The large and small error bars denote the absolute measurement accuracy and resolution, respectively. (a) Predicted $t/\tau_{\text{air}} = 4.38 - 0.00685T$; Measured $t/\tau_{\text{air}} = 4.33 - 0.00508T$, $R^2 = 0.973$; (b) Predicted $t/\tau_{\text{air}} = 4.49 - 0.00715T$; Measured $t/\tau_{\text{air}} = 4.48 - 0.00659T$, $R^2 = 0.987$. 
Figure B.3 Predicted and measured $t/t_{air}$ and $K_a$ vs. temperature ($T$) for a dry coarse sand using (a) 0.33 m (water content, $\theta_v = 0.090 \text{ m}^3 \text{ m}^{-3}$) and (b) 0.30 m ($\theta_v = 0.119 \text{ m}^3 \text{ m}^{-3}$) long probes. Each data point is the mean value (± SE) of two to eight TDR readings. Standard errors are smaller than the symbol. The large and small error bars denote the absolute measurement accuracy and resolution, respectively. (a) Predicted $t/t_{air} = 2.22 - 0.00117T$; Measured $t/t_{air} = 2.23 - 0.001057$ (regression line not shown), $R^2 = 0.868$; (b) Predicted $t/t_{air} = 2.60 - 0.00216T$; Measured $t/t_{air} = 2.56 - 0.000397$, $R^2 = 0.315$. 
**Figure B.4** Predicted and measured $t/t_{air}$ and $K_a$ vs. temperature ($T$) for loam (water content, $\theta_v = 0.487 \text{ m}^3 \text{ m}^{-3}$) using a 0.58 m probe. Each data point is the value of one TDR reading. The large and small error bars denote the absolute measurement accuracy and resolution, respectively. Predicted $t/t_{air} = 5.76 - 0.01087T$; Measured $t/t_{air} = 5.70 - 0.00829T$, $R^2 = 0.904$. 
Figure B.5 Predicted and measured $t/t_{\text{air}}$ and $K_a$ vs. temperature ($T$) for saturated peat (water content, $\theta_v = 0.810 \, \text{m}^3 \, \text{m}^{-3}$) using a 0.30 m probe. Each data point is the mean value ($\pm$ SE) of two to nine TDR readings. Standard errors are smaller than the symbol. The large and small error bars denote the absolute measurement accuracy and resolution, respectively. Predicted $t/t_{\text{air}} = 7.76 - 0.017427T$; Measured $t/t_{\text{air}} = 7.62 - 0.010717T$, $R^2 = 0.982$. 
saturated sand (26% and 8% for the 0.33 m and 0.58 m probes, respectively), 24% for the loam, and 39% for the peat (Figs B.2, B.4 & B.5). These differences between slopes are reduced by 7–8% if the measured relation between \( t/t_{\text{air}} \) in distilled water and temperature (Fig. B.1) is used instead of Eqn B.3 to predict the dielectric behavior of each soil.

My results suggest that the change in dielectric constant of free water with temperature is greater than that for water held in a soil matrix. This appears to be more pronounced in fine-textured and organic soils which usually have large active surface areas and surface charges. The first layers of water in physical contact with the soil particles have a dielectric constant lower than bulk water because of constrained rotational freedom. It is possible that in matrices with appreciable surface-water interactions more thermal energy must be imparted to the system to bring about a decrease in dielectric constant. The dielectric properties of bound water have been discussed for frequencies in the microwave region by Wang & Schmugge (1978), Dobson et al. (1985) and Hallikainen et al. (1985). Topp et al. (1980) also reported attempts to quantify the dielectric behavior of absorbed water using the TDR technique. Dirksen & Dasberg (1993) found that the low bulk density associated with fine-textured soils also contributes to the lowering of the composite dielectric constant. Clearly, the effects of soil matrix on \( K_c \) warrant further investigation.

**Absolute errors in soil water content**

We calculated the absolute errors in estimates of soil water content that would arise should changes in soil temperature not be taken into account. These were calculated for \( \theta_v \) between 0 and 0.80 m\(^3\) m\(^{-3}\) and soil temperature ranging from 5 to 50 °C. Predicted absolute errors in \( \theta_v \) per °C slightly increase with temperature. This second-order effect, which occurs because the relation between \( K_{\text{water}} \) and temperature is non-linear (Eqn B.3), is shown by the error bar for the predicted curve in Fig. B.6. In this analysis, I
Figure B.6 Predicted and measured absolute errors in water content (per °C) vs. soil water content. Each data point is the mean value (± SD) of all the absolute errors in water content observed in each soil sample. Predicted error = 0.00263$\theta_v$; Measured error = 0.00175$\theta_v$, $R^2 = 0.936$. The error bar beside the predicted curve represents the standard deviation in predicted absolute errors in water content per °C.
assumed that in a dry soil (i.e. when \( \theta_v = 0 \)) there is no absolute error attributable to temperature and thus regression lines fitted to both the predicted and measured data were forced through zero (Fig. B.6). Measured errors were 33% lower than those predicted. The slopes of the regression lines for the predicted and measured data for all soils are 0.00263 and 0.00175 \( \text{m}^3 \text{m}^{-3} \text{°C}^{-1} \), respectively.

This analysis shows that changes in soil temperature, if not accounted for, can produce significant errors in soil water measurements. For example, a 15 °C change in mean soil temperature in a 0.4 m soil profile with an average \( \theta_v \) of 0.15 \( \text{m}^3 \text{m}^{-3} \) would introduce an absolute measurement error of 0.004 \( \text{m}^3 \text{m}^{-3} \) (Fig. B.6), the equivalent of 1.6 mm of water. Similarly, a 0.021 \( \text{m}^3 \text{m}^{-3} \) absolute measurement error (equivalent to 8.4 mm of water) would result from a 15 °C temperature change in a 0.4 m peat profile with an average \( \theta_v \) of 0.80 \( \text{m}^3 \text{m}^{-3} \). These apparent changes in soil water correspond to a 2.6% relative error in water content.

I recommend that if there are large temperature gradients or changes in temperature within a soil profile over the measurement period, soil water contents measured using TDR should be corrected for temperature to obtain maximum accuracy. From a practical point of view, the differences found between measured and predicted soil water content errors are small and therefore either of the regression equations presented in Fig. B.6 could be used.

**SUMMARY**

My experimental data clearly indicate that temperature has a small but significant effect on the composite dielectric constant of wet soils. Furthermore, these results show that the dielectric mixing model (Eqn B.2) described by Alharthi & Lange (1987) and Roth *et al.* (1990) can be used to predict the changes in \( K_c \) with variations in soil temperature. Failure to account for changes in soil temperature can lead to errors, particularly at high soil
Appendix B - Temperature-dependent measurement errors in TDR

Water contents. Although measured errors in soil moisture content were consistently lower than those predicted by the mixing model, for practical purposes, either the empirical or theoretical relationships can be used to correct the effect of soil temperature on dielectric measurements because the absolute differences between theory and measurements are relatively small. These results suggest that in a soil matrix, the temperature dependence of the dielectric constant of water behaves differently than that of bulk water, particularly in fine-textured and organic soils. The effect of soil texture on the dielectric properties of wet soils warrants further investigation.

REFERENCES


Appendix C

A whole-plant cuvette system to measure short-term responses of conifer seedlings to environmental change
A whole-plant cuvette system to measure short-term responses of conifer seedlings to environmental change


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Received July 12, 1993

Summary

A computer-controlled whole-plant cuvette system is described that allows precise and independent control of temperature (± 0.05 °C), vapor pressure (± 0.02 kPa), CO₂ concentration (± 2 µmol mol⁻¹) and photosynthetic photon flux density (± 5 µmol m⁻² s⁻¹), and allows the continuous measurement of net photosynthesis and transpiration rates. Vapor pressure is controlled by circulating chamber air through a CaSO₄ desiccant column supported on a digital balance. Transpiration rate is calculated from the change in desiccant mass with time. Photosynthesis rate is measured by integrating the output of a mass flow controller used to inject CO₂ into the chamber to compensate for that assimilated by the plant.

The control system can be driven by set points that can be varied, for example, as a function of time, or held constant. We were able to simulate weather data obtained from climate stations and accurately follow, in real time, the output of sensors measuring outside conditions.

Experiments on well-watered one- and two-year-old nursery-raised western red cedar (Thuja plicata Donn.) and white spruce (Picea glauca (Moench) Voss) seedlings showed that if the mean daily temperature was increased from 20 to 22 °C with vapor pressure remaining constant at 1 kPa, CO₂ concentrations must almost double to compensate for the decrease in net photosynthesis rate.

Keywords: photosynthesis, temperature, transpiration, vapor pressure.

Introduction

Even though there is no general consensus on the degree and distribution of warming resulting from increased CO₂ in the atmosphere, or whether a global warming signal has already been detected (Schneider 1990), there is a growing interest in determining the effects on plant performance of increased CO₂ in conjunction with other stress factors such as increased temperatures, vapor pressure deficit and water deficits (Chaves and Pereira 1992).

Physiological responses to changes in environmental variables in natural conditions are often measured using porometers and estimates of whole-plant transpiration or photosynthesis rates are extrapolated from measurements made on single leaves. However, it is very difficult to isolate the effects of individual variables on stomatal behavior (Jarvis 1976, Livingston and Black 1987, Grossnickle and Russell 1991). Furthermore, this approach does not provide a continuous measure of plant response and is difficult because of the large amount of sampling required to account for microclimatic and within-plant physiological variability.

Whole-plant enclosure systems used in the field, whether flow-through types that measure the change in water vapor and CO₂ concentrations between incoming and
outgoing air, or transient types that measure the rate of change of gas concentrations, generally have little or no climate control. The portable field system described by Graham et al. (1989) although representing a considerable advance in system design, was not able to control temperatures to better than within 3 °C of ambient temperature. Open-top systems used to study the effects of increased CO₂ on productivity under natural conditions (Norby et al. 1992) do not allow good control or regulation of other variables such as temperature and vapor pressure.

Some control of environmental variables can be achieved in growth chambers or indoor cuvettes, but no system has been described that provides adequate simulation of natural conditions. Existing systems are typically used in studies where environmental variables are held constant. This paper describes a computer-controlled plant enclosure system that allows the precise and independent regulation of air temperature, vapor pressure, CO₂ concentration and photosynthetic photon flux density (PPFD), and the simulation of outdoor ambient conditions while providing continuous measurements of whole-plant photosynthesis (Pₚₚ) and transpiration (E) rates. We show how this system can be used to determine whether short-term effects of increased CO₂ concentrations on conifer seedling productivity and water use are offset by small increases in air temperature and vapor pressure deficit.

Materials and methods
The cuvette system is made up of five parts or subsystems; a computer-based data acquisition and control system, the seedling enclosure and housing with attached air circulation and temperature control systems, a humidity control system that operates independently of the temperature control system and also provides a continuous measure of E, a CO₂ control system that maintains chamber CO₂ at a predetermined set point and provides a continuous measure of Pₚₚ, and a PPFD control system.

1. Data acquisition and control
The generation of control signals and data logging is implemented using data acquisition and control software (Workbench P.C. Strawberry Tree inc., Sunnyvale, California, USA) run on an IBM-PC. The analog signals are accepted by a 16-channel, 16-bit analog to digital board (ACPC-16-16, Strawberry Tree), which also has digital I/O lines that are used to drive the solenoid valves and pumps. A 12-bit analog, 8-channel output board (ACAO-12-8, Strawberry Tree) provides the analog control signals for the mass flow controllers and water bath.

2. Enclosure, air circulation and temperature control
Two sizes of seedling enclosures are used, both made from polycarbonate cylinders (0.635 cm wall thickness). The larger cylinder (0.2 m O.D. and 0.3 m long) is designed to accommodate one- or two-year-old seedlings, whereas the smaller cylinder (0.14 m O.D. and 0.2 m long) accommodates younger seedlings. Each cylinder has removable top and bottom plates. The lower plate has a 1.27 cm diameter hole at its center and an adjustable slot to accommodate a seedling stem. A
seal around the stem is made with Plasticine. Gas sampling and injection tubing (0.635 cm O.D. 0.40 Dekeron, Dekeron Instrument Components, Aurora, Ohio, USA), as well as signal cables for the sensors inside the chamber enter through the cylinder walls (Figure 1).

Data presented in this paper were collected using the larger cylinder, even though the best control of environmental variables and highest measurement resolution are obtained when using the smaller cylinder.

Air is circulated through a total volume of 0.037 m$^3$ (this includes the large seedling enclosure, heat exchanger and humidity control system) at approximately 0.025 m$^3$ s$^{-1}$ by means of a 12 V D.C. fan (V609L, Micronel, Vista, California, USA). Measurements with a hot wire anemometer indicated that with a large seedling (projected needle area of 0.063 m$^2$) enclosed in the chamber, wind speeds are typically 2–2.5 m s$^{-1}$, which corresponds to a boundary layer conductance of approximately 0.17 m s$^{-1}$ (Landsberg and Thom 1971). The lowest wind speeds of 0.4 m s$^{-1}$, measured in a small region near the bottom of the chamber, correspond to a boundary layer conductance of 0.05 m s$^{-1}$.

An external diaphragm pump (TD-3LS, Brailsford and Co. Inc. Rye, New York, USA) provides a continuous flow of chamber air (0.02 dm$^3$ s$^{-1}$) to a dual detector infrared gas analyzer (IRGA) (LI-6262 Li-Cor Inc., Lincoln, NE, USA) for the measurement of CO$_2$ concentration and water vapor pressure. An output voltage is sent from a barometric sensor (PTA 427, Vaisala Sensor Systems, Ontario, Canada) to the IRGA to correct for changes in atmospheric pressure.

The chamber temperature is maintained at a selected value (set point) by an air to water heat exchange system. Air is circulated through 12 parallel thin-walled (0.0095 m O.D. 0.305 m long) copper tubes (the inside surfaces of which are nickel plated to minimize vapor and CO$_2$ adsorption) and cooled by the cross flow

![Figure 1. Schematic of the whole-seedling cuvette and environmental control system (not to scale) where M and S denote mass flow controllers and normally closed, two-way solenoid valves, respectively.](image-url)
(0.28 dm$^3$ s$^{-1}$) of water circulated through a refrigerated water bath (RTE-110D, Neslab Instruments Inc. Portsmouth, New Hampshire, USA). A negative feedback control system (Figure 2) is used to regulate the chamber temperature. A set point temperature is used to drive the proportional, integral and derivative (PID) controller of the water bath. This is compared to the actual chamber temperature measured by a fine wire thermocouple. The difference (error) is subtracted from the original set point and averaged over 30 s to give a new output set point so that, for example, if the actual chamber temperature is higher than the original set point (resulting in a negative error), the output set point is lowered and the bath temperature reduced. Conversely, if the chamber temperature is lower than the set point, a positive error is generated, the output set point is increased and the bath temperature raised.

The chamber temperature can be changed by adjusting the set point, for example, by generating a set point as a function of time. Alternatively, the set point can be driven by ambient temperature measured by a sensor outside the chamber or building, or by values read from a data file.

3. Humidity control and transpiration

Chamber water vapor pressure is controlled by a system similar to that described by Graham et al. (1989). Transpired water is absorbed by a column of CaSO$_4$ (8 mesh "Drierite") supported on a balance with 1 mg resolution and 2 kg capacity (PM 2500 Mettler Instrument Corp., Highstown, New Jersey, USA). The output of the balance is sent by means of an RS-232 port to the computer (typically every 60 s). The desiccant and balance are housed in a sealed polycarbonate chamber.

When the cuvette vapor pressure exceeds a specified set point, a small DC fan (V589, Micronel) is activated and air diverted through a normally closed (19.05 mm I.D.) solenoid valve (S4 in Figures 1 and 3a) (Ascoelectric, Brantford, Ontario, Canada) to the desiccant column and then back to the chamber. Precise control of vapor pressure is achieved either by adjusting the flow rate of air through the desiccant column by varying the voltage to the DC fan, or with a pulse modulation routine that varies the duration of the pulse used to drive the solenoid valves and fan (i.e., the pulse duration increases with increasing transpiration rate). As with the

![Figure 2. Block diagram of the temperature control algorithm where T is temperature and PID is proportional-integral-derivative control. The circle with the adjacent + and - signs indicates that Z is subtracted from Y to produce Y'. The coefficients a and b are fixed and are used to scale the output signal (Z) to a control voltage (10 mV °C$^{-1}$) for the water bath PID.](image-url)
WHOLE-PLANT CUvette SYSTEM TO MEASURE RESPONSES OF CONIFER SEEDLINGS

4. Carbon dioxide control and net photosynthesis

The CO$_2$ concentration in the cuvette (CO$_2$cuv) is controlled by a compensatory feedback system (Figure 3b). The measured concentration is continuously compared...
to a specified set point concentration (CO$_2$sp). When CO$_2$cuv is less than CO$_2$sp, cylinder CO$_2$ (0.5% CO$_2$ in N$_2$) is injected through a solenoid valve (S1, Figure 3b) into the cuvette to balance that assimilated by the plant. The injection rate is controlled and monitored by a mass flow controller and meter (Tylan model FC-260, Tylan Corp., Carson, California, USA). Net photosynthesis is calculated as the product of the integrated (typically 30 s) output of the mass flow meter and the injected gas concentration. Increased control of CO$_2$cuv is obtained by using a pulse-width modulation routine that adjusts the duration of the pulse driving the injection solenoid, and by varying the control signal voltage to the mass flow controller so that both the pulse driver and control voltage are proportional to $P_n$.

During the night or at other times when $P_n$ is negative and CO$_2$cuv is greater than a specified set point (typically set 5 μmol mol$^{-1}$ above CO$_2$sp), cuvette air is pumped (TD-3LS, Brailsford and Co. Inc.) by means of solenoids S2 and S3 through a soda lime column to remove CO$_2$ (Figure 3b). Respiration is calculated as the product of the flow through the scrubber and CO$_2$cuv (it is assumed that the soda lime removes all CO$_2$ from the air passing through it). Excellent control of CO$_2$cuv can be obtained by employing pulse-width modulation and proportional voltage control in the respiration routine.

5. PPFD Control

Photosynthetic photon flux density is controlled with a feedback control system as described by Livingston (1994). A Plexiglas tank is interposed between the light source (high-pressure sodium lamp), and PPFD changed by varying the level of dyed liquid in the tank. The amount of liquid pumped into or drained from the tank to a reservoir is a function of the difference (error) between a defined set point value of PPFD and that measured in the cuvette. The set point can be varied as a function of time, follow the output of a quantum sensor measuring ambient PPFD or be driven by values of PPFD read from a data file. Within the 0.4 to 0.64 μm wave band, the dye acts as a neutral density filter so no change in spectral distribution occurs. Photosynthetic photon flux density in the cuvette can be controlled to within 5 μmol m$^{-2}$ s$^{-1}$ when the set point is held constant or changed slowly (< 5 μmol m$^{-2}$ s$^{-1}$). Errors increase to approximately ± 20 μmol m$^{-2}$ s$^{-1}$ when the set point is changed more rapidly (to simulate intermittent cloud).

The entire cuvette system, with the exception of the water bath, is enclosed in an air-conditioned (Panasonic CW-500RK, Matsushita Electric of Canada, Mississauga, Ontario) cabinet. The air conditioner, which had a cooling capacity of 1470 W, is vented to the outside of the building.

Plant material

One- and two-year-old nursery raised western red cedar (Thuja plicata Donn.) and white spruce (Picea glauca (Moench) Voss) seedlings were used in all experiments. The seedlings were transplanted to PVC cylinders (0.15 m I.D. and 0.40 m long) filled with fine sand. Soil water was measured daily with time domain reflectometry by waveform subtraction techniques and probes with remotely switched diodes as
described by Hook et al. (1992). Soil water was maintained between 0.09 and 0.11 m³ m⁻³ throughout the measurement period. Transpired water (as estimated from the change in weight of the desiccant column) was replaced, by watering, every two to three days.

Results and discussion

Environmental control

The ability to control air temperature, vapor pressure and PPFD independently allowed us to simulate climate data (hourly averages) obtained from a number of different climate stations, and to accurately follow the output of sensors placed on the roof of our building.

The heat exchange system was capable of controlling the cuvette temperature to within 0.05 °C under a full heat load and with a varying set point (Figure 4a). This degree of control was possible over a wide range of temperatures (0 to > 35 °C). There were slight (less than 0.25 °C) excursions in cuvette temperature when the light source was switched on or off to provide an abrupt change in PPFD, but these were corrected within 20 minutes of the change. When PPFD was controlled to simulate natural conditions, there was no detectable change in cuvette temperature.

Typically, vapor pressure could be controlled to within 0.02 kPa over a wide range of vapor pressures (Figure 4b) and transpiration rates (0 to 1.5 mmol m⁻² s⁻¹, data not shown). Best control was obtained when the cuvette temperature was maintained between 5 and 30 °C. The uncertainty in cuvette vapor pressure translates into a storage capacity of 5.5 mg (as predicted by the ideal gas law and assuming a cuvette temperature of 20 °C and a volume of 0.037 m³).

There was excellent control of cuvette CO₂ concentration (typically better than ± 2 µmol mol⁻¹) over a wide range of CO₂ concentrations (Figure 4c) and net photosynthesis rates (0 to 15 µmol m⁻² s⁻¹, data not shown). A small error in £ was introduced by the injection of the CO₂/N₂ gas mixture. However, worst-case calculations made with the assumption that the injected gas displaces an equal volume of chamber air with a vapor pressure of 1.5 kPa, indicated that, at very high rates of photosynthesis (10 µmol m⁻² s⁻¹), £ would be overestimated by 0.072 g h⁻¹ (or 0.03 mmol m⁻² s⁻¹, assuming a plant with a projected needle area of 0.04 m²).

There was excellent agreement (within 4%) between cumulative transpiration (over periods ranging from 1 to 16 h) as measured by the change in desiccant weight and that estimated from the change in weight of a potted plant that was weighed (to 0.1 g) before and after placement in the cuvette. Cuvette estimates were consistently lower indicating that there was some storage of water on surfaces within the cuvette system. This was confirmed by experiments over a wide range of temperatures where the rate of water vapor efflux was measured after a step change in air water vapor pressure in the chamber. Typically, water desorption occurred for almost 2 h following the step change. Calculations indicated that at low £ (0.1 mmol m⁻² s⁻¹), transpiration rate could be overestimated by 10% following a step change from 2.0
Figure 4. Time versus (a) chamber temperature, (b) vapor pressure and vapor pressure deficit (VPD), and (c) chamber CO₂ concentration. Errors for each variable were calculated as the difference between the set point and measured value. The arrows on the x axis of (a) indicate when the light source was switched on (F) and off (X). Air temperature was sampled every minute for 1 h before the lights were turned on and 1 h after they were switched off. At all other times, air temperature and the other variables were sampled once every 5 min. Set point chamber temperature was 24 + 6(sin 0.5236t) where t is time in hours. Chamber vapor pressure was adjusted to maintain a set point VPD of 1.5 kPa. Set point CO₂ concentration was 330 μmol mol⁻¹ for 8 to 10 h and then 275 + 75(sin 0.5236t + 3.14). Photosynthetic photon flux density was 1400 μmol m⁻² s⁻¹.

to 0.5 kPa. For smaller step changes or higher E, errors were correspondingly lower. We found no significant improvement when the chamber walls were lined with polypropylene (propafilm) indicating that polycarbonate has relatively low water adsorption.

Experiments in which CO₂ evolution was measured following a step change in chamber CO₂ concentration indicated that errors resulting from the storage of CO₂ on chamber surfaces were not significant. We were also able to measure rapid changes in $P_n$ following changes in PPFD (Livingston 1994), indicating that CO₂ storage effects were minimal.
**Effects of increased temperature and vapor pressure deficit**

Figure 5a gives a typical time course of temperature and PPFD used in all experiments. Chamber CO₂ concentration was held constant at 330 μmol mol⁻¹. When the mean (24 h) temperature was increased from 20 to 22 °C and the VPD held constant at 1 kPa, there was a 6 and 8% reduction in daily $P_n$ for white spruce (data not shown) and western red cedar, respectively. However, when the vapor pressure was held constant (at 1 kPa) and VPD was allowed to vary with chamber temperature, there was an even more dramatic drop in daily $P_n$ (Figure 5b) with an increase in air temperature from 20 to 22 °C. This decrease in $P_n$ was a direct result of a corresponding decrease in stomatal conductance. White spruce was slightly less sensitive to increased VPD than western red cedar, and showed a 26% decrease in daily $P_n$. Daily $P_n$ in western red cedar decreased by 31% for the same increase in temperature. Preliminary experiments have shown that, over the short-term, CO₂ concentrations would have to almost double to compensate for the decreased $P_n$ brought about by a 2 °C increase in temperature, if there was no concomitant increase in vapor pressure.

![Figure 5a](image)

Figure 5. (a) Time course of chamber temperature and photosynthetic photon flux density (PPFD). The mean chamber temperature was 22 °C. (b) Time course of net photosynthesis rate ($P_n$) of a two-year-old western red cedar seedling (projected needle area = 0.0485 m²) for two daily mean temperatures (20 and 22 °C) when the vapor pressure was varied to maintain a constant vapor pressure deficit (VPD = 1 kPa) or was held constant at 1 kPa and VPD followed the air temperature. In all cases the diurnal course of PPFD was as in (a).

**Acknowledgments**

This research was supported, in part, by grants from the National Science and Engineering Research Council of Canada, the Science Council of British Columbia and the B.C. Ministry of Forests.
References


Appendix D

Response of transpiration and photosynthesis to a transient change in illuminated foliage area for a *Pinus radiata* D. Don tree
Response of transpiration and photosynthesis to a transient change in illuminated foliage area for a Pinus radiata D. Don tree

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ABSTRACT

Sudden but transient changes in the fraction of illuminated foliage area in a well-watered 7-year-old Pinus radiata D. Don tree were imposed by completely covering either the upper 22% or the lower 78% of the foliage for periods of up to 36 h. Measurements of transpiration flux density (ET), tree conductance (gT), stomatal conductance (gs) and net photosynthesis (A) were made to test the hypothesis that compensatory responses would occur in the remaining illuminated foliage when the cover was installed. When the lower foliage was covered there was an immediate decrease in gs. However, when tree conductance was normalized with respect to the illuminated leaf area (gs*), it increased between 50 and 75%, depending on the value of air saturation deficit (D). The effect was also apparent from concurrent measurements of increases in gT and A up to 59 and 24%, respectively, for needles in the top third of the crown. When the cover was removed these effects were reversed. The changes in the lower foliage when the upper foliage was covered were much smaller. Changes in bulk needle water potential were small. It is suggested that the observed responses occurred because of a perturbation to the hydraulic pathway in the xylem that could have triggered the action of a chemical signal to regulate stomatal conductance and photosynthesis.

Key-words: Pinus radiata D. Don; Pinaceae; conductance; hydraulic effects; photosynthesis; stomata; transpiration.

INTRODUCTION

As the distribution of irradiance and foliage area in canopies varies diurnally and seasonally, changes in stomatal conductance (gs) are linked closely with carbon uptake. Although it has been proposed that the regulation of gs by environmental variables may be such that transpiration is minimized for a given amount of carbon uptake (Cowan & Farquhar 1977), field measurements suggest that this hypothesis does not hold for conifers (Fites & Teskey 1988). Much of the variability in gs can be explained using phenomenological models of stomatal behaviour in relation to environmental variables (Jarvis 1976), and further progress in coupling changes in gs with rates of photosynthesis (A) for individual leaves (Collatz et al. 1991; Leuning 1995) and canopies (Leuning et al. 1995) has been achieved using semi-empirical models that incorporate the physiology of the processes.

Stomatal conductance may also depend on gradients of water potential within the plant. There is a linear dependence of the gradient in water potential between roots and leaves on the flux of water through the plant (Richter 1973), and regulation of the flux by changes in gs maintains leaf water potential within set limits (Sperry, Alder & Eastick 1993). Meinzer & Grantz (1990) showed that stomatal and hydraulic conductances in sugarcane (Saccharum spp., hybrid) are coordinated as a plant develops, to maintain leaf water potential constant over a range of plant sizes and environmental conditions.

It has been proposed that gs is regulated by chemical signals transported in the transpiration stream during plant development (Meinzer & Grantz 1991) or as the soil dries progressively (Davies & Zhang 1991; Tardieu & Davies 1993). The contention is that the chemical signal originates in the roots. Reductions in gs, independent of evaporative demand and bulk leaf water potential, resulted from partially severing the stem, partially removing the roots or cooling the roots in Abies amabilis seedlings (Teskey, Hinkley & Grier 1983). Since the responses occurred within a few minutes of the treatments, they were attributed to hydraulic effects associated with changes in transpiration, rather than the action of a chemical signal. Following Passioua & Munn (1984), pressure applied to the roots of tree seedlings sealed in a chamber reversed rapidly a decrease in gs resulting from soil drying, reduced hydraulic conductance or increased evaporative demand (Fuchs & Livingston 1996; Saliendra, Sperry & Comstock 1995). This suggests that woody plants are not fully responsive to signals generated in the roots. In tall trees, this is possibly because a long time lag would be needed to transmit a chemical signal from the roots to the stomata (Saliendra et al. 1995). However, the rapid transmission of a hydraulic change that triggers the generation of a chemical signal in the leaves to regulate gs is consistent with the observations (Malone 1993; Tardieu & Davies 1993).
Changes in $g_s$ and $A$ in response to hydraulic perturbations resulting from fluctuations in environmental variables could provide a homeostatic mechanism to maintain the water and carbon balances for whole plants. Generally, investigations of homeostatic compensation in $A$ and $g_s$ by the folage remaining on trees after the size of the carbon source is reduced have been restricted to the long-term effects of foliage removal (Tschaplinski & Blake 1989; Syverson 1994; Singh & Thompson 1995). In a recent study with Thuja plicata Donn. (ex D. Don) seedlings, where the illuminated foliage area was reduced by transient shading, the proportional reductions in photosynthesis and transpiration for whole seedling were less than the change in foliage area (Pepin & Livingston 1994). This suggests that compensation in $A$ and $g_s$ occurred in the foliage remaining fully illuminated. These results imply that such compensatory effects could occur in field conditions throughout the day as different parts of the crown become illuminated and shaded. Such effect could be significant when attempting to scale fluxes of CO$_2$ and water vapour from individual leaves to canopies.

In this paper an experimental treatment was designed to manipulate the illuminated proportion of foliage area for a large tree growing in a plantation forest by covering either the upper or the lower branches. Measurements of transpiration, $g_s$, $A$, and water potential were made at short intervals as the treatment was reversibly imposed. The objective was to determine the magnitude of the changes in carbon and water fluxes in relation to the change in foliage area and to elucidate the physiological mechanisms involved.

**MATERIALS AND METHODS**

**Experimental site**

Measurements were made on a Pinus radiata D. Don tree located in Balmoral Forest (lat. 42°50'S, long. 172°42'E, alt. 260 msl), 100 km north-west of Christchurch, New Zealand. The site was flat, consisting of a shallow, stony silt loam soil with a coarse fragment (> 2 mm diameter) fraction of 0.2. Before the trees were planted the site was ploughed to a depth of 1 m.

Mean annual rainfall at the site is 658 mm, uniformly distributed throughout the year, and the mean annual temperature is 10.8 °C with an annual range of 32.5 to −7.8 °C.

The stand of trees was planted in 1987 at a spacing of 2 m within the rows (east-west direction) and 4 m between the rows. At the time of measurements in March 1994 (late summer), the tree crowns within the rows were beginning to overlap near the base, but there was a gap of 1–2 m between the rows where there was no foliage. The tree selected for the study was 7 years old, 6-2 m tall, unpruned with a stem basal (sapwood) diameter of 151 mm. The horizontal area of the tree crown at its widest point projected down to ground level was 4.4 m$^2$. The foliage area for the tree, measured following the procedures of Beets & Pollock (1987), was 1.19 m$^2$, 56% of which had grown during the current year. Foliage areas in this paper are expressed on an all-surfaces basis. A scaffold was erected around the tree to allow access to the upper branches.

Measurements of air temperature, air saturation deficit ($D$, mmol mol$^{-1}$), ambient CO$_2$ concentration ($C_a$) and solar irradiance were made above the tree canopy and recorded as half-hourly averages.

**Measurements of transpiration, stomatal conductance, photosynthesis and water potential**

Transpiration flux density for the whole tree ($E$, mmol m$^{-2}$ s$^{-1}$) was measured as the mass flow of sap in the stem below the lowest branch cluster using the steady-state, null-balance technique (Cermák, Démí & Penka 1973; Pearcy, Schulze & Zimmermann 1989). The sapwood was heated by electrodes to a constant 2 °C ($T_a$) above the temperature of unheated sapwood at the same height but 0.2 m distant ($T_w$), using a continuously recording electronic system (UP Umweltanalytische Produkte, Munich, Germany). If $P$ (s$^{-1}$) is the power input to maintain the temperature difference, then

$$E = kP[L_{c_w}(T_w - T_a)],$$

where $L_a$ is the area of ground occupied by the tree (m$^2$), $c_w$ is the specific heat of water (J mol$^{-1}$ °C$^{-1}$) and $k = C/d(e - 1)$, where $C$, $d$ and $e$ are the stem circumference, the distance between electrodes and the number of electrodes, respectively. The power input is the difference between the power supplied and losses due to convection by sap flow and radial conduction and heat loss from the tree stem to the surrounding air. The stem was insulated 0.5 m above and below the point of insertion of the electrodes with 200-mm-thick fibre-glass covered with aluminized mylar sheeting. Conduction and radial heat losses were assumed to be constant and equal to the minimum power input when sap flow was close to zero at night (Kellihier et al. 1992).

Stomatal conductance ($g_s$, mmol m$^{-2}$ s$^{-1}$) and rates of net photosynthesis ($A$, mmol m$^{-2}$ s$^{-1}$) were measured on current-year needles distributed throughout the upper third of the tree crown using a porometer (model LI-6200 with an 0.25 dm$^2$ chamber; Li-Cor Inc., Lincoln, NE, USA). Twelve shoots growing in all directions around the tree were labelled and needles on these shoots were measured repeatedly. For each measurement, six needles were positioned in parallel across the chamber width in a configuration similar to that described by Edwards (1989). Six shoots were in the upper, fully illuminated part of the crown and six were located immediately below the part of the crown which was covered, at heights between 3-7 and 4-4 and 2-4 and 3-6 m, respectively. Simultaneous measurements of incident quantum irradiance ($Q$) were made with a sensor attached to the chamber. The manufacturer's calibrations of relative humidity, flow rate and temperature were checked independently at the start of the measure-
ment period and the calibrations for CO₂ concentration were checked twice daily.

Measurements of A and gₛ were analysed using the semi-empirical model described by Leuning (1995) which relates stomatal conductance to A and measurements of air saturation deficit (Dₛ) and the CO₂ concentration (Cₑ) at the leaf surface, where

$$gₛ = gₛ₀ + \frac{aA}{(Cₑ - \Gamma)(1 + Dₛ/Dₛ₀)}.$$  (2)

$$gₛ$$ is the stomatal conductance to CO₂ transfer (= gₛ/1 - 6, where 1 - 6 is the ratio of the diffusivities of water and CO₂ in air) and gₛ₀ is the residual conductance at the light compensation point (= 6 mmol m⁻² s⁻¹). \(\Gamma\) is the CO₂ compensation point (= 50 µmol mol⁻¹). Dₛ₀ is a parameter to describe the sensitivity of gₛ to Dₛ and \(a\) is related to the intercellular CO₂ concentration (Cₑ) at saturating irradiance, where \(1/a = 1 - Cₑ/Cₑ^*\).

Water potential was measured on three individual fascicles from shoots located close to those used for the porometer measurements, using a miniature pressure chamber (Roberts & Fourt 1977). The fascicles were removed, sealed in a tube containing moist air and used for the measurements immediately.

**Experimental protocol and the calculation of tree conductance**

Sudden but transient changes in the illuminated foliage area of the tree, lasting for up to 36 h, were imposed during a 14 d period. These changes were made by erecting or removing plastic panels that enclosed either the upper 22% or the lower 78% of the foliage on the tree. The panels were supported from scaffolding surrounding the tree, and they could be erected or removed within several minutes. The panels were white on the exterior and painted black on the interior. They covered the top and sides of the enclosed foliage, leaving a gap at the base to allow air circulation. The top of the lower shade was covered with forest litter to prevent reflection of radiation to the upper foliage. Intermittent measurements showed that the foliage inside the panels was in darkness and air temperature did not increase more than a few degrees above that outside.

During the measurement period the weather was predominantly fine and dry except for parts of 18–20 March, when 48 mm of rain fell, and 26 March, when a further 11 mm of rainfall occurred. Average volumetric water content in the top 0.2 m of soil, measured using time domain reflectometry (Hook et al. 1992), was 0.15 m³ m⁻³ during the period with a range between 0.12 and 0.20 m³ m⁻³. Determination of the water release curves from soil cores in the laboratory indicated that at these volumetric water contents the tree was not under soil water stress.

Using a simplification of the Penman–Monteith equation that is appropriate for aerodynamically rough coniferous forest canopies (McNaughton & Black 1973), tree conductance (gₛ, mol m⁻² s⁻¹) was calculated as

$$gₛ = E/D.$$  (3)

If Lₗ is the area of ground occupied by the tree and Dₛ is the illuminated foliage area then the tree conductance for the tree, normalized with respect to illuminated foliage area, \(gₛ'\), is

$$gₛ' = gₛLₛ/Dₛ.$$  (4)

The terms \(gₛ\) and \(gₛ'\) include stomatal, boundary layer and eddy diffusive aerodynamic conductances since calculations were made between the height of the 'average' stomata in the canopy and the height of the measurement of D above the canopy (Thom 1972). However, further calculations showed that the boundary layer and eddy diffusive conductances were relatively large. Similarly, in Eqn 2 it was assumed that \(Dₛ = D\) and \(Cₑ = Cₑ^*\).

**RESULTS**

During late summer when the measurements were made, days were generally dry and partially cloudy but intermittent periods of rain occurred on three of the days. Maximum air temperature and air saturation deficit reached 25.0 °C and 22.4 mmol mol⁻¹ in the afternoons of 17 and 15 March, respectively. Minimum air temperature was -0.6 °C shortly before dawn on 22 March. Day length was 12 h, starting at about 0700 h NZST.

The vertical distribution of foliage area density and maximum stomatal conductance (gₛmax) for fully illuminated foliage at different depths in the tree crown were determined as part of a companion study (N.J. Livingston et al., manuscript submitted to Plant, Cell and Environment). There was a linear increase in gₛmax from 55 to 77 mmol m⁻² s⁻¹ with increasing height in the crown from 1.1 to 4.4 m, respectively. However, the change in gₛmax with depth in the canopy was proportionally much smaller than the vertical change in foliage area density.

Tree conductance for the fully illuminated tree was high at the morning and decreased throughout the day until early afternoon when a minimum was reached. Maximum gₛ was 1-2 mol m⁻² s⁻¹ and the diurnal decrease closely followed the increase in D (Fig. 1). When the upper 22% of the foliage was covered, there was a very small decrease in gₛ, particularly in conditions in which D was low. This was reversed when the cover was removed, but the effect was somewhat confounded by changes in D (Fig. 1). A clear effect attributable to the presence of the cover was not detectable. However, when the lower 78% of the crown was covered, gₛ was much less and remained roughly constant throughout the day. The early morning peak and the decrease in conductance throughout the day were much less apparent (Fig. 1). When the lower cover was put in place or removed, there was a rapid change in conductance.

In conditions of high irradiance above the canopy (> 500 µmol m⁻² s⁻¹) gₛ decreased with increasing D and
Figure 1. Half-hourly calculations of tree conductance ($g$, from Eqn 3) for six days during the measurement period. Corresponding values of air saturation deficit ($D$) above the tree canopy are also shown. The open symbols are for when the whole crown was uncovered and the closed symbols are for when either the upper 22% (15 and 16 March) or the lower 78% (21, 22 and 23 March) of the foliage was covered.

the slope of the relationship was apparently greater when the tree was not covered (Fig. 2a). However, when conductance was normalized in relation to the fraction of foliage area illuminated ($g'$, Eqn 4), the slopes of the two lines became similar (Fig. 2b). There was some separation of the data for 17 and 23 March when the tree was fully illuminated, and for 21 and 23 March when the lower crown was covered. This was attributable to greater values of $D$ in the later period. However, median $g'$ within each range of $D$ was always greater when the lower part of the tree crown was covered (Fig. 2b, Table 1).

Measurements of stomatal conductance on needles distributed in the upper, fully illuminated crown also showed an increase when the lower crown was covered, supporting

Figure 2. Calculations of (a) tree conductance ($g$, from Eqn 3) and (b) tree conductance normalized with respect to illuminated foliage area ($g'$, from Eqn 4) in relation to air saturation deficit ($D$) when the tree crown was fully illuminated (open symbols) and when the lower 78% of the foliage was covered (closed symbols). The symbols refer to the days shown in Fig. 1. Following Leuning (1995), the lines for $g$ and $g'$ were fitted to the function $g = g_{\text{max}} (1 + D/D_0)^{-1}$ using a non-linear least-squares procedure. The parameters $g_{\text{max}}$ (maximum conductance) and $D_0$ (see Eqn 2) for the fully illuminated crown and for when the lower crown was covered are (a) 3-0, 3-0, 0-5 and 14-4 and (b) 112-3, 3-0, 79-1 and 14-4, respectively.

the calculations of \( g'_s \). However, the increase in \( g_s \) when the lower crown was covered was less than the increase in \( g'_s \) at all ranges of \( D \) (Fig. 3, Table 1). Rates of photosynthesis were greater in needles in the upper crown when the lower crown was shaded (Fig. 4). The increase in median \( A \) reached 24% at saturating irradiance, similar to the increase in \( g_s \) (Table 1). Further analysis of the data following Eqn 2, using values of \( D_p \) obtained from Fig. 3, shows a linear relationship between \( g_s \) and the right-hand-side term including \( A, C_1 \) and \( D \), with no clear separation between the data when the lower crown was fully illuminated and covered (Fig. 5).

When the lower crown was covered or the cover was removed, changes in \( g_s \) and \( A \) in the foliage in the upper and lower parts of the crown occurred rapidly. For example, when the cover was removed after being in place for 1-5 d, on a clear day when irradiance was high and \( D \) was steady between 8 and 10 mmol mol\(^{-1}\), \( g_s \) and \( A \) in the upper part of the crown decreased within 1 min. This was followed by a slight recovery to values lower than those before the cover was removed (Fig. 6). The changes in the lower crown started immediately after the shade was removed but were much slower, with the increase in \( g_s \) continuing for up to 1 h. The increase in \( A \) followed the change in \( g_s \) and reached a new maximum after about 0-75 h.

The changes in \( E, g_s \) and \( A \) were not associated with sudden large changes in needle water potential when the cover was put in place or removed. For example, measurements during the same period as that shown in Fig. 6 confirm that needle water potential in the upper crown continued to fall after the cover was removed, but was similar to measurements made on a control tree nearby (Fig. 7).

**DISCUSSION**

Measurements of \( g_{\text{max}} \) and maximum photosynthetic rate (\( A_{\text{max}} \)) during the period are similar to those reported for

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Table 1. Tree conductance normalised with respect to illuminated foliage area (\( g'_s \), mmol m\(^{-2}\) s\(^{-1}\)), stomatal conductance \( g_s \), mmol m\(^{-2}\) s\(^{-1}\)) and rate of photosynthesis (\( A \), mmol m\(^{-2}\) s\(^{-1}\)) in the fully illuminated upper part of the tree crown, when the lower 78% of the tree crown was illuminated or covered. Values are shown for low and high ranges of air saturation deficit (\( D \)). Measurements of \( g'_s \) and \( g_s \) are restricted to conditions when irradiance was greater than 500 mmol m\(^{-2}\) s\(^{-1}\) and measurements of \( A \) are restricted to conditions when irradiance was greater than 1500 mmol m\(^{-2}\) s\(^{-1}\). Median values are shown with lower (left) and upper (right) interquartile limits in parenthesis.

<table>
<thead>
<tr>
<th>( D ) range (mmol mol(^{-1}))</th>
<th>Fully illuminated</th>
<th>Lower crown covered</th>
</tr>
</thead>
<tbody>
<tr>
<td>( g'_s )</td>
<td>6-10</td>
<td>(26) 31 (36)</td>
</tr>
<tr>
<td>( g_s )</td>
<td>6-10</td>
<td>(49) 60 (77)</td>
</tr>
<tr>
<td>( g_s )</td>
<td>12-16</td>
<td>(24) 45 (61)</td>
</tr>
<tr>
<td>( A )</td>
<td>&lt;16</td>
<td>(34) 4 5 (5 5)</td>
</tr>
</tbody>
</table>

Figure 3. Stomatal conductance (\( g_s \)) in relation to air saturation deficit (\( D \)) for needles distributed between 3-7 and 4-4 m in the top part of the crown when the tree crown was fully illuminated (open symbols) and when the lower 78% of the foliage was covered (closed symbols). The measurements were made using a porometer and the symbols refer to the days shown in Fig. 1. The lines were fitted using the function given in Fig. 2 where the parameters \( g_{\text{max}} \) and \( D_p \) for the fully illuminated crown and for when the lower crown was covered are 169-5, 4-7 and 165-8, 8-6, respectively.

Figure 4. Measurements of photosynthesis (\( A \)) in relation to quantum irradiance (\( Q \)) made with the porometer on the same needles as those shown for \( g_s \) in Fig. 3. The lines were fitted using the non-rectangular hyperbole function of Jones (1983) where the coefficients \( p \) and \( q \) are related to the initial slope and \( A_{\text{max}} \), respectively. For the fully illuminated crown and when the lower crown was covered, the values for \( p \) and \( q \) are 0-028, 10-9 and 0-023, 7-7, respectively.
The measurements of $g_{\text{max}}$ were made on fully illuminated foliage at the branch tips and values of $g_s$ would have been less in the inner, shaded parts of the lower crown. Scaling the measurements of $g_s$ for each layer $i$, of foliage area $L_i$, in the canopy to calculate canopy conductance ($= \Sigma [g_{sL}L_i]$) needs to incorporate the variability between cohorts of foliage when making comparisons with conductances derived from whole-tree measurements of sap flow rate. When the lower 78% of foliage was covered, it is likely that this was equivalent to a smaller reduction in 'effective' foliage area and transpiration. Therefore, part of the apparent change in conductance by the illuminated foliage when the cover was installed (Fig. 1) is probably attributable to the use of actual foliage area in calculating $g_s^*$ (Eqns 3 & 4). However, measurements of $g_s$ and $A$ confirm that compensation effects, independent of errors associated with estimating the distribution of effective foliage area, did occur in the upper foliage. This is consistent with the changes in median $g_s$ being less than those for $g_s^*$ when the cover was installed or removed (Table 1). The differences between these data suggest that the lower 78% of foliage area may have contributed between 55 and 65% of total $E$, being greater when $D$ was high. When the lower

![Figure 5. The relationship between stomatal conductance ($g_s$) and $A/(C_r-F[D/D_0])$ for the data shown in Figs 3 and 4. The terms are defined in Eqn 2 with $C_r = C_r$ and $D_0 = D$. The values of $g_s$ shown are for water vapour transfer. To convert these into values for CO$_2$ transfer ($g_{sCO_2}$) they should be divided by 1.6.](image)

**Table 2.** Values of maximum stomatal conductance ($g_{\text{max}}$) and maximum rate of photosynthesis ($A_{\text{max}}$) for Pinus radiata trees growing in field conditions. Values are presented on an all-surfaces foliage area basis.

<table>
<thead>
<tr>
<th>$g_{\text{max}}$ (mmol m$^{-2}$ s$^{-1}$)</th>
<th>$A_{\text{max}}$ (µmol m$^{-2}$ s$^{-1}$)</th>
<th>Tree age (years)</th>
<th>Conditions</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>67</td>
<td>4.8</td>
<td>9</td>
<td>irrigated</td>
<td>Benecke (1980)</td>
</tr>
<tr>
<td>100</td>
<td>5.4</td>
<td>11</td>
<td></td>
<td>Kuppers et al. (1987)</td>
</tr>
<tr>
<td>49</td>
<td>1.8</td>
<td>5</td>
<td>irrigated</td>
<td>Attiwell et al. (1982)</td>
</tr>
<tr>
<td>95</td>
<td>3.3</td>
<td>7</td>
<td></td>
<td>Sheriff et al. (1986)</td>
</tr>
<tr>
<td>75</td>
<td>-</td>
<td>11</td>
<td></td>
<td>Whitehead &amp; Kellihier (1981)</td>
</tr>
<tr>
<td>125</td>
<td>7.3</td>
<td>14</td>
<td>irrigated</td>
<td>Thompson &amp; Wheeler (1992)</td>
</tr>
<tr>
<td>70</td>
<td>-</td>
<td>7</td>
<td></td>
<td>Whitehead et al. (1994)</td>
</tr>
<tr>
<td>80</td>
<td>6.0</td>
<td>7</td>
<td></td>
<td>This study</td>
</tr>
</tbody>
</table>

Transient changes in illuminated foliage area

![Diagram](image)

Figure 7. Measurements of needle water potential in the upper crown when the crown was fully illuminated (O) and when the lower crown was covered (•) on 22 March. Also shown are measurements made on four adjacent, untreated trees (V). Values shown are means of three measurements ± standard error.

Foliage was covered, it contributed very little to transpiration. The values of $g_s$ measured with the porometer on lower foliage inside the cover were near zero, confirming that the stomata were closed. Some water loss would have occurred through cuticular transpiration, but this would have been small. Air saturation deficit inside the cover was generally lower than that outside and this would have reduced water loss further.

The marked sensitivity of conductance to $D$ (Fig. 3) provides an explanation for the observed variability in $g_s$ within the canopy. Other studies have also shown that $D$ is the principal environmental variable regulating $g_s$ in Pinus radiata, since irradiance is only important when values are less than about 200 $\mu$mol m$^{-2}$ s$^{-1}$ (Thompson & Wheeler 1992; Whitehead et al. 1994). By coupling this response with measurements of photosynthesis, Leuning (1995) quantitatively explored the close relationship between $A$ and $g_s$ at the leaf scale. The lack of separation for the data between the treatments in Fig. 5 suggests that the relationship between $g_s$ and $A$ for foliage in the upper crown remained the same when the lower branches were covered. It follows that the value for $C_1$ did not change with the treatment.

Although $E$ and $g_s$ were reduced when the lower crown was covered, when normalized with respect to the foliage area remaining illuminated, $g_s^1$ increased (Fig. 2). The value of $E$ was relatively conservative throughout the range of $D$ when the lower crown was covered, but the increase in $g_s^1$ was greater when $D$ was low (Table 1). Consequently, under conditions more favourable for stomatal opening, there was greater compensation in $g_s^1$ when the cover was put in place. This suggests that there was a compensatory response to a reduction in carbon fixation resulting from the covering of the lower crown. This response was not transitory but $g_s^1$ remained greater until the effect was reversed by removal of the cover. Calculations of $g_s^1$ on 22 March (Fig. 1) during the period when the cover was removed show that there was a transient overshoot in $E$. This appeared to result from the imbalance between the slower increase in $g_s^1$ for the foliage in the lower crown and the more rapid decrease in $g_s$ for the foliage in the upper crown (Fig. 6).

The compensatory effects were associated principally with the foliage in the upper crown. It is clear from measurements of $g_{\text{max}}$, $A_{\text{max}}$, nitrogen concentration and independent estimates of $C_1$ from measurements of carbon isotope abundance (N.J. Livingston et al., manuscript submitted to Plant, Cell and Environment) that the physiological capacity of the foliage decreased with depth in the crown, even in well-illuminated conditions. The compensation effects were not detectable when the upper 22% of the foliage was covered (Fig. 1), in contrast with those when the lower 78% of foliage was covered. This may be explained by the inability of the lower foliage to respond because of lower physiological capacity (Hinekley et al. 1994).

When the cover was placed over the lower foliage or removed, the change in $g_s$ was very rapid and fully reversible (Fig. 1). The rapid change was clearly due to an increase or decrease in $E$ from the lower foliage, regulated by $g_s$ as shown in Fig. 6. After more than 24 h in darkness, $g_s$ and $A$ in the lower foliage increased immediately when the cover was removed and continued to increase for $\approx 1$ h. Such a slow stomatal response is typical for conifers, where time constants for the change in $g_s$ following a step change in irradiance are up to an 1 h, depending on the length of the previous dark period (Whitehead & Teskey 1995). The decrease in $g_s$ in the upper foliage when the cover was removed was instantaneous, with no time lag, since this was not associated with a change in irradiance or $D$.

Stomatal conductance is regulated by changes in the water flux density within the tree, which accommodates the supply of water from the roots with atmospheric transpiration demand, represented by $D$ (Monteith 1995). The rapidity and reversibility of the stomatal response to covering part of the tree crown provides compelling evidence for a second, co-occurring mechanism to account for the compensation effects, associated with a perturbation in the hydraulic pathway in the xylem (Teskey et al. 1983). The nature and timing of the response is similar to the observed increases in $g_s$ when the roots of woody plants are pressurized, thus reversing the effects of decreased $g_s$ due to dry soil (Fuchs & Livingston 1996), high $D$ or reduced hydraulic conductivity in the stem (Saliendra et al. 1995). Hydraulic signals resulting from sudden changes in transpiration provide a rapid means of communication within the plant, since the velocity of the pressure wave can approach the speed of sound in water (Malone 1993). This could trigger the release of a chemical messenger, probably abscisic acid (ABA), in the apoplast (Hartung & Slovik)
which acts on the guard cells. The rapid nature of the response and the large size of the xylem transport system suggests that it is unlikely that the chemical signal could be activated in the roots and transported to the foliage (Salienla et al. 1995). Maximum water fluxes in the tree stem on 17 and 23 March approached 2 dm$^3$ h$^{-1}$. This was equivalent to a velocity of less than 0.2 m h$^{-1}$. Even if only part of the sapwood conducted water at this rate, the velocity is far too slow to support the contention that a chemical messenger could be transported from the roots to the stomata within a few minutes (Fig. 6). Recent modeling of stomatal response to conditions of soil drying supports an explanation incorporating both hydraulic and chemical signals to account for the observations made on plants during conditions of soil drying (Tardieu & Davies 1993).

Although the changes in needle water potential observed in our tree when the lower branches were covered (Fig. 7) were small, changes in turgor potential, sufficient to result in stomatal opening, could have occurred. Such changes could have been masked by the measurement of bulk leaf water potential using the pressure chamber (Sperry et al. 1993).

Our results suggest that rates of transpiration and photosynthesis in parts of a tree crown may be regulated at different times through the day by hydraulic effects resulting from partial shading of different parts of the crown, by changes in water supply to the roots, and by changes in hydraulic conductance and capacitance of the xylem pathway. Branches may thus not be autonomous in terms of water and carbon balances, although Sprugel, Hinckley & Schaap (1991) concluded that the degree of hydraulic autonomy in branches increases with age.

This may, at least in part, explain the high variability found commonly in relationships between $g_i$, $A$ and environmental variables from field studies. In particular, if different parts of the crown are contributing dynamically to transpiration, hysteresis in the relationships between total transpiration and environmental variables in large trees could develop. This leads to complications when scaling from leaves to canopies using diurnal models of water vapour and carbon dioxide exchange between forest stands and the atmosphere.

CONCLUSION

There were substantial increases in $g_i$ and $A$ in the upper foliage when the lower foliage was covered, and the response was rapid and fully reversible. This effect occurred at the leaf and canopy scales and was attributable to a change in $g_i$ of up to 60%. There was no apparent change in $C$ for the upper foliage when the lower foliage was covered. When foliage in the upper crown was covered, the magnitude of the response in the lower foliage was much less. This suggests that the ability to respond is proportionally related to the physiological capacity of the foliage, reflected in values of $g_{i,max}$, $A_{i,max}$ and nitrogen concentration. This is interpreted as a compensatory response by the tree for the loss of carbon resulting from the temporary reduction in photosynthesizing foliage area. The rapidity of the changes provides strong evidence that the response results from the propagation of a signal along the hydraulic pathway. This possibly triggers the action of a chemical messenger within the foliage that acts on the stomatal guard cells. This effect is likely to occur in different parts of tree crowns as they become shaded throughout the day. This could partly account for the difficulties in modelling diurnal changes in $g_i$ and $A$ and the observations of hysteresis in relationships between $E$ and environmental variables.

ACKNOWLEDGMENTS

Funding for this work was provided by the New Zealand Foundation for Research, Science and Technology and the National Science and Engineering Research Council of Canada. We are grateful to Carter Holt Harvey Forests Ltd. for allowing access to Balmorel Forest, to R. Leunig for helpful discussion and to G.N.D. Rogers for assisting with biomass measurements.

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Received 25 September 1995; received in revised form 16 February 1996; accepted for publication 27 February 1996