INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI®

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

DISSERTATION

Hydraulic Regulation and Control of Photosynthesis in *Pinus ponderosa*

Submitted by
Robert M. Hubbard
Graduate Degree Program in Ecology

In partial fulfillment of the requirements
for the degree of Doctor of Philosophy
Colorado State University
Fort Collins, Colorado
Fall 2000

UMI Number: 3002082

UMI Microform 3002082

Bell & Howell Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

COLORADO STATE UNIVERSITY

August 1, 2000

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY ROBERT MARSH HUBBARD ENTITLED HYDRAULIC REGULATION AND CONTROL OF PHOTOSYNTHESIS IN *Pinus ponderosa* BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Eugene A Kelly

Frederick W Smith

Dan Binkley

Advisor

Michael G Ryan

Co-Advisor

Dan Binkley

Department Head/Director

ABSTRACT OF DISSERTATION

HYDRAULIC REGULATION AND CONTROL OF PHOTOSYNTHESIS IN *Pinus ponderosa*

Foresters and ecologists have long recognized that all trees eventually stop growing significantly taller, and all forests decline in net primary productivity after canopy closure. The hydraulic limitation hypothesis suggests that these patterns can be explained by the fact that as trees grow larger and older, whole tree leaf specific hydraulic conductance (K_L) declines. If stomatal control acts to maintain constant leaf water status, lower K_L will cause lower stomatal conductance and photosynthesis, reducing carbon gain in old, tall trees. I performed field and laboratory studies to test several physiological aspects of this hypothesis and to examine how hydraulics affect stomatal behavior and leaf gas exchange in ponderosa pine. My specific hypotheses were: 1) K_L is lower in tall (~33m and 230 yrs old) compared to short (~10m and 45yrs old) ponderosa pine trees causing decreased stomatal conductance (g_s) and photosynthesis (A) throughout the day; 2) reducing or increasing KL in trees or branches will cause a proportional change in stomatal conductance and photosynthesis and 3) longer path lengths cause K_L to be lower at the tops versus the bottom of open grown, old tree crowns. Our study site was an open grown stand of ponderosa pine growing on the east side of the Oregon Cascades. In testing hypothesis 1, I found that K_L at the end of the summer of 1995 (calculated from leaf water potential and leaf gas exchange measurements on one-year old needles) was 44% lower in old compared to young trees. Whole-tree sap flux per unit leaf area averaged 53% lower in old compared to young trees. In old trees, stomatal conductance (g_s) and assimilation (A) exhibited a steeper decline with air saturation deficit (D) for the entire summer. Mean values for g_s and A were approximately 32% and 21% lower respectively for old and young trees at typical midday D values (2.5-3.0 kPa). Removal of 50% of the foliage from a set of experimental branches on old trees caused g_s and A to decline less steeply with D in early summer, but values were not significantly different from controls in late summer. Cutting transverse notches in branches on young trees had no effect on the response of g_s and A with D. Leaf nitrogen content and photosynthetic capacity were identical suggesting differences in g_s and A between old and young trees were not caused by differences in photosynthetic capacity. These results suggest that lower K_L limits stomatal conductance and photosynthesis in tall, old open grown ponderosa pine at our site.

If stomatal control acts to maintain constant leaf water status during transpiration, and if lower stomatal conductance reduces assimilation, hypothesis two suggests that reducing K_L will result in a proportional decline in g_s and assimilation. To vary K_L, we systematically reduced stem hydraulic conductivity (k) of well-watered ponderosa pine seedlings (*Pinus ponderosa*) using air injection to induce cavitation while simultaneously measuring the response of canopy gas exchange in the laboratory under constant light and D. Short-statured seedlings (< 1 m tall) and hour-long equilibration times promoted steady-state flow conditions. We found that g_s, transpiration, A, and K_L all declined with decreasing k ($P < 0.001$). This response was independent of bulk leaf water potential

which remained constant near −1.5 MPa, except at the extreme 99% reduction in *k* when it fell to −2.1 MPa. Because stomata closed and bulk leaf water potential (Ψ_{leaf}) was maintained as K_L declined, stomatal conductance and assimilation were directly proportional to K_L ($R^2 > 0.90$). These results suggest that stomata in ponderosa pine seedlings exhibit a linear response to changes in K_L and that changes in K_L affect whole plant carbon gain.

To assess the effects of path length within individual tree crowns (hypothesis 3), we measured leaf gas exchange, foliar $\delta^{13}C$, branch sap flux, and K_L on branches at two different heights (~ 10 and 25m) within four tall (~ 30m) ponderosa pine. We found no difference in leaf gas exchange, branch sap flux or K_L between the upper and lower canopy of our study trees. We also found significantly lower leaf area to sapwood ratios (A_l:A_s) in branches from the upper compared to the lower canopy ($P = 0.03$). A_l:A_s averaged 0.17 ($m^2\ cm^{-2}$) and 0.27 in the upper and lower canopy respectively. Leaf specific branch conductance did not differ with canopy height ($P = 0.24$). These results indicate that increases in path length may not cause lower K_L in ponderosa pine at our site because ponderosa pine may mitigate the effects of increased path length by changing the ratio of transpirational to transport tissues.

In general, data from this dissertation support the hydraulic limitation hypothesis. Lower K_L in old compared to young ponderosa was associated with lower g_s and A, and there is convincing evidence from the laboratory study that ponderosa pine stomata regulate to maintain a constant leaf water potential and that the relationship between g_s with K_L is linear. Although changes in the ratio of leaf area to sapwood area may mitigate the effects of increased path length in single ponderosa pine, there is good

evidence that lower K_L causes lower g_s and A in old compared to young ponderosa at our site (this study and Ryan et al. 2000). Explaining these differences will require future research to focus on how other hydraulic properties such as sapwood permeability, increased branch length and, or larger number of branch junctions change with tree age and size.

Robert Marsh Hubbard
Graduate Degree Program in Ecology
Colorado State University
Fort Collins, CO 80523
Fall 2000

Acknowledgements

There are a number of people who contributed mightily to the completion of this work. Mike Ryan, my advisor, deserves special thanks and gratitude. His mentorship and guidance were instrumental in helping me complete this project and I may have learned more from watching him do science than any other single event during my program. In addition, Mike allowed me to enjoy the benefits (and struggles) of full time employment while pursuing a PhD and ultimately juggled the conflicting tasks of boss and advisor with much grace. Barbara Bond, although not an official member of my committee, certainly functioned as a mentor during my program. Barb provided guidance, intellectual and moral support for which I am most grateful. I am especially indebted to both Barb and Mike for providing me the opportunity to work on "their favorite hypothesis". Many thanks to Dan Binkley, my co advisor, who taught me a great deal about thinking critically and not letting alternative explanations get in the way of rigorous hypothesis testing. Likewise, Dan's insights into the science of ecology and his obvious joy in living life as an ecologist has been an inspiration. Thanks also to my committee who provided helpful, critical comments on experimental design and results. Rudy King provided invaluable advice on statistical design and data analysis. Special thanks to John Sperry and Volker Stiller who provided intellectual and logistical support for the experiment described in Chapter III. Thanks also to the rest of the Sperry lab for welcoming me as part of the group. Dave Woodruff and Bob Lewis were exceptional field assistants, worked many long hours and tolerated me as a novice scientist forging

my way through decision making and collecting field data during two field seasons. Thanks to Holly Barnard for moral support and help with lots of logistical details. Thanks to the agencies providing funding for this research: the USDA (NRICGP#9401021 and 97-35101-4376) and the USDA Forest Service, Rocky Mountain Research Station. Finally, to Megan, my wife and best friend. Thanks for all your love, support and for tolerating me through this process. I couldn't have done without you.

Dedication

To Noah Canyon, you are the light of my life

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION 1

CHAPTER II

EVIDENCE THAT HYDRAULIC CONDUCTANCE LIMITS PHOTOSYNTHESIS IN OLD *PINUS PONDEROSA* TREES 4

- Summary 5
- Introduction 6
- Materials and Methods 10
  - Site Description 10
  - Experimental design and treatment installation 10
  - Statistics 14
- Results 15
- Discussion 16

CHAPTER III

STOMATAL CONDUCTANCE AND PHOTOSYNTHESIS VARY LINEARLY WITH PLANT HYDRAULIC CONDUCTANCE IN PONDEROSA PINE 27

- Summary 28
- Introduction 29
- Methods 32
  - Plant material 32
  - Vulnerability curve 32
  - Treatments 33
  - Data Analysis 37
- Results 38
- Discussion 40

CHAPTER IV

THE EFFECT OF BRANCH HEIGHT ON LEAF GAS EXCHANGE, BRANCH HYDRAULIC CONDUCTANCE AND BRANCH SAP FLUX IN OPEN GROWN PONDEROSA PINE 51

Summary....52
Introduction....53
Materials and Methods....55
  Gas Exchange Measurements....56
  Branch Sap Flux Measurements....57
  $\delta^{13}C$ measurements....58
  K_{Ltree} measurements....58
  $K_{Lbranch}$ measurements....58
  Leaf area, sapwood area and $\delta^{13}C$ measurements....59
  Data Analysis....60
Results....61
Discussion....63

CHAPTER V

SUMMARY AND SYNTHESIS....71

Where are we now?....72
Where do we go from here?....74

REFERENCES....80

LIST OF FIGURES

Figure 2.1 Average hourly leaf specific hydraulic conductance (K_L) (A), leaf water potential (ψ) (B), and stomatal conductance (g_s) (C) on days 254-256 for old (closed circles) and young (open circles) trees. Error bars are ± 1 standard error. Average leaf specific hydraulic conductance (LSC) was 44% lower in old compared to young trees ($P < 0.001$). Average LSC for old trees declined slightly ($P = 0.045$) during the day, with most of the decline occurring before 11:00 p.m. Average LSC of young trees did not change significantly throughout the day. Leaf water potential was not significantly different between branches of old and young trees ($P = 0.21$) and average stomatal conductance declined more steeply throughout the day in old compared to young trees ($P< 0.01$). 22

Figure 2.2 Mean hourly whole-tree sap flux per m2 projected leaf area (PLA) during days 254, 255, and 256 for old (closed circles) and young (open circles) ponderosa pine trees. Error bars are ± 1 standard error. Mean sap flux was 53% lower in old trees than in young trees for the three-day time period. 23

Figure 2.3 Mean hourly leaf specific conductance ($K_{L\bullet}$) (calculated from Equation 7) for old (closed circles) and young (open circles) ponderosa pine trees. Error bars represent ± 1 standard error. Mean $K_{L\bullet}$ was 63% lower in old trees than in young trees. The contribution of height to $K_{L\bullet}$ in old and young trees was 20 and 10% respectively. 24

Figure 2.4 (A) Light-saturated stomatal conductance (g_s) and (B) CO_2 assimilation (A) versus air saturation deficit (D) for 1-year old needles on control branches from old (closed circles) and young (open circles) *Pinus ponderosa* trees. The slopes of the responses of g_s and A with D of needles from branches on old trees were significantly more negative than those on young trees ($P < 0.01$). Values of R^2 for g_s versus D for old and young trees were 0.55 and 0.34, respectively. The response of A with D had R^2 values of 0.69 for old trees and 0.43 for young trees. 25

Figure 2.5 Light-saturated response of stomatal conductance (g_s) (Figure A) and assimilation (A) (Figure B) for 1 year old needles on defoliation treatment branches in early summer. Regression line for control branches (solid line) is shown for comparison. The slope of the light saturated response of g_s and A with D in defoliation treatments (triangles) was significantly less negative than control branches ($P < 0.01$). R^2 values for the response of g_s with D of foliage from treatment branches in early summer was 0.42. R^2 values for the response of A with D of foliage from treatment branches in early summer was 0.78. 26

Figure 3.1 Possible steady-state responses of stomatal conductance (g_s) to changes in whole-plant leaf-specific hydraulic conductance (K_L) assuming a) Ohm's law analogy for fluid flow through the soil-plant hydraulic continuum, b) constant soil water potential, c) constant leaf-air vapor pressure deficit, and d) negligible influence of leaf boundary layer on leaf conductance to water vapor. Dotted line: no stomatal response associated with no regulation of bulk leaf water potential (Ψ_{leaf}), solid line: directly proportional stomatal response for g_s below a physiological maximum (g_{max} arrow, y axis) associated with perfect regulation of bulk Ψ_{leaf}, dashed line: curvilinear response associated with progressively stronger regulation of Ψ_{leaf} as it becomes increasingly negative with reduced K_L. 46

Figure 3.2 Vulnerability curve for stem xylem of ponderosa pine seedlings showing mean percent loss of stem hydraulic conductivity ($\%k_{loss}$) (open circles, n= 6) vs. the xylem pressure (Ψ_{xylem}). The best fit to the vulnerability curve (solid line) was a Weibull function : $\%k_{loss} = 100 - 100 * e^{(-(-P_{total}/b))^{f}}$ (see text for parameter values). Solid triangles represent mean percent loss of stem hydraulic conductivity measured after air injection (n = 2) at the actual pressure difference across the pit membrane (air injection pressure - Ψ_{xylem}). This pressure difference is equivalent to the Ψ_{xylem} axis of the vulnerability curve which assumes ambient air pressure. Dotted lines represent 95% prediction limits for the Weibull fit. 47

Figure 3.3 Solid line: typical pattern of stomatal conductance (g_s) and assimilation (A) in response to each injection pressure (3.5, 4.5, and 5.5 MPa, duration indicated on upper x axis) (solid line). Dotted line: Typical control plant (n=3) showing the response of g_s and A to bark and phloem removal plus injection chamber installation (but no injection). 48

Figure 3.4 Mean leaf water potential (Ψ_{leaf}, open circles), stomatal conductance (g_s, closed circles), transpiration (E, closed triangles), assimilation, (A, closed diamonds), and leaf specific hydraulic conductance (K_L, closed squares) versus predicted percent loss of stem hydraulic conductivity (n = 6). Error bars are ± 1 *se*. 49

Figure 3.5 Stomatal conductance (g_s) and assimilation (A) versus whole plant leaf specific conductance (K_L) for each seedling (n=6) at each injection pressure. Different symbols represent each separate seedling. The regression equations are $g_s = 4.5 + 496.86 * K_L$, and $A = 4.06 + 64.79 * K_L$. R^2 for g_s and A versus K_L is 0.91 and 0.90 respectively. The intercept was not different from zero ($P > 0.05$) and the slope did not differ from ($\Psi_{soil} - \Psi_{leaf}$)/leaf to air mole fraction difference (c. 0.032) ($P > 0.05$). 50

Figure 4.1 Stomatal conductance (g_s) and net photosynthetic assimilation (A) versus air saturation deficit (D) by canopy position for three ponderosa pine at the Black Butte study site. All measurements were averaged by 0.25 D class increments. The response of foliage from the upper and lower canopy did not differ for g_s ($P = 0.82$) or A ($P = 0.35$). R^2 for g_s in the upper canopy was 0.43 and 0.46 for the lower canopy. R^2 for A was 0.44 and 0.51 in the upper and lower canopy respectively. Error bars are ± 1 standard error for the variation among trees. 67

Figure 4.2 Mean hourly branch sap flux per unit leaf area (Q_L), per unit sapwood area (Q_{sw}) and mean hourly branch conductance (G_t) for days 191 – 211. Fifteen minute values were average by hour for days 191-211. Differences with canopy position were not significant for any of the three parameters ($P = 0.62$, $P = 0.12$, and $P = 0.98$ for Q_L, Q_{sw} and G_t respectively. Error bars are ± 1 standard error for the variation among branches. 68

Figure 4.3 Mean hourly sap flux per unit leaf area (Q_L) and whole branch conductance (G_t) versus D for days 191 – 211. Fifteen minute values were average by 0.25 kPa class. There was no significant difference with canopy position for either parameter ($P = 0.41$ and $P = 0.77$ for Q_L and G_t respectively). Error bars are ± 1 standard error. 69

Figure 4.4 Mean leaf specific conductance branch conductance (K_{Ltree}) (A) and mean leaf area to sapwood area ratio A_l:A_s (B) at the top and bottom of the canopy. K_{Ltree} did not differ with canopy position ($P = 0.48$) and A_l:A_s was significantly lower at the top versus bottom of the canopy ($P = 0.03$). Error bars are ± 1 standard error. 70

CHAPTER I

Introduction

Most forests exhibit a decline in net primary productivity (NPP) after canopy closure and all trees eventually stop growing significantly taller. Although forest ecologists universally recognize these patterns of forest and tree growth, we have not found a conservative, explanatory mechanism. Historically, increased woody tissue respiration costs was attributed to the decline in NPP (Whittaker and Woodwell 1967, Yoda et al. 1965) but this view is not supported by the available evidence (Ryan and Waring 1992.) Other possible causes include decreased nutrient supply (Binkley et al. 1995, Murty et al. 1996), increased allocation to below ground structures (Gower et al. 1995, Grier et al. 1981) and reduced leaf area (Long and Smith 1992). A more detailed treatment of possible mechanisms for declining NPP in closed canopy forests and cessation of height growth is given in Ryan and Yoder (1997), Ryan et al. (1997) and Gower et al. (1996).

Ryan and Yoder (1997) have recently offered the hydraulic limitation hypothesis to explain cessation of height growth and the decline in NPP in closed canopy forests. The hydraulic limitation hypothesis suggests that leaf-specific hydraulic conductance is lower in older, taller forests because the flow path from soil to leaves increases with height and branch length. Consequently, if stomata regulate transpiration to maintain leaf water potential from falling below a cavitation threshold (Jones and Sutherland 1991, Tyree and Sperry 1988) and the minimum Ψ_{leaf} is the same for any sized tree, stomata in taller trees will close earlier in the day relative to shorter, younger trees, decreasing their overall net carbon gain. Evidence supports age and height related declines in leaf specific hydraulic conductance (Mencuccini and Grace 1996a, Pothier et al. 1989b, Schäfer et al. 2000) and a large body of work has focused on stomatal response to environmental and physiological factors in an effort to explain stomatal behavior. Much of this later work is consistent with the hypothesis that stomata close in response to factors that will cause xylem water potential to fall below a cavitation threshold. Because changes in leaf specific hydraulic conductance can affect plant water status, stomata have been found to respond to experimental manipulations of hydraulic conductance involving induction of xylem cavitation (Sperry and Pockman 1993), root pruning (Meinzer and Grantz 1990, Teskey et al. 1983), notching of stem xylem (Sperry et al. 1993), freeze-thawing of stems (Hammel 1967), and defoliation (Pataki et al. 1998). When it has been measured, the response occurs within minutes of the manipulation, and bulk leaf water status can remain nearly constant during the experiment (Saliendra et al. 1995, Sperry et al. 1993, Teskey et al. 1983).

This dissertation tested some of the physiological underpinnings of the hydraulic limitation hypothesis and more broadly, examined fundamental aspects of how plants regulate stomatal conductance and the effect this has on plant carbon gain. Specifically, I tested three hypotheses: 1) leaf specific hydraulic conductance is lower in tall, old (~33m and 230 yrs) compared to younger, shorter (~10m and 45yrs) ponderosa pine trees causing decreased stomatal conductance and photosynthesis throughout the day; 2) altering leaf specific hydraulic conductance in trees or branches will cause a proportional change in stomatal conductance and photosynthesis and 3) a longer path length causes leaf specific hydraulic conductance to be lower at the tops than the bottom of old tree crowns resulting in lower stomatal conductance and assimilation.

Chapters two through four are individual studies with ponderosa pine that describe the results of this hypothesis testing. Chapter two describes a field study designed to determine if hydraulic limitation to photosynthesis occurs at the tops of tall old ponderosa pine. In this study, I determined the diurnal relationship of leaf gas exchange at the tops of tall and short ponderosa with air saturation deficit (*D*) and manipulated the leaf specific hydraulic conductance of branches to see if this altered leaf gas exchange. Chapter three describes a laboratory experiment that defined the relationship of stomatal conductance and photosynthesis with leaf specific hydraulic conductance in ponderosa pine seedlings. The study described in chapter four examined the effect of path length on stomatal conductance, branch sap flux and carbon gain in single trees. I also determined how changes in hydraulic architecture such as the ratio of leaf area to sapwood area might mitigate the effect of increased path length. Finally, I provide a brief summary and suggestions for future work to more fully test the hydraulic limitation hypothesis and determine its applicability across species.

CHAPTER II

Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees.

ROBERT M. HUBBARD

USDA Forest Service
Rocky Mountain Research Station and
Graduate Degree Program in Ecology, Colorado State University
240 West Prospect Rd
Fort Collins, CO 80526

BARBARA J. BOND

Oregon State University
154 Peavy Hall
Corvallis OR 97331

MICHAEL G. RYAN

USDA Forest Service
Rocky Mountain Research Station and
Graduate Degree Program in Ecology, Colorado State University
240 West Prospect Rd
Fort Collins CO 80526

1999 Tree Physiology (19) 165-172

Summary

We tested the hypotheses that hydraulic conductance is lower in old (ca.250 y, 30m tall) compared to young (ca. 40y, 10m tall) *Pinus ponderosa* Laws. trees and that lower hydraulic conductance of old trees reduces their photosynthesis. Hydraulic conductance at the end of the summer of 1995 calculated from leaf water potential and leaf gas exchange measurements on one-year old needles was 44% lower in old compared to young trees growing in a mixed age-class stand on the east slope of the Oregon Cascades. Whole-tree sap flux per unit leaf area averaged 53% lower in old trees compared to young trees and mean hydraulic conductance calculated from sap flux and water potential data was 63% lower in old trees than in young trees. For the entire summer, stomatal conductance (g_s) and assimilation (A) declined much more steeply with air saturation deficit (D) in old trees than in young trees. For both old and young trees, mean g_s and A were approximately 32% and 21% lower, respectively at typical midday D values (2.5-3.0 kPa). We hypothesized that if hydraulic conductance limits g_s and A, then increasing or decreasing the leaf specific conductance of a branch would result in proportional changes in the response of g_s and A with D. Removal of 50% of the foliage from a set of experimental branches on old trees caused g_s and A to decline less steeply with D in early summer, but values were not significantly different from controls in late summer. Cutting transverse notches in branches on young trees had no effect on the response of g_s and A with D. Leaf nitrogen content and photosynthetic capacity were identical suggesting that differences in g_s and A between old and young trees were not caused by differences in photosynthetic capacity.

Introduction

Water transport in trees is regulated by the hydraulic conductance of the soil-root-shoot-leaf pathway, and whole-tree hydraulic conductance is a function of the path lengths and permeability of these tissues. Because stomatal conductance (g_s) and photosynthesis (A) depend on the transport of water from soil to leaf to atmosphere, changes in whole-tree hydraulic conductance may affect leaf gas exchange.

Leaf specific hydraulic conductance of the flow path from soil to leaf (K_L), can be described by Darcy's law as

$$K_L = F_L / (\psi_{soil} - \psi_{leaf}) \qquad (1)$$

where F_L is the flux of liquid water per unit leaf area in the xylem tissue, and ψ_{soil} and ψ_{leaf} are the soil and leaf water potentials, respectively. For conifer leaves that are well coupled to the atmosphere and when leaf temperature is equal to air temperature, the transpirational flux from leaf to atmosphere can be described as

$$E_L = g_s D \qquad (2)$$

where E_L is the transpiration flux per unit leaf area, g_s is stomatal conductance to water vapor per unit leaf area and D is the air saturation deficit. Under steady state conditions, F_L is approximately equal to E_L such that K_L can be described by combining equations 1 and 2

$$K_L = g_s D / (\psi_{soil} - \psi_{leaf}) \qquad (3)$$

Trees undergo significant changes in size, growth form and physiology as they proceed from seedlings to maturity to old age (Ryan et al. 1997, Ryan and Yoder 1997). If these changes significantly reduce K_L, then rearranging equation 3 to solve for g_s

$$g_s = K_L (\psi_{soil} - \psi_{leaf})/D \tag{4}$$

predicts that g_s will be reduced in old compared to young trees (if the water potential gradient and vapor pressure deficits are equal). Therefore, effects of hydraulic conductance on g_s may be important when comparing net carbon assimilation (A) in old and young trees because stomata control the flux of CO_2 from the atmosphere into the leaf.

Tree age and height may both affect K_L (hereinafter we refer only to "old trees" and "young trees", recognizing that size increases along with age). Old trees often have lower assimilation rates per unit leaf area than young trees (Grulke and Miller 1994, Kull and Koppel 1987, Schoettle 1994, Yoder et al. 1994) and there is evidence that whole tree hydraulic conductance declines as trees grow older and larger (Köstner et al. 1996, Mattson-Djos 1982). The lower hydraulic conductance in older trees may be caused by the increased length of the pathway that water must travel from soil to leaf caused by increased height and branch length (Waring and Silvester 1994). Other possible causes include lower xylem conductivity in roots, stems, and branches, as well as changes in root surface area and permeability. Ryan and Yoder (1997) hypothesized that with diurnal increases in D, lower hydraulic conductance of old trees causes stomatal closure earlier in the day in old trees compared to young trees, leading to lower assimilation rates in the old trees (the hydraulic limitation hypothesis). They suggested that hydraulic limitation may explain both why all trees eventually reach a maximum height as well as the decline in forest stand net primary production commonly observed after canopy closure.

Several studies have shown that changes in K_L can affect stomatal behavior. Reducing whole plant hydraulic conductance by notching (Sperry and Pockman 1993), injecting air into xylem conduits (Sperry et al. 1993), or root pruning (Teskey et al. 1983) resulted in almost immediate stomatal closure. Other studies have shown that experimental alterations in leaf turgor induced by root pressurization, independent from soil or atmospheric water status, results in reversible changes in g_s (Fuchs and Livingston 1996, Saliendra et al. 1995). Furthermore, improving K_L of foliage either by foliage removal (Meinzer and Grantz 1990) or by shading (Whitehead et al. 1996) results in increased g_s and assimilation (A).

To understand how K_L can limit g_s and A in older, larger trees consider how hydraulic conductance changes with path length. Pothier et al. (1989b) described hydraulic conductance (K) as:

$$K = (k_s A)/(L\eta) \qquad (5)$$

where k_s is the sapwood permeability, A is sapwood cross-sectional area per unit leaf area, L is the length of the flow path and η is the viscosity of the xylem water. Consider two hypothetical trees, Tree A is 10m tall and Tree B is 40m tall. Each tree is identical with respect to the parameters in equation 5 except for L such that hydraulic conductance for Tree B is 4 times lower than Tree A. If ψ_{soil} is 1 MPa, Tree B would have to maintain a ψ_{leaf} that is approximately 4 times lower than Tree A to maintain the same flux of water from soil to leaf (Equation 1). However, there is a limit to the negative pressure that can be maintained in the xylem. Significant negative pressures do occur in xylem tissue (Holbrook et al. 1995, Pockman et al. 1995) but it has been suggested that stomata close when leaf water potential reaches a critical value beyond which the water column will cavitate (Jones and Sutherland 1991, Tyree and Sperry 1988). If this is true, Tree B in our example cannot maintain a ψ_{leaf} 4 times lower than that of Tree A and must reduce its stomatal opening to prevent cavitation which in turn limits the diffusion of CO_2 into the leaf.

Understanding the link between hydraulic conductance and photosynthetic performance of old and young trees may be particularly important for modeling forest growth and response to climate change. Physiological forest process models for trees or stands typically simulate A and g_s using mechanistic or empirical sub-models and scale up to the canopy level using leaf area, e.g. BIOMASS (McMurtrie et al. 1992), FOREST-BGC (Running and Smith 1991, Running and Coughlan 1988), HYBRID (Friend and Schugart 1993), FORGRO (Mohren et al. 1984) and MAESTRO (Wang and Jarvis 1990). An assumption common to all of these models (but see Williams et al. 1997) is that foliage from old and young trees of the same species, growing on the same site, are identical with respect to their photosynthetic response to air saturation deficit (given equal illumination, leaf nitrogen concentration, and energy balance). However, if K_L is lower in old compared to young trees, then assimilation per unit leaf area may be different for old and young trees and may significantly affect annual carbon gain.

Our objective in this study was to estimate K_L of old and young *Pinus ponderosa* (Laws.) trees using leaf gas exchange and leaf water potential measurements and determine if tree age and size affects K_L and in turn g_s and A. We also experimentally altered K_L of branches on old and young trees and determined the effect on g_s and A.

Our specific hypotheses are:

1) Leaf specific hydraulic conductance is lower in old compared to young trees causing g_s and A in old trees to be significantly lower throughout the day.

2) Increasing the K_L of branches on old trees by removing foliage will shift the short-term response of g_s and A of the remaining needles toward that of the young trees. Likewise, reducing the K_L of branches on young trees by notching will shift the short-term response of g_s and A toward that of old trees.

Materials and Methods

Site Description

Our study site, which has been described by Yoder et al. (1994) is a mixed-age ponderosa pine stand near Black Butte, OR (N44° 25′ W121° 40′). The site is located at an elevation of 1032 m with deep volcanic ash derived soils. The site receives most of its annual 450mm of precipitation during the winter months, and summers are hot and dry with long periods of consecutive sunny, cloudless days. Many years of partial harvesting and thinning at this site have resulted in an open stand and most trees receive full sunlight throughout the day. Tree height ranges from 3 to 35m and tree age ranges from less than 30 to greater than 250 years old.

Experimental design and treatment installation

We selected two old and three young trees for our experiments. The old and young trees were located approximately 50 m apart. The old trees had a mean height of

33m and were 230 years old whereas the young trees had a mean height of 12m and were 40 years old.

We used scaffolding to sample the upper third of the canopies, and installed three treatments (control, 50% defoliation of a branch, and 4 notches cut through 50% of the xylem tissue) on south-facing branches of old and young trees in late June 1995. Each treatment was replicated on four branches for a total of twelve experimental branches in old and young trees. For the defoliation treatments, we removed every other fascicle in all needle age classes except current-year needles. We did not remove current year needles because the buds were just beginning to expand at the time of treatment installation and removing any needles would have damaged those remaining. The amount of leaf area removed (50% of the June 1995 leaf area) was verified by determining the dry weights of removed needles and of the remaining needles at the end of the experiment. We decreased K_L using a notching technique described by Sperry et al. (1993). The notching treatment used four overlapping transverse notches through 50% of the branch diameter at the base of each branch. Wooden dowels prevented the wind from breaking the notched branches.

To test Hypothesis 1, we collected water potential and gas exchange data in September 1995 (days 254-256). We measured both ψ_{leaf} and predawn ($\psi_{predawn}$) water potential using a pressure chamber (PMS, Corvalis OR) for individual fascicles on the treatment branches concurrently with the leaf gas exchange measurements described below. We used $\psi_{predawn}$ from the young trees (adjusting for gravitational potential) to estimate ψ_{soil} for both old and young trees. Based on the water potential, leaf gas exchange data, and measured D at our site, we used Equation 3 to calculate K_L for the flow path from soil to leaf for old and young trees. We determined the effect of gravity on K_L by subtracting the gravitational component from equation 3 as

$$K_L = g_s D / (\Psi_{soil} - \Psi_{leaf} - h\rho g) \quad (6)$$

where hρg is the gravitational pull on a column of water of height, h and density ρ.

Additionally, we measured whole tree sap flux at the base of each of our experimental trees using the heat balance method (Cermák et al. 1973, Cermák and Kucera 1981). We measured sap flux every minute and stored 15-minute means using a P6 sap flux meter and data logger (Ecological Measuring Systems Ltd., Brno, Czech Republic). We insulated the gauges 0.5m above and below the measurement point with foam and reflective aluminum sheeting to protect the measurement point from ambient temperature fluctuations. Plastic sheeting sealed to the trunk covered the insulation to protect the gauges from moisture.

Sap flux and water potential data were used to calculate an independent estimate of hydraulic conductance in old and young trees. We calculated the leaf specific hydraulic conductance from soil to leaf ($K_{L}\bullet$) as:

$$K_{L}\bullet = F_{tree} / (\Psi_{soil} - \Psi_{leaf}) \quad (7)$$

where F_{tree} is mean hourly sap flux (kg h-1 m-2 of projected leaf area (PLA)). We did not make adjustments for lag times between sap flux and transpiration (e.g. Čermák et al. 1982) because sap flux did not change significantly for the hours for which we had water potential data (0900-1500). We assumed that Ψ_{leaf} was similar throughout the canopy and determined the effect of gravity on $K_{L}\bullet$ as in Equation 6.

To estimate leaf area for old and young trees, we developed a branch diameter and leaf area relationship at a logging site near our experimental area. We sampled 4 branches each from the upper, middle, and lower portions of the canopy of freshly cut ponderosa pine that were similar in size to our experimental trees. We measured branch diameter and determined dry weight of all the needles on the branch. Leaf area for individual fascicles was determined for the sampled branches by a regression of weight and leaf area on a sub-sample of 10 needles per age class from each branch. We determined leaf area for our experimental trees by measuring the branch diameter of every branch on old and young trees. The leaf area for each branch was determined using separate regression equations for old and young trees described above and summed for the entire tree.

If water supply to the foliage of old trees is reduced relative to young trees, photosynthesis will be more limited by stomatal conductance than by enzymatic capacity for photosynthesis. Therefore older trees should show less discrimination to ^{13}C and the δ^{13}C value of the cellulose in old tree foliage would be higher (less negative) than that of young trees (e.g. Evans et al. 1986, Farquhar et al. 1989). We extracted cellulose from foliage on control branches in old and young trees with a method described by Wise et al. (1945). These branches were in well-ventilated canopies and the branches received minimum shading throughout the day. The extracted cellulose was analyzed by mass spectrometry at the Stable Isotope Facility at the University of Waikato, New Zealand.

To determine the response of leaf gas exchange with D, we measured g_s and A diurnally on 1-year old foliage of control and treatment branches. Measurements were taken July through September on year days 197-199, 210-212, 226, 244 and 254-256.

We began each set of measurements at approximately 0700h and measured gas exchange hourly until approximately 1600h, unless clouds or high winds caused us to stop measuring early. We measured photosynthesis concurrently on old and young trees with two open system infrared gas analyzers (Field et al. 1989, LCA3 or LCA4, Analytical Development Company). Before each set of diurnals, the gas analyzers were calibrated with standard gas for CO_2, and a dew point generator (LI-610, LI-COR Inc.) for H_2O vapor. Because photosynthesis in ponderosa pine saturates at photosynthetically active radiation (PAR) above 800 μmol m^{-2} (Hadley 1969), we measured photosynthesis at PAR greater than 1000 μmol m^{-2} on foliage that was in full sunlight for at least 20 minutes prior to measurement. We determined leaf temperature in the cuvette by energy balance and determined the response of g_s and A with D using the natural variation of D in a day. We measured three control and three treatment branches for each set of hourly diurnal measurements.

Statistics

For our estimates of K_L and $K_{L\bullet}$, we used the mean hourly values for all three days of our gas exchange, water potential and sap flux data (Days 254-256, Equations 3 and 7). Significant differences ($\alpha = 0.05$) were determined by one way analysis of variance (Neter et al. 1990).

To test Hypotheses 1and 2, we examined the response surfaces of g_s and A with D for old and young control and treatment branches. We examined several non-linear models but found that a linear regression model best described our data. We tested for differences in slopes using analysis of co-variance and determined the significance ($\alpha = 0.05$) of the interaction between the regression lines of interest (Neter et al. 1990). One of the assumptions of linear regression is that the y-values are statistically independent of one another. Residuals for regressions of g_s and A with D were randomly scattered around zero indicating no bias from lack of independence.

Results

At the end of the summer (Days 254-256), mean K_L calculated from leaf gas exchange, ψ_{leaf} and ψ_{soil} measurements (Equation 3) was 51% lower in old trees compared to young trees (Figure 2.1A, $P < 0.01$). The effect of gravity accounted for approximately 20% of K_L in old trees compared with 10% in young trees.

Mean daily sap flux per unit leaf area during Days 254-256 was 53% lower in old trees than in young trees (Figure 2.2, $P < 0.01$) and $K_{L\bullet}$ calculated from sap flux measurements and water potential data (Equation 7) was 62% lower in old trees compared to young trees (Figure 2.3, $P < 0.001$). Mean projected leaf area was 150 m^2 for old trees and 24.9 m^2 for young trees. Leaf area to sapwood area ratios were significantly lower in old compared to young trees (0.09 and 0.14 m^2 /cm2 sapwood, respectively). The effect of gravity on $K_{L\bullet}$ was similar to its effect on estimates of K_L- approximately 20 and 10% of old-tree and young tree $K_{L\bullet}$, respectively. Both estimates of leaf specific conductance (K_L and $K_{L\bullet}$) indicated lower conductance in old trees than in young trees: however, K_L averaged 50 and 37% higher than $K_{L\bullet}$ for old and young trees, respectively.

During the summer, g_s declined more steeply with D in old trees than in young trees (hypothesis 1) (Figure 2.4A, $P < 0.01$). Because CO_2 diffusion into the leaf is limited by reduced g_s (Wong et al. 1985), we also observed a decline in carbon assimilation with increasing D in both old and young trees, with a significantly steeper decline in old trees

(Figure 2.4B, $P< 0.01$). At typical mid-day D values (2.5 to 3.0 kPa), mean g_s and A were 32% and 21% lower respectively in control branches of old trees compared to young trees. At the end of the summer, we found that the mean $\delta^{13}C$ of cellulose taken from foliage on control branches was significantly higher in old compared to young trees $-23.4^{o}/_{oo}$ and $-25.9^{o}/_{oo}$ ($P < 0.01$).

Defoliation treatments of branches on old trees resulted in a short-term increase in the response of g_s and A with D on the remaining needles on the branch (Figure 2.5A, 2.5B $P < 0.01$). However, measurements of g_s and A later in the summer showed no difference between treatment and control branches ($P > 0.05$). Defoliation treatments of branches on young trees did not change the response of g_s and A with D relative to controls ($P > 0.05$, data not shown).

Our efforts to reduce K_L by notching branches in old and young trees did not yield expected results. Notched branches on young trees showed the same response of g_s and A with D as controls (data not shown $P > 0.05$). Notching treatments on old tree branches caused two of the replicates to die and the remaining two showed no response to treatment.

Discussion

Our estimates for K_L and $K_{L\bullet}$.were much lower in old compared to young trees (Figure 2.1A and 2.3), supporting our first hypothesis. However for both K_L and $K_{L\bullet}$, the effects of gravity only accounted for 10%-20% of the differences between old and young trees. We suggest that the longer path length that water must flow from soil to leaf in the older, taller trees causes the remaining 80% to 90%. Old trees in this study were approximately three times taller than young trees and supported longer branches throughout the crown. Furthermore, Zimmerman (1983) has shown that branch junctions have lower conductivity when compared to the rest of the flow path such that the larger number of branch junctions in old trees may also contribute to lower K_L in old trees.

Estimates of K_L (estimated from gas exchange measurements) were 50% and 37% higher than estimates of $K_{L\bullet}$ (estimated from whole tree sap flux) for old and young trees, respectively. The gas exchange measurements were made on 1-year-old needles, whereas whole-tree sap flux measurements estimated flow to the entire canopy, including older age class needles. We suggest the lower estimate for $K_{L\bullet}$ compared with K_L reflects higher resistance of the soil to leaf pathway for the > 1-year-old needles.

Mean daily sap flux per unit leaf area (F_L), was significantly lower in old compared to young trees (Figure 2.2). Because water and carbon cycles are linked through canopy conductance, differences in F_L suggest lower canopy carbon gain in old compared to young trees. Additionally, equation 1 (using our estimates of ψ_{soil} and ψ_{leaf}) suggests that these differences are caused by lower mean K_L in the old trees than in the young trees.

Our measurements of leaf gas exchange throughout the summer show that g_s and A decline more steeply in old compared to young ponderosa pine trees supporting our first hypothesis (Figure 2.4A and 2.4B). Taken together with our estimate of K_L and the higher $\delta^{13}C$ values in old tree foliage, these data suggest that hydraulic conductance limits photosynthesis in old ponderosa pine. However the possibility exists that differences may be partially attributable to differences in ψ_{leaf} or in photosynthetic capacity between old and young trees at our site.

We did not measure Ψ_{leaf} with our early season measurements of leaf gas exchange so we can only speculate that the pattern of lower leaf specific hydraulic conductance in old tree was present in early as well as late summer (Figure 2.1A). However, Yoder et al. (1994) found that diurnal leaf water potential did not vary significantly between old and young ponderosa pine trees at our experimental site in early August. Additionally, in a separate experiment, there was no significant difference in early season predawn and midday Ψ_{leaf} between old and young ponderosa pine trees at a nearby study site (Ryan et al. 2000). Based on these findings and our data at the end of the summer that directly links a steep diurnal decline in leaf g_s of old trees with decreasing hydraulic conductance (Figures 1A, 1C and 3), we suggest that the pattern in Figure 2.4 is most probably caused by differences in hydraulic conductance, not differences in Ψ_{leaf}.

Differences in the response of g_s and A with D for old and young trees do not appear to be the result of differences in photosynthetic capacity. Photosynthetic capacity is strongly linked to leaf nitrogen content because of the large nitrogen content of Rubisco, the primary carboxylating enzyme in C_3 photosynthesis (Field and Mooney 1986). We found no significant difference in leaf nitrogen concentration of 1-year old needles from young and old trees (1.12 and 1.07% respectively, $P > 0.05$). Similarly, in a separate study, we found no significant difference in leaf nitrogen concentration or specific leaf area between old and young trees at our site (Bond et al., unpublished data). According to the Farqhuar model of photosynthesis (Farquhar et al. 1980), the initial slope of assimilation (A) versus the internal CO_2 concentration of the leaf (C_i) is determined by the efficiency of carboxylation by Rubisco. Yoder et al. (1996) showed no significant difference in the initial slope of A/C_i curves from foliage of our experimental trees suggesting there is no difference in Rubisco activity of foliage between old and young trees at our site.

Lower g_s and A for values of D above 1.0 kPa indicate that there may be a significant difference in annual carbon gain between old and young ponderosa pine if a large potion of the growing season experiences saturating irradiance and high air saturation deficits. PAR values were greater than saturation (800 µmol m^{-2}) for approximately 72% of the 1996 growing season (April 1-September 31). During 32% of the time when irradiance was above 800 µmol m^{-2}, D was greater than 2 kPa. Calculations from the relationships in Figure 2.3 suggest that g_s and A for a sunlit needle at the top of an old tree would be at least 22% and 11% lower respectively than a sunlit needle at the top of a young tree when $D = 2.0$ kPa. Therefore, lower g_s and A for the old tree needle would be apparent for at least 25% of the growing season with actual differences being proportionally larger at higher air saturation deficits. These differences may translate into significantly lower annual carbon gain for old trees and forests depending on the light environment of the canopy and how K_L changes with canopy position.

If the lower g_s and A in old trees is a result of lower hydraulic conductance, then experimentally increasing the K_L of individual branches on old trees should cause an increase in the response of g_s and A with D (Equation 4, Hypothesis 2). For old trees in early summer, the slopes of g_s and A versus D for defoliation treatments was significantly less than that of controls (Figure 2.5A, and 2.5B) There was no difference between defoliation treatments and controls for young trees (data not shown).

We did not measure ψ_{leaf} concurrently with our leaf gas exchange measurements until the end of the experiment; however, during the early season measurements, Ψ_{leaf} of treatment branches was either lower, higher, or similar to that of the control branches. If ψ_{leaf} for treatment branches was lower than that of the controls, g_s may have increased as a result of the increase in $\Psi_{\mathrm{leaf}} - \Psi_{\mathrm{soil}}$ (Equation 4) and we could observe the same pattern present in Figures 5A and 5B. If Ψ_{leaf} for treatment branches was higher or equal to control branches, then the K_L of the remaining foliage on defoliation treatment branches must have been increased by the treatment (Equation 4), causing the observed pattern of increased g_s and A. Because we did not measure ψ_{leaf}, these data are only consistent with our hydraulic limitation hypothesis and not a direct test. However, recent experiments on ponderosa pine in the foot hills near Fort Collins, CO suggest that removing needles on individual branches has no effect on $\psi_{\mathrm{pre\text{-}dawn}}$ or mid-day ψ_{leaf} of the remaining foliage (Hubbard, unpublished data).

Late in the summer there was no significant difference in the response of g_s and A with D between defoliation treatments and controls. The seasonal response of the defoliation treatment may be because we did not remove current-year needles as part of our defoliation treatments. Removing 50% of the leaf mass on defoliation treatment branches at the beginning of the summer resulted in a 50% increase in branch K_L. However, as current-year needles expanded throughout the summer, the differences in K_L between treatment and control branches may have become progressively less.

Our efforts to alter K_L by notching an individual branch in old and young trees were unsuccessful. We saw no difference in the response of g_s and A with D and notching killed two of our replicate branches on old trees. We conclude that notching is a less precise technique for modifying K_L than is defoliation because we could not determine the actual decrease in K_L caused by the notching treatment. The notching treatment may not significantly reduce K_L of young tree branches because there is a redundancy in the sapwood of ponderosa pine branches. Branch conductivity measurements of notched branches support this hypothesis because leaf specific conductivity of notched branches was not significantly different from controls ($P = 0.51$, data not shown).

Our experimental manipulations of K_L on branches in old and young trees were designed to examine the short-term response of g_s and A to changes in K_L. However, results from these treatments do not reflect how hydraulic conductance may change with increased size and age or how long term changes in hydraulic conductance affect leaf gas exchange. As trees grow larger, xylem permeability and sapwood per unit leaf area may increase, somewhat compensating for longer path lengths (Carlquist 1975, Pothier et al. 1989b). These changes may partially mitigate lower hydraulic conductance and steeper response of g_s and A with D, but the extent to which this occurs and over what time scales is a complicated and open question.

In summary, we have shown that K_L and $K_{L\bullet}$ are lower and that g_s and A decline more steeply as a function of D in old, tall trees compared to young short trees. The short term responses of g_s and A of needles from defoliation treatments on branches of old trees declined less steeply with D compared to that of controls suggesting that increasing K_L in old trees leads to increases in g_s and A. Although these data do not prove that g_s and A are limited by hydraulic conductance in ponderosa pine, they are consistent with the hydraulic limitation hypothesis.

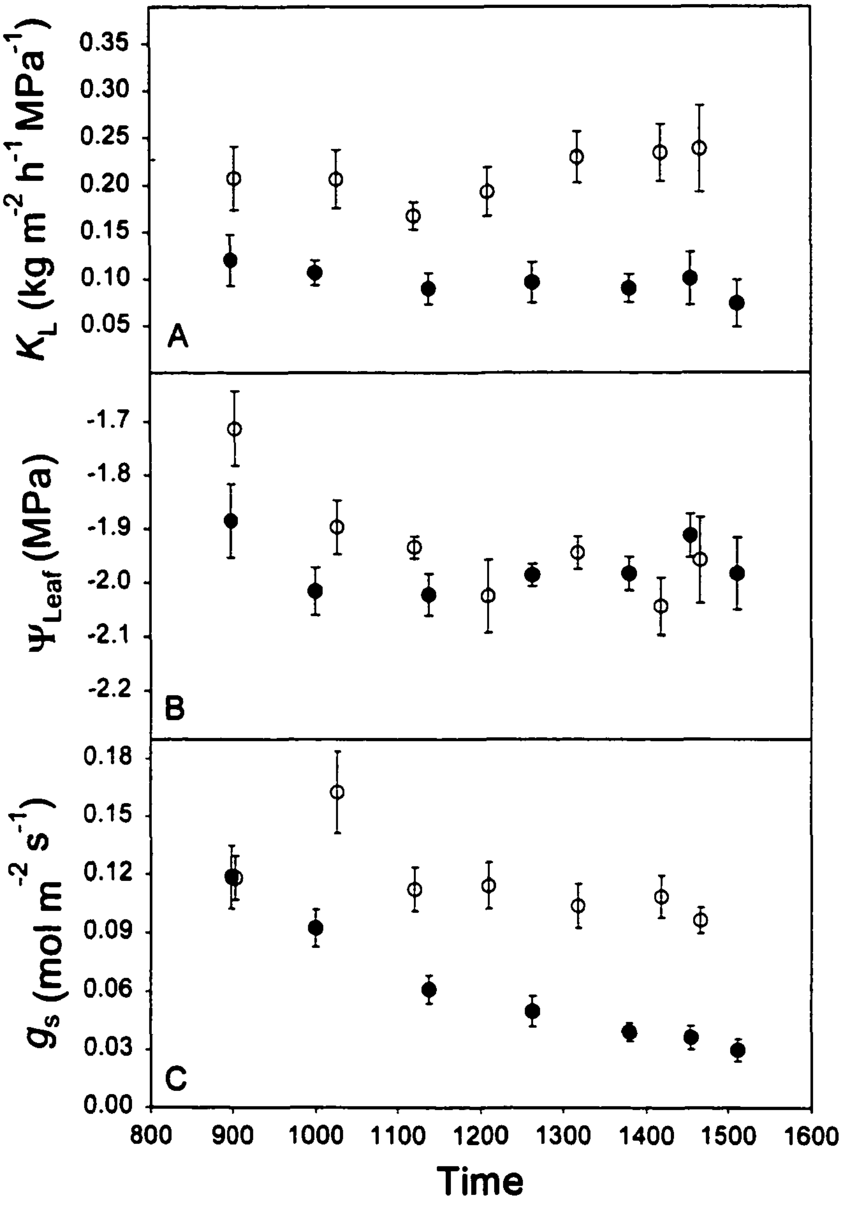

Figure 2.1 Average hourly leaf specific hydraulic conductance (K_L) (A), leaf water potential (ψ) (B), and stomatal conductance (g_s) (C) on days 254-256 for old (closed circles) and young (open circles) trees. Error bars are ± 1 standard error. Average leaf specific hydraulic conductance (LSC) was 44% lower in old compared to young trees ($P < 0.001$). Average LSC for old trees declined slightly ($P = 0.045$) during the day, with most of the decline occurring before 11:00 p.m. Average LSC of young trees did not change significantly throughout the day. Leaf water potential was not significantly different between branches of old and young trees ($P = 0.21$) and average stomatal conductance declined more steeply throughout the day in old compared to young trees ($P < 0.01$).

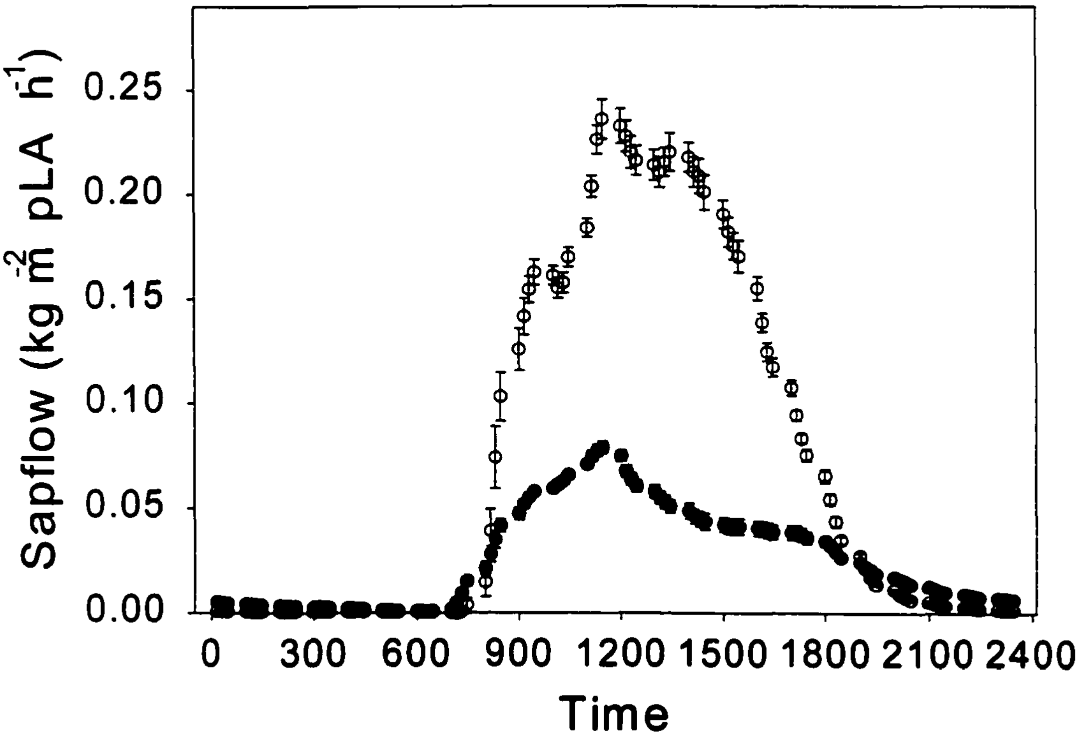

Figure 2.2 Mean hourly whole-tree sap flux per m^2 projected leaf area (PLA) during days 254, 255, and 256 for old (closed circles) and young (open circles) ponderosa pine trees. Error bars are ± 1 standard error. Mean sap flux was 53% lower in old trees than in young trees for the three-day time period.

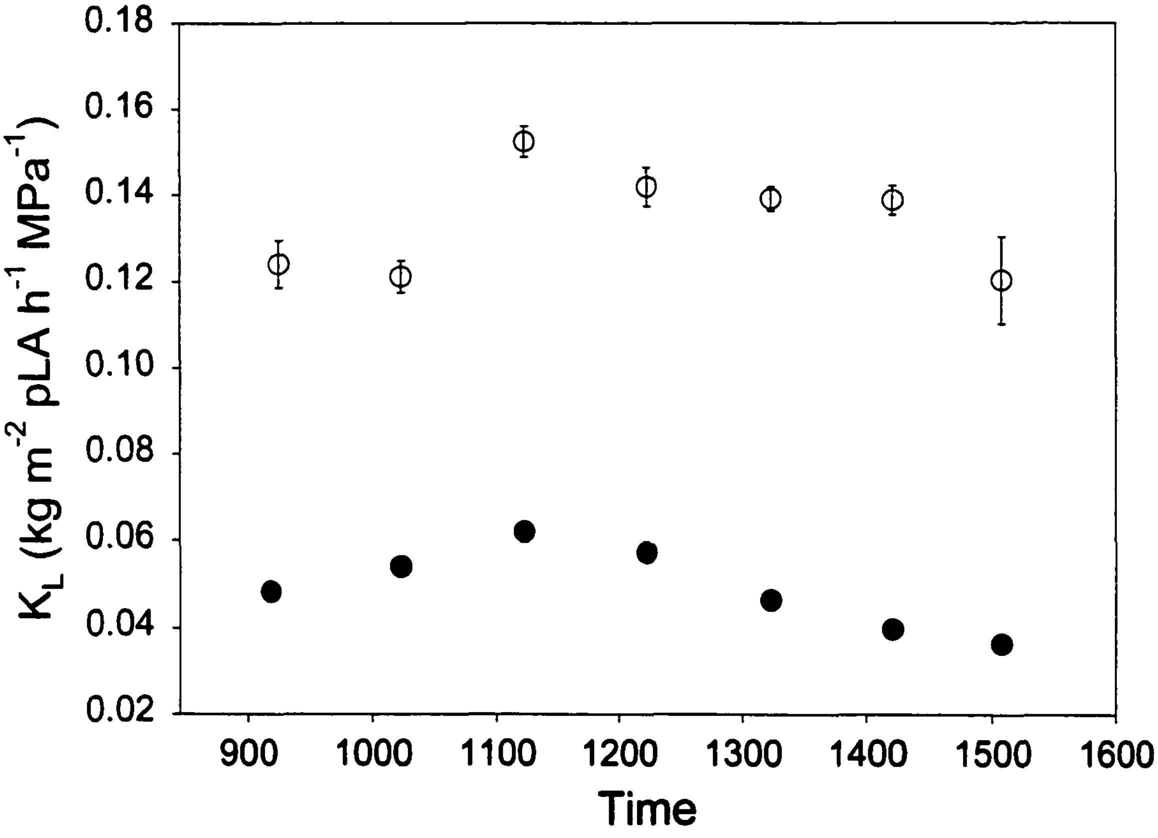

Figure 2.3 Mean hourly leaf specific conductance ($K_{L\bullet}$) (calculated from Equation 7) for old (closed circles) and young (open circles) ponderosa pine trees. Error bars represent ± 1 standard error. Mean $K_{L\bullet}$ was 63% lower in old trees than in young trees. The contribution of height to $K_{L\bullet}$ in old and young trees was 20 and 10% respectively.

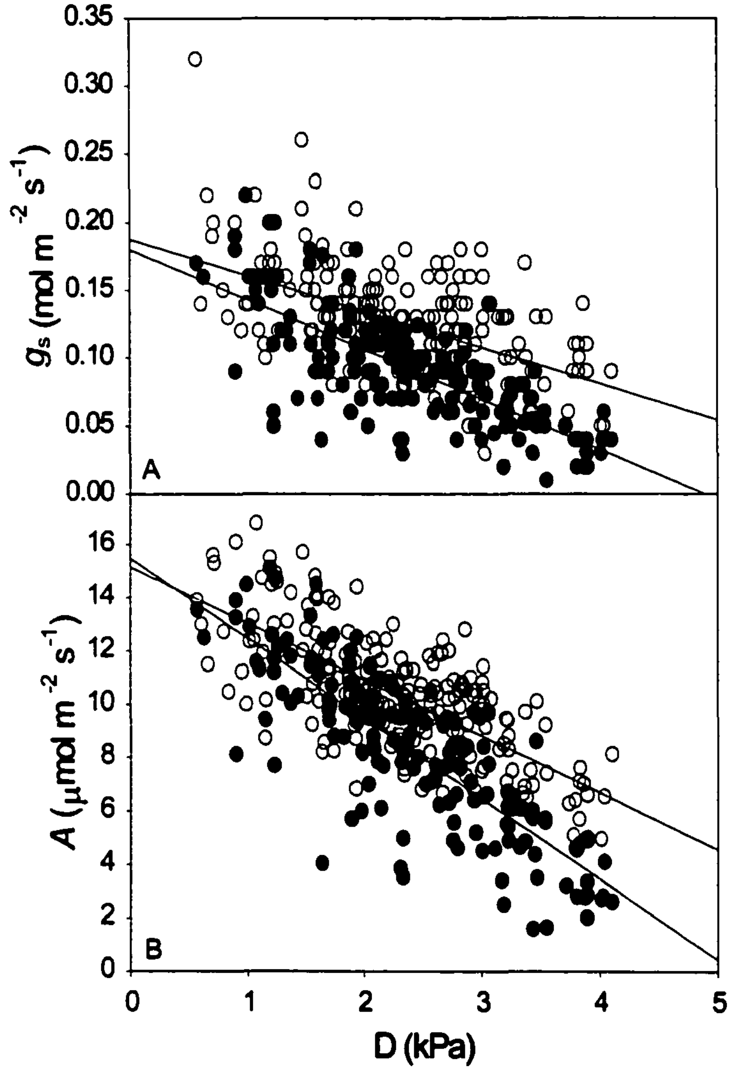

Figure 2.4 (A) Light-saturated stomatal conductance (g_s) and (B) CO_2 assimilation (*A*) versus air saturation deficit (*D*) for 1-year old needles on control branches from old (closed circles) and young (open circles) *Pinus ponderosa* trees. The slopes of the responses of g_s and *A* with *D* of needles from branches on old trees were significantly more negative than those on young trees ($P < 0.01$). Values of R^2 for g_s versus *D* for old and young trees were 0.55 and 0.34, respectively. The response of *A* with *D* had R^2 values of 0.69 for old trees and 0.43 for young trees.

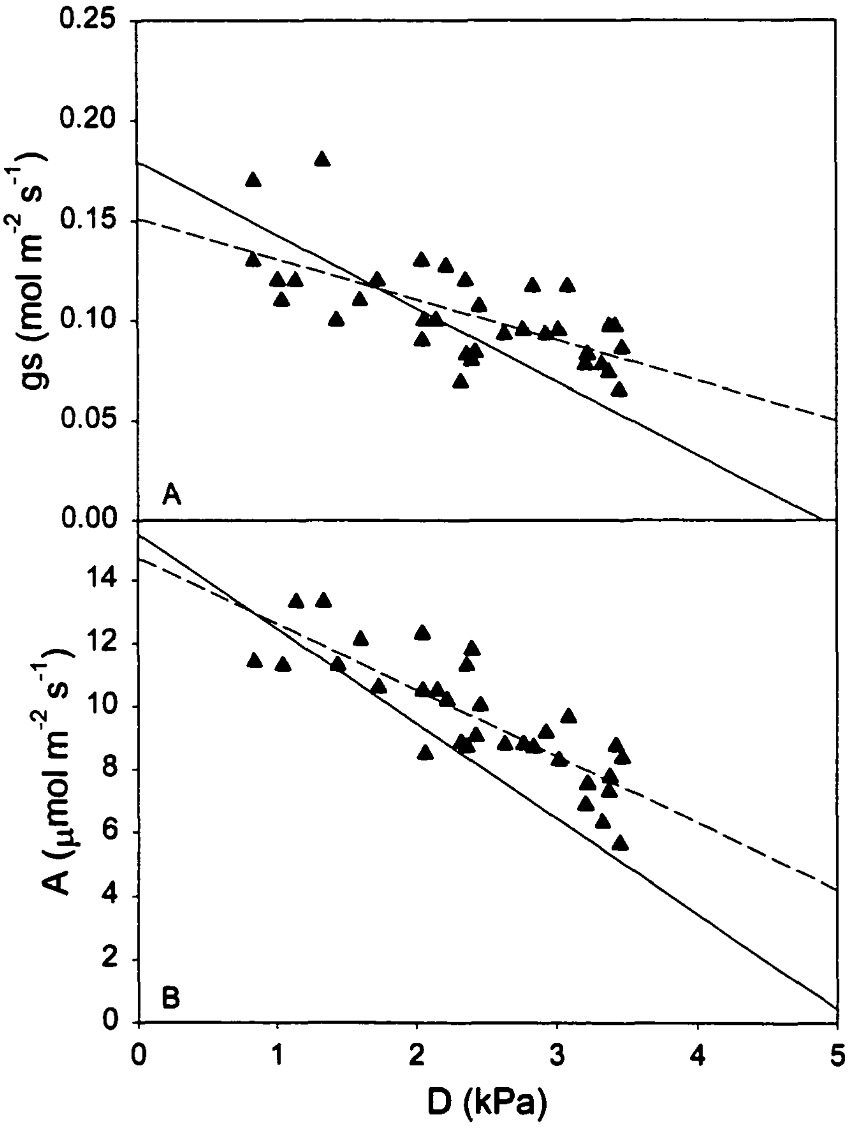

Figure 2.5 Light-saturated response of stomatal conductance (g_s) (*Figure* A) and assimilation (*A*) (Figure B) for 1 year old needles on defoliation treatment branches in early summer. Regression line for control branches (solid line) is shown for comparison. The slope of the light saturated response of g_s and *A* with *D* in defoliation treatments (triangles) was significantly less negative than control branches ($P < 0.01$). R^2 values for the response of g_s with *D* of foliage from treatment branches in early summer was 0.42. R^2 values for the response of *A* with *D* of foliage from treatment branches in early summer was 0.78.

CHAPTER III

Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine.

ROBERT M. HUBBARD
USDA Forest Service
Rocky Mountain Research Station and
Graduate Degree Program in Ecology, Colorado State University
240 West Prospect Rd
Fort Collins, CO 80526

VOLKER STILLER and JOHN S. SPERRY
Biology Department
University of Utah
257S 1400E
Salt Lake City, UT 84112

MICHAEL G. RYAN
USDA Forest Service
Rocky Mountain Research Station and
Graduate Degree Program in Ecology, Colorado State University
240 West Prospect Rd
Fort Collins, CO 80526

***Plant, Cell and Environment* (Accepted)**

Summary

Plants regulate their water status via stomatal control of transpirational water loss. Recent work has shown that stomata and assimilation are responsive to changes in the hydraulic conductance of the soil to leaf pathway (K_L), but no study has quantitatively described this relationship under controlled conditions where steady-state flow is promoted. Under steady-state conditions, the relationship between stomatal conductance (g_s), water potential Ψ and K_L are assumed to follow the Ohm's law analogy for fluid flow. When boundary layer conductance is large relative to g_s, the Ohm's law analogy leads to $g_s = K_L\ (\Psi_{soil} - \Psi_{leaf})/D$. Consequently, if stomatal control acts to maintain constant leaf water status during transpiration, and if reduced stomatal conductance limits assimilation, we hypothesized that reducing K_L would cause a linear decline in g_s and assimilation. To vary K_L, we systematically reduced stem hydraulic conductivity (k) of well-watered ponderosa pine seedlings (*Pinus ponderosa*) using air injection to induce cavitation while simultaneously measuring the response of canopy gas exchange in the laboratory under constant light and air saturation deficit (D). Short-statured seedlings (< 1 m tall) and hour-long equilibration times promoted steady-state flow conditions. We found that stomatal conductance, transpiration, assimilation, and K_L all declined with decreasing k ($P < 0.001$). This response was independent of bulk leaf water potential which remained constant near −1.5 MPa, except at the extreme 99% reduction in k when it fell to −2.1 MPa. Because stomata closed and bulk Ψ_{leaf} was maintained as K_L declined, stomatal conductance and assimilation were directly proportional to K_L ($R^2 > 0.90$), suggesting changes in K_L may affect plant carbon gain.

Introduction

A large body of work has shown convincing, yet sometimes conflicting evidence that stomata respond to a variety of water relations parameters under light-saturating conditions. Response mechanisms have been investigated for humidity, transpiration, and soil moisture. The humidity response may actually be a transpiration response (Mott and Parkhurst 1991) mediated by complex negative feedback via the water status of cells associated with the stomatal apparatus (Cowan 1995, see also Dewar 1995, Franks et al. 1997, Monteith 1995, Mott and Buckley 1998). This response can occur without a change in bulk leaf water status (Saliendra et al. 1995, Willmer, 1996). The response to soil moisture may involve chemical signals originating in the root system in response to soil drying, that are carried to the foliage in the transpiration stream where they induce stomatal closure (Loewenstein and Pallardy 1998, Tardieu and Simonneau 1998, Zhang and Davies 1989). However, other evidence suggests that the soil moisture response may in some cases be similar to the humidity response, mediated by negative feedback in a population of sensing cells in the leaf that cause a stomatal response without observable changes in bulk leaf water status (Fuchs and Livingston 1996, Saliendra et al. 1995).

Less work has been done on stomatal responses to hydraulic conductance. Natural variation in whole-plant leaf specific hydraulic conductance (K_L) with development (Hubbard et al. 1999, Meinzer and Grantz 1990, Mencuccini and Grace 1996a, Saliendra et al. 1995) through partial defoliation by storm events (Oren et al., *In Press*), and between species (Meinzer et al. 1995) has shown K_L to be positively related to stomatal conductance. Stomata have also been found to respond to experimental manipulations of hydraulic conductance involving induction of xylem cavitation (Sperry and Pockman 1993), root pruning (Meinzer and Grantz 1990, Teskey et al. 1983), notching of stem xylem (Sperry et al. 1993), freeze-thawing of stems (Hammel 1967), and defoliation (Hubbard et al. 1999, Pataki et al. 1998). When it has been measured, the response occurs within minutes of the manipulation, and bulk leaf water status can remain nearly constant during the experiment (Saliendra et al. 1995, Sperry et al. 1993, Teskey et al. 1983).

The link between stomatal conductance (g_s) and K_L arises from the fact that under photosynthetic conditions, stomata operate to enhance photosynthesis on the one hand while avoiding dehydration induced damage on the other. Such damage includes excessive cavitation and disturbance to cellular water relations and biochemistry. As a result, plant water status under photosynthetic conditions is regulated to permit the transpiration that necessarily accompanies stomatal opening but is kept from falling below damaging levels to prevent disruption of function. Consequently, stomata respond to K_L because changes in K_L influences plant water status (particularly leaf water status at the downstream end of the flow path).

The relationship between Ψ, g_s, and K_L under steady-state conditions is described by the Ohm's law analogy for fluid flow (Tyree and Ewers 1991). If boundary layer conductance is large relative to g_s, the Ohm's law analogy leads to:

$$g_s = K_L C \qquad (1)$$

where $C = \Delta \Psi / \Delta w$. The $\Delta \Psi$ is the water potential difference between bulk soil and leaf ($\Psi_{soil} - \Psi_{leaf}$) driving the flow (assuming a negligible effect of gravity on $\Delta \Psi$), and Δw is the vapor pressure deficit. Several possible relationships between K_L and g_s at steady-state are consistent with Equation 1, depending on how stomata regulate Ψ_{leaf}. These are illustrated in Figure 3.1 assuming constant Ψ_{soil} and Δw for simplicity. If stomata do not regulate Ψ_{leaf}, then there would be no expected response to K_{L} (Figure 3.1, dotted line). In contrast, if they regulate Ψ_{leaf} at a constant value, g_s and K_{L} would be directly proportional with a slope equal to C (Figure 3.1, solid line). If regulation of Ψ_{leaf} increased as it became more negative with decreasing K_{L}, an intermediate curvilinear response would be expected (Figure 3.1, dashed line). Previous studies have shown that g_s changes proportionally to single step changes in K_{L} (e.g. Saliendra et al. 1995). However, no study has varied K_{L} systematically under controlled, steady-state flow conditions to characterize the full relationship between K_{L} and g_s that follows from Equation 1 and the mode of Ψ_{leaf} regulation.

The relationship between liquid and vapor phase conductance may be part of the reason for the decline in forest productivity with age (Ryan et al. 1997, Ryan and Yoder 1997). Older forests are taller forests, and K_{L} and g_s have been shown to decrease with increasing size and age in many species (Hubbard et al. 1999, Mencuccini and Grace 1996b, Saliendra et al. 1995, Schäfer et al. 2000, Yoder et al. 1994). If leaf water potential is constrained from dropping with increasing tree height, g_s should decline and increasingly limit photosynthetic assimilation (A). The fraction of $\Delta\Psi$ required to hold water against gravity would also become significant in taller trees, reducing g_s beyond that expected from Equation 1. Reduced g_s and A have been documented with height (Hubbard et al. 1999, Schäfer et al. 2000) yet no study has directly evaluated the response in A to manipulations of K_{L} under controlled conditions.

This study focuses on how stomata of ponderosa pine (*Pinus ponderosa*) seedlings respond to a systematic decrease in K_L achieved by induction of cavitation in stem xylem under controlled conditions (i.e. light, Ψ_{soil}, and Δw held constant). Our objective was to determine which of the alternative relationships between gas exchange, $\Psi_{leaf,}$ and K_L shown in Figure 3.1 occurred in these plants. In addition, we measured the corresponding relationship between A and K_L to assess the importance of hydraulic considerations for plant carbon gain. In accomplishing these objectives, we also determined the direct relationship between gas exchange and leaf water status and the changes in stem conductivity caused by air injection. The use of conifer seedlings and long equilibration times for determining gas exchange parameters were chosen to minimize boundary layer conductance effects and to promote the steady-state flow conditions to which Equation 1 and Figure 3.1 apply.

Methods

Plant material

We selected 20 ponderosa pine seedlings with similar branching patterns and leaf area from local nurseries. The seedlings were between 0.75 and 1.0m tall and were 4-5 years old. The seedlings were held in the University of Utah greenhouse and watered twice daily to avoid water stress. Plants were exposed to ambient sunlight throughout the day with temperature and relative humidity ranging between 20 - 28 °C and 40 - 60% respectively.

Vulnerability curve

To manipulate the cavitation level in stems of intact seedlings (see Treatments) we needed to know the vulnerability curve for the stem xylem. A vulnerability curve defines the relationship between hydraulic conductivity (*k*) and xylem pressure. As xylem pressure becomes more negative, cavitation events increase, causing a decrease in *k*. We used the centrifugal force method (Alder et al. 1997) to measure the vulnerability of stem xylem in six seedlings. Each seedling was cut at the root collar under water and a 142 mm segment was removed from the stem between the root collar and the first whorl of branches. The *k* (kg m s^{-1} MPa^{-1}) was determined as the quotient of the mass flow rate of water through the segment (kg s^{-1}) and the pressure gradient (MPa m^{-1}) associated with the hydrostatic head causing flow through the segment. Conductivity differs from conductance in being a length-specific measure. Initial *k* (k_i) was determined for each segment. The segment was then centered on a centrifuge rotor and spun for three minutes along its long axis. Spin rates corresponded to xylem pressures of -1 and -2 MPa and were then decreased by 0.5 MPa increments to -6.0 MPa (Figure 3.2). After spinning, *k* was re-measured for each segment and the percentage loss of *k* (k_{loss}) determined as the percent decline from k_i.

Treatments

Each seedling was brought into the lab the night before treatment. The canopy of each plant was sealed into a Teflon lined Plexiglas cuvette and fine wire type T thermocouples were attached to five needles distributed evenly throughout the canopy. Photon flux density averaged 1500 μmol m^{-2} s^{-1} and was supplied with two 1000 W Na-vapor HID lamps. Two fans circulated air inside the cuvette and temperature was controlled with a circulating water bath through a heat exchanger in the cuvette. We monitored CO_2 and water vapor concentrations using an open gas exchange system (Field, Ball & Berry 1989). Reference and sample CO_2 and water vapor concentrations were determined using two LICOR 6252 (LICOR INC.) infrared gas analyzers. The CO_2 concentration within the cuvette was kept near 350 ppm and air saturation deficit was held at about 2.8 kPa (leaf-air mole fraction difference of c. 0.032). Boundary layer conductance of birch leaves under cuvette wind speed was previously determined to be 1000 mmol s^{-1} m^{-2} (Saliendra et al. 1995). This would be even greater for needle-leaved conifers, so boundary layer conductance was assumed to have a negligible influence on total leaf conductance to water vapor. Canopy level stomatal conductance (g_s) was determined from transpiration (E) measurements, and canopy assimilation rate (A) was derived from CO_2 consumption. Calculations for the gas exchange parameters and a more detailed description of the gas exchange system are given in Saliendra et al. (1995).

We used an air-injection method (Cochard et al. 1992, Salleo et al. 1992, Sperry and Saliendra 1994) to induce cavitation in the stem and reduce the stem and plant hydraulic conductance. Normally, cavitation occurs when the xylem pressure drops low enough to pull air into the functional xylem conduits (the air seeding mechanism Zimmermann 1983). In the air injection technique, cavitation is induced by raising the air pressure around the stem high enough to push air into the conduit network. To inject the air, we removed a 10 cm section of bark and phloem to restrict air pressure to the xylem tissue and bolted a steel split chamber with a compressible gasket around the stem. The gasket provided a nearly air tight seal. A pressure regulator held injection pressures of 3.5, 4.5, and 5.5 MPa constant despite occasional small leaks from the chamber. These injection pressures were chosen because the vulnerability curve indicated they would cause significant cavitation in stem xylem.

The experimental protocol is summarized in Figure 3.3 (solid line). Before treatments began, we estimated Ψ_{soil} from predawn Ψ_{leaf} measurements on three fascicles using a pressure chamber (Scholander et al. 1965). To begin treatments, we turned on the gas exchange system and allowed the seedling to acclimate for at least one hour or until g_s, E, and A were stable, and steady-state flow conditions were likely to be established in these short plants. After these parameters were stable, we measured Ψ_{leaf} on three fascicles and calculated the initial K_L from Equation 1. We then pressurized the stem injection chamber to 3.5 MPa for thirty minutes. The injection chamber was depressurized, and g_s, E, and A were again allowed to stabilize for at least one hour to promote a new steady-state equilibrium (Figure 3.3). Afterwards, we re-measured Ψ_{leaf} and calculated the change in K_L caused by the injection. We repeated this sequence for injection pressures of 4.5 and 5.5 MPa. Soil moisture for the entire treatment cycle was kept at field capacity. We used three control plants to determine if bark and phloem removal and the attachment of the air injection chamber affected leaf gas exchange independent of any induction of cavitation (Figure 3.3, dotted line). For each control plant, we measured g_s, E, A, and Ψ_{leaf} under the same environmental conditions as our treatment plants until these parameters were stable (at least one hour). Then we removed the bark and phloem and attached the air injection chamber. Afterwards, we monitored leaf gas exchange and Ψ_{leaf} for the duration of a typical treatment cycle (approximately 7 hours).

Gas exchange parameters (g_s, E, A) are reported as leaf specific rates. We determined leaf area for each seedling by removing and weighing all of the needles from the canopy after treatment. Projected leaf area (m^2) was determined from a leaf area:weight regression developed from similar seedlings. Fifty fascicles were removed from each of six seedlings and area determined by measuring the length and width of each needle with a digital micrometer.

Our methods of measuring g_s and K_L were not completely independent, because both g_s and K_L were determined in part from measurements of transpiration in the gas exchange cuvette. For this reason, we also evaluated the gas exchange response to independent estimates of the loss of stem conductivity caused by air injection (k_{loss}). Measuring k_{loss} directly was a destructive measurement that could only be made at the end of the 5.5 MPa injection when the experiment was finished. Consequently, we estimated k_{loss} for the stem segment within the injection chamber at all injection pressures using the seedling vulnerability curve. To make this estimation, we needed to know both the injection pressure *and* the xylem pressure at the time of injection. The difference between these pressures represented the total pressure difference driving air into the vascular system. The k_{loss} was then predicted using this total pressure difference as the equivalent of the xylem pressure axis of the vulnerability curve (which was conducted at ambient air pressure). We estimated the Ψ_{xylem} during injection as the mid-point between Ψ_{soil} and pre-treatment Ψ_{leaf} (Linton, Sperry & Williams 1998) calculated as

$$\Psi_{xylem} = \left(\Psi_{leaf} + \Psi_{soil}\right)/2 \qquad (2)$$

We subtracted this (negative) value from the injection pressure (0, 3.5, 4.5, or 5.5 MPa) to estimate the total pressure difference causing cavitation. We fit a Weibull function to our vulnerability curve data (see *Data Analysis*) and used this function to predict k_{loss} from the total pressure difference.

To test our estimates of k_{loss} from Ψ_{leaf}, Ψ_{soil} and the vulnerability curve, we directly measured k_{loss} in 6 separate plants (2 ea at 3.5, 4.5, and 5.5 MPa). After subjecting the plants to the same environmental conditions as our treatment plants, we measured k_{loss} after air injection at the specific pressure and compared it to k_i based on a branch diameter vs. k_i relationship developed on 12 separate ponderosa pine seedlings.

Data Analysis

We fit a Weibull function to our vulnerability curve data to describe the relationship between k_{loss} and Ψ_{xylem}. The specific function is given as:

$$\%k_{loss} = 100 - 100 * e^{(-(-P_{total/b}))^{c}} \qquad (3)$$

where b and c are constants generated by the curve fitting procedure, and P_{total} is the pressure difference causing cavitation. The function was fit and 95% prediction intervals generated using non-linear regression (Proc Nlin (SAS 1997)). We tested for normality and homogeneity of variance and found that our vulnerability curve data did not violate either of these assumptions.

We analyzed the response of leaf water potential and gas exchange parameters to changes in K_L using a general linear model, (Proc Mixed (SAS 1997)). The seedling was our sampling unit. However, because we measured leaf gas exchange with changes in K_L at four different applied pressures (0,3.5,4.5, and 5.5 respectively) on the same seedling, we used a repeated measures analysis. This analysis assumes a constant correlation among measurements on the same seedling (a standard compound symmetric covariance model). We found no violation of the assumptions of normality and homogeneous variance for this analysis. The response of leaf gas exchange and K_L to changes in k_{loss}

were analyzed using analysis of variance in the same repeated measures framework as described above.

Results

The Weibull function that best described our vulnerability curve for stem xylem was:

$$\%k_{loss} = 100 - 100 * e^{(-(-P_{total}/4.3))^{5.4}} \tag{4}$$

The R^2 for this function (Figure 3.2) was 0.92. The curve indicated that cavitation in stems of these seedlings occurred at xylem pressures between –3 and –6 MPa (at ambient air pressure).

Using the vulnerability curve (Equation 4) and estimates of Ψ_{xylem} during injection (Equation 2), we predicted that k_{loss} from air injections of 3.5, 4.5, and 5.5 MPa was 46, 92, and 99% respectively (Figure 3.4). When we tested these predictions by destructively sampling two plants for each pressure we found actual k_{loss} to be 62, 87, and 99% respectively. Measured values fell within the 95% prediction intervals for the Weibull fit (Figure 3.2).

The three control seedlings showed no effect of bark and phloem removal or attachment of the injection chamber on g_s or A either immediately after the treatment ($P = 0.52$) or throughout the 7 hour duration of the experiment (Figure 3.3, dotted line). In contrast, the injected seedlings showed an abrupt decline in g_s and A within minutes after each 30 minute injection (Figure 3.3, solid line). Interestingly, there was no response during the injection. These declines stabilized at lower values of g_s and A within half an hour after the injection treatment.

Stable values of g_s, E, and A after injection showed a significant decline ($P < 0.01$) with independent estimates of k_{loss} caused by injection (Figure 3.4; top three panels). The decline was essentially linear until the 99% k_{loss} extreme (caused by the 5.5 MPa injection) when values decreased even more abruptly. The decline in gas exchange parameters was associated with the maintenance of a constant mean Ψ_{leaf} ($P = .24$) near c. -1.45 MPa up until the 99% k_{loss} extreme when Ψ_{leaf} dropped abruptly to –2.1 MPa (Figure 3.4, top panel). Predawn Ψ_{leaf} averaged –0.4 MPa and was not different between seedlings ($P > 0.05$).

The bottom panel of Figure 3.4 shows the decline in K_L calculated from Equation 1 ($K_L = E / \Delta \Psi$) that was associated with the k_{loss} in the stem from the air injection. The decline in K_L paralleled the decline in gas exchange (Figure 3.4, upper three panels), and was approximately linear until the 99% k_{loss} point at which K_L dropped more abruptly. The non-linearity of the overall relationship indicated that to alter the conductance of the entire flow path by a given fraction, the stem segment conductivity had to be reduced disproportionately. For example, a 50% reduction in K_L required a 90% reduction in stem conductivity. This non-linearity is expected if the loss of conductivity caused by the injection did not spread equally throughout the vascular system. In support of this, air was seen to escape from the stem outside of the chamber suggesting that the high air pressures required to cause the k_{loss} dissipated with distance from the chamber. Another factor contributing to the non-linearity is the fact that the initial conductivity per unit leaf area is typically at least an order of magnitude greater in the main stem than in the lateral branches and leaves (Tyree and Ewers 1991, Zimmermann 1978). Consequently, stem conductivity must be lowered substantially before it can become the limiting conductance in the plant and significantly influence K_L.

In keeping with the closely parallel response of leaf gas exchange and K_L to k_{loss} of the stem, the summary relationship between g_s and K_L was linear ($R^2 = 0.91$). The intercept of this relationship was not significantly different from zero ($P > 0.05$) and the slope was not significantly different from $\Delta\Psi$ divided by the leaf to air mole fraction difference (c. 0.032) ($P > 0.05$). This result follows directly from the fact that Ψ_{leaf} was regulated at a constant value throughout most of the experiment (Figure 3.4, top panel) and because Equation 1 was used to calculate K_L. Assimilation (A) also showed a strong linear relationship to K_L (Figure 3.5, lower panel; $R^2 = 0.90$).

Discussion

Our results showed that ponderosa pine seedlings maintained constant bulk Ψ_{leaf} as k and K_L were systematically decreased by the induction of cavitation to all but the lowest values (Figs. 4,5). According to the Ohm's law analogy for steady-state flow through the continuum, this result requires a directly proportional response of g_s to K_L when Ψ_{soil} and Δw are constant, as in our experiments (Figs. 1, 5). Although it would have been desirable to measure K_L independently of g_s as has been done previously for step-changes in K_L (Saliendra et al. 1995, Sperry et al. 1993) the destructive nature of these measurements made it impossible to measure the complete response on single plants. In addition, these destructive measurements of leaf and soil-root system conductances may not reflect *in situ* conductance because of changes in flow path and cavitation associated with measurement conditions (Saliendra et al. 1995, Tyree et al. 1994b)

The lack of independence between the K_L and g_s measurement does not apply to several corollary results. Unlike g_s, A was measured independently from K_L, yet was also proportional (Figure 3.5). Because of the strong relationship between g_s and A (Wong et al. 1985) it is unlikely that the proportionality of g_s versus K_L was an artifact of autocorrelation between the two parameters. The decline in gas exchange was also strongly related to independent measures of stem conductivity (Figure 3.4). Finally, the fact that Ψ_{leaf} remained constant despite the reduction in stem conductivity (Figure 3.4, top panel) requires that g_s and K_L be directly proportional under steady-state conditions (Equation 1), which were likely to prevail in our study given the short stature of the seedlings and the hour long equilibration periods before measurement (Figure 3.2).

Other studies have shown similar stomatal response to a single step reduction in K_L. Sperry and colleagues (Sperry et al. 1993, Sperry and Pockman 1993) found that reducing K_L in *Betula occidentalis* by notching or air injection lowered g_s and E relative to control plants while Ψ_{leaf} remained constant. Similarly, Teskey, Hinkley, and Grier (1983) found that reducing the flow path of water in *Abies amabilis* via root pruning caused almost immediate stomatal closure with no change in Ψ_{leaf}. Similar conclusions were reached by Meinzer & Grantz (1990) in sugarcane. Other studies have shown that stomata close in response to lower conductance of the soil to leaf pathway via soil drying to prevent xylem cavitation (Sparks and Black 1999, Tyree et al. 1994a). By evaluating Ψ_{leaf} regulation in response to successive reductions in K_L in single plants we show that the stomatal response remains linear over a broad range of K_L, and that this response is also associated with a linear relationship between A and K_L.

The simplest explanation for the rapid response of g_s to K_L (Figure 3.2) is that stomata are not responding directly to K_L, but to the consequent change in water status within the leaf (Saliendra et al. 1995). According to this explanation, a reduction in K_L causes a momentary reduction in water status of at least a portion of the leaf tissue. Through pressure-volume changes in sensing cells or even transient cavitation within leaf veins (Salleo et al. 2000), the change in water status "signals" a reduction in stomatal conductance (perhaps via ABA signaling within the leaf). The reduction in stomatal conductance returns leaf water status to its original level. At the bulk tissue level, these small fluctuations in leaf water status in time and space are masked, so that bulk Ψ_{leaf} remains approximately constant. This small-scale feedback loop is analogous in principle to what has been proposed for stomatal regulation in response to changes in atmospheric moisture (e.g. Cowan 1995). Evidence for some form of leaf-level feedback in the K_L response is indicated by experiments showing that if leaf water status is experimentally increased (by root pressurizing), the stomatal closure to reduced K_L can be reversed (Saliendra et al. 1995). The fact that leaf water potential eventually did drop in response to the most extreme reduction in K_L (99% k_{loss}, and > 75% K_L loss; Figure 3.4, bottom) suggests there is a limit to the regulatory response of stomata when near complete closure was approached.

A large body of evidence exists for root signaling in connection with stomatal regulation (Tardieu and Davies 1993, Zhang and Davies 1989). However, any root signal involvement in response to changes in K_L (Meinzer et al. 1991) seems unlikely in our study for two reasons. First, the seedlings were well-watered throughout the experiment and second, the changes in K_L were induced *downstream* from the roots making it very unlikely they could perceive the change via alteration of water status and respond in kind.

It was interesting that we observed no decline in gas exchange until after the 30 minute injection treatment was terminated (Figure 3.3), whereas it is quite likely that the reduction in stem conductivity was significant within the first 10 minutes based on results from isolated stem segments (Sperry and Saliendra 1994). While this may reflect the lag time in the stomatal response to changes in K_L, it seems unlikely because in stem notching experiments, the stomatal response occurred within minutes of the reduction in conductance (Sperry et al. 1993). It is possible that the continual release of water caused by the progressive cavitation in the stem xylem was negating the effect of reduced stem conductivity on leaf water status. Only when the injection ceased would the full effect of reduced conductivity be felt in the leaf as an incipient reduction in water status. More detailed measurements of leaf water status during the injection process would be necessary to link small scale (in time and space) changes in leaf cell water potentials to these kinds of experimental manipulations.

Our study did not directly address the adaptive significance of regulating Ψ_{leaf} at c. −1.5 MPa in these seedlings. *A priori* we might expect it to relate to the avoidance of some form of damage from exposure to more negative values. In some previous studies, this damage was linked to extensive cavitation that would occur if pressures dropped below the regulated value (e.g. Meinzer et al. 1992, Saliendra et al. 1995). However, if the vulnerability curve for stem xylem represents that for leaf xylem, at Ψ_{leaf} of −1.5 MPa, these seedlings would have a 1.5 MPa cushion from incipient cavitation which was initiated at −3 MPa (Figure 3.2). More often than not, however, there is substantial variation in cavitation resistance along the xylem (Hacke and Sauter 1996). In *Psuedotsuga menziesii*, for example, smaller distal branches were more vulnerable than thicker proximal ones, and root xylem is particularly vulnerable (Sperry and Ikeda 1997). In addition to the avoidance of cavitation, there are other possible negative consequences of reduced Ψ_{leaf} including pressure-volume relations of leaf cells and organelles. However, we did not address the likelihood of such damage in this study.

Recently, Ryan and Yoder (1997) have proposed the hydraulic limitation hypothesis for reduced productivity observed in aging forest stands. This hypothesis suggests that as trees grow older and taller, K_{L} declines causing stomates to close earlier in the day (relative to younger, shorter trees) to preserve Ψ_{leaf} regulation. They propose that this may explain the well documented decline in stand NPP after canopy closure and why all trees eventually reach a maximum height. Although several studies have provided support for the hypothesis (Hubbard et al. 1999, Ryan et al. 2000), a critical test of the hydraulic limitation hypothesis requires that imposing a decrease in K_{L} should result in decreased carbon gain. As K_{L} declined after air injection in this study, A declined linearly suggesting a direct link between K_{L} and A, in support of Ryan and Yoder's hypothesis. Although our results were short-term responses to experimental reductions in K_{L}, they are consistent with apparent responses to longer-term developmental changes in K_{L}. Hubbard et al. (1999) found that old versus young ponderosa pine trees had 50% lower K_{L} with 25% lower A during mid-day. This is quite similar to our short-term observations in ponderosa pine seedlings where a 50% lower K_{L} resulted in 33% lower assimilation rates (Figure 3.5). These results suggest there is little acclimation in A vs. g_s relationships or in Ψ_{leaf} regulation in response to changes in K_{L} in this species. Understanding how changes in hydraulic conductance change with stand and tree age will be important for developing better models of forest growth (e.g. Williams *et al.* 1997) and predictions of forest response to climate change.

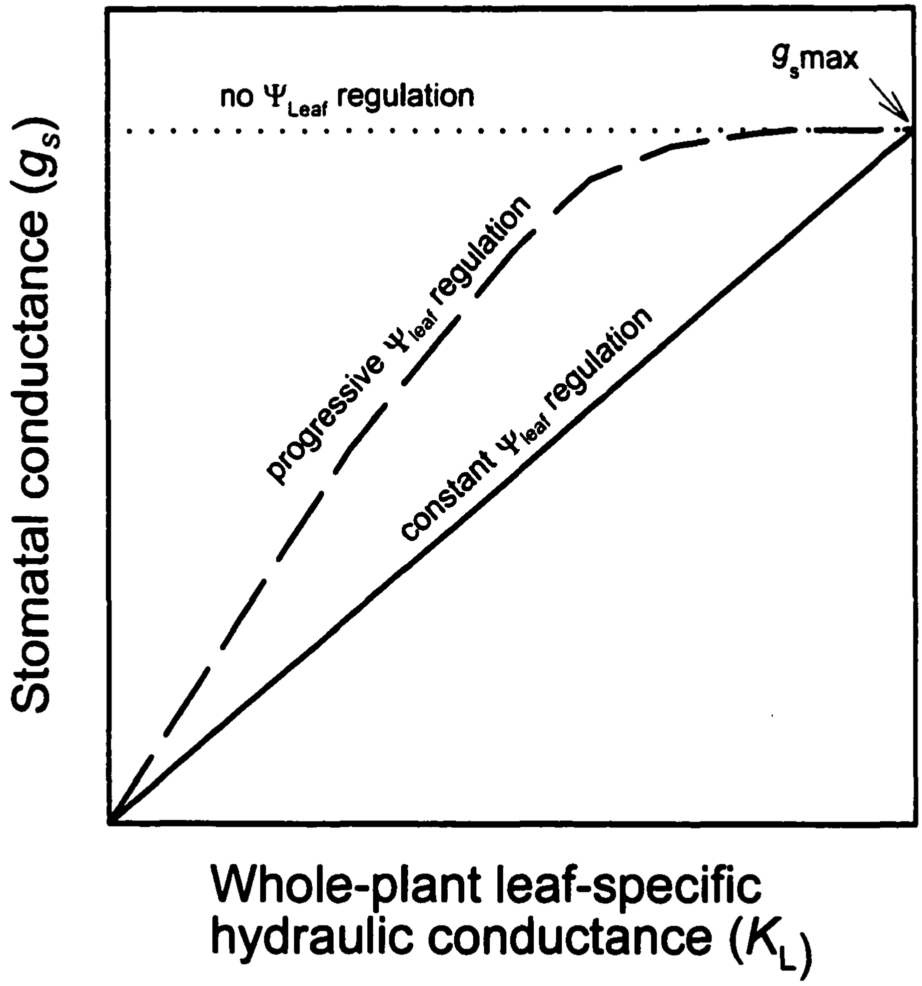

Figure 3.1 Possible steady-state responses of stomatal conductance (g_s) to changes in whole-plant leaf-specific hydraulic conductance (K_L) assuming a) Ohm's law analogy for fluid flow through the soil-plant hydraulic continuum, b) constant soil water potential, c) constant leaf-air vapor pressure deficit, and d) negligible influence of leaf boundary layer on leaf conductance to water vapor. Dotted line: no stomatal response associated with no regulation of bulk leaf water potential (Ψ_{leaf}), solid line: directly proportional stomatal response for g_s below a physiological maximum (g_{max} arrow, y axis) associated with perfect regulation of bulk Ψ_{leaf}, dashed line: curvilinear response associated with progressively stronger regulation of Ψ_{leaf} as it becomes increasingly negative with reduced K_L.

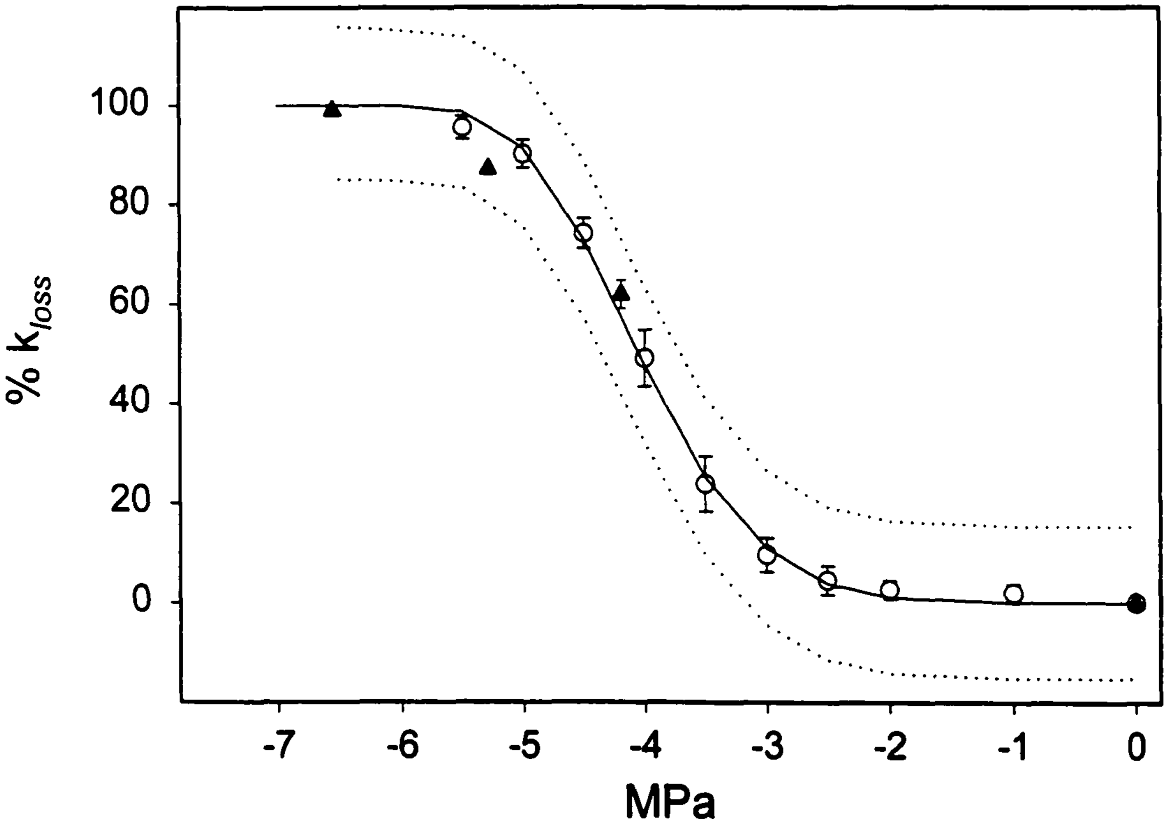

Figure 3.2 Vulnerability curve for stem xylem of ponderosa pine seedlings showing mean percent loss of stem hydraulic conductivity ($\%k_{loss}$) (open circles, n= 6) vs. the xylem pressure (Ψ_{xylem}). The best fit to the vulnerability curve (solid line) was a Weibull function: $\%k_{loss} = 100 - 100 * e^{(-(-P_{total}/b))^{f}}$ (see text for parameter values). Solid triangles represent mean percent loss of stem hydraulic conductivity measured after air injection (n = 2) at the actual pressure difference across the pit membrane (air injection pressure - Ψ_{xylem}). This pressure difference is equivalent to the Ψ_{xylem} axis of the vulnerability curve, which assumes ambient air pressure. Dotted lines represent 95% prediction limits for the Weibull fit.

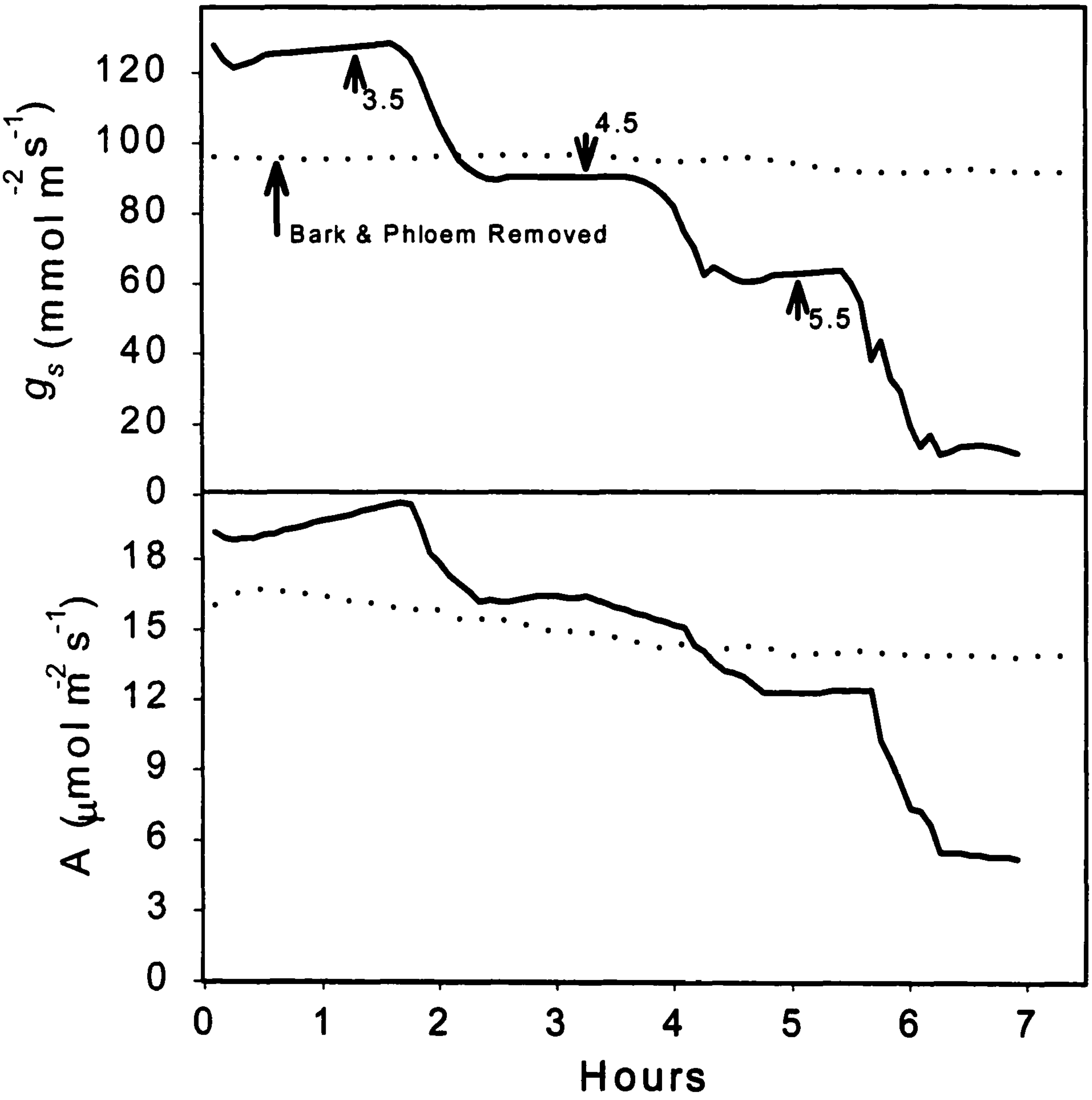

Figure 3.3 Solid line: typical pattern of stomatal conductance (g_s) and assimilation (A) in response to each injection pressure (3.5, 4.5, and 5.5 MPa, duration indicated on upper x axis) (solid line). Dotted line: Typical control plant (n=3) showing the response of g_s and A to bark and phloem removal plus injection chamber installation (but no injection).

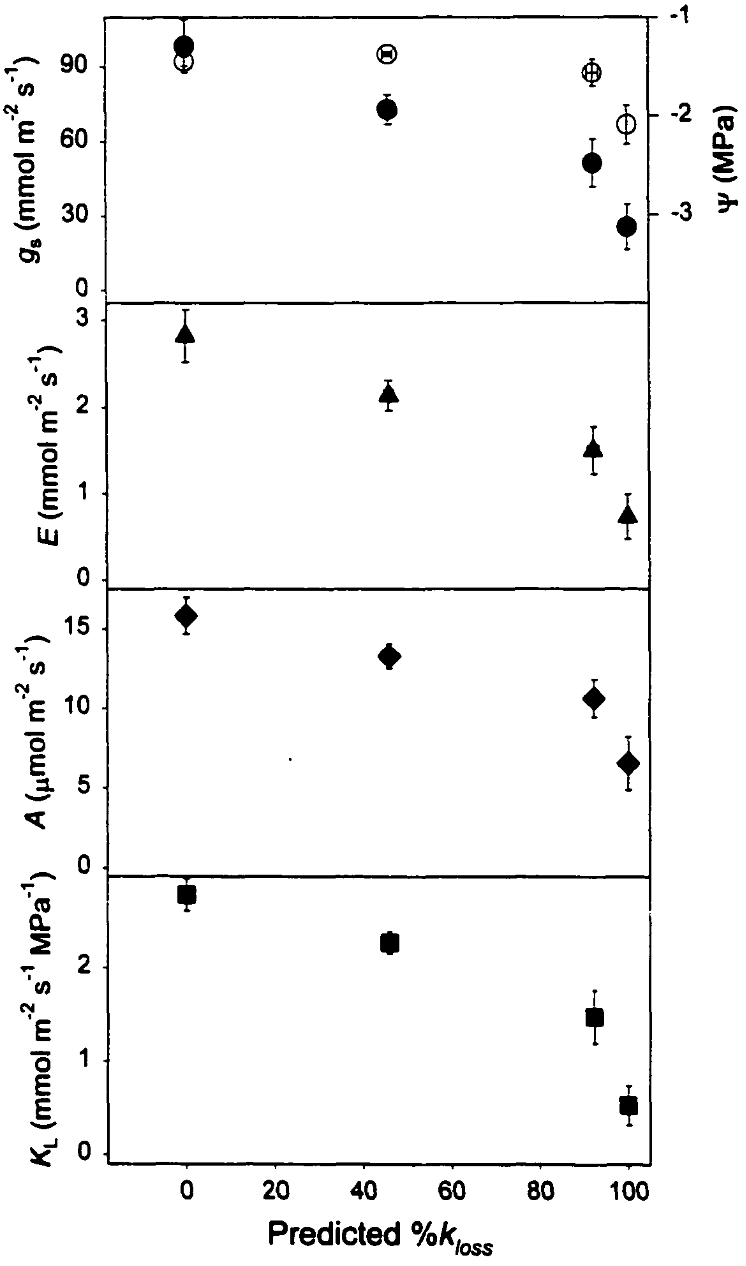

Figure 3.4 Mean leaf water potential (Ψ_{leaf}, open circles), stomatal conductance (g_s, closed circles), transpiration (E, closed triangles), assimilation, (A, closed diamonds), and leaf specific hydraulic conductance (K_L closed squares) versus predicted percent loss of stem hydraulic conductivity (n = 6). Error bars are ± 1 *se*.

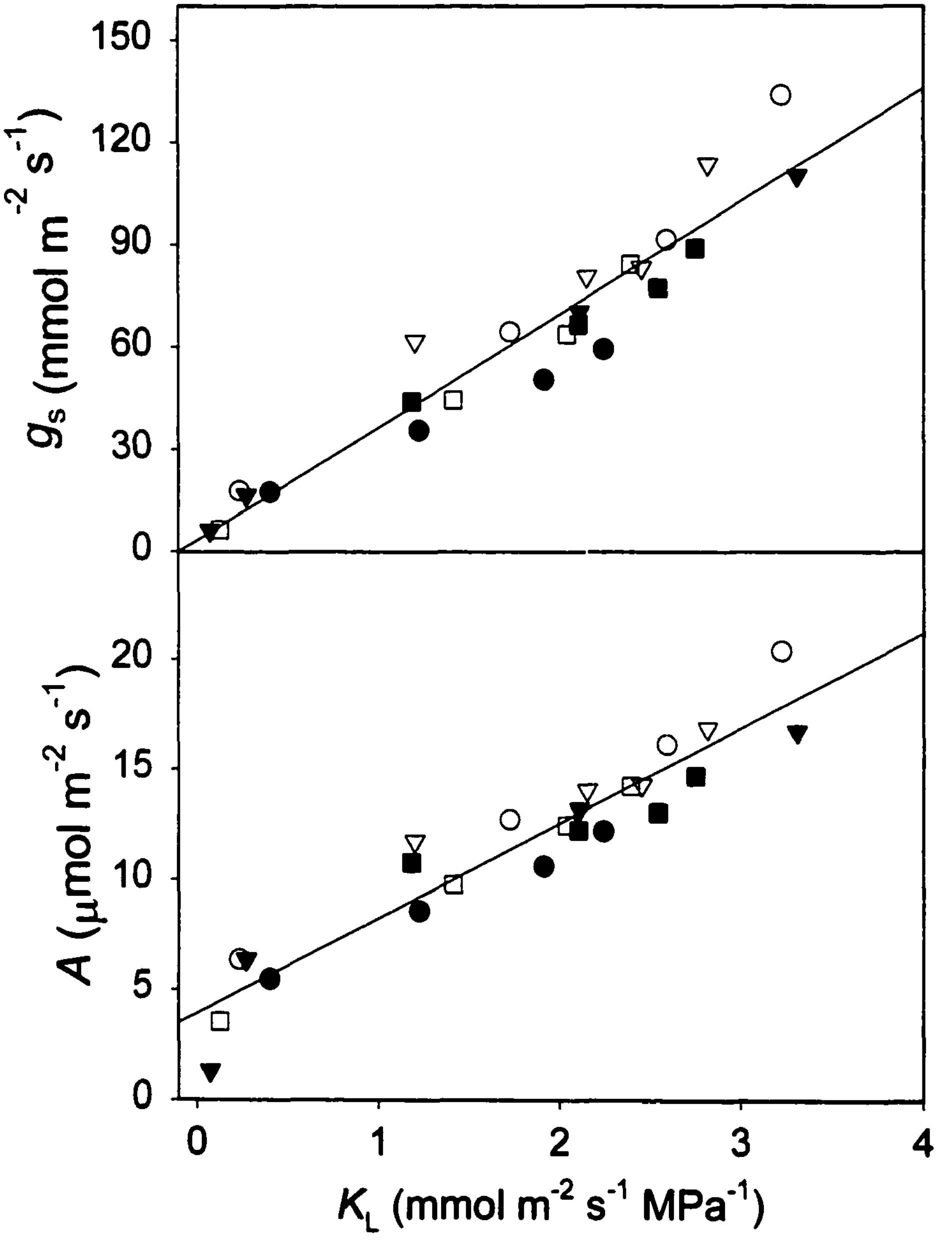

Figure 3.5 Stomatal conductance (g_s) and assimilation (A) versus whole plant leaf specific conductance (K_L) for each seedling (n=6) at each injection pressure. Different symbols represent each separate seedling. The regression equations are $g_s = 4.5 + 496.86 * K_L$, and $A = 4.06 + 64.79 * K_L$. R^2 for g_s and A versus K_L is 0.91 and 0.90 respectively. The intercept was not different from zero ($P > 0.05$) and the slope did not differ from ($\Psi_{soil} - \Psi_{leaf}$)/leaf to air mole fraction difference (c. 0.032) ($P > 0.05$).

CHAPTER IV

THE EFFECT OF BRANCH HEIGHT ON LEAF GAS EXCHANGE, BRANCH HYDRAULIC CONDUCTANCE AND BRANCH SAP FLUX IN OPEN GROWN PONDEROSA PINE

ROBERT M. HUBBARD
USDA Forest Service
Rocky Mountain Research Station and
Graduate Degree Program in Ecology, Colorado State University
240 West Prospect Rd
Fort Collins, CO 80526

BARBARA J. BOND
Oregon State University
154 Peavy Hall
Corvallis OR 97331

RANDY S. SENOCK
College of Agriculture and Forestry
University of Hawaii
Hilo, HI 96720

MICHAEL G. RYAN
USDA Forest Service
Rocky Mountain Research Station and
Graduate Degree Program in Ecology, Colorado State University
240 West Prospect Rd
Fort Collins CO 80526

For Submission to TREES

Summary

Recent experimental evidence has shown that stomata respond to changes in the hydraulic conductance of the flow path from soil to leaf suggesting that this may affect stomatal conductance and photosynthesis in branches of different heights in open grown, tall tree canopies. Our objective in this study was to determine if leaf gas exchange, branch sap flux, leaf specific hydraulic conductance, foliar $\delta^{13}C$ and ratios of leaf area to sapwood area within branches were dependent on branch height within the crowns of four individual open grown ponderosa pine (*Pinus ponderosa* Laws.) trees. Branch heights in the upper and lower crown were ~25m and 10m respectively. We found no difference in leaf gas exchange, branch sap flux or leaf specific hydraulic conductance from soil to leaf between the upper and lower canopy of our study trees. We also found significantly lower ratios of leaf area to sapwood area (A_l:A_s) in branches from the upper compared to the lower canopy. A_l:A_s averaged 0.17 (m^2 cm^{-2}) and 0.27 in the upper and lower canopy respectively. Differences in the leaf specific conductance of branches in the upper crown versus the lower crown were small and not significantly different. Other studies at our site indicate lower hydraulic conductance, lower sap flux, lower whole tree canopy conductance and lower photosynthesis in old compared to young trees. However, data from this study show that height induced increases in path length do not account for hydraulic limitation in these old, tall ponderosa pine. Instead, hydraulic limitation may be the result of other factors including increased branch length, reduced sapwood permeability or an increased number of branch junctions in the older, taller trees.

Introduction

Recent work suggests that stomata are responsive to changes in the hydraulic conductance of the flow path from soil to leaf. Whole-plant leaf specific hydraulic conductance (K_L) has been shown to be positively related to stomatal conductance as K_L changes with development (Hubbard et al. 1999, Meinzer and Grantz 1990, Mencuccini and Grace 1996a, Saliendra et al. 1995) and between species (Meinzer et al. 1995). Stomata also respond to experimental manipulations of hydraulic conductance involving induction of xylem cavitation (Hubbard et al. 2000, Sperry and Pockman 1993), root pruning (Meinzer and Grantz 1990, Teskey et al. 1983), notching of stem xylem (Sperry et al. 1993), freeze-thawing of stems (Hammel 1967), and defoliation (Hubbard et al. 1999, Pataki et al. 1998). More recently, Hubbard et al. (2000) showed that both stomatal conductance and photosynthesis varied linearly with K_L in ponderosa pine seedlings. When it has been measured, the response occurs within minutes of the manipulation, and bulk leaf water status can remain nearly constant during the experiment (Hubbard et al. 2000, Saliendra et al. 1995, Sperry and Pockman 1993, Teskey et al. 1983).

These studies suggest a tight link between liquid and vapor phase conductances arising from the fact that stomata control plant water status to allow photosynthesis on the one hand and to prevent dehydration induced damage from excessive transpiration on the other. Preventing dehydration induced damage requires that stomata close below a critical leaf water potential preventing cavitation of the water column (Jones and Sutherland 1991, Tyree and Sperry 1988). Since changes in hydraulic conductance can alter flow and thus plant water status, stomata respond to changes in K_L.

K_L in trees depends upon the path length from soil to leaf (Pothier et al. 1989a) suggesting that g_s may also vary with tree height. Whitehead et al. (1984) proposed a simple hydraulic model to illustrate the interrelationships between sapwood permeability (k), the pressure difference between the ends of the flow path ($\Delta\Psi$), leaf area (A_l), sapwood area (A_s), air saturation deficit (D), stomatal conductance (g_s) and path length (l):

$$\frac{Al}{A_s} = \frac{k(\Delta\Psi)}{l\eta D g_s} \tag{1}$$

Rearrangement of equation 1 to solve for g_s gives:

$$g_s = \frac{A_s}{A_l} \bullet \frac{k(\Psi_{soil} - \Psi_{leaf})}{l\eta D} \tag{2}$$

This model suggests that g_s will be inversely proportional to the length of the flow path unless there are changes in A_l:A_s, sapwood permeability, $\Delta\Psi$, or some combination thereof. Schaffer et al. (2000) recently used a similar hydraulic model to explain variations in whole canopy conductance (G_t) with height in European beech. Changes in A_l:A_s in their study partially compensated for the effects of height on g_s.

Other investigators have found that A_l:A_s at breast height decreases with height for individual trees and these studies have recently been reviewed by McDowell et al. (*In Review*). In general, a decrease in A_l:A_s with tree height may mitigate the effects of increased path length by decreasing the ratio of transpirational to transport tissues.

Few studies have examined the effects of increased height on K_L, g_s and A within a tree canopy. The length of the flow path from soil to leaf can vary substantially in trees with deep canopies with potentially large differences in g_s and K_L at the bottom versus the top of the tree. Most studies that have examined leaf level gas exchange with canopy depth have focused on changes in microclimate and or light interception (e.g. Ellsworth and Reich 1993, Rijkers et al. 2000, Schoettle and Smith 1999) such that we have little information of the direct effect of canopy height on leaf level gas exchange. Understanding how branch height affects stomatal conductance and photosynthesis may improve our mechanistic models of tree and forest growth and response to climate change.

Whitehead et al. (1984) showed that their hydraulic model also explained differences in stem internode A_l:A_s and the foliage above the internode within a single tree. However, because there is good evidence that resistance in branches can be much greater than in the stem (Tyree and Ewers 1991, Yang and Tyree 1994, Zimmermann 1983) branch A_l:A_s could be an equally important hydraulic adjustment to increased path length from the bottom to the top of the canopy.

Our goal in this study was to assess the impact of branch height within a tree on branch A_l:A_s, K_L, sap flux, g_s, photosynthesis (A) and the foliar $\delta^{13}C$ ratio in ponderosa pine growing in an open stand on the east side of the Oregon Cascades. We reasoned that the increased length of the flow path from the bottom to the top of the canopy would cause proportional differences in branch sap flux, g_s, A and K_L that might be partially mitigated by changes in the A_l:A_s ratio of branches.

Materials and Methods

Our study site is located near Black Butte Oregon USA (N44° 25′ W121° 40′) at an elevation of 1030m. Many years of partial thinning and harvesting have resulted in an open stand (LAI < 1) of ponderosa pine (*Pinus ponderosa* Laws.) with most trees receiving full sunlight throughout the day. The old, tall trees at our site are approximately 36m tall and 290 years old. There is a sparse understory consisting of bitterbrush (*Purshia tridentata* (Pursh) DC.), snowbrush (*Ceanothus velutinus* Doug.), Manzanita (*Arctostaphylos patula* Greene) and several species of bunch grass. The soil at our site is a deep sandy loam derived from volcanic ash and classified as a light-colored andic Inceptisol.

Our site has cool, wet winters and warm, dry summers and most of the precipitation falls during the winter months. Mean annual precipitation is 360 mm with about only 17% falling between May and September (Oregon Climate Service, Oregon State University, Corvallis, OR USA).

Gas Exchange Measurements

We used two methods of canopy access for our gas exchange measurements. We erected scaffolding beside one tree (T1) to a height of 25m allowing access to the upper and lower third of the canopy. For this tree we measured leaf gas exchange on 6 branches each at heights of 10 and 20m on days 190, 191, 194 and 195. We used a hydraulic lift to access the upper and lower third of 3 other ponderosa pine (T2 – T4) trees. Using the lift, we measured leaf gas exchange on 4 branches each at heights of ~10 and 25m on day of year 202, 203 and 204 for these three trees.

Gas exchange measurements were made using a LICOR 6400 (LICOR Inc.) open system infrared gas analyzer (Field et al. 1989). We measured g_s, transpiration (E), and A on one-year old intact needles under natural, saturating photosynthetically active radiation (PAR > 1000 μmols m^{-2}, Hadley, 1969). Relative humidity and CO_2 concentration within the chamber was kept near ambient using the automated control of the LICOR 6400. Branches were exposed to full sunlight for at least 30 minutes prior to measurement.

Branch Sap Flux Measurements

We measured whole branch sap flux (Q) at the canopy of T1 using heat balance sap flux gauges described in detail by Senock and Ham (1993). We measured 10 branches each at 10 and 20m. Branches were chosen that represented the sunlit portion of the crown through most of the day. The sap flux gauges were installed on branches approximately 2 cm in diameter following Senock and Ham (1993). Each gauge was insulated with polyurethane foam and wrapped with aluminum foil insulating the gauge from solar radiation.

We calculated Q (kg s^{-1}) as:

$$Q = E - E_v - E_r/[c(T_{so} - T_{si})] \qquad (4)$$

where E represents the heat energy supplied to the gauge, E_v is the apical and basal heat energy transferred by conduction along the stem axis, E_r is the heat energy transported by the mass flow of water, c is the specific heat of water (J kg^{-1} K) and $T_{so} - T_{si}$ is the temperature difference between the water flowing into and out of the heated segment.

Sap flux measurements were collected each minute, averaged and stored every 15 minutes using a data logger (21x, Campbell Scientific INC., Logan UT). We measured branch Q from 0300 through 2100 on days 191 – 211. We estimated heat loss from conduction (E_v) on a humid night prior to day 191 assuming zero flow through the branches. For the measurement period, we turned off the power from 2100 through 0300 to conserve battery power, which was charged using solar panels. Rates for the sap flux measurements are expressed per unit projected leaf area (Q_L m^2) and per unit sapwood area (Q_{sw} cm^2).

$\delta^{13}C$ measurements

If higher resistance reduces water supply to the foliage in the upper canopy, photosynthesis will be more limited by stomatal conductance than by enzymatic capacity for photosynthesis. Therefore, foliage from the upper crown position should show less discrimination to ^{13}C in these open, well-ventilated canopies and the δ^{13}C value of the cellulose in upper canopy foliage should be higher (less negative) than that of the lower canopy (e.g. Evans et al. 1986, Farquhar et al. 1989). We extracted cellulose from one year old foliage on branches from the upper and lower canopy of our sample trees using a method described by Wise et al. (1945). The extracted cellulose was analyzed for δ^{13}C by mass spectrometry at the Stable Isotope Facility at the University of Waikato, New Zealand.

K_{Ltree} measurements

We estimated the resistance of soil to leaf for one-year old needles at the top and bottom of trees T2-T4 using Equation 1. Ψ_{soil} was estimated from predawn Ψ_{leaf} measurements corrected for the hydrostatic gradient. We measured g_s and Ψ_{leaf} on each of 2 branches between 1100 and 1300. Afterwards, branches were removed and transferred back to the laboratory to determine K_L branch.

K_{Lbranch} measurements

At the end of our gas exchange measurements for trees T2-T4, we destructively sampled 2 branches each from the upper and lower canopy of each tree (10 and 20m).

We re-cut the branches under water and transported back to lab with cut ends immersed in water.

In the laboratory, we connected the branch to a potometer and allowed the branch to transpire under low light conditions (PAR ~400 μmol m^{-2}). Ψ_{leaf} was measured on 3 fascicles after steady state flow was reached (~ 30 minutes) and $K_{Lbranch}$ calculated as:

$$K_L = F/\Delta P \qquad (5)$$

where F is the flow of water through the branch (ml min^{-1}), ΔP is the difference between Ψ_{leaf} and the Ψ of water in the potometer reservoir exposed to atmosphere (0).

Leaf area, sapwood area and $\delta^{13}C$ measurements

We sampled 10 branches per canopy position for T1 and 2 branches per canopy position for trees T2 –T4 (as part of the $K_{Lbranch}$ measurements). After the respective measurements on the intact branches were complete, we harvested all of the branch needles, dried them at 65 °C for 48 hours and weighed them to the nearest 0.1 g. Projected leaf area was determined by a leaf area vs. weight relationship developed at the same site (Ryan et al. 2000).

For T2-T4, after $K_{Lbranch}$ was determined, we removed a 5 cm section of the branch stem from the proximal end to measure functional and total sapwood area (cm^2). We connected the stem segment to a gravity flow system with a pressure head of 0.01MPa and allowed filtered (0.22 μm) 0.1% (w/v) safranin dye (Sperry and Sullivan 1992) dye to flow through the stem segment. When dye was apparent on the distal end of the segment, we removed a thin disk from the middle of the segment and measured the total and stained sapwood area using an image analysis system (Image Pro, Media Cybernetics, 1995).

Data Analysis

Any differences in the relationship of leaf gas exchange parameters with D were assessed by averaging g_s, E, and A by 0.25 D class for all of our trees and measurement days. We analyzed the response of leaf gas exchange with D using a general linear model (Neter et al. 1990). The individual branch was our sampling unit but because we measured leaf gas exchange on the same branch through the course of each day we used a repeated measures analysis. This analysis assumes a constant correlation among measurements on the same tree (a standard compound symmetric covariance model).

Differences in branch sap flux with height (n=10) were assessed with two relationships. First, we estimated the average diurnal pattern by computing hourly averages for each gauge over the measurement period. Second, we estimated the response of Q_L and G_t with D by averaging all gauges by 0.25 kPa increments for all the days during our measurement period. For both relationships, we assessed differences using an independent samples t test (Ramsay and Silverman 1997). For this test, there was no violation of the assumptions of normality and homogeneity of variance.

Because we had to turn off the power at night, we were unable to asses the role of capacitance in our assumption that $Q_L = E$ for our derivation of G_t. However, in a study that included tree T1, Ryan et al. (2000) found that the effect of capacitance on Q_L was relatively small (~12% of daily flows) and did not affect diurnal patterns of sap flux in ponderosa pine at our study site.

For each branch we assumed $Q_L = E$ and calculated whole branch conductance (G_t) as:

$$G_t = Q_L / D \qquad (6)$$

where Q_L is xylem sap flux per unit leaf area and D is air saturation deficit in partial pressure units (kPa/kPa). Branch G_t represents the conductance of the average leaf on the branch. Although G_t includes both branch conductance g_b and aerodynamic conductance g_a (Köstner et al. 1992) we assume that G_t roughly equals g_b because these ponderosa pine canopies are well coupled to the atmosphere and g_a is typically at least an order of magnitude greater than g_b in coniferous forests (Granier et al. 1996, Lindroth 1985).

Differences in K_L tree and $K_{Lbranch}$ were determined using a paired samples t test (Ramsay and Silverman 1997). For A_l:A_s (n=4), K_{Ltree} and $K_{Lbranch}$ (n=3), the tree was our sampling unit.

Results

The response of g_s, and A with D did not vary with height in the canopy ($P = 0.83$ and $P = 0.34$ respectively; Figure 4.1A, 4.1B). Maximum g_s was 160 mmol m^{-2} s^{-1} and maximum A was approximately 16 µmol m^{-2} s^{-1}. At high D, g_s declined to approximately 60 mmol m^{-2} s^{-1} and A declined to approximately 6 µmol m^{-2} s^{-1}. The linear correlation coefficient (r) for g_s and A vs. D at both canopy positions was 0.82 and 0.66, respectively.

Measurements of branch sap flux and total branch conductance showed little variation with canopy height. Mean diurnal Q_L did not vary between the upper and lower canopy ($P = 0.62$, Figure 4.2). Although diurnal Q_{sw} at 20m had consistently lower values, the differences compared to Q_{sw} at 10m were not significant ($P = 0.12$, Figure 4.2). Mean Q_L increased steadily in the morning hours, fluctuated around a maximum value of about 3.5 mmol m^{-2} s^{-1} during late morning and early afternoon hours and then steadily declined later in the day (Figure 4.2). Mean diurnal G_t peaked around 0800, steadily declined throughout the day (Figure 4.2) and did not differ with branch height in the canopy ($P = 0.90$). The response of branch sap flux and total branch conductance with D was likewise similar with canopy height. Mean Q_L peaked around 1.75 kPa for both upper and lower canopy positions and remained relatively constant at higher levels of D ($P = 0.41$) (Figure 4.3). Although G_t appeared slightly higher for the branches in the upper canopy at low D values, the differences were not significant (P=0.77, Figure 4.3).

Stable isotope values of carbon also did not vary with canopy height ($P = 0.88$). $\delta^{13}C$ from one-year old needles was – 22.99‰ and –23.04‰ for the upper and lower canopy respectively.

Measurements of K_{Ltree} and $K_{Lbranch}$ did not differ with canopy height ($P = 0.56$ and 0.48 respectively). $K_{Lbranch}$ was 0.14 kg h^{-1} MPa^{-1} m^{-2} at the top of the canopy and 0.11 kg h^{-1} MPa^{-1} m^{-2} at the bottom. Values for K_{Ltree} at the top and bottom of the canopy were 0.14 and 0.15 kg h^{-1} MPa^{-1} m^{-2} respectively (Figure 4.4A).

The leaf area to sapwood ratio varied significantly with canopy height of the branch in our four experimental trees with branches at ~20m exhibiting a much lower A_l:A_s than branches from ~10m in the canopy ($P = 0.03$, Figure 4.4B). Values for A_l:A_s in the upper and lower canopy were 0.17 and 0.27 (m^2 cm^{-2}) respectively.

Discussion

We found that branch height in the canopy had no effect on the response of g_s and A with D in ponderosa pine at our site (Figure 4.1). The pathway from soil to leaf for branches in this study was a little more than twice as long to the upper versus the lower branches. The Whitehead model (Equation 2) suggests that g_s will be lower in the upper versus the lower canopy unless k_s, $\Delta\Psi$ or A_l:A_s change in concert with path length. We measured Ψ_{leaf} and Ψ_{soil} for a subset of our gas exchange measurements to estimate K_{Ltree} for trees T2-T4. Staining our branch segments suggests that permeability was similar with branch height and when we accounted for the height induced gravitational potential (0.01 MPa m^{-1}), there was also no difference in $\Delta\Psi$ with branch height. We used these data and predicted g_s for branches at 10m and 25m using Equation 2. There was no difference in predicted versus measured g_s ($P = 0.36$). Likewise our measurements of $K_{Lbranch}$ and K_{Ltree} showed no difference with canopy height ($P = 0.56$ and 0.48 respectively). Taken together, these data suggest that decreased branch A_l:A_s with height completely compensates for increased path length in these trees.

Most other studies that have examined leaf gas exchange throughout a canopy profile have focused on the light environment of the foliage and not the effect of changes in hydraulic conductance with path length (e.g. Ellsworth and Reich 1993, Rijkers et al. 2000, Schoettle and Smith 1999). However, Cochard et al. (1997) examined the vertical profile of leaf gas exchange in similar environmental conditions within 15-18m tall ash trees (*Fraxinus excelsior* L.) and found little variation in values of g_s, and E with canopy position. Likewise, Oliveira et al. (1996) found that g_s did not vary with crown position in cork oak (*Quercus suber* L.). These data are consistent with the patterns found in this study but the cause cannot be attributed to changes in A_l:A_s because this parameter was not measured in either of these studies.

Our branch sap flux data provide independent support for the lack of variation in leaf gas exchange with crown position in our study trees. The response of Q_L and Q_{sw} with time and D was no different in the upper compared to lower canopy (Figs 2&3). Our estimate of G_t (the average stomatal conductance on the branch) also indicates that vapor phase conductance does not differ with canopy height.

The stable carbon isotope signature of foliage provides a time-integrated measure of plant water status (Farquhar et al. 1989). Under similar environmental conditions, $\delta^{13}C$ signature from foliage at the top of the canopy should be higher (less negative) than at the bottom of our study trees if lower K_L at the top of the canopy reduces g_s and A. Because $\delta^{13}C$ was identical with foliage position, we suggest that this provides further evidence of similarity of g_s (and consequently A) with canopy position.

At our site, these open grown ponderosa appear to mitigate the effects of increased path length from soil leaf by reducing A_l:A_s in upper canopy branches. There are relatively few studies that have examined how branch A_l:A_s changes with canopy height (Oren et al. 1986) and only one study to our knowledge that attempts to determine which component of A_l:A_s is changing: leaf area, sapwood area or both. Cochard et al. (Cochard et al. 1997) found that branch leaf area declined in the upper canopy of *Fraxinus excelsior* trees presumably in response to increased xylem resistance. How the shift in branch A_l:A_s occurs suggests different strategies for how plants deal with increased hydraulic constraints with height growth. In our study, differences in branch diameter were small and not statistically different, allowing us to compare the relative amounts of leaf area and sapwood per branch in the upper and lower canopy. We found that leaf area decreased from 0.7 to 0.5 m^2 and sapwood area increased from 2.4 to 3.6 cm^2 from the bottom to the upper canopy; however neither of these changes were significant. An argument for changes in A_l:A_s resulting from changes solely from decreased leaf area or increased sapwood quickly becomes circular. For trees in our study however, both parameters seem to be changing in concert, leading to lower branch A_l:A_s in the upper canopy.

In another study that examined differences in growth patterns between high and low vigor ponderosa pine growing near Flagstaff AZ., Kaufmann (1995) used a branch sapwood area to leaf area relationship from five crown positions to estimate total leaf area. Although these specific data were not published, an initial analysis indicates a linear decline in branch A_l:A_s with height (P <0.01), supporting the findings from this study.

Ryan and Yoder (1997) proposed the hydraulic limitation hypothesis for the reduced productivity observed in aging forest stands. This hypothesis suggests that as trees grow older and taller, K_L declines causing stomates to close earlier in the day (relative to younger, shorter trees) to preserve Ψ_{leaf} regulation. They propose that this may explain the well-documented decline in stand NPP after canopy closure and why all trees eventually significantly slow their height growth.

Hydraulic limitation to water flux and leaf gas exchange does appear to occur in old compared to young ponderosa pines at our site. In a previous study, Hubbard et al. (1999) found lower K_L and steeper decline of g_s and A with D at the tops of tall versus short ponderosa pine. Interestingly, the slopes of the relationships of g_s and A with D in their study were not significantly different from those found here ($P = 0.14$, (1999). In a separate study at our site, Ryan et al. (2000) showed that whole tree sap flux and canopy conductance was lower in tall versus short ponderosa. In both studies, it was suggested that increased height my have significantly contributed to the hydraulic limitation in the taller trees. Data from this study shows height does not contribute to differences within a single tree. Instead, hydraulic limitation may result from other factors that change with height and age, possibly including longer branch length, decreased sapwood permeability and or increased number of branch junctions.

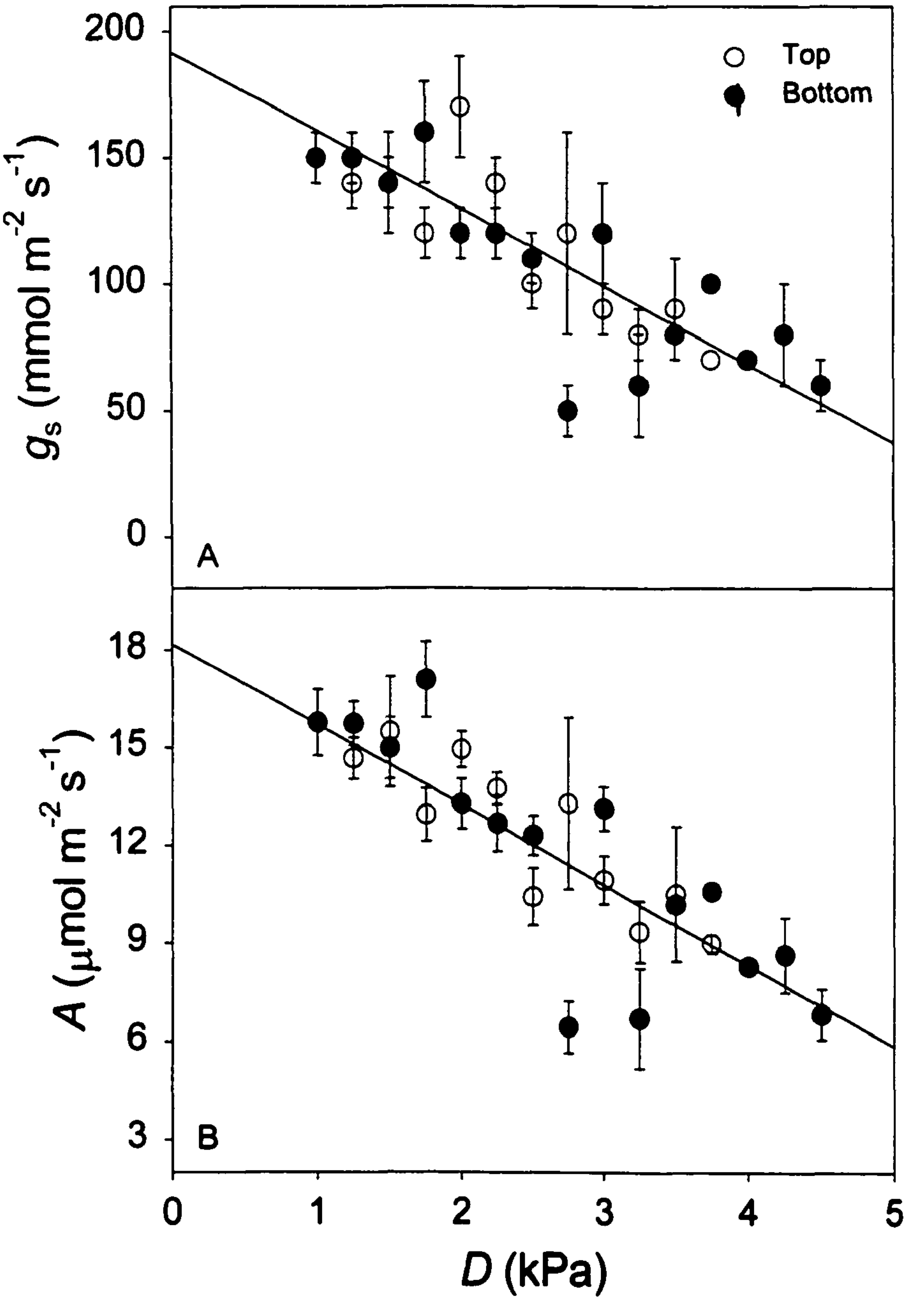

Figure 4.1 Stomatal conductance (g_s) and net photosynthetic assimilation (A) versus air saturation deficit (D) by canopy position for three ponderosa pine at the Black Butte study site. All measurements were averaged by 0.25 D class increments. The response of foliage from the upper and lower canopy did not differ for g_s ($P = 0.82$) or A ($P = 0.35$). R^2 for g_s in the upper canopy was 0.43 and 0.46 for the lower canopy. R^2 for A was 0.44 and 0.51 in the upper and lower canopy respectively. Error bars are ± 1 standard error for the variation among trees.

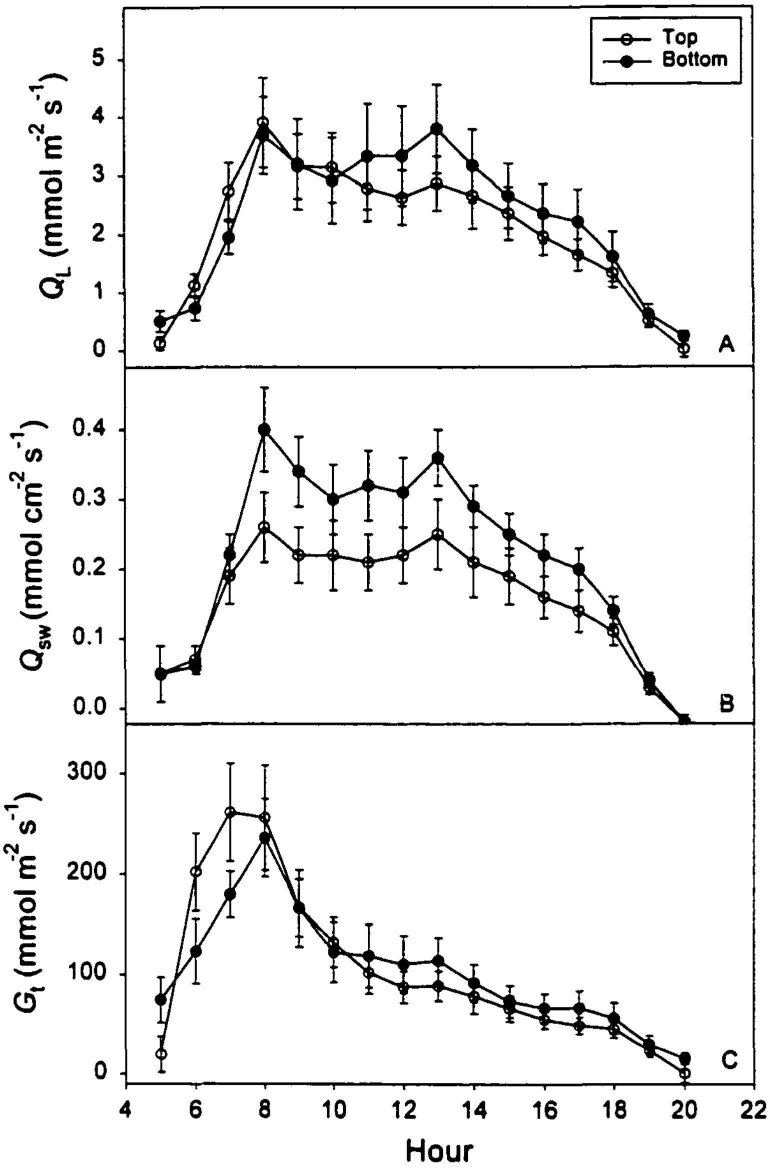

Figure 4.2 Mean hourly branch sap flux per unit leaf area (Q_L), per unit sapwood area (Q_{sw}) and mean hourly branch conductance (G_t) for days 191 – 211. Fifteen minute values were average by hour for days 191-211. Differences with canopy position were not significant for any of the three parameters ($P = 0.62$, $P = 0.12$, and $P = 0.98$ for Q_L, Q_{sw} and G_t respectively. Error bars are ± 1 standard error for the variation among branches.

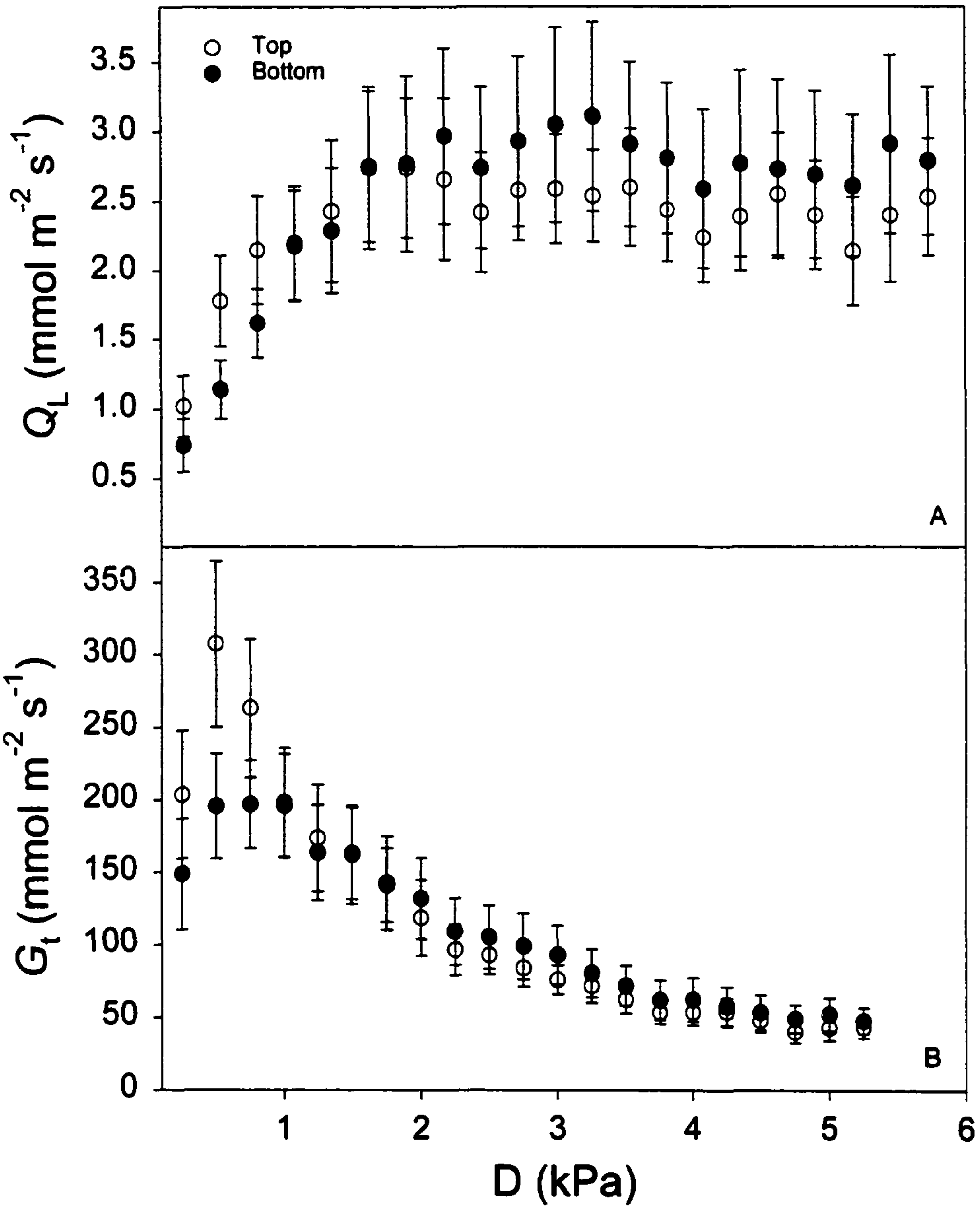

Figure 4.3 Mean hourly sap flux per unit leaf area (Q_L) and whole branch conductance (G_t) versus *D* for days 191 – 211. Fifteen minute values were average by 0.25 kPa class. There was no significant difference with canopy position for either parameter ($P = 0.41$ and $P = 0.77$ for Q_L and G_t respectively). Error bars are ± 1 standard error.

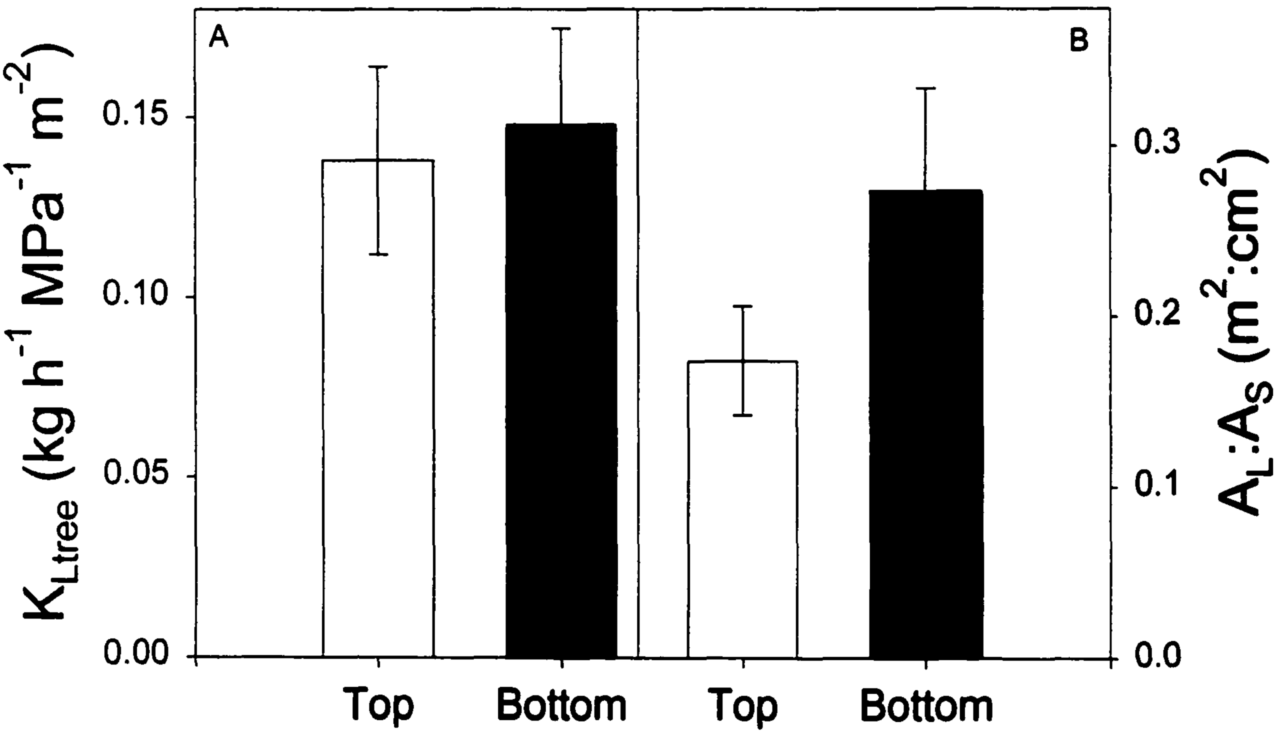

Figure 4.4 Mean leaf specific conductance branch conductance (K_{Ltree}) (A) and mean leaf area to sapwood area ratio A_l:A_s (B) at the top and bottom of the canopy. K_{Ltree} did not differ with canopy position (P = 0.48) and A_l:A_s was significantly lower at the top versus bottom of the canopy (P = 0.03). Error bars are ± 1 standard error.

CHAPTER V

Summary and Synthesis

If the hydraulic limitation hypothesis can explain cessation of height growth and the commonly observed decline in NPP after canopy closure, there are three basic physiological components that must be operating as trees grow taller and older. First, as trees increase in stature, K_L must decline and remain lower in tall, old compared to short, young trees. Second, stomata in the old trees must close in response to the increased resistance thereby maintaining Ψ_{leaf} above a critical level and avoiding cavitation. Third, this stomatal closure must decrease net carbon assimilation.

In this dissertation I show that for ponderosa pine, all of the above factors are present and may act to reduce growth per unit leaf area in tall/old trees, providing support for the hydraulic limitation hypothesis. In addition, these data provide further evidence that plants control stomata to maintain plant water status above a minimum level. In chapter two, I show that older trees have lower K_L and that stomata close at lower D thereby limiting A. K_L was 44% lower in old trees than in young trees. In old trees, g_s and A declined much more steeply with D for the entire summer. Mid-day g_s and A at typical mid-day values of D (2.5 – 3.0 kPa) were 32% and 21% lower respectively for old and young trees. In addition, increasing branch leaf specific conductance by removing 50% of the foliage from a set of experimental branches increased g_s and A suggesting stomata respond directly to change in K_L. In chapter 3, precise reductions of K_L using air injection resulted in a linear decline in g_s and A offering further support for the hypothesis. In chapter 4, g_s and A do not differ with branch height in the canopy. I also show that branch A_l:A_s decreases higher in the canopy suggesting ponderosa pine mitigate increase path length and resistance by changing the ratio of transport to transpirational tissues. The study in chapter one and the study by Ryan et al. (2000) suggest that hydraulic limitation occurs in old tall trees at the Black Butte site. The study in chapter four shows that increases in height are mitigated by decreased branch A_l:A_s with height. Therefore, other factors (perhaps longer branch length, decreased sapwood permeability and increased number of branch junctions) may change with height imposing hydraulic constraints on the older, taller trees.

Where are we now?

Since this research was started, several investigators have been examining hydraulic limitations to growth in a number of other species. Kolb and Stone (Kolb and Stone 2000), found lower morning and afternoon g_s and A was associated with lower K_L in old growth pine compared to sapling and pole size trees. Fredericksen et al. (1996) found lower rates of g_s and A in the upper grown of black cherry trees relative to younger shorter trees. Although they did not directly measure K_L their data from Ψ_{leaf} and g_s suggest lower K_L in the older trees. Schäfer et al. (2000) found lower sap flux per unit leaf area and lower canopy conductance in tall compared to short European beech. Scientists in Oregon have collected leaf level gas exchange and sap flux data indicating lower E, g_s and A from old, tall individual Douglas fir and white oak trees (Barbara Bond, personal communication). Ryan and Whitehead (pers. com.) found K_L was lower at the tops of tall versus short mountain beech trees in New Zealand and lower K_L was associated with a steeper decline of g_s and A with D.

Although most studies examining hydraulic limitation to growth generally support the hypothesis, several studies offer contradictory evidence. Phillips et al. (in prep) found no evidence for hydraulic limitation in two tropical species in Panama. However these trees did not appear to regulate Ψ_{leaf} and the tall and short trees were in very different microenvironments. Barnard et al. (in prep) likewise found that sap flux and leaf gas exchange per unit leaf area was not different in old tall Eucalyptus trees relative to younger, shorter trees on the same site; however they did observe a decrease in A_l:A_s in the older trees.

New hypotheses that strive to explain universal pattern and process in ecosystems are often controversial and the hydraulic limitation hypothesis is no exception. Becker et al. (2000) suggest that the hydraulic limitation does not explain cessation of height growth or decline in NPP because trees adjust the ratio of transpirational to transport tissues thereby maintaining a constant K_L during a lifetime. Furthermore they suggest that genetic signals trigger height growth stoppage in old tall trees as competition for light decreases. In their responses, Mencuccini and Magnani (2000)and Bond and Ryan (Bond and Ryan 2000) show that although we need to more fully examine the effects of changes in hydraulic architecture with age and consider below ground processes, the hydraulic limitation hypothesis is still useful construct to examine limitations to height growth and the decline in NPP with stand age. Available data and the debate initiated by

Becker et al. (Becker et al. 2000), point toward future research directions for examining hydraulic limits to tree growth.

Where do we go from here?

Although studies to date generally provide support for the hydraulic limitation hypothesis, we are a long way from showing that hydraulic limitation causes trees to stop growing in height and old forests to decline in above ground NPP. Future research should focus on the following four areas: 1) continue to characterize differences in K_L and leaf gas exchange in individual trees across a wide rage of species and site types 2) determine how hydraulic architecture changes with species and site type 3) characterize differences in stand level canopy conductance with age across species and site types and relate these differences to age related differences in NPP and 4) develop methods and test how changes in belowground structure and allocation affect overall K_L .

Testing the hydraulic limitation hypothesis on individual trees is necessary if we hope to determine if the physiological factors listed at the beginning of this chapter are operating during tree growth and across species and site types. Still, there are several problems associated with gas exchange and sap flux measurements in individual trees. A primary concern is finding the appropriate metric to express rates of conductance, assimilation and sap flux. All of the rates expressed here are on a per unit leaf area basis which seems appropriate given the open grown nature of our study site. However, comparisons of rates per unit leaf area in denser or closed canopy stands may be problematic. In these stands, differences in light absorption and or microclimate may confound comparisons between old and young trees because all the leaves on the tree may not be physiologically similar. In conifers that may retain needles for many years, leaf age may also make comparisons of rates per unit leaf area unrealistic. Testing and determining alternative metrics (light absorption for example) for comparisons should be a prime research focus in the future.

Several investigators have pointed out the importance of changes in hydraulic architecture in determining the effect of hydraulic constraints on tree growth (Becker et al. 2000, Magnani et al. 2000, Mencuccini and Magnani 2000). A growing body of evidence suggests that trees may at least partially mitigate the effects of increased height via reduced A_l:A_s (both stem to total canopy and within branches) as well as with increased sapwood permeability. However several studies suggest that these changes are insufficient to fully mitigate the effects of decreased K_L in old trees (Hubbard et al. 1999, Magnani et al. 2000, McDowell et al. *In Review*). The problem is few studies measure changes in hydraulic architecture in concert with physiological parameters (e.g. g_s, A and K_L). Future studies in individual trees should incorporate measurements of hydraulic architecture with physiological measurements.

Studies of individual trees are important for investigating the role of hydraulic constraints on tree growth but the hydraulic limitation hypothesis strives to explain the decline in stand level NPP after canopy closure. Clearly stand level comparisons of K_L, water use and assimilation are necessary, however they are difficult to obtain. Several areas of research offer promise in this regard; stable isotope ecology, eddy covariance measurements and examining stream flow data from old and young forests on gauged watersheds.

If water supply to the foliage of old trees is reduced relative to young trees, photosynthesis will be more limited by stomatal conductance than by enzymatic capacity for photosynthesis given equal illumination and isotopic ambient CO_2 values. Therefore older trees should show less discrimination to ^{13}C and the $\delta^{13}C$ value of the cellulose in old tree foliage would be higher (less negative) than that of young trees (e.g. Evans et al. 1986, Farquhar et al. 1989). This represents a potentially powerful tool for examining differences in leaf gas exchange between old and young trees of the same species. For example, if we can select samples not influenced by shading, leaf samples taken for $\delta^{13}C$ analysis at the tops of tall and short trees should reflect differences in g_s caused by differences in K_L. However, stable isotope data I collected for this dissertation suggests other factors may also be important.

In 1995 I collected one year old foliage from the tops of my experimental trees (Chapter 1). For those samples, $\delta^{13}C$ was significantly higher in foliage from old trees relative to young (-23.4‰ and –25.9‰ respectively ($P < 0.01$)). These data suggested that it would be possible to expand my sample size and determine if the hydraulic effect observed in a few trees in 1995 persisted in a larger portion of the stand. In 1998, I collected one year old foliage samples from the tops of nine old and nine young trees at the Black Butte site. Stable carbon isotope analysis of these samples indicated no difference between old and young trees in $\delta^{13}C$ signature (-22.2‰ and –22.4‰ respectively ($P = 0.91$).

What could cause the discrepancy in $\delta^{13}C$ values between years? Differences appear to be driven by heavier $\delta^{13}C$ values (more discrimination) for foliage from young trees. Its possible that old and young trees alike experienced a drought in 1997 causing higher stomatal limitation in the young trees than normal; leading to more negative $\delta^{13}C$ values. However, precipitation data does not support this argument. Foliage collected in

1995 was formed during 1994 and foliage collected during 1998 was formed in 1997. If current photosynthate is used to build new foliage, the precipitation during the previous winter may affect the $\delta^{13}C$ signature of the newly built foliage because these trees rely heavily on winter recharge of the soil water. For the 1994 foliage, I examined precipitation data for the months of November and December 1993 and January through May 1994. For the 1997 foliage, I examined to November and December 1996 and January through May 1997. Precipitation for the winter and spring preceding the formation of the 1994 and 1997 foliage was 146 mm and 377 mm respectively. Kozlowski (Kozlowski 1992) suggests that developing needles can be strong sinks for stored carbohydrate. In this case, precipitation for the entire growing season prior to foliage formation may be more important. Precipitation for the years preceding formation of the 1994 and 1997 foliage was 390 mm and 591 mm respectively. Whether new foliage is formed using current or stored photosynthate (or some combination), the photosynthate used to build foliage in 1994 and 1997 was assimilated when the trees were likely experiencing very different water status. However, a "wetter" 1997 does not explain the heavier $\delta^{13}C$ values for 1997 foliage from young trees. At the very least these data illustrate that hydraulic limitations of g_s and A may vary substantially between years and we must consider this possibility when assessing the extent to which hydraulics limit growth in individual trees and forests. The use of stable isotopes may still be a valuable tool for examining stand level hydraulic limitation, but these data must be accompanied by a clear understanding of other factors affecting $\delta^{13}C$ of foliage.

Comparisons of stand water use on a watershed basis is a potentially useful tool for determining stand level hydraulic limitation. There are a number of gauged watersheds with forests of different ages (e.g. HG Andrews, Hubbard Brook and Coweeta Hydrologic lab) where comparisons may be possible. One of the only published comparisons comes from Australia where stream flow declined ~50% from *Eucalyptus regnans* catchments when old 100m trees were replaced with a new forest after a large fire. Leaf area differences between the two forest ages was insufficient to explain the higher stream flow from catchments with older trees (Watson et al. 1999). Examining data from gauged watersheds offers considerable promise for testing the hydraulic limitation hypothesis.

Virtually all of the studies examining hydraulic limitation in forests have been conducted on above ground components because it is the increase in path length of above ground structures that seemed most important. Recent work suggests that below ground processes may be equally important when assessing the role of hydraulic limitation on tree and forest growth. Magnani et al. (2000) suggest that a functional homeostasis may occur in trees as path length increases. Using a combination of modeled and actual data, they show that for Scots pine, there is a shift in the balance between transpiring foliage and absorbing roots as trees grow taller and larger. However they also point out that for their modeling runs, changes in this ratio along with changes in A_l:A_s were insufficient to completely counteract the effect of increased path length. Further evidence for the importance of below ground processes comes from modeling work on root xylem vulnerability and resistance. Sperry et al. (1998) suggest that transpiration in trees is limited by either loss of hydraulic conductivity in the root system or above ground stem xylem depending on A_l:A_s ratio. Although the amount, permeability and conductance of root systems is clearly important in assessing the role of hydraulic limitation to growth, these parameters are difficult to measure. Future research should focus on new methods for measuring these parameters and below ground processes should be included in future studies of hydraulic limitation to forest and tree growth.

References

Becker, P., F.C. Meinzer and S.D. Wullshleger. 2000. Hydraulic limitation of tree height: a critique. Functional Ecol. 14:4-11.

Binkley, D., F.W. Smith and Y. Son. 1995. Nutrient supply and declines in leaf area and production in lodgepole pine. Can. J. For. Res. 25:621-628.

Bond, B.J. and M.G. Ryan. 2000. Comment of 'Hydraulic limitation of tree height:a critique' by Becker, Meinzer and Wullschleger. Functional Ecol. 14:135-140.

Carlquist, S. 1975. Ecological strategies of xylem evolution. University of California Press, Berkeley, 259 p.

Cermák, J., M. Deml and M. Penka. 1973. A new method of sap flow rate determination in trees. Biologia Plantarum 15:171-178.

Cermák, J. and J. Kucera. 1981. The compensation of natural temperature gradient at the measuring point during the sap flow rate determination in trees. Biologia Plantarum 23:469-471.

Cochard, H., P. Cruiziat and M.T. Tyree. 1992. Use of positive pressure to establish vulnerability curves. Plant Physiol. 100:205-209.

Cochard, H., M. Peiffer, K. Le Gall and A. Granier. 1997. Developmental control of xylem hydraulic resistances and vulnerability to embolism in *Fraxinus excelsior* L.: impacts on water relations. J. Exp. Bot. 48:655-663.

Cowan, I.R. 1995. As to the mode of action of guard cells in dry air. *In* Ecophysiology of Photosynthesis. Eds. E.D. Schulze and M.M. Caldwell, Springer-Verlag, New York, pp 205-229.

Dewar, R.C. 1995. Interpretation of an empirical model for stomatal conductance in terms of guard cell function. Plant Cell Environ. 18:365-372.

Ellsworth, D.S. and P.B. Reich. 1993. Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. Oecologia 96:169-178.

Evans, J.R., T.D. Sharkey, J.A. Berry and G.D. Farquhar. 1986. Carbon isotope discrimination measured concurrently with gas exchange to investigate CO2 diffusion in leaves of higher plants. Aust. J. Plant Physiol. 13:281-292.

Farquhar, G.D., J.R. Ehleringer and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Ann. Rev. Plant Physiol. 40:503-537.

Farquhar, G.D., S. von Caemmerer and J.A. Berry. 1980. A biochemical model of photosynthetic CO2 Assimilation in leaves of C3 species. Planta 149:78-90.

Field, C. and H.A. Mooney. 1986. The photosynthesis-nitrogen relationship in wild plants. *In* On the economy of plant form and function. Ed. T.J. Givnish, Cambridge University Press, Cambridge, pp 25-55.

Field, C.B., J.T. Ball and J.A. Berry. 1989. Photosynthesis: principles and field techniques. *In* Plant Physiological Ecology: Field methods and instrumentation. Eds. R.W. Pearcy, J.R. Ehleringer, H.A. Mooney and P.W. Rundel, Chapman and Hall, New York, pp 209-252.

Franks, P.J., I.R. Cowan and G.D. Farquhar. 1997. The apparent feedforward response of stomata to air vapour pressure deficit: information revealed by different experimental procedures with two rainforest trees. Plant Cell Environ. 20:142-145.

Fredericksen, T.S., K.C. Steiner, J.M. Skelly, B.J. Joyce, T.E. Kolb, K.B. Kouterick and J.A. Ferinand. 1996. Diel and seasonal patterns of leaf gas exchange and xylem water potentials of different-sized *Prunus serotina* Ehrh. trees. For. Sci. 42(3):359-365.

Friend, A.D. and H.H. Schugart. 1993. A physiology-based gap model of forest dynamics. Ecology 74:792-797.

Fuchs, E.E. and N.J. Livingston. 1996. Hydraulic control of stomatal conductance in Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] and alder [*Alnus rubra* (Bong)] seedlings. Plant Cell Environ. 19:1091-1098.

Gower, S.T., J.G. Isebrands and D.W. Sheriff. 1995. Carbon allocation and accumulation in conifers. *In* Resource Physiology of Conifers., Academic Press, Inc., pp.

Gower, S.T., R.E. McMurtrie and D. Murty. 1996. Aboveground net primary production decline with stand age: potential causes. TREE 11:378-382.

Granier, A., P. Biron, B. Köstner, L.W. Gay and G. Najjar. 1996. Comparisons of xylem sap flow and water vapour flux at the stand level and derivation of canopy conductance for Scots pine. Theoretical and Applied Climatology 53:115-122.

Grier, C.C., K.A. Vogt, M.R. Keyes and R.L. Edmonds. 1981. Biomass distribution and above- and below-ground production in young and mature *Abies amabilis* zone ecosystems of the Washington Cascades. Can. J. For. Res. 11:155-167.

Grulke, N.E. and P.R. Miller. 1994. Changes in gas exchange characteristics during the life span of giant sequoia: implications for response to current and future concentrations of atmospheric ozone. Tree Physiol. 14:659-668.

Hacke, U.G. and J. Sauter. 1996. Drought-induced xylem dysfunction in petioles, branches, and roots of *Populus balsimifera* (L.) and *Alnus glutinosa* (L.) Gaertn. Plant Physiol. 111:413-417.

Hadley, E.B. 1969. Physiological ecology of *Pinus ponderosa* in Southwestern North Dakota. Am. Mid. Nat. 81(2):289-315.

Hammel, H.T. 1967. Freezing of xylem sap without cavitation. Plant Physiol. 42:55-66.

Holbrook, M.N., M.J. Burns and C.B. Field. 1995. Negative xylem pressures in plants: A test of the balancing pressure technique. Science 270:1193-1194.

Hubbard, R.M., B.J. Bond and M.G. Ryan. 1999. Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees. Tree Physiol. 19:165-172.

Hubbard, R.M., V. Stiller, M.G. Ryan and J.S. Sperry. 2000. Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. Plant Cell Environ. *In Press.*

Jones, H.G. and R.A. Sutherland. 1991. Stomatal control of xylem embolism. Plant Cell Environ. 14:607-612.

Kaufmann, M. 1995. To live fast or not: growth, vigor and longevity of old-growth ponderosa pine and lodgepole pine trees. Tree Physiol. 16:139-144.

Kolb, T.E. and J.E. Stone. 2000. Differences in leaf gas exchange and water relations among species and tree sizes in an Arizona pine-oak forest. Tree Physiol. 20:1-12.

Köstner, B., P. Biron, R. Siegwolf and A. Granier. 1996. Estimates of water vapor flux and canopy conductance of Scots Pine at the tree level utilizing different xylem sap flow methods. Theoretical and Applied Climatology 53:105-113.

Köstner, B.M.M., E.-D. Schulze, F.M. Kelliher, D.Y. Hollinger, J.N. Byers, J.E. Hunt, T.M. McSeveny, R. Meserth and P.L. Weir. 1992. Transpiration and canopy conductance in a pristine broad-leaved forest of *Northofagus*: an analysis of xylem sap flow and eddy correlation measurements.}. Oecologia 91:350-359.

Kozlowski, T.T. 1992. Carbohydrate sources and sinks in woody-plants. Botanical Review 58:107-222.

Kull, O. and A. Koppel. 1987. Net photosynthetic response to light intensity of shoots from different crown positions and age in *Picea abies* (L.) Karst. Scand. J. For. Res. 2:157-166.

Lindroth, A. 1985. Canopy conductance of coniferous forests related to climate. Water Resour. Res. 21:297-304.

Loewenstein, N.J. and S.G. Pallardy. 1998. Drought tolerance, xylem sap abscisic acid and stomatal conductance during soil drying: a comparison of young plants of four temperate deciduous angiosperms. Tree Physiol. 18:421-430.

Long, J.N. and F.W. Smith. 1992. Volume increment in *Pinus contorta* var. *latifolia*: the influence of stand development and crown dynamics. For. Ecol. Manage. 53:53-64.

Magnani, F., M. Mencuccini and J. Grace. 2000. Age-related decline in stand productivity: the role of structural acclimation under hydraulic constraints. Plant Cell and Environment 23:251-263.

Mattson-Djos, E. 1982. The use of pressure-bomb and porometer for describing plant water status in tree seedlings. *In* Vitality and quality of nursery stock--Proceedings of Nordic symposium. Ed. P. Puttonen, Hyytiala, pp 45-57.

McDowell, N., H. Barnard, B.J. Bond, T. Hinckley, R.M. Hubbard, H. Ishii, B. Köstner, F.C. Meinzer, J.D. Marshall, F. Magnani, N. Phillips, M.G. Ryan and D. Whitehead. *In Review.* The relationship between tree height and leaf area:sapwood area ratio. Oecologia.

McMurtrie, R.E., H.N. Comins, M.U.F. Kirschbaum and Y.P. Wang. 1992. Modifying existing forest growth models to take account of effects of elevated CO_2. Aust. J. Bot. 40:657-677.

Meinzer, F.C., G. Goldstein, P. Jackson, N.M. Holbrook, M.V. Butierrez and J. Cavelier. 1995. Environmental and physiological regulation of transpiration in tropical forest gap species: the influence of boundary layer and hydraulic conductance properties. Oecologia 101:514-522.

Meinzer, F.C., G. Goldstein, H.S. Neufeld, D.A. Grantz and G.M. Crisosto. 1992. Hydraulic architecture of sugarcane in relation to patterns of water use during plant development. Plant Cell Environ. 15:471-477.

Meinzer, F.C. and D.A. Grantz. 1990. Stomatal and hydraulic conductance in growing sugarcane:stomatal adjustment to water transport capacity. Plant Cell Environ. 13:383-388.

Meinzer, F.C., D.A. Grantz and B. Smit. 1991. Root signals mediate coordination of stomatal and hydraulic conductance in growing sugarcane. Aust. J. Plant Physiol. 18:329-338.

Mencuccini, M. and J. Grace. 1996a. Developmental patterns of above-ground hydraulic conductance in a Scots pine (*Pinus sylvestris* L.) age sequence. Plant Cell Environ. 19:939-948.

Mencuccini, M. and J. Grace. 1996b. Hydraulic conductance, light interception and needle nutrient concentration in Scots pine stands and their relations with net primary productivity. Tree Physiol. 16:459-468.

Mencuccini, M. and F. Magnani. 2000. Comment on 'Hydraulic limitation of tree height: a critique' by Becker, Meinzer and Wullschleger. Functional Ecol. 14:135-140.

Mohren, G.M.J., C.P. Van Gerwen and C.J.T. Spitters. 1984. Simulation of primary production in even-aged stands of douglas fir. For. Ecol. Manage. 9:27-49.

Monteith, J.L. 1995. A reinterpretation of stomatal responses to humidity. Plant Cell Environ. 18:357-364.

Mott, K.A. and T.N. Buckley. 1998. Stomatal heterogeneity. J. Exp. Bot. 49:407-417.

Mott, K.A. and D.F. Parkhurst. 1991. Stomatal responses to humidity in air and helox. Plant Cell Environ. 14:509-515.

Murty, D., R.E. McMurtrie and M.G. Ryan. 1996. Declining forest productivity in aging forest stands: a modeling analysis of alternative hypotheses. Tree Physiol. 16:187-200.

Neter, J., W. Wasserman and M.H. Kutner. 1990. Applied linear statistical models: regression, analysis of variance and experimental design. Irwin, Boston, MA, 547 p.

Oliveira, G., C. Werner and O. Correia. 1996. Are ecophysiological responses influenced by crown position in cork-oak? Annales Des Sciences Forestieres 53:235-241.

Pataki, D.E., R. Oren and N. Phillips. 1998. Responses of sap flux and stomatal conductance of *Pinus taeda* L. trees to stepwise reductions in leaf area. J. Exp. Bot. 49(322):871-878.

Pockman, W.T., J.S. Sperry and J.W. O'Leary. 1995. Sustained and significant negative water pressure in xylem. Nature 378 (14):715-716.

Pothier, D., H.A. Margolis, J. Poliquin and R.H. Waring. 1989a. Relation between the permeability and the anatomy of jack pine sapwood with stand development. Can. J. For. Res. 19:1564-1570.

Pothier, D., H.A. Margolis and R.H. Waring. 1989b. Patterns of change of saturated sapwood permeability and sapwood conductance with stand development. Can. J. For. Res. 19:432-439.

Ramsay, J.O. and B.W. Silverman. 1997. Functional Data Analysis. Springer, Berlin Heidelberg New York, 310 p.

Rijkers, T., T.L. Pons and F. Bongers. 2000. The effect of tree height and light availability on photosynthetic leaf traits of four neotropical species differing in shade tolerance. Functional Ecol. 14:77-86.

Running, S. and G.T. Smith. 1991. Forest-BGC, A general model of forest ecosystem processes for regional applications.2. Dynamic carbon allocation and nitrogen budgets. Tree Physiol. 9:147-160.

Running, S.W. and J.C. Coughlan. 1988. A general model of forest ecosystem processes for regional applications I. Hydrological balance, canopy gas exchange and primary production processes. Ecol. Modeling 42:125-154.

Ryan, M.G., D. Binkley and J.H. Fownes. 1997. Age-related decline in forest productivity: pattern and process. Advan. Ecol. Res. 27:213-262.

Ryan, M.G., B.J. Bond, B.E. Law, R.M. Hubbard, D. Woodruff, E. Cienciala and J. Kucera. 2000. Transpiration and whole-tree conductance in ponderosa pine trees of different heights. Oecologia 124:553-560.

Ryan, M.G. and R.H. Waring. 1992. Maintenance respiration and stand development in a subalpine lodgepole pine forest. Ecology 73:2100-2108.

Ryan, M.G. and B.J. Yoder. 1997. Hydraulic limits to tree height and tree growth. Biosci. 47:235-242.

Saliendra, N.Z., J.S. Sperry and J.P. Comstock. 1995. Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis*. Planta 196:357-366.

Salleo, S., T.M. Hinckley, S.B. Kikuta, M.A. LoGullo, P. Weilgony, T.-M. Yoon and H. Richter. 1992. A method for inducing xylem emboli *in situ*: experiments with a field-grown tree. Plant Cell Environ. 15:491-497.

Salleo, S., A. Nardini, F. Pitt and M. LoGullo. 2000. Xylem cavitation and hydraulic control of stomatal conductance in Laurel (*Laurus nobilis* L.). Plant Cell Environ. 23:71-79.

Schäfer, K.V.R., R. Oren and J.D. Tenhunen. 2000. The effect of tree height on crown level stomatal conductance. Plant Cell Environ. 23:365-377.

Schoettle, A.W. 1994. Influence of tree size on shoot structure and physiology of *Pinus contorta* and *Pinus aristata*. Tree Physiol. 14:1055-1068.

Schoettle, A.W. and W.K. Smith. 1999. Interrelationships among light, photosynthesis and nitrogen in the crown of mature *Pinus contorta* ssp. *latifolia*. Tree Physiol. 19:13-22.

Scholander, P.F., H.T. Hammel, E.D. Bradstrees and E.A. Hemmingsen. 1965. Sap pressure in vascular plants. Science 148:339-346.

Senock, R.S. and J.T. Ham. 1993. Heat balance sap flow gauge for small diameter stems. Plant Cell Environ. 16:593-601.

Sparks, J. and R. Black. 1999. Regulation of water loss in populations of *Populus trichocarpa*: the role of stomatal control in preventing xylem cavitation. Tree Physiol. 19:453-459.

Sperry, J.S., F.R. Adler, G.S. Campbell and J.P. Comstock. 1998. Limitation of plant water use by rhizosphere and xylem conductance: results from a model. Plant Cell Environ. 21:347-359.

Sperry, J.S., N.N. Alder and S.E. Eastlack. 1993. The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. J. Exp. Bot. 44:1075-1082.

Sperry, J.S. and T. Ikeda. 1997. Xylem cavitation in roots and stems of Douglas-fir and white fir. Tree Physiol. 17:275-280.

Sperry, J.S. and W.T. Pockman. 1993. Limitation of transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*. Plant Cell Environ. 16:279-287.

Sperry, J.S. and N.Z. Saliendra. 1994. Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. Plant Cell Environ. 117:1233-1241.

Sperry, J.S. and J.E.M. Sullivan. 1992. Xylem embolism in response to freeze-thaw cycles and water stress in ring-porous, diffuse-porous, and conifer species. Plant Physiol. 100:605-613.

Tardieu, F. and W.J. Davies. 1993. Root-shoot communication and whole-plant regulation of water flux. *In* Water Deficits: plant responses from cell to community. Ed. W.J. Davies, BIOS Scientific Publishers, Lancaster, UK, pp 147-162.

Tardieu, F. and T. Simonneau. 1998. Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modeling isohydric and anisohydric behaviors. J. Exp. Bot. 49:419-432.

Teskey, R.O., T.M. Hinckley and C.C. Grier. 1983. Effect of interruption of flow path on stomatal conductance of *Abies amabilis*. J. Exp. Bot. 34:1251-1259.

Tyree, M.T. and F.W. Ewers. 1991. Tansley Review No. 34. The hydraulic architecture of trees and other woody plants. New Phytol. 119:345-360.

Tyree, M.T., K.J. Kolb, S.B. Rood and S. Patino. 1994a. Vulnerability to drought-induced cavitation of riparian cottonwoods in Alberta: a possible factor in the decline of the ecosystem? Tree Physiol. 14:455-466.

Tyree, M.T. and J.S. Sperry. 1988. Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Plant Physiol. 88:574-580.

Tyree, M.T., S. Yang, P. Cruiziat and B. Sinclair. 1994b. Novel methods of measuring hydraulic conductivity of tree root systems and interpretation using AMAIZED. Plant Physiol. 104:189-199.

Wang, Y.P. and P.G. Jarvis. 1990. Description and validation of an array model - MAESTRO. Agric. For. Meteorol. 51:257-280.

Waring, R.H. and W.B. Silvester. 1994. Variation in foliar 13C values within the crowns of *Pinus radiata* trees. Tree Physiol. 14:1203-1213.

Watson, F.G.R., R.A. Vertessy and R.B. Grayson. 1999. Large-scale modelling of forest hydrological processes and their long-term effect on water yield. Hydrological Processes 13:689-700.

Whitehead, D., W.R.N. Edwards and P.G. Jarvis. 1984. Conducting sapwood area, foliage area, and permeability in mature trees of *Picea sitchensis* and *Pinus contorta*. Can. J. For. Res. 14:940-947.

Whitehead, D., N.J. Livingston, F.M. Kelliher, K.P. Hogan, S. Pepin and T.M. McSeveny. 1996. Response of transpiration and photosynthesis to a transient change in illuminated foliage area for a *Pinus radiata* D. Don tree. Plant Cell Environ. 19:949-957.

Whittaker, R.H. and G.M. Woodwell. 1967. Surface area relations of woody plants and forest communities. Am. J. Bot. 54:931-939.

Williams, M., E.B. Rastetter, D.N. Fernandes, M.L. Goulden, G.R. Shaver and L.C. Johnson. 1997. Predicting gross primary productivity in terrestrial ecosystems. Ecol. Appl. 7(3):882-894.

Wise, L.F., M. Murphy and A.A. D'Addieco. 1945. Chlorite holocelulose, its fractionation and bearing on summative wood analysis and on studies on the hemicelluloses. Section of the Technical Association of the Pulp and Paper Industry 122:11-19.

Wong, S.C., I.R. Cowan and G.D. Farquhar. 1985. Leaf conductance in relation to rate of CO2 assimilation III. Influences of water stress and photoinhibition. Plant Physiol. 78:830-834.

Yang, S. and M.T. Tyree. 1994. Hydraulic architecture of Acer saccharum and A. rubrum: comparison of branches to whole trees and the contribution of leaves to hydraulic resistance. J. Exp. Bot. 45:179-186.

Yoda, K., K. Shinozaki, H. Ogawa, K. Hozumi and T. Kira. 1965. Estimation of the total amount of respiration in woody organs of trees and forest communities. J. Biol. (Osaka) 16:15-26.

Yoder, B.J., M.G. Ryan, R.H. Waring, A.W. Schoettle and M.R. Kaufmann. 1994. Evidence of reduced photosynthetic rates in old trees. For. Sci. 40:513-527.

Zhang, J. and W.J. Davies. 1989. Abscisic acid produced in dehydrating roots may enable the plant to measure the water status of the soil. Plant Cell Environ. 12:73-81.

Zimmermann, M.H. 1978. Hydraulic architecture of some diffuse-porous trees. Can. J. Bot. 56:2286-2295.

Zimmermann, M.H. 1983. Xylem Structure and the Ascent of Sap. Springer-Verlag, New York, 139 p.