

AN ABSTRACT OF THE THESIS OF

Jeanne A. Panek for the degree of Doctor of Philosophy in Forest Science and Plant Physiology presented on June 9, 1995. Title: A Stable Carbon Isotope Approach to Distinguish Climate Stress From Other Imposed Stresses in Coniferous Forests

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To understand the effect of human-induced stresses on forests, there is a need for a method to separate effects of imposed stress from effects of natural climate stress. I developed an approach to predict forest response to climate stress using as indicators stable carbon isotopes in tree foliage and growth-rings. This approach required understanding and modelling the relation between climate and isotope abundance in tree tissue. Isotope abundance is highly variable within and between trees. Before modelling, it was necessary to identify important sources of this isotope variability to ensure that I included the major components in the model. Six study sites across a climate gradient in Oregon incorporated the broad range of climate types necessary to explore $\delta^{13}\text{C}$ variability, and to establish and test the model. Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) was common to all sites across the gradient.

Patterns of $\delta^{13}\text{C}$ variability within the canopy of Douglas-fir trees implied that xylem hydraulics pose important limitations to carbon uptake. Branch length was significantly correlated with $\delta^{13}\text{C}$ in the foliage at branch tips, which suggested that stem hydraulics were involved in the relation, as branch length is a measure of path length of

water movement. The importance of hydraulic properties in $\delta^{13}\text{C}$ variability was confirmed by measuring branch hydraulic, specific, and leaf-specific conductivity of the same branches in which $\delta^{13}\text{C}$ was measured. A strong inverse relation between specific and leaf-specific conductivity and foliar $\delta^{13}\text{C}$ was found, as predicted by theory, and confirmed on several age-classes of foliage.

The model which best predicted annual variability in $\delta^{13}\text{C}$ in foliage and tree rings over a range of climate types included environmental constraints to stomata and xylem hydraulic properties. The model predicted $\delta^{13}\text{C}$ in foliage west of the Oregon Cascade Mts. extremely well. East of the Cascades, site means were well-characterized, but annual variability was not. Annual variability in tree-ring $\delta^{13}\text{C}$ was poorly characterized by the model, probably because annual variation in whole-canopy hydraulics was inadequately described by the hydraulic measure. Refinements for improving the tree-ring $\delta^{13}\text{C}$ relation are suggested.

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**A Stable Carbon Isotope Approach to Distinguish Climate Stress From Other Imposed
Stresses in Coniferous Forests**

by

Jeanne A. Panek

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If a thesis is really meant to describe what I learned in graduate school, then mine would recount facts only briefly, and would instead be a story of characters and their scientific context. This, then, would be my virtual thesis. I have spent much of my time here at OSU trying to expand the intellectual and emotional space that I inhabit. There have been a number of people who have helped me in that endeavor, and who have shaped my slant on science. Like all scientists, my scientific approach and my conclusions exist in a context—my own personal perspective. Mine strays outside of conventional science into other disciplines. This perspective, like the people who have helped me, must be acknowledged.

My parents, by giving me an unquestioning trust which inspired freedom and creativity in me, helped me build my personal framework and the perspective that I bring to science. Their support of my goals, regardless of whether they share or understand them, has always been the hallmark of their love of me. Their strength has imbued me with the courage to push my limits. Without their confidence, my own would have been shattered long ago. Jon and Jessie Panek fueled me with their enthusiasm for my endeavors, regardless of how improbable. Tara Panek offered her support and love in every way possible and was truly *always* there for me.

Dr. William Perry, my first mentor, is the wisdom that I know exists in the world, he is my model, my encouragement, and the evidence to my belief that there is a greater whole person that I can become. Dr. Perry has an undefinable quality - more than

wisdom, I have discovered. He is a magician with the human spirit. With a few well-chosen thoughts and actions, he transforms the mundane into the poetic and lets out the creative spirit in those he touches.

My second mentor, Dr. Robert Stallard, has encouraged the budding scientist in me since I first came into his office at Princeton and left with a heavy pile of papers and books. I didn't know then that he would continue to advise and inspire me for 14 more years. He has taught me, over the years since then, that the best path is not always the most obvious one, and that much can be learned through listening and observing.

I have come to understand that every sentence from Dr. Richard Waring is a like a door, push a little and you step over the threshold into a profound knowledge. I deeply respect Dr. Waring's ability to adjust the focal length of his intellect at will, and see clearly at many scales. He integrates—not only across disciplines, but across the rift between basic and applied science, between science and the public, and between the ivory tower and the community. He is an inspiration to me, as I seek to apply my ideas to real world problems but maintain the credibility of my scientific peers.

Dr. Barbara Yoder is a bodhisattva to whom I owe a great deal. A bodhisattva is a person who, at the threshold of personal enlightenment, turns to give a helping hand to others. She understood my enthusiasm and my frustrations, as a colleague, as a friend, as a woman, as a humanist, better than anyone else in the department. Her open door and open mind, and her unwillingness to treat me as less than a colleague, buoyed my confidence and helped me through the hardest parts of graduate school.

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have explored alone. Sometimes he shone the light on me, and helped me to see myself without judgement.

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No scientific paper should be read without understanding the approach of its author. There is a person behind the science, a person with a rich and varied experience. This we bring to our work. For there are no truths in science, only personal perspectives which arise from the context of our live's collective knowledge. To criticize my work is to practice science. To judge my perspective is to practice philosophy. To question my context is to exercise psychology. Science does not stand alone. The reader is welcome to critique all that I am, for

*We must not only have the courage of our convictions, but have the courage to question our convictions.*¹

¹ Nietzsche, Friedrich W. 1955. *Beyond Good and Evil*. Gateway Editions, H. Regnery Co., Chicago. 239pp.

Science is, at its core, creative but has become increasingly reductionist over time. The narrowness of reductionist thinking can be akin to using words alone to describe a sunset when there exists so many modes of expression, like pigment, pen, clay, stone, song, sign language... Similarly, to understand the context of human-imposed stresses on our forests I must also seek to understand the current human condition and its dependencies on natural resources. I draw from outside science, from philosophy, economics, sociology, and on the experiences of every person I can, including some I will never meet. I sit quietly in the woods and listen. I stand on mountaintops, on the cusp of earth and sky where the earth stops but the effects of people do not, and look out over a changing landscape and up into a changing sky. It is not difficult to see that

*The trail of the human serpent is over all.*²

This is a process of looking for answers. This, for me, is part of being a scientist. This context is not explicit in the chapters to come. There is no room for that there. This thesis follows convention, except for here, in this shadow of my virtual thesis.

² James, William. 1987. Pragmatism: A New Name for Some Old Ways of Thinking. In, William James Writings, 1902-1910. Literary Classics of the United States, Inc., NY.

CONTRIBUTION OF AUTHORS

The research work of Dr. Richard Waring, in collaboration with Dr. Warwick Silvester, provided the framework around which I designed the study presented in Chapter 2. Dr. Waring was instrumental in the editing phase of each manuscript.

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A Stable Carbon Isotope Approach to Distinguish Climate Stress From Other Imposed Stresses in Coniferous Forests

Chapter 1

1.1 INTRODUCTION

Forests are a significant component of global ecosystem stability and an important part of the global carbon cycle, therefore it is important to monitor their vitality in response to increasing human-induced stresses. These stresses are not isolated, but rather fit into a context of natural stresses under which forests continually grow. To distinguish the magnitude and timing of imposed stress from that of natural stress, there is a need for a method to separate the two. Understanding forest response to imposed stress offers a unique challenge when the impact is widespread or when the impact predates existing monitoring efforts. In these situations, establishing a control against which to gauge any imposed stress response is difficult.

In this thesis, I develop an approach to establishing a baseline of forest response to climate stress as separate from other imposed stresses which 1) allows researchers to sample retroactively to a time prior to an imposed disturbance, and 2) creates a baseline which could serve as a control where no control site can be established. The approach utilized the relation between stresses which reduce stomatal conductance and stable carbon isotope abundance in trees. The approach used a forest growth model, FOREST-BGC (Running and Coughlan 1988), to model tree physiological response, particularly stomatal conductance, to climate stresses. To develop an approach that was

effective over a range of climates, I established sites across a steep climate gradient in Oregon from the coast to the high desert. To compare sites to each other, I measured a common tree species across all sites. Douglas-fir (*Pseudotsuga menziesii* (Mirb) Franco) was ideal because it was common to all sites and because the low boundary layer resistances around the needles of conifers link them tightly to their atmospheric environment (Jarvis and McNaughton 1986). This tight coupling between leaf and atmosphere simplifies the interpretation of carbon-isotope signals.

To use stable carbon isotopes as indicators of forest climate stress required understanding and modelling the relation between tree isotope abundance and climate. Carbon isotope abundance is highly variable both within and between trees. This variability will be detailed in the remainder of this introduction. Before modelling, it was necessary to understand underlying causes for isotope variability to ensure that I included the important elements in the model. Recent evidence of a high correlation between branch length and foliar isotope abundance in a mesic climate (Waring and Silvester 1994) encouraged me to test the generality of the relationship across a range of climates. Chapter 2 reports a study which showed that branch length was significantly correlated with carbon isotope abundance in Douglas-fir foliage, but that the strength of the relation declined with increasing site aridity across the transect. This relation suggested that xylem hydraulic factors were the underlying mechanism responsible for the isotope variation found. Chapter 3 describes research which demonstrated that, indeed, specific and leaf-specific conductivity explained a high proportion of the variation in Douglas-fir across the climate gradient, which was also a gradient of

hydraulic conductivity. Finally, in Chapter 4, I use the results of Chapter 3 to model isotope abundance in multiple age-classes of foliage and tree-rings using a measure of hydraulic conductivity and tree physiological response to climate factors.

This approach to establishing a baseline of forest response to climate stress addresses the need for a retroactive and independent control. Through the use of multiple age-classes of foliage and tree-rings, the approach allows for retroactive sampling to a previous time period. Isotopic abundance as a function of tree response to climate alone can be modelled at many sites and serve as a control against which to compare the actual abundance of isotope present. The difference between the two should indicate the timing and magnitude of a non-climatic, imposed stress.

1.2 STABLE ISOTOPES

The use of stable carbon isotopes has brought to the field of ecophysiology a new tool with which to explore patterns and processes in the environment, and with which to understand organism-environment relationships. Stable carbon isotopes are useful in forest research to understand tree responses to environment. Trees are both long-lived organisms and organisms which lay down permanent growth every year, which allows for direct comparison between tree growth, isotope assimilation, and the environment in which the tree grew.

The abundance of carbon isotopes in foliage and tree-rings is the result of several influential factors, which operate at three distinct, but interdependent levels - the stand

level, the individual level, and the process level. In this introduction I will provide background information on how isotopes fractionate and are therefore useful in ecophysiological studies. Then I will describe the major known causes of variability in carbon isotope composition of woody plants at all three levels of variability.

1.2.1 Isotopes - background information

Isotopes are elements of the same species which have different numbers of neutrons in their nuclei, therefore have different masses and vary in their chemical equilibria and reaction kinetics. These differences between isotope species are what provide the useful application to woody plant research. The chemical equilibrium between two different molecules, for example $^{13}\text{C}^{16}\text{O}_2$ and $^{12}\text{CH}_4$, depends on the isotopic composition of each individual molecule. The composition determines the activities of the molecules depending on the ratio of heavy to light isotope, and can be described with the following equation:

$$\Delta G_{total} = RT \ln \frac{\frac{\alpha_1 H(p)}{\alpha_2 L(p)}}{\frac{\alpha_3 H(r)}{\alpha_4 L(r)}} \quad (1)$$

where ΔG_{total} is $\Delta G_H - \Delta G_L$, the difference between the change in free energy due to the heavy isotope and the difference due to the light isotope, R is the gas constant, T is the temperature in degrees Kelvin, and α_{1-4} are the activities of H, the heavy isotope or L, the light isotope, p is the product and r is the reactant. Diffusion is an example of an

equilibrium process (O'Neil 1986).

Reaction kinetics also differ between molecules, depending on their heavy to light isotope ratios, which affects their fundamental vibrational frequencies and, therefore, their dissociation energies. Lighter molecules are less stable and their bonds break more easily than heavy molecules. For example $^{12}\text{C}-^{12}\text{C}$ dissociates more easily than $^{12}\text{C}-^{13}\text{C}$, which dissociates more easily than $^{13}\text{C}-^{13}\text{C}$ (see discussion in O'Neil 1986). An example of a kinetic process which fractionates ^{13}C from ^{12}C is the assimilation of $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ by the C_3 plant enzyme ribulose biphosphate oxygenase carboxylase (rubisco).

Within the lighter isotopes—C, H, O and N—the differences in isotopic composition between molecules are usually very small because the absolute abundances of ^{13}C , D, ^{18}O and ^{15}N are quite small. These small differences can reveal significant and important patterns, therefore instruments must be able to measure isotope abundances with great precision. Carbon isotope analysis precision is usually about $\pm 0.1\text{‰}$ (Leavitt and Long 1986). Amplification of these small numbers is achieved by reporting isotope ratio values relative to some standard of known isotopic composition, and further amplified by using a ‰ (per thousand) scale, by this equation:

$$\delta(\text{‰}) = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000 \quad (2)$$

where R is the ratio of the heavier isotope to the lighter and the standard used for carbon isotope analysis is a fossil Belemnite from the Pee Dee formation. A $\delta^{13}\text{C}$ value of 0 ‰, for example, has the same isotopic composition as the standard. A value of -10 ‰ means the sample has 10 fewer ^{13}C atoms per thousand C atoms than the standard. The

average terrestrial abundance of ^{13}C is 1% of all carbon.

Plant $\delta^{13}\text{C}$ values are always negative and are therefore difficult to manipulate mathematically. For this reason Farquhar (1989) suggested the use of discrimination notation, Δ , (Eq. 3) which represents the difference in $\delta^{13}\text{C}$ between the source (usually the atmosphere for plants) and the sink (the plant). This notation, while generating positive numbers, suffers because the reported isotopic abundance is no longer associated with the original standard. As atmospheric $\delta^{13}\text{C}$ values drop (see below), reference to a standard becomes increasingly important. For that reason, abundances and not discrimination values will be reported in research results.

1.2.2 Process-level variability

Process-level events are responsible for internal isotopic variation in plants, therefore are manifest at all scales of variation, including stand and individual scales. The fractionation processes within the plant are the key to the interpretation of all carbon isotope patterns in trees.

The fractionation of $\delta^{13}\text{C}$ during photosynthesis was first observed by researchers interested in the carbon isotope ratios of organic materials. They noted two distinct classes of plant $\delta^{13}\text{C}$ ratios, most at around -27 ‰, but some near -12 ‰. This difference later was recognized as resulting from the different carboxylating enzymes for C_3 and C_4 plants respectively. CAM plants are intermediate between C_3 and C_4 plants (see review by O'Leary 1981).

Carbon isotope fractionation during photosynthesis has been modelled by

Farquhar et al. (1982) using the equation:

$$\Delta = \delta^{13}C_{plant} - \delta^{13}C_{air} = a \frac{c_a - c_i}{c_a} + b \frac{c_i}{c_a} \quad (3)$$

where Δ is discrimination, a is the fractionation due to diffusion (-4.4 ‰), b is the fractionation due to carboxylation (-30 ‰ for C3 plants), and c_a and c_i are the atmospheric and internal CO₂ concentrations respectively. Δ and c_i/c_a are linearly related (Farquhar et al. 1989). When c_i is small, Δ tends toward -4.4 ‰. When c_i approaches c_a , Δ approaches -30 ‰. This model assumes that fractionation due to carboxylation is constant, and that fractionation in photorespiration, dark respiration, and in translocation is small. The utility of this model lies in the ability to link the stomatal aperture to the assimilation of ¹³C. Factors known to affect stomatal conductance therefore affect plant $\delta^{13}C$ concentrations. For example, low temperatures and nutrient deficiencies increase stomatal conductance and result in reduced $\delta^{13}C$, while a restricted CO₂ or water supply decrease conductance and increase $\delta^{13}C$ (Tieszen and Boutton 1989). Stresses like high ozone concentrations, known to affect stomatal aperture, also result in increased plant $\delta^{13}C$ (Greitner and Winner 1988). This model is the foundation to the use of plant isotope analysis in the study of the effects of environmental stress on plants.

The $\delta^{13}C$ composition of various plant tissue metabolites differ, demonstrating that enzyme discrimination is not limited to rubisco. Cellulose has been found to be consistently lighter than whole plant tissue by 1-3 ‰ (Leavitt and Long 1986; O'Leary 1981). Cellulose is the tissue most frequently used for isotope analysis. Plant lipid $\delta^{13}C$

is much more negative than whole plant $\delta^{13}\text{C}$, usually by about 5 ‰, but can exceed 9 ‰ (Park and Epstein 1961; O'Leary 1981). It is not clear what happens to the enriched $\delta^{13}\text{C}$ fraction left behind by discrimination within plant tissue, although Park and Epstein (1961) suggest that lipid formation may be the reason that CO_2 in plant respiration has a greater $\delta^{13}\text{C}$ concentration than the whole plant.

1.2.3 Stand level variability

Broad climatic, topographical and geographical features cause variation in forest isotopic ratios on a large scale. Long-term temporal factors, like seasonality, also affect isotope ratios. Anthropogenic inputs have had a more recent and profound influence on isotopic ratios. These factors are manifest either directly or indirectly. Direct effects are those which alter the isotopic composition of trees by directly influencing their physiology. Indirect effects alter the isotopic composition of gases and nutrients which are then assimilated into the trees. Both direct and indirect factors affect the variability in isotopic composition at the stand level or higher.

1.2.3.1 Direct effects

Many carbon isotopes studies on trees have focused on the relationship between climate factors and $\delta^{13}\text{C}$ because of the utility of reconstructing temporal trends in climate from annual rings (Lipp et al. 1991, Leavitt and Long 1991, Tans and Mook 1980, Freyer and Belacy 1983). Because stomatal conductance is linked to external environmental conditions such as soilwater content (Livingston and Spittlehouse 1994,

Tardieu and Davies 1993), vapor pressure deficit (Lange et al. 1971, Running 1980), temperature (Hinckley et al. 1978, Kaufmann 1982), and ozone concentrations (Martin and Sutherland 1990), $\delta^{13}\text{C}$ in tree rings varies with these factors and reflects past site conditions. Despite the attention given to these environmental factors, a significant amount of unexplained variability in $\delta^{13}\text{C}$ still exists. In each of these studies, the reported variability confounding the $\delta^{13}\text{C}$ signal is as great, or greater, than the signal itself.

Climatic effects on general tree growth and ring-width size, and those on isotopic composition are sometimes correlated, so that climate variability affecting tree growth also affects the assimilation of isotopes (Fritts 1982). To find an undistorted climate signal, Fritts (1982) stresses the importance of sampling from one species in one habitat type, such as a north vs. a south aspect.

1.2.3.2 *Indirect effects*

Elevation causes changes in the isotopic composition of the atmosphere by decreasing the partial pressure of atmospheric CO_2 . $\delta^{13}\text{C}$ values in forest stands of the same species rise with elevation due to the reduced ratio of c_i to c_a (Körner et al. 1988). This pattern is opposite to that expected by increased precipitation with elevation. When environment is controlled through common garden experiments the trend reverses (Marshall and Zhang 1993, Read and Farquhar 1991).

Temporal trends exist in atmospheric $\delta^{13}\text{C}$ which contribute to stand-level variability. While it is evident that global CO_2 concentrations are rising (Post et al.

1990), measured $\delta^{13}\text{C}$ concentrations are dropping. Numerous studies using tree ring $\delta^{13}\text{C}$ values, report declines of about 2 ‰ since 1850. This is corroborated by independent Antarctic ice-core records dating back to 1740. Friedli et al. (1986) report pre-industrial $\delta^{13}\text{C}$ ice concentrations of -6.5 ‰, while present values are measured at -7.8 ‰, a change of -1.3 ‰. The timing and magnitude of the change suggests it is a result of the burning of fossil fuels, whose emissions have much lower $\delta^{13}\text{C}$ concentrations, roughly -27 ‰ (Fry 1989, Peterson and Fry 1987, Francey and Farquhar 1982, Wigley 1982). Farquhar et al. (1989) reported an annual cycle of 10 ppm in CO_2 and 0.2 ‰ $\delta^{13}\text{C}$ in the northern hemisphere associated with seasonality, and a 2 ‰ daily and annual variation in the atmospheric $\delta^{13}\text{C}$ values of metropolitan areas.

1.2.4 Individual Level Variability

Variability in isotopic composition at the individual level includes factors which make single trees different from each other, as well as those factors which lead to variability within one tree, such as circumferential variation and variation with height. These are superimposed on the variation caused by the large-scale factors discussed above. Once laid down ^{13}C remains immobile, therefore $\delta^{13}\text{C}$ values reflect the conditions at the time of wood production.

Micro- meteorological and -topographical effects are analogous to the climate and geographical effects discussed above but they vary on a smaller scale. Such factors affect trees at the individual level. The isotopic response of the tree depends very much on the morphology of the canopy, and on the local mixing of air within the canopy. A

field-grown tree is growing in a different microenvironment than a tree growing amidst others in the middle of a forest, and its isotopic composition will reflect this. Leavitt and Longé (1986) cite six studies in which $\delta^{13}\text{C}$ varied from 0.5 to 3 ‰ between trees.

The choice of tree species will affect tree-ring studies. Conifers, in general, have higher $\delta^{13}\text{C}$ than deciduous trees. Xylem hydraulic properties vary between diffuse-porous and ring-porous trees, and between conifer species (Kramer and Kozlowski 1979). In conifers, water moves through the bordered pits of many small tracheids, which confer high resistances to flow. Conifers generally exhibit a spiral pattern of water movement upward which is thought to more effectively distribute water to all parts of the crown (Vité and Rudinsky 1959). As the cambium is an outward growth from the same primordia as xylem, carbon comprising the growth ring is translocated from the many parts of the crown to the stem which should greatly influence the circumference variability of isotopes about one growth ring.

Circumference variability has been noted by several authors. Leavitt and Long (1986) reported different $\delta^{13}\text{C}$ concentrations on the north and south aspects of the same tree ring. They suggested that the different light levels associated with those aspects were responsible, and that the direction of the change was consistent with the Farquhar et al. (1982) model. Greater sun exposure leads to a lower internal CO_2 concentration, thus $\delta^{13}\text{C}$ values should be higher on the southern side, as they found. O'Leary (1981) reviewed reports of minor light intensity effects causing both small increases and decreases in fractionation. Waring and Silvester (1994) reported that $\delta^{13}\text{C}$ in foliage varied with aspect such that sun-exposed foliage had higher $\delta^{13}\text{C}$ values than shade-

exposed. Sternberg et al. (1989), however, found that shading was not responsible for lower $\delta^{13}\text{C}$ values in woody tissue. Several researchers have noted that while $\delta^{13}\text{C}$ varied significantly around the circumference of a group of rings, the ring-to-ring relationship of $\delta^{13}\text{C}$ is similar for different radial transects (Tans and Mook 1980, Long 1982). Some researchers address the problem of circumference variability by sampling from several locations on a given tree ring, or by using the entire ring for analysis.

Isotopic composition varies in different parts of the tree. Leavitt and Long (1986) found that the $\delta^{13}\text{C}$ concentration of ring-wood cellulose was 1-2 ‰ heavier than needles of the same age. Twigs were found to be lighter than needles by 2-3 ‰. They did not find a consistent trend in tree-ring $\delta^{13}\text{C}$ values with height.

One widespread pattern of isotopic variability has generated much debate. Woody tissue closer to the ground has lower (more negative) $\delta^{13}\text{C}$ concentrations. Likewise, the inner growth rings of trees have consistently lower $\delta^{13}\text{C}$ values than the outer growth rings, referred to as the 'juvenile effect' (Francey and Farquhar 1982). This effect is consistent with the Farquhar et al. (1982) model which states that the internal concentration of CO_2 in plants controls discrimination during photosynthesis. In the shaded and high humidity understory environment, plants open their stomata wider, increasing the internal CO_2 concentration, leading to greater discrimination and therefore lower $\delta^{13}\text{C}$ values within the plant. However, the isotope trend is also consistent with the observation that $\delta^{13}\text{C}$ respired from microorganisms and plants is much lighter than atmospheric $\delta^{13}\text{C}$. Sternberg et al (1989), measuring ambient $\delta^{13}\text{C}$ concentrations concurrently with those in tree tissue, reported a gradient of increasing $\delta^{13}\text{C}$ with

distance from the ground in a tropical forest where decomposition rates are high. Ambient $\delta^{13}\text{C}$ values near the ground were 2.5 ‰ more negative than those at 25m. This trend was also reflected in leaf tissue $\delta^{13}\text{C}$ concentrations at the same heights. Trees grown in a well-ventilated screen-house under shade cloth did not exhibit lower $\delta^{13}\text{C}$ values than the surrounding air.

1.2.5 Conclusion

Carbon isotope abundance in tree tissue is highly variable. Some of the variability can be used to follow physiological, individual and stand-level processes. Some of the variability can be avoided with proper sampling techniques. Some unexplained variation still exists, however. The relation between climate and stable carbon isotopes in trees would improve if a major source of unexplained variation in $\delta^{13}\text{C}$ were identified.

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Chapter 2

Carbon-isotope variation in Douglas-fir foliage: improving the
 $\delta^{13}\text{C}$ -climate relationship

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2.1 ABSTRACT

The natural abundance of stable carbon isotopes in the annual rings of forest trees is used as a tracer of environmental changes such as climate and atmospheric pollution. Although tree-ring $\delta^{13}\text{C}$ varies by about 2‰ from year to year, variability within the foliage can be as high as 6‰. Recent studies showed that branch length affected stomatal response, which influenced the integrated foliar $\delta^{13}\text{C}$ signal. To improve the ability of $\delta^{13}\text{C}$ to predict climate, we examined the relationship between branch length and foliar $\delta^{13}\text{C}$ at four sites across a steep climatic gradient in Oregon, USA, using *Pseudotsuga menziesii* (Mirb.) Franco. The transect spanned the boundary between the ranges of the coastal variety, *P. menziesii* var. *menziesii* (three sites), and the Rocky Mt. variety, var. *glauca* (one site). At the most maritime site, branch length explained 76% of within-site variation of 5‰, whereas at the harshest site branch length accounted for only 15%.

We considered the possibility that cavitation in the water-conducting xylem obscures the branch length effect in the harsher climates. Cavitation, as measured by dye perfusion, was most extensive at sites where the branch length effect in the coastal variety was weakest. Trees at the site with the most substantial cavitation displayed seasonal xylem refilling.

Branch length standardization significantly improved the relationship between $\delta^{13}\text{C}$ and climate. With standardization to constant length, $\delta^{13}\text{C}$ values were significantly related to the degree that climatic variables, as modelled with a forest growth simulation

model, constrain transpiration ($R^2=0.69$, $P<0.0001$). Without standardization, the R^2 was 0.27. We conclude that sampling standard-length branches, or tree-rings from trees of similar shape and size, is desirable when seeking correlations between isotopic composition and climate.

2.2 INTRODUCTION

Stable carbon isotopes in the annual rings of forest trees have the potential to contribute to our understanding of historic patterns of climate and pollution stress. A major difficulty in interpreting the stable carbon isotope composition of trees, however, is the large amount of variation within individuals. Foliar $\delta^{13}\text{C}$ can vary by as much as 6‰ (Leavitt and Long 1986, Waring and Silvester 1994). Annual ring $\delta^{13}\text{C}$ varies around a tree's circumference by as much as 1.5‰ (*Pinus edulis*, Leavitt and Long 1986) to 4‰ (*Quercus rubra*, Tans and Mook 1980). Nevertheless considerable research effort has focused on correlating the isotopic composition of annual rings to climate and other environmental factors (Tans and Mook 1980, Freyer and Belacy 1983, Lipp et al. 1991, Leavitt and Long 1991). In particular, variation in $\delta^{13}\text{C}$ has been linked to changes in soil water content (Livingston and Spittlehouse 1993), precipitation, temperature (Tans and Mook 1980, Freyer and Belacy 1983), and pollution (Martin and

³ $\delta^{13}\text{C}$ is an index of the ratio (R) of ^{13}C to ^{12}C in relation to the standard, Pee Dee Belemnite, such that

$$\delta^{13}\text{C} (\text{‰}) = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000 \quad (1)$$

Sutherland 1990). In each of these studies, the reported variability in the $\delta^{13}\text{C}$ signal was as great, or greater, than the difference of around 2‰ attributed to the environment.

The large variation within individuals makes it difficult to assign small differences in tree ring $\delta^{13}\text{C}$ to changes in climate or pollution.

We hypothesized that much of the within-tree $\delta^{13}\text{C}$ variation may be attributed to hydraulic properties of the water-conducting system of stem and branches. Reductions in a tree's hydraulic conducting properties would tend to increase stomatal sensitivity to the evaporative demand of the atmosphere and to soil drought. If hydraulic factors in the stem and branches contribute to more rapid stomatal closure without a concomitant decline in photosynthesis, the concentrations of CO_2 within the mesophyll decline, discrimination decreases, and photosynthetic products become enriched in ^{13}C (Farquhar et al. 1982).

A highly variable feature within forest trees is the pathlength of water movement from soil to foliage. Although trunks may represent a large portion of the pathlength, branches contribute a greater resistance to water conduction per unit length than trunks because they are composed of denser wood (Ewers and Zimmermann 1984a,b). For a given evaporative demand and hydraulic conductivity, increasing pathlength (branch length) increases xylem tension, in accordance with an Ohm's Law analogy of water movement, because $\Delta P/\text{pathlength}$ must remain constant:

$$F = k_h \times \frac{\Delta P}{\text{pathlength}} \quad (2)$$

where F is flux of water, k_h is the hydraulic conductivity and ΔP is the pressure difference between the soil and the evaporative surface of the leaf. Predawn xylem tension is inversely proportional to maximum stomatal conductance (Running 1976, Grieu et al. 1988, Cienciala et al. 1994). Thus, because stomatal constraint decreases discrimination, the foliage at the tips of longer branches should be enriched in ^{13}C compared with shorter branches.

The higher resistances in branches render them more susceptible to cavitation, because branches experience greater tensions than trunks at the same transpiration rate (Zimmermann 1978, Tyree and Sperry 1989). Cavitation occurs (1) when water in the vascular system cannot be supplied at rates to match transpiration, or (2) when the column freezes, creating air bubbles that disrupt water transport after thawing. Cavitation reduces the functional cross-sectional area available for water conduction, causing increased resistance to water flow. This leads to stomatal constraint and therefore should increase enrichment of ^{13}C .

Recent measurements on sun-exposed foliage from *Pinus radiata* trees showed that branch length correlated linearly with $\delta^{13}\text{C}$ over lengths of 0.5 to 10 m (Waring and Silvester 1994). The pine plantations were located on the North Island of New Zealand where frost and drought are rare. Under such favorable growth conditions, xylem water tensions reflect primarily pathlength resistances to water movement through a nearly saturated conducting system. In harsher climates, cavitation, not pathlength, may be the major source of resistance in the hydraulic system.

To further our understanding of the natural variation in $\delta^{13}\text{C}$, we designed a

study across a steep climate gradient in Oregon, USA, extending from coastal rainforest to continental woodlands in the rain shadow of the Cascade Mts. We sampled branches from a single species of conifer common to all sites — *Pseudotsuga menziesii* (Mirb.) Franco. A conifer species was selected because of the high resistance to flow conferred by tracheids in series and because the low boundary layer around needles links the tree more tightly to its environment (Jarvis and McNaughton 1986). We used *P. menziesii* because it has a range that extends over a broad array of climate types and because growth does not tap starch reserves stored the previous year. Only current-year photosynthate is utilized (Webb and Kilpatrick 1993).

We hypothesized that, across the transect, branch length would be an important source of variation in foliar $\delta^{13}\text{C}$, but that cavitation at the more xeric sites might mask the effect of branch length. The objective of testing this hypothesis was to develop a means of standardizing sampling procedures to tighten the relationship between $\delta^{13}\text{C}$ and environment. Based on differences in climate along the gradient, as quantified by a forest growth model (Running and Coughlan 1988), we analyzed the ability of branch length standardization to improve the utility of $\delta^{13}\text{C}$ in predicting climate differences.

2.3 METHODS

2.3.1 Site selection

Four sites (A–D) were selected along a steep climatic gradient in Oregon (Figure 2.1) to span a range of climates. Air mass trajectories at these sites come from the

Pacific Ocean and thus they are relatively free of pollutants. Three of the four sites along this transect were first described by Gholz (1982) and later used by the NASA Oregon

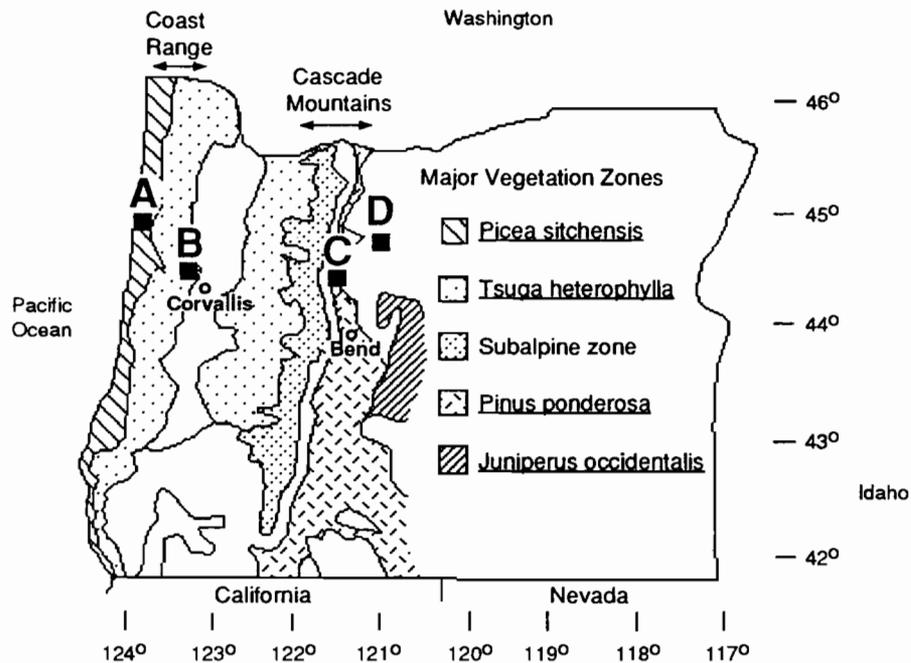


Figure 2.1. The sites used in this study represent a climatic gradient across Oregon (see Table 1). Douglas-fir (*Pseudotsuga menziesii*) is common to all sites.

Terrestrial Ecosystem Research project (Peterson and Waring 1994, Runyon et al. 1994). Table 2.1 describes salient features of the study sites. Further detail is provided by Runyon et al (1994). The coastal site at Cascade Head (A), like the New Zealand site studied by Waring and Silvester (1994), rarely experiences freezing temperatures or drought. Recent random amplified polymorphic DNA (RAPD) analysis has revealed that

the stand of Douglas-fir at site D is a western relic of the Rocky Mt. variety, *Pseudotsuga menziesii* var. *glauca* (Aagaard 1995, in press).

Table 2.1. Climate characteristics at the study sites

Site	Location	PPT ¹	Climate features
A	Cascade Head	244	summer rain common, frequent fog, freezes rare
B	Willamette Valley	107	summers hot and dry, occasional mid-winter freezes
C	Jefferson Wilderness	36	rainshadow of Cascade Mts., summers hot and dry, winters frozen
D	Grizzly Mt.	34	rainshadow of Cascade Mts., summers hot and dry, winters frozen

1. Annual average precipitation 1971-1992 in cm. (Oregon Climate Service, Corvallis, OR).

2.3.2 Sampling and analysis of $\delta^{13}\text{C}$

Four mature trees were selected at each site. Each tree was open-grown, with full southern sun exposure. Fully mature 1992 foliage (1993 at site D) was sampled from the tips of south-facing branches within 4 m of the ground to minimize trunk resistance and hydrostatic effects on water movement. Two to six branches were sampled per tree, depending on the number available with a southern aspect. Branch sizes ranged from 1 to 11 m. Four to five needles from the top of the terminal shoot were sampled and dried to constant weight at 70°C. Needles were ground by hand to pass a 40-mesh screen, and analyzed for whole tissue $\delta^{13}\text{C}$ with a continuous flow elemental analyzer (Carlo Erba

Analyser-NA1500) interfaced to an isotope mass spectrometer (Europa Scientific Tracermass). Analysis required 3-4 mg of sample to be encapsulated in tin foil, and introduced singly into the combustion reactor, which is kept at 1020°C and enriched with oxygen. The resulting combustion gases are swept through a reduction reactor, a water filter, and on to a gas chromatograph (GC) column by the carrier gas helium. The CO₂ gas is separated from the other gases in the GC column. A subsample of the CO₂ is transferred into the mass spectrometer for the measurement of ¹³C abundance. All analyses were run against the CSIRO sucrose standard and calculated in relation to the Pee Dee Belemnite standard, with variation of analysis less than 0.2‰.

2.3.3 Cavitation and refilling

To determine the extent of cavitation in the branches at each site, a single branch 3.5-4.5 m long was cut from the south-facing side of each of the same four trees described above at the time of seasonal extremes (1) in the fall of 1993 at the end of the drought period and (2) in the spring of 1994, when the soil water supply was near maximum (Runyon et al. 1994). The cut end of the branch was sealed with silicone and the branch transported to the laboratory in a dark plastic bag. The end was recut under water and placed in 0.05% safranin dye filtered to 0.22µm. The branch was allowed to draw safranin until the dye had fully saturated the functional conducting area 10 cm above the cut base. The percent conducting cross-sectional area was measured 5 cm above the branch end by cutting the branch again. The exposed surface was leveled with a razor blade. The conducting area was measured with an image processor (NIH Image

1.52 software), with a Motion Analysis Video Imaging System, Pulnix Video Control Unit and lens attached to a Macintosh computer. Heartwood areas, dyed area and total cross-sectional area of branches were identified in the images. Potential conducting area was defined as total area minus heartwood area.

2.3.4 Characterization of climatic constraints

We assessed stomatal limitations on gas exchange (CO_2 and water vapor) during the growing season at each site using a forest growth model, FOREST-BIOGEOCHEMISTRY (Running and Coughlan 1988). We define stomatal limitation as the percent reduction from maximum stomatal conductance at any given irradiance. Reduction in potential transpiration was then determined as the cumulative consequence of these constraints. FOREST-BGC models a water balance for a forest stand based on climate inputs (temperature, precipitation, relative humidity, solar radiation) on a daily time-step, and state variables characteristic to each site such as leaf area index and soil water capacity. FOREST-BGC constrains stomatal conductance in a hierarchy, first taking into account limitation due to soil drought, then vapor pressure deficit and finally air temperature (detailed in Running 1984). To quantify the difference in stomatal constraints at each site during a defined growing season due to each of these factors, the sum of reductions in conductance attributed to each factor were computed and compared to maximum stomatal conductance at the same irradiance.

We used FOREST-BGC to model the sum of daily transpiration during a growing season and to compare that value against potential transpiration when stomata

were not constrained. These calculations provided a seasonally integrated ratio of actual to potential transpiration that served as an index of limitations to gas exchange. We recognized that stomatal response differs slightly for the two varieties of *P. menziesii*, however we made no accommodations for these differences in modelling. The model has previously provided good agreement with seasonal predictions of predawn plant water potentials and with observed growth in forests near sites A–C (Running 1994).

Twenty-three years of climate data served to evaluate annual variation in the climatic constraints to stomata at each site. Because we sampled 1992 foliage, we used 1992 climate data for estimates of potential transpiration where available. These data did not exist for sites C and D, so the 23-year average was substituted. Temperature and precipitation data for all sites were available from the Oregon State Climate Center (G. Taylor, Oregon Climate Service, Corvallis, OR, USA). Relative humidity (RH) and solar radiation (SR) were modelled from temperature and precipitation data with a climate model, MT-CLIM (Glassy and Running 1994). We compared measured to modelled RH and SR for 365 days in 1990 to validate MT-CLIM. Both RH and SR were significantly correlated with modelled (For RH: Site A, $R^2=0.97$; site B, $R^2=0.96$; For SR: Site A, $R^2=0.91$; site B, $R^2=0.90$; For all, $P<0.0001$). Measured and modelled values were not significantly different from each other ($P<0.05$).

In some tree species, starch reserves from the previous year contribute to the construction of current-year leaves, complicating the relationship between foliar $\delta^{13}\text{C}$ and climate. In *P. menziesii*, however, current-year growth is derived exclusively from current-year photosynthate, although this includes starch reserves temporarily deposited

in twigs and older needles (Webb and Kilpatrick 1993). For the purposes of determining which climatic conditions constraining stomata were relevant to the foliar $\delta^{13}\text{C}$ value, we defined the start of the growing season as the time when current-year starch reserves began to accumulate, i.e., when modelled photosynthesis exceeded modelled respiration. The growing season terminated August 20, which corresponds to the cessation of shoot elongation and the onset of budset.

2.4 RESULTS

2.4.1 *Branch length effects on $\delta^{13}\text{C}$*

At a given site, $\delta^{13}\text{C}$ varied with branch length (Figure 2.2). At site A, branch length explained 76% of the site $\delta^{13}\text{C}$ variation ($P < 0.0001$). Branch length resolved 32% and 41% of $\delta^{13}\text{C}$ variation at sites B and C respectively ($P < 0.05$, $P < 0.005$). There was no correlation at site D ($R^2 = 0.15$, $P = 0.21$). Regression lines at each site were compared by analysis of covariance. The slopes of the regression lines were not significantly different at any of the sites ($P < 0.0001$) and the residuals were randomly distributed about the regression line. The mean minimum $\delta^{13}\text{C}$ expected at each site was estimated from the intercept of the regression line, when branch length=0. Intercepts were similar at sites A and B. At sites C and D, the intercepts were significantly higher (less discrimination) than at the western sites and the intercept at site C was more negative than at site D ($P < 0.05$). The range of $\delta^{13}\text{C}$ variation explained by branch length at site A was 5.2‰. The range of isotopic values at the other sites was smaller than at site A,

primarily because maximum branch length decreased across the gradient from wet to dry sites.

2.4.2 Climatic effects on $\delta^{13}\text{C}$

The modelled constraints to stomata, defined as the percent reduction in stomatal conductance from maximum, differed significantly among the three sites ($P < 0.05$; Figure 2.3). At site A, stomata were constrained less than 7% from maximum due to temperature, vapor pressure deficit (VPD) or soil water. At site C, the most climatically stressful site for *P. menziesii* var. *menziesii* along the transect, stomata were constrained 57% from maximum, primarily by freezing temperatures and protracted soil drought. Site D, representing the westernmost edge of the range of *P. menziesii* var. *glauca*, was similar to site C except that the soil drought was twice as severe. As a result, total constraint to stomata predicted from the model approached 70%. Site climatic constraints were compared using a Fisher's Protected LSD. At the 0.05 significance level, all sites were significantly different relative to their temperature and VPD constraints except C and D, whereas soil drought constraints differed at all sites except A and B. This analysis of the climatic constraints to stomata at each of the sites in part quantifies the sources of constraint to stomata that reduce potential transpiration. Isotope values were standardized to a common branch length of 4.0 m by regressing $\delta^{13}\text{C}$ on branch length (Figure 2.2) and adding the predicted value from the regression to the residual. This method was possible because the residuals were randomly distributed with respect to branch length. The adjusted values, now

independent of branch length, were highly correlated with the index of limitation to gas exchange, the ratio of actual to potential transpiration ($R^2=0.69$, $P<0.0001$). The more constrained the stomata, measured as a proportion of potential transpiration, the higher the $\delta^{13}\text{C}$ in the foliage at the end of the branches of identical length (Figure 2.5b).

Standardized mean $\delta^{13}\text{C}$ values were significantly different between all sites except A and B (Fisher's Protected LSD, $\alpha=0.05$).

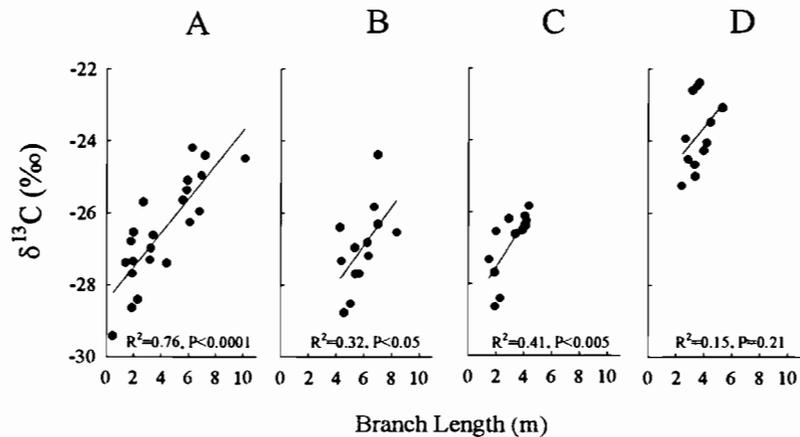


Figure 2.2. The relationship between $\delta^{13}\text{C}$ in foliage and branch length was significantly correlated within site, however the strength of the relationship decreased at the driest site (site D). The slope of the regression lines were not significantly different at any of the sites ($P<0.0001$). Intercepts were similar at sites A and B, but the C and D sites were significantly less negative ($P<0.05$).

2.4.3 Cavitation and refilling

Maximum functional sapwood in south-facing branches differed across the four sites and by season (Figure 2.4). Site A branches at full saturation utilized 97% of their

potential cross-sectional area for water conduction. Potential conducting area is the full branch cross-sectional area minus the heartwood area. In the fall, when predawn water potential values were most negative, the branches at sites B, C, and D utilized 91, 53, and 77% of their cross-sectional area for conduction, respectively. Spring refilling of embolized tracheids occurred only at site C, where the loss of conducting area after the protracted summer drought was greatest. Refilling brought these branches up to 88% capacity, similar to the value at site B. Fall and spring functional conducting cross-sectional areas differed significantly only at site C ($|T|=6.9$, $P<0.0001$). The ratio of leaf area to the area of spring, saturated functional sapwood was constant across all sites, $42.9 \pm 3.0SE$, $R^2=0.91$, $P<0.0001$ (Chapter 3), but the cavitation-reduced functional sapwood areas at site C were outliers to this relationship with leaf area.

2.5 DISCUSSION

2.5.1 Branch length effects on $\delta^{13}C$

Across the environmental gradient, where modelled climatic constraints to stomata differed by as much as 63%, the mean isotopic composition of *P. menziesii* foliage varied by 4.0‰ (Figure 2.5). This is roughly twice the magnitude of climatic effects on $\delta^{13}C$ reported elsewhere (Tans and Mook 1980, Freyer and Belacy 1983, Leavitt and Long 1991); however, if only sites with *P. menziesii* var. *menziesii* are considered, the range is 2‰, which is consistent with literature values.

Within-site variation was larger than between-site variation. Branch length explained 76% of foliar $\delta^{13}\text{C}$ variability at the climatically favorable site A. The range of $\delta^{13}\text{C}$ at this site—5.2‰—approached the 6‰ value reported by Waring and Silvester (1994) for *Pinus radiata* growing at a moist, maritime site in New Zealand. The relationship between branch length and $\delta^{13}\text{C}$ within a site diminished at harsher sites.

These results suggested that it is important to standardize branch lengths when comparing $\delta^{13}\text{C}$ within and between sites. This is demonstrated by comparing the relationship between $\delta^{13}\text{C}$ and proportion potential transpiration across sites with and without standardization. Variation about the mean isotope value at each site decreased with standardization. The ability to detect differences between climates increased in part because of decreases in variation and in part due to shifts in the mean $\delta^{13}\text{C}$ value. Without standardization, the relationship between climate and $\delta^{13}\text{C}$ had an R^2 of 0.27 ($P < 0.0001$). After standardization, the R^2 was 0.69 ($P < 0.0001$). Without standardization, only site D mean $\delta^{13}\text{C}$ was significantly different from the other sites. With standardization, all sites but A and B were significantly different. Thus $\delta^{13}\text{C}$ is a better predictor of climate effects on trees when branch lengths are standardized, that is when $\delta^{13}\text{C}$ is corrected for pathlength (Figure 2.5).

2.5.2 Climate effects on $\delta^{13}\text{C}$

Not all stomatal constraints influence foliar $\delta^{13}\text{C}$ similarly. FOREST-BGC predicted constraints based on VPD, soil drought and temperature (Figure 2.3), but VPD and soil drought only partially limit stomatal conductance, whereas sub-freezing night

temperatures close stomata for an extended period after temperature recovery (Running 1984). Without gas exchange, discrimination cannot occur, thus freezing temperatures shouldn't influence isotope abundance as much as VPD and soil drought. Stepwise regression (for maximum adjusted R^2) of each stomatal constraint on $\delta^{13}\text{C}$ values standardized to branch length showed that temperature was not significant to the relationship, whereas both VPD and soil drought were.

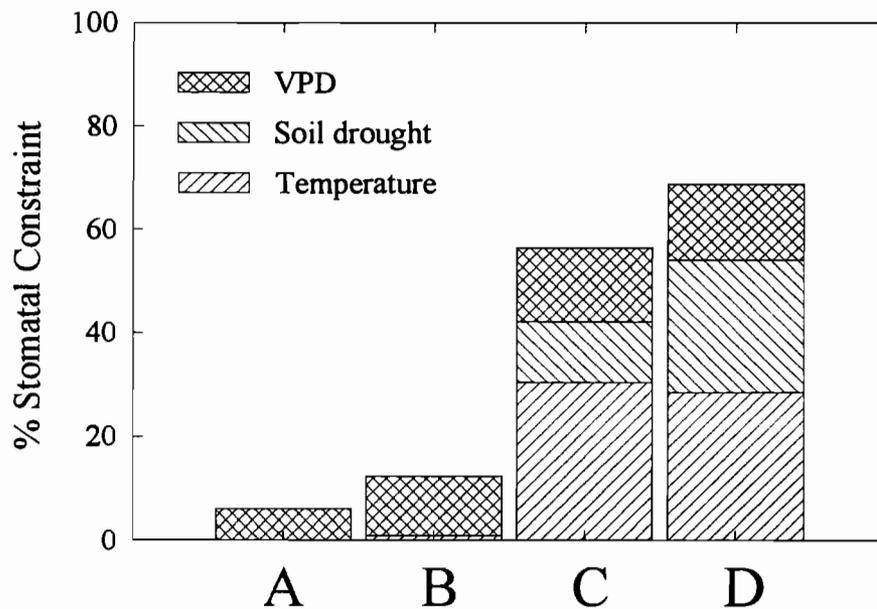


Figure 2.3. The modelled climatic constraint to stomata, defined as the proportional reduction in stomatal conductance from maximum, show the degree to which soil drought, temperature, and vapor pressure deficit reduced stomata conductance at each site.

2.5.3 Cavitation and refilling

Most sites experienced some permanent cavitation. The degree of cavitation increased with increasing climate stress across the transect (Figure 2.4). Functional sapwood area is a component of hydraulic conductivity. Data from a subsequent study with an expanded transect showed that, independent of branch length, branch hydraulic conductivity normalized by distal leaf area was correlated with $\delta^{13}\text{C}$ in terminal foliage

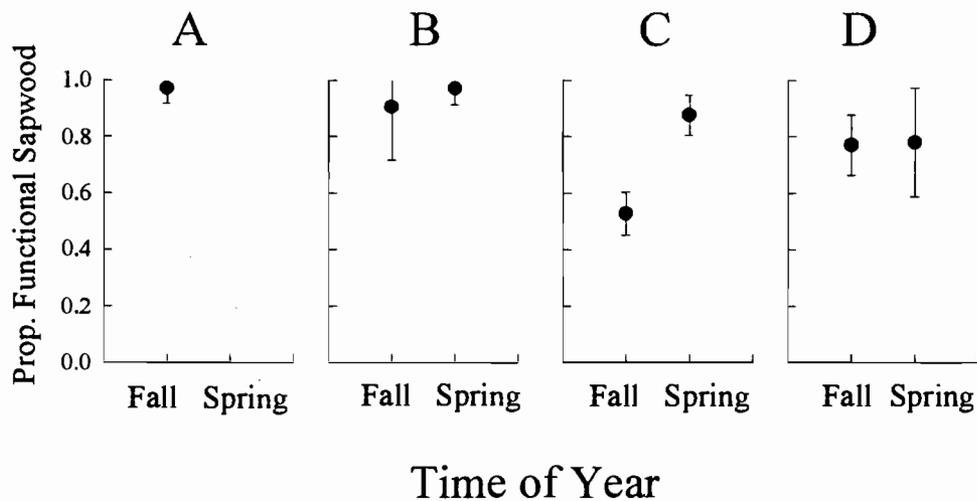


Figure 2.4 The conducting cross-sectional area in *P. menziesii* branches varied both spatially and seasonally across the climatic transect. Proportion of conducting cross-section was measured as a fraction of total cross-sectional area. Site A was saturated in the fall, so was not remeasured in the spring. Spring is significantly different from fall at site C (T-test, $P < 0.0001$). Error bars represent ± 1 SD.

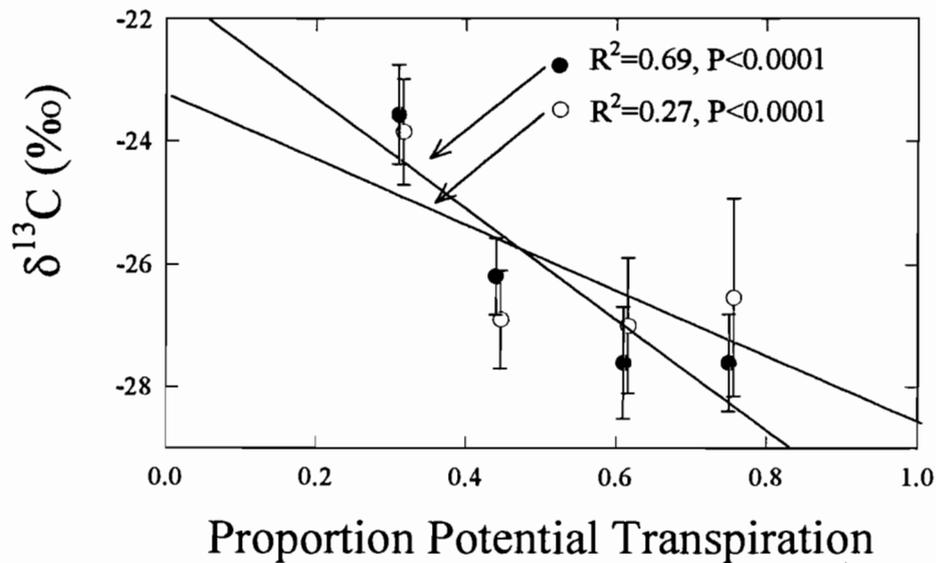


Figure 2.5. Climate effects were well correlated with $\delta^{13}\text{C}$ only when branch lengths were standardized. Hollow circles show the relationship between proportion of potential transpiration in 1992 at each site and $\delta^{13}\text{C}$ of all branches of various lengths from 1–11 m. Filled circles show the same relationship after branch lengths have been standardized to 4 m. Symbols represent the mean of all branches at a site. Error bars represent ± 1 SD. Regression lines are calculated through all values, not the means. Symbols are offset for ease of interpretation.

across the transect (Chapter 3). Damage to the conducting xylem corresponds to the degradation of the relationship between $\delta^{13}\text{C}$ and branch length. We speculate that as branches grow longer in the harsher climates, given the same evaporative demands and soil drought status, xylem tension will increase to a critical threshold at which point limited cavitation occurs, reducing k_h (see Eqn. 2), and causing the relationship between branch length and $\delta^{13}\text{C}$ to degrade. Values of $\delta^{13}\text{C}$, in this case, would be less negative

than predicted by branch length. This is consistent with the observation that the intercept of the regression line became less negative at the most cavitated site. Trees can protect themselves from reaching critical xylem tensions through stomatal regulation. Sperry (1995) suggested, however, that some trees might utilize limited cavitation at drought prone sites as a mechanism to conserve soil water and to insure against severe loss of soil conductance around the root zone, which is critical to the uptake of water.

At site C, where *P. menziesii* var. *menziesii* reaches its eastern distributional limits, the functional cross-sectional area was reduced by 34% at the end of the drought period (Figure 2.4). Trees at site C were the only trees to experience seasonal refilling. We would expect this degree of cavitation to render trees more sensitive to atmospheric humidity deficits and soil drought, and to result in transpiration rates more limited than those predicted by the model. By the spring, however, when most photosynthesis occurs, functional sapwood area at site C had recovered so that the sapwood area:leaf area ratios were similar at all sites. Thus $\delta^{13}\text{C}$ values in foliage and new wood at site C are unlikely to be affected by seasonal cavitation.

The general ability of trees to refill cavitated elements remains an open question. Evidence to support the ability of xylem elements to refill after cavitation has been found in the field for species known to have positive root pressure, such as sugar maple (Sperry et al. 1988) and wild grapevines (Sperry et al. 1987). Indirect evidence for refilling in other trees comes from fluctuations in relative water content (Chalk and Bigg 1957, Waring and Running 1978). In the laboratory, recovery has been observed, but only at very low xylem tensions (Dixon et al. 1984, Sobrado et al. 1992, Edwards et al. 1994).

Holbrook (1995) suggests that seasonal refilling may be a feature that conifers have but other trees do not.

Widely recognized patterns of $\delta^{13}\text{C}$ variation in trees are consistent with branch length and cavitation effects. The juvenile effect, a pattern of more negative $\delta^{13}\text{C}$ values in the inner growth rings of trees than in the outer rings, has been attributed to both shading (Francey and Farquhar 1982) and to an understory environment containing high concentrations of respired and thus isotopically lighter CO_2 (Sternberg et al. 1989, Jackson et al. 1993). Growth ring carbon represents an integration of photosynthate from a large portion of the canopy. Younger trees tend to have shorter branches overall, thus we expect them to have more negative tree-ring $\delta^{13}\text{C}$ values than older trees. The eventual loss of the juvenile effect through time may represent the cessation of height growth and the extension of branches as well as the loss of functional sapwood to cavitation in more mature trees.

Pseudotsuga menziesii var. *glauca* exhibited very different patterns of $\delta^{13}\text{C}$ and cavitation than *P. menziesii* var. *menziesii*. The $\delta^{13}\text{C}$ values of the population of var. *glauca* at site D were less negative than those of var. *menziesii*, but were comparable to those reported elsewhere for the variety (Martin and Sutherland 1990, Marshall and Zhang 1993). The sapwood in this population did not exhibit extensive cavitation. Sorensen (1979) studied the transition zone of these two Douglas-fir varieties along the eastern end of this transect in detail. He noted that the species as a whole is sensitive to water stress and that drought has been an important selection pressure in defining its range. Vulnerability to cavitation may be a critical factor that limits the range of var.

menziesii (Yoder, pers. comm.). Krahmer (1961) reported that var. *menziesii* has larger tracheid lumens than var. *glauca* and a higher permeability to creosote during lumber preservation treatments. In general, plants adapted to arid zones tend to have smaller-diameter conducting elements and are thus less vulnerable to cavitation (Tyree and Sperry 1989). This may explain the different patterns observed between var. *glauca* and var. *menziesii*.

The $\delta^{13}\text{C}$ patterns observed are relevant to the overall physiology of *P. menziesii*, but may also apply in general to conifers and diffuse-porous trees because of high resistance to flow through the stem. The $\delta^{13}\text{C}$ patterns indicate that leaves of shorter branches maintain higher internal CO_2 concentrations on average (Farquhar et al. 1982) and that these leaves are less water-use efficient than leaves on longer branches of the same tree. Waring and Silvester (1994), in a pruning experiment on *P. radiata*, showed that photosynthetic rates, stomatal conductance and carbon-isotope discrimination were substantially increased in 0.5 m versus 3.0 m long branches. Yoder et al. (1994) have shown that large, mature conifers maintain 30% lower photosynthetic rates than younger individuals in the same stands. These older trees support longer branches. Thus, accumulating evidence indicates that the length of the branches that form a tree's crown play a role in carbon uptake by the tree.

2.6 CONCLUSIONS

Although standard sampling protocols attempt to minimize variability associated with tree age, they have ignored the effects of branch length on carbon isotope discrimination. In this study, branch length accounted for up to 76% of within-site $\delta^{13}\text{C}$ variability and normalizing for branch length improved the $\delta^{13}\text{C}$ -climate relationship by 42% across sites. The importance of branch length on foliar $\delta^{13}\text{C}$ decreased as cavitation in the sapwood increased. Because tree ring carbon represents an integration of carbohydrates derived from parts of the entire tree crown, we suggest that trees of similar shape and size be sampled when seeking relations between carbon isotopes and environment. A daily analysis of carbon uptake patterns with a simple water balance model proved valuable in identifying the relative integrated constraints of soil drought, atmospheric humidity deficits and unfavorable temperatures.

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Chapter 3

Correlations between stable carbon-isotope abundance and hydraulic conductivity in Douglas-fir across a climate gradient in Oregon, USA.**3.1 ABSTRACT**

Stomatal conductance in trees is related separately to both foliar carbon-isotope abundance and stem hydraulic properties. I hypothesized, by combining these relations, that carbon-isotope abundance in foliage should vary with limitations to water movement through supporting branches. To obtain natural differences in hydraulic properties within one species, I sampled Douglas-fir branches (*Pseudotsuga menziesii* (Mirb.) Franco) from six sites across a climate gradient in Oregon, USA, for foliar carbon isotope abundance and stem hydraulic attributes. I quantified climate-induced stomatal limitations, expressed as reduced potential transpiration, across the gradient using a forest growth model. Foliar stable carbon-isotope abundance showed a strong inverse relationship with branch specific conductivity (hydraulic conductivity per unit functional sapwood area) and leaf-specific conductivity (hydraulic conductivity per unit leaf area) across the climate gradient. Foliar stable carbon-isotope abundance was correlated with modelled reductions in potential transpiration, however the addition of leaf-specific conductivity improved the correlation by more than 30%. Combined, leaf-specific conductivity and climate-induced stomatal constraints explained 84% of the variation in foliar isotope abundance in 1994 foliage. This model was then confirmed on foliage

classes 1990–1993. These correlations between stable isotope abundance and hydraulic properties have two important implications. First, foliar carbon isotope abundance may serve as a marker of long-term constraints to water movement within individual trees. Second, climate-isotope models, widely employed to reconstruct past climates, can be improved by taking into account sources of variation caused by hydraulic constraints on carbon uptake.

3.2 INTRODUCTION

Stomatal conductance in trees is related to foliar carbon-isotope abundance and to stem hydraulic properties through separate mechanisms. The combination of these separate relations indicates that stem hydraulic properties should influence carbon-isotope uptake. Stomatal conductance is related to stable carbon-isotope abundance because stomatal aperture controls the diffusion of CO₂ into the leaf, and thus influences the internal CO₂ concentration (c_i). At high c_i , the carboxylating enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) discriminates against ¹³C in favor of the lighter and more abundant ¹²C. Rubisco discrimination declines with c_i , thus as c_i decreases higher concentrations of ¹³C are assimilated into tissue and $\delta^{13}\text{C}$ increases (Farquhar et al. 1982, O'Leary 1993):

$$\delta^{13}\text{C}_{plant} \left(\text{‰} \right) = \delta^{13}\text{C}_{atm} - a - (b-a) \frac{c_i}{c_a} \quad (1)$$

where $\delta^{13}\text{C}$ is expressed in units of parts per thousand (‰), a is the discrimination against ^{13}C from diffusion, $\approx 4.4\text{‰}$, b is the discrimination caused by rubisco, $\approx 30\text{‰}$, and c_a is the atmospheric CO_2 concentration.

Stomatal conductance in trees is related to stem hydraulic conductivity because stomata respond to tension in the xylem. The upper limit to daily stomatal conductance is negatively correlated with predawn xylem tension (Running 1976, Grieu et al. 1988, Angell and Miller 1994). Stomatal conductance declines below that limit in response to a critical threshold of xylem tension (Sperry et al. 1993, Sperry and Pockman 1993) or to decreased hydraulic conductivity (Saliendra et al. 1995). Xylem tension results from the combined effects of evaporation, the availability of soil water, and from the conductivity of the pathway through which water moves. Assuming steady-state conditions with no influence of capacitance, an Ohm's Law analogy quantifies the relationship between hydraulic conductivity (κ_h , $\text{kg m s}^{-1} \text{MPa}^{-1}$) of the xylem and the tension of the water in the xylem,

$$\kappa_h = \frac{F}{\left(\frac{\Psi_{leaf} - \Psi_{soil}}{\Delta x} \right)} \quad (3)$$

where F is the flux of water (kg s^{-1}), $(\Psi_{leaf} - \Psi_{soil} / \Delta x)$ is the water potential gradient between the leaf and the soil (MPa) per unit distance the water travels (m). κ_h is independent of cross-sectional area and represents the amount of water that passes a

point regardless of the diameter of the stem at that point. Specific conductivity of a stem (κ_s , $\text{kg s}^{-1} \text{MPa}^{-1} \text{m}^{-1}$) is κ_h normalized by the area of conducting tissue in the stem cross-section, and is a measure of stem porosity. It is a function of the number and diameter of functional conduits and thus is highly dependent on stem cross-sectional area. Leaf-specific conductivity (LSC, $\text{kg s}^{-1} \text{MPa}^{-1} \text{m}^{-1}$) is stem κ_h normalized by the leaf area distal to the stem, and is a measure of the efficiency of water movement to evaporative surfaces (Tyree and Ewers 1991).

Assuming that photosynthetic capacity does not vary with hydraulic parameters, and that atmospheric $\delta^{13}\text{C}$ is similar for all sampled foliage, $\delta^{13}\text{C}$ in foliage should be an inverse function of specific and leaf-specific conductivity in trees (see Appendix for rationale and equations). Foliar $\delta^{13}\text{C}$ should increase with smaller LSCs because decreased stem conductivity results in greater stomatal closure, which increases $\delta^{13}\text{C}$. It is important to note that no isotopic discrimination occurs when stomata are closed because all internal carbon of both isotopic species is taken up (Eq. 1).

Circumstantial evidence exists for a relationship between hydraulic parameters and $\delta^{13}\text{C}$ in trees. Water availability and air humidity have long been known to effect tree $\delta^{13}\text{C}$ abundance (Tans and Mook 1980, Freyer and Belacy 1983). A simple site water balance model explained up to 68% of tree-ring $\delta^{13}\text{C}$ variability in Douglas-fir (Livingston and Spittlehouse 1993). Recent studies showed strong correlations between stable carbon-isotope abundance in foliage and the length of branches carrying water to that foliage (Waring and Silvester 1994, Panek and Waring, in press). These studies suggested that increased pathlength, represented by branch length, contributed to

lowering hydraulic conductivity, thereby decreasing stomatal conductance and increasing ^{13}C uptake. In these studies, branches were close to the ground to minimize the effect of tree bole length on water movement. In large trees, the pathlength represented by the bole figured substantially in total pathway resistance, and isotope abundance in foliage was linearly related to bole+branch length (Yoder and Panek, in review).

Several patterns of isotope variability in trees suggest underlying hydraulic mechanisms. The aspect effect, where foliage on the sun-exposed side of the tree is more enriched in ^{13}C (Leavitt and Long 1986, Waring and Silvester 1994) can be explained by an increased evaporative demand on the sun-exposed side, which causes loss of water and therefore stomatal constraint sooner than on the shaded side. That conifers tend to have higher $\delta^{13}\text{C}$ than deciduous trees can be explained by the less conductive elements of the hydraulic pathway leading to greater stomatal constraint. The juvenile effect (Francey and Farquhar 1982, Leavitt and Long 1988), can be explained by an increase in branch length and thus a decrease in root-shoot conductances as trees age.

To investigate the relations between hydraulic conductivity and foliar $\delta^{13}\text{C}$, I sampled from six sites across an established climate gradient in Oregon where I had previously determined that hydraulic conductivity varied naturally. Douglas-fir foliage (*Pseudotsuga menziesii* (Mirb.) Franco) was sampled because it was common to all sites and because the low boundary layer resistances around needles of conifers link them tightly to their atmospheric environment (Jarvis and McNaughton 1986). This tight coupling between leaf and atmosphere simplifies the interpretation of carbon-isotope

signals. I determined hydraulic conductivity, specific conductivity, leaf-specific conductivity, and stem cavitation in one internodal segment of the lowest branches on the tree as a surrogate for the entire hydraulic pathway. Although stomatal response is influenced by the cumulative conductance of the entire pathway, I reasoned that branches represent the major resistance to water movement as long as branches are close to the ground (Zimmermann 1978, Tyree et al. 1994). Furthermore, branches serve as sensitive indicators of losses of conductance due to cavitation because, according to the segmentation theory of tree hydraulics, trees appear to preserve the conductivity of their more vital trunks at the expense of their branches (Zimmermann 1978, Sperry 1995). Finally, I quantified stomatal constraints due to soil drought, vapor pressure deficit, and freezing at each site using a forest growth model (Running and Coughlan 1988).

3.3 MATERIALS AND METHODS

3.3.1 Site selection

Six sites were chosen along a transect in Oregon which was established by Gholz (1982) and recently served as the primary focus of a NASA project involving modelling forest growth from satellite (Goward et al. 1994, Peterson and Waring 1994). This study expands on the four sites used in Panek and Waring (in press)—sites A,B,C, and D—to include sites A/B and B/C (Figure 3.1).

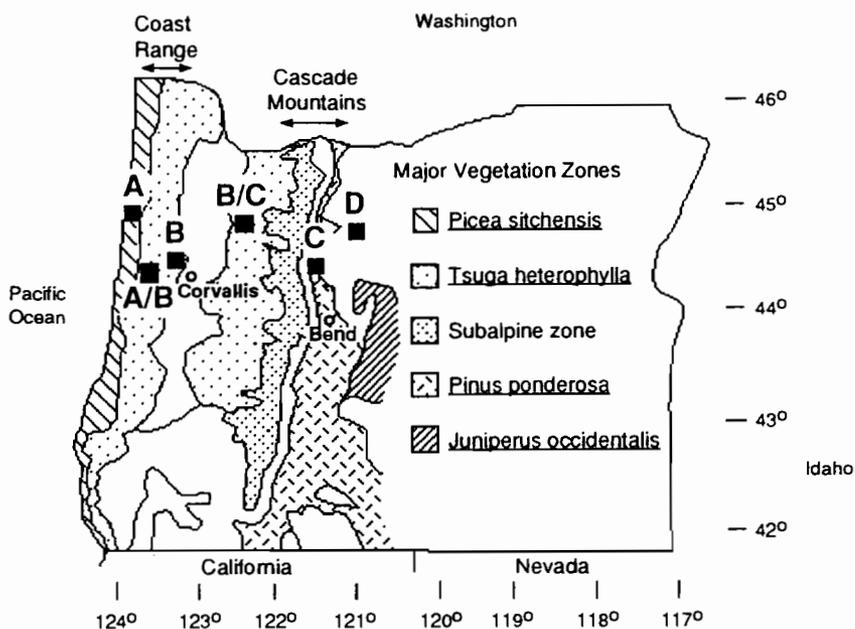


Figure 3.1. Study sites spanned a steep climate gradient across Oregon, USA, from a wet maritime to a dry continental climate.

These new sites represent zones of climate transition identified by Panek and Waring (in press), which were chosen to clarify the nature of the relation between $\delta^{13}\text{C}$ and plant hydraulics. Site A/B is in the Oregon Coast Range on a flank of Marys Peak and was not part of the original Oregon Transect. Site B/C is in the foothills of the Oregon Cascade Range, referred to as the Scio site elsewhere (Runyon et al. 1994, Yoder and Panek, in review). All site characteristics are documented in Runyon et al. (1994) and Panek and Waring (in press) except site A/B (Table 3.1). Site A/B is at 274 m elevation, and receives an average annual rainfall of 165 cm and experiences occasional mid-winter

freezes. The Douglas-fir stand was planted in 1963 (R. Worthean, pers. comm., Rock Creek Water Plant, Philomath, OR).

3.3.2 *Sample selection*

Four Douglas-fir with fully sun-exposed, south-facing branches were selected at each site. One branch of standard length 3.5–4.5 m long was excised from the south side of each tree in early August 1994 after the terminal shoot had fully elongated. Hydraulic conductivity and functional sapwood area were measured immediately in the field at sites B/C, C, and D, because these sites were far from the laboratory (details follow). Branch ends were sealed with silicone and branches were transported back to the laboratory in opaque plastic bags from the other sites and measured the same day. Most branches had some degree of cavitation, so sites were remeasured after winter and spring rains had fully resaturated soils and stems to determine whether cavitating elements had refilled. A comparison of fall and spring 1993 sapwood areas had previously been made at sites C and D (Panek and Waring, in press), so sites A/B, B, and B/C were remeasured in the spring of 1994. Carbon-isotope abundance was measured on cellulose from the current year's foliage at the terminus of the same branch (described below).

Table 3.1. Climate characteristics at the study sites.

Site	Location	PPT ¹	Climate features
A	Cascade Head	243	summer rain common, frequent fog, freezes rare
A/B	Marys Peak	164	summers hot and dry, occasional mid-winter freezes
B	Willamette Valley	106	summers hot and dry, occasional mid-winter freezes
B/C	Scio	136	summers hot and dry, frequent mid-winter freezes
C	Jefferson Wilderness	37	rainshadow of Cascade Mts., summers hot and dry, winters frozen
D	Grizzly Mt.	30	rainshadow of Cascade Mts., summers hot and dry, winters frozen

1. Climate data summarized from 1970-1994 records courtesy of the Oregon Climate Service, Corvallis, OR. Annual average precipitation is in cm.

Branches at site B were not excised in the summer because they were needed for a concurrent experiment. The terminal shoots were stripped of foliage immediately, however, for carbon isotope analysis. Branches were harvested the following spring for hydraulic measurements. Summer functional sapwood values were back-calculated by subtracting from the spring values the mean differences between spring and summer values at the adjacent sites. κ_s values were similarly back-calculated. LSC and κ_h values were then determined from κ_s , functional sapwood areas, and leaf areas.

3.3.3 *Measurement of xylem hydraulics*

Hydraulic conductivity was determined on 5 cm segments cut from the 1990 internode of the excised branch, by perfusing the stem with degassed 0.07% hydrochloric acid filtered to 0.22 μm (W. Pockman, pers. comm., protocol from J. Sperry laboratory, Univ. Utah, Salt Lake City). A pressure gradient of 0.01 MPa m^{-1} was applied and flow rate measured by 1) in the laboratory, collecting solution with a pre-weighed vial filled with absorbent paper, or 2) in the field, timing solution flux through a graduated pipette.

After κ_h was determined, the HCl solution was replaced with a 0.05% solution of safranin stain filtered to 0.22 μm , to identify the functional sapwood. Stained cross-sectional area, heartwood area, and total cross-sectional area were measured with an image analyzer (NIH Image 1.52 software) with a Motion Analysis Video Imaging System, Pulnix Video Control Unit and lens. Functional sapwood area was defined as total stained cross-sectional area minus heartwood area. To calculate κ_s , κ_h was divided by the functional sapwood area.

To determine leaf areas, needles were stripped from the branch distal to the segment measured. The leaf area of subsamples was determined with an AgVision image processing system (Decagon Devices, Inc., Pullman, WA, USA). All foliage was dried to constant weight at 70°C. Ratios of leaf area to dry weight were established on leaf-area subsamples. Total branch leaf areas were calculated by multiplying total leaf dry weights by the relation of leaf area to dry weight. LSC was calculated by dividing κ_h by total leaf area above the node where κ_h was measured.

3.3.4 *Characterization of climatic constraints*

I modelled growing season stomatal constraints due to climate at each site with a forest growth model, *FOREST-BIOGEOCHEMISTRY* (Running and Coughlan 1988). I defined stomatal constraint as the percent reduction from maximum stomatal conductance at the recorded irradiance due to vapor pressure deficit (VPD), soil drought, and freezing temperatures (Panek and Waring, in press). Transpiration was modelled, and the ratio of transpiration with the collective stomatal constraint to potential transpiration without stomatal constraint (T/PT) was calculated. *FOREST-BGC* models water balance in a forest stand by using climate inputs (temperature, precipitation, relative humidity, solar radiation), species characteristics, and site characteristics. Twenty-five years of meteorological data were available to characterize the reduction in stomatal conductance at each site on a daily time-step over the growing season (1970-1994, G. Taylor, Oregon Climate Center, Oregon State Univ., Corvallis). The cumulative growing season constraints from 1990-1994 were averaged to represent the constraints important to the hydraulic conductivity of branches from internode 1990 to present.

The growing season was defined as beginning when current year's starch reserves began to accumulate, i.e. when modelled photosynthesis exceeded modelled respiration. In some species of tree, starch reserves from the previous year contribute to the construction of current year's leaves, which complicates the relationship between foliar $\delta^{13}\text{C}$ and climate. In Douglas-fir, however, the current year's growth comes exclusively from the current year's photosynthate (Webb and Kilpatrick 1993). The end of the

growing season was defined as August 20, which corresponds to a conservative estimate of budset.

3.3.5 *Isotope analysis*

The terminal shoot was stripped of foliage for isotope analysis. Internodal segments representing past terminal shoots for the years 1993 through 1990 were also stripped of foliage, except at site A where trees retained foliage only as far back as 1991. Age-classes within trees were processed and analyzed individually. Needles were ground with a Wiley mill to pass a 40-mesh screen. The cellulose fraction was isolated by using the protocol from the University of Utah Stable Isotope Research Facility (S. Phillips, pers. comm., Univ. Utah, Salt Lake City), a method modified from Wise et al. (1946). Stable carbon-isotope abundance was measured at the University of Waikato Stable Isotope Facility, New Zealand, by using a continuous flow analyzer connected to a GC/MS (Europa Tracermass). For details, see Chapter 2. All analyses were run against the CSIRO sucrose standard and calculated in relation to the Pee Dee Belemnite standard, with variation of analysis less than 0.2‰.

3.4 RESULTS

3.4.1 *Xylem hydraulic parameters*

The sites across the transect spanned a range of values of specific and leaf-specific conductivities. Spring site κ_s and LSC decreased with increasing distance from

the coast (Figure 3.2), corresponding to a general increase in site aridity. A trend of increasing seasonal cavitation was observed from west to east across the transect in var.

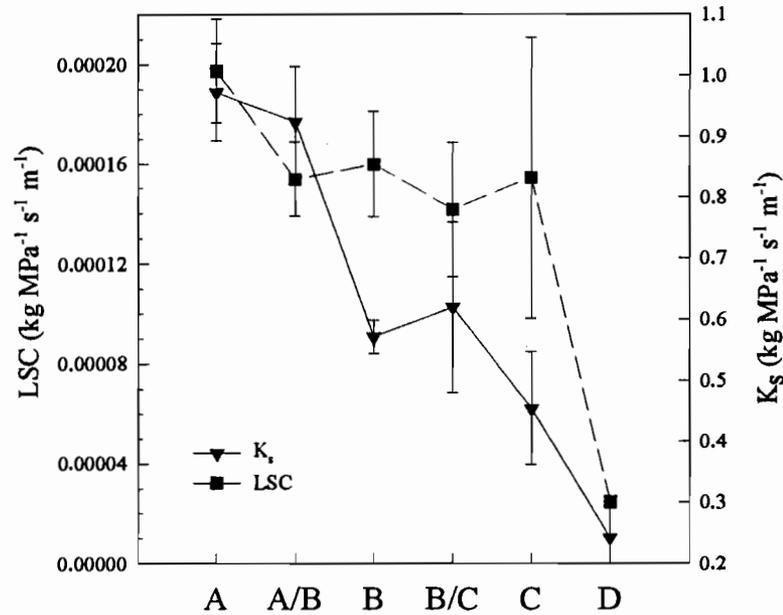


Figure 3.2. Mean hydraulic parameters of 1990 internodal segments from Douglas-fir branches ($n=4$) at six sites across the study transect from west to east. Error bars represent ± 1 SE.

menziesii, which was not correlated with temperature, VPD, or drought constraints on stomata (Figure 3.3). Differences between dry-season values and spring values were significant at sites B/C and C only (T-test, $\alpha=0.05$, $P=0.05$ and 0.0002 , respectively).

The relation between stem functional sapwood area to leaf area distal to the stem, across all sites, was highly significant (slope= 42.9 ± 3.0 SE, $R^2=0.91$, $P<0.0001$). Dry season

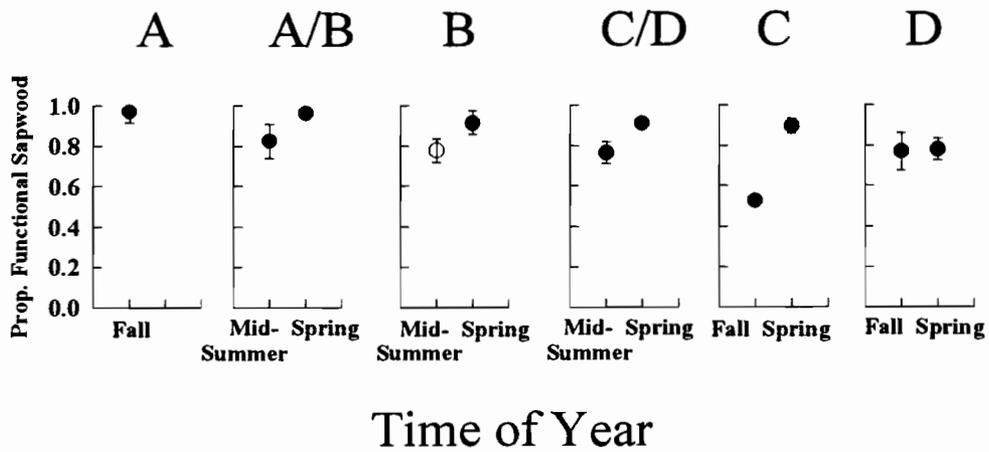


Figure 3.3. Functional sapwood as a proportion of total sapwood was measured at the beginning and end of the growing season. Seasonal reversible cavitation in branches increased toward the eastern extent of the range of *P. menziesii* var. *menziesii*. Site D was an isolated western population of var. *glauca*. Symbols represent the means of four branches. The open symbol at site B represents mid-summer values which were back-calculated (see text). Error bars represent ± 1 SE.

sapwood areas, however, were not related to leaf area. The number of foliage age classes retained by var. *menziesii* increased from four years at the coast to eight at site C. The trees at site D retained seven years of foliage.

Site D represents an isolated, western extension of the range of *P. menziesii* var. *glauca* (Aagaard et al., in press), which is common to the drier climate of the arid west. Plants adapted to arid zones tend to have water-conducting elements with smaller diameters than plants from more mesic areas and would therefore be expected to have lower conductivities (Tyree and Sperry 1989). A lumber preservation study showed the

permeability of var. *glauca* to creosote impregnation was lower than var. *menziesii* because of smaller tracheid lumens (Krahmer 1961). I found that the mean κ_s and LSC of branches at site D was significantly lower in each year from 1990 to 1994, and the $\delta^{13}\text{C}$ was significantly less negative, than the means of branches at any of the other sites (Fishers Protected LSD, $\alpha=0.05$).

3.4.2 *Climate constraints*

Stomatal constraints during the growing season caused by freezing, drought, and vapor pressure deficit (VPD) increased across the transect from the coast inland. VPD constraints were similar at all the sites, but drought-induced stomatal closure was only important from site B/C eastward (Figure 3.4). Freezing temperature contributed to stomatal constraint east of the Cascades only. Unless freezing induces xylem embolism its effects on stomata can be largely ignored. Freezing temperatures close stomata (Running 1994) and, because there can be no isotopic discrimination when stomata are closed, freezing constraint contributes little to foliar $\delta^{13}\text{C}$ values.

3.4.3 *Relations between climatic constraints, xylem hydraulic parameters, and $\delta^{13}\text{C}$*

Foliar $\delta^{13}\text{C}$ from the 1994 age class spanned from -19.56 to -26.53 ‰ across the transect, a range of 7 ‰. Results from forest growth simulations showed that climate factors in 1994 reduced stomatal conductance, and thus potential transpiration, to varying degrees below maximum at each site. The proportion of actual to potential

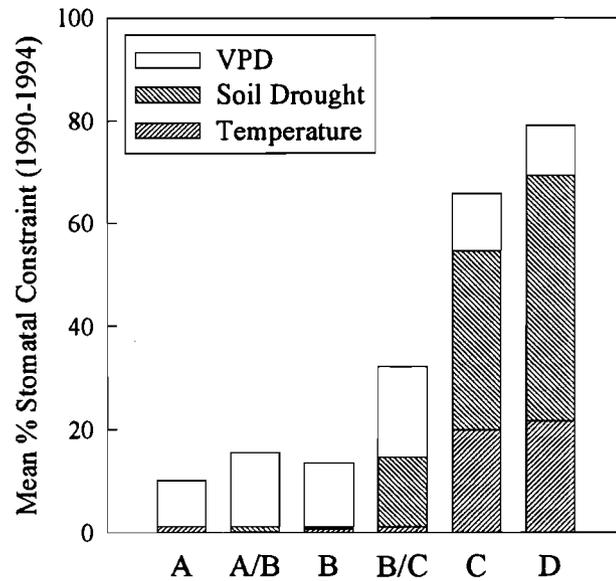


Figure 3.4. Cumulative growing season stomatal constraints at each site estimated from FOREST-BGC and site climate data from years 1990 to present. Constraints were measured as a proportion of maximum stomatal conductance at the recorded irradiance.

transpiration was linearly correlated with $\delta^{13}\text{C}$ across the transect ($R^2 = 0.50$, $P < 0.0001$).

κ_s and LSC were inversely correlated with $\delta^{13}\text{C}$, and highly significant (Figure 3.5, $R^2 = 0.73$, $P < 0.0001$; $R^2 = 0.84$, $P < 0.0001$, respectively). Cavitation alone, measured as proportional reduction from maximum conducting cross-sectional area, was poorly correlated with $\delta^{13}\text{C}$ ($R^2 = 0.03$, $P = 0.42$, data not shown).

A model including a combination of climatic constraints and xylem hydraulics did not explain more $\delta^{13}\text{C}$ variability than the hydraulic model alone. Using a stepwise

model selection technique (SAS Institute Inc. 1990) on all hydraulic and climate variables, the model which best fits the 1994 $\delta^{13}\text{C}$ data was 1/LSC or $1/\kappa_s$.

Model parameters are in Table 3.3.

Table 3.2. A comparison of different models explaining $\delta^{13}\text{C}$ variability in the most recent age-class (1994) of Douglas-fir foliage across the transect.

Model $\delta^{13}\text{C} =$	R^2	Coefficient	Intercept	Prob > T
1/LSC ($1/\text{kg s}^{-1} \text{MPa}^{-1} \text{m}^{-1}$)	0.84	0.0001	-26.16	0.0001
T/PT ¹ (dimensionless)	0.50	-4.9	-21.82	0.0001
1/LSC+T/PT :				
1/LSC	0.84	0.0001	-25.08	0.0001
T/PT		-1.22		0.7

1. T/PT is the ratio of actual to potential transpiration.

3.4.4 Testing the model

To test the validity of the regression model, as well as to assess improvement in prediction with the LSC model over the model using only stomatal constraints to transpiration, both models were applied to the age classes of foliage from 1990—the age-class of stem in which LSC was measured—through 1993. The 1/LSC model fits the years 1990 and 1991 very well, 1992 poorly, and 1993 well, but not as well as T/PT alone (Table 3.2).

Table 3.3. Parameter estimates for the model $\delta^{13}\text{C} = 1/\text{LSC}$ over foliage age-classes 1991-1993.

Year	R ²	Coefficient	Intercept	Prob > T
1990	0.80	0.00011	-26.43	0.0001
1991	0.79	0.00010	-26.14	0.0001
1992	0.44	0.00005	-24.43	0.0007
1993	0.69	0.00008	-26.16	0.0001

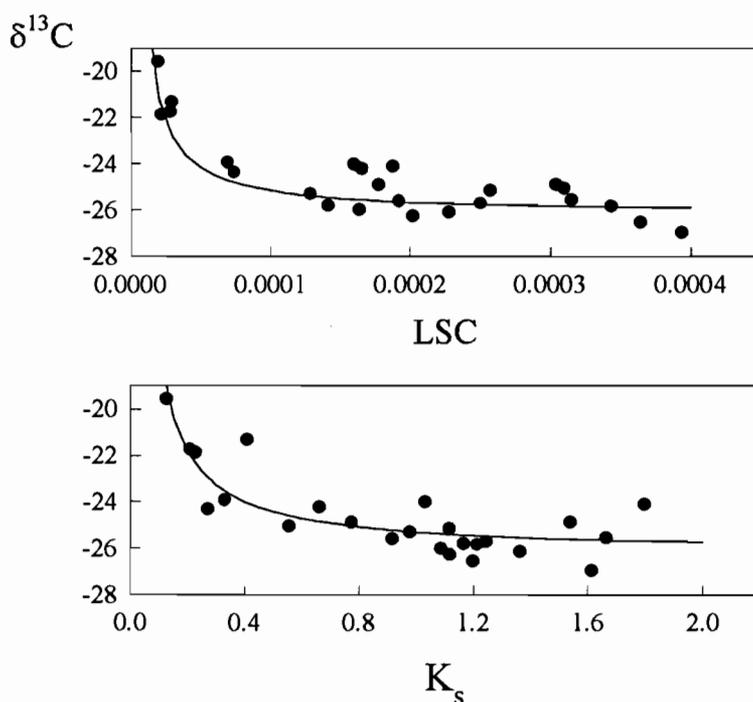


Figure 3.5. Relations between foliar $\delta^{13}\text{C}$ and hydraulic parameters in branches from Douglas-fir across the transect. Error bars represent ± 1 SE. κ_h is in units of $\text{kg m s}^{-1} \text{MPa}^{-1}$, κ_s and LSC are in units of $\text{kg s}^{-1} \text{MPa}^{-1} \text{m}^{-1}$.

3.5 DISCUSSION

3.5.1 Xylem hydraulic parameters

The mean κ_s and LSC of Douglas-fir found across the transect were similar to values found in other conifers. Sperry and Tyree (1990) reported κ_s values around 0.8 to 1.6 kg s⁻¹ MPa⁻¹ m⁻¹ in *Abies balsamea* and 0.4 to 0.8 kg s⁻¹ MPa⁻¹ m⁻¹ in *Picea rubens*. Tyree and Ewers (1991) reported LSC values of 5.7 to 27x10⁻⁵ kg s⁻¹ MPa⁻¹ m⁻¹ in the branches of *Tsuga canadensis*.

The mean κ_h , κ_s , and LSC of var. *glauca* branches were significantly lower than that of var. *menziesii* (Table 3.1), supporting wood permeability results reported by Krahmer (1961). κ_s and LSC values varied substantially across the entire transect, which suggested that tracheid number and diameter varied as well. Measures of hydraulic conductivity are dependent on the prevailing moisture regime at a site because smaller growth rings, containing smaller-diameter elements, form during periods of water stress (Bannan 1965) and at high xylem tensions (Rundel and Stecker 1977). Along the transect, soil drought limited stomatal conductance of Douglas-fir eastward of site B (Figure 3.4). It seems likely that drought was responsible for the differences in hydraulic parameters through its effects on tracheid size.

Some degree of reversible and irreversible cavitation existed in the branches at each site (Figure 3.3). A trend of increasing seasonal cavitation is observed from west to east across the transect in var. *menziesii*. There was no seasonal cavitation in var. *glauca*, although a limited amount of permanent cavitation was evident. Seasonal

refilling was observed at sites B/C and C. The results here provide strong support for the theory of Panek and Waring (in press) that cavitation increases in var. *menziesii* eastward across its range and may be the factor that limits its distribution. Both $\delta^{13}\text{C}$ and cumulative climatic constraints are measures of long-term, time-integrated processes. That the proportion of functional sapwood is not related to either $\delta^{13}\text{C}$ or climatic constraints to stomata, suggests that cavitation occurs episodically rather than slowly over time, and probably toward the end of the growing season. That cavitation is limited to growth rings 1991 and older suggests that a major cavitation event occurred in 1992 in response to the regional drought.

3.5.2 *Relation between climatic constraints, hydraulic parameters, and $\delta^{13}\text{C}$*

Foliar $\delta^{13}\text{C}$ abundance reflects seasonal changes in cavitation only if cavitation preceded periods of carbon uptake and foliar growth. At sites B/C and C, cavitation was observed in branches by the end of the growing season, but most carbon uptake occurs at the beginning of the growing season before the summer drought. Therefore foliar $\delta^{13}\text{C}$ values probably represent conditions prior to most cavitation and a somewhat higher pre-cavitation κ_s . If a majority of the growth occurred before cavitation, this also explains why the correlation between $\delta^{13}\text{C}$ and cavitation was poor.

The factor which best explained $\delta^{13}\text{C}$ variability across sites in the most recent age-class was branch LSC. Both LSC and κ_s were inversely correlated with $\delta^{13}\text{C}$ and their inverse transformations explained 84 and 73% of the variation in $\delta^{13}\text{C}$ across the transect, respectively. That across-site $\delta^{13}\text{C}$ variation is better explained by stem

hydraulics than by climatic constraints demonstrates the integrative quality of hydraulic measures. Stem hydraulics are not independent of site climate factors, as discussed. Relations between $\delta^{13}\text{C}$ and climatic constraints have been demonstrated for years (Tans and Mook, 1980; Freyer and Belacy, 1983). Livingston and Spittlehouse (1993) used a simple water balance model to show that seasonally accumulated transpiration alone accounted for 68% of variation in Douglas-fir tree-ring $\delta^{13}\text{C}$. Across the transect, T/PT regressed alone against $\delta^{13}\text{C}$ accounted for 50% of foliar $\delta^{13}\text{C}$ variability. The hydraulic measures, however, link these climate factors to the morphological and physiological responses within the tree—e.g., tracheid size and number, wood density, stomatal conductance—to explain the integrated response of a tree to climate. Climatic factors should be more important contributors to $\delta^{13}\text{C}$ variability when hydraulic factors vary less than climate factors, for example within an individual back through time in tree rings or foliage. Across the transect, however, hydraulic factors varied significantly.

The relation between $\delta^{13}\text{C}$ and LSC held when tested on age classes up to five years prior to present, confirming the results from 1994 foliage. The fit in 1992 was poor, however. In 1993 adding T/PT to the relation better predicted $\delta^{13}\text{C}$ than LSC alone. This suggests that in 1992 some factor disrupted the relation between foliar $\delta^{13}\text{C}$ and LSC, something which made the stomata much more sensitive to climate in the following year. Precipitation was very low across Oregon in 1992. Internode and needle length were much smaller than average at site C in 1992, especially on south-facing branches. I speculate that permanent cavitation due to the 1992 drought severely reduced the functional sapwood in branches at all sites. Stain patterns supported this.

Most cavitation measured across sites occurred in growth rings 1991 and older. I expect that the restricted flow of water to leaves caused stomata to be much more sensitive to drought and humidity during the following year, 1993. $\delta^{13}\text{C}$ values were less negative across the transect in 1992.

3.5.3 Integration of physiological processes

The strong correlation between foliar $\delta^{13}\text{C}$ and branch LSC suggests that constraints to water movement in the branches limit carbon uptake through a reduction in stomatal conductance. Because foliar $\delta^{13}\text{C}$ is a function of both carbon assimilation and stomatal conductance (Farquhar et al. 1989), the effects of hydraulic limitations on both processes must be considered. Many studies indicate that stomatal conductance is more sensitive to water limitations than is photosynthesis (Teskey et al. 1986, Seiler and Cazell 1990, Stewart et al. 1995). If LSC affected primarily assimilation, $\delta^{13}\text{C}$ should decrease with decreasing LSC because reduced assimilation decreases $\delta^{13}\text{C}$ (Eq. 5). The relation observed, however, was inverse which indicated that despite the effect of LSC on assimilation, reduction in conductance was so strong it dominated the relation.

Any factor, then, that reduces water movement through the root-shoot conducting pathway limits carbon uptake in the foliage. Reductions in LSC explain reductions in carbon uptake due to cavitation (Sperry and Pockman 1993), pathlength (Waring and Silvester 1994, Panek and Waring, in press, Yoder and Panek, in review), dense wood (Rundel and Stecker 1977) and reductions in photosynthetic and conductance rates as trees age (Yoder et al. 1994). Through its effect on carbon

exchange, changes in LSC effect a concomitant change in the $\delta^{13}\text{C}$ of foliage distal to the stem.

3.5.4 *Implications for $\delta^{13}\text{C}$ -climate relations*

Panek and Waring (in press) demonstrated that it is important to select trees of similar size and shape when seeking clean relations between $\delta^{13}\text{C}$ and climate. The research reported here showed that hydraulic parameters explain the relation between branch length and $\delta^{13}\text{C}$. Furthermore, hydraulic conductivity can vary among trees of the same size and shape depending on environment. These results strongly suggest the inclusion of hydraulic parameters in the use of $\delta^{13}\text{C}$ -climate models.

Additional experiments are needed where hydraulic factors are manipulated under controlled conditions to clarify the effects of κ_s , LSC, and climate factors on stomatal conductance, photosynthesis, and on $\delta^{13}\text{C}$.

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3.8 APPENDIX

Rationale for inverse relationship between $\delta^{13}\text{C}$ and leaf-specific conductivity.

$$\delta^{13}\text{C}_{leaf} = \delta^{13}\text{C}_{air} - a - (b-a) \frac{c_i}{c_a} \quad (1)$$

but because

$$\frac{c_i}{c_a} = - \frac{1.6 A}{g c_a} + 1 \quad (4)$$

therefore

$$\delta^{13}\text{C}_{leaf} = \delta^{13}\text{C}_{air} - a - (b-a) \left[1 - \frac{1.6 A}{g c_a} \right] \quad (5)$$

Also,

$$E = (\Psi_{soil} - \Psi_{leaf}) LSC \quad (6)$$

and

$$E = g (c_{wvi} - c_{wva}) \quad (7)$$

but because

$$g (c_{wva} - c_{wvi}) = 1.6 g (c_i - c_a) \quad (8)$$

these can be combined

$$LSC = \frac{1.6 g (c_i - c_a)}{\Psi_{soil} - \Psi_{leaf}} \quad (9)$$

solving for g ,

$$g = \frac{LSC (\Psi_{soil} - \Psi_{leaf})}{1.6 (c_i - c_a)} \quad (10)$$

Finally, substituting equation 10 into equation 1

$$\delta^{13}C_{leaf} = \delta^{13}C_{air} - a - (b-a) \left[1 - \frac{1.6 A}{c_a LSC (\Psi_{soil} - \Psi_{leaf})} \right] \quad (11)$$

where

$\delta^{13}C_{leaf}$ and $\delta^{13}C_{air}$ are the carbon isotope abundances of the foliage and atmosphere respectively

a is the discrimination due to diffusion into the leaf

b is the discrimination due to the carbon-fixing enzyme

c_i and c_a are the CO_2 concentrations inside and outside the leaf respectively

A is assimilation rate

g is stomatal conductance

E is transpiration rate

Ψ_{soil} and Ψ_{leaf} are the water potentials in the soil and leaf, respectively

LSC is the leaf-specific conductivity

c_{wvi} and c_{wva} are the concentrations of water vapor inside and outside the leaf, respectively

Chapter 4

A Stable Carbon Isotope Approach to Distinguish Climate Stress From Other Imposed Stresses in Coniferous Forests**4.1 ABSTRACT**

Stable carbon isotopes have the potential to be sensitive indicators of carbon uptake limitations in forest trees. In the mild climates of the Pacific Northwest, dendrochronologists have been unsuccessful at relating annual tree growth to variations in temperature and precipitation. This region is ideal for testing the sensitivity of stable carbon isotopes as indicators of environmental constraints on growth. I developed an approach using a water-balance model to predict daily constraints on stomatal conductance from climate, and compared these constraints to $\delta^{13}\text{C}$ values in Douglas-fir trees grown across a climate gradient in Oregon. I found that limitations on stomata during periods of leaf expansion correlated well with foliar $\delta^{13}\text{C}$. By including stem hydraulic properties with environmental factors, however, the relations were significantly improved. Annual growth-ring $\delta^{13}\text{C}$ was also correlated with predicted stomatal constraints, but not as well. Refinements are suggested that could improve the predictive power of the relation. This approach serves as a means of separating climate stresses from other imposed stresses by establishing a baseline of forest response to natural climatic stresses. This baseline serves as a control in areas where control sites cannot otherwise be established.

4.2 INTRODUCTION

4.2.1 *Rationale*

Humans are changing the global environment by increasing atmospheric concentrations of CO₂ and pollutants, by disrupting the nitrogen cycle and thus eutrophying the biosphere, and through land conversion (Vitousek 1994). Monitoring the state of earth's forests should be a priority in any effort to monitor the influence of these changes on global ecosystems. Forests are an important part of the stability of the global system and the role they play is complex. They are a major contributor to water and nutrient cycling (Shuttleworth 1988, Lindberg et al. 1986), they interact with the atmosphere and lithosphere (Schlesinger 1991), they modify the form and appearance of landscapes, and they also provide a number of products on which humans depend.

Separating human-imposed stresses from the natural stresses in which forests grow is an important first step to identifying the magnitude and timing of imposed stresses. Natural stresses that limit tree growth include temperature extremes, drought, lack of light, and high humidity deficits, as well as disturbances like fire, pathogens, and mechanical damage. Forest declines have been associated with anthropogenic pollution, including gases such as ozone (Johnson and Taylor 1989, Miller 1983) and SO₂ (Winner et al. 1985), acidic deposition such as cloud (Lovett et al. 1982), soil effects such as heavy metal toxicity (Shortle and Smith 1988) and concomitant nutrient deficiencies (Lange et al. 1987).

An innovative approach for separating the effects of human perturbation from natural variation is the Before-After-Control-Impact design with paired sampling, or BACIP sampling design (Stewart-Oaten et al. 1986, Osenberg et al. 1994). BACIP calls for concurrent sampling several times before and after perturbation at a control and an impact site. The difference in some environmental measure between the control and impact site on each site over time provides a gauge of the magnitude of the effect of the perturbation (Osenberg et al. 1994). The two major drawbacks of this sampling approach are 1) it requires the foresight to anticipate a perturbation at a given site and to sample appropriate environmental measures prior to disturbance, and 2) it requires the presence of a control near the perturbation. A method is needed, then, which allows researchers to retroactively obtain appropriate environmental measures of before-impact conditions. Furthermore, researchers need a means of establishing a control within a disturbed site.

Here I describe an approach to establishing a baseline of forest response to climate stresses in the midst of imposed stresses, using stable carbon isotope abundance in tree foliage and growth rings and a process-level forest growth model. Foliar $\delta^{13}\text{C}$ is analyzed, because foliage serves as the most direct link between the tree and its climatic environment, and is where carbon isotopes are taken up. Analyzing isotopes in tree-rings allows for retroactive sampling as far back as trees can be aged. The method was established and tested across a broad range of climates in Oregon, USA, for two varieties of a single species, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Carbon isotope abundance was measured in foliage and growth rings at six sites. A

forest growth model quantified stomatal constraints associated with drought, vapor pressure deficit, and freezing temperatures at each site for the years 1975 to 1994. The relation between $\delta^{13}\text{C}$ and climate-induced stomatal constraints was modelled, then the predictive power of the relationship was tested on an independent set of measurements.

4.2.2 Background

The abundance of $\delta^{13}\text{C}$ (an index of the ratio of $^{13}\text{C}/^{12}\text{C}$ against a standard)¹ changes in response to stresses that cause leaf stomata to close. During carbon fixation, carbon is twice fractionated—once as it diffuses into the leaf via the stomata and once by the carbon-fixing enzyme, rubisco (ribulose biphosphate carboxylase/oxygenase). In both cases the heavier isotope is discriminated against in favor of the lighter. Stomatal conductance and the rate of carbon assimilation control the internal CO_2 concentration, and thus the uptake of ^{13}C . When internal CO_2 concentrations drop, as when stomata are constricted due to stress, rubisco discrimination declines, and the ratio of $^{13}\text{C}/^{12}\text{C}$ in tree tissue rises. Thus, $\delta^{13}\text{C}$ provides an indication of how stress limits stomatal conductance and affects carbon fixation during cellulose formation (Farquhar et al. 1982, O'Leary 1993) :

5. $\delta^{13}\text{C}$ is an index of the ratio (R) of ^{13}C to ^{12}C in relation to the standard, Pee Dee Belemnite, such that

$$\delta^{13}\text{C} \text{ (}\text{‰}\text{)} = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000 \quad (1)$$

$$\delta^{13}C_{leaf} = \delta^{13}C_{air} - a - (b-a) \frac{c_i}{c_a} \quad (2)$$

where c_i and c_a are the leaf internal and atmospheric CO₂ concentrations respectively, a is the isotopic discrimination from diffusion (4.4‰) and b is the discrimination from carboxylation (30‰).

Dendrochronologists derive empirical relations between tree growth and climate. The relations are best in regions where growth is limited by drought or extremes in temperature. In the mild climates typical of the Pacific Northwest, however, correlations between indices of tree-ring width and drought are poor ($r < 0.16$, Meko et al. 1993). Functional growth models and more sensitive indicators of climate stress than ring-width are required in this region.

Process models provide a means of predicting forest response to environmental factors based on physiological principles. FOREST-BIOGEOCHEMISTRY estimates stand-level forest growth for any species given the availability of appropriate information for model parameterization (Running and Coughlan 1988). In this study, FOREST-BGC was used to predict reductions in stomatal conductance affected by climate and soilwater availability.

Climate factors controlling water availability and atmospheric humidity have long been known to affect tree $\delta^{13}C$ abundance (Tans and Mook 1980, Freyer and Belacy 1983). More recently, the underlying physiological responses have been investigated. Transpiration, estimated from a site water balance model, explained up to

68% of tree-ring $\delta^{13}\text{C}$ variability in Douglas-fir (Livingston and Spittlehouse 1993).

Strong correlations between stable carbon-isotope abundance in foliage and the length of branches carrying water to that foliage suggested that branch hydraulic properties influence isotopic uptake (Waring and Silvester 1994, Panek and Waring, in press). A strong correlation between foliar $\delta^{13}\text{C}$ and specific- and leaf-specific conductivity across a range of conductivities supports the importance of stem hydraulics (Chapter 2). Therefore, evidence has gathered which indicates that, by including hydraulic properties of trees in models, the relation between $\delta^{13}\text{C}$ and the climatic constraints affecting stomata can be greatly improved.

4.3 MATERIALS AND METHODS

4.3.1 Site selection

Six sites were chosen along a transect which cuts across a steep climatic gradient in Oregon from moist, maritime to arid, continental environments (Table 3.1, Figure 4.1). The transect was established by (Gholz 1982) and recently served as the focus of a NASA project involved with modelling forest growth from satellite (Peterson and Waring 1994, Runyon et al. 1994). Sites A/B and B/C represent zones of climatic transition between sites A, B, and C (Chapter 3).

4.3.2 Sample selection and analysis

Four Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) with fully sun-exposed, south-facing branches were selected at each site. One south-facing branch between 3.5–4.5 m in length was sampled from each tree. Tree heights and branch lengths were similar at all sites across the transect, however this required sampling older trees and older branches at sites C and D. The crown bottom of trees at sites A–B/C and D was within a meter of the ground, but at site C crown bottom was as high as 2 m. Needles

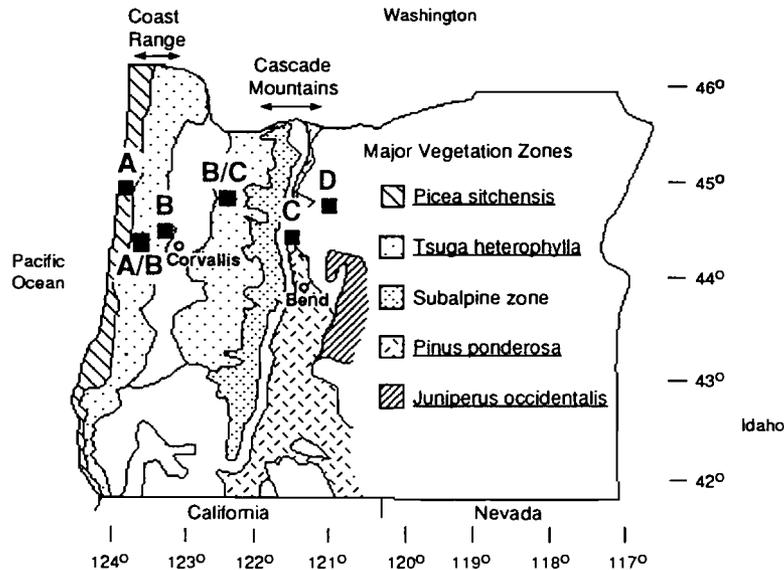


Figure 4.1. Site locations in Oregon, USA.

were stripped from the upper side of the primary axis of each sampled branch and sorted by age-class. Needles were dried to constant weight at 70°. Two tree cores were taken from the south side of each tree. One core was dried, mounted, and sanded for ring-width analysis. The other core was sectioned by year with the aid of a dissecting scope.

All needle age-classes were analyzed individually. Tree core segments were analyzed by annual age-classes from 1987 to 1994 for three out of four trees per site, and from 1976 (or from tree center) on the remaining sampled tree.

Needles were ground with a Wiley mill to pass a 40-mesh screen and core segments were ground to pass a 10-mesh screen. The cellulose fraction of foliage and core segments was isolated by methods established at the University of Utah Stable Isotope Research Facility (S. Phillips, pers. comm., Univ. Utah, Salt Lake City), which were modified from those described by Wise et al. (1946). Stable carbon-isotope abundance was measured at the University of Waikato Stable Isotope Facility, New Zealand, with a continuous flow analyzer connected to a GC/MS (Europa Tracemass). All analyses were run against the CSIRO sucrose standard and calculated in relation to the Pee Dee Belemnite standard, with variation of analysis less than 0.2‰. Details are in Chapter 2.

Mounted tree cores were analyzed with a tree-ring measuring system, which included a sliding incremental stage with measurement precision to 0.001mm (ACURITE, Jamestown, NY), an optical encoder (Metronics Quickcheck, Bedford, NH), and a Motion Analysis Video Imaging System attached to a standard PC. Analysis software was written by Paul Krusic, Tree-ring Laboratory, Lamont-Doherty Geological Observatory, Palisades, NY.

Tree-ring series were corrected for growth trends by fitting a quadratic or cubic polynomial to the ring-width data. The standard exponential decay curve (Fritts 1976) didn't fit the data well because the westernmost chronologies were short. Short series

are best fit with flexible splines (Holmes 1992). The splines represent the best-fit mean for any year. Residual differences between the mean and the observed represent the ring width corrected for the growth trend. Residuals were normalized by the mean value to enable cross-site comparisons.

4.3.3 Branch hydraulic parameters

Hydraulic properties were measured across the transect on the 1990 internode of the same branches sampled in a previous study (Chapter 3). From these data, specific and leaf-specific conductivity were calculated. Specific conductivity (κ_s) represents the flux of water per unit area of conducting sapwood at a given pressure difference across the sample. Leaf-specific conductivity (LSC) is the flux per unit leaf area distal to the segment at a given pressure difference across the sample. Flux (kg s^{-1}) was calculated as the time for a 0.07% HCl solution at 0.01 MPa pressure to move through a 5 cm segment. The HCl solution was then replaced with a safranin solution to stain the conducting portion of the cross-sectional area. Specific conductivity was determined by dividing flux by functional cross-sectional area. Leaf-specific conductivity was determined by dividing flux by the leaf area of all age-classes beyond the measured 1990 internode. Additional details are provided in Chapter 3.

4.3.4 Characterization of environmental constraints

At each site, constraints on stomatal conductance due to climate were assessed daily with the water-balance component of FOREST-BGC and summed over the growing

season. Stomatal constraint was expressed as the percent reduction from maximum stomatal conductance at the recorded irradiance (Panek and Waring, in press).

FOREST-BGC requires species and site characteristics, and daily climate data as input (temperature, precipitation, relative humidity, solar radiation). The model then predicted stomatal constraint separately due to vapor pressure deficit (VPD), soil drought, and freezing temperature daily, for 20 years of climate data (1975-1994, G. Taylor, Oregon Climate Center, Oregon State Univ., Corvallis, OR). I recognized that stomatal response differs slightly for the two varieties of Douglas-fir, however made no accommodation for differences in modelling. Additional details are provided in Chapter 2.

In some species of tree, starch reserves from the previous year contribute to the construction of current year's leaves, which complicates the relationship between foliar $\delta^{13}\text{C}$ and climate. In Douglas-fir, the current year's growth is derived exclusively from the current year's photosynthate. Starch reserves from the previous year are not utilized for foliar growth (Webb and Kilpatrick 1993). The modelled growing season was defined to begin when current year's starch reserves began to accumulate, i.e. when modelled photosynthesis exceeded modelled respiration. The end of the growing season was defined as August 20, the date at which budset had been observed at all sites.

The end of foliar growth does not coincide exactly with the end of tree-ring growth, so the growing season extended longer into the summer drought for tree rings than for foliage. Emmingham (1977) demonstrated that spring-wood formation in Douglas-fir in southwestern Oregon ends when foliar expansion ends, however latewood may continue to grow as late as the middle of September. This difference in growing

season was not accounted for during modelling, in part because springwood represents the majority of tree-ring growth. Summerwood, however, is recognized to be denser and may be a large percentage of the growth-ring biomass. Any resultant bias would appear as an underestimation of tree-ring $\delta^{13}\text{C}$ abundance.

4.3.5 Statistical Analysis

$\delta^{13}\text{C}$ was highly autocorrelated between years within site, in both foliage and tree-rings. To model the relationship between $\delta^{13}\text{C}$ and climatic constraints to stomata, it was necessary to determine the correlation structure between years and incorporate the autoregressive correlation into the model. Therefore a mixed linear model was used, which is a generalization of the standard linear model that allows for the analysis of data with several sources of variation (SAS Institute 1992).

The predictive power of the regression model was tested using the PRESS (Prediction Sum of Squares) statistic, which is recommended when sample sizes are small (Myers 1986). An independent dataset was created by jack-knifing, that is removing observations from the full dataset one at a time and using the remaining observations to model the deleted observation. The difference between the predicted and the observed value is the PRESS residual and is independent of the measured values. The PRESS statistic is the sum of squared residuals. An R^2 for prediction was determined from one minus the ratio of the PRESS statistic to the model sums of squares.

4.4 RESULTS

4.4.1 Variation in foliar $\delta^{13}\text{C}$

Trees at the eastern limit of the transect retained more age-classes of foliage than trees at the western limit of the transect. At site A, trees held foliage only four years, while at sites C and D trees held eight and seven years, respectively. Foliar $\delta^{13}\text{C}$

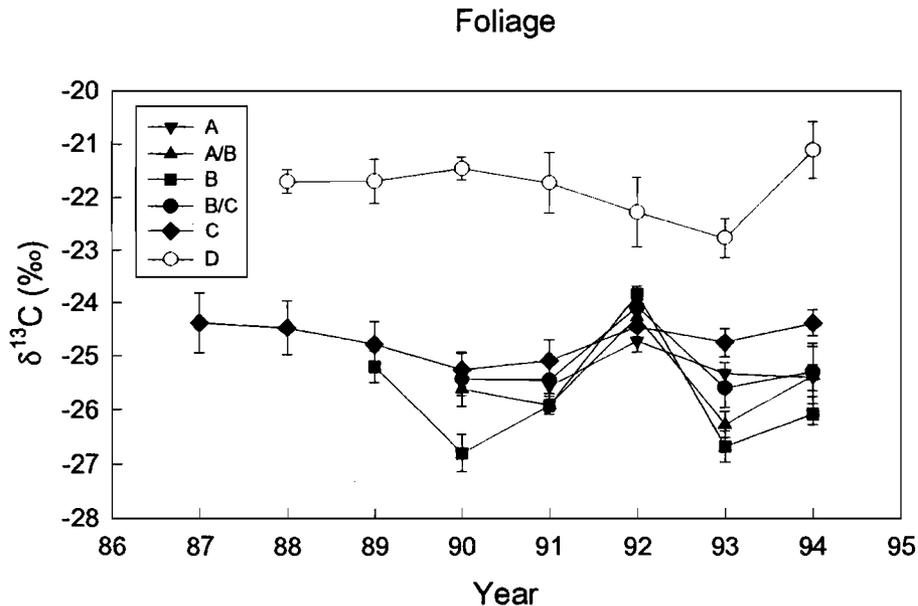


Figure 4.2. Annual variation in foliar $\delta^{13}\text{C}$ abundance. Western sites (A–B/C) retained fewer age-classes of foliage than eastern sites (C–D). Symbols represent the mean of four branches. Error bars represent ± 1 standard error.

abundance was significantly higher for all years at site D than at the other sites. In the eight years of foliage represented by trees along the transect, the largest deviation in $\delta^{13}\text{C}$

abundance occurred in a 1992 spike at all sites except site D (Figure 4.2). The 1992 age-class was missing in most branches at site C. Where 1992 foliage existed there, leaf length was markedly below that of other years. Internode length in 1992 was also noticeably shorter than in other years. This phenomenon was only observed at site C.

4.4.2 Variation in tree-ring $\delta^{13}\text{C}$

Tree-ring $\delta^{13}\text{C}$ exhibited a similar pattern to that of foliage (Figure 4.3). At site D tree-ring $\delta^{13}\text{C}$ was significantly higher than at the other sites in all years except 1993

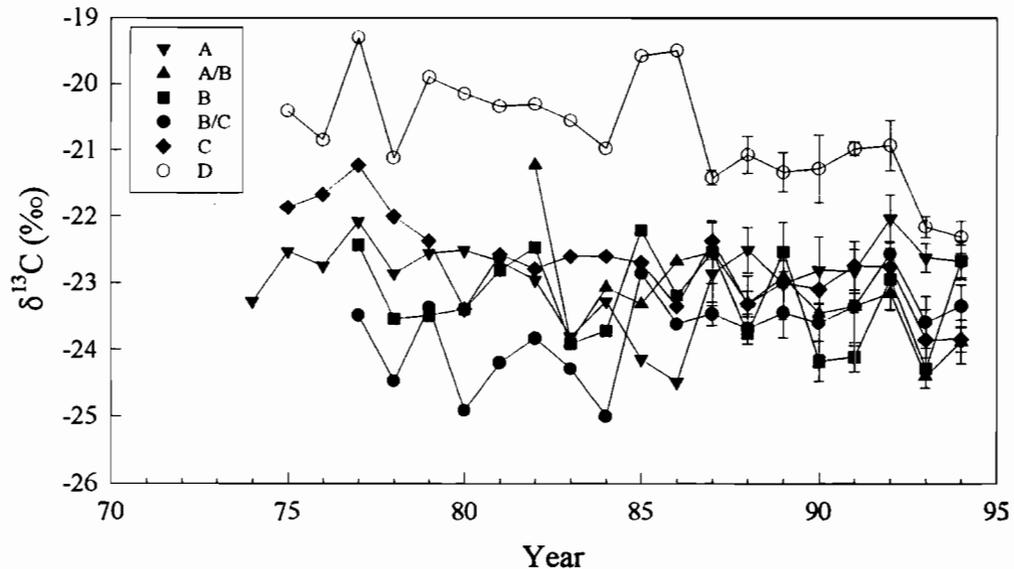


Figure 4.3. Annual variation in tree-ring $\delta^{13}\text{C}$ averaged from one increment core from each of four trees per site. Symbols represent the mean of four trees. Error bars represent ± 1 standard error. Points without error bars are values of a single tree at each site, analyzed for 22 years or to the center of the tree.

and 1994. The same abrupt increase occurred in 1992 across all sites. Foliar $\delta^{13}\text{C}$ and tree-ring $\delta^{13}\text{C}$, compared by year within individual trees, were linearly related (Figure 4.4), although the relation was weak ($R^2=0.42$, $P<0.0001$). Tree-ring $\delta^{13}\text{C}$ was, on average, 1.7‰ heavier than foliage.

Tree-ring width was not correlated with $\delta^{13}\text{C}$ within any growth year, including 1992, or over all growth years. Tree-ring width was not significantly correlated with precipitation, temperature, relative humidity, or solar radiation, either within site or over all sites, in 1992 or over all years.

4.4.3 Stomatal response to physical environment

Temperature, drought and VPD constraints on stomatal conductance increase dramatically from west to east across the transect (Figure 4.5). The integrated effects of VPD, drought, and temperature constraints to stomata are manifest as a drop in potential transpiration below maximum. The annual variation in the proportion of actual to potential transpiration (T/PT) at all sites from 1975 to 1994 is shown in Figure 4.6. Annual T/PT drops across the transect from west (site A) to east (site D), corresponding roughly to an increase in site aridity. A marked drop in T/PT was apparent at all sites but D in 1992, followed by an increase in 1993.

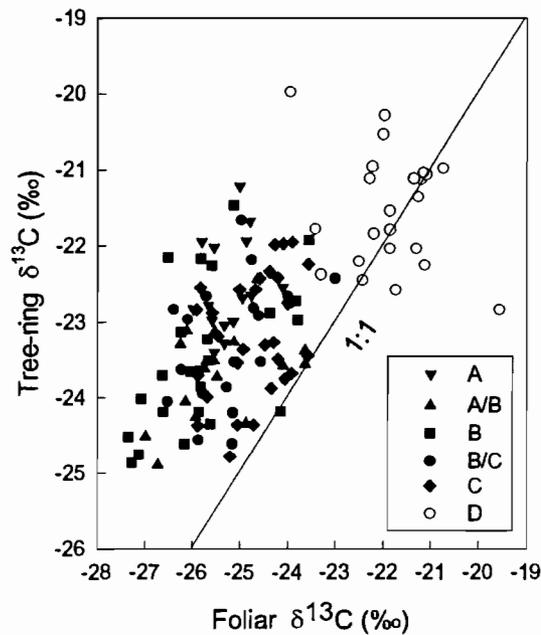


Figure 4.4. Foliar $\delta^{13}\text{C}$ and tree-ring $\delta^{13}\text{C}$, compared by year within tree, were linearly related, although the relation was not strong (tree-ring = $-12.39 + 0.42 \times \text{foliage}$, $R^2 = 0.42$, $P < 0.0001$). Tree-ring $\delta^{13}\text{C}$ was, on average, 1.7‰ heavier than foliage.

4.4.4 Effects of hydraulic properties on stomata

Hydraulic parameters were measured under saturated spring conditions and again at the end of the growing season when foliar elongation had ceased, as described in Chapter 2. The post-growing season values are of interest here, as they represent the cumulative effect of seasonal cavitation and thus correspond better to isotope values, which represent the cumulative seasonal integration of stomatal constraints. Both κ_s

and LSC of branches declined dramatically from west to east across the transect (Figure 3.2). The relation between $\delta^{13}\text{C}$ in foliage at the branch tip and both κ_s and LSC values was inverse, as predicted by theory, and highly significant across the transect (Figure 3.5).

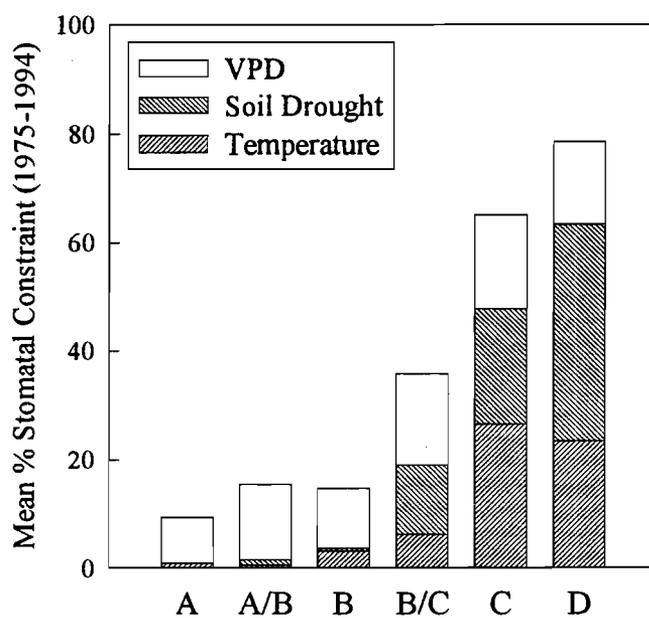


Figure 4.5. The mean modelled stomatal constraint due to VPD, soil drought, and temperature during the growing season for sites from west to east across the transect. Stomatal constraint is defined as the percent reduction from maximum stomatal conductance at the measured irradiance attributed to each environmental factor.

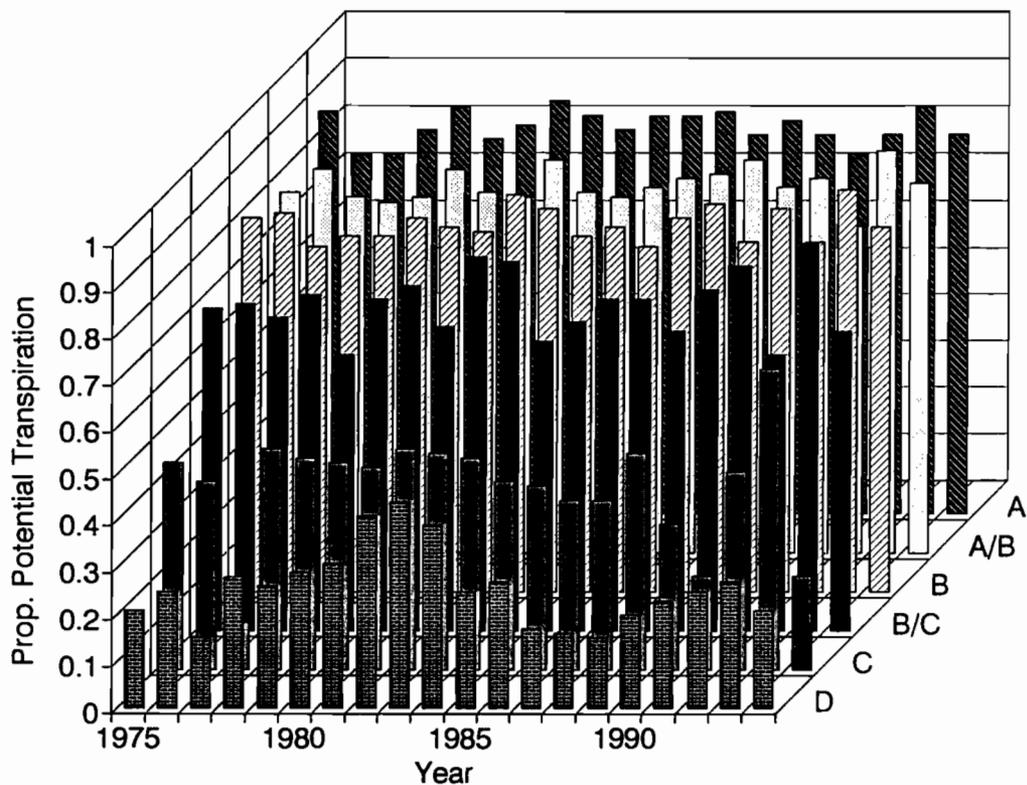


Figure 4.6. Annual variation in the proportion of potential transpiration modelled for the entire growing season for each site.

4.4.5 Regression model to predict $\delta^{13}C$

All data were averaged by site to develop a regression model relating foliar and tree-ring $\delta^{13}C$ to climatic constraints on stomatal conductance through time. Several mixed model covariance structures were tried, but the compound symmetry structure best fit the residuals and afforded the most predictive power because the variance in the correlation between years was the same regardless of year. Model fit was determined with Akaike's Information Criterion (AIC), which is dependent on the number of fit parameters. Fewer parameters will spuriously "improve" the AIC. Models with larger

AIC (in this case, less negative) values for the same number of parameters are preferred (SAS Institute Inc. 1992).

The variables that were tested in the model included proportion of actual to potential transpiration (T/PT), or combinations of the constraints to stomata from VPD, temperature, and drought, which specify the nature of the constraint more precisely than T/PT. The model that best explained the variation in foliar $\delta^{13}\text{C}$ over time included only VPD and drought ($P=0.006$, $P=0.001$ respectively, $\text{AIC}=-44.45$). Temperature was not a significant variable ($P=0.39$). Predicted vs. observed values are plotted in Figure 4.7a. All values cluster around the 1:1 line except those at site D.

When the inverse of specific conductivity was included in the model, full model AIC was improved ($\text{AIC}=-43.1$) and specific conductivity was highly significant ($P=0.01$). Furthermore, with $1/\kappa_s$ in the model, predicted values better fit observed values, including those at site D (Figure 4.7b). Predicted values are compared to measured values by site from west to east in Figure 4.9a. Values modelled without $1/\kappa_s$ are shown to demonstrate the importance of that variable in the model. Model parameters are in Table 4.1.

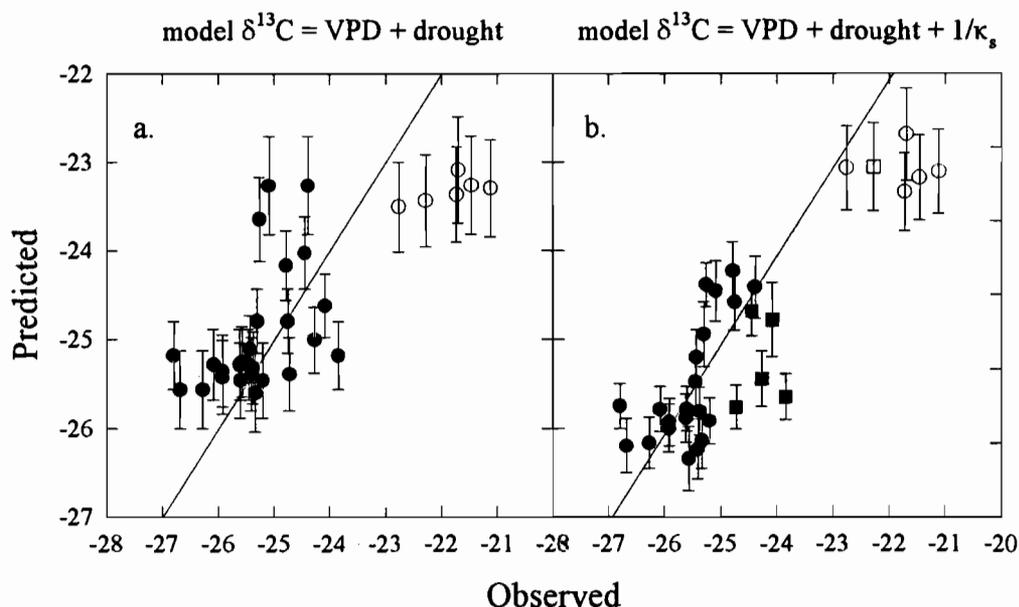


Figure 4.7. Predicted foliar $\delta^{13}\text{C}$ compared to measured. Solid symbols represent the mean of four trees per site of var. *menziesii*, open symbols represent var. *glauca*. Error bars are ± 1 standard error. (a) Prediction without κ_s (b) Prediction improvements with κ_s in the model.

The same approach was used to model tree-ring $\delta^{13}\text{C}$. Environmental constraints to stomata that best explained $\delta^{13}\text{C}$ variability were again VPD ($P=0.02$) and drought ($P=0.07$), $\text{AIC}=-52.03$ (Figure 4.8a). When $1/\kappa_s$ was included ($P=0.09$) full model AIC improved ($\text{AIC}=-51.6$) and site D predicted values were closer to observed values (Figure 4.8b), although both VPD and drought lost their significance. Predicted values for tree-rings were less accurate than foliar $\delta^{13}\text{C}$ predicted values (Figure 4.9b). Model parameters are presented in Table 4.1.

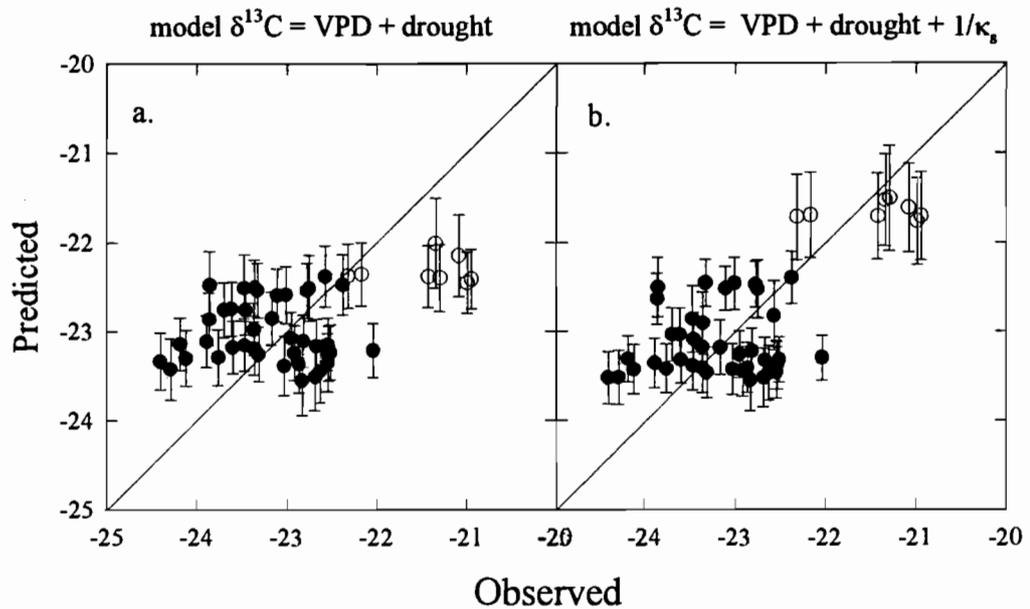


Figure 4.8. Predicted tree-ring $\delta^{13}\text{C}$ compared to measured. Solid symbols represent the mean of four trees per site of var. *menziesii*, open symbols represent var. *glauca*. Error bars are ± 1 standard error. (a) Prediction without κ_s (b) Prediction improvements with κ_s in the model.

4.4.6 Predictive capabilities of model

The PRESS residuals were calculated for the set of both foliar and tree-ring $\delta^{13}\text{C}$ values to determine the predictive ability of the model. The magnitude of PRESS residuals varied from site to site and from year to year within site. Residuals for foliar $\delta^{13}\text{C}$ ranged from 0.04 to 1.3‰ and were on average 0.67‰. They were highest at site D, which corresponds to the lowest measures of specific conductivity. A sensitivity analysis revealed that low κ_s , around $0.001 \text{ kg MPa}^{-1} \text{ s}^{-1} \text{ m}^{-1}$, generated the highest errors. At κ_s levels typical of var. *menziesii*, prediction errors ranged from 0.4–1‰

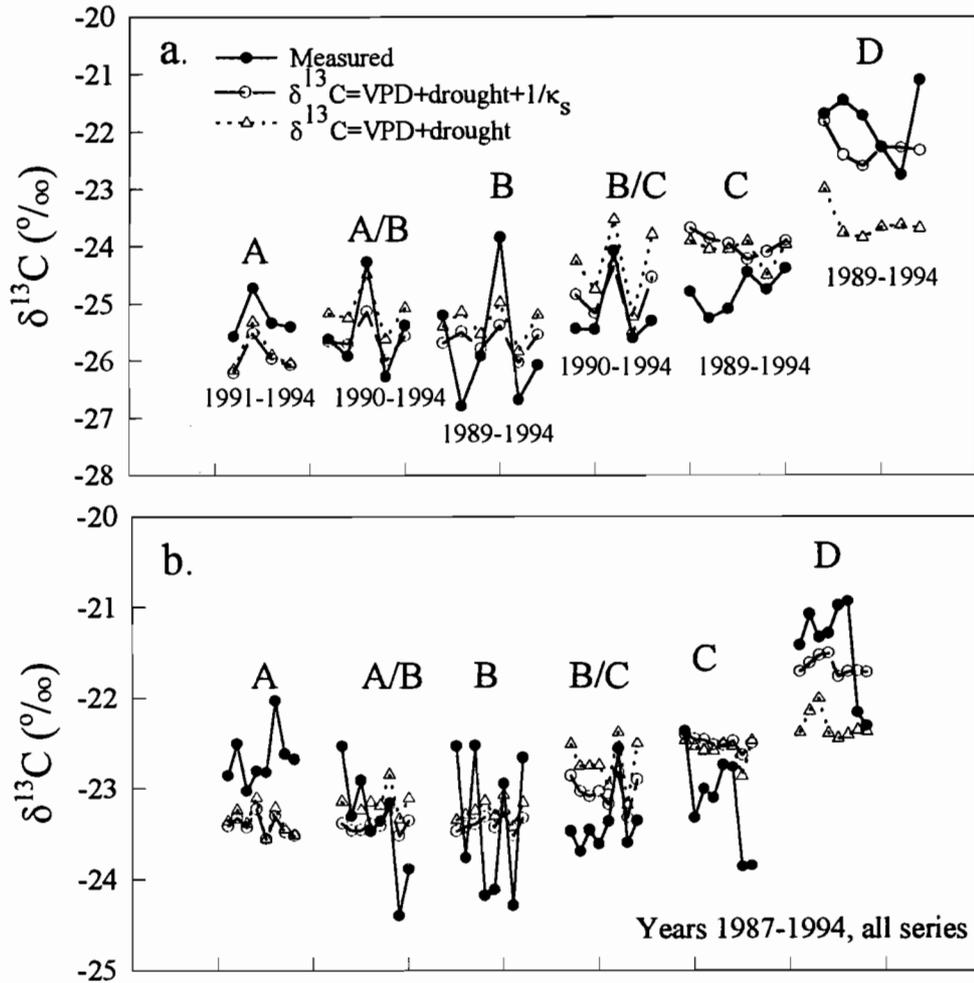


Figure 4.9. Annual predicted $\delta^{13}\text{C}$ values compared to measured $\delta^{13}\text{C}$ values. The three lines compare the annual variation of measured $\delta^{13}\text{C}$, $\delta^{13}\text{C}$ modelled with κ_s , and $\delta^{13}\text{C}$ modelled without κ_s , within and among sites (a) in foliage and (b) in tree-rings.

(Figure 4.10). Residuals for tree-ring $\delta^{13}\text{C}$ prediction ranged from 0.03 to 1.6‰ and averaged 0.58‰. The 95% confidence interval for foliar $\delta^{13}\text{C}$ prediction based on PRESS residuals varied from 0.5 to 1.4‰, and averaged 0.73‰. For tree-rings, the 95% confidence interval ranged from 0.5 to 1.3‰ and averaged 0.67‰. The R^2_{pred} was 0.72 for foliar $\delta^{13}\text{C}$ and 0.37 for tree-ring $\delta^{13}\text{C}$.

Table 4.1. Parameters for regression model relating $\delta^{13}\text{C}$ to climate constraints and specific conductivity in Douglas-fir across the transect.

Parameter	Estimate	Std. Error	Prob. > T	AIC ¹
Foliar $\delta^{13}\text{C}$				
Intercept	-27.05	0.572		-43.1
VPD	0.076	0.032	0.02	
Drought	0.026	0.012	0.03	
$1/\kappa_s$	0.550	0.209	0.01	
Tree-ring $\delta^{13}\text{C}$				
Intercept	-23.98	0.442		-51.6
VPD	0.029	0.022	0.1	
Drought	0.011	0.009	0.2	
$1/\kappa_s$	0.290	0.167	0.09	

1. Akaike's Information Criterion. See text for interpretation.

The number of trees to sample depends on the variability observed and on desired power to detect differences. In this study, to have a 95% probability (two-sided) that the mean was within 0.5‰ of the true mean required between 10 (site A) and 52 (site B)

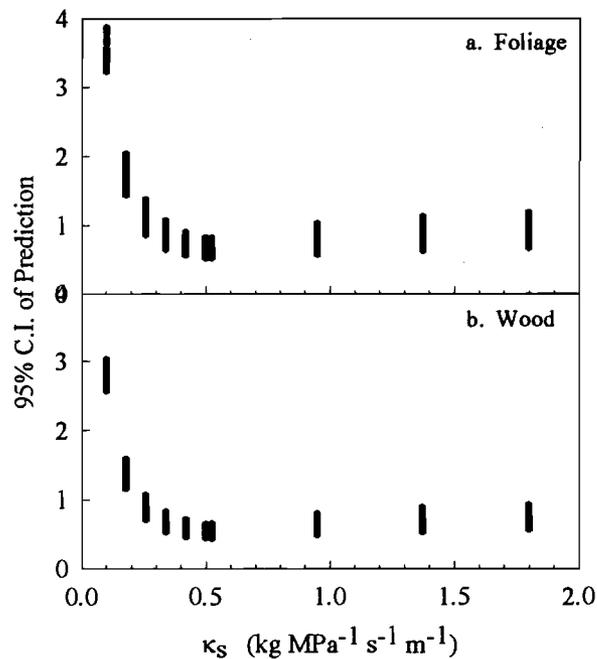


Figure 4.10. The 95% confidence intervals for model predictions from a sensitivity analysis of variable influence on error rates. Of the variables drought, VPD and κ_s , $\delta^{13}\text{C}$ abundance was most sensitive to κ_s , both (a) in foliage and (b) in wood.

trees per site using a power level of 0.90. In studies like this where not making a Type II error is important, decreasing power compromises the approach. One option is to increase sample size, but the expense of isotope analysis is severely limiting. Alternatively, more refined sampling of wood production during the growing season may yield additional precision and insight.

4.5 DISCUSSION

4.5.1 Variation in foliar $\delta^{13}\text{C}$

At site D the foliar $\delta^{13}\text{C}$ abundance of var. *glauca* was significantly less negative than in foliage along the rest of the transect, which is indicative of higher water use efficiencies (Farquhar et al. 1989). The branches of trees at site D had low stem hydraulic conductivities, typical of species adapted to arid regions. The unusually high $\delta^{13}\text{C}$ value in the 1992 foliage observed at all but site D was mirrored by an extreme drought recorded at all sites but D. $\delta^{13}\text{C}$ values in 1992 were less negative than model predictions, even when specific conductivity was included in the model (Figure 4.9a). It is likely that the severe drought caused cavitation in all branches of var. *menziesii* across the transect during 1992 (Chapter 2).

Foliar $\delta^{13}\text{C}$ correlated well with stomatal responses to VPD and drought, but not to freezing temperatures when stomata close completely. When stomata are closed no isotopic discrimination can occur. The ratio of actual to potential transpiration integrated all changes in stomatal conductance, including freezing temperature constraints. Because of this, VPD and drought constraints were better related to $\delta^{13}\text{C}$ values than was the transpiration index. In addition, transpiration was not directly correlated with photosynthesis, as observed at site D where water-use efficiencies differed from other sites.

By including κ_s with VPD and drought constraints, the model prediction improved significantly (Figures 4.7b, 4.9a), especially at site D. Further improvement

might be gained if κ_s were monitored annually instead of using one internodal segment as an index of hydraulic properties. When two varieties of Douglas-fir were compared, specific conductance played a particularly critical role in improving model prediction of $\delta^{13}\text{C}$.

4.5.2 Variation in tree-ring $\delta^{13}\text{C}$

Tree-ring $\delta^{13}\text{C}$ abundance was significantly correlated with foliar $\delta^{13}\text{C}$ abundance, but the relation was weak ($R^2=0.42$, $P=0.0001$). Foliar $\delta^{13}\text{C}$ may provide a tighter coupling to environmental factors which close stomata than tree-ring $\delta^{13}\text{C}$, especially if the effect is sufficient to restrict needle growth (Cleary 1971). Tree-ring $\delta^{13}\text{C}$ was 1.7‰ heavier than foliar $\delta^{13}\text{C}$, on average, consistent with a 1-2‰ difference reported for pines (Leavitt and Long 1986, Yoder et al. 1994). $\delta^{13}\text{C}$ is fractionated during translocation and wood-cellulose formation, and is thus subject to processes unrelated to climate (Deines 1980, O'Leary 1981). Furthermore, wood production continues after foliar elongation has ceased, thus integrates a different climate signal. Sampling only one core from one aspect probably did not contribute to the weak relationship between foliar and tree-ring $\delta^{13}\text{C}$ because, while there is significant circumference variability in tree-ring $\delta^{13}\text{C}$, trends through time are consistent from year to year (Tans and Mook 1980, Leavitt and Long 1986). On the other hand, when stem hydraulics are considered, one core may not fully represent processes occurring throughout the crown. Waring and Silvester (1994) showed that aspect influenced $\delta^{13}\text{C}$ abundance in the crown. Several studies

showed that branch length, and thus tree morphology, affected $\delta^{13}\text{C}$ (Waring and Silvester 1994, Panek and Waring, in press, Yoder and Panek, in review). In this study, the south side of trees were sampled with the objective of maximizing the chance of discerning yearly variation in $\delta^{13}\text{C}$ attributable to environmental factors. For foliage on branches, this approach was appropriate because each branch is essentially carbon-autonomous (Sprugel et al. 1991). Wood production in the tree stem, however, integrates the photosynthate from a large but unknown portion of the crown, favoring regions with higher stomatal conductance. The wood in the south side of the stem may not represent processes on the south side of the crown (Vité and Rudinsky 1959).

Although the fit between tree-ring $\delta^{13}\text{C}$ and climatic constraints to stomata was not as good as that for foliage, the general response was similar. Stomatal constraints from VPD and drought explained a significant amount of year to year variation in $\delta^{13}\text{C}$. Temperature again was not significant. Including κ_s improved the model fit across sites, and was critical to integrating the model across varieties.

Several factors may have contributed to the poor ability of the model to predict growth-ring $\delta^{13}\text{C}$. κ_s was determined for individual branches, however tree-ring $\delta^{13}\text{C}$ reflects carbon from many different parts of the tree crown. Whole tree hydraulics may better represent the hydraulic constraints to the entire canopy. The branches sampled, however, may serve as a lower limit of whole-tree conductance because branches confer a greater resistance to flow than trunks in the movement of water from root to leaf because they consist of denser wood (Zimmermann 1978). Lower branches in conifers usually have lower conductances than upper branches (Ewers and Zimmermann

1984a,b). Thus, by measuring south-facing bottom branches, the lowest conductances were sampled for each tree. Another reason growth-ring $\delta^{13}\text{C}$ may have been poorly modelled is that growth-ring $\delta^{13}\text{C}$ is not as tightly coupled to the climate environment as foliar $\delta^{13}\text{C}$. Carbon isotopes are further fractionated during translocation, which contributes to the variability of growth-ring $\delta^{13}\text{C}$. Finally, the growing season defined for foliage and used to estimate constraints to stomatal conductance underestimates the length of time during which stem growth occurs, as described above. Stem growth may continue for up to a month longer than foliar growth.

4.5.3 Relation between tree-ring $\delta^{13}\text{C}$ and tree-ring width

Ring-width indices were uncorrelated with tree-ring $\delta^{13}\text{C}$. Both tree-ring width and $\delta^{13}\text{C}$ represent indices of carbon-fixing processes, but in different ways. Ring-width represents the carbon remaining for increment growth after fixed carbon is used for fine roots, shoots, injury repair, and reproduction (Kramer and Kozlowski 1979, Waring and Schlesinger 1985). $\delta^{13}\text{C}$, on the other hand, is a permanent chemical signature of limitations on carbon fixation. Translocated carbon is further fractionated before it is incorporated into tree-ring tissue. As earlier dendrochronology studies have demonstrated, growth increment is a poor measure of environmental variation in Pacific Northwest forests (Meko et al. 1993).

4.5.4 Predictive capabilities

The prediction error of $\delta^{13}\text{C}$ in tree foliage and rings varied with environment. κ_s was the primary factor controlling error rates. When κ_s values were high the model precision was high. For κ_s above $0.3 \text{ kg MPa}^{-1} \text{ s}^{-1} \text{ m}^{-1}$, the 95% confidence intervals for both foliage and wood were consistently between 0.5 and 1‰ (Figure 4.10). When κ_s fell below $0.3 \text{ kg MPa}^{-1} \text{ s}^{-1} \text{ m}^{-1}$ the prediction error increased sharply.

At site A, within-site variability in foliar $\delta^{13}\text{C}$ was closely matched, however the trend was offset by 0.6‰ (Figure 4.9a). This suggested that the year-to-year response of trees to climate was predicted well, but that κ_s at the site may be generally lower than measured. At sites A/B and B, 1992 values of $\delta^{13}\text{C}$ were underestimated because cavitation that year affected hydraulic conductivity. Reasons for high errors in estimates of site B $\delta^{13}\text{C}$ in 1989 are unknown. Despite some inconsistencies, the model predicted foliar $\delta^{13}\text{C}$ well at westside sites. At sites C and D, the model represented the site mean well, but the trend was unexpected.

Mean site tree-ring $\delta^{13}\text{C}$ values were generally well-predicted, but the year-to-year variations were poorly estimated. Site A tree-ring $\delta^{13}\text{C}$, as in foliage, showed a reasonable trend but the modelled values were offset from the measured values. The general lack of good fit for year-to-year variation is probably due to the combined effects of small sample size, single core samples, and the lack of annual or whole-tree conductivity measurements.

The approach of predicting tree $\delta^{13}\text{C}$ based on climate stresses creates a control for areas where trees experience further stresses. The difference between $\delta^{13}\text{C}$ values predicted with the climate parameters and measured $\delta^{13}\text{C}$ values quantifies the magnitude of stomatal constraint due to the additional stress. With what certainty will this approach detect the added environmental stresses to stomata? Ozone and SO_2 are among the environmental factors which affect internal CO_2 concentrations, and thus $\delta^{13}\text{C}$, by disrupting net photosynthesis and stomatal conductance (Greitner and Winner 1988, Elsik et al. 1994). Elsik et al. (1994) found an increase of 1.5‰ in leaf and stem tissue of loblolly pine exposed to $2.5\times$ ambient concentrations of ozone. Martin and Sutherland (1990) attributed an increase of 2‰ in growth rings of Douglas-fir to high concentrations of SO_2 . In both cases, these $\delta^{13}\text{C}$ values fall outside the 95% confidence interval at sites A–C for all years, for predictions based on both foliage and wood. Therefore, 95% of the time, one could accurately identify growth years impacted by the pollutants ozone and SO_2 if the $\delta^{13}\text{C}$ responses reported for these pollutants were typical. Air pollution stress is usually chronic and there can be compensatory responses over time. This baseline approach can help identify the onset of the stress response by indicating when the measured $\delta^{13}\text{C}$ values begin to deviate from the predicted values. In the same manner, this approach can identify recovery with time.

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5. CONCLUSION

To adequately model the relation between climate and $\delta^{13}\text{C}$ it was necessary to identify and understand sources of variability in foliar and tree-ring $\delta^{13}\text{C}$. Patterns of $\delta^{13}\text{C}$ variability observed within the canopy of trees are consistent with the idea that xylem hydraulics contribute to $\delta^{13}\text{C}$ variability and therefore pose important limitations to carbon uptake. Branch length in Douglas-fir was found to be significantly correlated with $\delta^{13}\text{C}$ in the foliage at branch tips. This inferred, but did not directly confirm, that stem hydraulics were involved in the relation, as branch length is a measure of path length of water movement. Path length is an important component of hydraulic conductivity. The relation between $\delta^{13}\text{C}$ and branch length was strongest in climates where soilwater is abundant and VPD is low, and degenerates as climates become increasing arid.

The importance of hydraulic properties in $\delta^{13}\text{C}$ variability was confirmed by measuring branch hydraulic, specific, and leaf-specific conductivity in the same branches where $\delta^{13}\text{C}$ was measured, across a gradient of hydraulic conductivity. A strong inverse relation between specific and leaf-specific conductivity and foliar $\delta^{13}\text{C}$ was found, as predicted by theory, and confirmed on several age-classes of foliage. The relation across the transect was so strong, in fact, that the contributions of environmental constraints to $\delta^{13}\text{C}$ variability were overshadowed. Climate and stem hydraulics are not independent, however. Water limitations during stem growth limit the growth of cells, including

tracheids. Smaller tracheids reduce the conductivity of the hydraulic path. Thus the influence of hydraulic conductivity explains the effects of climate on $\delta^{13}\text{C}$. When hydraulic properties do not vary significantly, within an individual over time for example, then the effects of environment on $\delta^{13}\text{C}$ are evident.

A model to predict annual variability in $\delta^{13}\text{C}$ in foliage and tree rings over a range of climate types required both environmental constraints to stomata and xylem hydraulic properties to adequately describe $\delta^{13}\text{C}$ variability. The model predicted $\delta^{13}\text{C}$ in foliage west of the Oregon Cascade Mts. extremely well. East of the Cascades, site means were well-characterized, but annual variability was not. Annual variability in tree-ring $\delta^{13}\text{C}$ was poorly characterized by the model, probably because annual variation in whole-canopy hydraulics was inadequately described by the hydraulic measure.

Based on the results of Chapters 2 and 4, there exists another source of $\delta^{13}\text{C}$ variability in arid sites that was not measured in this set of studies. It was characterized by a poor correlation between branch length and $\delta^{13}\text{C}$ at the eastern extent of the transect, and by an inability to predict annual variability in foliar $\delta^{13}\text{C}$ well at those arid sites. Further study is needed to identify this source of variation, however it is worthwhile to speculate on potential sources here. The pattern of predicted versus observed (Figure 9) implies that while the mean variability between sites was well-modelled by including hydraulic parameters, the within-site variation was not. This $\delta^{13}\text{C}$ variability is a within-site response to year-to-year changes in climate, that is to VPD and soil drought. One of the big differences between eastside and westside sites is the nature of the soil. Eastside sites are sandy and porous. They don't hold water well. Westside

sites, having higher clay fractions, do hold water well. It is well-known that eastside Ponderosa pine and juniper have tap roots that allow them to access groundwater. The Douglas-fir that I sampled probably had access to groundwater because of their proximity to the Metolius River. FOREST-BGC (this version) makes no distinction between soil types and assumes that water used by the forest stand comes from precipitation. On the westside this was probably a fair assumption. On the eastside, precipitation probably had less to do with stomatal conductance than did the timing of snowmelt which recharged groundwater sources. Surges in groundwater availability would lag behind major precipitation events. If this hypothesis is true, then eastside trees should reflect their groundwater sources in their δD values, a hypothesis which is easily tested. Eastside trees should have more negative δD values than westside trees. The more arid the site, the more likely it is that a long-lived organism like a tree would tap into groundwater sources. To predict $\delta^{13}C$ adequately at arid sites, a water balance model must explicitly include changes in soilwater status which is not a result of either precipitation or evapotranspiration.

The approach to creating a baseline of forest response to climatic stress described in this thesis offers a potentially valuable addition to forest monitoring programs and to perturbation studies. It strengthens the weaknesses of the BACIP approach to quantifying the magnitude of an imposed perturbation by providing a means of retroactively sampling to a period prior to a known stress and by providing a method of creating a control in the midst of disturbance. Model parameters obtained from this

study (Table 4.2) should be applicable to many areas throughout the northwest because Douglas-fir covers a broad range of western forests, but the approach should apply to other conifer forest species. To create a baseline of forest response to climate stress requires a physiologically-based forest growth model, climate data, and branch hydraulic information. The model FOREST-BGC is recommended because it can be parameterized for any conifer species. Daily climate data is available from numerous sites throughout the country. Branch hydraulic data is not difficult to obtain (Chapter 2). Foliar $\delta^{13}\text{C}$ is recommended over tree-ring $\delta^{13}\text{C}$ for baseline comparison because of the tighter coupling to climate, and thus the better model fit. Furthermore it is much easier to measure branch hydraulic parameters than whole tree hydraulics. Not all trees retain multiple age-classes of foliage, however. The westernmost trees in this study retained only four age-classes. Ponderosa pine, another good candidate for baseline establishment because of its broad western distribution, retains only two age-classes. Therefore, there is a need for research to link tree-ring $\delta^{13}\text{C}$ to whole canopy hydraulic conductance to tighten up the relation between climatic constraints to stomata and tree-ring $\delta^{13}\text{C}$. This will enable retroactive sampling of disturbances as far back as the tree-ring record.

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