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

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Summary   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 $\delta^{13}C$ varies by about 2‰ from year to year, variability within the foliage can be as high as 6‰. Recent studies have shown that branch length affects stomatal response, which influences the integrated foliar $\delta^{13}C$ signal. To improve the ability of $\delta^{13}C$ to predict climate differences, we examined the relationship between branch length and foliar $\delta^{13}C$ in *Pseudotsuga menziesii* (Mirb.) Franco from four sites across a steep climate gradient in Oregon. The transect spanned the boundary between the ranges of the coastal variety, *P. menziesii* var. *menziesii* (three sites), and the Rocky Mountain variety, *P. menziesii* 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% of this variation.

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 $\delta^{13}C$ and climate. With standardization to constant length, $\delta^{13}C$ values were significantly related to the degree that climatic variables, as modeled 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.

Keywords: hydraulic conductivity, recovery, transpiration, tree rings, xylem cavitation.

Introduction

Stable carbon isotopes in the annual rings of forest trees have the potential to contribute to our understanding of climate and pollution patterns. However, a major difficulty in interpreting the stable carbon isotope composition of trees is the large variation within individuals. Foliar $\delta^{13}C$ can vary by as much as 6‰ (Leavitt and Long 1986, Waring and Silvester 1994) and annual ring $\delta^{13}C$ varies around a tree's circumference by as much as 1.5‰ (*Pinus edulis* Englem., Leavitt and Long 1986) to 4‰ (*Quercus rubra* L., Tans and Mook 1980). Nevertheless, considerable research has been 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 $\delta^{13}C$ 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 Sutherland 1990). In each of these studies, the reported variability in the $\delta^{13}C$ signal was equal to or greater than the 2‰ difference attributed to environment. The large variation within individuals makes it difficult to assign small differences in tree-ring $\delta^{13}C$ to changes in climate or pollution.

We hypothesized that much of the within-tree $\delta^{13}C$ 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 path length of water movement from the soil to foliage. Although trunks may represent a large portion of the path length, branches contribute a greater resistance to water conduction per unit length than trunks because they are composed of denser wood (Ewers and Zimmermann 1984a, 1984b). For a given evaporative demand and hydraulic conductivity, increasing path length (branch length) increases xylem tension in accordance with an Ohm's Law analogy of water movement because ΔP/path length must remain constant:

$$F = k_h \left(\frac{\Delta P}{\text{path length}} \right), \qquad (1)$$

where F is the 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, resulting in 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* D. Don trees showed that branch length correlated linearly with δ^{13}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 path length resistances to water movement through a nearly saturated conducting system. In harsher climates, cavitation, not path length, may be the major source of resistance in the hydraulic system.

To further our understanding of the natural variation in δ^{13}C, we designed a study across a steep climate gradient in Oregon, extending from coastal rain forest to continental woodlands in the rain shadow of the Cascade Mountains. We sampled branches from *Pseudotsuga menziesii* (Mirb.) Franco because its range extends across the climate gradient and its growth utilizes only current-year photosynthate (Webb and Kilpatrick 1993). In addition, conifers have high resistance to flow, conferred by tracheids in series, and a low boundary layer around needles, which links the tree more tightly to its environment (Jarvis and McNaughton 1986).

We hypothesized that, across the transect, branch length would be an important source of variation in foliar δ^{13}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 δ^{13}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 δ^{13}C in predicting climate differences.

Methods

Site selection

Four sites (A–D) were selected along a steep climate gradient in Oregon (Figure 1). Air mass trajectories at these sites come from the Pacific Ocean and thus 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 Terrestrial Ecosystem Research project (Peterson and Waring 1994, Runyon et al. 1994). Table 1 describes salient features of the study sites. Further details are 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 Mountain variety, *P. menziesii* var. *glauca* (Aagaard et al. 1995).
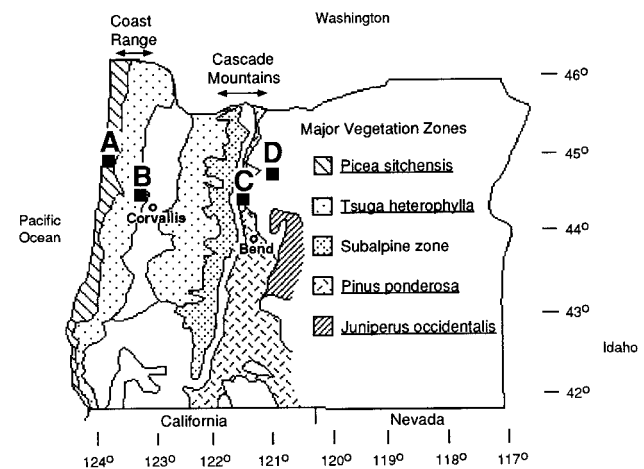

Figure 1. The sites used in this study represent a climate gradient across Oregon (see Table 1). Douglas-fir (*P. menziesii*) is common to all sites.

Table 1. Climate characteristics at the study sites.

Site	Location	PPT[1]	Climate features
A	Cascade Head	244	Summer rain common, frequent fog, rarely freezes
B	Willamette Valley	107	Summers hot and dry, occasional midwinter freezes
C	Jefferson Wilderness	36	Rain shadow of Cascade Mts., summers hot and dry, winters frozen
D	Grizzly Mountain	34	Rain shadow of Cascade Mts., summers hot and dry, winters frozen

[1] Annual average precipitation from 1971 to 1992 in cm (Oregon Climate Service, Corvallis,OR).

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

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 $\delta^{13}C$ with a continuous flow analyzer connected to a GC–MS (Europa Scientific Tracermass, Europa Scientific Ltd., Crewe, U.K.). 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‰.

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 was transported to the laboratory in a dark plastic bag. The end was recut under water and placed in 0.05% safranin dye filtered to 0.22 μm. 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 public domain software, National Institute of Health, USA) and video imaging system (Motion Analysis Inc., Eugene, OR). 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.

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 defined 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 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). At each site during a

defined growing season, the difference in stomatal constraints due to each of these factors was quantified by computing and comparing the sum of reductions in conductance attributed to each factor 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 modeling. 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).

Annual variation in the climatic constraints to stomata at each site were evaluated based on 23 years of climate data. 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). Relative humidity (RH) and solar radiation (SR) were modeled from temperature and precipitation data with a climate model, MT-CLIM (Glassy and Running 1994). We compared measured to modeled RH and SR for 365 days in 1990 to validate the MT-CLIM model. Both RH and SR were significantly correlated with the corresponding modeled values (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 modeled 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 $\delta^{13}C$ 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 $\delta^{13}C$ value, we defined the start of the growing season as the time when current-year starch reserves began to accumulate, i.e., when modeled photosynthesis exceeded modeled respiration. The growing season terminated on August 20, which corresponds to the cessation of shoot elongation and the onset of bud set.

Results

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

At a given site, $\delta^{13}C$ varied with branch length (Figure 2). At Site A, branch length explained 76% of the variation in $\delta^{13}C$ ($P < 0.0001$). Branch length resolved 32 and 41% of $\delta^{13}C$ variation at Sites B ($P < 0.05$) and C ($P < 0.005$), respectively. There was no correlation at Site D ($R^2 = 0.15$, $P = 0.21$).
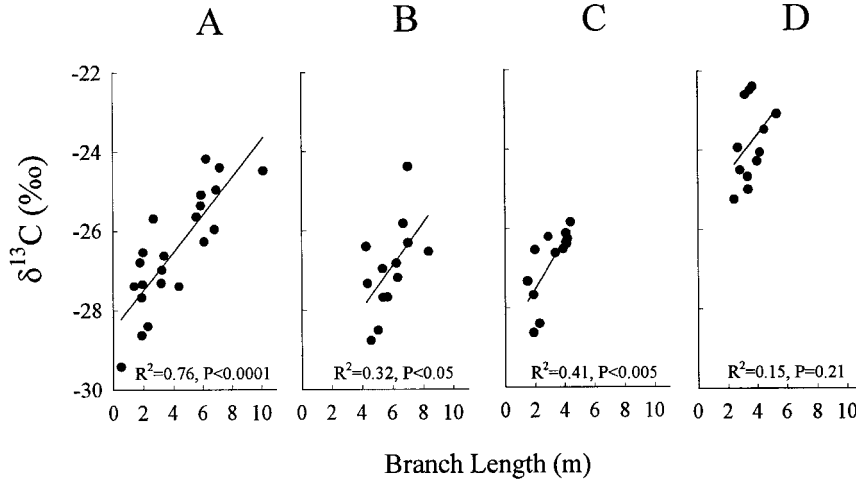
Figure 2. The relationship between $\delta^{13}C$ in foliage and branch length was significantly correlated within a site; however, the strength of the relationship declined at the eastern sites. The slopes of the regression lines were not significantly different at any of the sites ($P < 0.0001$). Intercepts were similar at Sites A and B, but at Sites C and D, the intercepts were significantly less negative ($P < 0.05$).

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 $\delta^{13}C$ 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 $\delta^{13}C$ 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.

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

The modeled constraints to stomata, defined as the percent reduction in stomatal conductance from the maximum value, differed significantly among the four sites ($P < 0.05$, Figure 3). At Site A, stomata were constrained less than 7% from maxi-

mum due to temperature, vapor pressure deficit (VPD) or soil water. At Site C, which has the harshest climate for *P. menziesii* var. *menziesii* along the transect, stomata were constrained 57% from maximum, primarily by freezing temperatures and prolonged 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 Sites C and D, whereas soil drought constraints differed at all sites except Sites A and B. This analysis of the climatic constraints to stomata at each of the sites quantifies the sources of constraint to stomata that reduce potential transpiration.

Isotope values were standardized to a central branch length of 4 m by regressing $\delta^{13}C$ on branch length (Figure 2) and adding the predicted value to the residual. This procedure was valid because the residuals were randomly distributed with respect to branch length. The adjusted values were independent of branch length and 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 $\delta^{13}C$ in the foliage at the end of the branches of identical length (see Figure 5b). Standardized mean $\delta^{13}C$ values were significantly different between all sites except Sites A and B (Fisher's Protected LSD, $\alpha = 0.05$).
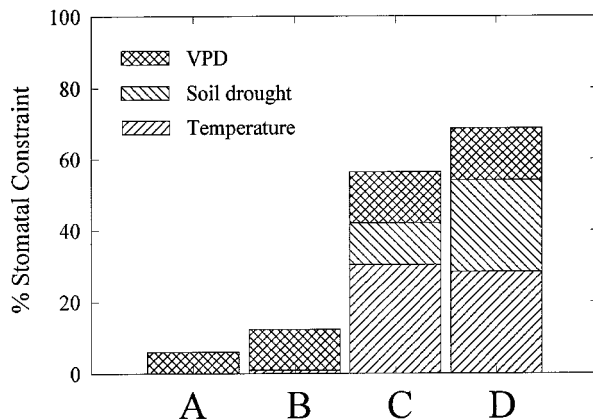

Figure 3. The modeled climatic constraints to stomata, defined as the proportional reduction in stomatal conductance from the maximum value, show the degree to which soil drought, temperature and vapor pressure deficit reduced stomatal conductance at each site.

Cavitation and refilling

Maximum functional sapwood in south-facing branches differed across the four sites and by season (Figure 4). Site A branches at full saturation utilized 97% of their potential cross-sectional area for water conduction. 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 prolonged summer drought was
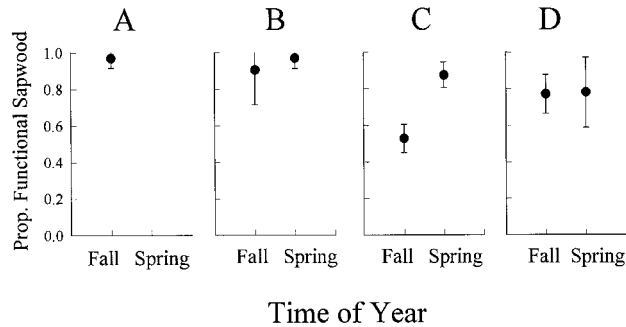
Figure 4. The conducting cross-sectional area in *P. menziesii* branches varied both spatially and seasonally across the climatic transect. The proportion of conducting cross-sectional area was measured as a fraction of the total cross-sectional area. Site A was saturated in the fall, so was not remeasured in the spring. Spring values were significantly different from fall values at Site C (*t*-test, $P < 0.0001$). Error bars represent $\pm$ 1 SD.

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 saturated functional sapwood produced in the spring was constant across all sites (42.9 ± 3.0 SE, $R^2 = 0.91$, $P < 0.0001$; results not shown), but the cavitation-reduced functional sapwood areas at Site C were not related to leaf area.

Discussion

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

Across the environmental gradient, where modeled climatic constraints to stomata differed by as much as 63%, the mean isotopic composition of *P. menziesii* foliage varied by 4.0‰
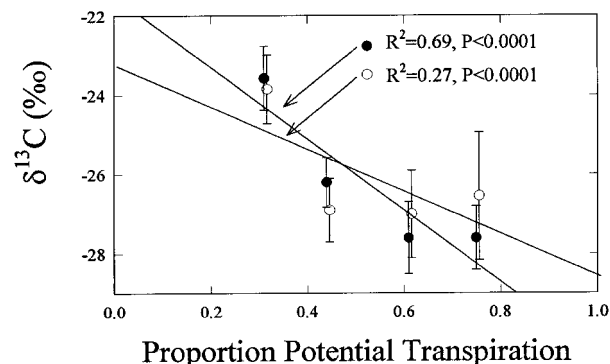

Figure 5. Climate effects were well correlated with $\delta^{13}C$ only when branch lengths were standardized. Open circles (○) show the relationship between proportion of potential transpiration in 1992 at each site and $\delta^{13}C$ of all branches of various lengths from 1 to 11 m. Solid circles (●) show the same relationship after branch lengths were standardized to 4 m. Symbols represent the mean of all branches measured at a site. Error bars represent $\pm$ 1 SD. Regression lines were calculated through all values, not the means. Symbols are offset for ease of interpretation.

(Figure 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 values in the literature.

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

These results suggest that it is important to standardize branch lengths when comparing $\delta^{13}C$ within and between sites. This is demonstrated by comparing the relationship between $\delta^{13}C$ and 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 because of decreases in variation and shifts in the mean $\delta^{13}C$ value. Without standardization, the relationship between climate and $\delta^{13}C$ had an R^2 of 0.27 ($P < 0.0001$); after standardization, the R^2 was 0.69 ($P < 0.0001$). Without standardization, mean $\delta^{13}C$ at Site D only was significantly different from the other sites. With standardization, all sites but Sites A and B were significantly different. Thus $\delta^{13}C$ is a better predictor of climate effects on trees when branch lengths are standardized; that is, when $\delta^{13}C$ is corrected for path length (Figure 5).

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

Not all stomatal constraints influence foliar $\delta^{13}C$ similarly. FOREST-BGC predicted constraints based on VPD, soil drought and temperature (Figure 3), but VPD and soil drought only partially limit stomatal conductance, whereas subfreezing night temperatures close stomata for an extended period after temperature recovery (Running 1984). Without gas exchange, discrimination cannot occur, thus freezing temperatures should not influence isotope abundance as much as VPD and soil drought. Stepwise regression (for maximum adjusted R^2) of each stomatal constraint on $\delta^{13}C$ values standardized to branch length showed that temperature was not significant to the relationship, whereas both VPD and soil drought were.

Cavitation and refilling

Most sites experienced some permanent cavitation. The degree of cavitation increased with increasing climate stress across the transect (Figure 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 $\delta^{13}C$ in terminal foliage across the transect (Panek, unpublished data). Damage to the conducting xylem weakens the relationship between $\delta^{13}C$ and branch length. We speculate that in the harsher climates, given the same evaporative demands and soil drought status, as branches grow longer, xylem tension increases to a critical threshold at which point limited cavitation occurs, reducing k_h (see Equa-

tion 1) and causing the relationship between branch length and $\delta^{13}C$ to lessen. Values of $\delta^{13}C$, 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 was least negative at the site where trees suffered the most cavitation. Trees can protect themselves from reaching critical xylem tensions through stomatal regulation. Sperry (1995) suggests, however, that some trees might utilize limited cavitation at drought-prone sites as a mechanism to conserve soil water and 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 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 limit transpiration rates more than 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, it is unlikely that $\delta^{13}C$ values in foliage and new wood at Site C were affected by seasonal cavitation.

The ability of trees to refill cavitated elements is questionable, although some supporting evidence 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 of conifers, but not of other trees.

Widely recognized patterns of $\delta^{13}C$ variation in trees are consistent with branch length and cavitation effects. The juvenile effect, a pattern of more negative $\delta^{13}C$ values in the inner growth rings of trees than in the outer rings, has been attributed to both shading (Francey and Farquhar 1982) and 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 $\delta^{13}C$ 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, and the loss of functional sapwood to cavitation in more mature trees.

Pseudotsuga menziesii var. *glauca* exhibited very different patterns of $\delta^{13}C$ and cavitation than *P. menziesii* var. *menziesii*. The $\delta^{13}C$ 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 in detail the transition zone of these two

Douglas-fir varieties along the eastern end of this transect. 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* (B.J. Yoder, personal communication). 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 thus are less vulnerable to cavitation (Tyree and Sperry 1989). This may explain the different patterns observed between var. *glauca* and var. *menziesii*.

The $\delta^{13}C$ 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 $\delta^{13}C$ patterns indicate that leaves of shorter branches maintain higher internal CO_2 concentrations on average (Farquhar et al. 1982), and that these leaves have a lower water use efficiency 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 that support longer branches maintain 30% lower photosynthetic rates than younger trees in the same stand. Thus, accumulating evidence indicates that branch size plays a role in carbon uptake by conifers.

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 $\delta^{13}C$ variability, and normalizing for branch length improved the $\delta^{13}C$–climate relationship by 42% across all sites.

The importance of branch length on foliar $\delta^{13}C$ decreased as cavitation in the sapwood increased. Because tree ring carbon represents an integration of carbohydrates derived from different parts of the tree crown, we suggest that trees of similar shape and size be sampled when determining relationships 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.

References

Aagaard, J.E., S.S. Vollmer, F.C. Sorensen and S.H. Strauss. 1995. Mitochondrial DNA products among RAPD profiles are frequent and strongly differentiated among races of Douglas-fir. Mol. Ecol. In press.

Chalk, L. and J.M. Bigg. 1957. The distribution of moisture in the living stem of Sitka spruce and Douglas-fir. Forestry 29:5–21.

Cienciala, E., A. Lindroth, J. Èermák, J. Hällgren and J. Kuèera. 1994. The effects of water availability on transpiration, water potential and growth of *Picea abies* during a growing season. J. Hydrol. 155:57–71.

Dixon, M.A., J. Grace and M.T. Tyree. 1984. Concurrent measurements of stem density, leaf and stem water potential, stomatal conductance and cavitation on the sapling *Thuja occidentalis* L. Plant Cell Environ. 7:615–618.

Edwards, W.R.N., P.G. Jarvis, J. Grace and J.B. Moncrieff. 1994. Reversing cavitation in tracheids of *Pinus sylvestris* L. under negative water potentials. Plant Cell Environ. 17:389–397.

Ewers, F.W. and M.H. Zimmermann. 1984*a*. The hydraulic architecture of eastern hemlock (*Tsuga canadensis*). Can. J. Bot. 62:940–946.

Ewers, F.W. and M.H. Zimmermann. 1984*b*. The hydraulic architecture of balsam fir (*Abies balsamea*). Physiol. Plant. 60:453–458.

Farquhar, G.D., M.H. O'Leary and J.A. Berry. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentrations in leaves. Aust. J. Plant Physiol. 9:121–137.

Francey, R.J. and G.D. Farquhar. 1982. An explanation of $^{13}C/^{12}C$ variations in tree rings. Nature 297:28–31.

Freyer, H.D. and N. Belacy. 1983. $^{13}C/^{12}C$ Records in northern hemispheric trees during the past 500 years—anthropogenic impact and climatic superpositions. J. Geophys. Res. 88:6844–6852.

Gholz, H.L. 1982. Environmental limits on aboveground net primary production, leaf area, and biomass in vegetation zones of the Pacific Northwest. Ecology 63:469–481.

Glassy, J.M. and S.W. Running. 1994. Validating diurnal climatology logic of the MT-CLIM model across a climatic gradient in Oregon. Ecol. Appl. 4:248–257.

Grieu, P., J.M. Guehl and G. Aussenac. 1988. The effects of soil and atmospheric drought on photosynthesis and stomatal control of gas exchange in three coniferous species. Physiol. Plant. 73:97–104.

Holbrook, N.M. 1995. Stem water storage. *In* Plant Stems: Physiology and Functional Morphology. Ed. B.L. Gartner. Academic Press, San Diego, pp 151–174.

Jackson, P.C., F.C. Meinzer, G. Goldstein, N.M. Holbrook, J. Cavelier and F. Rada. 1993. Environmental and physiological influences on carbon isotope composition of gap and understory plants in a lowland tropical forest. *In* Ecological Aspects of Carbon Isotope Variation. Eds. J.R. Ehleringer, A.E. Hall and G.D. Farquhar. Academic Press, San Diego, CA, pp 131–140.

Jarvis, P.G. and K.G. McNaughton. 1986. Stomatal control of transpiration: scaling up from leaf to region. Adv. Ecol. Res. 15:1–49.

Krahmer, R.L. 1961. Anatomical features of permeable and refractory Douglas-fir. For. Prod. J., September, pp 439–441.

Leavitt, S. and A. Long. 1986. Stable-carbon isotope variability in tree foliage and wood. Ecology 67:1002–1010.

Leavitt, S. and A. Long. 1991. Seasonal stable-carbon isotope variability in tree rings: possible paleoenvironmental signals. Chem. Geol. 87:59–70.

Lipp, J., P. Trimborn, P. Fritz, H. Moder, B. Becker and B. Frenzel. 1991. Stable isotopes in tree ring cellulose and climatic change. Tellus 43B:322–330.

Livingston, N.J. and D.L. Spittlehouse. 1993. Carbon isotope fractionation in tree rings in relation to the growing season water balance. *In* Ecological Aspects of Carbon Isotope Variation. Eds. J.R. Ehleringer, A.E. Hall and G.D. Farquhar. Academic Press, San Diego, CA, pp 141–153.

Marshall, J.D. and J. Zhang. 1993. Altitudinal variation in carbon isotope discrimination by conifers. *In* Ecological Aspects of Carbon Isotope Variation. Eds. J.R. Ehleringer, A.E. Hall and G.D. Farquhar. Academic Press, San Diego, CA, pp 187–199.

Martin, B. and E.K. Sutherland. 1990. Air pollution in the past recorded in width and stable isotope composition of annual growth rings of Douglas-fir. Plant Cell Environ. 13:839–844.

Peterson, D.L. and R.H. Waring. 1994. Overview of the Oregon Transect Ecosystem Research project. Ecol. Appl. 4:211–225.

Running, S.W. 1976. Environmental control of leaf water conductance in conifers. Can. J. For. Res. 6:104–112.

Running, S.W. 1984. Documentation and preliminary validation of H2OTRANS and DAYTRANS, two models for predicting transpiration and water stress in western coniferous forests. USDA Forest Service, Rocky Mt. For. Range Exp. Stn., Fort Collins, CO, 25 p.

Running, S.W. 1994. Testing FOREST-BGC ecosystem process simulations across a climatic gradient in Oregon. Ecol. Appl. 4:238–247.

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

Runyon, J., R.H. Waring, S.N. Goward and J.M. Welles. 1994. Environmental limits on net primary production and light-use efficiency across the Oregon Transect. Ecol. Appl. 4:226–237.

Sobrado, M.A., J. Grace and P.G. Jarvis. 1992. The limits to xylem embolism recovery in *Pinus sylvestris* L. J. Exp. Bot. 43:831–836.

Sorensen, F.C. 1979. Provenance variation in *Pseudotsuga menziesii* seedlings from the var. *menziesii*–var. *glauca* transition zone in Oregon. Silvae Genet. 28:37–119.

Sperry, J.S. 1995. Limitations on stem water transport and their consequences. *In* Plant Stems: Physiology and Functional Morphology. Ed. B.L. Gartner. Academic Press, San Diego, pp 105–124.

Sperry, J.S., J.R. Donnelly and M.T. Tyree. 1988. Seasonal occurrence of xylem embolism in sugar maple *(Acer saccharum)*. Am. J. Bot. 75:1212–1218.

Sperry, J.S., N.M. Holbrook, M.H. Zimmermann and M.T. Tyree. 1987. Spring refilling of xylem vessels in wild grapevine. Plant Physiol. 83:414–417.

Sternberg, L., S.S. Mulkey and S.J. Wright. 1989. Ecological interpretation of leaf carbon isotope ratios: influence of respired carbon dioxide. Ecology 70:1317–1324.

Tans, P.P. and W.G. Mook. 1980. Past atmospheric CO_2 levels and the $^{13}C/^{12}C$ ratios in tree rings. Tellus 32B:268–283.

Tyree, M.T. and J.S. Sperry. 1989. Vulnerability of xylem to cavitation and embolism. Annu. Rev. Plant Physiol. Mol. Biol. 40:19–38.

Waring, R.H. and S.W. Running. 1978. Sapwood water storage: its contribution to transpiration and effect upon water conductance through the stems of old-growth Douglas-fir. Plant Cell Environ. 1:131–140.

Waring, R.H. and W.B. Silvester. 1994. Interpreting variation in carbon isotopes within tree crowns. Tree Physiol. 14:1203–1213.

Webb, W.L. and K.J. Kilpatrick. 1993. Starch content in Douglas-fir: diurnal and seasonal dynamics. For. Sci. 39:359–367.

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

Zimmermann, M.H. 1978. Hydraulic architecture of some diffuse-porous trees. Can. J. Bot. 56:286–2295.