

## GENETIC VARIATION IN SEEDLING WATER-USE EFFICIENCY AS ESTIMATED BY CARBON ISOTOPE RATIOS AND ITS RELATIONSHIP TO SAPLING GROWTH IN DOUGLAS-FIR<sup>1</sup>

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### ABSTRACT

Stable isotope ratios of carbon in plant tissues have been theoretically and empirically linked to water-use efficiency (WUE). Older genetic tests offer the opportunity to reconstruct relative seedling WUE using the ratio of <sup>13</sup>C to <sup>12</sup>C in juvenile growth rings. Variation in discrimination against <sup>13</sup>C can then be related to variation in mature tree growth. Genetic variation in carbon isotope ratios was assessed for varieties, populations within varieties, and families within populations of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and related to source environment and tree size. Earlywood was sampled from the fourth annual ring of 19-year-old trees from four populations, two from each variety (var. *menziesii* and var. *glauca*), growing in a common garden experiment. Coastal genotypes from wetter source environments consistently discriminated less against <sup>13</sup>C, and thus had higher WUE's, than did interior genotypes from drier source environments. The differences between the two varieties were small but significant ( $P < 0.05$ ). Similarly, within varieties, populations from the wetter source consistently discriminated more against <sup>13</sup>C, although the differences were small ( $0.10 < p < 0.05$ ). Larger trees exhibited less discrimination against <sup>13</sup>C (higher WUE), on average ( $r = 0.38$ ;  $p < 0.0001$ ). Average stable carbon isotope composition of populations was closely related to mean annual precipitation at the source ( $R^2 = 0.99$ ;  $p < 0.01$ ).

**Keywords:** *Pseudotsuga menziesii*, stable carbon isotope ratios, water-use efficiency

### INTRODUCTION

Over much of its range, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) occupies sites that receive little or no summer precipitation; consequently, it must be adapted to tolerate drought stress (FRANKLIN & WARING 1981). Climate change may increase the frequency, intensity and duration of drought in this region (e.g., FRANKLIN *et al.* 1991). Genetic variation in the ability to survive and grow under drought conditions has been documented both between the two major geographic races (the coastal var. *menziesii* and interior or Rocky Mountain var. *glauca*) (FERRELL & WOODWARD 1966; PHARIS & FERRELL 1966) and among sources within var. *menziesii* (WHITE 1987; JOLY *et al.* 1989). These studies have largely focussed on growth, survival, phenology and biomass allocation, with limited physiological assessments. One physiological component that may be important to the ability of plants to survive and grow under drought conditions is wa-

ter-use efficiency (WUE): the ratio of CO<sub>2</sub> assimilation to transpirational water loss.

WUE has most commonly been assessed by measuring leaf CO<sub>2</sub> and H<sub>2</sub>O gas exchange simultaneously on individual leaves or small groups of leaves. However, such instantaneous measurements may not adequately reflect the long-term WUE of whole plants. It is also difficult to screen a large number of individuals over a short period (e.g., for ranking genotypes in a breeding program) by this method. Stable isotope ratios of carbon offer a means of integrating WUE over time.

The atmospheric ratio of <sup>12</sup>C:<sup>13</sup>C is approximately 89:1 (EHLERINGER & RUNDEL 1989). This ratio is commonly expressed in  $\delta$  notation relative to a carbonate standard; for the atmosphere,  $\delta^{13}\text{C}$  averages  $-8$  per mil.  $\delta^{13}\text{C}$  is lower in tissues of C<sub>3</sub> plants, typically  $-25$  to  $-35$  per mil, primarily because of slightly slower diffusion rates for CO<sub>2</sub> containing the heavier isotope and discrimination against <sup>13</sup>C by ribulose biphosphate carboxylase oxygenase in photosynthesis (BERRY 1989). Carbon dioxide diffusion and carboxylation also

affect the ratio of intercellular to atmospheric partial pressures of CO<sub>2</sub> ( $C_i/C_a$ ); thus, isotope fractionation can be modelled as a function of  $C_i/C_a$  (e.g., FARQUHAR *et al.* 1989). Experimental evidence with several C3 plant species has demonstrated that <sup>13</sup>C discrimination is proportional to  $C_i/C_a$  (e.g., EHLERINGER *et al.* 1985; EVANS *et al.* 1986). The  $C_i/C_a$  ratio, in turn, is inversely correlated with 'intrinsic' water-use efficiency (WUE, mol H<sub>2</sub>O transpired per mol C fixed) (e.g., FARQUHAR *et al.* 1982). Variation in the leaf-to-air vapor pressure difference can cause differences in actual WUE for a given intrinsic WUE (e.g., FARQUHAR & RICHARDS 1984). However, at a constant vapor pressure deficit and atmospheric isotope ratio (or when all plants are exposed to the same fluctuations in environmental conditions), plants with higher WUE discriminate less against <sup>13</sup>C plants than do plants with lower WUE.

Little research has been conducted on genotypic variation in  $\delta^{13}\text{C}$  in conifers (FLANAGAN & JOHNSEN 1995; ZHANG *et al.* 1993; ZHANG *et al.* 1994). ZHANG and others (1993) found significant differences in discrimination of carbon isotopes among Douglas-fir genotypes grown in a common garden. In their study, most variation was within the interior (Rocky Mountain) variety. They did not find significant differences between the coastal and interior varieties; however, samples of the coastal variety came from relatively dry sites on the east side of the Cascade Mountains, considered to be in the transition zone between the two varieties (LI & ADAMS 1989). Interestingly, the slow-growing populations from the southern Rockies had the highest discrimination against <sup>13</sup>C (i.e., the lowest WUE), and there was a significant positive correlation between altitude of the seed source and WUE. The authors suggest that these relationships, which are opposite to those for plants grown and assessed *in situ* (MARSHALL & ZHANG 1993), confer homeostasis in gas-exchange physiology across altitudinal gradients.

If genetic differences exist in Douglas-fir for WUE as assessed by  $\delta^{13}\text{C}$ , and if they are predictive of the ability of trees to grow under water stress, then they could be useful for screening genotypes in breeding programs. In addition, knowledge of the genetic relationship, if any, between growth and  $\delta^{13}\text{C}$  will make it possible to evaluate the impact on WUE of selecting for growth rate alone.

Carbon isotope ratios in annual growth rings provide a permanent record of the early history of woody plants. In addition to discrimination during photosynthesis, the <sup>12</sup>C:<sup>13</sup>C composition of wood can be affected by shifts in the isotopic composition of atmospheric CO<sub>2</sub> or by the chemical composition of wood (lignin, for example, is about 3 per mil lighter than cellulose). However, if these sources of variation can be accounted for, the isotopic composition of carbon in

annual growth rings can provide an index of differences in WUE in the past. This may be a powerful tool for investigating relationships between growth and physiology of forest trees. Physiological investigations are generally restricted to seedlings, although it is the growth of mature trees that is of interest. Over the past several decades, many provenance trials have been installed with forest tree species; many of these are now reaching maturity and excellent records of tree growth have been compiled for them.

We have taken advantage of such a trial in this study, and extracted growth rings from the juvenile stages. By comparing the <sup>12</sup>C : <sup>13</sup>C composition of earlywood in the juvenile stage with growth of more mature trees, we sought to determine whether a relationship could be demonstrated between one aspect of juvenile physiology (seasonally-integrated WUE) and later growth. We conducted this study to address the following objectives: (1) Determine if there is genetic variation among varieties, populations and families of Douglas-fir for stable carbon isotope ratios; (2) assess the relationship between annual precipitation and stable carbon isotope ratios; and (3) assess the relationship between juvenile stable carbon isotope ratios and sapling growth.

## MATERIALS AND METHODS

In 1975, the open-pollinated progeny of 15 Douglas-fir parent trees (i.e., 15 open-pollinated families) were planted in a common garden test near Monmouth, Oregon. Eight of the families were from the interior (Rocky Mountain) variety of Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) in Idaho, and seven of the families were from the coastal variety (var. *menziesii*) in Oregon. Of the eight Idaho families, four came from Clarkia (latitude 47°00' N, longitude 116°15' W, elevation 1,160 m, average annual precipitation about 950 mm) and four came from Moscow (46°44' N, 117°00' W, 915 m, 600 mm annual precipitation). The seven Oregon families consisted of five from the vicinity of Lacombe, on the eastern edge of the Willamette Valley at the base of the Cascade Range (44°35' N, 123°38' W, 245 m, 1,400 mm annual precipitation), and two from Valsetz (44°35' N, 122°42' W, 365 m, 2,900 mm annual precipitation). All of the sources typically receive little late-spring and summer rainfall in their native environment. The test site, on the western edge of the Willamette Valley (44°40' N, 123°25' W, 125 m), receives moderate annual precipitation (1,150 mm) with little late-spring and summer rainfall.

The families were planted in five-tree row plots of each family in each of five replications. The seedlings were 2 years old at the time of planting (1+1 stock). Dead seedlings were replaced with seedlings of the

same families in 1976. All trees were measured for height in 1975 1979 1982 1988 and 1992, and for diameter in 1988 and 1992. By 1992 (at the age of 19), trees from coastal sources had outgrown those from the interior sources, causing light to be suppressed in the latter. Sampling earlywood rings formed at age 4, rather than foliage, allowed  $\delta^{13}\text{C}$  to be estimated for tissue formed prior to the onset of severe competition. In the winter of 1992–93, wood cores were sampled at approximately 30 cm above the ground from a maximum of three trees in each five-tree family row plot in three of the five replications. We collected samples from the same height and aspect on all trees to control for spatial variation in  $\delta^{13}\text{C}$  (LEAVITT & LONG 1986). Mortality in some plots resulted in fewer than three trees being sampled from some families in some replications. The number of trees sampled per family over all three replications ranged from 5 to 9.

Earlywood from the fourth annual growth ring was dissected from the wood core. This usually corresponded to the second growth ring from the center, but was verified for each core by counting rings from the bark. Individual earlywood samples were homogenized by grinding in liquid nitrogen, then dried at 70°C for 48 h. Samples were stored in glass vials and shipped to the University of Utah's Stable Isotope Ratio Facility for Ecological Research for analysis of isotopic ratios. The carbon in samples was converted to carbon dioxide, and the relative abundance of the isotopes  $^{13}\text{C}$  and  $^{12}\text{C}$  relative to a Peedee Belemnite (PDB) standard was determined in a mass spectrometer. The results were expressed in  $\delta^{13}\text{C}$  units:

$$\delta^{13}\text{C} = (R_{\text{sam}}/R_{\text{std}} - 1) \cdot 1000 \quad [1]$$

where  $R_{\text{sam}}$  and  $R_{\text{std}}$  are  $^{13}\text{C}/^{12}\text{C}$  ratios for the sample and standard, respectively.

Many investigations of carbon isotope ratios in plants utilize extracted cellulose rather than whole tissue, because, as mentioned earlier, the chemical composition of the tissue can affect the signal. In this study we measured  $\delta^{13}\text{C}$  of whole earlywood in preparation for large scale genetic trials, where cellulose extraction might not be realistic. We removed latewood from the samples to minimize variation in lignin: cellulose composition. In order to validate the use of whole tissue, we analyzed the relationship between  $\delta^{13}\text{C}$  of whole earlywood samples and extracted cellulose for a small subsample of the trees included in this in this study. Oils and resins were removed with a soxhlet procedure, then holocellulose was isolated with an acetic acid sodium chlorite treatment using methods first described by WISE *et al.* (1945). Both whole earlywood samples and extracted cellulose were ship-

ped to the Stable Isotope Facility at the University of Waikato, New Zealand for analysis.

### Analysis

Data were analyzed by the SAS (1985) General Linear Model procedure. The following model was used to assess genetic variation in growth and  $\delta^{13}\text{C}$ :

$$Y_{ijklm} = m + R_i + V_j + RV_{ij} + P(V)_{k(j)} + RP(V)_{ik(j)} + F(P(V))_{l(k(j))} + RF(P(V))_{il(k(j))} + e_{ijklm} \quad [2]$$

where  $m$  is the overall mean,  $R_i$  is the effect of the  $i^{\text{th}}$  replication,  $V_j$  is the effect of the  $j^{\text{th}}$  variety,  $RV_{ij}$  is the interaction effect between the  $i^{\text{th}}$  replication and the  $j^{\text{th}}$  variety,  $P(V)_{k(j)}$  is the effect of the  $k^{\text{th}}$  population in the  $i^{\text{th}}$  variety,  $RP(V)_{ik(j)}$  is the interaction effect between the  $i^{\text{th}}$  replication and the  $k^{\text{th}}$  population,  $F(P(V))_{l(k(j))}$  is the effect of the  $l^{\text{th}}$  family in the  $k^{\text{th}}$  population in the  $j^{\text{th}}$  variety,  $RF(P(V))_{il(k(j))}$  is the interaction effect between the  $i^{\text{th}}$  replication and the  $l^{\text{th}}$  family within the  $k^{\text{th}}$  population in the  $j^{\text{th}}$  variety, and  $e_{ijklm}$  is the residual. Relationships between tree size and  $\delta^{13}\text{C}$  were assessed by estimating Pearson correlation coefficients between these traits for individual trees (phenotypic), family means, and population means. The consistency of genotypic rankings for  $\delta^{13}\text{C}$  for whole earlywood and extracted cellulose was assessed by estimating the phenotypic correlation between the two.

### RESULTS

Genetic material from wetter sources consistently had lower discrimination against  $^{13}\text{C}$  (*i.e.*, greater or less negative values of  $\delta^{13}\text{C}$ ), and thus higher implied WUE's, than did materials from drier sources (Table 1). The differences between the two varieties were small but significant (Table 2): the coastal variety (*var. menziesii*) had an average  $\delta^{13}\text{C}$  of  $-24.94$  per mil (s.e. = 0.11), compared to  $-24.39$  (0.08) for the interior variety (*var. glauca*). This relationship also held for populations within varieties (Fig. 1 A). In the interior variety, families from the moister *Clarkia* source showed, on the average, less discrimination against  $^{13}\text{C}$  than did families from the drier, slower-growing *Moscow* source. Similarly, although represented by only two open-pollinated families, the *Valsetz* population, from an extremely wet source, on the average discriminated against  $^{13}\text{C}$  less than did the *Lacomb* source.

There was considerable variation for  $\delta^{13}\text{C}$  within all populations. As a result of this within-population variation, mean annual precipitation for sources was only weakly predictive of  $\delta^{13}\text{C}$  values for individual trees (adjusted  $R^2 = 0.139$ ,  $p < 0.01$ ). However, mean  $\delta^{13}\text{C}$  for sources was strongly and linearly related to

**Table 1** Population means and family ranges for  $\delta^{13}\text{C}$  and for height 6 and 19 years after planting

Variety Population	$\delta^{13}\text{C}$ (per mil) mean (family range)	6-year height (m) mean (family range)	19-year height (m) mean (family range)
<i>var. glauca</i>			
Moscow, ID	-25.05 (-24.89 - -25.09)	1.14 (1.05-1.21)	7.29 (6.68-7.67)
Clarkia, ID	-24.88 (-24.66 - -25.03)	1.14 (1.05-1.21)	8.82 (8.43-9.34)
<i>var. menziesii</i>			
Lacomb, OR	-24.66 (-24.14 - -25.12)	1.97 (1.79-2.13)	14.73 (14.13-15.13)
Valsetz, OR	-24.11 (-23.50 - -24.27)	2.02 (1.90-2.14)	14.68 (14.67-14.69)

**Table 2** Results of an analysis of variance of  $\delta^{13}\text{C}$ , 6-year height, and 19-year height, with F-statistics for sources of variation and associated significance levels

Source of variation	Degrees of freedom	F-value		
		$\delta^{13}\text{C}$	Height 6	Height 19
Replication	2	8.02 ***	2.12 <sup>ns</sup>	8.71 ***
Variety	1	49.35 *	50.50 *	621.19 **
Replication $\times$ variety	2	0.36 <sup>ns</sup>	2.31 <sup>ns</sup>	2.34 <sup>ns</sup>
Population (variety)	2	5.28 +	5.93 +	10.06 *
Replication $\times$ population (variety)	3	0.87 <sup>ns</sup>	1.05 <sup>ns</sup>	1.93 <sup>ns</sup>
Family (population (variety))	11	0.92 <sup>ns</sup>	1.06 <sup>ns</sup>	0.84 <sup>ns</sup>
Rep. $\times$ family (population (variety))	22	1.63 +	0.88 <sup>ns</sup>	1.70 *
Error	71			

1) significance level associated with F-statistics: ns – non-significant, +  $P < 0.10$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

mean annual precipitation ( $R^2 = 0.995$ ,  $p < 0.01$ ). The relationships between source elevation and  $^{13}\text{C}$  discrimination were not clear. Across all four sources, the phenotypic (individual-tree) relationship was negative but very weak (higher-elevation populations showed more discrimination; adjusted  $R^2 = 0.060$ ,  $p < 0.01$ , Fig. 1 B). Within each of the varieties, however, the higher-elevation population showed less discrimination (higher WUE) than did the lower-elevation population.

Family means for  $^{13}\text{C}$  discrimination varied more for the coastal variety (-25.12 to -23.5 per mil) than for the interior variety (-25.09 to -24.66 per mil). The differences in tree size between varieties were large and significant: by age 19, the coastal families averaged almost twice the height of the interior families. There were significant ( $p < 0.05$ ) differences in tree size between populations within the interior variety, with families from the drier Moscow area significantly shorter than those from the moister Clarkia area at age 19.

The average  $\delta^{13}\text{C}$  value for whole wood and extracted cellulose differed considerably, with means of -24.99 and -22.79 per mil, respectively. However, a high Pearson correlation coefficient indicates the stability of rankings of individual trees for  $\delta^{13}\text{C}$  was quite strong

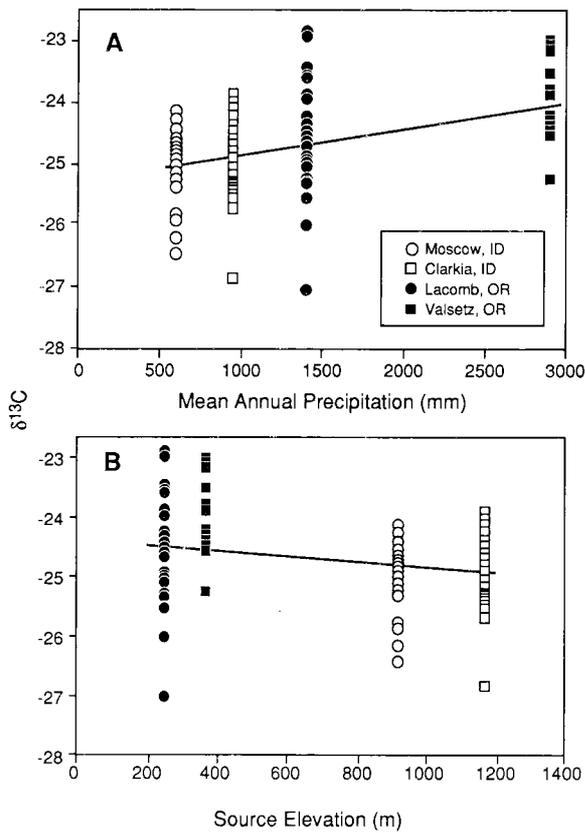
between the two methods ( $r = 0.89$ ;  $p < 0.01$ ), despite the small sample size.

The analysis of variance (Table 2) revealed significant differences ( $p < 0.05$ ) between varieties, and weaker ( $0.10 > p > 0.05$ ) differences among populations within varieties, for  $\delta^{13}\text{C}$ . Differences among families within a population for  $\delta^{13}\text{C}$  were not significant. These results were similar to those for height at 6 and 19 years, with strong differences between varieties in average size and smaller differences among populations within varieties.

The phenotypic relationship between  $^{13}\text{C}$  discrimination and tree size was significant (6-year height,  $r = 0.35$ ,  $p < 0.0001$ ; 19-year height,  $r = 0.38$ ,  $p = 0.0001$ ; Fig. 2B) with larger trees exhibited less discrimination against  $^{13}\text{C}$  (higher WUE), on the average. This phenotypic relationship was similar within both the Lacomb population ( $r = 0.39$ ,  $p < 0.01$ ) and the Moscow population ( $r = 0.38$ ,  $0.05 < p < 0.10$ ). Within both the Clarkia and Valsetz populations, the phenotypic correlations between growth and  $\delta^{13}\text{C}$  were not significant.

## DISCUSSION

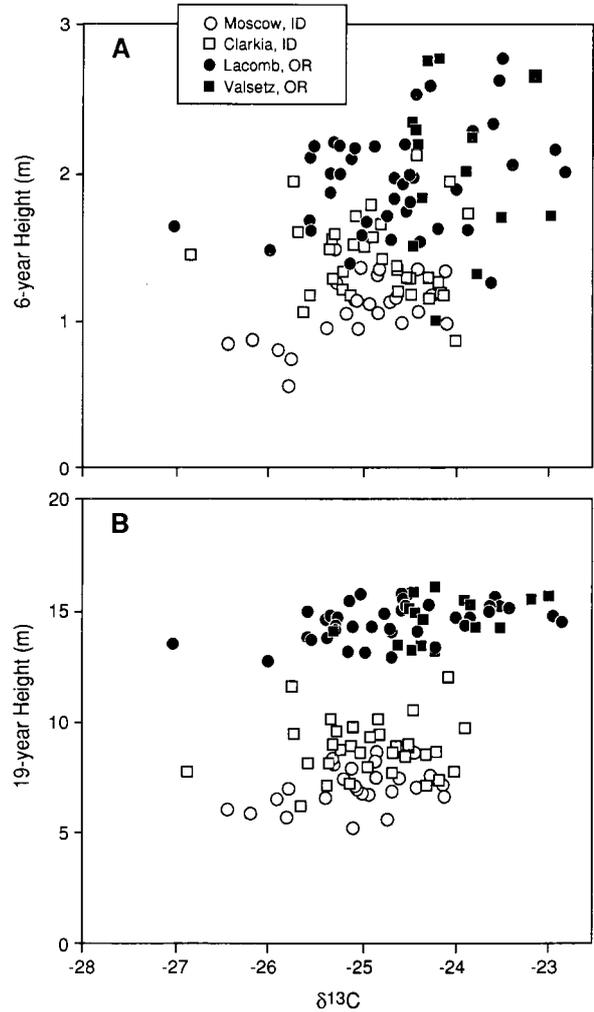
If plants are under selective pressure for WUE in arid environments (COHEN 1970; FISCHER & TURNER 1978;



**Figure 1** Relationships between stable carbon isotope ratios and seed source environments. Higher (less negative) values for  $\delta^{13}\text{C}$  indicate less discrimination against  $^{13}\text{C}$  and higher water-use efficiency (see text for details). (A) Mean annual precipitation of source environments versus  $\delta^{13}\text{C}$  of individual trees in a common garden test near Monmouth, Oregon (mean annual precipitation 1,150 mm). (B) Source elevation versus  $\delta^{13}\text{C}$  of individual trees in a common environment.

PASSIOURA 1982), isotope discrimination in plants adapted to arid environments should be lower than in plants adapted to wet environments when grown in a common garden. Also, if WUE is at the expense of growth (e.g., ORIAN & SOLBRIG 1977), a positive relationship is expected between discrimination against  $^{13}\text{C}$  and growth. However, these hypotheses were not supported in this study. Similarly, DELUCIA and SCHLESINGER (1991) found that drought-tolerant trees and shrubs (i.e., those able to maintain positive photosynthesis at low water potentials) had low WUE (high discrimination against  $^{13}\text{C}$ ) compared with less tolerant plants.

The theoretical basis for carbon isotope discrimination during photosynthesis of  $\text{C}_3$  plants is well established. As long as all photosynthesizing leaves have source air with the same isotopic composition and vapor pressure, the theory predicts a linear relationship



**Figure 2** Phenotypic relationships between stable carbon isotope ratios and tree size. (A)  $\delta^{13}\text{C}$  versus 6-year height. (B)  $\delta^{13}\text{C}$  versus 19-year height. Plots are for individual trees in all populations.

between  $\delta^{13}\text{C}$  and photosynthetic WUE. It is highly unlikely that there were significant variations among genotypes in source air at any point during this study. Douglas-fir needles have a high boundary conductance, and the spacing and stature of trees should promote enough turbulence to keep the air well mixed. However, we cannot rule out the possibility that differences in phenology among the genotypes led to differences in the atmosphere during periods of peak photosynthetic activity.

While differences in either stomatal conductance or in the enzymatic capacity for photosynthesis could result in variation in stable carbon isotope ratios, differences in stomatal behavior are a likely cause of the observed genetic differences in  $\delta^{13}\text{C}$  in Douglas-fir. CONDON *et al.* (1987) showed that stomatal variation was the cause of variation in  $\delta^{13}\text{C}$  for genotypic differences in wheat. In another study containing similar seed

sources to those in the current study, we found that stomatal conductance of two-year old seedlings of a wet coastal source of Douglas-fir was much more variable over a diurnal course than interior-dry populations in a nursery experiment (YODER *et al.*, unpublished data 1994). This change in stomatal conductance resulted in significant diurnal variation in internal CO<sub>2</sub> concentration in the coastal population, whereas little change in internal CO<sub>2</sub> occurred in the interior populations through a dry day. Over the same diurnal course, coastal sources had similar maximum photosynthetic rates (as measured with a LiCor 6200: see YODER & WARING 1994) to interior sources, thus it is unlikely that there was a significant difference in enzymatic capacity for photosynthesis among the sources.

The relative differences between the environment of the experimental planting site and the source environments offer one explanation for our results. The annual precipitation of the planting site at Monmouth, Oregon, is intermediate between the Oregon and Idaho sources. These precipitation patterns may be indicative of overall differences in the "droughtiness" of these sites (where "droughtiness" is a combination of soil moisture and atmospheric humidity), although we recognize that both seasonal distribution of rainfall and soil characteristics such as soil depth, texture, and organic matter content can greatly affect available soil water and thus droughtiness. Trees of coastal origin may be adapted to a more mesic environment, and respond to the Monmouth site with more conservative water use, whereas the inland sources may respond to the same environment as though it were moist. For example, the coastal trees may initiate stomatal closure at a relatively high vapor pressure or soil water potential as compared with trees from interior sources. Possible differences in biomass allocation to roots and shoots, root structure, hydraulic architecture, shoot or cambial phenology, and leaf morphology could all affect the way stomata and ribulose biphosphate carboxylase oxygenase activity respond to the availability of moisture in the air and soil.

ZHANG *et al.* (1993) observed a positive relationship between source elevation and  $\delta^{13}\text{C}$  in a common garden test. We observed this trend between populations within each of the varieties, but it did not hold across varieties. For this limited sample of populations, the relationship between mean annual precipitation and  $\delta^{13}\text{C}$  was stronger than that between source elevation and  $\delta^{13}\text{C}$ .

Quantitative genetic variation in  $\delta^{13}\text{C}$  has also been observed genotypes of many crop species, including wheat, barley, bean and peanut genotypes (ACEVEDO 1993; FARQUHAR *et al.* 1989; FARQUHAR & RICHARDS 1984; EHDAIE *et al.* 1993; WHITE 1993). For some species, samples collected from tissue produced early in the growth season exhibit higher heritabilities for  $\delta^{13}\text{C}$

than do those collected later in the year, possibly because of greater environmental variation under conditions of limited water availability (RICHARDS & CONDON 1993). Genotype  $\times$  environment interaction for  $\delta^{13}\text{C}$  is usually low or non-significant when plants are grown under normal field conditions; however, in more contrasting environments, interaction can be considerable.

The relationship between  $\delta^{13}\text{C}$  (and therefore WUE) and growth may be positive or negative, depending on whether variation in  $\delta^{13}\text{C}$  is a function primarily of differences in photosynthetic capacity, stomatal conductance, or both (HUBICK *et al.* 1986; VIRGONA *et al.* 1990). If variation in WUE is primarily a function of stomatal conductance, in an environment where soil moisture is not severely limited, high-WUE genotypes may have increased stomatal control, resulting in lower CO<sub>2</sub> fluxes through stomata, lower intercellular partial pressures of CO<sub>2</sub>, and less net photosynthesis. However, if genetic variation in WUE is determined primarily by photosynthetic rates, then genotypes with high photosynthetic rates will have higher WUE's.

The empirical relationship between  $\delta^{13}\text{C}$  and biomass in field studies in water-limited environments has been inconsistent and difficult to interpret (RICHARDS & CONDON 1993). There are reports of positive correlations between isotope discrimination and growth or yield (*e.g.*, CONDON *et al.* 1987; FARRIS & LECHOWICZ 1990; JOHNSON *et al.* 1990; EHDAIE *et al.* 1991; WHITE 1993; ACEVEDO 1993; DONOVAN & EHLERINGER 1994), negative correlations (*e.g.*, HUBICK *et al.* 1986; CONDON *et al.* 1990; WRIGHT *et al.* 1993; DONOVAN & EHLERINGER 1994; FLANAGAN & JOHNSEN 1995), or no correlation at all (*e.g.*, DONOVAN & EHLERINGER 1994; FLANAGAN & JOHNSEN 1995). The relationship has also been found to be environment-specific (DONOVAN & EHLERINGER 1994; FLANAGAN & JOHNSEN 1995). The positive relationship we found between tree growth and WUE was surprising because rapid growth is often considered to be wasteful of critical resources such as water (GRIME 1979). One possible explanation is that faster-growing individuals may carry a larger proportion of leaf area in the form of juvenile needles, which probably have a different WUE than do mature needles.

It may be that WUE plays a minor role in drought tolerance in Douglas-fir. Several mechanisms contributing to drought tolerance have been documented for this species, including growth phenology and biomass allocation to roots (WHITE 1987; JOLY *et al.* 1989). Crop species have shown a positive correlation between time of maturation and WUE as measured by  $\delta^{13}\text{C}$ , possibly because early-flowering genotypes complete flowering prior to the onset of drought and thus are not at a selective advantage with higher WUE (HALL *et al.*

1993; RICHARDS & CONDON 1993). In the same manner, drought-avoidance mechanisms such as shoot and cambial phenology may play a more important role than does WUE in drought tolerance in Douglas-fir. However, the fact that more variation exists for  $\delta^{13}\text{C}$  in the coastal variety than in the interior variety supports the theory that WUE has been under greater selective pressure in the latter than in the former.

Most current studies of stable carbon isotope ratios of wood conduct analyses on extracted cellulose, rather than on whole wood samples, as further discrimination against  $^{13}\text{C}$  can occur in plant metabolism after photosynthesis. While absolute values of whole wood and extracted cellulose  $\delta^{13}\text{C}$  differ significantly, the whole earlywood samples appeared quite reliable for the purpose of ranking the relative discrimination levels of different Douglas-fir genotypes. Similarly, a regression of cellulose  $\delta^{13}\text{C}$  against whole wood  $\delta^{13}\text{C}$  of *Pinus contorta* genotypes showed a very strong relationship between the two ( $R^2 = 0.98$ ; HOLOWACHUK 1993). Whole wood samples containing latewood may be less reliable than earlywood samples for estimating  $\delta^{13}\text{C}$  due to the higher lignin content of latewood. The procedure for extracting cellulose from wood samples is labor intensive and time consuming, and approximately doubles the cost of assessing  $\delta^{13}\text{C}$  in wood samples. If this step can be avoided, it greatly increases the potential applicability of  $\delta^{13}\text{C}$  assessment in tree improvement programs if WUE is found to be an important trait.

While significant genetic variation exists for WUE as estimated by  $\delta^{13}\text{C}$ , differences among populations of Douglas-fir are not easy to understand or interpret. Stable isotope ratios of carbon will require further investigation if forest researchers are to understand the role that WUE plays in trees' ability to survive and grow in water-limited environments, as well as the utility of these ratios for tree improvement programs.

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