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Differences in physiology and growth between coastal and inland varieties of Douglas-fir seedlings in a common garden☆

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Abstract

In a common garden study, seedlings of *Pseudotsuga menziesii* (Douglas-fir) var. *menziesii* (coastal) from Lacomb, Oregon and *P. menzeisii* var. *glauca* (interior) from Clearwater National Forest, Idaho, and their F_2 hybrids were grown in nursery beds in the coastal climate near Corvallis, OR. The coastal variety was from an elevation of 245 m with a mean annual rainfall of 1400 mm. The interior variety was from an elevation of 871 m and a mean annual rainfall of 600 mm. Height, stem diameter, and bud burst percent were determined. Metabolic heat rate and respiration rate were measured on apical meristems at 30, 35, and 40 °C. Similar tissue was dried, ground, combusted, and analyzed for carbon isotope ratios. The two varieties differed from one another in growth traits, bud burst, carbon isotope ratios, and respiration traits. The F_2 hybrid progeny of the varieties had isotope ratios similar to the interior variety, but respiration traits of the hybrids were similar to the coastal variety. Respiratory heat rate and height growth were the only significant trait differences found between families within varieties. The faster growing coastal variety showed less carbon isotope discrimination relative to the slower growing and more stressed (when grown at Corvallis) interior variety.

Keywords: Adaptation; Calorespirometry; Carbon isotopes; Douglas-fir; *Pseudotsuga menziesii*; Temperature

1. Introduction

The Cascade mountain range in Oregon causes air masses moving inland from the Pacific and Gulf of Alaska to deposit much of their moisture on the western slopes and valleys, giving this region a wet, mild climate with little temperature variation. East of the mountains there is less rainfall and the temperature is much more variable and continental. Douglas-fir (*Pseudotsuga menzeisii*) is an important species in forestry for timber production in these and other regions. *P. menzeisii* var. *menzeisii* grows rapidly and attains the greatest size in the moist Pacific coastal ranges where it is known as the coastal variety. *P. menzeisii* var. *glauca*, known as the inland variety, grows on the eastern slope of the Cascades and extends into the Rocky Mountains of Idaho where the climate is much drier and has more variable temperatures [1].

Direct measurements of plant growth rates in terms of volume, length, net photosynthate, etc. provide little information concerning the mechanism of adaptation of metabolism to an environment. To derive the mechanism, metabolic properties such as respiration rate and efficiency must be measured as functions of environmental variables [2]. Respiratory responses to temperature have been measured for different populations of the shrubs *Eurotia lanata* [3] and *Artemesia tridentata* [4], the grass *Bromus tectorum* [5], and tree species such as redwood (*Sequoia*) [6], *Larix laricinia* [7], *Pinus ponderosa* [8,9], and *Eucalyptus globulus* [10]. Respiratory, and thence growth, rate responses to tempera-

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Table 1
Plant material and origin

Variety	Family	Elevation (m)	Mean annual rainfall (mm)
Coastal	C1: Lacomb-1 × Lacomb-10, a control pollinated family from Lacomb, Oregon	245	1400
	C2: Lacomb-3 × Lacomb-11, a control pollinated family from Lacomb, Oregon	245	1400
Interior	l1: Open-pollinated from dry Idaho location on the Clearwater National Forest, Idaho	871	600
	l2: Open-pollinated from dry Idaho location on the Clearwater National Forest, Idaho	871	600
Hybrid	A × B: (Idaho Dry-10 × Lacomb-12) × (Idaho Wet-18 × Lacomb-11); C × D: (Idaho Dry-3 × Lacomb-10) × (Idaho Dry-10 × Lacomb-12)		

ture among populations and subpopulations appear to match the temperature profile of the native environment during the growth season so as to maximize growth when water is available [5]. In this study, respiratory traits were measured at 30, 35, and 40 °C because the varieties were expected to differ most in the upper temperature extremes as do the native climates.

For Douglas-fir, genetic variation in drought hardiness [11] has been noted for seed from different sites. Zhang and Marshall [12] studied 25 populations of Douglas-fir representing coastal, northern interior, and southern interior populations. Populations differed significantly in specific leaf area, photosynthetic rate, stomatal conductance and carbon isotope fractionation when grown in a common garden. Aitken et al. [13] studied two populations each from two Douglas-fir varieties (var. *menziesii* and var. *glauca*) growing at their native sites. Genotypes from coastal populations had a smaller carbon isotope fractionation, and thus appeared to be less stressed.

This study tests the hypothesis that the two varieties of Douglas-fir, when grown in a common garden, retain adaptive differences in growth and metabolism reflective of the site of origin of the seed. Also, inheritance of growth and respiratory traits as well as photosynthetic traits reflected in carbon isotope discrimination were examined in F_2 hybrid progeny.

2. Materials and methods

2.1. Plant materials

Of the coastal variety (i.e. var. *menziesii*), the U.S. Forest Service Pacific Northwest study included two control pollinated families from Lacomb, Oregon. Of the Rocky Mountain variety (i.e. var. *glauca*) two open-pollinated families were collected from "dry" Idaho locations in the Clearwater National Forest. In addition, four F_2 hybrid families between these varieties, four coastal backcross families, and four interior backcross families, for a total of 16 families, were included in the USFS study.

Plant material used in this study was selected from the larger USFS study and includes the four control families and two of the four F_2 hybrid families (Table 1). Although this sample is statistically marginal in representing both the varieties and families, the families selected for this study were chosen because they were expected to reveal maximum differences in growth, morphological and phenological traits. They are thus also expected to be the most likely to show significant adaptive differences in carbon isotope discrimination and respiratory metabolism.

In May 1995, stratified seeds were sown into a raised nursery bed (18 m long × 1.2 m wide × 1 m deep), filled with a sandy loam soil, at the Forestry Sciences Laboratory on the Oregon State University campus in Corvallis. The experimental design was a split-plot with three replications. Each replication consisted of four randomized main plots and five sub-plots randomized within each main plot. One group of three crosses on one F_1, seed parent (F_2 + coastal backcross + interior backcross) plus 1 each coastal and interior control group for a total of five families were planted in the sub-plots. Each sub-plot contained two rows of 12 seedlings (24 seedlings total), each with one border tree on each side, or 14 seedlings across a row, with a spacing of 12 cm between rows and 10.8 cm between seedlings within rows.

In early February 1997, a majority of the seedlings were lifted for destructive measurement of biomass and other traits for the USFS study. The trees left in the bed included the six families used in this study and four additional families representing the backcrosses. The lifting of rows and additional thinning within rows was done carefully to minimize the effects of irregular spacing between rows within replications. Finally, each replication contained two rows of four-tree subplots for each of the ten families that were allowed to grow for the following three additional years for a total of 5 years after sowing (i.e. until 2000).

Buds breaking dormancy, called bud break or bud burst, was determined by counting the number of buds breaking dormancy on 6 May in comparison with bud burst on 13 May. The numbers were expressed as percent of the total number of buds at each date.

Growth traits, measured as shoot height and diameter at the stem base, were determined at regular intervals.

2.2. Calorimetric measurements

Metabolic measurements were made at three times, in triplicate, on 12 seedlings from each of the six families included in this part of the study during the months of May and early June 2000. On each date, approximately 3–5 cm long, actively growing second-order lateral shoot-apices were collected from the upper whorls of the 72 trees. The tissues were stored at approximately 5 °C until calorimetric measurements were made, usually with a few hours. Metabolic experiments were conducted with an MC-DSC calorimeter (Calorimetry Sciences Corporation, Pleasant Grove, UT, USA, model 4100), following the methods of Criddle and Hansen [2,14]. Approximately 1 cm long sections of the apical meristem, with subtending developing stem and needles, were placed in the calorimeter ampoules. Metabolic heat rate (R_q) was measured first, then the CO_2 rate was measured by adding a 40 μL vial of 0.4 M NaOH to the ampoule, after which the vial was removed and the R_q measurement repeated. Measurements were made at 30, 35, and then 40 °C. CO_2 produced by the respiring tissues was absorbed by the NaOH to produce carbonate and additional heat ($-108.5\ kJ\ mol^{-1}$) at a rate proportional to the CO_2 production rate.

2.3. Carbon isotope ratio measurements

In August 2000, similar samples were collected and dried overnight in a vacuum oven at 65 °C, and ground to a powder. Ten milligrams of the dried and powdered material was combusted at 800 °C in an evacuated, sealed quartz tube with copper oxide as the oxygen source. Carbon isotope ratios ($^{13}C/^{12}C$) of the CO_2 in the tube were measured with a Finnegan Delta Plus isotope-ratio mass spectrometer. Ratios were expressed in parts per thousand (‰) relative to the PeeDee Belemnite (PDB) standard [15]. Carbon from stressed plants has more negative $\delta\ ^{13}C$ values than carbon from less stressed plants.

2.4. Statistical analysis

Statistical analyses were done with SAS [16] using the general linear models function and Duncan's multiple range tests.

3. Results and discussion

For this study, Douglas-fir seedlings were raised in a common garden near Corvallis, OR. The seeds came from two populations each of the coastal and interior varieties and F_2 hybrids between them (Table 1). Traits measured in this study are listed with their units of measurement in Table 2. Respiration, growth, time of bud burst, and carbon isotope discrimination were chosen as traits likely to be sensitive to interactions between environment and genetic adaptation to a given locality. While the two varieties differed the most in growth traits (height and diameter), respiratory heat rates at all three temperatures, carbon isotope discrimination, and bud burst also showed differences between varieties (Table 3, third column). Families within the varieties (Table 3, fifth column) differed only for metabolic heat rates measured at 30 and 35 °C and height, suggesting that even small sample sizes were able to detect genetic differences in these traits. Replication-by-variety and replication-by-family was largely absent for all measurements indicating reproducibility among plants of the same parentage.

Table 2
Description of traits used in this study

Traits	Symbols used for statistical analysis[a]
Carbon isotope discrimination (‰)	$\delta\ ^{13}C$
Metabolic heat rate at 30 °C ($W\ mg^{-1}$ dry wt)	Q30
Metabolic heat rate at 35 °C ($W\ mg^{-1}$ dry wt)	Q35
Metabolic heat rate at 40 °C ($W\ mg^{-1}$ dry wt)	Q40
CO_2 production rate at 30 °C ($pmol\ s^{-1}\ mg^{-1}$ dry wt)	C30
CO_2 production rate at 35 °C ($pmol\ s^{-1}\ mg^{-1}$ dry wt)	C35
CO_2 production rate at 40 °C ($pmol\ s^{-1}\ mg^{-1}$ dry wt)	C40
Height (cm)	HT
Diameter (mm)	DIA
Bud burst percent on 6 May	BB1
Bud burst percent on 13 May	BB2

[a] See Tables 3–5.

In Table 4, means of the two varieties and the F_2 hybrid progeny are compared. For $\delta\ ^{13}C$, the F_2 hybrid progeny was similar to the interior variety. Both the hybrid and interior variety had significantly more negative values than the coastal variety. When means of respiratory traits (i.e. heat and CO_2 rates) differed, the progeny was similar to the coastal variety. These results suggest the genes controlling photosynthesis came from the interior variety, but genes controlling respiration in the F_2 hybrid progeny came from the coastal variety. For growth traits (i.e. height and diameter), the progeny was intermediate between the varieties and for bud burst, the progeny was more similar to the coastal than to the interior variety.

Because of the high correlation between metabolic heat and CO_2 rates measured at different temperatures, Q30 and C30 were chosen to represent these traits in the correlation analyses. Similarly, HT and BB1 were chosen to represent growth and bud burst traits. Correlation was done at three levels: individual tree, replication means and family means. Results are given in Table 5. At individual and replication mean level, correlations between $\delta\ ^{13}C$ and respiration

Table 3
Sources of variation (degrees of freedom) and their *F*-values for carbon isotope discrimination, respiration, growth, and bud phenology traits (see Table 2) of two Douglas-fir varieties and their hybrid progeny

	Replication (2)	Variety (2)	Replication × variety (4)	Family (variety) (3)	Replication × family (variety) (6)
δ ^{13}C	10.77*	4.23+	1.08	0.02	2.06+
Q30	8.77*	3.29+	0.66	3.72+	1.99
Q35	13.93*	8.16*	0.44	4.21+	1.74
Q40	25.15**	10.61*	0.47	2.45	0.71
C30	7.18*	1.65	0.62	1.18	1.31
C35	12.59*	4.64	0.68	0.49	1.32
C40	2.95	0.11	0.44	0.28	1.78
HT	9.83*	194.1***	0.38	3.98+	2.10
DIA	18.20**	206.7***	0.28	0.61	1.50
BB1	4.54+	6.25+	0.50	1.13	0.95
BB2	0.13	4.32+	0.81	0.40	1.32

Values in parenthesis indicate trait.
+ Significance of *F*-value, $P < 0.10$.
* Significance of *F*-value, $P < 0.05$.
** Significance of *F*-value, $P < 0.01$.
*** Significance of *F*-value, $P < 0.001$.

Table 4
Estimated means (ranges) of metabolic and growth traits (see Table 2) of two varieties of Douglas-fir and their F_2 progeny and results of Duncan's multiple range tests on the means[a]

Traits	Coastal variety	F_2 progeny	Interior variety
δ ^{13}C	−27.43 A (−29.99 to −23.79)	−29.10 B (−33.13 to −24.99)	−29.10 B (−33.62 to −25.14)
Q30	18.58 B (12.29–24.00)	18.55 B (10.16–33.68)	22.21 B (14.06–46.93)
Q35	21.19 B (13.26–26.54)	21.43 B (12.65–31.92)	25.70 A (15.26–49.24)
Q40	22.09 B (14.18–31.22)	21.39 B (14.35–30.13)	25.09 A (13.73–42.90)
C30	47.56 A (13.86–71.37)	44.91 A (19.67–76.61)	53.11 A (13.17–103.0)
C35	38.55 B (10.52–80.07)	39.97 B (06.85–72.26)	51.06 A (22.31–88.31)
C40	17.41 A (00.00–39.08)	18.44 A (00.00–37.20)	18.87 A (00.00–48.90)
HT	204.3 A (167.0–270.0)	144.7 B (69.00–185.0)	106.0 C (76.00–150.0)
DIA	37.58 A (26.00–52.00)	26.75 B (18.00–32.00)	23.96 C (20.00–34.00)
BB1	31.42 B (00.00–100.0)	52.29 A (00.00–100.0)	27.63 C (00.00–98.00)
BB2	75.08 A (10.00–100.0)	82.21 A (02.00–100.0)	53.50 B (00.00–100.0)

[a] For a given trait, mean values differ significantly ($P < 0.10$) among the varieties and F_2 progeny when letters against means are different.

Table 5
Estimated correlationsa between paired traits on the basis of individual trees, replication means, and family means when two varieties and their hybrid are considered together

Paired traits		Individual tree (N = 66–72)	Replication mean (N = 18)	Family mean (N = 6)
δ ^{13}C	Q30	0.29*	0.25	−0.32
	C30	0.32**	0.52*	−0.15
	HT	0.35**	0.37+	0.88*
	BB1	−0.24*	−0.37+	−0.31
Q30	C30	0.59***	0.77***	0.94**
	HT	−0.17	−0.35	−0.43
	BB1	−0.32**	−0.43+	−0.47
C30	HT	−0.09	−0.18	−0.32
	BB1	−0.27*	−0.32	−0.41
HT	BB1	−0.27*	0.09	−0.02

+ Significance of correlation, $P < 0.10$.
* Significance of correlation, $P < 0.05$.
** Significance of correlation, $P < 0.01$.
*** Significance of correlation, $P < 0.001$.

(Q30, C30) were generally low and positive, i.e., trees that showed the highest respiration rates had the greatest isotope fractionation. However, this relationship did not hold at the family mean level. A consistent and significant relationship between isotope fractionation and height was found across all levels, supporting previous findings [13] that the faster growing coastal variety showed less carbon isotope discrimination than the slower growing interior variety. Specific heat rate at 30 °C (i.e. Q30) is, of course, highly correlated with the CO_2 rate at 30 °C (i.e. C30). Q30, C30, and height (HT) were correlated with bud burst at the individual tree level, but only Q30 was correlated with the replication mean bud burst. No significant correlation was found with family means.

4. Conclusions

Coastal and interior varieties of Douglas-fir were grown in a common garden in a coastal environment. The two varieties differed significantly in growth traits (height and diameter), respiration traits (heat rate), carbon isotope discrimination, and bud burst. Families within the varieties differed significantly in metabolic heat rate and height, suggesting that even small sample sizes were able to detect genetic differences in these traits. The faster growing coastal variety showed less carbon isotope discrimination than the slower growing interior variety. F_2 hybrid progeny from the two varieties were similar to the interior variety in isotope ratios, but similar to the coastal variety for respiration traits. The results show that the Douglas-fir varieties do not acclimate to the change from the interior to a coastal climate, but retain the metabolic traits that are genetically adapted to their native climates. F_2 hybrid progeny between the two varieties are intermediate in growth traits, but have the photosynthetic traits of the interior variety and the respiratory traits of the coastal variety.

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