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Stress and respiration traits differ among four geographically distinct *Pinus ponderosa* seed sources☆

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Abstract

This study tested the hypotheses that plant respiration and photosynthesis are genetically adapted to the native climate and that plant metabolism has little ability to acclimate to a non-native climate. Seeds from four geographically distinct seed sources (Oregon, Willamette and Deschutes and California, Mendocino and Eldorado) were used to grow 2-year-old seedlings of ponderosa pine (*Pinus ponderosa* var. *ponderosa*) in a common garden near Corvallis, Oregon. Respiratory heat and CO_2 rates were measured on elongating shoot tips at five temperatures from 15 to 35 °C. Heat and CO_2 rates did not differ significantly among the four seed sources when compared at a given temperature. Arrhenius temperature coefficients of heat and CO_2 rates of the Deschutes plants were significantly greater than those of the other three sources. Deschutes has the coldest, driest and most variable climate of the four sources. Carbon isotope ratios obtained on the same samples showed greater fractionation in the Mendocino plants than in the other three sources. Mendocino is a coastal site and the results show these plants experienced more stress when grown in the interior common garden site. Because acclimation to growth in a common garden did not erase significant differences in respiratory and photosynthetic properties, the results show that respiration and photosynthesis are genetically adapted to the native climate of the seed source.

Keywords: Calorimetry; Carbon isotopes; *Pinus ponderosa*; Temperature; Respiration; Adaptation

1. Introduction

Ponderosa pine (*Pinus ponderosa*) is one of the most abundant tree species in western North America. The species range extends from southern British Columbia to northern Mexico and from the Pacific Coast to the Black Hills of South Dakota [1]. Variety *ponderosa* extends from the northern Rockies in British Columbia and Idaho down the west coast through California, and variety *scopulorum* extends from Montana south into Mexico [2]. Variety *ponderosa* is further divided into three distinct geographic races, Pacific, Northern Plateau, and Southern California. Considerable genetic variation exists within these races for growth, stem form, needle morphology, leader growth phenology, monoterpenes, isozymes, cold hardiness, and drought hardiness [2–5]. Geographic races or seed sources from interior climates or from drier sites such as inland mountains, southern latitudes, and south facing slopes are generally more drought tolerant than races or sources from more mesic or coastal sites [4–6]. Coastal and mid elevation seed sources usually grow faster than more interior and high elevation sources [7,8].

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Although temperature and rainfall are the two most important climatic factors controlling growth and adaptation in ponderosa pine, the underlying physiological mechanisms of adaptation are unclear [6]. This study tests the hypothesis that respiratory heat rate (R_q), CO_2 production rate (R_{CO_2}), and their temperature dependencies (μ_q) and (μ_{CO_2}) in ponderosa pine are correlated to the temperature patterns of the native climates of different populations. Further, a hypothesis that the degree of stress associated with growth of plants in a climate that differs from the native climate of the population can be detected with carbon isotope fractionation was tested. To test these hypotheses, we determined the extent of variation in respiration traits and carbon isotope discrimination among four populations of ponderosa pine plants grown in a common garden from seed from geographically distinct seed sources. Previous studies have found relationships between respiration traits and climatic factors among different geographic sources of coast redwoods, eucalyptus, and many other plant species [9–15]. This study extends the climate-respiratory traits correlation to an important species in North American forestry.

Small, but readily measurable, differences in fractionation of the stable isotopes of carbon indicate variation in plant stress. Fractionation of plant carbon occurs during carbon dioxide assimilation in photosynthesis and is due to preferential uptake of ^{12}C over ^{13}C [16]. Discrimination against ^{13}C increases as stress increases (i.e., $\delta^{13}C$ values become more negative), and the isotope ratio in plant tissues thus changes in response to changes in environment. Thus, if plants do not acclimate to the climate at the common garden site, we expect isotope ratios in plants grown in a common garden to be correlated with differences in climate between the common garden and the seed sources.

2. Materials and methods

2.1. Sample collection

The seedlings used in this study were part of a larger study exploring geographic genetic variation of ponderosa pine from California, Oregon and Washington. Four geographically distinct sites, two from California and two from Oregon were chosen for this study. Nine families from each of the four seed sources, for a total of 36 families, were sampled. Families came from a range of elevations and climates within the region of the seed source (Table 1). The seedlings were grown in raised nursery beds in Corvallis, Oregon. Four trees from each of the 36 families were sampled at the beginning of their third growing season. Respiratory traits and carbon isotope ratios were thus measured on a total of 144 samples (4 seed sources × 9 families per seed source × 4 replications).

Samples of elongating leader shoots were taken four times over a time period from 50% to complete elongation, or up to pinfeather stage (i.e., when needles begin to elongate from the base of the shoots). Samples were taken between 8 and 9 am and immediately placed in 15 mL plastic centrifuge tubes with cold, half-strength Hoagland's solution containing 1% sucrose covering the cut section of the shoot. The vials were placed in a Styrofoam ice chest and shipped by overnight delivery to Brigham Young University, Provo, Utah, where the chest was opened and the samples stored in a cold room at 5 °C until calorespirometric measurements were completed (within 2–3 days after receipt). Respiratory rates of individual samples remained constant during the 3–4-day measurement period.

Table 1
Mean and range for geographic origin and climate data[a] (averaged for the years 1961–1990) for four ponderosa pine seed sources

Origin/climate		Oregon sources		California sources	
		Willamette Valley (WV)	Deschutes (DS)	Mendocino (MN)	Eldorado (EL)
Longitude (W)	LONG	122.9	121.6	122.9	120.5
Latitude (N)	LAT	44.6	44.2	39.7	38.8
Elevation (m)	ELE	171	1155	1219	1200
Annual precipitation (mm)	PRECIP	1166 (1000–1281)	558 (264–1125)	1360 (949–1782)	1324 (1231–1579)
Date of last spring frost, days since January[a]	SPRFRST	116 (107–132)	170 (163–174)	116 (104–129)	108 (103–117)
Date of first fall frost, days since January 1	FLLFRST	296 (291–300)	249 (234–254)	295 (288–306)	311 (302–319)
Frost-free period (days)	FRSTFREE	180 (159–192)	79 (69–91)	179 (159–203)	203 (185–215)
January daily minimum temperature (°C)	JANMIN	0.69 (0.21–1.3)	−6.45 (−8.4 to −5.2)	0.25 (−2.4 to −1.6)	−0.29 (−1.4–0.73)
July daily maximum temperature (°C)	JULMAX	26.66 (26.3–27.6)	26.53 (22.9–28.9)	29.19 (27.4–32.6)	30.45 (29.6–31.5)

[a] Based on the average of nine locations, one for each family in each seed source. Climate data obtained from PRISM, a climate model used to interpolate climate data from weather stations in mountainous terrain.

2.2. Calorespirometic measurements

Measurements were made in Hart Scientific model 7707 and Calorimetry Sciences Corp. model 4100 heat-conduction, multi-cell, differential scanning calorimeters operated in the isothermal mode [17]. The apical 2 mm of tissue, which produced unstable heat rates, was cut off and discarded. The remaining 1 cm long apical section, including the apical meristem with subtending stem, but not needles, was then cut off and placed in a 1 cm^3 ampule and heat and CO_2 production rates measured at temperatures of 15, 20, 25, 30, and 35 °C. The Arrhenius temperature coefficients of R_q (μ_q) and R_{CO_2} (μ_{CO_2}) were calculated for a temperature range from 15 to 30 °C, where nearly all measurements fit a linear plot of log (rate) versus reciprocal of the Kelvin temperature. Enthalpic growth rates ($R_{SG}\Delta H_B$) were calculated with the respiratory growth model published previously [18,19].

2.3. Carbon isotope ratio measurements

After calorespirometric measurements were made, the samples were dried in a vacuum oven at 70 °C for determination of dry mass and carbon isotope ratio. Carbon isotope ratios were determined by placing about 2 mg of dry sample in a tin capsule that was then crimped to a bead and placed in the carousel of an elemental analyzer coupled to a combustion furnace (1700–1800 °C) and gas chromatograph. A fixed volume of oxygen is injected into the helium carrier gas at the start of the analytical cycle to combust the sample and tin capsule, copper wires then absorb the excess oxygen. The gases from combustion of the sample, N_2, CO_2, H_2O, and SO_2, are separated on the gas chromatograhic column, detected by a thermal conductivity detector, and analyzed by continuous-flow, ratio mass spectroscopy (Finnegan Delta plus). Aspartic acid, barium carbonate, and lithium carbonate standards were used to calibrate the mass spectrometer for carbon isotope ratio determinations. Carbon isotope ratios were determined for 173 samples.

2.4. Statistical analysis

Statistical analyses for the calorespirometric measurements were done using SAS and the general linear models statistical function [20]. Arrhenius temperature coefficients of R_q and R_{CO_2} were estimated as the slopes of the natural logarithms of R_q and R_{CO_2} regressed on the reciprocal of Kelvin temperatures. Source means were compared using Duncan's multiple range test.

The multivariate relationship between the five climatic factors (annual precipitation, date of last spring frost, date of first fall frost, January daily minimum temperature, and July daily maximum temperature) and the two temperature coefficients of respiration were determined by canonical correlation analysis. Two canonical variates, u_x and v_x, were constructed such that u_x is the linear combination of the five climatic factors and v_x is the linear combination of the two temperature coefficients. The first pair of canonical variates (u_1 and v_1) was chosen to maximize the correlation between them. The second pair (u_2 and v_2) was uncorrelated with the first pair.

3. Results

3.1. Calorespirometric results

There was no significant difference in absolute values of metabolic heat (R_q) and CO_2 production rate (R_{CO_2}) at each temperature for any of the seed sources (Table 2). The Deschutes seed source differed significantly ($P < 0.01$) from the other seed sources in having larger temperature coefficients of both R_q and R_{CO_2} (Table 3).

A graph of the specific enthalpic growth rate $R_{SG}\Delta H_B$, calculated from R_{CO_2} and R_q values [18,19] against temperature shows that growth rates are the same for all four seed sources from 15 to 30 °C, but at 35 °C, the two California seed sources show only a small or no decrease in growth rate while growth rates of the two Oregon sources are greatly decreased

Table 2
Results from the analysis of variance for seed source and family within seed source differences in respiration (R_q, R_{CO_2}) and temperature coefficients of respiration (μ_q, μ_{CO_2}) for ponderosa pine seed sources measured at different temperatures

Trait	T (°C)	F-values and tests of significance (degrees of freedom)			
		Seed source (3)	Pr > F	Family within seed source (32)	Pr > F
Metabolic heat rate (R_q)	15	2.35	0.0914	0.76	0.8161
	20	1.52	0.2292	0.84	0.7117
	25	0.27	0.8433	0.86	0.6859
	30	0.42	0.7295	0.78	0.7797
	35	0.38	0.7672	0.94	0.5720
CO_2 production rate (R_{CO_2})	15	2.15	0.1139	0.95	0.5482
	20	1.49	0.2361	0.98	0.5075
	25	0.34	0.7973	0.72	0.8564
	30	0.67	0.5785	0.71	0.8631
	35	1.17	0.3369	0.96	0.5408
Temperature coefficient of R_q (μ_q)	15–30	8.87	0.0002	0.77	0.8037
Temperature coefficient of R_{CO_2} (μ_{CO_2})	15–30	5.02	0.0058	0.67	0.8984

Table 3
Estimated seed source means, family ranges, and coefficients of variation for temperature coefficients of R_q (μ_q) and R_{CO_2} (μ_{CO_2}) of four ponderosa pine seed sources

Source	μ_q[a] (kK)			μ_{CO_2}[a] (kK)		
	Mean	Range	CV	Mean	Range	CV
WV	4.56 bc	3.17–5.82	15	6.25 b	1.28–13.17	35
DS	5.24 a	3.11–8.36	19	7.50 a	2.94–13.42	27
MN	4.83 b	3.29–6.53	15	6.43 b	3.28–12.61	28
EL	4.41 c	1.49–6.57	22	6.39 b	3.68–9.88	21

[a] When letters against the means of seed sources are different, sources differed significantly in μ_q and μ_{CO_2} at $P < 0.05$. Also see Table 2.

(Fig. 1). Considering the average maximum temperature in California is about 30 °C while the average maximum temperature in Oregon is lower at 26 °C (Table 1), this result demonstrates the plants are closely adapted to the temperature of the native climate.

The correlations between temperature coefficients and climatic factors were moderate and significant, and the correlations among climatic factors were generally high and significant (Tables 4 and 5). Thus all the climate factors were important.

A plot of v_1 (canonical variate of temperature coefficients of respiration) against u_1 (canonical variate of climatic factors), Fig. 2, indicates that the respiratory temperature

Table 5
First pair of canonical variates (u_1, v_1) and their correlations with climatic factors and temperature coefficients of respiration[a]

Correlation of u_1 with climatic factors	r	Correlation of v_1 with temperature coefficients of respiration	r
PRECIP	−0.64	μ_q	0.61
SPRFRST	0.68	μ_{CO_2}	0.56
FLLFRST	−0.70		
JANMIN	−0.27		
JULMAX	−0.65		

[a] Climatic factors and temperature coefficients of respiration are defined in Tables 1 and 3, respectively.

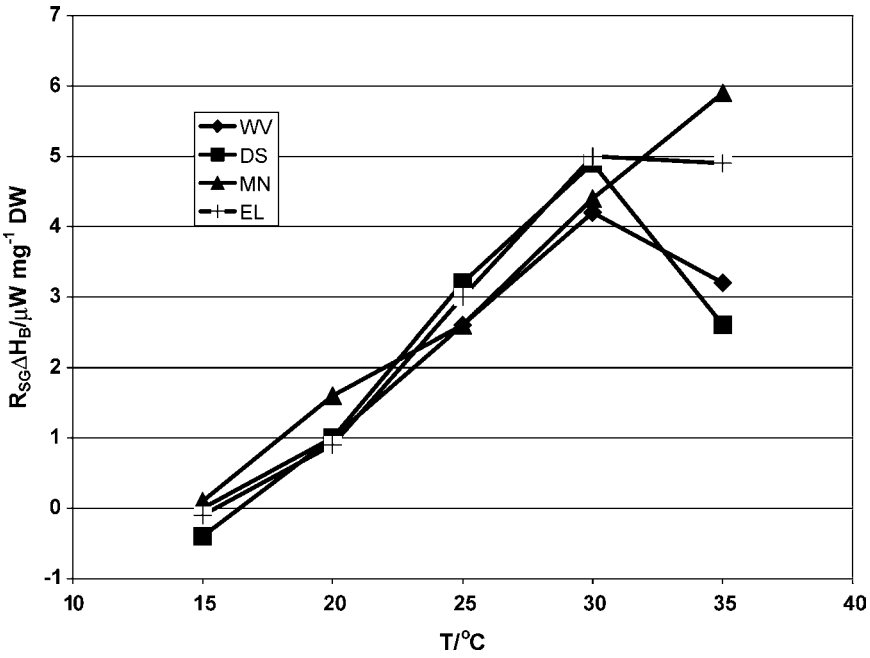

Fig. 1. Specific enthalpic growth rate vs. temperature for each of the four seed sources. Note the separation at 35 °C of the California (EL and MN) and Oregon (DS and WV) sources which are adapted to maximum daily temperatures of 30 and 26 °C, respectively.

Table 4
Family mean correlations[a] between temperature coefficients, between climatic factors, and between temperature coefficients and climatic factors[b]

	μ_q	μ_{CO_2}	PRECIP	SPFRST	FLFRST	FRSTFREE	JANMN
μ_{CO_2}	0.28						
PRECIP	−0.50**	−0.52**					
SPRFRST	0.55**	0.54**	−0.77**				
FLLFRST	−0.60**	−0.52**	0.80**	−0.98**			
FRSTFREE	−0.58**	−0.53**	0.79**	−1.00**	0.99**		
JANMIN	−0.56**	−0.48**	0.73**	−0.94**	0.91**	0.93**	
JULMAX	−0.33*	−0.09	0.29	−0.55**	0.58**	0.57**	0.38*

[a] Significance of correlations: *$P < 0.05$; **$P < 0.01$.
[b] Climatic factors and temperature coefficients of respiration are defined in Tables 1 and 3, respectively.

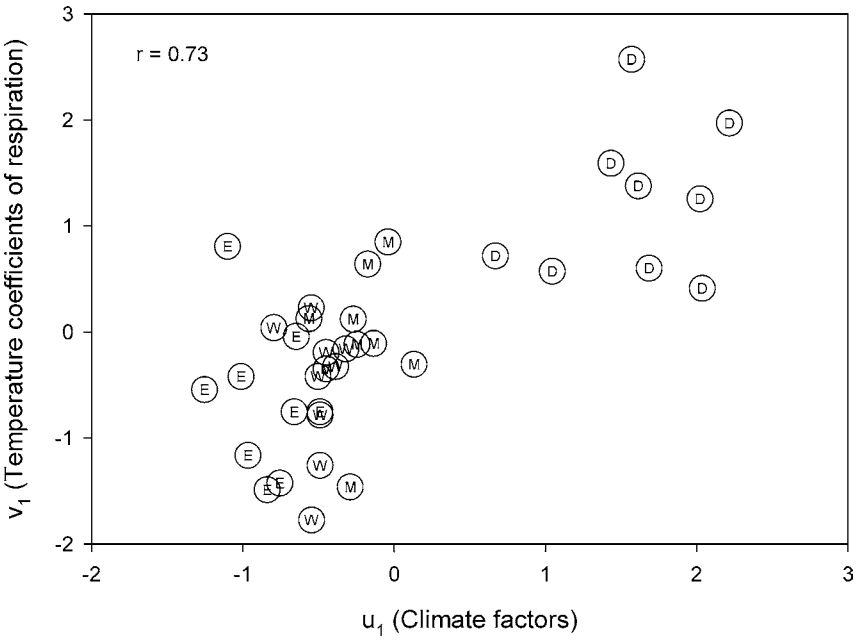

Fig. 2. Canonical variate of temperature coefficients of respiration (v_1) plotted against a canonical variate of climatic factors (u_1) for four seed sources of ponderosa pine: D, Deschutes (OR); E, Eldorado (CA); M, Mendocino (CA); and W, Willamette Valley (OR).

Table 6
Carbon isotopic values for each of the four seed sources, δ^{13}C ‰ vs. PDB standard

Seed source	Minimum	Maximum	Median	Mean ± S.D. of mean
DS, OR	−32.3	−27.9	−29.4	−29.6 ± 0.3
WV, OR	−31.1	−28.0	−29.1	−29.2 ± 0.3
EL, CA	−32.9	−26.7	−29.8	−29.9 ± 0.3
MN, CA	−33.0	−29.2	−30.4	−30.6 ± 0.3

coefficients of all nine families of the Deschutes seed source are distinct from those of the other sources. This plot shows no differentiation among the other seed sources.

3.2. Carbon isotope ratio results

δ^{13}C values for the four ponderosa pine seed sources are given in Table 6. The Mendocino source differs from the other values at about an 80% confidence level. The results thus indicate that plants from the Mendocino source experienced more stress during growth in the common garden at Corvallis than did those from the other sources.

4. Discussion

Considerable genetic variation in respiration traits exists between the Deschutes seed source and the other three Ponderosa pine seed sources. Both μ_q and μ_{CO_2} were significantly larger for plants grown from seed from the Deschutes region than from the other sources. The climatic factors in the Deschutes region also are significantly different from those in the other three seed source regions. The association between climatic factors and temperature coefficients of respiration agrees with earlier studies showing an increase in μ_q with elevation and latitude [11]. Furthermore, the temperature coefficients of R_q (μ_q) and R_{CO_2} (μ_{CO_2}) have been shown to be sensitive to moisture stress, with μ_q being more sensitive than μ_{CO_2} [21]. The greater sensitivity of μ_q and a finer differentiation among the four seed sources in μ_q is also noted in this study. Also consistent with the findings of this study, many earlier studies have also reported larger μ_q values for populations adapted to extreme climate temperatures [10,15] or growth sites with limited moisture availability [22]. Also consistent with earlier findings [15], the correlation between family means of μ_q and μ_{CO_2} is not significant in this study. This suggests that different sets of genes control the temperature sensitivities of R_q and of R_{CO_2}.

The relationship between temperature coefficients and native climates is not unexpected. Metabolic heat is produced mainly in catabolism [14], CO_2 from both catabolism and anbolism. Changes in the rates of production of heat and CO_2 within a tissue in response to a temperature change thus indicate changes in both the absolute and relative activities of metabolic pathways [12,14,15]. For example, the alternative oxidase pathway is known to increase R_q relative to R_{CO_2} in floral organs or in foliage tissues exposed to cold temperatures [23–25]. The ratio R_q/R_{CO_2} and its temperature dependence do not vary significantly among the seed sources in this study. Thus, the larger μ_q and μ_{CO_2} in the Deschutes seed source probably indicates larger temperature coefficients for both the cytochrome and alternative oxidase pathways in the Deschutes seed source compared to the other seed sources.

Another property that can be calculated from the heat and CO_2 rates is the growth rate in terms of the rate of accumulation of energy in anabolic products, that is $R_{SG}\Delta H_B = 455R_{CO_2} - R_q$ [13,18]. This enthalpic growth rate is not significantly different among the seed sources at temperatures from 15 to 30 °C, and shows an apparently linear increase with increasing temperature in this range. At 30 °C, $R_{SG}\Delta H_B$ ranges from 4.2 to 5.0 for the four seed sources. But the response differs among the seed sources at 35 °C. At this temperature, the two seed sources with the highest summer temperatures, Mendocino and Eldorado (see Table 1), also have the highest growth rates (5.9 and 4.9 μW per mg dry weight, respectively). The Deschutes and Willamette Valley sources have the lowest growth rates (2.6 and 3.2 μW per mg dry weight, respectively) at 35 °C. This respiration parameter thus may be useful in selection for high temperature tolerance.

The plants grown from the Mendocino seed source have more negative $\delta^{13}C$ values than the other three seed sources. Mendocino is a coastal site and these seeds were brought to an inland site to be grown. These plants apparently experienced more stress as they were growing because they are adapted to a wetter coastal climate than the climate at Corvallis where they were grown.

5. Conclusion

Measurable differences in the temperature effects on respiratory properties and carbon isotope ratios were found among four geographically distinct sources of *Ponderosa pine*. This study thus clearly shows a strong adaptation of metabolic properties to native climate, with temperature and moisture regimes probably being the most important factors. The results further show that ponderosa pine has limited ability to acclimate to a non-native climate within the species range. Also, $R_{SG}\Delta H_B$, μ_q and μ_{CO_2} may be useful as markers for screening genotypes suitable for particular conditions.

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