

Climate-related genetic variation in drought-resistance of Douglas-fir (*Pseudotsuga menziesii*)

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Abstract

There is a general assumption that intraspecific populations originating from relatively arid climates will be better adapted to cope with the expected increase in drought from climate change. For ecologically and economically important species, more comprehensive, genecological studies that utilize large distributions of populations and direct measures of traits associated with drought-resistance are needed to empirically support this assumption because of the implications for the natural or assisted regeneration of species. We conducted a space-for-time substitution, common garden experiment with 35 populations of coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) growing at three test sites with distinct summer temperature and precipitation (referred to as 'cool/moist', 'moderate', or 'warm/dry') to test the hypotheses that (i) there is large genetic variation among populations and regions in traits associated with drought-resistance, (ii) the patterns of genetic variation are related to the native source-climate of each population, in particular with summer temperature and precipitation, (iii) the differences among populations and relationships with climate are stronger at the warm/dry test site owing to greater expression of drought-resistance traits (i.e., a genotype $\times$ environment interaction). During midsummer 2012, we measured the rate of water loss after stomatal closure (transpiration_{min}), water deficit (% below turgid saturation), and specific leaf area (SLA, cm² g⁻¹) on new growth of sapling branches. There was significant genetic variation in all plant traits, with populations originating from warmer and drier climates having greater drought-resistance (i.e., lower transpiration_{min}, water deficit and SLA), but these trends were most clearly expressed only at the warm/dry test site. Contrary to expectations, populations from cooler climates also had greater drought-resistance across all test sites. Multiple regression analysis indicated that Douglas-fir populations from regions with relatively cool winters and arid summers may be most adapted to cope with drought conditions that are expected in the future.

Keywords: climate change, genecology, minimum conductance, minimum transpiration, specific leaf area, water deficit

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Introduction

Terrestrial plants have evolved complex systems of water management and water conservation that utilize a suite of adaptive morphological traits (e.g., small vessel elements, thick waxy cuticles, low specific leaf areas) and physiological processes (e.g., hormonal signaling, stomatal action), which collectively control plant-water relations and drought-resistance (Edwards *et al.*, 1982; Chaves *et al.*, 2003). Large differences have developed among plant taxa in their abilities to cope with moisture stress as a result of climate-related natural selection (Chaves *et al.*, 2002; Reich *et al.*, 2003). Even within species, there can be substantial variation in adaptive traits among populations originating along climate gradients to conserve water (White, 1987). As global temperatures increase and drought conditions

become increasingly prevalent (Easterling *et al.*, 2000; IPCC, 2007), the persistence of species may depend on those populations that are inherently pre-adapted to warmer, drier climates (Aitken *et al.*, 2008). To better understand and adapt to the impacts of climate change, it is critical to understand how intraspecific populations have differentiated with respect to their abilities to conserve and manage their water resources.

Isohydric vascular plants typically close their stomata during periods of low soil water availability or high vapor pressure deficits (Zwiazek & Blake, 1989; Edwards & Dixon, 1995; Chaves *et al.*, 2003; McDowell *et al.*, 2008). For the majority of studies on plant-water relations and drought-resistance, there is an underlying assumption that water loss following stomatal closure is negligible because the cuticle serves as an effective barrier to water transpiration (Edwards *et al.*, 1982). However, there is often continued water loss through incomplete stomatal closure (Kerstiens, 1996b; Barbour

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et al., 2005) and conductance of water through the cuticle (the continued water loss is hereafter referred to as transpiration_{min}) (Schönherr, 1982; Burghardt & Riederer, 2003). While the impact of transpiration_{min} may be relatively low during periods of high water availability (Kerstiens, 1996a), high rates of transpiration_{min} during periods of drought may increase the occurrence of xylem cavitations and eventually lead to drought-induced tree mortality (Edwards *et al.*, 1982; Hadley & Smith, 1983; Burghardt & Riederer, 2003). In addition to transpiration_{min}, water deficit and specific leaf area (i.e., the amount of leaf surface area per unit of leaf mass that is available for water loss) are also directly linked to drought tolerance and moisture stress (Grace, 1990; Fernández *et al.*, 1999), and collectively these three traits represent different short- and long-term components of plant drought-resistance (Levitt, 1980). Few studies on plant-water relations have measured these traits together; none of which we are aware have simultaneously examined their genetic variation among intraspecific populations.

Coast Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*) is one of the most ecologically and economically important forest species throughout the western USA and in many parts of Europe (Kleinschmit & Bastien, 1992). Using locally adapted seed sources for reforestation of Douglas-fir in the USA has been the *modus operandi* for many decades because of strong clinal variation in growth along environmental gradients both *in situ* and in common garden experiments (Campbell, 1979, 1986; Rehfeldt, 1989; St Clair *et al.*, 2005). In addition to growth, studies of Douglas-fir have found that traits associated with adaption to moisture stress are correlated with local climate at the locations where seeds originated (source-climate), indicating climate-related natural selection (Ferrell & Woodard, 1966; Pharis & Ferrell, 1966; Aitken *et al.*, 1995; Kavanagh *et al.*, 1999; Gould *et al.*, 2012). However, future generations of trees will likely experience novel climates to those experienced by previous generations due to climate change, thus bringing into question the paradigm that 'local is best' for reforestation of Douglas-fir.

Common garden studies conducted across multiple test sites with differing temperature and precipitation regimes can be used to understand how environmental conditions interact with among-population genetic variation to influence drought-resistance traits in trees (St Clair *et al.*, 2005; Wang *et al.*, 2010). These data provide us with key information for modeling the effect of climate change on natural populations of Douglas-fir, and for developing management options for responding to climate change (Joly *et al.*, 1989; Stonecypher *et al.*, 1996; Anekonda *et al.*, 2002; White *et al.*, 2007).

However, for these studies to have meaningful results with regard to climate change, they require (i) a pool of source-populations that originate across a large portion of the natural distribution of a species, and (ii) test sites that span a range of climates to allow inferences based on space-for-time substitution (i.e., warm/dry test sites represent future conditions, Wang *et al.*, 2010). We conducted a set of three common garden experiments on coast Douglas-fir using 35 populations collected from seven geographic regions to test the hypotheses that (i) there is large genetic variation among populations and regions in traits associated with drought-resistance of Douglas-fir, (ii) the patterns of genetic variation are associated with the native source-climate of each population, in particular with summer temperature and precipitation, and (iii) the differences among populations and relationships with climate are larger and stronger at the warmest and driest test site owing to greater expression of drought-resistance traits (i.e., a genotype $\times$ environment interaction). In conducting this research, we empirically test the assumption that populations originating from warmer and drier summer environments are better adapted to cope with conditions that are expected in the future.

Materials and methods

Experimental design

A common garden experiment was replicated three times in the Pacific Northwest region of USA (Fig. 1). The common gardens (hereafter referred to as 'test sites') were part of a larger reciprocal transplant study known as the Douglas-fir Seed Source Movement Trial (SSMT), which included a total of nine test sites. We selected these three test sites because they provided a range of environmental conditions with regard to summer temperature, summer precipitation, and summer heat-to-moisture index (Table 1). The southern-most test site was located in southern Oregon, and had a combination of relatively high summer temperatures and low summer precipitation compared to the other test sites. The moderate and cool/moist test sites were located in northern Washington, with differences in summer temperature and precipitation largely attributable to differences in elevation (1450 vs. 2800 m, respectively; Table 1). During the summer growing season in the Pacific Northwest, warmer summer temperatures are negatively correlated with summer precipitation ($r = 0.65$), whereas minimum winter temperatures were uncorrelated with summer temperatures or precipitation (see Figure S1 for graphs of correlations among climate variables). The three test sites are hereafter referred to as 'cool/moist', 'moderate', and 'warm/dry' test sites.

The sampling design we used for collection of seeds were based on results from an earlier genecology study conducted across the study area (St Clair *et al.*, 2005). Seeds were collected across seven regions in Washington, Oregon, and

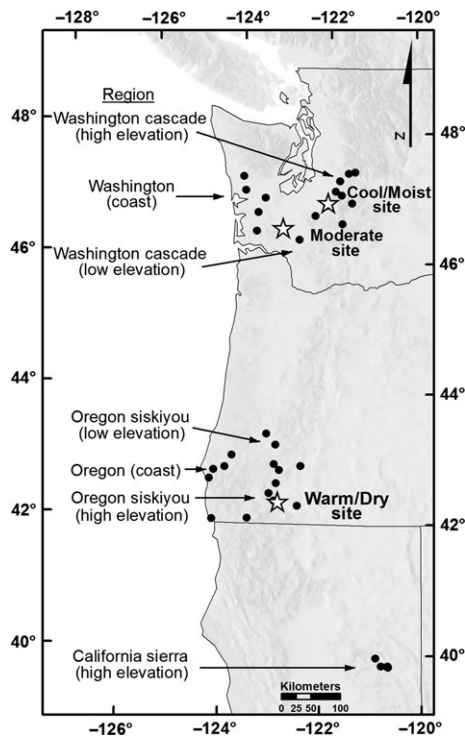


Fig. 1 Locations for each of the three common garden test sites (stars) and locations where seeds were collected for each of 35 populations of coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) (●) from seven regions (five populations per region). Each region is described as 'high elevation', 'low elevation', and 'coast' to indicate the relative locations of each region within each state.

California (Fig. 1; see Table S1 for supporting regional geographic and climate data). In each region, five populations separated by at least 20 km were selected. Each population consisted of two half-sib families each originating from a dominant or codominant parent tree. The two parents in each population were at least 100 m of each other, but in a similar microenvironment as determined by the same elevation and aspect. The latitude, longitude, and elevation at each collection location were used to estimate climate parameters (hereafter referred to as 'source-climate') using a spline model from Rehfeldt (2006) and ClimateWNA (Wang *et al.*, 2012) (see Table S2 for supporting populations' geographic and climate data).

In April 2007, seeds were sown into styroblocks filled with potting soil. Seedlings were transplanted to larger containers after one growing season and then outplanted to the test sites after two growing seasons. Trees were planted in a complete block design with trees from the same region planted in plots within each block. Blocks were replicated four times at each test site. Trees were arranged in four rows with five trees per row; trees were spaced 2.7 m apart. Each block had a buffer zone of trees to avoid edge effects. After 4 years of growth and mortality, there were 1–4 trees remaining for each population in each block, with a minimum of $n = 7$ trees per population per site (≈ 280 trees per site) surviving for measurements of plant response variables during summer 2012.

Table 1 Geographic and climatic descriptions of three, coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) common garden test sites (cool/moist, moderate or warm/dry) during the five-year period (August 2008–August 2012), and the conditions 30 days prior to tissue sampling

Geography/climate	Cool/moist site	Moderate site	Warm/dry site
Latitude (N)	46°56'54"	46°32'49"	42°20'56"
Longitude (W)	122°00'27"	122°59'24"	122°56'21"
Elevation (m)	853	442	418
5-year MAT (°C)	7.1	9.8	12.5
5-year T_{Min} (°C)	−1.1	−0.6	−1.6
5-year T_{Max} (°C)	21.1	25.9	32.4
5-year mean annual precipitation (mm)	1910	1648	521
5-year growing season precipitation (mm)	400	272	85
5-year summer heat-to-moisture index	42.1	72.3	291.8
30-day mean temperature (°C)	16.0	16.0	20.7
30-day cumulative precipitation (mm)	24.9	28.2	20.0
30-day mean relative humidity (%)	82.0	80.5	62.8

Abbreviations: MAT, mean annual temperature; T_{Min} , mean minimum temperature during the coldest month; T_{Max} , mean maximum temperature during the warmest month. 30-day data were recorded *in situ* at each site; other variables derived from ClimateWNA and Rehfeldt (2006).

Tissue sampling and response variables

Samples were collected in the field during midsummer (late July/early August) 2012 at each of the three test sites. For each tree, we first identified two typical twigs from the mid portion of the crown on the south side of the tree. Each twig was clipped 8 cm from the tip of the terminal bud around midday, placed in an airtight plastic bag, and stored in a cooler for transport. One twig was used for measurements of transpiration_{min} and specific leaf area, and the other was used for measurements of water deficit.

We assessed transpiration_{min} as the rate of water loss under conditions of maximum stomatal closure through measurements of leaf-drying curves from the clipped twigs (Hygen, 1951; Cape & Percy, 1996; Anfodillo *et al.*, 2002). The base of each twig was sealed with warmed grafting wax. Twigs were initially weighed and then placed in a warm, lighted area, and reweighed every 24 h for 7 days. As the twigs dried, there was an initial, rapid drop in water mass followed by a linear rate of water loss. The slope of the linear portion of the leaf-drying curve was considered the rate of transpiration_{min} (also referred to in the literature as 'minimum transpiration' or 'minimum conductance'), and is associated with stomatal

closure (Marks & Lechowicz, 2007) and the turgor loss point (Burghardt & Riederer, 2003). After the final weighing, needles and twigs were separated and dried at 65 °C for 72 h, and then reweighed (± 0.1 mg). A subsample of 8 fresh needles was removed from each fresh twig prior to any drying and used to determine fresh leaf area using image processing software, dried, and then weighed (± 0.1 mg). Specific leaf area (SLA) was calculated as fresh leaf area (cm^2) divided by dry leaf mass (g).

The second twig that was collected from each tree was initially weighed after collection (fresh weight) and then placed back into a plastic bag with 2 ml of distilled water added; bags were then resealed. Twigs were angled such that the base of each twig was submerged in water. Twigs were then placed in a refrigerator for 6 days to allow full hydration of twigs. Following hydration, twigs were removed from bags, blotted dry, and immediately weighed (turgid weight). Twigs were then dried at 65 °C for 72 h, and then reweighed (dry weight). Water deficit was calculated as (turgid weight–fresh weight)/(turgid weight–dry weight)*100 (Barrs, 1968). Other measures of plant-water status (e.g., predawn xylem water potential, conductance, and hydraulic conductivity) have relatively large sampling-time requirements. Thus, those measures of plant-water status limit replication across multiple tests sites. Transpiration_{min}, water deficit, and SLA are parameters of plant-water status that are intrinsically related to water loss, water conservation, and stress tolerance (Muller *et al.*, 2011), can be quickly collected for many individuals, and are a consequence of morphological development and physiological adjustments integrated over time (Barrs, 1968).

Data analysis

To assess differences in traits associated with drought-resistance (i.e., transpiration_{min}, water deficit, SLA) for Douglas-fir among common garden test sites, populations and regions, we conducted a mixed model analysis of variance (ANOVA) (SAS, v9.4). Test site and population or regions were fixed factors; block and plots within block (for population tests only) were random factors. In this context, ‘region’ refers to the set of populations from the same geographical areas that were expected to exhibit similar traits. Separate ANOVA models were fit to evaluate population-level or region-level differences. When effects of ANOVA were significant at $P = 0.05$, Tukey Honestly Significant Difference tests were used to compare regional means within each test site.

To evaluate if variation in transpiration_{min}, water deficit, or SLA was associated with geographic or climate gradients, we calculated Pearson correlation coefficients between plant response variables and the physical locations (latitude, longitude, elevation) or climate variables of the locations where seeds for each population were collected (i.e., source-climate). Six biologically relevant source-climate variables were chosen to represent a range of climate conditions (Isaac-Renton *et al.*, 2014), including mean annual temperature (MAT), mean minimum temperature during the coldest month ($T_{\min}$), mean maximum temperature during the warmest month ($T_{\max}$), mean annual precipitation (MAP), mean summer precipitation

(MSP, 1 May to 30 September) and summer heat-to-moisture index [SHM, ($T_{\max}/(\text{MSP}/1000)$)]. Climate variables $T_{\min}$ and $T_{\max}$ were derived from Rehfeldt (2006), and all other variables came from ClimateWNA (Wang *et al.*, 2012). We performed separate correlations for each site to understand, if potentially adaptive relationships differed under conditions of greater moisture stress (i.e., at the warm/dry test site) (White, 1987). We also conducted stepwise multiple regressions on trait values at each site with source-climate variables to understand how multiple climate variables relate to drought-resistance and to model genetic variation in traits across the Pacific Northwest. Regression models for each trait with the highest coefficient of determination (R^2) among sites were used to map traits across the landscape using the grid algebra function (Raster Calculator/Map Algebra) in ArcGIS 10.1 (ESRI, Redlands, California, USA) (St Clair *et al.*, 2013).

To determine if plant traits associated with transpiration_{min}, water deficit, and SLA were related to relative height and diameter growth rates, we conducted correlations between plant variables at each tests site. Height and diameter growth increments were calculated as the difference between end-of-season 2011 and 2012 measurements (collected as part of the larger SSMT study); incremental height and diameter growth was then normalized (scaled to total height and diameter at the beginning of the 2012 season, respectively), to give relative growth rates for 2012 (Gould *et al.*, 2012). We used the correlation coefficients (r values) to compare the direction and strengths of relationships among traits. All tests met the assumptions of normality and homoscedasticity of error variance.

Results

Trait variation among test sites, populations, and regions

The three traits associated with drought-resistance that we measured differed in the effects of test site, population or region, and their interaction (Table 2). Transpiration_{min} differed most strongly by test site (based on F -values), but also differed among populations and regions, and interactions of test site and population or region were significant (Table 2). Specifically, values of transpiration_{min} were similar at the cool/moist and moderate test sites, but were approximately 40% lower at the warm/dry site (Fig. 2a). At the moderate and warm/dry (but not cool/moist) test sites, populations from the California Sierra (Calif. high) region had the lowest transpiration_{min} compared to the other regions (Fig. 3a–c). Water deficits were similar among test sites (Fig. 2b) and populations, but did differ significantly (albeit relatively weakly) among regions and the interaction of test site and population or test site and region (Table 2). Populations from the California Sierra region had the lowest water deficit at the cool/moist site, highest at the warm/dry site, and intermediate at the moderate site (Fig. 3d–f). Values of SLA were affected by test site, population and region, but their interactions

Table 2 F-values from two-factor analysis of variance testing for the effects of common garden test site (cool/moist, moderate, warm/dry) and populations (35) or region (7) on coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) traits associated with drought-resistance

Plant trait	Test site × Population			Test site × Region		
	Test site (TS)	Population (Pop)	TS × Pop	TS	Region (Reg)	TS × Reg
Transpiration _{min}	40.35***	4.71***	1.55**	41.29***	20.07***	4.68***
Water deficit	1.84	1.45	1.46*	2.03	3.73**	3.69***
SLA	11.23**	14.70***	0.27	10.99**	70.15***	0.14

SLA, specific leaf area.

Significance of correlation coefficients: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

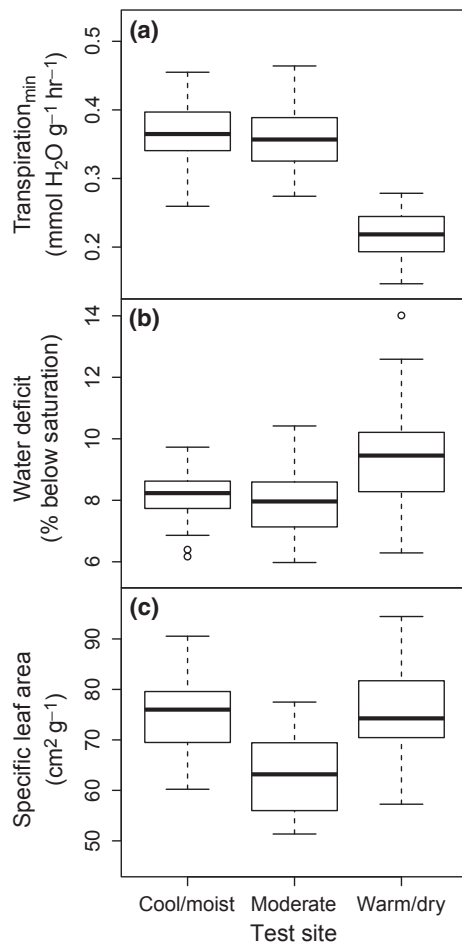


Fig. 2 Box-and-whisker plot of transpiration_{min}, water deficit, and specific leaf area for each of three common garden test sites (cool/moist, moderate, and warm/dry). The bottom and top of the boxes represent the 25th and 75th percentile, respectively; the middle line represents the median. Whiskers extend to the most extreme data point unless they are more than 1.5 box-lengths long; observations outside of this range are plotted as circles.

were not significant (Table 2). Values of SLA were generally 15% lower at the moderate test site compared to the cool/moist or warm/dry site (Fig. 2c). Population and regional effects were especially strong on values of

SLA compared to their effects on transpiration_{min} and water deficit (based on F -values in Table 2), and were lowest for populations from the California Sierra region, followed by Oregon regions, and then were highest for the Washington regions, particularly the Washington Coast region (Fig. 3g–i).

Trait-geography/climate relationships

All drought-resistance traits varied along geographic and climate gradients, although the strengths of the relationships differed among traits (Table 3). At all three sites, variation in population means of transpiration_{min} were negatively correlated with elevation and positively correlated with T_{min} and longitude (Table 3; Fig. 4a). At the warm/dry site only, transpiration_{min} was negatively correlated with T_{max} and SHM (Fig. 4b,c), and positively with latitude, MAP and MSP (Table 3). In general, variation in population means of water deficit was less associated with geographic or climate variables compared to transpiration_{min} or SLA, particularly at the warm/dry test site (Table 3). At the cool/moist and moderate (but not warm/dry) test sites, population means of water deficit were positively correlated with T_{min} (Fig. 4d) and negatively related to longitude, elevation, T_{max} , and SHM (Table 3; Fig. 4d–f). At the warm/dry site only, water deficit was only negatively correlated with latitude (Table 3). Values of SLA had relatively strong relationships to geographic and climate variables compared to transpiration_{min} and water deficit, and were positively correlated with latitude, longitude, T_{min} , MAP, and MSP, negatively correlated with elevation, T_{max} , and SHM, and unrelated to MAT at all three test sites (Table 3).

All three drought-resistance traits varied along multivariate climate gradients, as indicated by relatively high R^2 from regression analyses. The models with the highest R^2 for transpiration_{min} came from the warm/dry test site, for water deficit from the cool/moist test site, and for SLA from the moderate test site, and were respectively:

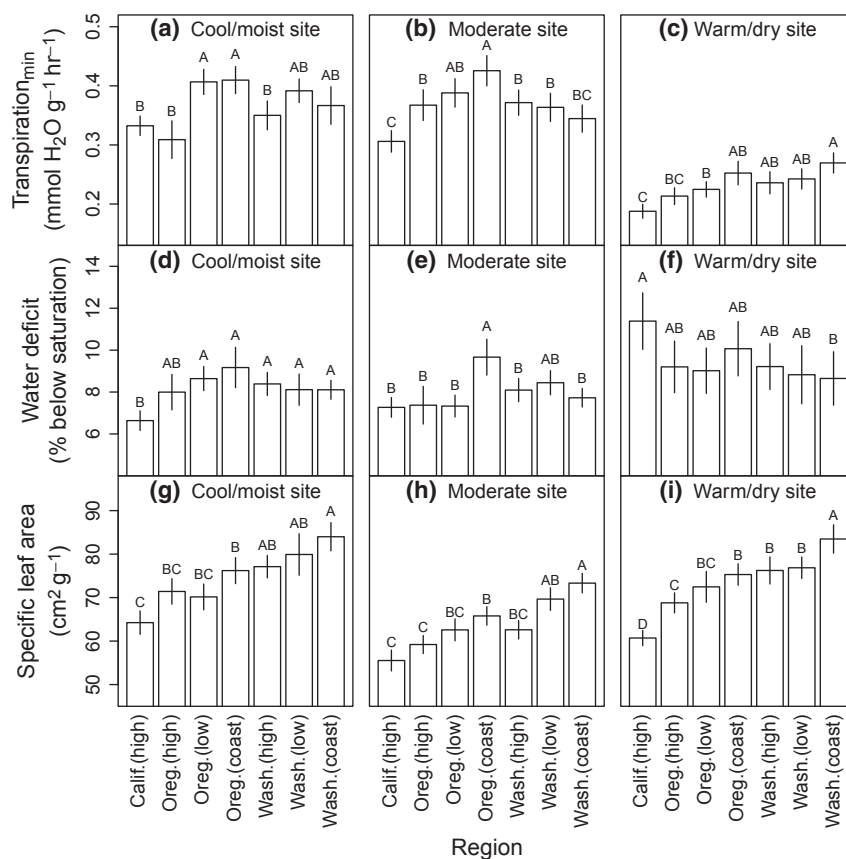


Fig. 3 Regional means (± 1 SE) of transpiration_{min} (a–c), water deficit (d–f) and specific leaf area (g–i) for coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) populations (five populations per region) at each of three common garden test sites. Each region is described as 'high', 'low', and 'coast' to indicate the relative locations and elevations of each region within each state. Within each panel of seven bars, different capitalized letters indicate significant differences between regions following ANOVA (ANOVA results in Table 2).

$$\text{Transpiration}_{\min} = 0.193 + 0.0067 * T_{\min} + 0.000117 * \text{MSP} (R^2 = 0.66)$$

$$\text{Water deficit} = 11.09 + 0.347 * T_{\min} - 0.275 * \text{MAT} (R^2 = 0.60)$$

$$\text{SLA} = 56.89 - 0.115 * \text{SHM} + 1.813 * \text{MAT} (R^2 = 0.68)$$

Transpiration_{min} rates were relatively high for populations from climates with warmer winter temperatures and with more summer precipitation, as indicated by the positive coefficients associated with $T_{\min}$ and MSP. For transpiration_{min}, the partial r^2 values for $T_{\min}$ and MSP were 0.41 and 0.24, respectively, indicating $T_{\min}$ had a relatively strong effect on transpiration_{min}. Water deficits were greatest for populations from climates with relatively high winter temperatures but cool mean annual temperature. For water deficit, the partial r^2 for $T_{\min}$ and MAT were 0.40 and 0.20, respectively. Specific leaf area was relatively high for populations with cooler, wetter summers (indicated by the negative coefficient

with SHM), and with higher MAT. For SLA, the partial r^2 for SHM and MAT were 0.47 and 0.21, respectively. When trait values were modeled across the Pacific Northwest, the lowest values (i.e., greatest drought-resistance) for all of the traits were located along the far eastern edge of the range of coast Douglas-fir (Fig. 5 for transpiration_{min}; water deficit and SLA not shown), particularly in the California Sierra region and along the Cascade Crest. Moderately low values were located on the leeward side of the coast mountains in Oregon and Washington. The highest values for all traits occurred along the Pacific coast, and were particularly high in the north Washington coast region, except for populations in the rain shadow of the Olympic Mountains on the Olympic peninsula, which had relatively low transpiration_{min}.

Trait–trait relationships

Transpiration_{min}, water deficit and SLA were all correlated with relative height and diameter growth,

Table 3 Pearson correlation coefficients for relationships between transpiration_{min}, water deficit and specific leaf area (SLA) and geographic/climate variables associated with the location where seeds for each population were collected at each of three common garden test sites (cool/moist, moderate, and warm/dry) with 35 coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) populations

Test site	Geography/ climate	Transpiration _{min}	Water deficit	SLA
Cool/ moist	Lat.	0.21	0.34*	0.75***
	Long.	0.41*	0.64***	0.45**
	Elev.	-0.68***	-0.58***	-0.64***
	MAT	0.48**	0.18	-0.07
	T _{Min}	0.67***	0.64***	0.49**
	T _{Max}	-0.12	-0.37*	-0.64***
	MAP	0.20	0.32	0.54***
	MSP	0.19	0.30	0.61***
	SHM	-0.24	-0.50**	-0.67***
Moderate	Lat.	0.13	0.20	0.71***
	Long.	0.53**	0.35*	0.50**
	Elev.	-0.41*	-0.41*	-0.77***
	MAT	0.33*	0.21	0.15
	T _{Min}	0.52**	0.47**	0.63***
	T _{Max}	-0.11	-0.35*	-0.50**
	MAP	0.16	0.39*	0.55***
	MSP	0.13	0.31	0.63***
	SHM	-0.25	-0.41*	-0.68***
Warm/ dry	Lat.	0.57***	-0.42*	0.74***
	Long.	0.63***	0.27	0.54***
	Elev.	-0.71***	0.22	-0.67***
	MAT	0.22	0.02	0.01
	T _{Min}	0.69***	-0.20	0.57***
	T _{Max}	-0.51**	0.23	-0.61***
	MAP	0.64***	-0.24	0.55***
	MSP	0.56***	-0.38	0.64***
	SHM	-0.60***	0.30	-0.73***

Abbreviations: Lat.=latitude; Long.=longitude; Elev.=elevation; MAT=mean annual temperature; T_{Min}=mean minimum temperature during the coldest month; T_{Max}=mean maximum temperature during the warmest month; MAP=mean annual precipitation; MSP=growing season precipitation (April–September); SHM=summer heat-to-moisture index.

Variables were derived from ClimateWNA and Rehfeldt (2006). Significance of correlation coefficients: **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

although the strengths of the relationships differed among traits and were test site-specific (Table 4). Values of transpiration_{min} were positively correlated with height growth and diameter growth, but only at the cool/moist test site (Table 4). Water deficit was unrelated to height growth at any of the sites, but was positively correlated with diameter growth at the cool/moist test site (Table 4). Values of SLA were positively correlated with diameter growth at all three sites, but unrelated to height growth (Table 4). Values of SLA

were also positively correlated with transpiration_{min} at all three sites, and had a negative relationship with water deficit at the moderate and warm/dry (but not cool/moist) test sites (Table 4). Transpiration_{min} and water deficit were positively correlated at the cool/moist and moderate test sites, and negatively related at the warm/dry site (Table 4).

Discussion

Our experimental approach using multiple common gardens revealed that traits related to drought-resistance of Douglas-fir were strongly influenced by short-term environmental conditions among test sites, long-term genetic differentiation among populations, and the interaction of environmental conditions with genotypes.

Under drought conditions, isohydric species such as Douglas-fir rely on a number of fast-acting and long-term physiological mechanisms to maintain stable water potentials and thus avoid xylem cavitations (Mcdowell *et al.*, 2008). Among our three common gardens, all of the populations growing at the warmest and driest test site had the lowest transpiration_{min}, indicating an environmental-induced response to moisture stress at the species-level (Tognetti *et al.*, 1997; Fernández *et al.*, 1999; Villar-Salvador *et al.*, 1999). To minimize water loss through transpiration_{min}, plants typically undergo structural changes, such as cuticle thickening (Rook, 1973) or an increase in biomass allocation to roots relative to shoots (Bloom *et al.*, 1985). Alternatively, differences in phenological development may have contributed to the differences that we observed in transpiration_{min} among test sites, with warm growing temperatures initiating earlier development of latewood, which may be more resistant to water loss than earlywood (Domec & Gartner, 2002; Eilmann *et al.*, 2013). Independent of the mechanisms involved, reduced transpiration_{min} rates at the warm/dry test site exhibited by all populations appeared to be a plastic response that can help cope with future climates (Davis & Shaw, 2001; Rehfeldt *et al.*, 2001; Westfall & Millar, 2004).

There was also considerable genetic variation in drought-resistance among populations originating along climate gradients. We found that populations originating from warmer, drier climates generally had lower values of transpiration_{min}, water deficit and SLA. The genetic trend in transpiration_{min} followed a similar pattern as the environmental trend that we observed across our three test sites (i.e., low transpiration_{min} at the warm/dry test site and from warm/dry source-climates), which is a classic example of cogradient variation in drought-resistance along both environmental

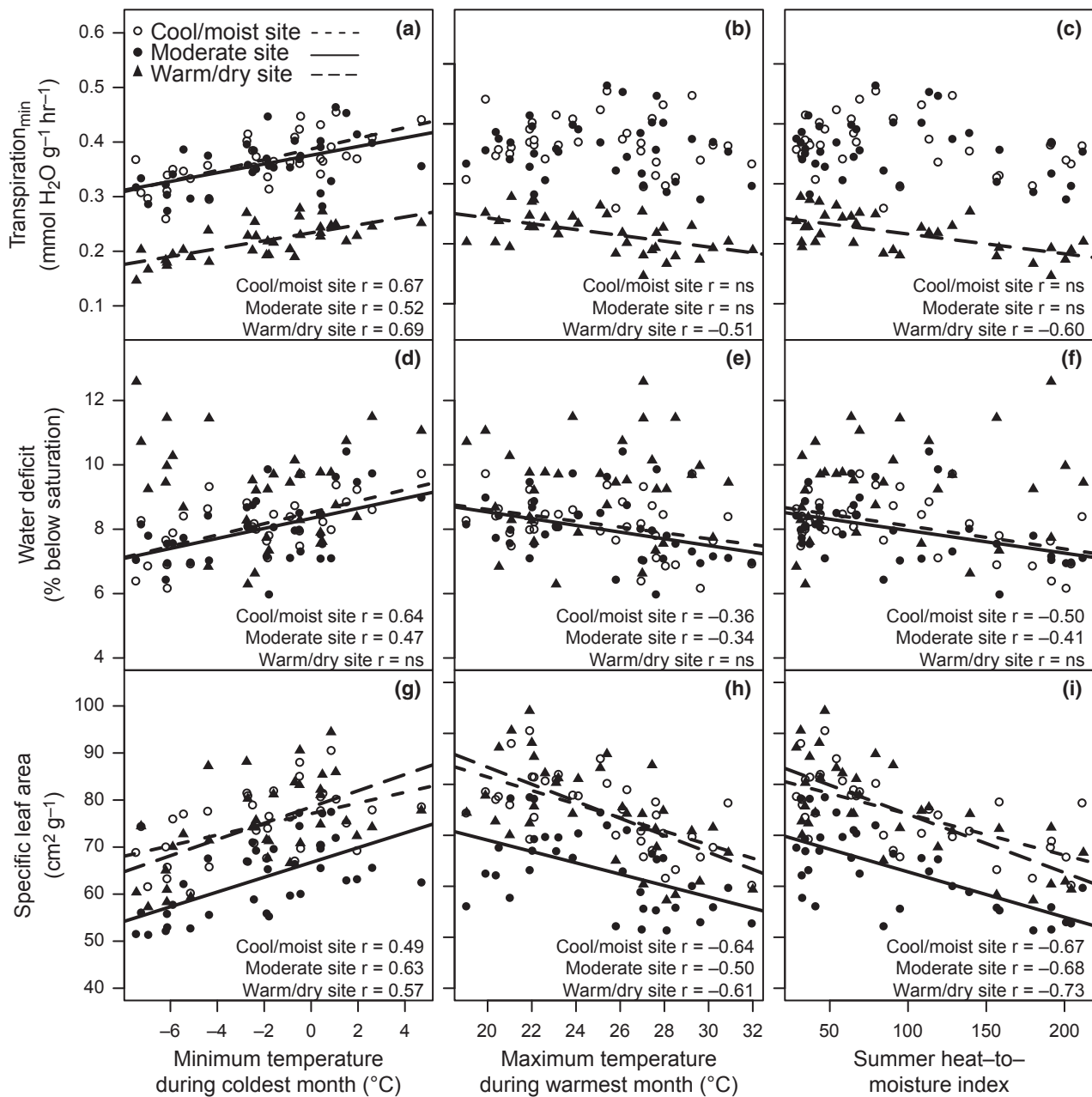


Fig. 4 Relationships between transpiration_{min} (a–c), water deficit (d–f) and specific leaf area (g–i) for 35 coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) populations and climate variables (minimum temperature during the coldest month, maximum temperature during the warmest month, summer heat-to-moisture index) of the location where seeds for each population were collected (source-climate) at each of three common garden test sites [cool/moist (○), moderate (●), warm/dry (▲)]. Lines with associated *r* values indicate significant correlations ($P < 0.05$) between plant traits and climate variables for each test site separately, whereas nonsignificant (ns) relationships have no line. Correlations follow Table 3.

and genotypic gradients (Kremer *et al.*, 2014). Cogradients in plant traits typically accelerates adaptation to changing climate conditions, which may particularly favor the persistence of those Douglas-fir populations at the southerly, warm edges of its distribution.

Even though populations from warm climates had relatively high drought-resistance, populations originating from the coolest climates also exhibited increased drought-resistance. Other common garden studies involving Douglas-fir have consistently reported a strong correlation between cool tempera-

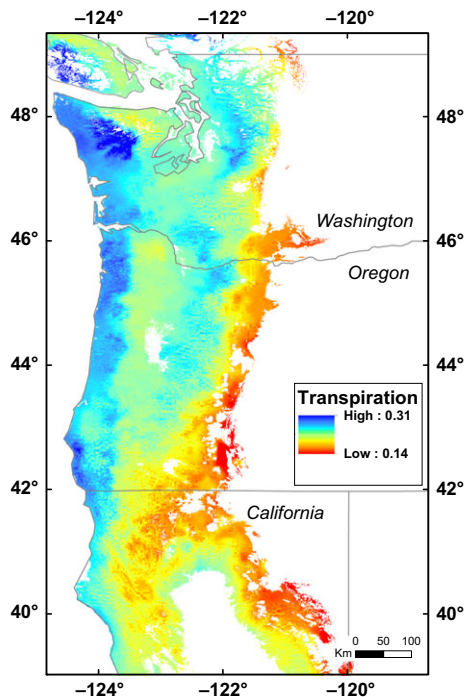


Fig. 5 Geographic genetic variation in projected rates of transpiration_{min} (Transpiration) of coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) in the Pacific Northwest, USA. Lower values represent population with lower rates of water loss (i.e., greater drought-resistance).

tures (or elevation) and variation in a number of morphological and physiological traits associated with adaption to stress (Ferrell & Woodard, 1966; Pharis &

Ferrell, 1966; Sorensen, 1983; White, 1987; Rehfeldt, 1989; Zhang *et al.*, 1993; Aitken *et al.*, 1995; St Clair *et al.*, 2005; Gould *et al.*, 2012). Trees growing in cool climates, such as at high elevations, are frequently exposed to high vapor pressure deficit, abrasive ice crystals and regular freeze-thaw events, which have been linked to increased transpiration_{min}, needle death and drought-induced mortality (Baig & Tranquillini, 1980; Tranquillini, 1982; Hadley & Smith, 1983; Gardingen *et al.*, 1991; Anfodillo *et al.*, 2002; Mayr *et al.*, 2002). Therefore, it is consistent for cool-climate populations to be protected against winter-desiccation by evolving mechanisms to increase drought-resistance (Schreiber & Riederer, 1996; Anfodillo *et al.*, 2002).

The relationships between transpiration_{min} and water deficits with summer aridity were weaker compared to the relationships to winter minimum temperatures (Table 3). Also, in the multiple regression of transpiration_{min} on climate variables, the contribution of minimum winter temperature to transpiration_{min} was relatively large compared to that of mean summer precipitation, indicating that winter conditions may have been more important than summer aridity in influencing genetic variation in drought-resistance. Nevertheless, our best models clearly revealed how drought-resistance was a function of both winter minimum temperatures and summer aridity, demonstrating how multiple climate conditions can impose selection pressure to increase drought-resistance across a species distribution (St Clair *et al.*, 2005; Isaac-Renton *et al.*, 2014). For coast Douglas-fir, our findings indicated that

Table 4 Pearson correlation coefficients for relationships between plant traits associated with drought-resistance and growth for 35 coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) populations at each of three common garden test sites (cool/moist, moderate, and warm/dry)

Test site	Traits	Transpiration _{min}	Water deficit	SLA	Height	Basal diameter
Cool/moist	Transpiration _{min}	—	—	—	—	—
	Water deficit	0.51**	—	—	—	—
	SLA	0.40*	0.31	—	—	—
	Height	0.53**	0.27	0.31	—	—
	Basal diameter	0.43**	0.61***	0.46**	0.42*	—
Moderate	Transpiration _{min}	—	—	—	—	—
	Water deficit	0.67***	—	—	—	—
	SLA	0.34*	−0.43*	—	—	—
	Height	−0.17	−0.19	0.18	—	—
Warm/dry	Basal diameter	0.20	−0.13	0.40*	−0.28	—
	Transpiration _{min}	—	—	—	—	—
	Water deficit	−0.44**	—	—	—	—
	SLA	0.80***	−0.45**	—	—	—
	Height	−0.17	−0.19	−0.18	—	—
	Basal diameter	0.20	−0.12	0.40*	−0.28	—

SLA, specific leaf area.

Values for height and basal diameter were calculated as the incremental increase in size during the 2012 growing season, corrected for initial plant size at the beginning of the season. Significance of correlation coefficients: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

those populations from relatively high elevations, such as the California Sierra mountains and along the Cascade Crest in Oregon and Washington, are likely to be relatively more drought-resistant, especially compared to coastal populations. However, for populations growing at lower elevations in the rain shadow of larger mountains, such as east of the Coast Range, summer aridity (as opposed to temperature or elevation) appears to be a more important driver of increased drought-resistance. Even within regions there was considerable variation in drought-resistance, which has considerable value for assisted migration and for tree improvement programs to cope with future drought (Stonecypher *et al.*, 1996).

Using multiple common gardens provides a unique opportunity to understand if and how environmental conditions influence the expression of genes and resulting phenotypes. In our study, the relationship between drought-resistance traits and minimum winter temperatures were evident at all three common gardens, even though the tissues we sampled were new growth, and therefore had not experienced winter conditions. Consequently, selection pressure from cool temperature climates may be one of the innate drivers of variation among populations in drought-resistance, independent of current environmental conditions. In contrast, the relationships between transpiration_{min} and maximum summer temperatures, aridity, and precipitation were evident only at the warm/dry test site, indicating that warm, dry environmental conditions triggered the expression of specific genes to limit water loss (Osakabe *et al.*, 2014). Other common garden studies with Douglas-fir have not consistently found strong relationships between drought-resistance and summer temperature or precipitation of source-climate, or interactions between environmental conditions and among populations genetic variation (Joly *et al.*, 1989; Zhang *et al.*, 1993; Stonecypher *et al.*, 1996; Eilmann *et al.*, 2013). The differences between studies may have occurred because our warm/dry test site was significantly warmer and drier during the study period than historical climate norms. The implication is that novel climate conditions, such as those that are expected to occur with climate change, could trigger the expression of stress-induced traits that would otherwise remain unexpressed, although further genetic research is necessary on the induction and regulation of genes associated with drought-resistance (Bray, 1997; Osakabe *et al.*, 2014).

Selection for tree populations with faster growth rates and greater yield potential is a fundamental goal of modern breeding programs (Stonecypher *et al.*, 1996; Blum, 2005). However, traits that confer faster growth typically reduce stress tolerance (Chapin, 1980). A clas-

sic example is higher SLA, which increases light-capturing and gas-exchange area for photosynthesis, but also increases surface area for water loss (Grace, 1990). Indeed, we observed positive relationships between SLA, transpiration_{min} and growth. Under wetter conditions, plants may employ a water-spending strategy with relatively high SLA and transpiration_{min} for faster growth rates to cope with competition (Cohen, 1970). Moreover, growth rates may be enhanced by higher rates of transpiration_{min} because it promotes nutrient uptake, prevents excess turgor, improves oxygen supply for respiration and prevents build-up of respiratory CO₂ in leaves (Marks & Lechowicz, 2007 and citations within). In line with our data, the positive relationship between transpiration_{min}, SLA and growth were more evident under relatively well-watered conditions at the cool/moist and moderate (but not warm/dry) test sites.

Our experimental design utilizing three common gardens with distinct differences in summer temperature and precipitation revealed strong differentiation among populations in their abilities to cope with drought stress. Moreover, each of the common gardens exhibited site-specific trends, indicating the strong influence of environmental conditions on the expression of genes and the resulting phenotypes. This research has many implications for coast Douglas-fir ecology as it relates to climate change. First, as temperatures warm and climate envelopes shift upward in latitude and elevation (Mckeeney *et al.*, 2007), those populations from higher elevations and northern latitudes appear to have 'built-in' adaptations to cope with drought conditions that will likely occur with greater frequency and intensity. This innate stress-coping strategy of cool-climate populations may reduce the negative impacts of future drought on Douglas-fir growth and survival, and thus, slow down shifts in the species' distribution that are expected to occur with climate change (Mckeeney *et al.*, 2007). Second, populations from warm and arid climates were also relatively drought-resistant, but only under relatively warm and arid environmental conditions. Therefore, southerly and lower elevation populations that are at risk of local extirpation may have greater resistance to drought conditions than previously indicated by earlier common garden studies with Douglas-fir. Consequently, the genetic differentiation of Douglas-fir that has occurred through natural selection may favor the persistence of Douglas-fir populations at both the leading and trailing edges of its distribution under future climate scenarios. Finally, those populations with a combination of cool winters and dry summers appear to be the most resistant to drought, and therefore may be key resources to incorporate into natural and assisted forest regeneration strategies for the future.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Correlations among climate variables of the location where seeds for each population were collected.

Table S1. Geographic descriptions and their associated climate data for 7 regions where coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) seeds were collected.

Table S2. Geographic descriptions and their associated climate data for 35 populations of coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*).