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Impact of climate change on cold hardiness of Douglas-fir (*Pseudotsuga menziesii*): environmental and genetic considerations

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Abstract

The success of conifers over much of the world's terrestrial surface is largely attributable to their tolerance to cold stress (i.e., cold hardiness). Due to an increase in climate variability, climate change may *reduce* conifer cold hardiness, which in turn could impact ecosystem functioning and productivity in conifer-dominated forests. The expression of cold hardiness is a product of environmental cues (E), genetic differentiation (G), and their interaction (G × E), although few studies have considered all components together. To better understand and manage for the impacts of climate change on conifer cold hardiness, we conducted a common garden experiment replicated in three test environments (cool, moderate, and warm) using 35 populations of coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) to test the hypotheses: (i) cool-temperature cues in fall are necessary to trigger cold hardening, (ii) there is large genetic variation among populations in cold hardiness that can be predicted from seed-source climate variables, (iii) observed differences among populations in cold hardiness *in situ* are dependent on effective environmental cues, and (iv) movement of seed sources from warmer to cooler climates will increase risk to cold injury. During fall 2012, we visually assessed cold damage of bud, needle, and stem tissues following artificial freeze tests. Cool-temperature cues (e.g., degree hours below 2 °C) at the test sites were associated with cold hardening, which were minimal at the moderate test site owing to mild fall temperatures. Populations differed 3-fold in cold hardiness, with winter minimum temperatures and fall frost dates as strong seed-source climate predictors of cold hardiness, and with summer temperatures and aridity as secondary predictors. Seed-source movement resulted in only modest increases in cold damage. Our findings indicate that increased fall temperatures delay cold hardening, warmer/drier summers confer a degree of cold hardiness, and seed-source movement from warmer to cooler climates may be a viable option for adapting coniferous forest to future climate.

Keywords: assisted migration, climate transfer distance, cold acclimation, cold damage, conifer, frost, genecology, reciprocal transplant

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Introduction

The ability to survive and thrive in cool climates is one of the key adaptations that allow conifers to dominate temperate and boreal forests throughout the world (Sakai & Larcher, 1987; Bannister & Neuner, 2001). In these forests, conifers play the principal role in regulating ecosystem functioning and productivity, driving successional trajectories, and defining habitat conditions for a suite of forest species (Okland & Eilertsen, 1996; Franklin *et al.*, 2002; Diekmann, 2003). A reduction in the capacity to tolerate cold temperatures as a result of climate change (Cannell, 1985; Hänninen, 1991) could have severe, negative impacts on ecosystems that rely on the continued health and productivity of conifers (Guo *et al.*, 2001; Lindner *et al.*, 2010). Therefore, understanding the impacts of climate change on cold hardiness of economically and ecologically important species such as coast Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*) is imperative for developing management strategies to cope with future climate.

The process of cold hardening in fall is triggered by shortened photoperiod and cool temperatures (Weiser, 1970; Greer & Warrington, 1982; Senser & Beck, 1982; Beck *et al.*, 2004; Baxter, 2014). Because photoperiod will not be affected by climate change, most coniferous species will always develop a limited level of cold hardiness during the onset of fall (Greer *et al.*, 1989). However, cool-temperature cues are much more effective in

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acclimating plants to subfreezing temperatures (Lavender *et al.*, 1968; Timmis & Worrall, 1975; Greer & Warrington, 1982; Öquist *et al.*, 2001; Ishizuka *et al.*, 2015). Thus, warmer temperatures during fall may delay cold hardening (Guak *et al.*, 1998). Prior to cold hardening, conifers are especially at risk to cold injury from extreme frost events (Timmis *et al.*, 1994), which will continue to occur in the future even with climate change (I.P.C.C., 2013). Consequently, a combination of warming temperatures and increased climate variability may render conifers *more* susceptible to cold injury.

Assisted migration of species and populations (also referred to as 'seed sources') involves moving organisms from their historical range to new locations, and is a potential strategy for adapting forests to future climates (Richardson *et al.*, 2009). Assisted migration is based on the concept that, due to rapid shifts in climate, local populations will have suboptimal growth and increased mortality due to adaptational or migrational lag times (Davis & Botkin, 1985; Mátyás & Yeatman, 1992; Lynch & Lande, 1993). Therefore, populations should be transferred from their current locations to areas that are predicted to have optimal climate in the future (Rehfeldt *et al.*, 1999, 2014b). In terms of cold hardiness, populations transferred from warmer to cooler climates should experience more cool-temperatures cues in fall, thus facilitating the cold hardening process and reduce fall cold injury. However, transferred populations will also experience cooler winter minimum temperatures (in the short term), which may increase the risk of winter cold injury owing to maladaptation (Rehfeldt, 1983; St. Clair & Howe, 2007). This potential consequence was realized in 1985, when 300–400 km^2 of translocated *Pinus pinaster* (from the Iberian Peninsula to France) experienced severe cold injury following an extreme frost event (Benito-Garzón *et al.*, 2013). Nevertheless, as winter climate warms, the short-term risk to maladaptation should correspondingly decline as well. Consequently, the planning horizon, transfer distance, and level of acceptable risk all need careful consideration when developing seed transfer guidelines (Howe *et al.*, 2003; Gray *et al.*, 2011).

Common garden genecology tests provide the opportunity to identify how seed sources have diverged in their ability to withstand cold temperatures as a result of climate-related natural selection (St. Clair, 2006). However, single common garden studies have limitations because actual cold hardiness *in situ* is a function of both genetic (G) *and* environmental (E) cues (Burdon, 1977; Cooper & Delacy, 1994). In addition, genotype × environment interactions (G × E) can be common (Jermstad *et al.*, 2003), meaning that up to 50% of variation in traits could be attributable to E or G × E (Campbell & Sorensen, 1978; Howe *et al.*, 2003). Relatively few common garden studies looking at cold hardiness of conifers have used multiple test environments to integrate the effects of G, E, and G × E, which is necessary to fully understand the mechanisms driving phenotypic expression (Hermann & Lavender, 1968; Monserud & Rehfeldt, 1990; O'neill *et al.*, 2000).

Widely distributed species that may be subject to assisted migration, such as coast Douglas-fir, are ideal for common garden studies because the species range spans diverse climatic conditions (e.g., minimum cold month temperatures from −9.7 °C to +10.7 °C), leading to high genetic differentiation among populations (Campbell & Sorensen, 1973; Kleinschmit & Bastien, 1992; Rehfeldt *et al.*, 2014c; Bansal *et al.*, 2015). We conducted a common garden study replicated three test environments on coast Douglas-fir using 35 populations collected from seven geographic regions to test the hypotheses: (i) cool-temperature cues in fall are necessary to trigger the induction of cold hardening, (ii) there is large genetic variation among populations in cold hardiness that can be predicted from seed-source climate variables, (iii) observed differences among populations in cold hardiness *in situ* are dependent on effective environmental cues, and (iv) movement of seed sources from warmer to cooler climates increases the risk of cold injury. This research will allow us to better understand, predict, and plan for the impacts of climate change on conifer cold hardiness.

Materials and methods

Experimental design

We evaluated cold hardiness using artificial freeze tests from samples collected in three of nine common gardens of a Douglas-fir reciprocal transplant study (called the Douglas-fir Seed-Source Movement Trial, SSMT) in the Pacific Northwest region of USA (Fig. 1). We selected these three common gardens (hereafter referred to as the cool, moderate, and warm test sites) to provide the greatest range of environmental test conditions with regard to annual temperature and precipitation (Table 1). The cool and moderate test sites were in Washington, and the warm site was in Oregon. In comparison with other sites, the northern most (cool) test site was at the highest elevation and had the longest, coolest winters and cool summers. The moderate test site was lower in elevation than the cool site (442 vs. 853 m, respectively; Table 1) and closer to the ocean and therefore had a relatively coastal climate. The southernmost (warm) test site had a more continental climate, with relatively warm annual and seasonal temperatures, less precipitation, and minimal precipitation as snow compared to the other test sites. Notably, the ending date of the frost free period and number of frost free days were similar between the moderate and warm test sites.

The seed sources used for the study were chosen based on an earlier genecological study with Douglas-fir (St. Clair *et al.*,

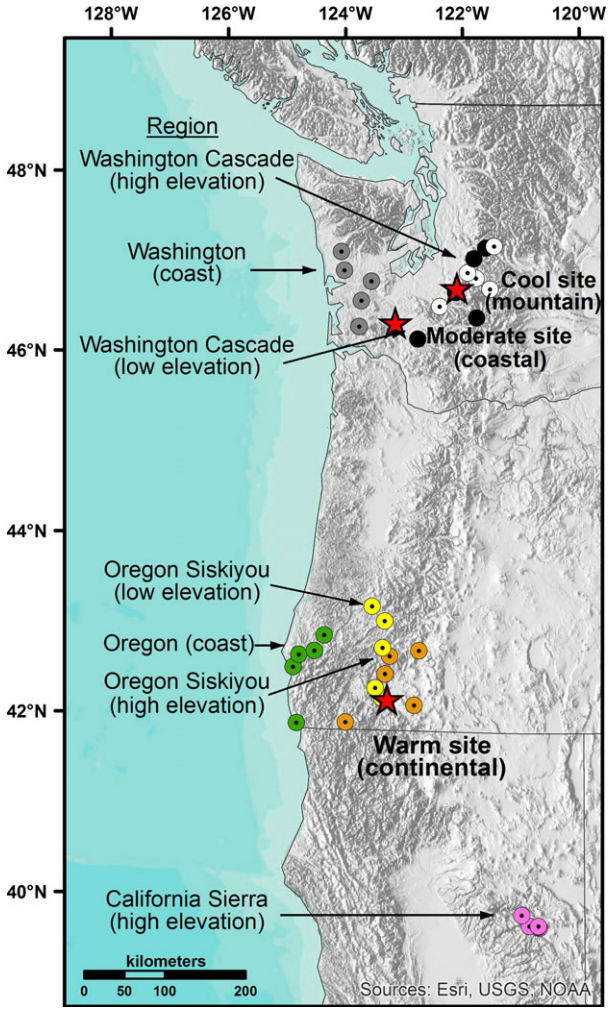

Fig. 1 Locations for each of the three common garden test sites (stars) and locations where seeds were collected for each of 35 populations (circles) of coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) from seven regions (five populations per region). The cool, moderate, and warm tests sites are labeled as 'mountain', 'coastal', or 'continental', respectively, to describe their respective climate types. Each region is labeled as 'high elevation', 'low elevation' and 'coast' to indicate the relative locations of each region. Populations from a region are color coded as follows: Washington Cascade (high elevation) = white; Washington Cascade (low elevation) = black; Washington (coast) = gray; Oregon Siskiyou (high elevation) = orange; Oregon Siskiyou (low elevation) = yellow; Oregon (coast) = green; California Sierra (high elevation) = pink.

2005) and for comparison to a companion study on drought resistance using the same study design and study trees (Bansal *et al.*, 2015). Seeds were collected from 35 populations across seven regions (five populations per region) in Washington, Oregon, and California (Fig. 1; see Methods S1 for scheme of experimental design). Each population consisted of two open-pollinated parent trees within a stand in a similar microclimate (same aspect and elevation), but separated by at least 100 m to minimize relatedness. Progeny from each parent tree were tracked separately (referred to as families). The latitude, longitude, and elevation at each source location and test site were used to estimate climate parameters using ClimateWNA (Wang *et al.*, 2012) (Table 1; see Tables S1 and S2 for regional and population climate data).

Table 1 Geographic and climatic descriptions of three, coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*), common garden test sites (cool, moderate, or warm) during the five-year study period (2008–2011) that the trees were growing at the field sites. The cool, moderate, and warm tests sites are labeled as 'mountain', 'coastal', or 'continental', respectively, to describe their respective climate types

Geography/ climate	Cool site (mountain)	Moderate site (coastal)	Warm site (continental)
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
Fall photoperiod (min)	632	633	646
MAT (°C)	7.1	9.8	12.5
MCMT (°C)	0.0	2.3	3.3
FFP (days)	144	201	203
eFFP (DOY)	282	308	307
PAS (mm)	261	74	13
EMT (°C)	−21.7	−17.1	−16.3
MWMT (°C)	15.9	18.0	23.2
TD (°C)	15.9	15.7	19.9
MAP (mm)	1910	1648	521
MSP (mm)	400	272	85
SHM (°C mm^{-1})	42	72	292
CMD (mm)	229	294	747

Abbreviations: MAT, mean annual temperature; MCMT, mean cold month temperature; MWMT, mean warm month temperature; TD, continentality (MWMT-MCMT); FFP, frost free period; eFFP (DOY), ending day of year of frost free period; PAS, precipitation as snow; EMT, extreme minimum temperature; MAP, mean annual precipitation; MSP, mean summer precipitation (May–September); SHM, summer heat-to-moisture index (MWMT/(MSP/1000)); CMD, aridity (Hargreaves climatic moisture deficit). All data were obtained from ClimateWNA (Wang *et al.*, 2012). Photoperiod data were for 15 October calculated on www.internetsv.info/PhotoPeriodC.html .

In 2008, two-year-old container-grown trees were planted in a complete block design with trees from the same region planted randomly in plots within each block. Blocks were replicated four times at each test site with a buffer zone of trees to avoid edge effects. Within each plot, trees were arranged in four rows with five trees per row at a 2.7 m square spacing.

Tissue sampling and cold hardiness tests

Branch samples were collected on two sampling dates in fall 2012 from each of the three common gardens. The first

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sampling date was chosen to correspond to the expected date of first fall frost for the cool test site (termed 'early fall', October 4, 2012), and the second sampling date occurred between the expected day of first fall frost for the moderate and warm test sites (termed 'late fall', October 30, 2012) as determined from ClimateWNA. Prior to the first and second sampling date, the cool test site experienced 10 and 75 h below 2 °C, respectively, and the warm test site experienced 0 and 6 h of temperatures below 2 °C, respectively. The moderate test site did not experience any degree hours below 2 °C prior to either sampling date.

Within each block, two trees per population (one from each of the two families) were selected for tissue sampling. From each tree, we clipped 6- to 8-cm-long shoot tips from four, healthy lateral branches that were positioned at the mid-portion of the crown on the northeast side of the tree. Branches that were undergoing second flush were avoided when possible because of the influence of second flushing on cold hardiness (Anekonda *et al.*, 1998). Branch samples were sealed in airtight plastic bags and stored in a cooler until freeze tests were conducted 1–5 days after collection.

We assessed cold hardiness of samples using standard freezer-test methods commonly used in forestry to predict seedling tolerance to subfreezing temperatures and extreme cold events (Warrington, 1980; Ritchie, 1984; Rietveld & Tinus, 1987; Anekonda *et al.*, 2000). Extreme cold events also drive natural selection for cold hardiness and cause the resultant genetic variation among population. Branch samples (bud, needle, and stem tissues separately) were exposed to a range of subfreezing temperatures in a programmable freezer, which was followed by visual assessment of cold damage as the percentage of each tissue type showing injury. In addition, we calculated bud LT_{50} (the temperature at which 50% of tissues were damaged), which corresponded well with mean damage scores (Fig. S1; see Methods S2 for details on freeze test methods and LT_{50} calculations). LT_{50} allows managers an alternative measure to visualize how extreme cold events might affect cold injury across the landscape.

Data analysis

We conducted a mixed-model two-way analysis of variance (ANOVA) (SAS, v9.4, SAS Institute Inc., Cary, NC, USA) to assess differences in cold damage for bud, needle, and stem tissues among common garden test sites, populations, and their interactions. Test site and population were fixed factors; block nested within test sites and plots nested within block were random factors. Separate ANOVAS were conducted for each of the two sampling dates in early and late fall. We conducted an additional two-way ANOVA on bud LT_{50} to assess the effects of sampling date, test site, and their interactions because bud LT_{50} values were comparable across sampling dates. Mean damage scores by population for each tissue type across all four temperatures and bud LT_{50} were used as the response variable for statistical analyses.

To evaluate whether variation in cold damage was associated with climate for each population where seeds were collected (i.e., seed-source climate), we performed correlation and multiple regression analyses between tissue damage scores and seed-source climate variables (R version 3.0.1; R Core Development Team, Vienna). Pearson correlation coefficients were significantly different than zero for $|r| > 0.33$ at $P < 0.05$, for $|r| > 0.43$ at $P < 0.01$, and for $|r| > 0.53$ at $P < 0.001$. Seed-source climate variables were chosen to represent the range of annual and seasonal climate conditions with biological relevance and limited autocorrelation, including MAT (mean annual temperature), MCMT (mean cold month temperature), eFFP (ending date of the frost free period), PAS (precipitation as snow), MWMT (mean warm month temperature), TD (continentality, MWMT-MCMT), and CMD (Hargreaves climatic moisture deficit, a measure of aridity when evaporative demands exceed available soil moisture). Warm season variables were used because summer conditions and soil moisture stress have been linked to cold hardiness (Blake *et al.*, 1979; Villar-Salvador *et al.*, 2013). Damage scores were constrained between 0 and 100% damage and modeled using generalized linear models with a beta error distribution and a logit link function. Bud LT_{50} values were modeled using linear models (with Gaussian error distributions). Models were selected based on AIC_c scores. We performed separate analyses for each test site and sampling date to understand whether potentially adaptive relationships differed under conditions of greater cold stress. The regression equation predicting bud LT_{50} at the cool test site in late fall was used to map genetic variation in cold hardiness across the Douglas-fir range in the Pacific Northwest in ArcGIS v10.1 (ESRI, Redlands, CA, USA).

We performed correlations to determine whether cold damage was related to other plant traits including height, diame-

Table 2 Results from two-way ANOVAS (*F* and *P* values) testing for the effects of common garden test site (cool, moderate, and warm), population (n = 35), and their interaction in early fall (October 4, 2012) and late fall (October 30, 2012) on cold damage of bud, needle, and stem tissues of coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*)

	Early fall						Late fall					
	Bud		Needle		Stem		Bud		Needle		Stem	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Site	0.49	0.633	6.07	0.037	3.53	0.103	6.35	0.033	6.93	0.028	1.33	0.333
Population	6.38	<0.001	2.79	<0.001	4.82	<0.001	7.11	<0.001	3.86	<0.001	3.27	<0.001
Site × Population	1.81	<0.001	1.33	0.061	1.29	0.100	1.56	0.013	1.61	0.008	0.99	0.521

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ter, date of bud set and bud burst, transpiration$_{min}$ (a measure of the rate of water loss after stomatal closure), and leaf mass area (LMA, represents leaf mass relative to leaf surface area for water loss relative). Height, diameter, and phenological measurements were collected in 2012 as part of the larger SSMT study. Bud set was recorded only at the moderate and cold test sites in 2012. Transpiration$_{min}$ and leaf mass area (LMA) were collected as part of a companion study on the same populations earlier in the 2012 growing season to assess drought resistance of Douglas-fir (Bansal *et al.*, 2015). We used the correlation coefficients (r values) to compare the direction and strengths of the correlations.

The effect of transferring populations from their native seed-source climate to our test site conditions on tissue damage was assessed by correlating damage scores to the climate transfer distance. Climate transfer distance was calculated as the difference between test site and seed-source climate variables. Climate variables for test sites were averaged over the 5-year period (2008–2012) that the study trees were growing in the field, and were obtained from ClimateWNA. Separate correlations were conducted for each tissue type and sampling date; the addition of a quadratic term in the models did not significantly improve model fit. All tests met the assumptions of normality and homoscedasticity of error variance.

Results

Variation in cold hardiness among test sites, populations, and sample dates

Test sites did not differ significantly for cold damage in the early fall except for needle tissue, which was very high (nearly 100%) at the warm test site (Table 2, Fig. 2). Test sites were significantly different for bud damage and needle damage in the late fall with greater damage found at the moderate site (Fig. 2). Populations differed significantly for cold damage of bud, needle, and stem tissues

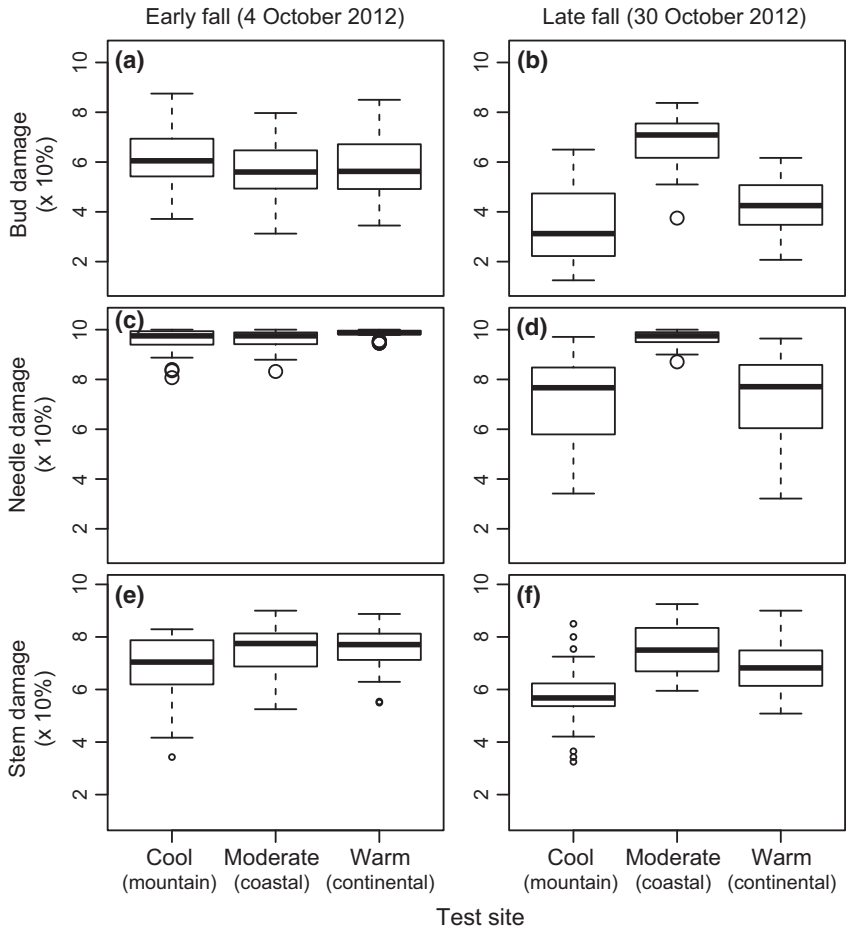

Fig. 2 Box-and-whisker plot of bud (a, b), needle (c, d), and stem damage (e, f) for each of three common garden test sites following freeze tests in early and late fall. The cool, moderate, and warm tests sites are labeled as 'mountain', 'coastal', or 'continental', respectively, to describe their respective climate types. 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.

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Table 3 Pearson correlation coefficients (r values) for relationships between cold damage of buds, needles, and stem tissues and climate variables associated with the location where seeds for coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) were collected (n = 35 populations). Separate correlations are shown for each sampling date (early fall (October 4, 2012) and late fall (October 30, 2012)) at each of three common garden test sites

		Bud		Needle		Stem	
Test site	Climate	Early fall	Late fall	Early fall	Late fall	Early fall	Late fall
Cool	MAT	**0.72**	**0.62**	0.49	**0.70**	**0.60**	0.50
	MCMT	**0.81**	**0.79**	0.47	**0.63**	**0.69**	**0.68**
	eFFP	**0.78**	**0.78**	0.45	0.47	**0.72**	**0.64**
	PAS	**−0.59**	−0.50	−0.37	**−0.64**	**−0.54**	−0.39
	MWMT	0.34	0.17	0.38	**0.66**	0.29	0.10
	TD	**−0.61**	**−0.73**	−0.20	−0.15	−0.52	**−0.66**
	CMD	0.04	−0.18	0.33	**0.57**	−0.10	−0.06
Moderate	MAT	0.53	**0.59**	**0.53**	0.17	**0.60**	**0.58**
	MCMT	**0.62**	**0.67**	0.42	0.10	**0.67**	**0.57**
	eFFP	**0.62**	**0.70**	0.37	0.14	**0.66**	0.55
	PAS	−0.50	**−0.64**	**−0.63**	−0.20	**−0.63**	−0.51
	MWMT	0.24	0.37	**0.60**	0.32	0.32	0.50
	TD	−0.49	−0.42	0.03	0.16	−0.46	−0.22
	CMD	0.04	0.10	0.41	0.31	0.02	0.35
Warm	MAT	0.46	**0.71**	0.45	**0.63**	0.47	**0.57**
	MCMT	0.48	**0.70**	0.38	0.50	0.50	**0.58**
	eFFP	0.48	0.58	0.33	0.38	0.38	**0.69**
	PAS	−0.40	**−0.61**	−0.24	**−0.55**	−0.45	−0.43
	MWMT	0.19	0.50	0.39	**0.70**	0.15	0.36
	TD	−0.37	−0.35	−0.09	0.04	−0.43	−0.34
	CMD	−0.16	0.32	0.21	**0.63**	−0.07	0.02

Abbreviations: MAT, mean annual temperature; MCMT, mean cold month temperature; eFFP, ending date of the frost free period; PAS, precipitation as snow; MWMT, mean warm month temperature; TD, continentality (MWMT-MCMT); CMD, aridity (Hargreaves climatic moisture deficit). Climate variables were derived from ClimateWNA. Correlations were significantly different than zero for $|r| > 0.33$ at $P < 0.05$, for $|r| > 0.43$ at $P < 0.01$, and for $|r| > 0.53$ at $P < 0.001$ and are in bold text (n = 35 for each correlation).

on both sample dates in the early and late fall (Table 2). The population × site interaction was significant for bud damage on both sampling dates, although the F-values for the interactions were considerably less than the F-value for population differences; thus, cold damage of populations may have somewhat different ranks dependent upon the test site, but the differences were not large and the direction of the relationships remained consistent. Bud LT_{50} decreased at all three test sites between the first and second sample dates (−15.8 ± 0.3 and −20.9 ± 0.6 °C, respectively; $F = 239.26$, $P < 0.001$). There was also a significant site × date interaction for bud LT_{50} ($F = 20.65$, $P < 0.001$), which was due to relatively high bud LT_{50} temperatures at the moderate test site (−19.0 ± 0.7 °C) compared to the cool (−22.2 ± 0.5 °C) and warm (−21.5 ± 0.4 °C) test sites, but only on the second sample date.

Damage-climate relationships

Populations from cooler climates had less cold damage; damage scores from all three tissues positively correlated with MAT, MCMT, and eFFP, and negatively with PAS (Table 3, Fig. 3). Climate variables related to warm summer temperatures (MWMT) and aridity (CMD) generally had weaker relationships to cold damage compared to winter climate variables. Even with relatively high damage scores for needles, there was sufficient variation in damage scores to identify relationships between cold damage and seed-source climate variables. Needle damage was positively correlated to MWMT and CMD, particularly during the second sampling date in late fall at the warm test site. Bud and stem (but not needle) damage was negatively correlated to TD, and the strengths of the negative correlations were stronger at the cool test site than at the moderate or warm test sites.

In the multiple regression analyses, MCMT or eFFP was included in the majority of the models, with populations from locations with colder winters and earlier frost free periods having less cold damage or lower LT_{50} temperatures (Table 4). The variable CMD with a negative coefficient was included in models for bud and stem tissue indicating that populations from more

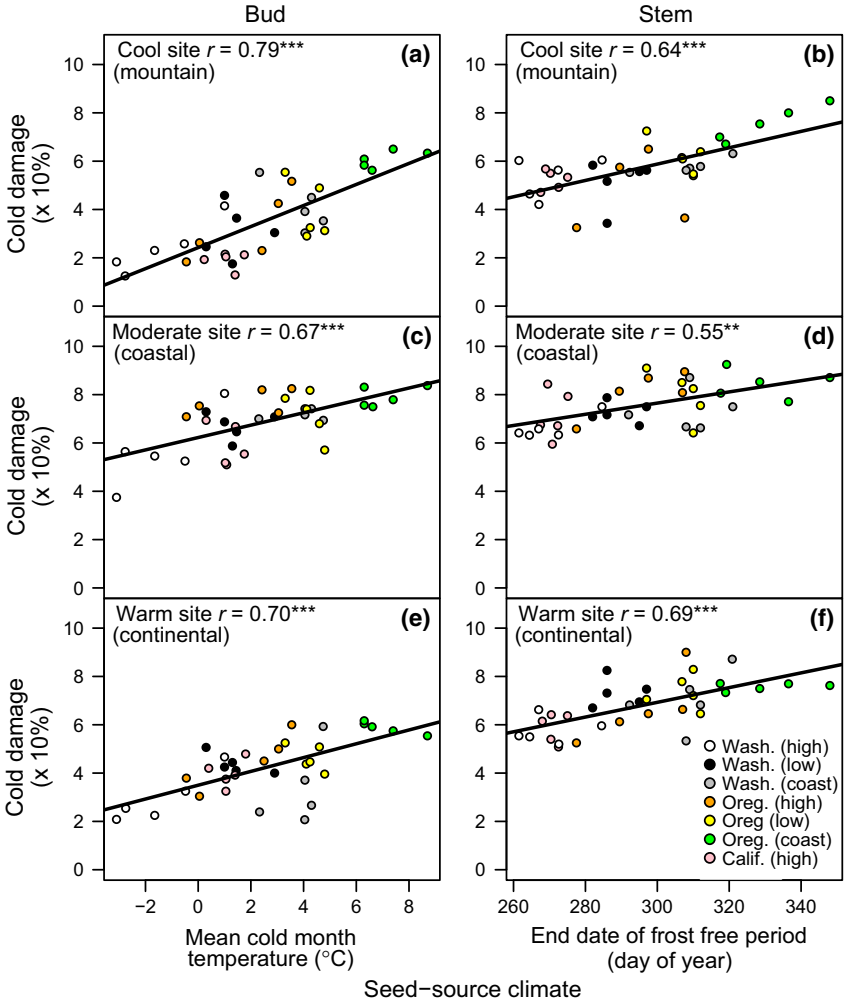

Fig. 3 Relationships between bud damage and mean cold month temperature (left panels), and between stem damage and the ending date of the frost free period (right panels) of the location where seeds for each population were collected (seed-source climate) for 35 coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) populations at each of three common garden test sites (cool, moderate, and warm). The cool, moderate, and warm tests sites are labeled as 'mountain', 'coastal', or 'continental', respectively, to describe their respective climate types. Populations from a region are color coded as following legend in panel f. Damage scores were from the sampling date in late fall (October 30, 2012). Lines with associated *r* values indicate significant correlations between damage scores and climate variables for each test site separately. Significance of correlation coefficients: $^{**}P < 0.01$ and $^{***}P < 0.001$. Correlations follow Table 3.

arid climates had less cold damage or lower LT_{50} temperatures (Table 4). Regression equations with the highest explanatory values were strongest from the cool test site after both sampling dates and from the warm test site after the second sampling, and weakest from the moderate test site after both samplings and the warm test site after the first sampling.

Geographic models of predicted bud LT_{50} temperatures showed populations that were predicted to be most cold hardy were located in areas with cold winter climates, such as the eastern mountainous regions along the Cascade Crest and the California Sierra Nevada (Fig. 4). Relatively more cold hardy populations were also located in relatively dry regions with cool winters, such as throughout the mountains of the California Pacific Coast Ranges. The least cold hardy populations were located along the Pacific coast, particularly in California and southern Oregon. Populations at lower elevations of the Cascades and Sierras, as well as inland coastal mountains in Oregon and Washington, were predicted to have intermediate LT_{50} temperatures, with hardier populations located on the leeward side of the coastal mountains.

Damage-trait relationships

Bud, needle, and stem damage all correlated positively with basal diameter and height, indicating that

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Table 4 Results from regression and coefficient of determination (R^2) values at three common garden test sites for relationships between bud, needle, and stem damage, and bud LT_{50} with seed-source climate of 35 populations of coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). Damage scores were logit-transformed. Removing climate variables in italics increased model AIC*c* by >2 and bolded variables increased AIC*c* by >10, with larger increases in AIC*c* indicating greater explanatory power of the predictor variable

Site	Date	Tissue	Regression equation	R^2
Cool	Early fall	Bud	0.23 + 0.16(**MCMT**)−0.0004(CMD)	0.68
		Needle	2.71 + 0.15(*MCMT*)	0.30
		Stem	−2.28 + 0.30(**MWMT**)−0.002(**CMD**)−0.09(*TD*)	0.60
		LT_{50} bud	−15.63 + 0.38(**MCMT**)−0.001(CMD)	0.52
	Late fall	Bud	−0.53 + 0.18(**MCMT**)−0.0008(CMD)	0.71
		Needle	3.61 + 0.004(**CMD**)−0.24(**TD**)	0.60
		Stem	2.79–0.16(**TD**) + 0.0009(*CMD*)	0.62
		LT_{50} bud	−40.89 + 0.09(**eFFP**)−0.45(*MWMT*)	0.51
Moderate	Early fall	Bud	−3.70 + 0.013(**eFFP**)	0.38
		Needle	2.92 + 0.002(*eFFP*)−0.002(*PAS*)	0.19
		Stem	−2.05 + 0.011(*eFFP*)−0.0007(PAS)	0.47
		LT_{50} bud	−26.46 + 0.036(*eFFP*)	0.24
	Late fall	Bud	−2.22 + 0.011(*eFFP*)−0.0006(PAS)	0.55
		Needle	2.17 + 0.002(**CMD**)+0.002(**eFFP**)	0.23
		Stem	−2.86 + 0.013(**eFFP**)+0.001(*CMD*)	0.45
		LT_{50} bud	−13.65 to 0.31(TD)	0.06
Warm	Early fall	Bud	−4.24 + 0.33(**MWMT**)−0.003(**CMD**)	0.39
		Needle	1.10 + 0.17(*MWMT*)	0.19
		Stem	8.6–0.3(**TD**)+0.4(**MWMT**)−0.03(*eFFP*)-0.002(*CMD*)	0.47
		LT_{50} bud	−9.1 to 0.46(*TD*)	0.17
	Late fall	Bud	−0.50 + 0.13(**MCMT**)	0.49
		Needle	−4.23 + 0.002(CMD)+0.016(eFFP)	0.52
		Stem	−7.95 + 0.024(**eFFP**)+0.14(*TD*)−0.0006(CMD)	0.56
		LT_{50} bud	−20.65 to 0.005(**PAS**)	0.34

Abbreviations: LT_{50}, temperature that 50% of tissues are cold damaged; MCMT, mean cold month temperature; CMD, aridity (Hargreaves climatic moisture deficit); MWMT, mean warm month temperature; TD, continentality (MWMT-MCMT); eFFP, ending frost free period; PAS, precipitation as snow; climate variables were derived from ClimateWNA.

populations with greater growth rates generally experienced more cold damage (Table 5). Date of bud set was positively related with cold damage of bud and stem tissues (but less so for needle), primarily at the moderate test site in early fall. Budburst generally had weak or no significant correlative relationship with cold damage. $Transpiration_{min}$ was positively related to cold damage, particularly in early fall at the cool test site for bud and stem damage (Table 5). Leaf mass area was generally uncorrelated to cold damage.

Climate transfer distance

Cold damage of all tissues generally increased or was unchanged when moving populations from a warmer seed-source climate to a cooler growing environment. The effects of climate transfer distance on tissue damage were most apparent (i.e., steepest slopes and highest *r* values) when considering the differences between test site and seed-source MCMT (Fig. 5), whereas transfer effects of eFFP, PAS, MWMT, or CMD had minimal or no impact on tissue damage (data not shown). The impact of MCMT transfer distance was stronger for buds than for needles or stems, and stronger at the first compared to second sampling date (Fig. 5). For buds in early fall, moving seed sources result in 2–3% increase in cold damage on average for every 1 °C decrease in MCMT of the planting environment.

Discussion

As climate changes, cool-temperature cues that are needed for cold hardening may occur later in the fall due to warming temperatures (Abatzoglou *et al.*, 2013), thus dramatically increasing risk to stand-level cold damage from fall frost events (Duffield, 1956; Grier, 1988). Typically, populations located at the southern edge of a species' distribution with relatively 'warm' climate are predicted to be at greatest risk to the effects

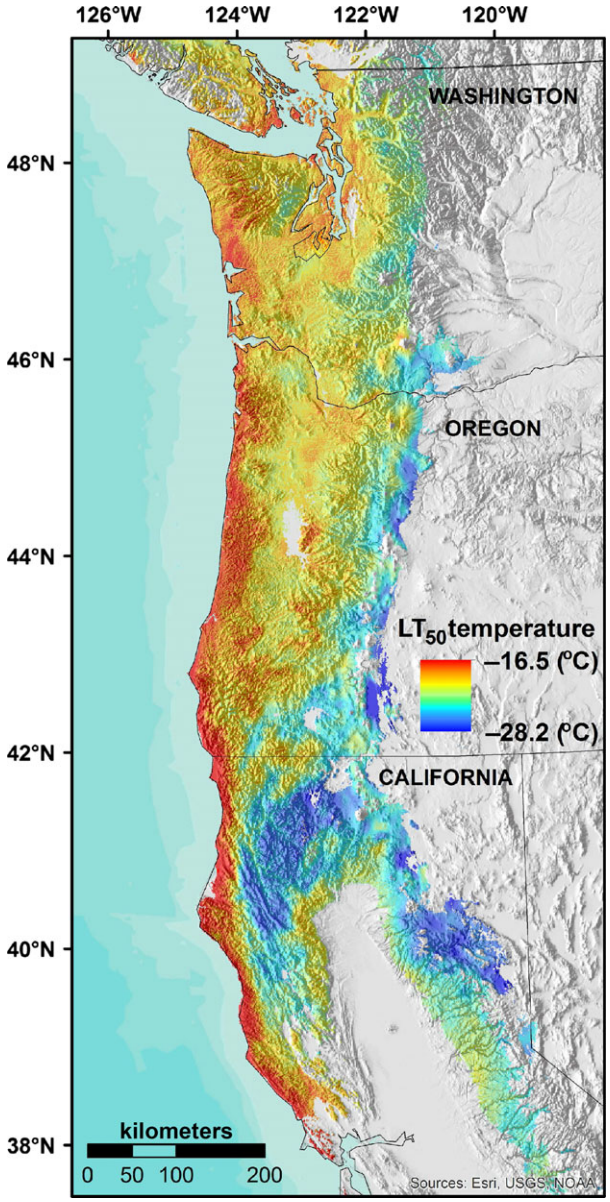

Fig. 4 Geographic genetic variation of bud LT_{50} temperatures of coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) in the Pacific Northwest, USA. The regression model used to predict bud LT_{50} temperatures was from the late fall (October 30, 2012) sampling date at the cool (mountain) test site following Table 4.

of climate warming (Gray *et al.*, 2011; Rehfeldt *et al.*, 2014a), although some studies have found differing trends with Douglas-fir (Chen *et al.*, 2010). We found that trees growing in a more central location with a moderate climate had the fewest cool-temperature cues and therefore greater cold damage compared to the sites located in warmer or cooler test environments. Consequently, changes in climate that are specific to minimum temperatures during the fall season will likely have the greatest impacts on risk to cold damage. Moreover, these results demonstrate how quantification of risks needs to account for underlying biological mechanisms and complex plant–climate relationships that are often more influenced by season-specific environmental conditions than annual averages.

If the expression of traits among seed sources is context dependent on test environmental conditions (i.e., G × E interaction), then developing models predicting the impacts of climate change on cold hardiness based on single common garden studies may have limited scope (Burdon, 1977; Campbell & Sorensen, 1978). In our study, the expression of cold hardiness among populations was relatively strong at the coolest test site, but was nevertheless generally similar across test sites (i.e., strong G effects and weak G × E interactions). Mean cold month temperature of the seed-source nearly always had the strongest relationship to cold damage (i.e., plants originating from cooler climates had 2–3 times less cold damage than those from warmer climates), even at the moderate test site that had minimal cool-temperature cues. The importance of cool, winter temperatures as a potential driver of natural selection has been confirmed not only for cold hardiness (Benowicz *et al.*, 2001; Bower & Aitken, 2006; St. Clair, 2006), but also for many other adaptive and growth traits in Douglas-fir and in other conifers (Campbell, 1979; Oleksyn *et al.*, 1998; Rehfeldt *et al.*, 2014c; Bansal *et al.*, 2015). As local climates warm, populations may be 'over-adapted' with respect to their genetically based tolerance to minimum winter temperatures, and therefore, have less winter cold injury. This potential scenario is demonstrated well in Fig. 5, in which a transfer of populations from cooler seed-source climates to planting environments with warmer winters had lower damage scores.

Developing seed transfer guidelines that are oriented toward future climate typically involves moving populations from warmer to cooler climates such as up in elevation or in latitude (Balduman *et al.*, 1999; St. Clair & Howe, 2007), perhaps beyond their natural ranges (i.e., assisted migration) (Mclane & Aitken, 2011). For contemporary populations of Douglas-fir, optimal growing conditions are predicted to be 500–1000 m above their current elevational range by 2060 (St. Clair & Howe, 2007; Rehfeldt *et al.*, 2014b). In the short term (i.e., prior to 2060), an upward shift would cause transferred populations to experience cooler fall and winter temperatures (depending on the transfer distance) than those that they have evolved under. Our results showed how cooler fall temperatures can improve cold hardening and effectively reduce cold injury, whereas cooler winter temperatures have potential to increase cold injury. Nevertheless, the potential increase in cold injury appears relatively small and short term (Fig. 5).

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Table 5 Pearson correlation coefficients (*r* values) for relationships between cold damage of bud, needle, and stem tissues on two sampling dates [early fall (October 4, 2012) and late fall (October 30, 2012)] and other traits at each of three common garden test sites (cool, moderate and warm) with 35 coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) populations

		Bud		Needle		Stem	
Test site	Traits	Early fall	Late fall	Early fall	Late fall	Early fall	Late fall
Cool	BD	**0.57**	**0.57**	0.46	0.12	**0.71**	0.38
	HT	0.38	0.36	0.14	−0.37	0.46	0.22
	BS	0.24	0.24	0.01	−0.40	0.27	0.12
	BB	0.01	−0.06	−0.20	**−0.59**	0.03	−0.17
	$Transp_{min}$	**0.59**	0.51	0.360	0.15	**0.57**	0.45
	LMA	−0.32	−0.35	−0.09	0.39	−0.39	−0.16
Moderate	BD	0.45	**0.54**	0.20	0.13	0.52	0.41
	HT	0.48	0.39	−0.02	−0.15	0.49	0.13
	BS	0.48	0.22	0.05	−0.24	0.46	−0.12
	BB	0.00	−0.11	−0.13	−0.28	0.08	−0.42
	$Transp_{min}$	0.29	0.41	0.07	0.10	0.39	0.43
	LMA	−0.30	−0.15	0.11	0.32	−0.28	0.11
Warm	BD	0.47	0.42	0.40	0.46	0.46	0.5
	HT	0.38	0.22	0.20	0.02	0.52	0.30
	BS*						
	BB	0.08	−0.39	−0.31	**−0.62**	−0.06	0.03
	$Transp_{min}$	**0.56**	0.00	0.10	−0.32	0.32	0.45
	LMA	−0.33	−0.01	−0.12	0.44	−0.38	−0.22

Abbreviations: BD, basal diameter (2012); HT, height (2012). BS, bud set (2012); BB, bud burst (2012); $transp_{min}$, minimum transpiration (water loss through cuticle after stomatal closure); LMA, leaf mass area; data for $transp_{min;}$ and LMA were obtained from Bansal *et al.* (2015); *BS data not collected. Correlations were significantly different than zero for $|r| > 0.33$ at $P < 0.05$, for $|r| > 0.43$ at $P < 0.01$, and for $|r| > 0.53$ at $P < 0.001$ and are in bold text ($n = 35$ for each correlation).

For example, a transfer of −3 °C resulted in increased bud damage by only 5–10%. Moreover, winter temperatures are expected to increase relatively quickly in the Pacific Northwest, USA (Abatzoglou *et al.*, 2013), thereby shortening the period of risk to winter cold injury from assisted migration. Consequently, the long-term benefits of optimal climate for growth and greater fall cold hardiness appear to outweigh the short-term costs of increased winter injury from assisted migration.

When modeling cold hardiness of Douglas-fir across its niche, it was clear that topography played an important role in shaping the genetic structure of cold hardiness across the landscape, with high elevation, mountainous regions having greater cold hardiness than coastal or lowland regions (Fig. 4). This geographic pattern of cold hardiness across the Douglas-fir range was remarkably similar to the pattern observed for traits associated with drought resistance (Bansal *et al.*, 2015). At the ecophysiological level, we found that cold damage and drought resistance were inversely related, meaning that populations with relatively high tolerance to cold stress *also* had high tolerance to drought stress, similar to studies on other tree species (Blödner *et al.*, 2005; Schreiber *et al.*, 2011). This seemingly paradoxical phenomenon is likely driven by common selection pressures to cope with desiccation stress (Tranquillini, 1982; White, 1987). During a freeze event, the formation of intercellular ice causes cavitations and cellular dehydration, similar to the effects of drought (Levitt, 1980; Palta & Li, 1980; Hacke & Sperry, 2001). Accordingly, there are several common physiological mechanisms to cope with both stressors (Palta & Li, 1980; Thomashow, 1999; Schreiber *et al.*, 2011; Whalley & Knight, 2013). Previous studies have suggested that climate-related natural selection to cope with winter desiccation has been an indirect driver of adaptation to summer drought for Douglas-fir (White, 1987). However, our best models consistently included both winter and summer seed-source climate variables, demonstrating how selection pressure from both climate extremes likely contributed to the common pattern of genetic geographic differentiation in drought and cold hardiness (Rehfeldt, 1977; Hacke & Sperry, 2001; Schreiber *et al.*, 2011).

Our study shows how the level of cold hardiness expressed by any given population is a product of short-term environmental cues and long-term climate-related natural selection. These findings have several implications for the ecology and management of coast Douglas-fir as the climate changes. First, as

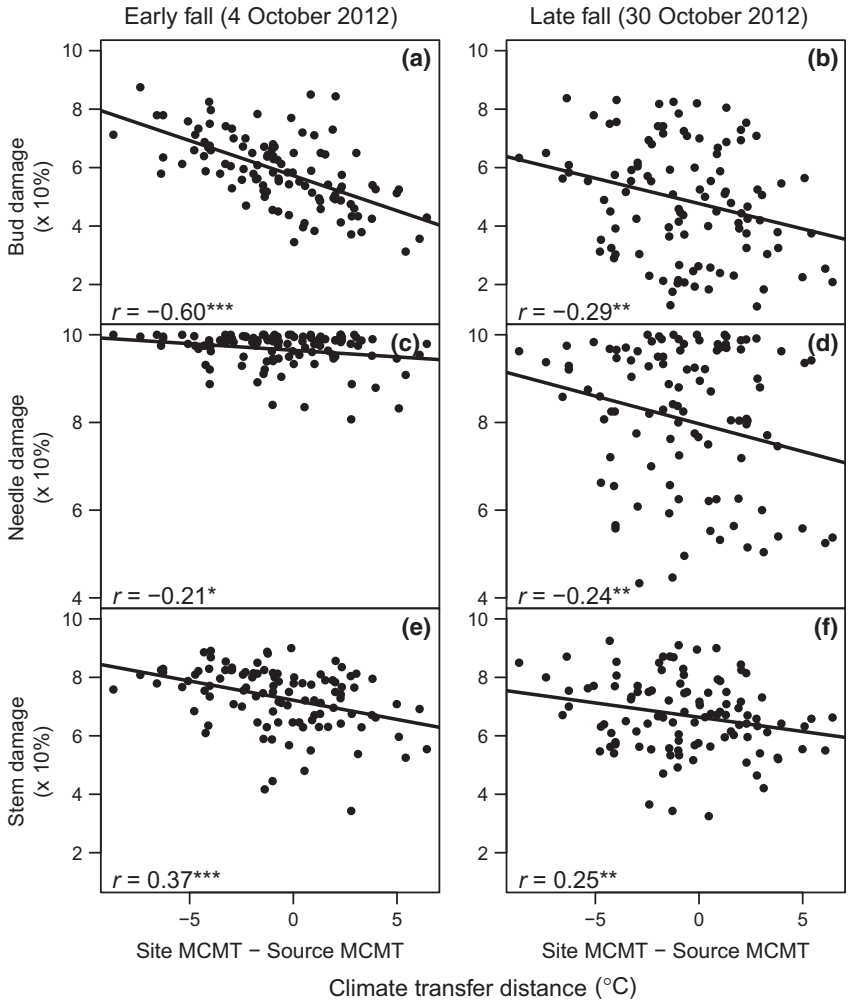

Fig. 5 Bud (a, b), needle (c, d), and stem (e, f) damage as a function of the difference between mean cold month temperature (MCMT) of the common garden test sites and seed-source MCMT (Climate transfer effect) for 35 coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) populations following freeze tests in early (October 4, 2012, left panels) and late (October 30, 2012, right panels) fall. Lines with associated *r* values indicate significant correlations between damage scores and the climate transfer distance. Significance of correlation coefficients: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

the environmental cues that trigger cold acclimation disappear, populations will have delayed cold hardening and increased risk to unseasonal frost events. However, contrary to traditional assumptions, the populations at greatest risk to delayed hardening are not necessarily from regions with the warmest mean annual temperatures, but instead are those from areas with relatively moderate fall seasons. Second, our study design with multiple common gardens demonstrated that the development of cold hardiness was largely influenced by the seed-source climate (i.e., strong G effect), which was consistent across test environment (i.e., limited G × E interactions). The fact that cold hardiness did not exhibit strong G × E interactions gives us increased confidence in the models that predict variation in Douglas-fir cold hardiness across its niche, which facilitates the development and viability of seed transfer guidelines. Finally, even though seed-source movement from warmer to cooler climates does increase the risk to winter cold injury, it appears that coast Douglas-fir populations can tolerate moderate transfer distances without succumbing to high levels of cold damage. This finding supports the prospect of using assisted migration as a viable management strategy to respond to concerns of climate change for coniferous forests. Clearly, season-specific changes in climate have unique impacts on the future of conifer cold hardiness, and therefore, need to be explicitly considered when predicting and planning for the impacts of climate change.

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Supporting Information

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

Table S1. Geographic descriptions and the associated climate data for seven regions where coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) seeds were collected.
Table S2. Geographic descriptions and the associated climate data for 35 populations of coast Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*).
Methods S1. Scheme of experimental design.
Methods S2. Freeze-test protocol and LT_{50} calculations.
Figure S1. Relationships between the temperature that 50% of tissue damage (LT_{50}) and mean damage of bud, needle and stem tissue in early and late fall.

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