Genecology and Phenotypic Selection in Whitebark Pine (*Pinus albicaulis*)
and Ponderosa Pine (*Pinus ponderosa*) Under Warm-Dry Climate

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Dedication

For my loving parents

Sandra and Dennis Warwell

Who gave me all that I needed to succeed

Abstract

To study patterns of adaptation to the contemporary climate and phenotypic selection under future predicted climate in whitebark pine (*Pinus albicaulis*), seedlings from 49 populations representative of the interior northwestern USA were grown in two low-elevation, common-garden field tests over a 12-year period. The primary objectives were to: 1) assess genetic variation in growth and survival among populations (Chapter 1); 2) model this genetic variation in relation to climate at the seed source (Chapter 1); 3) describe phenotypic selection on growth rhythm (Chapter 2); and 4) model growth rhythm in relation to climate of seed source (Chapter 2). Results showed moderate genetic divergence in growth and survival among populations that corresponded with climatic clines. Populations originating from locations with earlier spring warming exhibited greater inherent growth under favorable conditions, while populations originating from locations with lower spring precipitation exhibited greater survival under drought conditions. Selection on measures of growth rhythm was detected and varied between experimental sites and among years. More complex selection on measures of growth rhythm was observed on the experimental site with more favorable growing conditions. Curved directional selection and stabilizing selection tended to occur in earlier years and was chiefly associated with mortality in the year of expression. Differences among seed sources in growth rhythm were mild and were explained to a moderate extent ($r^2 = 0.08 - 0.28$) by variation in temperature or precipitation among seed origins. Individuals originating from milder climates tended to elongate slightly longer through the growing season and exhibit slightly higher shoot elongation rates.

To study phenotypic selection of ponderosa pine (*Pinus ponderosa* Doug.) in response to predicted future climate, seedlings were grown from seed in the field using three drought-imposed treatments under a climate warmer and drier than the climates of seed origins. The seedlings originated from 36 maternal trees from Priest River Experimental Forest, ID and 3 provenances (8 maternal trees per provenance) representative of the interior northwestern USA. The primary objectives were to 1) evaluate the magnitude, form and temporal dynamics of selection on seed and seedling traits in response to variation in the timing of growing-season drought for a single provenance and (2) assess differences in selection among provenances representative of the interior northern USA (Chapter 3). Differences in selection among treatments and provenances on traits associated with drought adaptation were detected and described. For the Priest River provenance, mortality differed among families within provenances. Selection via differential mortality in the year of emergence was particularly strong, while selection in subsequent years was far less pronounced. The form of selection was influenced by timing of drought. Differences in the magnitude and form of selection were also detected among provenances. In addition, patterns of variation for selection among provenances corresponded with dryness of seed origin. These results suggested that during the seed-to-seedling stage, populations within the region will likely differ in their selection response to changes in the timing of growing-season drought.

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Chapter 1: Genetic variation in whitebark pine (*Pinus albicaulis* Engelm.) in the interior northwestern U.S.A: joint analysis of 12- and 10-year survival and growth of 49 populations in common gardens

Abstract

Whitebark pine (*Pinus albicaulis* Engelm.) seedlings from 49 populations

representative of the interior northwestern USA were grown in two low-elevation,

common garden field experiments over a 12-year period. The primary objectives of this

study were to 1) assess genetic variation in growth and survival among the populations

and 2) model this genetic variation in relation to climate at the seed source. Significant

variation among populations was detected for mean annual apical shoot elongation. In

addition, significant variation among populations was detected for survival and mean

height of 14 year-old trees, 10 and 12 years after outplanting. No statistically significant

($P < 0.05$) differences among populations were detected for unconditional expected value

for height which was considered to be the best available approximation of fitness.

Expected values of height were unconditional because they explicitly included survival

over multiple years. A negative trend was apparent for fitness in relation to annual spring

precipitation (SPRP) at seed source. Survival was statistically significant and negatively

related to SPRP at seed source. SPRP accounted for 13.2 % of variation in survival

among populations. Mean annual apical shoot elongation was statistically significant ($P

< 0.05$) and negatively related to the Julian date that summed daily mean temperature $>

5°C$ reached $100°C$ (D100) at the seed source. D100 accounted for 21.3% of population

variation for expected growth under favorable conditions (growth potential). Clinal

variation for annual apical shoot elongation and height generally increased in a southeast

to northwest direction and with decreasing elevation. Conversely, survival and fitness generally decreased in northwest to southeast direction. Clinal patterns were assessed using regression analysis to provide seed transfer guidelines which are directly applicable to forest management decisions regarding gene conservation and reforestation. Reasonable confidence in population differentiation for survival under warm dry climate required ± 26.6 mm SPRP among climate of seed sources. While expected growth under favorable growing conditions (growth potential) required a separation of approximately ± 7.4 Julian days for D100 which is equivalent to about ± 210 m in elevation between seed sources.

Introduction

Many woody plant species are composed of populations that are adapted to a portion of the ecological breadth occupied by the species as a whole. This adaptive variation results from environmental selection acting on inherent variation that originates from processes of evolution which are subject to historic contingency (Morgenstern, 1996). Climate is a principal agent of selection. Its influence on adaptive patterns within plant species is supported by an abundance of common garden studies, including the seminal works of Turesson (1925) and Clausen and Hiesey (1958).

In general, these studies describe patterns of genetic variation in suites of intercorrelated traits within plant species that correspond with climatic variation along geographic gradients. For example, length of growing season and temperature tend to decline with increasing elevation. In accord, progeny collected from populations at

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higher elevations tend to exhibit lower annual height growth and increased cold tolerance in comparison to progeny originating from lower elevations when grown together at a low elevation. Conversely, sources from low elevation tend to exhibit greater annual height growth and reduced cold tolerance in comparison to high-elevation sources when grown together at a high elevation (Campbell, 1979; Rehfeldt, 1989; Rehfeldt, 1991).

The extent that populations within a particular tree species are adapted to environmental variation varies. Tree species have been found to range from generalist, where genetically based phenotypic plasticity is predominant, to specialist, where adaptation to a specific range of environmental conditions is predominant (see Levins, 1968; Rehfeldt, 1994a). For example, in the interior northwestern USA, Douglas-fir (*Pseudotsuga menziesii*) exhibits a specialist mode of adaptation, where its populations exhibit genetic differentiation detectable at a scale of approximately ±100 m in elevation in response to climatic variation (Rehfeldt, 1989). In contrast, western white pine (*Pinus monticola*) appears to exhibit more phenotypic plasticity in relation to climate, and genetic differentiation has not been detected among its interior northwestern populations in a study using similar statistical power (Rehfeldt, Hoff, and Steinhoff, 1984).

Most past genecology studies which assessed intraspecific genetic variation in relation to the environment were motivated to inform reforestation practices for commercial reforestation. Consequently only a few mostly economically important tree species were assessed in relatively productive environments and rarely under harsh climates were survival and growth could be substantially limited (Matyas and Yeatman, 1992). The threat of ongoing rapid climate change, however, necessitates the expansion

of genecology understanding to include more ecologically important plant species as well as the ability to predict their genetic response for survival and growth under potentially new conditions.

Whitebark pine (*Pinus albicaulis* Engelm.) is an upper subalpine species that occupies the cool-wet climate extreme among forest ecosystems of the western USA (Rehfeldt, Ferguson, and Crookston, 2008). It is considered both a keystone (Tomback and Kendall, 2001) and foundational species (Ellison et al., 2005). Its timberline populations modulate snow run off (Farnes, 1990), while its relatively large wingless seeds, which are dispersed predominantly by Clark's nutcracker (*Nucifraga columbiana*) (Tomback, 1978; Hutchins and Lanner, 1982), are also a major food source for many species of mammals and birds (Mattson, Kendall, and Reinhart, 2001).

Whitebark pine populations have been in decline since the mid-20th century due to fire suppression, which has altered successional patterns in its northern Rocky Mountain range, and the introduction of an exotic fungus, white pine blister rust (*Cranartium ribicola*), which has caused mass mortality range-wide (Tomback, Arno, and Keane, 2001). In addition rapid ongoing climate in the North American Pacific Northwest is predicted to lead to warmer winters with drier summers through the century (Mote and Salathé, 2010) and is projected to nearly eliminate whitebark pine's realized climate niche among USA populations by mid-21century (Warwell, Rehfeldt, and Crookston, 2007). In consideration of these and other factors, whitebark pine was assigned by the U.S. Fish and Wildlife Service (2011) to the Endangered Species Act

Candidate List and declared endangered by the Canadian federal government in accordance with the Species at Risk Act (COSEWIC, 2012).

The extent of genetic variation in quantitative traits of whitebark pine and its relationship to climate and geographic distribution have been previously assessed in only a few short-term common garden studies conducted in nursery environments using potted containers or raised beds. These studies report genetic variation among populations for total height (Mahalovich, Burr, and Foushee, 2006; Hamlin et al., 2008; Bower and Aitken, 2008 ; Hamlin, Kegley, and Sniezko, 2011), root and shoot dry mass, date of needle flush (Bower and Aitken, 2008), cold hardiness (Bower and Aitken, 2006; Mahalovich, Burr, and Foushee, 2006; Bower and Aitken, 2008), needle color (Hamlin, Kegley, and Sniezko, 2011) and rust resistance (Mahalovich, Burr, and Foushee, 2006).

The present study examined genetic variation in: 1) early growth under relatively favorable conditions (ie. growth potential) and 2) survival, and fitness over 12 and 10 years under warm-dry climate among whitebark pine populations in the interior northwestern USA, using seedlings from 49 representative populations grown in two low-elevation common garden field experiments. In the present study, unconditional expected height was used as the best available approximation of fitness. Expected values of height were unconditional because they explicitly included survival over multiple years with modeled distribution for either survival in a given year or height in 2012 dependent on the preceding year (see Shaw et al., 2008). In contrast, inferences of total height that do not consider prior mortality which are typically used to assess population performance are considered conditional. Unconditional expected height was evaluated using joint

analysis of survival and mean height of survivors to provide a more complete measure of population fitness than either fitness measure separately.

The primary objectives of this study were to 1) assess genetic variation in growth and survival among whitebark pine populations under conditions where environmental stress is limited and under climate where temperature and moisture availability is approximate to expected future climate change; and 2) model this genetic variation in relation to climate of seed source. Understanding how genetic variation within a species is related to the environment is critical to effective threat assessment, gene conservation and seed transfer guidelines. This work provides insight into the pattern of variation for growth and survival with respect to climate that directly informs gene conservation and reforestation decisions.

Materials and Methods

Plant Materials

The present study used seeds from wind-pollinated cones, collected during 1991 to 1997 from 49 wild whitebark populations in the interior northwestern USA (Fig.1-1, Table 1-1). Collections were made from 5-20 trees per population; however, eight populations were represented by bulk cone collections in which the total number of contributing maternal sources is unknown (Table 1-1). All populations were represented by cones collected in a single year with the exception of Heaven's Gate and Burke Summit, Idaho which used collections from two consecutive years (1995 and 1996). Each population was spatially defined as trees occurring together on a mountain top ridge

and/or in a basin within approximately 3.2 km in horizontal distance and 122 meters in elevation.

Seeds were germinated in 164-ml Ray Leach Cone-tainer™ super cells (Stuewe and Sons, Corvallis, Oregon, USA) in a greenhouse in Lewiston, Idaho in 1998. Following germination, seedlings were maintained in a shelter house in Moscow, Idaho. Seedlings that were later used for Experiment 2 (see below), were transplanted into 656-ml Deepot containers (Stuewe and Sons, Corvallis, Oregon, USA) in summer, 2000.

Planting Sites, Design and Procedure

Common garden field experiments of 46 (Experiment 1) and 42 (Experiment 2) populations, with 39 populations common to both experiments, were outplanted in forest openings at Priest River Experimental Forest in April 24-26, 2000 and October 2-5, 2001, respectively (Fig.1-1, A1-1, and A1-2). The surrounding forest was *Tsuga heterophylla*/*Clintonia uniflora* habitat type (Cooper et al., 1991) at both sites. Both experiment sites were located at relatively low-elevations (671m) where nonnative climate was drier than most seed sources and substantially warmer than all seed sources (Table 1-1). In addition, late summer drought conditions occurred periodically at the experimental sites while drought is hypothesized to rarely occur in the sampled range (Weaver, 2001). Experiment 1 (Exp. 1) was established to assess variation in growth and survival under generally favorable growing conditions. The Exp. 1 site (lat. 48.352°N, long.-116.848°W, elev. 671m) was located in a field that had been used as a forest nursery for decades. Soil was characterized as a coarse, loamy, mixed frigid Andic

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Xerochrept soil (Staff, 1996). During the study from 2001 to 2012, mean annual air temperature and annual precipitation at Exp. 1 site were 6.9°C and 825 mm, respectively (Table 1-2). Air temperature was measured using DS1922 Thermochron® temperature data loggers (Maxim integrated, San Jose, California, USA) with a ±1°C accuracy. These data loggers were located inside radiation shields, product #7714 (Davis Instruments, Hayward, California, USA) which were mounted on fence posts at a height of 1.3716 m (4.5 ft). No less than two data loggers were used at each experiment site for the study duration.

Experiment 2 (Exp. 2) was established to assess variation in growth and survival under growing conditions that contrasted with the Exp. 1 site and more closely resembled whitebark pine's high elevation habitat. The Exp. 2 site (lat. 48.332°N, long. -116.8477°W, elev. 671m) was established on a bench adjoining the Priest River approximately 2.2 km south of the Exp. 1 site. The site was previously used as a Civilian Conservation Corps (CCC) camp and parade ground in the 1930s. The soil was characterized as an ashy, glassy, frigid, Andic Fragiudalf, "Mission series" soil (formally: medial, frigid Ochreptic Fragixera) (Page-Dumroese, 1993). This soil type is characteristically poorly drained and shallow (~25.4 mm to ~50.8 mm) to a fragipan where moisture penetration and root growth are restricted. In addition, the soil at this planting site was embedded with many large (ca. 10-20 mm diameter) cobble stones. These rocky soil conditions were more representative of whitebark pine's native conditions than the deeper and better drained soil at the Exp. 1 site (see Weaver, 2001). The mean monthly air temperature in Exp. 2 was generally cooler than Exp. 1. Monthly

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mean air temperature in 17 months between 2004 -2006 was 6.65°C which was 0.25°C cooler than at the Exp. 1 site (Table A1-1). Exp. 2 site precipitation was similar to Exp. 1 site.

Both experiments used a randomized, complete block design with an initial planting of 30 trees per population. Populations were organized in row-plots with of five trees within each of six blocks in Exp. 1, and row-plots with 10 trees within each of three blocks in Exp. 2. Individual trees in Experiments 1 and 2 were planted using a dibble tool at a spacing of 1 m x 1 m and 35.56 cm x 33.02 cm (14 in x 13 in), respectively. Spacing in Exp. 1 was greater because the planting was intended to be used as an orchard following study conclusion. A border row of non-measured whitebark pine seedlings was planted in each site to equalize competitive effect on measured trees.

Extraneous environmental influences were intensively managed in both experiments. Fencing was used to exclude ungulates and burrowing mammals. Weeds were regularly plowed and removed by hand through 2005. Supplemental irrigation was applied in late summer as needed in 2003 and 2004 on both sites. Thinning to prevent competitive interaction among trees was not necessary despite close planting in Exp. 2 (35.56 cm x 33.02 cm) due to species' characteristic slow growth and high mortality prior to crowding.

Growth and Survival Data and Climate Estimates

Total above ground height measurements on 14 year-old trees were tallied in fall 2012 for both experiments. Survival was tallied in fall 2001-2005, 2011 and 2012, in

Exp. 1 (planted in spring 2000), and 2003-2006, 2010-2012 in Exp. 2 (planted in fall, 2001).

For each population's geographic origin, monthly precipitation and minimum, maximum and average temperature at the source location were inferred using ANUSPLINE version 4.3 as a function of latitude, longitude and elevation (Rehfeldt, 2006 and available at http://forest.moscowfsl.wsu.edu/climate/). These data were then used to calculate derived values for 26 climate variables of demonstrated importance in biology that represent the timing and interaction of temperature and moisture (Rehfeldt, 2006) (Table 1-3). The calculation for these point estimates of climate used data from weather stations in wester n United States and southwestern Canada that were normalized for the period of 1961-90 (Environment Canada, 1994; United States Department of Commerce, 1994).

Statistical Analysis

The degree of differentiation among populations for survival and unconditional expected height was assessed with aster analysis (Geyer, Wagenius, and Shaw, 2007; see Shaw et al., 2008) using the reaster function (Geyer, 2013) in R (R Core Team, 2012), which permits unified analysis of multiple life history stages with appropriate statistical distributions. In particular, the analysis jointly modeled survival to 2012 as well as unconditional expected height to 2012 (Fig.1-2). Conventional inferences of height are conditional on measures of survivors only and do not explicitly take mortality into

account. In the present study, the term unconditional expected height is used in the sense that height is inferred with mortality explicitly accounted for. Thus survival and unconditional expected height were modeled to 2012 using survival beginning in 2001 for Exp. 1 and 2003 for Exp. 2, and Experiments 1 and 2 combined (Fig.1-2). Survival was modeled using Bernoulli and height was modeled using normal distributions. Following the first stage (initial survival), each successor variable was modeled using its predecessor's sample size. Analysis of survival alone excluded height in the final node and retained survival for the year of interest and survival for the year(s) prior to the year of interest. For example, Exp. 1 aster model for survival in 2012 analysis included survival data for all available years from spring 2001 to fall 2012. In contrast, Exp. 1 aster model for survival in 2001 included only survival data for spring and fall 2001 (Fig. 2).

The degree of differentiation among populations for mean apical shoot elongation in 2005 and mean height in 2012 were assessed using generalized linear mixed-effects models (GLMM) using the lmer function (Bates, Maechler, and Bolker, 2013) in R (R Core Team, 2012). The objective of these analyses was to assess growth of survivors independent of previous mortality. Thus, GLMM were used rather than aster models because aster models require a minimum of two nodes or variables which would have required including mortality. The (GLMM) form for analysis of mean apical shoot elongation in 2005 and mean height in 2012 was:

$Y_{ijk} = (U + E_i + B(E)_{ij} + P_k + EP_{ik} + e_{ijk})$

where Y_{ijk} = is the observed mean of the kth population, in the jth block nested within the ith experiment, U is the overall mean, E_i is the experiment effect, $B(E)_{ij}$ is the effect of block nested within experiment, P_k is the population effect, EP_{ik} is the interaction of the ith experiment and the kth population and e_{ijk} is the random residual error. For both aster and GLMM analysis of the degree of differentiation among populations for their respective response variables (unconditional expected height, survival, mean height, and mean annual apical shoot elongation), population and experiment were treated as continuous fixed effects, while block within experiment and the interaction of population and experiment were treated as random effects. Statistical significance of fixed effects was tested by comparing the likelihoods of nested models.

The procedure used to evaluate the relationships between fitness measures and climate of seed origin assessed each experiment independently and followed the statistical framework presented by Leites et al. (2012a). The GLMM form for the analysis was:

$$Y_{ijkl} = (U + E_i + I_j + B_k + P_l + e_{ijkl})$$

where Y_{ijkl} is the observed plot mean of the kth population, in the lth block, U is the overall mean, E_i is the environment (climate or geography) at seed source effect, I is the height in 2001, B_k is the block effect, P_l is the population effect, and e_{ijkl} is the random residual error. Exp. 2 excluded height in 2001. Climate and geography of seed origin and

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in Exp. 1, height in 2001 were included as continuous fixed effect, continuous fixed

covariates, while effects for block and population were treated as random effects. Here

climate or geography of seed source (E_i) and population (P_l) jointly account for

differences among populations. The inclusion of a population-level random effect

accounted for population level effects not explained by climate or geography of seed

source. Including this random effect was necessary to account for within-group

correlation.

Climate patterns of Genetic Variation

The process used to select climate variables and identify the best model proceeded

as follows. Forward stepwise selection was used to identify environmental variables that

were significantly associated with each response variable (unconditional expected height,

survival, mean height, and mean apical shoot elongation). Twenty six climate (Table 1-3)

and three geographic variables (latitude, longitude and elevation) were tested using

maximum likelihood. Significant ($P < 0.05$) variables were retained. Those variables that

were highly correlated (Pearson's correlation coefficient ≥ 0.7) with the climate variable

in the best model were eliminated. The process was then repeated, adding a single

variable from the remaining pool to the best model to test two models using two

variables.

The procedure used to describe seed zones for practical application, followed

Rehfeldt's (1983b) floating seed zone model approach. The estimation of mean within-

population variance used residual error variance divided by the number of blocks for each

experiment. The square root of the estimation of mean within-population variance provided a mean within-population standard deviation. Confidence intervals were calculated at the significance level 0.2 to reduce the probability of committing a Type II error. For gene conservation applications, Type II errors are as great if not a greater concern than Type I errors (Brosi and Biber, 2009). Confidence intervals were used to identify the climatic distance per unit that reasonably distinguished two populations with respect to a particular trait. A population's performance for traits examined (survival, annual mean apical shoot elongation, and mean height) was considered to include its mean plus upper and lower confidence interval. Two populations were considered to be significantly different when one population's mean was outside of the confidence interval of the other. The formula used for confidence intervals was:

Upper $C.I._{0.2} = T_{\Delta/2} * sqrt((REV))/b)$ and Lower $C.I._{0.2} = - (T_{\Delta/2} * sqrt((REV))/b))$

Where $C.I._{0.2}$ is the confidence interval, $T_{\Delta/2}$ is the value of 't' at the desired significance level (0.02); REV (an estimate of the root within-population variance) is the residual error variance and 'b' is the number of blocks). GLMM was used to calculate residual error variance for survival.

Throughout the analysis, row-plot means for GLMM and sums for aster models were analyzed as the experimental unit to account for row-tree plot structure within blocks (Lambeth, Gladstone, and Stonecyhper, 1983). The study data set excluded plant response data from the first growing season following outplanting in order to allow time

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for establishment. Due to mortality during this establishment period, the number of trees

for each population in each experiment varied (Table 1-1). In total, 1153 trees and 963

trees were assessed in Exp. 1 and Exp. 2, respectively.

Results

After 12 and 10 years of growth in the field under climate warmer and drier than

seed source, mean height for the 14 year-old trees was 1553 mm and 1103 mm with 39%

and 40% survival for Experiments 1 and 2, respectively (Table 1-4).   Differences

between the two experiments were statistically significant for mean height (Table 1-5)

but not survival (Table 1-6).

Initial tree height following the first year after outplanting was only available for

Exp. 1. Initial mean tree height in 2001 was not a significant predictor of mean apical

shoot elongation in 2005 (Table 1-5), survival to 2012 or unconditional expected height

in 2012 (Table 1-6). Therefore differences in height at the start of the study did not

appear to be related to observed differences in performance later in the study. Mean

height in 2001 was a significant predictor of mean height in 2012 (Table 1-5). However,

mean height in 2001 explained only 9% of variation in mean height in 2012 (Fig. A1-3).

Population differentiation

In the joint analysis of Experiments 1 and 2, populations differed in their survival

response between experiments, as revealed by a highly significant ($P < 0.0001$)

interaction between experiment and population (Table 1-6). Populations with the lowest

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survival and unconditional expected height in Exp. 2, performed similarly in Exp. 1. In contrast, populations with the lowest shoot elongation in Exp. 1 performed similarly in Exp. 2 (Fig. A1-4).

To account for differences among populations between experiments, Experiments 1 and 2 were assessed independently in all subsequent analyses. Populations differed significantly for all response variables (Table 1-5 and Table 1-6). In Exp. 1, survival among populations varied from 8% to 75%, while mean apical shoot elongation in 2005 varied from 73.3 mm to 164.5 mm and mean height in 2012 varied from 904 mm to 2144 mm. In contrast, populations in Exp. 2 ranged from 19% to 77% survival and 46.4 mm to 136.5 mm for mean apical shoot elongation in the 2005, and 188 mm to 1,243 mm for mean height in 2012.

Environmental clines

Forward stepwise multiple regression analysis detected significant relationships between survival and climate and geography of the seed source in Exp. 2 but not Exp. 1 (Table 1-6 and Table 1-7). Differences among populations in mean apical shoot elongation in 2005 and mean height in 2012 were significantly related to climate and geography of the seed source in both Experiments 1 and 2 (Table 1-5 and Table 1-7). Model performance did not improve with additional predictors hence models with a single environmental predictor were chosen for all variables.

In Exp. 1, mean height for all populations was adversely affected by early drought stress at the planting site. Such adverse effects of stress were exemplified by an early

decline in survival to 68% by fall 2002. Mean apical shoot elongation in 2005 varied significantly and was negatively related to annual dryness and winter coldness at the seed source (ADIMMINDD0) (Table 1-7). A similar pattern was detected for mean height in 2012 which differed significantly among populations and was negatively related with the estimated annual ratio of growing season precipitation to mean annual precipitation (PRATIO) at the seed source. PRATIO accounted for 9.8% of the variation in mean height among populations. ADIMMINDD0 and PRATIO are highly correlated (r=0.935) among climate of the seed sources. The geographic pattern of increasing ADIMMINDD0 and PRATIO which corresponds with genetic variation for apical shoot elongation in the study region generally followed a trend in which growth increased with source location from southeast to northwest direction and with decreasing elevation in correspondence with increasing ADIMMINDD0 (Fig. A1-5) and PRATIO (Fig. A1-6).

In Exp. 2, survival declined significantly with increase in annual spring precipitation (SPRP) at the seed source (Table 1-7, Fig. 1-3). SPRP accounted for 13.2 % of variation in survival among populations, and survival was significantly related to SPRP beginning in fall 2006 but not before. These results correspond with a major decline in survival (from 88% to 50%) that occurred in fall 2006 following a drier than average summer (Table 1-2). The general geographic pattern of genetic variation for survival in the study region is a cline of increasing survival in a generally northwest to southeast direction in correspondence with decreasing SPRP (Fig.1-4).

In Exp. 2, mean height varied significantly in relation to the Julian date the sum of degree-days >5°C reached 100 (D100) at the seed source. D100 is a measure of the

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timing of spring warming. Height growth decreased with increasing D100 (later spring warming) at the seed source (Table 1-7, Fig.1-5), and D100 accounted for 21.3% of population variation for mean apical shoot elongation conditional on survival, but only 6.6% for mean height in 2012. Similar to results from Exp. 1, mean apical shoot elongation generally increased among sources in correspondence with decreasing D100 from a southeast to northwest direction (Fig.1-6). Geographic variables explained about the same amount of variation among populations for growth and survival as the associated climate variables. However, one exception was observed in Exp. 2, where elevation at seed source and D100 explained 21.4% and 6.6% of variation in mean height conditional on survival, respectively.

Discussion

The present study detected moderate genetic divergence in growth and survival among populations of whitebark pine that corresponded with climatic clines. In particular, populations originating from locations with earlier spring warming exhibited greater inherent growth under favorable conditions, while populations originating from locations with lower spring precipitation exhibited greater survival under drought conditions. Very few studies have assessed genetic variation in growth and survival for whitebark pine (Bower and Aitken, 2008). The present study is exceptional because it characterizes variation among whitebark pine populations for inherent growth expected under relatively favorable conditions as well as growth and survival performance through

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age 14 years under none-native climate that was approximate to predicted future climate for the present distribution of whitebark pine.

The present study was established at a relatively low-elevation (671m) research site where climate differed substantially in comparison to the typical, high-elevation habitat of whitebark pine (Table 1-1). In comparison to the warmest seed source among study populations (Beaver Creek, Idaho), the common garden sites were warmer in mean annual temperature by 4.2°C in Exp. 1 and 3.9°C in Exp. 2. In addition, soil in whitebark pine habitat is hypothesized to rarely exhibit drought characteristics (Weaver, 2001); however, dry conditions were frequently observed in most summers at both experiment sites during the study period.   Thus, results of this study may not necessarily indicate performance in the native habitat. Nevertheless, in common garden studies, significant phenotypic variation among populations that is associated with climate at seed origin is indicative of genetic divergence that reflects response to natural selection mediated by conditions at the seed origin (Reich et al., 2003).

Survival

Survival is a complex trait that reflects performance in relation to environment. Detecting differences in survival among populations under field conditions is largely dependent on the occurrence of environmental stresses which often take place infrequently within a range of intensity necessary to differentiate performance among populations (Rehfeldt, 1983a). Consequently field trials conducted over many years are generally best for evaluating survival (Morgenstern, 1996).

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In Exp. 1, survival declined episodically in association with dry years in 2002 and 2006-2011 with 39% survival in 2012 (Table 1-4). Differences among populations in survival did not correspond with climatic or geographic gradients among the sources. The failure to detect a geographic or climatic pattern may have resulted from early microenvironmental stress that exceeded threshold tolerance of all representative populations over most of the site.

Genetic variation for survival among populations was detected in Exp. 2. Results for differences in population survival in Exp. 2 suggest that whitebark pine populations vary with respect to adaptation to moisture stress. At the Exp. 2 site, soil conditions were rocky with a shallow fragipan, which can limit root growth and the availability of moisture. Survival on the site was relatively stable from year to year; survival nonetheless declined episodically to 40% by 2012 (Table 1-4). Much of this mortality (37%) occurred following the growing season in 2006 which was warmer and dryer than previous years for the Exp. 2 site (Table 1-2). In conjunction with the drier growing season in 2006, survival differed significantly among populations and was negatively associated with the estimated amount of annual spring precipitation (SPRP) at the seed origin (Table 1-7). In a region-wide study comparing populations in common gardens, Bower and Aitken (2008) also identified variation in survival in relation to precipitation patterns among populations originating from the Cascade Range in Oregon and California, but they did not detect variation for survival among populations from the Northern Rockies. The contrasting results of the present study may be due to differences between study environments in association with the timing of seedling development.

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Growth Potential

Following Rehfeldt (1990) the term "growth potential" represents an individual trait that is used to describe the inherent capacity for height growth expressed under favorable conditions where stress from the environment approaches zero. To assess growth potential, the present study used early apical shoot elongation in advance of substantial cumulative environmental stress associated with long warm-dry summers. In particular, apical shoot elongation in 2005 in Exp. 2 was reasoned to be the best available representation of growth potential because drought stress appeared to be low from 2003 through 2005 at the site. Supplemental irrigation had been applied in 2003 and 2004 and growing season precipitation in 2005 was relatively high with respect to the study period (Table 1-2). Furthermore, no mortality occurred from 2004 through 2005. Assessing growth potential using shoot elongation in a year that followed favorable conditions in multiple previous years was critical because conditions during bud formation in a given year are important in determining the shoot length of the following year for many temperate and boreal zone pines (Clements, 1970; Van Den Berg and Lanner, 1971; Kozlowski, Torre, and Marshall, 1973; Lanner, 1976; Junttila and Heide, 1981; Salminen and Jalkanen, 2005). In contrast, soil conditions at the Exp. 1 site appeared to be more favorable for growth however seedlings on the site were stressed early in the experiment following a warm-dry summer in 2002.

Growth potential differed significantly among populations (Table 1-5) and was negatively related to the Julian date that summed daily mean temperature $> 5°C$ reached

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100°C (D100) at the seed source (P < .01) in Exp. 2 and the interaction of annual dryness index with summed daily mean temperatures below 0°C (ADIMMINDDO) at the seed source in Exp. 1 (Table 1-7). These results are consistent with findings of Bower and Aitkens (2008) who observed increasing height growth with mean annual temperature of the seed origin of two year-old seedlings from populations in the Rocky Mountain region. A positive relationship between growth potential and temperature of seed origin has also been reported for several other forest tree species including western larch (*Larix occidentalis*) (Rehfeldt, 1995), Engelmann spruce (*Picea engelmannii*) (Rehfeldt, 2004), Douglas-fir (*Pseudotsuga menziesii*) (St Clair, Mandel, and Vance-Borland, 2005; Rehfeldt et al., 2014) eastern white pine (*Pinus strobus*) (Joyce and Rehfeldt, 2013) and ponderosa pine (*Pinus ponderosa*) (Rehfeldt et al., 2014).

Height growth measured during the juvenile stage under limited stress (i.e. growth potential) is predictive of mature height on favorable sites that are relatively stress-free. For example, Rehfeldt (2004) accounted for 80% of the variation among Scots pine (*Pinus sylvestris*) populations' normalized to 13-year heights at 46 sites located in favorable growing conditions scattered across Russia, using measures of growth potential from 2-year-old Scots pine seedlings grown in a greenhouse in Moscow, Idaho. In the present study, variation in growth potential assessed at age 7 years in 2005 accounted for at most 21.1% variation in mean height at age 14 years. The relative weakness of this relationship can be interpreted as a general indication that the cumulative stress over time imposed by study environment impaired growth of individuals within most populations.

Fitness

Both survival and relative height are closely related to fitness. Survival that allows successful reproduction is a fundamental component of fitness. In addition, small population size from low survival can lead to increased inbreeding. Small populations also have an increased potential for loss of allele diversity through random drift which can reduce a population's capacity to adapt to selection (see Reed, 2005) and have a greater risk of extinction due to environmental fluctuation, allele effects and demographic stochasticity (Wootton and Pfister, 2013). Relative height is positively associated with fitness. In forest trees, greater relative height confers physiological advantages such as increased access to resources and greater area for reproductive structures (see Mcgraw and Wulff, 1983).

In assessments of fitness, joint analyses of multiple fitness related traits can provide a more complete and therefore more realistic approximation of fitness. However, statistical analyses of fitness have typically been hindered by non-normal distributions that violate the assumptions of standard parametric approaches. Thus, fitness of long lived forest trees has normally been assessed using independent analysis of survival and/or height data. The present study used aster analyses, a newly available statistically rigorous approach to jointly assess final height and survival. Inferred values from the analyses are effectively estimates of the expected value of height at age 14 years taking into account mortality over the 12 (Exp. 1) and 11 (Exp. 2) year study periods. For example, by age 14 trees originating from a seed source in Powell Mine, Montana had

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11% lower survival than trees from a seed source in Beaver Creek, Idaho but the mean height of the surviving trees from Powell Mine exceeded that of trees from Beaver Creek by 339 mm. Taking both measures into account, the aster analysis estimated the unconditional expected height of both populations to be 546 mm. In this case, the aster analysis explicitly accounted for the higher survival of the Beaver Creek population in conjunction with the higher mean height of survivors of the Powell Mine population.

Fitness assessed using unconditional height at age 14, did not differ significantly among populations in either experiment (Table 1-6). However, a trend of differentiation among population means for fitness was apparent. In particular, fitness tended to increase with declining annual spring precipitation (SPRP) at seed origin in experiment 2 (Fig.1-7). The inability to detect significant differences for fitness among populations may result from low statistical power. Specifically, the use of row tree plots in the study design limited statistical power (Lambeth, Gladstone, and Stonecyhper, 1983) and provided no statistical advantage for the aster analyses. In support of this possibility, mean differences for height conditional on survival were substantial ranging from 154 mm to 1356 mm in Exp. 1 and 100 mm to 770 mm in Exp. 2.

Without question, assessing variation in fitness among long-lived trees based on 12- and 10-years field growth is tentative and must be interpreted with caution. However, studies of relatively short duration do capture variation expressed during the early life stages when forest trees are more susceptible to climate-related mortality (Namkoong, Usanis, and Silen, 1972; Namkoong and Conkle, 1976). Even so, in some cases where long-term provenance experiments have been conducted, selection has been

observed at intervals past 50 years (see Bakker, Moore, and Covington, 2006). Thus,

provenance experiments of longer duration may yield different results.

Geographic Distribution of Environmental Clines

Map projections of the timing of spring warming (D100) are representative of

geographic distribution for clinal variation of growth potential and revealed a general

trend of increasing growth potential from the southeast to the northwest and with

decreasing elevation (Fig.1-6). Conversely, map projections of annual spring

precipitation (SPRP) which are representative of clinal variation for survival and fitness

under warm-dry climate revealed a trend of decline in these traits in a generally

southwest to northwest direction (Fig.1-4). Thus seed sources from locations with low

SPRP toward southern Idaho and Wyoming appear to represent better-adapted

populations for warmer, dryer climates. Notably, high growth potential and high survival

under warm-dry climate were not predicted to be mutually exclusive. For example,

climate habitat in the Bitterroot Mountains along the north-central border of Idaho and

Montana was predicted to have populations that exhibited relatively high survival under

warm-dry climate and high growth potential. To be sure, these interpretations address

climate and do not take biotic interactions into considerations.

Map projections of clinal variation also suggest that considerable genetic variation

may reside in the Blue Mountains in northwestern Oregon (Fig.1-4 and 1-6). However,

the genetic variation from seed sources in this region cannot be confirmed by this study,

because the Blue Mountains extend beyond the geographic distribution of the sampled study region.

Management implications

A fundamental objective of reforestation management is to plant trees that are adapted to the planting site. Results from genecology studies are directly applicable to and are used extensively toward meeting this objective (Mahalovich, 1995; Johnson et al., 2004; Ying and Yanchuk, 2006). Recently, genecology methods have been applied to meet grassland restoration objectives (St Clair et al., 2013). Several analytical approaches (e.g. floating seed transfer and focal point seed zones) have been devised in order to judge how far seed from a population can be moved while minimizing the risk of maladaptation. These approaches interpret clinal variation in terms of natural selection and are based on Falconer's equation for the response to selection (Morgenstern, 1996). In addition, these approaches assume that local populations are best adapted and become less adapted as they move further from the local environment. Results from the present study indicate that reasonable confidence in population differentiation for growth potential requires a separation of approximately ± 7.4 Julian days for D100 between climate of seed sources. This climate range corresponds with an elevation range of approximately ± 210 m within the study region. Reasonable confidence in population differentiation for survival under a warmer and drier climate than seed source requires a separation of approximately ± 26.6 mm SPRP between seed sources. In comparison with other western forest species, these results suggest that the whitebark pine is as an

intermediate between specialist and generalist mode of adaptation for growth potential (see Levins, 1968; Rehfeldt, 1994a). Other species with similar intermediate modes with respect to growth potential in the study region include Engelmann spruce (Rehfeldt, 1994b), ponderosa pine (*Pinus ponderosa*) (Rehfeldt, 1991) and western larch (Rehfeldt, 1982).

Ultimately, informed management decisions will require guidelines that explicitly consider both species adaptive relationship with climate as presented above and potential climate change specific to the location and management objectives (see Rehfeldt and Jaquish, 2010; Joyce and Rehfeldt, 2013). Clinal variation identified and described in the present study can be directly applied to inform such guidelines.

Table 1-1. Geographic and genetic representation of Whitebark pine (*Pinus albicaulis*) population seed source, number of maternal families per population, trees per population, geographic location and predicted climate of seed source and mean 12-year (2001-2012) observed climate of study site at Priest River Experimental Forest (PREF)

Provenance	No. Families	No. Trees Exp. 1	No. Trees Exp. 2	Lat. °N	Long. °W	Elev. (m)	PRATIO	D100	SPRP (mm)	ADIMMIND0	MAP (mm)	MAT (°C)
Sawtel Peak	10	25	25	44.54	111.42	2545	0.481	175	118	119.3	734	-0.8
W.F. Cabin	8	28	-	44.82	111.87	2683	0.537	181	118	122.4	663	-1
Palmer Mtn.	Bulk	28	19	45.05	110.58	2774	0.513	187	134	98.9	786	-2.2
Goldstone Pass	9	29	26	45.15	113.55	2561	0.604	176	93	186.6	429	0
Lone Mtn.	10	28	26	45.27	111.42	2743	0.523	184	140	96.5	792	-1.4
Heaven's Gate	11	17	21	45.37	116.49	2438	0.352	183	155	61.3	1005	0.4
Little Bear	8	27	25	45.4	111.28	2621	0.532	180	142	99.0	784	-0.7
Tin Cup	9	25	28	45.47	114.4	2396	0.471	176	109	125.5	613	0.2
S. Baldy Mtn.	10	25	20	45.47	111.91	2743	0.556	183	138	103.4	710	-1
Gospel Peak	10	19	26	45.62	115.94	2499	0.366	185	150	65.0	982	-0.3
Burnt Knob	11	28	26	45.7	114.98	2499	0.400	185	131	83.4	837	-0.5
Quartz Hill	9	22	28	45.7	112.93	2438	0.621	170	105	162.1	467	0.6
Mudd Ridge	11	29	25	45.88	113.45	2408	0.571	174	107	148.0	506	0.2
Pilot Knob	12	25	21	45.9	115.7	2252	0.374	180	143	70.7	941	0.4
Coyote Mdws.	6	21	30	46.07	113.83	2451	0.506	181	121	113.2	629	-0.2
Mt. Emerine	Bulk	24	-	46.2	113.65	2560	0.509	183	129	103.6	662	-0.6
Cable Mtn.	Bulk	27	23	46.2	113.2	2439	0.563	176	123	123.0	577	0.2
Diablo Mtn.	11	25	-	46.3	114.61	2256	0.407	177	129	82.4	826	0.4
Forty Cr. Crazies	Bulk	-	16	46.31	110.48	2438	0.559	169	166	86.7	791	0.8
Willow L.O.	7	21	-	46.32	113.9	2499	0.478	183	133	94.1	719	-0.4
Powell Mine	Bulk	30	26	46.34	113.07	2438	0.559	176	130	114.7	606	0.2
St. Mary's	9	27	26	46.5	114.2	1972	0.448	160	118	101.4	687	2
Indian Post Office	10	25	19	46.53	115.05	2134	0.363	175	132	67.6	953	0.8
Daisy Pass	Bulk	21	20	46.55	113.8	2134	0.495	165	126	105.8	655	1.3
Beaver Ridge	11	26	24	46.56	114.44	2225	0.410	175	129	80.6	822	0.6
Blacklead Mtn.	16	24	26	46.64	114.85	2195	0.372	177	131	68.5	931	0.6
Freeze Out	10	28	21	47.01	116.03	1804	0.319	161	130	50.4	1048	2.5
Snow Bowl	10	25	26	47.02	113.98	2396	0.427	182	146	71.3	869	-0.2
Stark Mtn.	11	23	21	47.08	114.57	2145	0.377	173	129	67.9	906	0.8
Little Joe	11	28	16	47.15	115.23	2150	0.328	177	121	55.2	1027	0.7
Squaw Peak	10	25	25	47.15	114.37	2121	0.392	170	131	70.8	876	1
Burke Summit	11	26	26	47.5	115.7	1877	0.294	163	99	49.8	1037	2.2
Sheep Shed Mt	8	25	23	47.52	112.79	2317	0.533	176	160	84.3	749	0.4
Beaver Creek	10	24	34	47.63	115.74	1722	0.301	158	104	50.1	1021	2.7
Thomas Peak	11	26	22	47.73	114.83	1872	0.357	162	113	65.8	880	1.8
7 Point Mtn.	8	26	-	47.82	115.39	1747	0.322	159	104	58.8	937	2.2
Our Lake	7	-	23	47.84	112.81	2286	0.538	174	156	83.5	744	0.6
Baree Mtn.	11	26	16	47.95	115.56	1829	0.318	163	112	52.8	1011	1.8
Gizborn Mtn.	11	28	21	48.35	116.75	1689	0.332	158	164	41.5	1210	2.3
Schweitzer Area	9	28	29	48.36	116.63	1920	0.329	170	167	39.2	1269	1.1
Lunch Peak	10	28	22	48.37	116.19	1941	0.326	171	151	42.1	1208	1
Big Mtn.	20	30	23	48.49	114.34	1829	0.397	161	143	61.3	943	1.4
Copper Butte	Bulk	26	-	48.7	118.46	2165	0.413	182	128	61.0	795	0.1
Farnham Ridge	7	13	18	48.84	116.51	2042	0.345	182	177	37.3	1338	0
Farnham Peak	5	13	8	48.85	116.5	1768	0.348	164	169	40.9	1263	1.4
Sullivan L.O.	9	25	27	48.88	117.24	2175	0.370	186	183	36.7	1288	-0.6
Glacier Nat. P.	6	27	-	48.93	114.855	1841	0.391	165	136	60.4	928	0.8
Salmo L.O.	7	-	23	48.97	117.1	2092	0.364	184	193	34.6	1380	-0.4
Frozen Lake	Bulk	27	13	48.98	114.7	1646	0.418	157	135	67.3	862	1.8
Total	**400**	**1153**	**963**									
Study Site PREF (2001-2012)				48.35	116.83	671	0.454	138	132	-	825.1	6.9

Notes: Lat. = latitude, Long. = longitude, Elev. = elevation, see Table 1-3 for explanation of climate variables. Study was irrigated in 2003 – 2004. Exp. 1 = common garden experiment 1, Exp. 2 = common garden Experiment 2

Table 1-2. Observed climate for Priest River Experimental Station (latitude 48.35°N, longitude 116.83°W and elevation, 971m)

Year	PRATIO	SPPR (mm)	GSP (mm)	MAP (mm)	MTCM (^{0}C)	MAT (^{0}C)	MTWM (^{0}C)
2001	0.44	161.29	326.39	744.73	-3.16	6.99	18.69
2002	0.37	113.79	249.68	673.86	-2.13	6.67	19.24
2003	0.45	105.92	343.41	759.97	-3.11	7.50	19.29
2004	0.52	144.02	395.48	762.25	-3.28	7.49	18.73
2005	0.57	128.52	412.75	730.00	-5.50	6.71	18.09
2006	0.34	139.19	315.72	926.34	-3.21	7.39	20.08
2007	0.33	68.07	257.30	768.60	-5.41	7.05	21.14
2008	0.43	73.91	328.42	768.10	-6.71	5.98	18.04
2009	0.47	115.32	341.12	728.22	-6.29	6.37	19.07
2010	0.53	166.12	516.64	969.26	-3.22	6.90	17.16
2011	0.48	217.42	416.81	869.95	-4.53	6.01	17.19
2012	0.50	152.40	595.88	1200.40	-2.80	7.73	20.34
Mean	**0.45**	**132.16**	**374.97**	**825.14**	**-4.11**	**6.90**	**18.92**

Notes: (see Table 1-3 for additional explanation). Data available from National Climate Data Center (NCDC), the National Weather Service (NWS), and Priest River Experimental Forest (PREF) records.

Table 1-3. Climate variables used in this study.

adi— Annual dryness index, dd5/map

adimmindd0— adi*mmindd0

d100 — Julian date the sum of degree-days >5 degrees C reaches 100

dd0 — Degree-days <0 degrees C (based on mean monthly temperature)

dd5 — Degree-days >5 degrees C (based on mean monthly temperature)

fday — Julian date of the first freezing date of autumn

ffp — Length of the frost-free period (days)

gsdd5 — Degree-days >5 degrees C accumulating within the frost-free period

gsp — Growing season precipitation, April to September

map — Mean annual precipitation

mat — Mean annual temperature

mmax — Mean maximum temperature in the warmest month

mmindd0 — Degree-days <0 degrees C (based on mean minimum monthly temperature)

mmin — Mean minimum temperature in the coldest month

mtcm — Mean temperature in the coldest month

mtwm— Mean temperature in the warmest month

pratio — Ratio of growing season precipitation to mean annual precipitation, ie. gsp/map

sday — Julian date of the last freezing date of spring

sdi— Summer dryness index, gsdd5/gsp

sdimmindd0 — sdi*mmindd0

smrpb — Summer precipitation balance: (July+August+September)/(April+May+June)

smrsprpb — Summer/Spring precipitation balance: (July+August)/(April+May)

smrp — Summer precipitation: (July+August)

sprp — Spring precipitation: (April+May)

tdiff — temperature differential: mtwm – mtcm

winp — Winter precipitation: (November+December+January+Febuary)

Table 1-4. Total survival (%) by year for whitebark pine (*Pinus albicaulis*) common garden Experiments (Exp) 1 and 2. Mortality in the first year after outplanting was excluded to allow time for establishment. Therefore survival was represented as 100% at the start of each experiment in the following year.

	2001 spring	2001 fall	2002 fall	2003 sping	2003 fall	2004 fall	2005 fall	2006 fall	2010 fall	2011 fall	2012 fall
Exp 1	100%	96%	68%	-	64%	63%	63%	-	-	44%	39%
Exp 2	-	-	-	100%	93%	87%	86%	49%	42%	41%	40%

Table 1-5. Summary of model comparison testing the effects of experiment (Exp), climate and geographic origin of seed source and, height in 2001 on mean apical shoot elongation in 2005 and mean height in 2012. Random effects correspond with the respective null model in each row.

Response Variable	Term	Model df	Model Chisq	Chi df	P-value (> chisq)	Random Effects: Variance Term	
Exp 1 and 2:							
Mean apical shoot	Null	5		-		Exp:Population	69.96
elongation in 2005	Exp	6	9.3262	1	0.0022 **	Population	252.69
						Block	121.28
						Residual	1325.61
Mean height	Null	5		-		Exp:Population	7959
in 2012	Exp	6	10.879	1	0.0010 **	Population	10855
						Block	11172
						Residual	89415
Exp 1:							
Mean apical shoot	Null	4		-		Population	360.6
elongation in 2005	ADIMMINDD0	5	6.4322	1	0.0112 *	Block	174.68
	Latitude	5	7.6606	1	0.0057 **	Residual	1747.28
	Height 2001	5	2.6151	1	0.1059		
Mean height	Null	4		-		Population	25347
in 2012	PRATIO	5	3.971	1	0.0463 *	Block	10409
	Elevation	5	2.3506	1	0.1252	Residual	110999
	Height 2001	5	9.7495	1	0.0018 **		
Exp 2:							
Mean apical shoot	Null	4		-		Population	89.573
elongation in 2005	D100	5	79.335	1	0.003707 **	Block	10.297
	Elevation	5	8.422	1	0.01224 *	Residual	525.358
Mean height	Null	4		-		Population	4996.7
in 2012	D100	5	4.2009	1	0.0404 *	Block	8086.1
	Elevation	5	6.5015	1	0.01078 *	Residual	57744.8

Note: Significant climate or geographic predictors indicate significant differences among populations for the response variable. The effect of each response variable was tested against the null model for each predictor variable. Null models exclude variables listed directly below the term. Null model forms were Response = Exp + Block + Population + Exp:Population for Experiments 1 and 2 and Response = Block + Population for experiments 1 or 2. Random effects were from respective models with the following form: Climate variable + Exp + Block + Population + Exp:Population for Experiments 1 and 2 and Climate variable + Exp + Block + Population for experiment 1 or 2. See Table 1-3 for explanation of climate variables. Combined experiments use 39 populations common to both. * P < .05, ** P < .01, *** P < .001

Table 1-6. Results from aster model comparisons testing for the effects of survival and unconditional expected height in 2012 in Experiments (Exp) 1 and 2 and their combination. Random effects correspond with the respective null model in each row.

Response Variable	Term	Model df	Model Deviance	Test df	Test Deviance	Test P-value	Random Effects: Square Roots of Variance Components (P-values are one-tailed)	Estimate	Std. Error	P-value
Exp 1 & 2:										
Survival	Null	6	-4502.9	-	-		Block	0.6793	0.1703	< 0.0001 ***
	Exp	7	-4502.8	1	0.11291	0.7369	Population	0.3184	0.1151	0.0028 **
							Test:Population	0.3793	0.1	< 0.0001 ***
Unconditional	Null	6	-4646.6	-	-		Block	0.0027	3.591	< 0.0001 ***
expected height	Exp	7	-4646.3	1	0.30795	0.5789	Population	0	NA	NA
							Test:Population	0	NA	NA
Exp 1:										
Survival	Null	8	-3124.4	-	-		Block	0.8443	0.2542	< 0.0001 ***
	MMAX	9	-3123.4	1	1.0539	0.3046	Population	0.6479	0.104	< 0.0001 ***
	Latitude	9	-3123.8	1	0.6129	0.4337				
	Height 2001	9	-3123.7	1	0.7157	0.3976				
Unconditional	Null	8	-3291.9	-	-		Block	0	NA	NA
expected height	PRATIO	9	-3291.4	1	0.90495	0.4692	Population	0	NA	NA
	Latitude	9	-3291.3	1	0.16535	0.4255				
	Height 2001	9	-3288.7	1	1.2658	0.0726				
Exp 2:										
Survival	Null	8	-2512.5	-	-		Block	0.1575	0.09792	0.0528
	SPRP	9	-2507.3	1	5.1998	0.0226 *	Population	0.2474	0.11041	0.012 *
	Latitude	9	-2507.3	1	5.0491	0.0229 *				
Unconditional	Null	8	-2515.3	-	-		Block	0	NA	NA
expected height	SPRP	9	-2516.9	1	1.5466	0.2136	Population	0	NA	NA
	Elevation	9	-2516.6	1	0.31294	0.5759				

Note: Significant climate or geographic predictors indicates significant differences among population for the response variable. The most significant geographic and climate variable for each response variable are presented. In addition, square roots of variance components for random effects from the most significant model are presented. Null models exclude variables listed directly below the term. The effect of each predictor variable was tested against the null model for each predictor variable. For joint analyses of Experiments 1 and 2 random effects are estimated for models with the following form: Response = Exp + Block + Population + Exp:Population. For analysis of the experiments separately, the model was of the form, Response = Climate variable + Block + Population. See Table 1-3 for explanation of climate variables.
*P < .05, ** P < .01, *** P < .001

Table 1-7. Results of multiple regression analyses that describe genetic variation in whitebark pine (*Pinus albicaulis*) in response to climate of seed source.

	Independent Climate Variable	R²	Model Intercept	Model slope	Independent Geographic Variable	R²	Model Intercept	Model slope
Experiment 1								
Height in 2012	PRATIO*	0.098	1804	-821.9	-	-	-	-
Apical growth in 2005	ADIMMINDDO*	0.098	141.523	-0.304	Latitude *	0.1	-304.53	8.988
Experiment 2								
Survival in 2012	SPRP*	0.132	5.178	-0.0074	Latitude*	0.15	5.778	-0.138
Height in 2012	D100*	0.066	2146.681	-6.029	Elevation*	0.15	1557.02	-0.214
Apical growth in 2005	D100**	0.213	235.290	-0.874	Elevation**	0.2	132.448	-0.022

Note: ***** $P < .05$, ****** $P < .01$. See Table 1-3 for explanation of climate variables.
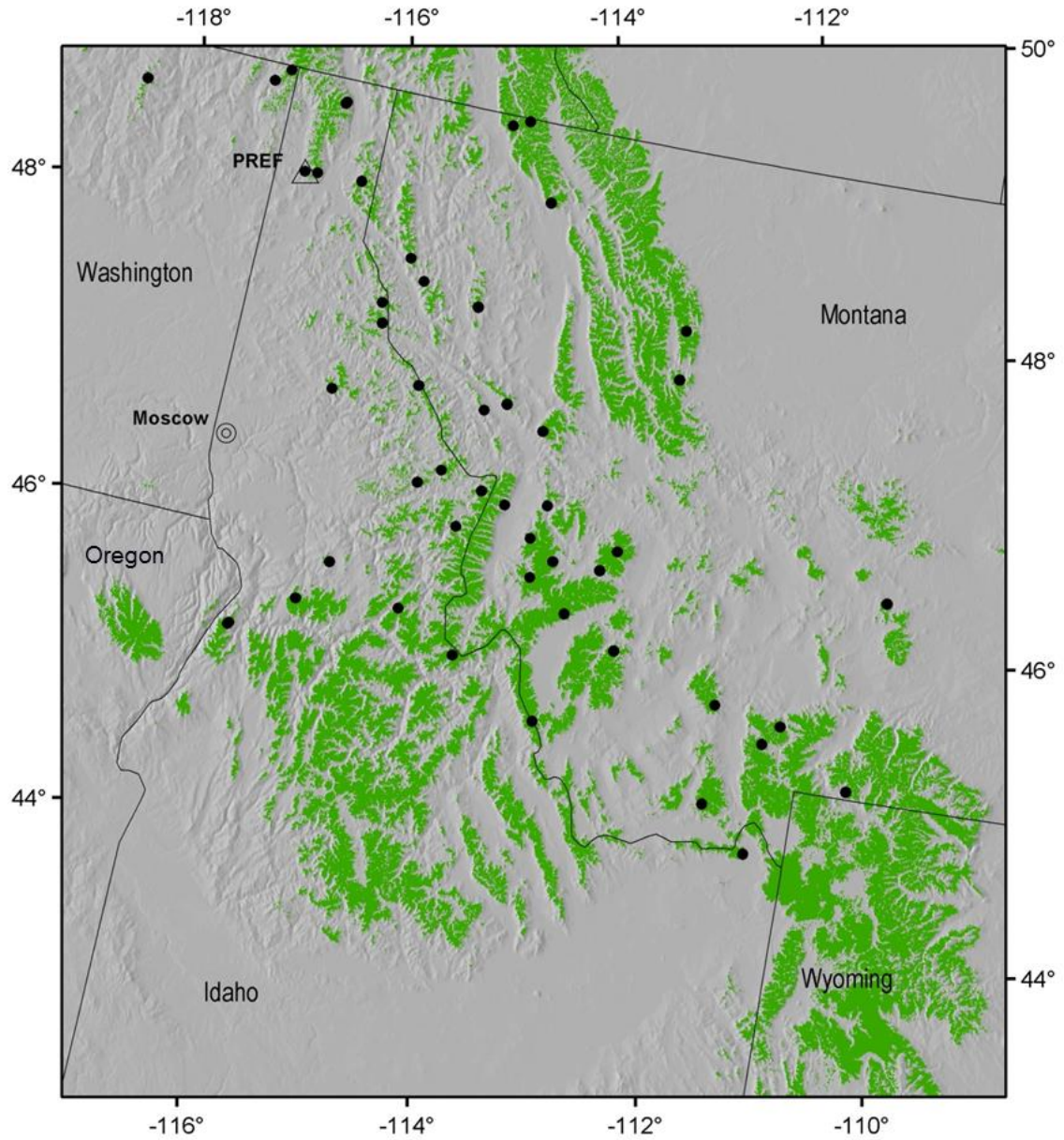
Figure 1-1. Location of whitebark pine (*Pinus albicaulis*) populations (●) and study site (Δ). Green area indicates estimated distribution using predicted realized climate niche of whitebark pine (Warwell *et al.* 2006).

A

Present		Survival		Survival		Survival		Survival		Survival		Survival		Survival
Spring 2001	→	Fall 2001	→	Fall 2002	→	Fall 2003	→	Fall 2004	→	Fall 2005	→	Fall 2011	→	Fall 2012

↓
Height
Fall 2012

B

Present		Survival		Survival		Survival		Survival		Survival		Survival		Survival
Spring 2003	→	Fall 2003	→	Fall 2004	→	Fall 2005	→	Fall 2006	→	Fall 2010	→	Fall 2011	→	Fall 2012

↓
Height
Fall 2012

C

Present		Survival		Survival		Survival		Survival		Survival
Spring 2003	→	Fall 2003	→	Fall 2004	→	Fall 2005	→	Fall 2011	→	Fall 2012

↓
Height
Fall 2012

Figure 1-2. Graphical models for full aster analysis unifying population survival and mean height data to assess fitness for (A) Experiment 1, (B) Experiment 2 and (C) Experiments 1 and 2 combined. Each node represents a component of life history while arrows represent the dependent association between predecessor and successor life-history components.
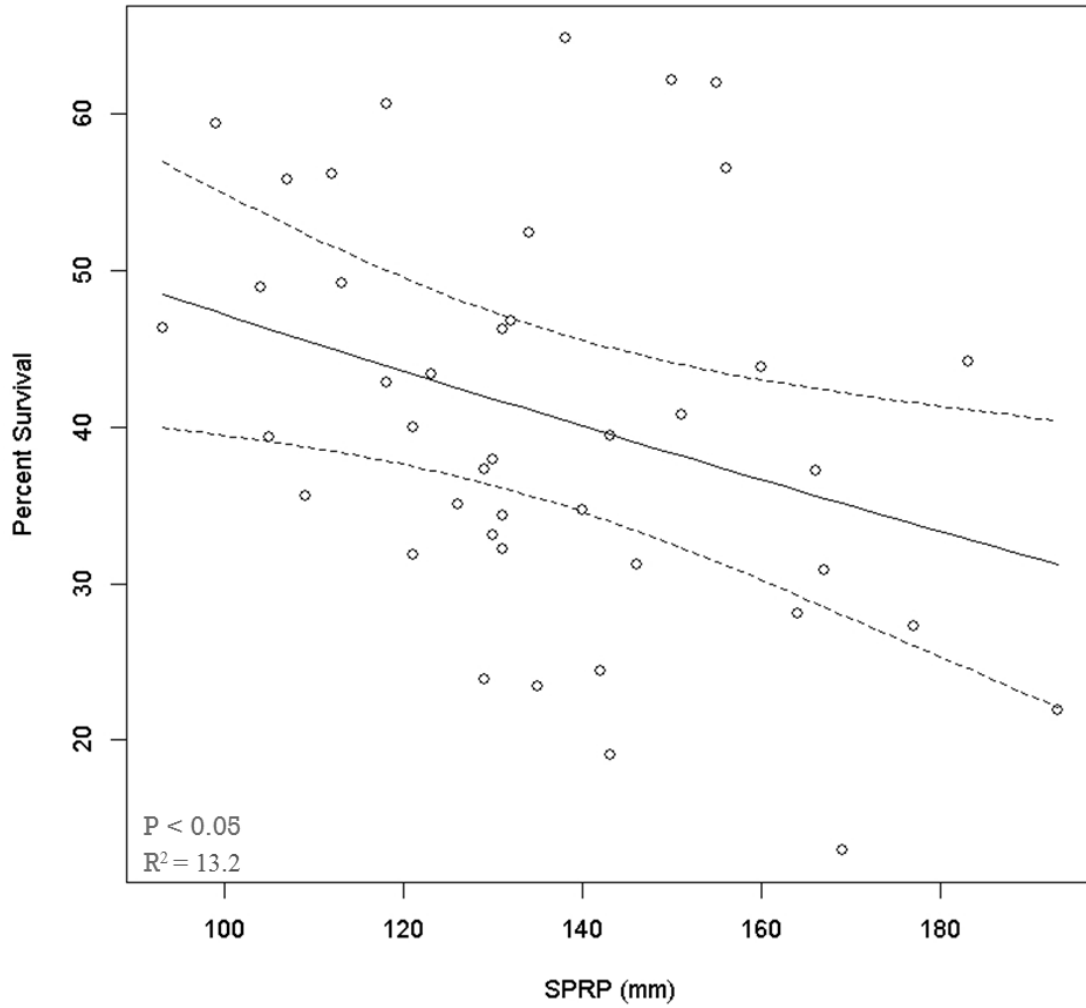
Figure 1-3. Experiment 2, predicted percent survival of whitebark pine (*Pinus albicaulis*) populations (open circles) plotted in relation to spring precipitation (SPRP: mm) at population origin (line and 95% CI).
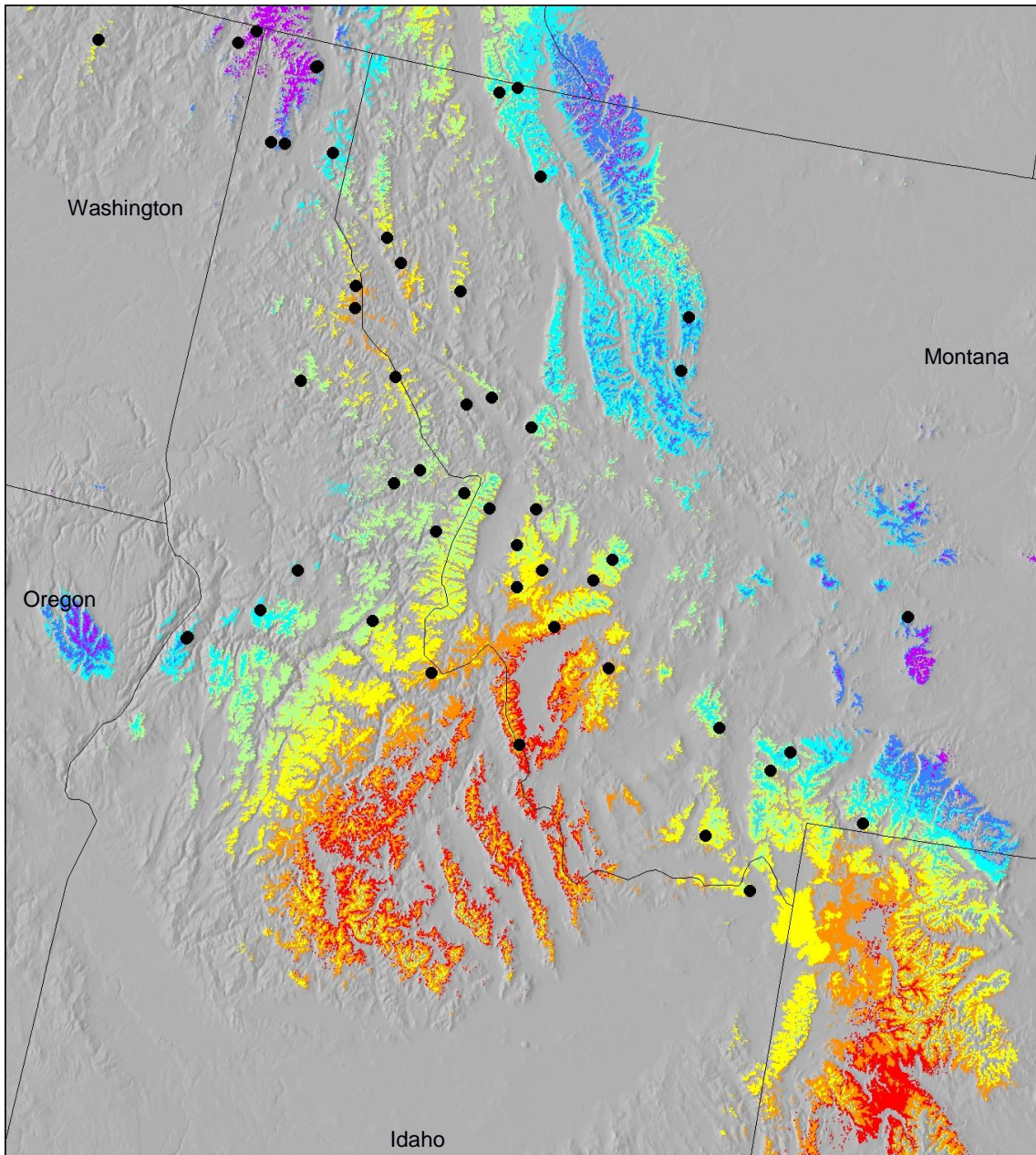
Figure 1-4. Geographic distribution of annual spring precipitation (SPRP) projected over predicted contemporary climate niche of whitebark pine (*Pinus albicaulis*) in interior northwestern USA. Red = 35 – 91 mm, orange = 91 – 106 mm, yellow = 106 – 120 mm, light blue = 136 – 152 mm, dark blue = 153-167 mm, purple = 168-401 mm and (●) indicates location of population.
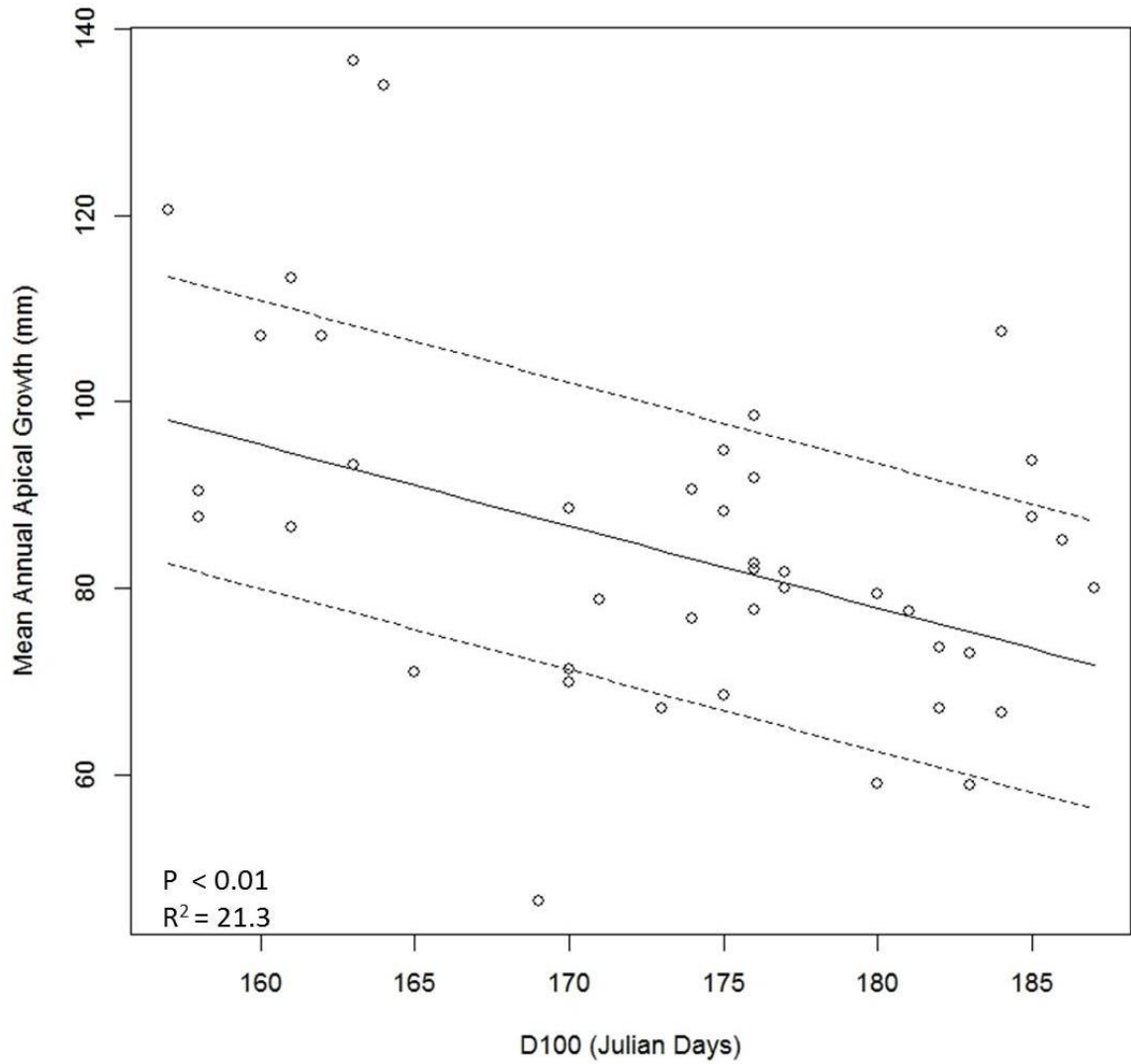
Figure 1-5. Experiment 2, predicted mean annual apical shoot elongation of whitebark pine (*Pinus albicaulis*) plotted in relation to Julian date when sum of degree-days > 5°C reaches 100 (D100) at the seed source in the study region (line and 95% CI) and by population (open circles).
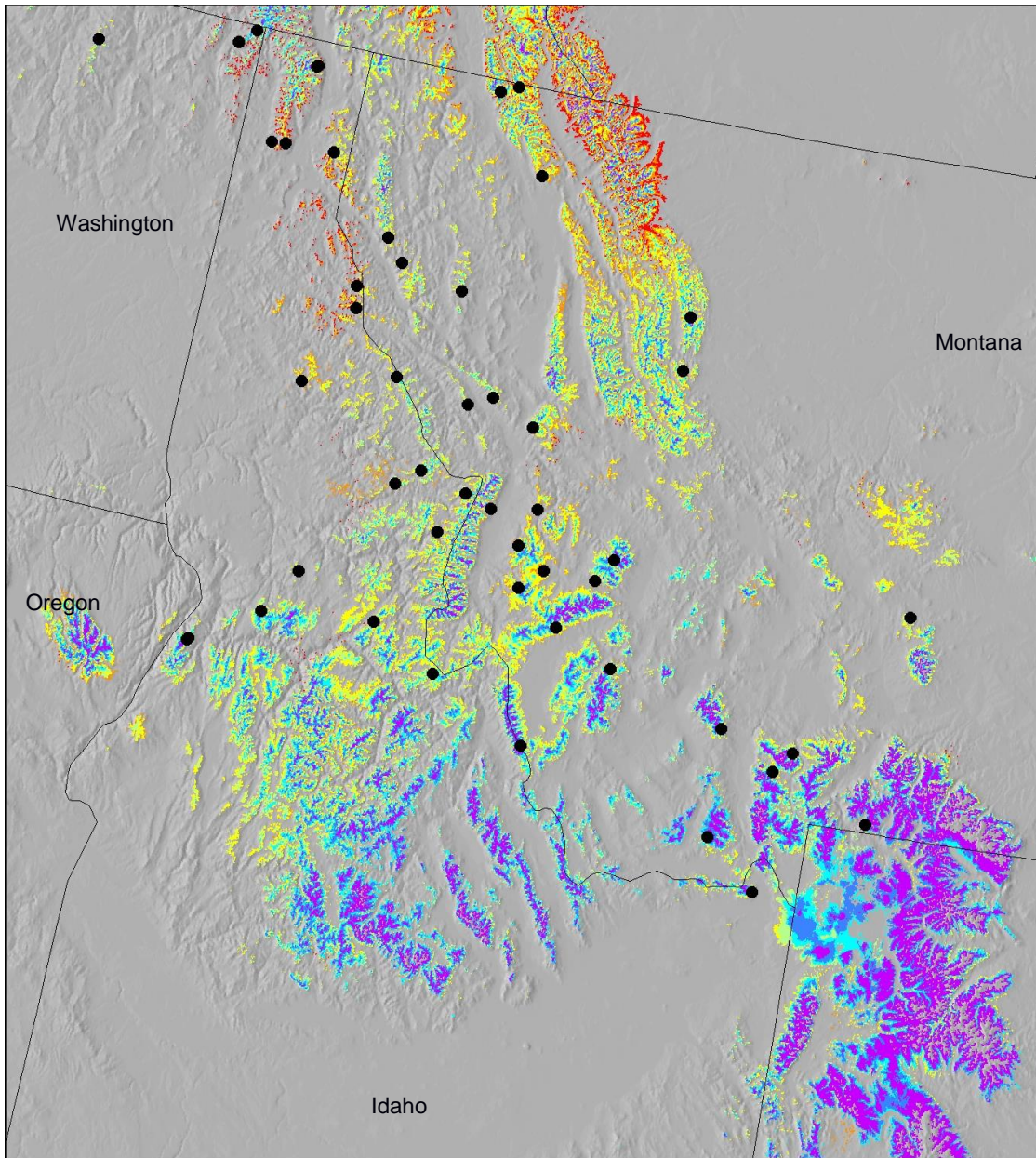
Figure 1-6. Geographic distribution of Julian date when the sum of degree-days > 5°C reaches 100 (D100) projected over predicted contemporary climate niche of whitebark pine (*Pinus albicaulis*) in interior northwestern USA. Red = 7 – 45, orange = 46 – 69, yellow = 70 – 94, green = 94 –120, light blue = 120 – 147, dark blue = 148 – 172 and purple = 173–299 for Julian Dates and (●) indicates location of population.

P < 0.21
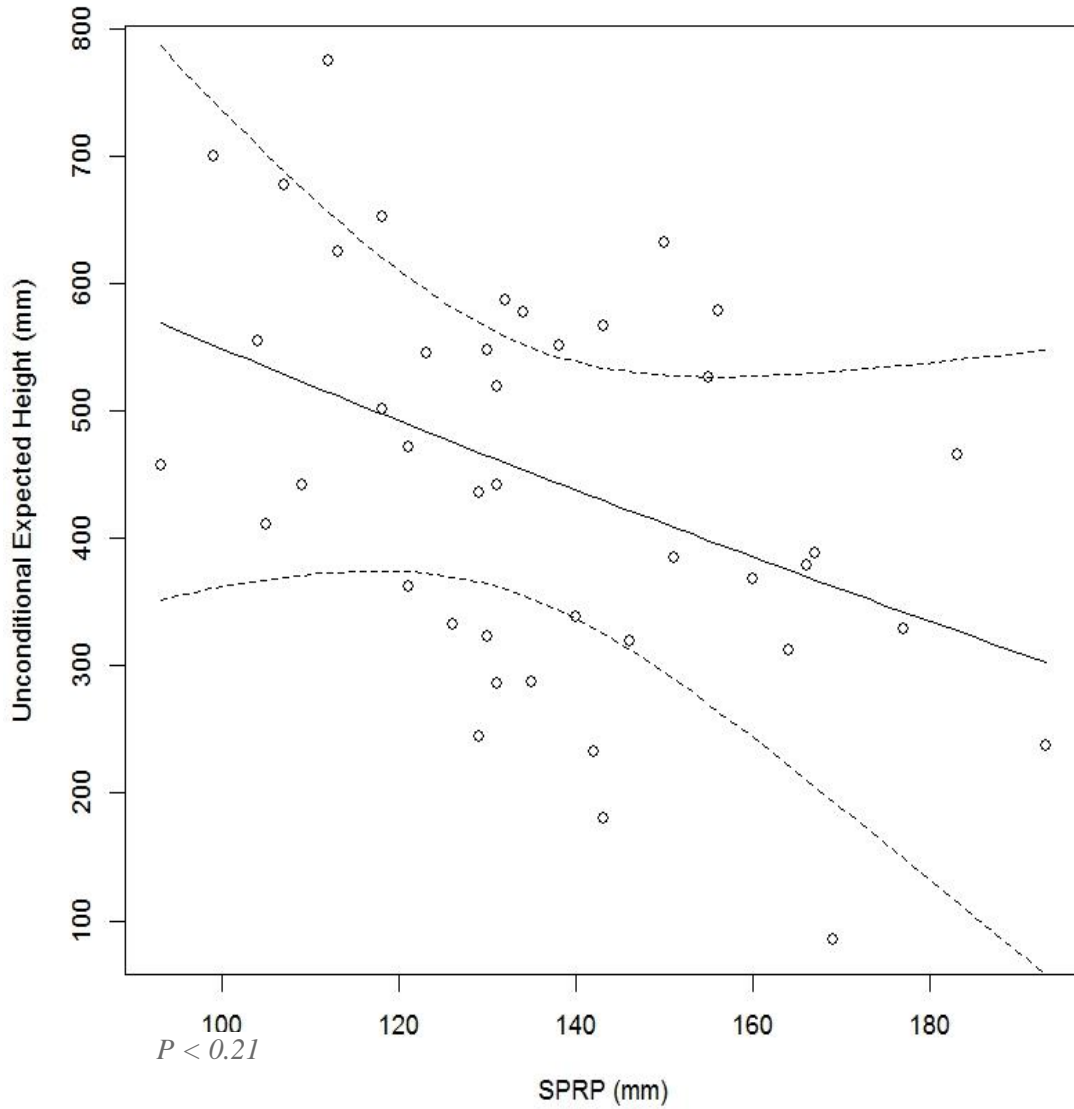
Figure 1-7. Experiment 2, Summed unconditional expected height of 14 years-old whitebark pine (*Pinus albicaulis*) populations plotted in relation to the mean annual spring precipitation (SPRP) of the population's seed source (line and 95% CI) and by population (open circles).

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Chapter 2: Phenotypic selection and genecology of growth rhythm in whitebark pine (*Pinus albicaulis* **Englem.**) grown in common gardens under warm-dry climate over 12 years.

Abstract

Growth rhythm represents the timing of annual plant growth and development in relation to the environment. Growth rhythm that is well synchronized with the local climate is understood to confer adaptation in plant species. Rapid ongoing climate change threatens to desynchronize growth rhythm for many plant populations, yet knowledge of how plant populations will respond through selection on growth rhythm is limited. Therefore, to evaluate phenotypic selection on growth rhythm, seedlings from 49 populations of whitebark pine (*Pinus albicaulis* Engelm.), representative of the interior northwestern USA were grown in two common garden field experiments over a 12-year period under climate (+ 4.4°C to 9.1°C mean annual temperature) approximating projected climate change in the present century for the sampled species range. In addition, variation in growth rhythm and climate of seed origin was examined to clarify how growth rhythm varies over the study region and describe contemporary patterns of likely adaptation to climate. Height at the end of the study was used as the best available measure of fitness. Survival and the unconditional expected value for height were dependent on timing of apical shoot elongation rates within and among growing seasons. Comparison of models using survival versus unconditional expected height as the proxy for fitness showed that differential survival strongly influenced selection on shoot elongation, a measure of growth rhythm. Analysis of the form and magnitude of selection detected directional

selection, curved directional selection, and stabilizing selection. The form and magnitude of selection on timing and rate of shoot elongation varied between experimental sites and among years. More complex selection was observed on the experimental site with more favorable growing conditions. Curved directional selection and stabilizing selection tended to occur in earlier years and was chiefly associated with mortality in the year of expression. Differences among seed sources in growth rhythm were mild and were explained to a moderate extent ($r^2 = 0.08 - 0.28$) by variation in temperature or precipitation among seed origins. Individuals originating from milder climates tended to elongate slightly longer through the growing season and exhibit slightly higher shoot elongation rates. Results are directly applicable to forest management decisions regarding gene conservation and reforestation.

Introduction

Differences in growth rhythm among populations within many woody plant species have long been observed and interpreted as a key intraspecific adaptation to climate. Growth rhythm refers to the timing and duration of sequential, annual developmental events that are expressed by an organism in a particular environment (cf. Dietrichson, 1964; Rehfeldt, 1992; Howe et al., 2003). For many forest trees in temperate and boreal climates, the expression of growth rhythm begins with the timing of dehardening in the spring and includes the timing of root elongation, shoot elongation, leaf expansion, bud development, diameter growth, lignification, pollen development, cone/flower development and concludes with cold acclimation. Plants that are well

adapted to their climate conditions complete growth and development within the growing season prior to onset of seasonal drought or extreme cold temperatures. In the interior northwestern North America, environmental limits of the growing season are defined by rising temperature during the spring and drought and/or cold temperature events later in the summer and or fall.

Growth rhythm is influenced by environmental and genetic factors (Partanen and Beuker, 1999; Partanen, 2004). Temperature has been identified as the most important environmental factor regulating the annual cycle of plants (Sarvas, 1972). For most temperate and boreal woody plant species, initiation of annual growth is determined by an accumulation of winter chilling and spring warming over time, whereas timing of bud set has been shown to be influenced by a combination of temperature and photoperiod, along with soil moisture, nutrition and light quality (see Howe et al., 2003). Response of the timing of annual growth to these environmental factors varies genetically within and among species (e.g. Chuine, Belmonte, and Mignot, 2000). Moderate to high levels of heritability have been detected within many species for many measures of growth rhythm (see Ekberg, Eriksson, and Weng, 1985).

The role of growth rhythm as a key trait that confers adaptation to climate has been well established. Variation in growth rhythm among populations within many species has been observed to correspond to the local climate of origin even when plants are growing under novel climate as with many common garden studies. For example, in provenance tests, early cessation of apical shoot elongation or bud set has been observed in seed sources originating from locations characterized by distinct late-summer drought

(e.g. Bouvarel, 1961; Joly, Adams, and Stafford, 1989) or early fall frost (Howe et al., 2003; Savolainen et al., 2007). Reduced vigor, increased injury and/or increased mortality have been observed in populations with growth rhythms that were poorly synchronized with annual timing of the local climate (e.g. Rehfeldt, 1983c; White, 1987; Skrøppa and Kohmann, 1997).

Ongoing rapid climate change is predicted to substantially alter climates throughout the 21st century (IPCC, 2013b). In particular, the accelerated rate of climate change threatens to disrupt the synchrony of growth rhythm of woody plant populations with local climate. When populations are subjected to climate that exceeds their physiological capacity, they will genetically adapt and/or shift their ranges through seed migration into areas with suitable climate or undergo extirpation (Lynch and Lande, 1993; Davis and Shaw, 2001; Rehfeldt, Wykoff, and Ying, 2001; Davis, Shaw, and Etterson, 2005). Over the long term, the persistence of a population is determined by the process of evolutionary adaptation. Evolutionary adaptation is expected when the traits under selection vary within a given population and are heritable (Darwin, 1859; Endler, 1973; Falconer and Mackay, 1996). Therefore, evolutionary trajectory may be estimated when information about selection on the phenotype and trait inheritance is available. However, this information is limited for tree species subjected to rapid climate change. In particular, little is known about how selection acts on growth rhythm during juvenile growth, a period when trees are more susceptible to climate-related stress in comparison to later years (Namkoong, Usanis, and Silen, 1972; Namkoong and Conkle, 1976).

The present study investigates phenotypic selection on growth rhythm in whitebark pine (*Pinus albicaulis* Engelm.). Whitebark pine is of particular interest given its status as a keystone (Tomback and Kendall, 2001) and foundational (Ellison et al., 2005) species in subalpine forest ecosystems of western North America. This species has been in decline since the mid-20th century (Tomback, Arno, and Keane, 2001) and has consequently been placed on the United States, Endangered Species Act Candidate List (U. S. Fish and Wildlife Service, 2011) and declared endangered by the Canadian Federal government in accordance with the Species at Risk Act (COSEWIC, 2012).

Whitebark pine has historically occupied the cool-wet climatic extreme among forest ecosystems in western North America. However, the climate of northwestern North America has recently become generally warmer with drier summers than the previous century (Kunkel et al., 2013), and this trend is predicted to continue through the 21st century (Mote and Salathé, 2010). Consequently, whitebark pine's contemporary (1961-1990) realized climate niche is projected to be eliminated by mid-century for almost all of its distribution in the USA (Warwell, Rehfeldt, and Crookston, 2007).

As a first step to investigate how forest trees may evolve under ongoing climate change, the present study assessed the form of phenotypic selection on growth rhythm among whitebark pine individuals grown under a climate that was warmer and drier than their climate of origin. In particular, the study objective was to quantify form, magnitude of selection over multiple growing seasons on growth rhythm during the more vulnerable juvenile stage under a climatic regime that approximates predicted climate late in the present century for the study region (see Mote and Salathé, 2010). In addition,

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variation in growth rhythm among populations and its relation to climate at the source

location were assessed to clarify how growth rhythm varies over the study region and to

assess contemporary patterns that likely resulted from adaptation to climate.

Materials and Methods

Wind-pollinated cones were collected from natural populations of whitebark pine

in the interior northwestern USA (Fig. 1-1). Seeds were sampled from approximately 10

trees per provenance. In total, seedlings from 49 provenances were outplanted in two

common garden field experiments at Priest River Experimental Forest (lat 48°21' N, long

116°41', 671m).

Common Garden Experiments 1 and 2

To assess seedlings growth and survival under generally favorable growing

conditions, Experiment 1 (Exp. 1) was planted in spring 2000 with 2 year-old seedlings

from 46 provenances (Fig. A1-1). Soil at the site was well drained and tilled prior to

planting. During the study period, mean annual air temperature (MAT) was 6.9°C which

was warmer than the warmest and coldest seed origins by 4.4^0C and 9.1^0C MAT,

respectively. Mean annual precipitation was approximately 825.4 mm (Table 2-1).

To assess seedlings growth and survival under growing conditions that contrasted

with Exp. 1 site and more closely resembled whitebark pines high elevation habitat,

Experiment 2 (Exp. 2) was planted in fall, 2001 with 3 year-old seedlings from 42

provenances (Fig. A1-2). Exp. 2 shared 39 populations in common with Exp. 1. The

Exp. 2 site was located in a cold draw 2.2 km from Exp. 1 in rocky soil that was shallow and poorly drained. Over the study period MAT at the Exp. 2 site was 0.25 °C cooler than the Exp. 1 site while MAP was the same (825.4 mm). See Chapter 1 for more detailed description of seed collection and study site descriptions.

Design and Procedure

A randomized complete block design with populations represented in 5-tree row-plots in each of 6 blocks was used in Exp. 1 and 10-tree row-plots in each of 3 blocks was used in Exp. 2. In Exp. 1 and Exp. 2, individual trees were planted using dibble tools at a spacing of 1 m x 1 m and 35.56 cm x 33.02 cm, respectively. A border row was planted in each site to equalize competitive effects on measured trees. High mortality prior to crowding and characteristic slow growth obviated competitive interactions and the need to thin throughout the study period. Obvious mortality due to damage from insect herbivory and deer browse was omitted from the study.

To focus on trait expression after effects of transplanting had dissipated, measures of plants during the first growing season following outplanting were not used. Mortality before spring 2002 was ignored and subsequent survival was recorded in fall 2002-2005, 2011 and 2012 in Exp. 1. In Exp. 2, mortality before spring 2003 was ignored with subsequent survival recorded in fall 2003-2006, 2010-2012. Measurements of annual incremental apical shoot elongation were recorded approximately weekly to bi-weekly from late March through July in 2002-2005 in Exp. 1 and in 2003-2006 in Exp. 2. Total height was measured in 2012 in both tests.

Because sequential events of annual development tend to be intercorrelated (Dietrichson, 1964), the timing and rate of apical shoot elongation were used as proxies for growth rhythm. Apical shoot elongation represents the expansion of plant cells formed at the conclusion of the previous growing season which normally occurs in each growing season following free growth in the first year (Romberger, 1963). Rates of apical shoot elongation for each tree were calculated by dividing the difference in shoot height between consecutive measurement dates within a growing season by the duration of the respective interval. This procedure produced a data set that represented apical shoot elongation of individual trees over each growing season as a series of shoot elongation rates corresponding to intervals of time. The number of intervals varied by year from 4 to 8 (Table 2-2). Characterization of growth rhythm using logistic functions to interpolate apical elongation over the growing season was considered. However, this approach was ruled out due to poor fit, particularly for either initiation or cessation of shoot elongation, resulting from the paucity of intra-seasonal incremental measurements and high variability in elongation rate that is typical of shoot elongation under field conditions.

Observations to calculate timing of apical shoot elongation were only available for 2005 and 2006. Therefore their inclusion in analysis was limited to these years. The interval where elongation was first detected between incremental height measurements was used to approximate the timing of initiation of apical shoot elongation, while the interval where growth between incremental height measurements was no longer positive was used to approximate the timing of cessation. New bud growth continues after apical

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shoot elongation has concluded. This phase of growth was approximated and excluded

by comparing the final measurement that excluded bud growth with earlier measures that

included bud growth. Timing of cessation of shoot elongation was determined to be the

period where the final measure of height to bud base was equal to or greater than the

earliest measure of height to the apical shoot tip. Duration of elongation was calculated

by subtracting the timing of cessation from the timing of initiation. The precision for

measures of initiation, cessation and duration was limited by length of measurements

intervals (see Table 2-2).

Statistical Analysis

Aster models (Geyer, Wagenius, and Shaw, 2007; Geyer and Shaw, 2008) permit

statistically rigorous, unified analysis of multiple life history stages. To evaluate

selection, the relationship between fitness and timing of annual rate, cessation, initiation

and duration of apical shoot elongation was assessed with aster models using the reaster

function (Geyer, 2013) in R (Team, 2013) following a general multivariate selection

approach (Lande and Arnold, 1983). Unconditional estimates of final height were used

as the best available proxy for fitness, and were obtained because they explicitly included

survival through multiple years. For example, normal distributions were used to model

final height in 2012 conditional on survival to that year, and Bernoulli was used to model

survival through each of multiple intervals concluding in 2012 and beginning with

seedlings present in 2002 (Fig. 2-1A) and 2003 (Fig. 2-1B). To model survival alone,

only survival in 2012 beginning with seedlings present in 2002 (Fig. 2-1C) and 2003

(Fig. 2-1D) for Exp. 2 were used. Comparisons between survival alone and unconditional expected height were used to assess the influence of total height alone and inversely survival alone on inferred fitness.

Selection analysis requires a census of the trait of interest prior to selection and in the present analysis, elongation rates were treated as unique to the growing season they were expressed in. Therefore, mortality prior to the growing season when rate and timing of shoot elongation was expressed was not included in the respective model of survival or unconditional expected height. For example, the analysis of selection on elongation rates expressed in 2005 used inferred survival and unconditional expected height in 2012 beginning with seedlings present in spring 2005.

Selection gradients (partial regression coefficients) were estimated to characterize the relationship between fitness and timing of apical shoot elongation for each experiment using the following procedure. First, Pearson's correlation coefficients for elongation rates expressed in each interval were compared within growing seasons. Only one of any group of highly correlated ($r^2 \geq 0.70$, determined *a priori*) intervals for elongation rate was retained to eliminate serious multicollinearity (see Mitchell-Olds and Shaw, 1987). In each experiment, at least four intervals for elongation rate were available in each growing season for the analysis following this step. Next, backward stepwise regression was used to identify which intervals for elongation rate in each growing season were significant predictors of survival to 2012 alone and also of unconditional expected height in 2012. For each test, linear selection gradients (β_i), and quadratic selection gradients (γ_{ii}) for elongation rate intervals were treated as fixed effects while

block, population and row within block were treated as random. Statistical significance of fixed effects was tested by comparing the likelihoods of nested models using maximum likelihood. Significant ($P < 0.05$) variables were retained. The detection of quadratic terms indicated curvature in the fitness surface. When more than one quadratic term was significant the cross-product pairs of traits were included in the model and treated as a fixed effect. Cross-product terms (γ_{ij}) represent correlational selection gradients, which indicate selection on one trait that varies depending on the second trait (Phillips and Arnold, 1989; Brodie III, Moore, and Janzen, 1995; Blows and Brooks, 2003). Therefore correlational selection gradients indicate nonlinear selection along axes that are not parallel to the axes of either trait.

A complete assessment of phenotypic selection using regression-analyses requires visualization of the data to determine how well the data fit the hypothesized model and identify potential unexpected patterns or problems in the data (Mitchell-Olds and Shaw, 1987). Bivariate nonlinear selection gradients were visualized using selection surfaces. For each selection surface, fitness, whether as survival or unconditional expected height, was represented using contours, while the rates of apical shoot elongation for identified growing season intervals were represented on the x and y axes. The steepness of the gradient defined by the relative closeness of contour lines described the magnitude of selection for a given point on the fitness surface. Observed phenotypes were superimposed to make clear the range of the data in relation to the estimated surfaces. Higher order interactions were not plotted due to increased complexity of visualizing shapes in more than 3 dimensions.

Additional phenotypic selection analyses were performed to assess the temporal dynamics of selection. In particular, the influence of mortality in a particular year from among multiple years concluding with 2012 (Fig. 2-1) on the form and magnitude of selection on timing of apical shoot elongation in a specific year was assessed by comparing the fitness surfaces that included and excluded mortality of a particular year.

Analysis of the degree of differentiation among populations for timing of elongation rates and evaluation of the relationship between the populations' climate of origin and elongation rates followed methods detailed in Chapter 1. In brief, the analysis followed statistical framework presented by Leites et al. (2012b). The analysis used generalized linear mixed-effects models (GLMM) using the lmer function (Bates, Maechler, and Bolker, 2013) in R (Team, 2013) to evaluate the relationship between measures of growth rhythm and climate of seed source. The GLMM form for the analysis was:

$$Y_{ijk} = (U + C_i + B_j + P_k + PR_{kl} + e_{ijk})$$

where Y_{ijk} = is the observed trait value of the ith climate variable of population origin effect, of the kth population, in the jth block, U is the overall mean, C_i is the climate of population origin effect, B_j is the block effect, P_K is the population effect, PR_{kl} is the interaction of the kth population and the lth row and e_{ijk} is the random residual error. The climate of seed source was treated as a fixed effect, while effects for block, population and the interaction of population and row were treated as random. A total of 26 climate

variables (see Chapter 1) were tested by comparing the likelihoods of nested models using maximum likelihood to identify the best model.

Results

Phenotypic variability of growth rhythm, height and survival

The magnitudes of apical shoot elongation rates and their timing ranged broadly among individual trees over five consecutive growing seasons in each experiment (Table 2-3). Even so, most shoot elongation occurred during a 2 to 3 week period in late May of each year. As a whole, apical shoot elongation rates tended to increase with yearly development. Elongation rates in Exp. 1 were greater than Exp. 2 throughout the study period. Total above ground mean height of survivors in 2012 differed significantly ($P < 0.05$) between experiments (1553 mm, Exp. 1 and 1103 mm, Exp. 2). Survival to 2012 was 39% and 40% in experiments 1 and 2, respectively. Survival declined substantially in single episodes in the fall following relatively dry growing seasons in 2002 in Exp. 1 and 2006 in Exp. 2 (Table 2-1 and Table 2-3).

Timing of initiation, cessation, and duration of elongation were highly correlated. Higher elongation rates were correlated with earlier initiation, later cessation and longer duration overall. In addition, these aspects of growth rhythm were more highly correlated with shoot elongation rates earlier rather than later in the growing season (Table 2-4). Variation in the timing of initiation of shoot elongation spanned 2 weeks, while timing of cessation of shoot elongation, varied by as much as 3 weeks.

Finally, total duration of shoot elongation for the majority of individuals ranged from approximately 7 to 10 weeks.

Selection on timing and rate of apical shoot elongation

Survival and unconditional expected height of seedlings in 2012 were dependent on timing of the rate of apical shoot elongation. In Exp. 1, the linear, nonlinear and bivariate nonlinear terms for the rate of apical shoot elongation in 2002 exhibited early and late in the growing season were significant predictors of survival and unconditional expected height in 2012 (Table 2-5). The shape of the fitness surface for survival (Fig. 2-2A) and unconditional expected height (Fig. 2-2C) in 2012 in relation to elongation rates in 2002 was defined by a saddle separating two peaks and two valleys indicating disruptive selection. The first optimum for fitness corresponded with low early-season and high late-season elongation rates while the second optimum for fitness corresponded with high early-season and low late-season elongation rates.

Observed elongation rates from 2002 plotted on the fitness surface for survival show elongation rates well represented from 0 mm/day to a broad saddle spanning intermediate elongation rates early and late in the growing season (Fig. 2-2A). In addition, observed elongation rates for early-season elongation rates are reasonably represented spanning the gradient across the upper saddle. Together these patterns represent a curvature over the fitness surface in the direction of selection. Thus curved directional selection favoring low early-season and high late-season elongation rates was observed. However few observations occur on or leading to the second fitness optimum

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corresponding with low early-season and high late-season elongation rates. Thus curved directional selection favoring high early-season and low late-season elongation rates was weakly supported. Observed elongation rates from 2002 plotted on the fitness surface for unconditional expected height (Fig. 2-2C) occurred over a range of the fitness surface that was similar to the distribution of observations for the fitness surface using survival described above. However, few observations were available to support curved directional selection against high early- and late-season elongation rates. Thus, when total height was considered along with survival, curved directional selection favoring low early-season elongation rates with high late-season elongation rate was observed, while curved directional selection against phenotypes with high early-season and high late-season elongation rates was not observed.

In Exp. 1, in later years, linear coefficients for rates of apical shoot elongation in 2003, 2004 and 2005 were statistically significant in relation to survival and unconditional expected height in 2012 (Table 2-5). Nonlinear coefficients were statistically significant for shoot elongation rates in 2003 and 2004 for survival, and 2003, 2004 and 2005 for unconditional expected height in 2012 (Table 2-5). When observed measures of growth rhythm were plotted in relation to modeled fitness surfaces using survival as a proxy for fitness, curved directional selection favoring high early-season and low late-season elongation rates in 2003 (Fig. 2-4A) and 2004 (Fig. 2-4B) was observed. When observed measures of growth rhythm were plotted in relation to modeled fitness surfaces using unconditional height as a proxy for fitness, selection favoring intermediate early-season elongation rates (stabilizing selection) and high late-season

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elongation rates (directional selection) in 2003 (Fig. 2-4D) was observed. In contrast, only directional selection was observed for shoot elongation in 2004 and 2005 in relation to unconditional expected height (Fig. 2-4E, and F). In addition, observations of timing of initiation or cessation, with late-season elongation rate of seedlings in 2005 in relation to survival to 2012 showed that phenotypes that started elongation earliest were favored by selection (Table 2-5, Fig. 2-6).

In Exp. 2, the linear and nonlinear coefficient for the rate of apical shoot elongation exhibited late in the 2003 growing season was statistically significant in relation to survival and unconditional expected height in 2012 (Table 2-6). The plotted relationship showed that as late-season elongation rates increased the expected percent survival (Fig. 2-3A) and unconditional expected height (Fig. 2-3C) increased to an intermediate optimum with an approximately symmetric decline thereafter. The corresponding 95% confidence interval for expected percent survival and unconditional expected height widened with increasing elongation rates. Thus selection favoring increasing elongation rate up to an intermediate optimum was well supported, while evidence of stabilizing selection or selection against the increasingly higher late-season elongation rates was weaker.

In Exp. 2, as observed in Exp. 1, the direction and magnitude of selection on elongation rates expressed among growing seasoned differed. In particular, shoot elongation rates in 2003 for both linear and nonlinear coefficients were statistically significant in relation to survival and unconditional expected height in 2012 (Table 2-6). When observed measures of growth rhythm were plotted in relation to modeled fitness

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surface that used survival as the proxy for fitness, selection favoring increasing early-season and decreasing late-season elongation rates was observed (Fig. 2-5A). In contrast, only linear coefficients for elongation rates early and late in 2005 and 2006 growing season were statistically significant in relation to survival and unconditional expected height in 2012 (Table 2-6). When observed measures of growth rhythm were plotted in relation to modeled fitness surfaces, directional selection favoring increasing early- and late-season elongation rates was observed (Fig. 2-5B, C, E, and F). Analysis of growth rhythm in 2005 and 2006 included timing of shoot initiation, cessation and duration; however traits were not statistically significant ($P > 0.05$) in relation to survival or unconditional expected height in Exp. 2 when elongation rates were included in the models.

In both experiments in all years (Fig. 2-2 through 2-6) the magnitude of selection which is represented by the steepness of the gradient or regression line varied across phenotypes. Strong selection was consistently observed on phenotypes with low early- and late-season elongation rates.

Temporal variation of selection

The form of selection on measures of growth rhythm differed over time in both tests (Table 2-5 and 2-6). In addition, the form of selection on elongation expressed in a given year was influenced by mortality that differed among growing seasons. For example, **w**hen mortality in 2002 in Exp. 1 was excluded from the fitness regression for elongation rate in 2002, nonlinear and bivariate covariates were no longer detected (P

<0.05). These results indicated that curved directional selection observed on elongation

rates early in the growing season resulted from mortality in fall 2002, while subsequent

mortality-related selection from 2003 to 2012 resulted only in directional selection on

elongations rates exhibited in 2002 (Fig. 2-2B and 2-3D). Likewise in Exp. 2, when

mortality from 2003 and 2004 was excluded from fitness regression for elongation rates

in 2003, nonlinear covariates were no longer detected (P <0.05). This result indicates that

stabilizing selection on late elongation in 2003 resulted from mortality which occurred in

fall 2003 and fall 2004, while subsequent mortality-related selection from 2005 to 2012,

resulted only in directional selection on elongation rates exhibited in 2003 (Fig. 2-3B and

2-4D).

Population differentiation and climatic distribution of elongation rates

Timing and rate of apical shoot elongation differed significantly among

populations in relation to climate of seed source (Table 2-7). Model performance did not

improve with multiple climate predictors, hence single climate predictors were used for

all models. In Exp. 1, the ratio of summer precipitation to mean annual precipitation

(PRATIO) of seed source was negatively associated with and explained 32% of the

variation for apical shoot elongation rates expressed late in the 2005 growing season

(Julian days 131-147). While mean annual precipitation (MAP) of seed source was

positively associated with and explained 8% of the variation in apical shoot elongation

expressed even later in the 2005 growing season (Julian days 147-153, 2005). For the

study region, PRATIO generally increased and MAP generally decreased from northwest

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to southeast direction. The coldness of winter measured in degree-days $< 0°C$ (based on mean minimum monthly temperature, MMINDD0) was negatively associated with and explained 28% of the variation for duration and 26% of the variation for timing of cessation of apical shoot elongation. In the study region, increasing MMINDD0 tends to occur from a north to south direction and with increasing elevation. Thus seed sources from generally more northwesterly and lower elevation origins, where a higher proportion of annual growing season precipitation and milder winters occur, tended to exhibit higher apical shoot elongation and grew longer in Exp. 1.

Climate predictors for rate and timing of shoot elongation differed between experiments although in general the same seed sources were favored at both sites. Therefore, in Exp. 2, the spring date when the sum of degree-days above $5°C$ reached 100 degree days (D100) was negatively associated with and explained 13% of variation in early-season (Julian days 108-118, 2005) and late-season (Julian days 132-145, 2005) apical shoot elongation rates. D100 also explained 12% of the variation in duration and 17% of the variation in timing of shoot cessation. For the study region, increasing D100 tends to occur in a southeast to northwest direction and with decreasing elevation. Thus seed sources from generally more northwesterly and lower elevation where earlier spring warming (D100) occurs tended to grow faster and grow longer at the harsher Exp. 2 site (see Chapter 1, Fig. 1-6).

Discussion

The present study detected selection (Tables 2-5 and 2-6) and described its form, magnitude and temporal dynamics (Figs. 2-3 through 2-7) for measures of growth rhythm in whitebark pine (*Pinus albicaulis*) trees grown 12 and 10 years in the field under a climate warmer and drier than the climate of seed origin. The form and magnitude of selection on timing and rate of shoot elongation varied between experimental sites and among years. Directional selection, curved directional selection, and stabilizing selection were detected. Curved directional selection and stabilizing selection were chiefly associated with mortality in the year of trait expression in earlier years (Fig. 2-2 and Fig 2-3). The magnitude of selection was consistently strong against phenotypes with low early- and late-season shoot elongation rates in both experiments and over time. Modest heritability has been reported for measures of growth rhythm in whitebark pine (Bower and Aitken, 2006). In addition, the climate of the study sites approximated future predicted climate of the present century for whitebark pine's contemporary distribution ($+ 4.4^0$C to $+9.1^0$C) in the interior US Rocky Mountains. Observed phenotypic selection is therefore likely indicative of the direction and magnitude of short term evolutionary response of whitebark pine under predicted climate change.

Assessing selection using survival vs. unconditional expected height as measures of fitness

A measure of fitness is required to assess selection on growth rhythm using regression analysis. Fitness, however, is a difficult trait to measure directly particularly in

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long lived trees which generally require several decades to generate offspring following germination. Thus, unconditional expected height was used as a proxy for fitness. Unconditional expected height, explicitly accounted for both total height at a particular time and survival to that time. Therefore, it is interpreted in the present study to represent a more complete assessment of fitness in comparison with survival alone. Indeed, survival to successful reproduction is fundamental to fitness while greater relative height confers a fitness advantage in forest trees by, for example, allowing for greater reproductive surface and access to resources (see Mcgraw and Wulff, 1983).

In the present study, the extent that tree height in 2012 influenced estimates of fitness using unconditional expected height in relation to growth rhythm was assessed by comparing models using survival alone versus unconditional expected height which included survival. In general, the modeled relationship between growth rhythm and fitness using either survival or unconditional expected height was similar (Figs 2-3A vs. 2-3C and Figs 2-4A vs. 2-4C). Thus survival to 2012 and not tree height in 2012 was the fitness component of primary importance for the inferred relationship between fitness and growth rhythm.

Variation in selection over time and between sites

Variation in temporal dynamics of selection within several natural populations has been reported (Siepielski, DiBattista, and Carlson, 2009; Morrissey and Hadfield, 2012). Because the form of selection can vary substantially over time, accounting for temporal dynamics in selection is important. Indeed, for the present study, the magnitude and

direction of selection on shoot elongation expressed in a single year differed in relation to yearly mortality over the 12 year study period. Curved directional and stabilizing selection on shoot elongation rates in a particular year were chiefly associated with mortality that occurred in the same year (Table 2-5 and 2-6; Figs. 2-3 and 2-4). In addition, the form and magnitude of selection also varied for shoot elongation rates expressed in different years. More complex forms of selection were generally detected on shoot elongation in increasingly earlier years (Figs. 2-5 and 2-6). The observed patterns undoubtedly represent change in the selection environment over time associated with tree development and yearly fluctuation in weather. These results demonstrate the influence of early mortality on the direction of phenotypic selection within plant populations and underscore the importance of assessing selection in long lived plant species over multiple years.

Stabilizing selection is expected to occur frequently in nature (Crow and Kimura, 1970), however its detection has been relatively scarce among empirical studies (Kingsolver et al., 2001). Shaw and Geyer (2010) suggest that in addition to methodological problems outlined by Travis (1989), statistical bias among some applications of Lande and Arnold's (1983) ordinary least squares (OLS) methods to quantify natural selection may also contribute to a lack of detection for stabilizing selection. In particular, attempted adherence to assumptions of normality for the distribution of fitness in OLS analysis generally limited such analyses to consider only components of fitness over single episodes of selection. Even under these simplified conditions estimates of selection gradients using best quadratic approximation in OLS

methods can be misleading when the population's trait mean differs substantially from the trait value that confers highest or lowest fitness (Shaw and Geyer, 2010). Therefore, it is noteworthy that stabilizing selection was detected in the present study which used aster analysis to explicitly included selection over multiple episodes of selection spanning much of the critical stage of early tree growth and development in relation to climate in a single, unified analysis.

The magnitude and direction of selection among seedlings in experiments 1 and 2 were similar. These similarities were likely due to the similarity in selective environments between tests. In particular, selection at both tests occurred on juvenile stage growth and development under a similar warm-dry climate with characteristic late summer drought. Nonetheless, the pattern of selection on growth rhythm was generally more complex in Exp. 1 than Exp. 2. Soil conditions appear to be the primary difference in selection environment. In comparison with Exp. 2, the less restrictive soil conditions in Exp. 1 appear to have supported greater expression of variation in apical shoot elongation rates and correlated traits among individuals which allowed for greater phenotypic variation for selection to act on. In contrast, the more restrictive soils conditions in Exp. 2 appear to have limited growth expression uniformly among individuals resulting in less phenotypic variation for selection to act on. Interestingly, the restrictive soil conditions in Exp. 2, are more representative of whitebark pine's native habitat. Therefore, these results suggest that restrictive soils may contribute to relaxed selection in whitebark pines's native habitat and may explain to some extent the mildness of climatic clines detected for measures of growth rhythm (Table 2-7). However, the

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present study cannot confirm this apparent relationship. Greater confidence toward

understanding the relationship between environmental stress and selection on plant traits

requires study designs that assess selection under replicated environmental stress (e.g.

Stanton, Roy, and Thiede, 2000; Chapter 3).

Direct Selection and Mechanism of Selection

Direct selection occurs when variation in a selected trait is causally related to

variation in fitness. While the present study detects and describes selection on growth

rhythm, whether selection is acting directly on the rate of apical shoot elongation and its

timing or indirectly through direct selection on a correlated trait(s) is uncertain. This

condition exists because analyses using a fitness regression approach cannot discount the

possibility that observed selection on a trait is due to selection on an unobserved and

phenotypically correlated trait(s) (Lande and Arnold, 1983). Nevertheless, increased

confidence can be attained with careful consideration of the traits included in an analysis

and their functionality (Mitchell-Olds and Shaw, 1987).

Results from the present study provide insight into potential mechanisms of

selection on growth rhythm. In all years where mortality occurred, selection was

predominantly against phenotypes with overall (early-and late-season) slow rates of shoot

elongation. Selection against slower phenotypes did not appear to be related to

competition. Inter-species competition was intensively controlled while intra-species

competition noted as overtopping or crown closure was rarely observed due to slow

growth, spacing, and high mortality in early years. Instead, the corresponding smaller

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size of slower growing phenotypes may have limited their ability to withstand environmental stress associated, to some extent, with the nonnative warm-dry environment. In addition, the detection of nonlinear forms of selection in only early years in both experiments, despite variation in selection environment over time and between sites suggests that stabilizing selection was closely associated with greater sensitivity to environmental stress during earlier development. To be sure, greater resolution of causality would require further studies which either manipulate the environment or the phenotype.

Genecology of Growth Rhythm

In the present study, a substantial range of variation in timing and rate of apical shoot elongation was observed within all populations. Indeed, as much or greater variation in growth rhythm is commonly found within populations than among populations of temperate and boreal forest tree species (see Ekberg, Eriksson, and Weng, 1985). Even so, climatic clines for variation among population means for measures of growth rhythm have been detected and described in many temperate forest tree species. These clines are characteristic of particular species and range from steep (ex. Pseudotsuga menziesii, St Clair, Mandel, and Vance-Borland, 2005) to mild (ex. Larix occidentalis, Rehfeldt and Jaquish, 2010) or not detectable (ex. Pinus monticola, Rehfeldt, Hoff, and Steinhoff, 1984). In the present study, mild differentiation in mean rate and timing of apical shoot elongation was detected among populations in relation to climate of seed source ($r^2 = 0.08 - 0.28$). Seed sources with slightly faster and slightly

longer growing phenotypes originate from locations where winters are milder (decreasing MMINDD0 and/or D100) and precipitation is more balanced and greater over the year (increasing PRATIO and/or MAP, respectively). This climate tends to occur in an increasing southeast to northwest direction over the study region. These results are consistent with clinal variation for growth potential reported in Chapter 1.

The apparent mildness of clinal variation of growth rhythm among populations in whitebark's native habitat may be explained by several mechanisms. For example, growth rhythm in whitebark may exhibit a moderate degree of adaptive phenotypic plasticity. This explanation has been proposed to explain intermediate steepness in clines detected among populations for several species in the interior northwest (Rehfeldt, 1994a). Under this condition, the same or similar genotypes would exhibit a moderate range of favorable phenotypes in response to variation in environmental stress in the native habitat thereby limiting selection on genetic variation (Lewontin, 1957; Bradshaw, 1965; Schlichting, 1986; West-Eberhard, 1989; Scheiner, 1993). Alternatively or in addition, selection pressure on growth rhythm may be mild over the study region. For example, differences in selection on growth rhythm in response to the timing and severity of cold events in the interior northwest may be minimal. Indeed growth rhythm among whitebark pine populations appears to be well adapted to cold with only mild differences detected for cold injury among regions in relation to timing of cold acclimation in the spring and predominantly in the fall (Bower and Aitken, 2006). In contrast, selection observed in the present study was under warm-dry and drought conditions predicted to rarely occur in the sampled range of whitebark pine habitat (Rehfeldt, Ferguson, and

Crookston, 2008). Mild-clines could also result from strong selection from varying microclimates over small geographic distances within population origins that are effectively repeated among population origins. However, despite detailed investigations, little evidence to support microclimate variation using topography as a surrogate has been detected for species interpreted as conveying physiological specialization (Psuedotsuga menziesii, Rehfeldt, 1974) or high phenotypic plasticity (Pinus monticola, Rehfeldt, 1979) for growth rhythm. Thus this possibility seems less likely. Finally, genetic variability within populations is expected to be enhanced by migration and mutation. In whitebark pine, gene flow via pollen facilitated by wind and via seed dispersal largely by Clark's nutcracker (*Nucifraga columbiana* Wilson) occurs broadly among populations, although gene flow is predominantly local (Richardson, Klopfenstein, and Brunsfeld, 2002), as it is in terrestrial plant species in general (see Ellstrand, 1992). Nonetheless, even limited amounts of pollen migration among perennial species can help maintain non optimal genes in a population and inhibit local adaptation (Antonovics, 1968; Lenormand, 2002). Continued research, including assessing phenotypic selection in whitebark pines' contemporary native habitat is needed to elucidate the extent that any of these mechanisms or others may influence genetic diversity for growth rhythm among and within populations.

Conclusion

The present study assesses fitness as a measure of survival and height at the end of 12-years following the first growing season after outplanting. This period is a critical

phase of establishment when juvenile trees are more susceptible to climate stress than later periods (Namkoong, Usanis, and Silen, 1972; Namkoong and Conkle, 1976). Nonetheless, extrapolation of a selection response based on survival or unconditional expected height assumes that the inferred relationship remains constant over time. In addition, results do not account for potential influences of additional abiotic and biotic interactions in whitebark pine's native habitat under rapid climate change. For example, the impact of climate change on deleterious biotic interactions such as with mountain pine beetle (*Dendroctonus ponderosae*) and whitebark pine blister rust (*Cronartium ribicola*) are uncertain. Thus, broad interpretations of phenotypic evolution under nonnative climates should be made with caution. Continuing research is needed to examine these limitations as well as phenotypic evolution of growth rhythm and other key adaptive traits during seed emergence and early establishment (see Chapter 3), and in relation to reproduction and gene flow.

Under ongoing rapid climate change, the distributions of plant species from lower elevations are predicted to shift upward in elevation (Rehfeldt et al., 2006a). Indeed, previous reports suggest that shifts in the elevation of many plant species have already occurred in some montane ecosystems (e.g. Beckage et al., 2008; Kelly and Goulden, 2008; Lenoir et al., 2008; Feeley et al., 2011). This condition may lead to competitive exclusion of relatively slow growing whitebark pine regardless of its degree of adaptation to warm-dry climate. Whitebark pine already occupies the uppermost timberline over much of its range. Elevations beyond the timberline are characterized by poorly formed soils or lack soil altogether such that whitebark pine may run out of room to expand its

range upward. In addition, whitebark pine may be required to exceed estimated historic range shifts of 10-50 km per century for tree species (see Davis and Shaw, 2001) by an order of magnitude to track contemporary climate northward (Malcolm et al., 2002). Thus results from the present study do not appear to contradict arguments for active gene conservation strategies including human assisted migration.

Table 2-1. Observed climate for Priest River Experimental Station at latitude 48.35°N, longitude 116.83°W and elevation, 971m from National Climate Data Center (NCDC), the National Weather Service (NWS), and USDA Forest Service, Priest River Experimental Forest records.

Year	PRATIO	SPPR (mm)	GSP(mm)	MAP (mm)	MAT (°C)	MTCM (°C)	MTWM (°C)
2001	0.44	161.29	326.39	744.73	6.99	-3.16	18.69
2002	0.37	113.79	249.68	673.86	6.67	-2.13	19.24
2003	0.45	105.92	343.41	759.97	7.50	-3.11	19.29
2004	0.52	144.02	395.48	762.25	7.49	-3.28	18.73
2005	0.57	128.52	412.75	730.00	6.71	-5.50	18.09
2006	0.34	139.19	315.72	926.34	7.39	-3.21	20.08
2007	0.33	68.07	257.30	768.60	7.05	-5.41	21.14
2008	0.43	73.91	328.42	768.10	5.98	-6.71	18.04
2009	0.47	115.32	341.12	728.22	6.37	-6.29	19.07
2010	0.53	166.12	516.64	969.26	6.90	-3.22	17.16
2011	0.48	217.42	416.81	869.95	6.01	-4.53	17.19
2012	0.50	152.40	595.88	1200.40	7.73	-2.80	20.34
Mean	**0.45**	**132.16**	**374.97**	**825.14**	**6.90**	**-4.11**	**18.92**

PRATIO = ratio of growing season precipitation to total precipitation, SPRP=Spring Precipitation, GSP=growing season precipitation, MAP=mean annual precipitation, MAT= mean annual temperature, MTCM=mean temperature in the coldest month, MTWM=mean temperature in the warmest months (see Chapter 1 for additional explanation)

Table 2-2. Intervals for timing of apical shoot elongation rates in Julian dates for each year used in aster model comparisons testing for Experiments 1 (Exp. 1) and Experiment 2 (Exp. 2).

Year	Early-Season				Late-Season						
	Exp.1										
2001	98-127				127-142	142-158	158-171				
2002	108-127				127-142	142-149	149-225				
2003	104-111	111-122			122-142	142-149	149-155	155-161	161-189		
2004	100-119	119-125			125-141	141-155	155-191				
2005	67-96	96-112	112-119		119-131	131-147	147-153	153-167	167-230		
	Exp. 2										
2003	105-120	120-129	129-135		135-148	148-155	155-170				
2004	106-114	114-119	119-126		126-141	141-156	156-195				
2005	96-108	108-118	118-125		125-132	132-145	145-152	152-165	165-215		
2006	95-101	101-109	109-115	115-122	122-129	129-136	136-143	143-150	150-157	157-164	164-232

Table 2-3. Apical shoot mean elongation rate, standard deviation (σ), maximum elongation rate, mean apical shoot elongation and survival by Experiment (Exp.) and year.

	Mean Rate (σ) (mm/day)	Max. Rate (mm/day)	Interval of total growing season used to calculate elongation rate in Julian dates	Fall Survival
Exp. 1				
2000	-	-	-	100 %
2001	-	-	-	96 %
2002	1.05 (0.51)	2.41	108 – 149	68 %
2003	1.69 (0.84)	4.20	104 – 149	64 %
2004	2.87 (1.20)	5.78	100 – 141	63 %
2005	2.39 (1.00)	4.92	96 – 147	63 %
2011	-	-	-	44 %
2012	-	-	-	39 %
Exp. 2				
2002	-	-	-	100 %
2003	0.92 (0.53)	3.00	105–155	93 %
2004	1.35 (0.77)	4.32	106–156	87 %
2005	1.78 (0.85)	4.76	96–152	86 %
2006	1.73 (0.66)	3.80	95–150	49 %
2010	-	-	-	42 %
2011	-	-	-	41 %
2012	-	-	-	40 %

Table 2-4a. Experiment 1, Person product moment correlations for intervals of apical shoot elongation rates for 2002 and 2005 and approximate timing of apical shoot initiation, cessation and duration Intervals are coded by last digit of their respective year followed by last Julian day of the interval, while the first interval of each year code the first and last Julian date of the interval.

	2/108-127	2/142	2/149	2/225	5/67-96	5/112	5/119	5/131	5/147	5/153	5/167	5/230	5/INIT	5/CES
2/108-127														
2/142	0.73***													
2/149	0.59***	0.65***												
2/225	0.33***	0.35***	0.61***											
5/67-96	0.08	0.06	0.02	0.00										
5/112	0.22***	0.13**	0.14**	0.11*	0.61***									
5/119	0.22***	0.16***	0.18***	0.14**	0.66***	0.87***								
5/131	0.17***	0.16***	0.20***	0.13**	0.67***	0.85***	0.93***							
5/147	0.11*	0.13**	0.29***	0.34***	0.51***	0.67***	0.75***	0.80***						
5/153	0.16***	0.12**	0.29***	0.49***	0.18***	0.30***	0.34***	0.33***	0.38***					
5/167	0.09*	0.09*	0.19***	0.29***	0.08	0.19***	0.23***	0.19***	0.34***	0.33***				
5/230	0.13**	0.12**	0.16***	0.11*	0.05	0.18***	0.18***	0.18***	0.18***	0.12**	0.40***			
5/INIT	-0.13**	-0.15***	-0.15***	-0.07	0.53***	0.60***	0.63***	0.68***	0.57***	0.18***	0.12**	0.07		
5/CES	-0.04	-0.06	-0.01	0.08	0.56***	0.67***	0.71***	0.73***	0.71***	0.34***	0.35***	0.21***	0.92***	
5/DUR	-0.04	-0.06	-0.02	0.07	0.60***	0.68***	0.72***	0.75***	0.72***	0.35***	0.33***	0.21***	0.90***	0.99***

Note: **Init**, initiation, **Ces**, cessation , **Dur**, duration, * $P < .05$, ** $P < .01$, *** $P < .001$

Table 2-4b. Experiment 2, Person product moment correlations for intervals of apical shoot elongation rates for 2003 and 2006 and approximate timing of apical shoot initiation, cessation and duration Intervals are coded by last digit of their respective year followed by last Julian day of the interval, while the first interval of each year code the first and last Julian date of the interval.

	3/105-120	3/129	3/135	3/148	3/155	3/170	6/95-101	6/109	6/115	6/122	6/129	6/136	6/143	6/150	6/157	6/164	6/232	6/Init	6/Ces
3/105-120																			
3/129	0.57***																		
3/135	0.73***	0.73***																	
3/148	0.66***	0.65***	0.74***																
3/155	0.28***	0.19***	0.27***	0.52***															
3/170	0.13*	0.17**	0.17**	0.29***	0.45***														
6/95-101	0.02	0.10	0.08	0.02	0.05	0.08													
6/109	0.08	0.11	0.06	0.04	0.01	0.02	0.66***												
6/115	0.27***	0.23***	0.24***	0.24***	0.06	0.10	0.12*	0.14*											
6/122	0.27***	0.26***	0.26***	0.26***	0.07	0.11*	0.20***	0.31***	0.77***										
6/129	0.26***	0.28***	0.29***	0.28***	0.10	0.12*	0.21***	0.33***	0.74***	0.82***									
6/136	0.24***	0.25***	0.24***	0.26***	0.04	0.12*	0.19**	0.24***	0.69***	0.84***	0.81***								
6/143	0.27***	0.29***	0.28***	0.40***	0.19***	0.18**	0.13*	0.19***	0.56***	0.74***	0.73***	0.77***							
6/150	0.15**	0.21***	0.23***	0.42***	0.38***	0.21***	0.02	0.06	0.25***	0.39***	0.31***	0.31***	0.67***						
6/157	0.08	0.07	0.11	0.24***	0.48***	0.25***	-0.04	0.06	0.06	0.17**	0.14*	0.09	0.35***	0.72***					
6/164	0.10	0.03	0.07	0.14*	0.39***	0.18**	-0.04	0.02	0.09	0.13*	0.15**	0.11	0.17**	0.30***	0.61***				
6/232	-0.08	-0.11	-0.09	-0.11	-0.03	-0.06	-0.04	-0.03	0.10	0.13*	0.18**	0.17**	0.09	-0.01	0.08	0.32***			
6/Init	0.11	0.04	0.08	0.08	0.04	0.03	-0.30***	-0.11	0.12*	0.11	0.10	0.11*	0.14*	0.11	0.06	0.03	0.04		
6/Ces	0.07	0.02	0.07	0.10	0.18**	0.08	0.04	0.17**	0.19***	0.27***	0.25***	0.23***	0.32***	0.36***	0.42***	0.40***	0.22***	0.58***	
6/Dur	0.05	0.06	0.07	0.09	0.17**	0.09	0.47***	0.43***	0.22***	0.34***	0.31***	0.28***	0.32***	0.32***	0.36***	0.34***	0.17**	0.19***	0.83***

Note: **Init**, initiation, **Ces**, cessation, **Dur**, duration, * P < .05, ** P < .01, *** P < .001

Table 2-5. Experiment 1, aster model comparison test results for the effects of timing of measures of growth rhythm within a given year on survival through 2012 beginning in the respective year and unconditional expected height in 2012 that included survival to 2012 beginning in the respective year.

Response	Year	Timing (Julian dates) of the rate of apical shoot elongation and cessation		Selection gradient	Standard error	Model df	Model Deviance	Test df	Test P-value	Random Effects: Square Root of Variance Components (P-values are one-tailed)	Estimate	Std. Error	P-value
2012 Survival	2002	Full Model				12	1437.1	-	-	Block	7.34E-01	2.29E-01	0.0007 ***
		108-127	β	4.79E+00	1.11E+00	9	1393.4	3	< 0.0001 ***	Population	6.24E-01	1.22E-01	< 0.0001 ***
		142-149	β	9.66E-01	2.29E-01	9	1394.5	3	< 0.0001 ***	Population: Row	0	NA	NA
		$(108-127)^2$	γ_{ii}	-3.63E-01	1.18E+00	10	1414.9	2	< 0.0001 ***				
		$(142-149)^2$	γ_{ii}	4.47E-03	5.19E-02	10	1417.8	2	0.0001 ***				
		108-127 * 142-149	γ_{ij}	-1.09E+00	3.50E-01	9	1397.1	3	< 0.0001 ***				
	2003	Full Model				11	2336	-	-	Block	8.04E-01	2.54E-01	0.0008 ***
		104-111	β	1.95E+00	5.09E-01	8	2305.8	3	< 0.0001 ***	Population	7.21E-01	1.31E-01	< 0.0001 ***
		142-149	β	3.95E-01	1.30E-01	8	2324.5	3	0.0010 **	Population: Row	NA	NA	NA
		$(104-111)^2$	γ_{ii}	-2.06E-01	2.11E-01	9	2326.3	2	0.0078 **				
		$(142-149)^2$	γ_{ii}	-1.06E-02	1.59E-02	9	2326.8	2	0.0100 *				
		104-111 * 142-149	γ_{ij}	-1.57E-01	9.28E-02	10	2332.8	1	0.0728 *				
	2004	Full Model				11	2790	-	-	Block	7.68E-01	2.44E-01	0.0008 ***
		119-125	β	1.13E+00	2.45E-01	8	2707.4	3	< 0.0001 ***	Population	8.19E-01	1.45E-01	< 0.0001 ***
		141-155	β	8.49E-01	2.75E-01	8	2771	3	0.0002 ***	Population: Row	0.01501	0.01435	0.115531
		$(119-125)^2$	γ_{ii}	-5.93E-02	2.92E-02	9	2769.6	2	< 0.0001 ***				
		$(141-155)^2$	γ_{ii}	-4.92E-03	4.42E-02	9	2777.2	2	0.0017 **				
		119-125 * 141-155	γ_{ij}	-1.70E-01	5.09E-02	10	2777.4	1	0.0004 ***				
	2005	Full Model				7	2538.8	-	-	Block	7.59E-01	2.42E-01	0.0009 ***
		131-147	β	7.20E-02	1.55E-02	5	2515.3	1	< 0.0001 ***	Population	7.95E-01	5.53E+00	< 0.0001 ***
										Population: Row	0.01718	0.807	0.2098
	2005	Full Model				9	2586.7	-	-	Block	7.96E-01	2.54E-01	0.0009 ***
		96-112	β	6.99E-01	3.65E-01	6	2582.6	3	0.0434 *	Population	8.13E-01	1.49E-01	< 0.0001 ***
		131-147	β	3.50E-01	7.77E-02	6	2549.7	3	< 0.0001 ***	Population: Row	0.0175	0.01484	0.1193
		Shoot initiation	β	-2.67E-02	4.24E-03	7	2538.8	2	< 0.0001 ***				
		$(131-147)^2$	γ_{ii}	-1.27E-02	3.88E-03	6	2575.0	2	< 0.0006 ***				
	[a]2005	Full Model				9	2589.7	-	-	Block	8.17E-01	2.59E-01	0.0008 ***
		131-147	β	1.64E+00	5.01E-01	6	2531.8	3	< 0.0001 ***	Population	8.10E-01	1.48E-01	< 0.0001 ***
		Shoot cessation	β	-6.46E-01	1.47E-01	6	2538.8	3	< 0.0001 ***	Population: Row	0.01678	0.01525	0.1356
		$(131-147)^2$	γ_{ii}	-8.45E-03	4.73E-03	6	2566.7	2	< 0.0001 ***				
		$(Shoot\ cessation)^2$	γ_{ii}	2.40E-03	5.85E-04	7	2563.9	2	< 0.0001 ***				
		131-147 * Shoot cessation	γ_{ij}	-8.84E-03	3.54E-03	8	2583.0	1	0.0096 **				
2012 Unconditional Expected Height	2002	Full Model				12	1542.4	-	-	Block	4.65E-04	1.44E-04	0.0006 ***
		108-127	β	3.16E-03	7.17E-04	9	1505.3	3	< 0.0001 ***	Population	4.21E-04	7.62E-05	< 0.0001 ***
		142-149	β	7.02E-04	1.43E-04	9	1461.7	3	< 0.0001 ***	Population: Row	0	NA	NA
		$(108-127)^2$	γ_{ii}	-3.99E-04	6.61E-04	10	1520.9	2	< 0.0001 ***				
		$(142-149)^2$	γ_{ii}	7.58E-06	2.89E-05	10	1525.5	2	0.0001 ***				
		108-127 * 142-149	γ_{ij}	-6.51E-04	1.86E-04	9	1504.8	3	< 0.0001 ***				
	2003	Full Model				7	2445.7	-	-	Block	5.39E-04	1.67E-04	0.0006 ***
		104-111	β	1.43E-03	3.04E-04	6	2401.9	1	< 0.0001 ***	Population	5.10E-04	8.38E-05	< 0.0001 ***
		142-149	β	1.35E-04	2.66E-05	6	2417.2	1	< 0.0001 ***	Population: Row	0	NA	NA
		$(104-111)^2$	γ_{ii}	-2.88E-04	9.52E-05	7	2436.1	1	0.0019 **				
	2004	Full Model				8	2925.1	-	-	Block	5.54E-04	1.72E-04	0.0006 ***
		119-125	β	7.20E-04	1.50E-04	6	2796.2	2	< 0.0001 ***	Population	5.28E-04	8.61E-05	< 0.0001 ***
		141-155	β	2.95E-04	4.72E-05	7	2881.6	2	< 0.0001 ***	Population: Row	0	NA	NA
		$(119-125)^2$	γ_{ii}	-4.13E-05	1.66E-05	7	2925.1	1	< 0.0094 **				
	[a]2005	Full Model				7	2623.3	-	-	Block	4.52E-04	1.42E-04	0.0007 ***
		131-147	β	-9.08E-06	2.90E-05	5	2586.6	2	< 0.0001 ***	Population	5.34E-04	8.12E-05	< 0.0001 ***
		147-153	β	1.06E-04	2.94E-05	6	2607.8	1	< 0.0001 ***	Population: Row	0	NA	NA
		$(131-147)^2$	γ_{ii}	4.06E-06	1.78E-06	6	2617.9	1	0.0202 *				

β= linear selection gradient, γ_{ii} = quadratic effects, γ_{ij} = cross products, full models = use variables listed for each year and the effect of each predictor variable was tested against the full model for each predictor variable, a = timing of shoot initiation, duration, and cessation were included in testing of elongation rates.

Table 2-6. Experiment 2, aster model comparison test results for the effects of timing of measures of growth rhythm within a given year on survival through 2012 beginning in the respective year and unconditional expected height in 2012 that included survival to 2012 beginning in each respective year. Only fixed effects that were significant are shown.

Response	Year	Timing (Julian dates) of the rate of shoot elongation and cessation		Selection gradient	Standard error	Model df	Model Deviance	Test df	Test P-value	Random Effects: Square Root of Variance Components (P-values are one-tailed)	Estimate	Std. Error	P-value
2012													
Survival	2003	Full Model				10	361.45	-	-	Block	2.62E-01	1.31E-01	0.0230 *
		135-148	β	1.1939368	0.2063	9	298.77	2	< 0.0001 ***	Population	2.73E-01	1.46E-01	0.0304 *
		$(135\text{-}148)^2$	γ_{ii}	-0.19843	0.04978	10	344.89	1	< 0.0001 ***	Population: Row	6.93E-03	6.75E-02	0.4591
	2004	Full Model				12	874.22	-	-	Block	2.76E-01	1.44E-01	0.0274 *
		119-126	β	1.7729482	1.01227	9	858.94	3	0.0016 **	Population	3.07E-01	1.60E-01	0.0277 *
		126-141	β	0.5908392	0.24159	9	862.84	3	0.0098 **	Population: Row	1.36E-02	5.16E-02	0.3963
		$(119\text{-}126)^2$	β	0.7158586	1.07466	10	863.45	2	0.0046 **				
		$(126\text{-}141)^2$	γ_{ii}	0.0098163	0.04046	10	867.11	2	0.0285 *				
		119-126 * 126-141	γ_{ij}	-0.640837	0.38386	9	856.85	3	0.0006 ***				
	[a]2005	Full Model				8	1259.2	-	-	Block	2.54E-01	1.34E-01	0.0286 *
		108-118	β	0.494153	0.21084	7	1253.5	1	< 0.0169 *	Population	3.08E-01	1.41E-01	0.0145 *
		132-145	β	0.2375592	0.06217	7	1244	1	< 0.0001 ***	Population: Row	1.37E-02	3.38E-02	0.3428
	[a]2006	Full Model				7	1334.3	-	-	Block	2.11E-01	1.17E-01	0.0353*
		109-115	β	0.6237743	0.21816	6	1325.8	1	0.0037 **	Population	1.81E-01	1.90E-01	0.1705
		143-150	β	0.2289682	0.05024	6	1312.5	2	< 0.0001 ***	Population: Row	2.15E-02	1.93E-02	0.1318
2012	2003	Full Model				10	392.94	-	-	Block	2.16E-04	1.06E-04	0.0209 *
Unconditional		135-148	β	1.08E-03	1.76E-04	9	298.17	2	< 0.0001 ***	Population	0	NA	NA
Expected Height		$(135\text{-}148)^2$	γ_{ii}	-1.54E-04	4.06E-05	10	377.94	1	0.0001 ***	Population: Row	0	NA	NA
	2004	Full Model				9	899.77	-	-	Block	1.96E-04	1.05E-04	0.0316 *
		126-141	β	7.27E-04	1.19E-04	7	786.28	2	< 0.0001 ***	Population	1.44E-04	1.54E-04	0.1752
		$(126\text{-}141)^2$	γ_{ii}	-4.92E-05	1.70E-05	8	872.67	1	< 0.0045 **	Population: Row	0	NA	NA
	[a]2005	Full Model				8	1347	-	-	Block	1.66E-04	9.89E-05	0.0466 *
		108-118	β	4.93E-04	1.70E-04	7	1338.5	1	< 0.0034 **	Population	1.99E-04	1.20E-04	0.0494 *
		132-145	β	3.55E-04	5.27E-05	7	1297.5	1	< 0.0001 ***	Population: Row	0	NA	NA
	[a]2006	Full Model				7	1402.1	-	-	Block	0	NA	NA
		109-115	β	7.62E-04	1.83E-04	6	1385	1	< 0.0001 ***	Population	0	NA	NA
		143-150	β	3.07E-04	4.12E-05	6	1342.1	2	< 0.0001 ***	Population: Row	0	NA	NA

β= linear selection gradient, γ_{ii} = quadratic effects, γ_{ij} = cross products, full models = use variables listed for each year and the effect of each predictor variable was tested against the full model for each predictor variable, a = timing of shoot initiation, duration, and cessation were included in testing of elongation rates.

Table 2-7. Results of multiple regression analyses that describe genetic variation for timing of apical shoot elongation rates relatively early and late in the 2005 growing season (Julian dates) and timing of apical shoot elongation in response to climate of seed source.

Response Variable	Term	Model df	Model Chisq	Chi df	P-value (> chisq)	Random Effects: Term	Variance	r^2
Experiment 1:								
131-147	Null	5	-	-		Prov:Row	1.5664	
	PRATIO	6	5.8042	1	0.016 *	Population	1.785	0.32
						Block	0.5043	
						Residual	20.5254	
147-153	Null	5	-	-		Prov:Row	1.00E-15	
	MAP	6	4.0496	1	0.0442 *	Population	0.0185	0.08
						Block	0.1139	
						Residual	7.973	
Initiation	Null	5	-	-		Prov:Row	0.851	
	SPRP	6	7.4253	1	0.1305	Population	5.07E-13	NA
						Block	2.15E-75	
						Residual	12.68	
Cessation	Null	5	-	-		Prov:Row	1.1404	
	MMINDD0	6	7.4253	1	0.0063 **	Population	0.9655	0.26
						Block	2.7024	
						Residual	26.5926	
Duration	Null	5		-		Prov:Row	1.0548	
	MMINDD0	6	17.021	1	0.0003 ***	Population	2.1069	0.28
						Block	0.3921	
						Residual	73.9617	
Experiment 2:								
108-118	Null	5	-	-		Prov:Row	0.028897	
	D100	6	6.0186	1	0.014 *	Population	0.008839	0.13
						Block	0.00122	
						Residual	0.241669	
132-145	Null	5	-	-		Prov:Row	0.08686	
	D100	6	4.0496	1	0.0152 *	Population	0.17948	0.13
						Block	0	
						Residual	2.82965	
Initiation	Null	5	-	-	0.2359	Prov:Row	0.02891	
	mtcm	6	1.4047	1		Population	0.039	NA
						Block	0.04341	
						Residual	0.30144	
Cessation	Null	5	-	-		Prov:Row	1.5772	
	D100	6	9.042	1	0.0026 **	Population	2.3054	0.17
						Block	0.6649	
						Residual	30.8007	
Duration	Null	5	-	-		Prov:Row	2.392	
	D100	6	17.021	1	0.0117 *	Population	2.929	0.12
						Block	1.247	
						Residual	42.602	

Null model does not include a climate predictor

A.

Present Spring 2002 → Survival Fall 2002 → Survival Fall 2003 → Survival Fall 2004 → Survival Fall 2005 → Survival Fall 2011 → Survival Fall 2012 → Height Fall 2012

B.

Present Spring 2003 → Survival Fall 2003 → Survival Fall 2004 → Survival Fall 2005 → Survival Fall 2006 → Survival Fall 2010 → Survival Fall 2011 → Survival Fall 2012 → Height Fall 2012

C.

Present Spring 2002 → Survival Fall 2002 → Survival Fall 2003 → Survival Fall 2004 → Survival Fall 2005 → Survival Fall 2011 → Survival Fall 2012

D.

Present Spring 2003 → Survival Fall 2003 → Survival Fall 2004 → Survival Fall 2005 → Survival Fall 2006 → Survival Fall 2010 → Survival Fall 2011 → Survival Fall 2012

Figure 2-1. Examples of graphical models used in aster analysis unifying survival over time and total height data and survival over time to assess fitness. Each node represents a component of life history while arrows represent the dependent association between predecessor and successor life-history components. Survival was modeled as a Bernoulli random variable and total height as normal random variable. **Graph A** shows the model used to predict unconditional height in fall 2012 which included survival from spring 2003 to fall 2012 in Experiment 1. **Graph B** shows model for height in fall 2012 which included survival from Spring 2003 to Fall 2012 in Experiment 2. **Graph C** shows the model used to predict survival in 2012 beginning with seedlings present in spring 2002 in Experiment 1. **Graph D** shows model for survival in 2012 beginning with seedlings present in spring 2003 in Experiment 2.
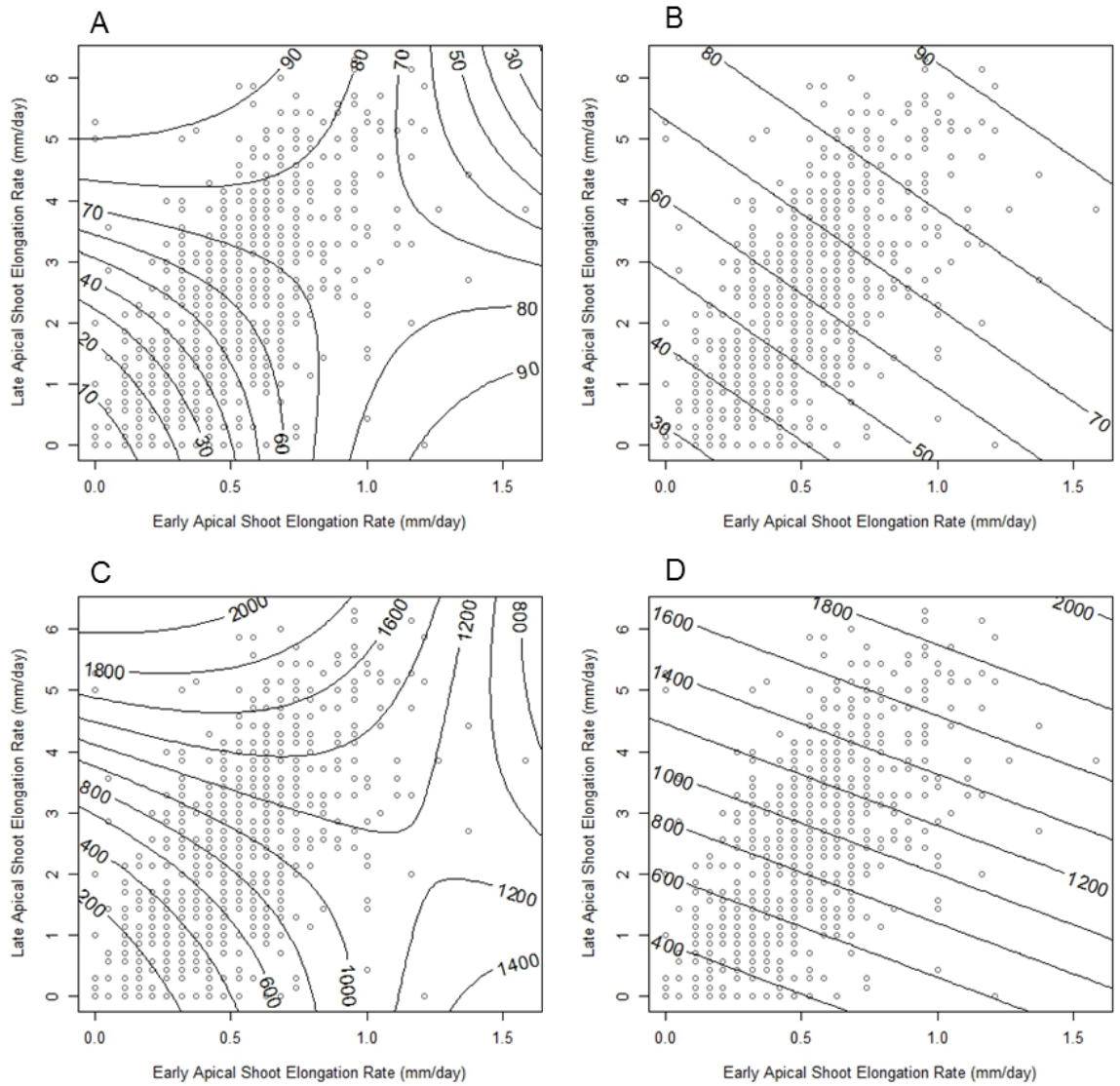
Figure 2-2. Experiment 1, fitness surface showing observed (open circles) apical shoot elongation rates (mm/day) early (Julian dates 108-127) and late (Julian dates 142-149) in the 2002 growing season in relation to modeled (contour lines) percent survival to 2012 that includes (A) or excludes (B) mortality prior to 2003 and unconditional height (mm) in 2012 that includes (C) or excludes (D) mortality prior to 2003.
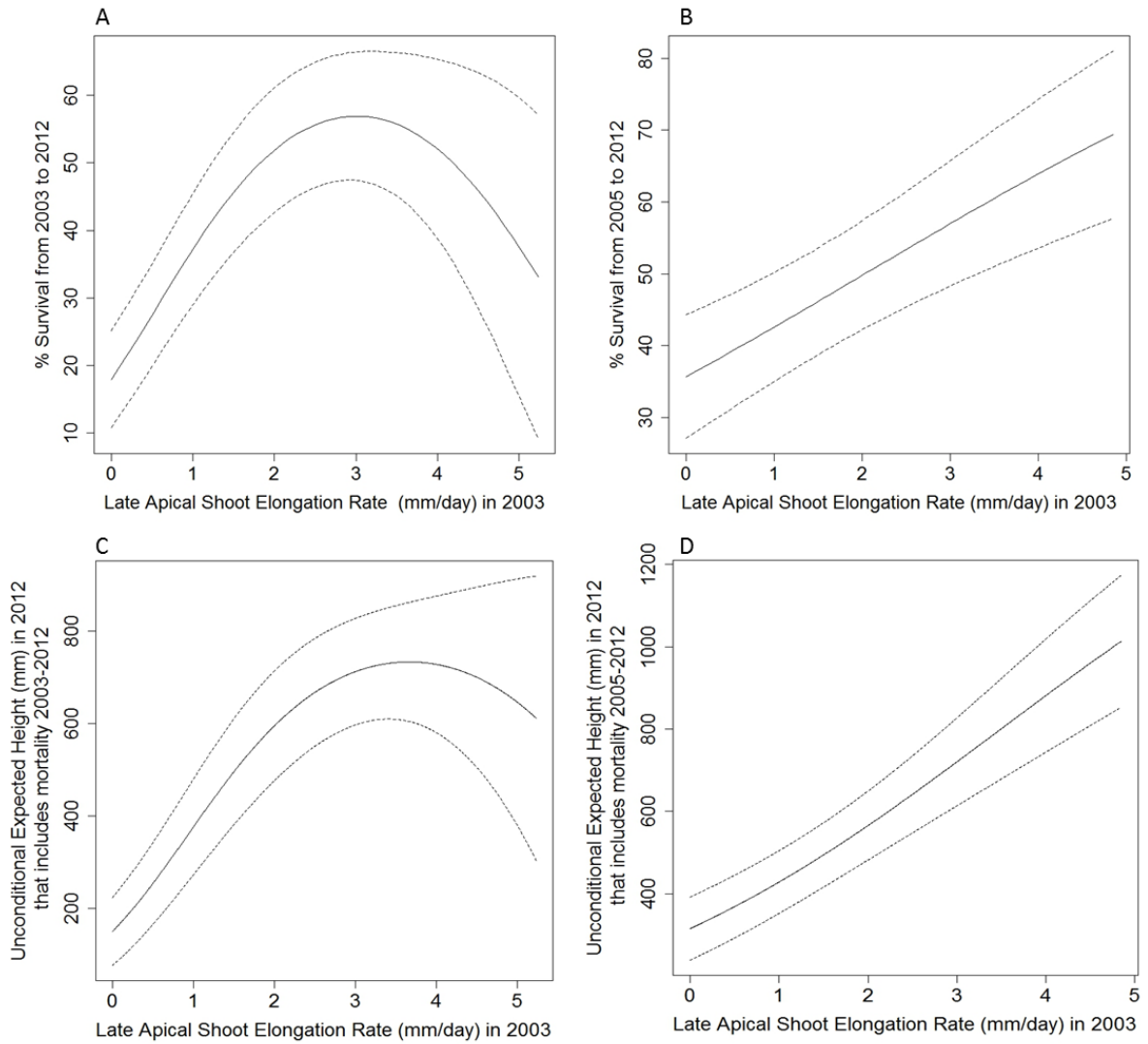
Figure 2-3. Experiment 2, modeled relationship between apical shoot elongation rates (mm/day) in 2003 (Julian dates 135-148) in relation to percent survival to 2012 that includes (A) or excludes (B) mortality prior to 2005 and unconditional height (mm) in 2012 site that includes (C) or excludes (D) mortality prior to 2005. Dotted lines represent 95% confidence intervals.
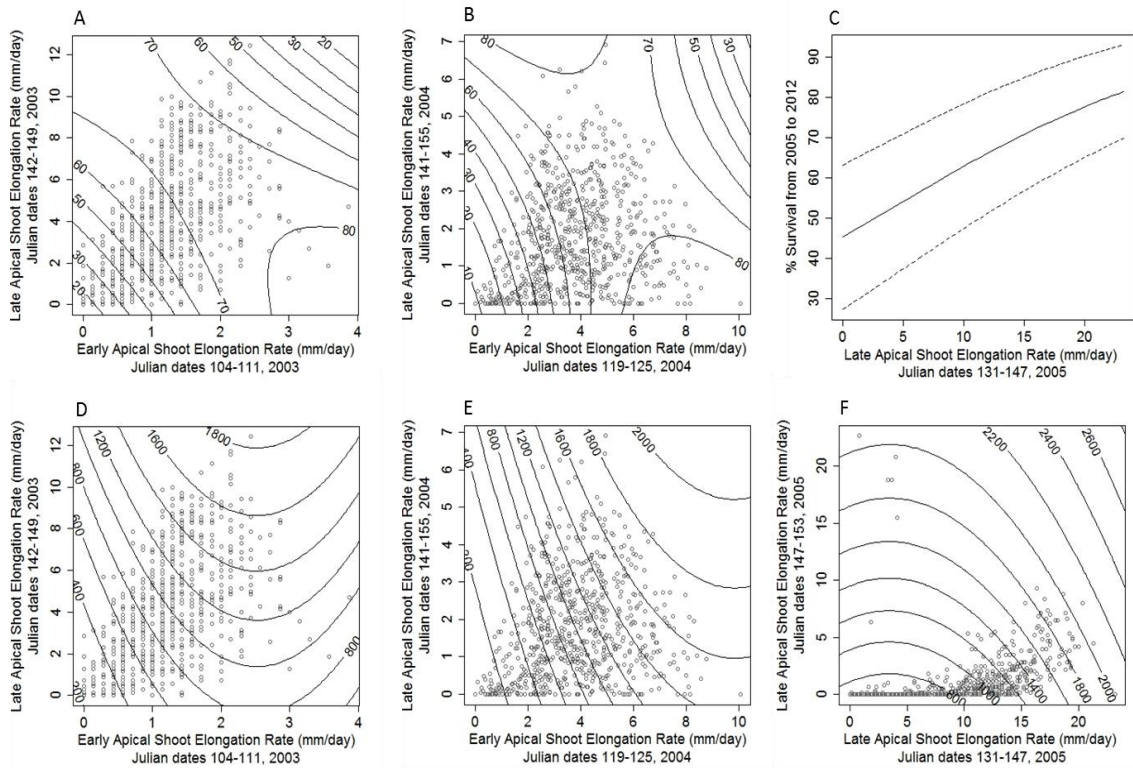
Figure 2-4. Experiment 1, fitness surfaces showing observed (open circles) apical shoot elongation rates (mm/day) in 2003 (A and D), 2004 (B and E) and 2005 (F) in relation to survival (contour) from (A) 2003-2012, and (B)2004-2012 and unconditional expected height (contour) in 2012 that includes survival from (D) 2003-2012, (E) 2004-2012, and (F) 2005-2012. Plot C shows fitness relation for only late apical shoot elongation because no other interval was statistically significant (P < 0.05) in relation to survival. Dotted lines represent 95% confidence intervals.
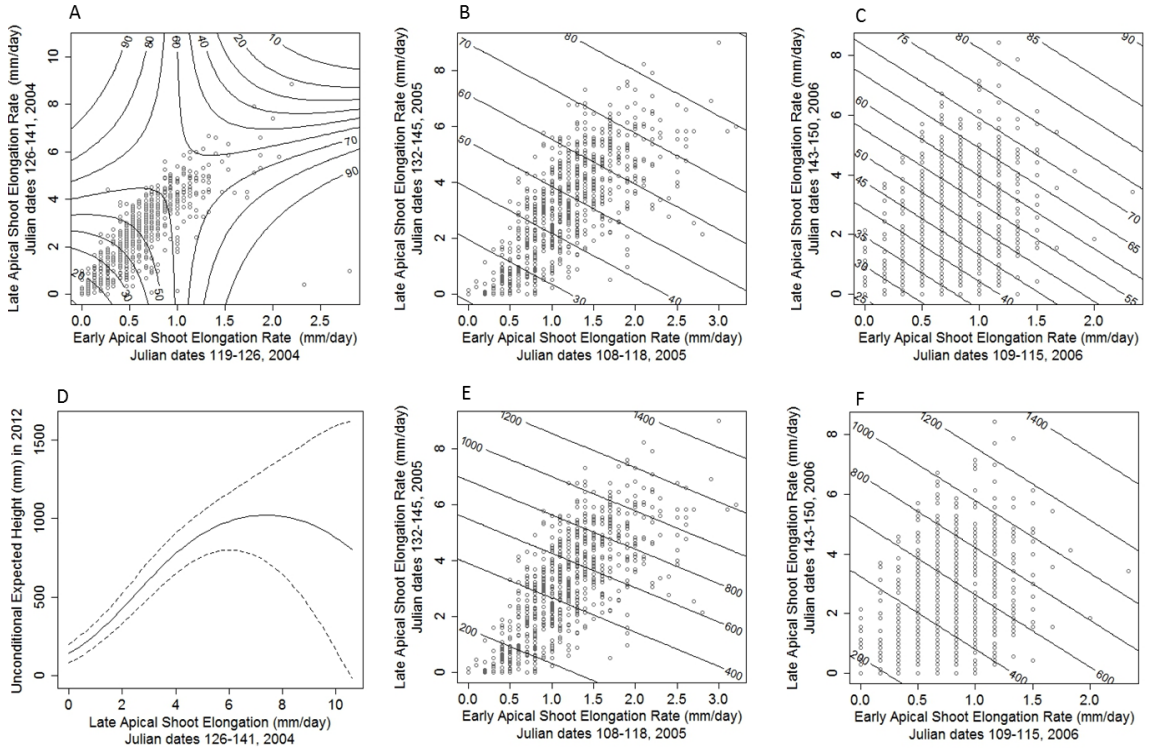
Figure 2-5. Experiment 2, fitness surfaces showing observed (open circles) apical shoot elongation rates (mm/day) in 2003 (A), 2004 (B and E) and 2005 (C and F) in relation to survival (contour) from (A) 2003-2012, (B)2004-2012, and (C) 2005 and unconditional expected height (contour) in 2012 that includes survival from (E) 2004-2012, and (F) 2005-2012. Plot D shows fitness relation for only late apical shoot elongation in 2004 because no other period was statistically significant (P < 0.05) in relation to survival. Dotted lines represent 95% confidence intervals.
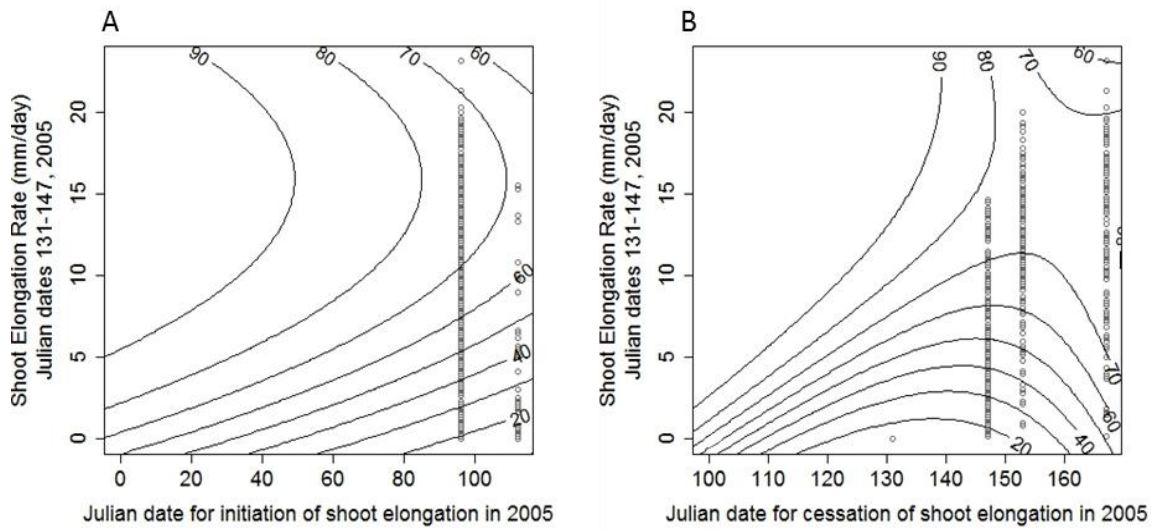
Figure 2-6. Experiment 1, fitness surfaces showing observed (open circles) measures of apical shoot elongation rates (mm/day) and initiation (A) or cessation (B) of apical shoot elongation of individual seedlings in relation to survival from 2005 to 2012 (contour lines). These figures do not show shoot elongation rates for all periods within the 2005 growing season that were statistically significant in relation so survival from 2005 to 2012.

Chapter 3: Unified life history analyses of phenotypic selection on ponderosa pine (*Pinus ponderosa* Doug.) seed and seedling traits in the field under three experimentally manipulated drought treatments

Abstract

Drought has the potential to act as a strong filter that selects against substantial genetic variability within forest tree populations during seedling emergence and early development. Thus selection during this period may have strong effects on population genetic architecture and adaptation. Yet knowledge of this process is limited and particularly so in relation to ongoing climate change. To evaluate differences in selection during early life stages, ponderosa pine (*Pinus ponderosa* Doug.) was grown from seed in two common-garden field experiments under climate warmer and drier than seed origins and under three experimentally imposed drought treatments. The first experiment used seed from 36 wild maternal trees originating from a single provenance at Priest River Experimental Station, Idaho to assess variation within provenances. The second experiment used seed from eight wild maternal trees originating from three climatically disparate provenances in the inland northwestern USA Rocky Mountains to assess variation among provenances. Drought treatments were imposed in the first growing season in both experiments by either eliminating irrigation in an otherwise arid environment in late June (Mid- and Late-Summer drought) or late July (Late-Summer drought) or continuing irrigation through the growing season (No drought). Aster models were used to infer fitness as a function of expected survival over multiple years and expected height that explicitly considered survival over multiple years. These inferred fitness proxies were than used in a fitness regression approach to assess selection on

mean density of seed (mg/ml), date of emergence, and timing of shoot elongation. The

analysis detected and quantified selection on seedling traits in the year of emergence with

substantially less pronounced selection in the subsequent two years. The form of

selection was influenced by timing of drought. Notably, when irrigation was maintained

through the growing season, directional selection favored increasingly earlier emergence

dates.   In contrast, when drought was imposed early, stabilizing selection favored

intermediate emergence dates that were relatively early and directional selection favored

increasingly high seed density. In addition, directional selection favored increasingly

higher early-season 2nd year, shoot elongation rates regardless of drought timing. The

form of selection also varied among seed source by drought treatment and the pattern of

variation corresponded with dryness of seed origin. Results thus suggest that during the

seed to young tree stage populations within the interior northwest region are differentially

adapted to growing season dryness and will likely differ in their selection response to

change in the timing of growing season drought. Findings from this study are directly

applicable to forest management decisions regarding reforestation, gene conservation and

assisted migration.

Introduction

Forest tree populations produce astronomical numbers of genetically variable

seeds which are commonly subject to high mortality during emergence and seedling

development (Haig, Davis, and Weidman, 1941; Leck, Parker, and Simpson, 2008) and

mortality during these stages is often drought related (Haig, Davis, and Weidman, 1941;

Moles and Westoby, 2004). In addition, emergence and early juvenile stages are more

susceptible to climate-related mortality than during later life stages (Namkoong, Usanis, and Silen, 1972; Namkoong and Conkle, 1976). Therefore, although mortality is not necessarily selective, drought related mortality has the potential to act as a strong selective filter during early life stages that may in turn have a greater effect on a population's genetic constitution, adaptation, and performance than mortality during later life stages.

Evolutionary response is expected when selection acts on a heritable trait that varies within a population (Darwin, 1859; Endler, 1986; Falconer and Mackay, 1996) and short term evolutionary response to selection can be estimated when knowledge of selection on the phenotype and trait inheritance are available (Falconer and Mackay, 1996). The analysis of natural selection on phenotypic variation represents a first step in predicting evolution of quantitative traits in response to selection. Phenotypic selection studies generally detect trait selection and describe its direction and magnitude in response to a selection pressure. Study results may also be used to gain insight into possible mechanisms of selection (Mitchell-Olds and Shaw 1987).

The present study investigates phenotypic selection from emergence to age three in ponderosa pine (*Pinus ponderosa* Doug.). Ponderosa pine is of particular interest given its status as both an economic and ecologically important tree species in western USA (Van Hooser and Keegan, 1992). The species naturally occurs across a broad geographic distribution that spans much of western USA and southern British Columbia (Fig. 3-1, insert) (Little, 1971; Perry, 1991) and occurs in both warm-moist and warm-dry climate (Rehfeldt, Ferguson, and Crookston, 2008). Throughout its distribution, ponderosa

pine defines the lower tree line and represents the warm, dry limits of the coniferous forest and is among the most drought-tolerant native tree species (Steele, 1992). Nonetheless, drought poses a significant barrier to early seedling establishment in ponderosa pine (Curtis and Lynch, 1957; Foiles and Curtis, 1965) and may act as a primary selection pressure throughout its distribution (Weber, 1988).

Studies of ponderosa pine have provided considerable evidence that populations possess potential to adapt to changes in drought dynamics. For example, intra-population genetic variation for traits associated with survival in response to variation in moisture availability has been observed in seed weight, color (Van Deusen and Beagle, 1970; Ager and Stettler, 1983; Weber, 1988), date of germination or emergence (Jenkinson, 1977; Feret, 1982; Bai, Thompson, and Broersma, 2000), tap root length, stem height (Jenkinson, 1975; Feret, 1982) root/shoot biomass ratios (Feret, 1982; Mcmillin and Wagner, 1995) stomatal density, and two measures of water use efficiency, the ratio of net photosynthesis over needle conductance to water vapor and carbon isotope discrimination (Olivas-García, Cregg, and Hennessey, 2000).

There is also substantial evidence that ponderosa pine has adapted to variation in moisture availability across its distribution, which suggests that selection has operated on trait variation in ponderosa pine. For example, seed collected from apparent moisture gradients showed corresponding differences in relative germination speed (Weber and Sorensen, 1992), in germination percentage under moisture stress conditions (Moore and Kidd, 1982), and seed weight was negatively correlated with moisture gradients (Ager and Stettler, 1983). In addition, height growth of three year-old seedlings representing

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138 provenances was greatest for seed sources originating from dryer locations on a xeric common-garden site while seed source originating from wetter locations were tallest on a mesic common-garden site (Rehfeldt, 1986b). To be sure, some proportion of the phenotypic variation associated with environment of origin may also be explained by maternal effects (Roach and Wulff, 1987), carryover effects (e.g. Lopez et al., 2003) and cultural effects such as timing of cone collection (Schopmeyer and United States. Forest Service., 1974).

However, basic knowledge of the form of natural selection on young trees is much less developed then for annual plants. In particular, knowledge of the magnitude and direction of natural selection under drought conditions during emergence and early juvenile stages in forest trees is very limited. Yet increased knowledge would advance the understanding of tree species distributions, colonization capability and range shifts. In application, such knowledge is critical for accurate prediction of species range response modeling, gene conservation and seed transfer guidelines under ongoing rapid climate change. In the case of forest tree species, this consideration is particularly relevant to western North America, which is expected to be subjected to warmer and dryer climates (IPCC, 2013a) and greater temporal frequency and spatial extent of growing-season drought events through the 21st century (Sheffield and Wood 2008).

Therefore, as a first step in an investigation of how forest tree populations may evolve under ongoing climate change, the present study assessed natural selection in ponderosa pine during early life stages following emergence in the field under climate warmer and drier than seed origins and under 3 experimentally imposed drought

treatments. In particular, the present study addressed two questions: (1) what is the magnitude, form and temporal dynamics of selection on seed and seedling traits in response to variation in the timing of growing-season drought for a single representative provenance from the inland northwestern USA Rocky Mountains and (2) how does selection on seed and seedling traits in response to variation in the timing of growing-season drought differ among provenances from this region.

Materials and Methods

Planting Site

Phenotypic selection of ponderosa pine during early seedling establishment under warm-dry climate and experimentally imposed drought was evaluated using two common-garden experiments in the field. The experiments were established at the Washington State University-Dryland Experimental Station in Lind, Washington (47°00′12″N, 118°33′46″W, elev. 491 m) (Fig. A3-1). The study site was considerably warmer and precipitation was substantially lower than climate of the sampled population origins (Table 3-1). Soil was Ritzville silt loam which is characteristically well drained (Whitney, 1916). In addition, microhabitat characteristics (aspect, solar radiation dynamics, and wind shelter) of the planting site were well suited to ponderosa pine regeneration as described by Munger (1917) and Heidmann (1992).

Experiment 1

Experiment 1(Exp.1) was designed to assess phenotypic and genetic variation within a single population. Seeds were from wind-pollinated cones, collected in fall 1989 from 36 wild trees with crown positions that were either dominant or codominant relative to the forest canopy in five natural stands at Priest River Experimental Forest, Idaho (Fig. 3-1). Sampled trees were located within a two-mile (~3.2 km) radius of each other at elevations between 800 to 950 m and represent a single altitudinal provenance or seed zone. The same seed collection was used by Marshall, Rehfeldt, and Monserud (2001) to assess family differences among seedlings for height growth and photosynthetic traits in common-gardens in their local environment. In addition, the seed collection was from the same maternal trees used by Marshall and Monserud (1996) for tree ring analysis.

Experiment 2

Experiment 2 (Exp.2) was designed to assess phenotypic and genetic variation in the inland northwestern region USA. Seed were from wind-pollinated cones, collected in 1982 from eight wild trees from each of three representative localities in the inland northwestern USA Rocky Mountains. The provenances (geographic origin of seed source) were selected based on adaptive differences in growth and shoot phenology (Rehfeldt, 1991) and disparity among their climate and geography of origin (Table 3-1). The seed sources were from the same collection used by Rehfeldt (1992), who used them to estimate genetic variances and covariances for traits describing growth, growth potential and shoot elongation.

Design and Procedure

Both common-garden experiments were sown together in the same week in the fall of 2008 and allowed to stratify over winter *in situ*. Seeds were first sterilized in 10% bleach solution (one part laundry bleach [5.25% sodium hypochlorite] to nine parts tap water) for 10 minutes with hand agitation, then rinsed and soaked 24hrs under running water to insure imbibition. Seed were sown in pairs at a uniform depth of 1.5 cm every 1.5 cm within rows and 20 cm between rows. Two seeds were planted per position to increase likelihood of consistent spacing. A total of 17,280 and 7,200 seed were sown in Exp. 1 and Exp. 2, respectively. Prior to the implementation of the Mid- and Late-Summer drought treatment (see below), seedlings were thinned to one per position by removing the eastern most seedling.

Both experiments used a randomized complete block design, with five blocks and three drought treatments randomized within each block. The experiments were interplanted by block and treatment so that for example, Block 1, Treatment 1 in Exp. 1 and Exp. 2 were spatially adjacent. Each family was represented in each treatment within block by two row plots of eight seedlings in Exp. 1 and two row plots with four seedlings in Exp. 2. Precipitation was very low with warm air temperatures at the study site through the growing season (Table 3-1) (WSU-DRS, 2015). Thus, seedlings that did not receive supplemental water during growing season months (April – October) were expected to experience drought conditions particularly from June to September 2009 when precipitation averaged 0.41 cm per month. Initially, all treatments were watered weekly beginning in early spring 2009 prior to emergence. This procedure was intended

to approximate high spring moisture availability which has been positively associated with natural patterns of concentrated seedling recruitment for ponderosa pine (League and Veblen, 2006). The No drought treatment was watered weekly through the growing season. The Mid- and Late-Summer drought treatment was imposed by eliminating irrigation in the last week of June, 2009. Finally, a Late-Summer drought treatment was imposed by eliminating irrigation in the last week of July, 2009. The timing of these treatments correspond to contemporary patterns of drought stress predicted to occur at the geographic origins of the study populations (Rehfeldt et al., 2006b; Appendix A). All irrigation was applied uniformly using timed hand-applications of well water. Because drought treatments within blocks were closely located, moisture-proof barriers were placed above and below ground between adjacent treatments during irrigation to maintain integrity of drought treatments. No irrigation was applied following 2009.

Extraneous environmental influences were intensively managed in both tests. Fencing was used to exclude ungulates and burrowing mammals from the small (0.02 ha) study site. In addition, hardware cloth was placed over seed beds from seed to early emergence stage to prevent predation from birds and small rodents. Rodent trapping was required during seedling emergence to prevent foraging, and insecticide (imidacloprid: 0.47% taufluvalinate: 0.61% and tebuconazole: 0.65%) was applied shortly after emergence to prevent damage from grubs. Weeds were removed weekly by hand. Data were collected for seedling emergence date; total height, shoot growth increment, bud and lammas shoot growth and survival over three growing seasons from emergence in Spring 2009 to the conclusion of the 2011 growing season. Mean family seed density

(mg/ml) was measured using the mean density of 20 seed in 5 independent measures for each family.

Statistical Analysis

Aster models (Geyer, Wagenius, and Shaw, 2007; Geyer and Shaw, 2008) applied using the reaster function (Geyer, 2013) in R (Team, 2013) were used to assess treatment, family and provenance effects on survival and final height. Aster models were also used to assess selection on seedling traits using an otherwise standard multivariate selection approach (Lande and Arnold, 1983). Aster models were used because they permit statistically rigorous, unified analysis of components of fitness expressed during multiple life history stages (Geyer, Wagenius, and Shaw, 2007; Geyer and Shaw, 2008). Unconditional estimates of final height were used as the best available proxy for fitness, and were obtained because they explicitly include survival through multiple years. In particular, Bernoulli distributions were used to model survival following seedling emergence in Spring 2009 to Fall 2011 (Fig. 3-2A) and Spring 2010 to Fall 2011 (Fig. 3-2B) and then normal distributions were used to model final height in 2011 conditional on survival to that year beginning in Spring 2009 (Fig.2C) and Spring 2010 (Fig.2D).

Analysis of selection on seedling traits under each drought treatment used the following procedure. First, model comparison using Log-likelihood ratio tests with a forward stepwise regression approach was used to determine whether treatment, family and provenance effects influenced survival and unconditional expected height. Then, Pearson's correlation coefficients between seedling traits were compared for each

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treatment in each test to identify how traits were interrelated and to eliminate serious

multicollinearity ($r^2 \geq 0.70$) (see Mitchell-Olds and Shaw, 1987) in regression analysis.

Correlation analysis used only live trees in 2011. Finally, two sets of significance tests

were performed. The first set tested whether family mean seed density (mg/ml), and date

of emergence were significant predictors of survival from emergence in 2009 to 2011

(Fig. 3-2A) and 2010 to 2011 (Fig. 3-2B) and unconditional expected height in 2011 that

included survival beginning in 2009 (Fig. 3-2C) and 2010 (Fig. 3-2D). Results of tests

that included and excluded mortality in 2009 were compared to deduce the effect of

mortality in 2009 on selection. The second set, tested whether family mean seed density

(mg/ml), rate of shoot elongation in early-season (March 6 to May 7) and mid- to late-

season (May 8 –Sept 28) were significant predictors of unconditional height in the 2011

that included survival beginning in 2010 (Fig. 3-2D). An analysis of selection on a given

trait requires a census of that trait prior to mortality. Yet shoot elongation could not be

assessed until 2010 when it was expressed for the first time. Therefore in this case, it was

necessary to exclude mortality in 2009. In addition, emergence date was excluded from

this analysis because its inclusion allowed fewer observations which limited statistical

power. In both sets of analyses, linear selection gradients (β_i), quadratic selection

gradients (γ_{ii}) and cross products (γ_{ij}) for seedling traits were treated as fixed effects

while block, row plot within block and family were treated as random. Statistical

significance of fixed effects was tested by comparing the likelihoods of nested models

using maximum likelihood. Significant ($P < 0.05$) variables were retained. The detection

of quadratic terms indicated curvature in the fitness surface. When the quadratic term for

more than one trait was significant, the cross-product functions of the traits were included as a fixed model term (see Blows and Brooks, 2003). Cross-product terms (γ_{ij}) represent correlational selection gradients which indicate selection on one trait that varies depending on the second trait.

A complete assessment of phenotypic selection using regression-analyses requires visualization of the data in relation to the hypothesized model to determine what range is supported by observation and to identify potential unexpected patterns or problems in the data (Mitchell-Olds and Shaw, 1987). Expected fitness (survival and unconditional expected height) was represented using contours while predictor variables family mean density (mg/ml), emergence date, and timing of the magnitude of elongation rate) were represented on the x and y axes. Finally, fitness surfaces were superimposed on the observed data to determine the range of the hypothesized model that was supported by empirical data. Higher order interactions were not plotted due to increased complexity of visualizing shapes in more than three dimensions.

Results

Survival and Mean Height by Experiment

A total of 10,165 seedlings representing 58.8% of the planted seeds emerged in Exp. 1, while 4,564 seedlings representing 63.3% of planted seeds emerged in Exp. 2. After culling one seedling at positions where two seedlings emerged 7,955 and 3,331 seedlings remained in Exp. 1 and Exp. 2, respectively. These seedlings represented the initial study population. By fall 2011, the initial study population for Exp. 1 and Exp. 2

had undergone 36% (sd= 0.48, se=0.1, n=6869) and 46% (sd= 0.48, se=0.5, n=2899) mortality while survivors exhibited a mean height of 260 mm (sd= 6.32, se=0.1, n=4367) and 270 mm (sd= 104.58, se=1.58, n=4367), respectively. Notably, about 94% of total mortality over the study period in either experiment occurred in 2009 during the growing season in which emergence occurred. Seedling mortality during this period appeared to be associated entirely with drought stress. Dead seedlings were desiccated in appearance and absent of signs of herbivory or physical damage. This conclusion was further evidenced by a positive correlation between mean daily evapotranspiration and timing of mortality where mean evapotranspiration rates explained 52.3% of variation in timing of mortality in both experiments combined (Fig 3).

Treatment x Family and Treatment x Provenance Effects

Significant differences in fitness measured as survival and unconditional expected height were detected among drought treatments in Exp. 1 (Table 3-2) and Exp. 2 (Table 3-3). Fitness measures were lowest in the Mid- and Late-Summer drought treatment and highest in the Late-Summer drought treatment (Fig. 3-4). In Exp. 1, statistical significance was also detected between families and drought treatments (Table 3-2). Most families exhibited the highest measures of fitness in the Late-Summer drought treatment however several families performed best in either the Mid- and Late-Summer or No drought treatment. In Exp. 2, there were also statistically significant interactions between provenance and treatments and between families within provenance and treatments (Table 3-3). Statistically significant variation among provenances within drought

treatments was not detected ((P >0.05), Fig. 3-5). For subsequent analyses of phenotypic selection, family effects were accounted for as random effects, and in Exp. 2 each provenance was analyzed separately.

Trait Variation and Correlations

Family mean seed density varied from 5.6 to 12.4 mg/ml in Exp. 1 and 8 to 11.5 mg/ml for 3 Mile, 10.4 to 11.4 mg/ml for Priest River, and 10.1 to 11.2 for Indian Prairie in Exp. 2. Seedlings emerged over a 40 day period beginning April 10, 2009 (Julian date 100). A single seedling emerged the following year but was not included in this analysis. In Exp. 1, mean emergence date occurred on Julian date 114 (sd= 6.32, se=0.1, n=3892). In Exp. 2, mean emergence date differed significantly among provenances (P<0.0001). Seeds originating from increasingly drier origins emerged earlier. In particular, mean emergence occurred on Julian date 114 (sd=5.89, se=0.26, n=498), 115 (sd= 6.45, se=0.29, n=498), 116 (sd=6.22, se=0.28, n=478), for Indian Prairie, 3Mile, and Priest River provenances, respectively. In addition, timing of seedling emergence differed significantly among families within provenance (P<.0001) in Exp.1 and Exp. 2 suggesting that this relationship is influenced to a degree by genetic and/or maternal effects. The mean rate of total shoot elongation was 0.24 mm/day and 0.19 mm/day in experiments 1 and 2, respectively.

In Exp. 1, trait correlations were generally similar among treatments (Table 3-4). Family mean seed density was not well correlated with any other traits. Emergence date was significantly correlated with most traits, with the strongest correlations in the late-

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summer drought treatment. Early-season elongation rates were significantly correlated ($P < 0.05$) with all traits examined except mean seed density. Notably elongation rates early in the growing season were weak and negatively correlated with late growing season elongation rates. Thus phenotypes that elongated more rapidly early in the season exhibited only a slight tendency to elongate relatively slower late in the season. In addition, shoot elongation late in 2010 growing season was moderate and positively correlated with bud and lammas shoot length in 2011.

In Exp. 2, trait correlations differed among provenances and the Indian Prairie and 3 Mile provenances were the most dissimilar (Table 3-5). Even so, early-season elongation rates were significantly correlated ($P < 0.05$) with most traits for all provenances. Trait correlations also differed among treatments; however these differences may have results from low sample size (n=36 to n=57) in the correlation analysis. This is a likely explanation because differences among treatments were not detected in Exp. 1, which used more than 8 times more observations for the Priest River provenance than represented in Exp. 2. In both experiments, family mean seed density and emergence date were either not significantly correlated or were weakly correlated with other measured traits (Table 3-4 and 3-5).

Selection on Seed Density and Emergence Date

In Exp. 1, survival from emergence in 2009 to 2011 in the Late-Summer drought treatment was dependent on family mean seed density (Table 3-6). In addition, unconditional expected height in 2011 that included survival beginning in 2009 was

dependent on mean seed density in the Mid- and Late-Summer drought treatments in both Exp. 1 (Table 3-6) and for the 3 Mile provenance in Exp. 2. In each of these cases, only mild directional selection that favored increasing mean seed density was detected. No evidence for selection on mean seed density was detected for the No drought treatment. When mortality in 2009 was omitted from the aster analysis, significant variation ($P <$ 0.05) in relation to either survival or unconditional height in 2011 was not detected (Table 3-6). Thus selection on mean density resulted from mortality in the first year in Exp 1.

In both experiments, linear and quadratic terms for emergence date were significant predictors of survival to 2011 beginning with emergence in 2009 and unconditional expected height in 2011 that included survival beginning from emergence in 2009 (Table 3-6 and 3-7). However, the statistical significance ($P < 0.05$) of these terms varied between response variables and among treatments in Exp.1 and among provenance by treatment in Exp. 2. Notably, no statistically significant ($P < 0.05$) relationship was detected between any predictor term and survival for Indian Prairie provenance in the Mid- and Late- Summer drought and Late-Summer drought treatments or Priest River provenance in the No drought treatment (Table 3-7). In addition, no statistically significant ($P < 0.05$) relationship was detected between any predictor term and unconditional expected height for the Indian Prairie provenance in the Mid- and Late-Summer drought treatment (Table 3-7). Thus, selection was not detected in any of these cases.

In Exp. 1, fitness surfaces plotted in relation to observed seed density and

emergence date showed phenotypes distributed over several contours leading to and

occupying the optimum (highest expected survival or height) thus directional selection

favoring earlier emergence dates and increasing seed density was observed (Fig. 3-6). In

addition, phenotypes were observed beyond the optimum for emergence date in both the

Mid- and Late- Summer and Late-Summer drought treatment, thus stabilizing selection

favoring intermediate emergence dates was observed in these treatments. Survival and

unconditional expected height declined as emergence date increased away from the

optimum. The rate of decline or magnitude of selection differed by drought treatment and

between fitness measures. For fitness assessed using survival, the magnitude of selection

on seed density was greatest in the No drought treatment and least in the Late-Summer

drought treatment (Fig. 3-6, top row). In contrast, for fitness assessed using unconditional

expected height, the magnitude of selection on emergence date was similarly high in both

the late-Summer and No drought treatments and least in the Mid- and Late-Summer

drought treatment (Fig. 3-6, bottom row). Differences between fitness surfaces for

survival versus unconditional expected height for the same treatment are interpreted as

indicating the effect of selection on total height among survivors.

In Exp. 2, when fitness surfaces for survival were plotted in relation to observed

mean seed density and emergence date, directional selection favoring increasingly earlier

emergence dates (Fig. 3-7A, B, C, E, and I) and stabilizing selection (Fig. 3-7D) favoring

an intermediate emergence date was observed. In contrast, when fitness surfaces using

unconditional expected height as the measure of fitness were plotted in relation to

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observed mean seed density and emergence date, directional selection favoring

increasingly earlier emergence dates (Fig. 3-8B, C, E, and I) and increasing seed density

(Fig. 3-8A) and stabilizing selection favoring an intermediate emergence date (Fig. 3-8H)

was observed.

Selection on Seed density and Timing and Magnitude of Shoot Elongation

Fitness measured as unconditional expected height in 2011 that included survival

beginning in 2010 was dependent on the rate of apical shoot elongation in 2010.

Selection on seed density was not detected which was consistent with results from the

above analysis of selection on seed density and emergence date when mortality in 2009

was also omitted.

In Exp. 1, linear and quadratic terms and the cross products for the timing of

shoot elongation were significant predictors of fitness (Table 3-8). The shapes of the

fitness surfaces were generally consistent across all drought treatments (Fig. 3-9).

Phenotypes with increasingly higher early-season growth rates and decreasingly lower

mid- to late-season growth rates were expected to exhibit the highest fitness. Fitness

surfaces plotted in relation to observed rates of shoot elongation showed phenotypes well

represented across contour lines toward and occupying or near the optimum of increasing

early-season growth rates, however only phenotypes in the No drought treatment were

represented across defined contour toward the optimum for mid-to-late season elongation

rates. Thus, directional selection favoring increasing early-season growth rate was

observed in all drought treatments while directional selection favoring decreasing mid-to

late-seasons growth rates was only observed in the No drought treatment. Notably, no phenotypes occurred beyond the optimum in any treatment; thus, stabilizing selection was not observed.

In Exp. 2, linear and quadratic terms for the timing of shoot elongation were significant predictors of fitness (Table 3-9). Notably, late-season elongation was not statistically significant in relation to unconditional expected height for the 3 Mile provenance in the Mid- and Late- Summer drought treatments or the Priest River provenances in any treatment. The shape of the fitness surface differed among provenances and treatments (Fig. 3-10). Phenotypes with increasingly higher early-season rates were expected to exhibit the highest fitness in most cases (Fig. 3-10A, D, E, G, and I). In contrast, stabilizing or plateauing selection favoring intermediate yet relatively high early-season elongation rates were expected for the 3 Mile (Fig. 3-10B) and Indian Prairie provenances (Fig. 3-10H) in the Late-Summer drought treatment and 3 Mile (Fig. 3-10C) and Priest River (Fig. 3-10F) provenances in the No drought treatments. In addition, phenotypes with increasingly higher late-season elongation rates were expected to exhibit the highest fitness in most cases (Fig. 3-10B, C, H, and I). In contrast, lower late-season elongation rates were expected to exhibit the highest fitness for the Indian Prairie provenance in the Mid- and Late-Summer drought treatment (Fig. 3-10G). Fitness surfaces plotted in relation to observed rates of shoot elongation showed phenotypes spanned much of the defined contour for directional selection favoring increasing early-season elongation rates. Thus, directional selection favoring increasing early-season growth rate was observed in all cases (Fig. 3-10). Phenotypes were less

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broadly represented over the defined contour lines for directional selection favoring increasing late-season elongation and particularly in the optimums. Even so, directional selection favoring increasing early-season elongation rate was observed for the 3 Mile, (Fig. 3-10B and C) and Indian Prairie provenances (Fig. 3-10H and I) in the Late-Summer (Fig. 3-10B) and No drought treatments and directional selection favoring decreasing late-season elongation rates for the Indian Prairie provenance in the Mid- and Late-Summer Drought treatment (Fig. 3-10G). Few observations were shown beyond the optimums where stabilizing selection was expected. Thus stabilizing selection on early-season shoot elongation was only weakly supported.

Discussion

The present study detected and described selection on traits associated with drought adaptation in ponderosa pine seedlings grown under three experimentally imposed drought treatments (Mid- and Late-Summer, Late-Summer and No) at a field site that was warmer and drier than seed origins. For the Priest River provenance, mortality differed among families. Selection via differential mortality in the year of emergence was particularly strong, while selection in subsequent years was far less pronounced. The magnitude and form of selection was influenced by timing of drought. Notably, when irrigation was maintained through the growing season, directional selection favored increasingly earlier emergence dates. In contrast, when drought was imposed early in the growing season, stabilizing selection favored relatively early but intermediate emergence dates and directional selection favored phenotypes with

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increasing seed density. In addition, directional selection favored increasing early-season, 2nd year, shoot elongation rates regardless of drought timing. The magnitude and form of selection also varied among provenances within the inland northwestern USA Rocky Mountains by drought treatment. In addition, patterns of variation for selection among provenances corresponded with dryness of seed origin. Results thus suggest that during the seed-to-seedling stage, populations within the region will likely differ in their selection response to changes in the timing of growing season drought.

Previous studies have found evidence of substantial genetic contributions to variation in seed size (Weber, 1988) germination timing (Weber, 1988; Weber and Sorensen, 1992) and shoot elongation rates (Rehfeldt, 1986b, a). Therefore, to the extent that the traits examined are heritable or correlated with heritable traits, results suggest that selection associated with differences in timing of drought during seedling establishment and early growth may substantially influence adaptation to local climate and genetic architecture among natural populations. In addition, results of the present study can be used with knowledge of additive genetic variance to predict evolutionary response to selection in the short term. Therefore, results may provide insight into how populations in the interior northwest will respond through evolution to increased warm-dry climate characterized by mid-to late-summer droughts. This knowledge is particularly relevant given that climate for western North America is expected to become warmer and dryer, with an increase in the temporal frequency and spatial extent of growing-season drought events through the 21st century (Sheffield and Wood, 2008).

The present study assessed natural selection during seedling emergence and early growth using seedlings that overwintered as seed, germinated, and grew entirely in the field. Therefore results more closely reflect selection under conditions that approximate natural regeneration in comparison with studies that use seedlings grown in growth chambers or greenhouse conditions and/or later outplanted into the field. In particular, root systems of seedling grown from seed at the study site were unimpeded by pots or artificial boundaries. This was important because initial root development is critical for seedling drought survival (Bates, 1923) and the tap root of ponderosa pine can penetrate soil at an average of 58 cm in the first growing season (Larson, 1963). Therefore the use of alternative methods such as raised beds or containers would likely obscure natural selection associated with variation in root development.

Assessing and interpreting selection during establishment and growth in the first three years

Direction and magnitude of selection on a trait can be assessed by examining the relationship between a given trait and fitness within a population. Fitness is generally defined as the number of offspring successfully contributed to the next generation. Measuring fitness can be difficult particularly in long lived trees. Consequently, most studies that assess fitness for tree species use measures of survival and or height as proxies. Survival is a fundamental component of fitness, while relative height can contribute to fitness by, for example, increased access to resources and greater reproductive surface (see Mcgraw and Wulff, 1983). In the present study, survival and

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unconditional expected height to age three were each used as proxies for fitness. Because

unconditional expected height considers survival as well as tree height, it is a more

complete proxy for fitness than either survival or height alone. However, selection due to

differential survival following age three is likely common (e.g. Chapter 2) and changes in

early relative height among families have been observed in ponderosa pine between age

20 and 25 years (Namkoong and Conkle, 1976). Therefore projection of fitness beyond

the three year-old span assessed in the present study represents an extrapolation and

should be interpreted with caution.

Selection analysis using a fitness regression approach can identify whether a trait

is under indirect selection but cannot confirm that a trait is under direct selection without

meeting the unlikely condition of including all correlated traits in the analysis (Lande and

Arnold, 1983). Nonetheless, insight into possible mechanisms of selection can be gained

through analyses that include a carefully considered range of traits with regards to their

function (Mitchell-Olds and Shaw, 1987).

Selection on seed density

Seed density and seed weight appear to act independently although they have both

been shown to affect seed vigor (Hoy and Gamble, 1985). In both experiments,

directional selection favoring increasing mean seed density was only detected in the

imposed drought treatments and only in association with mortality in the 1st year (Table

3-6 and Fig. 3-6, Table 3-7 and Fig. 3-8A). Given that seed density is positively

associated with seedling vigor (Krieg and Bartee, 1975; McDonald Jr, 1975; Hoy and

Gamble, 1985) results from the present study suggest that observed selection favoring increasing seed density (Fig. 3-6 and 3-8A) may have resulted from direct selection favoring increasing seed vigor under increasingly moisture limiting conditions in the growing season of emergence. These finding agree with similar observations reported for studies among annual crop species examined in laboratory tests or grown under moisture limiting conditions (e.g. Hoy and Gamble, 1985; Gardner and Vanderlip, 1989). Seed morphological traits which are largely under maternal control (Roach and Wulff, 1987) tend to be more uniform within families but vary substantially among families within populations (Ager and Stettler, 1983; Weber, 1988). Thus selection on seed density might contribute to adaptive structure expressed as clinal or ecotypic variation where increasing seed density corresponds with increasing dryness of growing season at seed origin. Indeed, increasing seed weight has been shown to be correlated with increasing elevation that parallels decreasing precipitation (Ager and Stettler, 1983). In the present study no trend was apparent among provenance mean density and growing season moisture at seed origin. However, Exp. 2 used only three provenances with eight families each, therefore sample size may not have been great enough to detect a trend.

Selection on emergence date

Early emergence date has been positively associated with increased growth and survival in ponderosa pine grown under nursery conditions (Mexal and Fisher 1987), in Scots pine (*P. sylvestris*) grown in experimental drought treatments imposed across three microhabitats in natural mountain forests in south-east Spain (Castro, 2006) and among a

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wide variety of plant life forms over a broad range of environments (see Verdu and Traveset, 2005). Hypotheses explaining this apparently common relationship have been grouped into two broad categories that ascribe to either environmental effects or maternal/genetic effects (Jones, Allen, and Sharitz, 1997). Environmental explanations associate increased growth and survival among earlier emergents to greater access to ephemerally available resources such as water or nutrient (Jones and Sharitz, 1989). In contrast, maternal/genetic explanations attribute greater growth and survival among earlier emergence to adaptive genotypes or traits such as seedling vigor which may be correlated with early emergence (Mcdaniel, 1969; Dunlap and Barnett, 1983).

In the present study growing conditions were more favorable in spring and early summer. Therefore the environmental hypothesis would predict directional selection favoring an increasingly earlier date of emergence as drought timing occurred increasingly earlier in the growing season. Surprisingly however, selection favored intermediate dates of emergence in drought-imposed treatments but not the No drought treatment (Fig. 3-6, 3-7, and 3-8). These results do not support a purely environmental explanation and instead suggest that the direction of selection on date of emergence was influenced by genetic/maternal factors and/or selection on correlated traits.

The present study also detected a positive trend for earlier emergence in association with increasingly drier seed origins which suggests that timing of seed emergence may be clinal. This finding corresponds with Weber and Sorensen (1992) who presented evidence that speed of germination among central Oregon provenances varied

positively with decreasing precipitation at the seed source which was inferred from geographic variables.

Selection on timing of the magnitude of shoot elongation rate

Directional selection favoring increasing early-season shoot elongation rates was observed in all drought treatments in both experiments (Fig. 3-9 and 3-10). In addition, selection favoring decreasing mid- to late-season elongation rates observed in Exp. 1 which used seed exclusively from Priest River, while selection was favored increasing elongation rates in Exp. 2 even for the Priest River provenance (Fig. 3-10E, F, H, and I). The difference in results between experiments may have resulted from the smaller range of elongation rates represented in Exp. 2 which likely resulted from the overall smaller sample size used in Exp. 2 in comparison with Exp. 1.

Growth rhythm represents the timing of an intercorrelated suite of annual growth processes in relation to annual climatic events (c.f. Dietrichson, 1964; Rehfeldt, 1992; Howe et al., 2003). The synchronization of growth rhythm with local climate is a key component of adaptation in many forest tree species (Morgenstern, 1996). Because shoot elongation rates are a component of growth rhythm, the detection of selection on these traits suggests that selection likely acted on many other components of growth rhythm such as date of bud set and timing of dormancy as well. Indeed, in the present study early season elongation rate was well correlated with most measured traits (Tables 3-4 and 3-5). Shoot elongation rates were significantly correlated with emergence date (Table 3-4 and 3-5B). Thus, selection on either trait appeared to have indirectly magnified selection

on the other. Unfortunately, regression analysis that included mortality in 2009 could not also include shoot elongation rates because this trait was not expressed for the first time until 2010. Therefore the shoot elongation rates of seedlings that died in 2009 could not be accounted for. This invisible fraction may have a substantial effect on selection of timing of shoot elongation and growth rhythm in general (see Mojica and Kelly, 2010). Further investigation may benefit from general approach used by Mojica and Kelly (2010) who assessed the effect of selection on the invisible fraction by using individuals with independently established genotype and phenotypic expression.

Management implication and Conclusion

Findings from this study are relevant to reforestation, gene conservation and assisted migration. In particular, study results have the potential to assist managers in early identification of better performing phenotypes under a warm-dry climate characterized by mid-to late-summer droughts. The present study showed that phenotypes with relatively high seed density, an intermediate but relatively early emergence date and high 2nd year early-season shoot elongation exhibited the greatest observed fitness after three years in the field under a climate warmer and drier than at the seed origins. However, the present results were conducted in only one environment, and although biotic interactions were controlled, their effects were not assessed. Therefore, natural selection on these same traits under wild conditions could differ substantially even under similar climate conditions.

Results suggest that natural selection specific to drought conditions in the year of emergence may influence genetic architecture and local adaptation. However, when seedlings grown under nursery conditions are outplanted, selection specific to the site in at least the first year is circumvented and it is unclear to what extent genetic architecture and adaptation over time may differ as a consequence. Further research is needed to assess the importance of natural selection in the first year and to determine how this may differ over time from populations planted with nursery stock.

A quantitative genetic analysis assessing change in genetic architecture among drought treatments and evolutionary response was not addressed here. Data used in the present study will be used to assess these factors, and in particular, estimate additive genetic variance, heritability and change in population mean fitness to be presented in a companion paper. In addition, ongoing testing is assessing subsequent variation in selection beyond the initial three year period addressed in this first paper.

Table 3-1. Population origins and their predicted climatic means (Rehfeldt 2006) and the study sites[x] location and its observed mean climate for 2008-2011 (WSU-DRS 2015)

	Longitude	Latitude	Elevation (m)	Mean Annual Temperature (°C)	Mean Annual Precipitation (mm)	Mean Growing Season Precipitation (Apr - Oct)
3 Mile, ID	116.22°W	44.98°N	1341	5.4	661	239
Priest River Exp. For., ID	116.82°W	48.35°N	808	6.4	819	287
Indian Prairie, MT	114.17°W	46.47°N	1219	6.1	428	217
Lind Dryland Exp. Sta., WA[x]	118.57°W	47°N	501	9.8	269	125

Table 3-2. Experiment 1, results from aster model comparison testing for the effects of drought treatment and family, on survival to 2011 and seedling unconditional expected height in 2011 that included survival beginning with emergence in 2009.

Response	Terms	Model df	Model Deviance	Test df	Test P-value	Random Effects: √ of Variance Components (P-values are one-tailed)	Estimate	Std. Error	p value
							Estimate	Std. Error	p value
Survival	Null	3	16671			Block	0.3169	0.1042	0.00118 **
	Drought Treatment	5	16866	2	< 0.0001 ***	Row	0	NA	NA
	Drought Treatment	5	16866			Block	0.3548	0.1163	0.00114 **
	Drought Treatment:Family	110	17072	105	< 0.0001 ***	Row	0	NA	NA
Unconditional	Null	3	16671			Block	0.001461	0.0004707	0.000953 ***
Expected Height	Drought Treatment	5	16981	2	< 0.0001 ***	Row	0	NA	NA
	Drought Treatment	5	16981			Block	0.001555	0.0005007	0.000947 ***
	Drought Treatment:Family	110	17241	105	< 0.0001 ***	Row	0	NA	NA

Table 3-3. Experiment 2, results from aster model comparison testing for the effects of timing of drought treatment (TRT), provenance (PROV) and family within provenance (FAM), on survival to 2011 and seedling unconditional expected height in 2011 that included survival beginning with emergence in 2009.

Response	Terms	Model df	Model Deviance	Test df	Test P-value	Random Effects : √ of Variance Components (P-values are one-tailed)		Estimate	Std. Error	p value
Survival	Null	3	5850.9			Block		0.3629	0.122	0.00147 **
	TRT	5	5732.3	2	< 0.0001 ***	Row Plot		0	NA	NA
	TRT	5	5732.3			Block		0.3656	0.1229	0.00146 **
	TRT + TRT:PROV	11	5756.2	6	0.0005 ***	Row Plot		0	NA	NA
	TRT + TRT:PROV	11	5756.2			Block		0.3831	0.1285	0.00143 **
	TRT + TRT:PROV + TRT:PROV:FAM	89	5850.9	78	0.095454	Row Plot		0	NA	NA
Unconditional Expected Height	Null	3	5742.0			Block		0.001854	0.00061	0.00109 **
	TRT	5	5811.8	2	< 0.0001 ***	Row Plot		0	NA	NA
	TRT	5	5811.8			Block		0.001875	0.00061	0.00109 **
	TRT + TRT:PROV	11	5851.6	6	< 0.0001 ***	Row Plot		0	NA	NA
	TRT + TRT:PROV	11	5851.6			Block		0.0019732	0.00064	0.00107 **
	TRT + TRT:PROV + TRT:PROV:FAM	89	5980.2	54	0.0003 ***	Row Plot		0	NA	NA

Null = excludes treatment, provenance and/or family effects. Random effects are from top model among each model comparison pair.

Table 3-4. Experiment 1, Person product moment correlations for family mean seed density (Density), emergence date in the 1st year (E Date), rate of shoot elongation during March 6 – May 7: early-growing season: (EER), May 8-June 2: mid-growing season (MER), June 3 - Sept 28: late-growing season (LER), and the total growing season (Total Rate) in 2010, apical bud length and lammas growth in fall 2010 (Bud10) and 2011 (Bud11) in the Mid- and Late-Summer (n=335), Late-Summer (n=490), and No (n=437) drought treatments. Correlations consider only live trees in 2011.

	Density	E Date	EER	MER	LER	Total Rate	Bud10
Mid- and Late-Summer Drought Treatment							
Density							
E Date	-0.04						
EER	-0.03	-0.19**					
MER	0.01	-0.10	0.57***				
LER	-0.05	0.05	-0.17*	-0.03			
Total Rate	-0.03	-0.17*	0.91***	0.78***	0.12*		
Bud10	0.10	-0.12*	0.25***	-0.11*	-0.07	0.13*	
Bud11	0.09	-0.14*	0.20**	0.05	0.19**	0.22***	0.44***
Late-Summer Drought Treatment							
Density							
E Date	-0.11*						
EER	0.06	-0.20***					
MER	0.03	-0.07	0.63***				
LER	-0.06	0.06	-0.10*	-0.07			
Total Rate	0.04	-0.15**	0.93***	0.81***	0.12*		
Bud10	-0.01	-0.21***	0.39***	0.00	-0.01	0.28***	
Bud11	-0.08	-0.08	0.22***	0.03	0.24***	0.22***	0.52***
No Drought Treatment							
Density							
E Date	-0.02						
EER	-0.05	-0.15*					
MER	0.01	-0.11*	0.61***				
LER	-0.08	0.13*	-0.29***	-0.09			
Total Rate	-0.05	-0.13*	0.94***	0.80***	-0.06		
Bud10	-0.05	-0.05	0.48***	0.11*	-0.13*	0.39***	
Bud11	-0.02	0.00	0.17**	0.05	0.22***	0.19***	0.46***

Note: * P < .05, ** P < .01, *** P < .001

Table 3-5A. Experiment 2, 3 Miles, Idaho provenance, Person product moment correlations for family mean seed density (Density), emergence date in the 1st year (E Date), rate of shoot elongation during March 6 – May 7: early-growing season: (EER), May 8-June 2: mid-growing season (MER), June 3 - Sept 28: late-growing season (LER), and the total growing season (Total Rate) in 2010, apical bud length and lammas growth in fall 2010 (Bud10) and 2011 (Bud11) in the Mid- and Late-Summer (n=36), Late-Summer (n=44) and No (n=43) drought treatments. Correlations consider only live trees in 2011.

	Density	E Date	EER	MER	LER	Total Rate	Bud10
Mid- and Late-Summer Drought Treatment							
Density							
E Date	0.03						
EER	0.44*	0.11					
MER	0.35*	-0.15	0.64***				
LER	-0.25	-0.32	-0.13	0.07			
Total Rate	0.42*	0.00	0.96***	0.82***	0.04		
Bud10	0.12	0.19	0.04	-0.25	-0.23	-0.08	
Bud11	-0.22	-0.12	-0.29	-0.54**	0.15	-0.37*	0.57**
Late-Summer Drought Treatment							
Density							
E Date	0.28						
EER	0.10	-0.10					
MER	0.06	0.18	0.71***				
LER	0.06	0.27	-0.39*	-0.23			
Total Rate	0.12	0.07	0.92***	0.82***	-0.06		
Bud10	0.09	-0.30*	0.43*	-0.06	-0.16	0.27	
Bud11	0.15	0.20	0.07	0.05	0.49**	0.24	0.22
No Drought Treatment							
Density							
E Date	-0.18						
EER	0.15	-0.18					
MER	0.03	0.15	0.27				
LER	0.30*	0.13	-0.22	0.17			
Total Rate	0.27	-0.09	0.87***	0.55**	0.23		
Bud10	0.30	-0.47*	0.49**	-0.04	-0.01	0.43*	
Bud11	0.14	-0.11	0.39*	0.17	0.14	0.46*	0.52**

Note: * P < .05, ** P < .01, *** P < .001

Table 3-5B. Experiment 2, Priest River, Idaho provenance, Person product moment correlations for family mean seed density (Density), emergence date in the 1st year (E Date), rate of shoot elongation during March 6 – May 7: early-growing season: (EER), May 8-June 2: mid-growing season (MER), June 3 - Sept 28: late-growing season (LER), and the total growing season (Total Rate) in 2010, apical bud length and lammas growth in fall 2010 (Bud10) and 2011 (Bud11) in the Mid- and Late-Summer (n=38) Late-Summer (n=45) and No (n=47) drought treatments. Correlations consider only live trees in 2011.

	Density	E Date	EER	MER	LER	Total Rate	Bud10
Mid- and Late-Summer Drought Treatment							
Density							
E Date	0.07						
EER	0.01	0.16					
MER	-0.25	0.09	0.62***				
LER	0.08	-0.16	-0.11	-0.13			
Total Rate	-0.05	0.10	0.91***	0.76***	0.21		
Bud10	0.45*	-0.08	-0.11	-0.08	0.13	-0.07	
Bud11	0.15	-0.26	-0.24	-0.09	0.52**	-0.03	0.61***
Late-Summer Drought Treatment							
Density							
E Date	-0.07						
EER	0.01	-0.28					
MER	0.06	-0.03	0.70***				
LER	-0.04	-0.01	-0.36*	-0.39*			
Total Rate	0.02	-0.26	0.95***	0.76***	-0.12		
Bud10	0.01	-0.29	0.44*	0.05	-0.09	0.37*	
Bud11	0.12	-0.22	0.01	-0.29	0.59***	0.11	0.36*
No Drought Treatment							
Density							
E Date	-0.37*						
EER	0.32*	-0.40*					
MER	-0.08	-0.06	0.41*				
LER	0.18	0.00	0.08	0.01			
Total Rate	0.25	-0.29*	0.86***	0.67***	0.44*		
Bud10	0.28	-0.37*	0.47**	-0.16	-0.04	0.23	
Bud11	0.37*	-0.24	0.43*	-0.08	0.51**	0.45*	0.46*

Note: * P < .05, ** P < .01, *** P < .001

Table 3-5C. Experiment 2, Indian Prairie, Montana provenance, Person product moment correlations for family mean seed density (Density), emergence date in the 1st year (E Date), rate of shoot elongation during March 6 – May 7: early-growing season: (EER), May 8-June 2: mid-growing season (MER), June 3 - Sept 28: late-growing season (LER), and the total growing season (Total Rate) in 2010, apical bud length and lammas growth in fall 2010 (Bud10) and 2011 (Bud11) in the Mid- and Late-Summer (n=41), Late-Summer (n=57), and No (n=55) drought treatments. Correlations consider only live trees in 2011.

	Density	E Date	EER	MER	LER	Total Rate	Bud10
Mid- and Late-Summer Drought Treatment							
Density							
E Date	0.30						
EER	0.09	0.07					
MER	0.18	0.36*	0.51**				
LER	-0.04	0.08	-0.25	0.04			
Total Rate	0.13	0.21	0.90***	0.81***	-0.01		
Bud10	-0.40*	-0.37*	-0.30	-0.35*	0.16	-0.34*	
Bud11	-0.30	-0.36*	-0.41*	-0.40*	0.18	-0.44*	0.80***
Late-Summer Drought Treatment							
Density							
E Date	-0.27*						
EER	-0.01	-0.19					
MER	0.17	-0.10	0.69***				
LER	0.14	0.16	-0.20	-0.02			
Total Rate	0.08	-0.14	0.94***	0.85***	0.04		
Bud10	-0.10	-0.05	0.37*	-0.08	-0.08	0.23	
Bud11	-0.11	0.13	0.07	-0.04	0.32*	0.09	0.54***
No Drought Treatment							
Density							
E Date	0.20						
EER	-0.22	-0.21					
MER	-0.21	0.03	0.51***				
LER	0.20	0.25	-0.19	-0.13			
Total Rate	-0.20	-0.09	0.93***	0.72***	0.05		
Bud10	-0.37*	-0.07	0.52***	0.38*	-0.30*	0.47**	
Bud11	-0.13	-0.01	0.31*	0.31*	0.20	0.40*	0.65***

Note: * P < .05, ** P < .01, *** P < .001

Table 3-6. Experiment 1, results from aster model comparison testing for the effects of mean seed density (mg/ml), emergence date and its quadratic on survival though 2011 beginning in 2009 and 2010 and unconditional expected height (mm) in 2011 that included survival beginning in 2009 and 2010.

Drought Treatment Response and	Terms		Selection Gradient	Standard Error	Model df	Model Deviance	Test df	Test P-value	Random Effects: √ of Variance Components (P-values are one-tailed)	Estimate	Std. Error	p value
Mid- and Late-Summer												
Survival	Full model				6	2269.4	-	-	Block	1.0685	0.3456	0.0010 **
2009-2011	Mean Seed Density				5	2266.2	1	0.0704	Family	0.3393	0.0905	< 0.0001
	Emergence Date	β	6.45E-02	4.49E-02	4	2210.2	2	< 0.0001	Row Plot	0	NA	NA
	(Emergence Date)^2	γ_ii	-4.47E-03	1.41E-03	5	2258.5	1	< 0.0001				
Survival	Full model				6	3702.8	-	-	Block	0.0016	76.61211	0.5
2010-2011	Mean Seed Density				5	3704.9	1	0.2860	Family	0	NA	NA
	Emergence Date				4	3703.8	2	0.3151	Row Plot	0	NA	NA
	(Emergence Date)^2				5	3704.3	1	0.1811				
Unconditional	Full model				6	2201.1	-	-	Block	1.0685	0.3456	0.0010 **
Expected Height	Mean Seed Density	β	7.25E-04	2.98E-04	5	2196.6	1	0.0332 *	Family	0.3393	0.0905	< 0.0001
2009-2011	Emergence Date	β	1.28E-04	1.48E-04	4	2142.2	2	< 0.0001	Row Plot	0	NA	NA
	(Emergence Date)^2	γ_ii	-1.24E-05	4.80E-06	5	2193.8	1	0.0070 **				
Unconditional	Full model				6	3733.4	-	-	Block	0.00322	0.001064	0.00124 **
Expected Height	Mean Seed Density				5	3731.9	1	0.2260	Family	0.00205	0.000423	< 0.0001
2010-2011	Emergence Date	β	-2.70E-04	5.34E-05	4	3727.2	2	0.0459 *	Row Plot	0	NA	NA
	(Emergence Date)^2				5	3733.2	1	0.6979				
Late-Summer												
Survival	Full model				6	4899.9	-	-	Block	0.53132	0.18046	0.00162 **
2009-2011	Mean Seed Density	β	2.18E-01	8.45E-02	5	4895.2	1	0.0287 *	Family	0.28536	0.09448	0.00126 **
	Emergence Date	β	1.03E-01	4.29E-02	4	4793.9	2	< 0.0001	Row Plot	0	NA	NA
	(Emergence Date)^2	γ_ii	-6.10E-03	1.33E-03	5	4878.8	1	< 0.0001				
Survival	Full model				6	6404.0	-	-	Block	1.2157	0.4131	0.00163 **
2010-2011	Mean Seed Density				5	6401.4	1	0.1086	Family	0.3586	0.2438	0.0706
	Emergence Date				4	6401.3	2	0.2653	Row Plot	0	NA	NA
	(Date of				5	6402.5	1	0.2294				
Unconditional	Full model				6	4963.9	-	-	Block	0.00218	0.000724	0.00128 **
Expected Height	Mean Seed Density				5	4962.3	1	0.2082	Family	0.00128	0.000292	5.78e-06 ***
2009-2011	Emergence Date	β	3.61E-04	1.41E-04	4	4822.9	2	< 0.0001	Row Plot	0	NA	NA
	(Emergence Date)^2	γ_ii	-2.34E-05	4.62E-06	5	4936.4	1	< 0.0001				
Unconditional	Full model				6	6450.9	-	-	Block	0.00322	0.001065	0.00124 **
Expected Height	Mean Seed Density				5	6450.8	1	0.8303	Family	0.00205	0.000424	6.61e-07 ***
2010-2011	(Emergence Date)^2	β	-2.70E-04	5.34E-05	4	6423.8	2	< 0.0001	Row Plot	0	NA	NA
	(Date of				5	6449.8	1	0.2952				
No												
Survival	Full model				6	3175.6	-	-	Block	0.8942	0.2908	0.00105 **
2009-2011	Mean Seed Density				5	3174.6	1	0.3177	Family	0.155	0.1325	0.1211
	Emergence Date	β	0.12009	0.04819	4	3089.2	2	< 0.0001	Row Plot	0	NA	NA
	(Emergence Date)^2	γ_ii	-0.00684	0.00158	5	3156.1	1	< 0.0001				
Survival	Full model				6	4675.8	-	-	Block	0.00601	0.001936	0.000951 ***
2010-2011	Mean Seed Density				5	4674.6	1	0.2763	Family	0.00127	0.000393	0.000613 ***
	Emergence Date				4	4672.2	2	0.1637	Row Plot	0	NA	NA
	(Date of				5	4675.8	1	0.9171				
Unconditional	Full model				6	3279.9	-	-	Block	0.00391	0.00127	0.00104 **
Expected Height	Mean Seed Density				5	3279.3	1	0.4363	Family	0.00054	0.000358	0.06602
2009-2011	Emergence Date	β	4.30E-04	1.47E-04	4	3171.6	2	< 0.0001	Row Plot	0	NA	NA
	(Emergence Date)^2	γ_ii	-2.56E-05	5.15E-06	5	3252.7	1	< 0.0001				
Unconditional	Full model				6	4860.6	-	-	Block	0.00595	0.001917	0.000955 ***
Expected Height	Mean Seed Density				5	4860.3	1	0.5453	Family	0.00113	0.000413	0.003175 **
2010-2011	Emergence Date	β	2.86E-04	2.21E-04	4	4839.5	2	< 0.0001	Row Plot	0	NA	NA
	(Emergence Date)^2	γ_ii	-1.80E-05	7.80E-06	5	4855.1	1	0.0189 *				

β= linear selection gradient, γ_ii = quadratic effects , Full models included all presented variables listed directly under the term, Random effects are from models using emergence date and its quadratic or null model with no fixed effects in treatments where emergence date was not significant. Estimated selection gradients (partial regression coefficients) and standard errors represent results from models that used only seed density or only date of emergence.

Table 3-7. Experiment 2, results from aster model comparison testing by provenance for the effects of mean seed density (mg/ml), emergence date and its quadratic on survival though 2011 beginning in 2009 and unconditional expected height (mm) in 2011 that included survival beginning with emergence in 2009.

Drought Treatment / Provenance	Response	Terms		Selection Gradient	Standard Error	Model df	Model Deviance	Test df	Test P-value	Random Effects: √ of Variance Components (P-values are one-tailed)		Estimate	Std. Error	p value
Mid- and Late-Summer														
3 Mile, Idaho	Survival	Full model				6	157.9	-	-	Block		1.485876	0.558723	0.00391 **
		Mean Seed Density				5	157.7	1	0.0871	Family		0.001594	48.52068	0.49999
		Emergence Date	β	-0.07252	0.03191	4	151.8	2	0.0469 *	Row Plot		0	NA	NA
		(Emergence Date)^2				5	157.7	1	0.6611					
	Unconditional Expected Height	Full model				6	169.2	-	-	Block		0.006625	0.002528	0.00439 **
		Mean Seed Density	β	0.002043	6.91E-04	5	162.3	1	0.0086 **	Family		0	NA	NA
		Emergence Date				4	166.1	2	0.2107	Row Plot		0	NA	NA
		(Emergence Date)^2				5	169.1	1	0.7577					
Priest River, Idaho	Survival	Full model				6	152.5	-	-	Block		1.2143	0.4429	0.00306 **
		Mean Seed Density				5	149.7	1	0.0972	Family		0	NA	NA
		Emergence Date	β	0.28532	0.18214	4	138.7	2	0.0180 *	Row Plot		0	NA	NA
		(Emergence Date)^2	γii	-0.01171	0.00572	5	146.9	1	0.0010 **					
	Unconditional Expected Height	Full model				6	167.4	-	-	Block		0.005401	0.001904	0.0024 **
		Mean Seed Density				5	167.3	1	0.7697	Family		0	NA	NA
		Emergence Date	β	0.001149	5.71E-04	4	154.1	2	0.0013 **	Row Plot		0	NA	NA
		(Emergence Date)^2	γii	-4.6E-05	1.72E-05	5	161.2	1	0.0130 *					
Indian Praire, Montana	Survival	Full model				6	260.4	-	-	Block		0.004502	0.0016325	0.00291 **
		Mean Seed Density				5	259.99	1	0.5381	Family		0.001876	0.0008508	0.01374 *
		Emergence Date				4	259.49	2	0.6455	Row Plot		0	NA	NA
		(Emergence Date)^2				5	260.35	1	0.8807					
	Unconditional Expected Height	Full model				6	281.8	-	-	Block		0.9781	0.3652	0.0037 **
		Mean Seed Density				5	281.3	1	0.4630	Family		0.2809	0.3049	0.1785
		Emergence Date				4	280.3	2	0.4821	Row Plot		0	NA	NA
		(Emergence Date)^2				5	280.4	1	0.2328					
Late-Summer														
3 Mile, Idaho	Survival	Full model				6	212.6	-	-	Block		0.4989	0.2598	0.0274 *
		Mean Seed Density				5	212.6	1	0.8820	Family		0	NA	NA
		Emergence Date	β	-0.06828	0.02934	4	206.5	2	0.0465 *	Row Plot		0	NA	NA
		(Emergence Date)^2				5	212.6	1	0.9045					
	Unconditional Expected Height	Full model				6	226.5	-	-	Block		0.003272	0.001378	0.00879 **
		Mean Seed Density				5	223.5	1	0.0880	Family		0	NA	NA
		Emergence Date	β	-0.00032	0.00011	4	216.3	2	0.0061 **	Row Plot		0	NA	NA
		(Emergence Date)^2				5	225.5	1	0.3368					
Priest River, Idaho	Survival	Full model				6	398.6	-	-	Block		1.151	0.4262	0.00346 **
		Mean Seed Density				5	398.4	1	0.6800	Family		0	NA	NA
		Emergence Date	β	-0.00039	0.00012	4	391.1	2	0.0230 *	Row Plot		0	NA	NA
		(Emergence Date)^2				5	398.3	1	0.5854					
	Unconditional Expected Height	Full model				6	395.1	-	-	Block		0.003652	0.001414	0.0049 **
		Mean Seed Density				5	394.5	1	0.4620	Family		0	NA	NA
		Emergence Date	β	-0.00039	0.00012	4	382.4	2	0.0018 **	Row Plot		0	NA	NA
		(Emergence Date)^2				5	394.6	1	0.5091					
Indian Praire, Montana	Survival	Full model				6	995.5	-	-	Block		1.5481	0.5527	0.00255 **
		Mean Seed Density				5	995.5	1	0.8987	Family		0.3208	0.3847	0.20218
		Emergence Date				4	989.6	2	0.0527	Row Plot		0	NA	NA
		(Emergence Date)^2				5	993.6	1	0.1707					
	Unconditional Expected Height	Full model				6	992.1	-	-	Block		0.005296	0.001936	0.00311 **
		Mean Seed Density				5	992.0	1	0.7862	Family		0	NA	NA
		Emergence Date	β	-0.00019	6.5E-05	4	982.0	2	0.0065 **	Row Plot		0	NA	NA
		(Emergence Date)^2	β	0.001278	0.00054	5	985.7	1	0.0113 *					

Table 3-7. Continued

Drought Treatment Provenance	Response	Terms		Selection Gradient	Standard Error	Model df	Model Deviance	Test df	Test P-value	Random Effects: √ of Variance Components (P-values are one-tailed)	Estimate	Std. Error	p value
No Drought													
3 Mile, Idaho	Survival	Full model				6	200.8	-	-	Block	0.5363	0.2569	0.00184 *
		Mean Seed Density				5	200.8	1	0.9380	Family	0.1361	0.4608	0.3839
		Emergence Date	β	-0.11594	0.03186	4	185.7	2	0.0005 ***	Row Plot	0	NA	NA
		(Emergence Date)^2				5	200.7	1	0.7701				
	Unconditional Expected Height	Full model				6	201.2	-	-	Block	0.00266	0.001199	0.00133 **
		Mean Seed Density				5	200.7	1	0.4693	Family	0	NA	NA
		Emergence Date	β	-0.00037	0.00012	4	189.8	2	0.0034 **	Row Plot	0	NA	NA
		(Emergence Date)^2				5	200.8	1	0.5329				
Priest River, Idaho	Survival	Full model				6	440.1	-	-	Block	0.8066	0.32	0.00548 **
		Mean Seed Density				5	473.4	1	0.2946	Family	0.3852	0.2728	0.079
		Emergence Date				4	466.7	2	0.0664	Row Plot	0	NA	NA
		(Emergence Date)^2				5	474.8	1	0.6567				
	Unconditional Expected Height	Full model				6	456.7	-	-	Block	0.003819	0.0014445	0.0041 **
		Mean Seed Density				5	439.0	1	0.5339	Family	0.001518	0.0009328	0.0518
		Emergence Date	β	-0.00035	0.00012	4	446.1	2	0.0061 **	Row Plot	0	NA	NA
		(Emergence Date)^2				5	456.2	1	0.4973				
Indian Praire, Montana	Survival	Full model				6	572.4	-	-	Block	0.8101	0.3177	0.00539 **
		Mean Seed Density				5	571.7	1	0.1327	Family	0.4296	0.2418	0.03783
		Emergence Date	β	-0.10928	0.03199	4	556.1	2	0.0003 ***	Row Plot	0	NA	NA
		(Emergence Date)^2				5	569.9	1	0.3776				
	Unconditional Expected Height	Full model				6	595.6	-	-	Block	0.004673	0.0017134	0.00319 **
		Mean Seed Density				5	594.1	1	0.2166	Family	0.001544	0.0008754	0.03887 *
		Emergence Date	β	-0.00034	0.00011	4	580.4	2	0.0005 **	Row Plot	0	NA	NA
		(Emergence Date)^2				5	591.8	1	0.0516				

β= linear selection gradient, Full models included all presented variables listed directly under the term. The effect of each predictor variable was tested against the full model. Random effects are from model using density term and significant terms for emergence date. Random effects are from null model where no other traits are statistically significant.

Table 3-8. Experiment 1, results from aster model comparison testing the effects of mean seed density (mg/ml), early- and late-season shoot elongation rates (mm) in 2010 and their quadratic and cross products on unconditional expected height (mm) in 2011that included survival from 2010 through 2011.

Drought Treatment Response	Terms		Selection Gradient	Standard error	Model df	Model Deviance	Test df	Test P-value	Random Effects: ν of Variance Components (P-values are one-tailed)	Estimate	Std. Error	p value
Mid- and Late-Summer	Full Model				8	8581.8	-	-	Block	0.002793	0.0009312	0.00135 **
	Mean Seed Density				7	8581.5	1	0.6073	Family	0.001091	0.0004329	0.00588 **
	Early Elongation Rate (EER)	β	0.037034	0.00202	5	8157.4	3	< 0.0001 ***	Row Plot	0	NA	NA
	(EER)^2	γ_{ii}	-1.24E-02	2.62E-03	6	8548.5	2	< 0.0001 ***				
	Late Elongation Rate (LER)	β	0.054848	0.01899	7	8538.2	1	< 0.0001 ***				
	(LER)^2	γ_{ii}	-0.05984	0.03188	6	8574.0	2	0.0200 *				
	(EER*LER)	γ_{ij}	-0.018475	0.01226	7	2894.8	1	0.1297				
Late-Summer	Full Model				8	12555	-	-	Block	0.002637	0.0008804	0.00137 **
	Mean Seed Density				7	12555	1	0.8122	Family	0	NA	NA
	Early Elongation Rate (EER)	β	0.032198	0.00279	5	12076	3	< 0.0001 ***	Row Plot	0	NA	NA
	(EER)^2	γ_{ii}	-0.007143	0.00241	6	12526	2	< 0.0001 ***				
	Late Elongation Rate (LER)	β	0.07547	0.01082	5	12469	1	< 0.0001 ***				
	(LER)^2	γ_{ii}	-0.075715	0.02678	6	12537	2	0.0002 ***				
	(EER*LER)	γ_{ii}	-0.033985	0.0112	7	12545	1	0.0023 **				
No	Full Model				8	9986.1	-	-	Block	0.002589	0.000888	0.00177 **
	Mean Seed Density				7	9986.1	1	0.9742	Family	0.000486	0.0004885	0.15976
	Early Elongation Rate (EER)	β	0.02914	0.0024	5	9570.7	3	< 0.0001 ***	Row Plot	0	NA	NA
	(EER)^2	γ_{ii}	-0.006663	0.00192	6	9932.9	2	< 0.0001 ***				
	Late Elongation Rate (LER)	β	0.067832	0.00949	7	9912.5	1	< 0.0001 ***				
	(LER)^2	γ_{ii}	-0.061617	0.02495	6	9957.9	2	< 0.0001 ***				
	(EER*LER)	γ_{ii}	-0.042933	0.00979	7	9966.8	1	< 0.0001 ***				

β= linear selection gradient, γ_{ii} = quadratic effects, γ_{ij} = cross products, Full models included all presented variables listed directly under the term. The effect of each predictor variable was tested against the full model for each predictor variable. Selection gradients, standard error and random effects are from models that include only significant terms.

Table 3-9. Experiment 2, results from aster model comparison testing the effects of mean seed density (mg/ml), early- and late-season shoot elongation rates (mm) in 2010 and their quadratic and cross products on unconditional expected height (mm) in 2011 that included survival from 2010 through 2011.

Drought Treatment Provenance	Terms		Selection Gradient	Standard Error	Model df	Model Deviance	Test df	Test P-value	Random Effects: √ of Variance Components (P-values are one-tailed)	Estimate	Std. Error	p value
Mid- and Late-Summer												
3 Mile, Idaho	Full Model				8	671.6	-	-	Block	0.002935	0.001287	0.0113 *
	Mean Seed Density				7	670.7	1	0.3485	Family	0	NA	NA
	Early Elongation Rate (EER)	β	0.060148	0.01042	5	633.4	3	< 0.0001 ***	Row Plot	0	NA	NA
	(EER)^2				6	669.9	2	0.4371				
	Late Elongation Rate (LER)				5	668.4	1	0.3678				
	(LER)^2				6	671.6	2	0.9958				
	(EER*LER)				7	671.6	1	0.9403				
Priest River, Idaho	Full Model				8	938.9	-	-	Block	0.003449	0.001521	0.0117 *
	Mean Seed Density				7	938.2	1	0.3970	Family	0	NA	NA
	Early Elongation Rate (EER)	β	0.024641	0.00408	5	904.7	3	< 0.0001 ***	Row Plot	0	NA	NA
	(EER)^2				6	938.5	2	0.8341				
	Late Elongation Rate (LER)				5	938.1	1	0.8475				
	(LER)^2				6	938.4	2	0.7708				
	(EER*LER)				7	938.8	1	0.7783				
Indian Praire, Montana	Full Model				8	1564.4	-	-	Block	0.00527	0.002046	0.00500 **
	Mean Seed Density				7	1562.5	1	0.1666	Family	0.003006	0.001105	0.00327 **
	Early Elongation Rate (EER)	β	0.02515	0.00374	5	1510.3	3	< 0.0001 ***	Row Plot	0	NA	NA
	(EER)^2				6	1562.3	2	0.3556				
	Late Elongation Rate (LER)	β	0.07651	0.03062	5	1554.8	1	0.0220 *				
	(LER)^2	γii	-0.24825	0.09674	6	1554.8	2	0.0084 **				
	(EER*LER)				7	1564.2	1	0.6729				
Late-Summer												
3 Mile, Idaho	Full Model				8	938.6	-	- .	Block	0.001092	0.001167	0.175
	Mean Seed Density				7	938.2	1	0.5442	Family	0	NA	NA
	Early Elongation Rate (EER)	β	0.043485	0.0084	5	873.9	3	< 0.0001 ***	Row Plot	0	NA	NA
	(EER)^2	γii	-1.63E-02	7.53E-03	6	932.5	2	0.0471 *				
	Late Elongation Rate (LER)	β	0.032589	0.01623	5	930.7	1	0.0469 *				
	(LER)^2				6	934.9	2	0.1542				
	(EER*LER)				7	937.9	1	0.4140				
Priest River, Idaho	Full Model				8	1358.9	-	-	Block	0	NA	NA
	Mean Seed Density				7	1358.8	1	0.7417	Family	0	NA	NA
	Early Elongation Rate (EER)	β	0.030955	0.00297	5	1257.5	3	< 0.0001 ***	Row Plot	0	NA	NA
	(EER)^2				6	1353.2	2	0.0585				
	Late Elongation Rate (LER)				5	1355.4	1	0.3241				
	(LER)^2				6	1358.1	2	0.6934				
	(EER*LER)				7	1358.2	1	0.3993				
Indian Praire, Montana	Full Model				8	2284.2	-	-	Block	0.003994	0.00152	0.00429 **
	Mean Seed Density				7	2284.1	1	0.7564	Family	0	NA	NA
	Early Elongation Rate (EER)	β	0.041762	0.0085	5	2173.9	3	< 0.0001 ***	Row Plot	0	NA	NA
	(EER)^2	γii	-0.018033	0.00771	6	2274.8	2	0.0089 **				
	Late Elongation Rate (LER)	β	0.052063	0.01103	5	2274.8	1	0.0246 *				
	(LER)^2				6	2283.9	2	0.8711				
	(EER*LER)				7	2284.0	1	0.6782				

Table 3-9. continued

Drought Treatment Provenance	Terms		Selection Gradient	Standard Error	Model df	Model Deviance	Test df	Test P-value	Random Effects: V of Variance Components (P-values are one-tailed)			
No												
3 Mile, Idaho	Full Model				8	908.0	-	-	Block	0.003095	0.001544	0.0225 *
	Mean Seed Density				7	907.8	1	0.6318	Family	0	NA	NA
	Early Elongation Rate (EER)	β	0.060148	0.01042	5	838.8	3	< 0.0001 ***	Row Plot	0	NA	NA
	(EER)^2	γ_{ii}	-3.14E-02	1.01E-02	6	895.0	2	0.0012 **				
	Late Elongation Rate (LER)	β	0.059963	0.01746	7	892.0	1	0.0012 **				
	(LER)^2				6	903.9	2	0.1298				
	(EER*LER)				7	905.8	1	0.1379				
Priest River, Idaho	Full Model				8	1179.2	-	-	Block	0.002872	0.00147	0.0254 *
	Mean Seed Density				7	1179.1	1	0.7524	Family	0	NA	NA
	Early Elongation Rate (EER)	β	0.055676	0.01022	5	1112.5	3	< 0.0001 ***	Row Plot	0	NA	NA
	(EER)^2	γ_{ii}	-0.026009	0.00902	6	1170.9	2	0.0159 *				
	Late Elongation Rate (LER)				5	1174.2	1	0.1718				
	(LER)^2				6	1178.6	2	0.7417				
	(EER*LER)				7	1178.6	1	0.4448				
Indian Praire, Montana	Full Model				8	1651.4	-	-	Block	0.004425	0.00164	0.00347 **
	Mean Seed Density				7	1651.0	1	0.5403	Family	0	NA	NA
	Early Elongation Rate (EER)	β	0.022943	0.00274	5	1580.4	3	< 0.0001 ***	Row Plot	0	NA	NA
	(EER)^2				6	1645.4	2	0.0501				
	Late Elongation Rate (LER)	β	0.046759	0.01077	5	1626.6	3	< 0.0001 ***				
	(LER)^2				6	1649.4	2	0.3784				
	(EER*LER)				7	1651.2	1	0.7087				

Note: Significant terms indicate significant differences among phenotypes for unconditional expected height. Full models included all presented variables listed directly under the term. The effect of each predictor variable was tested against the full model for each predictor variable. Selection gradients, standard error and random effects are from models that include only significant terms.
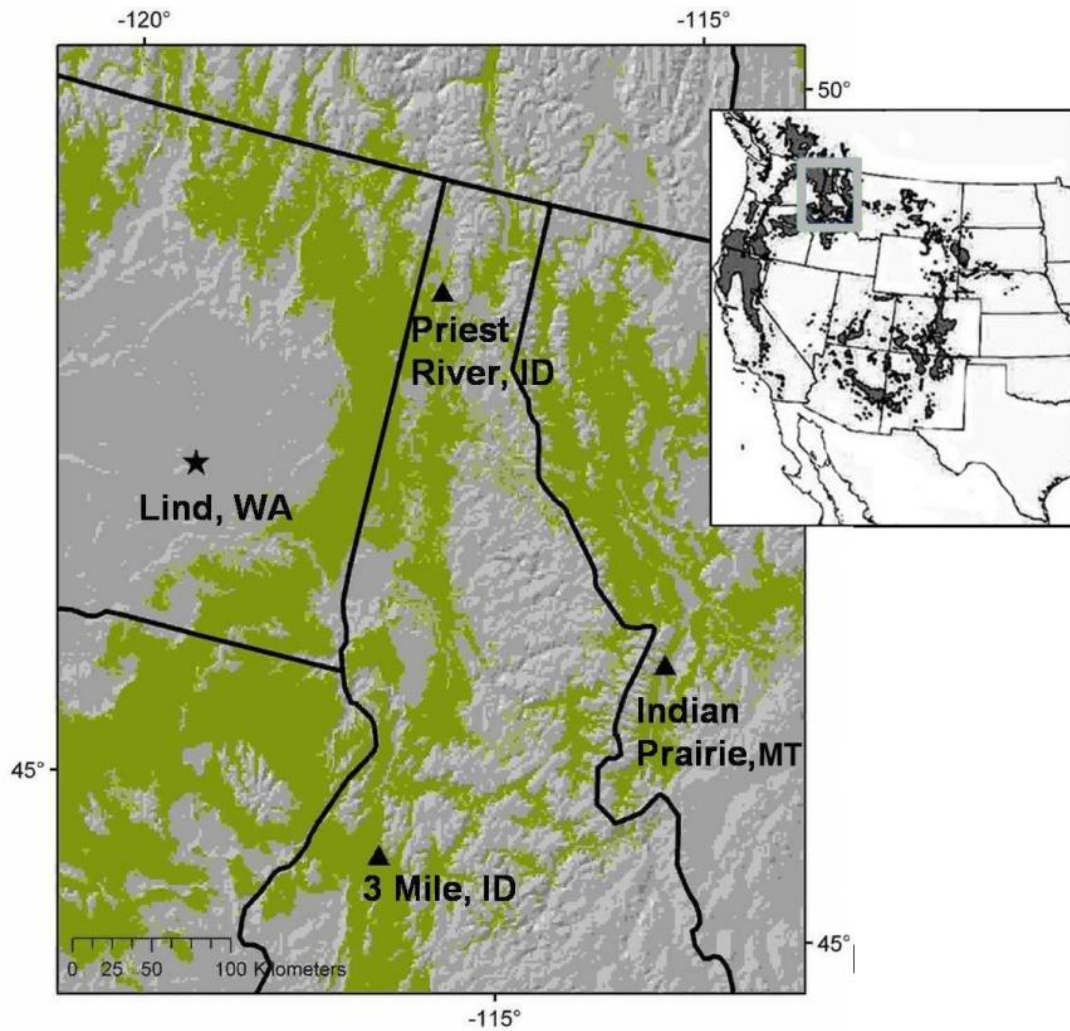
Figure 3-1. Location of provenances (▲) and study site (★). Green indicates predicted realized climate niche of ponderosa pine (*Pinus ponderosa*) (Rehfeldt et al. 2006). Upper right insert shows the range-wide distribution of ponderosa pine (Little 1971) with the southern range updated to reflect recent taxonomic reclassifications (Perry, 1991). The area represented in the larger image is indicated by the grey rectangle.
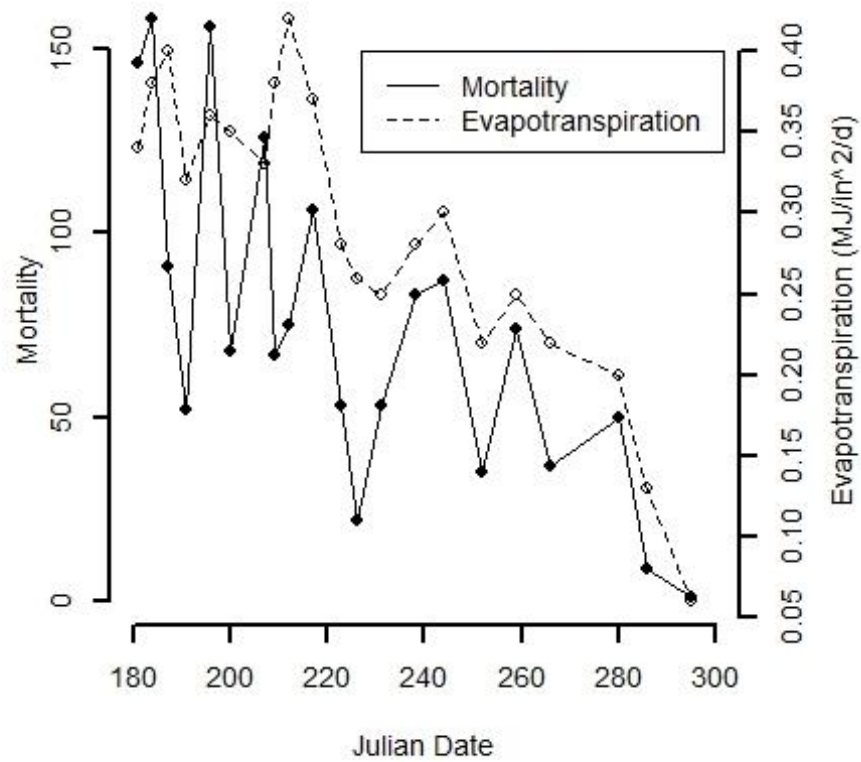
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A

Bernoulli   Bernoulli
Present Spring 2009 $\longrightarrow$ Survival Fall 2009 $\longrightarrow$ Survival Fall 2011

B

Bernoulli
Present Spring 2010 $\longrightarrow$ Survival Fall 2011

C

Bernoulli   Bernoulli   Normal
Present Spring 2009 $\longrightarrow$ Survival Fall 2009 $\longrightarrow$ Survival Fall 2011 $\longrightarrow$ Total Height
Fall 2011

D

Bernoulli   Normal
Present Spring 2010 $\longrightarrow$ Survival Fall 2011 $\longrightarrow$ Total Height Fall 2011

Figure 3-2. Graphical models for aster analysis unifying survival over time and survival over time and total height data to assess fitness. Each node represents a component of life history while arrows represent the dependent association between predecessor and successor life-history components. Survival was modeled as a Bernoulli random variable and total height as normal random variable. **Graph A** shows the model used to predict survival in 2011 beginning with seedlings present in Spring 2009. **Graph B** shows model for survival in 2011 beginning with seedlings present in Spring 2010. **Graph C** shows the model used to predict unconditional height in fall 2011 which included survival from Spring 2009 to Fall 2011. **Graph D** shows model for height in Fall 2011 which included survival from Spring 2010 to Fall 2011.

Figure 3-3. Timing of seedling mortality in relation to evapotranspiration (Kimberly-Penman equation, AgriMet 2015). Solid points represent number of seedlings tallied as dead on the corresponding date. Open circles indicate mean evapotranspiration for the period leading up to and including the corresponding date. Lines represent interpolations of the data and are shown to illustrate general trend. Julian days 180 = June 29, and 300 = October 27, 2009.
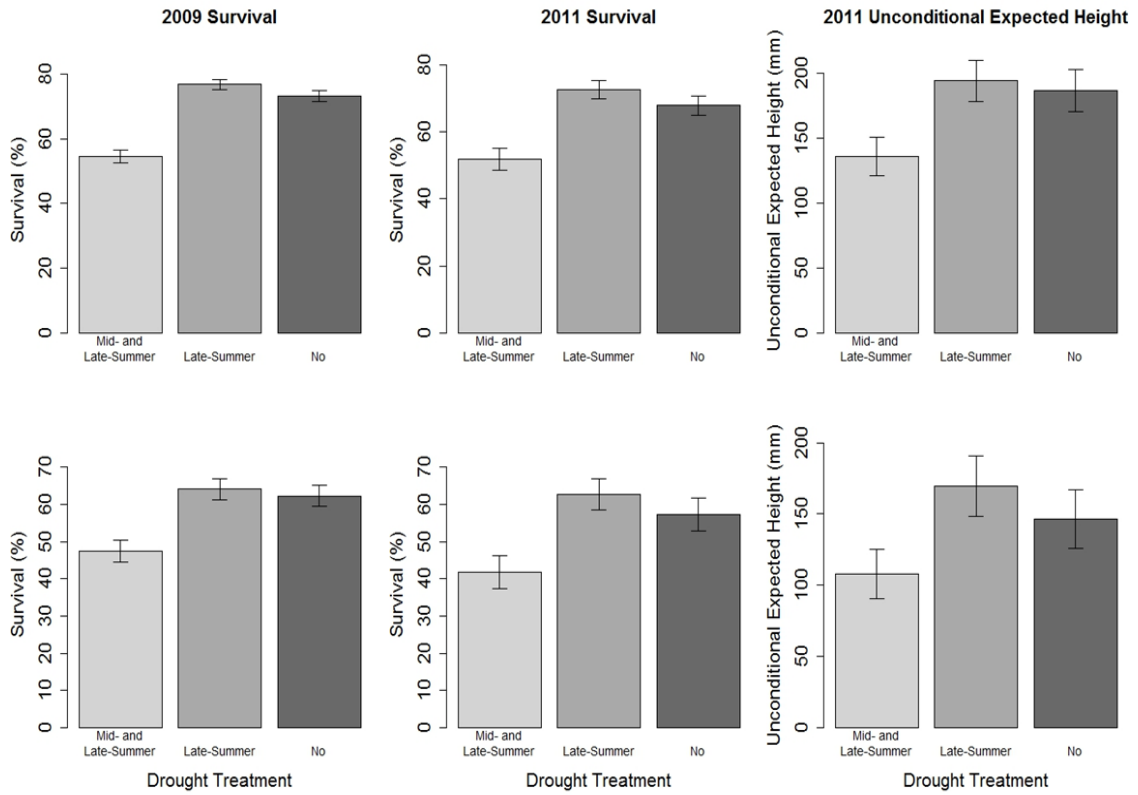
Figure 3-4. Modeled survival (%) (±1SE) in 2009 and 2011and unconditional expected height (mm) (±1SE) in 2011 by Mid- and Late-Summer, Late-Summer and No drought treatment in Experiment 1 (top row) and Experiment 2 (bottom row).
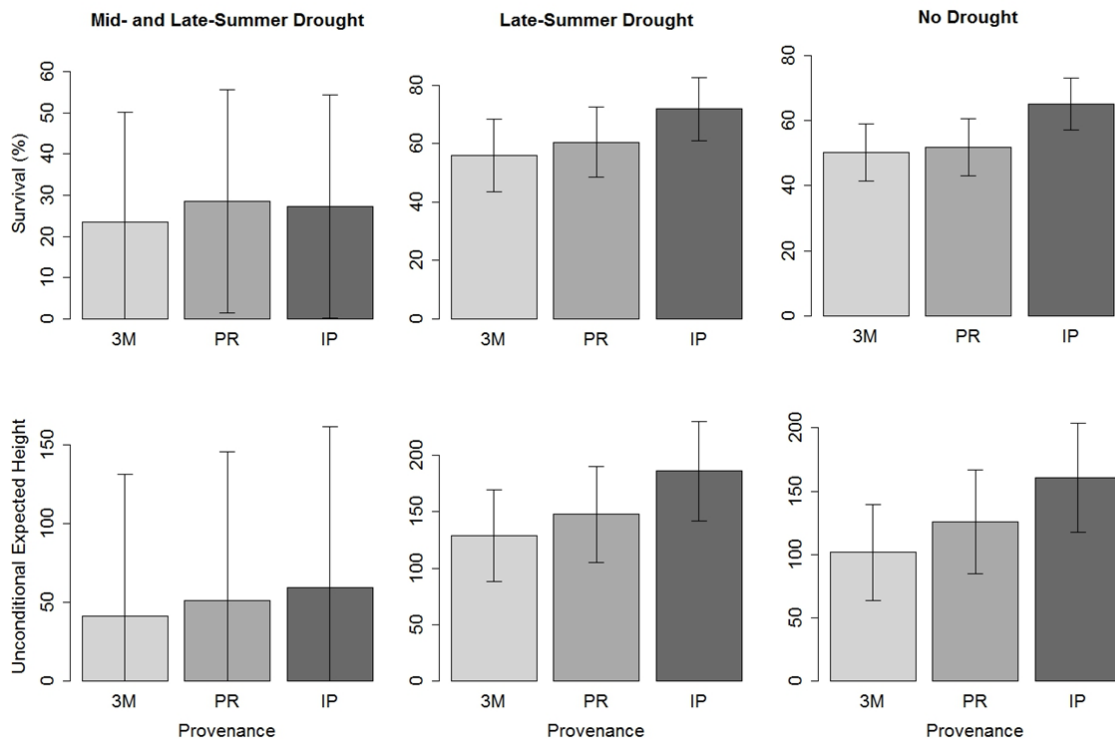
Figure 3-5. Experiment 2 modeled survival (%) (±1SE) from 2009 to 2011 and unconditional expected height (mm) (±1SE) in 201l that included survival beginning in 2009 among for 3Mile, Priest River and Indian Prairie provenances in the Mid-Summer, Late-Summer and No drought treatments.
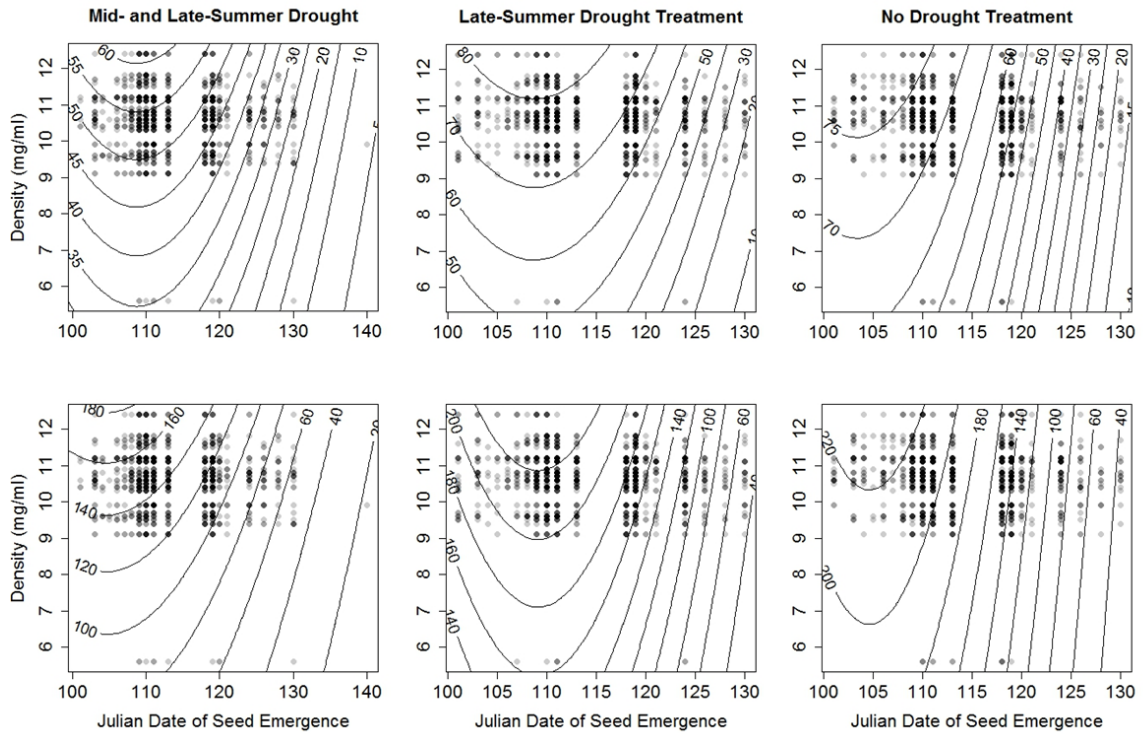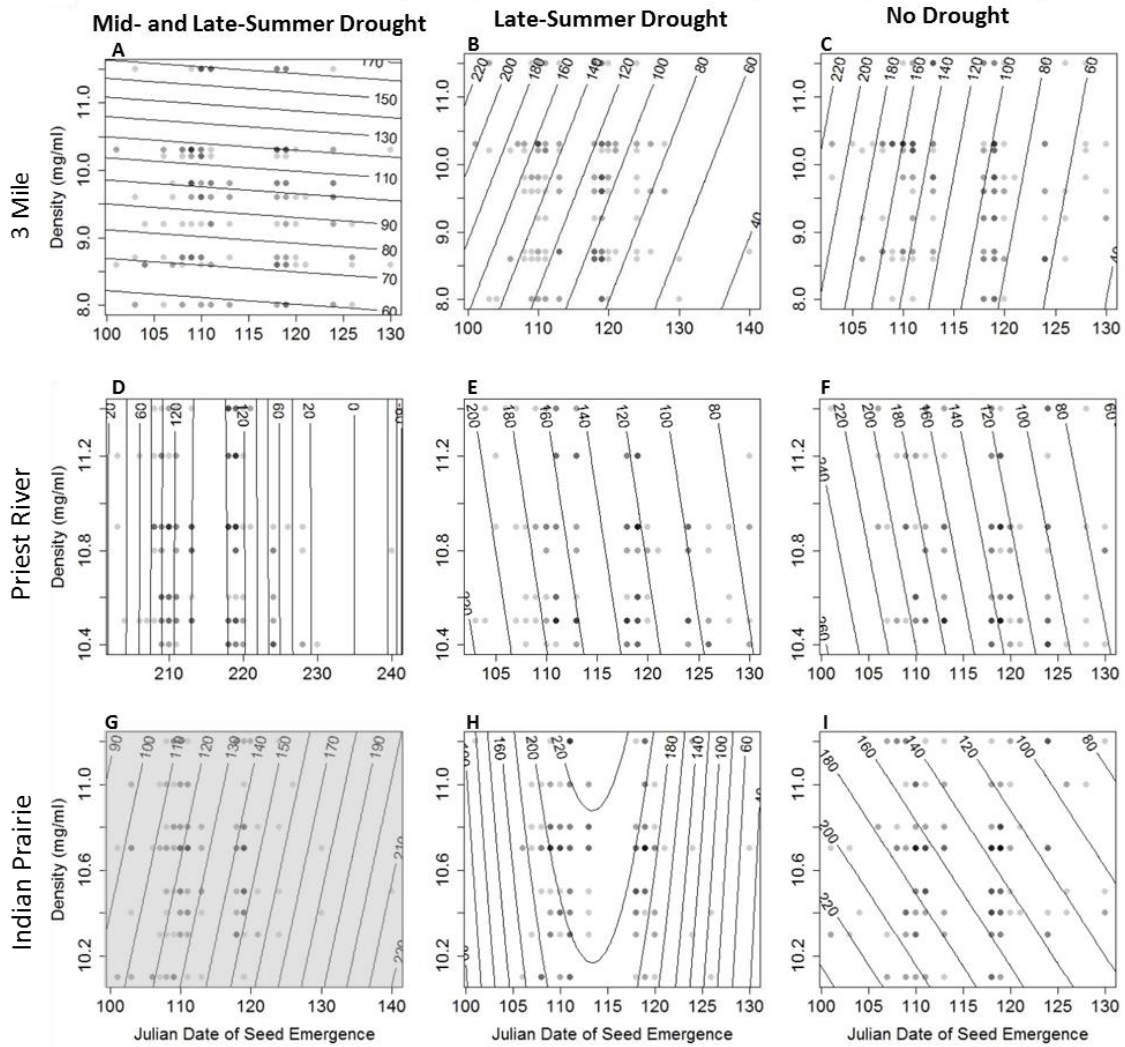
Figure 3-6. Experiment 1, fitness surfaces showing observed (circles) Julian data of emergence and mean density of seed (mg/ml) by family in relation to modeled (contour lines) survival from 2009 to 2011 (top row) and unconditional expected height (mm) in 2011 that included survival beginning in 2009 (bottom row) in the Mid- and Late-Summer, Late-Summer and No drought treatment. Increasing darkness of circles indicates higher amount of observations for the indicated measures.
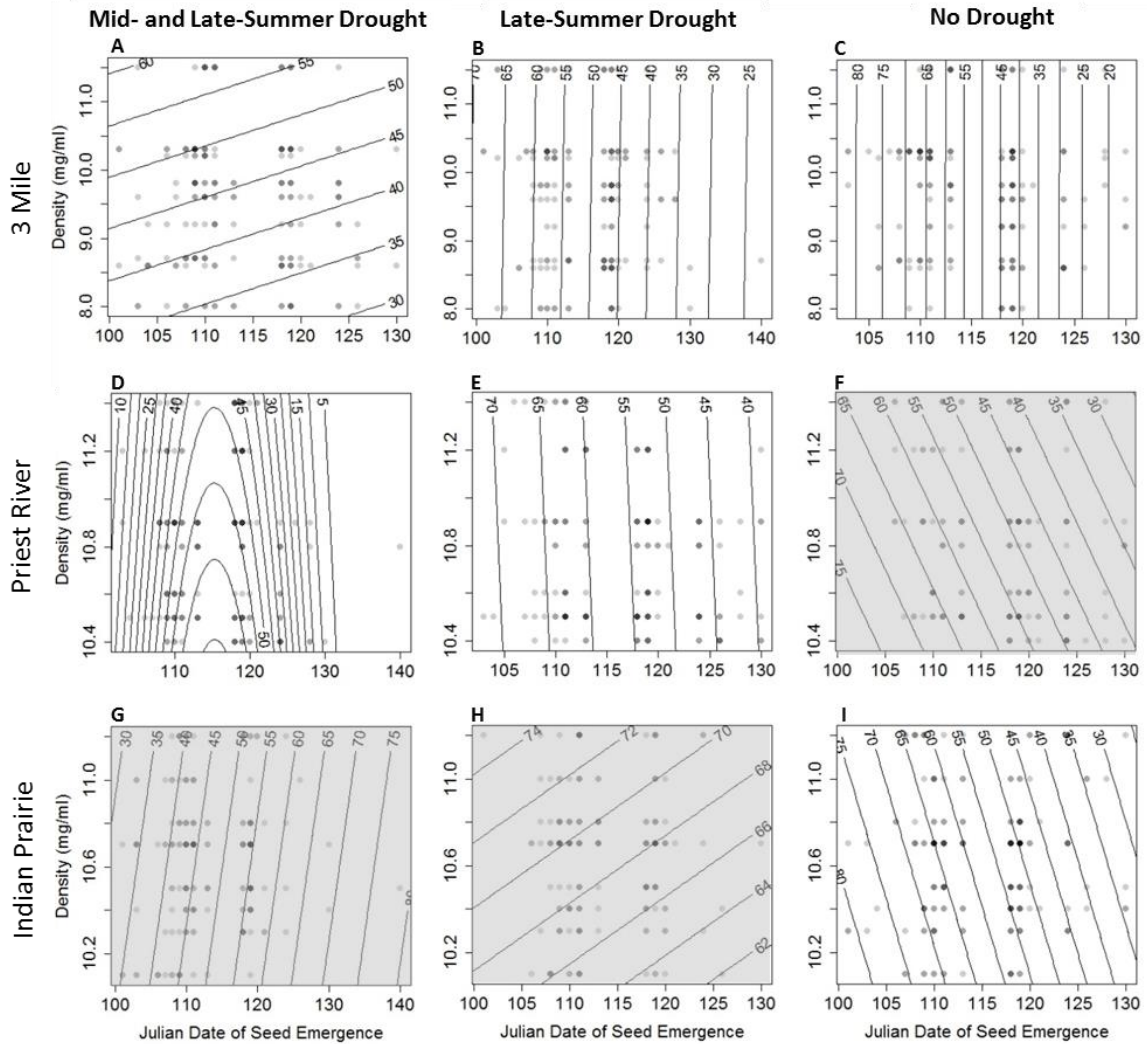
Figure 3-7. Experiment 2, fitness surfaces showing observed (circles) Julian data of emergence and mean density of seed (mg/ml) by family in relation to modeled (contour lines) survival (%) from 2009 to 2011 in the Mid- and Late-Summer, Late-Summer and No drought treatment for seed source from 3 Mile, Idaho, Priest River Experimental Forest, Idaho and Indian Prairie, Montana. Increasing darkness of circles indicates higher amount of observations for the indicated measures. Density was not statistically significant (P < 0.05) in any plot and shows only trends (see Table 3-7). Shaded plots (F, G and H) indicate where date of emergence was also not statistically significant (P < 0.05)

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Figure 3-8. Experiment 2, fitness surfaces showing observed (circles) Julian data of emergence and mean density of seed (mg/ml) by family in relation to modeled (contour lines) unconditional expected height (mm) in 2011 that included survival beginning in 2009 in the Mid- and Late-Summer, Late-Summer and No drought treatment for seed source from 3 Mile, Idaho, Priest River Experimental Forest, Idaho and Indian Prairie, Montana. Increasing darkness of circles indicates higher amount of observations for the indicated measures. Density but not date of emergence was statistically significant in plot A. Neither density nor date of emergence was statistically significant ($P < 0.05$) in plot G (shaded plot). Date of emergence but not density was statistically significant ($P < 0.05$) in all other plots (see Table 3-7).

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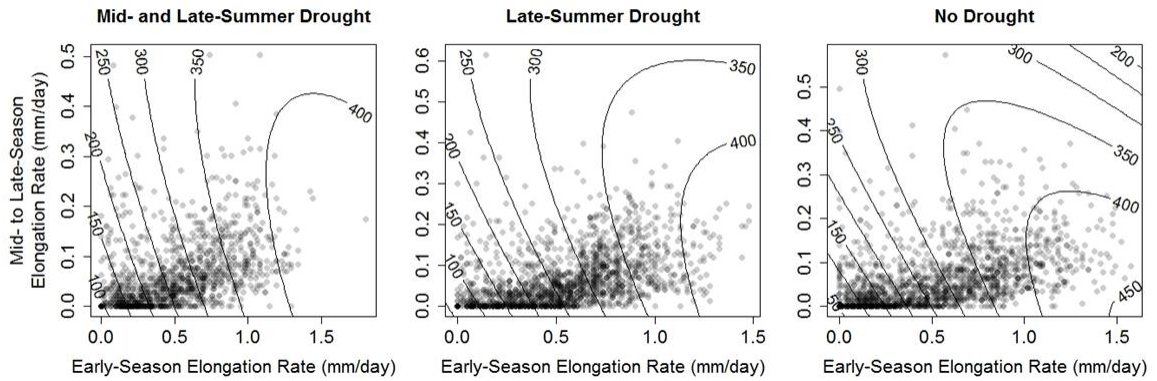
Figure 3-9. Experiment 1, fitness surfaces showing observed (circles) apical shoot elongation rates (mm/day) early (March 6 to May 7) and mid- to late-season (May 8 – Sept 28) in 2010 in relation to modeled (contour lines) unconditional expected height in 2011 that included survival beginning in 2009 in the Mid-Summer, Late-Summer and No drought treatment. Increasing darkness of circles indicates higher amount of observations for the indicated measures.
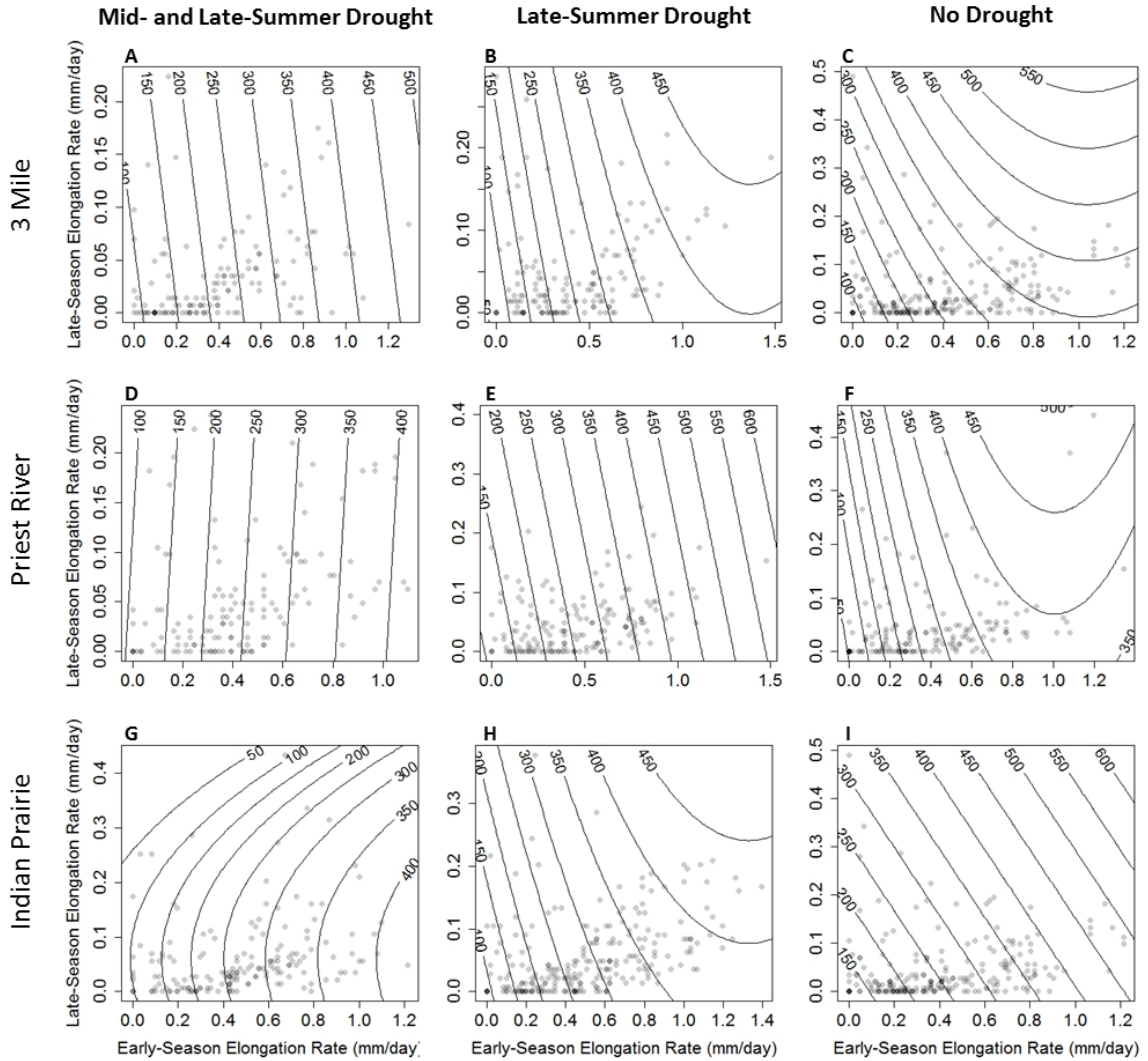
Figure 3-10. Experiment 2, fitness surfaces showing observed (circles) apical shoot elongation rates (mm/day) early (March 6 to May 7) and mid- to late-season (May 8 – Sept 28) in 2010 in relation to modeled (contour lines) unconditional expected height in 2011 that included survival beginning in 2009 in the Mid-Summer, Late-Summer and No drought treatment for seed source from 3 Mile, Idaho, Priest River Experimental Forest, Idaho and Indian Prairie, Montana. Increasing darkness of circles indicates higher amount of observations for the indicated measures. Late-season elongation rate was statistically significant ($P < 0.05$) for plots (E, F, G, H and, I see Table 3-9).

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Table A1-1. Monthly mean air temperature (°C) using measurements over a continuous 24-hour period and 8-hour, night-time period for Experiment 1 and Experiment 2 sites at Priest River Experimental Forest.

measure period	Year	Experiment	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
24 hours daily	2004	1	5.6	7.4	24.5	26.1	-	-	-	-	-	-
		2	5.1	7.4	24.4	26.1	-	-	-	-	-	-
	2005	1	-	-	-	-	-	-	24.6	21.3	14.6	5.9
		2	-	-	-	-	-	-	23.7	20.6	14.3	5.4
	2006	1	-	7.3	11.2	15.1	19.8	16.8	12.4	-	-	-
		2	-	7.3	11.2	15.2	19.9	16.6	12	-	-	-
8 hours daily	2004	1	0.16	2.8	24.3	25.8	-	-	-	-	-	-
(2300-0700)		2	-0.4	2.7	24.2	25.8	-	-	-	-	-	-
	2005	1	-	-	-	-	-	-	18.8	18.8	13.9	4.4
		2	-	-	-	-	-	-	17.9	18.2	13.5	3.8
	2006	1	-	2	5.3	9.6	11.7	8.9	5.5	-	-	-
		2	-	1.9	5.1	9.5	11	8.1	4.5	-	-	-

Figure A1-1. Experiment 1, Priest River Experimental Forest, whitebark pine (*Pinus albicaulis*) common garden, summer 2000

Figure A1-4. Experiment 2, Priest River Experimental Forest, whitebark pine (*Pinus albicaulis*) common garden, fall 2001
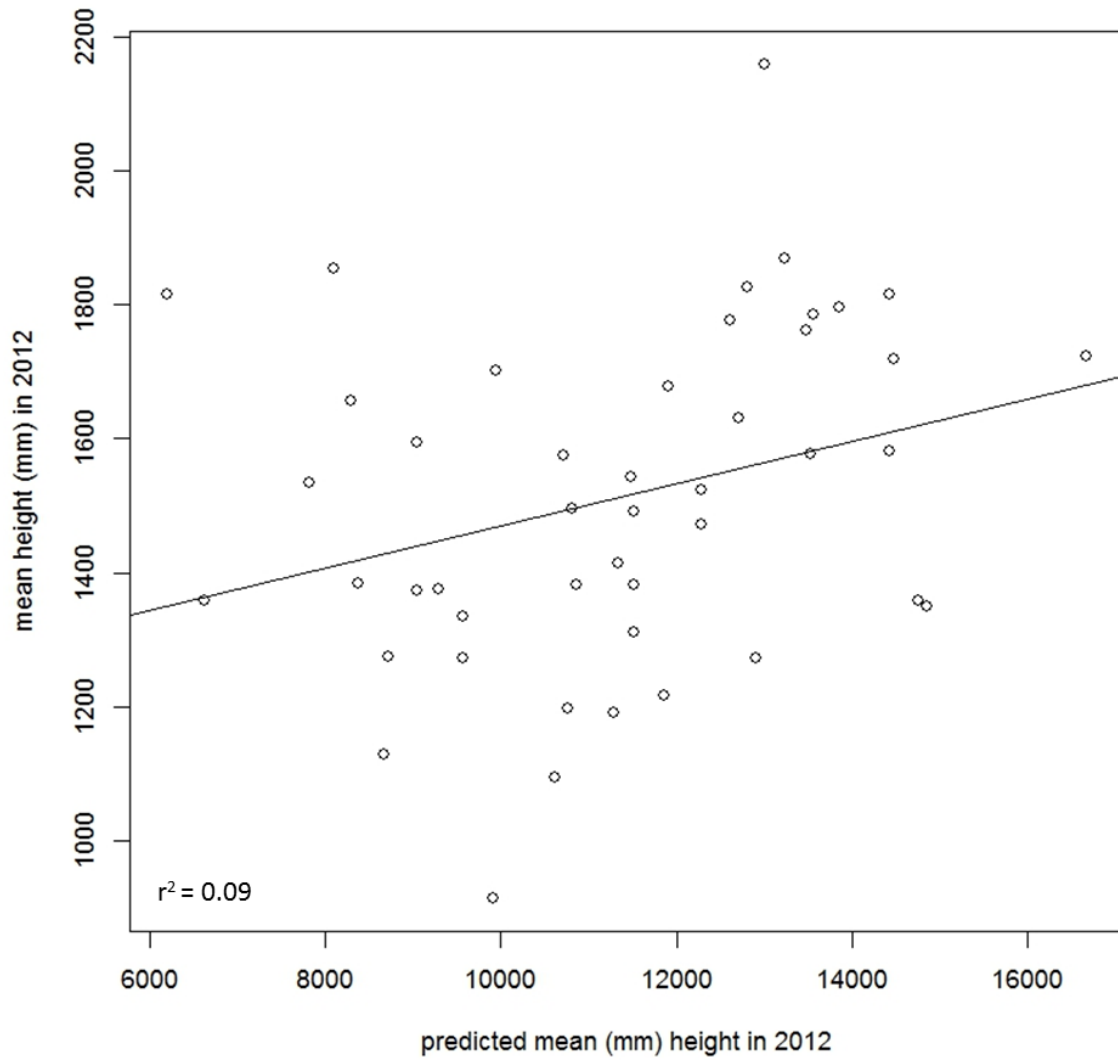
Figure A1-3. Experiment 1, mean height in 2012 versus predicted mean height in 2012 using height in 2001 at start of study as the single predictor for whitebark pine (*Pinus albicaulis*) populations.
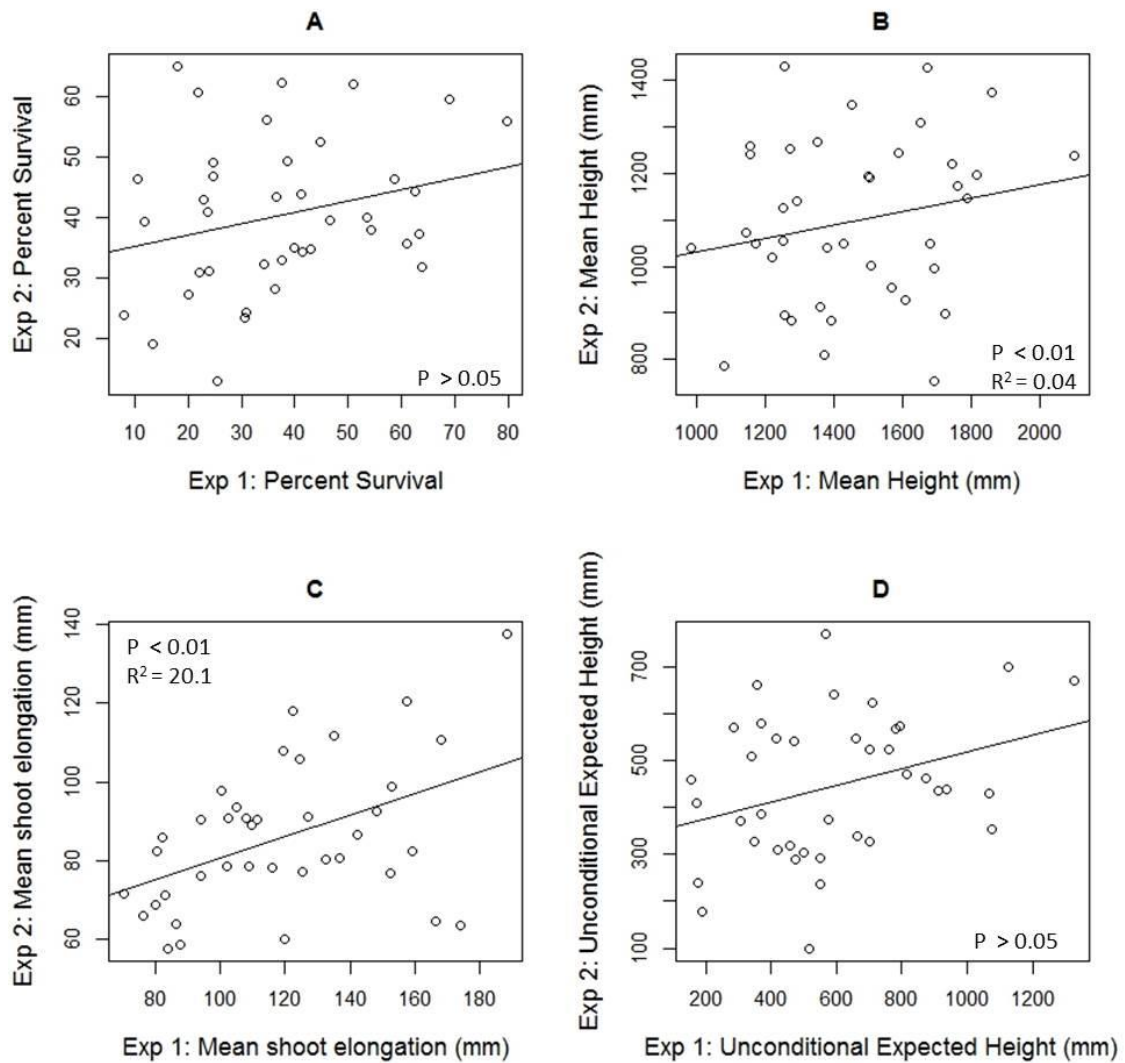
Figure A1-4. Scatter plots of whitebark pine (*Pinus albicaulis*) population predicted performance in Experiment 1 versus Experiment 2 for percent survival in 2012 (A), mean height in 2012 (B), mean apical shoot elongation in 2005 (C), unconditional expected height in 2012(D).
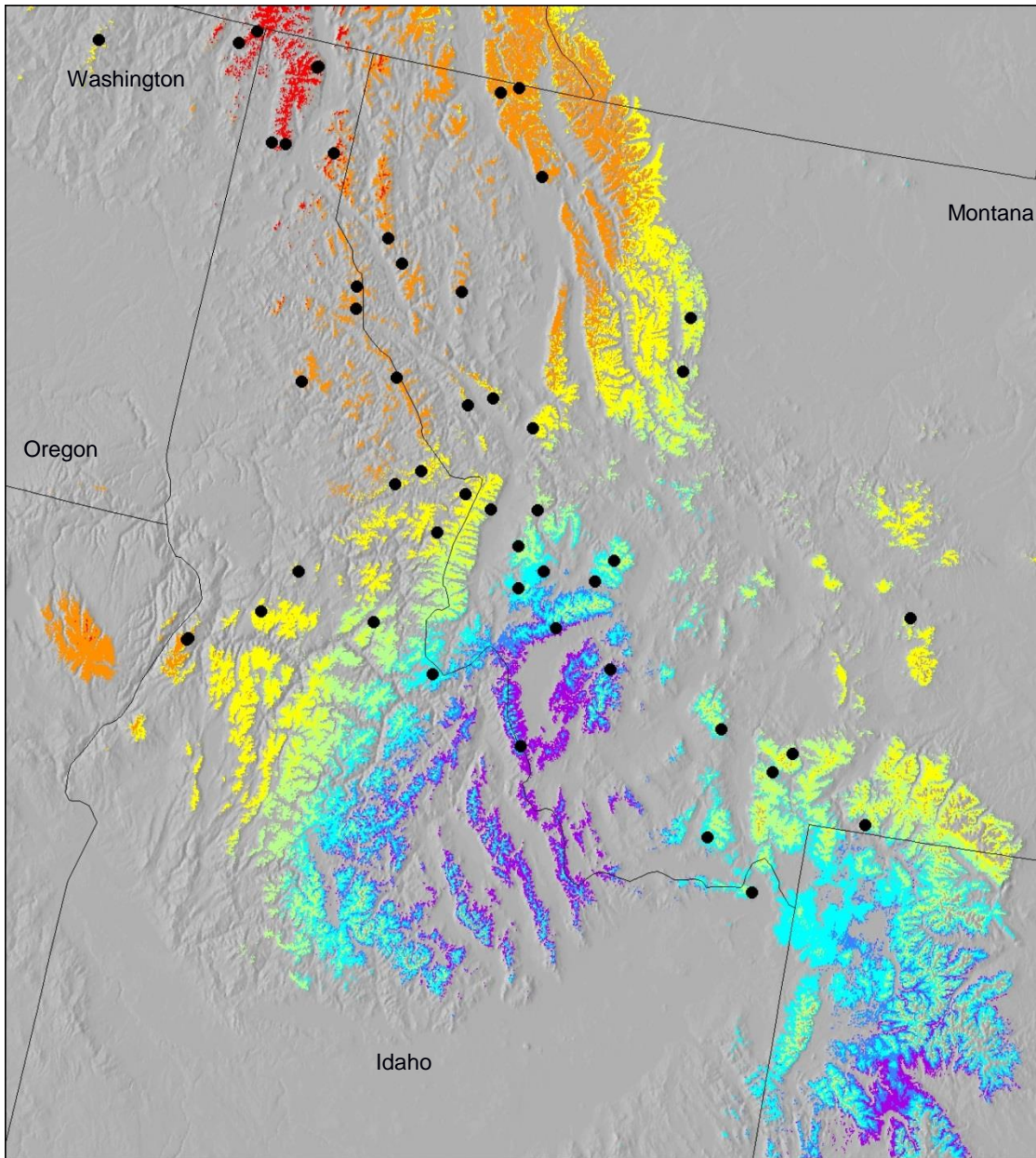
Figure A1-5. Geographic distribution for the interaction of annual dryness and winter coldness (ADIMMINDD0) projected over predicted contemporary climate niche of whitebark pine (*Pinus albicaulis*) in interior northwestern USA. Red = 7 - 45, orange = 45 - 70, yellow = 70 – 94, green = 94 - 120, light blue = 120 – 147, dark blue = 147 – 173, purple = 173 - 299 and (●) indicates location of population.
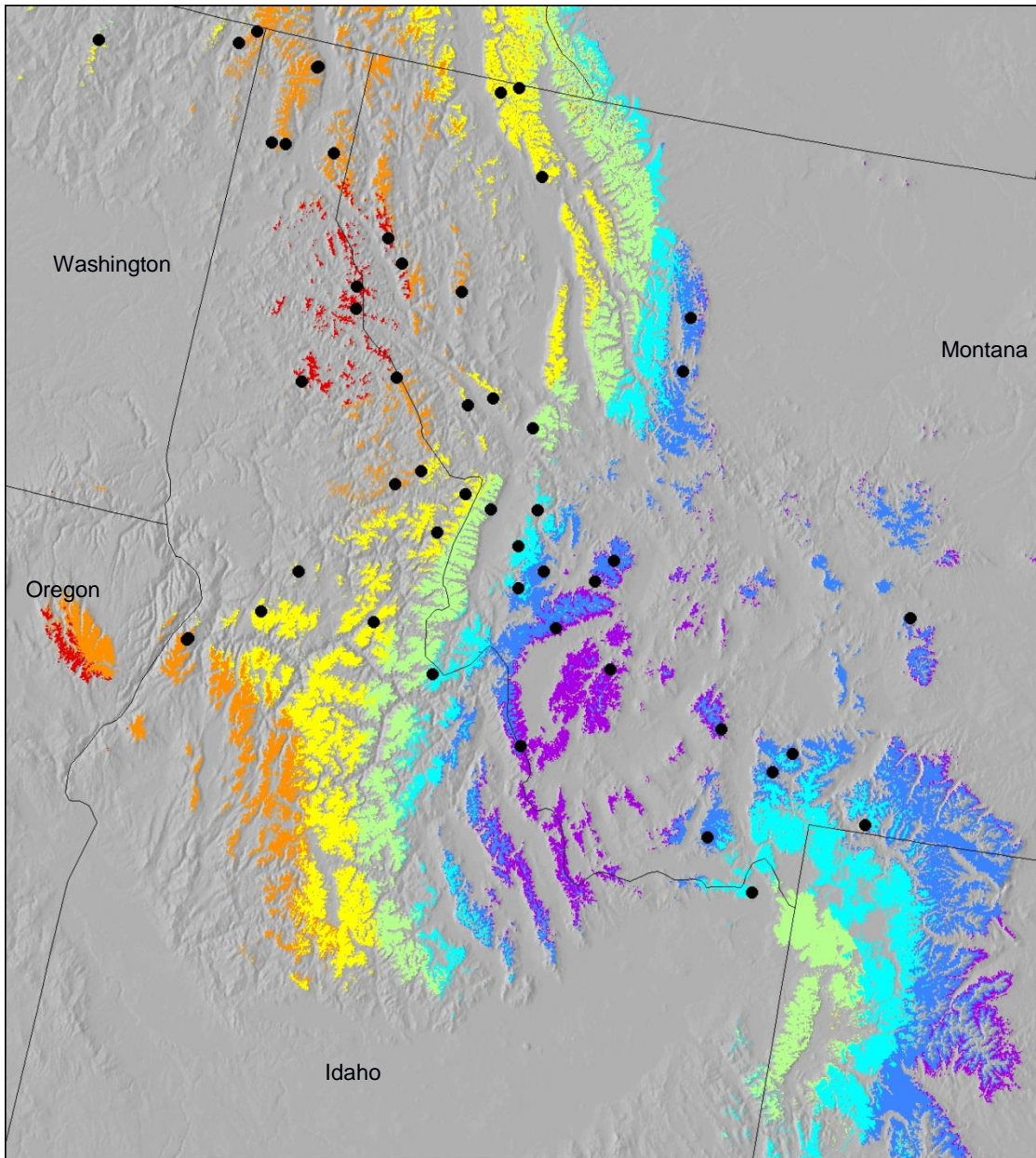
Figure A1-6. Geographic distribution for ratio of summer precipitation to mean annual precipitation (PRATIO) projected over predicted contemporary climate niche of whitebark pine (*Pinus albicaulis*) in interior northwestern USA. Red = 0.16 - 0.32, orange = 0.32 - 0.37, yellow = 0.38 – 0.42, green = 0.43 - 0.47, light blue = 0.48 – 0.52, dark blue = 0.53 – 0.58, purple = 0.59 - 0.70 and (●) indicates location of population.
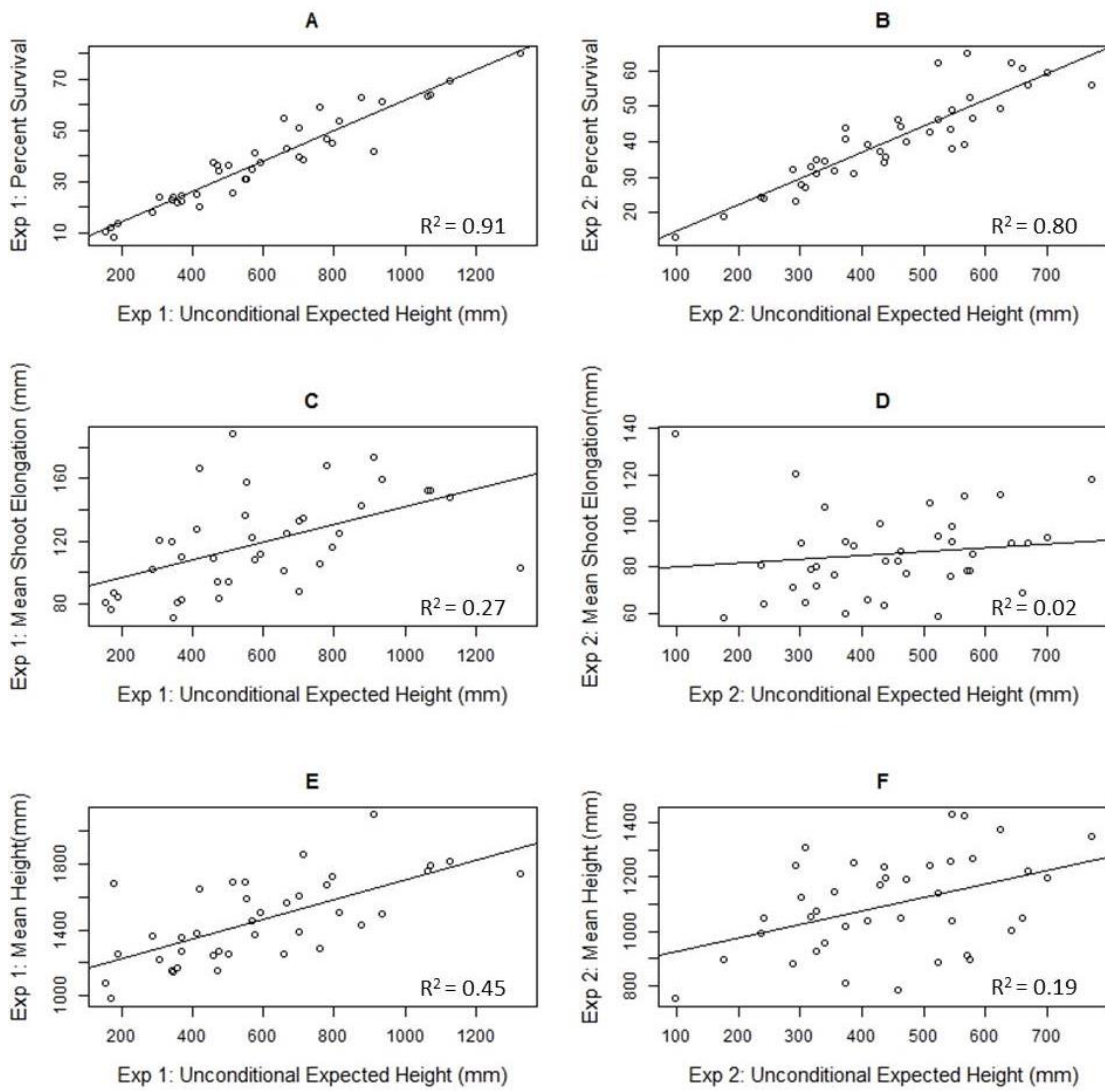
Figure A1-7. Scatter plots of whitebark pine (*Pinus albicaulis*) population predicted response for percent survival by unconditional mean height in 2012 Experiment 1 (A) and Experiment 2 (B), mean apical shoot elongation in 2005 by unconditional mean height in 2012 in Experiment 1 (C) and in Experiment 2 (D) and mean height in 2012 unconditional mean height in 2012 in Experiment 1 (E) and Experiment 2 (F).
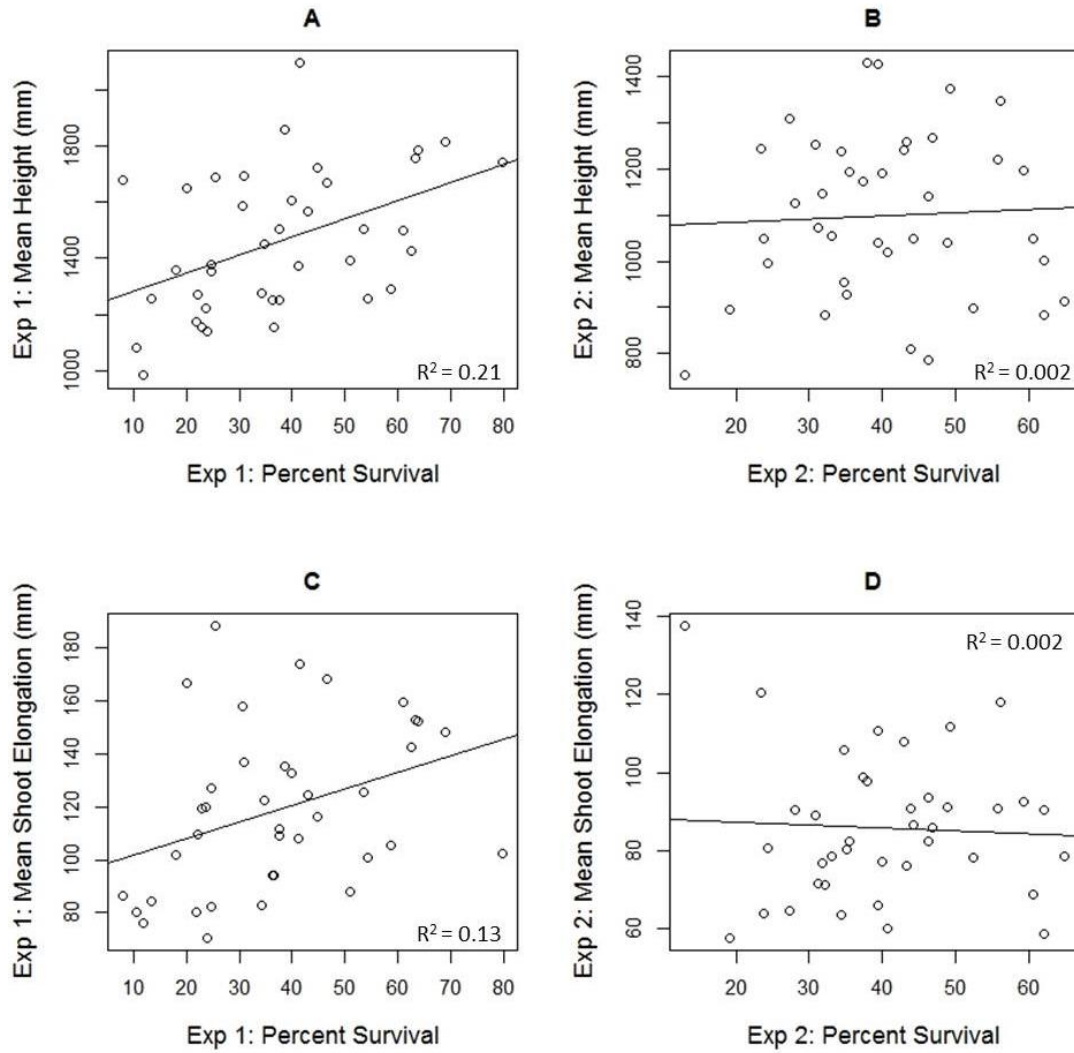
Figure A1-8. Scatter plots of whitebark pine (*Pinus albicaulis*) population predicted response for percent survival by mean height in 2012 in Experiment 1 (A) and Experiment 2 (B), and mean apical growth in 2005 in Experiment 1 (C) and in Experiment 2 (D).

Figure A3-1. Experiment 1 and 2, Washington State University, Lind Dryland
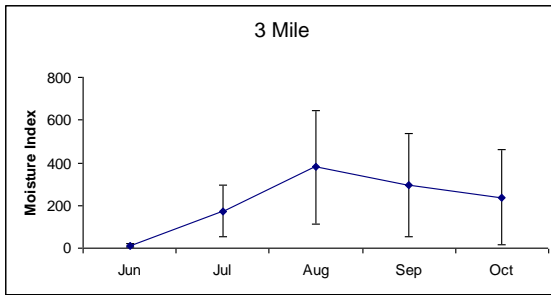Experimental Station, ponderosa pine (*Pinus ponderosa*) common garden, spring 2009
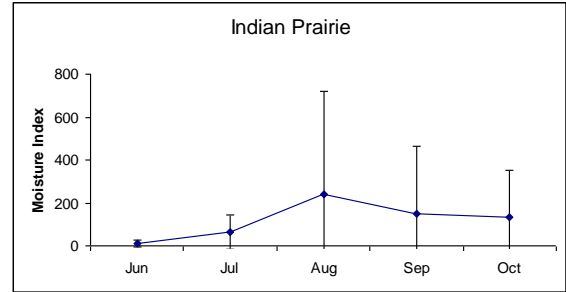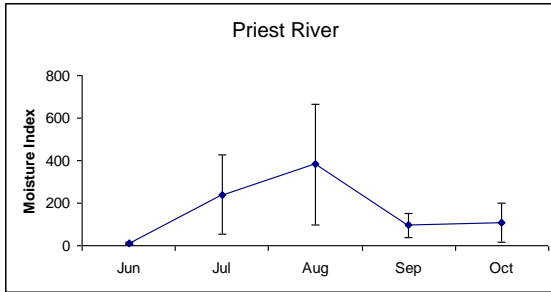
Figure A3-2. Mean and standard error for moisture index (1960-1982) for 3 representative inland northwestern USA provenances of ponderosa pine. Moisture index was calculated as a ratio of cumulative annual temperature over 5˚C to monthly precipitation (mm) and represents a measure of drought as it affects the plant (Tchebakova, Rehfeldt, and Parfenova, 2005).