DISSERTATION

THE ROLE OF DISTURBANCE AND LOCAL ADAPTATION IN ALTERING TREE REGENERATION RESPONSES TO CLIMATE CHANGE

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ABSTRACT

THE ROLE OF DISTURBANCE AND LOCAL ADAPTATION IN ALTERING TREE REGENERATION RESPONSES TO CLIMATE CHANGE

Anthropogenic climate warming is predicted to reorganize the communities seen on the landscape today as species migrate to stay within their preferred climate niche. However, for long-lived trees, it is uncertain whether migrations will be able to keep pace with the rapidly changing climate. In addition, adaptations to local climate that have evolved within subpopulations of a species may become obsolete or maladaptive under climate change. Natural disturbances such as wildfire and insect outbreaks in forests may help to accelerate range shifts by reducing competition from other plant species and creating favorable microsites for establishment of novel species. For landscapes that are not recovering after disturbance events, human-assisted movement of populations within species (assisted gene flow) has been proposed to pre-adapt the forest to future climate conditions.

In the first part of my dissertation, I analyze large-scale USDA Forest Service Forest Inventory and Analysis data from the interior western US and conduct a field study of trembling aspen in southern Colorado to understand how disturbances are interacting with climate change to alter species range shifts at broad and local scales. The first chapter of my dissertation shows that disturbances are catalyzing range shifts on a large-scale for the dominant tree species of the interior western US under both disturbed and undisturbed conditions, but mostly through reduced regeneration in hot and dry portions of species ranges rather than expansions into cooler and wetter areas. However, chapter 2 reveals that expansions are happening on more local scales for trembling aspen after wildfire, but not after beetle-kill mortality events. Therefore, this research shows that disturbance-facilitated species range expansions are limited to certain species and locations, which may not be enough to keep pace with climate warming.

The second part of my dissertation investigates the potential for assisted gene flow in ponderosa pine by examining differences between seedlings from different populations and mother trees in both the greenhouse under drought and the field under a variety of microsite conditions. This research reveals that population climate and watering regime are important determinants of seedling growth in the greenhouse, but that ponderosa pine seedlings from hotter and drier populations do not possess adaptations typically associated with drought-tolerance and do not perform well when planted at the cooler range margin. In addition, mother tree sensitivity to climate was influential in seedling water use efficiency in the greenhouse and in the sensitivity of seedlings to microclimate conditions in the field, making the case that targeting seed collections from specific mother trees may be warranted. Overall, my dissertation research provides insight into how future disturbance events are likely to influence regeneration at species range margins and provides valuable information for land managers seeking to implement assisted gene flow at the upper elevational margin of ponderosa pine forests.

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PREFACE

Anthropogenic climate warming is predicted to reorganize the communities seen on the landscape today as species migrate to stay within their preferred climate niche (Freeman et al., 2018; Lenoir and Svenning, 2015; Svenning and Sandel, 2013). The amount and way in which a species' distribution will shift with climate change depends on the ability of the species to both persist in its current range and migrate outside of its range (Lenoir and Svenning, 2015). A species able to persist and migrate will likely see an expanded range in the future whereas a species that is unlikely to persist nor migrate is expected to experience range contraction. Habitat suitability for tree species in the western US is generally predicted to increase in elevation and latitude as climate continues to warm and therefore, persistence at the warm range margin is expected to decline while migration from the cold range margin is expected to increase (Kemp et al., 2019; Rehfeldt et al., 2006). However, for long-lived trees, it is questionable whether migrations will be able to keep pace with the rapidly changing climate, as significant lags have already been documented (Gray and Hamann, 2013; Rees et al., 2020; Renwick and Rocca, 2015).

Lags in migration can occur for many reasons including dispersal limitations, biotic competition, or a lack of suitable microsites and/or weather conditions for regeneration (Alexander et al., 2018; Brooker, 2006; Brown et al., 2019; Davis and Shaw, 2001; Eriksson, 2000; Kroiss and HilleRisLambers, 2015). Natural disturbances such as wildfire and insect outbreaks in forests may help to accelerate range shifts by reducing competition from other plant species and creating favorable microsites for establishment of novel species (Allen and Breshears, 1998; Landhäusser et al., 2010; Wang et al., 2019). However, this is likely to be species- and disturbance- specific and dependent on the extent and severity of the disturbance event (Buma and Wessman, 2012). Disturbances are predicted to be altered by climate change as well (Abatzoglou and Williams, 2016; Alizadeh et al., 2021; Keyser and Westerling, 2019), therefore making it essential to understand how climate change and disturbance events will interact to shape species distributions in the future.

In addition to species ranges becoming misaligned with their climate niches, adaptations to local climate that have evolved for populations within species ranges may become obsolete or maladaptive under climate change (Maguire et al., 2018; Rehfeldt et al., 2014). As changes in climate accelerate, migrations from the species range margin of individuals with adaptations to cooler environments may not best prepare the regenerating forest for the warmer conditions to come (Hargreaves and Eckert, 2019). Therefore, disturbances may also present an opportunity for reforestation with genotypes better suited to future climate conditions, which is known as assisted gene flow (Aitken and Bemmels, 2016). Assisted gene flow has begun to be operationalized in forestry programs, where seed sources from lower elevations and latitudes are recommended to be planted at higher elevations and latitudes in anticipation of warming (MacKenzie and Mahony, 2021; Nagel et al., 2017; Young et al., 2020). However, there are still unanswered questions concerning which seed sources are best and if they will survive in the short term to reach the future climate they are adapted to (Park and Rodgers, 2023). In order to move species in a way that best prepares forests for future climate changes, we must understand what local adaptations they possess and how those adaptations will fare under novel conditions. The overarching goal of my dissertation is to understand how lags in tree species range shifts may be overcome, either through natural disturbances or assisted gene flow.

Research Outline

The first part of my dissertation research (Chapters 1 & 2) is focused on disturbance-facilitated range shifts in trees of the interior western US. In my first chapter I investigate this on a large-scale for many species by looking at the mismatch between adult tree and seedling distributions in climate space under disturbed and undisturbed conditions (Chapter 1). In my second chapter, I focus on upslope range expansion of trembling aspen (*Populus tremuloides*) to determine whether a high severity wildfire or bark beetle-induced mortality event facilitated seedling establishment upslope of trembling aspen's current elevational range (Chapter 2; Nigro et al. 2022).

The second part of my dissertation research (Chapters 3 & 4) concerns assisted gene flow in ponderosa pine (*Pinus ponderosa*), a species with significant documented regeneration failure in the lower elevations of its range and limited seed dispersal. My third dissertation chapter investigates differences between seedlings from distinct populations and mother trees under experimental drought and watered conditions in the greenhouse (Chapter 3). My fourth and final chapter expands this greenhouse study to the field, where seedling growth and survival is tracked for different populations and mother trees under a range of microclimate conditions within and at the upper elevational edge of ponderosa pine's current range (Chapter 4).

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CHAPTER 1

[1]DISTURBANCE FACILITATES TREE RANGE CONTRACTIONS IN THE INTERIOR WESTERN US

INTRODUCTION

Climate warming and associated increases in extent and frequency of high severity disturbance events will reshape the vegetation patterns seen on the landscape today (Millar and Stephenson, 2015), and are generally projected to result in poleward migrations and upward shifts in elevation of species ranges as they track changes in climate (Chen et al., 2011). Yet these climate change induced range shifts can manifest in several ways on the landscape. Expansion from the cool edge or contraction from the warm edge of species climatic niches can change niche margins, while shifts in abundance within a species' niche can occur without changing the cool or warm edge positions (Lenoir and Svenning, 2015). Species distribution modeling has largely relied on the assumption that species will maintain the same climatic niche in the future, thus necessitating their migration to sufficiently cooler areas as temperatures continue to warm (Malcolm et al., 2002; Pearson and Dawson, 2003). However, species ranges are not solely governed by temperature (Hargreaves *et al.*, 2014). Most studies have found that tree species ranges are contracting and that tree migrations are lagging behind those expected with climate warming, indicating that there are significant barriers to migration for trees (Gray and Hamann, 2013; Renwick and Rocca, 2015). This could be due to a lack of dispersal ability,

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or to microclimate buffering by persistent adult tree canopies preventing establishment of new species (Davis et al., 2019; De Frenne et al., 2013).

High severity disturbances can provide a catalyst for species range shifts by altering site and microsite conditions and reducing biotic competition for resources (Boisvert-Marsh and de Blois, 2021; Landhäusser et al., 2010). Variation in disturbance type and severity create unique environments that allow different types of colonizing species to establish (Buma and Wessman, 2012; Johnstone et al., 2016). For example, high severity fire eliminates biomass from the canopy and forest floor, creating high light, bare mineral soil environments that facilitate the establishment of well-dispersed shade-intolerant species with preference for seedbeds with little organic matter (Johnstone et al., 2010; Nigro et al., 2022). In contrast, tree mortality due to biotic agents, such as insects and pathogens, tends to be more species-specific and results in changes to canopy arrangement and complexity, while adding organic material to the forest floor (Atkins et al., 2020). Thus, the microenvironment created by insect or disease outbreaks may favor species that are more shade-tolerant and can germinate under thick litter layers, in contrast to those favored following high-severity fire or similar disturbance (Stevens-Rumann et al., 2015). Though recent work has highlighted the potential role of disturbances to catalyze range shifts of certain tree species (Hill and Field, 2021; Landhäusser et al., 2010; Nigro et al., 2022; Renwick et al., 2016), there is limited empirical data on how multiple disturbance events have influenced the magnitude and direction of tree species range shifts following recent warming at a continental-scale.

The goal of this study is to quantify the effects of disturbance events on range shifts of dominant tree species in the interior western United States using observational evidence from forested lands in the USDA Forest Service Forest Inventory and Analysis data. We compared climatic niches of adult trees and seedlings of the same species after fire, insect or disease outbreaks, and in undisturbed forests to determine whether dominant tree species are showing signals of range shifts, and whether these signals vary by disturbance type. We categorized FIA plots by the most recent disturbance experienced in the past 30 years and compared the climate of plots where seedlings were present (future niche) to that of plots where adults were present (current niche) for each species in each disturbance type. We represented variation in climate across plots in a 2-dimensional principal components analysis space, and delineated realized climatic niches for adults and seedlings using the positions in the principal components space of the plots where adults or seedlings of each species were present. We examined differences between climatic niche centers of adults versus their conspecific seedlings, as well as differences in the niche margins and overall niche size. We compared these differences across disturbance types, with the hypothesis that niche shifts would be larger in areas that experienced disturbances in the past 30 years compared to those without documented recent disturbance. We also evaluated how species traits influenced the amount of niche contraction or expansion experienced, with the hypothesis that shade-intolerant, wind dispersed species would expand more in disturbed areas than others.

METHODS

Forest Inventory and Analysis Plots

The USDA Forest Service Forest Inventory and Analysis (FIA) program surveys species composition of trees and seedlings across the United States each year using standard plot designs and data collection procedures (Burrill *et al.,* 2021). We focused on data from the Interior West Region of the United States (which includes ID, MT, WY, NV, UT, CO, AZ, and NM). Plots selected for analysis were all surveyed with the national standard plot design (Bechtold & Patterson, 2005; Burrill *et al.*, 2021), located on public land, and had no evidence of artificial regeneration (tree planting), resulting in 25,030 plots for analysis. The FIA program plots are meant to represent a 0.4-hectare sample area and consist of 4 circular subplots measuring 7.3 m in radius and arranged in a spoke pattern, with each plot spaced 36.6 m from the center plot. All trees greater than 12.7 cm in diameter at breast height (DBH) or diameter at root collar (DRC) are measured on each subplot. Within each subplot, all trees less than 12.7 cm in DBH/DRC are recorded within a microplot measuring 2.1 m in radius. Seedlings are defined as trees with a diameter less than 2.5 cm and at least 15.2 cm tall for conifers and 30.5 cm tall for hardwoods. The standard FIA protocol only collects tree and seedling data in "forested" plots, which must have had at least 10% tree cover now or before recent disturbance. We thus only included seedling and adult data from these "forested" FIA plots to ensure both adults and seedlings were surveyed in all plots analyzed.

Environmental data and principal components analysis

To examine the climatic niche of all plots in the analysis, we performed a principal components analysis (PCA) with eight key climatic variables extracted from ClimateWNA (Hamann et al. 2013): mean temperature of the coldest month (MCMT), number of frost-free degree days (NFFD), climatic moisture deficit (CMD), mean summer (May – Sept) precipitation (MSP), summer heat moisture index (SHM; calculated as mean temperature of the warmest month/mean summer precipitation/1000), winter precipitation (PPT_wt), precipitation as snow (PAS), and degree days below 0 degrees Celsius (DD_0). PCA proceeds from a correlation matrix with Pearson correlation coefficients, which relativizes all variables and assumes linear relationships between them (McCune & Grace, 2002). Therefore, the variables of PPT_wt, MSP, PAS, DD_0, and SHM were log-transformed to meet this assumption before analysis. The PCA was performed using the "prcomp" function in R version 4.1.3 (R Core Team, 2019).

Disturbance and species data

The disturbance history of each plot was compiled using FIA tree and condition data (Burrill *et al.*, 2021), LANDFIRE vegetation disturbance data (LANDFIRE, 2016) and Monitoring Trends in Burn Severity data (MTBS, 2018). When using the FIA tree data, we counted an FIA plot as disturbed if the FIA tree data indicated that it had 5 or more trees killed by one disturbance. The FIA condition data records disturbances (up to 6 per plot) if the disturbance was at least 0.4 hectares in size and damaged at least 25% of trees or area in the condition. The MTBS database included fires from 1984 – 2016 and the LANDFIRE vegetation disturbance database included all disturbances from 1999 – 2014 that were classified as medium to high severity. Plots were then categorized by the most recent disturbance experienced in 30 years prior to the most recent plot survey, which resulted in the groups: fire (n=3,875), insect/disease (n=4,409), harvest (n=464), wind (n=87), other (n=1,973), and undisturbed (n=14,222). If two or more disturbances were equally recent at a plot, the plot was assigned to the disturbance hypothesized to have the greatest ecological effects on the regeneration environment (fire > harvest > wind > insect/disease > other). In addition, all plots that burned in the past 10 years were included in the "fire" category, as recent burning is likely to control regeneration at a plot, regardless of subsequent disturbances. Both live and dead standing adult trees at each plot were counted in the presence/absence data to capture the adult presence prior to disturbance. If a plot had no disturbance detected in the 30 years prior to its most recent survey, then it was categorized as “undisturbed”. To have a robust sample size, only fire, insect/disease and undisturbed plots were analyzed in this study.

Species were chosen for this analysis based on their occurrence in the data; only species that had seedlings (which are less common than adults) present on at least 60 plots in two out of the three disturbance types were included. This resulted in fifteen species chosen for analysis, nine of which had over 60 plots in all disturbance categories and six of which were only analyzed for two of the disturbance categories (Table A1.1). In this study, the presence of adult trees (> 12.7 cm DBH) at a plot was considered the “current” forest species composition and the seedling (< 2.54 cm DBH) species composition data were considered the “future” forest composition, as seedling composition in the first 2-3 years post-disturbance has been found to be indicative of long-term establishment trends (e.g., Stevens-Rumann *et al.*, 2018). Plots surveyed from 1995 – 2018 were used for data on adult tree presence whereas only plots surveyed from 2009 – 2018 were used for data on seedling presence, to capture the most recent and youngest cohort of trees as an indication of future forest composition.

Shifts in niche centers

Here we defined the “climatic niche” as the 95% prediction interval around the set of points in two-dimensional PC space for adults and seedlings of each species in each disturbance type. Climatic niches were estimated by fitting Bayesian multivariate normal distributions to each unique species by age by disturbance combination using the function *siberMVN* in the package “siber” (Jackson et al., 2011), which uses the package “rjags” in R (Plummer et al., 2022). The model used in this function was:

$$y_i \sim multivariate\ normal(\boldsymbol{\mu}, \Sigma)$$

$$\boldsymbol{\mu} \sim normal(\boldsymbol{\mu_0}, \boldsymbol{\tau_0})$$

$$\Sigma \sim inverse\ Wishart(\Sigma_0, d)$$

Where $i = 1,\ldots,n$ with n being the sample size for each unique species by age by disturbance combination. The PC1 and PC2 values for each unique group were first z-score transformed. Since values in PC space were z-score transformed prior to model fitting, their modeled mean values should be close to zero and the modeled marginal variances should be close to 1; therefore, we assigned vague priors for the mean and variance parameters of the multivariate normal distribution (Jackson, 2021). The prior on $\boldsymbol{\mu}$ was normal with $\boldsymbol{\mu_0} = (0,0)$ and $\boldsymbol{\tau_0}$ (precision) = (0.001,0.001). An inverse-Wishart distribution with 2 degrees of freedom (d) and a 2x2 diagonal scale matrix (Σ_0) with 1's on the diagonals was used as the prior for the covariance matrix. The models were run for 10,000 iterations with the first 1,000 discarded as burn-in. Two chains were run, and the posterior distributions were thinned by 10. This resulted in 4,000 posterior estimates of $\boldsymbol{\mu}$ and Σ (the covariance matrix) for the multivariate normal distribution describing each species by age by disturbance combination. All trace plots of the MCMC output were examined to ensure that they had converged and were well-mixed after burn-in (Hobbs and Hooten, 2015). The 95% prediction ellipse (which contains 95% of the data) around the posterior estimates of each unique group was considered the climatic niche of that group (posterior estimates are plotted in Appendix 1).

We calculated the distance and angle between the centroids of the adult climatic niches and the seedling climatic niches for each disturbance type using the functions "siberCentroids" and "allCentroidVectors" in the R package "siber" (Jackson et al., 2011). We then took the median distance and angle for each and tested whether the distance and angle between centroids were significantly different ($\alpha = 0.05$) between different disturbance types using ANOVA, with either distance or angle as the response variable and disturbance type as the predictor variable. The response variables were log-transformed to meet the assumptions of ANOVA.

Shifts in niche margins

We investigated how both the niche margins and the niche centroid shifted across species and disturbance types to get a full picture of how species may be experiencing range shifts (i.e., are they expanding at the cool edge or contracting at the warm edge?). To investigate shifts in the niche margins, the posterior mean estimates of $\boldsymbol{\mu}$ and Σ for adults and seedlings of each species in each disturbance type were used to generate a new dataset with 5,000 observations using the function *mvrnorm* in R version 4.1.3 (R Core Team, 2022). The 2.5th and 97.5th quantiles for PC1 values and PC2 values of these new datasets were calculated to represent the edges of the 95% prediction ellipse. Centroids were estimated as described in the previous section.

The difference between adult and seedling centroids and niche margins on each principal component was calculated as follows:

$$Diff_{dqs} = \frac{(seedling_{dqs} - adult_{dqs})}{(adult_{d97.5s} - adult_{d2.5s})}$$

Where *d* represents the disturbance category, *q* represents the quantile of interest (2.5th, centroid, 97.5th), and *s* represents the species. The denominator of the fraction represents the adult niche range on that PC and was included so that differences in centroids and range margins between adults and seedlings were scaled to the adult niche size (as in Dobrowski et al., 2015), as we expect species with larger niches to be able to expand more than those with smaller niches.

We then used a one sample T-test or Wilcoxen signed rank test (for non-normal data) to test whether the average shift across all species for each quantile was significantly different from zero for each disturbance type (indicating a potential range shift across species). This involved eighteen (3 quantiles x 3 disturbance categories x 2 principal components) one sample, two-sided T-tests or Wilcoxon signed rank test where the data for each test consisted of the $Diff_{dqs}$ values for each species in each disturbance by quantile by principal component combination (n = 15 for undisturbed, n = 14 for insect/disease, and n = 8 for fire plots). Next, we compared shifts on each quantile across species between disturbance types using an ANOVA to evaluate whether climatic niche shifts differed under different recent disturbance histories. This involved six ANOVAs (3 quantiles x 2 principal components) in which the $Diff_{dqs}$ values within each disturbance category were compared, for every quantile x principal component combination. We only included the nine species that had data for all three disturbance categories in this test. All assumptions of ANOVA were met. If a significant p-value ($p < 0.05$) was found, a post-hoc Tukey HSD test was used to determine which pairwise comparisons were statistically significantly different.

Niche expansions and contractions

For each disturbance type, we determined the area of each adult niche, seedling niche, and overlap between the two using the function "bayesianOverlap" in the package "siber" (Jackson et al., 2011), which calculates the area within each 95% prediction ellipse and the area of overlap between seedling and adult niches for each disturbance type. This resulted in 4,000 estimates of each ellipse area and area of overlap, one for each of the posterior estimates. We then derived the amount of niche expansion and contraction for each estimate using the formulas:

$$expansion = \frac{A_s - A_o}{A_a}$$

$$contraction = \frac{A_a - A_o}{A_a}$$

Where A_s is the ellipse area of the seedling, A_a is the ellipse area of the adult, and A_o is the area of overlap between the two. This is similar to the concepts of “niche unfilling” and “expansion” used by Petitpierre *et al.* (2012) to describe invasive species niches but expresses expansion and contraction as proportions of the adult niche size, in order to scale the results across species with varying niche sizes. To calculate net niche expansion for each species and disturbance type, each estimate for niche contraction was subtracted from the corresponding estimate for niche expansion. We also found the 95% credible interval for these estimates by calculating the 2.5th and 97.5th quantiles of the net niche expansion values for each species in each disturbance type. The mean value of the posterior estimates for expansion, contraction, and net expansion were used in the following analyses.

A two-sample Wilcoxon test was performed to evaluate whether there was a significant difference between the amount of expansion and the amount of contraction across all species and disturbance types. For the remaining analyses, we only used the subset of nine species that had a large enough sample size of plots for inclusion of all three disturbance categories in the analysis. With these nine species, we compared expansion and contraction between disturbance types and sample sizes in separate ANCOVAs, with log-transformed expansion/contraction as the response variable and disturbance, sample size, and their interaction as the predictor variables. Interactions were examined with type-1 ANOVA. If no interaction was detected ($p > 0.05$), then the interaction term was removed, and significance of disturbance and sample size were assessed with type-2 ANOVA.

Shade tolerance scores for each species ($n = 9$) were taken from Niinemets & Valladares (2006), who defined shade tolerance as a plant’s potential for growth in the shade, or the minimum amount of light needed for growth. The shade tolerance scale ranges from 1 to 5, going from very intolerant to very tolerant, and roughly corresponds to these categories of percentage of full sunlight needed for growth: >50% (1), 25-50% (2), 10-25% (3), 5-10% (4), 2-5% (5) (Niinemets & Valladares, 2006; Table A1.1). The main dispersal mode (wind or animal) was determined for each species based on USDA Forest Service documentation (Burns & Honkala, 1990; Simonin, 2000). We also included the mean position on PC1 for each species as a trait to represent the general altitudinal/latitudinal range of the species (e.g., foothills vs. montane vs. subalpine species). Since climate moisture deficit (CMD) was strongly associated with PC1, we can infer that species with higher mean PC1 values live at higher elevations and latitudes (where it is colder and wetter) whereas species with lower PC1 values are lower elevation and latitude (where it is warmer and drier) species.

We ran six ANCOVA models to test whether shade tolerance, dispersal mode, or mean position on PC1 predict expansion and contraction in climatic niche. Each model contained the mean posterior estimate of expansion or contraction as the response variable. Disturbance category and either shade tolerance, dispersal mode, or mean PC1 position were included as fixed predictor variables, as well as the trait by disturbance interaction. Sample size (number of plots containing adults) for each category was also included as a fixed predictor variable and as an interacting effect with the trait variable to account for differences due to sample size in our analysis. Significance of interactions were evaluated with a type-1 ANOVA and if no interactions were significant, the interaction terms were removed from the model, and it was re-evaluated with a type-2 ANOVA. The corrected Akaike Information Criterion (AICc) for the model with sample size included was compared to that without sample size and the model with the lowest AICc was retained for final analyses. Based on this, all models predicting expansion retained the fixed effect of sample size, whereas none of the contraction models did.

RESULTS AND DISCUSSION

Climatic niche space

Variation in climate across the interior western US was well represented by the first two principal components of our principal components analysis (90.5% variation explained; Figure 1.1). PC1 explained most of the variance (74.4%) and was most correlated with climate moisture deficit (CMD; eigenvector coefficient = -0.40) and precipitation as snow (0.39). Therefore, FIA plots that fall toward the lower end of PC1 tend to be situated in hot and dry areas (high CMD), such as lower elevations and latitudes, whereas those on the high end are in cold and wet areas (low CMD), such as high elevations and latitudes. The second most explanatory axis of the interior west climate space (PC2) was most correlated with mean summer precipitation (-0.63) and summer heat moisture index (0.41). Therefore, most of the remaining variation in climate between plots is due to separation between hot/wet plots (lower PC2 values) and cold/dry plots (higher PC2 values).

Shifts in niche centers

To assess shifts in climatic niche center, a vector was drawn for each species in each disturbance type from the centroid of the adult climatic niche to the centroid of the seedling climatic niche. Shifts in centroid reveal how the probability of occurrence is shifting within the species' niche as a whole, whether due to expansions and contractions at the margins, or changes in density within the established niche (Lenoir and Svenning, 2015). Across all disturbances and species, seedling climatic niche centroids were shifted toward cooler areas than adult climatic

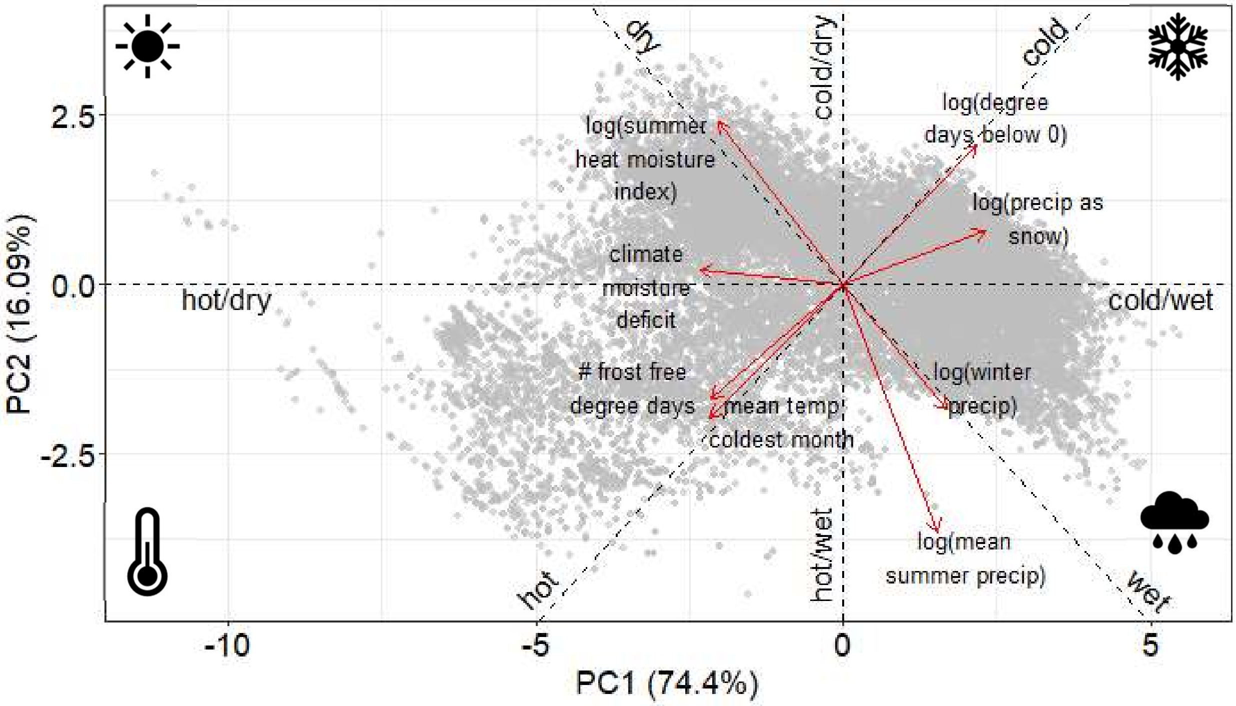

Figure 1.1. Climatic Niche Space. Biplot of principal components analysis (PCA) axes 1 and 2. PC1 explained 74.4% of the variance in climate variables and PC2 explained 16.09% of the variance. Climate variables are overlaid as red vectors on the plot indicating which climate variables are most correlated with which PC. Gray dots are plot scores for each FIA plot in the analysis (n = 25,030). Labelled dotted lines indicate the climate associated with each of the cardinal and ordinal directions on the plot.

niche centroids (Figure 1.2A). All species shifted away from hot and dry areas, with burned plots showing more species shifting to cold and wet areas and insect/disease plots showing more species shifting to cold, but dry areas, while species in undisturbed plots shifted to both wet and dry, cool areas (Figure 1.2B). Although not statistically different (ANOVA, F = 2.1, p = 0.15), this pattern may represent the different conditions that are produced by these disturbances. Whereas fires tend to increase runoff due to reduced vegetation and hydrophobic soil layers (Vieira et al., 2015), insect and disease outbreaks tend to increase soil moisture by reducing the number of trees taking up water and increasing coarse woody debris at the soil surface (Edburg et al., 2012; Morehouse et al., 2008). Therefore, shifts to dry areas may be facilitated after insect and disease outbreaks whereas post-fire regeneration is more likely to occur on sites that are wet. The main signal across all species and disturbance types was shifts away from hot areas; however, species are clearly responding in more nuanced ways to different precipitation environments, which aligns with other studies documenting precipitation-mediated range shifts (Crimmins et al., 2011; Fei et al., 2017; Serra-Diaz et al., 2016).

The distance that climatic niche centroids shifted did not significantly differ between disturbance types (Figure 1.2C), although disturbed areas did show slightly larger shifts than undisturbed areas. When shifts are considered separately along each principal component, however, we found that the difference between adult and seedling centroids was significantly different across disturbance types (Figure 1.3; PC1: ANOVA, $df = 2$, $F = 4.2$, $p = 0.03$; PC2: ANOVA, $df = 2$, $F= 3.5$, $p = 0.047$), and disturbed plots showed overall greater shifts than undisturbed plots (Figure 1.3). On PC1, species in both disturbance types showed significant shifts in centroid toward cool/wet areas and away from hot/dry areas whereas species' centroids did not shift in undisturbed areas (T-test, fire: $t = 4.8$, $p = 0.001$; insect.disease: $t = 3.0$, $p = 0.01$; none: $t = 1.7$, $p = 0.1$; Figure 1.3). On PC2, species in areas impacted by insect or disease showed centroid shifts away from hot/wet areas and toward cold/dry areas (T-test, $t = 4.6$, $p = 0.0005$), whereas the other two disturbance categories did not have significant shifts. The positive shifts in centroid observed in disturbed areas indicate that species ranges are shifting toward colder areas in these altered landscapes but does not indicate whether these changes are the result of expansions at the cool niche edge, contractions at the hot niche edge, or changes in density within the niche (e.g., Kelly and Goulden, 2008).

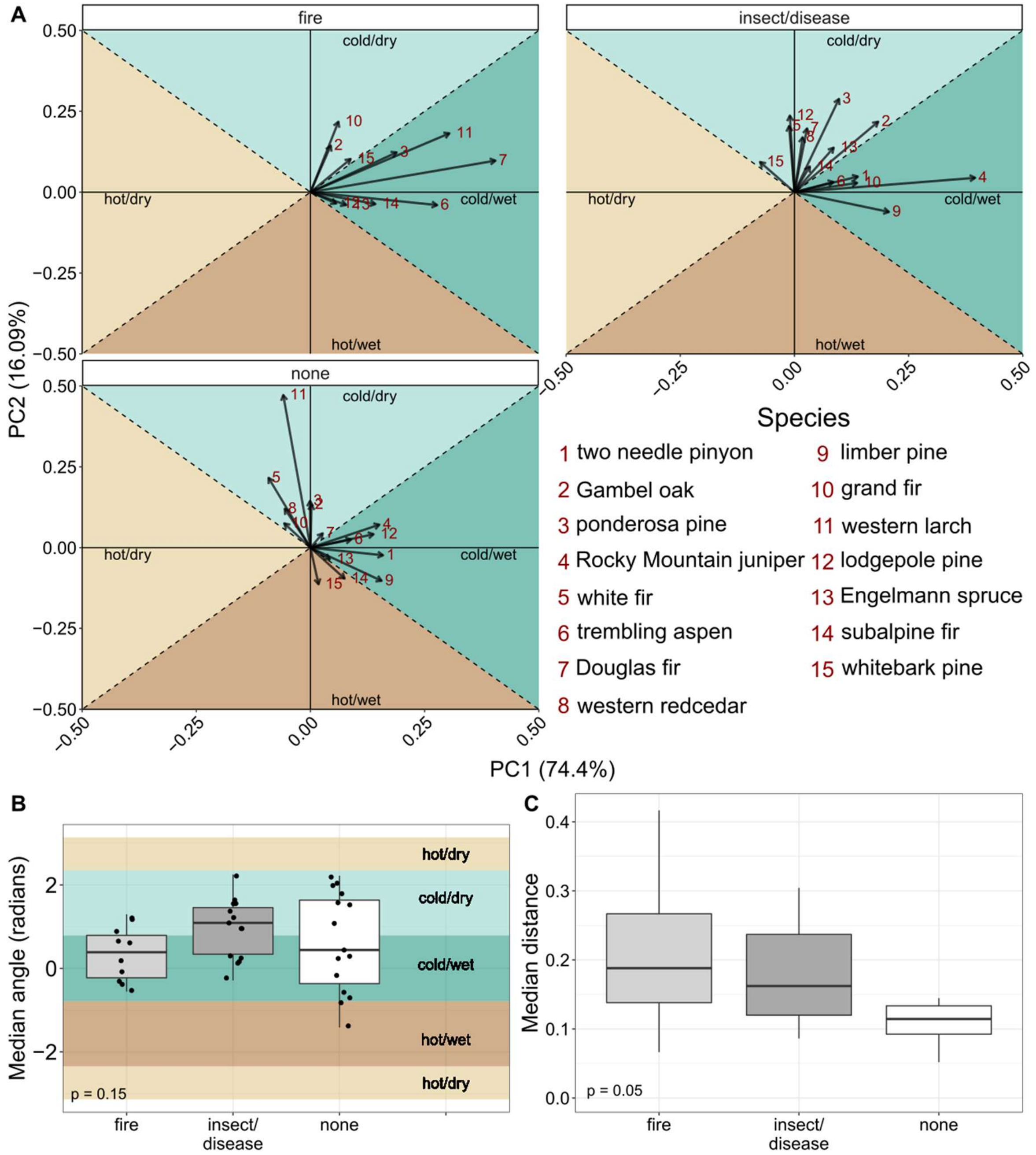

Figure 1.2. Shifts in Niche Centers. **A)** Median distance and direction between the centroid of adult (tails) and seedling niches (arrow heads) for each species (numbered) in each disturbance type (panel). Background shading corresponds to the four quadrants of the PCA biplot that represent hot/dry, hot/wet, cold/wet, and cold/dry conditions. **B)** Box plots showing the median angle and **C)** median distance between adult and seedling niche centroids in the three disturbance types analyzed. P-values from the ANOVAs comparing disturbance types are shown in the lower left of each figure.

Shifts in niche margins

We compared climatic niche margins (97.5th & 2.5th quantiles of each species' abundance along PC1 & PC2) between adults and seedlings to infer whether shifts in niche centers (Figure 1.2A) were due to expansions from the cool edge or contractions away from the hot edge of species niches. All niche centroid shifts toward cold areas were driven by contractions away from the hot niche margin, rather than expansions from the cold niche margin (Figure 1.3). For both PC1 and PC2, there were not significant shifts in the 97.5th quantile ($p > 0.07$), which represents the coolest range margin on both climate axes. However, there was a significant positive shift in the 2.5th quantile of PC1 for all disturbance types ($p < 0.05$; Figure 1.3), indicating that species are contracting away from hot and dry areas, but not migrating to cool and wet areas. Similarly, there was a positive significant shift on the 2.5th quantile of PC2 for plots in all disturbance types ($p < 0.006$). If species were migrating to new cooler areas due to climate warming, we would expect to see positive shifts on the 97.5th quantile, but we only saw positive shifts on the 2.5th quantile, indicating that tree species of the interior western US are failing to regenerate in the hot/dry and hot/wet regions of their range, and also failing to colonize areas cooler than their current climatic niche. The observed lack of expansion by treeline species may be artificially limited by data collection not occurring above treeline, but for all other species, the lack of cool-edge expansion is clear. Disturbances did not significantly differ in niche margin shifts, but burned areas seem to have the most potential for expansion at the cold/wet edge (95% confidence interval of [-0.004, 0.069]), compared to insect/disease (95% CI of [-0.05, 0.01]) and undisturbed areas (95% CI of [-0.04,0.01]).

The niche margin results contextualize the findings that niche centroids are shifting to cool areas by revealing that contractions away from hot areas dominate the climatic niche shifts of trees in the interior western US. This result makes it clear that examining the entire climatic niche, rather than just median position, is important in gaining a full picture of how a species may be shifting. For example, when looking at centroid shifts for each species (Figure 1.2), it appears that many species are shifting as expected toward cooler climates but examining shifts at the margins of climate space shows that mostly contractions away from the hottest climates, rather than expansions into cooler climates are driving these observed shifts (Figure 1.3). The combination of contractions at the hot edge, significant positive shifts in centroid, and no change in the cool edge for species in disturbed areas reveals that contractions are potentially more extreme following disturbance events, while expansions are limited. This is in accordance with the hypothesis that biotic interactions are more important in defining warm-edge limits, while abiotic conditions influence cool-edge limits more (Paquette and Hargreaves, 2021). Disturbances are clearly accelerating change at the warm-edge but are not adequately accelerating expansions beyond the cool-edge.

Field evidence for post-disturbance tree regeneration failure in hot and dry areas has been accumulating in the last several years across the western US (Coop et al., 2020; Davis et al., 2020; Stevens-Rumann and Morgan, 2019) and the lack of regeneration in cooler areas could be due to a variety of factors. First, it is reasonable to assume that contraction would happen faster than expansion in disturbed areas, since disturbances will kill individuals quickly, while new seedling establishment and regeneration will take much longer. Germination and survival of tree seedlings requires favorable weather, preferred microsites, and abundant seed to all coincide, and this can be rare at the margins of species niches (Kroiss and HilleRisLambers, 2015). Therefore, it is possible that not enough time has passed to see new tree establishment in cooler areas, especially for slow establishing species like whitebark pine and subalpine fir (Stevens-Rumann et al., 2018). Seedlings are also likely to have episodic establishment coinciding with preferred weather windows (Andrus et al., 2018; Brown and Wu, 2005), which may have not yet occurred in the time since disturbance. Tree seedling recruitment is often patchy (Astrup et al., 2008), which decreases the chance of FIA plots picking up all areas where seedlings are regenerating, especially given the small search area for seedlings in the FIA national standard plot design and the lack of plots above treeline (Burrill *et al.*, 2021). Despite these limitations, our current analysis implies that the 'extinction debt' (Dullinger et al., 2012) of the dominant tree species in the west is being 'paid,' but it remains to be seen whether regeneration can compensate for these losses.

Niche expansions and contractions

Seedling climatic niches were also evaluated for how much they contracted or expanded compared to the adult niche area. Expansion represents the area in climate space that is occupied by the seedling niche but was not previously occupied by the adult niche. In contrast, contraction represents the adult niche area that is no longer occupied by the seedling climatic niche. Despite differences in niche centroid shifts between disturbance types, the average amounts of niche expansion and contraction were not significantly different between disturbances (expansion: ANCOVA; $F = 1.7$, $p = 0.2$; contraction: ANCOVA; $F = 0.6$, $p = 0.6$). Across disturbance types, species experienced greater amounts of niche contraction than expansion (Wilcoxon signed rank test; $W = 174$, $p < 0.0001$; Figure 1.4). This is in accordance with other studies that have found ample evidence for range contractions of long-lived tree species (Bell et al., 2014; Renwick et al., 2016; Zhu et al., 2012). In addition, sample size had a significant effect on the amount of expansion, but not contraction, indicating that species with smaller sample sizes in the data

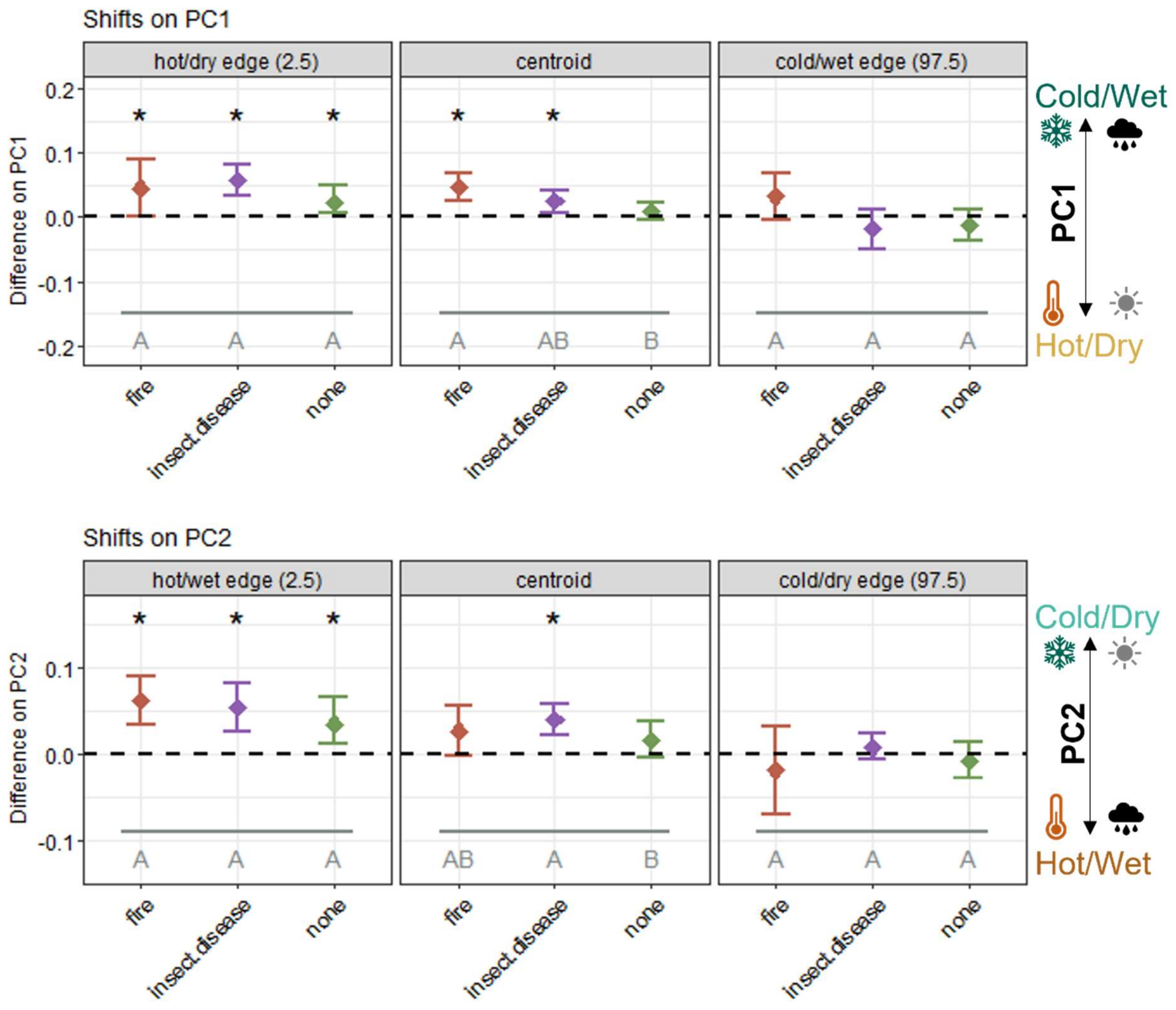

Figure 1.3. Shifts in Niche Margins. Diamonds show the sample estimate for mean (t-test) or pseudo-median (Wilcoxon signed rank test) difference between seedling and adult centroids and quantiles on PC1 (top) and PC2 (bottom) across all species analyzed (n = 15), within each disturbance type. Error bars show the 95% confidence interval associated with the sample estimate. Dotted horizontal line indicates where there is no difference between centroids or quantiles in adults vs. seedlings. Positive sample estimates (above the dotted line) for PC1 indicate that seedlings occupy cooler/wetter areas than adults. Positive sample estimates for PC2 indicate that seedlings occupy colder/drier areas than adults. Asterisks mark which shifts were significantly different from zero ($p<0.05$) and letters indicate if disturbance types were significantly different from each other (based on ANOVA), with different letters representing significant differences between disturbance types.

experienced greater expansions (ANCOVA; F = 12.3, p = 0.002). This is most likely an artifact of the analysis, as a 95% ellipse constructed with fewer data points will be more influenced by outliers. As such, expansions may be even rarer than this analysis suggests. This adds nuance to previous work documenting fire-catalyzed centroid shifts in tree species climatic ranges throughout the west (Hill and Field, 2021) – although disturbances are catalyzing shifts in climatic niche centroids, this is primarily driven by niche contractions. Species will need both contractions and expansions to remain within their ideal climatic niche as temperatures continue to warm, and there is little evidence to suggest that disturbances are catalyzing niche expansions on the large scale. However, the limited amount of seedling data in the FIA database may have obscured finer scale patterns in niche contractions and expansions, and therefore targeted field data collection is still needed to illuminate local level changes, especially in disturbed areas.

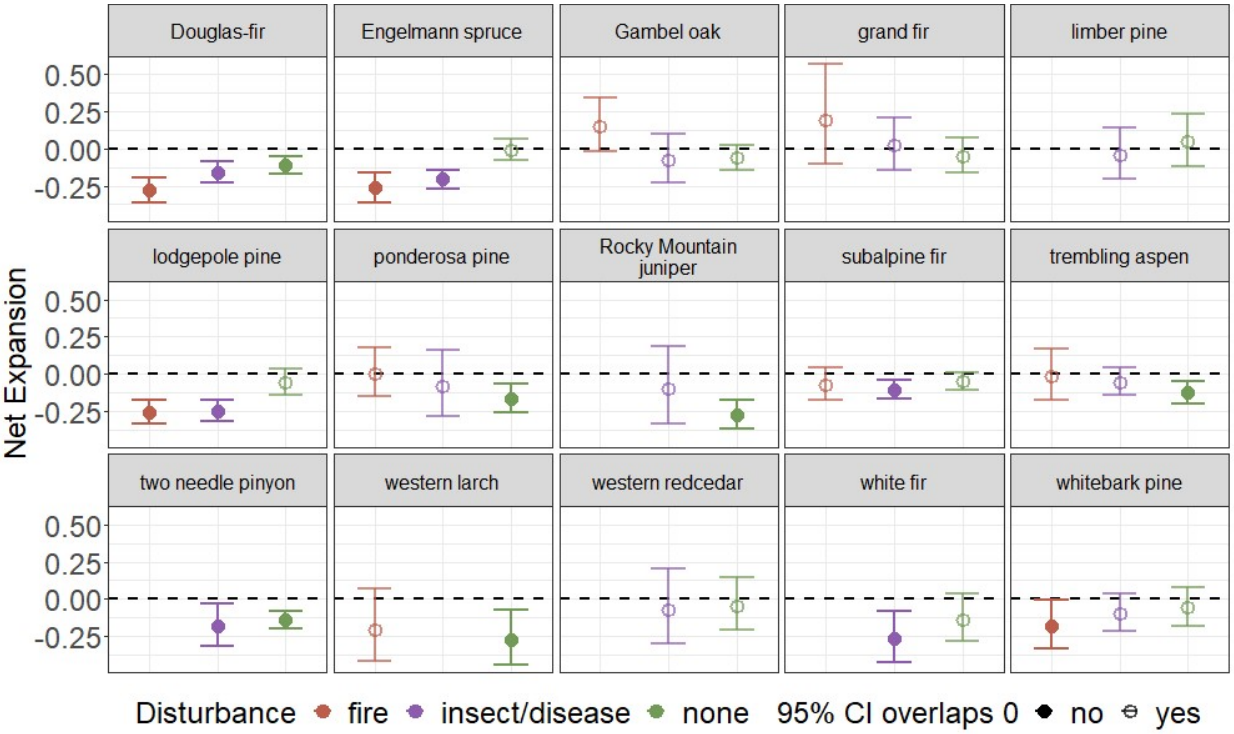

Figure 1.4. Net Niche Expansion. Net expansion (expansion minus contraction) of the seedling climatic niche relative to the adult climatic niche for each tree species. Points show the posterior mean net expansion estimate while error bars show 95% credible intervals around this estimate. The horizontal dashed line represents no net expansion. Estimates for which the 95% credible interval overlap 0 are partially transparent and represented with an open circle, while intervals that don't overlap 0 are opaque with closed circles. Positive values indicate more expansion of the seedling climatic niche than contraction (i.e., an overall niche expansion) whereas negative values indicate overall niche contraction.

Influence of species traits

Shade tolerance, dispersal mode, and their interactions with disturbance type did not significantly predict the amount of niche expansion or contraction by species in this study (Figure 1.5). The lack of interactive effects of traits and disturbance on range shifts was unexpected, as shade tolerance has been found to significantly affect seedling recruitment (Dobrowski et al., 2015) and dispersal distance has long been used as a predictor for migration potential (Aubin et al., 2016). However, germination and survival of many tree species is highly influenced by microtopographic variables and prior studies have shown that range shifts can be nuanced due to these local, small-scale conditions (Carnicer et al., 2021; Coop and Schoettle, 2009; Landhäusser et al., 2010). Our necessarily coarse disturbance attribution methods and climate data lacked the precision needed to detect trait and disturbance interactions, especially any due to microrefugia. The number of species in the traits analysis was also relatively small (n = 9) and patterns may be more apparent with the inclusion of more species. For example, undisturbed areas in our analysis showed slightly more expansion and less contraction for the most shade tolerant species (Figure 1.5), which would align with the hypothesis that shade tolerant species would be able to persist in and expand into undisturbed, intact forests better than species that need abundant light. The relatively small amounts of expansion evident among the dominant tree species of the western US may also mean it is too early to detect differences among species in expansion. As climate warming continues to accelerate and species are forced to migrate or perish, the influence of species traits may become more apparent.

The one factor that did influence climatic niche expansions and contractions was mean position on PC1 (i.e., habitat cold/wetness). Species that live in cooler and wetter areas (higher PC1 mean score) tended to expand less and contract more in burned areas than species that live

in hotter and drier climates (Figure 1.5). However, the positive trend in niche contraction for burned areas was mostly driven by the very small amount of contraction that Gambel oak experienced (Figure 1.5C). Therefore, habitat coldness/wetness seems to be most influential in predicting climatic niche expansion, with species that live in hotter and drier areas expanding their climatic niches more after disturbance than those that live in cool and wet areas.
Overall, this shows that higher elevation and latitude tree species are more at risk of niche contractions than species that live in lower elevations and latitudes, especially after disturbance events. This has been shown in other studies and attributed to the smaller amount of suitable climate area that exists at the tops of mountains and the fact that high elevation species are limited in how much higher they can migrate before the top of the mountain (Engler et al., 2011; Freeman et al., 2018). Climatic range expansions for cold-adapted trees may also represent tree-line advances in many areas, which can be limited by long processes like soil development and tree island establishment (Davis and Gedalof, 2018), and which our study was unable to assess.

Fire in particular may limit niche expansion in cold/wet environments, as the fire regime in these habitats includes mostly large, high severity fires that can eliminate all seed sources for new establishment, thereby increasing the time required for recovery (Turner et al., 1998). Given that expansion was limited for all species investigated in this study, the reduced expansion of cold-adapted species documented here could still be made up for in the future. The species in the coldest/wettest habitats in this analysis tend to take long to establish and grow slowly during relatively short growing seasons (Burns & Honkala, 1990; Stevens-Rumann et al., 2018). Therefore, there may not have been enough time for them to be detected in the FIA seedling surveys, which only record conifer seedlings if they are greater than 15 cm in height. Overall, the lack of correlation between species traits and niche contraction and expansion suggests that many interacting and overlapping factors are influential in tree establishment in new environments, and no one trait alone is the key to a species' migration potential.

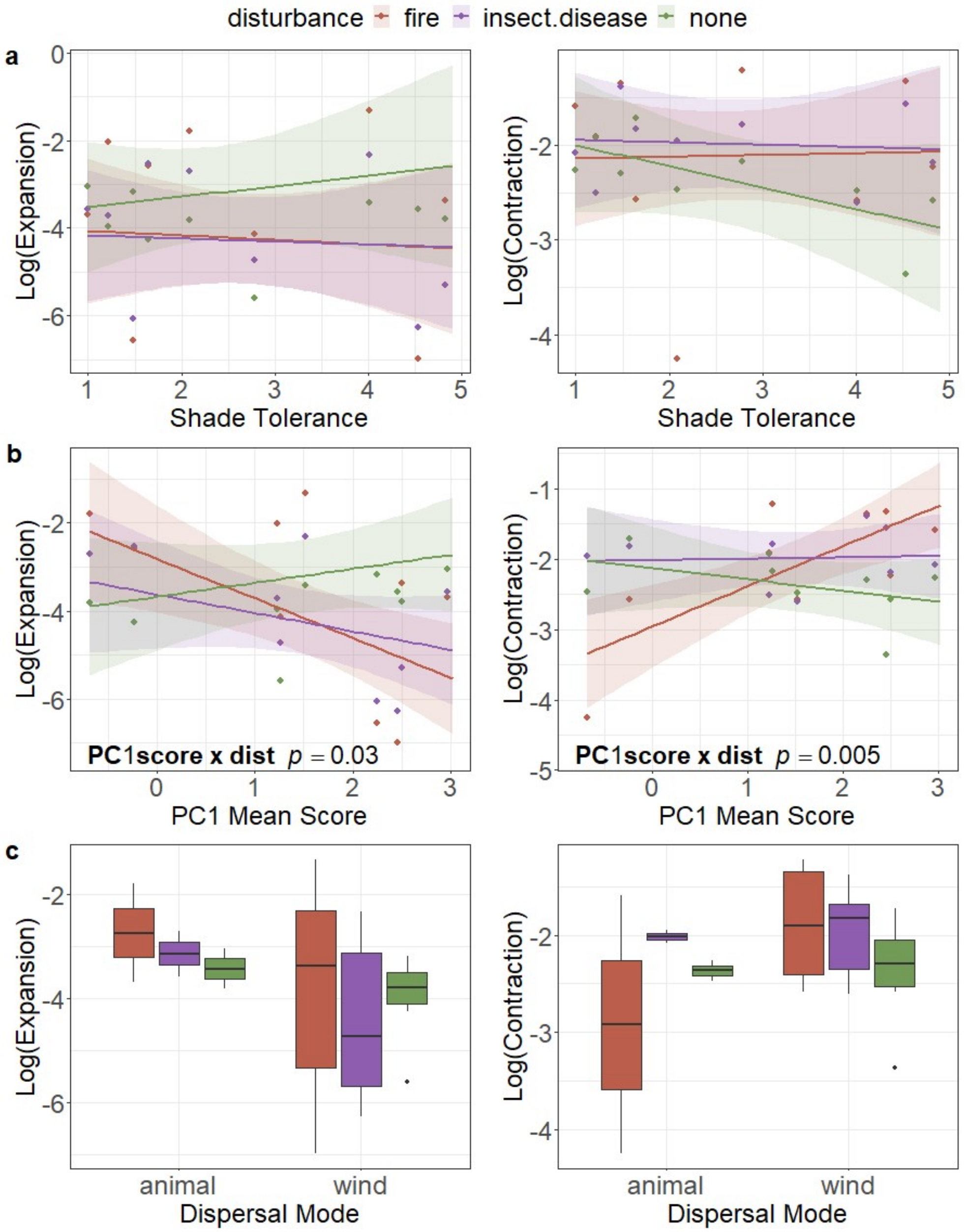

Figure 1.5. Effects of Traits on Niche Expansion. **A)** Effects of disturbance and shade tolerance, **B)** PC1 mean score, and **C)** dispersal mode on niche expansion (left) and contraction (right). Dots represent data and lines show linear model fit to the data, with shading representing 95% confidence intervals. All models of expansion included sample size as an additional fixed effect, which is held constant at its mean value for predictions in this plot. Lower PC1 mean score values represent species from hotter and drier climates whereas higher PC1 mean scores represent species from cooler and wetter climates. Model effects and their p-values are listed on the plots for variables that were statistically significant.

Conclusions

This study provides evidence that disturbance-facilitated niche shifts in trees of the western United States are currently driven by range contractions, rather than expansions. Most species are showing shifts away from hot areas, with greater shifts in places that have been disturbed. Despite the different microclimates that fire vs. insect and disease outbreaks create, tree species responded in similar ways to both. Regardless of disturbance type, niche contractions were dominant across species, with trends of positive net expansion present in only four out of 39 examined niche shifts (Figure 1.4). High elevation and latitude species are experiencing more contraction and less expansion than lower elevation and latitude species in disturbed areas, making them more at risk of local extirpation due to climate warming and increased disturbance frequency. Given that most of the recent disturbances in this study occurred within the last 20 years, the discouraging result that tree species are failing to regenerate at their warm climate edge and not expanding outside of their cool climate edge may be slightly premature if plots were surveyed too soon post-disturbance to capture regeneration that has yet to occur. However, targeted field studies have documented these same patterns (Renwick and Rocca, 2015) and climate warming only continues to accelerate. Therefore, trees may be ideal candidates for assisted migration programs that can help facilitate migration into new climate zones (Butt et al., 2021), especially for species whose ranges have already contracted significantly due to disturbance.

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CHAPTER 2

[2]WILDFIRE CATALYZES UPWARD RANGE EXPANSION OF TREMBLING ASPEN IN SOUTHERN ROCKY MOUNTAIN BEETLE-KILLED FORESTS

INTRODUCTION

Climate warming and associated increases in extent and frequency of high severity disturbance events will reshape the vegetation patterns seen on the landscape today (Millar & Stephenson, 2015). There is considerable evidence that changes in past climate prompted range shifts and genetic adaptation in plant species (Carter et al., 2017; Davis & Shaw, 2001); therefore, current and future increases in temperatures are expected to result in poleward migrations and upward shifts in elevation of species ranges as they track changes in climate (Chen, Hill, Ohlemüller, Roy, & Thomas, 2011; Harsch, Hulme, McGlone, & Duncan, 2009; Mamet, Brown, Trant, & Laroque, 2019). Evidence for recent upward migration of plants in North America and Europe in response to climate warming is extensive (Brusca et al., 2013; Chen et al.; Gottfried et al., 2012; Parolo & Rossi, 2008), though significant lags have been reported for long-lived woody species (Gray & Hamann, 2013; Lenoir, Gegout, Marquet, de Ruffray, & Brisse, 2008; Rees, et al., 2020; Renwick & Rocca, 2015).

Lags in elevational shifts of plants may occur because of geographic barriers (Davis & Shaw, 2001), biotic competition with resident plant communities (Brooker, 2006), lack of appropriate environmental conditions for seed production and/or germination (Brown et al.,

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[2] By Katherine M. Nigro, Monique E. Rocca, Mike A. Battaglia, Jonathan D. Coop, Miranda D. Redmond. Formatted for and published in *Journal of Biogeography.*

2019), and microrefugia or evolutionary adaptation allowing population persistence under seemingly unfavorable climate conditions (Alexander et al., 2018; Renwick & Rocca, 2015). Remnant populations that continue to persist as the climate becomes unfavorable can contribute to migration lags by maintaining environmental conditions that favor continued establishment of the remnant species, through competitive interactions and by reducing the availability of establishment microsites for newly colonizing species, especially those that are shade-intolerant (Eriksson, 2000; Svenning & Sandel, 2013). Long-lived tree species are especially likely to retain remnant populations as not only do they have long lifecycles, but adults are typically able to persist in a wider range of environmental conditions relative to seedlings, due to extensive carbon reserves and large root systems (Jackson, Betancourt, Booth, & Gray, 2009; Svenning & Sandel, 2013).

High-severity disturbances can remove some of these barriers to range shifts by reducing biotic competition, removing remnant populations, and altering abiotic conditions. Most forest disturbances cause adult tree mortality, which, when extensive, can eliminate the ability of the forest canopy to buffer the understory from temperature extremes (Arx, Pannatier, Thimonier, & Rebetez, 2013; Carlson, Sibold, & Negrón, 2021; Davis, Dobrowski, Holden, Higuera, & Abatzoglou, 2019). This is likely to result in the development of a plant community that is warmer adapted, as all regenerating species will be exposed to increasingly warmer temperatures under continued climate warming. In this way, inertia in tree species range shifts may be overcome rapidly, as the sudden removal of remnant tree populations produces novel climate conditions for regeneration and survival (Allen & Breshears, 1998; Dullinger et al., 2012; Lenoir & Svenning, 2015). Shade-intolerant species may require high-severity disturbance events at the leading edges of their ranges (the range margins expected to expand with climate warming) in order to overcome "establishment lags" and migrate with climate change (Alexander et al., 2018; Loehle, 2003). Observed and predicted increases in the extent and frequency of high-severity disturbance in the western US (Keyser & Westerling, 2019; Millar & Stephenson, 2015; Westerling, 2016) could therefore promote range expansion of shade-intolerant tree species that were previously unable to establish beneath closed forest canopies.

Variation in disturbance type, size and severity creates unique environments that allow different types of colonizing species to establish (Buma & Wessman, 2012). For a disturbance to catalyze a range shift of a particular species, it must create microsite conditions that align with the regeneration requirements of the migrating species and that species must have adequate dispersal capacity for propagules to reach areas within the disturbance footprint (Alexander et al., 2018). Therefore, disturbances that are large and cause abundant tree mortality are most likely to catalyze range shifts in species with high light requirements and widely dispersed seeds. Establishment of species that require reduced litter and organic layers for regeneration may be best promoted by wildfire, which can generate large expanses of bare mineral soil (Johnstone & Chapin, 2006). In contrast, disturbances such as insect outbreaks, disease, and drought can increase light and moisture availability but maintain or even increase dense litter layers adverse to the establishment of many species (Astrup, Coates, & Hall, 2008; Carlson, Sibold, & Negrón, 2020; DeRose & Long, 2010). Insect outbreaks are also often species specific such that only certain tree species are killed, whereas fires are more likely to cause mortality across species, resulting in different post-disturbance tree species composition. Accordingly, a mechanistic framework for severe wildfire to catalyze tree species range shifts is well developed; however, surprisingly few examples have been observed (Brice et al., 2020; Crausbay, Higuera, Sprugel, & Brubaker, 2017; Johnstone & Chapin, 2003; Wang et al., 2019), and we lack contrasts between the effects of fire and other disturbance types.

In this study, we assessed whether recent wildfires and bark beetle-caused tree mortality led to upward range expansion of trembling aspen (*Populus tremuloides* Michx., hereafter "aspen"), a widely distributed and ecologically important shade-intolerant species in North America. Aspen's life history traits suggest that range expansion at its leading edge may require a catalyst such as wildfire (Brown, Hansen, Keane, & Graumlich, 2006; Kulakowski, Veblen, & Drinkwater, 2004; Romme, Floyd-Hanna, Hanna, & Bartlett, 2001). Aspen's primary mode of reproduction is asexual, via resprouting from its roots in response to canopy mortality; however, it also has small, wind dispersed seeds and studies have documented aspen regeneration from seed after disturbance events (Fairweather, Rokala, & Mock, 2014; Kreider & Yocom, 2021; Landhäusser, Deshaies, & Lieffers, 2010; Quinn & Wu, 2001; Romme et al., 1997). Aspen seedling establishment is associated with moderate temperatures, high-light environments, thin soil organic matter layers, and microsites that capture moisture (Lafleur, Cazal, Leduc, & Bergeron, 2015; Landhäusser, Pinno, & Mock, 2019; McDonough, 1979; Schott, Karst, & Landhäusser, 2014). Thus, seedlings are most often found in severely burned areas, where these seedbed requirements and nurse objects (in the form of snags and down logs) are more prevalent (Coop & Schoettle 2009; Johnstone & Chapin, 2006; Turner, Romme, Reed, & Tuskan, 2003).

Bioclimatic modeling studies have projected an upward and northward shift in aspen's distribution (e.g., Gray & Hamann, 2013; Rehfeldt, Ferguson& Crookston, 2009; Worrall et al., 2013), yet few studies have documented upward range shifts (Landhäusser et al., 2010), especially in the context of multiple, high-severity disturbances. Through a field survey in burned and unburned beetle-killed forests in the southern Rocky Mountains, we sought to answer the following questions: 1) Is aspen's upper elevational limit expanding upslope, and does that vary as a function of disturbance type? and 2) What microsite conditions promote aspen upslope expansion within disturbances? We hypothesized that aspen has expanded upslope of its previous range limit via seed due to recent climate warming, that aspen seedling establishment at high elevations is greater in burned areas due to greater light levels and more bare mineral soil, and that aspen seedlings preferentially establish in microsites buffered by nurse objects, areas with less litter and less competing vegetation, and areas with higher light levels.

MATERIALS & METHODS

Study Area

Our study was conducted in the San Juan Mountains of southern Colorado, USA on the Divide Ranger District of the Rio Grande National Forest (Figure 2.1). The study area's mean annual temperature from 1981-2010 averaged 1.8°C and mean annual precipitation averaged 849 mm (PRISM Climate Group, 2012). Surveys were conducted between 3280 – 3770 meters in elevation. High elevation areas receive precipitation in the form of monsoonal rains from July – September and snow from October – April, with a short dry period from May – June, but precipitation can be highly variable from year to year (Goble, Doesken, & Joyce, 2016). Annual mean temperature in the study area has increased 0.8°C in the past 100 years, while annual minimum temperature has increased 1.8°C, and annual maximum temperature has experienced little change in the past century (National Climatic Data Center, 2020).

The study was focused within and around the Papoose and West Fork East fire scars which together burned approximately 32,954 hectares in 2013 as part of the lightning-ignited West Fork Fire Complex. The pre-fire forest composition was dominated by Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*), with a substantial aspen component occurring both as large stands spanning thousands of hectares and as small patches intermixed

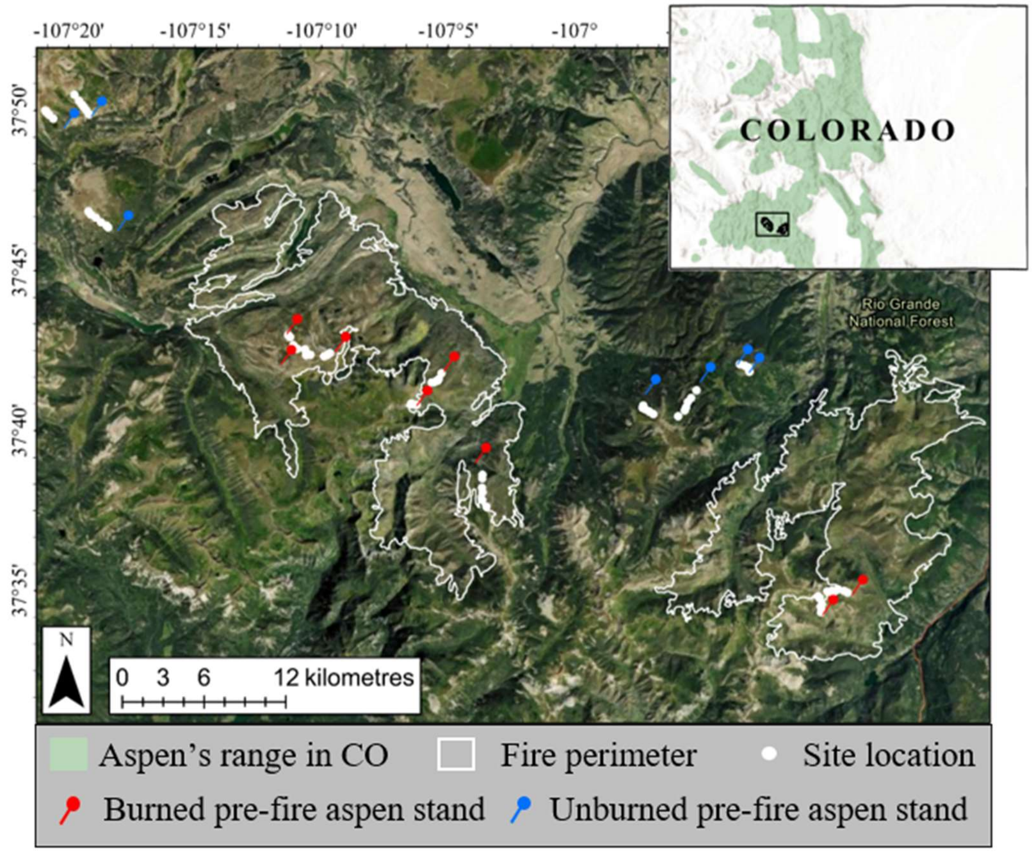

Figure 2.1. Map of the study area and aspen seedling survey locations in the Rio Grande National Forest, Colorado, USA. The rectangle on the locator map in the upper right corner encapsulates the West Fork East and Papoose fires (outlined in white), where the study was conducted. The main map displays the pre-fire aspen stands with a push pin symbol, where color denotes whether the stand was burned (red) or not (blue) in the 2013 West Fork Fire Complex. The satellite imagery displayed is the NAIP Imagery base map from ArcGIS Pro and the projection is Mercator Auxiliary Sphere.

within conifer forests. Areas above treeline are dominated by shrubland, dry tundra and grassland (LANDFIRE, 2012). The study area experienced extensive mortality of Engelmann spruce due to a spruce beetle (*Dendroctonus rufipennis*) outbreak, first detected in the area in 2004 (Carlson, Sibold, Assal, & Negrón, 2017). By the time of the fire, over 88% of the area that would burn contained large contiguous patches of beetle-killed trees and the adjacent unburned areas similarly had extensive spruce beetle-caused tree mortality (USDA Forest Service, 2013;

Savage, Lawrence, & Squires, 2017). The West Fork Fire Complex caused further tree mortality, resulting in many areas with no or very low overstory tree cover (Carlson et al.; Savage et al.). Aspen stands near the species upper elevational limit that existed before the West Fork Fire Complex (referred to hereafter as pre-fire aspen) were selected for sampling. These aspen stands likely established anywhere from 140 – 300 years ago, as pre-EuroAmerican settlement fire rotations in high-elevation spruce-fir forest types were centuries long (300 years or more) and studies from the area have dated the most recent large-scale stand-replacing fire to have occurred in the 1870's (Margolis, Swetnam, & Allen, 2007; Romme et al., 2001; Romme, Floyd, & Hanna, 2009; Sibold, Veblen, & González, 2006). Therefore, the pre-fire aspen stands in this study likely established when climate was more than 0.8°C cooler than the current average (National Climatic Data Center, 2020).

Determination of pre-fire upper elevational limit

To determine the upper elevational limit of aspen in the study area prior to the 2013 wildfires, high-resolution aerial imagery (ArcGIS World Imagery Basemap January 2014 release; 0.3-m resolution) was assessed to identify aspen presence/absence from 7,494 circular plots placed along 520 randomly generated upslope transects that spanned from 3200 m elevation to the highest mountaintops in the study region (see Appendix 2 for details). We tested the accuracy of our ability to visually determine aspen presence by field sampling a subset of these plots (n = 400) for aspen presence (at least one aspen tree within the plot). We determined our overall classification accuracy (total number of correctly classified plots divided by all plots) was 94%, with most classification errors being those of omission (see Appendix 2), likely due to the challenges of detecting smaller, subdominant canopy aspen. Overall, the high classification accuracy indicates that our visual assessment of aerial imagery was effective at detecting aspen presence but was limited to canopy-dominant or co-dominant aspen stands.

The upper elevational limit of pre-fire adult aspen in the study area was calculated in two ways. First, using all sample plots containing aspen, the "upper inner fence" of the data was calculated as:

Q3 (third quartile elevation) + 1.5 x IQR (interquartile range)

to capture the upper limit of the data without including mild outliers (Ott & Longnecker, 2015). Using this approach, we determined that prior to the 2013 wildfires, the elevational limit of aspen in our study area was 3506 m, and only 10 plots containing aspen occurred above this limit (99th quantile). Second, the maximum elevation where adult aspen were identified (3568 m) was used as a more liberal estimate of the pre-fire upper limit.

Seedling Surveys

To assess whether aspen upslope expansion has occurred following fire across our study area, we field-sampled a total of 47 burned and 41 unburned sites across eight burned and seven unburned elevational transects (Figure 2.1). Although the unburned sites did not experience mortality from the West Fork Fire Complex, all were impacted by the spruce beetle outbreak of the 2000's and therefore still experienced severe canopy mortality before our survey. From the aerial imagery analysis, we identified locations where aspen existed at or near its conservative upper limit (3506 m) prior to the fire. From the locations of each of these aspen stands, an elevational transect was established running upslope to the top of the mountain or a total increase of 240 m in elevation, whichever occurred first, maintaining the same aspect. The first site on each elevational transect was established immediately upslope and adjacent to the pre-fire aspen stand, though one of these first sites was determined to be hazardous and therefore excluded from analysis. Subsequent sites were established every 40 m of elevation gain on the transects, which translated to 82 – 1,226 m of horizontal distance on the ground. Minor adjustments to this protocol were necessary on a few sites to avoid hazardous conditions or to keep the elevational transect in its intended burn category. Slope, aspect, elevation, latitude, and longitude were recorded for each site in the field. Linear distance to the nearest live adult aspen stand was calculated in ArcMap using post-fire (2016 & 2018) aerial imagery to find the nearest stands. At each site, belt transects measuring 50-m long by 8-m wide were established horizontally across the slope and searched for aspen juveniles (<1.4-m tall). The exception to this was at the pre-fire aspen stand sites (the lowest site on each elevational transect), where variable-width belt transects measuring 50 m in length were established. These belt transects were a minimum of 0.5-m wide and were widened by 0.5 m until at least 14 aspen juveniles were found, or a maximum width of 8 m was surveyed. This was done to account for the large number of aspen suckers at these pre-fire aspen-dominated sites.

For each aspen juvenile found at a site, we recorded whether it was a seedling or sucker by gently excavating the roots. Aspen suckers sprout from lateral roots that can usually be found within 10 cm of the soil surface and can extend up to 30 meters away from the parent stem (Day, 1944; USDA, NRCS, 2020). Therefore, the presence of a lateral root within 10 cm of the soil surface on a juvenile surrounded by other aspen juveniles indicated sucker status. Seedlings were characterized by multiple fine roots with no thick lateral root and generally occurred in isolation away from clumps of other aspen juveniles (as in Fairweather et al., 2014). In addition, one to five of the aspen seedlings found at the sites were excavated across each elevational transect and were confirmed to have a branching, fine root system characteristic of aspen established from seed (Day, 1944). The height and diameter at soil surface were measured for each juvenile encountered, and any browse damage was noted. Microsite characteristics within 5 cm of the rooting location were recorded for each juvenile, including the presence or absence of a nurse object (defined here as rocks and logs > 5-cm diameter), the substrate type, the presence and depth of litter, and the presence or absence of vegetation. Canopy cover above the juvenile was measured with a spherical densiometer.

Across the 50-m length of the belt transect, the line point-intercept method was used to quantify the abundance of nurse objects, substrates, litter, understory vegetation, and canopy cover at the site. A pin flag was dropped every 0.5 m and all vegetation touching the pin flag (or touching the imaginary line ascending from the pin flag) was recorded according to functional group (grass, forb, shrub, tree) and status (live or dead). If multiple species of the same functional group touched the pin, the functional group was only recorded once for that point. Substrate type (soil, duff, boulder – rocks > 20-cm diameter and not easily lifted, or coarse woody debris (CWD) – wood > 20-cm diameter and not easily lifted), and presence of rocks (> 5-cm diameter and easily lifted), wood (> 5-mm diameter and easily lifted) and litter at each point was also recorded. The percent cover of all vegetation, substrate types and nurse objects at each site was calculated by dividing the number of points where the vegetation/substrate was recorded by the total number of points sampled (n = 100) and multiplying this by 100. A point classified as "bare soil" in the analysis had to have a substrate of soil and no litter, duff, moss, rock, or wood present at the point. Stand structure was additionally measured in three 100-m^2 circular plots (5.64-m radius) centered on the middle of the belt transect at 7 m, 25 m, and 43 m. The diameter at breast height (DBH), height, and status (live or dead) was recorded for each tree within the circular plot.

Statistical Analyses – Upslope expansion and fire effects

Aspen upslope expansion was evaluated at local and regional levels. Local expansion was indicated by aspen seedlings growing above the pre-fire aspen stand identified on each elevational transect. The elevation of the pre-fire aspen stand on each elevational transect was subtracted from the maximum elevation where aspen seedlings were found on that elevational transect to calculate local expansion. The difference between the pre-fire adult aspen upper elevational limit and post-fire seedling elevational limit across the study area was used to quantify regional upward expansion. The upper inner fence of the elevations where aspen seedlings were found in the field was 3740 m and exceeded the maximum recorded elevation of seedlings in the field due to the small number of observations (n = 15) and lack of outliers. More seedling observations would be needed to determine if this is an appropriate estimate of seedling upper elevational limit; therefore, we used a more realistic estimate of the seedling upper limit (the actual maximum recorded elevation) to quantify regional expansion. Due to the retrospective nature of this study and limited satellite imagery resolution, the upper limit of aspen juveniles prior to the fire in burned areas is unknown; however, if seedlings did exist above the pre-fire adult elevational limit before the fire, they would represent the same generation of aspen reproduction as the seedlings documented in this study (only 6 years post-fire) and would be picked up in our surveys of unburned areas.

Linear mixed effects models (LMMs) were used to assess the effect of disturbance type (burned/beetle-impacted vs. unburned/beetle-impacted) on several site characteristics hypothesized to influence aspen seedling establishment. Only data from sites surveyed above the pre-fire aspen stands were used to characterize the upslope environment under burned and unburned beetle-impacted conditions. The predictor variable in all models was burn category, represented as a fixed effect. Elevational transect was included as a random intercept to account for clustering among sites within each elevational transect and to account for differences due to position within the burned landscape. The response variables tested were distance to nearest seed source, % canopy cover, % duff, % bare soil, % CWD, and % understory vegetation. Assumptions of linear regression were checked for all models before analysis. Percent CWD, duff, and bare soil were log-transformed before analysis to meet assumptions of normality. Percent dead trees from the stand structure plots and live Engelmann spruce canopy cover (from Savage et al. (2017) data) were calculated for unburned/beetle-impacted and burned sites with trees to compare canopy loss due to spruce beetle alone vs. beetle-kill and wildfire. All models were performed in the 'lme4' package in R version 3.6.1 (Bates, Mächler, Bolker, & Walker, 2014).

A generalized linear mixed effects model (GLMM) was used to assess the influence of site characteristics on aspen seedling abundance. No seedlings were found in unburned/beetle-impacted sites; therefore, we analyzed burned sites above the pre-fire aspen stands only. The number of aspen seedlings encountered in the 50 x 8-meter belt was used as the response variable and potential predictor variables included distance to nearest seed source, % bare soil, % CWD, % understory vegetation, heat load index, elevation, and burn severity at the site. Percent canopy cover and % duff were not considered for predictor variables in the GLMM because there was little variation between burned sites in these variables and the GLMM was aimed at teasing apart the factors driving variable aspen seedling abundance among burned sites. Heat load index was calculated following Lutz, Wagtendonk, & Franklin (2010) (adapted from McCune & Keon, 2002) and integrates latitude, slope, and aspect into a unitless index of solar radiation at a site. Burn severity was represented by the relativized differenced Normalized Burn Ratio (RdNBR) (Miller & Thode, 2007) using data obtained from Monitoring Trends in Burn Severity (MTBS Project, 2018; Eidenshink et al., 2007). All predictor variables were centered (mean subtracted from value) and scaled (centered value divided by its standard deviation) to ease model convergence and comparison between variables. Elevational transect was included as a random intercept. The model was fit to the seedling count data with a Poisson error structure. We performed parametric bootstrapping to generate 1,000 new datasets based on the model to assess model fit. The true number of zero responses in the data were captured by the simulated datasets, indicating no zero-inflation. The sum of squared Pearson residuals from the bootstrap samples also captured the sum of squared residuals from the actual data, indicating that there was no overdispersion and the Poisson error structure was appropriate (Harrison et al., 2018). We therefore used a GLMM with Poisson error structure using the 'lme4' package in R (Bates et al., 2014). We performed variable selection for this model by comparing all subsets of models with the five potential predictor variables listed above that we hypothesized would be most important in driving aspen seedling abundance and selected the final model as the one with the lowest AICc.

Statistical Analyses – Microsite association of aspen seedlings

Interaction index (I), as described in Armas, Ordiales, & Pugnaire (2004), was used to measure aspen seedling associations with bare soil, nearby vegetation, and nurse objects:

$$\mathrm{I} = \frac{D_m - D_0}{D_m + D_0}$$

Where D_m is the density of seedlings using the microsite of interest and D_0 is the density of seedlings not using the microsite of interest. This index was used due to its robust statistical properties (Armas et al. 2004) and its previous use in assessing microsite facilitation of seedlings (Redmond, Weisberg, Cobb, Clifford, & Barger 2018; Redmond & Barger, 2013). Density was calculated as the number of seedlings in a certain microsite divided by the available area of that microsite within the belt transect (as measured using the line point-intercept method). Values of I range from -1 to +1, with negative values indicating a negative association with the microsite and positive values indicating a positive association. Values close to zero indicate no association. Sites were only analyzed if the expected number of seedlings established in the microsite of interest by chance (proportion of microsite in the belt transect x number of aspen seedlings found) equaled or exceeded 0.9. This cutoff was used to ensure that the interaction index reflected actual association of microsites with seedlings rather than just a lack of availability of that microsite on the belt transect (e.g., Redmond & Barger, 2013). This allowed us to analyze 11 sites for bare soil association, 11 sites for vegetation association, and 10 sites for nurse object association. Bare soil included burned and unburned bare mineral soil with no litter, duff, moss, rock or wood presence, nurse objects included both rocks and CWD greater than 5-cm diameter, and nearby vegetation included both understory grasses and forbs in this analysis (shrubs were not found near aspen seedlings at any sites). Assumptions of normality were checked for all variables and two-way, one-sample t-tests were used to evaluate whether interaction index values for bare soil, vegetation and nurse objects were significantly different than zero ($P < 0.05$).

We expected nurse objects to facilitate seedling establishment more at climatically extreme sites (e.g., Conver, Yarwood, Hetherington, & Swann, 2020; Drezner, 2007); therefore, the interaction index for nurse object association at each site was used as a response variable in a multiple regression model (as in Redmond et al., 2018) to investigate the influence of topographic variables on nurse association. The interaction index used here is linear and continuous within its range and is therefore safe to use in statistical operations (Armas et al., 2004). Heat load index, elevation and their interaction were considered as predictor variables for the model. Model selection was performed by evaluating AICc of models including all subsets of the predictor variables, using the 'dredge' function in the 'MuMin' package in R (Barton, 2019). The model with the lowest AICc value was selected and used for inference.

RESULTS

Disturbance effects on upslope expansion

We found evidence for upslope aspen expansion via aspen seedling establishment; notably, this expansion only occurred in beetle-impacted sites that burned (Figure 2.2). In total, 80 aspen seedlings were found on 15 upper elevation sites within burned areas. Average seedling density across these sites was 133 (± 24.7 SE) seedlings per hectare. Aspen suckers were encountered only on the pre-fire aspen sites, averaging 4400 (± 1539 SE) suckers per hectare on burned/beetle-impacted sites and 6443 (± 2437 SE) on unburned/beetle-impacted sites. The elevation difference between the pre-fire adult aspen clone at a site and the highest seedling found at that site (local expansion) ranged from 73 – 395 vertical meters (Figure 2.2). The highest aspen seedling found was at 3678 m on an east facing slope (Figure 2.3), which was 172 m higher than the conservative pre-fire regional elevational limit of 3506 m, and 110 m higher than the more liberally estimated pre-fire regional limit of 3568 m.

Relative to unburned/beetle-impacted sites, burned/beetle-impacted sites above the pre-fire aspen stands had longer distances to nearest live adult aspen (df = 13.2, t = -3.1, $P = 0.008$), less canopy (df = 13.6, t = 2.4, $P = 0.029$), understory vegetation (df = 13.2, t = 3.0, $P = 0.011$), and duff cover (df = 13.6, t = 3.4, $P = 0.005$), and more bare soil cover (df = 12.2, t = -2.4, $P =$

0.03) (Figure 2.4). Percent CWD was not significantly different between burned and unburned

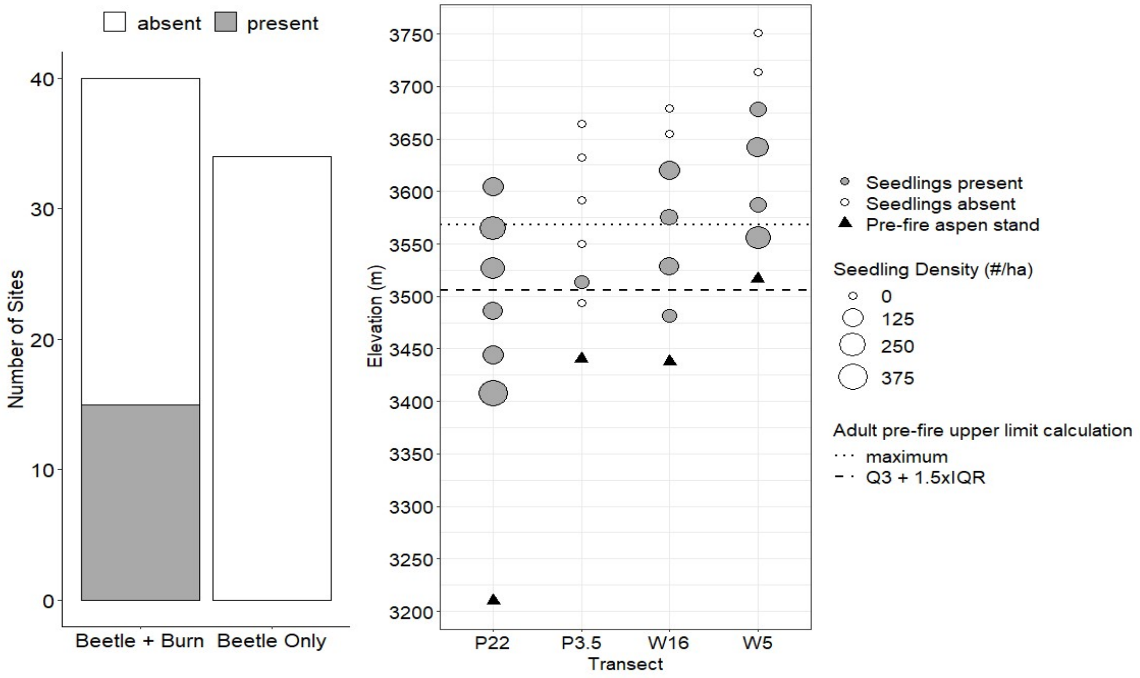

Figure 2.2. Left panel: Number of sites where aspen seedlings were found (gray) vs. not found (white) for beetle-impacted and burned vs. beetle-impacted only (unburned) sites in the San Juan Mountains, CO, USA. Right panel: Aspen seedling presence and density (# of seedlings per hectare) at sites surveyed on all elevational transects where aspen seedlings were found. Black triangles represent the pre-fire aspen stands on the elevational transect and the circles represent all sites surveyed on each elevational transect where color indicates aspen seedling presence (gray) or absence (white) and size indicates the density of aspen seedlings found at each site. Horizontal lines show the two estimates of the pre-fire adult aspen upper limit calculated in the aerial imagery analysis: maximum elevation (dotted) and Q3 + 1.5 x IQR (dashed).

Figure 2.3. Photo of the highest aspen seedling found in the field survey (top left) and the burned site where it was found (3678 m in elevation) in the San Juan Mountains, CO, USA.

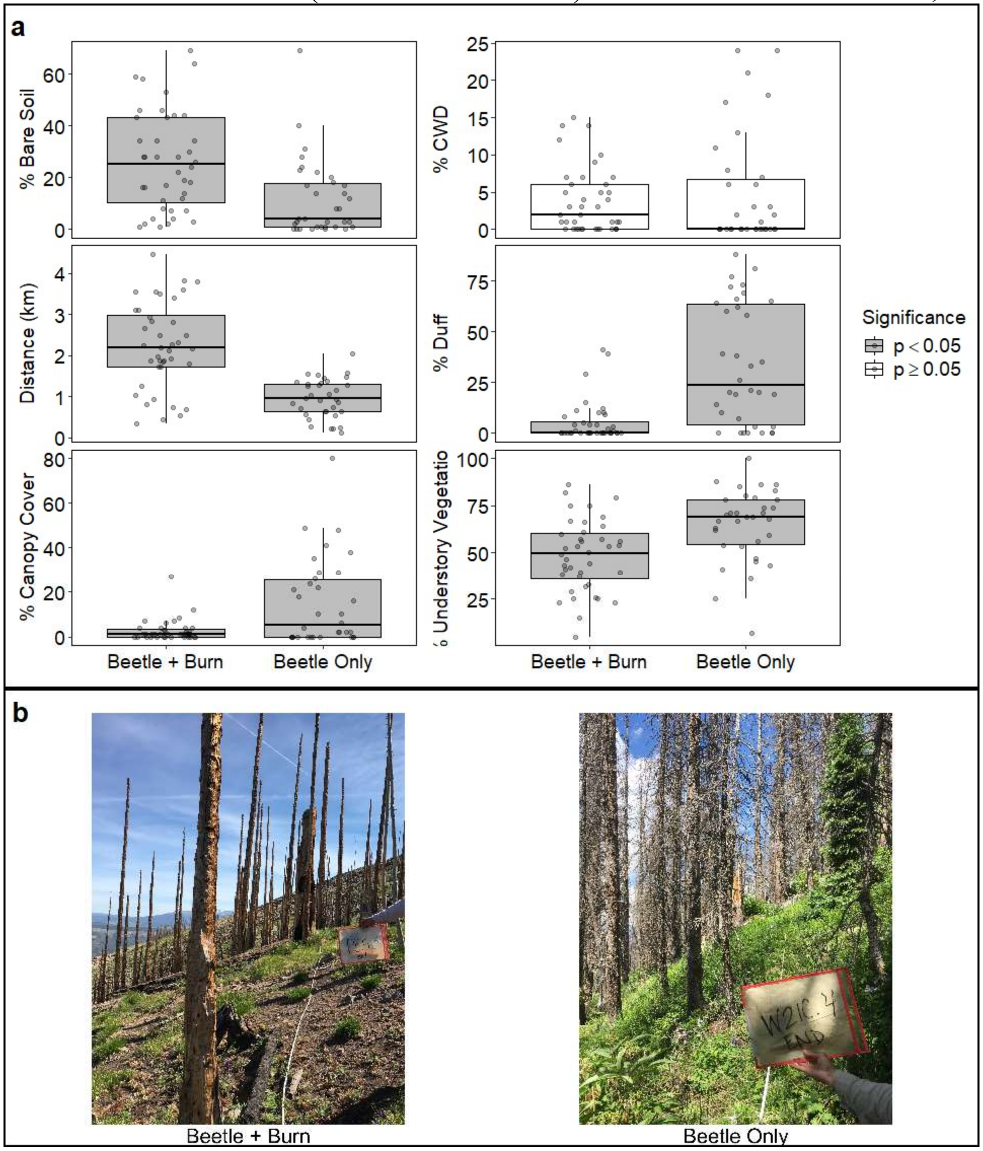

Figure 2.4. Differences in site characteristics between beetle-impacted and burned and beetle-impacted only (unburned) sites in the San Juan Mountains, CO, USA. A) Box plots shown in grey (% bare soil, distance to seed source, % duff, % canopy cover and % understory vegetation) are significantly different ($P < 0.05$) between the site types. Boxes bound the first and third quartiles of the data and the horizontal line indicates the median. The vertical lines indicate the lowest and highest observations within 1.5 x IQR (inter-quartile range) of the first and third quartiles. Data for each site is shown in points; 40 burned sites and 34 unburned sites were evaluated. B) Photograph of a beetle-impacted and burned site (left) and a beetle-impacted only site (right).

beetle-impacted sites (*P* > 0.9). Mean live Engelmann spruce cover, as computed in Savage et al.

(2017) for plots with trees, was only 5.2% (± 0.4% SE) in unburned/beetle-impacted sites and averaged 0.8% (± 0.3% SE) in burned/beetle-impacted sites. Percentage of dead trees of all species, as measured in the stand-structure plots, averaged 46.4% (± 0.05% SE) in the unburned/beetle-impacted sites and 95.7% (± 0.02%) in the burned/beetle-impacted sites, while six unburned and three burned beetle-impacted sites contained no trees. All site characteristics were uncorrelated to distance to the fire perimeter, indicating that edge effects likely did not influence the results.

Along the burned elevational transects, upslope aspen expansion (i.e., aspen seedling establishment) was associated with CWD and elevation. There were five models with delta AICc values less than 2 (see Table A2.1 in Appendix 2), and the model with the lowest AICc included elevational transect as a random intercept and elevation, % CWD and distance to seed source as predictor variables, of which only elevation and % CWD were significant ($P < 0.05$). Elevation was negatively associated with seedling abundance, whereas % CWD positively predicted seedling abundance (Figure 2.5). Notably, the standard deviation in log-seedling abundance among elevational transects (2.9) was larger than the effects of elevation and % CWD, indicating high spatial variability in seedling abundance.

Microsite association of aspen seedlings

Aspen seedlings found across all sites occurred under an average canopy density of 6.8% (± 0.67 SE) with an average litter depth of 0.79 cm (± 0.13 SE). Fifty-two percent of the seedlings were nursed, 70% were within 5 cm of a forb or grass and 76% were browsed. Seedlings showed a positive association with microsites near understory vegetation (mean interaction index ± 1 SE = 0.35 ± 0.15; t = 2.4, df = 10, $P = 0.036$), with 9 out of 11 sites analyzed having a positive interaction index (Figure 2.6). Seedlings showed no association with

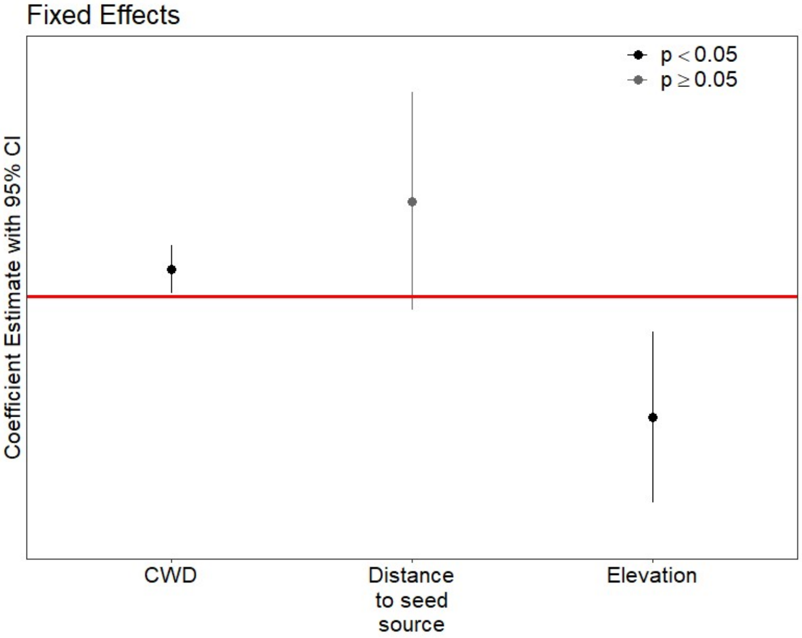

Figure 2.5. Results of the GLMM used to assess the effects of site characteristics on aspen seedling abundance in the San Juan Mountains, CO, USA. Elevation, % coarse woody debris (CWD) and distance to seed source were included as predictor variables in the model with elevational transect as a random effect. Points show the coefficient estimate of each fixed effect in the model and lines represent the 95% confidence interval for those effects. The red horizontal line is centered on zero; coefficients with lines that overlap zero are considered non-significant (gray dots), whereas those that don't overlap zero are considered significant (black dots).

bare soil (mean interaction index ± 1 SE = 0.15 ± 0.17; t = 0.92, df =10, *P* = 0.38; Figure 2.6).

Association with nurse objects (i.e., logs and rocks) strongly varied among sites (mean interaction index ± 1 SE = 0.16 ± 0.17), and a two-way t-test revealed no significant difference from zero (t=0.93, df=9, *P* = 0.38), indicating that seedlings did not have an overall association with nurse objects (Figure 2.6). This variability in nurse object association is likely driven by variability in local climatic conditions: both heat load index and elevation were significant predictors of nurse interaction index (t = -2.7, *P* = 0.029; t = 3.2, *P* = 0.014, respectively) with heat load exhibiting a negative effect and elevation exhibiting a positive effect on nurse interaction index (Figure 2.7). This implies that nurse objects are more beneficial at high

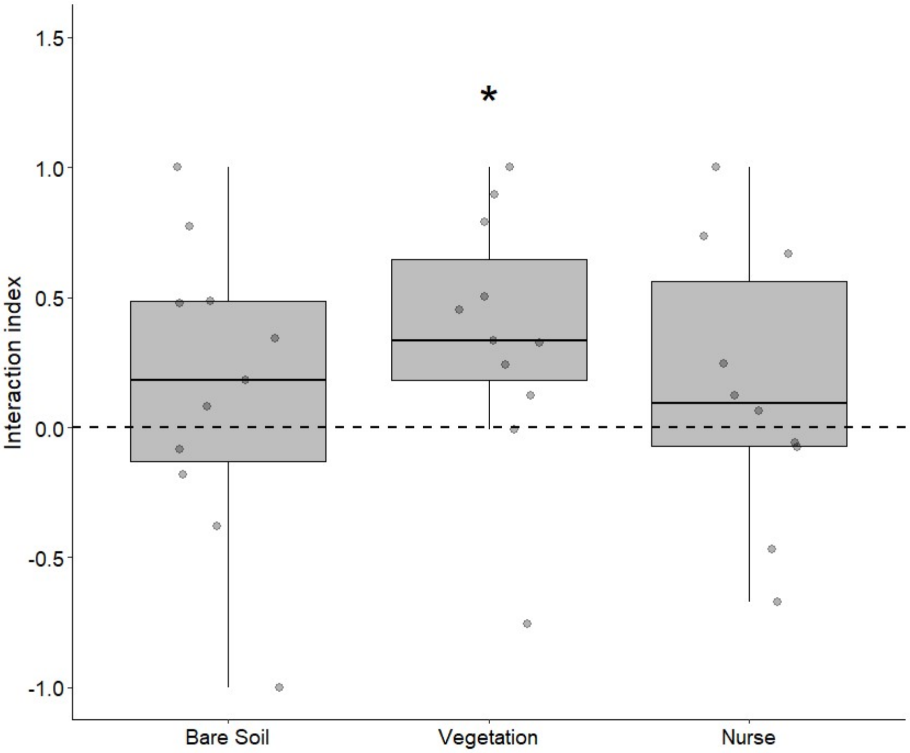

Figure 2.6. Interaction indices between aspen seedlings found in the San Juan Mountains, CO, USA and bare soil, vegetation, and nurse objects. Positive interaction indices represent microsites positively associated with aspen seedling establishment, with increasingly positive values representing an increasingly greater association. The dotted horizontal line shows the interaction index value (I = 0) that corresponds to no association of the given microsite. Boxes bound the first and third quartiles of the data and the solid horizontal line indicates the median. The vertical lines indicate the lowest and highest observations within 1.5 x IQR (inter-quartile range) of the first and third quartiles. Individual observations are shown as points; there were 11 sites analyzed for bare soil and vegetation interactions and 10 sites analyzed for nurse object interactions. The asterisk indicates the interaction index was significantly greater than zero ($P < 0.05$).

elevation sites and those with less solar radiation at the upper elevational extremes of aspen’s range.

DISCUSSION

This study documents disturbance-facilitated range expansion at the leading edge for a widespread wind-dispersed tree of North America and the specific conditions under which

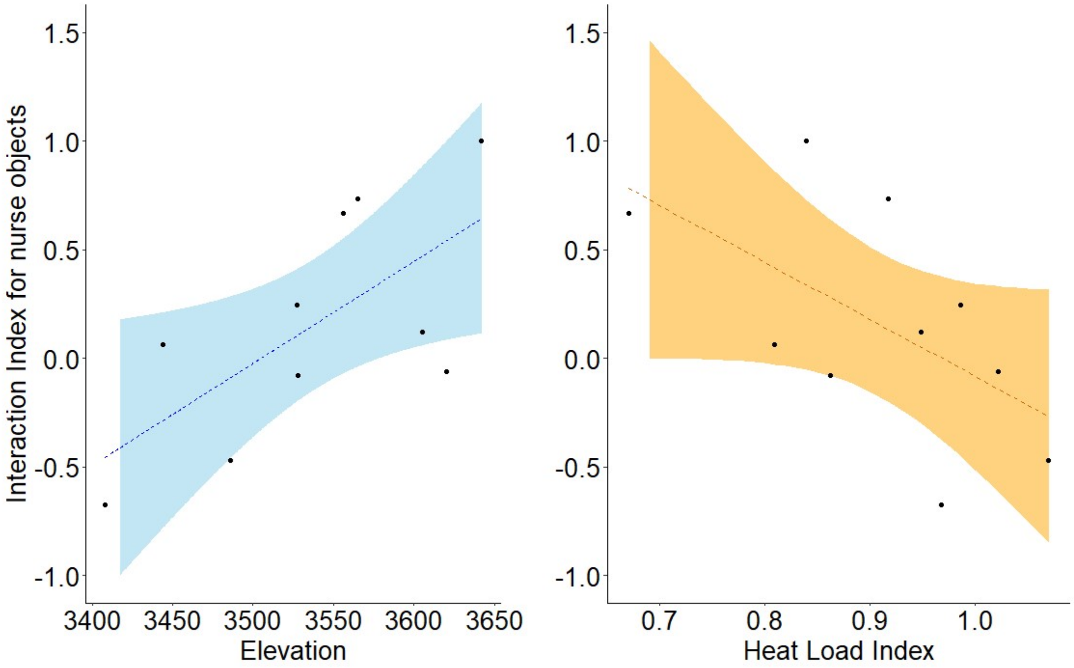

Figure 2.7. The predicted mean effect (shading shows 95% confidence interval) of elevation (left) and heat load index (right) on the interaction index for nurse objects and aspen seedlings in the San Juan Mountains, CO, USA. Closed circles show each data point rather than partial residuals. Both predictors were significant at the $P < 0.05$ level.

expansion is occurring. Aspen seedlings were found above the upper elevational limit of adult aspen across the study area, and only in areas where recent high-severity fire overlapped prior spruce beetle kill (Figure 2.2). The results presented here demonstrate that plant species range shifts may be catalyzed by some disturbance types, but not others, depending on establishment requirements. Wildfire resulted in low cover of duff, understory vegetation and canopy in these subalpine ecosystems (Figure 2.4), which likely facilitated aspen seedling establishment. Notably, unburned areas also experienced extensive canopy mortality from a spruce beetle outbreak, but this disturbance alone did not facilitate aspen seedling establishment above its upper elevational limit (Figure 2.2). Expansion at the leading edge is not only governed by the changing climate but can also be highly localized and dependent on the availability of specific microsite conditions needed for seedling establishment.

Keeping pace with climate change

Many studies have documented upward shifts in species optimum elevations due to climate warming, but hardly any tree species have been documented to migrate fast enough to keep pace with climate warming at their leading elevational edge (Lenoir et al., 2008; Lu, Liang, Wang, Babst, & Camarero, 2021; Renwick & Rocca, 2015). Migration lags for trees at the leading edge are often attributed to dispersal limitations, lack of suitable habitat, competition, and climatic constraints (Renwick & Rocca, 2015). Aspen seedling presence upslope of adult pre-fire stands in this study indicates that these barriers were overcome and that elevational tracking of changes in climate may be possible for aspen and other disturbance facilitated species under certain conditions. Post-fire dominance of aspen via resprouting has been predicted for sites in the study area where aspen previously existed (Andrus, Hart, Tutland, & Veblen, 2021); however, the fact that no suckers were found at sites above the pre-fire aspen stands in this study indicates that in addition to re-establishing dominance after conifer dominance, aspen's realized niche is also expanding. We also found elevation to be a negative predictor of aspen seedling abundance (Figure 2.5), indicating that the sites surveyed captured the upper elevational limit of aspen's current climate niche. With the reduction of competition and creation of necessary soil conditions, the West Fork fire complex reduced several barriers to migration. In addition, distance to seed source did not appear to limit aspen seedling establishment; distance to seed source was weakly *positively* associated with aspen seedling establishment (meaning more aspen establishment in areas further from seed sources, Figure 2.5), which corroborates other studies documenting long-distance dispersal (Landhäusser et al., 2010; Turner et al., 2003). This allowed aspen to expand its range into large burn patches and contrasts with other Rocky Mountain tree species, particularly conifers, for which post-fire regeneration has been repeatedly found to be closely tied to distance to live seed source, with little to no regeneration at distances exceeding 40 to 400 m (Stevens-Rumann & Morgan, 2019).

The adult aspen stands that make up the pre-fire upper elevational limit in this study represent the highest elevations, and thus coolest climates, where aspen was able to establish in the past. If we conservatively assume these stands established 100 years ago, climate warming in the area would have resulted in at least a 133-m upslope shift of the equivalent climate conditions under which those stands established [temperature decreases at the environmental lapse rate of approximately 6°C with every 1,000-m increase in elevation (Baker, 1944) and average annual temperature has increased 0.8°C in the past century; $0.8 \div 6 \times 1000 = 133$]. We found local expansion of 73, 161, 182 and 395 meters upward of pre-fire aspen stands and regional expansion of 110 – 172 m in elevation (Figure 2.2), suggesting that, following fire, aspen has expanded upwards in many settings to the extent expected due to climate warming in this region. However, four of the burned and all the unburned elevational transects showed no upslope expansion, indicating that fire is necessary, but not sufficient by itself to accelerate range shifts (suitable microsites are also needed). In their meta-analysis, Renwick & Rocca (2015) found no evidence of any tree species keeping pace with climate change at its leading elevational edge. Regionally, this seems to be the case for aspen as well, considering that much of the landscape is still unburned and that even in burned areas aspen seedling density was relatively low (133 seedlings/hectare on average among sites with seedlings). Aspen may be unique relative to other tree species in its ability to track changes in climate as quickly as they are occurring, though disturbance history and site factors clearly play a large part in range shifts, as discussed in the sections below.

Disturbances vary in their impact on species range shifts

Our results suggest that wildfire, but not beetle-kill alone, promotes upward range expansion of aspen and likely other shade-intolerant species with long distance dispersal capabilities. Insect outbreaks, drought and disease often cause mortality of only certain tree species and result in moderate increases in understory light conditions and often increased litter layer depth (Astrup et al., 2008; Carlson et al., 2020; DeRose & Long, 2010). These conditions have been shown to promote germination and advanced regeneration of shade-tolerant species that can germinate under dense litter layers (Renwick, Rocca, & Stohlgren, 2016), and even support aspen suckers (Diskin, Rocca, Nelson, Aoki, & Romme, 2011), but, as shown here, do not appear to facilitate aspen seedling establishment. In contrast, severe wildfire drives greater reductions of canopy cover than insect outbreaks and exposes bare mineral soil, which facilitates establishment of many shade-intolerant species that rely on high light for germination and growth (Johnstone, Hollingsworth, Chapin, & Mack, 2010; Scheller & Mladenoff, 2005) and require bare mineral soil or limited litter for successful establishment (Landhäusser et al., 2010). These two factors (high light and limited litter/duff cover) in burned areas (Figure 2.4) likely provided the conditions needed for aspen seedling establishment.

Our burned sites also experienced beetle-kill, so we cannot rule out whether the combination of wildfire and beetle kill, or just wildfire alone, was necessary for aspen seedling establishment. Yet several other studies have documented fire-facilitated establishment of aspen by seed, making it likely that wildfire was the primary catalyst of this upward range shift (Fairweather et al., 2014, Kreider & Yocom, 2021). Burn severity did not seem to impact aspen seedling abundance in this study as it has in others (Gill, Sangermano, Buma, & Kulakowski, 2017) but this is likely because most burned sites experienced near complete canopy mortality regardless of burn severity, because of prior beetle-kill. Our results emphasize that not all disturbance types are equal, supporting other studies documenting the key importance of particular disturbances, such as wildfire and forest harvesting, in facilitating range shifts of shade-intolerant species (Brice et al., 2020; Johnstone & Chapin, 2003; Landhäusser et al., 2010). We occasionally observed aspen seedlings in unburned/beetle-impacted areas while hiking to sites (see Appendix 2); however, this was a rare occurrence compared to burned areas, reflecting the data collected. Thus, regeneration of shade-intolerant species can occur after insect outbreaks, but this disturbance type is unlikely to catalyze range expansion for these species. However, stand replacing disturbance events can also serve as barriers to migration for species with limited dispersal capabilities (Rayfield et al., 2021; Thom, Rammer, & Seidl, 2017). Thus, spatially extensive disturbances may only be effective at facilitating range shifts among species like aspen with long distance dispersal capabilities.

Range shifts are highly localized

While the results of this study suggest a positive impact of high severity disturbance on range shifts for shade-intolerant species at the leading elevational edge, the patchiness of post-disturbance recovery found here points to the highly localized nature of range shifts across the landscape. Half of the burned elevational transects surveyed experienced no upward migration of aspen via seedlings (Figure 2.2), despite being equally as near to live seed sources and experiencing similar degrees of warming over the past century. This was likely due in part to the factors found to predict aspen seedling abundance at the site level, such as elevation and coarse woody debris (Figure 2.5). However, other unmeasured factors may have acted as constraints to seedling establishment across the elevational transect such as topography, wind patterns, or soil properties. For example, one of the elevational transects where upward migration was not recorded was the only burned elevational transect on a North-facing slope (where temperatures should be cooler), and another had most sites located on excessively rocky terrain (likely to have low soil water holding capacity). This adds support to the growing consensus that predicting range shifts based on bioclimate modeling alone is not sufficient to accurately predict where species will occur in the future. Rather, other topographic, edaphic, biological and stand structure data are needed to better predict where range shifts are likely (Benito Garzón, Robson, & Hampe, 2019; Bertrand, Perez, & Gégout, 2012; Brown et al., 2019; Ettinger & HilleRisLambers, 2013; Rehfeldt, Worrall, Marchetti, & Crookston, 2015).

Microsites can also play a large role in governing range expansions, further refining the climate niche a species can expand into. In this study, coarse woody debris cover was a positive predictor of seedling abundance (Figure 2.5) and nurse objects were associated with aspen seedlings at the highest elevations and coolest sites (Figure 2.7). Nurse objects have been found to support the establishment of plants above their upper elevational limit by providing protection from the high winds and excessive solar radiation at high altitudes, reducing exposure to frost, and increasing soil moisture via snowdrift accumulation on top of the objects (Germino, Smith, & Resor, 2002; Resler, Butler, & Malanson, 2005; Smith, Germino, Hancock, & Johnson, 2003). These modifications of the abiotic environment likely explain why aspen seedlings at the highest elevations and coolest sites were positively associated with nurse objects. Nurse objects can also promote seedling establishment by protecting seedlings from browsing (Ripple & Larsen, 2001) or simply acting as seed traps (Haussmann, McGeoch, & Boelhouwers, 2010). The association between nurse objects and aspen seedling establishment documented here supports prior studies on the importance of nurse objects in facilitating range shifts, especially for species that exist near treeline (Chen, Yang, Wang, Sun, & Schöb, 2020; Pyatt, et al., 2016). Since high severity fires increase the abundance of nurse objects on the landscape (in the form of downed trees and exposed rocks), they may facilitate range shifts of species to even higher elevations than they would have been able to establish in the absence of such microsites.

Seedlings as early signs of range shifts

Here we interpret aspen seedling establishment upslope of adult aspen stands as an early indication of a distributional shift. Yet, while seedlings are often used as early indicators of range shifts, their ecological niches can differ significantly from those of adults (Bell, Bradford, & Lauenroth, 2014; Ettinger & HilleRisLambers, 2013), and thus it is not yet clear whether these aspen seedlings will eventually reach maturity. Browsing by ungulates and defoliation by invertebrates can reduce growth and prevent juveniles of some tree species from reaching canopy dominance, thereby limiting range expansion (Van Bogaert, Jonasson, De Dapper, & Callaghan, 2009; Rogers & Mittanck, 2014; Smith, Fettig, & Bowker, 2016). Annual fluctuations in temperature and precipitation in high elevation areas are also likely to determine whether range expansions will persist. Average temperature is often used to predict habitat suitability for species, but extreme events are likely more important for determining seedling survival to maturity and the persistence of the population (Germain & Lutz, 2020). Using seedlings as proxies for future forest composition is helpful for predicting where range shifts may occur, but must be viewed as an early signal and not a guaranteed shift.

Conclusions

This study documents early evidence for wildfire, but not bark-beetle, catalyzed upward expansion of aspen's elevational range limit via seedling establishment. This is a promising sign that other shade-intolerant species with high dispersal capacity may be able to track rapid changes in climate, especially in areas experiencing recent high severity disturbances. Future studies addressing lower elevation limits and the impact of more site-specific factors on establishment will further elucidate how these range shifts will manifest across the landscape and influence future forest composition. Fire was overwhelmingly the most important factor in determining aspen seedling establishment success, but other microsite factors will play a role in determining the specific sites where seedlings can establish and larger patterns in climate and browse pressure will ultimately influence survival and growth into adult stands. As disturbance events become more frequent and severe (Millar & Stephenson, 2015), lags in upslope migrations for some tree species may be overcome.

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CHAPTER 3

RELATIVE INFLUENCES OF LOCAL ADAPTATION VS. MATERNAL SEED SOURCE IN WATER-STRESSED PONDEROSA PINE SEEDLING DEVELOPMENT

INTRODUCTION

Plant species distributions are highly dependent on climatic conditions, which partially dictate which species assemblages may exist at a site (Gleason, 1926). Paleoecological records show that species have historically migrated to align with climatic conditions suitable for their establishment and growth, resulting in the ecosystems seen on the landscape today (Birks and Birks, 2008; Carter et al., 2017; Wang et al., 2023). The rates at which temperatures are increasing under anthropogenic climate change are unprecedented, however, and the ability of plant species to track current changes in climate through migration is uncertain (Renwick and Rocca, 2015). For long-lived plant species such as trees, studies have already found significant range contractions and rates of migration that are too slow to keep pace with contemporary climate change (Gray and Hamann, 2013; Renwick and Rocca, 2015).

To combat the ever-increasing gap between tree distributions and their ideal climate conditions, assisted migration practices have been suggested (Dumroese et al., 2015; Martínez-Berdeja et al., 2019; O'Neill and Gómez-Pineda, 2021; Palik et al., 2022). Assisted migration in general refers to any movement of species by humans to combat climate change effects (Ste-Marie et al., 2011). Assisted gene flow in particular refers to moving populations of species within their already established range to align locally adapted populations with changing climate conditions (Pedlar et al., 2012; Ste-Marie et al., 2011). The practice of assisted gene flow is starting to become operationalized in federal forestry programs throughout the US and Canada (Palik et al., 2022; Pelai et al., 2021; Young et al., 2020), and therefore more information is needed on local adaptation in trees to make seed-sourcing decisions.

Tree species with broad geographic ranges experience a broad range of environmental conditions, making it likely that populations are locally adapted to the unique conditions at the site that they occupy (Savolainen et al., 2007). Provenance studies have demonstrated differences in tree growth due to geographic origin for the past century and were originally used to find optimum growth and yield of trees so that seed zones could be delineated for silvicultural practices (Hyun, 1955; Rehfeldt, 1993; Sorensen, 1983; Ying and Yanchuk, 2006). In recent years, there has been a renewed interest in provenance studies for informing assisted gene flow efforts, as several have shown that seeds collected from different populations of the same tree species may be differentially adapted to stressors such as heat and drought (George et al., 2016; Kamakura et al., 2021; Lachenbruch et al., 2021; Matías et al., 2016; Zas et al., 2020). Complicating differentiation due to seed provenance is evidence that maternal traits, or the genotype of the maternal tree, may also have influence over seedling growth traits (Castro et al., 2008; Kolb et al., 2016; Tíscar and Lucas, 2010), as can the maternal environment that the seed developed in (Borgman et al., 2014; Cendán et al., 2013; Johnsen et al., 2005; Vivas et al., 2013). If certain populations or mother trees are to be targeted for seed collection for reforestation, it is important to understand what is driving differentiation in seedling traits.

The goal of this study is to understand the relative importance of seed source population and maternal traits on interior ponderosa pine (*Pinus ponderosa* var. *scopulorum*) germination, growth, and survival under drought versus well-watered conditions. Interior ponderosa pine is an ecologically and economically important species in the western US whose regeneration potential is threatened by climate change impacts on drought, heat, and fire severity (Davis et al., 2019; Feddema et al., 2013; Kemp et al., 2019; Korb et al., 2019; Minott and Kolb, 2020; Savage et al., 2013; Simeone et al., 2019). The area suitable for interior ponderosa pine growth is predicted to decrease by 50-59% in the next 40 – 70 years and is expected to move upwards in elevation by 600 or more meters (Kemp et al., 2019; Maguire et al., 2018; Rehfeldt et al., 2014a, 2014c). Future habitat losses due to climate change are predicted to be substantially greater for the interior variety of ponderosa pine (var. *scopulorum*) than the coastal variety (var. *ponderosa*) (Maguire et al., 2018; Rehfeldt et al., 2014a), making it especially important to investigate the ways in which this variety will be able to adapt to climate change. Provenance studies have revealed significant variation in several growth, development, and physiological traits between interior ponderosa pine populations across the interior west (Kerr et al., 2015; Marias et al., 2016; Rehfeldt, 1993, 1991, 1990, 1986; Zhang and Cregg, 2005). Significant amounts of variation in drought-adaptive traits between individuals of ponderosa pine within the same population have also been found, with intrapopulation variation sometimes exceeding interpopulation variation (Kolb et al., 2016; Maherali et al., 2002; Warwell and Shaw, 2019). Thus, it is still uncertain to what extent assisted gene flow will enhance resilience of interior ponderosa pine forests in the future.

We performed a greenhouse experiment using seeds collected from 48 individual mother trees across eight populations that span a broad climatic gradient to assess how seed source population and mother tree traits differentially impact germination, growth, and survival of seedlings under drought. By assessing differences in seedling growth due to maternal tree and seed source population, this study clarifies the role that these different levels of variation play in seedling adaptations. We hypothesized that seedlings from hotter and drier populations would grow more and survive longer under drought conditions, due to local adaptation to drought. We also hypothesized that seedlings from mother trees with stronger correlations between growth and climate would be more adapted to drought and survive longer under drought stress.

METHODS

Seed collection and processing

Cones were collected from ten trees ("mother trees") of *Pinus ponderosa* var. *scopulorum* (hereafter, ponderosa pine) in each of eight sites sampled in 2019 that span a gradient of latitude (38.10850 - 40.69161 degrees), elevation (1747 - 2843 m), and climatic moisture deficit (15 - 272 mm.; Figure 3.1) to capture a broad range of climate variability. Mother trees were all mature trees within less than a 5-hectare area of each other. Cones were left to air-dry and open naturally in the lab and 90 seeds were weighed from each mother tree to obtain an estimate of average seed mass.

Population climate traits

Two variables were calculated to characterize the climate conditions of each population: climatic water deficit (CWD) and proportion monsoonal precipitation ("monsoonality"). Climatic water deficit was calculated in R using the CWD and AET function written by Redmond (2022), which uses a modified Thornthwaite water balance model from Lutz et al. (2010). In this function we used 30-year normal (1991-2020) monthly climate data from PRISM (PRISM Climate Group, 2022), aspect, slope, and elevation from the USGS 3D Elevation Program 1/3 arc-second digital elevation models (U.S. Geologic Survey, 2019), and soil available water capacity (0 – 150mm) from the Soil Survey Geographic Database (SSURGO) in

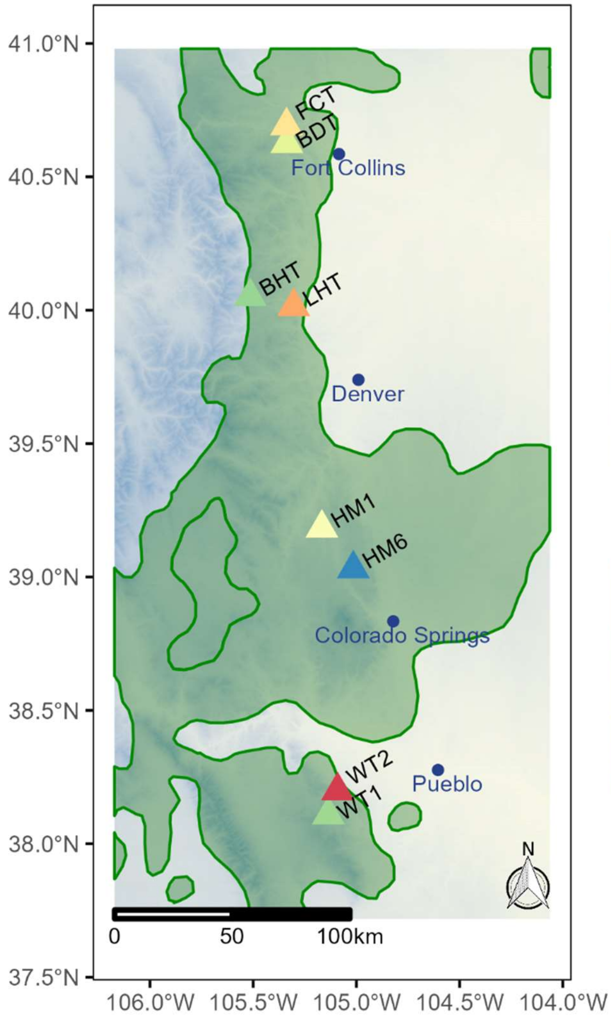

Figure 3.1. Locations of sites in Colorado where cones were collected in 2019, color coded by climatic water deficit (cwd). The current range of ponderosa pine is shaded in green and overlaid on an elevation map where darker blue indicates higher elevations and lighter beige indicates lower elevations.

SoilWeb Earth (O'Geen et al., 2017). Folded aspect was calculated as: 180 - |aspect – 180|, where aspect is in degrees (McCune and Keon, 2002). Climatic water deficit (CWD) is a measure of the evaporative demand that was not met by available water, where low values generally correspond to cooler and wetter sites and high values correspond to hotter and drier sites (Stephenson, 1998, 1990).

Monsoonality was calculated as the cumulative amount of precipitation received by each site in the months of July, August, and September (monsoon season) divided by the total amount of precipitation received in a year. Therefore, it represents the proportion of precipitation received during monsoon season. To calculate this, we used 30-year normal (1991-2020) averages from PRISM (PRISM Climate Group, 2022). In our study, sites with greater monsoonality tend to occur at more southern latitudes, with monsoonality of the four northernmost populations ranging from 0.26 – 0.30 and the four southernmost populations ranging from 0.36 – 0.38.

Mother tree traits

To characterize maternal tree traits, we used an increment borer (5 mm or 12 mm in diameter) to extract two cores from each maternal tree in November – December 2020. All of the following laboratory procedures were conducted using the methods described in Gonzalez (2022) and are briefly summarized here. In the lab, cores were air dried, mounted, and sanded with progressively finer sandpaper following standard dendrochronological techniques (Gonzalez, 2022). To determine radial annual xylem growth, 5mm cores were visually dated and cross dated using COFECHA, with any weak correlations being manually verified using local ponderosa pine ring width chronologies (Gonzalez, 2022; Rodman et al., 2021; Veblen et al., 2000), and International Tree-Ring Data Bank chronologies (https://www.ncei.noaa.gov/products/paleoclimatology/treering). Annual xylem growth was measured in mm for each year from 2000-2020. To measure carbon discrimination, tree cores were dried and the growth rings for each year from 2000 – 2020 were separated. The wood was then ground to a powder and samples from across years were homogenized, including an approximately equal amount of material from each year. Thus, the carbon discrimination values reported here represent average gas exchange patterns for each tree over 21 years (2000 – 2020). The homogenized samples were analyzed by the UC Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The δ13C values received from the lab were expressed relative to the international standard VPDB (Vienna Pee Dee Belemnite).

The carbon isotope discrimination ($\Delta^{13}C$‰) values for the mother trees in this study were calculated using this equation from Farquhar (1989):

$$\Delta^{13}C\text{‰} = \frac{\frac{\delta 13C_a}{1000} - \frac{\delta 13C_p}{1000}}{1 + \frac{\delta 13C_p}{1000}} x\ 1000$$

$\delta 13C_a$ is the isotopic ratio of ^{13}C to ^{12}C in atmospheric CO2 (assumed to be -8‰) and $\delta 13C_p$ is the isotopic ratio of ^{13}C measured in the mother tree wood tissue. Therefore, the higher the proportion of ^{13}C in the plant, the lower carbon discrimination will be. Since ^{12}C is preferentially used in photosynthesis over ^{13}C, higher proportions of ^{13}C in plant tissue are caused by long periods of stomatal closure, during which the plant uses up most of the ^{12}C that was already captured and is required to use ^{13}C until stomata reopen (Lambers et al., 2008). We scaled the carbon isotope discrimination value for each mother tree to the population by subtracting the mean and dividing by the standard deviation of carbon discrimination values for all mother trees in a population. This accounts for differences in moisture between sites, and accounts for any differences in atmospheric carbon isotope proportions between sites. Although carbon discrimination has been interpreted to represent long-term water use efficiency of plants (Lambers et al., 2008), when it is compared between individuals of the same species, it can also simply indicate the moisture conditions that the plant has experienced due to differences in microtopography within a site (Tei et al., 2019). For example, both mother trees that are not very responsive to drought (i.e., have less stomatal closure during drought) and those that are responsive (i.e., have more stomatal closure during drought) but just haven't experienced drought due to microtopography, would have similarly high carbon discrimination values. Therefore, in this study we interpret high carbon discrimination values as less stomatal closure over the 21 years of measurement (2000 – 2020), which could be a result of low water use efficiency or having greater access to moisture due to microtopography or neighborhood competition.

The second mother tree trait we calculated was growth-climate sensitivity, or the extent that mother tree annual ring growth is correlated with drought stress. To calculate this, we first detrended the annual growth ring measurements from Gonzalez (2022) using the classic smoothing spline from Cook & Peters (1981) with the *detrend.series* function from the R package *dplR* (Bunn, 2008), in order to remove non-climatic growth trends from the ring width data. The full time series of annual growth ring measurements for each mother tree was used in detrending to get the longest-term trend possible (timeseries ranged from 27 – 222 years). Using PRISM annual climate data for each site (PRISM Climate Group, 2022), we then calculated the forest drought stress index (FDSI), which is a combination of winter precipitation before the growing season, and vapor pressure deficit of the previous fall and current growing season (Williams et al., 2013). The FDSI values were scaled at the site-level such that positive values indicate years that are wetter than average whereas negative values indicate years that are drier than average at the site. We then calculated the Pearson correlation between the detrended growth value and the scaled FDSI value for the years 2000 – 2020 for each mother tree at each site. This correlation coefficient is what we termed the tree's "growth-climate correlation", where positive values indicate that the tree grows more when conditions are wet and less when conditions are dry and negative values indicate the opposite.

Greenhouse methods

Seeds were cold stratified by placing the seeds in mesh bags, covering them with wet vermiculite, and leaving them in a refrigerator at 2.7°C for 45 days, like Luna et al. (2008). Seeds from six mother trees from each of 8 populations (n = 48) were sown for use in the experiment. 28 Rayleach "conetainers" (SC10R) for each mother tree were sown with 2 seeds each, for a total of 56 seeds sown per mother tree. There was a total of 14 conetainer racks, each containing two conetainers from each mother tree. Conetainers were filled with a 1:1 ratio of field soil from our experimental plot (40.645001, -105.524180) and potting soil (as in Augustine and Reinhardt, 2019). Conetainers were watered once daily for the first 25 days after sowing (May 31st – June 25th) to allow time for all germination to occur. Conetainers were originally situated so that all racks to be included in the watered treatment were on one side of the bench and all to be included in the drought treatment were on the other. However, after 10 days we noticed that more germination was occurring on one side of the bench than the other (likely due to proximity to the swamp cooler). Therefore, racks were subsequently arranged so that they alternated treatment designations and were rotated from one side of the bench to the other every 10 days. Conetainers were checked daily for germination. After 25 days, if both seeds in a conetainer had germinated, the shortest seedling was plucked so that each conetainer only had one seedling. Conetainers were then redistributed across the racks so that there were similar sample sizes for each mother tree in both drought and watered treatments. Five mother trees that were sown had very low germination and were not used in the experiment, resulting in 43 mother trees from 8 populations at the start of the experiment (Table A3.1, Appendix 3). The racks were then set up on the greenhouse bench with 7 on one side and 7 on the other, alternating drought and watered treatments.

The drought treatment started on day 28 (June 28th, 2021) after sowing. The drought treatment method was modeled after Augustine & Reinhardt (2019) and is meant to simulate the dry down experienced throughout the summer in ponderosa pine forests of the southern Rocky Mountains. To start the drought treatment, all conetainers were watered to saturation and one conetainer per rack was weighed on day 25. Then, on day 28, the same conetainers were weighed again to obtain the weight after 3 days without watering. The difference between these two weights was calculated and interpreted to represent the water lost to evapotranspiration during the three days. Conetainers in the drought treatment were then watered with a pipette 80% of this "water lost to evapotranspiration" value, while those in the watered treatment were watered to saturation. One new conetainer from each rack was then weighed again. This continued every 3 days, with the drought treatment receiving 80% for the first 21 days, then 60% for the next 21 days, then 40% for the next 21 days, then 20% for the next 21 days, after which the drought treatment stopped receiving water for the rest of the experiment. The watered treatment was watered to saturation every 3 days for the duration of the experiment.

Immediately after the drought treatment started (June 28th – July 1st), we measured the length of each seedling's stem (from soil surface to bottom of cotyledon scar), three random cotyledons, and the longest three primary needles. Approximately 6 weeks after the drought treatment started (August 10th – 13th), the three longest primary needles were measured again, along with all true needles that had appeared. The length of all true needles was measured one final time on September 6th (10 weeks after the drought treatment started). We intended to measure root elongation as another seedling trait, but when we harvested seedlings just 43 days after sowing, most already had roots that reached the bottom of the conetainer. Therefore, the conetainers that seedlings were grown in inherently limited the depth and potentially volume of seedling roots, limiting our ability to evaluate how root structure would be altered under natural settings.

The seedlings in the drought treatment were last watered on September 20th, 2021 (12 weeks after the start of the drought treatment), while the watered treatment continued to be watered every 3 days to saturation. On October 1st-2nd, a subset of seedlings was harvested to measure the end of experiment above- and below- ground biomass. When possible, 3 seedlings from each mother tree and watering treatment were harvested, but exceptions were made where sample size was low (Table A3.1, Appendix 3). The above-ground biomass was separated from the below-ground biomass and dried in the oven at 60C for 3 days before being weighed. After watering stopped, seedling survival was monitored every 1-3 days. Seedlings were recorded dead when they had no visible green left on their needles. We stopped monitoring when all seedlings were recorded as dead.

Gas exchange measurements were taken 3 times throughout the course of the study on July 27th (4 weeks after start of drought treatment), September 1st (9 weeks after start of drought treatment), and October 4th (14 weeks after start of drought treatment & 2 weeks after watering stopped). Photosynthesis and conductance were measured with a LI-6400XT portable photosynthesis system (LI-COR Biosciences, Inc., NE, USA), which had a CO_2 concentration set to 400 ppm and the light source set to 1200 μmol m^{-2} s^{-1}. All measurements were taken between 10am and 3pm on one representative seedling from each mother tree in each treatment ($n = 86$). Due to the small size of the seedlings, all needles of the seedling were flattened and enclosed in the LI-6400 chamber and a picture was taken to estimate the leaf area within the chamber to use for calculation of photosynthesis and conductance rates. Throughout the duration of the experiment, the greenhouse temperature was set between 20 – 23°C during the day and 14 – 17°C at night.

Statistical Analyses

The measurements of seed mass, stem length, cotyledon length, root mass, shoot mass, and total biomass were all highly positively correlated (Pearson correlation > 0.8). Therefore, we only analyzed seed mass and total biomass as representative of these measurements, as well as germination timing, germination rate, root:shoot biomass ratio, instantaneous water use efficiency in July, September, and October, and survival time after watering ceased for droughted seedlings. Instantaneous water use efficiency (iWUE) is defined here as the rate of photosynthesis divided by the rate of stomatal conductance, as measured by the LICOR.

We first performed variance partitioning analyses to assess variation in these seedling traits across the two hierarchical scales: among mother trees within populations and among populations. To do this, we performed linear mixed effects models with the trait as the response variable and random effects of mother tree nested within population. The only fixed effect was either the intercept (for traits unaffected by the drought treatment) or treatment (for root:shoot ratio and total biomass). All traits were modeled using a Gaussian distribution using the *lmer* function except for germination rate, which was modeled as a binomial process using the *glmer* function in the package *lme4* (Bates et al., 2015), with the number of seeds that successfully germinated from each mother tree as the successes and the number that did not germinate as the failures. Residuals of all models were checked for normality and homoscedasticity. The variables seed weight and germination timing were log-transformed to conform to model assumptions. The variance components of the random effects of population and mother tree nested within population were compared within each model to assess how much variation in the seedling trait was due to the mother tree that the seed came from, versus the population that it belonged to. The random effects model for survival time (number of days after watering ceased that the seedling survived) with both population and mother tree as random effects was singular, likely because this trait did not vary much between populations and mother trees.

For all aforementioned traits, we also performed nested ANOVAs with population as a fixed effect and mother tree as a random effect to assess whether there were statistically significant differences between populations for each trait. For models without interactions, we used type-2 ANOVAs. For biomass traits, we added additional fixed effects of treatment and its interaction with population to these models and performed type-1 ANOVAs to test whether the interaction term was significant. If the interaction was not significant, we re-fit the model without the interaction term and used a type-2 ANOVA to assess significance of the individual fixed effects. If the interaction was significant, we compared confidence intervals between treatments to determine which populations experienced treatment effects. For gas exchange measurements, we did not have a large enough sample size to assess the effect of mother tree (n=1 per mother tree per treatment). Instead, we performed a two-way ANOVA with treatment, population, and their interaction as fixed effects, and the log-transformation of iWUE as the response variable. The response was log-transformed to meet the assumptions of ANOVA. After looking at differences between mother trees and populations, we wanted to determine if the climate of the source population was a driver of these differences. For each trait we fit a linear mixed effect model with the trait as the response variable, climatic water deficit (CWD) and monsoonality as fixed continuous effects, and mother tree nested within population as a random effect. For biomass and gas exchange traits, treatment was also included as a categorical fixed effect, interacting with both climate variables. We used a type-1 ANOVA to test whether there were any significant interactions, and then re-fit a new model that only included significant interactions, if any. If no interaction was detected between CWD and monsoonality, the new model included both variables without an interaction. All models were linear with Gaussian error structures, except for the model predicting germination rate, which was fit with a binomial error structure, and an observation-level random effect to account for overdispersion. Seed mass, germination timing, total biomass, and instantaneous water use efficiency in October were log-transformed to meet linear model assumptions.

We then looked at the relationship between mother tree traits and the population where the mother trees lived. To do this we fit separate models for mother tree climate-growth correlation and mother tree carbon discrimination, with a fixed effect of population. Data from all 80 mother trees from which seeds were collected were used in these analyses so that relationships were as reflective of reality as possible. If there was a significant effect of population, we then fit a model with the two population climate variables (CWD and monsoonality) as fixed, continuous effects.

To examine the effect of mother tree traits on seedling traits, we fit models with each seedling trait as the response variable and the two mother tree traits (climate-growth sensitivity and carbon discrimination) as continuous fixed effects with an interaction. Type-1 ANOVA was used to determine if there was a significant interaction, and if there was no interaction, simpler models with the two mother tree traits as separate fixed effects were fit. If interactions were significant, mother tree within population was also included as a random effect in all models to account for the measurements made on multiple seedlings from the same mother tree, except for iWUE which only had one mother tree measured per treatment. Instead, iWUE in July, September, and October included population as a random effect.

RESULTS

Differences in seedling traits

For all traits except those related to germination, most of the trait variation (62 – 98 %) was explained by population with considerably less of the variation (2-38%) explained by mother trees (Figure 3.2). In contrast, variances in germination timing and germination rate were more influenced by mother tree identity (54 – 75%).

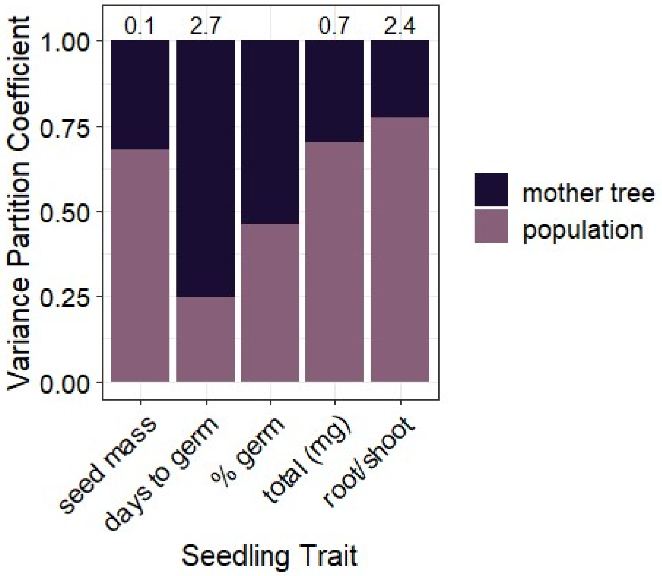

Figure 3.2. Variance components of models for each seedling trait with enough replication (x-axis). Variance components of population (pop) and mother tree are expressed as a proportion of the total variance between populations and mother trees. Residual variance (where applicable) is shown as a proportion above the relevant measurement, calculated by dividing total residual variance by the sum of the variance attributed to all other effects. Therefore, a value of 1 would mean that residual variance was equal to the amount of variance partitioned to all other variables. Germination rate does not have residual variance as it was fit with a binomial model. Residual variance is likely low for seed mass as it was averaged for each mother tree rather than assessed for each seed individually. Residual variance in germination timing was likely influenced by initial position in the greenhouse.

Seed weight, germination rate and timing, and root:shoot ratio all significantly differed among populations ($P < 0.05$ for all; Figure 3.3; Figure 3.4). Total biomass also varied by population, and there was a significant population by treatment interaction ($P < 0.0001$; Figure 3.4). Seedlings from LHT, HM1, and WT2 had significantly more total biomass in the watered treatment than the drought treatment (Figure 3.4), whereas seedlings from all other populations did not differ significantly in total biomass between the two treatments. The watered treatment resulted in higher root:shoot ratios (ANOVA, df= 1, $p < 0.001$), but there was no treatment by population interaction (Figure 3.4). Survival time (in droughted seedlings only) did not significantly differ between populations ($P = 0.5$).

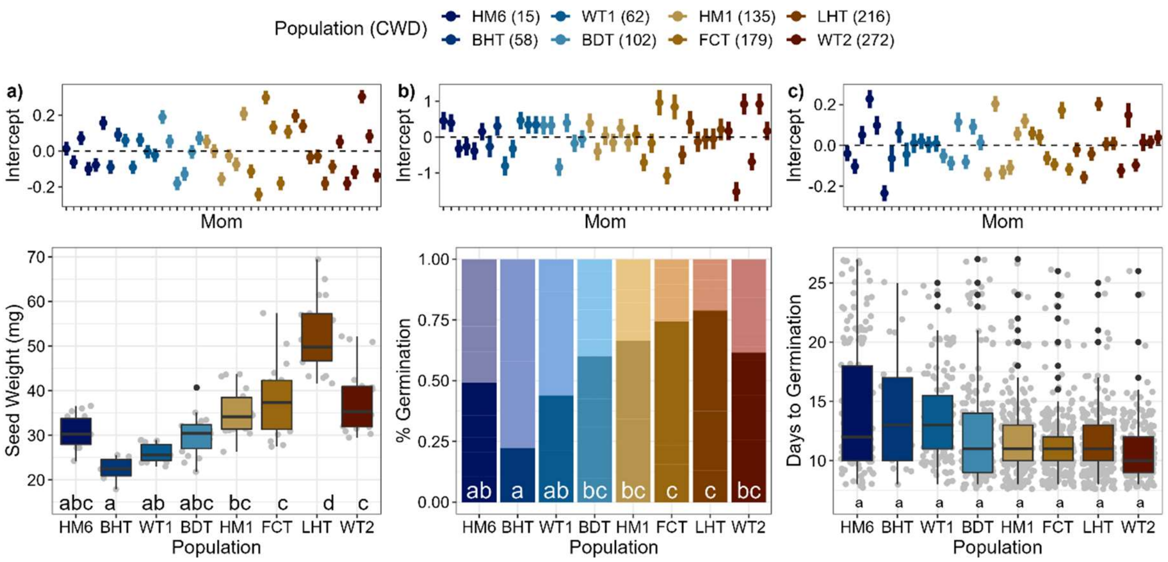

Figure 3.3. Data and model results for a) seed weight, b) germination rate, and c) germination timing. Populations are arranged from lowest climatic water deficit (CWD) to highest (cold/wet to hot/dry). Gray points in the background of box plots show actual data. Letters below boxplots indicate statistical significance, as evaluated with a nested ANOVA where populations that do not share a letter are significantly different from each other. Random effect estimates and standard deviations for each mother tree are shown in a separate plot above the main plot for each trait. For the plot of germination rate (b), dark bars indicate the percent of seeds that germinated, while light bars show percent that did not germinate.

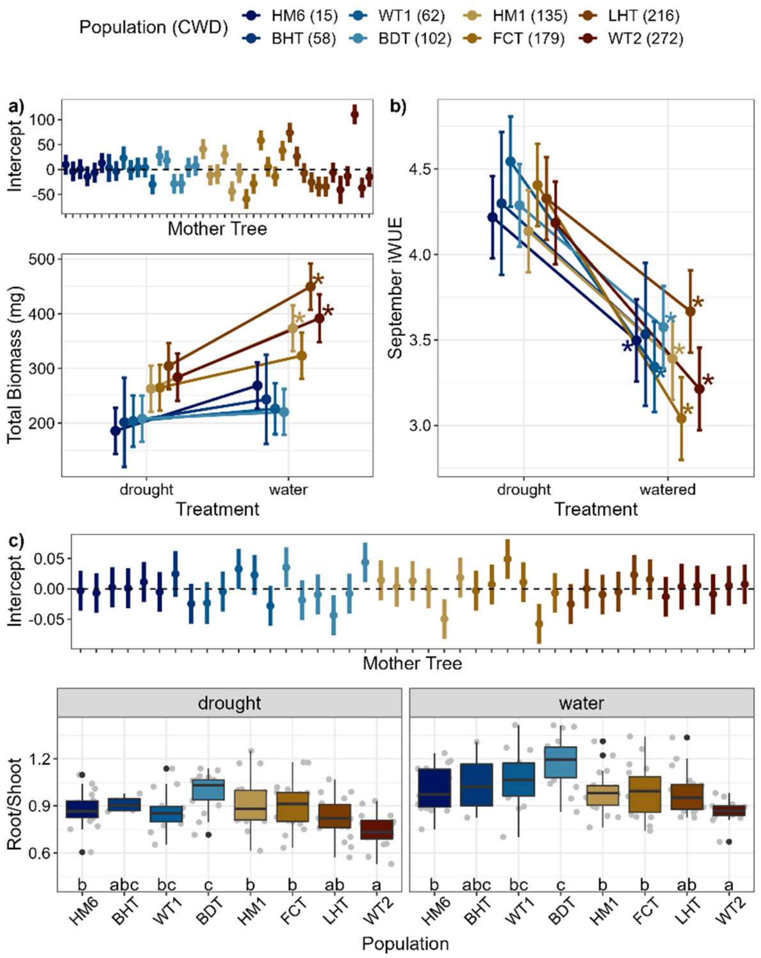

Figure 3.4. Data and model results for a) total biomass, b) instantaneous water use efficiency (iWUE) in September, and c) root:shoot biomass ratio. Populations are arranged from lowest climatic water deficit (CWD) to highest (cold/wet to hot/dry). Where interactions are significant (a & b), points represent model estimates for each population in each treatment, while error bars display 95% confidence intervals for the model fits. Asterisks indicate which populations had a significantly different mass in the watered treatment, compared to the drought. In c), gray points in the background of boxplots show actual data and letters below boxplots indicate statistical significance, as evaluated with a nested ANOVA where populations that do not share a letter are significantly different from each other. Where applicable (a & c), random effect estimates and standard deviations for each mother tree are shown in a separate plot above the main plot for each trait. Mother tree effects were not assessed for gas exchange as measurements were only taken on one mother tree per treatment.

Instantaneous water use efficiency differed significantly (P = 0.03) between populations in July (4 weeks after the start of the drought treatment) but did not differ between watering treatments (P = 0.15), nor was there a difference between populations in their response to watering (an interaction). However, iWUE in September (9 weeks after the start of the drought treatment) was significantly predicted by an interaction between population and treatment (type-1 ANOVA, $F = 197.68$, $P < 0.0001$). For all populations except BHT, confidence intervals in the drought treatment did not overlap those in the watered treatment, indicating that water use efficiency was significantly greater in the drought treatment. The treatment effect was particularly strong for seedlings from FCT and WT1 (Figure 3.4). When gas exchange was measured in October (2 weeks after watering had stopped), most seedlings in the drought treatment were not photosynthesizing at all, whereas iWUE of seedlings in the watered treatment was not different from previous dates (ANOVA, $F = 2.2$, $P = 0.12$), and populations did not differ from each other (ANOVA, $F = 1.4$, $P = 0.3$).

Population Climate Effects

CWD and monsoonality had a significant interaction in predicting seed mass (LMER, $t = -3.2$, $p < 0.05$) and germination rate (GLMER, $z = -2.1$, $p < 0.05$), where seeds from hotter and drier climates were larger and germinated more, especially in populations with less monsoonal precipitation (higher latitudes). CWD was a significant positive predictor of germination speed (LMER, $t = -3.8$, $p < 0.001$). There was a significant interaction between CWD and treatment in predicting total seedling biomass (LMER, $t = 3.2$, $p < 0.01$ for interaction), where seedlings from hotter and drier populations accumulated more biomass, especially in the watered treatment. Root:shoot ratio was also significantly predicted by CWD ($t = -2.5$, $p < 0.05$) and treatment ($t = 8.0$, $p < 0.001$), where seedlings from hotter and drier populations had lower root:shoot ratios in both treatment types, and root:shoot ratios were overall lower in the drought treatment (Figure 3.5).

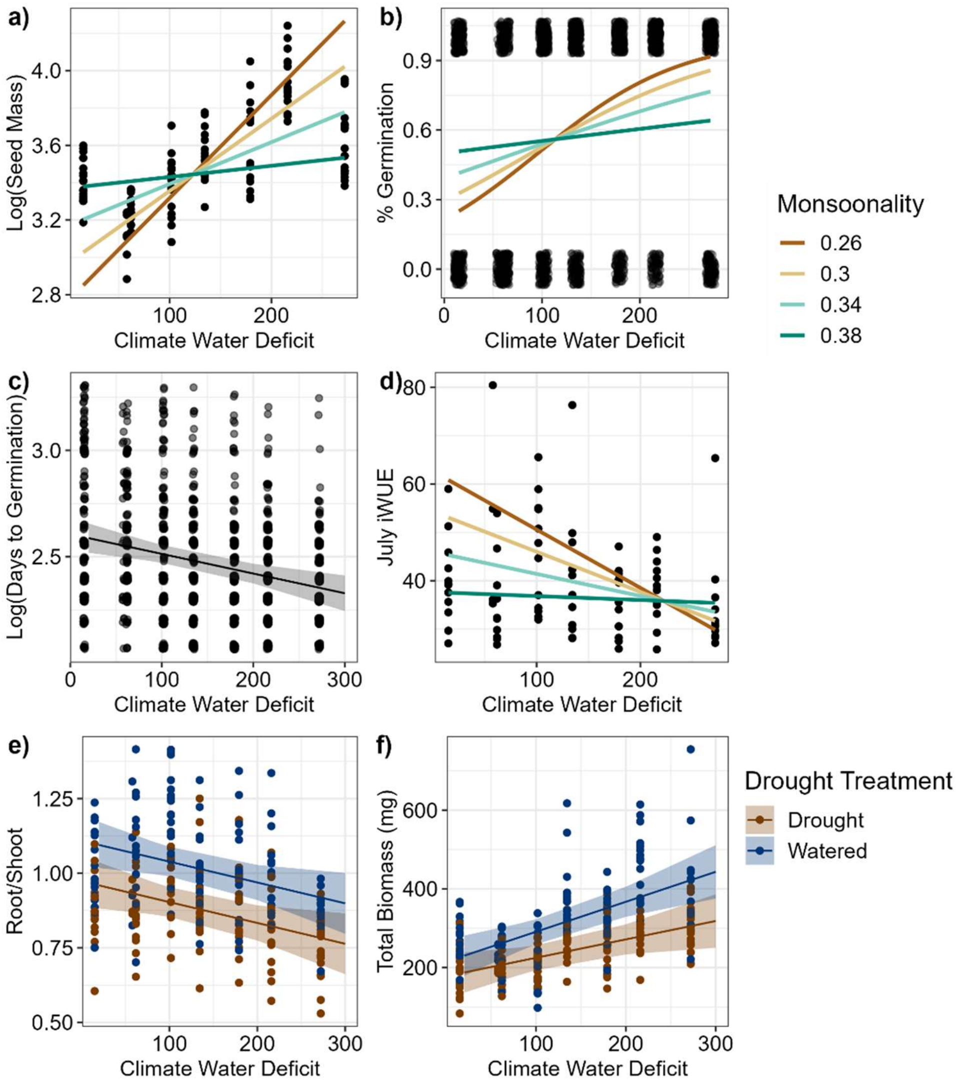

Figure 3.5. Model results and underlying data for significant effects identified in population models. Lines show model predictions, shading shows 95% confidence intervals for model predictions, and points represent actual data. Where monsoonality did not significantly interact with CWD, it was held at its average to generate the predictions for these plots.

Instantaneous water use efficiency in July (4 weeks after the start of the drought treatment) was significantly predicted by the interaction between CWD and monsoonality (t = 2.3, $p < 0.05$), but was unaffected by watering treatment (t = -1.2, p = 0.2) at that point in the study. In July, seedlings from cooler and wetter climates had higher water use efficiency when they were also from areas with low monsoonality (Figure 3.5). In September (9 weeks after the start of the drought treatment), there was no longer a significant effect of CWD (t = -1.4, p =

0.2), and only the treatment effect was significant ($t = -11.7$, $p < 0.001$), where seedlings in the drought treatment had greater water use efficiency than those in the watered treatment. In October (2 weeks after watering stopped), there were still no significant relationships between instantaneous water use efficiency and population climate in the seedlings that were still photosynthesizing (those in the watered treatment). Most seedlings in the drought treatment had stopped photosynthesizing by this point, so efficiency could not be tested in the drought treatment.

The models for population climate effects on germination rate, germination timing, and instantaneous water use efficiency in July and September resulted in singular fits, with estimates for the random effect of population near zero. This indicates that the random effect of population was not needed after using CWD and monsoonality to predict these seedling traits. For all other models, the standard deviation of the random effect of population was less than the coefficient estimates for significant effects in the models, indicating that population was not very influential as a random effect, after considering the effects of CWD and monsoonality.

Mother tree effects

The correlation coefficients used to assess mother tree growth-climate sensitivity ranged from -0.33 to 0.80, with a mean value of 0.34. As expected, this indicates that tree ring width is either relatively uncorrelated with FDSI (at the low end), or positively correlated (at the high end) where tree rings grow less when drought is severe. Population was significant in predicting mother tree climate sensitivity (ANOVA, $F = 8.2$, $p < 0.001$), with populations that receive more monsoonal precipitation (LM, $t = 4.8$, $p < 0.001$) and that have a higher CWD (LM, $t = 2.9$, $p < 0.01$) having greater growth-climate correlations (Figure 3.6). Carbon discrimination before being scaled to population, was significantly different between populations (ANOVA, F = 5.6, p < 0.0001), with populations that receive more monsoonal precipitation (LM, t = -5.9, p < 0.001) and that have a higher CWD (LM, t = -2.4, p < 0.05) having lower carbon discrimination values (more stomatal closure) than those with less monsoonal precipitation.

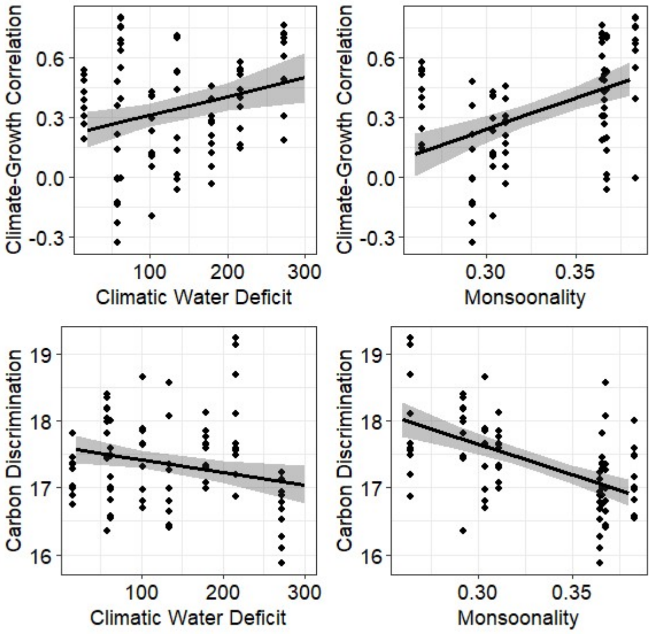

Figure 3.6. Relationship between population climate traits and mother tree traits. Higher climate-growth sensitivity indicates mother trees had high correlations between ring growth and the forest drought stress index, while high carbon discrimination indicates lower 21-year integrated water use efficiency or less stomatal closure. Model fit and 95% confidence intervals were predicted with the other model effect held at its average and are plotted over actual data.

Seed mass was significantly greater in mother trees with higher carbon discrimination (t = 2.1, p < 0.05; Figure 3.7). There was marginally significant interaction between mother tree traits in predicting germination (z = -1.96, p = 0.05), where germination was highest for seeds from mother trees with the highest carbon discrimination values and lowest growth-climate correlations. Neither mother tree trait was significant in predicting germination timing or root/shoot ratio (p > 0.1). Total biomass was marginally significantly predicted by mother tree carbon discrimination (t = 2.0, p = 0.057), with seedlings from mother trees with higher carbon discrimination accumulating more total biomass (Figure 3.7). Climate-growth sensitivity of mother trees was significant in predicting instantaneous water use efficiency in seedlings in July ($t = 3.1$, $p < 0.01$), where seedlings from more climate-sensitive mother trees were more efficient in their water use across both treatments (Figure 3.7). In October, the opposite trend was observed where growth-climate correlation negatively predicted iWUE of seedlings in the watered treatment ($t = -2.0$, $p < 0.05$). However, there were no significant mother tree traits in predicting water use efficiency of seedlings in September, when treatment effects were most pronounced.

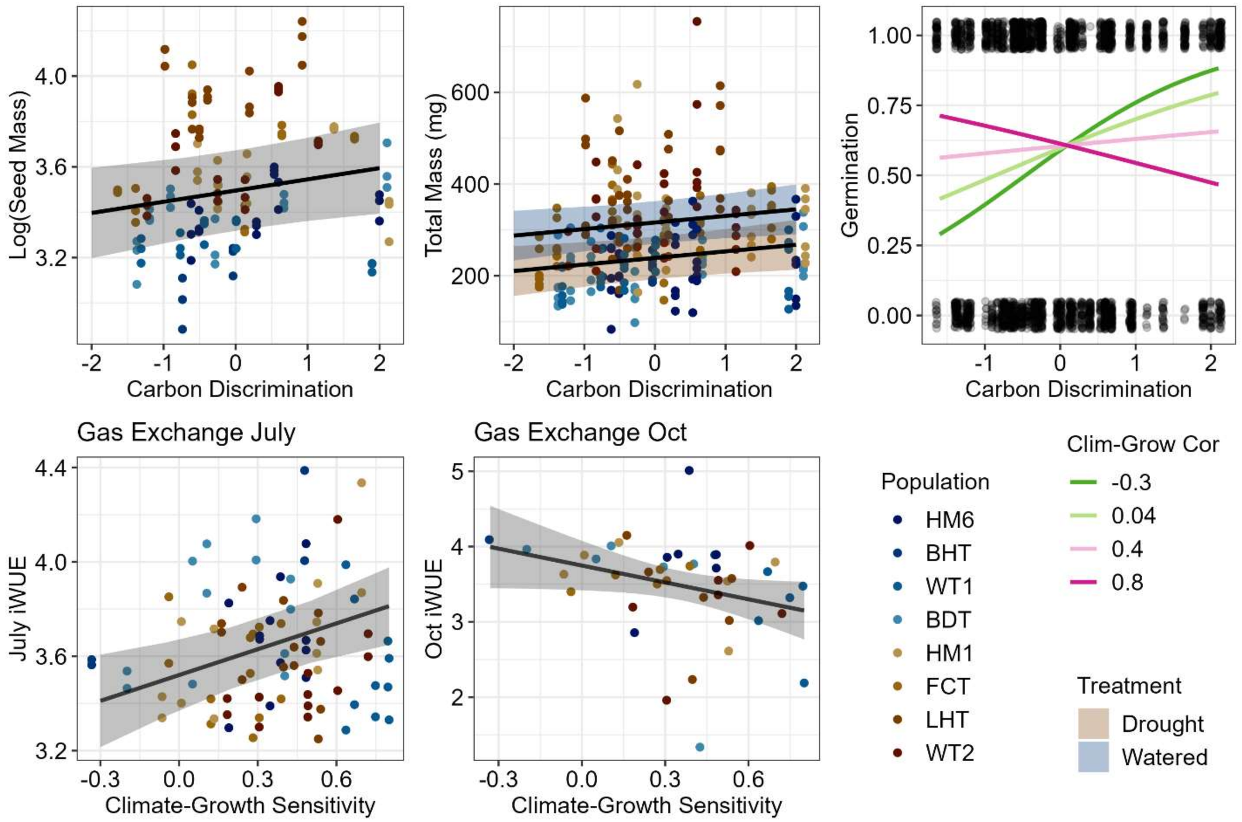

Figure 3.7. All significant model results and underlying data from mother tree models. Only significant trends are plotted. Lines show model predictions, shading shows 95% confidence intervals for model predictions, and points represent actual data, color-coded by population. Lower carbon discrimination indicates greater long-term water use efficiency in mother trees and higher climate-growth sensitivity indicates mother trees had high positive correlations between ring growth and the forest drought-stress index. In each plot, the mother tree trait not plotted was held at its average to generate model predictions.

DISCUSSION

Our results show that ponderosa pine seedling germination, growth, and physiological traits are variable across populations and maternal lines. Much of this variation is likely attributed to seed mass differences between populations and mother trees. Seeds from hotter and drier populations, and mother trees with greater carbon discrimination were larger and germinated faster, producing seedlings with greater total biomass, lower root:shoot ratios, and lower instantaneous water use efficiency under well-watered conditions. Mother tree climate-growth sensitivity was not correlated with seed mass but did predict patterns in instantaneous water use efficiency and germination rates in seedlings. Therefore, sensitivity to climate in mother trees may be passed to seedlings through mechanisms other than seed provisioning. Droughting seedlings in the greenhouse resulted in differences in biomass and instantaneous water use efficiency, with implications for drought conditioning in seed planting operations. In general, seeds from hotter and drier sources and more climate-sensitive mother trees did not show greater adaptation to drought in this study.

Drought effects across populations

As expected, the drought treatment resulted in seedlings with lower total biomass, consistent with other studies (Augustine and Reinhardt, 2019; Kerr et al., 2015). Drought treatment also reduced the root to shoot ratio of seedlings, which is thought to reduce drought-tolerance in the field (McDowell et al., 2008; Padilla and Pugnaire, 2007; Toca et al., 2022). Both root and shoot biomass increased under well-watered conditions, so the increased root to shoot ratio demonstrates that seedlings were investing the “excess” biomass produced in the well-watered treatment more into roots than shoots. Lower root to shoot ratios under drought have been found in other studies of ponderosa pine (Augustine and Reinhardt, 2019; Zhang et al., 1996), and may indicate that preconditioning seedlings to drought could have deleterious effects on their tolerance to desiccation after planting in the field.

On average, seedlings had greater instantaneous water use efficiency under drought than when well-watered, demonstrating the isohydric nature of ponderosa pine to adjust stomatal conductance when water is limited (Augustine and Reinhardt, 2019; Koepke and Kolb, 2013; Stout and Sala, 2003). The fact that only one seedling died in the drought treatment before watering ceased demonstrates the high resistance ponderosa pine has to drought. This also indicates that the drought treatments imposed in this study may not have been severe enough to fully test the range of adaptations to drought across the populations and mother trees studied. However, several differences were still apparent.

Population climate

Seed mass was significantly different between populations, with hot and dry climates producing heavier seeds. This variation in seed mass is likely what drove most of the differences between populations in other traits, as seed mass was highly correlated with all size-related traits, including total biomass, root biomass, shoot biomass, stem length, and cotyledon length. Variance in seed mass between populations and mother trees may be due to environmental conditions during cone initiation and seed production (i.e., maternal environmental effects), or could be genetically determined. Several studies of conifer species have also found that variation in germination and early seedling growth traits is correlated with variation in the maternal environment (Cendán et al., 2013; Johnsen et al., 2005), often due to variation in seed provisioning under variable climate conditions (Borgman et al., 2014; Tíscar and Lucas, 2010;

Vivas et al., 2013; Zas et al., 2013). We were unable to disentangle genetic vs. maternal environmental effects on seed mass in this study, as we only collected seeds from one year. However, another study on two sympatric pine species found significant variation among years in seed mass (Borgman et al., 2014) making it likely that both genetics and maternal environmental effects were at play here as well.

Seeds from hotter and drier populations were not only larger, but also germinated faster and had more successful germination, which is consistent with other studies (Cendán et al., 2013; Gao et al., 2021; McLane and Aitken, 2012; Warwell and Shaw, 2019; Weber and Sorensen, 1992). These larger seeds also grew into seedlings with more total biomass and lower root to shoot ratios. Contrary to our study, other studies have found that ponderosa seedlings from drier sites grow more slowly and are smaller (Dixit and Kolb, 2020; Kerr et al., 2015) or that population climate is less important than mother tree traits in predicting growth and biomass (Kolb et al., 2016). Interestingly, the root to shoot ratios of seedlings from hotter and drier climates were reduced, indicating that seedlings from bigger seeds invested more of their initial resources into shoot growth rather than root growth. This is an indication that using seeds from hotter and drier seed sources in reforestation may come with increased risk of desiccation in the field, as taller seedlings tend to have greater mortality (Grossnickle, 2012; Hill and Ex, 2020).

The reduced instantaneous water use efficiency of seedlings from hotter and drier climates under relatively well-watered conditions (July) was also contrary to previous studies that have found seedlings from drier sites to exhibit greater water use efficiency (Dixit et al., 2022; Marias et al., 2016) or no difference (Cregg, 1994; Maherali et al., 2002; Zhang et al., 1997; Zhang and Marshall, 1995). However, other studies have documented reduced carbon discrimination (and therefore increased water use efficiency) for tree populations from higher elevations, potentially due to reductions in stomatal conductance under low temperatures and/or differences in leaf structural traits that increase photosynthetic capacity and increase internal resistance to conductance (Balekoglu et al., 2023; Dixit et al., 2021; Hultine and Marshall, 2000; Marshall and Zhang, 1994). The fact that water use efficiency was the greatest in seedlings from sites with both low CWD and low monsoonality suggests that harsh site conditions may be driving this more conservative water use strategy. Alternatively, seedlings from hotter and drier climates may be more plastic and better able to take advantage of the available water that was still present at the July measurement. This was also the case with total biomass, as seedlings from hot and dry populations had greater increases in biomass with watering than seedlings from cold and dry populations. Increased stomatal conductance can also cool leaf surfaces in the face of heat stress (Marchin et al., 2022), so seedlings from hotter sites may be adapted to increase conductance when water is available. In contrast to the difference in biomass accumulation plasticity, there were no population differences in how much seedlings adjusted iWUE when the drought treatment intensified, indicating that all populations were equally plastic when it comes to stomatal closure under water limitations.

The fact that germination success was highest in populations from hotter and drier climates bodes well for the future of ponderosa pine management, as seeds from hotter and drier populations are likely to be used more in out-planting for climate-adaptive restoration (Rehfeldt et al., 2014b). Although speedy germination increases the risk of damage from late spring frost events, it has been shown to increase seedling success overall (Bianchi et al., 2019; Warwell and Shaw, 2019). Seeds from colder and wetter populations took longer to germinate and were less successful, but also had greater proportions of their biomass stored in the roots, which may be beneficial in stressful environments (Grossnickle, 2012). Since germination was defined in this study as the day in which the seedling emerged aboveground, it is possible that seedlings that were recorded as germinating later had already started accumulating significant biomass belowground before breaking the surface. Although it was a flaw in the execution of our study, it was interesting to note that seeds in racks closer to the swamp cooler in the greenhouse took longer to germinate, and this was exacerbated for seeds from cool and wet populations and from mother trees that are more responsive to their environment (low carbon discrimination and high climate-growth sensitivity). This un-planned observation hints that cooler and wetter populations may be producing seeds with more plasticity in germination time (Cagnacci et al., 2020; Fernández-Pascual et al., 2021; Weber and Sorensen, 1992), and that mother tree plasticity in growth and gas exchange may translate to other types of plasticity in seedlings. Differences in the germination environment could be explored more to further test these patterns.

Mother tree traits

Interestingly, variance in traits related to germination rate and speed of germination was more attributed to mother tree identity than population, but significant mother tree trait patterns were only found for germination rate. This implies that other mother tree traits may be more important in driving germination speed than the ones measured here. As discussed above, the maternal environment has been shown to influence seedling traits, not only through seed provisioning but also through epigenetic pathways which alter gene expression in the progeny, rather than the genetic code (Vivas et al., 2013). Given that seed mass was only moderately correlated with germination speed (Pearson correlation coefficient = -0.50), other maternal effects may be influencing this trait. There was also a lot of residual variation in germination speed that was not due to population or mother tree, demonstrating high individual variability in this trait as well.

Seed and seedling mass increased with increasing mother tree carbon discrimination (Figure 3.6). This may be indicative of the tradeoffs that ponderosa pine is known to experience between growth, defense, and reproduction (Gonzalez, 2022; Pearse et al., 2016). Greater carbon discrimination indicates that mother trees were either not adjusting stomatal openings very much to reduce water loss during drought, or that they did not experience much water scarcity over the last 21 years, which would allow them to photosynthesize more and invest more carbon in reproduction. However, mother trees that had lower carbon discrimination, and therefore a more conservative approach to water use, may have had less carbon available to invest in making large seeds. The fact that population CWD and mother tree carbon discrimination significantly influenced seed mass separately indicates that a gradient exists within sites in the types of seeds and seedlings that will result from individual mother trees. Effects on germination rate appeared to be separate to those on seed mass, where increased carbon discrimination only increased germination rate in mother trees that were insensitive to climate. Reduced growth-climate sensitivity could also indicate a tree that is not conservative in resource use and was therefore able to produce more viable seeds. Therefore, it may be important to target certain mother trees for cone collection, even within targeted populations.

In contrast to our hypothesis, mother tree carbon discrimination was not indicative of seedling instantaneous water use efficiency, or its plasticity in response to drought, as there were no significant effects of mother tree carbon discrimination on gas exchange measurements. This, combined with the fact that mother trees from cooler and wetter areas had higher carbon discrimination, indicates that mother tree carbon discrimination values were likely more affected by site water availability than the individual tree’s inherent physiological response to drought

(Tei et al., 2019). Dixit et al. (2022) found that carbon discrimination values from seedling needles were more indicative of seedling water use efficiency than “snapshot” measurements of gas exchange at one point in time. Therefore, it is also possible that our measurements of iWUE may not represent the full picture of seedling water use efficiency. There is a limited number of studies on the physiology of ponderosa pine new germinants (0-16 weeks old), and only one to our knowledge that has investigated multiple populations (Kerr et al., 2015). It has been suggested that seedlings may prioritize short-term gains in biomass accumulation over long-term investments in drought-adaptive traits early in their life, despite risking cavitation (Augustine and Reinhardt, 2019; Bond, 2000), which may also be why we did not see a relationship between mother tree carbon discrimination and seedling gas exchange at the early life stage examined here.

Mother tree growth-climate correlation, unlike carbon discrimination, was not correlated with seed mass, but was still significant in predicting seedling gas exchange traits, indicating a pathway other than seed provisioning for the influence of maternal family on progeny. Mother trees that had higher correlations between growth and climate tended to produce seedlings with greater instantaneous water use efficiency in July, but lower water use efficiency in October (Figure 3.7). A strong correlation between growth and forest drought stress index indicates greater mother tree plasticity in response to drought, as these trees reduce growth when water is scarce. It is also a more conservative water-use strategy, which is also represented by higher seedling instantaneous water use efficiency in July. It is unclear why this trend switched direction in October, as gas exchange in October was only analyzed for seedlings in the watered treatment so moisture conditions should have been similar to July. This did produce a lower sample size however, which may have influenced the results. Temperatures in the greenhouse may have also gotten slightly colder as fall started, which could influence seedling water use efficiency trends. Similar to population effects, the lack of interaction between treatment and mother tree traits indicates that plasticity in response to drought does not differ systematically with carbon discrimination or growth-climate correlation.

Caveats

The results of this study are directly applicable to growing seedlings in a greenhouse for out-planting but may not accurately reflect differences between seedlings grown in natural environments under different soil moisture conditions. Conetainer dimensions likely altered seedling structural characteristics in ways that would not manifest in the field and could be important for seedling drought-adaptation. Secondly, the drought treatment in this study may not have been severe enough for drought-adaptive traits to fully be expressed in seedlings. The fact that gas exchange was not affected by the drought treatment after 4 weeks indicates that watering to 60% of evapotranspiration was still plenty of water for ponderosa pine seedlings to continue functioning as normal. The effects of the drought treatment may have been greater if we had droughted the seedlings more severely for a longer time or implemented periods of no water followed by watering to measure the seedlings' ability to recover from complete dry down. Maherali (2002) also found limited effects of drought on biomass and hydraulic traits in ponderosa pine and attributed this to their drought treatment being too short or not severe enough. This study provides important insights on ponderosa pine in its first four months of life, but it is also possible that drought adaptations develop later in the seedling's life, after it has survived its first growing season (Bond, 2000). Therefore, integrated studies that follow seedlings from germination through several years of life will be most informative for whether drought adaptations will be expressed in the long-term.

Implications

This study demonstrates that certain traits of out-planted seedlings can be selected for by collecting seeds from particular populations and even mother trees. Seeds from hot and dry climates tend to be bigger and produce larger seedlings, with greater total biomass and less investment in roots relative to aboveground biomass. However, we also demonstrated that the conditions under which these seedlings are grown in the greenhouse before out-planting can be highly influential as well, with implications for the ability to increase out planting success by drought conditioning seedlings in the greenhouse (Sloan et al., 2020). Droughting seedlings can reduce total biomass overall and investment in roots, compared to shoots. Planting seedlings from hotter and drier climates than the planting site has been recommended to "pre-adapt" a site to future warmer temperatures, as it is assumed that these seedlings possess local adaptations to hot and dry conditions (Aitken and Bemmels, 2016; Rehfeldt et al., 2014b). We did not find any strong indication that seedlings from hotter and drier climates were more drought-adapted, in line with the findings of a recent attempt to operationalize assisted gene flow in the field (Young et al., 2020). Seedlings from colder and wetter climates increased their water use efficiency just as much as those from hotter and drier climates during drought and had higher root to shoot ratios than seedlings from hot and dry climates, which could indicate increased ability to deal with water stress (Grossnickle, 2012). In addition, there was no difference in populations in how long they were able to survive without water. The greater biomass of seedlings from hot and dry climates could also hinder their success after out-planting, as studies have shown that taller seedlings have lower survival in the field (Grossnickle, 2012; Hill and Ex, 2020). The one favorable trait that seedlings from hotter and drier populations possess is that their rates of germination were higher, and they germinated more quickly. Therefore, they should have more success in greenhouse growing operations. Warwell and Shaw (2019) also found that fitness in the field was higher for ponderosa pine seeds that emerged earlier in the spring, so this trait may be advantageous in natural settings as well.

Our study also confirms the findings of others that there is a lot of seedling trait variation attributed to mother tree identity, regardless of population (Kolb et al., 2016; Maherali et al., 2002; Warwell and Shaw, 2019). However, the mother tree characteristics that we investigated (growth sensitivity to climate and carbon discrimination) had only marginally significant relationships with seedling traits and may be hard to select for. Measuring carbon discrimination in trees requires testing field samples in a lab, which can be lengthy and expensive for managers. Correlations between growth and climate variables requires that a tree core is collected and processed with standard dendrochronological techniques, which also requires time and expertise. Therefore, it may be beneficial to identify other proxies or correlated traits that are more easily measured on mother trees that are candidates for cone collection. Seed mass was correlated with mother tree carbon discrimination, population climate, and a host of seedling traits, and therefore may be a reliable and easy to measure indicator of potential seedling success. Also, given the lack of strong associations between our measures of plasticity in mother trees and corresponding measures of drought tolerance in seedlings, it may be a better strategy to collect seeds from a wide variety of mother trees at each site, to capture as much genetic variation as possible. Similar to Kolb et al. (2016), we demonstrated that individual maternal seed sources can be significant drivers of variation in germination, seedling growth, and structural traits, in some cases more so than local adaptation to the seed source climate. However, the connection between these trait differences and drought survival remains unclear and increasing drought stress is not the only hurdle that trees will face in the future. Increasing heat and CO_2 in the atmosphere are also likely to affect ponderosa pine's ability to withstand drought (Callaway et al., 1994; Marias et al., 2016), and adaptations to heat extremes and carbon fertilization should also be considered in seed source selection. This study was one of the first to our knowledge to investigate mother tree traits in relation to seedling traits and thus, it would be beneficial to continue studying the links between mother trees and seedlings by investigating other traits and possible epigenetic effects across different seed production years.

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CHAPTER 4

MICROSITE CONDITIONS AND PLANTING METHOD MEDIATE EARLY SUCCESS IN ASSISTED GENE FLOW OF PONDEROSA PINE IN THE COLORADO FRONT RANGE

INTRODUCTION

In the western US, recent fires have been burning at increasingly high severities (Fornwalt et al., 2016; Keyser and Westerling, 2019; Parks and Abatzoglou, 2020), leaving large patches of the landscape far from viable seed sources (Chambers et al., 2016) and prompting managers to prioritize areas for reforestation (Stevens et al., 2021). Several recent studies have documented reduced tree regeneration at low elevations and latitudes due to climate warming shifting these areas outside of the climatic conditions suitable for tree growth (Chambers et al., 2016; Davis et al., 2019a; Dodson and Root, 2013; Feddema et al., 2013; Kemp et al., 2019; Woolman et al., 2022). With the rapidly warming climate and increased probability of future high severity fire in the western US (Keyser and Westerling, 2019), managers are likely to face more areas in need of reforestation and harder decisions concerning whether the climate is still suitable for the pre-fire species and if so, which seed sources should be used to ensure maximum restoration success in the future climate.

One key consideration that has risen to the forefront of reforestation efforts is the potential for assisted gene flow (aka assisted population migration), or the movement of locally adapted populations of a species to areas within its range that will become suitable for their adaptations in the future under accelerated climate warming (Dumroese et al., 2015; O’Neill and Gómez-Pineda, 2021; Palik et al., 2022; Ste-Marie et al., 2011). In this way, genotypes that are pre-adapted for future climate conditions may be added to the gene pool to enhance forest resilience under climate change (Aitken and Bemmels, 2016). For species with widespread distributions, it is common for populations to have different tolerances for and adaptations to the particular environment that they inhabit, known as local adaptation (Kawecki and Ebert, 2004; Savolainen et al., 2007). Even in areas where the future climate is predicted to remain suitable for a wide-ranging species, locally adapted populations are likely to become maladapted as climate change progresses (Maguire et al., 2018; Rehfeldt et al., 2014c). So far, recommendations for assisted gene flow of trees in the western US have included moving populations from hotter and drier sites to cooler and wetter sites, in anticipation of warming and exacerbated drought in the future (Aitken and Bemmels, 2016; Rehfeldt et al., 2014b).

There are still many uncertainties in how assisted gene flow and other types of assisted migration will work in the field. Tests of assisted migration in trees have been mixed, with some studies finding evidence that growth and survival increase at and beyond the upper elevational/latitudinal range limit, while others find evidence for continued growth and survival optimums near the center of the range (Park and Rodgers, 2023; Pedlar et al., 2023). Most studies are consistent in showing greater growth of populations from warmer climates compared to those from cooler climates when transferred to cooler climate zones (Martínez-Berdeja et al., 2019; Park and Rodgers, 2023; Sáenz-Romero et al., 2021). In some cases, planted seedlings experience a tradeoff between height growth and survival in new environments, especially for species that are planted outside of their current range limits (i.e., assisted range expansion or assisted species migration) (Muller et al., 2019). There has been some evidence for populations from lower elevations and latitudes containing greater drought and heat tolerance than those from high elevations (Dixit et al., 2021; Young et al., 2020), but this remains to be seen on a large scale. Precipitation environments, microclimate conditions, biotic competition, and herbivory have significantly influenced the outcome of many assisted migration field trials, highlighting the importance of microclimate and biotic factors in the success of these operations (Dixit et al., 2021; Martínez-Berdeja et al., 2019; McLane and Aitken, 2012; Muller et al., 2019; Palik et al., 2022; Sáenz-Romero et al., 2021).

It is still unknown how much the maternal origin of seeds will contribute to the success of seedlings in assisted gene flow efforts. Differences between populations in growth and development of trees in the western US have been well-documented (Kerr et al., 2015; Marias et al., 2016; Rehfeldt, 1993, 1991, 1990, 1986; Zhang and Cregg, 2005), but studies have also found significant amounts of variation in seedling drought-adaptive traits between seedlings from mother trees within the same population (Kolb et al., 2016; Maherali et al., 2002; Warwell and Shaw, 2019). Understanding the amount of variation in adaptation between seedlings in the same population gives managers an idea of the amount of variability within a site's seed collection. Given the trend towards more extreme climatic events, planting seedlings with a range of adaptations may help ensure future population resilience to late spring and early fall frosts, extreme drought, and other anomalous weather events (Buma et al., 2017; del Campo et al., 2020; Germain and Lutz, 2020; Lisella et al., 2022).

In this study, we assess the potential for assisted gene flow of interior ponderosa pine (*Pinus ponderosa var. scopulorum*; hereafter "ponderosa") in the Colorado Front Range by tracking the survival and growth of seedlings from disparate populations and individual mother trees across a range of post-fire microclimate conditions in the field. Suitable habitat for ponderosa pine is projected to decline in area and increase in elevation in the future (Kemp et al., 2019; Rehfeldt et al., 2014a, 2006). When intraspecific variation in local adaptation is considered, these estimates become more dramatic (Maguire et al., 2018; Rehfeldt et al., 2014b). Therefore, assisted gene flow has been proposed as a way to pre-adapt ponderosa pine forests to future climate conditions (Rehfeldt et al., 2014b). Here we test ponderosa pine seedling growth and survival after sowing seed and planting one-year-old seedlings in the field after wildfire. Planting greenhouse-grown seedlings represents how reforestation is typically performed while sowing seeds reflects a more natural establishment environment for ponderosa pine. The goals of this study were to 1) determine whether seedlings of ponderosa pine from populations with different climates and mother trees with different traits respond differently to post-fire microclimate conditions within and at the upper elevational margin of ponderosa's range and 2) evaluate differences in post-fire reforestation success between sowing seed vs. planting seedlings. We hypothesized that seedlings from hotter and drier climates would perform best in hotter and drier microclimates within ponderosa's range and that seedlings from cooler and wetter climates would perform best at the upper elevational range margin under cooler and wetter conditions. In addition, we hypothesized that seedlings from mother trees with drought adaptations would grow and survive better in drier microclimates. Provenance trials so far have been lacking tests at the margins of species climatic niches (Park and Rodgers, 2023); thus, this study provides important information about both planting at and collecting seeds from sites at the range margins of ponderosa pine in Colorado.

METHODS

Seed collection and mother tree traits

The same cone collections used in Chapter 3 were used for this study (Table 4.1). Therefore, all population climate traits (CWD and monsoonality) and mother tree traits (growth-climate correlation and carbon discrimination) were calculated using the same methods as Chapter 3. For this study, 5 mother trees from each population were tested, except for one population for which only 4 mother trees were tested (Table A4.1, Appendix 4). We tried to use as many of the same mother trees from the greenhouse experiment in this study as possible, but substituted other mother trees if we did not have enough seeds. Seeds were counted out for sowing and an average seed weight was obtained for each mother tree by dividing the total weight by the number of seeds counted (n = [592, 740]).

Table 4.1: Characteristics of the eight populations where cones were collected and the two field sites where the experiment was performed (mid-elev & high-elev). Climatic water deficit (CWD) and actual evapotranspiration (AET) are 30-year normal values covering the period 1981-2010. The distance from the mid elevation site to each population was used in the distance calculation.

Population code	Latitude	Longitude	Elevation (m)	30-year normal CWD (mm)	Proportion monsoon	Distance from experiment (km)
FC	40.69161	-105.338	1760.8	179.2	0.31	16.5
BD	40.625	-105.337	2477.5	101.8	0.30	16.0
BH	40.05141	-105.514	2828.7	57.9	0.29	66.1
LH	40.01375	-105.304	1747.0	215.9	0.26	72.7
HM1	39.18307	-105.167	2188.6	134.5	0.37	165.4
HM6	39.03066	-105.015	2821.7	15.1	0.37	184.7
WT2	38.19893	-105.091	2059.9	272.1	0.36	274.3
WT1	38.1085	-105.136	2843.0	62.0	0.38	283.8
Mid-elev	40.64676	-105.5241	2481.2	148.6	0.30	0
High-elev	40.61697	-105.4973	2706.7	26.5	0.29	4.0

Field Setup

The field experiment was conducted within and around the High Park fire scar in the Canyon Lakes District of the Roosevelt National Forest. The High Park fire burned 36,733 hectares in June of 2012; 21% burned at high severity. Two sites were established in spring of 2020, with the intention of one site firmly within the range of ponderosa pine in this area and one at the upper elevational limit of ponderosa pine's current range. To determine the current upper elevational range of ponderosa in the study area, we used the "Existing Vegetation Type" spatial product from the Landscape Fire and Resource Management Planning Tools program (LANDFIRE, 2016) and clipped it to the extent of the High Park Fire. We then extracted the highest elevation from within any area with ponderosa pine listed as one of the resident species. We found the minimum elevation of ponderosa in this area to be 1589 m and the maximum to be 2801 m, with a 97.5% quantile of 2472 m in elevation. These data, combined with accessibility considerations, guided our site selection. We established a "mid elevation" site at 2481 m in elevation, which is within but towards the upper elevational limits of ponderosa pine's current range in the area, and a "high elevation" site at 2707 m which is near the current upper elevation limit of ponderosa pine in the study area (Table 1). Both sites were on Northwest facing aspects and therefore represent the coolest climate conditions found at these elevations.

Six plots were established in high severity burned areas (100% adult tree mortality within a 5-m radius) at each elevation (hereafter "HH" – high elevation and "MH" – mid elevation). An additional six plots were established at the mid elevation site under partial canopy cover (<10% adult tree mortality in a 5-m radius) to test the effects of canopy buffering on seedling establishment (hereafter "ML"). Each plot consisted of four 1-m^2 quadrats arranged in a grid, two of which were randomly selected to be sown with seeds, and two of which were randomly selected to be planted with 1-year old seedlings the following year. In the first growing season (2020) before seedlings were planted, one of the empty quadrats at each plot was monitored for natural tree regeneration, but none was observed. The quadrats to be sown were cleared of all leaf litter, rocks and logs and gently raked to standardize the ground surface conditions across all plots. The quadrats were divided into two hundred 5 cm x 10 cm cells, and three seeds per cell were sown. A toothpick was placed next to each sown seed to distinguish them from any natural germination in the plot. In addition, we only analyzed survival and growth of seedlings that germinated during the first summer that seeds were sown (2020) to further ensure no natural germinants were mistaken for artificially sown seeds. Each mother tree was randomly assigned 10 adjacent cells within one of the two quadrats at each plot, for a total of 30 seeds sown per mother tree per plot. Some mother trees had limited seed quantities and thus had fewer seeds sown (Table A4.1, Appendix 4). All plots were enclosed within a cage made of hardware cloth to exclude seed predation. The cages were left on for the first two summers (2020 and 2021), with the lid left open during winter 2020 to avoid unnatural snow accumulation effects. Cages were then removed in September 2021 and left off for the remainder of the study. Herbaceous vegetation was weeded out of the plots each summer, to achieve cover of less than 15% in each plot.

Microsite measurements

We measured air temperature every 2 hours at each plot with a Thermochron iButton (iButtonLink, WI, USA) located at the center of the plot beneath a radiation shield attached to rebar and positioned 10 cm above the soil surface. This was done to measure air temperature experienced by small seedlings (Davis et al., 2019b). Canopy openness was measured by analyzing hemispheric photos taken before dawn on October 18th, 2021 with a fish-eye lens camera mounted within a self-leveling mechanism (24MP DSLR Compact OMount, Regent Instruments, Inc., Québec, Canada) and positioned roughly 15 cm above the ground. The photos were analyzed with WinSCANOPY Pro 2016a software (Regent Instruments, Inc., Québec, Canada). A soil sample from 0 – 15 cm depth from adjacent to each plot was collected in August 2021 and analyzed for soil texture, pH, and a suite of nutrients at the Colorado State Soil Testing

Lab. We measured soil percent volumetric water content (%VWC) for each plot with a Hydrosense II handheld soil moisture sensor (HS2, Campbell Scientific) approximately weekly in 2020 (June 2nd - August 6th), five times in 2021 (every 3 weeks from June 3 - September 3), and four times in 2022 (approximately monthly from May 18 - August 11).

Seed sowing, planting, and monitoring

Seeds were subjected to cold stratification before sowing in the field. Seeds of each mother tree were placed in separate mesh bags and covered with vermiculite in a plastic tub with drainage holes. The vermiculite was soaked with water and allowed to drain before being placed in a refrigerator at approximately 2.8°C. The seeds were left in the refrigerator for 31-40 days before being taken out and placed in plastic bags which were brought to the field and stored in a cooler until sowing. The seeds sown at the mid elevation site underwent 31 days of cold stratification and the seeds sown at the high elevation plots underwent 37-40 days of cold stratification. Seeds were sown at the mid elevation site from May 12th – 17th, 2020 and at the high elevation site from May 19th – 21st, 2020 by digging a shallow (3-5 mm deep) hole with a chopstick, placing the seed inside, and gently covering it with soil. All plots were briefly watered immediately after seed sowing, with a fine mist from a backpack sprayer so that their position in the soil was not disturbed.

Plots were watered for the first 6-7 weeks after sowing to simulate an "establishment year" for ponderosa pine, as germination pulses are known to be associated with above-average moisture availability (League and Veblen, 2006; Rodman et al., 2020; Rother and Veblen, 2017; Savage et al., 1996). The first round of watering occurred approximately a week after sowing, with all watering ceasing after July 10th, 2020. The watering scheme employed was modeled after that used by Rother et al. (2015). Plots were watered weekly so that they received precipitation equal to the weekly upper quartile of precipitation in the last 35 years (1985 – 2019) for that month, according to the nearest RAWS weather station at a similar elevation (Red Feather Colorado, elevation: 2499 m, lat: 40.798056, long: -105.572222). To do this, we calculated total precipitation received in the past 7 days before watering by extracting the PRISM daily total precipitation data (4km resolution; PRISM Climate Group) from each site location and summing it over the week. We then subtracted this from the upper quartile of weekly precipitation received in the last 35 years during the same month. We then watered plots to make up this difference. If the precipitation in the last 7 days met or exceeded historical upper quartile weekly precipitation (which occurred the week of June 7-13, 2020), we did not water that week.

The number of seedlings in each cell was recorded weekly from May 27th – August 6th, 2020, and the final count for the season was made on November 19th, 2020. The Cameron Peak Fire ignited on August 13th, 2020 and while it didn't burn our plots, closures prevented us from accessing them between August and November. We took the first height measurements of seedlings at the mid elevation plots on April 30th – May 1st, 2021 and at the high elevation plots on June 9th, 2021. The first season height measurements were made from the soil surface to the base of the cotyledons. Survival was monitored monthly in 2021 and heights were measured again on September 10-17, 2021, this time from the soil surface to the tip of the bud. In 2022, survival was monitored once in the spring (May 18-19, 2022) and once in the fall (September 26-29, 2022) and height from the soil surface to bud tip was measured on September 26, 2022. Greenhouse grown seedlings (approximately 1-year old) were planted in all plots in the spring of 2021, as soon as possible after snowmelt. This resulted in planting the mid elevation site from

May 6-8, 2021 and the high elevation site from May 24-26, 2021. One seedling per mother tree was planted in a randomly assigned position within one of two quadrats at each plot. Seedlings were planted 20 cm apart. The seedlings were grown in the greenhouse at Colorado State University; they were sown between June 10th - July 13th, 2020 in Ray Leach conetainers (SC10R) and were fertilized once per week with 20-10-20 NPK diluted to 125 ppm N from August 10, 2020 to February 24, 2021. The seedlings were then watered with "finisher" fertilizer every 3 days from February 24 – March 5 and put in a growth chamber in which the temperature and photoperiod were gradually lowered to simulate temperatures dropping in the fall. On February 26th, the photoperiod was 13 hours and the temperature was set at 53F at night and 63F during the day. On March 17, the photoperiod was reduced to 12 hours with 48F at night and 58F during the day. On March 23, the photoperiod was changed to 11 hours and temperatures set to 43F at night and 53F during the day. Finally, on April 5th, the temperatures were lowered to 38F at night and 48F during the day with an 11-hour photoperiod and remained at those settings until seedlings were removed for planting. Seedlings in the growth chamber were watered on March 17 and then were not watered again until planting.

Greenhouse seedlings were watered immediately after planting but were not watered again after that. After seedlings were planted, their initial height (from soil surface to bud tip) and diameter at soil surface (using calipers) were measured on June 3rd, 2021. Some seedlings had already died by this date, which we attributed to either outplanting stress or their condition prior to outplanting. Therefore, we did not include the seedlings that were dead at the time of monitoring on June 3rd in any analyses of planted seedling success. Planted seedling survival was monitored monthly during the growing season of 2021 and heights and diameters were measured again in the fall (September 17, 2021). Survival was monitored again in the spring (May 18) and fall (September 29) of 2022 and diameters and heights were measured in the fall as well. To account for the fact that diameter growth in a smaller seedling equates to a smaller amount of biomass accumulation than the same amount of diameter growth in a larger seedling, we used basal area growth as a measure of stem widening in this study. To calculate basal area growth, we calculated the basal area of the seedling (using its diameter) at the earlier time point (e.g., fall 2021), and subtracted it from the basal area of the seedling at the later time point (e.g., fall 2022). Height growth was calculated by taking the height of the earlier time and subtracting it from the later time for both sowed and planted seedlings. For some growth periods, height and basal area growth values were negative (height: n = [6 – 128] sowed, [35 – 194] planted; basal area: [4 – 155] planted), likely owing to measurement error or, in the case of height, changes in the soil surface level due to erosion or wildlife. We eliminated any seedlings that had a negative change in height growth during the period analyzed that was more negative than -1 cm and a negative change in diameter growth that was more negative than -1.5 mm, as these differences were large enough to either be errors or caused by something other than slight measurement differences. We left all other negative height and area growth measurements in the analyses as there were likely errors in the positive direction as well, and therefore eliminating all negative values would bias the data.

Errors in seed sowing at sites MH and ML prevented us from tracking mother trees in the populations WT1 and HM1 at these sites. Therefore, these two populations were removed from any analyses where effects of mother trees were analyzed as fixed in the model and in the variance partitioning analysis. We did, however, leave them in when models only included mother tree as a random effect, and we weren't directly comparing mother tree results because we are confident that the correct populations were sown and that only seeds from one mother tree were sown in each row. Therefore, incorrect mother tree identities in these two populations should not influence the random effect of mother tree greatly.

Analysis - Microsite characterization

We used type-2 ANOVAs to test for differences between sites (HH, MH, or ML) in 18 microsite variables. The variables included were elevation, folded aspect, slope, canopy openness, average, maximum and minimum June daily temperature, average volumetric water content (VWC), soil pH, soil electrical conductivity (mmhos/cm), % soil organic matter, soil nitrate (ppm), soil phosphorus (ppm), soil sulfate (ppm), soil potassium (ppm), and soil % sand, % silt, and % clay. A principal components analysis was also conducted to visualize differences between plots and sites (Appendix 4). Folded aspect was calculated as: 180 - |aspect – 180|, where aspect is in degrees (McCune and Keon, 2002). This makes aspect values range from 0 to 180, with northeast aspects having the lowest values and southwest aspects having the highest values. To summarize temperature variables, we took the mean, minimum and maximum value recorded each day at each plot and averaged this daily summary for each month in each plot. Monthly averages were highly correlated across months and years, and therefore we only used the June monthly average for analysis. We hereafter refer to these mean June daily temperature variables as the minimum, average, and maximum temperatures at each plot for simplicity. Volumetric water content was averaged across all measurements made from 2020 – 2022, as plot VWC values were highly correlated between most sampling times.

Analysis – differences in survival and growth between mother trees, populations, plots, and sites

To assess the relative influences of the nested variables of plot within site (HH, MH, or

ML) and mother tree within population, we fit random effects models and evaluated the proportion of variance partitioned to each random effect. Four models for sowed seedling survival were investigated: sowed seedling survival in the first year (survival of germinants in 2020 to spring 2021), second year (survival from spring 2021 to spring 2022), third summer (survival from spring 2022 to fall 2022), and total (survival of germinants in 2020 to fall 2022). Three models were investigated for sowed seedling height growth: 1) height growth from spring 2021 to fall 2021, 2) height growth from fall 2021 to fall 2022, and 3) total height growth from fall 2020 to fall 2022. For planted seedlings, we investigated 1st year survival, height growth and basal area growth (from outplanting to spring 2022), 2nd summer survival, height growth, and basal area growth (from spring 2022 to fall 2022) and total survival, height growth and basal area growth (from outplanting to fall 2022). Survival models were mixed effect Bernoulli models with binomial error structures, and as such residual variance could not be evaluated. Height and basal area growth models were linear mixed effects models with Gaussian error structures. The variance estimated for each random effect was divided by the sum of the variance estimated for all random effects to calculate the proportion of variance attributed to each random effect specified in the model. For linear mixed effects models, these proportions were calculated without the residual variance to reveal the relative variance estimated for each of the nested effects. Residual variance was then divided by the sum of the variance attributed to all other effects to see how it compared to the variance captured by all other random effects in the model. Variance partitioning analyses revealed that analyzing total survival and growth trends over the duration of the study was representative of the year-by-year trends, and thus we only analyzed total survival and growth patterns for both sowed and planted seedlings in all other analyses. We compared sowed seedling total survival and total height growth and planted seedling total survival, total height growth, and total basal area growth between sites (HH, MH, or ML) using a mixed effects ANOVAs with the measurement as the response variable, a fixed effect of site, and random effects of 1) plot and 2) mother tree nested within population. We then ran another suite of ANOVAs for each site separately with a fixed effect of plot and random effect of mother tree nested within population to determine whether there were significant differences between plots at each site in the measured variables. Finally, we ran an ANOVA with the interaction between site and population as a fixed effect and random effects of plot and mother tree to determine whether there were significant differences in the measured variables between seed source populations at each site. Survival models were mixed effect Bernoulli models with binomial error structures while growth models were linear mixed effects models with Gaussian error structures. When models did not converge with the interaction between site and population (sowed and planted seedling survival models), we ran separate models for each site. If planted seedling models did not converge with the random effect of mother tree, it was removed as there was only one seedling per mother tree planted at each plot. Tukey's HSD tests were used whenever an ANOVA was significant to determine which sites, plots, or populations were significantly different from each other.

Analysis – Effects of seed source and microclimate on seedling survival and growth

To assess the joint effects of seed source and microclimate on seedling survival and growth, models combining 1) population and microclimate variables, 2) mother tree trait and microclimate variables and 3) initial planted seedling size and microclimate variables were evaluated for the measurements of 1) sowed seedling total survival, 2) sowed seedling total height growth, 3) planted seedling total survival, 4) planted seedling total height growth, and 5) planted seedling total basal area growth. First, correlations between 5 microclimate variables (VWC, canopy openness, and min, max, and average temperature) within three site groupings were examined to determine all possible combinations of microclimate variables that did not include variables with a Pearson correlation of absolute value greater than 0.6 (Appendix 4). The sites examined were HH, MH, and the combination of MH and ML. The MH+ML grouping was always used unless ML plots were unable to be analyzed due to low sample size (sowed seedling height growth only). Site groupings were analyzed separately due to the differences in correlations between microclimate variables in each grouping (Appendix 4). Examination of correlations resulted in 8 model sets for the HH site, 10 model sets for the MH+ML site, and 4 model sets for the MH site (Appendix 4). Each model set contains a unique combination of microclimate variables to use in analyzing survival and germination responses of sowed seeds and planted seedlings.

A suite of candidate models was constructed to assess seed source population climate effects on seedling survival and growth in different microclimates. The two population climate variables (CWD and monsoonality) were included in all candidate models, as well as their interactions with all microclimate variables in each model set and random effects of 1) plot and 2) mother tree nested within population. Survival models were mixed effect Bernoulli models with binomial error structures while growth models were linear mixed effects models with Gaussian error structures. Planted seedling models included additional fixed effects of initial height and diameter at outplanting, which both interacted with all microsite variables in the model subset. For sowed seedling models, candidate models that did not converge were excluded from further consideration. For planted seedling models, if a model did not initially converge, the random effect of mother tree was removed, and the model was run again. If the model still did not converge, it was removed from further consideration. Variance inflation factors (VIFs) and model diagnostic plots were checked for all candidate models and VIFs were confirmed to be less than 3 for all included variables in each model. The remaining candidate models were then ranked according to AICc. All subsets of the model with the lowest AICc were then run using the function *dredge* in the *MuMIn* package in R (Bartoń, 2022) and the model with the lowest AICc was selected for final interpretation and analysis.

The same process as for population models was repeated for mother tree models, with the exception that the three mother tree traits (seed mass, carbon discrimination, and growth-climate correlation) were always included in the candidate models (rather than population variables). In addition, one of the mother trees was excluded from the analysis as the tree died after we collected seeds but before we were able to collect trait information. We also eliminated all sowed seedlings from the populations WT1 and HM1 at the MH and MH+ML sites, since mother tree traits were included in models as fixed effects. The same site groupings and model sets were used for these analyses as for the population analyses (Appendix 4).

Planted seedling initial height and diameter were included in all population and mother tree models to control for the effects of initial size. However, we also wanted to examine the effects of initial size and its interaction with microclimate variables on planted seedling survival and growth. To do this, we used the same process as for population and mother tree models, but only included initial height and diameter as the fixed effects in all models, in addition to their interactions with the microclimate variables in each model set. The same model selection process was used to determine the final best model to interpret trends from.

RESULTS

Microsite characterization

There were no significant differences between sites in soil pH, electrical conductivity, nitrate, sulfate, or phosphorus concentrations (ANOVAs, all $p > 0.2$), but all other microsite variables significantly differed by site (ANOVAs, all $p < 0.04$; Figure 4.1). By design, HH plots were higher in elevation than MH and ML plots. They also had greater slope angles, intermediate canopy openness, lower average temperatures, intermediate maximum temperatures, lower minimum temperatures, higher soil moisture, higher soil potassium, less organic matter, less sand, more silt, and more clay content (Figure 4.1). MH and ML plots significantly differed in canopy openness, temperature variables and soil organic matter. The partial shading at ML plots did not significantly increase average soil moisture.

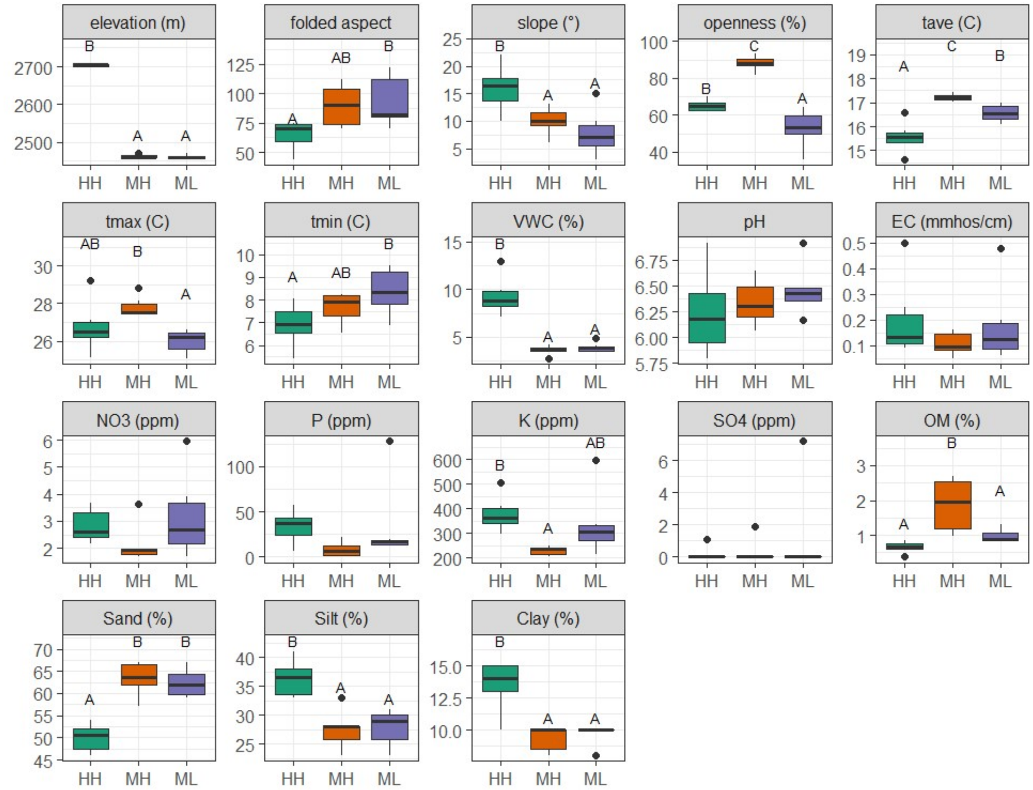

Figure 4.1. Boxplots for all 18 microsite variables (facets) examined across sites. Boxes bound the first and third quartiles of the data and the horizontal line shows the median. The vertical lines show the lowest and highest observations within 1.5 × IQR (inter-quartile range) of the first and third quartiles and points represent outliers. Plots with letters indicate variables that were

significantly different between sites, where sites that do not share a letter are statistically significantly different from each other.

Variance Partitioning

The relative proportions of variance attributed to mother tree, population, plot, and site for overall sowed seedling survival throughout the duration of the experiment strongly resembled those of the model for first year survival (Figure 4.2), indicating that the first year was most important in determining survival of sowed seedlings. Overall, plot contributed most to the differential survival of sowed seedlings across all time periods (61 – 80%), with mother tree identity growing in importance in years 2 (25%) and 3 (20%). In contrast, population contributed very little to variance in survival of sowed seedlings (0 – 7%). There was much more variance attributed to seed source population in sowed seedling height growth (3 - 62%), and little attributed to plot overall (12%).

For planted seedlings, variance partitioning for survival through the duration of the experiment was similar to variance partitioning of survival in the first year, where most of the variance in survival was due to plot (90 – 100%). Variance in height and basal area growth of planted seedlings was most attributed to site (23 – 74%) and plot (21 – 48%) conditions, with little variance attributed to mother tree (0 – 8%). Like sowed seedlings, population was more important for growth (4 – 31%) than survival (0 – 1%) of planted seedlings. It is important to note that residual variance in all growth models was equal to or greater than the variance attributed to all factors combined in these analyses, indicating that there was substantial individual-level variation in how much seedlings grew in this experiment.

Sowed Seedling Survival and growth

Survival of sowed seedlings was overall high throughout the experiment. However, there were significant differences between sites (ANOVA, df = 2, p < 0.001), plots (ANOVA , df = 5,

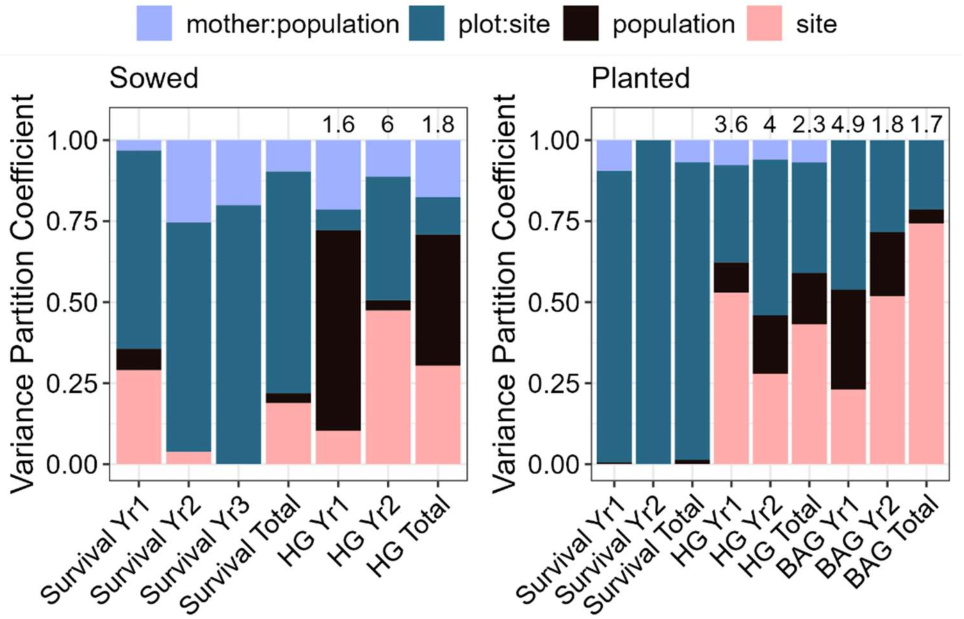

Figure 4.2. Relative variance estimated for nested random effects in random effects models for **left)** sowed and **right)** planted seedlings. The x-axis displays the model's response variable (HG = height growth, BAG = basal area growth) and the y-axis shows the relative proportion of variance attributed to each random effect. Residual variance (where applicable) is shown as a proportion above the relevant measurement, calculated by dividing total residual variance by the sum of the variance attributed to all other effects. Therefore, a value of 1 would mean that residual variation was equal to the amount of variation partitioned to all other variables. Survival models do not have residual variance as they were fit with Bernoulli models.

p < 0.001 for all sites) and seed source populations (ANOVA, HH: df = 7, p < 0.05, MH: df = 7, p = 0.2, ML: df = 7, p = 0.9) in total survival (Figure 4.3). Overall survival was highest at the HH site (75%), lower at the MH site (51%) and very low at the ML site (4.5%). Interestingly, the populations with the greatest survival differed between the HH site and the MH site (Figure 4.3). Few seedlings germinated at the ML site overall (n = 799 total, compared to 2,495 in MH plots and 3,737 in HH plots). At the ML site, plot 2 was the only plot with more than three (n = 26) surviving seedlings by the end of summer 2022, out of the ~1,137 seeds sown per plot in 2020. Interestingly, ML2 was an outlier in terms of soil nutrients among the plots at the mid elevation region (Figure A4.1, Appendix 4), which may be why germination and survival was highest there. Survival of germinated seeds from 2020 to the end of the summer in 2022 was mostly driven by survival in the first summer (2020), which was 88% at HH, 67% at MH and 10% at ML. In all subsequent seasons (winter and summer 2020, 2021, & summer 2022), survival was greater than 71% at each site, and greater than 88% at the MH and HH sites.

Height growth of sowed seedlings from spring 2021 to fall of 2022 was also significantly different between sites (ANOVA, df = 2, p < 0.001), plots within sites (ANOVA, HH & MH: df = 5, p < 0.001, ML: df = 5, p = 0.98), and populations (ANOVA, df = 7, p < 0.001) (Figure 4.3). In addition to having higher survival, seedlings at the HH site also grew more than seedlings at the MH and ML sites. In contrast, height growth differences between plots within each site did not follow the same trends as survival, with certain plots having comparatively low survival, but high growth (for example, MH2). There was not a significant interaction between site and population (ANOVA, df = 14, p = 0.6), indicating that the populations with the greatest growth had consistently greater growth across all sites.

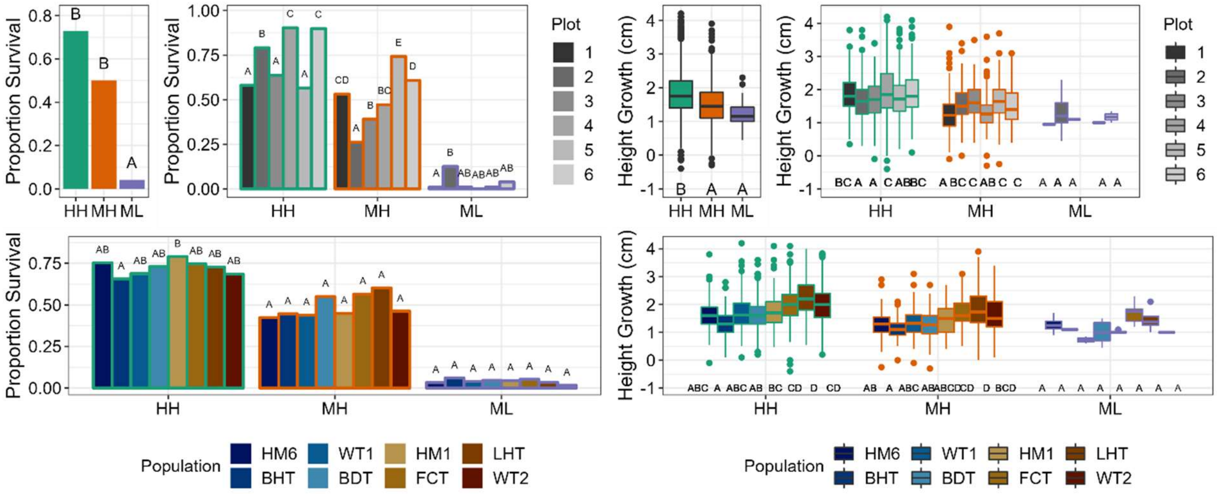

Figure 4.3. Differences between sites, plots, and populations in sowed seedling survival (left) and height growth (right). Letters denote significant differences between sites, plots within sites, and populations within sites, where shared letters indicate a lack of significant difference. Populations are ordered from lowest (dark blue) to highest (dark red) climatic water deficit.

The best population model for sowed seedling survival at the HH site included significant effects of the interaction between 1) plot VWC and population CWD ($z = 2.1$, $p < 0.05$), 2) plot canopy openness and population CWD ($z = 2.4$, $p < 0.05$), and 3) plot canopy openness and population monsoonality ($z = 3.1$, $p < 0.01$). Sowed seedlings at the HH site survived better in plots with greater canopy openness and higher soil moisture, and these trends were stronger for seedlings from sites with greater CWD (hotter and drier) and more monsoonality (Figure 4.4). The best mother tree model for sowed seedling survival at the HH site included significant interactions between 1) plot VWC and seed weight ($z = 2.1$, $p < 0.05$) and 2) plot canopy openness and mother tree growth-climate correlation ($z = 2.4$, $p < 0.05$). The VWC and seed weight interaction mimicked that of the interaction with population CWD, where survival of seedlings from mother trees with larger seeds was even more positively affected by plot soil moisture at the HH site than those from mother trees with smaller seeds. The second significant interaction revealed that seedlings from mother trees more sensitive to climate were more greatly affected by canopy openness at the HH site, than seedlings from less sensitive mothers. The best population and mother tree models for sowed seedling height growth at the HH site did not reveal significant interactions, but height growth was significantly greater for seedlings from hotter and drier populations ($t = 3.5$, $p < 0.05$) and larger seeds ($t = 9.0$, $p < 0.001$).

At the MH+ML site, the interaction between plot VWC and population CWD in predicting sowed seedling survival was significant ($z = -2.5$, $p < 0.05$), but in the opposite direction as at the HH site (Figure 4.5). Seedlings at the MH+ML site from hot and dry populations had worse survival in plots with greater soil moisture. Population models also revealed a significant negative effect of population monsoonality on survival at the MH+ML site ($z = -2.1$, $p < 0.05$), and both population ($z = 5.7$, $p < 0.001$) and mother tree ($z = 4.5$, $p < 0.001$)

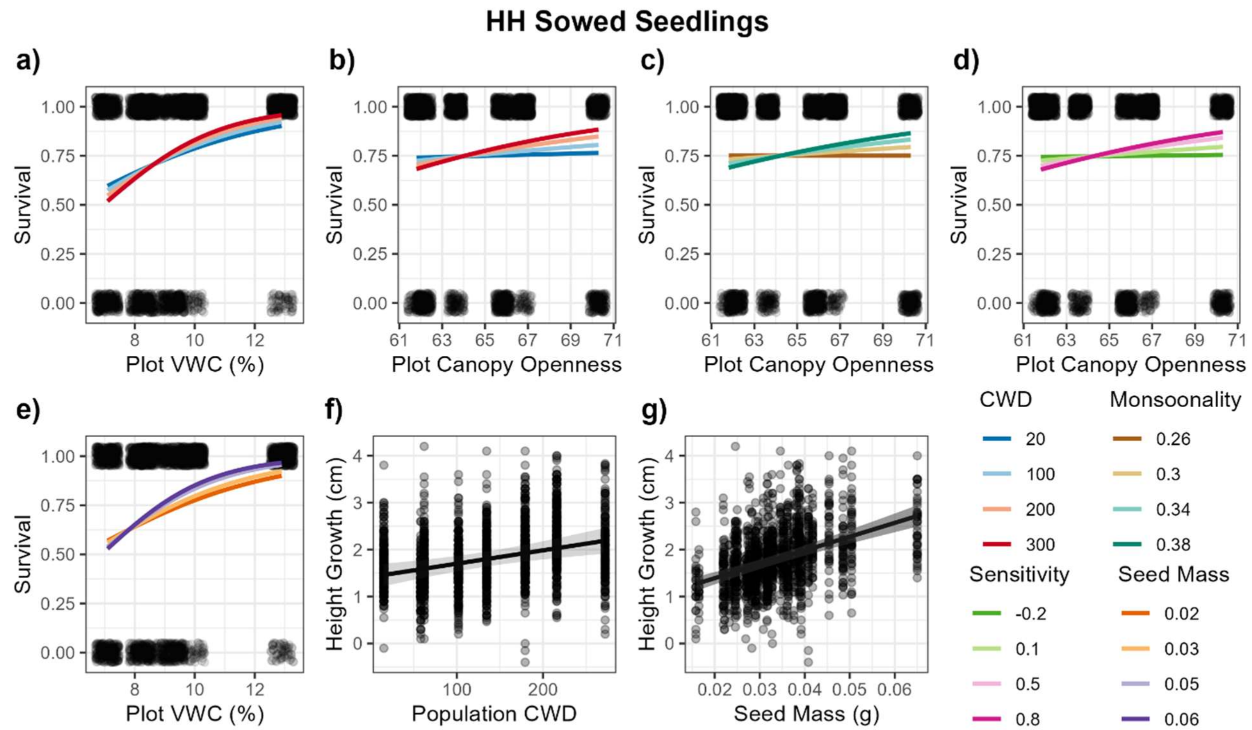

Figure 4.4. All significant ($p < 0.05$) effects of microclimate, population climate, and mother tree traits from models predicting sowed seedling survival and growth at the HH site are plotted. Points represent actual data while lines show model predictions holding all unplotted variables at their mean. Points are jittered in survival plots to better display all data points. When interactions were significant, the predicted trends for four levels of the interacting non-microclimate variable were plotted. 95% confidence intervals are represented with shading where interactions are not present.

models revealed a significant positive effect of canopy openness. There was also a significant positive effect of seed mass on survival at the MH+ML site ($z = 2.0$, $p < 0.05$). Like the HH site, sowed seedling height growth at the MH site was significantly greater for seedlings from hotter and drier populations ($t = 3.4$, $p < 0.05$). There was also a significant interaction between seed weight and plot maximum temperature ($t = -4.3$, $p < 0.001$), where bigger seeds grew more than smaller seeds where temperatures were more buffered (lower maximum temperatures), but height growth was similar in plots with more extreme temperature fluctuations (higher maximum temperatures).

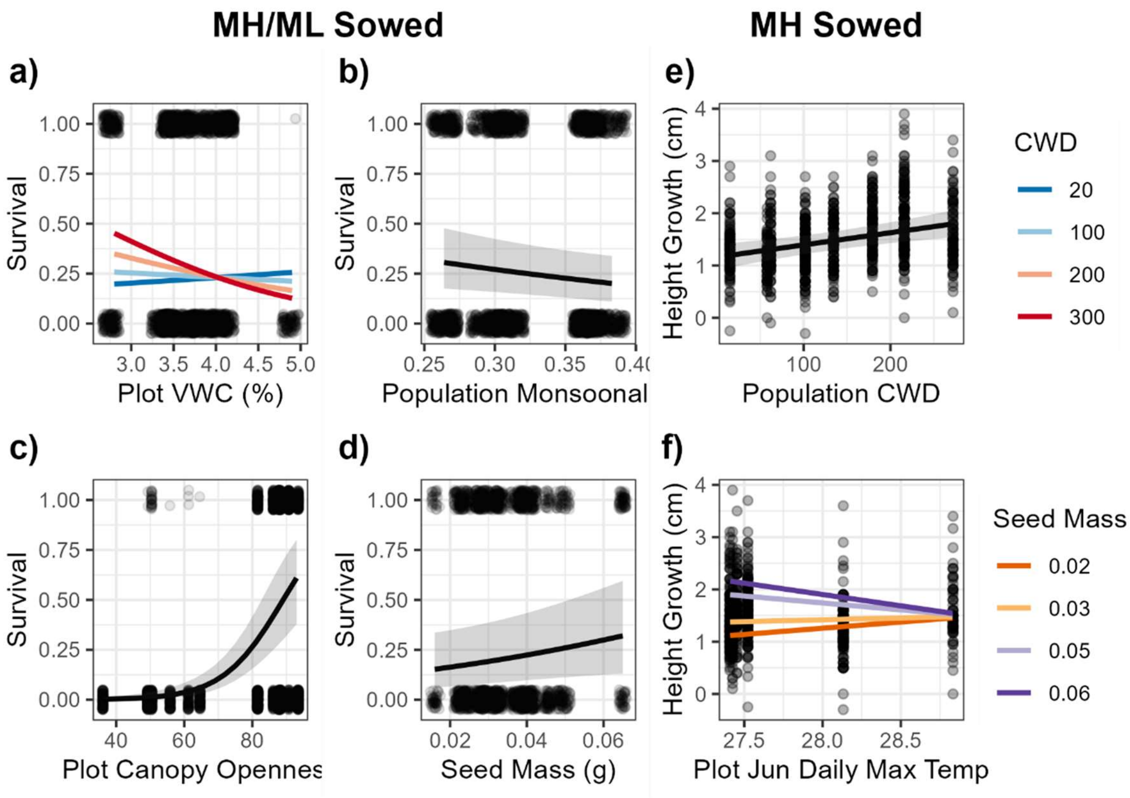

Figure 4.5. All significant ($p < 0.05$) effects of microclimate, population climate, and mother tree traits from models predicting sowed seedling survival and growth at the MH/ML site (a-d) or the MH site (e-f) are plotted. Points represent actual data while lines show model predictions holding all unplotted variables at their mean. Points are jittered in survival plots to better display all data points. When interactions were significant, the predicted trends for four levels of the interacting non-microclimate variable were plotted. 95% confidence intervals are represented with shading where interactions are not present. Canopy openness was significant in both population and mother tree models, but only the predictions from population models are plotted here as the relationships were very similar and the population model included more complete data.

Planted seedling survival and growth

Survival of planted seedlings was not significantly different between sites (ANOVA, df = 2, p = 0.4), but did differ between plots within sites (ANOVA, df = 5, $p < 0.001$ for all sites; Figure 4.6). Planted seedling survival rates were 71% at MH, 63% at HH and 52% at ML. As was the case for sowed seedlings, survival of planted seedlings only significantly differed between populations at the HH site (ANOVA, df = 7, $p < 0.01$), but not at either of the mid elevation sites (ANOVA, df = 7, MH: p = 0.9, ML: p = 0.4). The worst surviving populations at the HH site were from hot and dry populations, whereas the best surviving population was the one from the coldest and wettest population. There were significant differences between height growth of planted seedlings at different sites (ANOVA, df = 2, $p < 0.01$), plots (ANOVA, df = 5, $p < 0.01$ for all sites), and populations (ANOVA, df = 7, $p < 0.01$; Figure 4.6). Height growth in planted seedlings was elevated at the HH site and in general was highest in plots that also had the highest survival for planted seedlings. Tukey HSD tests revealed that differences between populations in height growth of planted seedlings were only significant at the HH and MH sites, with no differences apparent at the ML site.

Basal area growth of planted seedlings was significantly different between sites (ANOVA, df = 2, $p < 0.001$); it was greater at the HH site compared to the MH and ML sites (Figure 4.6). Basal area growth was only significantly different between plots at the MH site (ANOVA, df = 5, $p < 0.001$), where plots that had higher height growth and survival also had higher basal area growth (plots 2 and 5). There was a significant interaction between population and site (ANOVA, df = 14, $p < 0.05$), where populations only significantly differed in basal area growth at the HH and MH sites, and relative rankings of populations were different at each.

The best population model for predicting planted seedling survival at the HH site included a significant interaction between plot maximum temperature and population CWD ($z = 2.1$, $p < 0.05$; Figure 4.7). Seedlings from hotter and drier populations had less survival overall, especially in the colder plots. The best mother tree model for survival of planted seedlings at the HH site included a significant interaction between plot maximum temperature and mother tree climate sensitivity ($z = 3.0$, $p < 0.01$). Planted seedlings from mother trees that are more sensitive to climate had increased survival at plots with higher maximum temperatures, whereas the effect was not as strong for seedlings from mother trees less sensitive to climate. There was also a

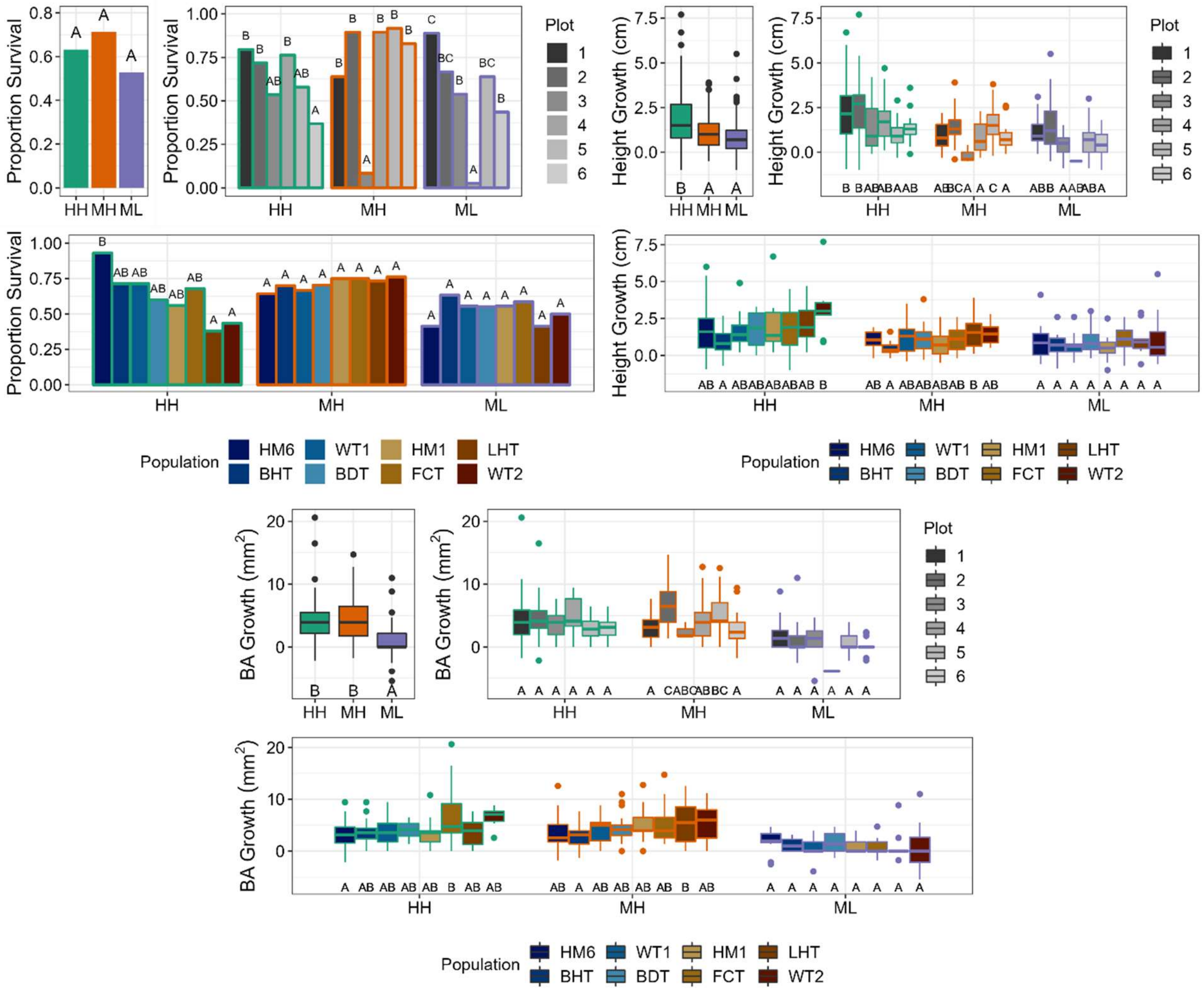

Figure 4.6. Differences between sites, plots, and populations in planted seedling survival (top left), height growth (top right), and basal area (BA) growth (bottom). Letters denote significant differences between sites, plots within sites, and populations within sites, where shared letters indicate a lack of significant difference. Populations are ordered from lowest (dark blue) to highest (dark red) climatic water deficit.

significant negative effect of seed weight (z = -2.1, $p < 0.05$) and a positive effect of minimum temperature (z = 2.1, $p < 0.05$) on survival of planted seedlings at the HH site. Despite the best population and mother tree models for planted seedling height growth at the HH site containing many variables, none were statistically significant in either model. The best population model for planted seedling basal area growth at the HH site included a significant effect of the interaction between population CWD and plot maximum temperature (t = 3.0, $p < 0.01$) where planted seedlings from hotter and drier populations had greater basal area growth in hotter plots whereas seedlings from cooler and wetter populations had less basal area growth overall and were less responsive to plot maximum temperature. The best mother tree model for basal area growth did not reveal any significant effects of mother tree traits nor their interactions with microclimate variables on planted seedling basal area growth.

At the MH+ML site, neither population climate nor mother tree traits interacted with microclimate variables to influence planted seedling survival nor height, likely due to the overwhelming effects of initial seedling size on planted seedlings at this site (see below). The only significant effect identified for planted seedlings at the MH+ML site was the effect of population CWD on basal area growth, where seedlings from hotter and drier climates had greater basal area growth across all microclimate conditions (Figure 4.7).

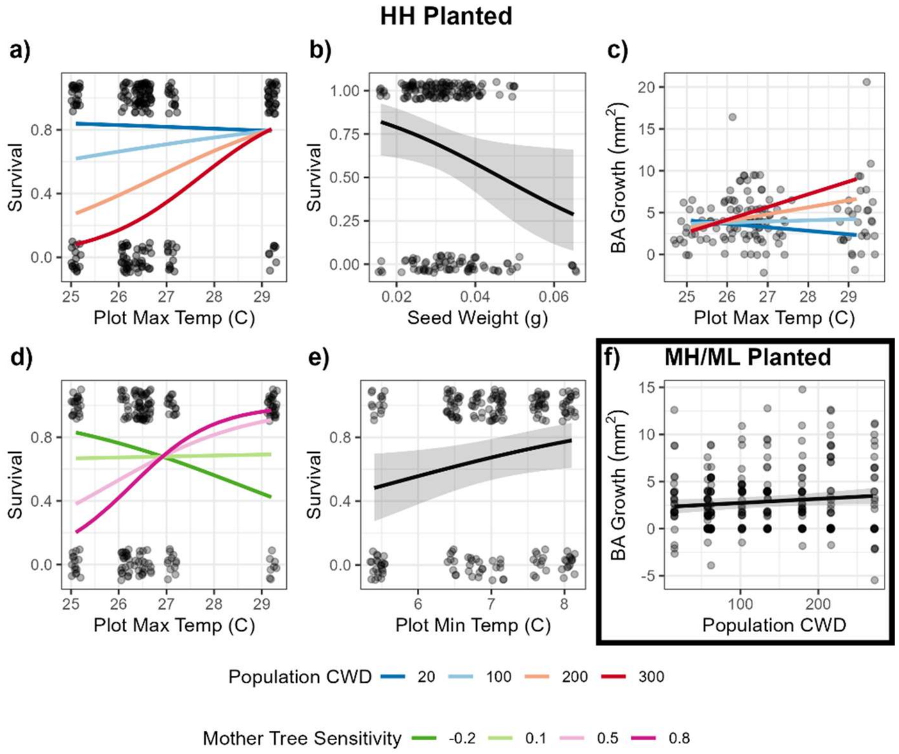

Figure 4.7. All significant ($p < 0.05$) effects of microclimate, population climate, and mother tree traits from models predicting planted seedling survival and growth at the HH site (a-e) and MH/ML site (f) are plotted. Points represent actual data while lines show model predictions holding all unplotted variables at their mean. Points are jittered in survival plots to better display

all data points. When interactions were significant, the predicted trends for four levels of the interacting non-microclimate variable were plotted. 95% confidence intervals are represented with shading where interactions are not present. BA = basal area.

The only significant effect of initial planted seedling size identified for the HH site was the interaction between plot average temperature and initial diameter on survival ($z = 2.0$, $p < 0.05$). Seedlings with larger initial diameters survived better in the hottest plots and survived worst in the coolest plots whereas seedlings with small initial diameters were less sensitive to plot average temperature. At the MH+ML site, there were many significant interactions identified between initial planted seedling size and microclimate that influenced survival and growth. Significant interactions identified in survival models were between 1) maximum temperature and initial diameter ($z = 3.0$, $p < 0.01$), 2) maximum temperature and initial height ($z = -2.5$, $p < 0.05$), and 3) average temperature and initial height ($z = 2.0$, $p < 0.05$). Significant interactions in height growth models were between 1) minimum temperature and initial diameter ($t = -3.2$, $p < 0.01$), 2) minimum temperature and initial height ($t = 3.2$, $p < 0.01$), and 3) average temperature and initial height ($t = 2.0$, $p < 0.05$). Basal area growth was significantly predicted by an interaction between maximum temperature and initial diameter ($t = 5.1$, $p < 0.001$). Seedlings with larger initial diameters had greater survival, height growth, and basal area growth in plots with higher maximum temperatures and lower minimum temperatures. Initially taller planted seedlings had lower survival than those that were initially shorter in plots with more extreme temperatures and that were colder on average. Similarly, height growth for taller seedlings was greater in hotter and more buffered plots and reduced at cooler and more extreme plots.

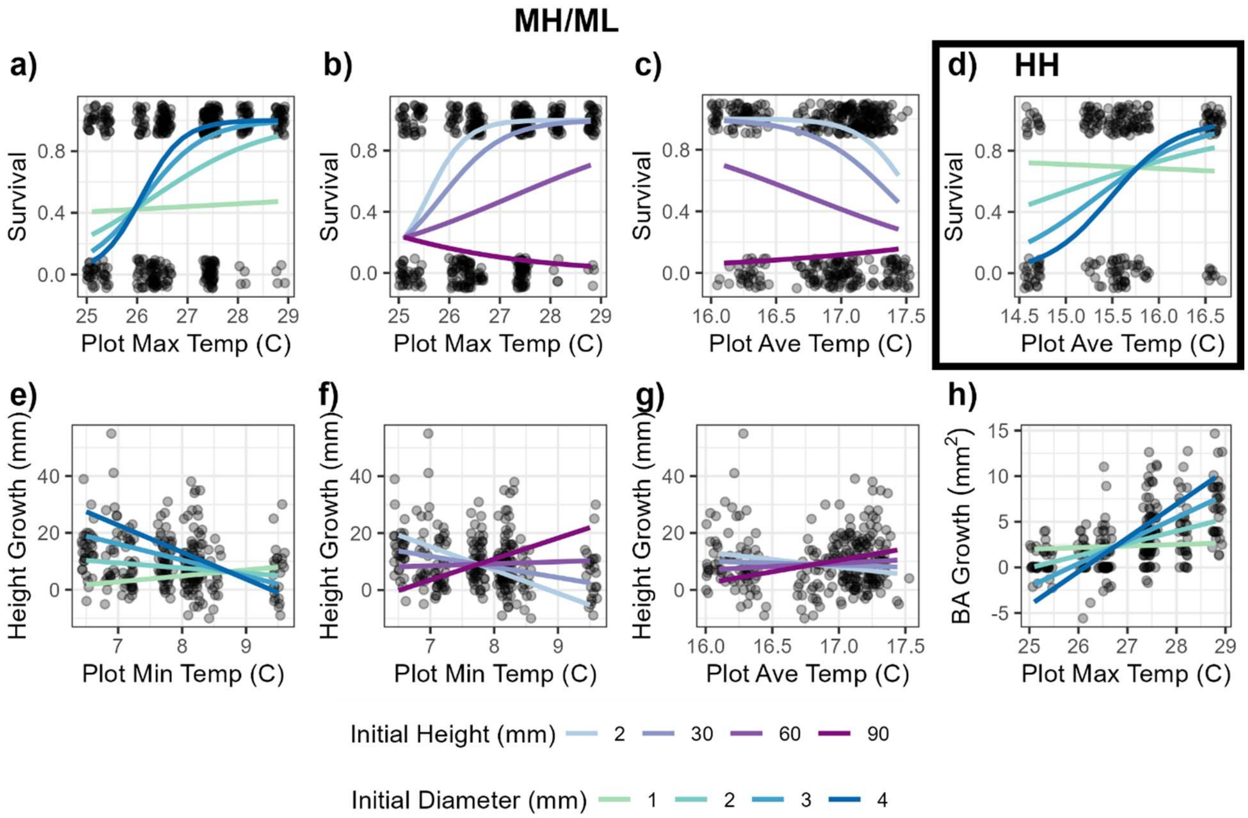

Figure 4.8. All significant ($p < 0.05$) interactions between microclimate and initial planted seedling size from all initial size models predicting planted seedling survival (a-d) and growth (e-h) at the HH site (d) and MH/ML site (a-c, e-h) are plotted. Points represent actual data while lines show model predictions holding all unplotted variables at their mean. Points are jittered in survival plots to better display all data points. When interactions were significant, the predicted trends for four levels of the interacting non-microclimate variable were plotted. 95% confidence intervals are represented with shading where interactions are not present.

DISCUSSION

This study is the only to our knowledge to track both population and mother tree effects on sowed and planted seedlings of ponderosa pine in the field. We found significant influences of elevation and microclimate on seedling survival and growth, which were moderated by seed source population and mother tree identity. Our results indicate that the upper range margin of ponderosa pine is climatically suitable for seedlings with a range of adaptations to different environments, thereby making assisted gene flow a feasible option for reforestation efforts. Differences between sowed and planted seedlings show that success will vary depending on the method of reforestation and the condition of seedlings before planting. It is important to note that since we simulated a wetter than average year for germination of our sowed seedlings, the microclimate trends identified here for sowed seedlings will be most relevant for “establishment years” or those years when spring and early summer precipitation is great enough to elicit abundant ponderosa germination.

Elevation effects

When a species occupies its full climatic niche, fitness is thought to decline towards the range edge where climatic conditions stray farther from optimal (Miller et al., 2020; Pironon et al., 2017). We found that sowed seedling germination, survival, growth and planted seedling height growth were greater at our high elevation site on the range edge than at the mid elevation site within the current geographic range of ponderosa pine. This implies that ponderosa’s current range limits do not align with its climatic niche limits, as has been found for other plant species (Bohner and Diez, 2020; Cagnacci et al., 2020; Gray and Hamann, 2013; Hargreaves et al., 2014; Martínez-Berdeja et al., 2019), likely due to recent accelerations in climate warming. Therefore, planting ponderosa at and beyond the high elevation range margin appears warranted and will likely be successful in sites such as ours with little herbivory and disturbance from small mammals (but see Dixit et al. (2021)). Our results for sowed seedlings imply that climate conditions at the high elevation range margin are also conducive for natural regeneration, but there are many additional barriers to natural establishment of ponderosa pine that we alleviated in our study (e.g., competition from other plants, seed dispersal and predation, and adequate early summer precipitation). Planted seedlings had similar survival rates at the high elevation site as compared to the mid elevation site and had much greater survival than sowed seedlings in partially shaded plots at the mid-elevation site. This suggests that 1-year old planted seedlings are better able to survive under a range of microclimate conditions than seedlings germinated from seed in the field. In our study and in others (Warwell and Shaw, 2019), survival in the first year has been found to be most important in determining longer-term survival, which makes it likely that planted seedlings were not as affected by site conditions because they had already been selected as individuals that survived their first year of growth in the greenhouse.

Our hypothesis that seedlings from cooler and wetter climates would be more successful at the high elevation site was only supported for 1-year old seedlings that were planted, and not for sowed seeds. Planted seedlings from hot and dry populations had lower survival at the high elevation site than planted seedlings from cool and wet populations, and were more affected by plot temperatures, with greater survival and basal area growth in hotter plots. This trend was not found for sowed seeds, which suggests that germinating under field conditions allowed sowed seedlings to survive better than when germinated under greenhouse conditions and planted out. This suggests that either 1) only seeds that would have good survival in the high elevation environment were cued to germinate there or 2) the high elevation environment resulted in seedling growth forms that were more conducive to survival. If 2) is true, phenotypic plasticity in ponderosa pine may be great enough to weaken the effects of local adaptation thus increasing climate change resilience, which has been found elsewhere (Baquedano et al., 2008; Bisbing et al., 2021). Most studies that find increased performance of edge populations at and beyond the upper range edge have used transplants (Hargreaves and Eckert, 2019; Lucas-Borja et al., 2021) and thus our study reveals that local adaptation effects may not be as strong when seeds are germinated in the field.

There was still some evidence for local adaptation in sowed seeds from hot and dry environments, as they were more responsive to microclimate conditions (Figure 4.4), but unlike planted seedlings they had overall greater survival in the high elevation environment. This indicates that germination conditions influence which seedling phenotypes are expressed (Cáceres et al., 2021) and thus conditioning seedlings in the greenhouse before outplanting operations could enhance reforestation success. Current recommendations for assisted gene flow of ponderosa pine involve planting seedlings from hotter and drier populations at cooler and wetter sites, in anticipation of climate warming (Aitken and Bemmels, 2016; Rehfeldt et al., 2014b). Our study shows that if this strategy is employed, survival rates may be lower than average. However, more success may be had if the seedlings are germinated and grown in the greenhouse under conditions that mimic those of the outplanting site so that their initial growth is better able to prepare them for the environment where they will eventually be planted.

Microsite effects

In addition to the effect of site elevation on seedling success, much of the variance in the survival of all seedlings and the growth of planted seedlings was partitioned to plots (Figure 4.2). This provides evidence that microclimate conditions are most important for influencing survival of natural regeneration, and they influence both survival and growth of artificial regeneration. We found that sowed seedling survival was highest in plots with greater canopy openness across both sites. This is in line with natural regeneration studies that have found increased regeneration with reduced adult tree basal area and canopy cover (Malone et al., 2018; Puhlick et al., 2012; Rodman et al., 2020). At the high elevation site, seedlings from populations with hotter and drier climates and greater monsoonality (lower latitudes) were able to take advantage of increased light availability more than seedlings from cooler and wetter and less monsoonal populations and at the mid elevation site, sowed seedlings from hotter and drier climates survived better under drier microclimate conditions. This could indicate that high population CWD and monsoonality promote genotypes that have increased propensity for plasticity in response to microclimate conditions. Indeed, studies of ponderosa and other tree species have found increased plasticity in genotypes from warmer and drier climates (Cooper et al., 2019; de la Mata et al., 2022). However, seedlings from populations with greater monsoonality did worse at the mid elevation site, demonstrating the potential importance of also matching precipitation environments for seedlings success (Warwell and Shaw, 2019).

At the mid elevation site, the trend of increased survival with increased openness was greatly driven by the extremely poor germination and survival in ML plots with greater canopy cover and more buffered temperatures (higher minimums and lower maximums). We initially hypothesized that these microclimate conditions would aid in the survival of ponderosa seedlings at this site by alleviating the stresses of hot and dry conditions (Crockett and Hurteau, 2021; Davis et al., 2019b), but this was not the case. In addition, some of the ML plots had similar or even greater canopy openness values than the plots at the high elevation site, where sowed seedling success was highest. Therefore, it seems that differences exist in how a high density of burned snags (in HH plots) vs. a live tree cluster (in ML plots) influences the regeneration environment. The only significant differences between ML plots and plots at the other sites involved reduced canopy openness and more buffered temperatures for ML plots. Therefore, it is likely that reduced access to light, without concomitant increases in soil moisture were ultimately detrimental to germination and survival. It is also possible that the buffered temperatures resulted in a lack of germination cue, thereby reducing germination of seeds. Interestingly, the ML plot that had the greatest survival across populations had much greater levels of nitrate, phosphorus, potassium, and sulfate in the soil compared to all other plots at the mid elevation site (Appendix 4). Therefore, elevated soil nutrients may partially mitigate the impacts of high canopy cover on ponderosa establishment, as they have been shown to increase regeneration in other studies (Owen et al., 2020; Puhlick et al., 2012). It is possible that the buffered temperatures under partial tree canopies would have been more beneficial for ponderosa seedlings at a lower elevation or more southerly facing site, as other studies have found remaining live trees to facilitate seedling success under drought conditions (Crockett and Hurteau, 2021; Redmond et al., 2018). However, under the moderate climate conditions of our mid elevation site, surviving tree islands after fire are likely critical for supplying seed sources, but more success will be had by seeds or seedlings that are established in high severity burn patches away from tree canopies.

At both the high and mid elevation sites, height growth of sowed seedlings was less influenced by microclimate conditions than survival was, and more driven by population climate. Seedlings from hotter and drier climates grew more than those from cooler and wetter climates at both sites and across microclimate conditions, which is consistent with our greenhouse experiment (Chapter 3) and has been found in other studies and for other tree species (Aitken and Bemmels, 2016; Bisbing et al., 2021; Hancock and Hughes, 2014; Rehfeldt, 1993; Vasey et al., 2023). Height growth of planted seedlings, however, was not affected by population climate, likely because initial height of the planted seedlings was already accounted for and contributed significantly to subsequent growth in the field (Figure 4.8). Planted seedlings from hotter and drier climates did however have significantly greater basal area growth at the mid elevation site and in plots with higher temperatures at the high elevation site, revealing that population effects may persist longer for basal area growth than for height growth. Interestingly, height growth of both sowed and planted seedlings was highest at the high elevation site, which is contrary to other studies of ponderosa pine that found intermediate elevations to have the greatest growth potential (Dixit et al., 2021; Puhlick et al., 2012).

Initial seedling size played a significant role in planted seedling survival and growth at the mid elevation site especially. In mid elevation plots that were not buffered by a partial canopy, seedlings that were initially shorter with larger diameters had greater survival. This is in line with other studies that have found reduced survival of taller planted seedlings due to greater desiccation risk (Chen and Nelson, 2020; Hill and Ex, 2020). Current greenhouse practices are also aimed at producing shorter and larger diameter seedlings for planting at droughty sites, due to their greater success in outplanting (Grossnickle, 2012). The effect of initial size on height growth of planted seedlings was also mediated by plot temperature, where shorter seedlings with larger diameters had comparatively greater height growth in more extreme plots, whereas taller seedlings had more height growth in the buffered plots. This indicates that partial canopy conditions may favor taller seedlings in terms of height growth, but the reduced survival makes this effect practically irrelevant. Interestingly, we did not find many significant effects of initial seedling size on outplanting success at the high elevation site, likely because cooler and wetter conditions reduced the chances of mortality from desiccation. Therefore, there is more leeway for variation in planted seedling size at sites where conditions are cooler and wetter.

Mother tree identity is clearly important in sowed and planted seedling survival as more variance was attributed to it than population in models predicting survival. This reflects other studies of ponderosa that have found significant intrapopulation variation in tree and seedling traits (Ager and Stettler, 1983; Kolb et al., 2016) At the high elevation site, we found that survival of sowed and planted seedlings from mother trees with greater growth-climate sensitivities was more sensitive to microclimate conditions. These results indicate that mother trees with greater sensitivity to climate conditions may produce seedlings that are more responsive to microclimate conditions. Mother trees with greater growth-climate correlations are trees that reduce basal growth when water is scarce and increase basal area growth when water is abundant. This reflects a physiological strategy that is highly plastic and influenced by external conditions. In contrast, mother trees whose growth is relatively uncorrelated with climate conditions may have a more static physiological strategy that remains consistent despite external factors. These same strategies are apparent in the seedlings in this experiment, where seedlings from more sensitive mother trees were able to benefit more when conditions were good, but struggled when conditions were less favorable. In contrast, seedlings from less sensitive mother trees were relatively consistent across microclimates but also less able to take advantage of beneficial conditions. Sowed seedlings from mother trees with greater seed weights also grew taller, which is consistent with our findings from the greenhouse experiment in Chapter 3.

Conclusions

This study reveals that climate conditions at the upper elevational range margin of ponderosa pine in northern Colorado have become more suitable for seedling regeneration than lower elevations previously considered to be the core of the range. Therefore, assisted range expansion efforts are warranted in places where natural regeneration is unable to succeed on its own. Additionally, using seeds from hotter and drier populations for reforestation at the upper range margin may increase success, but only under certain scenarios. Both sowed and planted seedlings from hotter and drier climates had greater growth, which is beneficial for outcompeting other plant species and reaching a fire-resistant size faster. But this will only be beneficial if seedlings survive. When seeds from hot and dry populations were sowed and germinated in the field, they survived well at both sites and were better able to take advantage of beneficial microclimate conditions at the high elevation site. However, when seedlings were planted as 1-year-olds, survival was greatly reduced for seedlings from hot and dry climates. Therefore, planting hotter and drier populations at and above the upper elevational range margin will likely only be successful with specific greenhouse conditioning of seedlings before planting. Sowing seeds has the potential for the greatest success, but only if early summer precipitation is above average or watering is implemented. In general, seed sowing is not employed as a method of artificial regeneration on a large scale due to the greater cost and time investment, and lack of success when broadcast seeding without watering is performed (Rietveld and Heidmann, 1976; Winters and van Diepen, 2023).

Another important finding from this study is that mother tree identity is often more influential in seedling survival than seed source population. Mother trees may be selected for different goals – seeds from less climate-sensitive mother trees are more likely to have consistent survival across different microclimates, whereas survival of seeds from climate-sensitive mother trees is more likely to vary based on microclimate conditions. Overall, differences between populations and mother trees had relatively subtle influences on survival and growth compared to differences in microclimate, underscoring the importance of light availability, temperature, soil moisture, and soil nutrients on ponderosa pine regeneration. Our study indicates that seedling survival in the first 3 years at the upper range limit can be relatively high for seedlings from a range of populations if microclimate conditions are suitable. However, extreme weather events can act as major selecting agents and the short time span of this study was unable to assess differences in resistance to extreme events between populations. This is an important future avenue of study for determining feasibility of assisted gene flow.

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CONCLUSION

This dissertation aimed to understand how lags in tree species range shifts may be overcome through natural disturbances and assisted gene flow. I used a variety of studies, from a large-scale data analysis to a field-based observational study, to experimental approaches in both the field and the greenhouse.

This research expands on previous work documenting range shifts in trees of the western US by explicitly examining the role of different types of disturbance events on broad scale changes in the distribution of tree regeneration. It also adds to the growing literature documenting fire-facilitated trembling aspen establishment from seed, which was previously thought to be a rare occurrence. Taken together, Chapters 1 and 2 reveal that disturbances have the potential to catalyze tree species range shifts, but range contractions are most common across the interior western US currently. They also demonstrate that wildfire vs. biotic disturbances produce different regeneration environments, which will interact with climate change to determine future species ranges. So far, range expansions into cooler locations seem to be highly localized and dependent on microsite conditions for the tree species studied here.

The importance of microsite conditions was further demonstrated in Chapter 4, where ponderosa seedling success in the field was most dependent on beneficial microsite conditions. Chapters 3 and 4 also demonstrated the variability in how these microsite conditions impact seedlings from different seed sources. Across the greenhouse and field study, seedlings from hotter and drier populations tended to grow larger, which could be beneficial in a natural regeneration setting, but is likely to increase risk of desiccation and reduce success of seedlings planted in the field. The field study further elucidated that the upper elevational range margin of ponderosa pine in northern Colorado may still be too cold for seedlings from hotter and drier populations planted there to be successful. However, seed sowing seems to reduce the differences in survival between populations and therefore may allow for greater assisted gene flow success.

This research represents significant advances in the field of forest regeneration and assisted migration by including one of the first studies to incorporate disturbance impacts on tree species range shifts and by documenting a range expansion in the field. Studies incorporating mother tree traits along with seed source provenance in predicting seedling survival and growth are currently rare and we tested the differences between these major levels of variation in both the greenhouse and the field, providing valuable information to land managers seeking to reforest with climate-adapted genotypes of ponderosa pine. However, there are still many questions left to answer, including if documented range shifts will persist into the future, whether species will adapt in place to changes in climate, and if climate change-adapted genotypes can withstand extreme events that are likely to occur. Therefore, continued efforts to understand the interplay between climate, disturbance, and adaptation will allow us to manage forests for more resilience in the uncertain future that they face.

APPENDICES

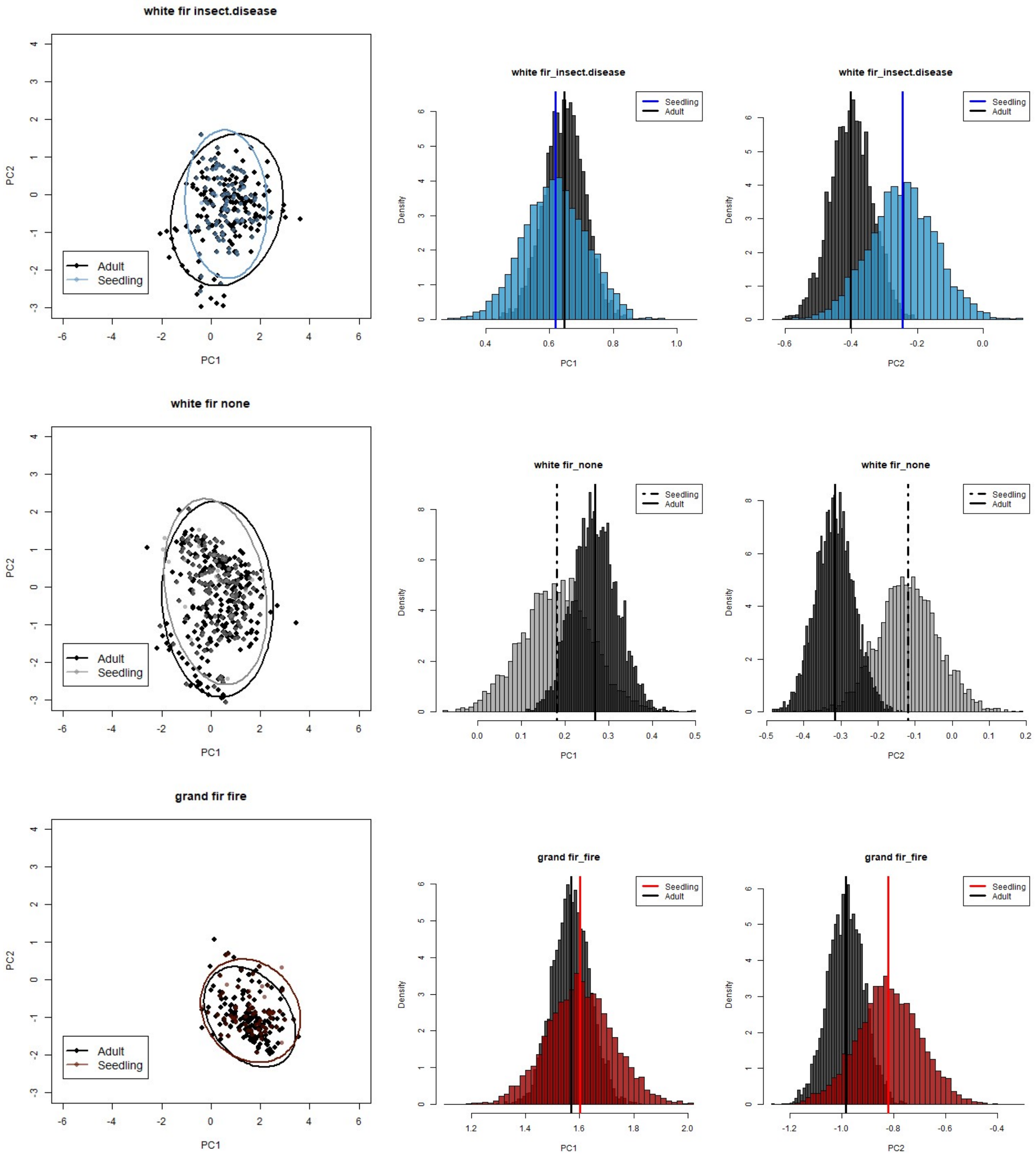
APPENDIX 1

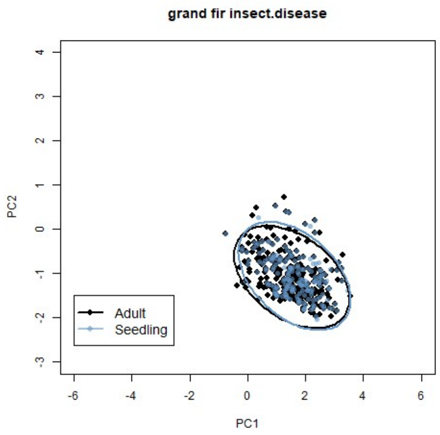
Table A1.1 Sample Size & Traits by Species. Sample size, shade tolerance score, dispersal mode, and PC1 mean score of all species analyzed in this study. Species by disturbance combinations with seedling sample size below 60 were not included in the analysis (highlighted).

Scientific Name	**Common name**	**Fire sample size**		**Insect/Disease sample size**		**Undisturbed sample size**		**Shade Tolerance**	**Dispersal Mode**	**PC1 mean**
		Seedling	**Adult**	**Seedling**	**Adult**	**Seedling**	**Adult**			
Abies concolor	white fir	27	155	95	248	161	384	4.33	Wind	0.33
Abies grandis	grand fir	67	211	190	418	419	654	4.01	Wind	1.52
Abies lasiocarpa	subalpine fir	381	1008	1348	1852	1501	2452	4.83	Wind	2.50
Juniperus scopulorum	Rocky Mountain juniper	31	213	62	187	282	1148	1.48	Animal	-0.22
Larix occidentalis	western larch	64	152	10	293	69	521	1.35	Wind	1.63
Picea engelmannii	Engelmann spruce	279	763	839	1724	1021	2380	4.53	Wind	2.46
Pinus albicaulis	whitebark pine	144	299	327	502	306	589	1	Animal	2.97
Pinus edulis	two needle pinyon	58	362	185	396	1056	2827	1.44	Animal	-1.83
Pinus contorta	lodgepole pine	480	1091	576	1770	536	1951	1.48	Wind	2.25
Pinus flexilis	limber pine	37	151	178	359	165	648	1.56	Animal	1.45
Pinus ponderosa	ponderosa pine	162	1096	72	466	345	1808	1.64	Wind	-0.24
Pseudotsuga menziesii	Douglas-fir	340	1385	720	1814	1160	3196	2.78	Wind	1.26
Thuja plicata	western redcedar	9	82	78	195	171	285	4.73	Wind	1.34
Populus tremuloides	trembling aspen	238	260	689	977	778	1327	1.21	Wind	1.23
Quercus gambelii	Gambel oak	268	388	209	269	914	1129	2.09	Animal	-0.69

Species x disturbance climate niches

Each of the following rows is devoted to one species by disturbance combination. The first plot shows the position of adults (black) vs. seedlings (red, blue, or grey) in the 2-dimensional PC space, where the seedling presence points are plotted on top of the adult presence points. The next two histograms show the posterior estimates of μ_1 (mean of PC1; left) and μ_2 (mean of PC2; right) from the Bayesian multivariate normal models for adults (black) and seedlings (red, blue, or grey), with posterior means shown as vertical lines. Therefore, the difference between the vertical lines approximates the shift in centroid on the respective principal component.

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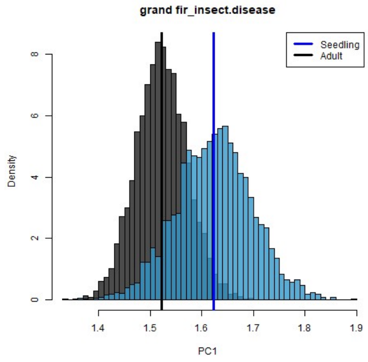
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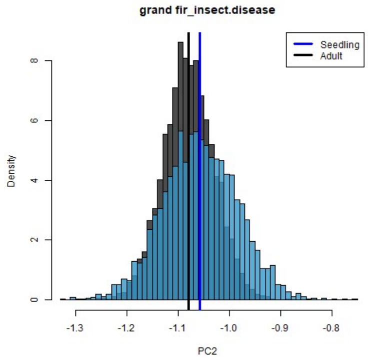
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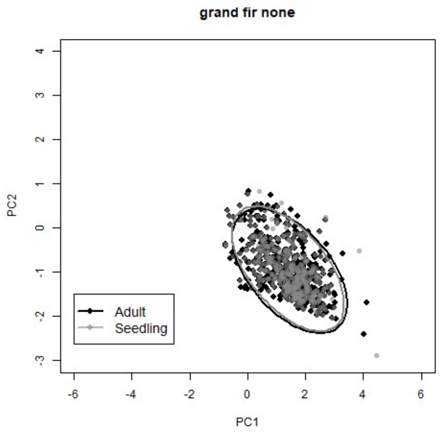
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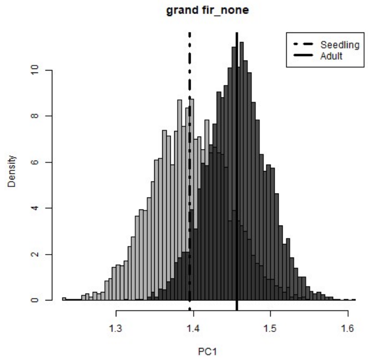
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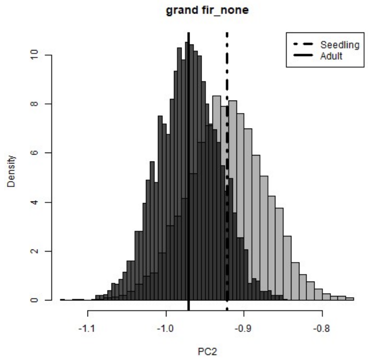
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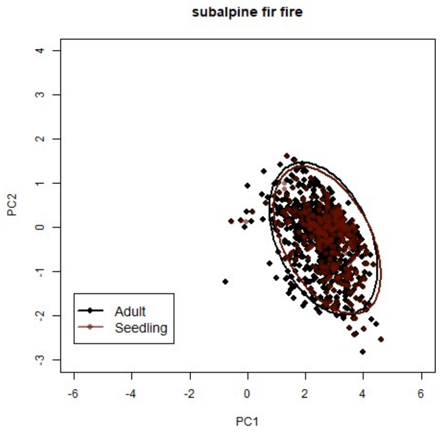
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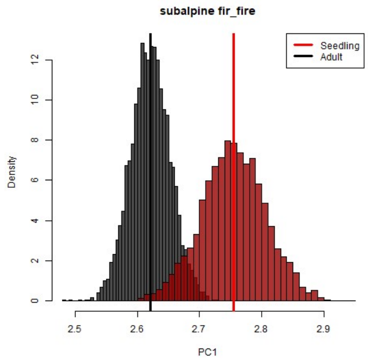
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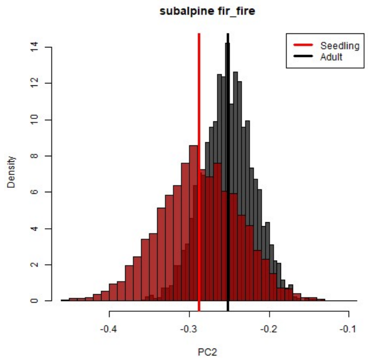
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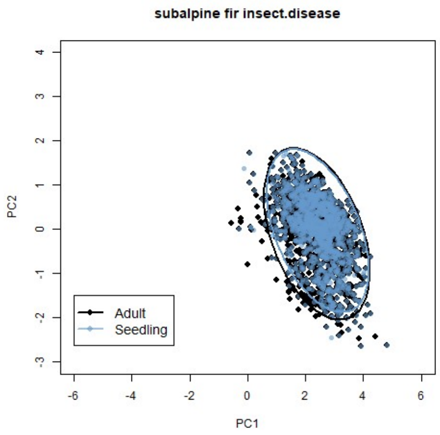
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PC1
Adult
Seedling
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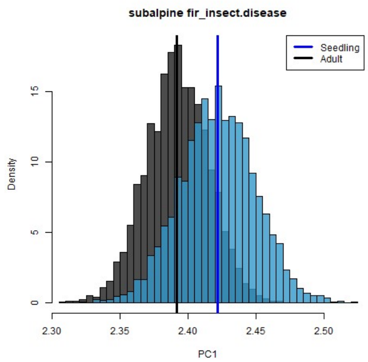
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Adult
Density
PC1
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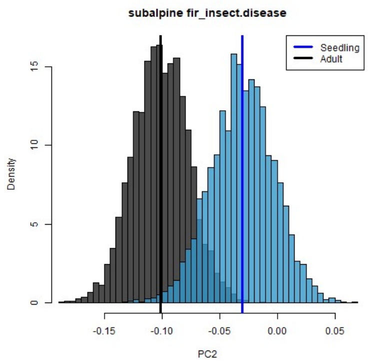
0 5 10 15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Adult
Density
PC2
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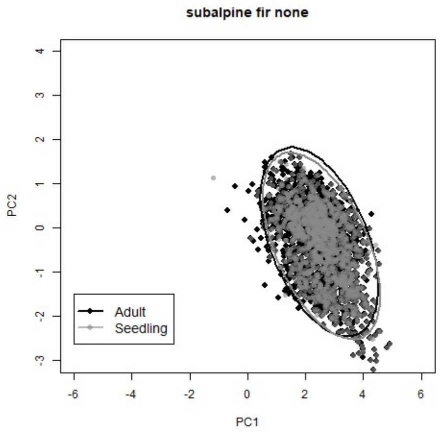
0 5 10 15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PC1
Adult
Seedling
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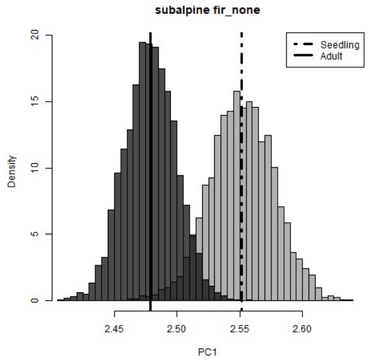
-3 -2 -1 0 1 2 3 4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Adult
Density
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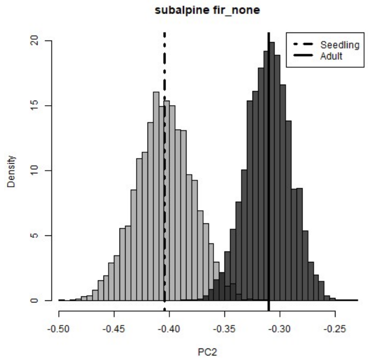
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Seedling
Adult
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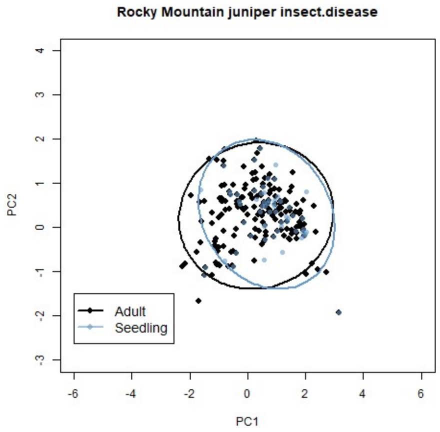
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PC1
Adult
Seedling
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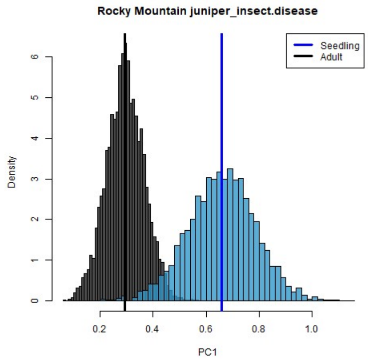
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Seedling
Adult
Density
PC1
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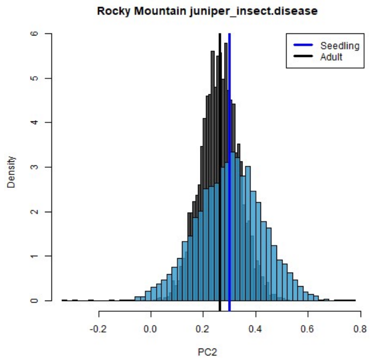
0 1 2 3 4 5 6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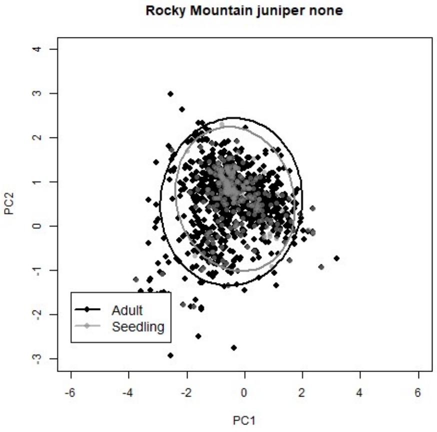
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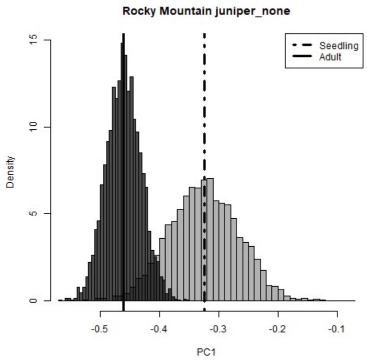
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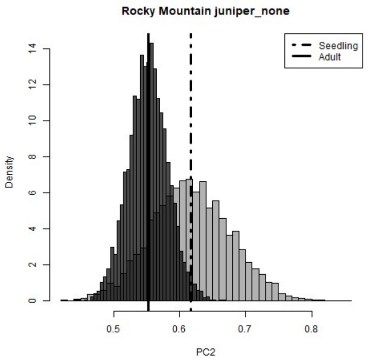
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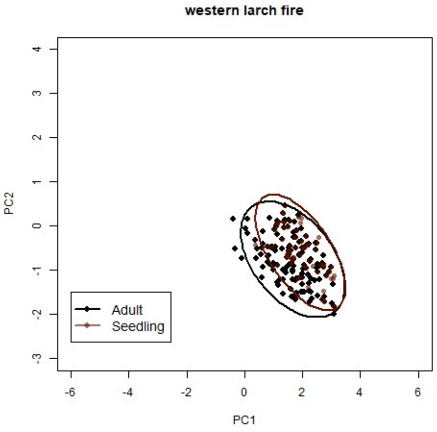
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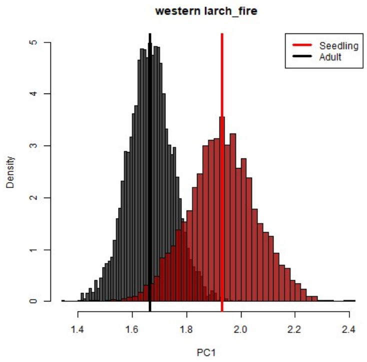
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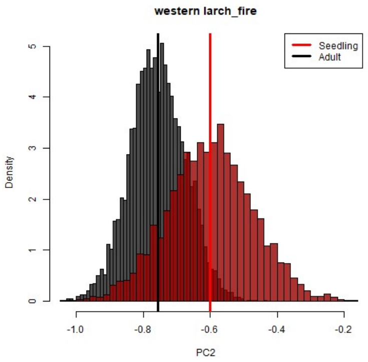
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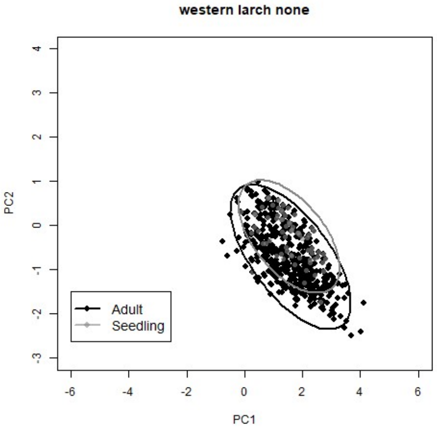
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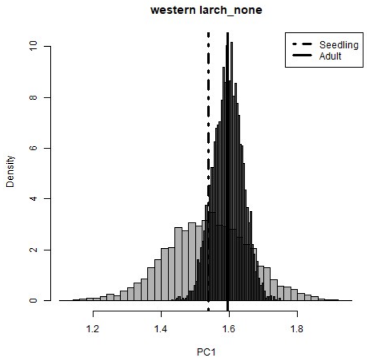
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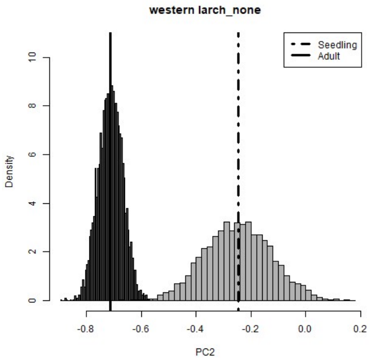
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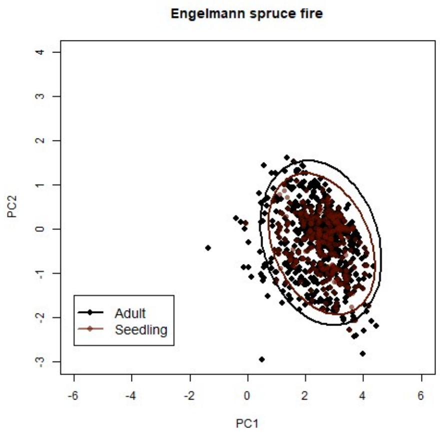
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PC1
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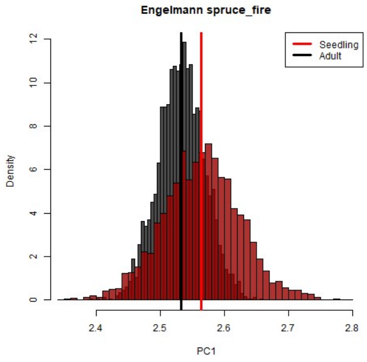
4
6
Adult
Seedling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Seedling
Adult
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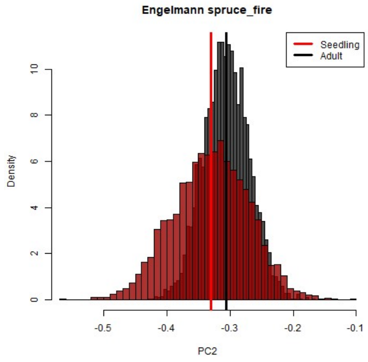
2.8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Seedling
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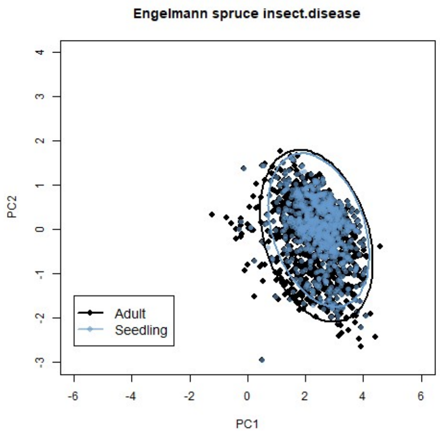
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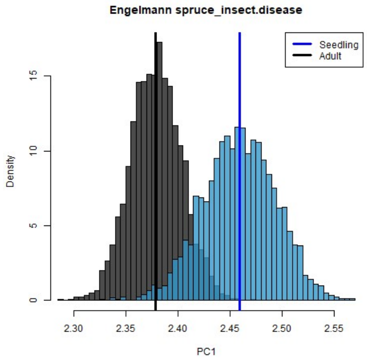
Adult
Seedling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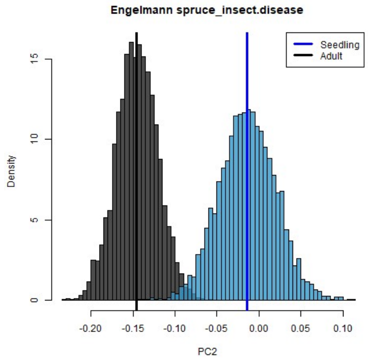
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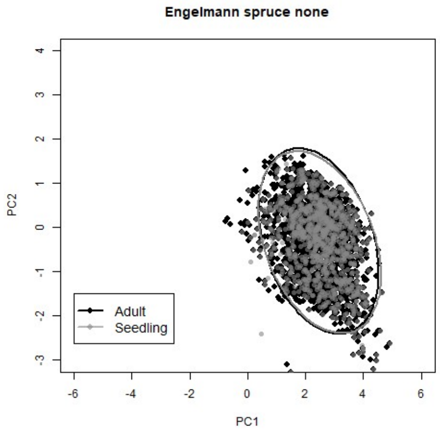
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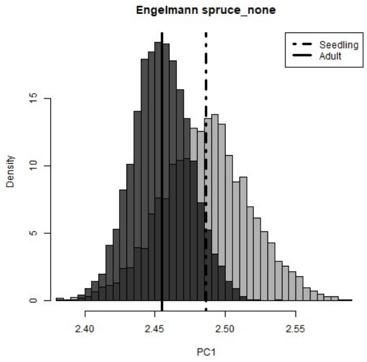
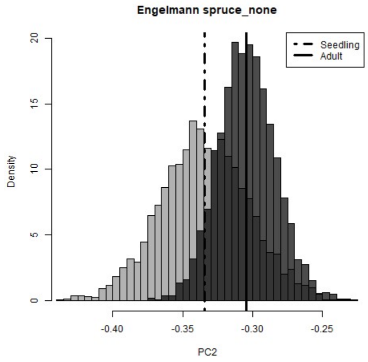
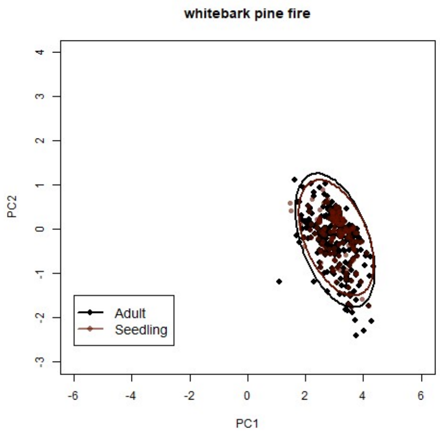
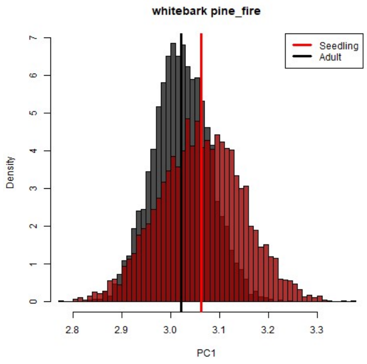
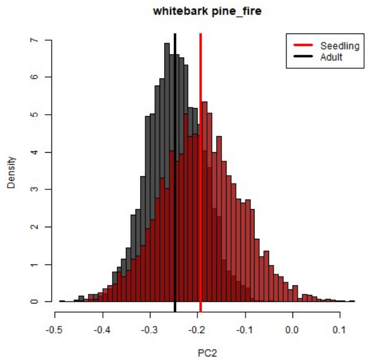
Adult
Seedling

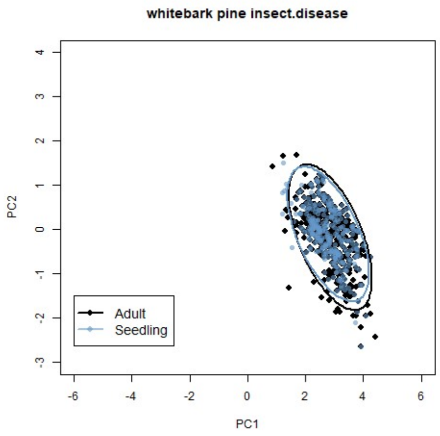

Engelmann spruce_none
Seedling
Adult
Density
PC1
0
5
10
15
2.40
2.45
2.50
2.55

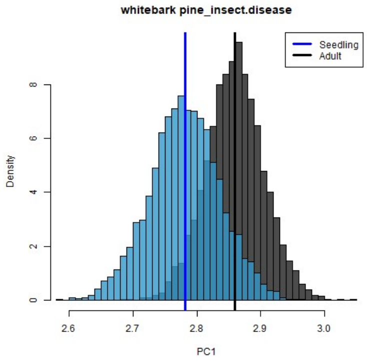

Engelmann spruce_none
Seedling
Adult
Density
PC2
0
5
10
15
20
-0.40
-0.35
-0.30
-0.25

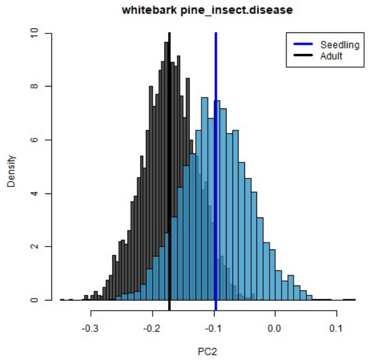

whitebark pine fire
PC2
4
3
2
1
0
-1
-2
-3
-6
-4
-2
0
2
4
6
PC1
Adult
Seedling

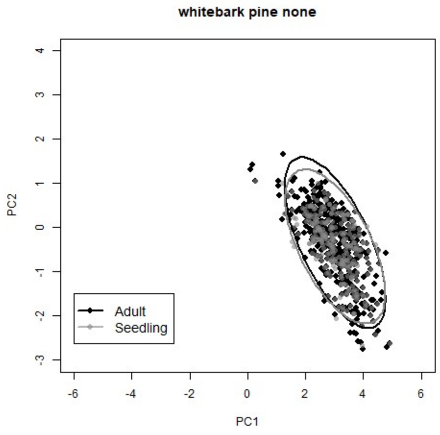

whitebark pine_fire
Density
7
6
5
4
3
2
1
0
2.8
2.9
3.0
3.1
3.2
3.3
PC1
Seedling
Adult

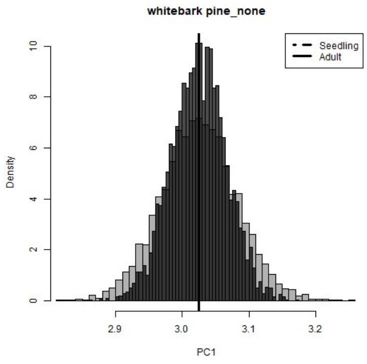

whitebark pine_fire
Density
7
6
5
4
3
2
1
0
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
0.1
PC2
Seedling
Adult

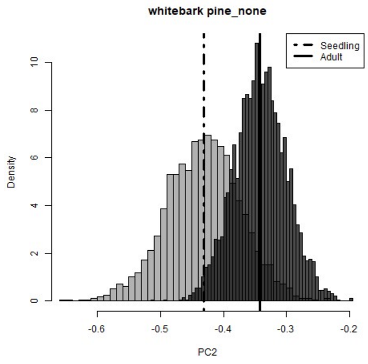

whitebark pine insect.disease
PC2
4
3
2
1
0
-1
-2
-3
-6
-4
-2
0
2
4
6
PC1
Adult
Seedling

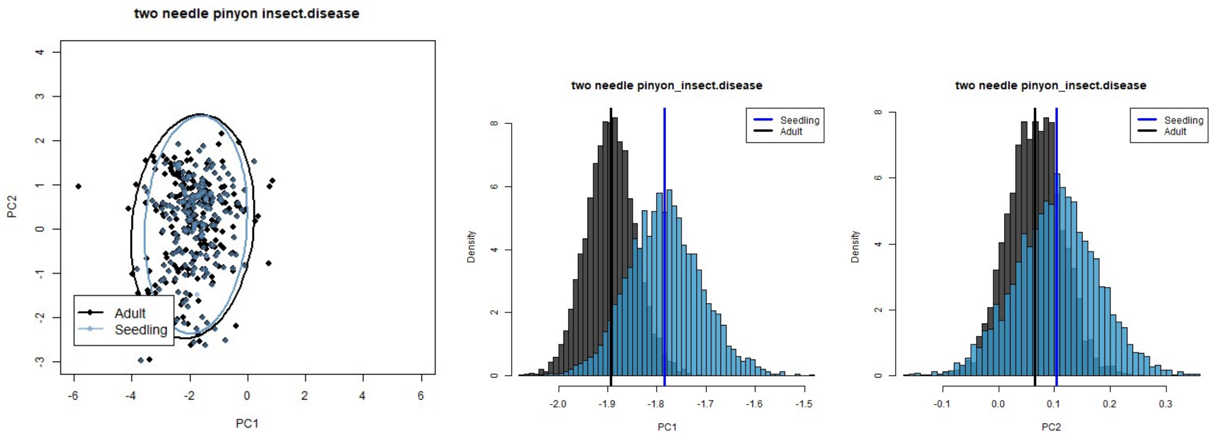

whitebark pine_insect.disease
Density
8
6
4
2
0
2.6
2.7
2.8
2.9
3.0
PC1
Seedling
Adult

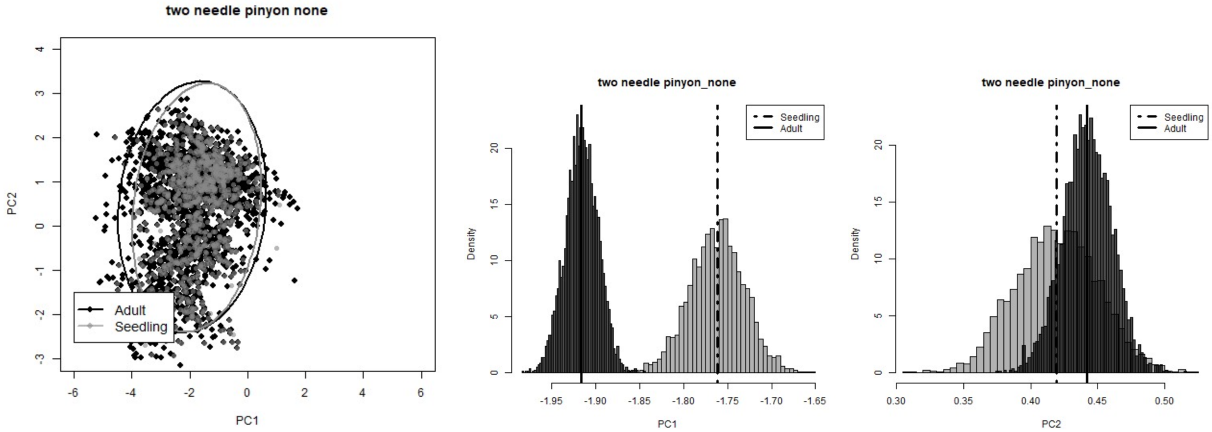

whitebark pine_insect.disease
Density
10
8
6
4
2
0
-0.3
-0.2
-0.1
0.0
0.1
PC2
Seedling
Adult

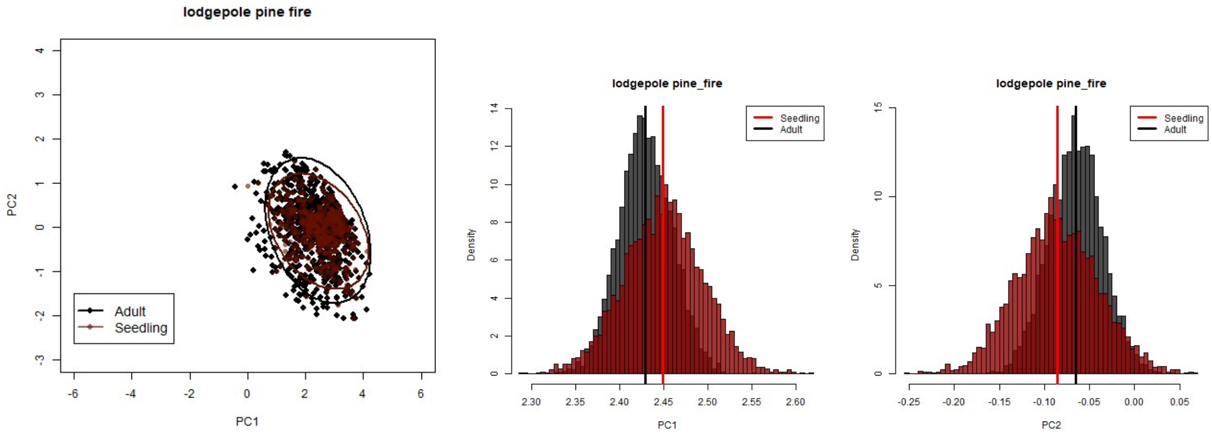

whitebark pine none
PC2
4
3
2
1
0
-1
-2
-3
-6
-4
-2
0
2
4
6
PC1
Adult
Seedling

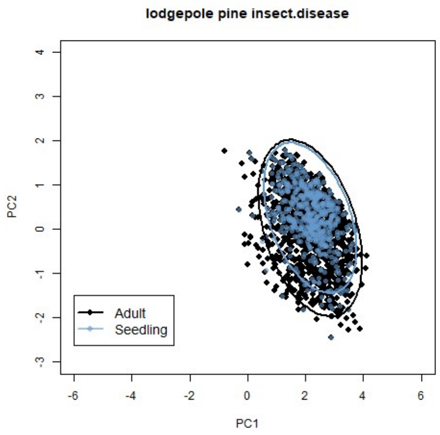

whitebark pine_none
Density
10
8
6
4
2
0
2.9
3.0
3.1
3.2
PC1
Seedling
Adult

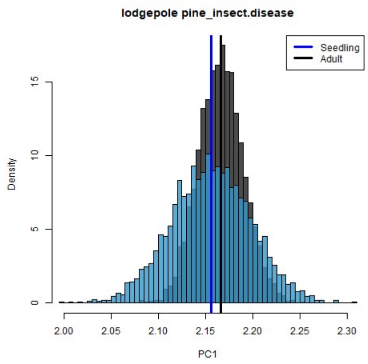

whitebark pine_none
Density
10
8
6
4
2
0
-0.6
-0.5
-0.4
-0.3
-0.2
PC2
Seedling
Adult

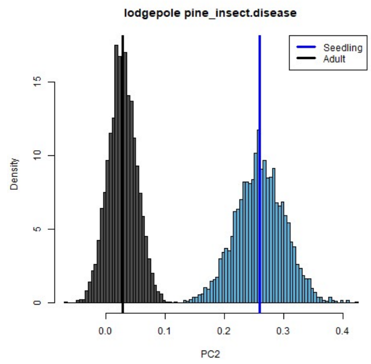

two needle pinyon insect.disease
PC1
PC2
Adult
Seedling
two needle pinyon_insect.disease
Density
PC1
Seedling
Adult
two needle pinyon_insect.disease
Density
PC2
Seedling
Adult

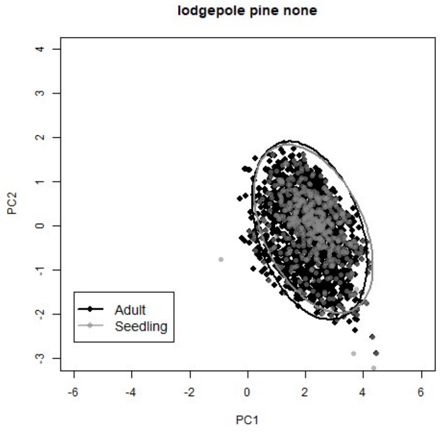

two needle pinyon none
PC1
PC2
Adult
Seedling
two needle pinyon_none
Density
PC1
Seedling
Adult
two needle pinyon_none
Density
PC2
Seedling
Adult

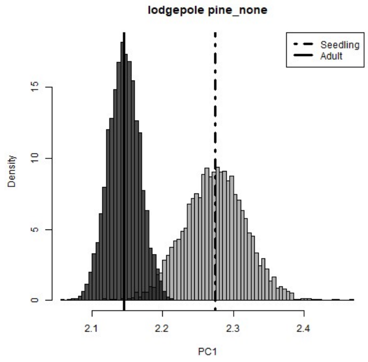

lodgepole pine fire
PC1
PC2
Adult
Seedling
lodgepole pine_fire
Density
PC1
Seedling
Adult
lodgepole pine_fire
Density
PC2
Seedling
Adult

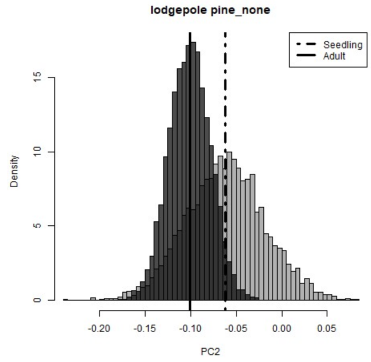

lodgepole pine insect.disease
PC2
PC1
-3
-2
-1
0
1
2
3
4
-6
-4
-2
0
2
4
6
Adult
Seedling

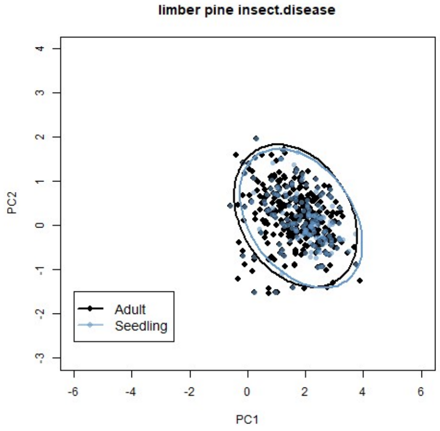

lodgepole pine_insect.disease
Seedling
Adult
Density
0
5
10
15
2.00
2.05
2.10
2.15
2.20
2.25
2.30
PC1

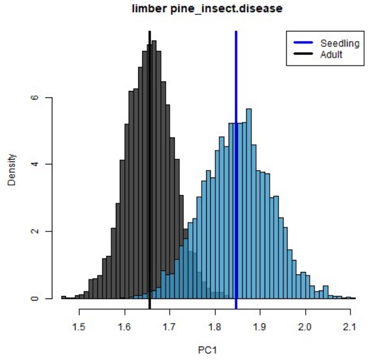

lodgepole pine_insect.disease
Seedling
Adult
Density
0
5
10
15
0.0
0.1
0.2
0.3
0.4
PC2

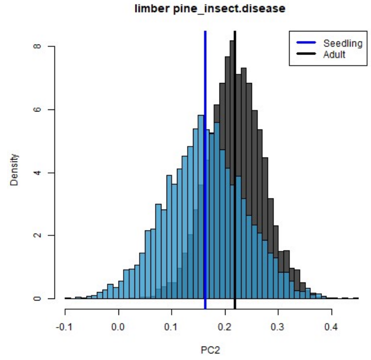

lodgepole pine none
PC2
PC1
-3
-2
-1
0
1
2
3
4
-6
-4
-2
0
2
4
6
Adult
Seedling

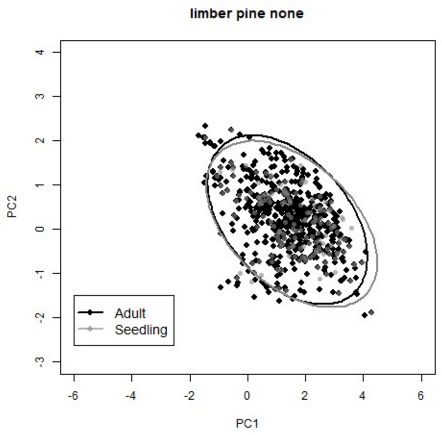

lodgepole pine_none
Seedling
Adult
Density
0
5
10
15
2.1
2.2
2.3
2.4
PC1

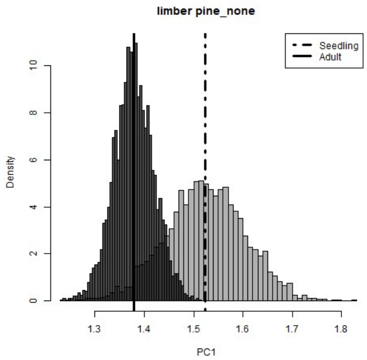

lodgepole pine_none
Seedling
Adult
Density
0
5
10
15
-0.20
-0.15
-0.10
-0.05
0.00
0.05
PC2

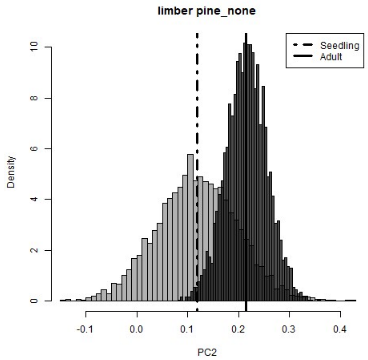

limber pine insect.disease
PC2
PC1
-3
-2
-1
0
1
2
3
4
-6
-4
-2
0
2
4
6
Adult
Seedling

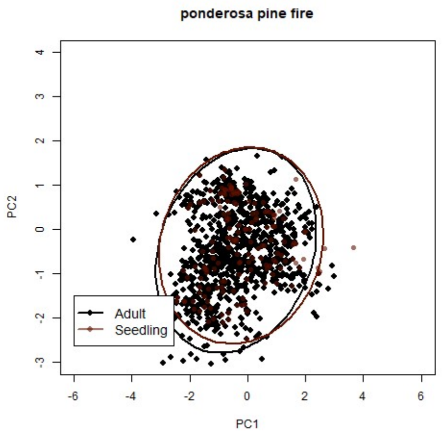

limber pine_insect.disease
Seedling
Adult
Density
0
2
4
6
1.5
1.6
1.7
1.8
1.9
2.0
2.1
PC1

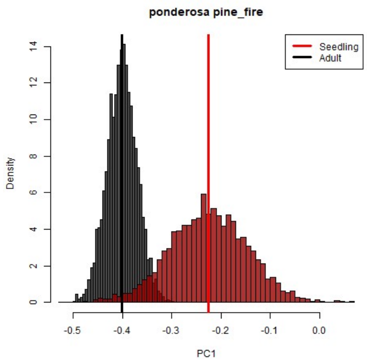

limber pine_insect.disease
Seedling
Adult
Density
0
2
4
6
8
-0.1
0.0
0.1
0.2
0.3
0.4
PC2

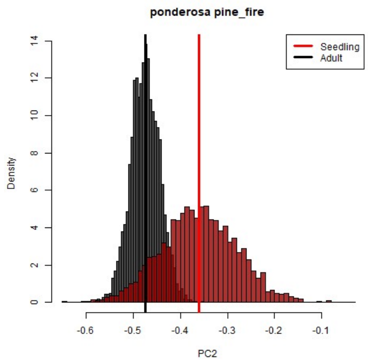

limber pine none
PC2
PC1
4
3
2
1
0
-1
-2
-3
-6
-4
-2
0
2
4
6
Adult
Seedling

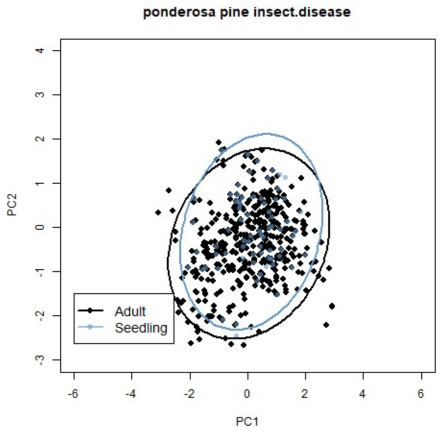

limber pine_none
Seedling
Adult
Density
PC1
10
8
6
4
2
0
1.3
1.4
1.5
1.6
1.7
1.8

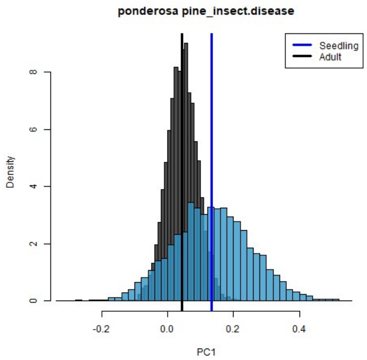

limber pine_none
Seedling
Adult
Density
PC2
10
8
6
4
2
0
-0.1
0.0
0.1
0.2
0.3
0.4

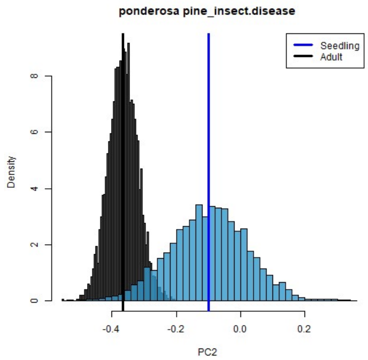

ponderosa pine fire
PC2
PC1
4
3
2
1
0
-1
-2
-3
-6
-4
-2
0
2
4
6
Adult
Seedling

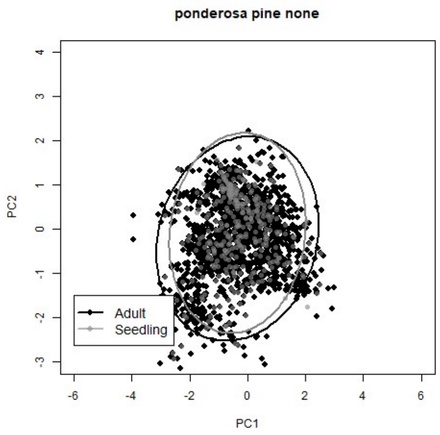

ponderosa pine_fire
Seedling
Adult
Density
PC1
14
12
10
8
6
4
2
0
-0.5
-0.4
-0.3
-0.2
-0.1
0.0

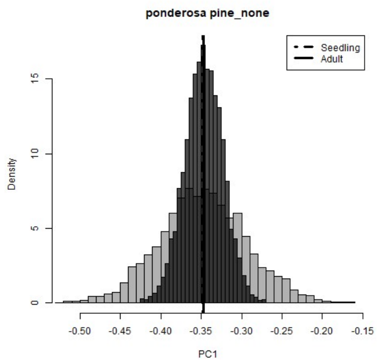

ponderosa pine_fire
Seedling
Adult
Density
PC2
14
12
10
8
6
4
2
0
-0.6
-0.5
-0.4
-0.3
-0.2
-0.1

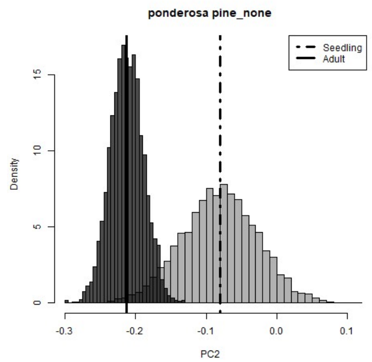

ponderosa pine insect.disease
PC2
PC1
4
3
2
1
0
-1
-2
-3
-6
-4
-2
0
2
4
6
Adult
Seedling

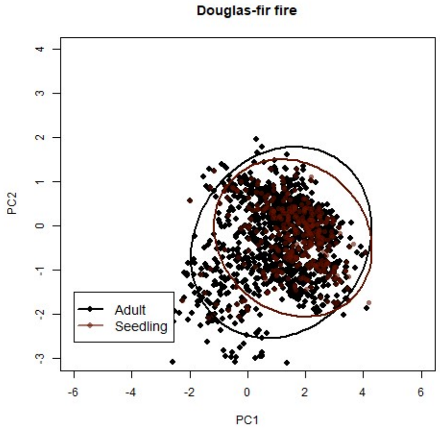

ponderosa pine_insect.disease
Seedling
Adult
Density
PC1
8
6
4
2
0
-0.2
0.0
0.2
0.4

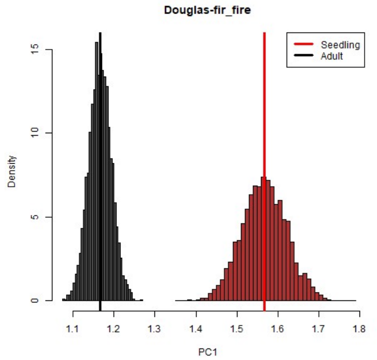

ponderosa pine_insect.disease
Seedling
Adult
Density
PC2
8
6
4
2
0
-0.4
-0.2
0.0
0.2

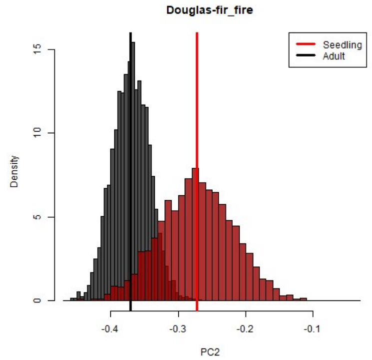

ponderosa pine none

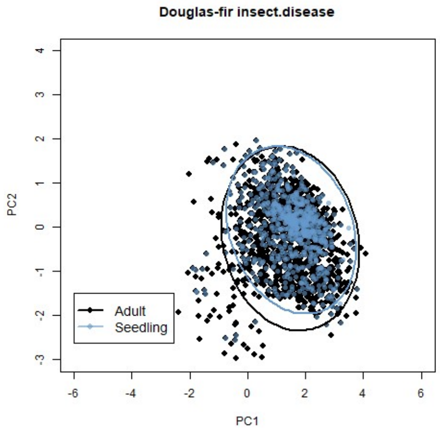

ponderosa pine_none

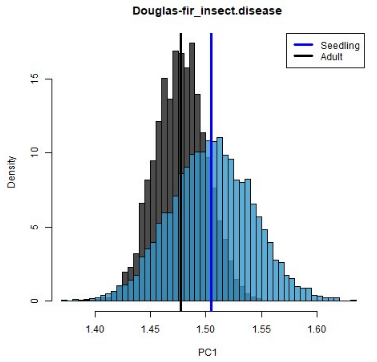

ponderosa pine_none

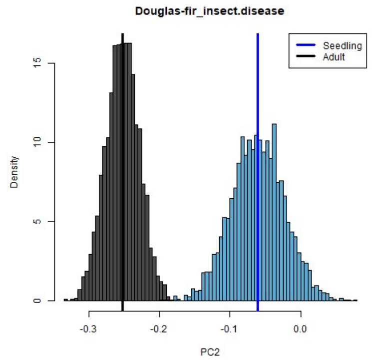

Douglas-fir fire

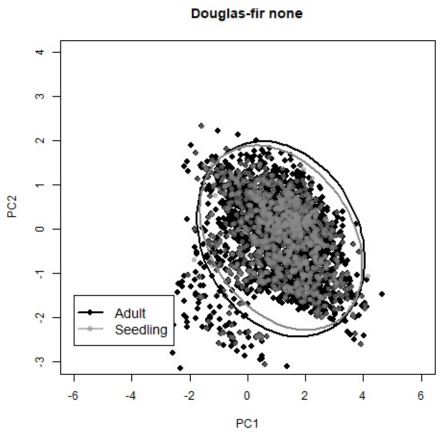

Douglas-fir_fire

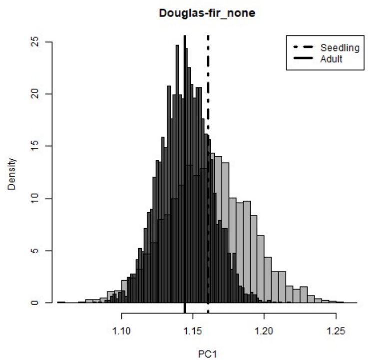

Douglas-fir_fire

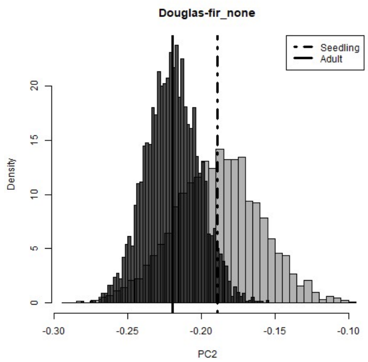

Douglas-fir insect.disease

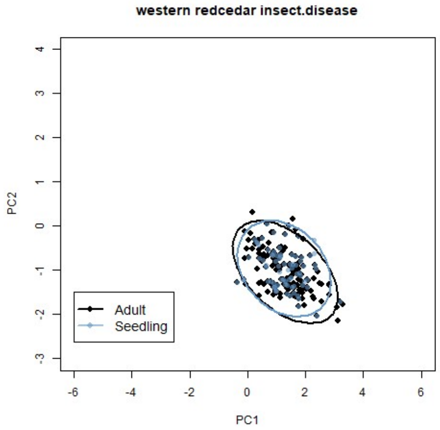

Douglas-fir_insect.disease

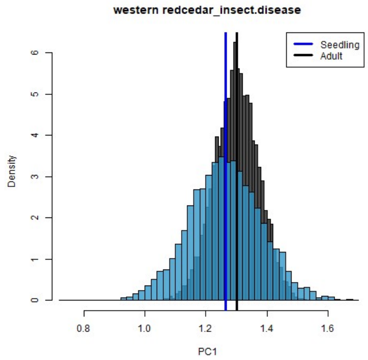

Douglas-fir_insect.disease

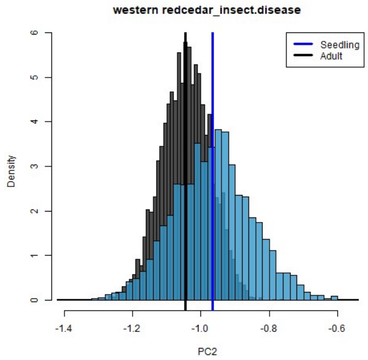

Douglas-fir none
PC2
PC1
Adult
Seedling
-6 -4 -2 0 2 4 6
-3 -2 -1 0 1 2 3 4

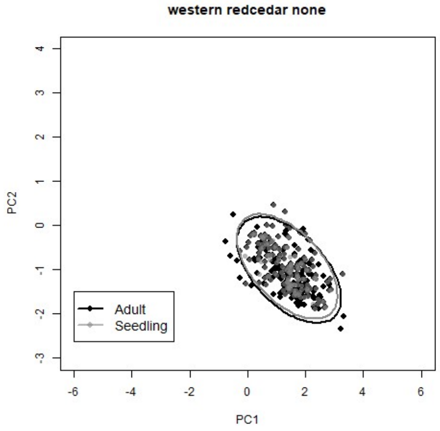

Douglas-fir_none
Seedling
Adult
Density
PC1
1.10 1.15 1.20 1.25
0 5 10 15 20 25

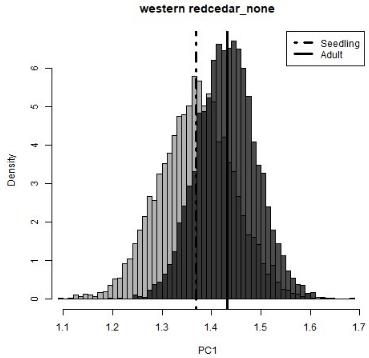

Douglas-fir_none
Seedling
Adult
Density
PC2
-0.30 -0.25 -0.20 -0.15 -0.10
0 5 10 15 20

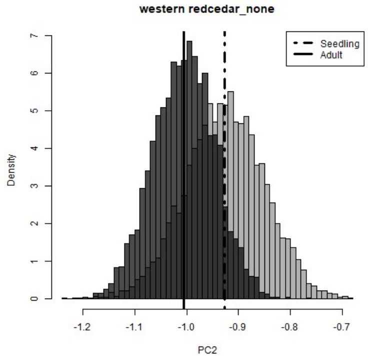

western redcedar insect.disease
PC2
PC1
Adult
Seedling
-6 -4 -2 0 2 4 6
-3 -2 -1 0 1 2 3 4


western redcedar_insect.disease
Seedling
Adult
Density
PC1
0.8 1.0 1.2 1.4 1.6
0 1 2 3 4 5 6


western redcedar_insect.disease
Seedling
Adult
Density
PC2
-1.4 -1.2 -1.0 -0.8 -0.6
0 1 2 3 4 5 6


western redcedar none
PC2
PC1
Adult
Seedling
-6 -4 -2 0 2 4 6
-3 -2 -1 0 1 2 3 4


western redcedar_none
Seedling
Adult
Density
PC1
1.1 1.2 1.3 1.4 1.5 1.6 1.7
0 1 2 3 4 5 6


western redcedar_none
Seedling
Adult
Density
PC2
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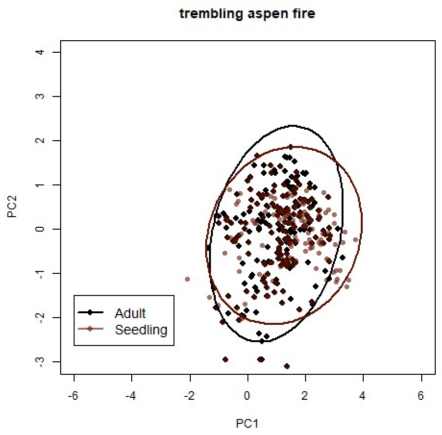
trembling aspen fire
PC2
PC1
Adult
Seedling
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-2
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-6
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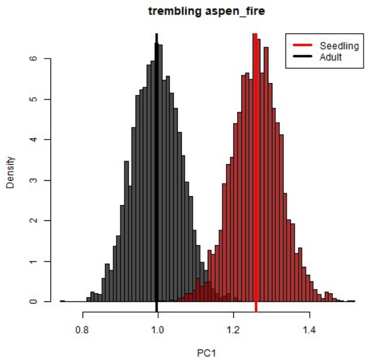
-2
0
2
4
6


trembling aspen_fire
Seedling
Adult
Density
PC1
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2
3
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6
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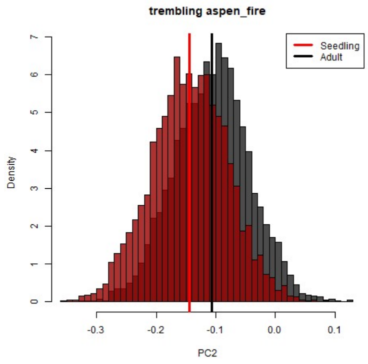
1.2
1.4


trembling aspen_fire
Seedling
Adult
Density
PC2
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2
3
4
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6
7
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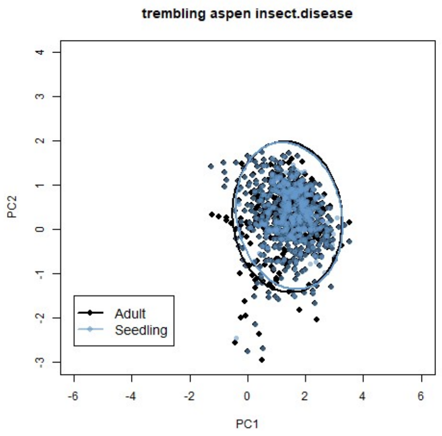
-0.1
0.0
0.1


trembling aspen insect.disease
PC2
PC1
Adult
Seedling
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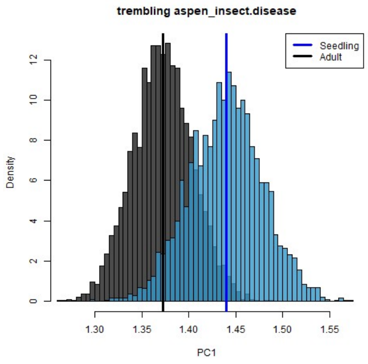
3
2
1
0
-1
-2
-3
-6
-4
-2
0
2
4
6


trembling aspen_insect.disease
Seedling
Adult
Density
PC1
0
2
4
6
8
10
12
1.30
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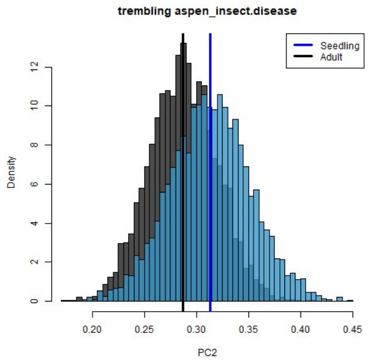
1.40
1.45
1.50
1.55


trembling aspen_insect.disease
Seedling
Adult
Density
PC2
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4
6
8
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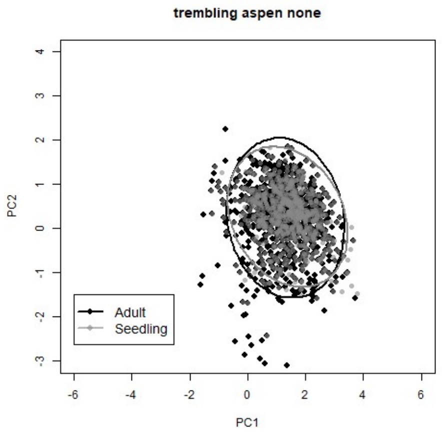
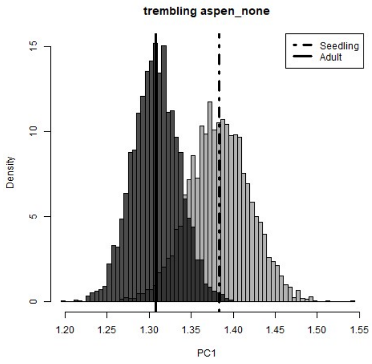
0.30
0.35
0.40
0.45


trembling aspen none
PC2
PC1
Adult
Seedling
4
3
2
1
0
-1
-2
-3
-6
-4
-2
0
2
4
6


trembling aspen_none
Seedling
Adult
Density
PC1
0
5
10
15
1.20
1.25
1.30
1.35
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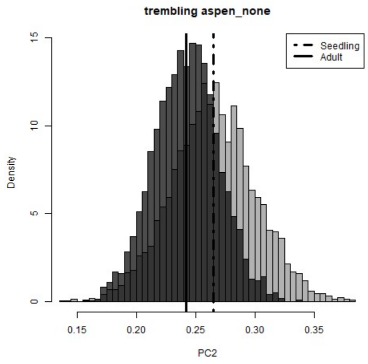
1.45
1.50
1.55


trembling aspen_none
Seedling
Adult
Density
PC2
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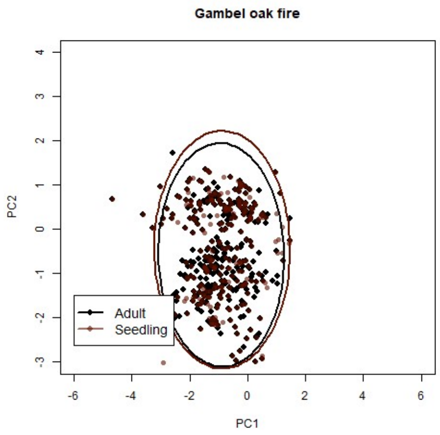
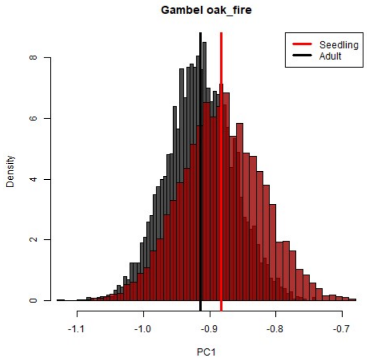
5
10
15
0.15
0.20
0.25
0.30
0.35


Gambel oak fire
PC2
PC1
Adult
Seedling
-6
-4
-2
0
2
4
6
-3
-2
-1
0
1
2
3
4


Gambel oak_fire
Seedling
Adult
Density
PC1
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-0.9
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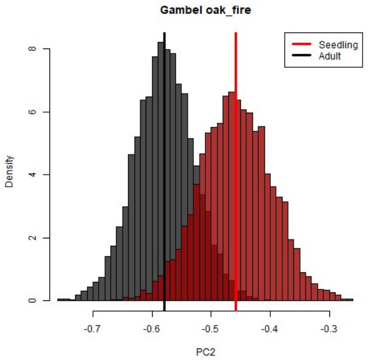
-0.7
0
2
4
6
8


Gambel oak_fire
Seedling
Adult
Density
PC2
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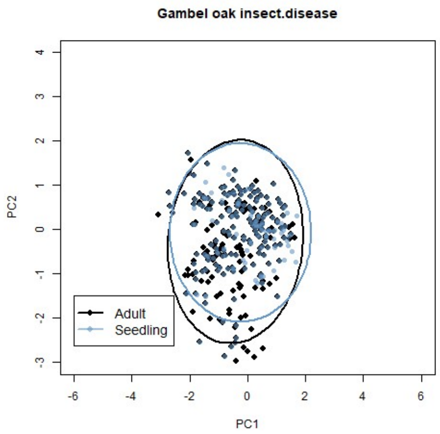
-0.5
-0.4
-0.3
0
2
4
6
8


Gambel oak insect.disease
PC2
PC1
Adult
Seedling
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-4
-2
0
2
4
6
-3
-2
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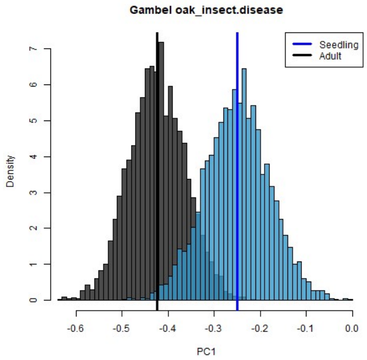
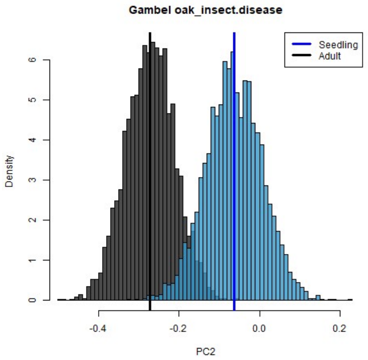
0
1
2
3
4


Gambel oak_insect.disease
Seedling
Adult
Density
PC1
-0.6
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
0
1
2
3
4
5
6
7


Gambel oak_insect.disease
Seedling
Adult
Density
PC2
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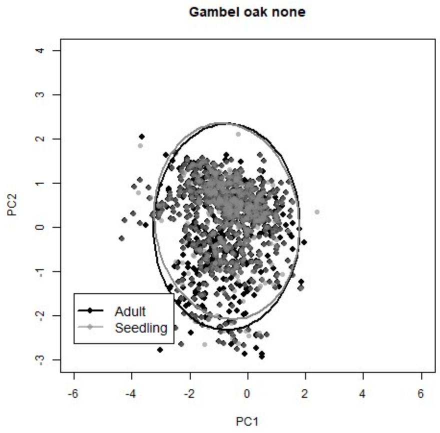
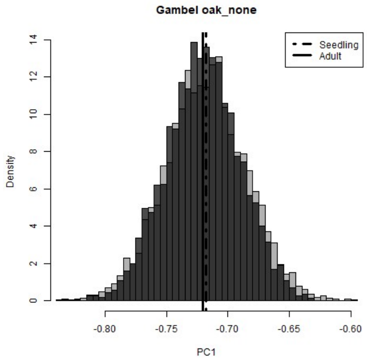
-0.2
0.0
0.2
0
1
2
3
4
5
6


Gambel oak none
PC2
PC1
Adult
Seedling
-6
-4
-2
0
2
4
6
-3
-2
-1
0
1
2
3
4


Gambel oak_none
Seedling
Adult
Density
PC1
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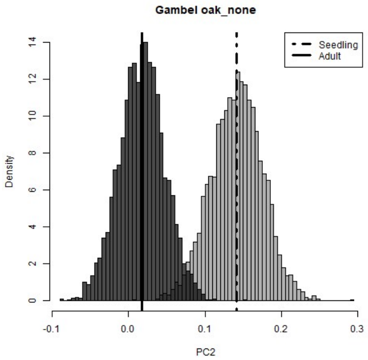
-0.75
-0.70
-0.65
-0.60
0
2
4
6
8
10
12
14


Gambel oak_none
Seedling
Adult
Density
PC2
-0.1
0.0
0.1
0.2
0.3
0
2
4
6
8
10
12
14

APPENDIX 2

Aerial imagery analysis: detailed methods

Pre-fire 2011 aerial imagery from the ArcGIS World Imagery Basemap (January 2014 release; 0.3-m resolution) was the primary source for assessment, but was supplemented with 2011 NAIP imagery (1-m resolution) if the primary source was obscured by presence of shadows, clouds, etc. To establish transects across the study area, we first generated a set of random points (n = 1,228) at 3200, 3300 and 3400 meters in elevation. Then, from each random point we established a transect that followed the terrain upslope, placing a circular plot (25-m radius) at horizontal increments of 60 m. We then selected a subset of these transects (n = 550) for sampling, as follows. To ensure that we sampled areas containing aspen, we evaluated all transects that intersected areas classified as aspen in the 2012 LANDFIRE existing vegetation type data (n = 181), which uses a combination of field data, satellite imagery, biophysical layers, and regression trees to produce a predictive model of vegetation cover on the landscape (LANDFIRE, 2012). We then added an additional random sample of transects (n = 369), for a total of 550 transects assessed. Circular plots along each transect were visually examined using the aerial imagery to determine if aspen occurred anywhere within the 25-m radius circle. If so, this point was counted as an "aspen present" point. In the case where circular plots overlapped due to intersecting lines, only the first plot viewed was used for analysis. All imagery was collected during the summer months, when aspen leaves were present and could be easily visually distinguished from much darker conifer needles. Aspen is the only tall broadleaf species in this area and therefore we had high confidence that it was correctly visually classified. It was distinguished from broadleaf shrub species by the presence of shadows (indicating tree status) in the aerial imagery as well as landscape context (e.g., alpine areas with high willow cover were not classified as aspen).

A total of 7,494 plots were classified across 520 unique transects; 1,266 of these plots (16.9%) were classified as aspen. The median elevation of plots classified as aspen was 3313 m and the maximum was 3568 m.

Aerial imagery analysis: accuracy assessment methods

In the summers of 2018 and 2019, a subset of plots (n = 400) were navigated to in the field using GPS coordinates, to test the accuracy of our aspen presence data derived from aerial imagery. Only plots outside of the fire perimeters that had not been disturbed (by fire or harvesting) since 2011 were used for field validation. Plots that were near roads and most easily accessible were selected. At each plot, we established a 25-m radius circular plot and recorded the presence or absence of canopy aspen within the plot. Overall classification accuracy (total number of correctly classified plots divided by all plots) was 94%. Producer's accuracy (the probability that a plot classified as aspen actually represents aspen on the ground) was 97.7% and user's accuracy (the probability of a plot containing aspen being correctly classified) was 86.2% (accuracy assessments from Congalton, 1991). Most of the errors in classification were errors of omission, where plots that were classified as "no aspen" actually had aspen on the ground (13.8%), which is consistent with another study that found more aspen in field surveys than reported from aerial imagery (Kaye et al. 2003). There was no significant elevational bias to errors of omission or commission and both were made at relatively low elevations (average elevation of plots with errors of omission = 3289 m ± 18.2 SE, average elevation of plots with errors of commission = 3336 m ± 86 SE).

Table A2.1: All models of aspen seedling abundance among burned sites sampled with delta AICc less than 2. The most parsimonious model that is described in detail in the Results is in bold. RdNBR = relativized differenced Normalized Burn Ratio.

Intercept	% Bare Soil	% CWD	Distance to seed source	Elevation	Heat Load Index	RdNBR	% Vegetation	df	AICc	Δ	weight
-2.00		**0.29**	**1.01**	**-1.28**				**5**	**120.08**	**0.00**	**0.30**
-1.81		0.29		-0.49			-0.24	5	120.70	0.62	0.22
-1.76		0.31		-0.56				4	120.71	0.63	0.22
-1.92		0.29	0.79	-1.07			-0.17	6	121.65	1.57	0.14
-1.80		0.26		-0.58		0.27		5	121.87	1.79	0.12

Aspen seedlings encountered in unburned / beetle-impacted areas

Although no aspen seedlings were found on the 40 unburned/beetle-impacted sites surveyed, aspen seedlings were encountered in unburned/beetle-impacted areas when hiking to and from the surveyed sites. The microsite characteristics of 46 of these seedlings were opportunistically measured. The average litter depth under these seedlings was 1.5 cm (± 0.1 SE), 65% were growing within 5 cm of a forb or grass, 22% were nursed (all but one by a rock) and 72% were browsed. Average canopy density above these seedlings was 26.4% (± 2.2 SE), which was not significantly different from the average canopy density across unburned/beetle-impacted sites that were surveyed within the same elevation range (27.3% ± 1.08 SE; t-test, t=0.16, df= 30.5, *P* =0.87). Sixty-three percent of the the seedlings were established in duff, the rest were established in soil. The average elevation of the seedlings was 3527 m (±7.1 SE), with the highest found at 3617 m.

REFERENCES

Congalton, R. G., (1991). A review of assessing the accuracy of classifications of remotely sensed data. *Remote sensing of environment*, 37, 35–46.

Kaye, M. W., Stohlgren, T. J., & Binkley, D. (2003). Aspen structure and variability in Rocky Mountain National Park, Colorado, USA. *Landscape Ecology*, 18, 591–603.

LANDFIRE (2012). Existing Vegetation Type (LANDFIRE 1.3.0) [data file]. Retrieved July 1, 2018, from https://landfire.cr.usgs.gov/evt.php

APPENDIX 3

Table A3.1. Number of seedlings at the start of the experiment in each treatment (WATER start & DROUGHT start), number of seedlings that died during watering, number of seedlings that were harvested in the two harvesting events (July & September) and the final sample size of live seedlings at the time that watering stopped (WATER end & DROUGHT end).

Mother tree	Ave % germ.	WATER start	DROUGHT start	WATER died	DROUGHT died	# harvested/trt July	# harvested/trt Sept	WATER end	DROUGHT end
BDT2	60.8	12	12		1	1	3	8	7
BDT4	59.1	11	11			1	3	7	7
BDT5	68.5	13	13			1	3	9	9
BDT7	64.4	12	12			1	3	8	8
BDT8	30.3	7	7			0	3	4	4
BDT9	58.2	11	12			1	3	7	8
BHT7	34.6	6	6			0	3	3	3
BHT9	17.4	4	5			0	1	3	4
FCT1	90.2	14	14			1	3	10	10
FCT2	41.3	11	11			1	3	7	7
FCT4	89.6	14	14			1	3	10	10
FCT5	69.6	13	13			1	3	9	9
FCT6	63.1	12	13			1	3	8	9
FCT9	83.0	13	14			1	3	9	10
HM1T1	74.4	13	13			1	3	9	9
HM1T10	76.6	13	14			1	3	9	10
HM1T2	58.4	11	11			1	3	7	7
HM1T4	65.1	12	12			1	3	8	8
HM1T7	67.5	11	12			1	3	7	8
HM1T8	68.6	11	11			1	3	7	7
HM6T1	58.3	11	12			1	3	7	8

Mother tree	Ave % germ.	WATER start	DROUGHT start	WATER died	DROUGHT died	# harvested/trt July	# harvested/trt Sept	WATER end	DROUGHT end
HM6T2	52.5	10	11			1	3	6	7
HM6T4	32.6	7	8			0	3	4	5
HM6T5	50.6	10	10			1	3	6	6
HM6T8	34.0	8	8			0	3	5	5
HM6T9	45.9	8	9			0	3	5	6
LHT116	69.2	13	14			1	3	9	10
LHT123M	72.9	12	13			1	3	8	9
LHT188	83.5	13	13			1	3	9	9
LHTI1300	87.0	14	14			1	3	10	10
LHTI204	74.3	12	13			1	3	8	9
LHTM71	84.0	13	13			1	3	9	9
WT1T12	49.3	11	12			1	3	7	8
WT1T14	51.8	10	11			1	3	6	7
WT1T15	61.6	11	11			1	3	7	7
WT1T4	21.2	5	5			0	2	3	3
WT1T7	29.9	7	7			0	3	4	4
WT2T10	84.2	13	14			1	3	9	10
WT2T2	66.4	13	13			1	3	9	9
WT2T4	67.3	12	12			1	3	8	8
WT2T6	40.0	9	9			0	3	6	6
WT2T8	90.0	13	13			1	3	9	9
WT2T9	23.9	4	5			0	1	3	4

APPENDIX 4

Sample Size

Table A4.1: Number of seeds sown for each mother tree in the experiment.

Population	Mother tree	# seeds sown	Population	Mother tree	# seeds sown
BD	BDT2	539	HM6	HM6T1	540
BD	BDT4	540	HM6	HM6T2	540
BD	BDT5	540	HM6	HM6T6	432
BD	BDT8	540	HM6	HM6T8	540
BD	BDT9	540	HM6	HM6T9	540
BH	BHT10	539	LH	LHT116	540
BH	BHT5	540	LH	LHT123M	539
BH	BHT6	539	LH	LHT188	540
BH	BHT7	540	LH	LHTI1300	540
BH	BHT8	540	LH	LHTM31	540
FC	FCT1	540	WT1	WT1T11	432
FC	FCT2	540	WT1	WT1T12	432
FC	FCT4	540	WT1	WT1T14	538
FC	FCT5	540	WT1	WT1T15	540
FC	FCT6	540	WT1	WT1T9	486
HM1	HM1T1	540	WT2	WT2T2	432
HM1	HM1T10	540	WT2	WT2T3	432
HM1	HM1T2	540	WT2	WT2T4	540
HM1	HM1T4	540	WT2	WT2T8	540
HM1	HM1T7	540			

Microsite PCA Analysis

To evaluate differences in microsite conditions between plots, we ran a principal components analysis (PCA) using the *prcomp* function in R. The variables included in the PCA were folded aspect, slope, canopy openness, average, maximum and minimum June daily temperature, average volumetric water content (VWC), soil pH, soil electrical conductivity (mmhos/cm), % soil organic matter, soil nitrate (ppm), soil phosphorus (ppm), soil sulfate (ppm), soil potassium (ppm), and soil % sand, % silt, and % clay. Folded aspect and summaries of temperature and volumetric water content were calculated as described in the manuscript.

The first three principal components were retained for analysis, as they had eigenvalues greater than one and the change in variance explained with each axis tended to level off after 3 components. PC1 explained 41.57% of the total variance, PC2 explained 19.77%, and PC3 explained 12.02%, for a cumulative of 73.4% of total variance explained (Figure A4.1). PC1 largely separated high elevation plots from mid elevation plots, with average temperature (0.33), % sand (0.32), potassium (-0.32), and soil moisture (-0.30) contributing most to the variance. The variables contributing most to variance on PC2 were sulfate (-0.42) and nitrate (-0.41), which mostly distinguished plot ML2 as an outlier with much greater concentrations of soil nutrients than other plots. Finally, variance on PC3 was most attributed to maximum temperature (-0.53) and canopy openness (-0.44), therefore separating HH and ML plots (lower maximum temperatures and openness) from MH plots (greater maximum temperatures and openness).

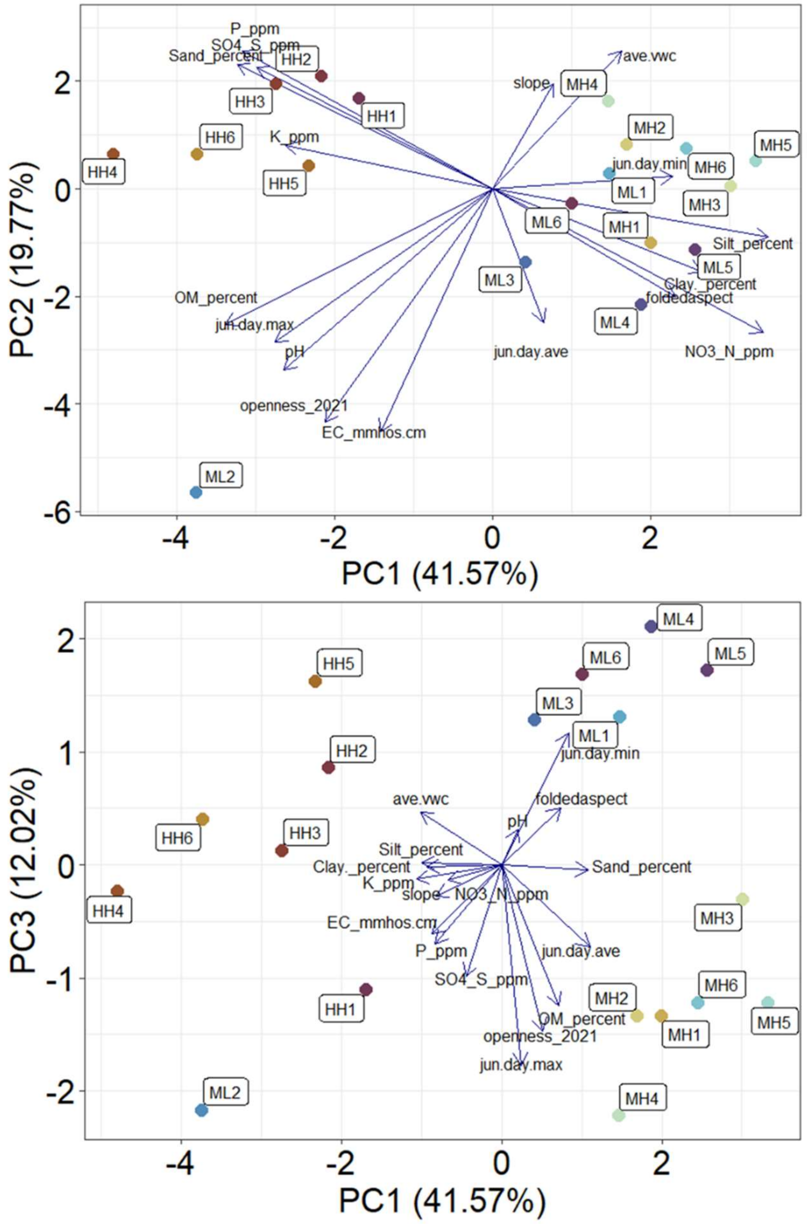


Figure A4.1. Biplot of principal components analysis (PCA) axes 1 and 2 (top) and 1 and 3 (bottom). Microsite variables are overlaid as blue vectors on the plot indicating which climate variables are most correlated with which PC. Points are plot scores for each field plot, labelled with the plot code.

Microclimate model sets

Below are all model sets used in analyses of population climate and mother tree trait interactions with microclimate for HH, MH, and MH+ML. Each numbered model represents a candidate model that was fit to survival and height growth data of sowed and planted seedlings, as well as basal area growth data of planted seedlings for each site grouping (HH, MH, and MH+ML) as indicated. Candidate models were constructed by selecting all combinations of microclimate variables that had correlations with an absolute value less than or equal to 0.6 within each site grouping (Figure A4.2). All candidate models were ranked according to AICc, with the model with the lowest AICc value retained for further analysis.

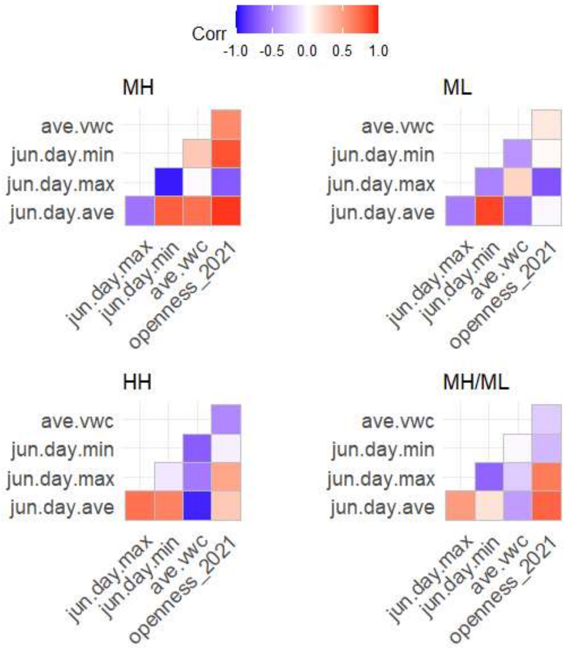


Figure A4.2. Pearson correlation matrices for microclimate variables at each site (MH, ML, HH) separately and the MH+ML site grouping.

Population models

HH

Sowed seedlings
#1
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +

```
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
#2
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +
#3
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
#4
scale(jun.day.min)*scale(cwd) + scale(jun.day.min)*scale(prop.monsoon) +
scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
#5
scale(jun.day.min)*scale(cwd) + scale(jun.day.min)*scale(prop.monsoon) +
scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +
#6
scale(jun.day.min)*scale(cwd) + scale(jun.day.min)*scale(prop.monsoon) +
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
#7
scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
#8
scale(jun.day.ave)*scale(cwd) + scale(jun.day.ave)*scale(prop.monsoon) +
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
```

Planted seedlings

```
#1
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +
scale(jun.day.max)*scale(height_outplant) + scale(jun.day.max)*scale(diam_outplant)+
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
scale(openness_2021)*scale(height_outplant) + scale(openness_2021)*scale(diam_outplant) +
#2
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +
scale(jun.day.max)*scale(height_outplant) + scale(jun.day.max)*scale(diam_outplant)+
#3
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
scale(openness_2021)*scale(height_outplant) + scale(openness_2021)*scale(diam_outplant) +
#4
scale(jun.day.min)*scale(cwd) + scale(jun.day.min)*scale(prop.monsoon) +
scale(jun.day.min)*scale(height_outplant) + scale(jun.day.min)*scale(diam_outplant)+
```

scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +
scale(jun.day.max)*scale(height_outplant) + scale(jun.day.max)*scale(diam_outplant)+
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
scale(openness_2021)*scale(height_outplant) + scale(openness_2021)*scale(diam_outplant) +
#5
scale(jun.day.min)*scale(cwd) + scale(jun.day.min)*scale(prop.monsoon) +
scale(jun.day.min)*scale(height_outplant) + scale(jun.day.min)*scale(diam_outplant)+
scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +
scale(jun.day.max)*scale(height_outplant) + scale(jun.day.max)*scale(diam_outplant)+
#6
scale(jun.day.min)*scale(cwd) + scale(jun.day.min)*scale(prop.monsoon) +
scale(jun.day.min)*scale(height_outplant) + scale(jun.day.min)*scale(diam_outplant)+
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
scale(openness_2021)*scale(height_outplant) + scale(openness_2021)*scale(diam_outplant) +
#7
scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +
scale(jun.day.max)*scale(height_outplant) + scale(jun.day.max)*scale(diam_outplant)+
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
scale(openness_2021)*scale(height_outplant) + scale(openness_2021)*scale(diam_outplant) +
#8
scale(jun.day.ave)*scale(cwd) + scale(jun.day.ave)*scale(prop.monsoon) +
scale(jun.day.ave)*scale(height_outplant) + scale(jun.day.ave)*scale(diam_outplant) +
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
scale(openness_2021)*scale(height_outplant) + scale(openness_2021)*scale(diam_outplant) +

MH + ML

Sowed Seedlings
#1
scale(jun.day.min)*scale(cwd) + scale(jun.day.min)*scale(prop.monsoon) +
scale(jun.day.ave)*scale(cwd) + scale(jun.day.ave)*scale(prop.monsoon) +
#2
scale(jun.day.min)*scale(cwd) + scale(jun.day.min)*scale(prop.monsoon) +
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
#3
scale(jun.day.min)*scale(cwd) + scale(jun.day.min)*scale(prop.monsoon) +
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
#4
scale(jun.day.min)*scale(cwd) + scale(jun.day.min)*scale(prop.monsoon) +
scale(jun.day.ave)*scale(cwd) + scale(jun.day.ave)*scale(prop.monsoon) +
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
#5
scale(jun.day.min)*scale(cwd) + scale(jun.day.min)*scale(prop.monsoon) +
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +

```
#6
scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +
scale(jun.day.ave)*scale(cwd) + scale(jun.day.ave)*scale(prop.monsoon) +
#7
scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
#8
scale(jun.day.ave)*scale(cwd) + scale(jun.day.ave)*scale(prop.monsoon) +
scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
#9
scale(jun.day.ave)*scale(cwd) + scale(jun.day.ave)*scale(prop.monsoon) +
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
#10
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
```

Planted Seedlings

```
#1
scale(jun.day.min)*scale(cwd) + scale(jun.day.min)*scale(prop.monsoon) +
scale(jun.day.min)*scale(height_outplant) + scale(jun.day.min)*scale(diam_outplant)+
scale(jun.day.ave)*scale(cwd) + scale(jun.day.ave)*scale(prop.monsoon) +
scale(jun.day.ave)*scale(height_outplant) + scale(jun.day.ave)*scale(diam_outplant) +
#2
scale(jun.day.min)*scale(cwd) + scale(jun.day.min)*scale(prop.monsoon) +
scale(jun.day.min)*scale(height_outplant) + scale(jun.day.min)*scale(diam_outplant)+
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
#3
scale(jun.day.min)*scale(cwd) + scale(jun.day.min)*scale(prop.monsoon) +
scale(jun.day.min)*scale(height_outplant) + scale(jun.day.min)*scale(diam_outplant)+
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
scale(openness_2021)*scale(height_outplant) + scale(openness_2021)*scale(diam_outplant) +
#4
scale(jun.day.min)*scale(cwd) + scale(jun.day.min)*scale(prop.monsoon) +
scale(jun.day.min)*scale(height_outplant) + scale(jun.day.min)*scale(diam_outplant)+
scale(jun.day.ave)*scale(cwd) + scale(jun.day.ave)*scale(prop.monsoon) +
scale(jun.day.ave)*scale(height_outplant) + scale(jun.day.ave)*scale(diam_outplant) +
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
#5
scale(jun.day.min)*scale(cwd) + scale(jun.day.min)*scale(prop.monsoon) +
scale(jun.day.min)*scale(height_outplant) + scale(jun.day.min)*scale(diam_outplant)+
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
scale(openness_2021)*scale(height_outplant) + scale(openness_2021)*scale(diam_outplant) +
```

```
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
#6
scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +
scale(jun.day.max)*scale(height_outplant) + scale(jun.day.max)*scale(diam_outplant)+
scale(jun.day.ave)*scale(cwd) + scale(jun.day.ave)*scale(prop.monsoon) +
scale(jun.day.ave)*scale(height_outplant) + scale(jun.day.ave)*scale(diam_outplant) +
#7
scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +
scale(jun.day.max)*scale(height_outplant) + scale(jun.day.max)*scale(diam_outplant)+
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
#8
scale(jun.day.ave)*scale(cwd) + scale(jun.day.ave)*scale(prop.monsoon) +
scale(jun.day.ave)*scale(height_outplant) + scale(jun.day.ave)*scale(diam_outplant) +
scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +
scale(jun.day.max)*scale(height_outplant) + scale(jun.day.max)*scale(diam_outplant)+
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
#9
scale(jun.day.ave)*scale(cwd) + scale(jun.day.ave)*scale(prop.monsoon) +
scale(jun.day.ave)*scale(height_outplant) + scale(jun.day.ave)*scale(diam_outplant) +
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
#10
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
scale(openness_2021)*scale(height_outplant) + scale(openness_2021)*scale(diam_outplant) +
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
```

MH – sowed seedling height growth only

```
#1
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +
#2
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
scale(jun.day.min)*scale(cwd) + scale(jun.day.min)*scale(prop.monsoon) +
#3
scale(ave.vwc)*scale(cwd) + scale(ave.vwc)*scale(prop.monsoon) +
scale(openness_2021)*scale(cwd) + scale(openness_2021)*scale(prop.monsoon) +
#4
scale(jun.day.max)*scale(cwd) + scale(jun.day.max)*scale(prop.monsoon) +
scale(jun.day.ave)*scale(cwd) + scale(jun.day.ave)*scale(prop.monsoon) +
```

Mother Tree Models

HH

Sowed Seedlings

#1
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*wuescaled +
scale(jun.day.max)*scale(fdsi.cor) +
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +
scale(openness_2021)*scale(fdsi.cor) +
#2
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*wuescaled +
scale(jun.day.max)*scale(fdsi.cor) +
#3
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +
scale(openness_2021)*scale(fdsi.cor) +
#4
scale(jun.day.min)*scale(g.seed) + scale(jun.day.min)*wuescaled +
scale(jun.day.min)*scale(fdsi.cor) +
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*wuescaled +
scale(jun.day.max)*scale(fdsi.cor) +
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +
scale(openness_2021)*scale(fdsi.cor) +
#5
scale(jun.day.min)*scale(g.seed) + scale(jun.day.min)*wuescaled +
scale(jun.day.min)*scale(fdsi.cor) +
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*wuescaled +
scale(jun.day.max)*scale(fdsi.cor) +
#6
scale(jun.day.min)*scale(g.seed) + scale(jun.day.min)*wuescaled +
scale(jun.day.min)*scale(fdsi.cor) +
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +
scale(openness_2021)*scale(fdsi.cor) +
#7
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*wuescaled +
scale(jun.day.max)*scale(fdsi.cor) +
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +
scale(openness_2021)*scale(fdsi.cor) +
#8
scale(jun.day.ave)*scale(g.seed) + scale(jun.day.ave)*wuescaled +
scale(jun.day.ave)*scale(fdsi.cor) +
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +
scale(openness_2021)*scale(fdsi.cor) +

Planted Seedlings

#1
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*wuescaled +
scale(jun.day.max)*scale(fdsi.cor) + scale(jun.day.max)*scale(height_outplant) +
scale(jun.day.max)*scale(diam_outplant)+
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +
scale(openness_2021)*scale(fdsi.cor) + scale(openness_2021)*scale(height_outplant) +
scale(openness_2021)*scale(diam_outplant)+

#2
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*wuescaled +
scale(jun.day.max)*scale(fdsi.cor) + scale(jun.day.max)*scale(height_outplant) +
scale(jun.day.max)*scale(diam_outplant)+

#3
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +
scale(openness_2021)*scale(fdsi.cor) + scale(openness_2021)*scale(height_outplant) +
scale(openness_2021)*scale(diam_outplant)+

#4
scale(jun.day.min)*scale(g.seed) + scale(jun.day.min)*wuescaled +
scale(jun.day.min)*scale(fdsi.cor) + scale(jun.day.min)*scale(height_outplant) +
scale(jun.day.min)*scale(diam_outplant)+
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*wuescaled +
scale(jun.day.max)*scale(fdsi.cor) + scale(jun.day.max)*scale(height_outplant) +
scale(jun.day.max)*scale(diam_outplant)+
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +
scale(openness_2021)*scale(fdsi.cor) + scale(openness_2021)*scale(height_outplant) +
scale(openness_2021)*scale(diam_outplant)+

#5
scale(jun.day.min)*scale(g.seed) + scale(jun.day.min)*wuescaled +
scale(jun.day.min)*scale(fdsi.cor) + scale(jun.day.min)*scale(height_outplant) +
scale(jun.day.min)*scale(diam_outplant)+
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*wuescaled +
scale(jun.day.max)*scale(fdsi.cor) + scale(jun.day.max)*scale(height_outplant) +
scale(jun.day.max)*scale(diam_outplant)+

#6
scale(jun.day.min)*scale(g.seed) + scale(jun.day.min)*wuescaled +
scale(jun.day.min)*scale(fdsi.cor) + scale(jun.day.min)*scale(height_outplant) +
scale(jun.day.min)*scale(diam_outplant)+
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +

```
scale(openness_2021)*scale(fdsi.cor) + scale(openness_2021)*scale(height_outplant) +
scale(openness_2021)*scale(diam_outplant)+
#7
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*wuescaled +
scale(jun.day.max)*scale(fdsi.cor) + scale(jun.day.max)*scale(height_outplant) +
scale(jun.day.max)*scale(diam_outplant)+
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +
scale(openness_2021)*scale(fdsi.cor) +  scale(openness_2021)*scale(height_outplant) +
scale(openness_2021)*scale(diam_outplant)+
#8
scale(jun.day.ave)*scale(g.seed) + scale(jun.day.ave)*wuescaled +
scale(jun.day.ave)*scale(fdsi.cor) + scale(jun.day.ave)*scale(height_outplant) +
scale(jun.day.ave)*scale(diam_outplant)+
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +
scale(openness_2021)*scale(fdsi.cor) + scale(openness_2021)*scale(height_outplant) +
scale(openness_2021)*scale(diam_outplant)+
```

MH + ML

Sowed Seedlings

```
#1
scale(jun.day.min)*scale(g.seed) + scale(jun.day.min)*wuescaled +
scale(jun.day.min)*scale(fdsi.cor) +
scale(jun.day.ave)*scale(g.seed) + scale(jun.day.ave)*wuescaled +
scale(jun.day.ave)*scale(fdsi.cor) +
#2
scale(jun.day.min)*scale(g.seed) + scale(jun.day.min)*wuescaled +
scale(jun.day.min)*scale(fdsi.cor) +
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
#3
scale(jun.day.min)*scale(g.seed) + scale(jun.day.min)*wuescaled +
scale(jun.day.min)*scale(fdsi.cor) +
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +
scale(openness_2021)*scale(fdsi.cor) +
#4
scale(jun.day.min)*scale(g.seed) + scale(jun.day.min)*wuescaled +
scale(jun.day.min)*scale(fdsi.cor) +
scale(jun.day.ave)*scale(g.seed) + scale(jun.day.ave)*wuescaled +
scale(jun.day.ave)*scale(fdsi.cor) +
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
#5
scale(jun.day.min)*scale(g.seed) + scale(jun.day.min)*wuescaled +
scale(jun.day.min)*scale(fdsi.cor) +
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +
scale(openness_2021)*scale(fdsi.cor) +
```

```
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
#6
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*wuescaled +
scale(jun.day.max)*scale(fdsi.cor)+
scale(jun.day.ave)*scale(g.seed) + scale(jun.day.ave)*wuescaled +
scale(jun.day.ave)*scale(fdsi.cor) +
#7
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*wuescaled +
scale(jun.day.max)*scale(fdsi.cor)+
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
#8
scale(jun.day.ave)*scale(g.seed) + scale(jun.day.ave)*wuescaled +
scale(jun.day.ave)*scale(fdsi.cor) +
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*wuescaled +
scale(jun.day.max)*scale(fdsi.cor)+
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
#9
scale(jun.day.ave)*scale(g.seed) + scale(jun.day.ave)*wuescaled +
scale(jun.day.ave)*scale(fdsi.cor) +
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
#10
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +
scale(openness_2021)*scale(fdsi.cor) +
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
```

Planted Seedlings

```
#1
scale(jun.day.min)*scale(g.seed) + scale(jun.day.min)*wuescaled +
scale(jun.day.min)*scale(fdsi.cor) + scale(jun.day.min)*scale(height_outplant) +
scale(jun.day.min)*scale(diam_outplant)+
scale(jun.day.ave)*scale(g.seed) + scale(jun.day.ave)*wuescaled +
scale(jun.day.ave)*scale(fdsi.cor) + scale(jun.day.ave)*scale(height_outplant) +
scale(jun.day.ave)*scale(diam_outplant)+
#2
scale(jun.day.min)*scale(g.seed) + scale(jun.day.min)*wuescaled +
scale(jun.day.min)*scale(fdsi.cor) + scale(jun.day.min)*scale(height_outplant) +
scale(jun.day.min)*scale(diam_outplant)+
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
#3
scale(jun.day.min)*scale(g.seed) + scale(jun.day.min)*wuescaled +
scale(jun.day.min)*scale(fdsi.cor) + scale(jun.day.min)*scale(height_outplant) +
scale(jun.day.min)*scale(diam_outplant)+
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +
scale(openness_2021)*scale(fdsi.cor) + scale(openness_2021)*scale(height_outplant) +
```

```
scale(openness_2021)*scale(diam_outplant)+
#4
scale(jun.day.min)*scale(g.seed) + scale(jun.day.min)*wuescaled +
scale(jun.day.min)*scale(fdsi.cor) + scale(jun.day.min)*scale(height_outplant) +
scale(jun.day.min)*scale(diam_outplant)+
scale(jun.day.ave)*scale(g.seed) + scale(jun.day.ave)*wuescaled +
scale(jun.day.ave)*scale(fdsi.cor) + scale(jun.day.ave)*scale(height_outplant) +
scale(jun.day.ave)*scale(diam_outplant)+
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
#5
scale(jun.day.min)*scale(g.seed) + scale(jun.day.min)*wuescaled +
scale(jun.day.min)*scale(fdsi.cor) + scale(jun.day.min)*scale(height_outplant) +
scale(jun.day.min)*scale(diam_outplant)+
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +
scale(openness_2021)*scale(fdsi.cor) + scale(openness_2021)*scale(height_outplant) +
scale(openness_2021)*scale(diam_outplant)+
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
#6
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*wuescaled +
scale(jun.day.max)*scale(fdsi.cor)+ scale(jun.day.max)*scale(height_outplant) +
scale(jun.day.max)*scale(diam_outplant)+
scale(jun.day.ave)*scale(g.seed) + scale(jun.day.ave)*wuescaled +
scale(jun.day.ave)*scale(fdsi.cor) + scale(jun.day.ave)*scale(height_outplant) +
scale(jun.day.ave)*scale(diam_outplant)+
#7
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*wuescaled +
scale(jun.day.max)*scale(fdsi.cor)+ scale(jun.day.max)*scale(height_outplant) +
scale(jun.day.max)*scale(diam_outplant)+
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
#8
scale(jun.day.ave)*scale(g.seed) + scale(jun.day.ave)*wuescaled +
scale(jun.day.ave)*scale(fdsi.cor) + scale(jun.day.ave)*scale(height_outplant) +
scale(jun.day.ave)*scale(diam_outplant)+
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*wuescaled +
scale(jun.day.max)*scale(fdsi.cor)+ scale(jun.day.max)*scale(height_outplant) +
scale(jun.day.max)*scale(diam_outplant)+
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
#9
scale(jun.day.ave)*scale(g.seed) + scale(jun.day.ave)*wuescaled +
scale(jun.day.ave)*scale(fdsi.cor) + scale(jun.day.ave)*scale(height_outplant) +
scale(jun.day.ave)*scale(diam_outplant)+
```

scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+
#10
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*wuescaled +
scale(openness_2021)*scale(fdsi.cor) + scale(openness_2021)*scale(height_outplant) +
scale(openness_2021)*scale(diam_outplant)+
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*wuescaled + scale(ave.vwc)*scale(fdsi.cor)+
scale(ave.vwc)*scale(height_outplant) + scale(ave.vwc)*scale(diam_outplant)+

MH – sowed seedling height growth only
#1
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*scale(fdsi.cor) + scale(ave.vwc)* wuescaled +
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*scale(fdsi.cor) +
#2
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*scale(fdsi.cor) + scale(ave.vwc)* wuescaled +
scale(jun.day.min)*scale(g.seed) + scale(jun.day.min)*scale(fdsi.cor) +
scale(jun.day.min)*wuescaled +
#3
scale(ave.vwc)*scale(g.seed) + scale(ave.vwc)*scale(fdsi.cor) + scale(ave.vwc)*wuescaled +
scale(openness_2021)*scale(g.seed) + scale(openness_2021)*scale(fdsi.cor) +
scale(openness_2021)*wuescaled +
#4
scale(jun.day.max)*scale(g.seed) + scale(jun.day.max)*scale(fdsi.cor) +
scale(jun.day.max)*wuescaled +
scale(jun.day.ave)*scale(g.seed) + scale(jun.day.ave)*scale(fdsi.cor) +
scale(jun.day.ave)*wuescaled +