12-2021

Methods to Improve Our Understanding of Aspen Regeneration and Aspen Distribution Across the Intermountain West

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Recommended Citation

Bidner, Robert Joseph Julius, "Methods to Improve Our Understanding of Aspen Regeneration and Aspen Distribution Across the Intermountain West" (2021). *All Graduate Theses and Dissertations*. 8336.
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METHODS TO IMPROVE OUR UNDERSTANDING OF ASPEN REGENERATION AND ASPEN DISTRIBUTION ACROSS THE INTERMOUNTAIN WEST

by

Robert Joseph Julius Bidner

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Ecology

Approved:

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2021

ABSTRACT

Methods to Improve Our Understanding of Aspen Regeneration and Aspen Distribution Across the Intermountain West

by

Robert J.J. Bidner, Master of Science

Utah State University, 2021

Major Professor: Dr. Karen E. Mock
Department: Wildland Resources

In the U.S. Intermountain West, quaking aspen (*Populus tremuloides*) is a widely distributed tree species of great ecological importance. Recent landscape-scale dieback events have invigorated interest in understanding the factors that influence aspen distribution, anticipating that this might help predict future persistence and inform management decisions. In my thesis, I explore two separate questions related to aspen ecology; 1) How is the distribution of aspen sexes distributed with respect to geospatial and climatic variables? and 2) How do above- and below-ground measurements of aspen predict suckering sized root mass and regeneration potential?

The first question in my thesis is motivated by the limited amount of research on the impact of sex-specific differences on aspen distribution. I used a molecular marker to determine sex in each of 1447 trees across 31 sites in the Intermountain West. Tree sampling was intentionally distributed across elevation and moisture gradients at each site. Results across sites showed a nearly 2:1 male:female sex ratio, and increasing elevation and mean annual precipitation were associated with a higher probability of an aspen tree being male. I also found a negative interaction between elevation and latitude. The skewed sex ratio across the landscape may be an indication of decreased persistence and/or greater mortality of female aspen clones after establishment.

The second question in my thesis is motivated by a lack of knowledge about how the root condition of aspen relates to regeneration potential and the above-ground condition. I sampled 23 aspen-dominated sites, mainly in southern Utah. I recorded above-ground measurements of stand conditions and collected aspen roots from 6 plots at each site. A subset of roots were suckered in a greenhouse experiment where the number of suckers and non-structural carbohydrates (NSC) were measured. I found correlations between suckering-sized root mass (SSrm) and shrub canopy cover (0.755), grass cover (-0.460), and individual tree crown dieback (-0.337). I also found correlations between phloem diameter (0.427) and total NSC in the whole root (0.193) with suckers/root surface area. I conclude that the use of stand- or root-level measurements in addition to standard silvicultural methods may improve prediction of aspen suckering response.

(99 pages)

PUBLIC ABSTRACT

Methods to Improve Our Understanding of Aspen Regeneration and Aspen Distribution Across the Intermountain West

Robert J.J. Bidner

Quaking aspen (*Populus tremuloides*) is the dominant broadleaf tree and an ecologically important species at upper elevations in the Intermountain West. Recent large-scale forest mortality events have raised questions about how physiological and climatic factors influence aspen's distribution across the western U.S. Aspen is particularly well-known for reproducing asexually from its root sprouts, leading to the formation of large clonal stands. In addition, as a wind-dispersed species, aspen sexual reproduction plays an important role in how it is distributed at a landscape scale. My research focuses on questions relating to both sexual and asexual reproduction of aspen.

My first research question was to determine how is aspen distributed by sex and climatic variables across the Intermountain West? My results indicated that there were nearly 2:1 male:female aspen across the landscape. These results indicate an overall male bias among established aspen in the Intermountain West, which may suggest male aspen clones are persisting longer or expanding more than female clones.

My second research question was to determine how well above- and below-ground measurements predict aspen suckering sized root mass and regeneration potential. Results indicated a few strong correlations between the mass of suckering-sized roots and understory associated species cover, as well as proportion of crown dieback. There were also strong correlations between root phloem diameter proportion and root carbohydrate measurements. These results suggest that the use of stand- or root-level measurements can improve prediction of aspen suckering response.

ACKNOWLEDGMENTS

First and foremost, I would like to express my gratitude to my advisor Karen Mock for her guidance and support throughout this project. Her mentorship helped me grow as a person during this experience and she has a level of dedication and skill that is unmatched, thank you for sticking through this with me Karen.

I would like to thank the other members of my committee for their input and time over the last few years. The expertise (and wit) of Simon Landhäusser was indispensable when it came to designing the greenhouse experiment and the carbohydrate analysis. The expertise provided by Jim Long and Darren McAvoy made designing and implementing the field work possible and I can't thank them enough. For the second chapter, I would like to thank Jon Mock, Alex Howe, Dave Soderberg, Torrey Rodgers, and Jim Vandygriff for collecting samples and traveling all over the Intermountain West, this was an incredible effort to gather that many leaves and it couldn't have been done without all of you. During the analysis, I would like to thank Sara Goeking for her input and technical expertise choosing climate variables and to Sue Durham, Will Pearse, and John Shaw for helping design and fine-tune the model. Statistical analysis is its own beast, and all of your help was invaluable. For the work described in the third chapter, I would like to thank both Randall Violett and the late Chad Reid for showing me around Cedar Mountain, connecting us with landowners, and for both of your banter and hospitality. Thanks are in order for my field crew Etta Crowley, Liam McAvoy, Cashe Rasmussen, and Savannah Tollefson for car camping on ranches, becoming experts on measuring aspen, and for your laughs and high spirits through all the tough field days. Thanks are in order for Mark Kreider as well, your trailer pizza was amazing, and my crew always appreciated the visits to your field camp near Brian Head. Thank you to the Cedar Mountain landowners, as well as the Fish Lake and Cache districts of the U.S. Forest Service. I would like to also thank Pak Chow for his work performing the NSC extraction and analysis. I am grateful for the financial funding through the Cedar Mountain Initiative. Finally, I cannot fully express how thankful I am for the support of my partner Karen Foley who help keep me going through all the ups-and-downs, I love you and am excited to begin our life together as well as my family, (especially my mom Jean, my dad Dave and my sister Katie) and friends through this entire journey, you all mean so much to me. I am excited to share and begin new journeys with all of you for many years to come.

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CHAPTER 1

INTRODUCTION

Quaking aspen (*Populus tremuloides*) is a dioecious tree species of conservation interest (Barnes 1966; Kemperman and Barnes 1976) and is the most broadly distributed tree species in North America (Little 1979) ranging across 48 degrees of latitude (Little and Viereck 1971) and at elevations ranging from 0-3650m (DeByle and Winokur 1985). Across the U.S. Intermountain West, aspen tends to be the dominant broadleaf tree at middle to upper elevations (Little and Viereck 1971; Burns et al. 1990). Aspen is associated with high levels of plant, bird, and insect biodiversity (Kuhn et al. 2011; Rogers and Mittanck 2014). Dramatic local mortality episodes in southwestern aspen (Huang and Anderegg 2012) and forecasts of decreases in aspen range (Rehfeldt et al. 2009; Worrall et al. 2013) have fueled uncertainty about the long-term persistence of aspen with climate change and associated episodic drought events (Anderegg et al. 2013b, a).

Aspen is known for its tendency to reproduce clonally following seedling establishment, and is considered to be an early successional species, with suckering pulses following disturbance (typically fire) that removes the above-ground biomass (Barnes 1966; DeByle and Winokur 1985). In western U.S. landscapes, presumably due to generally inhospitable conditions for seedling establishment, suckering frequently results in the establishment of large clones (Barnes 1966; Grant et al. 1992) including the famous “Pando” clone in central Utah (Kemperman and Barnes 1976; DeWoody et al. 2008). It should be noted however that most clones even in western U.S. landscapes seem to be small (e.g. under 1ha in size) and that stands frequently consist of multiple clones

(Hipkins and Kitzmiller 2004; Mock et al. 2008). While the importance and prevalence of aspen establishment by seed dispersal has been recognized recently in the western U.S. (Long and Mock 2012), most individual stems are the result of vegetative regeneration (suckering) (Schier 1973; McDonough 1985; Schier et al. 1985), and aspen management is generally focused on maintaining previously established clones. The goals of my research were to better understand and identify factors related to aspen ecology both across its distribution and at the local stand level.

In Chapter 2, I examined the distribution of male and female aspen clones at a continental scale. Improving the ability to manage aspen in the Intermountain West requires an understanding of the environmental and physiological factors influencing aspen's current distribution and how those factors will affect its future distribution. Sex-specific trait differences in dioecious tree species, and how those traits influence landscape distributional patterns, is particularly important as the impacts of climate change increase (Hultine et al. 2007; Landhäusser et al. 2010). Clonal species such as aspen can exhibit more asexual than sexual reproduction when the time between disturbances increases, which can limit the number of seedling establishment opportunities (Silvertown 2008). In dioecious plants, when the sexes are distributed differently due to physiological differences, the distance between sexes may also increase, potentially further decreasing the frequency of sexual reproduction (Obeso et al. 1998; Charpentier 2001; Mock et al. 2012). Within the *Populus* genus, sex-specific responses to water availability, salinity, and temperature have been documented (Xu et al. 2008; Chen et al. 2010) and could potentially favor one sex vs. another across the landscape. In Chapter 2, I asked whether the sexes in aspen were distributed randomly across the landscape at both large (continent-wide) and local scales, and whether particular climate variables were associated with any sex bias. To address this question, I used samples collected from 31 30km x 30km sites (1447 samples total) distributed across the western U.S. I expected to find sex distribution biases similar to those described by Grant and Mitton (1979), who found in a local study in Colorado that female aspen occupy lower elevation, riparian habitats (Grant and Mitton 1979).

In Chapter 3, I explored correlations between above- and below-ground factors that might be predictive of successful vegetative regeneration in aspen. Aspen possess a relatively shallow root system, with extensive lateral roots and a few “sinker” roots (Jones et al. 1985), with the vegetative asexual reproduction originating from the primordia on lateral roots within 0.15m of the soil surface (Baker 1925; Farmer 1962; Schier 1973; Schier and Campbell 1978). Traditionally, above-ground factors (browsing intensity, site index, overstory condition) are used to prioritize silvicultural treatments to regenerate aspen stands asexually (Sheppard 2001; Smith et al. 2011; Britton et al. 2016). However, it is unclear how well these above-ground stand measurements are correlated to below-ground conditions, and whether additional site or below-ground metrics should be considered when making regeneration treatment decisions. In Chapter 3, I performed a field study at 23 sites across Utah, measuring above- and below-ground factors in aspen stands and comparing them to metrics that could predict regeneration potential, including suckering-sized root mass (SSrm), non-structural carbohydrates (NSC), and phloem diameter proportion of roots within a stand. In the field, I explored relationships between the suckering sized root mass and a range of above-ground stand metrics. In the greenhouse, I assessed relationships between the suckering ability of root segments and how that ability relates to the NSC concentrations and phloem diameters in those roots. The results of both of these projects will help answer questions about aspen distribution and guide future research on aspen regeneration.

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CHAPTER 2

LANDSCAPE-SCALE DISTRIBUTION OF MALE AND FEMALE TREES IN ASPEN ACROSS THE WESTERN U.S.

Abstract

Dioecious plants can exhibit sex-specific physiological traits that manifest as distinct landscape scale distributions, putatively due to the energetic burden associated with seed production in females. In aspen (*Populus tremuloides*), when distribution is patchy and clones are large, a strong distributional sex bias could limit successful fertilization opportunities and influence landscape patterns of seed production. Variation in distribution by sex has only been described for aspen in limited, local-scale studies, with the ratio skewing higher towards males at higher elevations. Aspen sex ratios have not been assessed at a large spatial scale. I hypothesize that if seed production and physiological constraints limit female success in harsher habitats, the overall sex ratio of aspen genets would be male-biased at drier, warmer sites and at higher elevations. I sampled leaves from a total of 1447 trees at 31 sites across the Intermountain West. Tree sampling was intentionally distributed across elevational and moisture gradients within sites. Sex was determined using a genetic marker. Bayesian analysis of a Bernoulli generalized linear model (GLM) was used to assess the association between aspen sex ratios with elevation, latitude, heat load index, mean annual precipitation, and their interactions. Results at the study-wide scale indicate increasing elevation and mean annual precipitation were associated with a higher probability of an aspen tree being male. There was also a negative interaction between elevation and latitude. Latitude and heat load index were not associated with male probability. Site-level associations

between sex ratios and my predictors were highly variable and may have more to do with site-specific environments and histories than an overarching trend on the landscape.

Introduction

Differences between sexes in dioecious plant species have been recognized, documented, and exploited by humans since the beginnings of civilization (Darwin 1877; This et al. 2006; Terral et al. 2012). Many of these sex-specific differences are morphological, physiological, and ecological traits, often resulting in an energetic burden associated with seed production in females (Freeman et al. 1980; Gross and Soule 1981; Dawson and Ehleringer 1993; Espírito-Santo et al. 2003; Li et al. 2007; Barrett and Hough 2013). In some dioecious tree species, males have been found to flower at a higher frequency and have higher survival rates than females (Iszkuło and Boratyński 2011); females are often found in areas of higher quality, with less resource stress compared to their male counterparts, especially in arid environments (Freeman and McArthur 1982; Dawson and Ehleringer 1993; Li et al. 2007). Such ecological differences could result in distinct sex-specific landscape-scale distributions with consequences for plant-herbivore interactions, demography, and stand resilience (Lande 1980; Dawson and Bliss 1989; Cornelissen and Stiling 2005; Geber et al. 2012).

Understanding sex-specific trait differences in tree species and how they influence landscape species distributional patterns is particularly important as the impacts of climate change increase (Hultine et al. 2007; Landhäusser et al. 2010). For example, if female plants are disproportionately impacted by climate changes, their distributional changes may lead to spatial changes in ecosystem functions or resilience, even if the species distribution as a whole does not change. Additionally, the frequency and pattern of sexual reproduction are dependent on the dispersal ability of male gametes and the density of females within that dispersal radius (Eriksson 1989; Crawford and Balfour 1990; Ortiz et al. 2002). When male and female trees become segregated across large temperature or moisture gradients, for example, the chances of successful fertilization may decrease (Hultine et al. 2007; Nuñez et al. 2008). Pronounced environmental variation, potentially favoring one sex over another, is particularly evident in topographically complex landscapes (Dawson and Bliss 1989). The ecological impact of distributional differences in sexes and the prevalence of reproductive strategies needs to be considered when discussing a species that reproduces both sexually and asexually (Vallejo-Marín et al. 2010).

Clonal species often exhibit more asexual than sexual reproduction when the time between disturbances (and hence seedling establishment opportunities) increases (Silvertown 2008). In these situations, clones can become quite large (Kemperman and Barnes 1976; Grant and Mitton 1979; DeWoody et al. 2008), magnifying sex-distributional differences and their associated ecological functional differences. Clonal species also often have a higher spatial genetic structure when compared to non-clonal species (Kettenring and Mock 2012; Dering et al. 2015), potentially due to long-term persistence and disproportionate contribution of specific larger clones to local sexual reproduction (i.e. increased genetic drift). In dioecious plants, when clone sizes increase, so might the distance between sexes, potentially further decreasing the frequency of sexual reproduction (Obeso et al. 1998; Charpentier 2001; Silvertown 2008; Mock et al. 2012). However, sexual reproduction is still necessary for adaptation and long-term persistence of these populations, particularly in rapidly changing climatic conditions (Wilcock and Neiland 2002).

Non-homogeneous spatial distribution of sexes in clonal dioecious plants could be an important consideration in species distribution models. The use of such models to understand and predict species distributions in changing climates is an increasingly important aspect of ecological and economic forecasting (Elith and Leathwick 2009; Austin and Niel 2011). Predictions and management decisions based on species distribution models are particularly valuable for foundation species, which have cascading impacts when they experience range contractions or expansions (Pecl et al. 2017). If there is a predictable relationship between environmental factors and sex distribution in these species, that information can be used to improve models of species persistence, climate change impacts and restoration strategies.

Quaking aspen (*Populus tremuloides*) is a dioecious tree that reproduces both sexually and by asexual clonal propagation (Barnes 1966; Kemperman and Barnes 1976; Mock et al. 2008). Aspen is the most broadly distributed tree species in North America (Little 1979) ranging across 48 degrees of latitude (Little and Viereck 1971) and at elevations ranging from 0-3650m (DeByle and Winokur 1985). Across the U.S. Intermountain West, aspen tends to be the dominant broadleaf tree at middle to upper elevations (Little and Viereck 1971; Burns et al. 1990; Rogers et al. 2014). Aspen is associated with high levels of plant, bird, and insect biodiversity (Kuhn et al. 2011; Rogers and Mittanck 2014) and thus is a foundation species of great ecological importance. In the northern portions of the Intermountain West, aspen tend to occupy lower elevations and more southerly aspects, shifting to higher elevations and northerly aspects at the southern/southwestern end of its range (DeByle and Winokur 1985). In the southwestern portion of its range, aspen favors clonal reproduction, with examples of genets achieving sizes over 40 hectares (Kemperman and Barnes 1976). Aspen in the western U. S., south of the last glacial maximum, are also genetically distinct (Callahan et al. 2013), so genetic factors may also contribute to clone sizes and the infrequency of sexual reproduction in this portion of the species range. Dramatic local mortality episodes in southwestern aspen have fueled uncertainty about long-term persistence with climate change and associated episodic drought events. Climate-associated stress, particularly water stress (Anderegg et al. 2013a, b) has been implicated as a major proximate factor in these mortality events (Worrall et al. 2010; Huang and Anderegg 2012) but differential distribution, physiology, polyploidy, and mortality of aspen sexes may also be a factor.

Within the *Populus* genus, sex-specific responses to water availability, salinity, and temperature have been documented (Xu et al. 2008; Chen et al. 2010). Seed production, genet size, resource limitations, and physiological constraints can all potentially reduce female aspen survivability and reproductive success (Bourdeau 1958). In the Intermountain West, large local variation in elevation and aspect creates pronounced water and temperature disparities across aspen habitats. In *P. tremuloides*, one local-scale study in Colorado addressed the question of differential landscape distribution of sexes, and described a bias towards male aspen clones at higher elevations (Grant and Mitton 1979). If this pattern is general in southwestern aspen, elevational sex bias could be an explanatory factor in landscape patterns of mortality. However, aspen sex identification in Grant and Mitton (1979) was based only on field observations, which can be problematic as aspen often do not flower every year. Male-biased sex ratios are more commonly recorded for dioecious tree species when sex is recorded from flowering individuals, due to males flowering at a higher frequency (Field et al. 2013). The distribution of aspen sex ratio variation has never been described over a large spatial scale. In this study I sampled aspen populations in the Intermountain West across multiple local moisture and elevational gradients and used a genetic marker to determine individual sex rather than relying on flowering. Given the reproductive energetic burden on female trees and observations in other dioecious tree species, I hypothesize that the overall sex ratio of aspen genets on the landscape would generally be male-biased, and that this bias would be most pronounced in landscapes where drought stress risk is most pronounced. Alternatively, clonal persistence and relatively infrequent female flowering in southwestern aspen may buffer against these biases.

Materials and Methods

Aspen leaf samples were collected in the summer of 2016 (n = 1447) at 31 sites across the Intermountain West (Fig. 2.1, Table 2.1). Sites were chosen by first filtering to include only U.S. Forest Service land with high proportions of aspen cover type. 30km x 30km sites were then chosen prior to site visits to maximize distribution of sites across latitude and longitude and to assure road access and elevational variation within sites. Within each site, leaves were sampled from 21-50 mature (over ~5cm DBH) trees. Sampled trees were either separated by large geographic distances or taken from stands which were clearly separated by non-aspen vegetation, in order to minimize the probability of sampling the same clone more than once. An effort was made to distribute samples across both elevational and moisture gradients within sites (Table 2.2). Moisture levels were subjectively determined (“wet”, “dry”, “intermediate”) in the field based on plant composition and immediate proximity to surface water. An attempt was made to minimize “intermediate” sampling points and to spatially distribute approximately equal numbers of “wet” and “dry” sampling points. To maximize the number of samples collected over limited time, sampling was generally constrained to secondary road corridors of ~1 km width. Location and elevation were recorded using a Garmin Montana 610t GPS unit.

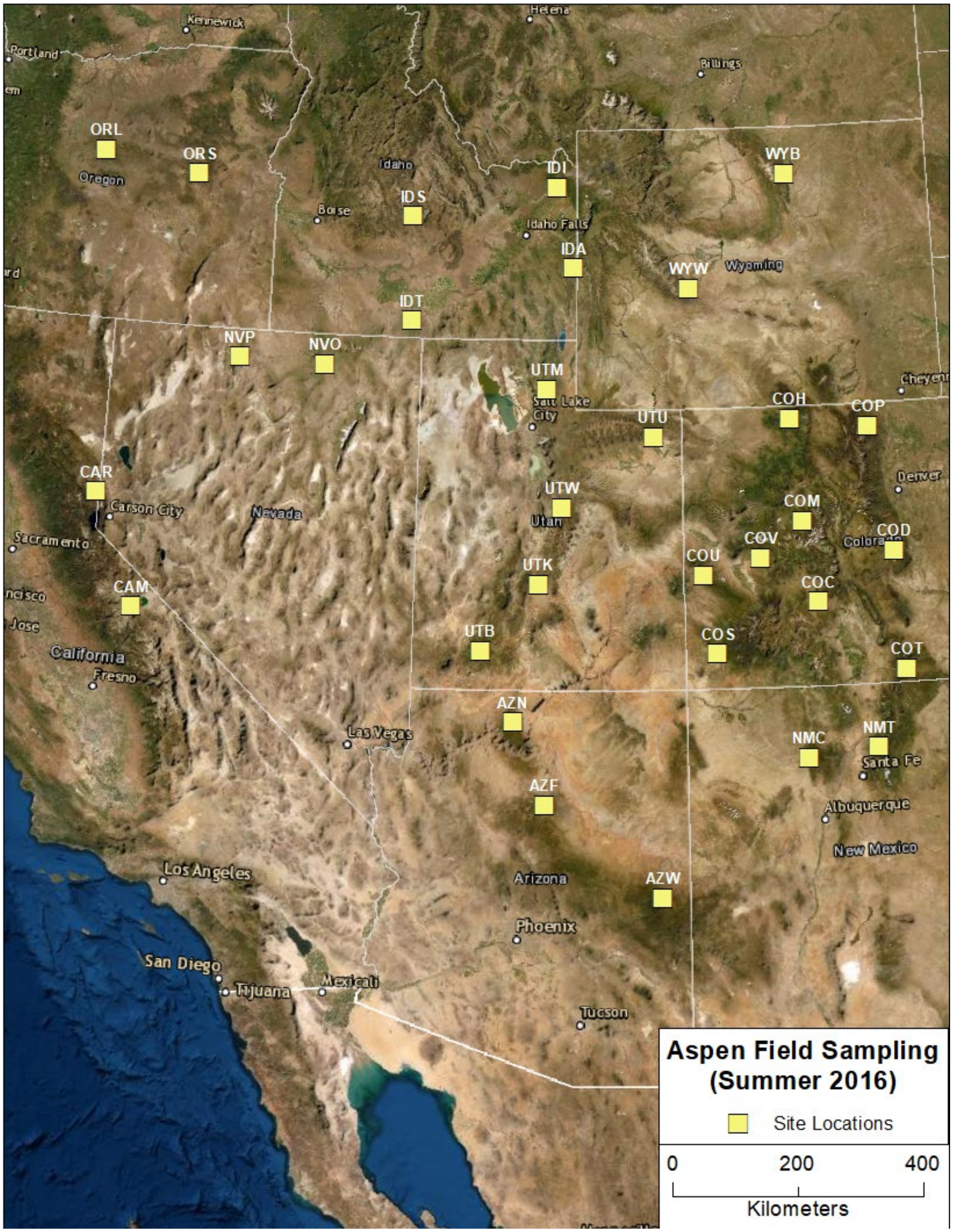

Figure 2.1 Location of 31 field sampling sites across the Intermountain West from the summer of 2016. Sites were 30km x 30km and were chosen prior to sampling. A total of 21-50 leaves were sampled from mature (>5 cm DBH) trees. Samples were collected across a range of elevational and moisture gradients present at each site.

Table 2.1 Elevation range, HLI range, latitude average of sampling points, and male:female ratio of sampled leaves at each of the sampling sites. Elevation and latitude were determined with GPS and checked using USGS 1/3 arc-second DEM's. HLI was calculated using folded aspect, slope, and latitude following methods in McCune and Keon (2002). Sex determination was made using the TOZ19 locus for *Populus* using methods from Pakull et al. (2015).

Site	N	Elevation Range (m)	HLI Range ln(Rad, $MJ/cm^{2/}yr^{1}$)	Latitude (decimal degrees)	Proportion of male Aspen (Ratio)
AZF	48	2197 - 2906	-0.488 - 0.053	35.3164	0.604
AZN	49	2189 - 2790	-0.261 - 0.048	36.4489	0.580
AZW	49	2605 - 2855	-0.307 - -0.011	33.9751	0.551
CAM	50	2170 - 2964	-0.328 - 0.077	38.0232	0.500
CAR	44	1715 - 2698	-0.489 - -0.010	39.3816	0.523
COC	49	2756 - 3249	-0.416 - 0.066	38.1577	0.612
COD	21	2083 - 3077	-0.551 - 0.007	38.7760	0.714
COH	49	2141 - 2782	-0.636 - 0.031	40.9225	0.653
COM	50	2348 - 2986	-0.496 - 0.024	39.3278	0.520
COP	50	2261 - 2985	-0.474 - 0.012	40.7788	0.640
COS	50	2286 - 3147	-0.272 - 0.054	37.6504	0.680
COT	49	2472 - 3317	-0.605 - 0.050	37.2997	0.755
COU	50	2492 - 2931	-0.352 - -0.012	38.5065	0.740
COV	50	2080 - 2732	-0.498 - 0.022	39.1943	0.760
IDA	28	1741 - 1904	-0.667 - -0.042	43.1155	0.429
IDI	50	1789 - 2204	-0.500 - -0.008	44.2978	0.500
IDS	42	1817 - 2462	-0.507 - -0.133	43.7868	0.571
IDT	48	1608 - 2229	-0.514 - 0.001	42.1811	0.708
NMC	50	2242 - 2872	-0.497 - 0.016	35.9751	0.840
NMT	50	2371 - 3148	-1.019 - 0.078	36.2007	0.620
NVO	50	1801 - 2270	-0.627 - -0.125	41.6429	0.700
NVP	32	1650 - 2347	-0.604 - -0.034	41.6720	0.531
ORL	50	1172 - 1959	-0.482 - -0.072	44.3743	0.720
ORS	49	1316 - 1789	-3.038 - -0.288	44.1260	0.592
UTB	50	2418 - 3145	-0.390 - -0.027	37.5885	0.600
UTK	50	2453 - 3143	-0.593 - -0.035	38.6611	0.780
UTM	49	1688 - 2718	-0.517 - 0.023	41.4079	0.776
UTU	48	2252 - 3002	-0.514 - -0.050	40.7425	0.625
UTW	50	2628 - 3083	-0.446 - 0.005	39.6678	0.660
WYB	49	1956 - 2803	-0.515 - -0.096	44.2300	0.490
WYW	49	2164 – 2893	-0.653 - -0.095	42.6030	0.612
All Sites	1446	1172 - 3317	-3.038 – 0.078	39.8066	0.638

Table 2.2 Sampled aspen grouped by elevation range in low, medium, and high categories with the probability of an individual being a male.

Category	N	Elevation Range (m)	Probability of male Aspen (Ratio)
Low	384	1172 - 2197	0.599
Mid	785	2200 - 2799	0.628
High	277	2800 - 3317	0.718
Total	1446	1172 - 3317	0.638

Table 2.3 Primer sequences used in sex identification of aspen including fluorophore tag, base pairs (BP), and annealing temperature (AT). Bold sections are additions from the M13R-1 universal primer sequence (Pakull et al. 2015).

Primer	Sequence 5'-3'	Fluorophore	BP	AT (°C)
TOZ19-1F-FAM	TTAGGTGCTGATGGTTTGGTAAAGCAG	6FAM	27	50
TOZ19-1R	CTTGCATGCAGATAGCCAACACAAGAATT	-	29	50
Control_rev-FAM	AATGAGCAGCTTCACGTTCCAACTCAACT	6FAM	29	50
Control_for	CTACCATGCTGAGTTTGAATTCTGGGTC	-	28	50
TOZ19-1F-HEX	TTAGGTGCTGATGGTTTGGTAAAGCAG	HEX	27	52
TOZ19-1R-4Control_for-4	**CCAT**CTACCATGCTGAGTTTGAATTCTGGGTC	-	32	52
TOZ19-1F-HEX	TTAGGTGCTGATGGTTTGGTAAAGCAG	HEX	27	52
TOZ19-1R-4	**CCAT**CTTGCATGCAGATAGCCAACACAAGAATT	-	33	52
TOZ19-1F-8PET	**ATGACCAT**TTAGGTGCTGATGGTTTGGTAAAGCAG	PET	35	55
TOZ19-1R-8	**ATGACCAT**CTTGCATGCAGATAGCCAACACAAGAATT	-	37	55
Control_for-8	**ATGACCAT**CTACCATGCTGAGTTTGAATTCTGGGTC	-	36	55
Control_rev-8PET	**ATGACCAT**AATGAGCAGCTTCACGTTCCAACTCAACT	PET	37	55

DNA was extracted from leaf samples using Qiagen DNeasy 96 Plant extraction kits. Sex determination was performed using the TOZ19 locus for *Populus*, which is only found in males (Pakull et al. 2015). A Control locus found in both males and females was used to differentiate between PCR failure and a negative fragment amplification (Pakull et al. 2015). To enable multiplexing of samples 3 separate primer sets were developed by modifying the 5' end of the TOZ19 and the Control locus primer sets. Fluorescent labels FAM (Applied Biosystems, 403169), HEX (Applied Bioscience, 403170), and PET (Applied Biosystems, 26-6679) were attached to the 5' end of each TOZ19 and control locus (Table 2.3). Additional bases from a modified M13R-1 universal primer was also added to the 5' end to vary fragment lengths identifiable when run on a capillary fragment analyzer (Table 2.3). Loci were amplified in 10uL reaction volumes containing 2x MyTaq HS Master Mix (Meridian Bioscience, BIO-25045), 0.5uM DMSO), 0.24uM of the TOZ19 forward and reverse primers, 0.16uM of the Control forward and reverse primers, and approximately 20ng template DNA. Both TOZ19 and the Control locus were amplified under similar conditions: 95°C for 3 min. initial denaturation, followed by 35 cycles at 95°C for 30 sec., a primer-set-specific annealing temperature for 40 sec (Table 2.3), and 72°C for 50 sec, followed by a final extension at 72°C for 10 min. I replicated 3-5 samples from within and across 96-well plates to provide plate-level quality control. All PCR amplifications were performed using an Applied Biosystems 2720 Thermal Cycler and run on an Applied Biosystems 3730 DNA Analyzer. Allele scoring was performed using Gene Marker v2.7 software (SoftGenetics). Any samples that did not have discernable peaks in the chromatograms, even after re-amplification (n=32/1479) were excluded from statistical analysis. Four of my samples failed to amplify, and 28 samples produced ambiguous results and were excluded from further analysis.

The assay was validated using 12 distinct clones from different locations in Utah in which sex was verified by observation of flower production in the field (4 males, 8 females). The validity of the assay was further confirmed in a recent study of aspen in Wisconsin (444 males, 249 females) which demonstrated 98% accuracy (Christopher Cole, University of Wisconsin, personal communication).

A Bayesian analysis of a generalized linear mixed-effects model (GLMM) was used to test the probability of an aspen being male to elevation, latitude, heat load index (HLI), and mean annual precipitation (MAP). My model structure was as follows, with "y" representing the probability of an aspen tree being male, with mean (θ) and variance (n) for individuals (i).

$$y_i \sim binom(\theta_i, n)$$

$$logit(\theta_i) \sim \beta_0 + \beta_1 x_{Elevation} * \beta_2 x_{Latitude} * \beta_3 x_{HLI} * \beta_4 x_{MAP} +$$
$$(\beta_5 x_{Elevation} * \beta_6 x_{Latitude} * \beta_7 x_{HLI} * \beta_8 x_{MAP} \parallel x_{site})$$

Values for the model parameters elevation ($x_{Elevation}$), latitude ($x_{Latitude}$), HLI (x_{HLI}), and MAP (x_{MAP}) were centered on their global mean to allow comparisons of these variables, which all have different scales. Each model parameter was drawn from distributions centered around the mean (p) and estimated variance (τ) of the data. The random effect (x_{site}) was modeled hierarchically, each with there own common mean and standard deviation.

$$\sim Normal\ (p, \tau)$$

The y-intercept (β_0), intercept for each variable (β_j), and the random effect (x_{site}) were given flat, “uninformative” priors. variance (n_i) was given a flat, “uninformative” beta prior. Specifying priors in this way is less of a concern with a large sample size.

$$\beta_i \sim Normal(0,1e^{-4})$$

$$b_i \sim Normal(0,1e^{-4})$$

$$n_i \sim beta(\alpha_0, \beta_0)$$

Latitude and elevation were determined from GPS locations and elevation were taken at each sampled tree using Garmin Montana 610 units. HLI is a direct measure of incident radiation using aspect extracted from digital elevation models (DEMs) (Buttrick et al. 2015). I calculated HLI following the method of (McCune and Keon 2002), which uses folded aspect, slope, and latitude. I chose HLI as an indicator of water stress risk because it is an estimator of the potential evaporative demand on the trees. I obtained data on aspect, slope, and latitude for each sample from USGS 1/3 arc-second DEMs from the National Elevation Dataset (U.S. Geological Survey 2002). MAP was extracted from 30-year normal climate data with a grid size of 800m (PRISM Climate Group 2004).

The data was analyzed in R 3.6.0 using the *rstan* and *rstanarm* packages (Brilleman et al. 2018; Goodrich et al. 2018; Stan Development Team 2019). I used a Bayesian approach with Markov Chain Monte Carlo (MCMC) sampling to account for uncertainty in the estimation of model parameters. I used uninformative prior distributions for the parameters due to the lack of studies on aspen sex distribution (Diggle et al. 1998; Zhao et al. 2006). I computed 4 MCMC chains with 2,000 iterations each, of which I discarded the first 1,000 iterations as a burn-in. Model convergence was confirmed the Gelman-Rubin statistic which is the ratio of the variance of the model parameters across all chains compared to the within-chain variance (Gelman et al. 1992). All Gelman-Rubin values were equal to 1, indicating convergence of the posterior distributions. Effect sizes and Bayesian 95% credible intervals were then calculated from the posterior distributions of the model parameters. I calculated a marginal probability (MP) value, which is the ratio of MCMC estimates that were different from 0 in a single direction (positive or negative) compared to all MCMC estimates for each model parameter. When an MP for a parameter is extremely high, e.g. >95%, I can say the results show a "significant" effect of the parameter on the probability of an aspen tree being male (Berger and Pericchi 1996).

Results

Across all 31 sites (i.e. globally) I found a nearly 2:1 (64%) probability of a sampled aspen tree being male, with the probability reaching just under 3:1 (72%) at elevations >3000m (occurring in nine sites, Table 2.4) (Fig. 2.2). A vast majority of sites (27 out of 31) had more males than females present overall. Of the four remaining sites, two had an even number of males and females and two had more females present than males (Table 2.1). When comparing site means, there is a weak positive correlation between elevation and probability of an aspen being male (Fig. 2.3). At the site level, most sites (22 of 31) exhibited an increase in male aspen as elevation increased, and all but one site had a sex ratio greater than 0.5 (Table 2.1).

Table 2.4 Bayesian estimates of the mean, 95% and 99% confidence intervals of individual model parameters and 2-way interaction terms. Variables were centered on global means and a one-unit change represents a change of one standard deviation.

Variable	Mean	1%	2.50%	97.50%	99%
Intercept	0.477	0.187	0.233	0.712	0.758
Elevation	0.37	0.002	0.066	0.682	0.759
Latitude	0.036	-0.291	-0.24	0.318	0.378
HLI	0.103	-0.547	-0.423	0.634	0.71
MAP	0.173	-0.113	-0.066	0.434	0.484
Elevation*Latitude	-0.28	-0.702	-0.623	0.046	0.121
Elevation*HLI	-0.102	-0.961	-0.806	0.621	0.744
Latitude*HLI	-0.023	-0.722	-0.619	0.572	0.697
Elevation*MAP	-0.055	-0.424	-0.358	0.227	0.281
Latitude*MAP	0.037	-0.386	-0.324	0.376	0.427
HLI*MAP	0.268	-0.389	-0.279	0.797	0.918

Bayesian analyses indicated that globally, increasing elevation (99% MP) and increasing MAP (92% MP) were associated with a higher probability of an aspen tree being male (Figs. 2.3 and 2.4). There was also a detectable (95% MP) negative interaction between elevation and latitude with higher probabilities of being male at lower elevations at higher latitudes and also at high elevations at lower latitudes (Fig. 2.5). There were no significant effects for latitude or HLI on the probability of a sampled tree being male, and no other interactions between the variables both globally and at the site level (Table 2.4). I found no significant results at the site level for any of the predictor parameters or interactions.

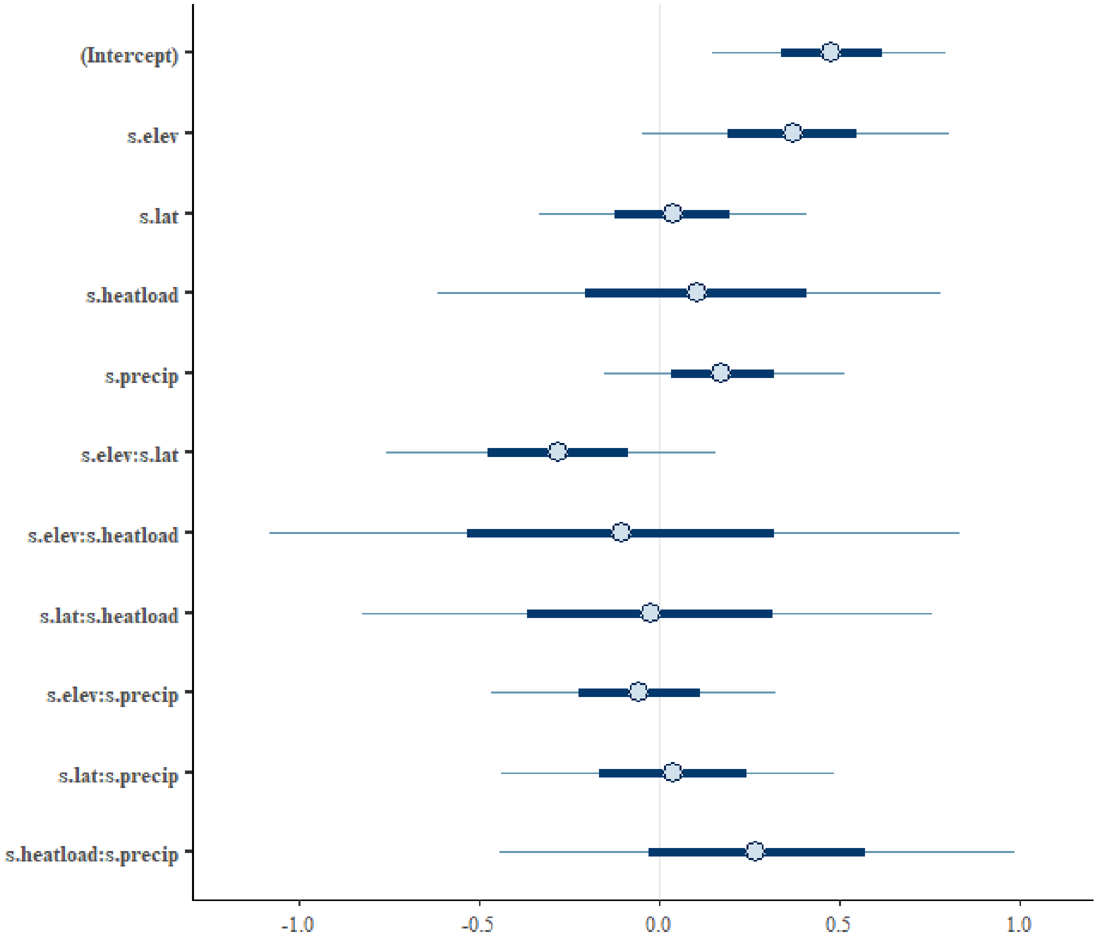

Figure 2.2 Posterior estimates of all single and 2-way interaction variables from the Bayesian generalized linear mixed model assessing the probability of an aspen being male. Variables are centered on their mean with values ranging from -1 to 1. The dark blue bars represent the 75% posterior predictive interval, while the light grey bar represents the 99% posterior predictive interval. Point estimates for each parameter are represented by the blue circle.

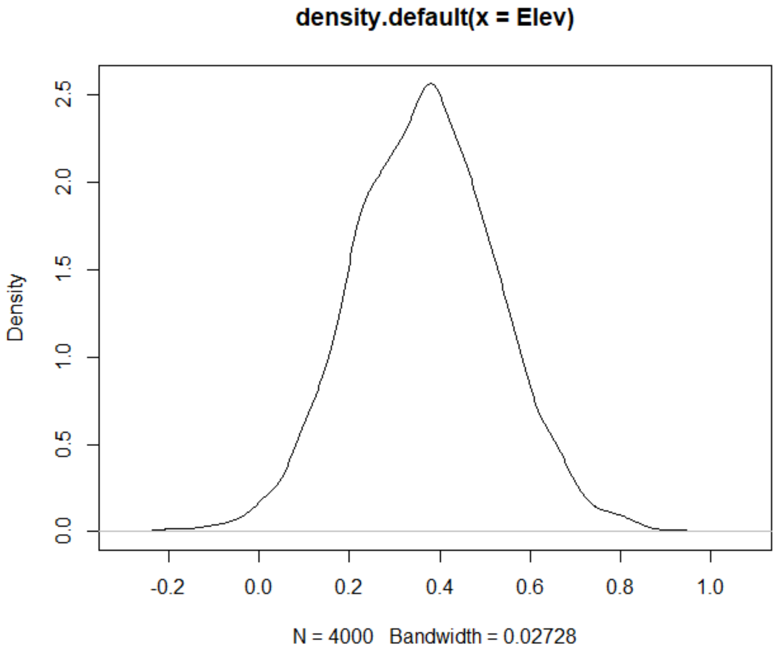

Figure 2.3 Posterior estimate of elevation from the Bayesian generalized linear mixed model. Over the 4000 iterations of the model, the marginal probability of an aspen tree being male increasing with increasing elevation was 99%.

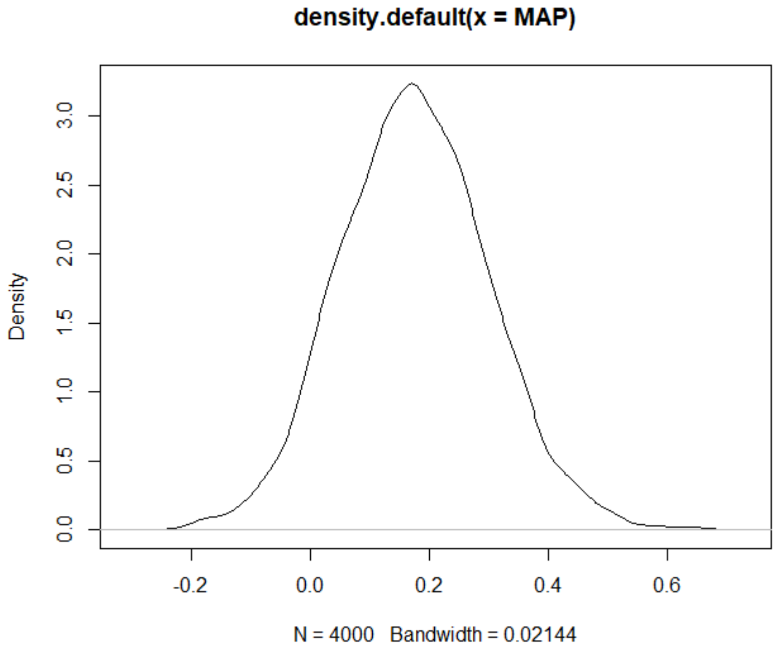

Figure 2.4 Posterior estimate of mean annual precipitation (MAP) from the Bayesian generalized linear mixed model. Over the 4000 iterations of the model, the marginal probability of an aspen tree being male increasing with increasing MAP was 92%.

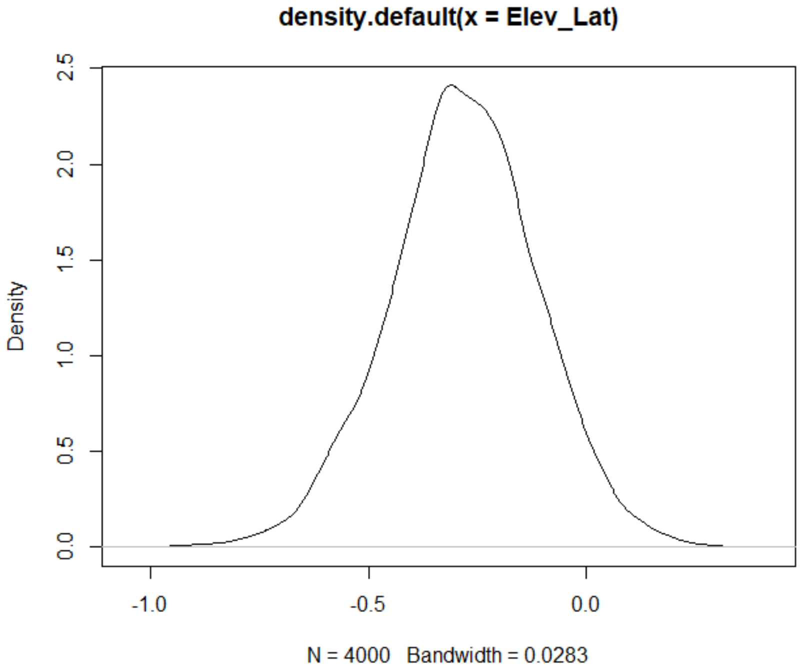

Figure 2.5 Posterior estimate of the interaction between elevation and latitude from the Bayesian generalized linear mixed model. Over the 4000 iterations of the model, the marginal probability of a negative interaction was 95%.

Discussion

Overall, I found a remarkably strong male bias in sampled aspen trees spanning the Intermountain West, including the southwestern populations. In dioecious clonal species, a male:female ratio differing from 1:1 suggests either (*i*) decreased persistence in one of the sexes following establishment and/or (*ii*) larger clone sizes in one of the sexes, presuming that sex ratios in seed crops are approximately 1:1. The 1:1 ratio in aspen seeds has been confirmed by using these same molecular methods to assess in seed crops (n=100) of two female aspen clones in northern Utah (Mock, Burney, and Walton, unpublished data). Male-biased sex ratios are most often explained by the additional energetic burden of reproduction in females, which may manifest as reduced carbohydrate reserves, chemical defenses, or growth, and/or a reduced ability to recover from drought-caused hydraulic failure (Tognetti 2012; Field et al. 2013; Lyu 2016). In general, female trees may be selected against as individuals in a population age, leaving a generally male-biased sex ratio that is not uncommon in the genus *Populus* (Xu et al. 2008; Petzold et al. 2013; Lei et al. 2017). I am not aware of studies assessing sex differences on increment growth, clonal size, clonal age, or mortality risk in common landscapes, but such studies could help elucidate the physiological factors driving this pronounced sex bias. If clone size is different between male and female aspen, then the probability of sampling the sex with larger clones may be greater, even if the number of clones is equal. Similarly, if there is a generally skewed sex ratio among triploids, my observed landscape-scale sex ratios may be related to, and perhaps due to, ploidy level. There is a known tendency for larger aspen clones to be triploid, meaning they may be overrepresented in my sampling (Van Buijtenen 1958; Mock et al. 2008). Relationships between clone size, ploidy, and sex should be considered in future studies.

I observed that the probability of an aspen being male increased across sites as elevations increased. This finding was consistent with the only previous study of aspen sex ratios at a landscape scale (Grant and Mitton 1979), although at the site level I found only a weak relationship between elevation and the probability of a sample being male. Notably, I also found a negative interaction between elevation and latitude. At higher latitudes, the probability of being male was increased at lower elevations than at lower latitudes. This pattern is likely associated with the latitudinal shift in elevational distribution described for other species (Hoch and Körner 2005; Shaw and Long 2007).

In general, increasing elevation is correlated with three general climatic trends: decreasing atmospheric pressure, decreasing temperature, and increasing solar radiation

(Körner 2007). Natural frosts that occur more often at higher elevations can damage leaves and vascular tissues, affecting plant growth and the ability to take up water and nutrients (Rixen et al. 2012; Ladinig et al. 2013; Neuner et al. 2020). The increased probability of being male at higher altitudes generally supports my hypothesis of female aspen occupying less stressful environments due to female reproductive energetic burden. Cold temperatures can also cause defects during the development of male gametophytic organs (De Storme and Geelen 2014) and lead to pollen sterility (Oliver et al. 2005) which may cause aspen to favor asexual reproduction at higher elevations. Additionally, exposure to ultra violet (UV) radiation at high elevations could potentially reduce plant productivity and inhibit photosynthesis (Vass et al. 2005; Takahashi and Badger 2011), but this can vary due to dynamic responses to UV by different species (Barnes et al. 1987, 2015; Tevini and Teramura 1989). Decreased atmospheric pressure at higher elevations can potentially lead to reduced leaf area and total biomass (Daunicht and Brinkjans 1992) however, reduce atmospheric pressure was shown to have no effect on the dry mass of *Tritticum aestivum* (Massimino and Andre 1999). While some of the climatic trends associated with increased elevation support my hypothesis, there may be environmental factors separate from elevation contributing to aspen sex distribution.

In addition to elevation, I observed a positive relationship between the probability of an aspen being male and MAP, contrary to my expectations. I expected environments with higher MAP to have a lower probability of an aspen being male due to female individuals in other woody plant species having higher stomatal conductance, basal area, and leaf area in high moisture environments (Dawson and Bliss 1989; Ward et al. 2002; Xu et al. 2008). I also observed no interaction between elevation and MAP, despite the

Intermountain West being dry at low elevations and humid at high elevations (Körner 2007). Precipitation at my study sites may be impacted more by the time between precipitation events, with evaporative demand increasing with longer in-between periods in these semi-arid environments (Lauenroth and Bradford 2009; Wise 2012). Overall the MAP result did not support my hypothesis that a male-biased sex ratio would be more pronounced at sites with a more pronounced drought risk.

My hypothesis that certain landscape indicators of potential water stress would be associated with aspen sex ratios was also not supported. Previous studies have shown female individuals of other woody plant species to be less water-use efficient (Dawson and Bliss 1989), occupying wetter habitats (Dawson and Ehleringer 1993) and having reduced photosynthetic capacity in drought conditions (Xu et al. 2008) compared to their male counterparts. Neither HLI or latitude explained the probability of being male or female, either within or across sites. HLI also failed to show interactions with elevation. There are a variety of possible explanations for the HLI result. First, HLI data may be unable to accurately characterize hydraulic stress in a local area, where local soil type, springs, and local topography may influence soil moisture. HLI is calculated at the individual tree level, but the factors contributing to HLI including slope and aspect are determined at a pixel size (10 m) larger than the individual tree. Estimates of water stress could be improved by adding a combination of other local measurements of environmental factors that modulate water availability, including soil type and subsurface subsidy (Love et al. 2019), or a variety of remotely-sensed parameters (Rao et al. 2019). Microsite variation in soil water-holding capacity may have a greater impact on aspen distribution than that predicted by more regional metrics. Second, female aspen in drier areas may not flower as frequently as those in more mesic sites, reducing their exposure to selection against female trees in those sites (Field et al. 2013). Third, my explanatory variables do not account for extreme episodic events, such as drought, heat, or pest outbreaks. These episodic events may be only loosely correlated with HLI and even MAP but may be important sources of differential stress on male vs. female trees. Fourth, various cumulative stressors (Anderegg et al. 2013b) may impact female clones more than male clones, causing a general paucity of females but not in a pattern that is related strongly to particular landscape metrics. Future studies assessing how sex ratio varies during different life stages (e.g. seed production, seedling establishment, and following specific stressful events) may provide insights to the sex ratio differences I observed in this study.

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CHAPTER 3

COMPARING BELOW-GROUND VS. ABOVE-GROUND METRICS OF STAND CONDITION AND REGENERATION POTENTIAL IN ASPEN

Abstract

Aspen (*Populus tremuloides*) regeneration in the Intermountain West is often accomplished through clearfelling, and stand condition at the time of harvest is often used to predict regeneration success. Stand condition is typically assessed using above-ground metrics. Since aspen regenerates via root suckering, below-ground metrics might be an informative adjunct to above-ground metrics, but are not generally used due to a larger labor investment and a lack of knowledge about how root conditions relate to regeneration potential and above-ground condition. The goals of this study were (1) to assess relationships between above-ground stand variables and the amount of shallow, suckering-sized roots, and (2) to determine whether the suckering ability of individual root segments was related to the carbohydrates present in the root.

In 23 aspen-dominated sites across Utah, I collected data on both above- and below-ground stand condition. For below-ground condition, I collected aspen root biomass and size class data from 6 sample trenches per site. As a surrogate for vegetative regeneration potential, a subset of roots from each trench were placed in vermiculite and allowed to sucker for five weeks in greenhouse conditions, and measured suckers per unit root surface area. Prior to suckering, I sampled the roots for non-structural carbohydrates (NSC) to assess how NSC concentration and the size of NSC pools related to stand condition and root suckering potential. Correlation analysis and principal component analyses (PCA) were used to compare above-ground measurements to suckering-sized root biomass (SSrm) and to compare below-ground variables to each other. A Least Absolute Shrinkage and Selection Operator (LASSO) regression was performed to find a predictive model for SSrm. Results indicated that ten of the thirteen above-ground variables had strong correlations with SSrm, and the PCA grouped variables along gradients of stand health and stand age. My LASSO regression resulted in a model with predictor variables that were prominent in the correlation analysis with the exception of dead and down cover. The correlation results for the below-ground variables showed strong relationships between phloem diameter and total and starch NSC measurements of the whole root. Contrary to my expectations, basal area was not correlated with other above-ground measurements. I conclude that the use of stand- or root-level measurements show potential to predict suckering response and could be a rapid method for evaluating an aspen stand's suckering potential and overall health.

Introduction

Quaking aspen (*Populus tremuloides* Michx.) is the most broadly distributed tree in North America and an ecologically important species in the U.S. Intermountain West (Burns et al. 1990; Rogers et al. 2014; Service et al. 1971). In the dry landscapes of western North America at mid to upper elevations, aspen is often the dominant deciduous tree species, and it supports high levels of plant and animal diversity (Barnes 1966; DeByle and Winokur 1985). In the western U.S., stand conditions in aspen tend to vary with elevation, latitude, and moisture gradients. At lower elevation, drier sites, fire suppression has increased conifer encroachment into aspen-dominated forests and increased the fire return intervals, but has largely not affected higher elevation, wetter sites which traditionally see large, infrequent fires (Hanna and Kulakowski 2012;

Kulakowski et al. 2013). Climate change is also impacting aspen forests, and is contributing to dramatic local-scale mortality events, particularly in the southwestern U.S. (Anderegg et al. 2013a; Worrall et al. 2010), and leading to projections of severe range constrictions in the coming century (Worrall et al. 2013, 2008). Increasing drought frequency and severity appear to be major mechanisms of these mortality events (Anderegg et al. 2013b; Huang and Anderegg 2012), exacerbated by ungulate herbivory and other factors (Kay and Bartos 2000; Rhodes et al. 2019).

Aspen is known for its tendency to reproduce clonally following seedling establishment, and is considered to be an early successional species, with suckering pulses following disturbance (typically fire) that removes the above-ground biomass (Barnes 1966; DeByle and Winokur 1985). In western U.S. landscapes, presumably due to generally inhospitable conditions for seedling establishment, suckering frequently results in the establishment of large clones (Barnes 1966; Grant et al. 1992), although most clones seem to be small (e.g. under 1ha in size) and stands tend to consist of multiple clones (Hipkins and Kitzmiller 2004; Mock et al. 2008). While the importance and prevalence of aspen establishment by seed dispersal has been recognized recently in the western U.S. (Long and Mock 2012), most individual stems are the result of vegetative regeneration (suckering) following the original establishment from seed (McDonough 1985; Schier 1973; Schier et al. 1985). Aspens possess a relatively shallow root system, with extensive lateral roots and “sinker” roots (Jones et al. 1985). Vegetative suckers typically originate from widespread lateral roots within 0.15 m of the soil surface (Baker 1925; Schier 1973; Schier and Campbell 1978). Suckers arise from lateral root primordia which are pre-formed on roots (Farmer 1962; Frey et al. 2003;

Schier 1973), suppressed by auxin from above-ground stems and stimulated by accumulation of cytokinin's produced by roots (Cline 1991; Schier 1981; Schmülling 2002; Wan et al. 2006). Nitrate concentrations in root are also known to promote cytokinin synthesis and release of suckers (Crawford 1995; Wan et al. 2006). Clonal differences in the abundance of primordia can be extensive (Zasada and Schier 1973). Once initiated, sucker growth can be influenced by a variety of other factors, including non-structural carbohydrate (NSC) levels in roots, environmental conditions, and genetic variation (reviewed in Frey et al. 2003). Carbohydrate starvation can lead to the loss of fine-root biomass and has been documented as a factor in canopy condition decline and tree mortality when coupled with hydraulic failure (Landhäusser and Lieffers 2002; Sevanto et al. 2014) potentially influencing aspen sucker survival. NSC storage pool size in living bark tissues is thought to influence suckering potential following disturbance, but NSC remobilization is still poorly understood in aspen (Wiley et al. 2019).

Concerns about persistence of aspen stands in much of the western U.S. (Worrall et al. 2013) has stimulated an increased interest in improved aspen regeneration and recruitment strategies to improve watershed protection, wildlife habitat, and conservation of biodiversity (DeByle and Winokur 1985; Krasnow and Stephens 2015; Long and Mock 2012; Shepperd et al. 2006). The primary management approach to aspen regeneration involves clearfelling or selective harvest of mature aspen, which can result in vigorous suckering of existing clones (DeByle and Winokur 1985). However, the success of these approaches depends on a number of factors, including pre-existing above- and below-ground stand conditions, ungulate herbivory pressure, and diseases (Britton et al. 2016; Jacobi et al. 1998; Kemperman and Barnes 1976). However, if vegetative regeneration fails, aspen can be lost from a site entirely if seed-based regeneration does not occur, as root resources necessary for suckering will have been depleted (Kitchen et al. 2019; Smith and Smith 2005; Smith et al. 2011; Worrall et al. 2008).

Managers generally predict the success of clearfelling or selective cutting on vegetative aspen regeneration based on above-ground stand measurements (browsing intensity, site index, overstory condition) (Britton et al. 2016; Sheppard 2001; Smith et al. 2011), with varying success. Examination of how well these metrics predict suckering potential and below-ground root mass, however, has not been directly addressed, since field studies measuring regeneration can be confounded by herbivory and other environmental factors. The goals of the study were to (1) assess relationships between above-ground stand variables and the amount of shallow, suckering-sized roots and (2) determine whether the suckering ability of individual root segments was related to the carbohydrates present in the root.

Materials and Methods

Site selection

The study was conducted in landscapes of northern, central, and southern Utah, where aspen stands in a broad range of conditions could be located and where aspen regeneration treatments are actively being considered. A total of 23 sampling sites were selected on Cedar Mountain (CM), located east of Cedar City, Utah, with additional sites at Monroe Mountain (MM) on the Fish Lake National Forest., and Logan Canyon (LC) on the Cache National Forest (Fig. 3.1). CM consists of mostly private land, while both

MM (Sevier Plateau) and LC (Bear River Range) are primarily public land (US Forest Service and Utah DNR Forestry, Fire, and State Lands). All sampling sites were located at mid-upper elevations, between 2100-2800 m, and receive the majority of precipitation during the winter months as snow. Both the MM and CM areas are also subject to monsoonal rainfall patterns during the summer. The forest types found at all sites were pure aspen or aspen-mixed conifer. The main conifer associates were subalpine fir (*Abies lasiocarpa*), Douglas-fir (*Pseudotsuga menziesii*), and Engelmann spruce (*Picea engelmannii*).

Sampling sites were selected to represent a spectrum of stand conditions, based on basal area (BA) and number of age classes present (Fig. 3.2, Table 3.3). For site selection purposes, BA was sampled using a 3m wedge prism from a random point that was >60m from the forest edge in the potential sampling area. The sites were also selected to have road access in order to accommodate root sampling equipment. Sampling was split into two phases to measure above- and below-ground characteristics. Above-ground measurements at all sites were taken after the trees had fully leafed out (June-July 2018) (Table 3.1). Below-ground measurements at all sites were taken once bud-set had begun and trees began storing NSC for next season (August-September 2018) (Table 3.2).

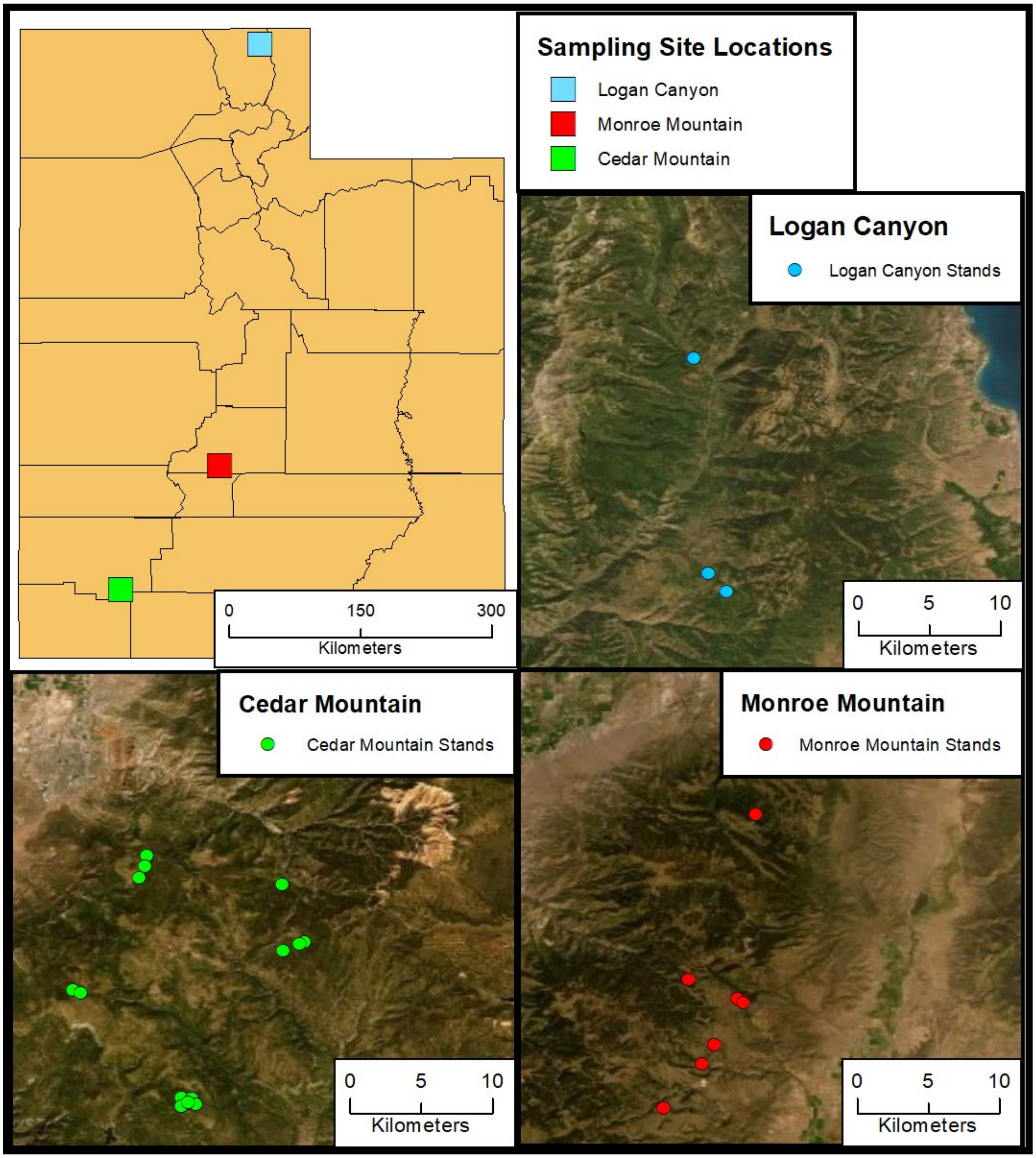

Figure 3.1 Location of 31 field sampling sites across 3 primary sampling locations in the Intermountain West. Within each sampling site, 6 plots were sampled for both above- and below-ground variables.

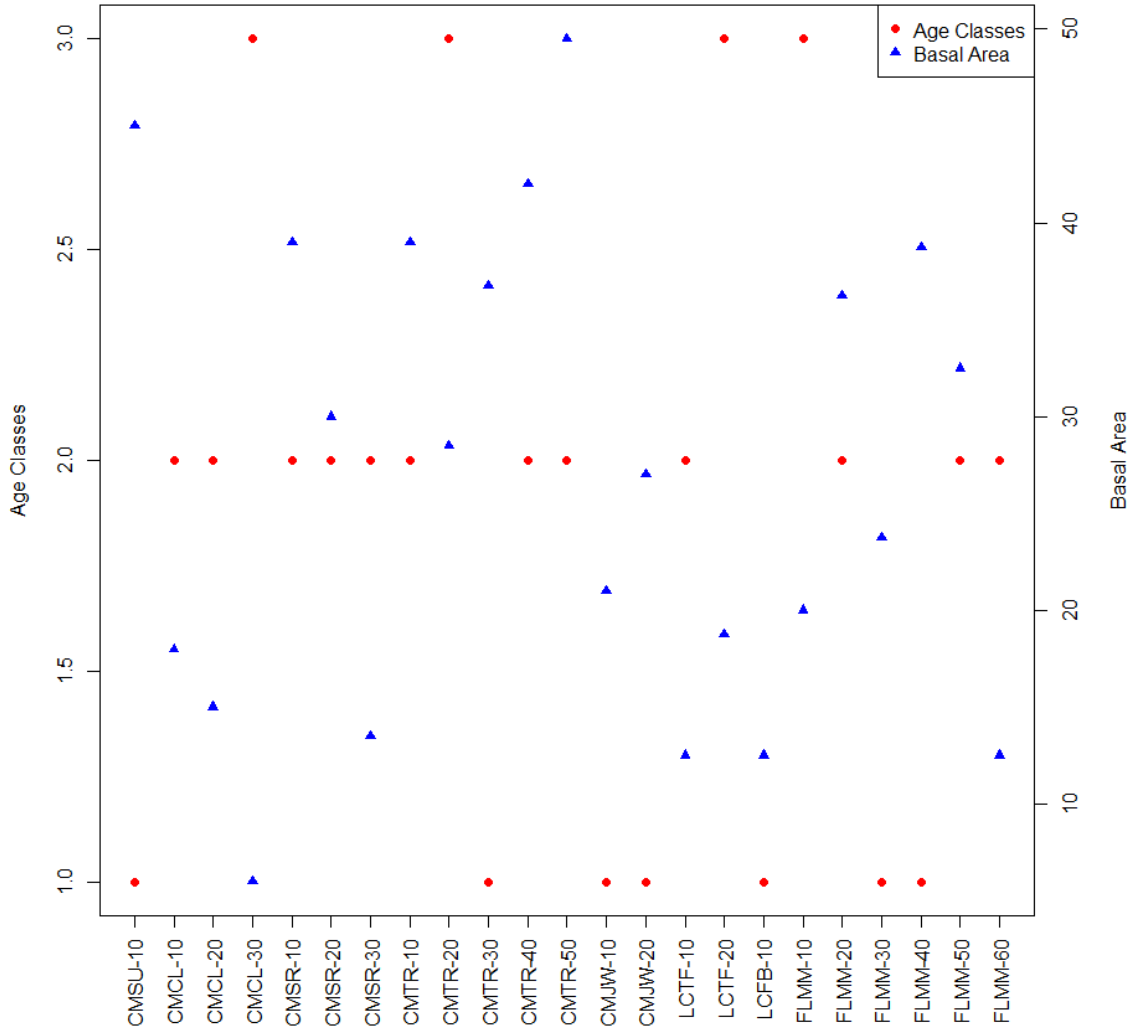

Figure 3.2 Visualization of Table 3.3, with number of age classes (understory, midstory, overstory) present on the left axis and basal area (BA)/m^2 at each site location on the right axis. Age classes were determined visually during the site selection process.

Table 3.1 List of above-ground aspen stand measurements taken at each plot (n=136) for all 23 sites (CM, MM, LC locations).

Trees > 10cm DBH ($100m^2$ plot)	Trees < 10cm DBH ($25m^2$ plot)	Shrub and Dead/Down Cover (10m transect)	Grass/Forb Cover (10m transect)
Live/Dead	Live/Dead	Species Identification	Daubenmire Cover Classes (%)
Species ID	Species ID	Cover (%)	
Height (m)	Height (m)		
DBH (cm)	Small Trees/ha		
Basal Area (m^2/ha)			
Large Trees/ha			
Uncompacted Live Crown Ratio (%)			
Crown Density (%)			
Crown Dieback (%)			

Table 3.2 Root-related measurements in aspen stands, taken from trenches at each of 136 plots. Plot Roots are measurements taken on all roots collected from each plot while Individual Roots are measurements taken on root segments selected from each plot for the greenhouse experiment.

Plot Roots	Individual Roots
Total Root Mass (Trm) (g)	Suckers/Root Surface Area (#/cm^2)
Suckering-Sized Root Mass (SSrm) (g)	Number of Suckers
	Number of Nodes
	Phloem Diameter Proportion (% of whole root)
	Total NSC Phloem (% by weight)
	Total NSC Whole Root (% by weight)
	Starch NSC Phloem (% by weight)
	Starch NSC Whole Root (% by weight)

Table 3.3 Number of age classes (understory, midstory, overstory) present and basal area (BA)/m^2 at each site location to demonstrate that a range of stand conditions was sampled.

Site	Age Classes	BA (m^2/ha)
CMSU-10	1	45
CMCL-10	2	18
CMCL-20	2	15
CMCL-30	3	6
CMSR-10	2	39
CMSR-20	2	30
CMSR-30	2	13.5
CMTR-10	2	39
CMTR-20	3	28.5
CMTR-30	1	36.75
CMTR-40	2	42
CMTR-50	2	49.5
CMJW-10	1	21
CMJW-20	1	27
LCTF-10	2	12.5
LCTF-20	3	18.75
LCFB-10	1	12.5
FLMM-10	3	20
FLMM-20	2	36.25
FLMM-30	1	23.75
FLMM-40	1	38.75
FLMM-50	2	32.5
FLMM-60	2	12.5

Above-ground sampling methods

At each site, a nested-fixed radius circular plot design was used for above-ground sampling, with a total of 6 plots placed at 30 m and 60 m along 3 transects that originated from the stand entrance adjacent to the road (Fig. 3.2). Within each large plot (100 m^2) mature trees, which was defined as being >10cm DBH, were sampled. Within the smaller, nested plot (25 m^2) the seedling and sapling trees, which was defined as being <10cm DBH were sampled. Cutoff diameters were determined from US Forest Service sampling protocols (Randolph 2011). Within each large plot, shrub, grass, and forb cover was sampled along a 10 m transect that was centered on the plot center and oriented in a random direction. Shrub cover was determined using the line intercept method, measuring the distance of the shrub crown canopy that overlapped the transect and recorded as a length along the transect (Lutes et al. 2006). Grass and forb cover were determined using a 20 × 50 cm Daubenmire square and recorded as 5% increment cover classes at each meter along one side of the transect that was randomly chosen.

Below-ground sampling methods

To excavate the roots for sampling, I used an Airspade 3000®, which uses compressed air to excavate soil with minimal root damage. At the center of each large plot, I excavated a 2 m long trench that was 0.3 wide and 0.2 m deep trench. The trench was placed as close to plot center as possible in a north/south direction and was located at least 1 m away from trees >10 cm DBH to avoid excessively large (non-suckering sized) roots. All aspen roots present within the trench were harvested using a hand pruner and stored in sealed plastic bags on ice until they were brought back to the lab. Once in lab, I gently removed soil from all roots with tap water, and roots were rinsed in a 5% bleach solution. Roots from each plot were separated into small (< 5 mm diameter), suckering-sized (5-25 mm diameter), and large (>25 mm diameter) categories. All roots were wrapped in damp paper towels for refrigerator storage (4°C) for 2 to several weeks in plastic bags.

Greenhouse suckering and root carbohydrate measurements

Instead of conducting clearfelling treatments and measuring sucker regeneration the following year, I conducted a greenhouse experiment to assess how the suckering ability of individual root segments was related to the carbohydrates present in the root. The response variable was the number of suckers per cm^2 root surface area based on the total surface area of each segment. This approach allowed us to avoid logistical problems with small-scale clearfelling and the confounding effects of post-treatment herbivory. For each plot (trench), 1-2 roots of suckering size (5-25 mm diameter, 10-30 cm in length) were selected. To establish the reserve conditions prior to suckering for each segment a small portion (<2.5 cm) was cut from each end of every selected root segment. The remaining root segment were then placed approximately 2-4 cm deep in trays containing 100% coarse vermiculite and left for 5 weeks on a misting bench to allow for sucker initiation (DeByle and Winokur 1985). The greenhouse was maintained at 20°C with natural lighting. While I measured light availability in the form of the various canopy measurements, the suckers were not measured in the field and received equal amounts of light in the greenhouse. Root segments were harvested just prior to suckers breaking the vermiculite surface. For each root segment, I counted the number of individual sucker stems and the number of suckering nodes (number of points >1cm apart from which one or more sucker was emerging). Root segment surface area was determined using the measurements of length and diameters of both ends of the root, using ImageMagick® software (The ImageMagick Development Team 2020). These values were used to calculate the surface area of a cylinder with those end dimensions. Root tissue that was visibly damaged was measured and subtracted from the calculation as I was only interested in the live tissue capable of suckering. I counted both the number of individual suckers and the number of suckering nodes because the number of suckers per node varied from 1-12. In a natural situation, only one to a few suckers per node would be expected to be able to contribute to regeneration. When two suckering root segments represented a plot (105 of 136 plots), the suckers per unit area were averaged.

Root samples taken for NSC analysis (see above) were randomly assigned to represent either whole root segment NSC analysis or just phloem NSC. Phloem thickness and whole root diameter were recorded from the segments used for NSC analysis and used to calculate a phloem to xylem thickness ratio. Both samples were dried in an oven at 68°C for 72 hours to ensure all moisture had been removed. Following drying, NSC segments were frozen in a -20°C freezer until they could be processed (ground) using a Wiley mill (40-mesh), placed in 50-ml glass vials, and shipped to the University of Alberta Department of Renewable Resources for analysis. Total NSC for the whole root and phloem segments were (separately) measured by initial extraction of soluble sugars in 80% ethanol followed by a phenol-sulfuric acid method (Landhäusser et al. 2018). Starch was measured by digestion with α-amylase (Sigma-Aldrich A3403) and amyloglucosidase (Sigma-Aldrich A1602) separately, while glucose hydrolysate was measured by PGO (Sigma-Aldrich P7119). Values for total NSC and starch were reported as a percentage of dry weight of the whole root or phloem sample and values for plots with two root segments were averaged.

Statistical methods

To explore relationships between suckering-sized root mass (SSrm) and above-ground variables, I first used a combination of principal component analysis (PCA) and correlation analyses. In the PCA, the data were standardized around the mean, and principal components (PC) with an eigenvalue value > 1 were used to summarize the results. The analysis was performed using the *FactoMineR* package in R 4.0.2 (Lê et al. 2008; R Core Team 2017). The correlation was performed using the Pearson's correlation coefficient (r) to measure the strength of the relationships between the variables and was also analyzed in R 4.0.2 (Freedman et al. 2007; R Core Team 2017). To assess the predictive effects of above-ground variables on SSrm, I also performed a LASSO (Least Absolute Shrinkage and Selection Operator) regression analysis. Parameter values were centered on their global mean to allow for comparisons across different scales for the LASSO regression. LASSO regression simultaneously minimizes the absolute value of the error and the number of coefficients (Tibshirani 1996). In a process called shrinking or regularization, LASSO uses the L1 arc-length (lambda), which is the sum of the absolute value of coefficients. LASSO starts with a very low lambda value to penalize coefficients and shrink their coefficient value to 0. Then lambda is slowly increased (decreasing the penalty) which slowly increases the absolute value of the coefficients. When a coefficient value being non-zero increases model fit, that coefficient is included into the model. I then compare the sequence of models tried at each value of lambda where a coefficient was made non-zero and find the one that balances complexity with explanatory power. Cross-validation was performed to determine the model from the LASSO regression that had the lowest mean-squared error (MSE). I report the mean standardized parameter estimates of the model with the lowest MSE, indicating the strength of the potential effect of each parameter on SSrm for every one standard deviation change. The data was analyzed in R 4.0.2 using the *glmnet* package.

To assess relationships between the suckers/root surface area and the NSC measurements taken on the roots suckered in the greenhouse, I performed PCA and correlation analyses on these variables. The analysis for both the correlations and PCA was done in a similar manner as the above-ground variables and SSrm, with the data standardized around the mean, using PCs with an eigenvalue value > 1 to summarize the PCA, and using the Pearson's correlation coefficient in the correlation analysis (Freedman et al. 2007; Lê et al. 2008; R Core Team 2017).

Results

Above-ground variables and SSrm

The selection of my sites covered a range of stand conditions (Table 3.3, Table 3.4). Stands were primarily comprised of aspen which constituted 98% of large trees and of small trees recorded across all my sampling sites (Table 3.5). Shrub cover in all sites was mostly dominated by mountain snowberry (*Symphoricarpos oreophilus*), representing 78% of all measured shrub canopy (Table 3.5).

The PCA biplot shows the relationships between the variable vectors in the first two orthogonal PCs (Fig. 3.3). The first 2 PCs combined to explain 44.1% of the variance in the data across sites (Table 3.6). The PCA showed that the first 5 PCs had an eigenvalue > 1 (Table 3.6). The largest contributors by percentage to the first PC (25.2% of the variance) included variables that were associated with stand health, which in order of loading included individual tree crown dieback, overstory canopy density, grass cover, and SSrm (Table 3.7). The second PC was defined by variables that indicate stand development stage, height of large trees, individual tree live crown, and BA (Table 3.7).

The correlation analyses and LASSO results had overlap on two variables, shrub canopy cover and large trees/ha, but neither were the strongest variable in the LASSO model, which was dead/down cover. In the correlation analyses, ten of the thirteen above-ground variables were correlated with SSrm with an r-value > 0.1 (Fig. 3.4, Table 3.7). The strongest correlations were with shrub canopy cover (0.755), grass cover (-0.460), individual tree crown dieback (-0.337), and large trees/ha (0.306) (Table 3.7). These results are generally consistent with the PCA biplot representation is generally consistent with the correlation matrix result, with where individual tree crown density, large trees, SSrm, and shrub canopy cover all having positive relationships with each other and having a negative relationship with individual tree crown dieback (Fig. 3.3). For the LASSO regression analysis, none of the plot variables had r-values > 0.9 with SSrm, so all above-ground variables were retained. The LASSO regression resulted in a best model that had six variables that were similarly prominent in the correlations (listed with their mean standardized parameter estimates): dead/down cover (2.37), shrub canopy cover (0.92), individual tree crown density (0.51), overstory canopy density (0.31), large trees/ha (0.004), and small trees/ha (0.00001) (Table 3.8, Fig. 3.5) A three-fold cross-validation was performed to determine the lambda sequence for the LASSO regression and compute model fit (Fig. 3.6). The best model was selected from the lambda value (2.606) that had the lowest model mean-squared error (439.5) (Fig. 3.6).

Table 3.4 Site-level averages of above-ground measurements and SSrm.

Plot Code	SSrm	Overstory canopy density (%)	Height large trees (m)	Individual tree live crown (%)	Individual tree crown density (%)	Individual tree crown dieback (%)	Large trees/ha
CMSU-10	89.87	78	14.1	23.4	34.3	2.9	1167
CMCL-10	33.4	74.8	15.4	25.1	33.7	2.3	267
CMCL-20	19.41	63.5	12.7	36.2	31.1	0.6	467
CMCL-30	51.5	68.1	9.9	41.7	38.4	4.3	467
CMSR-10	33.06	88.7	12	35.5	35.1	0.5	1000
CMSR-20	21.57	61.6	12.8	45.8	30.7	3.3	283
CMSR-30	23.21	78.7	16.4	37.4	36.6	0.8	350
CMTR-10	38.33	73.9	23	15.2	34	3.8	267
CMTR-20	30.88	80.7	11.6	29	27.6	0.8	650
CMTR-30	51.37	59.4	20.2	18.3	28.3	5.8	233
CMTR-40	4.53	67.2	19.4	13.8	20.4	1.7	233
CMTR-50	45.72	79.5	24.4	23.5	34.7	0	300
CMJW-10	11.35	38.8	14.6	11.5	19.3	9.2	317
CMJW-20	27.19	59.1	18.9	18.3	34.7	0.8	317
LCTF-10	87.85	61.3	10.1	33.2	34	0.8	350
LCTF-20	64.86	75	15.1	28.2	28.2	1.3	417
LCFB-10	20.82	55.7	9.1	32.6	29.2	4.2	417
FLMM-10	96.3	72.2	14.5	20.4	32.7	1.7	717
FLMM-20	72.46	75.5	17.8	19.1	34.7	0.3	550
FLMM-30	28.24	70.2	14.5	21.3	34.2	0.5	867
FLMM-40	44.54	70.4	12.3	27.4	38.1	1.8	917
FLMM-50	66.73	60.9	13	38.3	46.5	2.4	650
FLMM-60	69.28	62.2	10.9	23.1	23.7	0.3	550

Table 3.4 continued.

Plot Code	Grass cover (%)	Forb cover (%)	Height small trees (m)	Small trees/ha	Shrub canopy cover (%)	Dead/down cover (%)	BA (m2/ha)
CMSU-10	16.8	17	1.1	2933	25.9	8.5	45
CMCL-10	27.5	13.3	4.8	3067	0.3	7.6	18
CMCL-20	14.9	18.6	1.8	3133	7.1	6.8	15
CMCL-30	21.3	16.3	1.8	3933	1.6	5.1	9
CMSR-10	22.3	18.8	1.8	7467	1.2	4.6	39
CMSR-20	18.4	16.4	3.7	1933	0	5.3	30
CMSR-30	16.6	17.6	3.1	2200	0	8.4	13.5
CMTR-10	16.3	20.7	2	5400	0	6.9	39
CMTR-20	17	19.6	3	3267	1.6	4.5	28.5
CMTR-30	16.3	18.5	0.4	1133	3.5	10.4	36.8
CMTR-40	12.9	19.7	2	1467	11	2	42
CMTR-50	10.9	15.9	1.2	6867	3	7.9	49.5
CMJW-10	29.8	14.3	0.2	533	0	9.3	21
CMJW-20	25.8	15.3	0.1	667	0	5.9	27
LCTF-10	24	33	1.5	2100	39.5	2.5	12.5
LCTF-20	11.8	29.4	2.8	6067	25.4	6.8	18.8
LCFB-10	20.3	38.3	2.7	1800	22.8	2	12.5
FLMM-10	7.4	9	1.8	4467	42.3	8.9	20
FLMM-20	10.4	6.6	1.1	7133	37.7	3	36.3
FLMM-30	11.2	12	0.5	667	1.7	2.6	23.8
FLMM-40	15.8	8.7	1	1667	23.9	2.4	38.8
FLMM-50	7.8	3.8	0.6	1533	57.4	2.3	32.5
FLMM-60	16.9	14.9	1.4	8533	58.7	4.4	12.5

Table 3.5 Shrub canopy cover, overstory trees, and understory trees by species and their presence at sampling sites. Table 3.6: PC eigenvalues, variance explained, and the cumulative variance explained by each additional PC from the PCA of above-ground variables and SSrm.

Species Name	Common Name	Total Shrub Canopy (m)	% of All Recorded Canopy	# Sites Present
Amelanchier utahensis	Utah serviceberry	10.37	4.88	2
Artemisia spp.	sagebrush	4.94	2.33	5
Mahonia repens	creeping Oregon grape	1.51	0.71	4
Juniperus communis	common juniper	16.07	7.57	4
Prunus virginiana	chokecherry	0.82	0.39	1
Quercus gambelii	Gambel oak	0.6	0.28	1
Rosa woodsii	Wood's rose	9.97	4.69	6
Symphoricarpos oreophilus	mountain snowberry	168.11	79.15	17

Species Name	Common Name	Number of Large Trees	% of Large Trees	Number of Small Trees	% of Small Trees
Abies lasiocarpa	sub-alpine fir	4	0.006	5	0.004
Abies concolor	white fir	3	0.004	3	0.003
Juniperous scopulorum	Rocky Mountain juniper	4	0.006	2	0.002
Prunus virginiana	chokecherry	0	0.000	10	0.009
Populus tremulodies	quaking aspen	683	0.983	1124	0.981
Pseudotsuga menziesii	Douglas-fir	1	0.001	2	0.004

Table 3.6 PC eigenvalues, variance explained, and the cumulative variance explained by each additional PC from the PCA of above-ground variables and SSrm.

Dimension	Eigenvalue	Variance Explained (%)	Cumulative Variance Explained (%)
PC 1	3.53	25.23	25.23
PC 2	2.64	18.88	44.11
PC 3	1.99	14.25	58.36
PC 4	1.44	10.29	68.64
PC 5	1.12	8.03	76.68

Table 3.7 Variable contribution loading values (%) to each of the first two principal components (PC) from the PCA and r-values from the correlation analysis of the above-ground predictor variables and SSrm. Data are sorted by ecological grouping and their absolute value of r.

Variable (w/SSrm)	PC 1 (25.2%)	PC 2 (18.9%)	Ecological Grouping	r-value	Abs r-value
Large trees/ha	11.818	0.219	Aspen Tree Size/Density	0.306	0.306
Small trees/ha	7.886	0.269	Aspen Tree Size/Density	0.230	0.230
Height of small trees (m)	0.260	7.491	Aspen Tree Size/Density	-0.220	0.220
Height of large trees (m)	0.229	29.705	Aspen Tree Size/Density	-0.114	0.114
BA (m^2/ha)	2.118	20.744	Aspen Tree Size/Density	0.044	0.044
Individual tree crown dieback (%)	13.700	0.408	Canopy Condition	-0.337	0.337
Individual tree crown density (%)	9.940	0.626	Canopy Condition	0.249	0.249
Overstory canopy density (%)	12.170	0.585	Canopy Condition	0.104	0.104
Individual tree live crown (%)	1.353	20.968	Canopy Condition	0.056	0.056
Shrub canopy cover (%)	9.310	1.912	Competition	0.755	0.755
Grass cover (%)	12.651	2.873	Competition	-0.460	0.460
Forb cover (%)	3.927	6.981	Competition	-0.122	0.122
Dead/down cover (%)	2.614	7.202	Dead/Down	-0.054	0.054
SSrm	12.023	0.017	SSrm	1.000	1.000

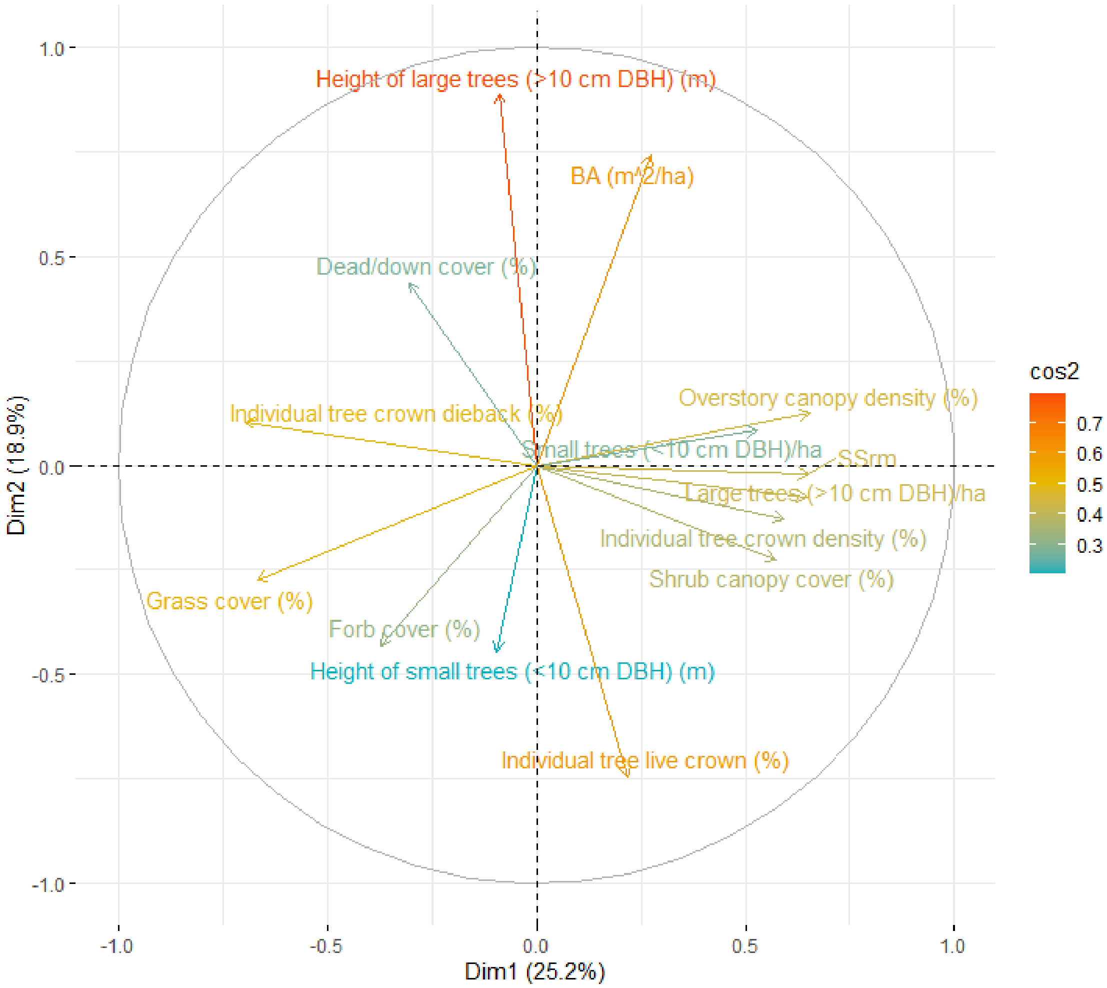

Figure 3.3 Plot of the first two principal components (PC) from the principal components analysis of above-ground predictor variables and suckering-size root mass (SSrm). Variable contributions to PC1 (x-axis) and PC2 (y-axis) are represented by their squared coordinates value (cos2).

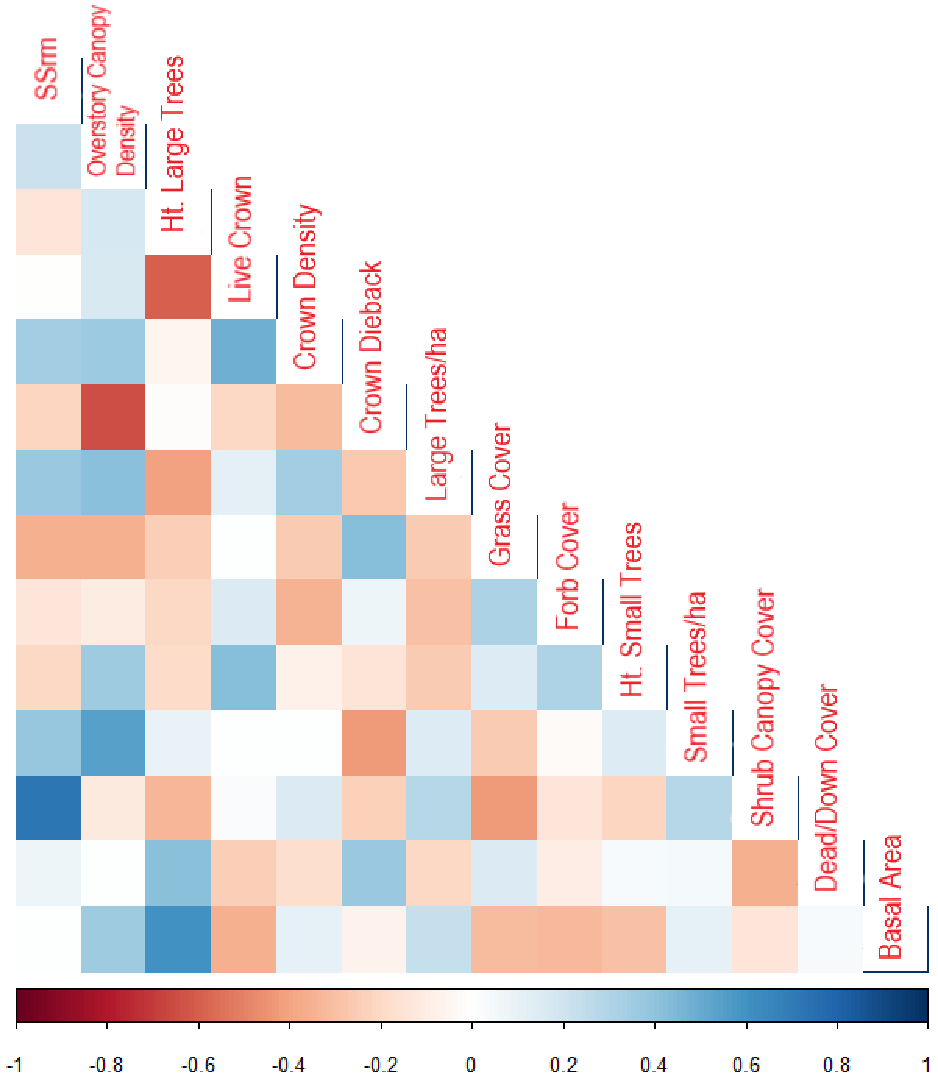

Figure 3.4 Correlation matrix of suckering-sized root mass (SSrm) (BG2) and all above-ground predictor variables. Each variable pair has an r-value that denotes a positive (blue) or negative (red) correlation. SSrm and above-ground variables r-values are listed in Table 3.7, with an |r| >0.1 denoting a potential relationship at the study sites.

Table 3.8 LASSO regression results for the model with the lowest absolute error and each standardized parameter estimate that was >0. All variables were standardized on their mean and standard deviation.

Coefficient	Estimate
Intercept	-22.47
Dead/Down cover (%)	2.37
Shrub canopy cover (%)	0.92
Individual tree crown density (%)	0.51
Overstory Canopy Density	0.31
Large trees/ha	0.004
Small trees/ha	0.00001

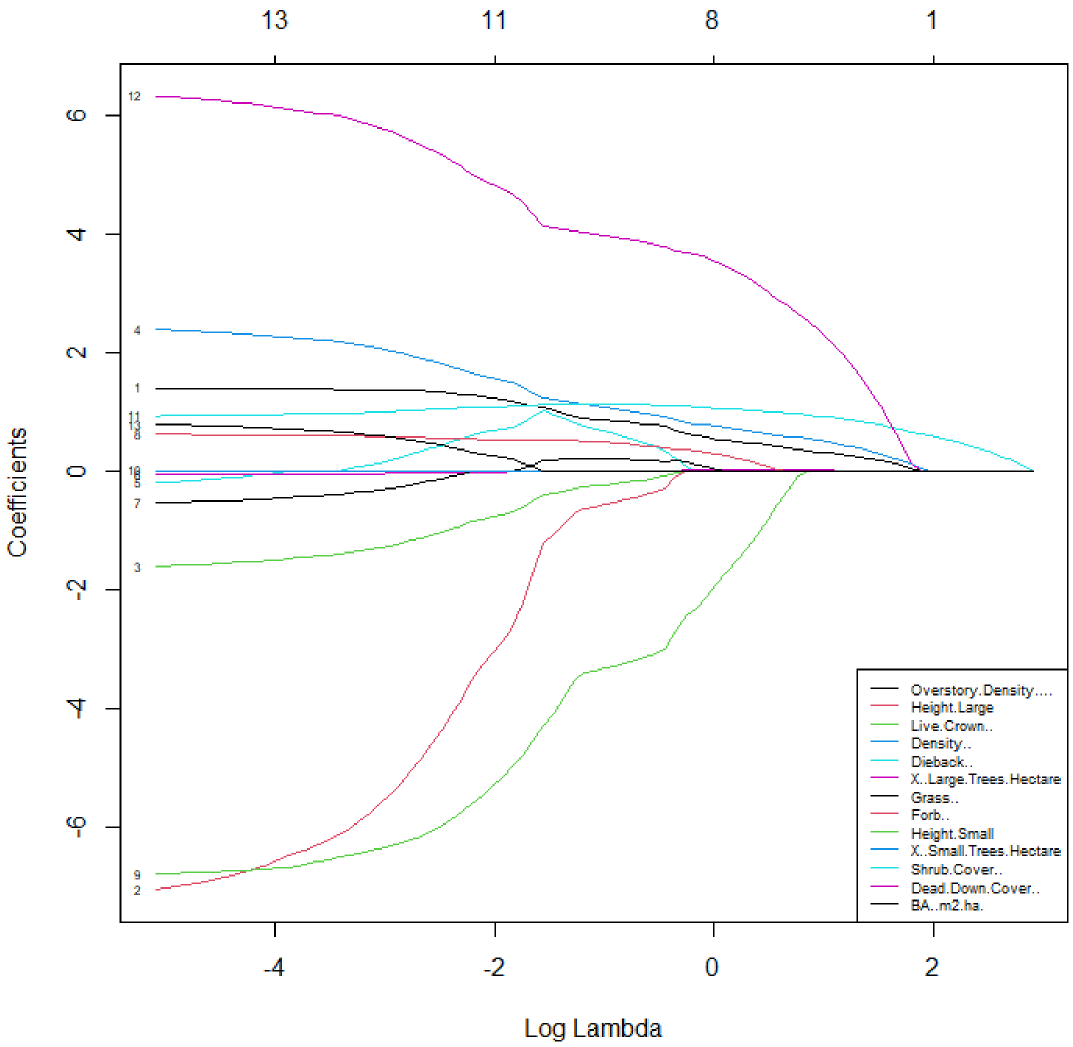

Figure 3.5 Model coefficient values of each model tried in the LASSO regression at each value of log(lambda). The scale on top indicates the number of variable coefficients that are incorperated in each model with a perticluar value of log(lambda). The left portion of the graph indicates models with a smaller penalty towards each coefficient and the right portion indicates models with a larger penalty.

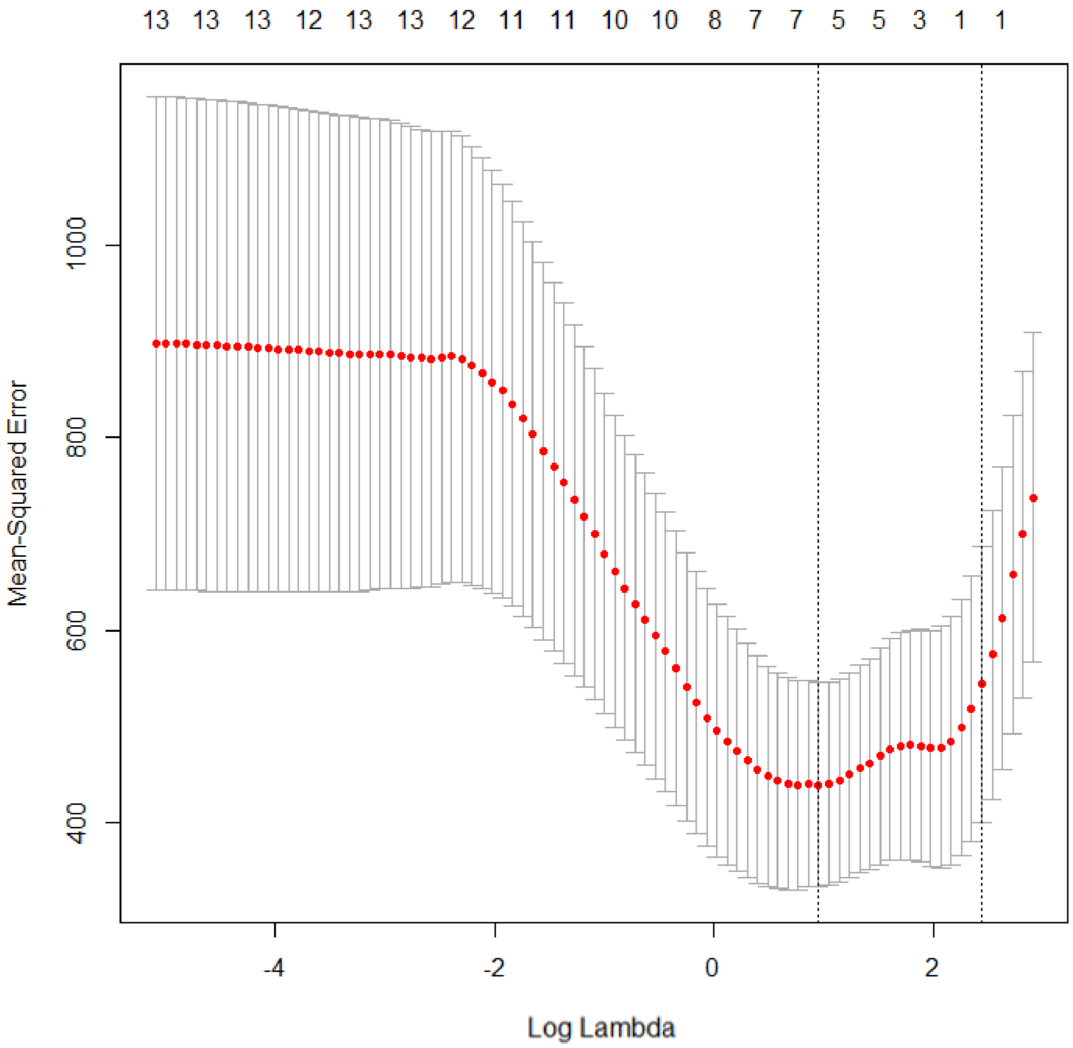

Figure 3.6 Cross-validation plot of the MSE of each model tried in the LASSO regression at each value of log(lambda). The scale on top indicates the number of coefficients that are incorporated in each model with a particular value of log(lambda). The leftmost vertical dashed line indicates the log(lambda) value and model with the lowest MSE. The vertical dashed line to the right indicates one SD from the best model.

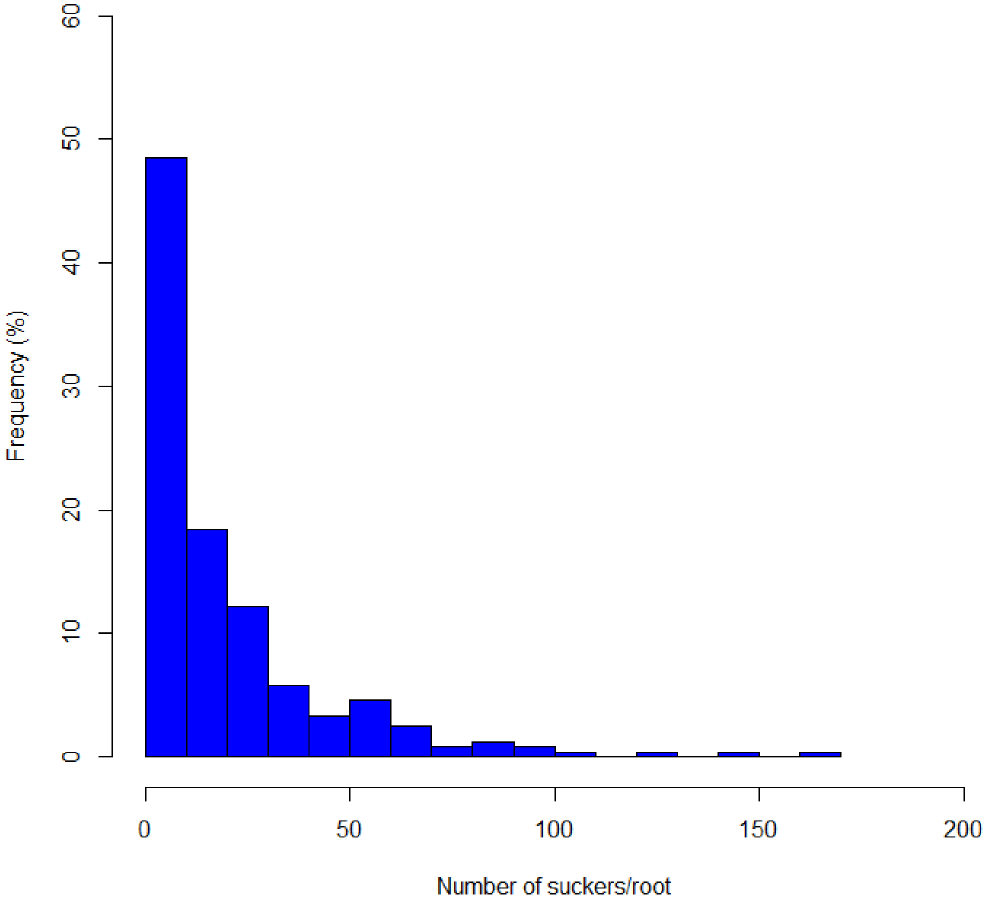

Figure 3.7 Density plot of the number of suckers recorded on each greenhouse root grouped into 10 suckers/root bins. The first bin includes roots where 0 suckers were recorded.

Table 3.9 PC eigenvalues, variance explained, and the cumulative variance explained by each additional PC from the PCA of below-ground variables and suckers/root surface area. Eigenvalue gives us a measure of how many variables-worth of information define each PC.

Dimension	Eigenvalue	Variance Explained (%)	Cumulative Variance Explained (%)
PC 1	3.09	51.51	51.51
PC 2	1.42	23.72	75.23

Table 3.10 Variable contributions (%) to each of the first 2 principal components (PC) from the PCA and the r-values from the correlation analysis of the below-ground variables and suckers/root surface area. The percentage for each PC is the amount of variance it explains in the data and is listed in the table header. Data are sorted by their correlation r-value with suckers/root surface area.

Variable	PC 1 (51.3%)	PC 2 (21.8%)	r-value	Abs r-value
Phloem Diameter Proportion (% total root diameter)	1.343	44.934	0.427	0.427
Total NSC Whole Root (% dry wt.)	25.193	0.666	0.193	0.193
Starch NSC Whole Root (% dry wt.)	25.313	3.466	0.086	0.086
Total NSC Phloem (% dry wt.)	22.863	0.011	0.081	0.081
Starch NSC Phloem (% dry wt.)	23.804	5.301	0.023	0.023
Suckers/Root Surface Area (#/cm^2)	1.484	45.622	1.000	1.000

Suckering and root carbohydrates

Greenhouse roots had varying levels of suckering, with the number of suckers per root ranging from 0-161 (Fig. 3.7). The PCA showed that only the first 2 PCs had an eigenvalue > 1 and that they explained 73.12% of the variance in the data (Table 3.9). The largest contributors by percentage to the first PC included all the NSC measurements, which collectively accounted for 98.2% of that dimension (Table 3.10). The second PC was defined by the other 2 variables which were phloem diameter (44.2), and suckers/root surface area (30.1) (Table 3.10). The PCA biplot shows the relationship between the variable vectors, with phloem diameter and suckers/root surface area having a positive relationship and the NSC measurements having positive relationships with each other (Fig. 3.8). Correlations between root variables and sucker/root surface area were generally quite weak, with three of the five variables having r-values > 0.1 (Fig. 3.9, Table 3.10). The strongest correlations I observed were phloem diameter proportion (0.427), total NSC whole root (0.193), and starch NSC whole root (0.086) (Table 3.10).

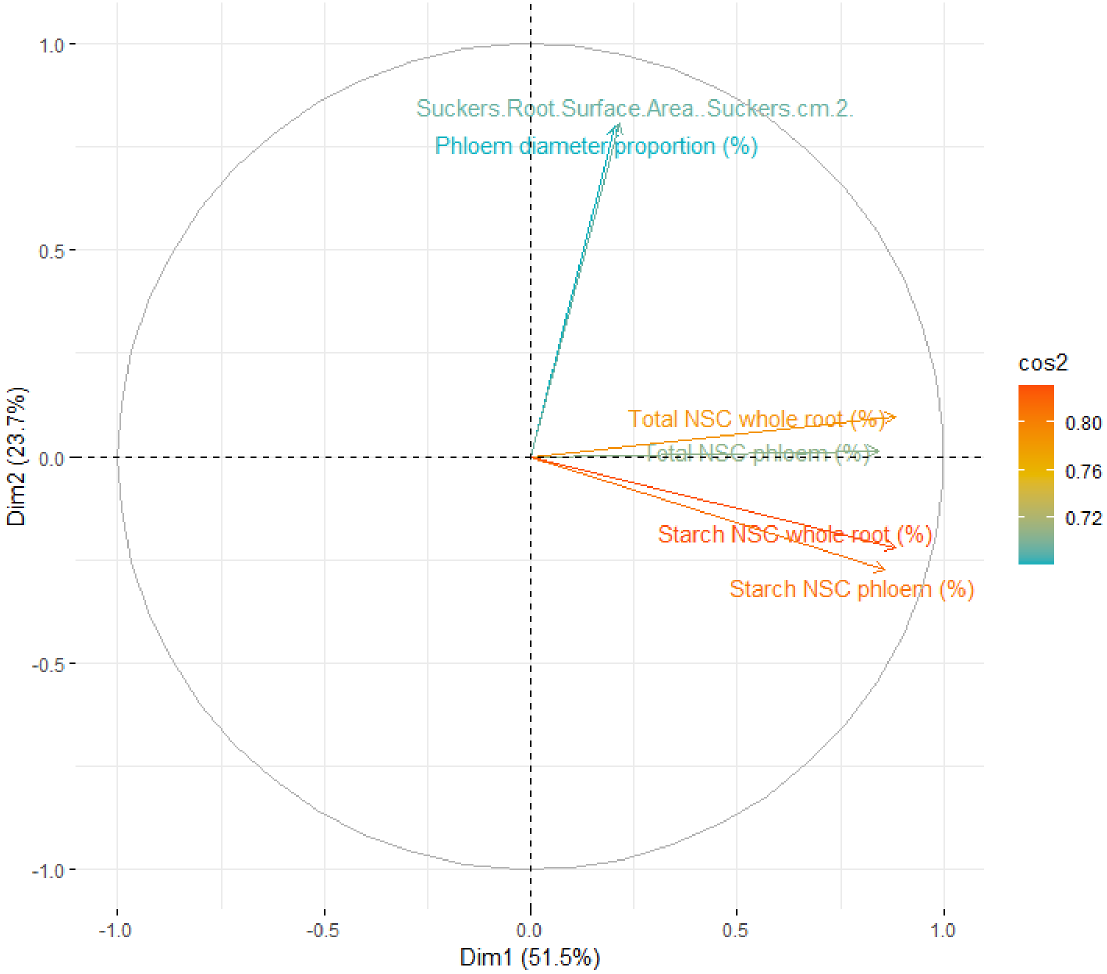

Figure 3.8 PCA biplot of the first two PC and each variables contributions and relationship to each other from the PCA of below-ground variables and suckers/root surface area. Variable contributions to PC1 (x-axis) and PC2 (y-axis) are represented by their squared coordinates value (cos2).

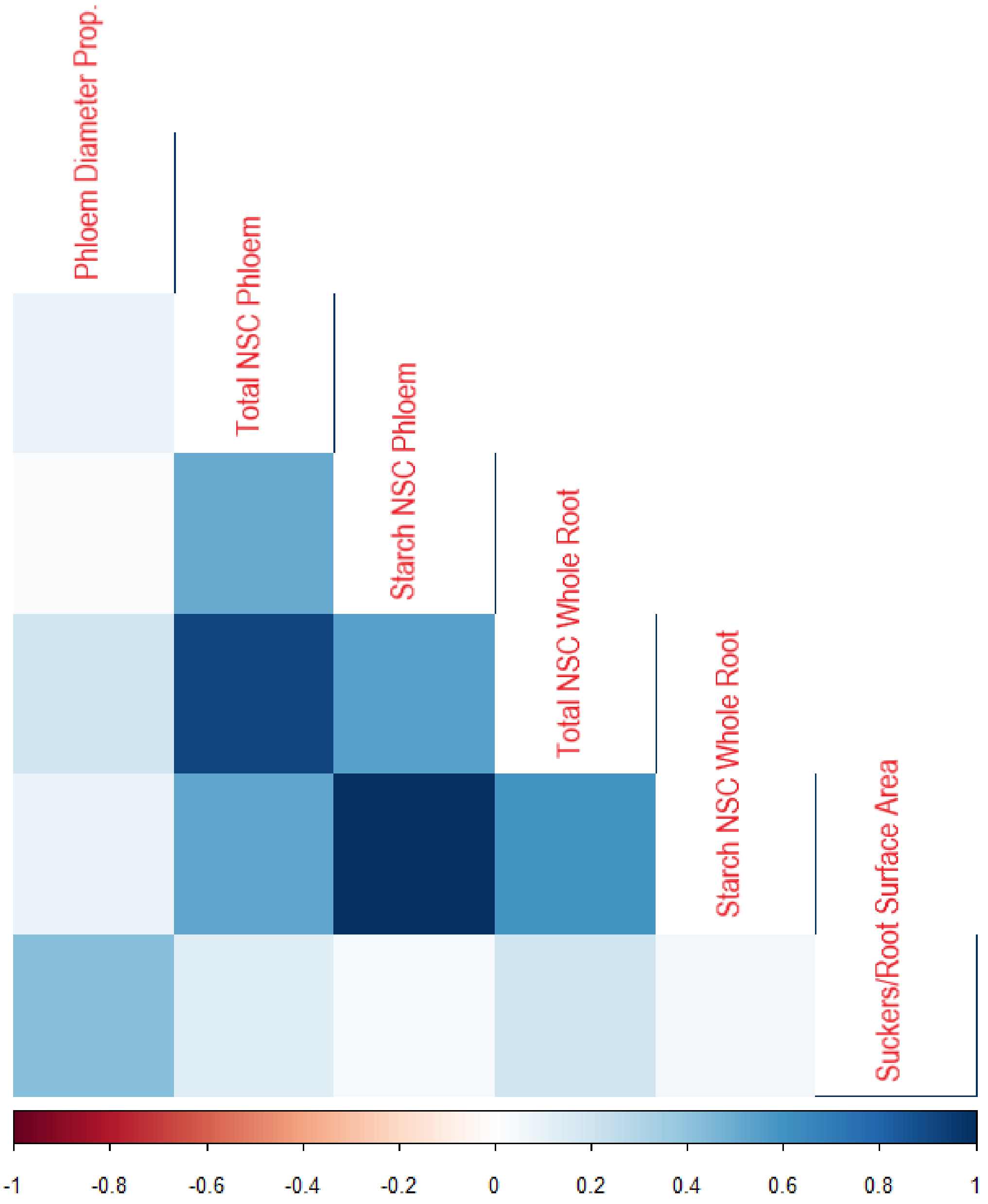

Figure 3.9 Correlation matrix of suckers/root surface area (#/cm^2), proportion of the root diameter that was phloem, and NSC measurements (% dry weight) on roots sampled from the study plots/sites. Each variable pair has an r-value that denotes a positive (blue) or native (red) correlation. Suckers/root surface area and root variables r-values are listed in Table 3.9, with |r| >0.1 denoting a potential relationship at the study sites.

Discussion

Concerns about increasingly high rates of mortality of mature aspen in western U.S forests have led to an increased interest in silvicultural treatments to regenerate aspen stands in a timely manner. Regeneration is commonly accomplished through clearfelling, which releases a new generation of suckers from existing root systems. However, regeneration following clearfelling is sometimes poor, leading to loss of the stand. If landowners and land managers could improve predictions of regeneration, more informed decisions could be made about the timing and extent of regeneration treatments in aspen. Regeneration failure due to post-treatment herbivory can be assessed (Britton et al. 2016), but such failure can also be due to a poor suckering response independent of herbivory (Long and Mock 2012). In this study, I examined relationships between (1) a range of easily-measured above-ground stand metrics and the biomass of suckering-sized roots (SSrm) in aspen stands, and (2) a set of root-specific parameters and the suckering response of those roots in a greenhouse setting.

Above-ground stand variables and suckering-sized root mass

In the PCA analysis, site characteristics varied in ways that were generally expected. Variables associated with stand health (e.g. crown density and density of large and small trees/ha, SSrm) were in generally opposite directions in the first dimension (25.2% of the variance) than variables associated with stand decline (e.g. dieback, grass cover). Measures of canopy dieback are already used as an indicator to measure stand condition (Randolph 2011) and short-term drought impact (Hogg et al. 2008) as stands that have been defoliated or accrued damage to their leaves become at-risk of carbon starvation (Landhäusser and Lieffers 2012). The second dimension (18.9% of variance) may be related to stand development stage, with the height of large trees, basal area, and dead/down cover having positive loadings, and the individual tree live crown and the height of small trees having negative loadings. In this dimension, the height of large trees had a strong positive loading, while the individual tree live crown had a strong negative loading. Surprisingly, shrub canopy cover varied in the same direction as indicators of stand health (Fig. 3.3, Table 3.6), and in the opposite direction of grass cover, suggesting fundamentally different competitive interactions with aspen for grass and shrub cover.

Correlations between above-ground variables and SSrm were generally weak, and the LASSO regression was mostly redundant with the correlation results. One surprising result in the LASSO regression was the strong model parameter estimate of dead/down cover, considering the weak correlation of this variable with SSrm. This finding may be an artifact of the other stand variables in combination but the weak correlations with these variables may mean the LASSO model did not perform well enough to predict dead/down cover. Interactions between variables were not considered in the LASSO regression model and could be explored in future studies. I found positive correlations between SSrm and both shrub canopy cover and large trees/ha, and negative correlations between SSrm and both grass cover and individual tree crown dieback. These findings were consistent with the PCA biplot. These four above-ground characteristics taken together may have value in predicting stand-level SSrm in field settings, and potentially in predicting suckering response to clearfelling. Other stand-level variables were only weakly correlated with SSrm. As mentioned above, the strong positive correlation between shrub cover and SSrm was unexpected, since shrubs and aspen should compete directly for water and nutrients. The majority of the total shrub cover across my sites was mountain snowberry (*Symphoricarpos oreophilus*) (79%) which is a common understory associate of aspen stands in the Intermountain West (DeByle and Winokur 1985; Warner and Harper 1972; Wasser and Shoemaker 1982). Except for wet riparian corridors, mountain snowberry generally does not occur in open canopy outside of the aspen understory, suggesting that aspen stands may facilitate the establishment of snowberry. It is possible that aspen and snowberry partition their root zones to minimize direct competition, although little research has been done on the subject. One study in Northern Utah sampled mountain snowberry roots up to a depth of 81cm, which was limited by a claypan, and roots did not extend beyond 31cm horizontally from the base of single stems (George and McKell 1978). Another possibility is that resource-rich sites can support both species well, although direct evidence for this is limited. Mountain snowberry and mountain big sagebrush (*Artemisia tridentata* spp. *vaseyana*) associations have been documented in Nevada, occurring primarily on productive, dark-colored soils (Tueller and Eckert Jr, 1987) but this has not been studied for aspen and mountain snowberry. The negative correlation between grass cover and aspen SSrm suggests that grass and aspen compete more directly for light, water, and soil resources (Bockstette et al. 2017; Donaldson et al. 2006). Future studies could address these mechanistic relationships more directly, and potentially could further improve predictions of suckering responses to clearfelling or other treatments.

Large trees/ha, small trees/ha, and individual tree crown density were all positively correlated with SSrm, and individual tree crown dieback negatively correlated with SSrm in aspen. These relationships were rather weak but were in the expected directions given the linkage between light-capturing capacity in the canopy and resources available for production and maintenance of root systems. The relationships between these variables was also apparent in the PC biplot (Fig. 3.3). Measurements of density and stocking, and canopy condition are already common practices for assessment of stand health, and their potential relationships with SSrm shown in this study are worth investigating across additional sites.

Root parameters and root suckering potential

The PCA of root-level variables resulted in my first PC having large positive loadings of all measurements of NSC and explained > 50% of the variance in the root data (Table 3.9). These results suggest are consistent with the current understanding that that root NSC pools are a potentially important way to describe root condition at the stand level. NSCs play a critical role in energy storage and metabolism, and osmoregulation of forest trees (Hartmann and Trumbore 2016). NSC measurements have been used to determine if carbon starvation was primarily involved in tree death (Adams et al. 2013; Landhäusser and Lieffers 2012) although the levels of NSC vary between tree tissues, biome, and seasons (Martínez-Vilalta et al. 2016). Measurements of NSC in below-ground tissues are an important tool in understanding the energy storage capacity of forest stands and sampling whole-tree NSC pools and factoring in the seasonality of these pools may give a better understanding of NSC dynamics (Hoch et al. 2003; Richardson et al. 2013) than what could be analyzed in this study. My findings provide more evidence that NSC concentrations are important for understanding the condition of a forest stand.

Suckers/root surface area and phloem diameter proportion in my second PC also had positive loadings and explained an additional 23% of the variance in my data (Table

3.9). In addition, phloem diameter proportion had a much stronger correlation with suckers/root surface area than measures of NSC (Table 3.10). A larger proportion of phloem in roots may be important in describing the vigor and health of a stand's root system, as it would allow for transportation of more remobilized NSCs once a suckering response is initiated and provide more storage for NSCs elsewise. A recent study found that resprouting aspen roots remobilized up to four times more NSC from phloem and inner bark tissues than from xylem (Wiley et al. 2019), and phloem is thought to provide a majority of remobilized NSC in suckering sized roots (Landhäusser and Lieffers 2003; Loescher et al. 1990).

In aspen the relationship between phloem diameter proportion and suckering potential has not been investigated, although whole root diameter has been shown to effect rooting efficiency and the timing of suckering (Stenvall et al. 2006). Total NSCs in the phloem and total NSCs in the whole root all had weak positive correlations with suckers/root surface area (Table 3.10). I expected a stronger relationship between my measurement of suckering potential and NSCs because NSC mobilization is critical to many physiological processes in woody plants (Hartmann and Trumbore 2016), and in aspen, sucker survival may be related to NSC reserves (Wachowski et al. 2014). My results suggest that the NSC concentrations alone may not be the strongest predictor of aspen suckering response. The storage pool size, in addition to concentration, of the living bark and delineation of the compounds in NSC storage pools should be considered to better understand aspen suckering potential (Wiley et al. 2019). Phloem diameter proportion of the whole root may provide a quick way to assess aspen roots in the field, and in conjunction with an analysis of NSC concentration can give a clearer picture of resources available for aspen suckering potential in a stand.

The greenhouse method for assessing aspen sucker production potential allowed me to isolate root traits as variables contributing to suckering, and to avoid the confounding impacts of herbivory on suckering in the field. Both ungulate and rodent herbivory can be major factors in sucker survival in field settings (Britton et al. 2016; Cantor and Whitham 1989; Howe 2017; Seager et al. 2013). A variety of other site conditions may not be well captured in the greenhouse method, including soil moisture, soil texture, and insect and soil microbial communities. The greenhouse method likely greatly overestimates the number of surviving suckers in a field setting, but I assumed that it was a reasonable measure of relative suckering potential among different roots. I suggest that future studies compare the greenhouse method with field observations post-treatment in areas with low herbivory pressure.

In this study I did not consider the genetic composition of my sampled trees or roots, but these factors may contribute to variance in regeneration success (Frey et al. 2003; Mock et al. 2012; Zasada and Schier 1973). For this study my goal was to identify stand-level parameters that could be associated with SSrm and useful in identifying suckering potential, and at a stand level, there can be one or many genets. It is possible that genetic differences among stands, especially single-clone stands, may confound interpretation of the variables I measured. It is also possible that root genotype may have a large impact on suckering ability, as suggested by Schier (1981) and this may have confounded my interpretation of root variables and their impact on suckering response. In future studies I recommend that genotype be considered to the extent practical.

Management recommendations

The multitude of biotic and abiotic forces and their interactions that can influence an aspen stand are complex, but landowners need an easy metric to predict post-treatment aspen regeneration. The implementation of stand- or root-level measurements to predict suckering response could be a rapid method for evaluating an aspen stand's suckering potential and overall health. My results suggest that a combination of the number of large trees/ha, canopy dieback, and grass and shrub cover may assist land managers in deciding whether to implement regeneration treatments or to improve stand health first. My results suggest that stands with > 500 large trees/ha, shrub cover of >~20%, < ~5% crown dieback, and grass cover of <~20% were associated with the greatest SSrm and potentially better regeneration (excluding herbivory issues). However, my sites represented only a small subset of possible aspen stand conditions, so these should only be considered coarse guidelines. The most accurate approach, however, would be to measure SSrm directly, using a series of shallow trenches. Although I used a compressor and AirSpade to dig 2 m * 0.3 m * 0.2 m trenches, these could be dug by hand, and aspen roots from 5-25 cm in diameter could be counted, and potentially collected, dried and weighed. Identifying aspen roots vs other roots would be an important aspect of this approach. In my study, I observed a range of 4.53-96.3g SSrm/trench. Although this range does not span the possible range of observations in aspen, it can at least provide some context. In future studies, measures of SSrm preceding regeneration treatments, followed by post-treatment regeneration assessments, could help provide more robust guidelines. Along with SSrm, the aspen phloem diameter proportion is a relatively simple and inexpensive measurement, and may also inform decisions about regeneration treatments and stand health. My results suggest that suckering-sized roots (5-25 cm diameter) with > 10% of the root diameter represented by phloem have a higher suckering potential. NSC measurements could also be useful but require that samples be submitted to a laboratory for analysis, and these measurements were not as predictive of suckering as phloem diameter proportion. While the management implications from this study are limited to mixed and pure aspen stands of the southwest, I suggest land managers include measurements of above- and below-ground factors suggested in this study in conjunction with monitoring herbivory in their stands (Britton et al. 2016). Further study is needed to validate these results by monitoring aspen regeneration in clearfelling treatments, including site preparation techniques used in these operations.

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CHAPTER 4

SUMMARY

My thesis research aimed to answer two separate questions about aspen ecology; 1) is aspen distribution male-biased across its range in the Intermountain West and how do climatic factors contribute to this distribution and 2) what were the relationships between above-ground variables and suckering sized roots and how does the root suckering ability of root segments relate to carbohydrates in those roots?

In Chapter 2, I determined sex in a set of 1447 aspen samples representing 31 sites across the western U.S., extracted climate variables using GIS techniques, and explored relationships between sex ratios and climate variables. My results indicated that aspen were generally male-biased across the landscape and that the ratio of male:female aspen increased with increasing elevation. I failed to find an impact of mean annual precipitation (MAP) and heat load index (HLI) on aspen sex ratios. These results suggest a potential decrease in persistence or clonal expansion in female aspen following establishment, compared to males. Male-biased sex ratios could occur if the energetic burden of reproduction in females results in decreased growth, chemical defenses, or their ability to recover from drought-induced hydraulic failure (Lyu 2016). In other studies, female trees in the genus *Populus* have been shown to have selective pressures against them in stressful environments (Xu et al. 2008; Lei et al. 2017). The impact of elevation on sex ratios suggests that female aspen may struggle to survive in more stressful site conditions. Generally, as elevation increases there is a decrease in atmospheric pressure and temperature and an increase in solar radiation (Körner 2007). Shorter growing seasons, colder temperatures, and more frequent frost events at higher elevations may also contribute to more stressful growing conditions (Rixen et al. 2012; Ladinig et al. 2013; Neuner et al. 2020). MAP was positively correlated with the probability of an aspen being male, and I did not observe any relationship with HLI. These measures in precipitation and radiation were useful in that they covered the entirety of my sampling area, but I lacked data on soil composition and water holding capacity, which would also be factors in environmental stress.

In Chapter 3, I sampled the above- and below-ground condition of aspen stands at sites across Utah. I also performed a greenhouse experiment to simulate the regeneration potential of aspen root segments and assessed relationships between suckering and root traits including non-structural carbohydrates (NSC). I found a correlation between several above-ground measurements of aspen and the suckering-sized root mass (SSrm) in stands. Future studies of these correlations at a larger spatial scale could determine if these potential relationships hold for aspen forests beyond my sites in Utah. A principal component analysis (PCA) showed that measurements generally associated with stand health versus those associated with decline generally varied in opposite directions, as expected. Some results were perplexing, including (i) strong correlations between multiple measures of understory cover and SSrm, and (ii) the lack of a correlation between basal area (BA) (a common measure used in forest management) and SSrm. My results indicate that some above-ground measures of aspen stands may be important in determining the amount of root mass below, and by extension the suckering potential in the stand. These findings can help land managers make more informed decisions on when (or if) they should perform regeneration treatments in particular aspen stands, although further validation of my results is necessary. I also found a correlation between the proportion of phloem in root cross-sections and the proportion of total NSC in roots by weight. Root NSC composition is known to be important in many physiological processes in woody plants (Hartmann and Trumbore 2016). My results showing a correlation between phloem diameter and NSC suggests that phloem diameter may be a useful variable in providing coarse estimates of root NSC. This result requires further validation before it is broadly useful.

The potential relationships between my above-ground variables and SSrm mostly displayed patterns consistent with the current understanding of aspen ecology but there were a few exceptions. My surprising positive correlation between shrub cover and SSrm may be due to the species present at my sites. The majority of the shrub cover was mountain snowberry (*Symphoricarpos oreophilus*) which is a known understory associate of mature aspen stands in this region of the Intermountain West (Warner and Harper 1972; Wasser and Shoemaker 1982; DeByle and Winokur 1985). The strong model parameter estimate of dead/down cover in the LASSO regression model also indicated that understory measures may be a proxy for other factors that impact aspen's SSrm. Until the relationships are better understood between the common measures of aspen's above-ground condition and SSrm, direct measurement of aspen root systems may offer more information to the condition of a stand. This is reinforced by the correlations I observed between the root phloem diameter proportion and the total NSC concentrations in the roots. Measurements of NSC are already an important tool to understand the energy storage capacity of forest stands, as they can represent the available energy for a stand to regenerate, but they are laboratory intensive and expensive. The ability to quickly sample aspen root segments in the field and measure their phloem diameter proportion may offer an easier tool to determining an aspen stand's NSC concentrations. The greenhouse experiment used in this study also offers a unique way to measure aspen's suckering potential without risking a failed silvicultural treatment. This method has the drawback of not properly capturing soil conditions and pests at a site. In addition, future studies should consider the genetic differences among stands, as these may confound many of the variables used determine aspen stand health. Further adjustments to the methods used in this study may offer important insight into aspen's suckering potential and could be incorporated into future silvicultural practices.

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