

ASSESSMENT OF A SEEDLING-BASED APPROACH TO ASPEN
RESTORATION IN THE INTERMOUNTAIN WEST

by

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ABSTRACT

Assessment of a Seedling-Based Approach to Aspen
Restoration in the Intermountain West

by

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In western North America, quaking aspen (*Populus tremuloides*) is an ecologically and culturally important tree species of great conservation interest, however severe droughts have caused widespread dieback of aspen over the past decade, and climate forecasts predict major range contractions across the western U.S. during the coming century. Current silvicultural methods for aspen regeneration rely on the generation of vegetative reproduction from root suckering, limiting genetic diversity of populations and precluding reforestation or afforestation efforts. Planting nursery-grown aspen seedlings could help overcome these limitations, yet it has received little attention in the U.S. Intermountain West (IW). In my thesis, I explored the use of a seedling-based approach to aspen restoration in an IW context.

I first assessed whether nursery protocols designed for boreal aspen will require modification for IW aspen by growing seedlings from two IW sources with a boreal

source in a common nursery environment. Seedlings from the two IW seed sources developed significantly smaller root-to-stem ratios and sequestered less carbohydrate and nutrient reserves compared to the boreal seed source. The two IW sources also differed from one another significantly, despite their relative geographic proximity. This suggests that while nursery protocols will need to be adapted for IW seed sources generally, sub-regional modifications may also be important.

In the second phase of my research, I planted 7,200 of the seedlings I grew in the nursery at 15 plots across three sites in southwestern Utah and monitored their growth, survival, and major contributing mortality factors. After two growing seasons overall seedling survival was only 10%, with apparent drought stress and small mammal herbivory the main causes of mortality. The majority of seedling survival occurred at just two plots where higher plant available water was observed during the early summer. The application of a wood mulch treatment in one of the plots also increased seedling survival to 62%, compared to no survival for seedlings without mulch. Despite the substantial mortality experienced in this study, these findings suggest that with the development of appropriate site selection and preparation techniques, seedling-based aspen restoration could become a viable management tool in the IW.

(100 pages)

PUBLIC ABSTRACT

Assessment of a Seedling-Based Approach to Aspen

Restoration in the Intermountain West

Alexander A. Howe

Quaking aspen (*Populus tremuloides*) is an important species ecologically and culturally in the western U.S., where it is one of the only broadleaf trees in the mostly conifer-dominated forests. Aspen management in the West has focused on regenerating existing stands vegetatively through root suckering, however this approach is restricted to locations where aspen currently exists and limits the genetic diversity of aspen populations. Planting nursery-grown aspen seedlings offers a potential method for overcoming these limitations, but it has received little attention in the U.S. Intermountain West (IW) to date. In order for this approach to be more broadly implemented, nursery protocols designed to grow high-quality aspen seedlings are needed, along with an understanding of what the major challenges to seedling establishment will be.

With the assistance of my committee, my research tested a seedling-based approach to aspen restoration in an IW context in two phases. I first used nursery protocols developed for boreal aspen to grow seedlings collected from IW sources in order to assess whether modification of the protocols would be necessary. I then planted the seedlings I grew at three sites in southwestern Utah and monitored their growth and survival over two years. Results from the nursery phase suggest that protocols will need to be modified in order to produce a more consistent response from IW aspen seedlings.

In the field, only 10% of the seedlings survived, though the majority of survival occurred in just two locations where soil moisture remained highest during the driest part of the early summer. These results provide useful information to direct future research and suggest that with a better understanding of appropriate site selection, seedling-based aspen restoration could still become a viable management tool in the IW.

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Thank you to the members of the Utah Conservation Corps crew that spent seven

weeks of their summer laboring on our plot exclosures, as well as Ryan Riddle and his crew at the Utah Division of Forestry, Fire & State Lands who performed the controlled burns for our plots. Thank you to Chad Reid with USU Iron County Extension for his helpful advice on site selection, and thanks to the Cedar Mountain landowners Burke Staheli, Thomas Bayles, and Heber and Barbara Meeks, whose interest in aspen conservation allowed me to undertake this project.

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Alexander A. Howe

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CHAPTER 1

INTRODUCTION

The effects of climate change are impacting forest ecosystems globally through increased tree mortality linked to higher temperatures and more intense droughts (Allen et al. 2010; 2015). In the western US, increased rates of tree mortality associated with drought stress have been documented across species and throughout the region (van Mantgem et al. 2009). With the effects of climate change likely to intensify, maintenance of resilient forest ecosystems will likely require increasingly proactive and novel approaches (Millar et al. 2007; Aitken et al. 2008).

One tree species of considerable conservation interest in the southwestern U.S. is quaking aspen (*Populus tremuloides* Michx.), the most widely distributed tree species in North America (Little 1971). Aspen serves an important ecological role as one of the only broadleaf species in the conifer-dominated forests of the West, where it supports increased biodiversity, provides an important source of forage for domestic livestock and wildlife, and possesses high cultural and aesthetic value (DeByle and Winokur 1985; Peterson and Peterson 1992). Aspen is also remarkable for its ability to reproduce vegetatively through root suckering, leading to the establishment of many large clones, particularly in the U.S. Intermountain West (IW) (Barnes 1966; Kemperman and Barnes 1976). Indeed, the largest known organism on earth is an aspen clone in central Utah called “Pando” (Kemperman and Barnes 1976; DeWoody et al. 2008). While aspen possesses a broad ecological niche, it has nevertheless experienced substantial declines in

recent years, particularly in southwestern portions of its range. Aspen dieback following a severe drought from 2000-2003 was so widespread that it received its own term, “Sudden Aspen Decline” (Rehfeldt et al. 2009; Worrall et al. 2013), and climate forecasting suggests aspen’s range in the western U.S. is likely to contract considerably over the coming century (Rehfeldt et al. 2009; 2015).

Aspen management in the western U.S. has focused primarily on maintaining established clones on the landscape through promotion of vegetative reproduction. Even-aged stand management practices using clearfelling or controlled burns are the preferred methods, aimed at producing a vigorous sprouting response to regenerate stands (Long and Mock 2012). While these aspen management practices became commonplace due to their relative ease of implementation (DeByle and Winokur 1985), they can have important drawbacks that are likely to challenge effective conservation efforts under climate change. For instance, the focus on asexual reproduction does not allow for the introduction of new genetic material. This has important ramifications for the resilience of aspen populations to changing environmental conditions. Exclusively promoting asexual regeneration also limits management actions to locations where aspen currently exists. While seedling establishment from dispersed seeds may provide an important mechanism for aspen regeneration into new habitat following disturbance (Landhäusser et al. 2010; Gill et al. 2017), aspen seeds have a short period of viability, and seedling establishment requires consistent and ample moisture and light (McDonough 1979). These requirements likely limit sexual reproduction in the more xeric regions of the IW. With drought severity and frequency likely to increase under climate change, appropriate

conditions for aspen seedling establishment may become even rarer in the future.

Instead of relying on natural seedling establishment, targeted planting with nursery-grown aspen seedlings could provide greater control over the management of aspen regeneration, while also addressing some of the limitations inherent in current management practices. In addition to promoting increased genetic diversity, planting seedlings could allow for restoration of aspen where it has been lost on the landscape, or for the establishment of new stands where climate suitability is predicted to be greatest (e.g. Millar et al. 2007; Aitken et al. 2008; Gray et al. 2011; Williams and Dumroese 2013). Additionally, strategic planting of aspen as fuel breaks around structures in the wildland-urban interface could be an important tool for limiting the damage of increasingly frequent wildfires due to the low flammability of aspen relative to conifers (Fechner and Barrows 1976; Fisher 1986).

Despite the potential advantages of utilizing planted seedlings for aspen management, this approach has received only limited consideration in the IW (Fisher 1986; Shepperd and Mata 2005). However, a need for effective reclamation techniques in the boreal forests of western Canada has driven more extensive research into the production and planting of aspen seedlings for disturbed sites (Martens et al. 2007; Landhäusser et al. 2012a; b; Pinno et al. 2012; Schott et al. 2013). These studies have focused on aspen seedling quality through production of stock targeted for marginal environments. In the drought-prone environments of the IW, aspen seedlings targeted for marginal environments are also likely to be a requisite for successful implementation of seedling-based restoration.

The purpose of my research was to assess the potential for seedling-based aspen restoration in the IW and identify major barriers to successful implementation. To accomplish this, I tested two important aspects of this management approach. In Chapter 2, I investigated whether a nursery protocol that was developed for aspen in the boreal region of western Canada would produce similar seedling characteristics with IW seed sources. Regional adaptation of nursery protocols is often necessary to achieve consistency of desired seedling stock characteristics, and I expected that differences between boreal and IW aspen could be particularly pronounced given the distinct phylogenetic boundary between populations in the two regions (Callahan et al. 2013). In Chapter 3, I conducted a field study using the nursery-grown aspen seedlings from Chapter 2. I planted seedlings from the three sources (Utah, New Mexico, and Alberta) at three sites in southwestern Utah, and monitored them over two growing seasons to assess seedling growth, survival rates, and significant mortality factors. The results of this research will help identify major hurdles and guide future studies on this novel approach to aspen management in the IW.

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CHAPTER 2

ASSESSING NURSERY PROTOCOLS FOR GROWING CONTAINERIZED ASPEN
SEEDLINGS SOURCED FROM INTERMOUNTAIN WEST CLONES**Abstract**

Management of the foundational species quaking aspen (*Populus tremuloides* Michx.) in western North America has long focused on promoting the vegetative regeneration of established clones. While planting nursery-grown aspen seedlings is becoming increasingly common as a forest reclamation tool in the boreal region, it has received little attention in the U.S. Intermountain West (IW). This approach to aspen restoration could provide land managers with new opportunities for afforestation or reforestation, as well as the ability to increase the genetic diversity of aspen populations. Effective implementation of seedling-based aspen restoration in the IW will require nursery protocols designed to maximize seedling quality for planting in marginal environments, however research on aspen nursery production to date has been concentrated on boreal aspen. The objective of this study was to assess whether a nursery protocol designed to maximize desirable traits in boreal aspen seedlings would produce similar traits in aspen seedlings sourced from the IW. I grew aspen seedlings in a common nursery environment with seed collected from clones in Utah and New Mexico, USA, and compared them to a boreal source from Alberta, Canada. IW seedlings differed significantly from the boreal seedlings, developing smaller root-to-stem ratios and sequestering less carbohydrate and nutrient reserves in their tissues. The Utah and New

Mexico seedling sources also differed significantly despite their relative geographic proximity. These results indicate that nursery protocols designed with boreal aspen will require regional modification in order to optimize seedling stock quality and trait consistency for IW aspen.

Introduction

Quaking aspen (*Populus tremuloides* Michx.) is a foundational species in western North America, where it supports high levels of biodiversity, provides important wildlife and domestic forage, and is prized for its aesthetic qualities (DeByle and Winokur 1985; Peterson and Peterson 1992). Aspen is well-known for its ability to reproduce asexually via root suckering, with healthy, established stands frequently exhibiting a vigorous suckering response to disturbance (Baker 1925; Schier and Smith 1979; Navratil and Bella 1990). By contrast, natural seed-based aspen reproduction in the drought-prone U.S. Intermountain West (IW) is frequently limited by seedling requirements for adequate soil moisture, moderate temperatures, and mineral substrates (McDonough 1979; Schott et al. 2014). Traditional silvicultural practices to regenerate aspen in the IW have long focused on promoting asexual reproduction of existing stands by inducing root suckering through coppicing or burning of the overstory (Long and Mock 2012). These methods have been favored due to their relative ease of implementation (DeByle and Winokur 1985). However, exclusive reliance on vegetative reproduction limits management options for IW aspen. For instance, it fails to introduce new genetic material, thereby constraining the adaptive potential of aspen populations. Crucially, it is

also limited to locations where aspen already exists, preventing establishment of new stands or reforestation of stands that are lost.

Planting nursery-grown aspen seedlings could offer an option for addressing these limitations, allowing for more proactive management approaches to aspen restoration. For example, assisted migration to unoccupied, climatically appropriate habitat could be implemented as a conservation strategy in response to climate change (Millar et al. 2007; Aitken et al. 2008; Gray et al. 2011; Williams and Dumroese 2013a). Strategic planting of aspen seedlings to create firebreaks within a forest or defensible spaces near a wildland-urban interface has also been suggested due to the low flammability of aspen stands compared to conifers (Fechner and Barrows 1976; Fisher 1986), although this has not yet been implemented in operational practices. While propagation of aspen from root cuttings is also possible (Schier 1978), obtaining cuttings from a wide variety of clones is both time- and cost-prohibitive, limiting the amount of genetic variation that can be introduced with restoration efforts.

Given the potential benefits of seedling-based aspen restoration for western forests, the scarcity of research to date on aspen planting stock and their quality is notable. In contrast, container-grown seedlings of economically important timber species (mostly conifers) have been used in silvicultural systems for decades, with nursery protocols developed and refined based on research and operational experimentation. For example, production of container-grown conifer seedlings in Oregon alone increased from under one million in 1970 to more than 35.2 million by 2016, with all important timber species in the Pacific Northwest successfully cultivated in this manner (Cleary et

al. 1978; Hernández et al. 2017). The main reasons aspen has not experienced the same level of attention as other tree species are likely threefold. For one, the market for aspen timber products in the IW has historically been limited compared to softwoods, and thus it has never been pursued for plantation style forestry (DeByle and Winokur 1985); second, the prevalence of vegetative reproduction following disturbance has made even-aged management with clearfelling the default silvicultural system for the regeneration of IW aspen (Long and Mock 2012); and third, there has been a longstanding perception in the western US that aspen reproduction is entirely vegetative with virtually no natural seedling establishment (Long and Mock 2012). Thus, silvicultural practice and research in the IW have only minimally pursued the use of aspen seedlings (but see (Fisher and Neumann 1987; Shepperd and Mata 2005).

The lack of protocols for growing aspen seedling stock could prove to be a significant barrier to successful implementation of seedling-based restoration in the IW. Arid summers and variable precipitation are common across the region and climate change is likely to exacerbate annual weather volatility and drought severity. Increased browsing pressure, particularly from elevated ungulate populations, has also challenged aspen regeneration in many areas (Kay and Bartos 2000; Rogers and Mittanck 2014; Britton et al. 2016; Smith et al. 2016). Given the potential challenges of the growing environment across much of the IW, the ability to produce seedlings with characteristics targeted specifically for the restoration site, i.e. the “Target Plant Concept” (Dumroese et al. 2016), may prove to be particularly important.

While there has been minimal research on nursery protocols for containerized

aspen in the US, reclamation of disturbed boreal sites in western Canada in recent years has fueled extensive research on the use and quality of planted aspen seedlings (Martens et al. 2007; Landhäusser et al. 2012a; b; Schott et al. 2013). Much of this research has focused on the identification of traits in aspen seedlings that correlate with higher outplanting success and performance. Unlike most conifer seedlings, for which aboveground traits like root collar diameter (RCD) and height are good indicators of seedling stock quality (Sutton 1979), root system traits appear to be better indicators of aspen stock quality. High root-to-stem ratios (RSR) and total non-structural carbohydrate (NSC) reserves in the roots were found to be more important predictors of aspen seedling outplanting performance than stem traits (Martens et al. 2007; Landhäusser et al. 2012a). Aspen has an indeterminate growth strategy, making it a challenge to develop robust root systems in a nursery setting where favorable conditions encourage seedlings to continue stem elongation throughout the growing season, allocating most of their resources to height growth, rather than to root growth and the storage of photosynthates and nutrients (Landhäusser et al. 2012a). To maximize RSR and root provisioning in aspen seedling stock, it was discovered that inducing premature bud set during the growing season allowed continued photosynthesis and fertilization, which increased the RSR, NSC and nutrient concentration of seedlings (Landhäusser et al. 2012a; Schott et al. 2013). The termination of shoot growth could be achieved using various methods, but the use of a hormonal shoot growth inhibitor provided the most consistent results (Landhäusser et al. 2012a).

In this study I investigated whether a nursery protocol designed to maximize

certain desirable characteristics such as RSR, NSC, and nutrient concentrations in boreal aspen seedlings would produce similar traits in IW aspen seedlings. I hypothesized that significant differences in these traits would develop between seedlings from the two regions following the nursery period due to the distinct phylogeographic boundary that exists between IW and boreal aspen populations (Callahan et al. 2013). To investigate this, I used a nursery protocol developed by the University of Alberta to grow seedlings in a common nursery environment. I grew a boreal aspen seedling source from Alberta, Canada alongside seedlings sourced from two regions in the IW (northern Utah and northern New Mexico, USA), which were chosen opportunistically based on seed collection logistics. In order to assess the response of each source to the nursery protocol, I measured seedling traits for the three sources during and at the completion of the nursery period. The primary goal of this study was to determine whether a common seedling production protocol can be used for both boreal and IW aspen, or whether the protocol will require regional adaptation in the IW in order to produce comparable seedlings traits.

Materials and Methods

Seedling nursery production

Aspen seed used in this study was collected from multiple open-pollinated clones in three geographically separate regions in the late spring of 2014. The IW sources originated from clones in Logan Canyon, Utah, USA (N 41°56'; W 111°31') and near Los Alamos, New Mexico, USA (N 35°53'; W 106°22'). The boreal source was collected

near Edmonton, Alberta, Canada (N 56°43'; W 113°31'). The Alberta (AB) and Utah (UT) sources were each collected from an estimated 15 different female clones, while the New Mexico (NM) source came from seven clones. A cursory germination test of 100 seeds per source showed consistently high germination across sources (>90%).

Starting in the spring of 2015, aspen seedlings were grown at the New Mexico State University John T. Harrington Forestry Research Center in Mora, NM. A total of 18,000 D16 Deepot® cells (Stuewe and Sons, Inc., Tangent, OR, USA) with cavities 17.8 cm deep and 5 cm in diameter (262 ml) were filled with a 2:1:1 mix of sphagnum peat moss, vermiculite, and Turface® clay granules, respectively. Prior to sowing, the media was irrigated until saturation and then misted with 100 g / 19 L of 10-30-20 NPK fertilizer. Cells were hand-seeded in the second week of March, each containing five to ten aspen seeds from one of the three sources described above, for a total of 6,000 cells per source. Racks holding 50 cells each were separated by seed source and randomly distributed throughout the greenhouse. For three weeks after sowing, the seedlings were misted for three to five minutes, five times per day. At the end of three weeks, cells were thinned down to one seedling and the watering schedule switched to a target dry-down of 85% of media field capacity. Weekly fertigation also began at this time with a 10-52-10 NPK solution (200 ppm), switching to a 15-30-15 NPK solution (200 ppm) after the first two weeks. Greenhouse conditions were maintained at 21-24°C during the day and 17-21°C at night. After seven weeks the temperature was changed to maintain a daily range of 15-18°C. Average humidity was 65% and high-intensity discharge lamps were used to maintain a 14 hour photoperiod.

Seedlings were moved to sub-irrigation tanks outside of the greenhouse nine weeks after germination, where they continued to receive the same schedule of watering and fertigation. At week 11, all seedlings were treated with an initial application of the shoot growth inhibitor hormone paclobutrazol (Bonzi®, Syngenta, Wilmington, DE, USA) at a concentration of 20 mg L⁻¹ by adding 5 mL Bonzi® per liter to the sub-irrigation tank and allowing the seedlings to soak in the solution for 15 minutes before draining. A second application of Bonzi® was conducted at week 14 following the above procedure. At week 18 the weekly fertilization was increased to 300 ppm of 15-30-15 NPK fertilizer for two weeks before increasing one final time to 400 ppm for two weeks. Fertilization was discontinued after week 21 and the watering schedule was changed to a target dry down of 75% of media field capacity at week 22 until seedlings naturally reached dormancy. Additionally, due to partial leaf defoliation on aspen seedlings beginning at the end of June, a 2:1 mixture of organic Insect Killing Soap Concentrate and End All® insecticide (Safer® Brand, Lititz, PA, USA) was hand-sprayed on all seedlings 1-2 times per week as needed until the end of August.

Seedling measurements

To determine how each seed source was developing prior to the hormone treatment, I destructively harvested 30 randomly subsampled seedlings per source the first week of June 2015 (12 weeks after germination). Height, RCD, root and shoot dry weight, leaf area and leaf dry weight were measured and each seedling was visually assessed for terminal bud set. After recording seedling height and RCD, I removed a leaf

from each seedling and digitally scanned it to save an image of the fresh leaf for subsequent area calculation using the program ImageJ (Schneider et al. 2012). I then removed the seedlings from their containers and washed the media from their root systems. The woody tissue was cut at the root collar before drying the stem, root, and leaf tissue of all seedlings in a 70°C oven for 48 hours. Dry weights of these tissues were used to calculate the RSR and specific leaf area (SLA, the ratio of leaf area to mass). Due to substantial foliar damage by insects and fungal infection during the mid-summer, which appeared to differ in severity by source, I performed a visual assessment of all racks of aspen seedling stock and quantified the percentage of damaged leaves per rack. The percent of leaves damaged by either fungus (predominately Marssonina leaf blight) or herbivorous insects was separately quantified and recorded by seedling source.

In order to assess the final stock quality produced in the nursery of each source, I again destructively harvested a total of 30 randomly subsampled seedlings per source in November (35 weeks post-germination) after the seedlings had abscised their leaves and were fully dormant. Seedling height, RCD, root and shoot dry weights were assessed, and terminal bud volume was estimated from measurements of the length and diameter of the bud (calculated as for an ellipsoid). Root volume was measured using water displacement (Olesen and Roulund 1971). I measured seedling root and shoot NPK and NSC with a different subsample of 50 seedlings per source that had undergone a final round of culling from the initial 18,000 seedlings to 7,200 seedlings prior to outplanting in a subsequent field study. Root and shoot tissue samples from each seedling were separated and dried at 70°C for 48 hours before grinding them through a 40-mesh (0.4 mm) screen with a Wiley

mill (Thomas Scientific, Swedesboro, NJ, USA). N concentration of the stem and root tissue was assessed using the Kjeldahl method (Kalra and Maynard 1991). P and K concentrations were analyzed using inductively-coupled plasma optical emission spectrometry (ICP-OES) following microwave digestion (EPA Method 3051, U.S. Environmental Protection Agency, Washington, D.C.). NSC concentrations were determined by extracting water soluble sugars three times from each sample with 80% ethanol at 95°C. Total sugar concentration in the ethanol extract was analyzed using phenol-sulfuric acid (Chow and Landhäusser 2004). Starch content was then determined from the residue remaining after sugar extraction using a digestion enzyme mixture of α -amylase and amyloglucosidase followed by the colorimetric measurement of the glucose hydrolysate using a peroxidase-glucose oxidase-o-dianisidine reagent (Chow and Landhäusser 2004).

Analyses

To assess differences in the response to the nursery protocols between the three aspen seed sources used in this study, randomized subsamples of 30 (June, 2015) and 50 seedlings per source (November, 2015) were destructively harvested and measured as described above. I analyzed differences in seedling characteristics between the seed sources using a one-way analysis of variance (ANOVA), and then used a post-hoc Tukey's test to evaluate pairwise comparisons between specific sources for statistical significance. Statistical significance was determined at $\alpha = 0.05$ and all ANOVAs were conducted using R 3.4.1 (R Core Team 2017) with packages "multcomp" (Hothorn et al.

2008) and “lsmeans” (Lenth 2016).

Results

Growth differences between seedling stocks were already apparent by the first destructive measurements taken the first week of June (12 weeks after germination). IW seedling stock favored early shoot growth over root growth, producing seedlings with significantly greater height ($F_{2,87} = 78.77, P < 0.001$), RCD ($F_{2,87} = 13.81, P < 0.001$), and shoot dry weight ($F_{2,87} = 31.66, P < 0.001$), but decreased root dry weight ($F_{2,87} = 17.58, P < 0.001$) compared to the boreal seedling source from Alberta (Table 2.1). This resulted in a significantly greater RSR in the Alberta seedlings of 3.8, more than double that of the IW seedlings (1.8 and 1.0, Utah and New Mexico respectively, $F_{2,87} = 99.27, P < 0.001$) (Table 2.1). This appeared to be driven at least partially by a higher rate of early bud set in the Alberta seedlings (63%) compared with Utah (23%) and New Mexico (3%), which was recorded at the time of destructive harvesting. Between the two IW seed sources, New Mexico seedlings displayed a significantly greater prioritization of shoot growth over root growth across all traits measured compared to Utah seedlings (Table 2.1). Susceptibility to foliar fungal infection also differed significantly between Utah and the other two sources, with 30% of Utah seedling leaves affected, but only 7% of New Mexico and 11% of Alberta affected ($F_{2,348} = 48.69, P < 0.001$). Foliar insect herbivory was significantly lower in the Alberta source compared to New Mexico source, but did not differ significantly from Utah seedlings (Alberta: 6%, Utah: 7.4%, New Mexico: 8.9%, $F_{2,348} = 11.06, P < 0.001$).

Table 2.1 Average (standard deviation) of morphological traits observed in seedling stock from first destructive subsampling (June 2, 2015, 12 weeks after germination)

Seed Source	Height (cm)	RCD (mm)	Root Dry Weight (g)	Stem Dry Weight (g)	Root:Stem Ratio	Specific Leaf Area (cm ² /g)
Alberta	11.8 a (3.3)	1.9 a (0.36)	0.67 a (0.21)	0.19 a (0.08)	3.8 a (1.1)	181 a (23)
Utah	19.2 b (5.1)	2.1 a (0.31)	0.54 b (0.17)	0.32 b (0.11)	1.8 b (0.6)	183 a (22)
New Mexico	29.1 c (7.0)	2.4 b (0.37)	0.41 c (0.12)	0.43 c (0.15)	1.0 c (0.4)	203 b (22)

Different letters connote significant differences ($\alpha = 0.05$) between seed sources ($n = 30$)

The two applications of the hormone treatment to all seedlings at weeks 11 and 14 affected seed sources differently. In the Alberta source, nearly all seedlings set bud that had not already done so and terminated additional shoot growth for the rest of the growing season after the second hormone treatment. In contrast, while most of both IW seed sources terminated shoot growth initially following the hormone treatments, the majority broke bud (terminal and/or axillary) or suckered from the roots and continued to grow new stem and leaf tissue late into the growing season. The result of these differing responses to the hormone treatment intensified the gap in the RSR between the boreal and IW seedlings, leading to a significantly higher average final RSR of 9.7 in the Alberta stock compared to the IW stock (3.4 and 2.3, Utah and New Mexico respectively, $F_{2,87} = 92.44$, $P < 0.001$) (Table 2.2). Differences in the RSR between sources were largely driven by the shoot size, with height ($F_{2,87} = 45.78$, $P < 0.001$) and RCD ($F_{2,87} = 30.94$, $P < 0.001$) differing significantly between all sources following the same trend observed at week 12 for the first destructive measurements (from smallest to largest: Alberta, Utah, New Mexico) (Table 2.2). Final average root dry weights between sources were more

similar by the end of the growing season (week 35) than during the middle (week 12), with Alberta and New Mexico nearly the same (2.6 g and 2.5 g respectively), but with both significantly greater than Utah (1.8 g, $F_{2,87} = 6.31$, $P = 0.003$) (Table 2.2).

Table 2.2 Average (standard deviation) of morphological traits observed in seedling stock from final destructive subsampling following seedling dormancy (November 6, 2015, 35 weeks after germination)

Seed Source	Height (cm)	RCD (mm)	Root Volume (mL)	Root Dry Weight (g)	Stem Dry Weight (g)	Root:Stem Ratio	Terminal Bud Volume (mm ³)
Alberta	13.0 a (4.7)	3.2 a (0.6)	12.4 ab (4.0)	2.6 a (1.0)	0.32 a (0.19)	9.7 a (3.5)	12.3 a (4.8)
Utah	20.4 b (8.3)	3.7 b (0.9)	10.0 a (3.7)	1.8 b (0.8)	0.62 a (0.42)	3.4 b (1.1)	12.6 a (5.4)
New Mexico	34.5 c (12)	4.8 c (0.9)	13.2 b (4.4)	2.5 a (1.0)	1.35 b (0.76)	2.3 b (1.4)	11.4 a (3.8)

Different letters connote significant differences ($\alpha = 0.05$) between seed sources ($n = 30$)

Seedling nutrient and NSC tissue concentrations also differed between the Alberta and IW seed sources by the end of the nursery period. Whole-plant N concentration was significantly higher in the Alberta seedlings (48.0 mg/g) compared to the IW seedlings (37.5 mg/g and 33.4 mg/g, Utah and New Mexico, respectively, $F_{2,53} = 33.66$, $P < 0.001$) as was P concentration (6.0 mg/g in Alberta seedlings, 4.6 mg/g in both IW seedlings, $F_{2,53} = 37.01$, $P < 0.001$) (Table 2.3). Only K concentration did not differ between sources ($F_{2,53} = 1.75$, $P = 0.183$) (Table 2.3). Similarly, stem NSC concentration was significantly higher in the Alberta seedlings than in IW seedlings ($F_{2,57} = 8.28$, $P < 0.001$), and while Alberta seedlings also had the greatest root NSC on average, these levels only differed significantly from the Utah source ($F_{2,57} = 10.14$, $P < 0.001$) (Table

2.3). Root NSC variability in Alberta seedlings was less than either IW source (Table 2.3).

Table 2.3 Average (standard deviation) of nutrient and carbohydrate reserves measured in seedling stock from final destructive subsampling following seedling dormancy (November 6, 2015, 35 weeks after germination)

Seed Source	N* (mg/g)	P* (mg/g)	K* (mg/g)	Root			
				NSC (%)	NSC (g)	NSC (%)	NSC (g)
Alberta	48.0 a (5.6)	6.0 a (0.57)	8.6 a (1.2)	33.3 a (2.8)	0.84 a (0.33)	16.2 a (2.7)	0.06 a (0.03)
Utah	37.5 b (5.8)	4.6 b (0.53)	8.7 a (1.4)	23.4 b (10)	0.48 b (0.31)	13.2 b (3.7)	0.10 b (0.06)
New Mexico	33.4 c (7.0)	4.6 b (0.53)	8.0 a (0.8)	28.0 ab (6.3)	0.65 ab (0.35)	12.9 b (1.6)	0.14 b (0.08)

Different letters connote significant differences ($\alpha = 0.05$) between sources (NSC: n = 20; Alberta NPK: n = 17; New Mexico NPK: n = 20; Utah NPK: n = 19)

*Nutrient concentrations for whole seedling (root and stem)

NSC = total non-structural carbohydrates

Discussion

IW aspen seedlings grown from Utah and New Mexico seed sources differed markedly in their response to a nursery protocol developed for boreal aspen when compared to seedlings from Alberta, Canada. The IW sources did not set bud with a uniform timing following two applications of the shoot growth inhibitor hormone paclobutrazol. Additionally, many of the seedlings that did initially set bud subsequently suckered from the root system or underwent lateral or terminal bud flush and continued shoot growth late into the growing season. The Alberta seedlings, by contrast, responded to the nursery protocol as previously described (Landhäusser et al. 2012a; b; Schott et al. 2013); uniformly setting and holding bud throughout the rest of the growing season,

leading to the development of elevated seedling RSR, root and shoot NSC, as well as whole-plant woody tissue concentrations of N and P. The highly variable response of the IW sources to the hormone treatment resulted in significantly lower average values and increased variance for these same seedlings traits.

Despite the inconsistent response of the IW seedlings to the protocol, the paclobutrazol hormone addition may still have had a marked effect on their growth. In a subsequent experiment growing the same three seed sources without the use of paclobutrazol the following year (n=12 seedlings/source, but seedlings were not moved outdoors and were grown in smaller Ray Leach Cone-tainer™ SC-10 cells, 164 mL), seedlings had a 23%, 33%, and 61% lower average root NSC concentration for Alberta, Utah and New Mexico seedling stock, respectively. The average RSR in these seedlings was also 79%, 68%, and 70% lower, respectively (Owen Burney, unpublished data). These differences suggest that the hormone treatment was at least partially effective in its goal of increasing RSR and NSC by limiting seedling shoot growth in favor of developing a larger root system with greater carbon reserves. It is also notable that both IW seedling stocks developed a final average RSR well over 2.0, and an average height under 40 cm, which are seedling traits associated with improved aspen seedling outplanting performance in marginal environments (Landhäusser et al. 2012a). Nevertheless, the timing and concentration of the hormone treatment will likely need to be optimized for IW seedlings in order to produce a more consistent bud set using this method.

Differences observed in final stock characteristics between the IW and boreal

seedlings in this study are not entirely surprising given the latitudinal distance of their origin and the phylogeographic boundary between aspen populations in the IW and elsewhere throughout their range (Callahan et al. 2013). More unexpected are the significant stock trait differences observed between the Utah and New Mexico seedling sources. These differences suggest that the variability between aspen populations in the IW may necessitate more local adaptation of nursery protocols to optimize seedling stock quality across the region. Despite their relative geographic proximity, significant genetic population structuring by geographic distance throughout the IW exists and may at least partially explain the differences between these two sources in response to the same nursery protocol (Callahan et al. 2013). Greater susceptibility to pathogens such as *Marssonina* leaf blight in the Utah source may also have impacted the photosynthetic efficiency of those seedlings, leading to reduced growth (Table 2.2) and more variable NSC accumulation (Table 2.3). However, pathogen susceptibility may have only intensified the differences between the two IW sources, as alternative growth strategies were already developing by week 12, with New Mexico seedlings prioritizing shoot development over roots compared to Utah seedlings (Table 2.1). Given these differences, testing of additional aspen seed sourced from populations across the IW will be an important step to determine the consistency of response to nursery protocols across the region. Additionally, testing alternative methods for increasing RSR should be explored. Artificially shortening day length through the temporary application of a blackout treatment midway through the growing season has also proven effective at increasing aspen seedling RSR and NSC through the induction of early bud set, but only when

seedlings are grown outdoors (Landhäusser et al. 2012a). A blackout treatment has the potential to be a lower cost alternative to the application of a hormone, but IW seedling sources may also respond differently to this treatment necessitating further experimentation. Additionally, top-pruning seedlings to a standardized height prior to outplanting has been demonstrated to increase establishment success of many hardwood tree species in marginal environments (South 1996), and this could be a useful interim technique for creating more uniform aspen seedling stock until nursery protocols are better refined.

The development of nursery protocols for growing quality seedlings is an iterative process that can take decades to refine. For example, concerted efforts to develop nursery best practices for the economically important southern pine species began in the 1920s and continued late into the century, resulting in survival rates for planted seedlings commonly exceeding 90% (Fox et al. 2007). Comparatively, the development of nursery protocols for the production of quality aspen seedling stock has only begun recently as its reclamation potential after oil sands mining in the boreal forest has been recognized (Martens et al. 2007; Landhäusser et al. 2012a; b; Pinno et al. 2012; Schott et al. 2013). With very little research on growing aspen from seed for restoration in the IW (Fisher 1986; Shepperd and Mata 2005), testing and refining nursery protocols for IW seed sources is a critical next step towards developing quality seedling stock that can be utilized with greater success in the marginal environments typical of the region.

Nursery production of aspen seedlings for outplanting could become an increasingly important tool for forest managers in the IW, particularly with anticipated

reductions in climatically suitable habitat for aspen across much of the region (Rehfeldt et al. 2009; Worrall et al. 2013). Seedling-based aspen restoration offers some distinct advantages over traditional silvicultural methods. It increases genetic diversity across the landscape, boosting resilience of aspen populations to both biotic and climatic stressors. It provides the ability to reforest decadent stands that have poor vegetative reproductive capacity, and even allows assisted migration through the establishment of aspen in new locations which are expected to be more favorable under climate change. Additionally, reforestation with aspen seedlings following severe, stand replacing fires or stand thinning treatments may be an important and underutilized strategy for breaking up forest fuel connectivity across the landscape, and for providing defensible space around structures in the wildland-urban interface (Fechner and Barrows 1976; Fisher 1986). Developing the nursery protocols to maximize aspen seedling quality for use in the drought-prone environments common to the IW is an important first step for realizing this potential, and this study highlights the need for continued work on this subject.

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CHAPTER 3
EXPLORING SEEDLING-BASED ASPEN RESTORATION
IN THE INTERMOUNTAIN WEST: BARRIERS
AND POTENTIAL SOLUTIONS

Abstract

With the effects of climate change expected to intensify over the coming century, land managers will increasingly require more proactive and novel approaches to conserve and restore threatened ecosystems and species. In the U.S. Intermountain West (IW), quaking aspen (*Populus tremuloides* Michx.) is a species of considerable conservation interest due to its ecological importance and cultural value. However, traditional silvicultural practices in the region, which focus exclusively on generating vegetative regeneration, limit restoration potential to locations where aspen is already established, and preclude more adaptive management approaches such as reforestation and assisted migration. Planting nursery-grown aspen seedlings could overcome these limitations, and while this approach has become increasingly utilized for land reclamation and afforestation in the boreal forests of western North America, it has received little attention in the IW. Using aspen seedlings grown from two IW seed sources and one boreal source, this study sought to explore the potential challenges of seedling-based aspen restoration in an IW landscape. I planted 7,200 nursery-grown aspen seedlings across 15 unirrigated plots at three sites in southwestern Utah, USA and monitored their growth and survival rates over the following two growing seasons. Overall seedling

survival was only 10% by the end of the study, with 50% of mortality due to apparent drought stress, 38% due to small mammal herbivory, and 2% due to unknown causes. 79% of the surviving seedlings were located in just two plots, where higher plant available water was observed during the early summer. Additionally, the application of wood mulch in one plot increased seedling survival to 62%, compared to zero percent survival in a non-mulched treatment. Together, these findings suggest that while drought conditions pose a significant barrier to seedling establishment, with the development of appropriate site selection and preparation techniques, seedling-based aspen restoration could become a viable management tool in the IW.

Introduction

Forests globally are experiencing elevated tree mortality associated with droughts and rising temperatures due to climate change (Allen et al. 2010; 2015). In the western US, an increase in the rate of background tree mortality linked to drought stress has been observed across forests and species (van Mantgem et al. 2009), and rapid, sub-continental scale die-off of forest overstory has been reported in response to severe, “global-change-type” drought (Breshears et al. 2005). One species of particular interest and conservation concern in western North America is quaking aspen (*Populus tremuloides* Michx.). Aspen has significant ecological and cultural value as one of the few broadleaf deciduous trees in the conifer dominated forests of the West, where it is considered a foundational species that supports increased biodiversity, provides an important forage source for wildlife and domestic livestock, and has high aesthetic value (DeByle and Winokur 1985;

Peterson and Peterson 1992). Aspen possesses the broadest distribution of any tree species in North America (Little 1971) and possesses the ability to reproduce vegetatively through root suckers, allowing rapid response following disturbance (Bartos and Meuggler 1981; Peterson and Peterson 1992; Frey et al. 2003).

Despite its broad ecological niche, aspen has experienced marked declines across much of western North America in recent years, particularly in the southern, drier portions of the species range. These declines have been attributed to a variety of potentially interconnected stressors, including drought, pathogens, conifer encroachment, and herbivory (Frey et al. 2004; Worrall et al. 2008; Rogers and Mittanck 2014). Additionally, a severe drought from 2000-2003 incited a rapid and widespread aspen dieback termed “Sudden Aspen Decline”, linked primarily to moisture stress and hydraulic failure, leaving surviving clones more vulnerable to future drought (Hogg et al. 2008; Anderegg et al. 2013; Worrall et al. 2013, 2015). Significant declines in aspen populations across the western US are projected to continue, with broad-scale climate envelope models predicting a substantial contraction and shift in the range of aspen over the coming century (Rehfeldt et al. 2009). Natural seedling establishment may prove an important mechanism for aspen to adapt to these changing conditions through successful colonization of new habitat following disturbance (Landhäusser et al. 2010; Long and Mock 2012; Gill et al. 2017). Instances of successful seedling establishment in the West have been noted, even in more xeric regions (Romme et al. 2005; Fairweather et al. 2014). However, aspen has a short period of seed viability and germinants require a combination of ample moisture, light, and a mineral soil seedbed, which are uncommon

across the western range of aspen (McDonough 1979). This suggests that successful seedling establishment is likely to be highly stochastic and difficult to plan for or manage. Thus, if we hope to support the persistence and resilience of this important species in the West, more active management approaches may be required (Millar et al. 2007; Aitken et al. 2008; Rogers et al. 2013).

In the western US, the default regeneration method for aspen has long been even-aged management using clearfelling or prescribed fire to induce vegetative reproduction (Long and Mock 2012). However, the reliance on vegetative reproduction has several important limitations. First, it only allows for the management of the existing aspen root system, meaning that once a stand or clone is lost from the landscape it is effectively lost forever, barring vegetative spread from another stand or natural seeding. Second, regeneration of stands is time-sensitive, as mature stands with poor natural regeneration eventually reach a “tipping point” of resilience, beyond which their ability to adequately sucker following disturbance or silvicultural treatment is diminished or lost as carbon reserves are depleted in the root system (Bartos and Campbell 1998). Third, by managing exclusively for vegetative reproduction, the genetic diversity of aspen stands remains static over time, limiting the adaptive evolution of aspen populations.

Production of nursery-grown aspen seedlings for targeted planting offers an alternative technique that could overcome some of these limitations and provide new options for management. In addition to restoring aspen stands in areas that had been previously occupied by aspen, more proactive management approaches could also be implemented such as establishing aspen in currently unoccupied, but climatically

appropriate habitats through assisted migration (e.g. Millar et al. 2007; Aitken et al. 2008; Gray et al. 2011; Williams and Dumroese 2013a). Creating forest firebreaks or establishing defensible space around structures in the wildland-urban interface with strategic aspen planting has also been proposed due to the low flammability of aspen stands compared to conifers (Fechner and Barrows 1976; Fisher 1986). Seedling-based aspen restoration additionally enhances the adaptive potential of aspen populations through increased genetic diversity, providing greater resilience to threats like herbivory, pathogens or drought.

While planting nursery-grown seedlings is hardly a novel silvicultural practice for other important tree species, this approach has received only cursory consideration or research in the West (i.e. Fisher 1986; Shepperd and Mata 2005). In recent years, however, the need for effective reclamation practices on disturbed sites in the boreal forests of western Canada has led to substantial research into the use and quality of planted aspen seedlings (Martens et al. 2007; Landhäusser et al. 2012a; b; Pinno et al. 2012; Schott et al. 2013). This work has focused on producing aspen seedlings that can effectively establish and grow on the typically nutrient- and carbon-poor capping soils found at reclamation sites, and has led to a greater understanding of the morphological and physiological seedling characteristics of aspen that enhance outplanting success in marginal environments.

Implementing a seedling-based approach to aspen restoration in the southwestern regions of North America will likewise involve outplanting into marginal environments. Unlike the boreal region, where most research on aspen seedlings has taken place to date,

aspen habitat within the Intermountain West (IW), defined as the region in the US between the Sierra Nevada and Cascade Range to the west and the Rocky Mountains to the east, is typically more topographically complex, with disjunct aspen communities across the landscape ranging in size from small patches to large stands (DeByle and Winokur 1985). This landscape heterogeneity presents additional challenges to aspen seedling establishment, as climate, hydrology and edaphic features can vary widely over relatively small spatial scales, greatly influencing vegetation communities and restoration potential. Aspen habitat in the IW also spans a region both lower in latitude and higher in elevation than the boreal, leading to increased solar radiation during the growing season that can dry soils more quickly. Additionally, locations where aspen has declined in recent years and are in the greatest need of restoration are also more likely to be predisposed to moisture stress (Worrall et al. 2013). While these factors may challenge effective implementation of seedling-based aspen restoration in the IW, it is currently unknown what the primary barriers, if any, will prove to be.

Given the paucity of research in the IW on the topic, the objective of this study was to explore the potential for seedling-based aspen restoration in the IW region and assess potential barriers to inform future research. In this study I planted nursery-grown aspen seedlings to assess seedling survival rates and contributing mortality factors during the first two years following outplanting. I used nursery protocols developed for planting boreal aspen on marginal reclamation sites (see Chapter 1) to grow three seedling stocks with seed sourced from two IW aspen populations and one boreal population. These seedlings were outplanted at three sites in southern Utah, USA where substantial aspen

decline has been documented in recent years (Worrall et al. 2013). I monitored the seedlings over the following two growing seasons to examine whether seedling source or outplanting environment were limiting factors through measurements of growth and assessment of mortality cause for all seedlings.

Methods

Seedling nursery production

Aspen seedlings used in this study were grown from three geographically separate seed sources. Two seed sources originated in the IW from 15 clones in Logan Canyon, Utah, USA (N 41°56'; W 111°31') and seven clones near Los Alamos, New Mexico, USA (N 35°53'; W 106°22'). The final source was collected from 10 separate clones near Edmonton, Alberta, Canada (N 56°43'; W 113°31'). All seed sources were collected from multiple open-pollinated clones in the spring of 2014. Beginning the following spring of 2015, 18,000 aspen seedlings (6,000 from each seed source) were grown at the New Mexico State University John T. Harrington Forestry Research Center in Mora, NM (N 35°58'33"; W 105°20'54") following nursery protocols detailed in Chapter 1. At the 23rd week following germination I selected 7,200 seedlings (2,400 per source) in preparation for outplanting, selecting against those with missing or small terminal buds and those that were particularly tall or short.

Assessment of the average initial seedling morphological characteristics and carbon and nutrient reserve status at the time of the original outplanting was accomplished by destructively harvesting a total of 50 subsampled seedlings per source,

which were randomly selected from the planting stock. I measured all of these seedlings for root collar diameter (RCD), height, root and stem dry weight, root volume, and terminal bud volume (calculated as for an ellipsoid using length and diameter measurements). In a subset of these (17-20 per source) I also measured root and stem non-structural carbohydrates (NSC) and NPK concentrations. I then removed seedlings from their containers and washed the media from their root systems before cutting the seedlings at the root collar and measuring root volume using water displacement (Olesen and Roulund 1971). Separated seedling stem and root tissues were then dried in a 70°C oven for 48 hours and the dry weights of these tissues were used to calculate the root-to-stem ratio (RSR) of each seedling. To measure seedling NPK and NSC, I used the dried root and stem tissue from a subset of the seedlings and ground them through a 40-mesh (0.4 mm) screen with a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA). The protocols used for estimating stem and root NPK and NSC concentrations are detailed in Chapter 1.

The seedling stock used in the study differed significantly by seed source (Alberta, Utah, and New Mexico) with respect to morphological characteristics (Table 3.1) and carbon and nutrient reserves (Table 3.2). Alberta seedling stock developed significantly smaller stems (measured by height, RCD, and stem dry weight) than either IW source, but produced the largest average root dry weight (Table 3.1). The resulting RSR of the Alberta source was 7.6, nearly three times greater than the IW sources (2.7 and 2.6, Utah and New Mexico respectively). This difference between sources was likely due to a more consistent and uniform early bud set in the Alberta source than the IW

sources in response to a shoot growth inhibitor hormone treatment (Chapter 1). The Alberta seed source also differed from the IW sources in nutrient and NSC tissue concentrations. Alberta sequestered significantly more nitrogen (N) (48.0 mg/g) compared to the IW seedlings (37.5 mg/g and 33.4 mg/g, Utah and New Mexico, respectively) as well as phosphorus (P) (6.0 mg/g in Alberta seedlings, 4.6 mg/g in both IW seedlings) (Table 3.2). Potassium (K) concentration was the only nutrient to not differ between sources. Similarly, stem NSC concentration was significantly greater in the Alberta seedlings than in IW seedlings, though total NSC content was actually the lowest in Alberta seedlings due to their small stems. Finally, while Alberta seedlings possessed the greatest root NSC on average, these levels only differed significantly from the Utah source (Table 3.2).

Table 3.1 Average (standard deviation) of pre-planting morphological characteristics of aspen seedling stock at the time of outplanting (October, 2015). Different letters connote significant differences ($\alpha = 0.05$) between seed sources (n = 50 for all variables)

Seed Source	Height (cm)	RCD (mm)	Root Volume (mL)	Root DW (g)	Stem DW (g)	Total DW (g)	RSR	TBV (mm ³)
Alberta	14.6 a (4.1)	3.1 a (0.5)	10.2 a (2.8)	2.4 a (0.8)	0.33 a (0.15)	2.7 a (0.9)	7.6 a (2.0)	12.1 a (4.8)
Utah	23.8 b (5.6)	4.2 b (0.7)	9.5 a (2.5)	1.9 b (0.6)	0.75 b (0.30)	2.6 a (0.8)	2.7 b (0.8)	16.4 b (5.9)
New Mexico	31.5 c (8.5)	4.6 c (0.7)	10.4 a (2.9)	2.3 a (0.8)	1.02 c (0.49)	3.3 b (1.1)	2.6 b (1.3)	13.1 a (3.6)

Different letters connote significant differences ($\alpha = 0.05$) between seed sources (n = 50 for all variables)

DW = Dry Weight

RSR = Root:stem ratio

TBV = Terminal Bud Volume

Table 3.2 Average (standard deviation) of nutrient and carbohydrate reserves measured in seedling stock from final destructive subsampling following seedling dormancy (November 6, 2015, 35 weeks after germination)

Seed Source	N* (mg/g)	P* (mg/g)	K* (mg/g)	Root NSC (%)	Root NSC (g)	Stem NSC (%)	Stem NSC (g)
Alberta	48.0 a (5.6)	6.0 a (0.57)	8.6 a (1.2)	33.3 a (2.8)	0.84 a (0.33)	16.2 a (2.7)	0.06 a (0.03)
Utah	37.5 b (5.8)	4.6 b (0.53)	8.7 a (1.4)	23.4 b (10)	0.48 b (0.31)	13.2 b (3.7)	0.10 b (0.06)
New Mexico	33.4 c (7.0)	4.6 b (0.53)	8.0 a (0.8)	28.0 ab (6.3)	0.65 ab (0.35)	12.9 b (1.6)	0.14 b (0.08)

Different letters connote significant differences ($\alpha = 0.05$) between sources (NSC: n = 20; Alberta NPK: n = 17; New Mexico NPK: n = 20; Utah NPK: n = 19)

*Whole-plant (stem and root) nutrient concentration

NSC = total non-structural carbohydrates

Nursery stock evaluation

In order to assess aspen seedling quality under non-limiting conditions, 33 seedlings were randomly selected from each source in October 2015 from the seedlings selected for out-planting. These seedlings were kept at 4°C during the winter, transplanted into 11.3 L pots containing a mix of 2:1:0.5 sphagnum peat moss, vermiculite, and sand and then grown first in a greenhouse (9 weeks) after which they were transferred to an outdoor shadehouse. Both the greenhouse and shadehouse were located on the Utah State University campus in Logan, UT, USA (N 41°45'; W 111°48'). I recorded initial seedling height and RCD in March 2016. Throughout the growing season, these seedlings were watered to field capacity daily and misted with 100 g / 38 L of 20-20-20 NPK fertilizer once weekly until the seedlings naturally entered dormancy in mid-October 2016. I measured the final RCD and height of each seedling before

destructively harvesting them to determine dry mass of roots and stems. Height and RCD growth (final – initial) were calculated for each seedling. Seedling root dry weight growth was also calculated using the average initial root dry weight for each source (Table 3.1).

Plot selection, preparation, and seedling outplanting

Site selection was conducted during June 2015 on Cedar Mountain (N 37°33'; W 113°02'), situated between Cedar City and Cedar Breaks National Monument (southwestern Utah, USA). 15 plot locations were chosen to serve as replicates for aspen seedling outplanting. These plots were split between three sites located within 9 km of each other at approximately 2,800 m above sea level. Site A is located in the Winnemucca soil series, while Sites B and C are located in the Faim series (Soil Survey Staff, USDA NRCS). At each site, five plots (27 m × 23 m) were chosen in close proximity. All plots were located in open meadows at least 15 m away from mature aspen stands to prevent root suckering from established clones within the plot. Individual plot locations were chosen to maximize the homogeneity of slope, aspect, and existing vegetative cover (primarily forbs and shrubs at Site A; grasses at Sites B and C) within plots and among plots at the same site.

Prior to planting, 2.5 m tall wildlife exclosures were constructed around each plot. I reduced existing vegetation within the exclosures using herbicide (Glyphosate, Roundup, Monsanto, St. Louis, MO, USA), first applied during the summer with a second application a month before outplanting in the fall of 2015. Thatch was removed following herbicide treatment using controlled burns performed by the Utah Division of

Forestry, Fire & State Lands immediately prior to outplanting. Following outplanting, new vegetation growing within 10 cm of aspen seedlings was hand-weeded at the beginning and end of both growing seasons during the study. Dominant vegetation that returned to the plots varied by site (Site A: showy goldeneye (*Heliomeris multiflora* Nutt.); Site B: Idaho fescue (*Festuca idahoensis* Elmer); Site C: Sandberg bluegrass (*Poa secunda* J. Presl) and hairy false goldenaster (*Heterotheca villosa* Pursh)). Due to the presence of Botta's pocket gopher (*Thomomys bottae*), identified by characteristic soil mounding in and near the plots, I conducted weekly trapping where fresh sign was encountered during the summer and fall preceding outplanting. Despite this initial effort, substantial pocket gopher herbivory of aspen seedlings occurred in several plots during the winter following outplanting (see Results), necessitating continued trapping and the use of buried poison gopher bait (Moletox® Mole & Gopher Killer, Bonide Products Inc., 6301 Sutliff Rd., Oriskany, NY, 13424) through the next two growing seasons (2016-17).

In October of 2015, aspen seedlings were outplanted in a grid with 1 m × 1 m spacing following a randomized complete block design. Eight blocks were planted per plot, with each block containing 20 seedlings of each source (Alberta, Utah, and New Mexico) planted in parallel rows. A total of 480 seedlings per plot and 7,200 seedlings across all 15 plots were planted in this manner. A 2 m buffer around the periphery of each plot was also planted with an additional row of aspen seedlings to reduce possible edge effects.

Plot environmental measurements

In order to examine how differences in abiotic factors at the plot-level could have influenced seedling survival and growth, edaphic conditions were monitored or measured during the study, including soil temperature, water content, texture, pH, salinity, P and K) concentrations (Table 3.3). Soil temperature was sampled hourly over the course of the experiment using HOBO 64K Pendant[®] Temperature Data Loggers (Onset Computer Corporation, 470 MacArthur Blvd., Bourne, MA 02532) buried 5 cm beneath the soil surface at the center of each plot. After observing substantial aspen seedling mortality to apparent drought conditions (see Results), I decided it would also be important to quantify soil moisture within each plot. I estimated soil water content using time domain reflectometry (TDR) at 30 cm depth with a TRIME-FM3 with Tube Probe T3 (IMKO Micromodultechnik GmbH, Am Reutgraben 2, 76275 Ettlingen / DE). These measurements were made weekly during the second summer of the study, from June through August 2017, using an installed PVC access tube with a sealed cap at the center of each plot. I assessed soil properties by using bulked soil samples taken from the top 20 cm of the soil profile at three locations within each plot. Soil particle size distribution was measured using particle fractionation with a hydrometer and graduated cylinder (Day 1965). Salinity and pH were quantified using a saturated soil paste (Rhoades 1982). Available P and K were estimated using an Olsen sodium bicarbonate extract followed by atomic absorption spectrometry to estimate K and an ascorbic acid/molybdate reagent and colorimeter to estimate P (Olesen and Sommers 1982).

Table 3.3 Soil characteristics at each of the 15 plots used for aspen seedling outplanting on Cedar Mountain, Utah, USA

Site	Plot	Avg. Soil Temp. (°C)	(%)				pH	Salinity (dS/m)	(mg/kg)		Average Water Content (%)			Average Plant Available Water (%)		
			Sand	Silt	Clay	WCT			P	K	Early	Late	Whole Summer	Early	Late	Whole Summer
A	A1	18.5	39	44	17	11.7	6.6	0.64	30	538	14.3	18.6	16.3	2.6	6.9	4.6
	A2	18.1	28	51	21	12.8	6.3	0.59	33	369	14.6	32.8	22.9	1.8	20.0	10.1
	A3	19.1	30	47	23	13.7	6.5	0.55	38	480	9.3	12.6	10.8	-4.4	-1.1	-2.9
	A4	19.2	30	47	23	13.7	6.4	0.25	33	456	14.8	21.3	17.8	1.1	7.6	4.1
	A5	19.7	27	48	25	14.5	6.1	0.23	40	380	13.6	17.8	15.5	-0.9	3.3	1.0
B	B1	17.2	23	43	34	19.0	6.0	0.19	9.3	205	24.8	24.7	24.7	5.8	5.7	5.7
	B2	17.8	23	39	39	21.8	6.0	0.21	14	312	17.4	19.6	18.4	-4.4	-2.2	-3.4
	B3	15.5	16	42	42	23.8	6.1	0.24	14	254	29.2	31.3	30.1	5.4	7.5	6.3
	B4	17.7	21	40	39	21.9	5.8	0.17	7.1	180	18.9	20.2	19.5	-3.0	-1.7	-2.4
	B5	17.4	16	41	43	24.4	5.9	0.18	6.5	192	21.0	29.8	25.0	-3.4	5.4	0.6
C	C1	17.4	11	28	61	36.2	7.5	0.4	27	899	39.4	39.5	39.4	3.2	3.3	3.2
	C2	18.7	21	40	39	21.9	6.1	0.21	17	352	25.7	38.5	31.5	3.8	16.6	9.6
	C3	18.3	14	37	49	28.3	7.5	0.45	16	221	41.4	45.3	43.2	13.1	17.0	14.9
	C4	16.9	16	41	43	24.4	6.5	0.35	44	487	23.7	26.6	25.0	-0.7	2.2	0.6
	C5	14.4	16	40	44	25.0	7.3	0.82	20	379	54.7	32.9	44.8	29.7	7.9	19.8

Avg. Soil Temp = June to September, 5 cm depth

WCT = Water Content Threshold (Volumetric water content at a soil matric potential of -1,500 kPa)

P = Available Phosphorus

K = Available Potassium

Early = June 17 – July 22, 2017

Late = July 30 – August 26, 2017

Soil particle size distributions and water content measurements were then used to calculate a metric of average plant available water (APAW) for each plot. This metric offers an estimate of how much the volumetric water content of the soil in a given plot was above or below the permanent wilting point for the first half of the summer, on average, adjusting for the soil texture of the plot. To estimate average plant available water, the water content that corresponds to a soil matric potential of -1500 kPa (the permanent wilting point) was first calculated for each plot based on its soil texture using the equation

$$\Psi = A\Theta^B \quad (1)$$

where Ψ = water potential in kiloPascals (kPa), $A = \exp[a + b(\% \text{ clay}) + c(\% \text{ sand})^2 + d(\% \text{ sand})^2(\% \text{ clay})] \times 100$, $B = e + f(\% \text{ clay})^2 + g(\% \text{ sand})^2 + g(\% \text{ sand})^2(\% \text{ clay})$, and Θ = volumetric soil water content (m^3 / m^3), $a = -4.396$, $b = -0.0715$, $c = -4.880 \times 10^{-4}$, $d = -4.285 \times 10^{-5}$, $e = -3.140$, $f = -2.22 \times 10^{-3}$ and $g = -3.484 \times 10^{-5}$ (Saxton et al. 1986).

Instead of solving for Ψ , however, -1500 was used as a constant for the water potential and Θ was calculated. This produced an estimate of the water content threshold (WCT), or the volumetric soil water content at which the soil in a given plot would be at the permanent wilting point based on its sand and clay content (Table 3.3). Next, the average water content of each plot was estimated by calculating the mean of the weekly TDR measures of volumetric soil water content throughout the summer (Table 3.3). Because the early summer tends to be much drier in this region until monsoonal precipitation typically begins in late July to early August, estimates of average water content in each plot were further divided into the early summer (June 17 – July 22) and the late summer

(July 30 – August 26) (Table 3). Finally, the WCT was subtracted from the average water content of each plot to arrive at an estimate of APAW (Table 3.3).

Temperature and precipitation on Cedar Mountain was estimated for the three months of the growing season (June-August) during 2016 and 2017 from PRISM climate data analysis (PRISM Climate Group) (Table 3.4). Overall, precipitation during the 2016 growing season was well below the 30-year average (81.2 mm vs. 119.3 mm), with only 4.1 mm of precipitation during June of 2016. While there was no precipitation recorded in June 2017, heavy monsoonal rains during late July and through August of 2017 accrued 154.8 mm of precipitation, well above the 30-year average of 97 mm for these two months.

Table 3.4 Precipitation and air temperature on Cedar Mountain, UT by month during the growing season (June-August) during both years of the study, 2016 and 2017 as well as 30-year averages for each month (PRISM Climate Group)

	June			July			August		
	30-year Ave.	2016	2017	30-year Ave.	2016	2017	30-year Ave.	2016	2017
Precipitation (mm)	22.3	4.1	0	42.7	28.1	77.2	54.3	49	77.6
Mean Temp (°C)	12.3	15.1	14.9	16	16.6	16.8	15	14.7	15.2

Seedling survival and growth measurements

Field performance of the aspen seedlings outplanted on Cedar Mountain was evaluated over two growing seasons following outplanting (2016-17). Each spring, prior to full leaf flush of the aspen seedlings (late May), I measured the RCD and height of all seedlings along with any stem dieback. All seedling mortality was recorded and a likely

cause was attributed. I assigned herbivory as the cause of mortality when there was evidence of adjacent pocket gopher activity (i.e. excavated soil mounds, tunnels, and winter soil casts), cleanly bitten stems at or below ground level, or holes where the seedling was planted. I assigned drought stress as the cause of mortality when seedlings appeared to have died due to insufficient soil moisture (i.e. signs of stem brittleness and darkening, and/or dry, discolored leaves or leaf-margins with no obvious stem damage or soil disturbance nearby). Seedlings that displayed drought stress symptoms were checked for obvious root herbivory by pulling the stem upward and assessing the resistance from the root system. Those that remained firmly anchored in the soil were recorded as drought stress mortality, while those that pulled out easily with a missing root system were assigned herbivory mortality. Finally, seedlings that displayed no clear mortality cause or could not be found were recorded as unknown.

While this basic assessment of seedling mortality cause was the only method logistically feasible, it is important to acknowledge two potential caveats it presents. First, it is possible that the cause of mortality in some seedlings was due to a combination of drought stress and herbivory. However, this would not have been discernable and I assume that these would have been a small proportion of the overall seedling mortalities. Second, because the mortality causes were considered mutually exclusive, it is possible that in the absence of herbivory, drought stress would have caused mortality for a given seedling, and vice versa.

I took a second round of measurements on all seedlings at the end of the first summer (late August 2016). In addition to the height and RCD, I measured the longest

shoot produced during that growing season at this time, based on the length of new stem tissue from the previous year's bud scar to the base of the stem's terminal bud. Finally, all new mortality was recorded and assigned a cause as described above. I repeated the same measurements of surviving seedling RCD, height, and longest shoot length along with an assessment of new mortality at the beginning and end of the growing season in 2017.

Mulching experiment

After observing nearly complete loss of the aspen planted at Site A during the first growing season (see Results), I conducted a follow-up experiment that explored the use of wood mulch as a ground cover to reduce evaporation from the soil. I grew new aspen seedlings during the summer of 2016 from the NM source in Ray Leach Cone-tainer™ SC10 cells (164 mL volume) (Stuewe and Sons, Inc., Tangent, OR, USA). In November 2016, I prepared mulch from dead aspen branches sourced from a stand near the experimental enclosure using a mechanical wood chipper.

Exclosure A5 was selected for the experiment because all original seedlings were lost during the first growing season, with the majority of mortality due to drought stress (Fig. 3.1). Sixteen paired plots were distributed evenly throughout the enclosure, consisting of a mulched plot next to a non-mulched plot. The mulched plots were covered with aspen wood mulch spread approximately 10 cm deep in a 2.5 m x 2.5 m square. Each non-mulched plot was located 2 m away from the paired mulched plot. I planted half of the aspen seedlings under the mulched plots with 50 cm x 50 cm spacing and

planted the other half in the same pattern, but without any ground cover in the non-mulched plot (16 seedlings per plot, 256 seedlings in total). In June 2017, I installed two PVC soil access tubes, one in a randomly selected mulched plot and one in the adjacent non-mulched plot, in order to estimate differences in APAW between the treatments across the growing season. I recorded individual seedling survival and growth at the end of the summer (August, 2017).

Analyses

Differences in seedling stock characteristics among the seed sources were analyzed using a one-way analysis of variance (ANOVA), followed by a post-hoc Tukey's test to evaluate pairwise comparisons between specific sources for statistical significance. Statistical significance was determined at $\alpha = 0.05$ and all ANOVAs were conducted using R v3.4.1 (R Core Team 2017) with packages "multcomp" (Hothorn et al. 2008) and "lsmeans" (Lenth 2016).

To evaluate field growth differences among the three seedling sources, I averaged seedling traits by source across all living seedlings in each block at plots C3 and C5, where adequate survival (46% and 73%, respectively) permitted analysis (see Results). Differences between sources were then analyzed using a one-way ANOVA and post-hoc Tukey's test as described above.

I examined predictors of aspen seedling survival (live/dead) in the field after two growing seasons using generalized linear mixed models (GLMM) with a binomial distribution and a logit link in R v3.4.1 using package lme4 (Bates et al. 2015). Predictors

were tested individually as fixed effects factors because the small number of replicates ($n = 15$) made testing multiple predictors simultaneously in a model inappropriate. I first tested seedling source and found no evidence of a significant effect on seedling survival, so I excluded source as a fixed effect factor in subsequent analyses. Additional predictors tested were initial seedling size at outplanting (height and RCD), and plot-level environmental variables (Table 3.3). An observation-level random intercept was used to accommodate potential overdispersion due to clustering of seedlings as grouped data. To assess the factors contributing specifically to drought-stress mortality, I excluded all seedling mortality attributed to small mammals or of unknown cause from all analyses. When the same models were run with drought-stress related seedling mortality excluded instead, none of the above factors showed a significant correlation with seedling survival, suggesting that small mammal herbivory was likely indiscriminate. I ran models with initial seedling RCD and height as predictors of seedling survival on a reduced dataset from plots C3 and C5 because these were the only plots with adequate survival as described above. Random intercepts were used with plot and source nested within plot as random effects. Site was not used as a random effect because both plots were located at the same site. All models were assessed for significance using a likelihood ratio test.

Analysis of the mulching experiment was also conducted with a binomial GLMM, using mulching treatment as a fixed effects factor. Random intercepts were used, with plot as the random effect. Because model convergence was not possible when run on the full dataset due to zero survival of seedlings in the non-mulched treatment, an intercept-only GLMM was applied to predict seedling survival for seedlings in the mulched

treatment only. This yielded an estimate of average survival across all eight mulched plots.

Results

Seedling growth under controlled conditions

Aspen seedlings from each source that were grown under controlled conditions in a shadehouse to assess seedling quality showed substantial growth in both above and belowground characteristics during the second growing season. Seedlings grew in height by an average of 77 cm, 69 cm, and 119 cm, and increased their RCD by 7.5 mm, 9.3 mm, and 12.3 mm in the Alberta, Utah, and New Mexico sources, respectively, with New Mexico seedlings displaying the greatest growth in both metrics (height, $F_{2,93} = 35.24$, $P < 0.001$; RCD, $F_{2,93} = 28.36$, $P < 0.001$) (Table 3.5). Belowground, average root dry weight in New Mexico seedlings also increased the most (78 g) compared to Alberta and Utah (55 g and 67 g, respectively), though this difference was not significant ($F_{2,93} = 2.82$, $P = 0.065$). Overall, this growth resulted in a more balanced RSR between seed sources, though these ratios still differed significantly ($F_{2,93} = 17.38$, $P < 0.001$). When comparing total dry weights between sources, New Mexico seedlings were also significantly larger than the other two sources ($F_{2,93} = 12.05$, $P < 0.001$) with an average weight of 145 g compared to 82 g and 108 g (Alberta and Utah, respectively). Only three seedlings were lost from this group in total during the growing season (two Utah and one New Mexico), which were culled due to spider mite infestation.

Table 3.5 Average (standard deviation) of aspen seedling morphological characteristics for each seed source after two growing seasons (2016) with regular watering and fertilization in a shadehouse

Seed Source	Final Height (cm)	Height Gain (cm)	Final RCD (mm)	RCD Gain (mm)	Total DW (g)	Stem DW (g)	Root DW (g)	Root DW Gain (g)	RSR
Alberta	89 a (28)	77 a (28)	10.5 a (2.5)	7.5 a (2.6)	82 a (61)	25 a (14)	57 a (48)	55 a (48)	2.3 a (1.0)
Utah	104 b (25)	69 a (25)	13.9 b (2.2)	9.3 b (2.3)	108 a (51)	39 b (15)	69 a (39)	67 a (39)	1.8 b (0.7)
New Mexico	148 c (22)	119 b (25)	16.5 b (2.6)	12.3 c (2.8)	145 b (44)	65 c (21)	80 a (27)	78 a (27)	1.3 c (0.3)

Different letters connote significant differences ($\alpha = 0.05$) between seed sources ($n = 33$ for Alberta, $n = 31$ for Utah, and $n = 32$ for New Mexico)

DW = Dry Weight

Root DW Gain = Final Root DW – Initial seed source average nursery Root DW (Table 3.1).

RSR = Root:Stem Ratio

Field performance

Overall survival (across all three sites) of the 7,200 aspen seedlings during the two growing seasons following outplanting was only 10%. Drought stress was likely responsible for mortality of 50% of the seedlings based on seedling physical appearance (see Methods), while small mammal herbivory accounted for 38% (Table 3.6 and Fig. 3.1). Three percent of seedlings could not be assigned a cause of mortality. Site A lost almost all seedlings (99%) during the first year post-outplanting, with over 31% lost to pocket gopher herbivory during the first winter under the snowpack, increasing to a total of 61% mortality due to small mammal herbivory by the end of the summer. Of the remaining seedlings, 38% were lost to drought stress by the end of the first summer and the remaining 1% were lost to drought stress during the second summer. Site B also

experienced heavy small mammal herbivory, with pocket gopher mortality during the first winter accounting for 18% of seedlings lost and increasing to 39% of seedlings lost to small mammals by the end of the second year. Loss to drought stress accounted for 37% of mortality the first year and increased to 56% by the end of the second year, leaving only 3% survival of all seedlings outplanted at Site B by the end of the study (Table 3.6 and Fig. 3.1). Small mammal herbivory was the least at Site C, accounting for 15% of seedling mortality by the end of the study. However, 52% of all seedlings outplanted at Site C were lost to drought stress, reducing the final survival rate to 27%.

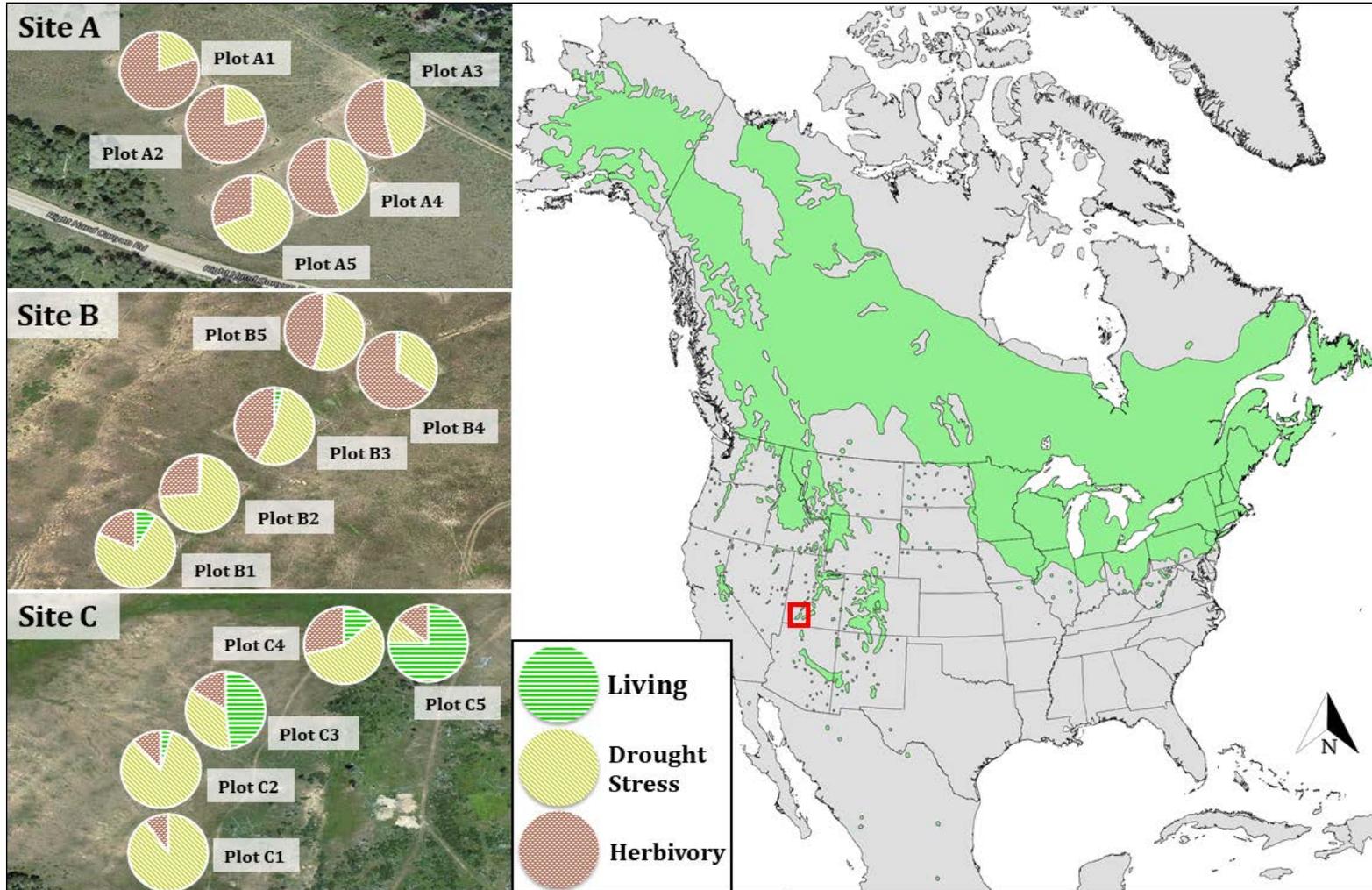


Fig. 3.1 Cumulative proportion of planted aspen seedlings living compared to dead due to either drought stress or herbivory within each plot across three study sites at the end of two growing seasons (480 total seedlings per plot). The distribution of aspen is shown in green (Little 1971), and the study location in southern Utah, USA is highlighted with a red box

Table 3.6 Final counts of seedling survival and mortality by plot. A total of 480 seedlings were planted at each plot

Mortality Cause	Plot														
	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	C1	C2	C3	C4	C5
Living	0	0	0	0	0	41	5	16	9	2	0	20	219	62	349
Drought Stress	96	104	220	214	333	335	347	257	149	252	422	367	158	260	46
Herbivory	384	376	256	266	147	85	126	202	302	214	46	53	73	129	68
Unknown	0	0	4	0	0	19	2	5	20	12	12	40	30	29	17
Survival	0%	0%	0%	0%	0%	9%	1%	3%	2%	0%	0%	4%	46%	13%	73%

While survival was poor in aggregate, 79% of all seedlings that did survive the two growing seasons during the study came from just two plots (C3 and C5, with 46% and 73% plot seedling survival respectively) (Table 3.6). Final aboveground seedling size for living seedlings at plots C3 and C5 differed significantly by source (RCD, $F_{2,45} = 21.77$, $P < 0.001$; height, $F_{2,45} = 35.28$, $P < 0.001$), with New Mexico the largest on average (7.0 mm RCD and 41.3 cm height), Utah in the middle (6.4 mm RCD and 33.9 cm height) and Alberta the smallest (5.2 mm RCD and 26.3 cm height) (Table 3.7). This pattern of stem size among the sources paralleled nursery observations (Table 3.1). While final stem size did vary, height, RCD, and shoot growth did not differ significantly among sources (height, $F_{2,45} = 1.99$, $P = 0.149$; RCD, $F_{2,45} = 2.47$, $P = 0.096$; LSG 2016, $F_{2,45} = 2.01$, $P = 0.146$; LSG 2017, $F_{2,45} = 0.91$, $P = 0.407$) (Table 3.7).

Table 3.7 Average (standard deviation) of final size and growth characteristics for surviving seedlings at Plots C3 and C5 after two growing seasons

Seed Source	Initial Height (cm)	Final Height (cm)	Height Growth (cm)	Initial RCD (mm)	Final RCD (mm)	RCD Growth (cm)	LSG 2016 (cm)	LSG 2017 (cm)
Alberta	12.1 a (2.0)	26.3 a (5.0)	14.1 a (5.5)	2.7 a (0.2)	5.2 a (0.6)	2.7 a (0.7)	15.8 a (4.5)	14.6 a (4.3)
Utah	22.5 b (3.4)	33.9 b (5.1)	11.4 a (4.4)	3.5 b (0.3)	6.4 b (0.8)	3.0 a (0.8)	16.7 a (5.4)	13.9 a (3.4)
New Mexico	30.5 c (4.2)	41.3 c (5.2)	10.8 a (5.2)	4.0 c (0.3)	7.0 c (0.6)	3.3 a (0.7)	20.0 a (5.4)	15.4 a (4.2)

Different letters connote significant differences ($\alpha = 0.05$) between seed sources ($n = 16$ for all variables)

LSG = Longest Shoot Growth

Seedling survival relationships

Of the edaphic factors measured (Table 3.3), only early summer average soil water content ($P = 0.031$, $\beta = 0.190$, $se = 0.072$), and early summer average plant available water (APAW) ($P = 0.006$, $\beta = 0.200$, $se = 0.060$) significantly correlated with seedling survival probability using a binomial GLMM. Fig. 3.2 is a visual representation of the model using APAW as the predictor, and illustrates that the probability of seedling survival increases with increasing APAW. The relationship of average water content to seedling survival was nearly identical.

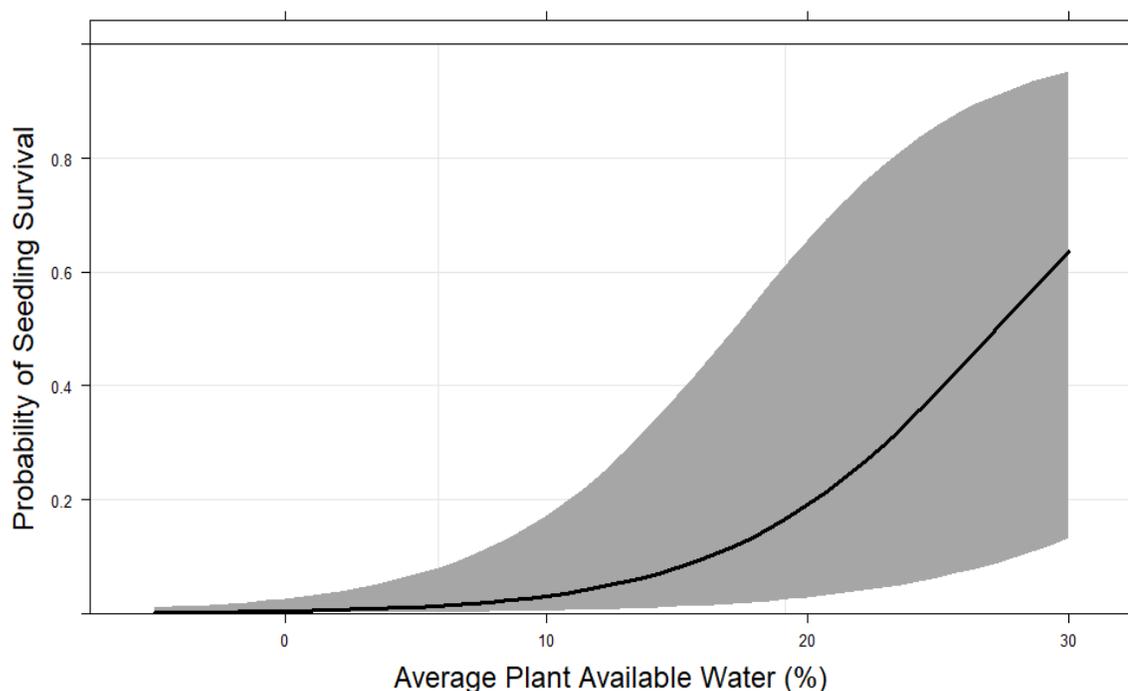


Fig. 3.2 Binomial GLMM model visualization of the plot-level relationship between early summer (June 17 – July 22) average plant available water (APAW) in 2017 to the proportion of surviving aspen seedlings after two growing seasons. The black line represents the model predicted values and the gray area represents standard error

At plots C3 and C5, where adequate seedling survival allowed for assessment, initial seedling height at the time of outplanting had a significant negative correlation with seedling survival probability for seedlings that were lost to drought stress using a binary GLMM ($P = 0.015$, $\beta = -0.030$, $se = 0.011$). A visualization of the model is depicted in Fig. 3.3. Initial seedling RCD, however, was not significantly correlated to seedling survival ($P = 0.776$, $\beta = 0.037$, $se = 0.131$). Seedling survival to drought stress also did not differ significantly by seedling source at these two plots ($P = 0.208$).

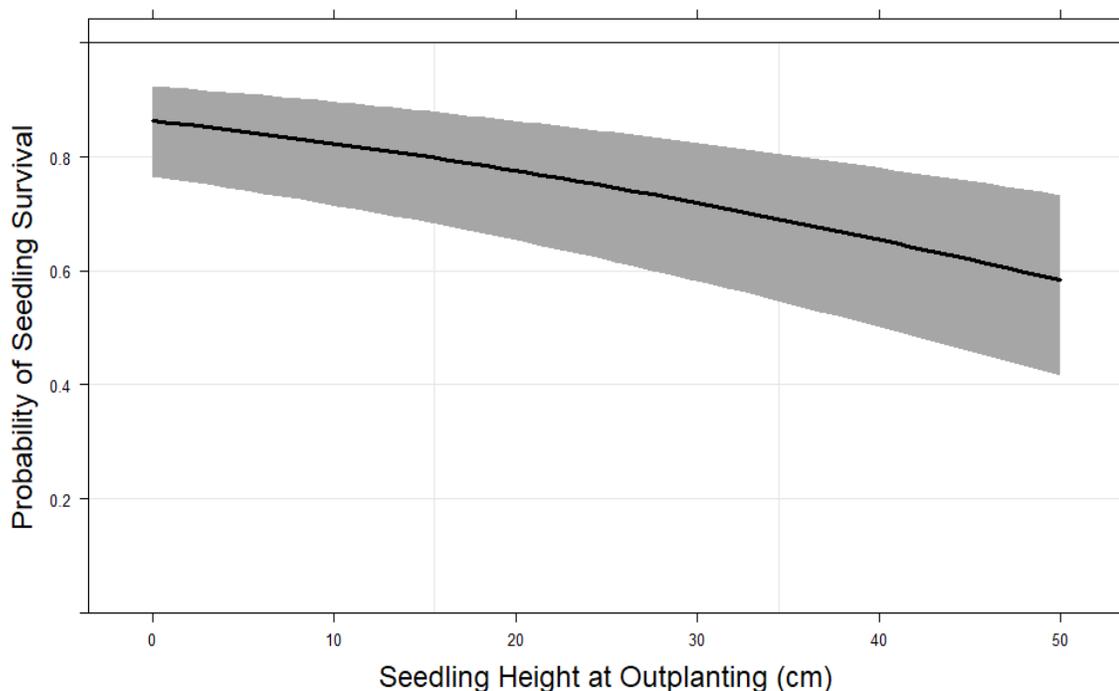


Fig. 3.3 Binary GLMM model visualization of the relationship between initial seedling height at the time of outplanting (October, 2015) and the probability that seedling survived to the end of the study (August, 2017) at plots C3 and C5. The black line represents the model predicted values and the gray area represents standard error

Mulching experiment

Results from the experiment using chipped aspen as mulch also indicate that APAW is an important factor impacting aspen seedling survival. Sixty-two percent (se = 8%) of the 128 mulched seedlings survived following their first summer compared to zero percent survival of the non-mulched seedlings. The mulching treatment substantially boosted soil moisture retention, with early summer APAW estimated at 14.8% in the monitored mulched block, compared to -0.9% in the non-mulched block just 2 m away.

Discussion

With only 10% survival of the 7,200 aspen seedlings initially planted, this study clearly demonstrates several significant barriers that will need to be addressed before seedling-based aspen restoration can be effectively implemented in the IW, particularly in drought-prone areas. The largest cause of mortality in my study appeared to be drought stress, accounting for the loss of nearly 50% of all seedlings. Seedling drought stress mortality (inferred from symptoms) was strongly correlated with low early summer APAW across many of the experimental plots (Fig. 3.2). Monsoonal precipitation patterns during the summer are characteristic of the study region, with dry early summers persisting until more regular rains begin in late July or August. During the first growing season in 2016, snowmelt occurred across the plots between mid-April to mid-May, which was followed by a dry and warm June and July during which precipitation was only 50% of the 30-year normal and the average temperature was 1.7°C above the 30-year normal (Table 3.4). These climatic conditions likely accelerated evaporation of soil

moisture during a critical period of initial seedling root expansion. Perhaps most suggestive of containerized aspen's requirement for consistent and adequate soil moisture following outplanting is the anomalous plot C5, where 73% of the seedlings survived to the end of the second growing season (48% of all seedling survival in the study). Plot C5 was inadvertently located just below an east-facing cliff that collected snowdrifts during the winter, creating a deep snowpack that persisted into late June during both years of the study. The extended melting of this snowpack ensured that most of the soil in Plot C5 remained fully saturated through the first half of summer until monsoonal precipitation typically began. The increased seedling survival at Plot C5 therefore suggests that designing site selection protocols to maximize growing season soil moisture retention could prove an effective method for increasing restoration success when working in drought-prone regions.

A lack of physical shade (e.g. logs or berms) within the plots likely contributed to the widespread drought stress seedlings experienced by increasing the rate of soil moisture loss. Partial evidence for this comes from the mulching trial conducted during the second growing season in which 62% of mulched aspen seedlings survived compared to 0% survival for seedlings planted without mulch. In addition to reducing evaporation and lowering soil temperatures, the mulch also visibly suppressed annual weeds, likely reducing both light and water competition for the aspen seedlings. Evidence from natural aspen seedling establishment in a similar IW environment suggests that coarse woody debris (CWD) may serve a similar role, acting as important shelter for aspen seedlings by ameliorating harsh microsite conditions, particularly during severe droughts (Fairweather

et al. 2014). Utilizing ground shelter to enhance aspen seedling survival could easily be accomplished in many restoration contexts in which CWD is present, such as after stand thinning treatments, controlled burns, or wildfires. Microsite topography could also play an important role in seedling survival by enhancing moisture collection and retention. For example, natural aspen seedling regeneration in the Canadian Rockies was most common in concave microsites (Landhäusser et al. 2010), while a similar preference was found at a mine reclamation site near Edmonton, Canada (Schott et al. 2014). Another study in the boreal mixedwood of northeastern Alberta, Canada, found that greater soil surface roughness was correlated with increased natural establishment of aspen seedlings at an oil sands mine reclamation site (Pinno and Errington 2015). Targeted planting in natural concave topographical features or artificially increasing soil surface heterogeneity through site preparation techniques like mechanical scarification could enhance survival for planted aspen seedlings.

Small mammal herbivory was the second most significant cause of seedling mortality, accounting for the loss of 38% of all seedlings. The vast majority of this herbivory is likely attributable to pocket gophers. Further, forty-nine percent of all herbivory mortality occurred during the winter, when only pocket gophers could physically access the seedlings beneath the snowpack. The remaining 51% of herbivory occurred during the two growing seasons of the study. In these cases, I almost always observed fresh sign of belowground pocket gopher activity (i.e. earth mounds from tunnel excavation) in the immediate vicinity. Past studies of the effect of pocket gopher herbivory on the suckering regeneration of established aspen clones in the IW have

produced mixed results. (Cantor and Whitham 1989) found a substantial increase in aspen vegetative sucker survival, recruitment, and growth rate when pocket gophers were removed from plots in northern Arizona, concluding that pocket gophers may act as a keystone species by limiting aspen to rocky outcrop refugia and maintaining deep soil mountain habitat as open meadows. However, Coggins and Conover (2005) performed a similar experiment in the same area as this study and found no effect of pocket gopher removal on aspen sucker regeneration. While the impact of pocket gophers on established aspen clone regeneration remains unclear, their extensive herbivory in this study, despite sustained removal efforts, strongly suggests that these fossorial rodents are a significant threat to aspen seedling establishment. However, it is important to note that the use of enclosures to prevent access by large herbivores may have increased small mammal utilization within the plots due to predator exclusion (Shepperd and Mata 2005). Nevertheless, quantifying local pocket gopher abundance through surveys of soil mounding and winter casting may provide a useful metric for assessing the relative suitability of a site for seedling-based aspen restoration. At high-priority sites where the cost could be justified (e.g. wildland-urban interface sites, campsites), protecting individual seedlings by wrapping the root system with a biodegradable mesh at the time of outplanting may offer a solution to aid establishment success (Baierlein 2015). Planting at lower densities and over larger areas, or in extensive recently burned areas, could also help reduce the intensity of pocket gopher herbivory.

None of the three aspen seedling sources (Alberta, Utah and New Mexico) had a clear advantage during establishment, despite the markedly different traits they developed

in the nursery (Tables 3.1 and 3.2). At plots C3 and C5, where seedling survival was greatest, all three sources displayed nearly identical survival and growth rates after two growing seasons. While conditions at the outplanting locations appeared to be too limiting to reveal adaptive differences at this early stage, it is likely that phenological and genetic differences among sources would result in differential survival and growth over the long term, making the selection of appropriate seed sources for a given site an important consideration. The similar performance among all three sources suggests that the primary factor limiting seedling survival in this study was the environment, not the seed sources themselves. This is supported by the near complete survival and robust growth rates that all three sources displayed when allowed to grow in non-limiting conditions (Table 3.5). Across seed sources, however, there was one metric that did appear to correlate with seedling survival: seedling height. Taller seedlings at the time of outplanting had reduced survival in plots C3 and C5 when looking only at seedlings that were lost due to drought stress (Fig. 3.3). This may be due to a root system that is inadequate for supporting the increased transpiration of a tall seedling with many leaves when conditions are stressful. Indeed, a high root-to-shoot ratio in aspen seedling stock has been shown to correlate with increased field performance (Martens et al. 2007; Landhäusser et al. 2012a). While developing the appropriate nursery protocols to maximize root-to-shoot ratio in aspen seedling stock should be an important goal (Chapter 1), top-pruning of seedlings to a standardized height prior to outplanting could be an interim method for improving survival of particularly tall seedlings in marginal environments (South 1996).

Seedling-based aspen restoration offers some notable benefits over traditional silvicultural techniques for managing aspen. Planting aspen seedlings increases the genetic diversity of aspen stands, improving the resilience of aspen populations to environmental stressors. This is true even with the limited seedling survival (10%) achieved in this study, which equates to 723 new aspen genotypes. This strategy also enables assisted migration, permitting the movement of aspen into habitat projected to be suitable under climate change, or for the sourcing of aspen genotypes adapted to warmer or dryer climates for a desired restoration site. Seedling-based aspen restoration could also be used to achieve broader management objectives. For example, planting aspen in stands with limited seedling or suckering recruitment could maintain forest cover and prevent conversion to non-forest. While natural aspen seedling establishment may become more common under increasingly frequent disturbance regimes in some areas of the IW (Gill et al. 2017), supplemental planting with aspen seedlings could provide greater control and increase the likelihood of adequate seedling establishment. Utilizing aspen seedlings for reforestation after stand replacing fires or fuels treatments may also be an important approach for breaking up forest fuel connectivity across the landscape and for creating defensible space around structures or campsites in the wildland-urban interface (Fechner and Barrows 1976; Fisher 1986).

It is also important to consider that unlike conifers, the ability of aspen to reproduce vegetatively means that even limited seedling survival can lead to long-term restoration success if seedlings sucker adequately. In the IW, clones originating from single seeds can spread to cover many ha (Mock et al. 2008). However, suckering ability

in aspen is highly variable among naturally established clones (Schier 1974) and from planted aspen seedlings (King and Landhäusser 2017), suggesting that initial establishment of varied genotypes is an important restoration target. Despite the significant barriers encountered that limited seedling establishment in this study, reasonable survival did occur at two of the fifteen plots. This suggests that with future research aimed at developing appropriate site selection and outplanting protocols, seedling-based aspen restoration could be a viable and valuable management tool in the IW.

Conclusions and Recommendations

Seedlot selection

In silviculture, ensuring the adaptation of seedling stock for a chosen planting site using seed transfer guidelines and seed zones is an established approach to seedlot selection (Ying and Yanchuk 2006; Morgenstern 2011). Development of appropriate seed transfer guidelines is often aided by the use of provenance trials for individual species, and while some trials have been conducted for aspen in western Canada (Gray et al. 2011; Hamann et al. 2011), there has been no such work done in the IW. This is problematic for widespread implementation of seedling-based aspen restoration in the IW, as a lack of provenance data is compounded by the topographic complexity of the montane habitats that define the range of aspen in the region. Additionally, high rates of triploid aspen clones in the IW have been documented (Mock et al. 2012), which could complicate seed collection efforts due to clone sterility.

The results of the nursery phase of this study suggested significant differences among aspen seedlings sourced from different parts of western North America, but mortality in the field phase was too pronounced to assess the degree of regional adaptation. However, future studies contributing to an improved understanding of the spatial scale of local adaptation in aspen will be important for optimizing seedlot selection in the IW. This information will be particularly helpful for improvement of web-based resources for matching seedlots with outplanting environments in the context of climate change (for a list see Williams and Dumroese 2013b).

Site selection

The seedling survival results from this study indicate that appropriate site selection is one of the most important factors governing successful aspen seedling establishment in the IW. While the topographic complexity of IW landscapes makes appropriate site and seedlot selection more challenging, restoration efforts can take advantage this complexity by considering small-scale areas of suitable habitat which are likely to persist as climates change (Rehfeldt et al. 2015). However, it is important to recognize that restoration efforts may be futile at sites where local climate increasingly departs from the fundamental niche of aspen. Local herbivory pressure by ungulates (Britton et al. 2016), rodents, and lagomorphs should also be taken into consideration when determining the restoration potential of a site.

The development of tools to identify and predict both suitable and unsuitable sites for aspen restoration efforts will be increasingly important as restoration needs increase.

Given the significance of adequate soil moisture for seedling survival in this study, utilizing a modeling approach to calculate average soil water balances across the landscape at fine spatial scales with topographic drivers offers a potential solution (Dilts et al. 2015; Rehfeldt et al. 2015). Additional site selection approaches could include: 1) using winter aerial imagery to predict areas where snow accumulation and drainage could increase local soil moisture, 2) planting aspen after large wildfires where herbivory pressure as well as competition for light and water are reduced, and 3) targeting plantings to create defensible space, particularly around the wildland-urban interface, where additional cost and labor for watering and seedling protection can be justified.

Site preparation

One of the most dramatic results of this study was the significant increase in aspen seedling survival with mulching. This finding suggests that with appropriate site preparation, seedling survival could be significantly increased, at least during the particularly vulnerable period of initial establishment. Evidence that natural aspen seedling establishment in the IW is aided by CWD during extreme droughts (Fairweather et al. 2014) suggests that targeting seedling planting near these features could provide benefits similar to mulching. However, CWD and mulch may differ in their benefits for planted aspen and their utilization should be further explored. Other methods to enhance aspen survival and performance through microsite amelioration could include soil surface preparation techniques like mechanical scarification to increase surface roughness and water penetration, or tilling, which could additionally reduce competition from sod-

forming grasses. Testing the efficacy of these site preparation treatments will be an important step towards developing outplanting protocols that maximize the success of seedling-based aspen restoration in the IW.

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CHAPTER 4

SUMMARY

My thesis research was conducted to assess a seedling-based approach to aspen management in the U.S. Intermountain West (IW). Traditional management of aspen in the region has relied on the promotion of asexual regeneration, but these methods have important limitations which may be mitigated to some extent by supplemental planting of nursery-grown seedlings. In order to better understand the potential challenges to implementation of seedling-based aspen restoration, I first tested whether nursery protocols developed for growing quality boreal aspen seedlings would also be effective when applied to IW seed sources. I then planted the nursery-grown seedlings at three sites in southwestern Utah and monitored their growth, survival, and causes of mortality over the following two growing seasons. The results of my research suggest that there are several important barriers that will need to be addressed before seedling-based aspen restoration can be implemented more widely in the IW.

In the nursery, aspen seedlings from two IW sources differed substantially from the boreal aspen seedlings in their response to the nursery protocol developed for boreal seedlings. One feature of the boreal protocol was the use of a shoot growth inhibitor hormone treatment to induce early bud set and limit stem development. This allows for continued root growth and the accumulation of nutrient and carbohydrate reserves in the seedlings, leading to seedling traits that have been linked to greater outplanting success for aspen on boreal reclamation sites (Martens et al. 2007; Landhäusser et al. 2012a).

However, both IW sources displayed an inconsistent response to the application of this hormone treatment, which produced more variable seedling characteristics in the IW sources compared to the boreal aspen seedlings, which uniformly set bud. The boreal aspen in my study developed an average root-to-stem ratio nearly three times greater than the IW sources, while also possessing a significantly higher concentration of nitrogen, phosphorous, and non-structural carbohydrates. However, both IW seedling sources did develop root-to-shoot ratios greater than 2.0 and heights below 40 cm, both of which are traits previously associated with greater outplanting performance (Landhäusser et al. 2012a). The differences in final stock traits between the boreal and IW seedlings are not entirely surprising given the latitudinal distance between their origins, as well as the phylogeographic boundary that exists between IW populations and those elsewhere throughout aspen's range (Callahan et al. 2013). Interestingly, significant trait differences also developed between the two IW seedling sources despite their relatively close geographic proximity. This variability between IW sources suggests that nursery protocols may require more local adaptation.

In the field-based portion of my research, aspen seedlings planted at three sites in 15 plots in southwestern Utah experienced substantial mortality over the two growing seasons they were monitored. Of the 7,200 seedlings initially planted, only 10% survived until the end of the study. The two major causes of mortality appeared to be drought stress and herbivory from small mammals, based on field observations. Drought stress mortality symptoms were significantly correlated with decreased early summer plant available water measured in the soils of each plot. This is likely due to the monsoonal

precipitation patterns typical of southern Utah, where summers tend to be dry until regular rainfall begins in late July or early August. Nearly half of all the seedlings that survived through the study were in just one plot, which was located downslope from a topographic feature on the landscape that collected snowdrifts during the winter, creating a deep snowbank that persisted into late June in both growing seasons during the study. The slow-melting snowbank allowed the plot to remain saturated well into the mid-summer when most other plots became quite dry.

The other major source of seedling mortality was small mammal herbivory, which was likely attributable primarily to pocket gophers (*Thomomys bottae*) based on field observations. Even with sustained removal efforts throughout the study, the substantial loss of seedlings to herbivory suggests that small mammals, and in particular pocket gophers, can pose a significant threat to aspen seedling establishment in the IW. However, it is important to note that predator exclusion, caused by the use of fencing to prevent plot access by large herbivores, may have inadvertently increased utilization by small mammals (Shepperd and Mata 2005), potentially confounding the relative importance of this mortality source. Regardless of the effect of fencing on small mammal use, the results of this study clearly indicate that local herbivory pressure needs to be taken into account and mitigated to the extent possible when selecting a site for restoration.

Despite the significant differences in seedling traits among seed sources observed in the nursery phase of my study, none of the three seed sources possessed a clear advantage once outplanted. All of the seed sources displayed nearly equal growth and

survival rates in the two plots with the greatest seedling survival, suggesting that environmental conditions were simply too limiting for adaptive differences between the sources to manifest. Nevertheless, appropriate seedlot selection is likely to be an important step for successful aspen restoration in the IW, particularly given the significant genetic structuring by geographic distance among aspen populations in the region (Callahan et al. 2013). Assessment of seedlots at a regional scale could be greatly aided by provenance trials in order to develop effective seed transfer guidelines (Morgenstern 2011).

The seedling survival results from my study indicate that appropriate site selection is one of the most important factors for successful aspen seedling establishment. The significant differences in seedling survival between neighboring plots highlight the challenge that topographic complexity presents for effective site selection in IW landscapes, though restoration efforts could also take advantage of this fine spatial scale variance through careful selection of small-scale areas of suitable habitat. Development of tools necessary to predict site suitability across the landscape will be increasingly important as restoration needs increase. Based on the results of this and other studies, adequate soil moisture is one of the most significant drivers of seedling establishment in IW landscapes, suggesting that use of water balance models that account for fine-scaled topography may offer a valuable approach to site selection (Dilts et al. 2015; Rehfeldt et al. 2015).

Implementation of certain site preparation and planting techniques may also improve restoration outcomes. In a side experiment that tested the effects of a mulching

treatment on seedling establishment (Chapter 3), aspen seedlings that were planted under a layer of aspen wood mulch survived at 62% after one growing season, compared to zero percent survival of aspen planted without mulch. While not the same as mulch, planting seedlings near coarse woody debris (CWD) could also be a viable strategy with aspen restoration due to the ameliorating effects of physical shade on the seedling microsite, and natural aspen seedling establishment in the IW has been observed in close proximity to CWD during severe drought (Fairweather et al. 2014).

Based on the results of my thesis, additional research aimed at addressing the major challenges encountered in these studies will be necessary before seedling-based aspen restoration can be implemented more widely in the IW. Nursery protocols will need to be refined and tested on a wide variety of IW seedling sources, while outplanting practices for appropriate site selection and preparation will need to be developed. However, despite the significant barriers that limited seedling establishment, it is important to consider that the ability of aspen to reproduce vegetatively means even limited initial survival has the potential to lead to long-term restoration success. Indeed, clones in the IW originating from a single seed have spread to cover many hectares (Mock et al. 2008). While refinements of this approach to aspen restoration are clearly necessary, the potential benefits it provides to management of this important species in the IW could prove substantial, particularly in the context of a changing climate.

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