



Exploring seedling-based aspen (*Populus tremuloides*) restoration near range limits in the Intermountain West, USA



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ABSTRACT

With the effects of climate change expected to intensify over the coming century, land managers will require more proactive and novel approaches to conserve and restore threatened ecosystems. In the US Intermountain West, quaking aspen (*Populus tremuloides* Michx.) is a foundation species of considerable conservation interest. However, traditional silvicultural practices in the region focus exclusively on its vegetative regeneration potential, limiting restoration efforts to locations where aspen is already established, and precluding approaches such as afforestation and assisted migration. Planting nursery-grown aspen seedlings could overcome these limitations. This approach has become common for forest land reclamation and afforestation projects in the boreal forests of western North America, but has received little attention in the US Intermountain West to date. In this study we explored the potential for seedling-based aspen restoration in an Intermountain West landscape. We planted 7,200 nursery-grown aspen seedlings across 15 unirrigated plots at three high elevation (~2,800 m) sites in southwestern Utah, USA and monitored their growth and survival rates for two growing seasons. Overall seedling survival was only 10% by the end of the study, with 49% of mortality due to apparent drought stress, 38% due to small mammal herbivory, and 3% due to unknown causes. 79% of the surviving seedlings were located in just two plots, where higher levels of soil moisture were present during the driest summer months. A subsequent test of wood mulch to retain moisture in one of the plots increased seedling survival to 62%, compared to 0% in a non-mulched treatment. Together, these findings indicate that seedling-based aspen restoration in these habitats was limited primarily by site conditions. For this approach to become operational, additional study on seedling quality, site selection and site preparation will be necessary.

1. 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 U.S., 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 trembling or 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 western U.S., 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). Aspen has the broadest distribution of any tree species in North America (Little, 1971) and primarily reproduces vegetatively through root suckering, allowing rapid regeneration 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 regions of North America in recent years, particularly in the southern, drier edges of its range in the US Intermountain West (Worrall et al., 2013). These declines have been attributed to a variety of potentially interconnected stressors, including drought, pathogens,

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conifer encroachment, herbivory and land management practices (Frey et al., 2004; Worrall et al., 2008; Rogers and Mittanck, 2014). Additionally, aspen has been experiencing episodic 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; Worrall et al., 2015). Significant declines in aspen populations across the Intermountain West 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 Intermountain West have been noted, even in more xeric regions (Romme et al., 2005; Fairweather et al., 2014), but conditions for germination and establishment in the Intermountain West are limited by climate, fire suppression, and ungulate herbivory. Thus, the use of nursery-grown seedlings, a common approach in most other forest tree species, may present an unexplored opportunity for aspen restoration (Landhäusser et al., 2019).

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 seedling establishment. Second, vegetative 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 (Shepperd et al., 2001; Shepperd and Smith, 1993). Third, by managing exclusively for vegetative reproduction, the genetic diversity of aspen stands remains static or declines over time, limiting the adaptive evolution of aspen populations. Solely promoting vegetative reproduction may also favor triploid aspen, which are common in the Intermountain West (Mock et al., 2012). Triploid aspen may have a growth advantage over diploids, but they also appear to be more susceptible to hydraulic failure in drought conditions (DeRose et al., 2015; Dixon and DeWald, 2015; Greer et al., 2017) and are expected to have very low fertility.

In recent years, the need for effective forest reclamation practices on disturbed sites in the boreal regions 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, 2012b; Pinno et al., 2012; Schott et al., 2013). This work has focused primarily on producing quality 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. Manipulating site conditions through site preparation and soil or surface amendment are other options that may enhance outplanting success for aspen, though these remain relatively unexplored (Landhäusser et al., 2019).

Implementing a seedling-based approach to aspen restoration in western montane 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 is typically more topographically complex, resulting in disjunct aspen communities across the landscape ranging in size from small patches to large stands (DeByle and Winokur, 1985). Additionally, locations where aspen has declined in recent years and are in the greatest need of restoration are 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 Intermountain West, it is currently unknown whether planting nursery-grown aspen seedlings can be an effective means to overcoming the low rate of natural seedling recruitment in marginal environments (Landis, 2011).

As a pilot study to address this question, we planted over 7000 nursery-grown aspen seedlings across three sites in southern Utah, USA where substantial aspen decline has been documented in recent years (Worrall et al., 2013). We sourced aspen seed from populations in three distinct geographical regions, two in adjoining southwestern US states and one in Alberta, Canada. These seedling sources were also involved in a study to grow seedlings following an established nursery protocol designed for aspen (Howe et al., 2020). Our goals with this study were to 1) assess seedling survival and likely mortality causes across seedling sources in unirrigated plots over two growing seasons following outplanting, and 2) measure the environmental and seedling morphological characteristics associated with seedling performance. After observing high seedling mortality rates associated with low soil moisture (see results), we conducted an additional trial to assess the efficacy of wood mulch as a soil surface amendment to reduce the risk of seedling desiccation during the initial establishment.

2. Materials and methods

2.1. Seedling nursery production

Aspen seedlings used in this study were grown from three geographically separate seed sources: (1) 15 separate clones (min 0.4 km, max 13 km distance) in Logan Canyon, Utah, USA (N 41°56'; W 111°31'), (2) seven separate clones (min 6.4 km, max 127 km distance) near Los Alamos, New Mexico, USA (N 35°53'; W 106°22'), and (3) 10 separate clones (min 1.2 km, max 15 km distance) near Edmonton, Alberta, Canada (N 56°43'; W 113°31') (Fig. 1). Each seed source is expected to contain a wide range of genetic variability because aspen are outcrossing and wind-pollinated. Due to the distinct phylogeographic boundary that exists between northern and southwestern aspen populations (Callahan et al., 2013), we selected two sources within the Intermountain West to provide latitudinal diversity within the region (Utah and New Mexico), while the Canadian source was included because it had a proven record of nursery-grown seedling establishment from previous research in the boreal region of western Canada (Landhäusser et al., 2012a, 2012b; Martens et al., 2007; Schott et al., 2013). We collected all seed sources in the spring of 2014. Beginning the following spring of 2015, we grew 18,000 aspen seedlings (6,000 from each seed source) at the John T. Harrington Forestry Research Center with New Mexico State University in Mora, NM (N 35°58'33"; W 105°20'54") (Fig. 1), following protocols originally optimized for aspen from Alberta, Canada (Howe et al., 2020). Seedlings were grown in D16 Deepot® cells (262 mL volume) (Stuewe and Sons, Inc., Tangent, OR, USA) using a 2:1:1 mixture of sphagnum peat moss, vermiculite, and Turface® clay granules by volume. At the 23rd week following germination we selected 7,200 seedlings (2,400 per source) to be used as the planting stock. We selected for seedlings with a large terminal bud and average height relative to other seedlings from the same source based on results indicating a correlation between these traits and increased first growing season height growth in a previous study of container-grown aspen seedlings (Landhäusser et al., 2012a).

2.2. Plot selection, preparation, and seedling outplanting

Our study was conducted on Cedar Mountain (N 37°33'; W 113°02') (Fig. 1), located between Cedar City and Cedar Breaks National Monument (southwestern Utah, USA). Cedar Mountain was chosen as the location for this study because it has experienced substantial aspen decline in recent decades (Rogers et al., 2010; Worrall et al., 2013) and is thus representative of Intermountain West landscapes which are



Fig. 1. Location of the three aspen seed sources (Utah, USA; New Mexico, USA; Alberta, Canada), nursery where the aspen seedlings were grown (Mora, New Mexico, USA), and outplanting location (Cedar Mountain, Utah, USA). The distribution of aspen is shown in green (Little, 1971). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

candidates for seedling-based aspen restoration.

Three sites were chosen based on an initial assessment of soil texture, proximity to existing aspen stands, and landowner interest in the study. The three sites were located within 9 km of each other at approximately 2,800 m asl. At each site, five plots (27 m × 23 m) were delineated in close proximity (< 300 m apart). All plots were located in open meadows at least 15 m away from mature aspen stands to prevent potential root suckering from established clones interfering with the planted seedlings. 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 exclosures were constructed with game fencing around each plot to prevent entry by wild and domestic ungulates. We 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 (*Helioneris 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, we 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 gopher bait within the exclosures (Moletox® Mole & Gopher Killer, Bonide Products

Inc., 6301 Sutliff Rd., Oriskany, NY, 13424) during the next two growing seasons (2016–17) following the manufacturer instructions.

In October of 2015, we outplanted aspen seedlings in a grid with 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. We also planted a 2 m buffer around the periphery of each plot with an additional row of aspen seedlings to reduce possible edge effects.

2.3. 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, and phosphorus (P) and potassium (K) concentrations (Table 1). Site A was located in the Winnemucca soil series (USDA NRCS) with predominantly loam textured soils within plots, while Sites B and C were located in the Faim series with higher clay contents (Table 1). Soil temperature was sampled hourly over the course of the experiment using HOBO 64 K 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 during the first growing season (see Results), we decided to quantify soil moisture within each plot. We 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, 76,275 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. We assessed soil properties by using bulked soil samples taken from the

Table 1

Soil conditions at each of the 15 plots used for aspen seedling outplanting on Cedar Mountain, Utah, USA.

Site	Plot	(%)								(mg/kg)		Average Water Content (%)			Average Plant Available Water (%)		
		Avg. Soil Temp. (°C)	Soil Texture	Sand	Silt	Clay	WCT	pH	Salinity (dS/m)	P	K	Early	Late	Whole Summer	Early	Late	Whole Summer
A	A1	18.5	Loam	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	Silt Loam	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	Loam	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	Loam	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	Loam	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	Clay Loam	23	43	34	19.0	6	0.19	9.3	205	24.8	24.7	24.7	5.8	5.7	5.7
	B2	17.8	Clay Loam	23	39	39	21.8	6	0.21	14	312	17.4	19.6	18.4	-4.4	-2.2	-3.4
	B3	15.5	Silty Clay	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	Clay Loam	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	Silty Clay	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	Clay	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	Clay Loam	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	Clay	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	Silty Clay	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	Silty Clay	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.

Table 2Precipitation 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, Oregon State University, <http://prism.oregonstate.edu>, created 1 Dec 2017).

	June			July			August		
	30-year Average	2016	2017	30-year Average	2016	2017	30-year Average	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

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).

Soil particle size distributions and water content measurements were then used to calculate a metric of average plant available water for each plot (Table 1). 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, 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 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). -1500 was used as a constant for the water potential (Ψ) and θ was calculated. This produced an estimate of the water content threshold, 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 1). 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. 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). Finally, the WCT was subtracted from the average water content of each plot to arrive at an estimate of average plant available water (Table 1).

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 2). 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.

2.4. Baseline seedling assessment

In order to assess nursery stock quality, we destructively harvested a total of 50 randomly selected seedlings per source, and measured morphological characteristics and carbon and nutrient reserve status. As an additional check on seedling quality, we assessed planting stock performance during the second growing season (2016) under non-limiting conditions. We randomly selected 33 seedlings from the planting stock of each source in October 2015 and stored them at 4 °C during the winter prior to transplantation into 11.3 L pots containing a mix of 2:1:0.5 sphagnum peat moss, vermiculite, and sand by volume. We then

grew them in a greenhouse for 9 weeks before transferring them to an outdoor shadehouse for the remaining growing season. Both the greenhouse and shadehouse were located on the Utah State University campus in Logan, UT, USA (N 41°45'; W 111°48'). Seedlings were watered to field capacity daily and misted with 100 g / 38 L of 20–20–20 NPK fertilizer once weekly until they naturally entered dormancy in mid-October 2016. Details of these measurements and results are provided in the [Supplementary Materials](#).

2.5. Seedling survival and growth measurements

Field performance of the aspen seedlings was evaluated over two growing seasons following outplanting (2016–17). Each spring, prior to full leaf flush of the aspen seedlings (late May), we measured the ground line diameter (GLD) and height of all seedlings along with any stem dieback. All seedling mortality was recorded and a likely cause was attributed based on field observations. We assigned herbivory as the cause of mortality when the stem was cleanly bitten at or below ground level, or there was a hole where the seedling was planted. Additionally, if there was evidence of pocket gopher activity within 2 m of the planting location of the seedling (i.e. excavated soil mounds, tunnels, and winter soil casts) we also assigned herbivory as the mortality cause. We assigned drought stress as the cause of mortality based on symptoms (i.e. leaf and stem tissue desiccation with no obvious insect or fungal pathogens, stem damage or nearby soil disturbance). Seedlings that displayed these 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 (i.e. pocket gophers). 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 possible that the cause of mortality in some seedlings was due to a combination of drought stress and herbivory.

We took a second round of measurements on all seedlings at the end of the growing season each year (late August 2016, 2017). Because aspen seedlings often added their most substantial growth from lateral buds, we additionally measured the longest shoot produced during that growing season based on the length of new stem tissue from the previous year's bud scar to the base of the terminal bud on that stem. All summer mortality was recorded and assigned a cause as described above.

2.6. Mulching trial

After observing nearly complete loss of the aspen planted at Site A during the first growing season (see Results), we conducted a follow-up trial that explored the use of wood mulch as a ground cover to reduce evaporation from the soil. We grew new aspen seedlings during the summer of 2016 using only the New Mexico seed source at the NMSU nursery in Mora, NM. The nursery approach differed from the main experiment in that the new seedlings did not receive an application of the shoot growth inhibitor paclobutrazol (Bonzi®, Syngenta, Wilmington, DE, USA) and were grown in smaller Ray Leach Cone-tainer™ SC10 cells (164 mL volume) (Stuewe and Sons, Inc., Tangent, OR, USA). We prepared mulch from dead aspen branches sourced from a stand near the experimental enclosure using a mechanical wood chipper.

Enclosure A5 was selected for the trial because all original seedlings were lost during the first growing season, with the majority of mortality due to drought stress (Fig. 2). Sixteen paired 2.5 m × 2.5 m plots were distributed evenly throughout the enclosure. Each pair consisted of a mulched plot next to a non-mulched plot. The mulched plots were covered with aspen wood mulch spread approximately 10 cm deep

across the plot. Each non-mulched plot was located 2 m away from the paired mulched plot. In November 2016, we planted half of the aspen seedlings in mulched plots with 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, we 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 average plant available water between the treatments across the growing season. We recorded individual seedling survival and growth at the end of the summer (August 2017).

2.7. Analysis

All data analysis was conducted with R v3.4.1 (R Core Team, 2018). Differences in pre-planting seedling stock characteristics among the seed sources were analyzed using a one-way ANOVA. To evaluate field growth differences among the three seedling sources, we 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.

We examined predictors of aspen seedling survival (live/dead) in the field after two growing seasons using a generalized linear mixed model (GLMM) with a binomial distribution and a logit link in R package lme4 (Bates et al., 2015). Predictors were tested individually as fixed effects factors due to the small number of replicates ($n = 15$). After finding no effect of seed source (see Results) we excluded source as a fixed effect factor in subsequent analyses. An observation-level random intercept was used to accommodate potential overdispersion (source nested within plot nested within site). We ran models with initial seedling GLD and height as predictors of seedling survival on a reduced dataset from plots C3 and C5 (plots with adequate survival, as described above). Random intercepts were used with source nested within plot as random effects. All models were assessed for significance using a likelihood ratio test.

3. Results

3.1. Baseline seedling assessment

Following nursery production, seedlings differed significantly by seed source (Alberta, Utah, and New Mexico) with respect to morphological characteristics (Table S1) and carbon and nutrient reserves (Table S2). Alberta nursery stock developed significantly smaller stems as measured by height, root collar diameter (RCD), and stem dry weight than either Intermountain West source, but produced the largest average root dry weight (Table S1). The resulting root-to-stem ratio (RSR) of the Alberta source was 7.6, nearly three times greater than the Intermountain West sources. Still, all sources developed an average RSR above 2.0 and an average height below 40 cm, which have both been identified as thresholds for increased outplanting success (Landhäusser et al., 2012a, 2012b; Martens et al., 2007).

In non-limiting conditions during the subsequent growing season, seedling height increased by an average of 77 cm, 69 cm, and 119 cm, RCD increased by 7.5 mm, 9.3 mm, and 12.3 mm, and average root dry weight increased by 55 g, 67 g, and 78 g in the Alberta, Utah, and New Mexico sources, respectively (Table S3). This growth resulted in a more balanced RSR between seed sources compared to initial measurements, though these ratios still differed significantly ($P < 0.001$). Overall, New Mexico seedlings were significantly larger than the other two sources with an average total dry weight of 145 g compared to 82 g and 108 g (Alberta and Utah, respectively, $P < 0.001$, Table S3). 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. These results indicate that the nursery stock seedling vigor was not a likely contributor to the high mortality we

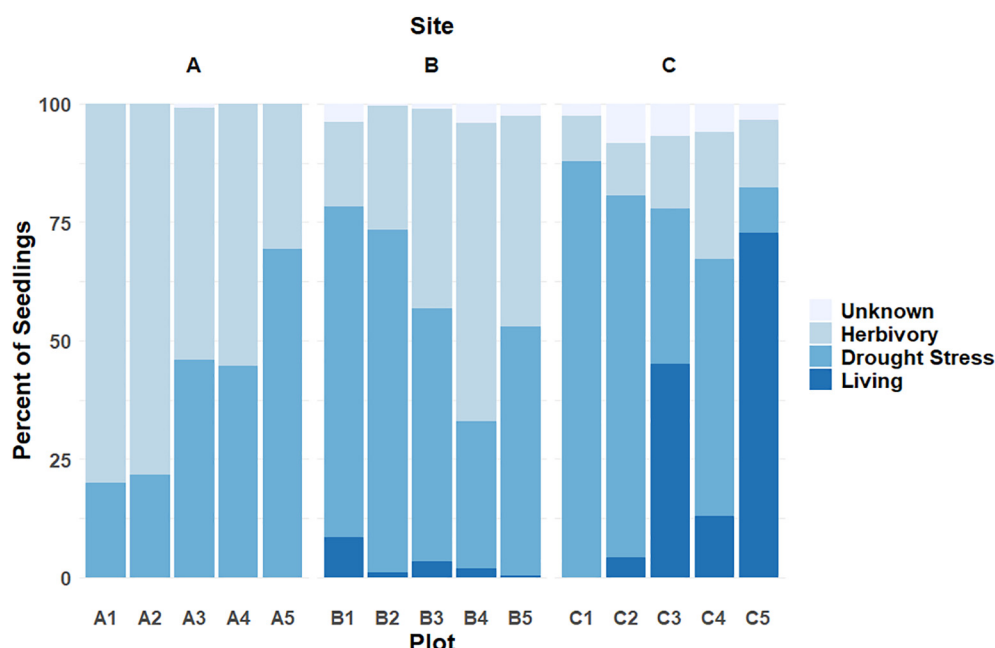


Fig. 2. Proportion of planted aspen seedlings living compared to dead due to either drought stress or herbivory within each plot across three study sites at the end of two growing seasons (480 total seedlings per plot).

observed in the field.

3.2. Field performance

Overall survival (across all three sites) of the 7,200 aspen seedlings at the end of two growing seasons following outplanting was only 10%. Mortality was due to drought stress (49%), small mammal herbivory (38%), or was not assignable (3%) (Fig. 2). Site A lost almost all seedlings (99%) during the first year post-outplanting, with over 31% lost to small mammal herbivory during the first winter under the snowpack attributable to pocket gophers, increasing to a total of 61% mortality due to small mammal herbivory by the end of the summer. Of the remaining Site A 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 mortality, increasing to 39% by the end of the second year. Drought stress accounted for 37% of mortality the first year, increasing to 56% by the end of the second year, leaving only 3% survival of all Site B seedlings by the end of the study (Fig. 2). In Site C, small mammal herbivory accounted for only 15% of seedling mortality by the end of the study. However, 52% of Site C seedlings were lost to drought stress, reducing the final survival rate to 27%.

While survival was poor in aggregate, 79% of all surviving seedlings came from just two plots (C3 and C5, with 46% and 73% plot seedling survival respectively) (Fig. 2). Final aboveground seedling size for surviving seedlings at plots C3 and C5 differed significantly by source (GLD, $P < 0.001$; height, $P < 0.001$), with New Mexico the largest on average (7.0 mm GLD and 41.3 cm height), Utah in the middle (6.4 mm GLD and 33.9 cm height) and Alberta the smallest (5.2 mm GLD and 26.3 cm height) (Table 3). This pattern of stem size among the sources paralleled nursery observations (Table S1). While final stem size varied, total growth in height, GLD, and longest shoot did not differ significantly among sources (height, $P = 0.149$; GLD, $P = 0.096$; LSG 2016, $P = 0.146$; LSG 2017, $P = 0.407$) (Table 3).

3.3. Seedling survival relationships

To assess the factors contributing specifically to drought-stress

mortality, we excluded all seedling mortality attributed to small mammals or of unknown cause from analyses of seedling survival. 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.

Of the edaphic factors measured (Table 1), only early summer average soil water content ($P = 0.031$, $\beta = 0.190$, $se = 0.072$), and early summer average plant available water ($P = 0.006$, $\beta = 0.200$, $se = 0.060$) significantly correlated with seedling survival probability. 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 ($P = 0.015$, $\beta = -0.030$, $se = 0.011$). Initial seedling GLD, 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 seed source at these two plots ($P = 0.208$).

3.4. Mulching trial

Results from the trial using chipped aspen as mulch also indicate that average plant available water was an important factor impacting aspen seedling survival. 62% ($se = 8\%$) of the 128 mulched seedlings survived following their first summer compared to 0% survival of the non-mulched seedlings. The mulching treatment substantially boosted soil moisture retention, with early summer average plant available water estimated at 14.8% in the monitored mulched block, compared to -0.9% in the non-mulched block just 2 m away.

4. Discussion

Outplanting success of aspen seedlings in our study appears to be severely limited by site conditions. With only 10% survival of the 7,200 aspen seedlings initially planted, our work highlights several significant barriers that will need to be addressed before seedling-based aspen restoration can be effectively implemented in the Intermountain West, particularly in drought-prone areas. The largest cause of mortality in our study appeared to be drought stress, accounting for the loss of

Table 3

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 GLD (mm)	Final GLD (mm)	GLD 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.

GLD = Ground Line Diameter.

**Fig. 3.** Aerial image showing location of Plot C5 (73% seedling survival) relative to late-melting snowpack that accumulated along an east aspect cliff (long white rectangle). Long arrow highlights the general direction of the flow from snowmelt downhill. Note: imagery acquisition date was July 1, 2019 (two years following study), however similar snow accumulations were noted during both summers of the study (2016 and 2017).

nearly 50% of all seedlings. Seedling drought stress mortality (inferred from symptoms) was strongly correlated with low early summer average plant available water across many of the experimental plots ($P = 0.006$). 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, summer precipitation was only 50% of the 30-year normal and the average temperature was 1.7°C above the 30-year normal (Table 2). These climatic conditions likely accelerated evaporation of soil moisture during a critical period of initial seedling root expansion following outplanting. Indeed, moisture stress has been implicated as a factor limiting the distribution of aspen at the xeric fringes of its range (Rehfeldt et al., 2009; Worrall et al., 2013). Perhaps most suggestive of a seedling 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 (Fig. 3). The extended melting of this snowpack, which drained downhill through Plot C5, ensured that most of the soil in the plot remained fully saturated through the first half of both summers until monsoonal precipitation typically began. Plot C3 also had relatively high seedling survival at 46%. Unlike Plot C5, Plot C3 was not located downslope from extended snowmelt, yet it maintained high soil moisture throughout the summer which appeared to be related to the high clay and silt content of its soil (Table 1). The increased seedling survival at Plots C5 and C3 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.

The importance of maintaining soil moisture was also illustrated in the mulching trial, in which 62% of aspen seedlings survived when soil was mulched compared to 0% survival for seedlings that had no mulch cover. In addition to reducing evaporation and lowering soil temperatures, the mulch also visibly suppressed forb and grass growth, likely reducing both light and water competition for the aspen seedlings. Evidence from natural aspen seedling establishment in a similar Intermountain West environment suggests that coarse woody debris may serve a similar role to mulch, acting as important shelter for aspen seedlings by ameliorating harsh microsite conditions, particularly during severe droughts (Fairweather et al., 2014). Using coarse woody debris to enhance aspen seedling survival could easily be accomplished in many restoration contexts, such as after stand thinning treatments, controlled burns, or wildfires. Microsite topography can 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). In natural seedling regeneration following a large fire near Cedar Mountain in 2017, seedlings were observed primarily in areas with coarse woody debris, under logs, and in natural depressions (K. Mock, personal observation). 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. 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, we 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 Intermountain West 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 and found no effect of pocket gopher removal on aspen sucker regeneration. While the impact of pocket gophers on aspen regeneration from well-established clonal root systems remains unclear, their extensive herbivory in this study, despite sustained removal efforts, strongly suggests that these fossorial rodents have the potential to drastically limit aspen seedling establishment. However, it is important to note that the use of exclosures may have increased small mammal activity within the plots due to predator exclusion (e.g. Shepperd and Mata, 2005), and the placement of plots in open meadows may have coincided with areas of locally higher pocket gopher density compared to adjacent forested areas (Cantor and Whitham, 1989). Quantifying local pocket gopher abundance, along with ungulate herbivory (Britton et al., 2016), may provide a useful metric for assessing the relative suitability of a site for seedling-based aspen restoration. Planting in extensive recently burned areas could also help minimize herbivory during the establishment period (Wan et al., 2014).

The similar poor performance among all three sources suggests that the primary factor limiting seedling survival in this study was the environment, which potentially masked differences among seed sources. None of the seedlings grown from the three aspen seed sources (Alberta, Utah and New Mexico) had a clear survival advantage during establishment, despite the markedly different traits they developed in the nursery (Tables S1 and S2; Howe et al., 2020). At plots C3 and C5, where seedling survival was greatest, all three sources displayed nearly identical survival and growth rates after two growing seasons. Site conditions at the outplanting locations were likely too limiting to reveal adaptive differences at this early establishment stage. Over the longer-term, phenological and genetic differences will likely result in differential survival and growth, making the selection of appropriate seed sources for a given site an important consideration. In addition, more attention and consideration need to be given to the nursery culture and planting windows of aspen seedlings to explore seedling characteristics and outplanting times advantageous for the harsh and seasonally dry site conditions common in the Intermountain West region.

Notably, there was one metric that did correlate with seedling survival across seed sources: initial seedling height. Taller seedlings at the time of outplanting had reduced survival in plots C3 and C5 when considering only seedlings that were lost due to drought stress. This may be due to an imbalance between the root system size and the shoot size, which might be inadequate for supporting the increased transpirational demands of taller seedlings. Indeed, a high root-to-shoot ratio in aspen seedling stock has been shown to correlate very well with increased field performance (Martens et al., 2007; Landhäusser et al., 2012a).

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 drier climates for a desired restoration site (Aitken et al., 2008; Millar et al., 2007). Using 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). Though the potential for aspen to be used as a natural fire break remains largely unexplored, there is evidence that aspen-dominated stands have low flammability and crown fire potential compared to conifer-dominated stands (e.g. Cumming, 2001), due in part to greater fuel moisture, lower surface fuel loading and canopy bulk densities, and fewer ladder fuels (DeByle et al., 1987).

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 (King and Landhäusser, 2018). In the Intermountain West, clones originating from single seeds can spread to cover many ha (Mock et al., 2008). Indeed, the restoration strategy of applied nucleation, which focuses on planting clusters of seedlings as focal areas for recovery (Corbin and Holl, 2012), could be particularly suited to the suckering potential of aspen. Despite the significant barriers encountered that limited seedling establishment in this study, reasonable survival did occur at two of the fifteen plots. We maintain that with future research aimed at developing appropriate nursery cultural practices as well as site selection and outplanting protocols, seedling-based aspen restoration could be a viable and valuable management tool in the Intermountain West.

CRediT authorship contribution statement

Alexander A. Howe: Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Simon M. Landhäusser:** Conceptualization, Methodology, Resources, Writing - review & editing. **Owen T. Burney Landhäusser:** Conceptualization, Methodology, Resources, Writing - review & editing, Funding acquisition. **James N. Long:** Methodology, Writing - review & editing. **Randall D. Violett:** Resources, Writing - review & editing. **Karen E. Mock:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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