

BIG SAGEBRUSH (*Artemisia tridentata*) IN A SHIFTING CLIMATE CONTEXT:
ASSESSMENT OF SEEDLING RESPONSES TO CLIMATE

by

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ABSTRACT

The loss of big sagebrush (*Artemisia tridentata*) throughout the Great Basin Desert has motivated efforts to restore it because of fire and other disturbance effects on sagebrush-dependent wildlife and ecosystem function. Initial establishment is the first challenge to restoration, and appropriateness of seeds, climate, and weather variability are factors that may explain success or difficulties in big sagebrush restoration efforts. This project provided several ways of assessing climate responses of big sagebrush seedlings during the critical establishment phase post-fire. We evaluated eleven different seed sources of big sagebrush from all three subspecies, dissimilar climates-of-origin, and different ploidy levels to assess how subspecies, cytotype, and climate-of-origin affect initial establishment of sagebrush in a common garden study. We assessed ecophysiological-climate adaptation as it relates to seedling performance using a suite of dependent variables, including: survival, growth, water balance, photosynthesis, and threshold freezing responses. Results indicate the importance of minimum temperatures to seedling establishment, and reveal a gradient of physiological responses to freezing that inform big sagebrush adaptation and functional diversity. We then used in-situ experimental warming to isolate minimum temperatures, and test the effects of warming on seedling physiological performance for the three dominant subspecies of big sagebrush: *A.t. tridentata*, *A.t. vaseyana*, and *A.t. wyomingensis*. Experimental warming further supported our minimum temperature hypothesis, indicating that warming may

alter seedling freezing response thereby affect growth and survival. In a third experiment, we evaluated how initial establishment of big sagebrush is influenced by management treatments on the herb layer, as post-fire rehabilitation frequently involves alterations of the plant community and soil. Results suggest that drill seeding combined with land management treatments that cause disturbance of the herb layer and soil surface may negatively affect sagebrush during the establishment phase. Also, seedlings from local seed or faster-growing populations had greater survival than seedlings from climates that differed from the experimental site. In summary, we provide experimental evidence for the importance of minimum temperatures and seed sources to big sagebrush ecology and management of sagebrush systems. As the climate warms, selection for population-specific freezing resistance mechanisms may alter subspecies distributions. Our data indicated that warming could increase relative abundance of *A.t. tridentata* compared to *A.t. wyomingensis* at our Birds of Prey National Conservation Areas study site on the lower Snake River plain. The underlying mechanism for this is greater stress overcome by changes in resource allocation from freezing protection to growth, as well as an extraction of deeper soil water resources in *A.t. tridentata*. Mortality of *A.t. vaseyana* appeared to relate to drought stress and greater vulnerability to minimum temperature exposure. Understanding differences in big sagebrush populations' ability to compete with different types and abundances of herbs as well as variation in freezing resistance mechanisms will contribute to appropriate seed selection for particular restoration sites. The implication is that selection of seed is critical for big sagebrush restoration success.

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INTRODUCTION

Climate shifts, invasion by exotic annuals, and wildfire have transformed or eliminated millions of hectares of the sagebrush steppe ecosystem during the past century. Rapid loss of big sagebrush (*Artemisia tridentata*) in Great Basin Desert has motivated efforts to restore it through seeding or planting. Despite large investment, restoration success has been mixed, often resulting in little sagebrush established. Initial establishment is the first challenge in restoration and a major obstacle associated with seeding big sagebrush is obtaining the appropriate taxonomic identity of seed sources from adapted sites. While the importance of local seed sources is recognized, the basis for climatic seed zones is still under development for sagebrush. Appropriateness of seed source, local climate, and weather variability are all factors that may help explain success or difficulties in sagebrush restoration efforts.

Big sagebrush displays high interspecific diversity within and among subspecies, and also has high diversity in responses to climate. Three dominant subspecies are widely recognized: *Artemisia tridentata* ssp. *tridentata* “basin,” *A.t.* ssp. *vaseyana* “mountain,” and *A.t.* ssp. *wyomingensis* “Wyoming.” Genetic and physiological variability of sages is also linked to ploidy level (two sets of chromosomes in somatic cells). *A.t. wyomingensis* is universally tetraploid whereas *A.t. vaseyana* and *A.t. tridentata* can be either diploid or tetraploid. Polyploidy and inter- and intraspecific hybridization are likely to be important mechanisms in big sagebrush adaptation and landscape dominance, but few studies have evaluated how this concept relates to restoration success.

Seeding or plantings of big sagebrush in restoration efforts are often accompanied by landscape treatments to reduce wildfire fuel loads and invasive species through mowing, herbicide applications, or rangeland drill seeding. The neighboring herbaceous layer can compete with sagebrush seedlings for water and other soil resources. Physiological water balance, soil water uptake, and soil water use vary significantly between big sagebrush subspecies, cytotypes, and populations, and therefore are potentially major contributing factors to big sagebrush climate adaptation. While these factors are normally considered with respect to how sagebrush relates to the abiotic environment, it is reasonable to expect that many of these attributes may also affect how sagebrush competes with different types and abundances of herbs.

Big sagebrush is a semi-evergreen shrub that remains physiologically active during a large portion of the growing season, and understanding the relationship between water balance and big sagebrush physiology is important to the research described here. In semiarid systems, water is the most limiting resource, and how temperature and timing of precipitation correspond to conditions of moisture and cold stress may affect shrub morphology and ecophysiological response. Walter-type climate diagrams illustrate the relationship between temperature and seasonal precipitation, and we created these diagrams for our study sites at Birds of Prey National Conservation Area (BOP NCA) in 2013 compared to historical averages from 1999-2010 (Figure 1). Walter's (1973) hypothesis suggests that woody plants and grasses compete for water in upper layers of soil in steppe systems, but shrubs like sagebrush have exclusive access to water sources in deep soil. In a normal year at BOP NCA, the typical climate has more months in water surplus than in water deficit; but at the same time, temperatures are low and can limit

plant physiology and development. Thus, experiments incorporating how big sagebrush responds to changes in climate (particularly minimum temperatures and precipitation) are critical for the advancement of sagebrush ecology.

Currently, there is limited understanding of big sagebrush ecology for establishing seed transfer zones under future climate regimes and a major pending question is whether minimum temperatures relate to seedling survival and establishment. Research indicates that temperatures can be limiting factors in geographic distribution of many desert species, but little is known about freezing responses in big sagebrush or how freezing response relates to survival. It is reasonable to expect that *A.t. vaseyana* is the most cold-adapted among the subspecies due to distribution at higher elevations where snowpack is common and winter conditions are more extreme. Further, tetraploid big sagebrush populations have double the genetic material of diploid populations and polyploidy may increase plasticity in physiological responses of big sagebrush to cold temperatures, as well as drought resistance and nutrient uptake. Post-fire restoration of big sagebrush requires more in-depth knowledge of basic biology on climate adaptation of big sagebrush.

Our primary objectives in this study were to assess climate responsiveness of seedlings of different populations on burned areas and relate ecophysiological-climate adaptation to seedling performance. We evaluated eleven different seed sources of big sagebrush from all three subspecies with dissimilar climates-of-origin and different ploidy levels to assess how subspecies, cytotype, and climate-of-origin affect initial establishment of sagebrush in a common garden study (Chapter 1). A suite of dependent variables were measured, including: survival, growth, water balance, photosynthesis, and

threshold freezing responses. Results indicated the importance of minimum temperatures to seedling establishment, and revealed a spectrum of physiological responses to freezing that inform big sagebrush adaptation and functional diversity. The effect of minimum temperatures on big sagebrush was investigated using experimental warming chambers in the field in Chapter 2. Experimental warming further supported our minimum temperature hypothesis (Chapter 2), and responses of survival, growth, and physiological performance to warming varied among of the three subspecies of big sagebrush. Low emergence occurred from seed sowed and emergence appeared to occur earlier in the year under warming compared to control frames (data is not shown) and was followed by high mortality. As of spring 2014, there were only six seedlings that emerged from seed and remained in warmed frames and no seedlings remained in control frames.

In a third experiment (Chapter 3), we assessed seedling responses to management treatments of herbs using the same seed sources described in Chapter 1. Seedlings were outplanted into pre-existing, landscape-scale treatments of herbs, including herbicide application, mowing, and drill seeding arranged in a full factorial design on three replicate blocks. Seedlings from local seed or faster-growing populations had greater survival than seedlings from climates that differed from the experimental site. Overall seedling survivorship was greater on plots that had treatments leading to herb-reduced cover (herbicide + mowing + no drill seeding) compared to plots that had been seeded with herbs, grasses, and sagebrush. Significant mortality of seedlings reduced our power to make inferences about how different sagebrush seed sources can compete with the surrounding herb layer.

Our results provide experimental evidence for the importance of minimum temperatures to big sagebrush ecology and management of sagebrush systems. Changes in minimum nighttime temperatures are directly associated with the greenhouse effect, and as the climate warms selection for population-specific freezing resistance mechanisms may alter subspecies distributions. Results suggest that warming could increase relative abundance of *A.t. tridentata* compared to *A.t. wyomingensis* at our Birds of Prey National Conservation Areas study site on the lower Snake River plain. The underlying mechanism for this is greater stress overcome by changes in resource allocation from freezing protection to growth, as well as enhanced drought adaptation in *A.t. tridentata*. High mortality of *A.t. vaseyana* was anticipated and did occur largely due to ill-adaptation to the study site. Mortality of *A.t. vaseyana* appeared to relate to drought stress and, ironically, greater vulnerability to minimum temperature exposure.

In conclusion, experimental warming did not cause a uniform climate response in big sagebrush. Climate responses in big sagebrush are variable, and interact synergistically with factors such as time, weather, microclimate, herb community, as well as subspecies, cytotype, and among populations. We saw strong selection for minimum-temperature response in a relatively cold and dry year (Figure 1a, Table 1), and population-specific freezing resistance mechanisms may emerge as important criteria for seed-source selection for restoration in the future. Further, understanding differences in the ability of big sagebrush to compete with different types and abundances of herbs will contribute to appropriate seed selection for particular restoration sites. The implication is that careful selection of seeds is important for big sagebrush restoration success.

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CHAPTER 1: INTRASPECIFIC VARIATION IN SAGEBRUSH SEEDLING RESPONSES TO POST-FIRE WEATHER INDICATES THE IMPORTANCE OF MINIMUM TEMPERATURES TO ESTABLISHMENT

Abstract

The loss of big sagebrush due to wildfire has motivated efforts to restore it through seeding or planting. A major obstacle associated with post-fire seeding of big sagebrush is obtaining the appropriate taxonomic identity of seed sources from adapted sites. Big sagebrush is a genetically diverse species that includes subspecies and cytotypes. Genetic and cytological differences are not trivial, and are likely to be important mechanisms in big sagebrush adaptation and landscape dominance. Our objective was to assess differences in climate responses of big sagebrush among eleven different seed sources that varied in subspecies identity, climates-of-origin, and ploidy level. A suite of dependent variables were measured, including: survival, seedlings height, water balance, photosynthesis, and freezing response thresholds. In a laboratory, we determined low temperature damage (F_v/F_{m50}), freezing point, and freezing resistance mechanisms (tolerance or avoidance) by comparing F_v/F_{m50} with freezing point (F_v/F_{m50} —freezing point). Survival of *A.t. tridentata* was greatest compared to other subspecies although the study site is considered an *A.t. wyomingensis* ecosite. Tetraploid cytotypes had consistently greater survival and height within subspecies (especially in *A.t. tridentata*) than diploids. Examination of freezing resistance mechanisms revealed that tetraploid *A.t. tridentata* is a freezing “avoider,” whereas all other big sagebrush are

considered freezing tolerant. “Avoiders” had less capacity to survive cold temperatures in March when minimum air temperatures were nearly identical to those in January. *A.t. vaseyana*, the most freezing tolerant among group, appeared to be adapted to higher elevation habitats insulated from cold temperatures by snow and therefore was more likely to suffer more negative effects of infrequent, intense freezing. *A.t. wyomingensis* and diploid ssp. *tridentata* had reduced freezing tolerance consistent with their lower elevational distributions. Other ecophysiological parameters measuring water balance indicated differences among subspecies and cytotypes, but differences in these factors did not explain patterns in mortality to the same extent as freezing response. Our results suggest that freezing resistance mechanisms vary among subspecies and cytotypes in tolerance or avoidance of frost, and may be important factors in seedling survival.

Introduction

Climate shifts, invasion by exotic annuals, plants, or grasses, and wildfire have transformed or eliminated millions of hectares of the sagebrush steppe ecosystem during the past century (West 1996; Anderson & Inouye 2001; Wisdom et al. 2003). Big sagebrush (*Artemisia tridentata* Nutt.) is the most common shrub across this landscape and an essential component of ecosystem patterns and processes (West 1983; Welch 2005; Prevéy et al. 2010). These shrubs prevent soil erosion, enhance nutrient and water cycling, and provide important forage for wildlife (Vale 1974; Welch 2005). Big sagebrush acts as critical habitat for a number of sagebrush-obligate and facultative obligate wildlife species, including the threatened greater sage-grouse (*Centrocercus urophasianus*) (Connelly et al. 2004; Crawford et al. 2004). The loss of big sagebrush and associated habitats is a major concern for biodiversity conservation and land

management of rangelands in the western United States (Manier et al. 2013; Shlaepfer et al. 2014).

Big sagebrush is not fire-adapted and decreased fire intervals have resulted in landscape-scale habitat loss. To mitigate this loss, seeding and planting of big sagebrush are increasingly common. Restoration success varies despite consistently large investment, often resulting in little sagebrush established (Shaw et al. 2005; Pyke 2011; Arkle et al. 2014). A major obstacle associated with restoring big sagebrush is obtaining the appropriate taxonomic identity of seed sources from adapted sites. Suitable seed sources may be essential for big sagebrush persistence (Lysne & Pellant 2004) because non-adapted seeds may differ in habitat, moisture, temperature, and germination requirements (Winward & Tisdale 1977; Barker & McKnell 1986; Harniss & McDonough 1975). While the importance of local seed sources is recognized (Mahalovich & McArthur 2004), the basis for climatic seed zones is still under development for big sagebrush. Appropriateness of seeds and climate and weather variability are all factors that may help explain success or difficulties in sagebrush restoration efforts (Shaw et al. 2005; Lysne & Pellant 2004).

Big sagebrush is highly variable in morphology, and three dominant subspecies are widely recognized in the sagebrush steppe of North America: *Artemisia tridentata* ssp. *tridentata*, *A.t.* ssp. *vaseyana* (Rydb.) Beetle, and *A.t.* ssp. *wyomingensis* Beetle and Young (Goodrich et al. 1985). *A.t. vaseyana* inhabits deep, well-drained soils at mid to high elevations and has access to summer precipitation. *A.t. tridentata* is distributed at lower to mid elevations in valleys and foothills that have deep soils (Mahalovich &

McArthur 2004). *A.t. wyomingensis* occupies lower elevations on drier sites with shallower or often rocky soils (Bonham et al. 1991; Shultz 2006).

Polyploidy, two sets of chromosomes in somatic cells, is also linked to morphological and genetic variation in big sagebrush. *A.t. wyomingensis* is universally tetraploid and *A.t. vaseyana* and *A.t. tridentata* can be either diploid or tetraploid (Bajgain et al. 2011). It is thought that polyploid cytotypes have adaptive traits enhancing resistance to drought, and can colonize areas with more extreme ecological conditions than diploid populations of the same species (Johnson et al. 1965; Grant 1971; Levin 1983; Ramsey & Schemske 1998). Little is understood about the advantages of polyploidy in sagebrush, but polyploidy and inter- and intraspecific hybridization are likely to be important mechanisms in big sagebrush adaptation and landscape dominance (Bajgain et al. 2011; Mahalovich & McArthur 2004; Richardson et al. 2012).

Plant recruitment and establishment is limited by a seedlings' ability to survive in the abiotic environment near the soil surface (Franco & Nobel 1988; Smith & Nowak 1990). In cold deserts, temperatures near the soil surface are typically lower and more variable than air temperatures at one meter above the ground, resulting in a colder more inconstant microclimate for seedlings than that experienced by mature plants (Nobel, 1988). Seedling response to freezing can be highly variable, and cold temperatures can be limiting factors in geographic distribution of many desert species (Nobel 1988; Loik & Nobel 1993, Loik & Redar 2003; Pockman & Sperry 1997). Seedlings may differ not only in ability to survive freezing, but also in physiological mechanisms employed to resist freezing damage, which may also impact survival.

Negative impacts of freezing on plants can be mitigated by using either avoidance or tolerance mechanisms (Levitt 1980; Schulze et al. 2005). Avoidance of freezing is achieved by depressing the temperature causing ice formation. Freezing tolerance is a plant's ability to survive extracellular freezing events (Sierra-Almedia & Lohengrin 2012; Larcher 2003). Freezing avoidance can be a risky strategy as it only protects plants from freezing damage for short periods of time (perhaps only hours) (Goldstein et al. 1985; Rada et al. 1985; Sierra-Almedia et al. 2010), and may incur high fitness costs if freezing is intense or occurs frequently (Medeiros et al. 2012). However, freezing tolerance may reduce growth and competitive ability in the absence of freezing (Medeiros et al. 2012). Trade-offs between growth rates, survival, and freezing resistance mechanisms likely exist and might provide key insight of selective pressure and diversification on big sagebrush subspecies, cytotype, and population distributions on the landscape.

The objective of this study was to evaluate intraspecific variation in ecophysiological responses of big sagebrush seedlings to minimum temperatures at Morley Nelson Birds of Prey National Conservation Area (BOP NCA). We evaluated how survivorship, growth, and freezing response varied among three subspecies, two cytotypes, and populations from different climates-of-origin. We hypothesized that local populations and subspecies would be better adapted to site conditions at BOP NCA than those from other environments. The Swan Falls Dam area (location of study plots) (Figure 2) of BOP NCA is designated a "Loamy 8-12" inch annual precipitation *Artemisia tridentata* ssp. *wyomingensis*/*Pseudoroegneria spicata* ssp. *spicata* ecological site (<https://esis.sc.egov.usda.gov/>, USDA NRCS), specifying predicted dominance of

A.t. wyomingensis, although it has mostly undergone conversion to early exotic annual or seeded perennial grasses. We predicted that the highest survival would be from *A.t. wyomingensis* populations local to the study area. We also predicted that tetraploid cytotypes would exhibit greater survival, growth, and response to stress than diploid cytotypes. Lastly, we predicted that ecophysiological differences in freezing response (both avoidance and tolerance of/to freezing) correspond with differences in survival and growth among the subspecies, cytotypes, and populations (and may vary among groups) of big sagebrush.

Methods and Materials

Plant Material and Germination

Eleven different seed sources were utilized to assess how subspecies, cytotype, and population affect initial establishment of sagebrush (Table 2). Seeds sources were initially selected to include all three big sagebrush subspecies based on locality to the Boise area. The remaining eight genotypes were selected from a wide range of genetic backgrounds and include both diploid and tetraploid ssp. *tridentata* and ssp. *vaseyana* cytotypes from non-local climates-of-origin (Table 3). We incorporated populations originating from drier or cool areas. Seeds used in the experiment were acquired from the same lots used in the common garden experiment and were in cold storage. Seed sources were genetically identified in Richardson et al. (2012), and while selected populations incorporate different climates-of-origin, all are from colder sites than BOP NCA one. Our selection of seed sources was limited by available quantities of seed.

Seedlings were grown outdoors at U.S. Geological Survey Snake River Field Station in Boise, Idaho (43°60'90.98"N, 116°21'12.01"W). Approximately 10 seeds were

sown into each 10 cm³ cone-tainer filled with native soils (silty loam) on August 10, 2012 and germination occurred within a week. We grew out approximately 50 seedlings per population for this experiment. Cone-tainers were rearranged periodically to limit the influence of microsite and watered one to two times daily depending on climate conditions. Seedlings were shifted to full sun and hardened before outplanting in fall 2012.

Site Conditions and Study Design

Plots were established in five separate blocks burned in summer of 2012 at BOP NCA, south of Boise, Idaho (Figure 2). The five fires (Poen, South Point, Swan Falls, Coyote, and Kave) represent a variety of pre-burn conditions in the sagebrush steppe. Poen was drill seeded with *Kochia prostrata* after burning in 1993. The South Point fire was also drill seeded after burning in 1998 but with Russian wildrye grass (*Psathyrostachys junceus* (Fisch.) Nevski). The Swan and Coyote areas had a mixed mosaic of cheatgrass understory and sagebrush, and the Kave area was considered to be intact sagebrush steppe prior to burning. Plant community post-fire was determined using point-line intercept, and percent vegetative cover was estimated in 20x20 cm² quadrats. Soils were assessed for texture analysis using a modified sedimentation technique (Gee & Bauder 1986), and percent soil organic matter was determined by weight loss-on-ignition using a muffle furnace (Schulte & Hopkins 1996). We quantified elemental composition of nitrogen and carbon in soils using a Costech flash combustion CHNSO analyzer (ECS 4010, Costech Analytical Technologies; Valencia, CA).

We used a randomized complete block design with each site acting as a block. Each block was fenced to prevent cattle disturbance and grazing on the plots. Seedlings

were outplanted in November 2012 and average seedling heights ranged from 22.5-38.3 mm. Ten seedlings per population were bare-rooted transplanted into plots approximately 10 cm apart, and populations were randomly assigned to rows. Holes for seedling plugs were dug with a 2-inch soil corer to minimize disturbance of the soil. All planting was completed within a span of three weeks. Plants received supplemental watering two times following outplanting, directing water to individual seedling stems.

Microclimate

Soil volumetric water content (θ , m^3m^{-3}) was determined using EC-5 volume soil moisture sensors connected to Em50 ECH₂O data loggers (Decagon Devices, Pullman WA), at 2-5 cm soil depth and at the soil surface (0-2 cm). Air and soil surface temperatures were recorded using HOBO H08-032-08 and Pendant UA-002-64 loggers (Onset Computer, Pocasset, MA), with sensors in two positions: the 5 cm above the soil to measure air temperatures affecting seedlings and directly under the soil to estimate (0-1 cm) surface temperature. Radiation shields were used for all above-ground sensors. All measurements were made hourly after out planting from November 2012 through May 2014.

Plant Growth and Survival

Height and survival were recorded for each seedling at the time of outplanting, November 2012, and then in monthly increments from February 2013 to May 2014. Mortality was assumed when the plant was gone or all foliage was missing and no re-greening occurred after rain. High mortality at 4 of the 5 blocks precluded determination of statistical significance in growth and physiology among groups across all blocks, and growth and physiological analyses were done only on the Poen block.

Physiological Measurements

Chlorophyll fluorescence was used to measure F_v/F_m as an indicator of seedling physiological stress levels in April 2013, November 2013, January 2014, and February 2014. F_v/F_m is the ratio of variable to mean fluorescence emitted from chlorophyll *a* of photosystem II (PSII) following dark adaptation, and indicates the yield of energy trapped in PSII (Maxwell & Johnson, 2000). A healthy, non-stressed leaf usually has a F_v/F_m value around 0.80, and this value declines under the influence of stress factors. F_v/F_m was measured at pre-dawn using a model 6400-40 fluorometer (Li-Cor, Inc., Lincoln, NE), $n=3$. Pre-dawn water potential was measured in June 2014 to assess plant-water status under warmed and control conditions. Three seedlings per population were clipped at the Poen block for leaf water potential (Ψ_{leaf}) measurements before 0500. Measurements of water potential were made with excised tissue using a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR).

Gas exchange measurements were made on seedlings in April 2014. All measurements were conducted between 1000 and 1600 local time using a 2 x 3 cm leaf chamber and external LED light source connected to portable, open-mode photosynthesis system (Li-Cor Model 6400, Licor Inc., Lincoln, NE). The chamber was clamped onto fully elongated leaves from seedlings of each population ($n=3$). Carbon dioxide flowing into the chamber was set to $400 \mu\text{mol m}^{-1}$ and vapor pressure matched to ambient levels for all gas exchange measurements. Measurements of photosynthesis were corrected for projected leaf area determined from digital photos taken immediately following gas exchange measurements and using image processing software (Image J; Scion, Fredrick, MD). Specific leaf area (SLA) was calculated as the average ratio of leaf area (cm^2) to

leaf mass (mg) for each population. Values of SLA were collected in September and December 2012, October 2013, and January 2014. Only values from October 2013 are reported here due to constraints obtaining plant material due to high mortality. Plant leaf area was determined using digital photos and image processing software (Image J; Scion, Fredrick, MD).

To assess freezing point, three to five excised leaves (collected in January and May 2014) from different plants of each population were placed on a ceramic thermoelectric module (CP14-127-06-L1-RT-W4.5/Laird Technologies, Earth City MO) attached to thermocouples whose readings were recorded by a data-logger at 5 second intervals (model CR7, Campbell Scientific, Logan UT) and chilled at 4°C per hour to -20°C in a regulated freezer. The temperature corresponding to the exothermic heat released during cell freezing was then identified as the “freezing point” (the temperature causing freezing) for each sample. Osmotic concentration and other traits affecting colligative properties of water cause depression of freezing point and thus inform about tolerance or avoidance of freezing. The same cooling system was used to measure F_v/F_{m50} , which in our study is the temperature causing 50% loss of chlorophyll *a* fluorescence. Literature on freezing responses in plants frequently uses the term “LT₅₀” (Boorse et al. 1998; Loik et al. 2000) to describe the temperature causing 50% loss of chlorophyll *a* fluorescence. However, we did not validate that 50% F_v/F_m equates to mortality and therefore use the term F_v/F_{m50} . Excised leaves were sealed in plastic bags and chilled at 4°C per hour to -5, -10, -12, -14, -16, -18, or -22°C. Chlorophyll fluorescence (F_v/F_m) was measured after samples warmed to room temperature in darkness.

This variable approximates light-use efficiency and thereby health and stress level of leaves (maximum value=0.8).

Freezing resistance mechanism (tolerance or avoidance) is determined by comparing the F_v/F_{m50} temperature (point of irreversible damage to the plant tissue) to the temperature where the plant tissue freezes (freezing point) (Sakai & Larcher 1987). A lower exotherm (more negative freezing point) along with an F_v/F_{m50} close to the freezing point indicate an emphasis on avoidance rather than tolerance of cellular freezing. Conversely, plants with significantly lower F_v/F_{m50} values relative to freezing point suggest a freezing tolerance strategy.

Plant Carbon Isotopes

Integrated measures of water-use efficiency over seedling lifetimes were assessed with carbon isotope (^{13}C) ratios. The isotopic ratio of plant-tissue carbon ($^{12}\text{C}/^{13}\text{C}$) indicates variation in the CO_2 concentration gradient between air and leaves during photosynthesis given similar environmental conditions, and thus indicates water-use efficiency (WUE), the amount of CO_2 assimilated per unit water diffusing through the stomata (Farquhar et al. 1982; Farquhar et al. 1989). Leaf samples were collected in December of 2013, dried in an oven at 65°C , ground to powder, and analyzed for $^{13}\text{C}/^{12}\text{C}$ using a Costech flash combustion ECS 4010 and Picarro cavity ring down spectrometer (Model 2020, Picarro Inc, Santa Clara, CA) at the U.S. Geological Survey Snake River Field Station. The $^{13}\text{C}/^{12}\text{C}$ ratio of samples relative to the international standard Vienna Pee Dee Belemnite (VPDB) are reported using delta notation, with samples more depleted in δ^{13} (more negative values) resulting from a smaller gradient of CO_2 between air and leaves and indicating less water-use efficiency (Farquhar et al. 1989).

Data Analysis

Sampling events for seedling survival occurred on a coarse time scale, meaning individual deaths were recorded in monthly intervals and actual time of death could differ up to 30 day between two seedlings. Thus, the time-to-death data is referred to as “tied” and is considered “interval-censored” (Allison 2010; Sun 1997). Interval censored survival data is most appropriately analyzed with conditional logistic models fit with complementary log-log link mathematical reconstructions (Hosmer & Lemshow 2003; Prentice & Gloeckler 1978; Allison 1982). Conditional logistic models could not be run over the entire observation period due to “complete separation,” a condition in which the dependent variable does not vary at some levels of the independent variable. Complete separation occurred because of little seedling mortality after June 2013. Therefore, we used interval-censored survival analysis to examine seedling survival only during months 3, 4, 5, and 6 (March-June 2013) when the most significant die-off occurred.

Conditional logistic models included survival (binomial) as a function of month, subspecies x cytotype, a single climate variable, and all their interactions as fixed factors. We fit conditional logistic models with generalized estimating equations (GEE, (Liang & Zeger 1986) to model the framework in the geepack (Yan 2002) library in R (R Development Core Team, 2011) to evaluate population-averaged effects of survivorship in lieu of a subject-specific approach. We ran five models and used model selection to determine relative importance of 1) growing degree days, 2) frost free days, 3) minimum air temperature, 4) minimum soil temperatures, and 5) days of soil surface VWC below $0.08 \text{ m}^3 \text{ m}^{-3}$ (which we designated to be a threshold between free versus tightly bound water in this system). All microclimate variables were considered correlated and five

separate models were run, each with a different microclimate variable. These models were then evaluated QIC (Pan 2001) in library MuMIn (Bartón 2013) to determine which microclimate factor explained the most variability in seedling survival. To interpret the final model, predicted probabilities of survival were calculated in SAS (9.4) using PROC GENMOD function.

We used survival analysis in the Survival library in R version 3.0.2 with the `coxph()` function (Therneau 2014) to address seedling survivorship throughout the entire observation period (due to limitations of conditional logistic models). We ran semi-parametric Cox proportional-hazard regression models to estimate the effects of subspecies, cytotype, and climate-of-origin on seedling hazards (or risk) of death (Cox 1972). The Cox analysis provides “hazards of death” for each factor, which are interpreted as decreased (less than 1) or increased (greater than 1) probabilities of death (Venables & Ripley 2002). Models were tested for assumptions and stratified by block (n=5) to account for random environment differences. Comparison of survival probabilities between subspecies, cytotype, and climate-of-origin groups were assessed with log-rank tests.

To address seedling growth, we performed a single multivariate analysis of variance (MANOVA) to evaluate height differences among cytotypes using PROC GLM with a repeated statement in SAS 9.4 (SAS Institute Inc, Cary, NC). Only seedlings that survived the entire observation period at the Poen block were used in this analysis, due to inadequate numbers of seedlings in other blocks. When the assumption of sphericity was violated, only multivariate test statistics were utilized. We evaluated differences in mean height by cytotype during months 3-6 (March to June 2013) when the most mortality

occurred using least square means with Bonferroni correction for multiple means comparisons. A-priori pairwise contrasts were used to evaluate differences in height between cytotypes within subspecies at the end of the observation period (May 2014).

We used one way (subspecies, cytotype, or climate-of-origin as fixed factor) analysis of variance (ANOVA) tests to address physiological responses of subspecies, cytotype, and climate-of-origin to freezing. Model assumptions for normality and homogeneity of variance were met unless otherwise noted. Linear regression determined F_v/F_{m50} values by fitting a line through temperatures where damage was indicated by reduced F_v/F_m . Freezing resistance mechanisms (avoidance or tolerance) were determined by assessment of the difference between F_v/F_{m50} and freezing point. All statistically significant results ($p < 0.05$) were evaluated using Tukey honestly significant difference criteria for pairwise comparisons. Tests were conducted in JMP 9.0.2 (SAS Institute Inc, 2010).

Results

Site Characteristics and Seedling Survival

Seedlings experienced high mortality, and by June of 2013, there was 10.9% survival at Coyote, 78.2% at Poen, 30.0% at Swan, and less than 5% survival at South Point and Kave. By May 2014, Poen had 71.8% survivorship whereas all other blocks experienced 100% mortality. Soils were generally considered silty, but the Poen block soils had 59.5% clay whereas all other blocks had less than 25% clay content. Soil characteristics, other than texture analysis, did not reveal any other significant differences between blocks (Table 4). The highest plant diversity was detected at the Poen block, but there were no significant differences between blocks in plant cover compositions

separated by functional groups in an ANOVA analysis (Table 5). Low survivorship at all other blocks reduced statistical inference for physiological analyses.

According to conditional logistic models, mortality patterns were more related to soil minimum temperatures (QIC=1378) compared to air minimum temperatures (QIC=1473), growing degree days $>10^{\circ}\text{C}$ (QIC=1462), or volumetric water content (QIC=1466). Survival probability of *A.t. tridentata* was positively related to minimum soil temperature in March, particularly for tetraploids (Table 6). In April and May, survival of diploid *A.t. tridentata* was also positively correlated to minimum soil temperature. However, survival of tetraploid groups in April and May were not linked to cold temperatures during those months (Figure 3). *A.t. wyomingensis* survival probability was also positively related to minimum temperatures in March, April, and June, but not in May (Table 6, Figure 3). Survival of *A.t. vaseyana* in March was not associated with survival for either cytotype, but positive correlations were detected for both groups in April, May, and June (Table 6, Figure 3).

Seedling survival within subspecies did not relate to climate-of-origin and minimum temperatures, and effects were statistically marginal but not considered significant. High mortality among the blocks reduced our ability to make inferences about climate-of-origin. Local climate *A.t. wyomingensis* seedlings had greater probability of survival relative to cool/wet climate-of-origin seedlings ($p=0.08$) during all months. Probability of survival of *A.t. tridentata* seedlings from drier climates was greater than local or cool/wet climates in March only ($p=0.09$). Local climate *A.t. vaseyana* survival probability was greater than that of cool/wet or dry climates-of-origin in May only ($p=0.075$, data not shown). While climate-of-origin did not have statistically

significant effects on survival, only three big sagebrush populations had 100% survivorship at the Poen block: IDW-2, local ssp. *wyomingensis* population, and MTW-3 and ORV-1 from relatively from cool/wet environments. MTW-3 was the only population that developed reproductive structures by the time the study ended in June 2014.

Seeding survival over the entire duration of the experiment was reduced for *A.t. vaseyana* relative to both *A.t. tridentata* and ssp. *wyomingensis* (Table 8, Figure 4). Survival of tetraploid groups was consistently greater than diploids for comparisons within subspecies (Figure 4): $\chi^2=6.8$, $p=0.009$ for *A.t. vaseyana* and $\chi^2=4.4$, $p=0.036$ for *A.t. tridentata*. Seedling survival over the entire duration of the experiment did not relate to climate-of-origin for any subspecies.

Growth

Growth patterns differed among subspecies and cytotypes (Wilks lambda =0.677, $F_{56, 235.56} = 2.09$, $p<.001$, Figure 5), but results are statistically marginal. Although the graph seems to suggest greater height among tetraploids, statistically significant height differences were detected between diploid and tetraploid *A.t. tridentata* (Figure 5) in March 2013 only. There were no significant differences between subspecies or cytotypes within subspecies at the end of the observation period in May 2014 (Figure 5).

To assess if height/survival trade-offs may have occurred, we compared height variation to relative differences in survival. Regression of growth and survival did not reveal any significant trends. However, diploid *A.t. tridentata* populations UTT-1 and ORT-2 had the greatest average heights of all populations and their survivorship was at 12% and 16% (61.3 mm and 57.57 mm respectively). Survival of other populations was

20-30%, and heights ranged from 30-45 mm. Survival of *A.t. wyomingensis* was 24%, and height averaged 46.5 mm.

Physiology

Chlorophyll fluorescence was not significantly different between subspecies or cytotypes in April 2013, November 2013, January 2014, and February 2014 (data not shown). Leaf $\delta^{13}\text{C}$ varied between subspecies and cytotype but significant differences were only detected between diploid *A.t. tridentata* and ssp. *vaseyana* $\delta^{13}\text{C}$. Diploid *A.t. vaseyana* $\delta^{13}\text{C}$ was -1.5 per mil more negative (less WUE) than in diploid $\delta^{13}\text{C}$ *A.t. tridentata* (Table 9, Figure 6, $p=0.029$). Pre-dawn water potential (Ψ_{leaf}) differed only among diploid and tetraploid *A.t. tridentata* (Table 9). Ψ_{leaf} of tetraploid *A.t. tridentata* was 1.4 MPa less negative than diploid *A.t. tridentata*. Photosynthesis measured in April 2014 did not differ among subspecies or cytotypes (Table 9). SLA measurements from October 2013 also did not differ between subspecies or cytotype (Table 9).

Freezing Response

Freezing points of *A.t. vaseyana* populations were 2-3°C significantly higher than for *A.t. wyomingensis* populations (Table 10, Figure 7a). *A.t. tridentata* had freezing points at $-10.51 \pm 0.71^\circ\text{C}$. No other significant differences were detected among subspecies x cytotype, except diploid populations of *A.t. vaseyana* had higher freezing points than *A.t. wyomingensis* and tetraploid *A.t. tridentata* (Table 9). Freezing point did not relate to climate-of-origin for any subspecies, but this result is potentially due to low sample size. Further, freezing point did not differ in subspecies, cytotype, or climate-of-origin for plant material collected in January (data not shown).

The temperature at which freezing damage occurred (lower F_v/F_{m50} temperatures indicate greater resistance to freezing) was significantly different among subspecies and cytotypes, but the range of F_v/F_{m50} among subspecies x cytotypes was much smaller than that observed for freezing point (Table 9, Table 10, Figure 7b). Among subspecies, *A.t. wyomingensis* had greater freezing resistance than both *A.t. vaseyana* and *ssp. tridentata* by approximately 1.1°C ($p=0.0017$). There were no differences in F_v/F_{m50} between cytotypes within each subspecies, but *A.t. wyomingensis* had 1.4°C greater freezing resistance than diploid *A.t. vaseyana* (Table 9). Freezing resistance did not relate to climate-of-origin for any subspecies.

The difference (in °C) between F_v/F_{m50} and freezing point (F_v/F_{m50} –freezing point) revealed variable freezing response strategies among subspecies and cytotypes (Figure 8). Tetraploid *A.t. tridentata* had a significantly higher F_v/F_{m50} –freezing point than tetraploid *A.t. vaseyana* (Table 10), indicating freezing avoidance and tolerance strategies respectively. F_v/F_{m50} –freezing point was not associated with climate-of-origin for any subspecies.

Discussion

The high mortality in our study relates to relatively cold and dry conditions in the 2012/13 winter and a dry spring 2013 (Figure 1b, Table 1). We attribute the high survivorship at Poen compared to the other four wildfires to be a result of high clay content in soils. This is potentially the result of subtle geomorphic differences among sites in an otherwise flat, plain landscape. The Poen study area is located on a terrace above an old stream channel, resulting in older age soils (Bull 1990) and higher clay composition of the soil.

Effects of Subspecies, Cytotype, and Climate-of-Origin on Overall Seedling Survival and Growth

Our results did not indicate greater overall survival of *A.t. wyomingensis* relative to *A.t. tridentata* and ssp. *vaseyana* (Figure 4, Table 8), which is in contrast to our hypothesis that local subspecies would have greater survival than other subspecies. *A.t. tridentata* had greater growth yet similar survival to *A.t. wyomingensis*. Enhanced success of *A.t. tridentata* (in an *A.t. wyomingensis* ecosites) may relate to greater stress overcome by extraction of deeper soil water resources or higher WUE in diploids ($\delta^{13}\text{C}$, Figure 6) and greater drought adaptation in tetraploids (Ψ_{leaf} , Table 9) within the subspecies. When water is limited, plants that use a finite water supply more efficiently should grow more rapidly (Wright et al. 1993). The rapid growth of *A.t. tridentata* may be a result of increased drought tolerance and adaptation in water-limited environments (Frank et al. 1986; Shultz 1986; Welch & McArthur 1986; Lambrecht et al. 2007), which enhanced overall survival. As anticipated, *A.t. vaseyana* (when considering both diploids and tetraploids together) experienced the highest mortality of all subspecies, perhaps due to native distribution at higher elevations where annual precipitation can be two or three times greater than the precipitation gradient throughout the elevational distribution of *A.t. wyomingensis* or ssp. *tridentata* (Miller et al. 1986; West 1988).

Polyploid cytotypes had higher survival and growth rates (depending on time of year) than diploids (Figure 3 & Figure 4), supporting our hypothesis and literature on polyploids in other shrubs and perennial forbs species (Lumaret et al. 1997). Increased capacity to occupy drier habitats among polyploids has been suggested or shown for other species (Baldwin 1941; Maherali et al. 2009; Manzaneda et al. 2012) and may

relate to resistance to drought-induced hydraulic failure (Hao et al. 2013). *A.t. wyomingensis* and ssp. *tridentata* exhibit greater resistance to cavitation and hydraulic failure than *A.t. vaseyana* (Kolb and Sperry 1999), but perhaps resistance to hydraulic failure increased with polyploidy, particularly for *A.t. vaseyana*, enhancing survival. Cytotype appears to be an important factor in explaining functional diversity, possibly even as important as subspecies itself for seedling establishment and survival.

We did not detect an effect of seed transfer (i.e., local or not) or climate-of-origin on big sagebrush seedling survival, although local seeds or those from warmer origins had greater growth. It was notable, in the relatively dry year of our study, that populations from warm/dry climates had no greater survival than populations from local and cool/wet climates. However, our ability to make inferences concerning the importance of climate-of-origin is limited. Significant mortality among all seedlings reduced our power to detect differences within population groups. The greater growth of the local and warmer-origin *A.t. tridentata* is consistent with Germino's (2014) seed source study.

Growth and Survival Tradeoffs?

Although intrinsic differences in height accompany different growth strategies of the subspecies, growth rates of seedlings of small stature should nonetheless be indicative of performance to some extent. *A.t. wyomingensis* was not taller than other subspecies, but is considered more stress-adapted and slow growing (Bonham et al. 1991). *A.t. wyomingensis* also may have evolved to achieve early maximum growth in the growing season and limit above ground growth as a mechanism of drought tolerance (Booth et al. 1990). Trade-offs between seedling height and survival are small and not statistically significant, but are indicative of subspecies establishment strategies. Diploid *A.t.*

tridentata populations UTT-1 and ORT-2 had greatest overall height of seedlings up until March 2014, but also greatest overall mortality. *A.t. tridentata*'s prolific seed production (Daubenmire 1975; Rosentreter 2004) may mitigate reproductive risk of an early establishment growth/survival trade-off.

Effects of Minimum Temperature on Seedling Survival

Exposure to late-winter extreme weather and subspecies variability in freezing response and growth all likely contributed to the pronounced mortality events occurring in spring 2013. Patterns in seedling survival from March to June 2013 as a function of minimum temperatures appeared related to physiological resistance to freezing via tolerance or alternatively avoidance mechanisms, supporting our hypothesis (Figure 3). Using the criteria for freezing avoidance versus tolerance outlined by Sakai and Larcher (1987), our data suggest big sagebrush's freezing resistance strategy is classified as freezing tolerance, except for tetraploid *A.t. tridentata*, an avoider (Figure 8). The relationship between F_v/F_{m50} and freezing point did not reveal a freezing response trade-off, meaning freezing tolerance or freezing avoidance mechanisms are not mutually exclusive (Figure 8a). However, plotting the relationship between freezing point and F_v/F_{m50} —freezing point revealed a gradient in the degree of freezing tolerance with tetraploid *A.t. vaseyana* being the most tolerant (Figure 8b).

F_v/F_{m50} —freezing point in *A.t. vaseyana* was most negative compared to all other cytotypes, indicating that it had strong tolerance of freezing but little compensatory ability to increase avoidance of ice formation in leaves. It would thus appear more likely to suffer more negative effects of freezing. The relatively higher elevations of *A.t. vaseyana* habitat, where snow and freezing temperatures are constant throughout the

duration of winter, might require seedlings to tolerate lengthy cold conditions, but while insulated by snow cover against harsh winter minimum temperatures. F_v/F_{m50} —freezing point in tetraploid *A.t. vaseyana* was even more negative (by 1.6°C) than diploid populations, suggesting polyploidy may enhance freezing tolerance. *A.t. wyomingensis* and diploid ssp. *tridentata* had relatively less freezing tolerance, which is consistent with their lower-elevational distributions.

Data collected for analysis of freezing response mechanisms occurred in May following significant mortality among seedlings (Figure 4), and perhaps revealed local selection for freezing response among subspecies and cytotypes. Freezing avoidance only protects plants for short periods of time (Goldstein et al. 1985; Rada et al. 1985), and if freezing point is not reached then the plant is not negatively affected by frost. Tetraploid *A.t. tridentata*, an “avoider,” had less capacity to cope with frost in March when minimum air temperatures were nearly identical to those in January (-10.21°C and -10.25°C respectively). Freezing events from April to June were episodic and less intense than in March (see Appendix A), and these conditions likely contributed to increased survival in tetraploid *A.t. tridentata*, as the plants freezing point was not reached during this period of time. Freezing avoidance may also correspond to high growth rates and drought resistance (Levitt 1980; Schulze et al. 2005); and from April to June, survival and growth of tetraploid *A.t. tridentata* was greatest relative to other groups.

“Avoiders” had the most mortality in March, whereas freezing “tolerators” experienced consistent mortality due to minimum temperatures from April to June. A putative investment in freezing tolerance at the expense of other growth (competitive) traits may limit the ability of seedlings to compete with faster growing species when

freezing events are infrequent (Medeiros et al. 2012) resulting in mortality. However, survival of *A.t. wyomingensis* (the least frost tolerant among “tolerators”) did not correspond to minimum temperatures in May, unlike other freezing tolerant subspecies and cytotypes. *A.t. wyomingensis* has the lowest freezing point (most negative) among freezing tolerant groups, and perhaps reduced mortality in May indicates an ability to avoid ice formation in leaves, similar to an “avoider.” The degree to which *A.t. wyomingensis* can tolerate freezing (Figure 8b) may mitigate for the use of both strategies pending weather conditions.

Ecophysiological parameters including water potential and leaf $\delta^{13}\text{C}$ indicate differences among subspecies and cytotypes in water balance, and these factors presumably contribute to patterns in mortality. Water potential (Ψ_{leaf}) differed between tetraploid *A.t. tridentata* and diploid ssp. *tridentata*, signifying that tetraploid *A.t. tridentata* is more drought adapted than diploids within the subspecies. It is likely that increased drought tolerance is associated with greater survival of tetraploid *A.t. tridentata*. Further, high leaf $\delta^{13}\text{C}$ (less negative) is expected to confer physiological benefits in desert environments. Low leaf $\delta^{13}\text{C}$ (most negative) in diploid *A.t. vaseyana* shows poor water-use efficiency in this cytotype, and is indicative of *A.t. vaseyana*’s adaptation to cooler and wetter environments. Tetraploid *A.t. vaseyana* had similar leaf $\delta^{13}\text{C}$ to both *A.t. wyomingensis* and tetraploid ssp. *tridentata*, and perhaps polyploidy enhanced drought tolerance in *A.t. vaseyana*, resulting in greater overall relative to diploids (Figure 6, Table 9). Differences in water balance among subspecies and cytotypes are evident and expected; however, survival models including water-related climate variables (VWC) did not explain patterns in mortality to the same extent as

minimum temperatures (from model selection). These findings indicate the importance of minimum temperatures and freezing responses in big sagebrush, explaining big sagebrush adaptation and functional diversity.

In summary, our research indicates that seedling mortality, growth, and response to freezing differ at the subspecies, cytotype, and population level. Physiological differences among the three subspecies were largely driven by cytotype within subspecies, indicating that cytotype may be as important as subspecies in explaining adaptation and functional diversity in big sagebrush. By evaluating sagebrush seedlings during their critical establishment phase, we were able to determine physiological responses contributing to population and subspecies distributions. Our hypotheses regarding cytotype and freezing response were supported; however, results indicate local seed sources may not demonstrate greater survival than those from other climates. We saw strong selection for minimum temperature response in a cold and dry year, and population-specific freezing resistance mechanisms may emerge as important criteria for seed source selection for restoration in the future.

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Tables

Table 1. Average precipitation (mm) and average temperature for 2013 compared to average temperature and precipitation from 1999-2010 at Boise WSFO Airport weather station

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2013 Average Precipitation (mm)	30.73	16.00	9.14	24.13	19.05	10.41	3.30	11.43	44.45	19.30	37.85	16.76
2013 Average Temperature °C	-6.89	1.92	7.25	9.97	16.11	21.08	27.47	25.69	19.22	10.08	4.31	-4.42
1999-2010 Average Precipitation (mm)	31.50	28.19	34.04	31.75	33.53	19.05	8.13	6.86	13.97	20.07	33.78	37.85
1999-2010 Temperature °C	-0.56	1.92	7.22	10.56	15.00	20.00	24.44	23.89	18.33	11.67	4.44	-0.56

Table 2. Description of big sagebrush seed sources utilized in this study

<i>Provenance</i>	<i>State</i>	<i>Location</i>	<i>Subspecies</i>	<i>Climate-of-origin</i>	<i>Ploidy</i>
IDT-2	ID	Orchard (Local)	Tridentata	Local	2N
BOP-W	ID	Birds of Prey	Wyomingensis	Local	4N
IDV-2	ID	(Local)	Vaseyana	Local	2N
NMT-2	NM	San Luis Mesa	Tridentata	Dry	4N
UTT-1	UT	Canyon B.	Tridentata	Cool/Wet	2N
IDW-2	ID	Orchard (Local)	Wyomingensis	Local	4N
ORT-2	OR	Echo	Tridentata	Warm/Dry	2N
MTW-3	MT	Montana	Wyomingensis	Cool/Wet	4N
ORV-1	OR	Lookout Mountain	Vaseyana	Cool/Wet	4N
IDW-3	ID	Sommer Camp	Wyomingensis	Dry	4N
IDV-3	ID	Stanley	Vaseyana	Cool/Wet	2N

Table 3. Climate information for seeds sources used in this experiment. BOPW is not included in the table and is considered to have a similar climate to IDW2, both are local and collected in similar areas.

Seed Source	Longitude	Latitude	Elevation (m)	Mean Annual Temp °C	Mean Annual Precip (mm)	Growing Season Precip (mm)	Mean Temp of the Coldest Month°C	Min Temp of the Coldest Month°C	Mean Temp of the Warmest Month°C	Max Temp of the Warmest Month°C	Frost Free Period (Days)	Growing Degree Days > 5°C
IDT2	-116.0081	43.3371	939	10.4	324	121	-1.6	-6.1	23.4	32.7	158	2633
MTW3	-108.7832	45.2066	1458	6.3	365	250	-6.7	-13.2	19.7	28.6	107	1809
UTT1	-112.5087	37.9933	2106	7	351	189	-3.9	-11.7	19.4	29.1	96	1804
NMT2	-107.1478	35.7572	1979	9.9	265	170	-2.3	-9.8	22.2	31.2	136	2516
IDW2	-116.0037	43.3274	977	10.2	333	122	-1.8	-6.3	23.1	32.4	155	2576
IDW3	-116.8531	43.4657	870	10	256	112	-2	-6.8	22.1	32.3	133	2471
IDV2	-115.9738	43.6776	1003	9.4	470	153	-2.6	-7.1	22.2	32	141	2383
IDV3	-114.6402	44.259	1766	4	339	153	-8.3	-15.1	16.5	27.7	51	1316
CAT2	-118.5704	37.7973	1974	8.9	250	89	-1.2	-8.5	20.5	29.6	123	1553
ORT2	-119.2076	45.7589	223	11.5	239	80	1	-3	22.8	31.6	176	2795
ORV1	-117.3233	44.4883	986	8.1	386	138	-4.4	-9.1	20.9	30.5	122	2115

Table 4. Percent carbon, nitrogen, and soil organic matter at 2 and 15 cm soil depth on 5 burned experimental blocks located at BOP NCA. Soil compositions did not differ significantly between blocks at $\alpha=0.05$.

Site	%N-2cm		% N-15cm		%C-2cm		%C-15cm		%SOM-2cm		%SOM-15cm	
	<i>Mean</i>	<i>SE</i>	<i>Mean</i>	<i>SE</i>	<i>Mean</i>	<i>SE</i>	<i>Mean</i>	<i>SE</i>	<i>Mean</i>	<i>SE</i>	<i>Mean</i>	<i>SE</i>
Coyote	0.075	0.012	0.062	0.008	0.659	0.187	0.617	0.113	1.291	0.195	2.155	0.217
Kave	0.104	0.012	0.063	0.009	1.117	0.161	0.577	0.129	1.569	0.294	1.454	0.300
Poen	0.081	0.011	0.063	0.008	0.770	0.145	0.590	0.120	0.956	0.088	1.950	0.238
SP	0.122	0.013	0.080	0.009	1.289	0.173	0.977	0.129	1.886	0.313	2.288	0.188
Swan	0.085	0.012	0.059	0.008	0.816	0.161	0.667	0.113	1.527	0.234	1.759	0.183
	F	<i>p</i> -value	F	<i>p</i> -value	F	<i>p</i> -value	F	<i>p</i> -value	F	<i>p</i> -value	F	<i>p</i> -value
	2.380	0.072	0.980	0.431	2.310	0.078	1.710	0.170	2.100	0.132	2.072	0.135

Table 5. Percent cover by functional group across all five blocks at BOPNCA.

<i>Site</i>	% Cover							
	<i>Bare Ground</i>	<i>Litter</i>	<i>Rock</i>	<i>Moss/Lichen</i>	<i>Native Grass</i>	<i>Cheatgrass</i>	<i>Forbs</i>	<i>Shrubs</i>
S.Point	45.5	24.3	5.0	0.0	20.8	0.0	3.9	0.0
Coyote	29.8	3.0	0.0	7.9	61.5	0.0	1.2	1.0
Swan	64.5	23.0	6.3	0.0	18.1	0.0	0.0	0.0
Poen	15.0	15.9	0.0	7.9	50.5	7.3	3.6	2.5
Kave	19.0	42.7	0.0	0.0	47.5	0.0	0.0	0.0

Table 6. Conditional logistic output of model comparing effects of month, cytotype, and minimum soil temperature on binomial response (alive or dead) of sagebrush seedlings. Positive coefficients show increased hazards of death relative to the “base level” of the explanatory variable to which all comparisons are made. Base levels are alphabetic; for cytotype the base level is *ssp. tridentata*, 2n; April is base level for month and for soil minimum temperature the base is 0°C. Block was treated as a clustering variable to account for site-specific heterogeneity. Significance of explanatory variables is given by the Wald statistic with respective *p*-values. Asterisks indicate level of significance: *=*p*>0.05, **=*p*>0.01, ***=*p*> 0.001.

Variable	Estimate	SE	Wald	<i>p</i> -Value	
(Intercept)	-0.377	0.162	5.390	0.020	*
Minimum Soil Temperature	0.218	0.039	31.020	0.000	***
<i>ssp. tridentata</i> , 4n	0.931	0.249	13.990	0.000	***
<i>ssp. vaseyana</i> , 2n	-0.172	0.379	0.210	0.650	
<i>ssp. vaseyana</i> , 4n	-0.152	0.314	0.230	0.630	
<i>ssp. wyomingensis</i>	0.168	0.133	1.590	0.208	
June	-3.216	1.483	4.700	0.030	*
March	1.710	0.465	13.530	0.000	***
May	0.317	0.339	0.870	0.350	
Minimum Soil Temp x <i>ssp. tridentata</i> , 4n	-0.226	0.099	5.200	0.023	*
Minimum Soil Temp x <i>ssp. vaseyana</i> , 2n	0.046	0.149	0.090	0.760	
Minimum Soil Temp x <i>ssp. vaseyana</i> , 4n	0.169	0.123	1.890	0.169	
Minimum Soil Temp x <i>ssp. wyomingensis</i>	0.139	0.051	7.390	0.007	**
Minimum Soil Temp x June	0.114	0.155	0.540	0.462	
Minimum Soil Temp x March	0.052	0.123	0.180	0.673	
Minimum Soil Temp x May	-0.185	0.088	4.440	0.035	*
<i>ssp. tridentata</i> , 4n x June	-0.702	1.829	0.150	0.701	
<i>ssp. vaseyana</i> , 2n x June	-1.303	1.413	0.850	0.357	
<i>ssp. vaseyana</i> , 4n x June	2.319	0.803	8.330	0.004	**
<i>ssp. wyomingensis</i> x June	0.364	1.647	0.050	0.825	
<i>ssp. tridentata</i> , 4n x March	0.095	0.359	0.070	0.791	
<i>ssp. vaseyana</i> , 2n x March	-0.568	0.485	1.370	0.242	
<i>ssp. vaseyana</i> , 4n x March	-0.561	0.694	0.650	0.419	
<i>ssp. wyomingensis</i> x March	0.016	0.247	0.000	0.949	
<i>ssp. tridentata</i> , 4n x May	0.221	0.413	0.290	0.593	
<i>ssp. vaseyana</i> , 2n x May	0.129	0.346	0.140	0.709	
<i>ssp. vaseyana</i> , 4n x May	-0.077	0.225	0.120	0.732	
<i>ssp. wyomingensis</i> x May	0.244	0.127	3.720	0.054	.
Minimum Soil Temp x <i>ssp. tridentata</i> , 4n x June	0.209	0.188	1.230	0.267	
Minimum Soil Temp x <i>ssp. vaseyana</i> , 2n x June	0.103	0.133	0.600	0.437	
Minimum Soil Temp x <i>ssp. vaseyana</i> , 4n x June	-0.320	0.066	23.540	0.000	***
Minimum Soil Temp x <i>ssp. wyomingensis</i> x June	-0.128	0.134	0.910	0.339	
Minimum Soil Temp x <i>ssp. tridentata</i> , 4n x March	0.614	0.136	20.290	0.000	***

Minimum Soil Temp x ssp. <i>vaseyana</i> , 2n x March	-0.255	0.172	2.200	0.138	
Minimum Soil Temp x ssp. <i>vaseyana</i> , 4n x March	-0.470	0.182	6.700	0.010	**
Minimum Soil Temp x ssp. <i>wyomingensis</i> x March	-0.124	0.069	3.230	0.072	
Minimum Soil Temp x ssp. <i>tridentata</i> , 4n x May	0.064	0.049	1.720	0.190	
Minimum Soil Temp x ssp. <i>vaseyana</i> , 2n x May	-0.047	0.138	0.120	0.732	
Minimum Soil Temp x ssp. <i>vaseyana</i> , 4n x May	-0.001	0.128	0.000	0.997	
Minimum Soil Temp x ssp. <i>wyomingensis</i> x May	-0.200	0.048	17.110	0.000	***

Table 7. Coefficients for calculating the probability of survival as a function of minimum soil temp. The coefficients are presented as the equation for the line (slope and intercept) holding ploidy and month constant for every one unit increase in minimum soil temperature (indicated by “t”). Standard errors of the coefficients are below the line equation. Superscript numbers next to standard errors indicate coefficients that are different from one another. Coefficients not connected by the same number are statistically different.

<i>Ploidy</i>	<i>March</i>	<i>April</i>	<i>May</i>	<i>June</i>
<i>ssp. tridentata</i> , 2n	1.34 + 0.27t (0.44) (0.12) ^{1,2}	-0.38 + 0.22t (0.16) (0.04) ²	-0.06 + 0.03t (0.23) (0.06) ¹	-3.60 + 0.33t (1.43) (0.13) ^{1,2}
<i>ssp. tridentata</i> , 4n	2.36 + 0.66t (0.30) (0.12) ³	0.55 + -0.01t (0.36) (0.13) ¹	1.09 + -0.13t (0.34) (0.07) ³	-3.37 + 0.32t (0.56) (0.05) ¹
<i>ssp. vaseyana</i> , 2n	0.62 + -0.03t (0.38) (0.07) ²	-0.53 + 0.39t (0.44) (0.16) ²	-0.29 + 0.20t (0.21) (0.03) ¹	-1.43 + 0.18t (0.86) (0.08) ¹
<i>ssp. vaseyana</i> , 4n	0.60 + 0.06t (0.31) (0.08) ²	-0.55 + 0.26t (0.50) (0.19) ²	-0.10 + 0.03t (0.23) (0.09) ¹	-5.07 + 0.48t (0.38) (0.03) ²
<i>ssp. wyomingensis</i>	1.52 + 0.29t (0.20) (0.07) ²	-0.21 + 0.36t (0.27) (0.09) ²	0.35 + -0.03t (0.20) (0.07) ²	-3.06 + 0.34t (0.96) (0.08) ¹

Table 8. Coefficients of survival model. Positive coefficients show increased hazards of death relative to the base level of the explanatory variable, which is *A.t. wyomingensis* for both subspecies and cytotype. Asterisks indicate level of significance: $*=p>0.05$, $**=p>0.01$, $***p>0.001$.

<i>Subspecies</i>		<i>Coefficient</i>	<i>Hazard</i>	<i>SE</i>	<i>z</i>	<i>p-Value</i>	
<i>A.t. tridentata</i>		0.141	1.151	0.110	1.285	0.199	
	<i>Cytotype</i>						
	ssp. <i>tridentata</i> , 2n	0.244	1.276	0.118	2.056	0.040	*
	ssp. <i>tridentata</i> , 4n	-0.124	0.884	0.175	-0.708	0.479	
<i>A.t. vaseyana</i>		0.371	1.449	0.121	3.079	0.002	**
	<i>Cytotype</i>						
	ssp. <i>vaseyana</i> , 2n	0.552	1.737	0.135	4.094	<0.001	***
	ssp. <i>vaseyana</i> , 4n	0.071	1.073	0.178	0.397	0.692	

Table 9. Average values (SE $\pm$ 1) and statistical results from one-way ANOVA tests comparing the effects of cytotype subspecies on seedling physiology. Those analyses that were statistically significant at $\alpha=0.05$ are shown in bold. Letters indicate means that are significantly different using Tukey HSD.

Variable	<i>ssp. tridentana</i>				<i>ssp. vaseyana</i>				<i>ssp. wyomingensis</i>		ANOVA	
	2n		4n		2n		4n		4n		Cytotype	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	F	p-value
WUE (δ^{13})	-27.204^A	0.309	-27.949 ^{AB}	0.438	-28.765^B	0.309	-27.930 ^{AB}	0.438	-28.031 ^{AB}	0.219	3.20	0.029
Freezing resistance (F_v/F_{m50}) °C	-15.441 ^{AB}	0.311	-15.137 ^{AB}	0.491	-15.078^A	0.311	-16.039 ^{AB}	0.490	-16.542^B	0.209	5.29	0.005
Freezing point °C	-9.673 ^{AB}	0.803	-12.610^B	1.270	-8.061^A	0.803	-7.441 ^{AB}	1.270	-10.995^B	0.541	4.41	0.010
SLA (cm ² /mg)	10.394	1.727	6.604	2.992	6.763	2.116	9.7360	2.992	8.333	1.496	0.61	0.669
Ψ_{leaf} (MPa)	-2.933^A	0.221	-4.300^B	0.382	-3.000 ^{AB}	0.296	-3.933 ^{AB}	0.382	-3.175 ^{AB}	0.191	3.41	0.022
Photosynthesis Amax($\mu\text{mol}^{-2}\text{s}^{-1}$)	10.260	1.195	11.698	1.952	10.263	1.512	8.850	1.952	9.115	0.976	0.48	0.751

Table 10. Freezing resistance of 11 populations of big sagebrush by subspecies and cytotype. Difference corresponds to F_v/F_{m50} – freezing point magnitude. Significantly different values notated with asterisks ($p < 0.05$) indicate a freezing tolerance strategy. Data correspond to mean values $\pm 1SE$. Due to limited plant material, provenance IDT2 consists of one individual. Six outlying exotherm temperatures were removed from means estimation and analysis.

<i>Provenance</i>	<i>Cytotype</i>	<i>Exotherm °C</i>	F_v/F_{m50}	<i>Difference</i>
IDT2	ssp. <i>tridentata</i> , 2n	-12.30	-15.52	-3.22
ORT2	ssp. <i>tridentata</i> , 2n	-9.70 ± 0.14	-15.06 ± 0.57	-5.22*
UTT1	ssp. <i>tridentata</i> , 2n	-8.32 ± 0.46	-14.89 ± 0.77	-6.11*
NMT2	ssp. <i>tridentata</i> , 4n	-12.61 ± 0.33	-15.33 ± 0.3	-2.39
IDV2	ssp. <i>vaseyana</i> , 2n	-8.12 ± 0.78	-15.50 ± 0.37	-6.60*
IDV3	ssp. <i>vaseyana</i> , 2n	-9.44 ± 1.67	-15.08 ± 0.66	-3.97*
ORV1	ssp. <i>vaseyana</i> , 4n	-7.44 ± 0.85	-15.80 ± 0.42	-7.51*
BOPW	ssp. <i>wyomingensis</i>	-13.13 ± 0.08	-16.75 ± 0.3	-3.55*
IDW2	ssp. <i>wyomingensis</i>	-10.70 ± 0.6	-15.86 ± 0.29	-4.56*
IDW3	ssp. <i>wyomingensis</i>	-11.40 ± 0.85	-16.65 ± 0.05	-4.40*
MTW3	ssp. <i>wyomingensis</i>	-7.58 ± 0.12	-17.21 ± 0.22	-9.51*

Figures

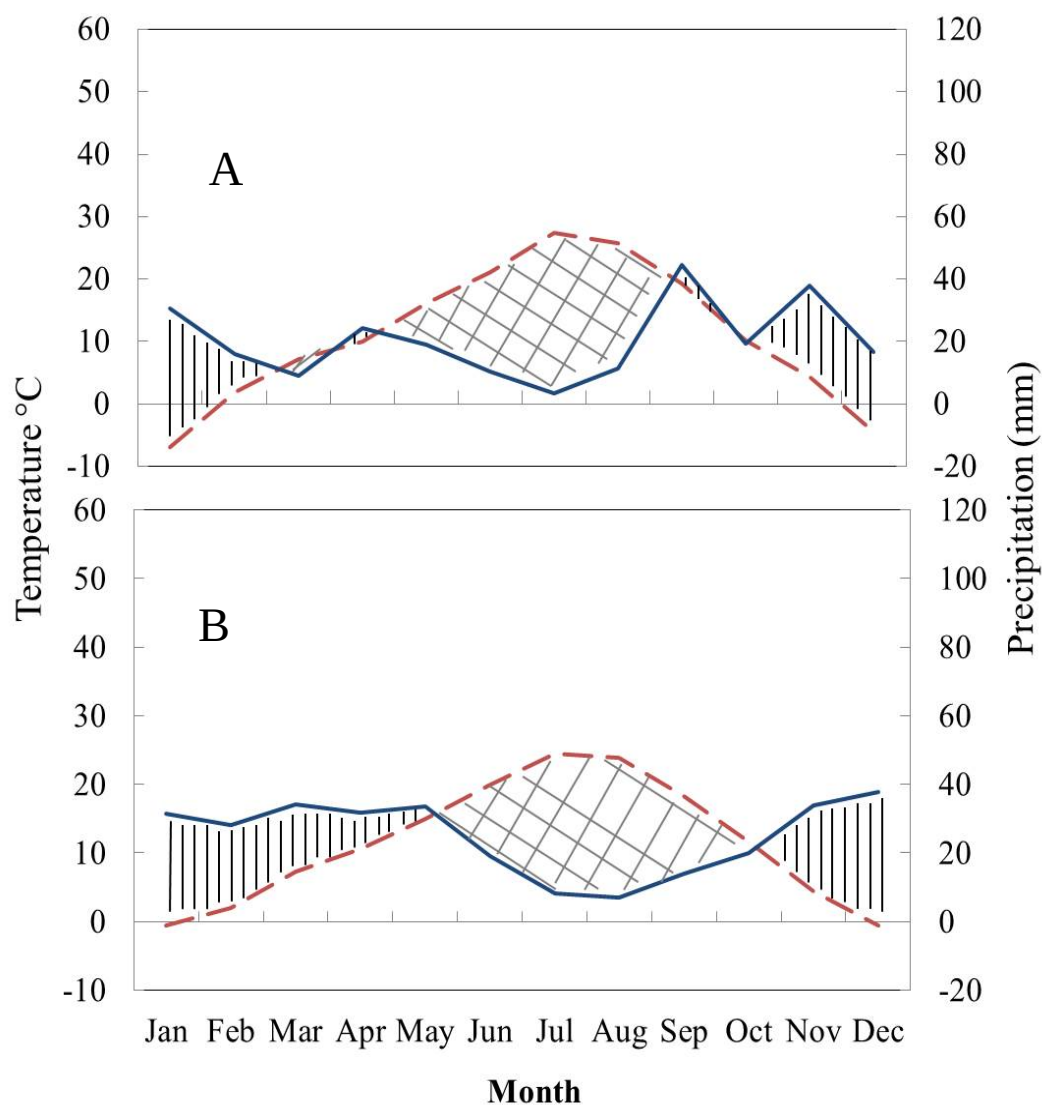


Figure 1. Walter-type climate diagram showing monthly average temperature (air) precipitation and Boise WSFO Airport weather station for (A) 2013 and (B) average values from 1981-2010. Dark vertical bars indicate periods when water is gained by the system and grey hatched areas indicate when the system experienced a net deficit of water. See Table 1 for values.

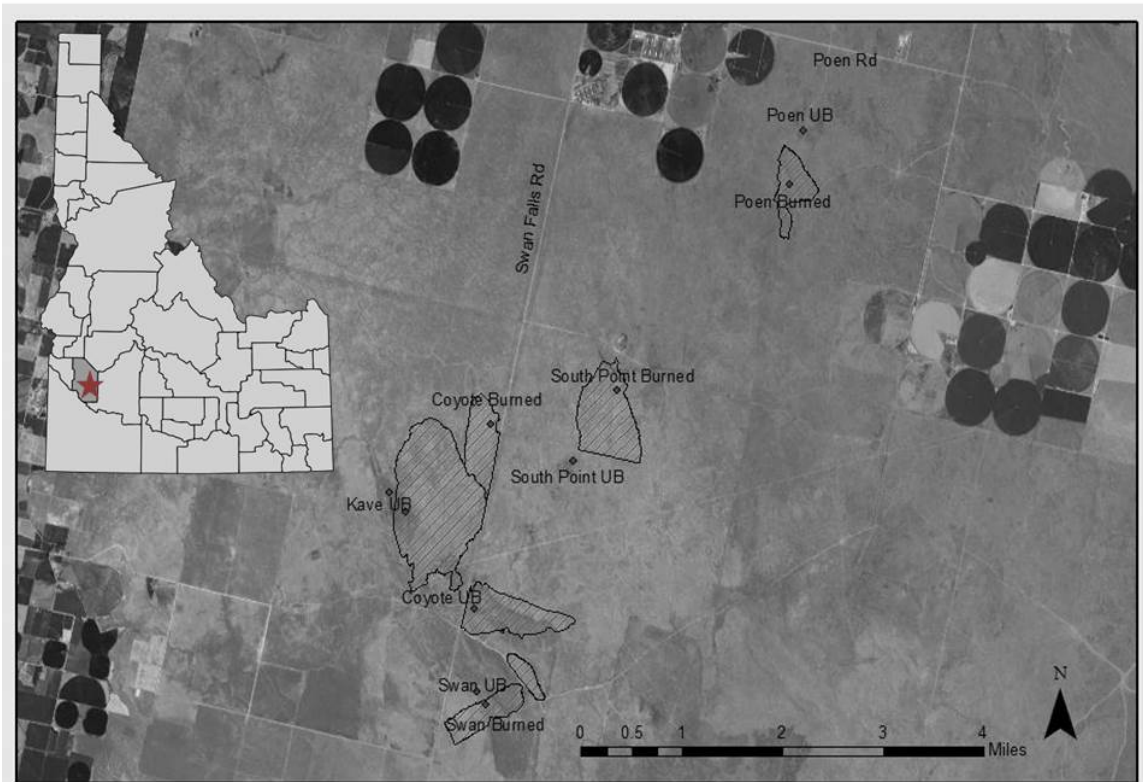


Figure 2. Map showing location of study site relative to location in southwestern Idaho. Hatched areas in the map are burned and “UB” refers to unburned sites not discussed in this chapter.

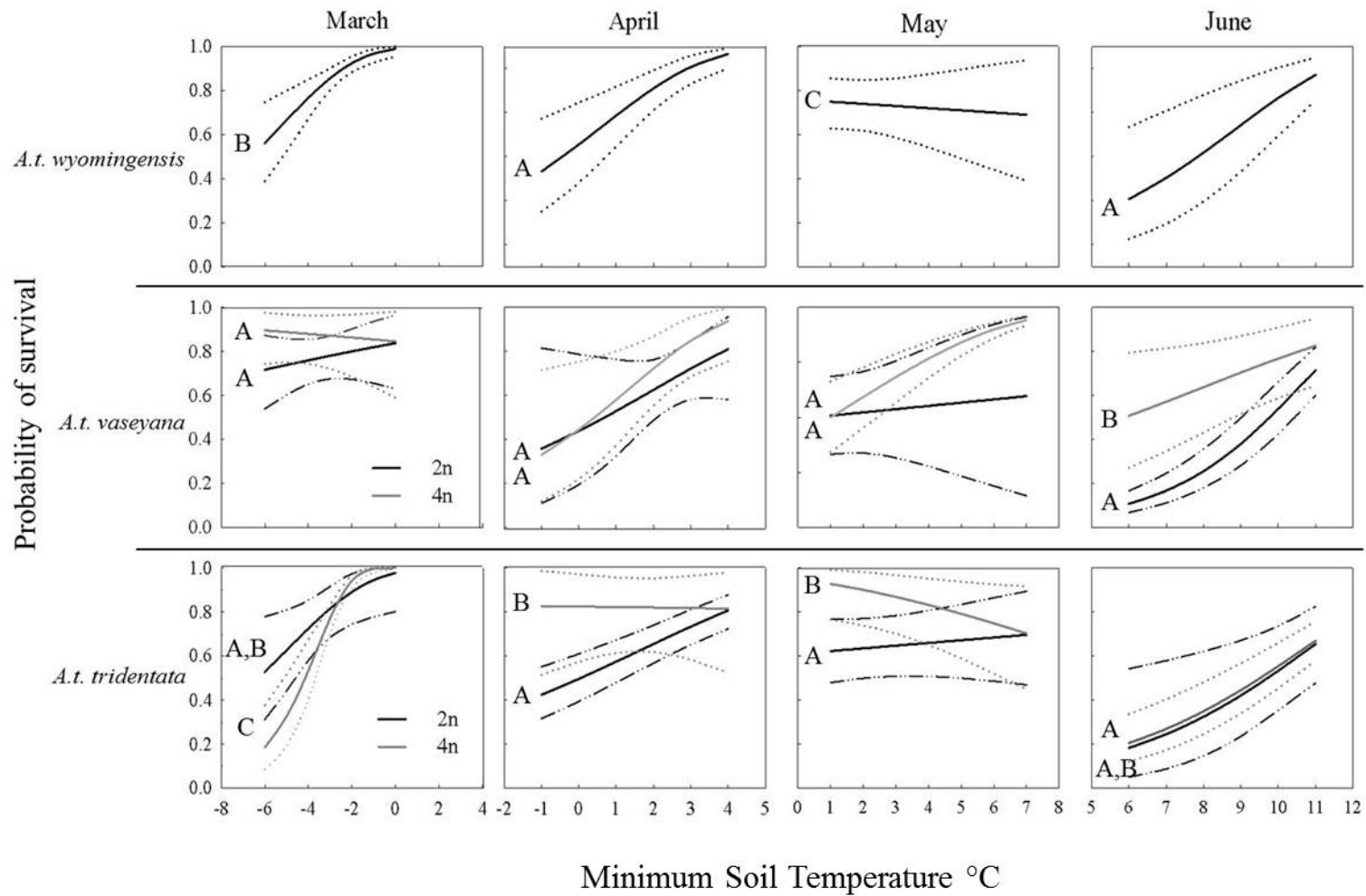


Figure 3. Binomial general linearized model showing predicated probability of survival (1=alive, 0=dead) for subspecies x cytotype as a function of minimum soil temperature in March, April, May, and June 2013. Letters indicate subspecies x cytotype significant differences at $\alpha=0.05$. See Tables 4 and 5 for statistics.

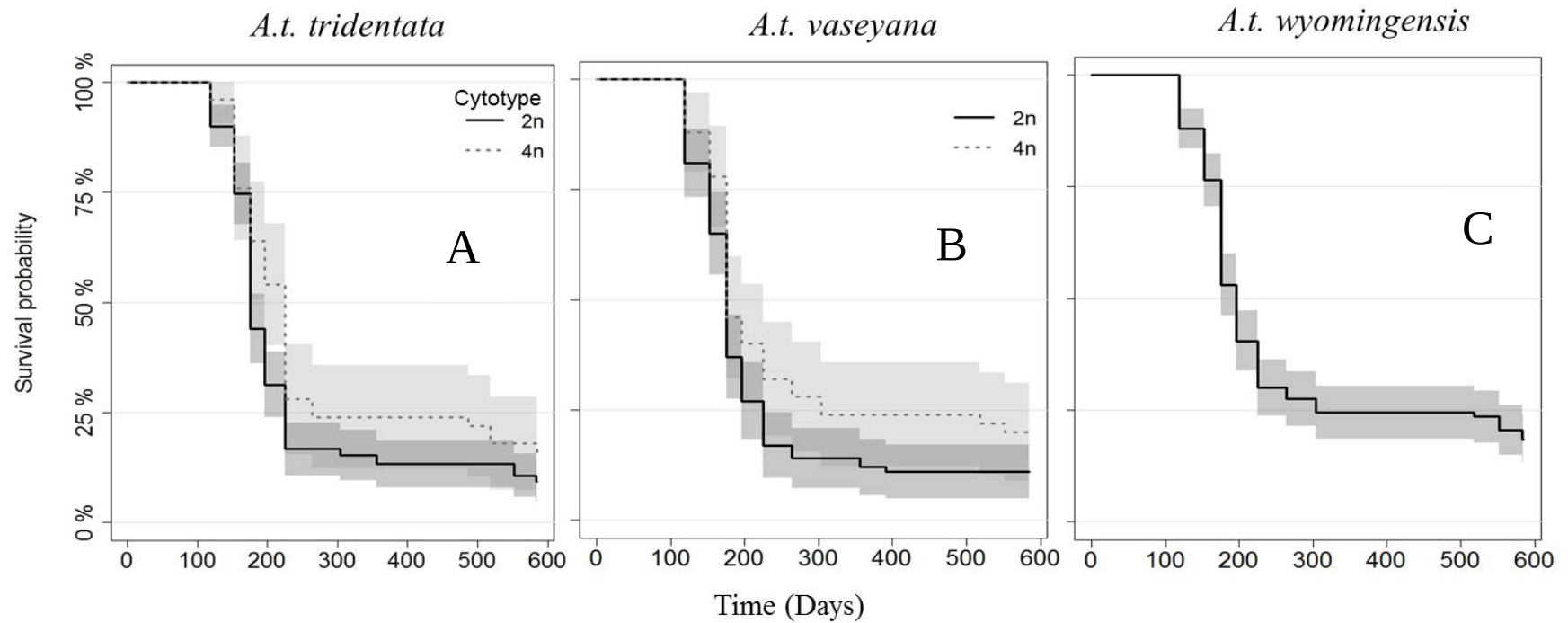


Figure 4. Non-parametric survival curves for all three subspecies of big sagebrush seedlings across all blocks from October 2012 to May 2014. Survival distributions by cytotype are different, using the log-rank test. For *A. t. tridentata*, $p=0.0.012$ (A); for *A. t. vaseyana*, $p=0.005$ (B); and no diploid cytotype for *A.t. wyomingensis* (C). Shaded intervals represent 95% confidence intervals.

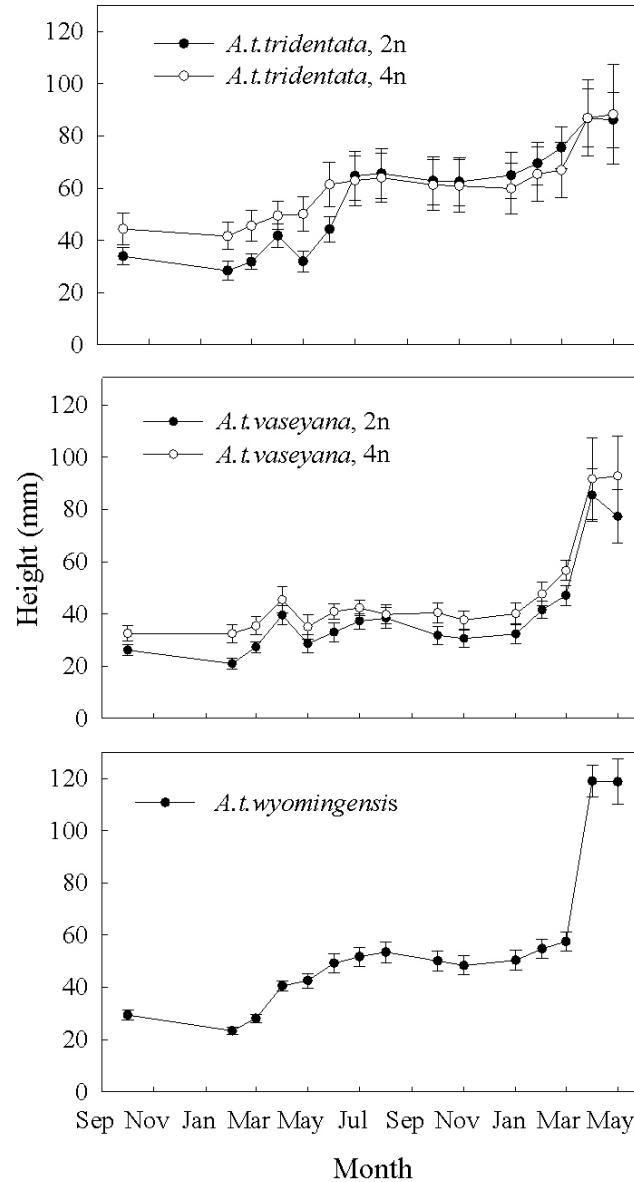


Figure 5. Average plant heights (mm) from the Poen block only October 2012 to May 2014 by subspecies and cytotype. Plants that died were removed from graph. At the end of the observation period, no significant differences were detected within subspecies by cytotype or between all three subspecies. Bars represent the SE of individual plants not plots. Statistically significant height differences were detected between diploid and tetraploid *A. t. tridentata* (Figure 5) in March 2013 only when evaluating differences in height from March to June 2013 when the most mortality was occurring.

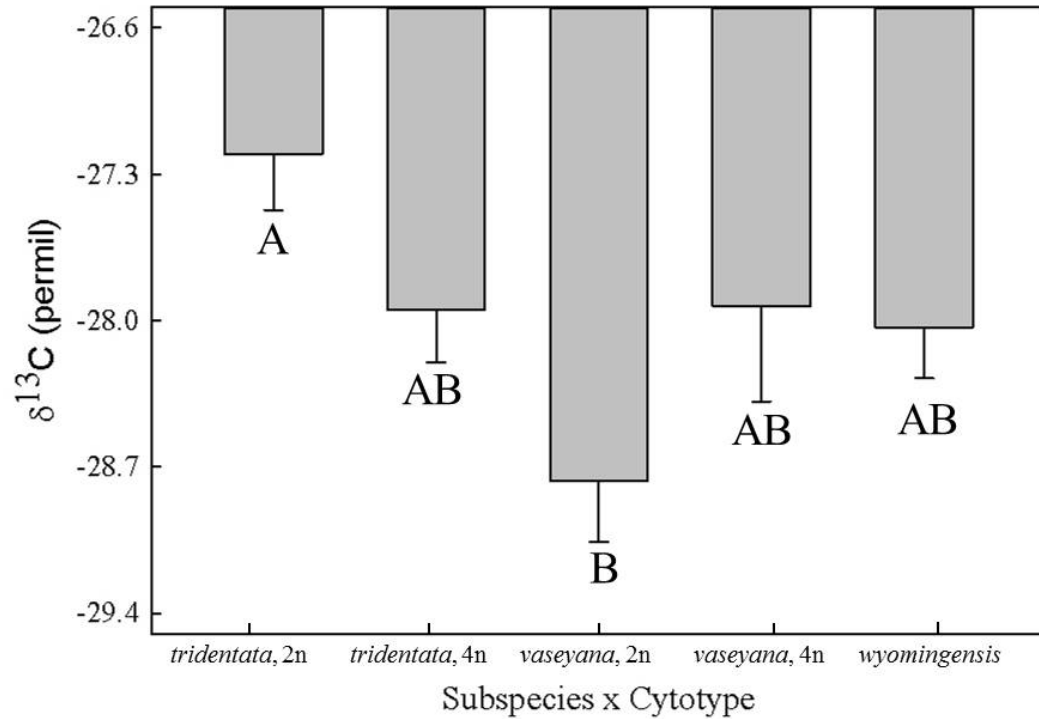


Figure 6. Variation in $\delta^{13}\text{C}$ of leaves of sagebrush by cytotype and subspecies in January 2014 ($p=0.029$). Letters indicate significant difference at $\alpha=0.05$ between groups. $\delta^{13}\text{C}$ was significantly less (indicating greater WUE) in diploid *A.t. tridentata* compared to diploid *A.t. vaseyana*. Error bars are SE.

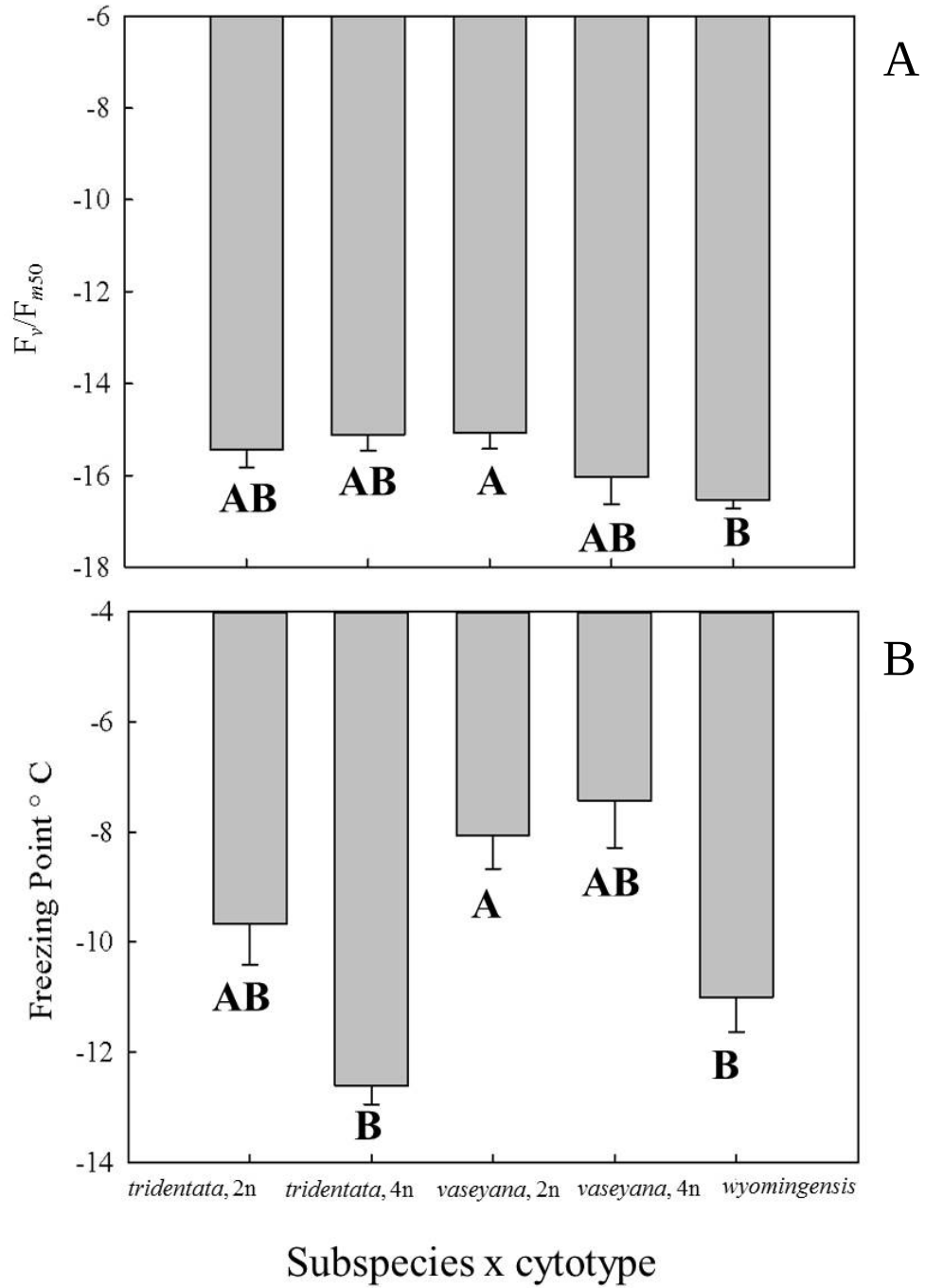


Figure 7. Variation in F_v/F_{m50} °C (A) and Freezing Point °C (B) between subspecies and cytotypes. Letters indicate significant difference at $\alpha=0.05$ between groups. See Table 9 for statistics.

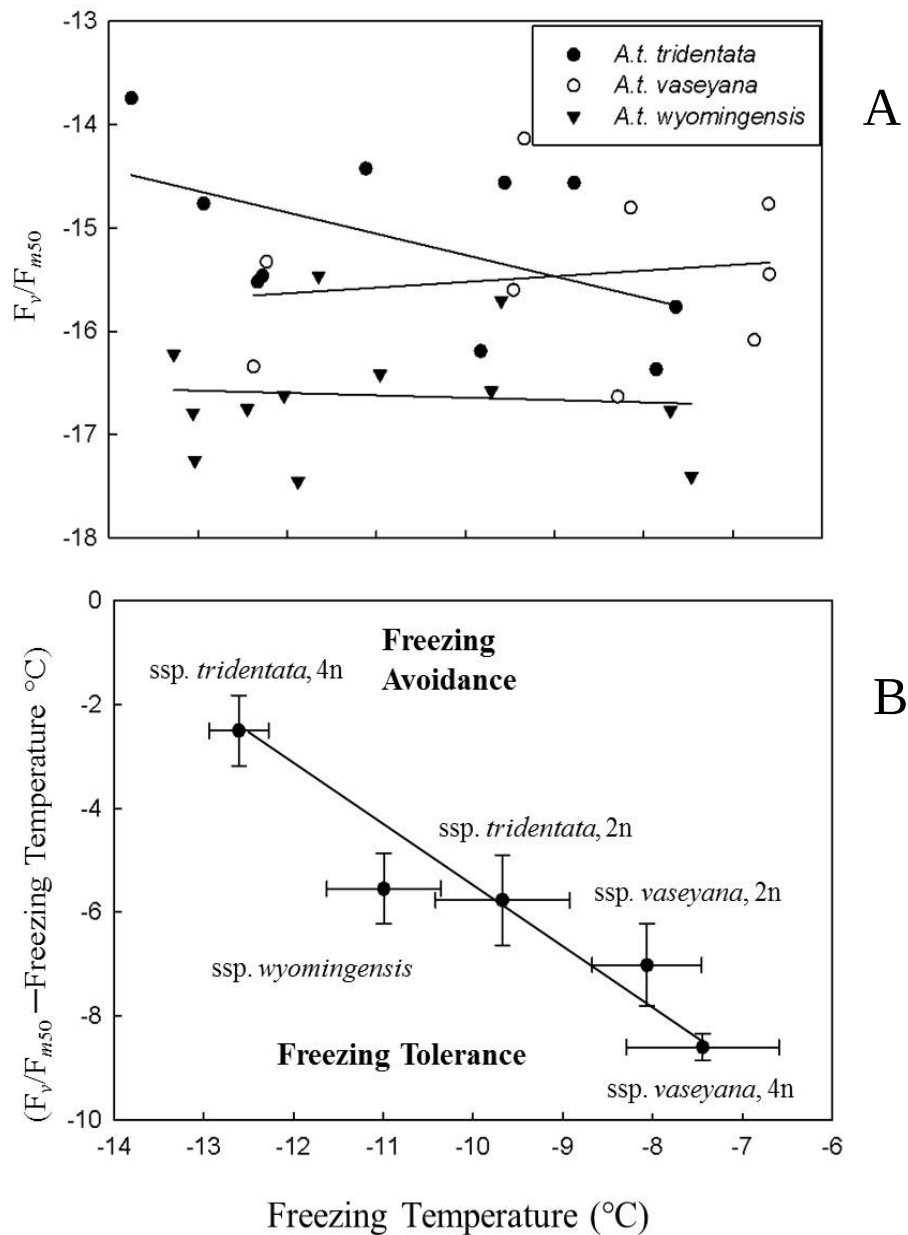


Figure 8. The relationship of temperature causing 50% loss of F_v/F_m (F_v/F_{m50}) to freezing point (A, all three regression lines $R^2 < 0.05$) in contrast to the relationship of the difference between F_v/F_{m50} and freezing point (" F_v/F_{m50} - Freezing Point $^\circ\text{C}$ ") to freezing point by subspecies and cytotype ($R^2 = 0.776$). Significant differences were detected between tetraploid *A.t. tridentata* and tetraploid ssp. *vaseyana*, only; however, these results indicate a spectrum of freezing response adaption. These data are following most mortality (Figure 4) and perhaps show selection of freezing avoidant vs. tolerant subspecies x cytotypes.

CHAPTER 2: EXPERIMENTAL WARMING SUPPORTS IMPORTANCE OF MINIMUM TEMPERATURE TO POST-FIRE ESTABLISHMENT

Abstract

Minimum temperatures are directly linked to the greenhouse effect, and are the only climate change variable predicted to increase with certainty. Correlative evidence suggests that responses of big sagebrush seedlings to minimum temperatures are particularly important for explaining adaption and diversity of big sagebrush (Chapter 1). We used *in situ* experimental warming to increase minimum temperatures, and test the effects of warming on seedling physiological performance for the three dominant subspecies of big sagebrush: *A.t. tridentata*, *A.t. vaseyana*, and *A.t. wyomingensis*. We measured a suite of ecophysiological parameters including survival, growth, water balance, photosynthesis, and freezing responses. Our results indicate that warming did not affect overall seedling survival; however, experimental warming did influence seedling survival as a function of time and weather. We found that warming increased survival probability of *A.t. tridentata* in late spring, likely due to shifts in resource allocation from freezing protection to greater growth. Survival of *A.t. wyomingensis* did not differ between warmed and control treatments, contrary to predicted enhancement of survival under warmed conditions. Experimental warming did not alleviate stress for *A.t. wyomingensis* seedlings due to greater frost resistance (most negative F_v/F_{m50}) than other subspecies, as well as local adaptation to the study site. However, growth of *A.t. wyomingensis* was reduced on warmed plots, indicating that warmer winters could result in slower growth patterns increasing overall time to establishment. High mortality of *A.t.*

vaseyana was anticipated due to ill-adaptation to the study site and appeared to relate to drought stress and greater vulnerability to minimum temperature exposure. Our results indicated that experimental warming may alter seedling freezing response and thereby affect growth and survival of big sagebrush. We concluded that freezing response should be considered when predicting changes in subspecies distributions, as well as in seed source selection for restoration.

Introduction

Most inferences on plant species distribution are drawn from correlations (i.e., species distribution models), but it can be difficult to separate biotic and disturbance effects. This is particularly true for species such as big sagebrush which is widely distributed and affected by fire and invasive species. Thus, there is an experimental need for isolation of climate effects on big sagebrush. In the last decade, millions of acres of sagebrush habitat have been burned by wildfire and reseeded with sagebrush, but restoration success is variable, often resulting in few sagebrush established (Arkle et al. 2014; Knutson et al. 2014). Species distribution models indicate that minimum temperatures are important factors in determining big sagebrush occurrences in the Great Basin Desert. Climate projections for the Great Basin Desert estimate a 2.5–3°C increase in mean temperatures and a decrease in number of days when overnight temperatures drop below freezing by mid-century (Abatzoglou & Kolden 2011). Increases in temperatures and shifts in frost lines are predicted to cause species distributions to move upward in elevation (Chambers et al. 2014).

Changes in temperature may also result in decreased abundance and increased patchiness in big sagebrush while shifting population and subspecies distributions upward

(Schlaepfer et al. 2012; Shafer et al. 2001; Bradley 2010). Determining how minimum temperatures affect big sagebrush establishment is a necessary component for improving efficiency of seeding, and could be critical for successful restoration efforts. Big sagebrush is a genetically diverse species that displays high diversity in its climate responses (as seen in Chapter 1), and the adaptive genetic variation of the species as it relates to climate shifts can be used to predict success of seeding efforts.

Big sagebrush (*Artemisia tridentata* Nutt.) has wide ecological amplitude and three subspecies are widely recognized: “mountain,” *A.t. vaseyana*; “basin,” *A.t. tridentata*; and “Wyoming,” *A.t. wyomingensis*. The subspecies align along environmental gradients of elevation, soil moisture, and soil texture. *A.t. tridentata* occupies deep, well-drained soils in valleys of the Great Basin; *A.t. wyomingensis* on drier, rockier soils from valleys to plateaus; and *A.t. vaseyana* at higher elevations (Bonham et al. 1991; Shultz 2006). While all three subspecies of *Artemisia tridentata* are well adapted to arid environments, the seedling physiological freezing response is variable but mostly unknown (Loik et al. 2004; Loik & Redar 2003; Lambrecht et al. 2007). Both *A.t. tridentata* and ssp. *vaseyana* are sensitive to freezing temperatures, but studies show increased freezing sensitivity of *A.t. vaseyana* when grown in low soil moisture conditions, resulting in reduced stomatal conductance and photosystem II (PSII) quantum yield (Lambrecht et al. 2007).

In situ experimental warming can simulate conditions associated with future climates and help predict shifts in distribution of big sagebrush communities as the climate warms. Increases in soil temperature may result in reduced plant ecodormancy over winter months, causing physiologically active plants to become more susceptible to

episodic freezing events in late winter (Larcher 2003). Conversely, earlier onset of the growing season may enhance freezing resistance due to more mature plant tissues (Taschler et al. 2004). Great Basin desert warming studies conducted at high elevations (subalpine) found positive correlations between increased temperatures and photosynthetic freezing tolerance in *A.t. vaseyana* (Loik et al. 2004), suggesting acclimation to colder temperatures due to earlier snowmelt. Minimum temperatures are directly linked to the greenhouse effect, and are the only climate change variable predicted to increase with certainty (IPCC 2014). In *A.t. tridentata*, no studies have explored how physiological response (e.g., freezing resistance, tolerance, and avoidance) relate to seedling survival, and are needed to predict how big sagebrush distributions will shift under future climate conditions.

The overall goal of this research was to assess the effects of simulated warming on seedling physiological performance for the three dominant subspecies of big sagebrush: *A.t. tridentata*, *A.t. vaseyana*, and *A.t. wyomingensis*. We evaluated how warming relates to survival and alters physiology and cold temperature responses among the three subspecies, which we expected to differ in adaptedness to the study sites, which were *A.t. wyomingensis* habitat. We hypothesized that *A.t. wyomingensis*, the most drought resistant of the subspecies, would have greater survival and physiological performance under warmed conditions. We also hypothesized greater enhancement of *A.t. tridentata* under warming, owing to greater water soil overcome by extraction of deeper soil water resources. Warming will exacerbate mortality in *A.t. vaseyana* due to normal distribution at higher elevations. We evaluated seedling response to warming through measurements of survival, growth, photosynthesis, water balance, and freezing response.

We then interpreted these results in terms of first-year establishment potential of big sagebrush seedlings in response to a warmed climate.

Materials and Method

Plant Material and Germination

Local seed sources representing all three subspecies were used to assess the effects of warming on subspecies survival and establishment. The local *A.t. tridentata* (diploid) was collected near Idaho National Guard Orchard Training Area (43°20'13.6"N, 116°00'29.2"W). *A.t. wyomingensis* (tetraploid) was collected at Birds of Prey National Conservation Area “BOP NCA” (43°14'58.6"N, 116°15'54.0"W) near Moore Road. *A.t. vaseyana* (diploid) came from the Boise National Forest north of Lucky Peak State Recreation Area (43°40'39.4"N, 115°58'25.7"W). Seedlings were grown outdoors at the U.S. Geological Survey Snake River Field Station in Boise, Idaho (43°60'90.98"N, 116°21'12.01"W). Approximately 10 seeds were sown into each 10 cm³ cone-tainer filled with native soils (silty loam) on August 10, 2012 and germination occurred within a week. Cone-tainers were rearranged periodically to limit the influence of microsite and watered one to two times daily depending on climate conditions. Seedlings were shifted to full sun and hardened before outplanting in fall. The three subspecies used in this experiment were also used as local seed sources in Chapter 1.

Site Conditions and Study Design

Plots were established in five separate blocks burned in summer of 2012 at BOP NCA, south of Boise, Idaho (Figure 2). The five fires (Poen, South Point, Swan Falls, Coyote, and Kave) represent a variety of pre-burn conditions in the sagebrush steppe. Poen was drill seeded with *Kochia prostrata* after burning in 1993. The South Point fire

was also drill seeded after burning in 1998 but with Russian wildrye grass (*Psathyrostachys junceus* (Fisch.) Nevski). The Swan and Coyote areas had a mixed mosaic of cheatgrass understory and sagebrush and the Kave area was considered to be intact sagebrush steppe prior to burning. Plant community post-fire was determined using point-line intercept, and percent vegetative cover was estimated in 20x20 cm² quadrats. Soils were assessed for texture analysis using a modified sedimentation technique (Gee & Bauder 1986). Percent soil organic matter was determined by weight loss-on-ignition using a muffle furnace (Schulte et al. 1996). We quantified elemental composition of nitrogen and carbon in soils using a Costech CHNSO analyzer (ECS 4010, Costech Analytical Technologies; Valencia, CA).

Experimental passive warming frames were installed in fall 2012 prior to outplanting to create the desired effect of warming (Figure 9). The warming frames are open-sided with a louvered roof, and increase long-wave solar radiation for plant and soil surfaces thereby increasing minimum nighttime temperatures by 2-4°C (Germino & Smith 1999 modified by Germino & Demshar 2008; Figure 9). The louvered roof consists of 8' x 4" acrylic strips 1/8" thick, mounted into 60° slots 2" apart to allow precipitation to pass through. Frame dimensions measure 2.4x2.4 meters² and 60 cm in height. Control chambers were also installed and had roofs of clear nylon string at the same spacing of the acrylic strips to mimic potential shading from the louvers in the warming chamber. Each block contained one control and one warmed plot.

We used a split-plot experimental design; warming was the whole plot factor and seedlings of the three different subspecies of big sagebrush were the sub-plot factor. Ten seedlings per population were bare-root transplanted into rows in plots approximately 10

cm apart, and populations were randomly assigned to rows. Holes were dug with a 2" soil corer to minimize disturbance of the soil. Seedlings were outplanted in November 2012 and average heights ranged from 28.8 to 32.6 mm. All planting was completed within a 3-week period. Plants received supplemental watering two times following outplanting, directing water to individual seedling stems.

Microclimate

Soil volumetric water content (θ , m^3/m^3) was determined using EC-5 volume soil moisture sensors connected to Em50 ECH₂O data loggers (Decagon Devices, Pullman WA), at 2-5 cm soil depth and at the soil surface (0-2 cm). Air and soil surface temperatures were recorded using HOBO H08-032-08 and Pendant UA-002-64 loggers (Onset Computer, Pocasset, MA), with sensors in two positions; the 5 cm above the soil to measure air temperatures affecting seedlings and directly under the soil to estimate (0-1 cm) surface temperature. Radiation shields were used for all above-ground sensors. All measurements were made hourly from November 2012 through May 2014.

Plant Growth and Survival

Height and survival were recorded for each seedling at the time of outplanting, November 2012, and then in monthly increments from February 2013 to May 2014. Mortality was assumed when a plant was gone or all foliage was missing and no re-greening occurred after rain. High mortality at 4 of the 5 blocks precluded determination of statistical significance in growth and physiology among groups across all blocks, and growth and physiological analyses were done only on the Poen block.

Physiological Measurements

Chlorophyll fluorescence was used to measure F_v/F_m as an indicator of seedling physiological stress levels in April 2013, November 2013, January 2014, and February 2014. F_v/F_m is the ratio of variable to mean fluorescence emitted from chlorophyll *a* of photosystem II (PSII) following dark adaptation, and indicates the yield of energy trapped in PSII (Maxwell & Johnson 2000). A healthy, non-stressed leaf usually has a F_v/F_m value around 0.80, and this value declines under the influence of stress factors. F_v/F_m was measured at pre-dawn using a model 6400-40 fluorometer (Li-Cor, Inc., Lincoln, NE), $n=3$. Pre-dawn water potential was measured in June 2014 to assess plant-water status under warmed and control conditions. Three seedlings per population were clipped at the Poen block for leaf water potential (Ψ_{leaf}) measurements before 0500. Measurements of water potential were made with excised tissue using a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR).

Gas exchange measurements were collected from seedlings in April 2014. All measurements were conducted between 1000 and 1600 local time using a 2 x 3 cm leaf chamber and external LED light source connected to portable, open-mode photosynthesis system (Li-Cor Model 6400, Licor Inc., Lincoln, NE). The chamber was clamped onto fully elongated leaves from seedlings by subspecies and treatment (i.e. 3-4 plants for each subspecies from both warmed and control plot). Carbon dioxide flowing into the chamber was set to $400 \mu\text{mol m}^{-1}$ and vapor pressure was matched with ambient levels for all gas exchange measurements. Measurements of photosynthesis were corrected for projected leaf area determined from digital photos taken immediately following gas exchange measurements and using image processing software (Image J; Scion, Fredrick, MD).

Specific leaf area (SLA) was calculated as the average ratio of leaf area (cm^2) to leaf mass (mg) for each treatment type and subspecies. Values of SLA were collected in September and December 2012, October 2013, and January 2014. Only values from October 2013 are reported here due to constraints obtaining plant material due to high mortality. Plant leaf area was determined using digital photos and image processing software (Image J; Scion, Fredrick, MD).

To assess freezing point, three to five excised leaves (collected in January and May 2014) from different plants of each population were placed on a ceramic thermoelectric module (CP14-127-06-L1-RT-W4.5/Laird Technologies, Earth City MO) attached to thermocouples whose readings were recorded by a data-logger at 5 second intervals (model CR7, Campbell Scientific, Logan UT) and chilled at 4°C per hour to -20°C in a regulated freezer. The temperature corresponding to the exothermic heat released during cell freezing was then identified as the “freezing point” (the temperature causing freezing) for each sample. Osmotic concentration and other traits affecting colligative properties of water cause depression of freezing point and thus inform about avoidance of freezing. The same cooling system was used to measure F_v/F_{m50} , which in our study is the temperature causing 50% loss of chlorophyll *a* fluorescence. Literature on freezing responses in plants frequently uses the term “LT₅₀” (Boorse et al. 1998; Loik et al. 2000) to describe the temperature causing 50% loss of chlorophyll *a* fluorescence. However, we did not validate that 50% F_v/F_m equates to mortality and therefore use the term F_v/F_{m50} . Excised leaves were sealed in plastic bags and chilled at 4°C per hour to -5, -10, -12, -14, -16, -18, or -22°C . F_v/F_m was measured after samples warmed to room temperature in darkness.

Plant Carbon Isotopes

Integrated measures of water-use efficiency over seedling lifetimes were assessed with carbon isotope (^{13}C) ratios. The isotopic ratio of plant-tissue carbon ($^{12}\text{C}/^{13}\text{C}$) indicates variation in CO_2 concentration gradient between air and leaves during photosynthesis given similar environmental conditions, and thus indicates water-use efficiency (WUE), the amount of CO_2 assimilated per unit water diffusing through the stomata (Farquhar et al. 1982; Farquhar et al. 1989). Leaf samples were collected in December of 2013, dried in an oven at 65°C , ground to powder, and analyzed for $^{13}\text{C}/^{12}\text{C}$ using a Costech flash combustion ECS 4010 and Picarro cavity ring down spectrometer (Model 2020, Picarro Inc, Santa Clara, CA) at the U.S. Geological Survey Snake River Field Station. The $^{13}\text{C}/^{12}\text{C}$ ratio of samples relative to the international standard Vienna Pee Dee Belemnite (VPDB) are reported using delta notation, with samples more depleted in δ^{13} (more negative values) resulting from a smaller gradient of CO_2 between air and leaves and indicating less water-use efficiency or a sample more depleted in δ^{13} (Farquhar et al. 1989).

Data Analysis

Sampling events for seedling survival occurred on a coarse time scale, meaning individual deaths were recorded in monthly intervals and actual time of death could differ up to 30 day between two seedlings. Thus, the time-to-death data is referred to as “tied” and is considered “interval-censored” (Allison 2010; Sun 1997). Interval-censored survival data is most appropriately analyzed with conditional logistic models fit with complementary log-log link mathematical reconstructions (Hosmer & Lemshow 2003; Prentice & Gloeckler 1978; Allison 1982). Conditional logistic models could not

accommodate the entire observation period due to “complete separation,” a condition in which the outcome does not vary at some levels of the independent variable. Complete separation occurred because of limited seedling mortality after June 2013. Therefore, we used interval-censored survival analysis to examine seedling survival only during months 3, 4, 5, and 6 (March-June 2013) when the most significant die-off occurred.

Conditional logistic models included survival (binomial) as a function of month, subspecies, minimum soil temperature, and all their interactions as fixed factors. The model statement could not use treatment (i.e., warmed or control) as a categorical factor because it had both positive and negative effects on survival depending on time of year. Specifically, warming in spring 2013 appeared to reduce mortality, whereas in late summer, increased temperatures might have exacerbated mortality. Minimum soil temperatures were significantly different between warmed and control plots using a two-way ANOVA (Table 11, Figure 10), and therefore the factor was treated a continuous variable in lieu of warmed/control categorical variable. We fit conditional logistic models with generalized estimating equations (GEE) (Liang & Zeger 1986) model framework in the geepack (Yan 2002) library in R (R Development Core Team, 2011) to estimate population-averaged effects in lieu of a subject-specific approach. To interpret the final model, predicted probabilities of survival were calculated in SAS (9.4) using the PROC GENMOD function.

We used survival analysis in the Survival library in R version 3.0.2 with the survfit() function (Therneau 2014) to address differences in seedling survival throughout the entire observation period (due to limitations with conditional logistic models). We used the non-parametric Kaplan-Meier survival estimator to test for significant

differences in mortality rates among seedlings within treatment groups (Kaplan & Meier 1958). Models failed to meet the assumption of proportional hazards for Cox regression analysis. Models were stratified by block (n=5) to account for random environment differences. Comparison of survival probabilities between warming treatment and subspecies were assessed with log-rank tests.

To address seedling growth, we performed a two-way (treatment by subspecies) multivariate analysis of variance (MANOVA) to evaluate height differences using PROC GLM with a repeated statement in SAS 9.4 (SAS Institute Inc, Cary, NC). When the assumption of sphericity was violated, only multivariate test statistics were utilized. We evaluated differences in mean height by treatment and subspecies during months 3-6 (March to June 2013) when the most mortality occurred using least square means with Bonferroni correction for multiple means comparisons. A-priori pairwise contrasts were used to evaluate differences in height by treatment within subspecies at the end of the observation period (May 2014).

To address seedling physiological response to warming, we used two-way (treatment and subspecies as fixed factors) analysis of variance (ANOVA) tests. Data transformations were conducted where necessary to meet model assumptions. All statistically significant results ($p < 0.05$) were evaluated using Tukey's Honestly Significant Difference criteria for pairwise comparisons. Tests were conducted in JMP 9.0.2 (SAS Institute Inc. 2010). Bonferroni corrections were used to correct for multiple means comparisons.

Results

Site Characteristics and Seedling Survival

Seedlings experienced high mortality, and by June of 2013 survival on combined control and warmed plots was 18.3% at Coyote, 71.67% at Poen, 17.54% at Swan, and less than 7% at both South Point and Kave wildfire site blocks. The high mortality in our study is due to relatively cold and dry conditions in the 2012/13 winter and a dry spring 2013. The Poen block soils had 59.5% clay whereas all other blocks had less than 25%. Soil characteristics, other than texture analysis, did not reveal any other significant differences between blocks (Chapter 1, Table 4). While Poen demonstrated the highest plant diversity, there were no significant differences between plant cover compositions separated by functional groups in an ANOVA analysis (Chapter 1, Table 4). Low survivorship at all other blocks reduced statistical inference for physiological analyses.

Seedling survivorship was not altered by experimental warming over the entire duration of the experiment ($\chi^2=0.429$, $p=0.513$). However, more seedlings survived under warmed conditions (22 seedlings) compared to the control (16 seedlings). The interaction between warming and subspecies survival was evaluated in independent models for the entire observation period, and survivorship correlated with warming for any subspecies (Figure 11, Table 12). Among subspecies, survival of both *A.t. tridentata* and *ssp. wyomingensis* was greater than *A.t. vaseyana* ($\chi^2= 20.6$, $p<0.001$). Variation in survival probability among sampling dates from March to June 2013 was positively related to minimum soil temperature in *A.t. tridentata* only in May (Figure 12, Table 13). Probability of survival was not associated with minimum soil temperatures for any other subspecies throughout the spring observation period (Table 13).

Growth

Growth patterns differed between treatment and subspecies groups (Wilks lambda = 0.006 $F_{70, 85.02} = 2.34$, $p < 0.001$, Figure 13). While the graph seems to suggest taller *A.t. tridentata* on warmed plots and taller *A.t. wyomingensis* on the control plots, height differences among treatments and subspecies were statistically marginal. Control *A.t. wyomingensis* and warmed *A.t. tridentata* were taller than warmed *A.t. vaseyana* in May 2013. Control *A.t. wyomingensis* was significantly taller than warmed *A.t. vaseyana* in June 2013. Height differences were also detected at the end of the study and warmed *A.t. tridentata* was significantly taller than control *A.t. tridentata*.

Physiology

Chlorophyll fluorescence was significantly different between treatment groups in April 2013 and January 2014. F_v/F_m of warmed seedlings was 9% greater in April 2013 and 24% greater in January 2014 than control seedlings (Table 14). F_v/F_m measured in April differed among subspecies, however no significant interactions were detected between treatment and subspecies; *A.t. wyomingensis* had significantly greater F_v/F_m (0.50 ± 0.01) than *A.t. vaseyana* (0.46 ± 0.01), and *A.t. tridentata* was no different than the other two subspecies (0.48 ± 0.01). These values are much lower than average F_v/F_m values in November 2013, January 2014, and February 2014, indicating high levels of plant stress. Significant mortality was also observed in April 2013. No significant interactions or differences among the subspecies were detected for F_v/F_m on other dates.

Leaf $\delta^{13}\text{C}$ did not detect a warming effect, but differed $\delta^{13}\text{C}$ between subspecies. *A.t. vaseyana* was -1.23 to -1.11 per mil more negative (less WUE) than *A.t. tridentata* and ssp. *wyomingensis* (Table 15, Figure 14). Pre-dawn water potential (Ψ_{leaf}) differed

among subspecies and treatments, but no significant interaction was detected overall (Table 15, Table 16). Experimental warming increased water potential of seedlings by 15% indicating better hydrated plants on the warmed plot. Ψ_{leaf} of *A.t. tridentata* was 0.13-0.45 MPa less negative (better hydrated) than both *A.t. vaseyana* and *ssp. wyomingensis* (Table 16, $p=0.01$), but no significant interaction between subspecies and treatment was detected. Photosynthesis measured in April 2014 did not differ among treatments or subspecies (Table 15, Table 16). We were unable to statistically evaluate SLA due to limited sample size but means are reported in Table 15.

Freezing point was not responsive to experimental warming for any subspecies. Freezing points of *A.t. vaseyana* were significantly higher ($-8.5 \pm 0.40^\circ\text{C}$) than *A.t. wyomingensis* ($-12.7 \pm 0.52^\circ\text{C}$) and *ssp. tridentata* ($-11.9 \pm 0.83^\circ\text{C}$) ($p < 0.01$, Table 16) in May 2014, but not in January 2014. F_v/F_{m50} of warmed seedlings increased by 1°C compared to the control seedlings (Table 16, Figure 15).

Discussion

Experimental Warming Affects Survival in Temporal Patterns

Warming did not affect overall seedling survival; however, experimental warming did influence seedling survival as a function of time and weather. Survival probability of *A.t. tridentata* had a positive relationship with minimum temperatures in May, indicating that seedlings on the warmed plot had increased survival relative to those on the control in support of our hypothesis (Table 13, Figure 12). Our results did not support our hypothesis of increased *A.t. wyomingensis* survival under warmed conditions. *A.t. wyomingensis* had greater survival on control than warmed plots, and this pattern emerged after a spring 2014 culling event (not statistically significant). As anticipated,

A.t. vaseyana experienced the most mortality of all subspecies, but warming did not mitigate mortality in spite of reduced seedling stress under warming in mid-winter (Table 14). The high mortality in our study relates to relatively cold and dry conditions in the 2012/13 winter and a dry spring 2013, and we attribute the high survivorship at the Poen block to be a result of high clay content in soils (Chapter 1).

Experimental Warming Causes Loss of Acclimation to Freezing

Exposure to low temperatures induces genetic, morphological, and physiological changes in plants, resulting in cold acclimation (Levitt 1972; Thomashow 1990; Guy 1990). Experimental warming resulted in a loss of cold acclimation in big sagebrush (Figure 15), but similar loss occurred for all subspecies. Chapter 1 suggested that the three subspecies tolerate extracellular freezing (although freezing points vary) (Figure 8), and it is therefore reasonable to infer that loss of acclimation might impact physiological changes among subspecies in a similar manner. However, here we observed variability in growth between subspecies on warmed and control plots, indicating that subspecies respond differently to a reduction in cold stress.

Sakai and Larcher (1987) recognized a trade-off between growth intensity and freezing resistance, and our results suggest these trade-offs may extend to include survival in big sagebrush. *A.t. tridentata* was consistently taller on warmed than control plots, and perhaps reduced investment in freezing resistance allowed greater allocation of resources for growth. Survival of *A.t. tridentata* was also greater on warmed than control plots (by 20%), likely indicating that reduced freezing resistance may also enable greater survival under mild temperature conditions, particularly because of *A.t. tridentata*'s rapid growth strategy. These results are consistent with studies of high-elevation plant species

that have shown trade-offs between growth rates and freezing resistance in seedlings (Körner & Alsos 2008; Sierra-Almedia et al. 2010)

An explanation for why *A.t. wyomingensis* had 30 to 53% greater average height on control plot versus warmed is perhaps due to earlier and increased physiological activity on warmed plots resulting in increased root growth. Warmer soil temperatures may have allowed earlier root growth, and perhaps the seedlings allocated more sugars to the roots at the expense of shoot growth. *A.t. vaseyana* seedlings had greater height on the warmed plots but similar survival between control and warmed plots, which is inconsistent with its presumed adaptation to colder climates. Native distribution for *A.t. vaseyana* is at relatively high elevations where snow and freezing temperatures are constant throughout winter.

Experimentally warmed seedling exhibited less light stress than control plants (in F_v/F_m measurements), corroborating the sensitivity of seedlings to minimum temperatures (at least in *A.t. tridentata*), and revealing sensitivity that was otherwise not evident in the survival of *A.t. wyomingensis* and ssp. *vaseyana*. Light stress occurs as a function of combined high light intensity and cold temperatures. The warmed plots had higher temperatures and thus reduced light damage to PSII in seedlings. *A.t. wyomingensis* was least light stressed in April 2013, independent of warming treatment and relative to the other three subspecies, perhaps indicating local adaption to climate at BOP NCA. Further, *A.t. wyomingensis* is the most frost resistant (most negative F_v/F_{m50}) among subspecies, and perhaps warming did not alleviate any stress among seedlings resulting in similar survival on warmed and control treatments. Greater freezing resistance associated with lower freezing points in *A.t. wyomingensis* (Chapter 1, Table 10) and reduced stress

(F_v/F_m) in its local environment may have selective advantages in environments like BOP NCA where freezing can be episodic and snow cover may be insufficient to act as insulation during late winter and early spring.

It is reasonable to predict *a priori* that *A.t. vaseyana* would be the most frost resistant among subspecies due to its normal distribution at higher elevations and therefore capable of surviving extreme cold weather events. In spite of high elevations, *A.t. vaseyana* may have limited exposure to freezing because it is normally insulated by snow cover. Our results suggest that *A.t. vaseyana* has a freezing tolerance strategy combined with weak freezing avoidance, indicating that episodic freezing may negatively impact young *A.t. vaseyana* seedlings. While the high mortality of *A.t. vaseyana* in our experiment is likely a function of drought stress coupled with minimum temperature exposure, increased minimum temperatures (due to climate change) may reduce snow cover at higher elevations and perhaps shift *A.t. vaseyana* distributions downslope.

Ecophysiological parameters measuring water balance (leaf $\delta^{13}\text{C}$, Ψ_{leaf}) indicated differences among subspecies. Differences in drought adaptation were consistent with Kolb and Sperry (1999), with *A.t. wyomingensis* being the most drought tolerant and *A.t. vaseyana* being the least drought tolerant among the subspecies. We did observe an increase in Ψ_{leaf} (less negative MPa) among all subspecies due to experimental warming, particularly in warmed *A.t. tridentata* and ssp. *wyomingensis*. Decreases in soil water content (Figure 10) under warmed conditions during hot summer months may favor plants with more drought tolerant water status. However, it is likely that decreased drought stress on the warmed plots is a result of plants utilizing deep soil water resource

pools during summer drought as seen in leaf $\delta^{13}\text{C}$. Experimental warming did not negatively affect water balance in any of the subspecies.

We did observe strong warming effects in growth parameters, which likely are a function of alteration to resource allocation among subspecies. Warmer winters may favor *A.t. tridentata* due to greater water stress overcome by extraction of deeper soil water (WUE), rapid growth, and higher competitive ability. Survival of *A.t. wyomingensis* is only marginally affected by variable climate; however, warmer winters could result in slower growth patterns, increasing overall time to establishment. As anticipated, *A.t. vaseyana* is not adapted to xeric climates like BOP NCA and cannot survive and establish in this climate. It seems likely that climate change will impact big sagebrush through a combination of earlier resumption of growth, spring drought, and frost events, which may act in concert to reduce overall survival.

In conclusion, experimental warming affected growth of all three subspecies differently, indicating that protection from freezing damage may lead to enhanced survival of some subspecies under some conditions, but neutral or negative impacts on other subspecies. Our hypothesis that experimental warming may alter seedling freezing response thereby affecting growth and survival was supported albeit differently for the different subspecies. We saw enhancement of *A.t. tridentata* in survival and growth, and reduced growth for warmed *A.t. wyomingensis*. Climate change is expected to increase the frequency and intensity of frosts in some habitats (Inouye 2000), and selection for freezing resistance mechanisms could become increasingly important. Freezing response should be considered when predicting changes in subspecies distributions, as well as in seed source selection for restoration.

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Tables

Table 11. Summary of two-way ANOVA results for minimum soil temperature due to warming treatment. Minimum soil temperature was approximately 2-4 degrees warmer on the warmed plot as compared to the control. All means were significantly different from one another using Tukey HSD. Asterisks indicate level of significance: $*=p>0.05$, $**=p>0.01$, $***p>0.001$.

Source of Variation	DF	F ratio	<i>p</i> -Value	
Treatment	1	1.541	0.3436	
Block	4	5.012	0.0891	
Month	3	4335.6	<.0001	***
Month x Treatment	3	14.02	<.0001	***

Table 12. Results of Kaplan-Meier survival analysis. Survival probabilities were calculated within subspecies by treatment groups. The log-rank test was used to compare survival curves. No significant differences were detected.

Subspecies	Treatment	N	Observed	Expected	χ^2	<i>p</i> -value
<i>A.t. wyomingensis</i>	Warmed	50	40	40.6	0.010	0.846
	Control	47	40	39.4	0.011	
<i>A.t. tridentata</i>	Warmed	50	47	43	0.371	0.262
	Control	50	39	43	0.371	
<i>A.t. vaseyana</i>	Warmed	50	46	48.1	0.150	0.443
	Control	50	44	41.3	0.176	

Table 13. Conditional logistic model parameters comparing effects of month, subspecies, and minimum soil temperature on binomial response (alive or dead) of sagebrush seedlings. Positive coefficients show increased hazards of death relative to the “base level” of the explanatory variable to which all comparisons are made. Base levels are alphabetic; for subspecies the base level is *ssp. wyomingensis*, April is base level for month, and for soil minimum temperature the base is 0°C. Block was treated as a clustering variable to account for site-specific heterogeneity. Significance of explanatory variables is given by the Wald statistic with respective *p*-values. Asterisks indicate level of significance: *=*p*>0.05, **=*p*>0.01, ****p*> 0.001.

	Estimate	SE	Wald	<i>p</i> -Value	
(Intercept)	-0.124	0.357	0.120	0.728	
<i>ssp. tridentata</i>	0.008	0.363	0.000	0.983	
<i>ssp. vaseyana</i>	-0.201	0.246	0.670	0.414	
March	-3.385	1.921	3.110	0.078	.
June	0.511	0.228	5.030	0.025	*
May	1.170	0.098	143.470	< 2e-16	***
Soil Minimum Temp	0.049	0.082	0.350	0.552	
<i>ssp. tridentata</i> x March	1.707	2.594	0.430	0.511	
<i>ssp. vaseyana</i> x March	-2.339	1.327	3.100	0.078	.
<i>ssp. tridentata</i> x May	0.287	0.328	0.770	0.382	
<i>ssp. vaseyana</i> x May	-0.075	0.522	0.020	0.886	
<i>ssp. tridentata</i> x June	-1.985	0.205	93.820	< 2e-16	***
<i>ssp. vaseyana</i> x June	-1.779	0.830	4.590	0.032	*
<i>ssp. tridentata</i> x Soil Minimum Temp	-0.065	0.105	0.380	0.536	
<i>ssp. vaseyana</i> x Soil Minimum Temp	0.085	0.208	0.170	0.684	
March x Soil Minimum Temp	0.304	0.252	1.460	0.227	
May x Soil Minimum Temp	-0.042	0.081	0.280	0.599	
June x Soil Minimum Temp	-0.232	0.076	9.370	0.002	**
<i>ssp. tridentata</i> x June x Soil Minimum Temp	-0.109	0.306	0.130	0.720	
<i>ssp. vaseyana</i> x June x Soil Minimum Temp	0.137	0.147	0.870	0.352	
<i>ssp. tridentata</i> x March x Soil Minimum Temp	0.106	0.139	0.580	0.445	
<i>ssp. vaseyana</i> x March x Soil Minimum Temp	-0.166	0.191	0.750	0.385	
<i>ssp. tridentata</i> x May x Soil Minimum Temp	0.480	0.068	50.060	0.000	***
<i>ssp. vaseyana</i> x May x Soil Minimum Temp	0.139	0.323	0.190	0.667	

Table 14. Statistical results from two-way ANOVA tests comparing the effects of treatment and subspecies on F_v/F_m . We used a Bonferroni correction to address multiple means comparisons and $\alpha=0.012$ for significance. Asterisks indicate level of significance: $*=p>0.012$, $**=p>0.001$, $***p>0.001$.

Source	2013					2014				
	April			November		January		February		
	F	<i>p</i> -value		F	<i>p</i> -value	F	<i>p</i> -value	F	<i>p</i> -value	
Treatment	20.629	<0.001	***	3.477	0.891	8.337	0.011	*	0.026	0.875
Subspecies	5.529	0.009	*	0.446	0.651	1.096	0.360		0.279	0.761
Treatment x Spp.	0.536	0.596		0.413	0.671	2.689	0.100		0.910	0.426

Table 15. Average values (SE $\pm$ 1) for measures of seedling physiology by treatment and subspecies. See Table 16 for statistics. No standard errors are available for SLA due to limited plant material. Leaves were pooled from 3 plants together in one sample, resulting in an n=1 per subspecies and treatment type. Statistics were not evaluated for this parameter.

Variable	<i>ssp. tridentana</i>				<i>ssp. vaseyana</i>				<i>ssp. wyomingensis</i>			
	Warmed		Control		Warmed		Control		Warmed		Control	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
WUE (δ^{13})	-28.031	0.359	-28.082	0.248	-29.289	0.266	-29.317	0.105	-28.551	0.118	-27.315	0.398
Freezing resistance (F_v/F_{m50}) °C	-14.808	0.277	-15.778	0.257	-15.256	0.463	-15.496	0.373	-15.115	0.297	-16.753	0.529
Freezing Temperature °C	-11.770	1.339	-12.330	1.810	-7.930	0.360	-8.123	0.780	-12.320	1.216	-13.126	0.077
Ψ_{leaf} (MPa)	-2.067	0.145	-2.650	0.176	-2.525	0.025	-2.450	0.050	-2.533	0.033	-3.083	0.109
SLA (cm ² /mg)	10.537		10.598		10.597		9.295		5.550		8.862	
Photosynthesis A_{max} ($\mu\text{mol}^{-2}\text{s}^{-1}$)	9.146	1.938	8.551	3.133	9.476	1.507	9.473	1.974	13.202	2.199	7.435	1.600

Table 16. Statistical results from two-way ANOVA tests comparing the effects of treatment and subspecies on seedling physiology. Those analyses that were statistically significant at $\alpha=0.05$ are shown in bold. Asterisks indicate level of significance: *= $p>0.05$, **= $p>0.01$, ***= $p>0.001$.

Variable	Subspecies			Treatment		ssp x Treatment	
	F	<i>p</i> -value		F	<i>p</i> -value	F	<i>p</i> -value
WUE (δ^{13})	17.027	<0.01	***	3.56	0.09		3.75 0.06
Freezing resistance (F_v/F_{m50}) °C	1.92	0.19		4.86	0.05	*	0.62 0.56
Freezing Temperature °C	12.13	<0.01	**	0.33	0.58		0.04 0.95
Ψ_{leaf} (MPa)	8.01	0.01	*	12.11	0.01	*	3.91 0.06
Photosynthesis A_{max} ($\mu\text{mol}^{-2}\text{s}^{-1}$)	0.40	0.67		0.52	0.49		2.62 0.11

Figures



Figure 9. Photo showing style of warming and control chambers. In photo, the leftmost chamber is the warming device, and two chambers having rainout shelters in the back row are rainout treatments for a related project. Inset picture shows cross section of roof design.

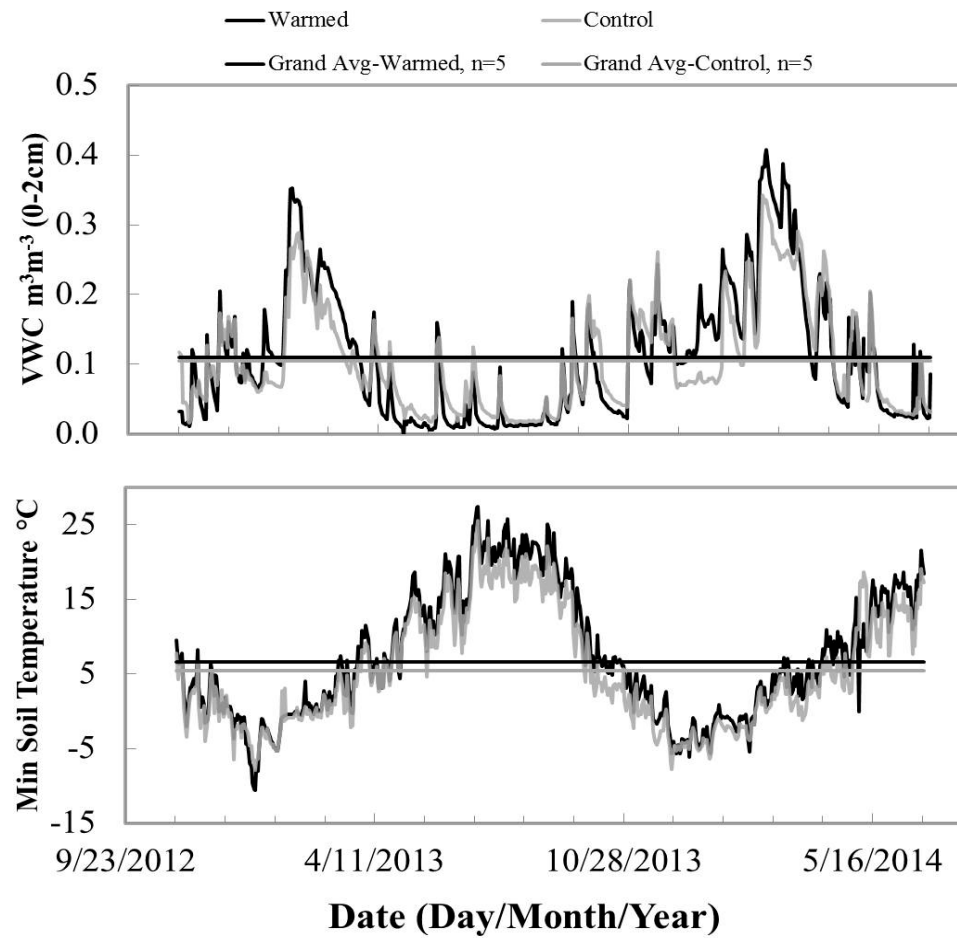


Figure 10. Minimum soil temperatures ($^{\circ}\text{C}$) and volumetric water content (m^3m^{-3}) under the warming frames and control plot

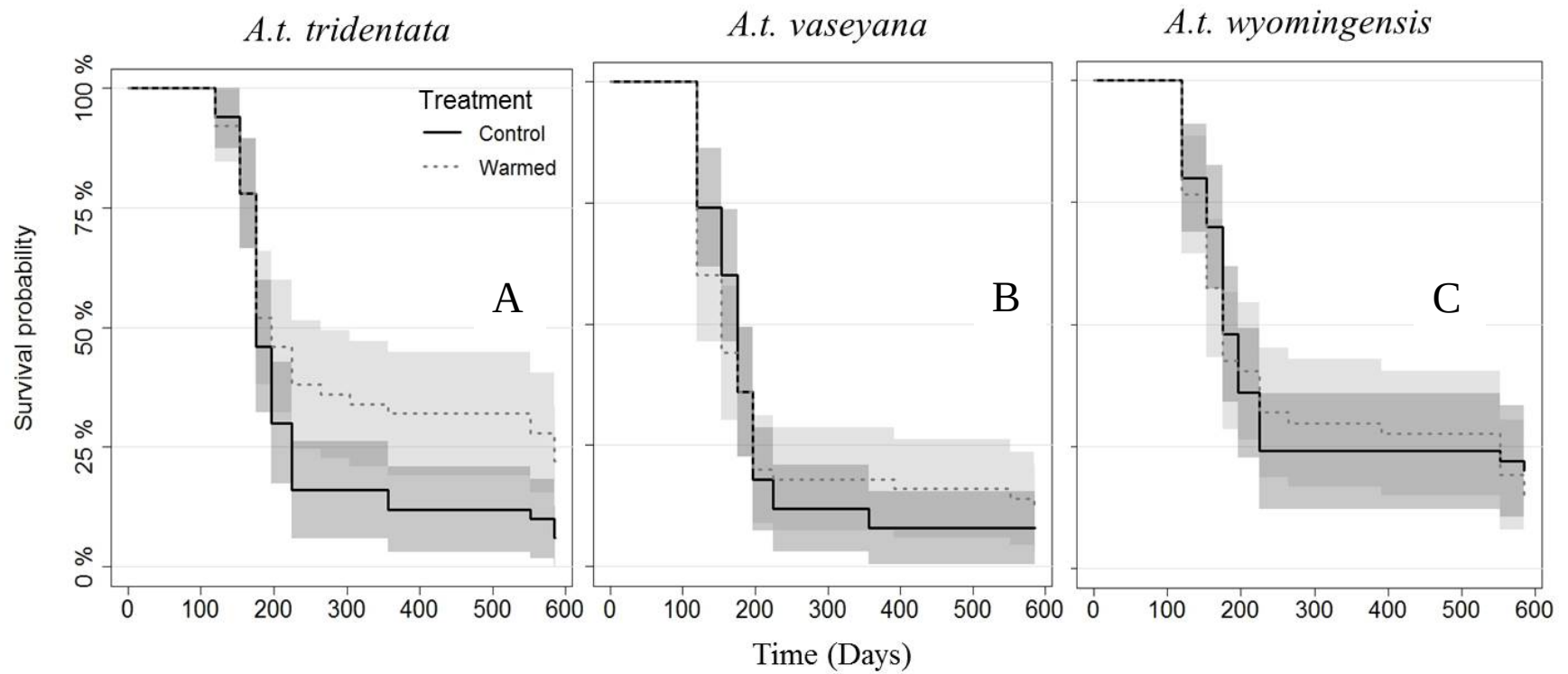


Figure 11. Non-parametric survival curves showing treatment effects by species: *A.t. tridentata* (A) ($\chi^2=1.3$, $p=0.262$), *A.t. vaseyana* (B) ($\chi^2=0.60$, $p=0.443$), and *A.t. wyomingensis* ($\chi^2=0.20$, $p=0.642$). There was no significant interaction effect of warming on subspecies. Shaded intervals represent 95% confidence intervals.

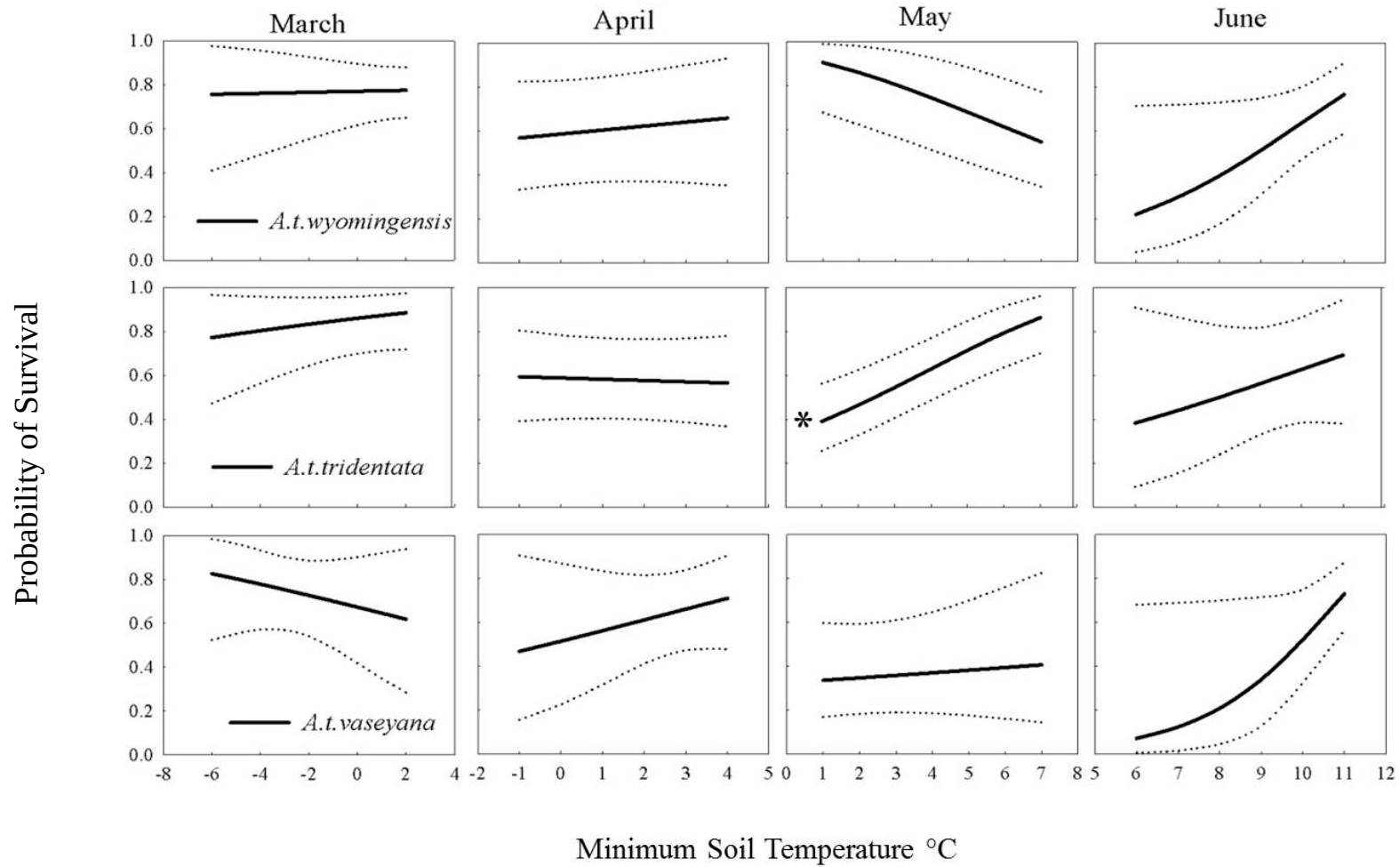


Figure 12. Binomial general linearized model showing predicated probability of survival (1=alive, 0=dead) for subspecies as a function of minimum soil temperature in March, April, May, and June 2013. Experimental warming is considered a continuous variable. Asterisks indicate significant differences in survival probabilities at $\alpha=0.05$.

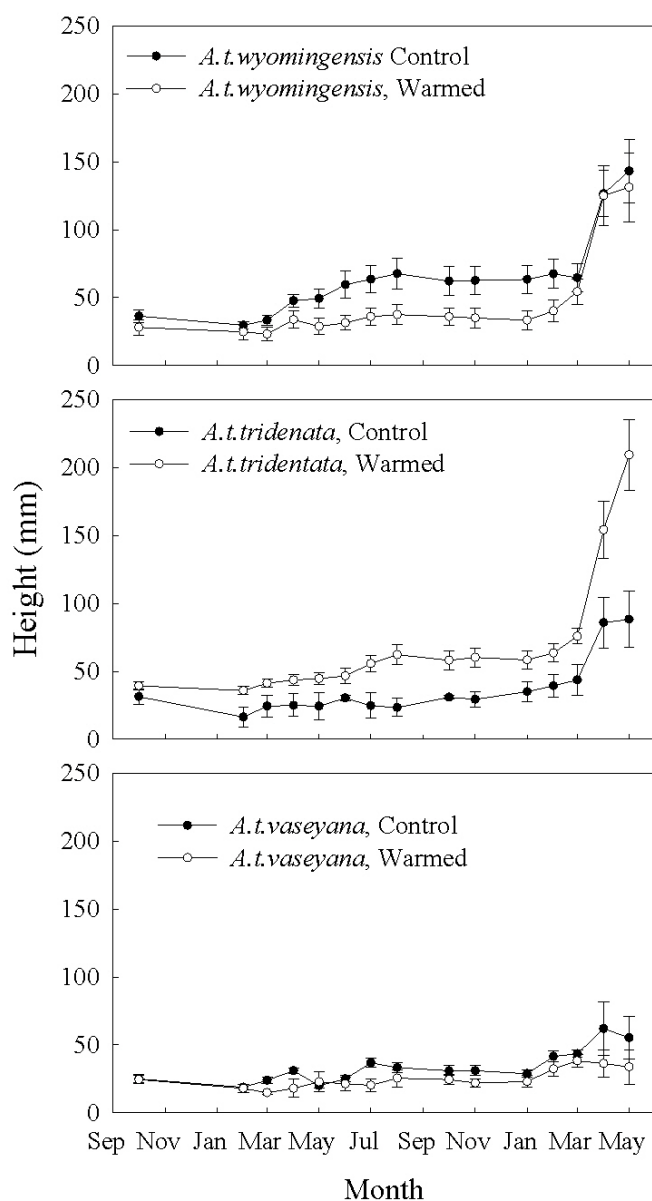


Figure 13. Average plant heights (mm) from the Poen block only October 2012 to May 2014 by subspecies and treatment. Plants that died were removed from graph. Bars represent the SE of individual plants not plots. At the end of the observation period (May 2014), significant differences were detected between warmed and control *A. t. tridentata* only using pairwise comparisons. Bars represent the SE of individual plants not plots.

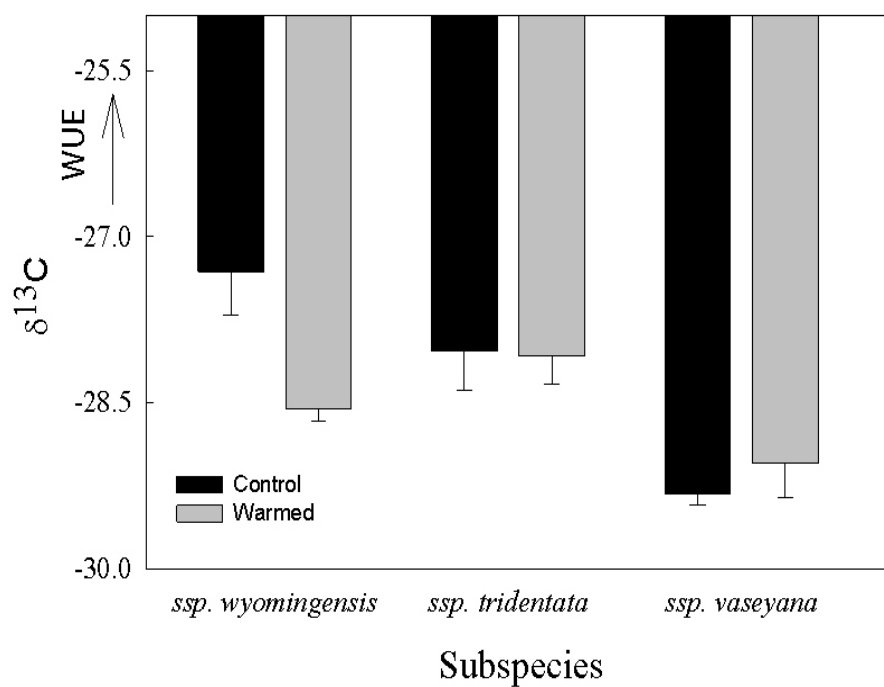


Figure 14. Variation in $\delta^{13}\text{C}$ of leaves of sagebrush by subspecies and treatment in January 2014 ($p=0.001$). There was significant effect of subspecies ($p=0.001$) but no significant interaction between subspecies and treatment. See Table 16 for statistics

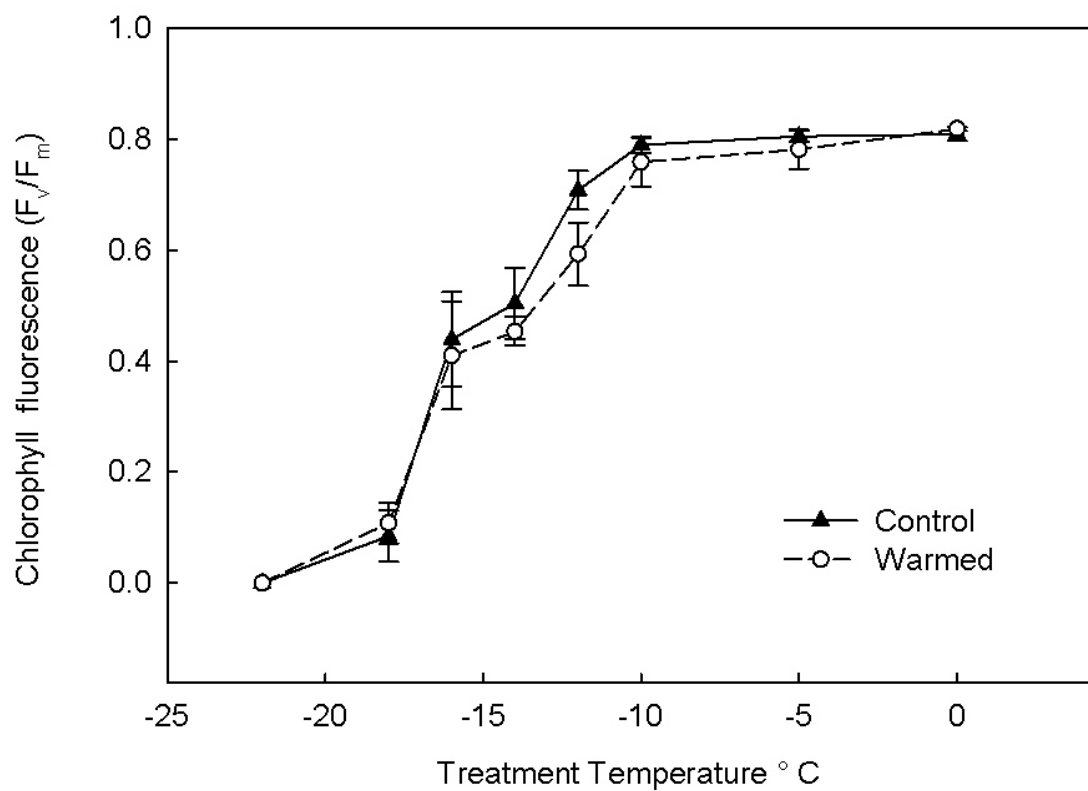


Figure 15. Chlorophyll fluorescence (F_v/F_m) as a function of air temperature for big sagebrush seedlings from experimentally warmed and control plots indicate a loss of cold acclimation for the warmed seedlings. Data are means $\pm$ 1 SE ($n=3$). See Table 16 for statistics.

CHAPTER 3: RESPONSE OF YOUNG SAGEBRUSH SEEDLING TO MANAGEMENT TREATMENTS OF HERBS

Abstract

Wildfires burn approximately 2 million hectares of sagebrush steppe per year in the Great Basin Desert, leaving large portions of the landscape devoid of big sagebrush shrubs. Big sagebrush is non-fire adapted and post-fire recovery is complicated by limited seed dispersal capabilities and short-term seed viability. The loss of big sagebrush has motivated efforts to restore it, and post-fire rehabilitation involves alteration of a variety of plant community, soil, and site conditions. Herbicide application, mowing, drill seeding with forbs and grasses, and combinations of any of these treatments may happen before seeding or out-planting big sagebrush. The objective of this study was to evaluate how initial establishment of big sagebrush is influenced by management treatments on the herb layer, and to determine how these effects vary among different populations (seed sources) of big sagebrush. We asked if polyploidy, growth strategy, or local adaption in big sagebrush populations relate to better ability to compete with the herb layer as a result of land management treatments. We evaluated seedling survival after outplanting, and results indicate that seeding combined with land management treatments that cause disturbance of the herb layer and soil surface may negatively affect sagebrush during the establishment phase. Survival probabilities increased for local and fast-growing populations of big sagebrush relative to other populations. Seedling growth strategy and

adaptation to local environment may increase seedling survival on rehabilitated lands after fire.

Introduction

Wildfires burn approximately 2 million hectares of sagebrush steppe per year in the Great Basin Desert (US National Interagency Fire Center, 2001–2012 eastern and western Great Basin; http://www.nifc.gov/fireInfo/fireInfo_stats_lightng.html accessed 22 September 2014) leaving vast areas of the landscape devoid of big sagebrush shrubs. Big sagebrush is not fire-adapted and post-fire recovery is complicated by limited seed dispersal capabilities and short-term seed viability (Cione et al. 2002; McIver & Starr 2001; Monsen & McArthur 1985). Studies suggest that sagebrush recruitment from existing seedbanks is dependent on favorable weather patterns (Perryman et al. 2001), and seed germination is variable due to differences in seed morphology and physiology as well as soil surface and microclimate conditions (Chambers 2000; Young & Evans 1989). As issues with wind erosion compound due to large-scale barren areas, big sagebrush seeds may be even more unlikely to germinate following deep burial in drifts (Jacobson & Welch 1987; Young & Evans 1989; but see Wijayratne & Pyke 2012). Thus, the rapid loss of sagebrush habitat across the Great Basin Desert has motivated efforts to restore it through seeding and planting, with many of these efforts occurring on recently burned sites impacted or threatened by exotic grass.

Typically, sagebrush seed is broadcast aerially or seeded in with a rangeland drill, and restoration success is mixed (Lysne & Pellant 2004) with opportunity for improvement. Containerized stock and outplanting are costly but more effective as this method overcomes the adverse conditions associated with seed germination (Beyers

2004; Lysne 2005). Aside from rehabilitation method, the seed source can also play a critical role in initial establishment as well as long-term persistence of sagebrush stands (Beyers 2004; Lysne 2005). Sagebrush is a genetically diverse species that also displays high diversity in its responses to the surrounding environment. Sagebrush populations can differ in attributes such as ploidy level, growth rate, stress responses, water-use efficiency, depth of rooting, phenology with respect to chilling, and in other related variables (Welch & Jacobson 1988; Lambrecht et al. 2007; Loik & Redar 2003, Maier et al. 2001; Wijayratne 2011). These factors are normally considered with respect to how big sagebrush relates to the abiotic environment, but all of these factors should also affect how sagebrush competes with different types and abundances of herbs. The growing environment is variable due to climate, weather, and surface conditions as they relate to density, diversity, and soil resource (water) use of herbs and grasses. For example, populations of big sagebrush having more rapid growth rates may result in less competition if large stature occurs before herbs establish significantly. Further, rooting depth (which varies among subspecies, see Welch et al. 1988; Lambrecht et al. 2007) affects effective moisture available to seedlings and may impact seedlings' ability to compete with dense herb layers like landscapes dominated by cheatgrass.

Post-fire rehabilitation typically occurs as a multi-staged operation. Herbicide application, mowing, drill seeding with forbs and grasses, and combinations of any of these treatments may happen before seeding or out planting big sagebrush. Forbs and grasses can differ in a number of ways, but very strong differences in soil resources can occur under cheatgrass compared to mixed native perennial grassland with some forbs (James et al. 2008; Prévey et al. 2010; Davies 2011). Sites dominated by cheatgrass

might have fleeting shallow soil water availability (Melgoza et al. 1990; Cline et al. 1977), and big sagebrush populations capable of quickly deploying a deep tap root in early spring might stand a better chance at competing in such conditions. Information like this would be valuable in seed selection for particular restoration sites.

The objective of this study was to evaluate how initial establishment of big sagebrush is influenced by management treatments of the herb and grass layer, and to determine how these effects vary among different populations of big sagebrush originating from different climates and ploidy levels. We evaluated which populations of big sagebrush have a better ability to compete with the herb layer as a result of land management treatments by evaluating seedling survival after outplanting. We predicted that fast-growing big sagebrush populations will have greater survival on treatments with increased exotic herb abundance (i.e., no herbicide treatment), whereas tetraploid populations will have greater survival overall due to enhanced tolerance to drought.

Materials and Methods

Plant Material and Germination

Seedlings used in this experiment consisted of 10 populations of *A. tridentata*, and were selected from a wide variety of genetic backgrounds. Seeds sources were initially selected to include all three big sagebrush subspecies based on locality to the Boise area. The remaining seven genotypes were selected from a wide range of genetic backgrounds and included both diploid and tetraploid ssp. *tridentata* and ssp. *vaseyana* cytotypes and populations from non-local climates-of-origin. We incorporated populations originating from drier or cool/wet areas (Table 17). Seedlings were grown outdoors at the U.S. Geological Survey Snake River Field Station in Boise, Idaho (43°60'90.98"N,

116°21'12.01"W). Approximately 10 seeds were sown into each 10 cm³ cone-tainer filled with native soils (silty loam) on August 10, 2012 and germination occurred within a week. Cone-tainers were rearranged periodically to limit the influence of microsite and watered one to two times daily depending on climate conditions. Seedlings were shifted to full sun and hardened before outplanting in fall. Plants overwintered (January to March 2014) at U.S. Forest Service Lucky Peak Plant Nursery's cold storage facility (45°79'82.1"N, 115°99'51.45"W).

Site Conditions and Study Design

The experimental plots (43°23'20.06"N, 116°23'21.94"W) were located in Birds of Prey National Conservation Area and consisted of a mixed cheatgrass and Sandberg's Bluegrass community on relatively flat terrain with loamy soils. Herbs and grasses established naturally post-fire and sagebrush was not present on the site due to historic fire regimes. Three replicate blocks received each of eight treatments resulting from a completely randomized combination of four management practices (no treatment, herbicide application, mowing, and mowing plus herbicide) and two seeding treatments (without and with seeding). The controls were the no-management treatment without seeding (Figure 16). All treatments were implemented in fall 2012 with the exception of grazing, which began in late winter/early spring of 2013. The plots measure 1 hectare in size with an untreated area of 1 hectare separating each plot. In each of the 48 hectare-sized plots were 9 sampling circles that measured 25 m in diameter, which left a 12.5 m buffer zone on all four sides of each plot (Figure 17). Seeding was conducted with a seed drill (7512 OTG Drill, Truax Company, New Hope, MN) as well as hand broadcast in

November 2012 (Appendix B). Herbicide treatment was applied in October 2013 using Imazapic at a rate of 6-4 ounces/acres with a calibrated boom sprayer.

Seedling outplanting began on March 16, 2013 and continued through April 10, 2013. We bare-root transplanted seedlings into small holes and outplanted into all 48 plots to ensure equal treatment effect across the plots. Seedlings received one liter of supplemental water after outplanting. Here we evaluated only the ungrazed plots because they all had the same populations of big sagebrush (Table 17). Specifically, four sagebrush populations were planted in each of the 9 sampling circles per plot, totaling 36 seedlings per plot (9 seedlings per population). Seedlings were planted in each of the cardinal directions in the circles, resulting in 12-15 m separation between seedlings (Figure 16). The four populations were *A.t. tridentata* subspecies, and included a local and a tetraploid population, as well as two populations that appear to have slower (CAT2) and faster growing strategies (ORT2) (Table 17). These observations for this final criterion were based on data collected from adult plantings in common gardens.

Plant Survival

Monitoring of survival of big sagebrush seedlings began on April 10, 2013 and occurred three subsequent times in May and July 2013, and March 2014. Mortality was assumed when a plant was gone or all foliage was missing and no re-greening occurred after rain.

Data Analysis

We used survival analysis in the Survival library in R version 3.0.2 with the coxph() function (Therneau 2014) to address seedling survivorship throughout the entire observation period. We ran semi-parametric Cox proportional-hazard regression models

to estimate the effects of management treatment, seeding, big sagebrush population, and all their interactions on seedling hazards (or risk) of death (Cox 1972). Sampling events for seedling survival occurred on a coarse time scale, meaning individual deaths were recorded in monthly intervals and actual time of death could differ up to 60 days between two seedlings. Thus, the time-to-death data is referred to as “tied” and is considered “interval-censored” (Allison 2010; Sun 1997). We monitored seedlings survival in four observation periods, April, May, and July 2013, as well as March 2014, and used maximum likelihood estimation for its appropriate treatment of censored data in Cox models (Lee 1992). The Cox analysis provided “hazards of death,” and hazard rates less than one indicate decreased instantaneous risk of mortality and rates greater than one indicated increased risk of mortality (Venables & Ripley 2002). Models were tested for assumptions and stratified by block (n=3) to account for random environment differences.

Results

Only 7 seedlings remained out of 864 within the exclosures as of March 2014; however, we were still able to detect interactions between management treatments, seedings, and big sagebrush population on seedling survival ($\chi^2=69.8$, $p<0.001$, Table 18). Seedling survivorship was greater on plots that had an array of treatments that lead to reduced herb cover (mowing, mowing+herbicide, no drill seeding of herbs) compared to plots that had been seeded (Figure 18), although substantive emergence of seeded herbs was not readily detected in the treatments (Figure 19). The risk of seedling mortality on seeded plots increased by 2-15 fold on treatments that were seeded compared to unseeded plots (Table 18). The local *A.t. tridentata* (IDT2) population had reduced hazards of death compared to other populations that included sources from warmer or drier sites and

also tetraploids (Figure 20). However, no significant differences in hazards of death were detected between local IDT2 and a fast-growing *A.t. tridentata* population, ORT2. ORT2 had the most survivors at the end of the experiment (3 out of 7 seedlings).

Discussion

Very high mortality occurred when precipitation fell far below average from April to June 2013. Timing of outplanting can affect survival (Shaw 2004), and dry spring conditions coupled with warm temperatures may have increased mortality at our study site. Seeded plots had greater abundance of herbs than non-seeded plots (evidence is reduced and based on descriptive statistics), and competition from native or invasive vegetation may negatively affect establishment (Boyd & Svejcar 2011; McAdoo et al. 2013). Non-seeded plots had greater bare soil cover likely resulting in greater proximity between neighboring herbs and seedlings (Figure 18). The reduced intensity of plant interactions in communities on non-seeded plots potentially increased water and nutrient availability for seedlings and thus survival. One study suggests that negative responses of *A.t. vaseyana* seedlings to neighboring herbs are not attributable to interactions over water availability, but likely result from interactions over factors like nutrients (DiCristina & Germino 2006). However, no research is currently available on responses of the other two subspecies to herb interactions to best of my knowledge. BOP NCA is considered an *A.t. wyomingensis* site and is significantly more arid and water-limited than higher elevation *A.t. vaseyana* sites. It is likely that competition with dense herb layers from seedlings may increase negative effects on big sagebrush seedlings at lower elevations, but mechanisms are uncertain and need to be discovered in future research.

Our ability to make inferences concerning the importance of climate-of-origin, growth strategy, and polyploidy is limited. Significant mortality among all seedlings reduced our power to detect differences within population groups and treatment types. Fast-growing big sagebrush (ORT2) did not have enhanced survival on treatments with greater herb cover, contrary to our hypothesis, and there was no support for greater success of tetraploid populations among all treatment types. Further, in the relatively dry year of our study, seedlings from warm/dry climates had no greater survival than local populations. However, survival probabilities increased for local and fast-growing populations relative to other populations and regardless of treatment, indicating that growth strategy and local adaptation may increase seedling survival post-fire.

In conclusion, our results suggest success of sagebrush planting may be influenced by accompanying land management treatments that alter the herb layers and soil surface (e.g., mowing and seeding) and negatively or positively affect sagebrush during the establishment phase. The herbaceous flora of the sagebrush steppe is highly variable due to exotic plant invasions and management actions. Seed source is considered critical for planting success and determining how big sagebrush populations compete with different types and abundances of herbs will contribute to appropriate seed selection for particular restoration sites.

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Tables

Table 17. Description of big sagebrush seed sources utilized in this study. **Bold seed sources indicate seedlings planted in full-factorial experimental design inside grazing exclosures.**

<i>Provenance</i>	<i>State</i>	<i>Location</i>	<i>Subspecies</i>	<i>Climate-of-origin</i>	<i>Ploidy</i>
IDT-2	ID	Orchard (Local)	Tridentata	Local	2N
BOP-W	ID	Birds of Prey	Wyomingensis	Local	4N
IDV-2	ID	(Local)	Vaseyana	Local	2N
NMT-2	NM	San Luis Mesa	Tridentata	Dry	4N
UTT-1	UT	Canyon B.	Tridentata	Cool/Wet	2N
ORT-2	OR	Echo	Tridentata	Warm/Dry	2N
MTW-3	MT	Montana	Wyomingensis	Cool/Wet	4N
ORV-1	OR	Lookout Mountain	Vaseyana	Cool/Wet	4N
CAT-2	CA	Benton B.	Tridentata	Dry	2N
IDV-3	ID	Stanley	Vaseyana	Cool/Wet	2N

Table 18. Coefficients of survival model. Positive coefficients show increased hazards of death relative to the base level of the explanatory variable which is control and not seeded for treatments types and IDT2 for population. Asterisks indicate level of significance: $*=p>0.05$, $**=p>0.01$, $***p>0.001$.

Variable	Parameter estimate	Hazard Ratio	SE	z	p-Value	
Herbicide	0.132	1.141	0.402	0.327	0.744	
Mow + Herbicide	0.568	1.764	0.409	1.388	0.165	
Mow + Herbicide	-0.326	0.722	0.397	-0.823	0.411	
Seeded	0.655	1.925	0.415	1.579	0.114	
CAT2	1.305	3.688	0.442	2.956	0.003	**
NMT2	0.966	2.627	0.425	2.271	0.023	*
ORT2	0.659	1.933	0.416	1.586	0.113	
Herbicide x Seeded	-1.075	0.341	0.576	-1.867	0.062	
Mow+Herbicide x Seeded	-1.011	0.364	0.586	-1.725	0.085	
Mow x Seeded	0.102	1.107	0.582	0.175	0.861	
Herbicide x CAT2	-0.794	0.452	0.608	-1.306	0.192	
Mow+Herbicide x CAT2	-1.460	0.232	0.608	-2.403	0.016	*
Mow x CAT2	-1.097	0.334	0.594	-1.846	0.065	
Herbicide x NMT2	-0.301	0.740	0.600	-0.502	0.616	
Mow+Herbicide x NMT2	-1.099	0.333	0.595	-1.848	0.065	
Mow x NMT2	-0.917	0.400	0.581	-1.578	0.115	
Herbicide x ORT2	-0.021	0.979	0.594	-0.036	0.971	
Mow+Herbicide x ORT2	-1.026	0.358	0.587	-1.748	0.080	
Mow x ORT2	-0.231	0.794	0.575	-0.401	0.688	
Seeded x CAT2	-1.465	0.231	0.619	-2.365	0.018	*
Seeded x NMT2	-1.664	0.189	0.598	-2.784	0.005	**
Seeded x CAT2	-0.951	0.386	0.595	-1.599	0.110	
Herbicide x Seeded x CAT2	1.865	6.455	0.855	2.181	0.029	*
Mow+Herbicide x Seeded x CAT2	2.308	10.055	0.861	2.681	0.007	**
Mow x Seeded x CAT2	1.536	4.648	0.852	1.803	0.071	
Herbicide x Seeded x NMT2	1.203	3.329	0.830	1.448	0.148	
Mow+Herbicide x Seeded x NMT2	2.742	15.522	0.850	3.227	0.001	**
Mow x Seeded x NMT2	2.018	7.524	0.841	2.400	0.016	*
Herbicide x Seeded x ORT2	0.707	2.029	0.835	0.848	0.397	
Mow+Herbicide x Seeded x ORT2	2.719	15.162	0.862	3.155	0.002	**
Mow x Seeded x ORT2	0.374	1.454	0.827	0.453	0.651	

Figures

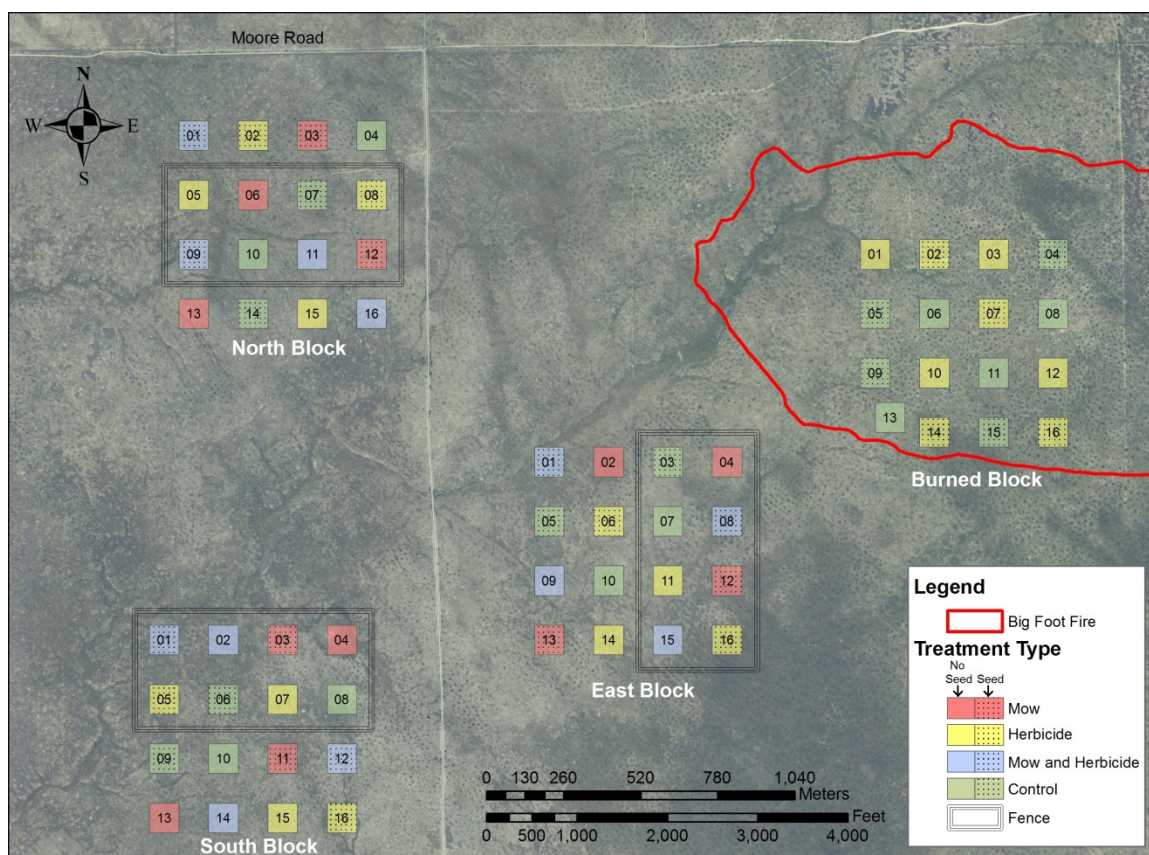


Figure 16. Experimental layout of management treatments of herbs. Map provided by D. Shinneman, USGS.

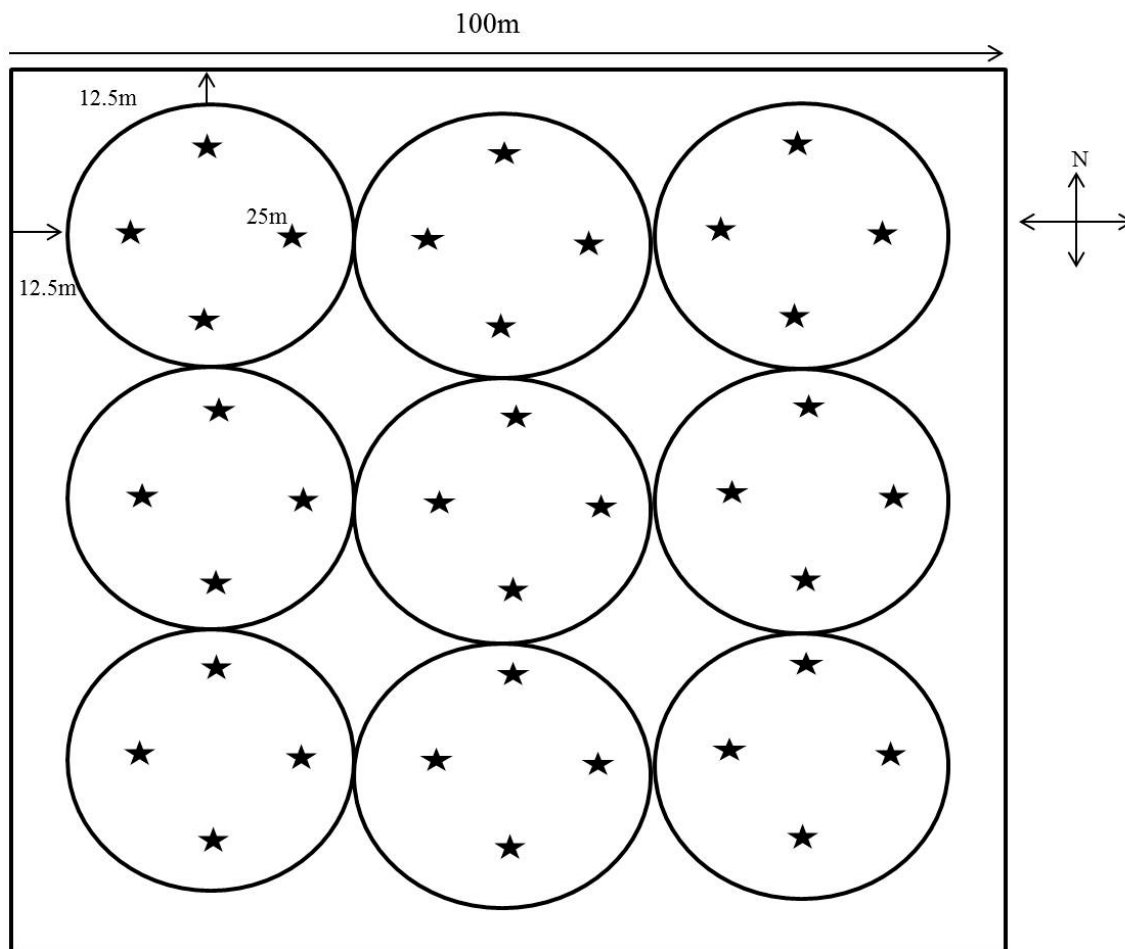


Figure 17. Experimental layout of seedling outplanting design within each treatment type at JFSP. Stars indicate location of outplanted seedling, $n=36$ per plot.

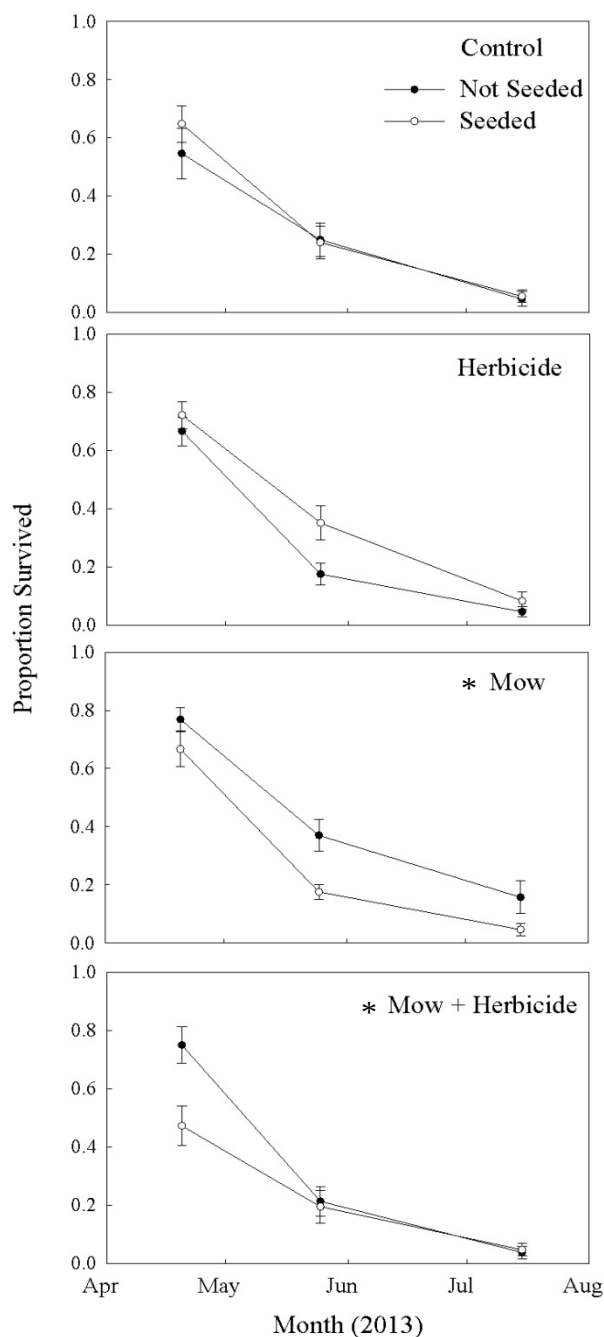


Figure 18. Differences in survivorship among 8 treatments in April, May, and June 2013. Results in Table 18 indicate significant interactions between seeding x mowing and seeding x mowing + herbicide. These results show cumulative survival among all populations by treatment type. High mortality of seedlings resulted in limited ability to evaluate differences in population survivorship. Asterisks indicate significant interactions in survival models. No patterns in mortality among treatments were detected in March 2014 and data is not graphed.

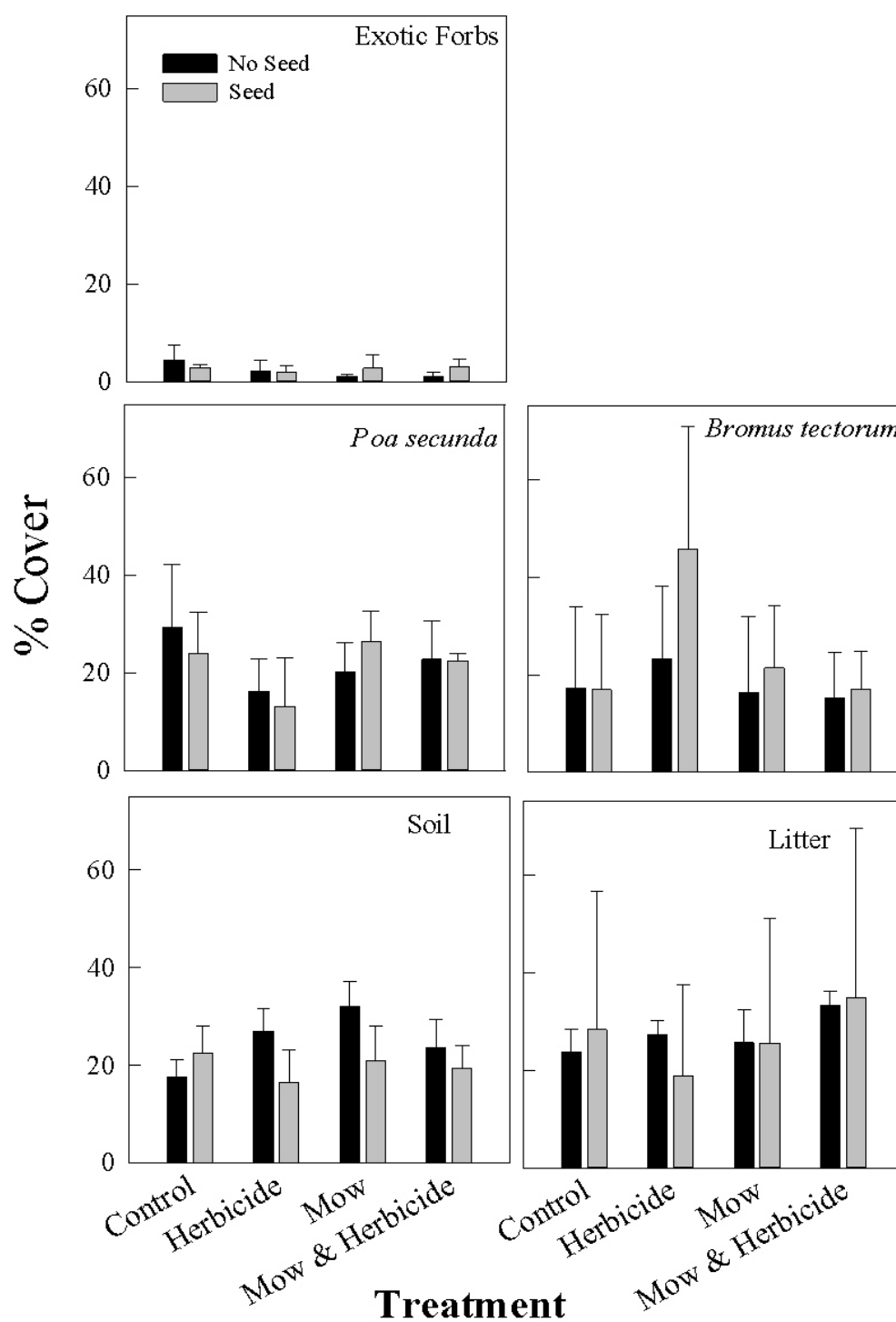


Figure 19. Mean plant community cover determined from line-point intercept in monitoring circles. Categories are exotic forbs (*Salsola tragus* and *Sisymbrium altissimum*), cheatgrass (*Bromus tectorum*), sandberg bluegrass (*Poa secunda*), bare soil, and litter. No significant differences were detected between treatment groups.

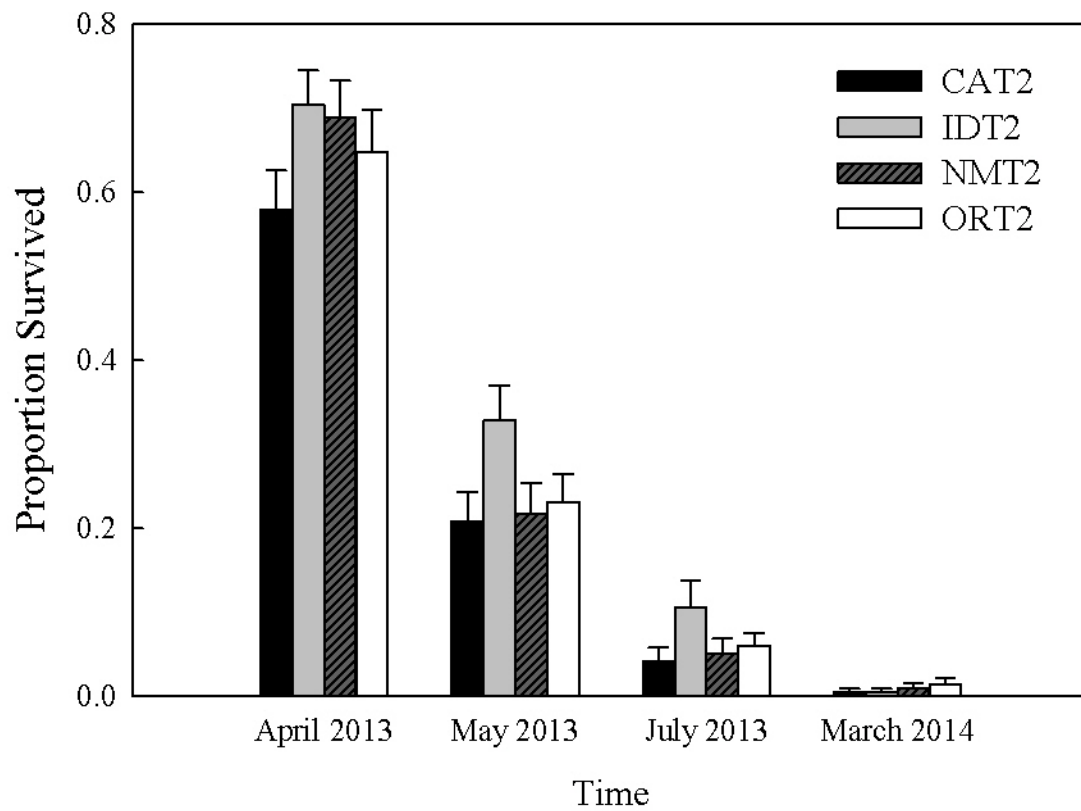
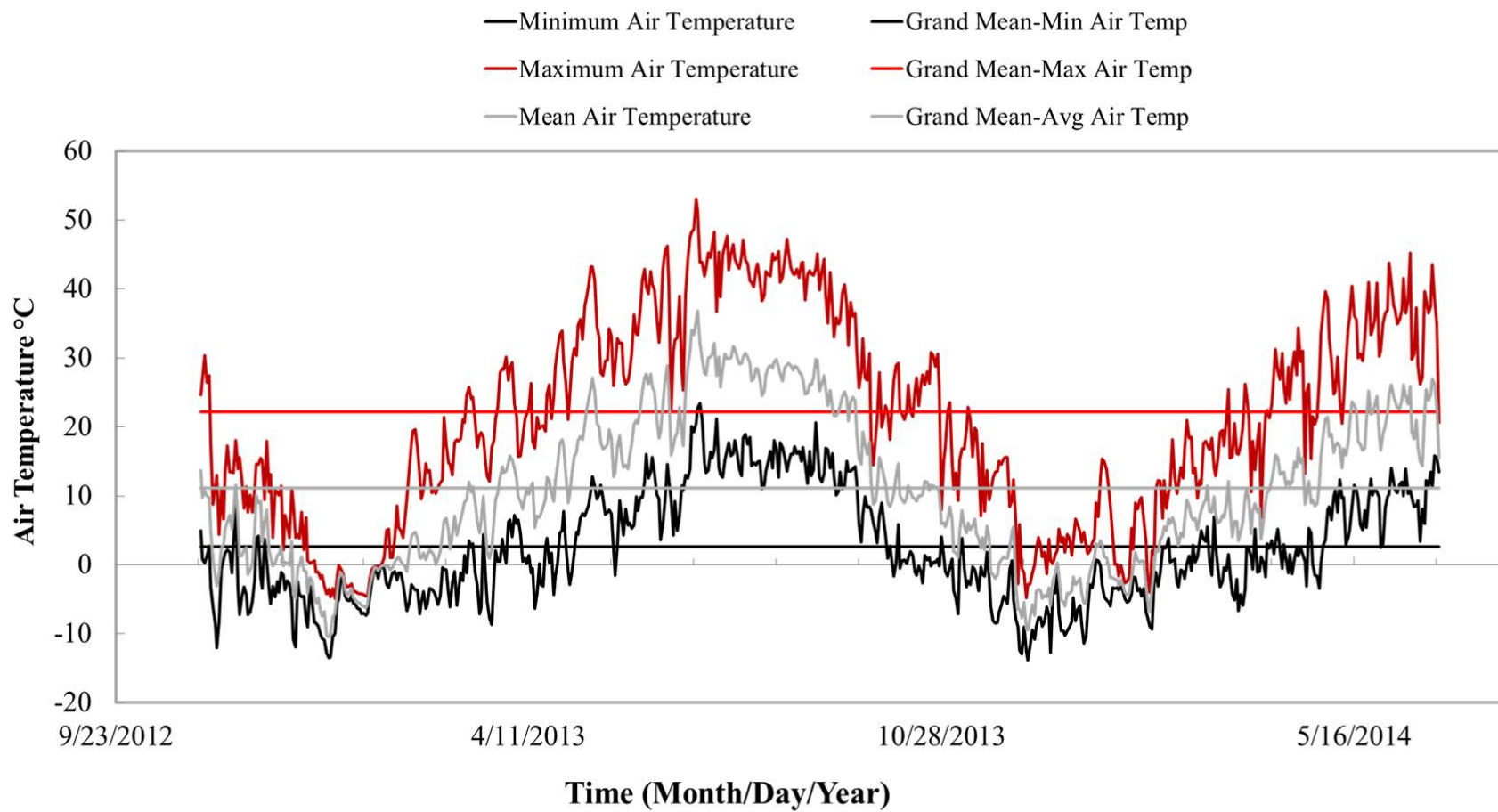
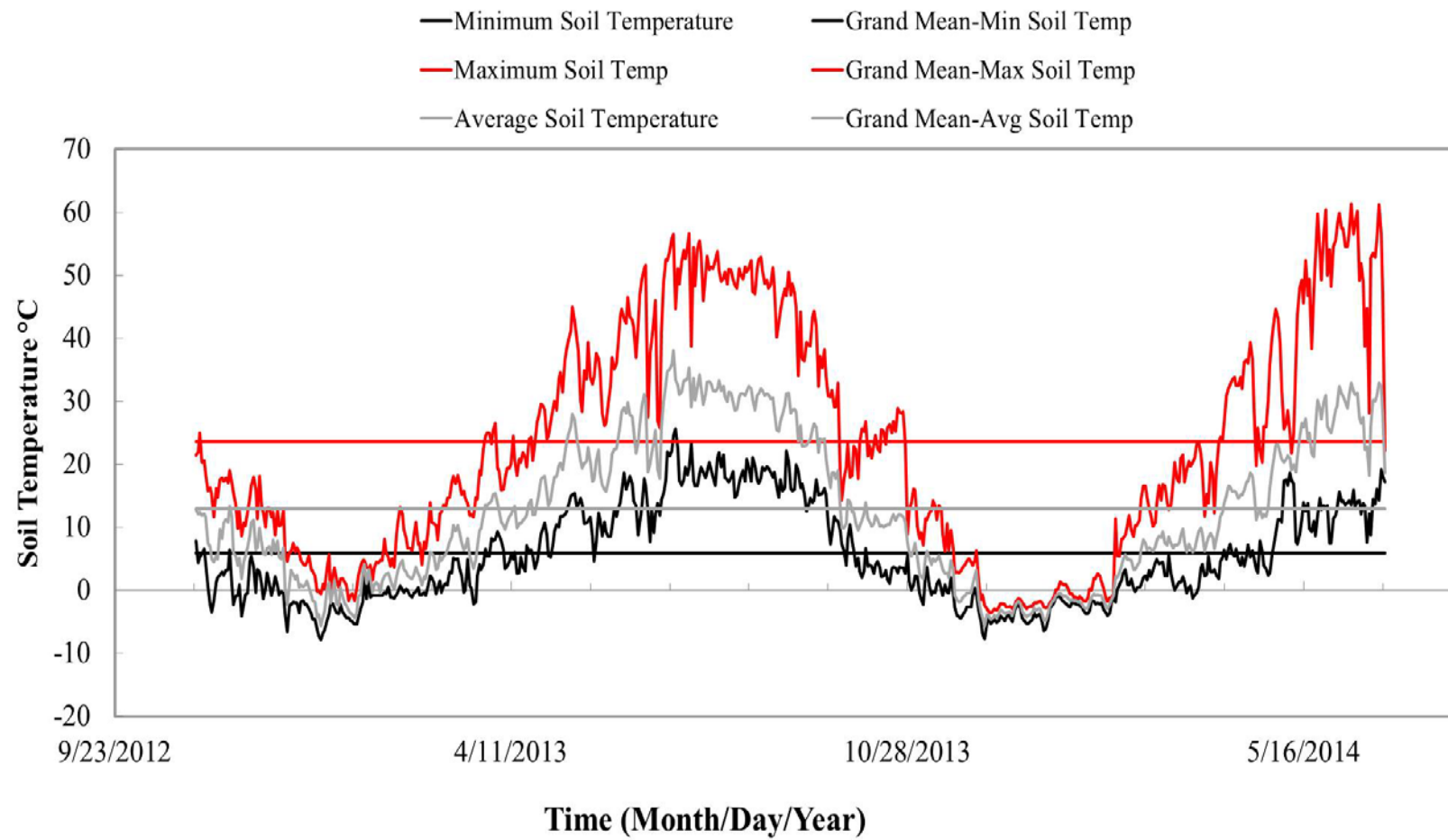


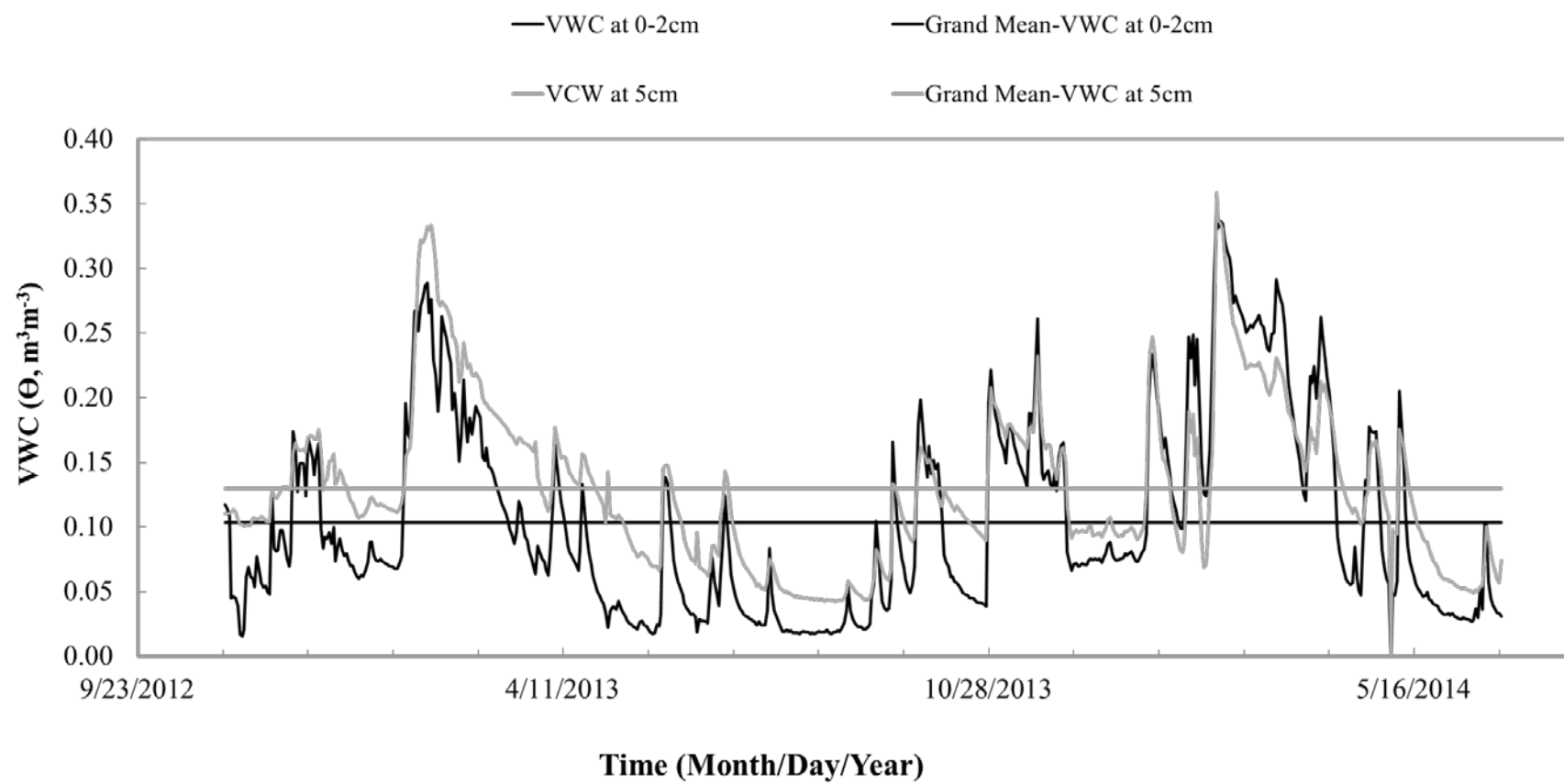
Figure 20. Differences in survivorship of four seed sources of *A.t. tridentata*. IDT2, the local seed source, comprised 35% of survivors in Fall 2013; however, only one IDT2 seedling remained in March 2014. ORT2 had greatest overall survivorship, with 3 survivors out of 7 total seedlings. n=216.

APPENDIX A

**Microclimate Data for Air, Soil, and Volumetric Water Content from All 5 Blocks
at BOP NCA. Graphs Show Climate Data from the Control Plots Only.**







APPENDIX B

Seed Mixes and Seeding Rates Used in Management Treatments at BOP NCA

Table B1. Species and seeding rates.

Plant Species	Common Name	PLS	PLS lbs/acre
Shrubs			
<i>Artemisia tridentata</i> ssp. <i>wyomingensis</i>	Wyoming big sagebrush	0.2836	0.936
<i>Ericameria nauseosa</i>	rubber rabbitbrush	0.4052	0.3975
<i>Krascheninnikovia lanata</i> (Lincoln Co. NV)	winterfat	0.7	0.25
Perennial Grasses			
<i>Poa secunda</i>	Sandberg bluegrass (Mt. Home)	0.8198	0.4
<i>Elymus elymoides</i> (Vale)	bottlebrush squirreltail	0.97	0.4
<i>Elymus wawawaiensis</i>	Snake River wheatgrass	0.75	0.35
Forbs			
<i>Astragalus filipes</i>	Threadstalk milk-vetch	0.57	0.1
<i>Achillea millefolium</i> (Eagle)	Eagle yarrow	0.9425	0.1461
<i>Chaenactis douglasii</i> or <i>Erigeron pumilus</i>	Douglas false yarrow		0.15
<i>Sphaeralcea munroana</i>	Munro globemallow	0.61	0.15
<i>Lomatium dissectum</i>	fern-leaf biscuitroot		0.1
<i>Machaeranthera canescens</i>	hoary aster		0.1
<i>Penstemon acuminatus</i>	sharp-leaf penstemon	0.57	0.1

Table B2. Seed mixes and species used in broadcast and drill seedlings at BOP NCA.

Broadcast Mix 1	<i>Artemisia tridentata</i> ssp. <i>wyomingensis</i> , <i>Ericameria nauseosa</i> , <i>Krascheninnikovia</i> <i>lanata</i> , <i>Poa secunda</i> , <i>Achillea</i> <i>millefolium</i> (Eagle)
Broadcast Mix 2	<i>Erigeron pumilus</i> , <i>Machaeranthera</i> <i>canescens</i> , <i>Penstemon acuminatus</i>
Drill Mix 1	<i>Elymus wawawaiensis</i> , <i>Elymus elymoides</i> (Vale)
Drill Mix 2	<i>Astragalus filipes</i> , <i>Lomatium dissectum</i> , <i>Sphaeralcea munroana</i>