Climate adaption and post-fire restoration of a foundational perennial in cold desert: insights from intraspecific variation in response to weather

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Summary

1. The loss of foundational but fire-intolerant perennials such as sagebrush due to increases in fire size and frequency in semi-arid regions has motivated efforts to restore them, often with mixed or even no success. Seeds of sagebrush *Artemisia tridentata* and related species must be moved considerable distances from seed source to planting sites, but such transfers have not been guided by an understanding of local climate adaptation. Initial seedling establishment and its response to weather are a key demographic bottleneck that likely varies among subspecies and populations of sagebrush.

2. We assessed differences in survival, growth and physiological responses of sagebrush seedlings to weather among eleven seed sources that varied in subspecies, cytotype and climates-of-origin over 18 months following outplanting. Diploid or polyploid populations of mountain, Wyoming and basin big sagebrush (*A. tridentata* ssp. *vaseyana, A. tridentata* ssp. *wyomingensis* and *A. tridentata* ssp. *tridentata*, respectively) were planted onto five burned sites that normally support *A.t. wyomingensis* with some *A.t. tridentata*.

3. *A.t. wyomingensis* had the most growth and survival, and tetraploid populations had greater survival and height than diploids. Seasonal timing of mortality varied among the subspecies/cytotypes and was more closely related to minimum temperatures than water deficit.

4. Temperatures required to induce ice formation were up to 6 °C more negative in 4n-*A.t. tridentata* and *A.t. wyomingensis* than in other subspecies/cytotypes, indicating greater freezing avoidance. In contrast, freezing resistance of photosynthesis varied only 1 °C among subspecies/cytotypes, being greatest in *A.t. wyomingensis* and least in the subspecies normally considered most cold-adapted, *A.t. vaseyana*. A large spectrum of reliance on freezing avoidance vs. freezing tolerance was observed and corresponded to differences in post-fire survivorship among subspecies/cytotypes. Differences in water deficit responses among subspecies/cytotypes were not as strong and did not relate to survival patterns.

5. *Synthesis and applications.* Low-temperature responses are a key axis defining climate adaptation in young sagebrush seedlings and vary more with cytotype than with subspecies, which contrasts with the traditional emphases on (i) water limitations to explain establishment in these deserts, and (ii) subspecies in selecting restoration seedings. These important and novel insights on climate adaptation are critical for seed selection and parameterizing seed transfer zones, and were made possible by incorporating weather data with survival statistics. The survival/weather statistics used here could be applied to any restoration planting or seeding to help elucidate factors contributing to success and enable adaptive management.

Key-words: climate response, fire, freezing responses, restoration, Sagebrush, seed zones, seedling survival, semi-arid landscapes, water stress, weather

Introduction

Changes in wildfire regimes have impacted many semi-arid landscapes globally, often diminishing native foundational species and creating a need to restore them. Increases in fire and invasives such as cheatgrass *Bromus tectorum* L. have transformed or eliminated millions of hectares of sagebrush steppe in the Western USA during the past century (Miller *et al.* 2011). The resulting decline of fire-intolerant perennials such as sagebrush and sagebrush-obligate wildlife species like the greater sage grouse *Centrocercus urophansianus* has motivated large efforts to plant or seed sagebrush and associated species (Jewell 2015). Restoring desirable perennials to these semi-arid ecosystems is challenging, and the success of establishing sagebrush has been mixed (Knutson *et al.* 2014).

Like many widespread species, big sagebrush is diverse and includes three widely recognized subspecies and two cytotypes (McArthur & Sanderson 1999; Richardson *et al.* 2012). Wyoming big sagebrush *Artemisia tridentata* ssp. *wyomingensis* is tetraploid and occurs in relatively warm and dry areas that can have shallow soils. Basin big sagebrush *A.t. tridentata* is diploid or tetraploid and occurs in deep, well-drained soils at lower elevations. Mountain big sagebrush *A.t. vaseyana* is diploid or tetraploid and occurs at relatively higher elevations that are cooler and have more precipitation. These subspecies do not resprout after burning, and their obligate seed strategy is complicated by short seed viability and dispersal distances (<few years or metres). The greatest invasion and fire issues are in *A.t. wyomingensis* sites, where effects of competition from exotic herbs and frequent unfavourable weather on seedling survival can compound seed limitations (e.g. Brabec *et al.* 2015). 'Exotic' subspecies are often seeded onto burn areas (e.g. *A.t. vaseyana* seeded into *A.t. wyomingensis* sites), and seed transfers from 300–600 km across climate gradients have been common (Germino 2014). Population differentiation in sagebrush is known across topographic gradients (McArthur & Sanderson 1999) and it follows that seed transfers among regions could affect seeding success.

Several studies reported differences in physiology, growth and survival among sagebrush subspecies (e.g. Kolb & Sperry 1999; Loik & Redar 2003). Polyploidy is considered important to climate adaptation and is known to be essential for warm-desert shrubs (e.g. *Larrea tridentata*) and polyploids are hypothesized to have greater stress resistance, particularly to water deficit (Hao *et al.* 2013). The importance of polyploidy to climate adaptation in big sagebrush has not been experimentally evaluated. Furthermore, big sagebrush ecosystems are cold deserts where periods of water availability are strongly constrained by low temperatures, and little is known about the relative importance of water and temperature on the different sagebrush subspecies/cytotypes to identify climate parameters necessary for seedling establishment

(Maier *et al.* 2001). Greenhouse trials with *A.t. tridentata* and ssp. *vaseyana* suggest differences in minimum-temperature responses could be important to subspecies distribution (Loik & Redar 2003; Lambrecht, Shattuck & Loik 2007); however, no field studies have characterized the variability in freezing responses among populations of *A. tridentata,* including all three subspecies and two cytotypes.

These information gaps on population variability and climate parameters are essential for understanding effects of seed transfer. While the use of seed transfer zone guidelines is long established in forest, crop and horticultural disciplines, their basis and application in desert environments is underdeveloped. Further, post-seeding weather is highly variable in cold deserts such as sagebrush steppe, and the timing of precipitation often overlaps with physiologically limiting temperatures and is suspected to cause high failure rates in seedings or plantings. Published literature on dryland restoration seldom incorporates the effects of weather variability on seedling establishment (Hardegree *et al.* 2011). Moreover, available literature tends to focus on successful restoration and so the most challenging restoration settings have little information to guide improvements, which may relate to a lack of statistical tools to draw inference from mortality patterns. Most ecological/demographic research has used Cox or Kaplan–Meier statistics which cannot incorporate time-varying factors such as weather. Statistical methods from epidemiology offer an underutilized means to relate post-seeding weather to mortality patterns in outplantings such as sagebrush. Initial seedling establishment and weather variability are cruxes for restoration of sagebrush steppe ecosystems and other semi-arid environments, and their consideration may improve restoration outcomes.

The objective of this study was to evaluate intraspecific variation in ecophysiological responses of big sagebrush seedlings to weather variation at the Morley Nelson Birds of Prey National Conservation Area (hereafter BOP NCA), with an emphasis on detecting population differences in threshold responses to water deficit and temperature. The BOP NCA is considered an *A.t. wyomingensis* site with some *A.t. tridentata*. We evaluated how survivorship, growth and freezing response varied among the subspecies/cytotypes and populations from different climates-of-origin (Table 1). We predicted that: (i) survival would be greater for the native *A.t. wyomingensis* than the other 'exotic' subspecies, (ii) *A.t. vaseyana* would be least impacted by low-temperature stress and most negatively affected by water deficit, (iii) tetraploid cytotypes would exhibit greater survival, growth and resistance to climate/weather stress than diploid cytotypes, and (iv) ecophysiological differences in freezing response (both avoidance and tolerance of/to freezing) or water stress would correspond with differences in weather-related survival patterns among the subspecies, cytotypes and populations of big sagebrush.

Table 1. Seed sources evaluated at BOP NCA in Idaho, USA. State abbreviations are ID = Idaho, NM = New Mexico, UT = Utah, OR = Oregon. The climate-of-origin column indicates the seed source climate type relative to BOP NCA, which is the 'local' site. See Table S1 for climate-of-origin information

Identity	State	Location	Subspecies	Climate-of-Origin (Relative to BOP NCA)	Ploidy
IDT-2	ID	Orchard (Local)	*tridentata*	Local	2N
NMT-2	NM	San Luis Mesa	*tridentata*	Dry	4N
UTT-1	UT	Canyon B.	*tridentata*	Cooler/Wetter	2N
ORT-2	OR	Echo	*tridentata*	Warmer/Dry	2N
IDV-2	ID	Lucky Peak	*vaseyana*	Cooler/Wetter	2N
ORV-1	OR	Lookout Mountain	*vaseyana*	Cooler/Wetter	4N
IDV-3	ID	Stanley	*vaseyana*	Cooler/Wetter	2N
BOP-W	ID	Birds of Prey (Local)	*wyomingensis*	Local	4N
IDW-2	ID	Orchard (Local)	*wyomingensis*	Local	4N
MTW-3	MT	Montana	*wyomingensis*	Cooler/Wetter	4N
IDW-3	ID	Sommer Camp	*wyomingensis*	Dry	4N

Materials and methods

PLANT MATERIAL AND GERMINATION

We used eleven different seed sources that varied in subspecies and cytotype, that were local or distant from BOP NCA and that included contrasting climates-of-origin and had been genetically identified populations in Richardson *et al.* (2012; Table 1). Seedlings were grown outdoors about 25 km from the planting/study site and at the same elevation. Seeds were sown into 164-mL Conetainers™ filled with native soils (silty loam) on 10 August 2012. Germination occurred after one week and seedlings were thinned to one plant per pot. Seedlings were periodically watered and rearranged to limit influence of microsite. Seedlings were germinated outdoors in a shady area and moved to a full-sun area during cold hardening in November 2012 prior to outplanting.

SITE CONDITIONS AND STUDY DESIGN

The study area in BOP NCA is classified as having loamy soil and receiving 19–29 cm annual precipitation. Native bunchgrasses such as *Pseudoroegneria spicata* (Pursh) A. Löve and *Achnatherum thurberianum* (Piper) Barkworth once co-dominated the BOP NCA with sagebrush, but the area is now dominated by mixes of *B. tectorum* and the native *Poa secunda* J. Presl as well as a wide range of other exotic annuals and biennials and naturalized perennials. These vegetation changes resulted from recurrent fires in recent decades and increases in cheatgrass that enhanced fire fuels before and especially following each fire. Perennial bunchgrasses are also often depleted by inappropriate grazing, and the sites have long seasons for livestock grazing excluding the two years following fire.

Study plots were established in five separate wildfire sites that burned in the summer of 2012 at BOP NCA, south of Boise, Idaho (see Fig. S1 in Supporting Information for site locations). The five test sites encompass a 13·3 km² flat area on the Snake River Plain and represent a variety of pre-burn conditions. The most northern site is located at 43·396°N, −116·365°W and the southern site is at 43·322°N −116·405°W, and is located 8·8 km away from the northern site. No climatic gradient was detected between the five site locations. Each site was fenced with the blocking unit containing all seed sources in randomized complete block layout. Seedlings were outplanted in November 2012, when their heights ranged 2·6–3·8 cm. At all five study locations, ten seedlings per population

were bare-root-transplanted into a small core holes arrayed in a stratified random layout with 10 cm spacing between seedlings. We added 100 mL of water to each seedling upon planting and again several weeks later (water is customary in BOP NCA). There was no above-ground vegetation at the time of outplanting (due to burning in summer 2012) and little recovery of herbaceous vegetation occured during the entire study period.

Edaphic properties were also evaluated to assess their potential to cause block effects. Soil texture was determined using a modified sedimentation technique on five cores per plot with 0–10 cm soil depth per core (Gee & Bauder 1986). From these same soil samples, percentage of organic matter was approximated by mass-loss-on-ignition using a muffle furnace (Schulte & Hopkins 1996), and nitrogen and carbon content using a flash combustion analyser (ECS 4010; Costech Analytical Technologies, Valencia, CA, USA).

MICROCLIMATE

Volumetric water content of soils (VWC, Θ, m³ m⁻³) was determined using electronic sensors (5-cm probes, model EC-5 and Em50 logger; Decagon Devices, Pullman, WA, USA), inserted horizontally directly below the soil surface (approximately 2-cm deep). Air and soil surface temperatures were recorded using HOBO™ H08-032-08 and Pendant UA-002-64 loggers (Onset Computer, Pocasset, MA, USA), with sensors in two positions: at 5 cm above the soil to measure air temperatures affecting seedlings, and directly beneath the soil surface spanning 0–2 cm depth. Radiation shields were used for all above-ground sensors. All measurements were made hourly after outplanting from November 2012 through May 2014. Growing degree days (GDD, °C day) were calculated for each site by determining the mean daily temperature ((Max Air Temp – Min Air Temp)/ 2) minus base temperature (10 °C). Growing degree days were then summed at each site for every month after outplanting. Frost-free days (FFD, °C day) were calculated as a count of the number of days per month over 0 °C. Minimum soil and air temperatures were the lowest temperature recorded within a month for both air and soil.

PLANT RESPONSES

Height and survival were recorded for each seedling upon outplanting in 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 regreening occurred after rain. Growth assessments included all seedlings that survived the entire observation period at the northern site ($n = 35$ plants for *A.t. wyomingensis*, $n = 14$ for 2n-*A.t. tridentata*, $n = 8$ for 4n-*A.t. tridentata*, $n = 11$ for 2n- *A.t. vaseyana*, $n = 10$ for 4n- *A.t. vaseyana*). Ecophysiological assessments included plant water status, water-use efficiency using carbon isotopes, specific leaf area, wood density, gas exchange, and threshold freezing and stress responses to climate/weather ($n = 3$). The unit of replication for seedling survival was many seedlings stratified within blocks (sites). Individual seedlings at the northernmost site were the unit of replication for growth and physiological measurements due to plant material limitations.

Chlorophyll fluorescence, specifically dark-adapted F_v/F_m, was measured in April 2013, November 2013, January 2014 and February 2014. F_v/F_m is the ratio of variable to maximum florescence emitted from chlorophyll *a* of photosystem II (PSII) and is the maximum intrinsic light-use efficiency of PSII. Chlorophyll *a* fluorescence from PSII is an indicator of physiological stress, specifically photosynthetic sensitivity to temperature stress, and decreases in F_v/F_m following a freezing result from destabilization or damage to chloroplastic membranes (Larcher 1995). F_v/F_m was measured at pre-dawn using a model 6400-40 fluorometer (LiCor, Inc., Lincoln, NE, USA). Pre-dawn water potential (Ψ_{leaf}) was measured once, on 28 June 2014, using a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR) fit with a 1/8 inch compression gasket. Wood density, an indicator of hydraulic resistance and vulnerability to cavitation, was determined by measuring the average diameter of a 1 cm length of stem with callipers and then obtaining the dry weight of the stem in June 2014. Greater wood density implies enhanced drought tolerance.

Gas exchange was measured in April 2014 between 10:00 and 16:00 h local time using a 2×3 cm leaf chamber and external LED light source connected to a portable, open-flow photosynthesis system (Model 6400; LiCor Inc.). The chamber was clamped onto fully elongated leaves from seedlings of each population. Photosynthesis is reported on a projected leaf area basis using photos and image analysis (ImageJ; Scion, Frederick, MD, USA). Specific leaf area (SLA) was also determined as leaf area (cm^2) to leaf mass (mg) for each population in January 2014.

To assess freezing point as a measure of freezing avoidance, three to five excised leaves from different plants of each population were placed on a ceramic thermoelectric module that emitted a voltage signal upon sensing the exothermic release of heat when ice formed (CP14-127-06-L1-RT-W4·5; Laird Technologies, Earth City, MO, USA). Leaf temperatures were measured with fine-wire thermocouples attached to leaves, and volt readings were recorded by a data logger at five-second intervals (model CR7; Campbell Scientific, Logan, UT), as the plate-to-leaf assemblies were 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).

To assess freezing resistance, we measured F_v/F_{m50}, which is the temperature causing 50% loss of chlorophyll *a* fluorescence in our study. Excised leaves were sealed in plastic bags and chilled at 4 °C per hour to -5, -10, -12, -14, -16, -18 or -22 °C, and chlorophyll florescence (F_v/F_m) was measured after samples warmed to room temperature in darkness.

Freezing-response strategies (tolerance vs. avoidance) can be distinguished by comparing the F_v/F_{m50} temperature (point of irreversible damage to the plant tissue) to the temperature when 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 avoidance rather than tolerance strategy for dealing with cellular freezing. Conversely, a significantly lower F_v/F_{m50} value relative to freezing point suggests a freezing-tolerance strategy.

PLANT CARBON ISOTOPES

Carbon isotope ratios ($^{13}C/^{12}C$) provide an integrated measure of water-use efficiency (WUE, photosynthesis/stomatal conductance). Leaf samples were collected in December 2013, dried in an oven at 65 °C, ground to powder and analysed for $^{13}C/^{12}C$ using a Costech flash combustion ECS 4010 and laser spectrometer (Model 2020; Picarro Inc., Santa Clara, CA, USA) and reported on a delta scale relative to Vienna Pee Dee Belemnite ($\delta^{13}C$; less negative values indicate greater WUE; Farquhar, Ehleringer & Hubick 1989).

STATISTICS

Survival

The five blocks were replicates for two survival analyses, one that evaluated survival over the entire study period (Cox) and a second that evaluated survival–weather relationships among months within the study period (logistic regression). Sampling events for seedling survival occurred on a coarse time-scale, meaning individual deaths were recorded in monthly intervals and actual time of death occurred within these intervals. Thus, the time-to-death data are referred to as 'interval-censored' and either must be corrected using an estimation method in Cox regression or alternatively analysed using conditional logistic models fit with a complementary log–log link function (Allison 1982). Cox models cannot incorporate time-varying factors such as weather data, and thus, we used logistic models to analyse survival–weather relationships. However, the logistic model could not evaluate survival over the whole period because of 'complete separation', a condition in which the dependent variable (survival) does not differ at some level of the independent variable; that is, most seedlings died prior to June 2013. Therefore, we used logistic regression to examine seedling survival–weather relationships only during March–June 2013, when the most significant die off occurred.

The semi-parametric Cox proportional hazard regression model was used to estimate the effects of subspecies, cytotype and climate-of-origin on seedling hazards (i.e. risk) of death with the coxph() function (R v.3·0·2; R Core Team 2013, Therneau 2014). The Cox analysis provides 'hazards of death' for each factor which are interpreted as decreased (<1) or increased (>1) probabilities of death. Comparison of survival probabilities between subspecies, cytotype and climate-of-origin groups were assessed with log-rank tests.

In the logistic modelling, survival (binomial) was modelled as a function of month, subspecies/cytotype, a single climate variable, all their interactions as fixed factors, and sites as blocks, for the dates up to June 2013. We fit the models with the generalized estimating equations model framework (GEE; Liang & Zeeger 1986) in geepack library in R (Yan 2002) to examine the population-averaged effects of seed source on survival in lieu of

investigating subject-specific effects like those obtained from generalized linear mixed models (GLMs). The microclimate variables were correlated and so separate models were run for each factor, including (i) GDD, (ii) FFD, (iii) minimum air temperature, (iv) minimum soil temperature and (v) days of soil surface VWC below 0·08 m^3 m^{-3}. In the absence of rain, we observed soils drying readily to 0·08 m^3 m^{-3} VWC and greater time or evaporative demand was required to cause further drying below this threshold. This indicated that a soil VWC near 0·08 m^3 m^{-3} is a transition between free vs. tightly bound water and thus water stress. We compared the five-model candidate set using the relative ranking of QIC values in R library MuMIn (Barton 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.

Seedling growth and physiology statistics

Seedlings at the northern block were the unit of replication for statistics on growth and physiology, due to high mortality at other blocks or the logistical challenge of completing physiological sampling across all the distant blocks in a practical time frame to allow comparison. We used one-way ANOVAs to determine significance of differences in seedling height (on the final observation date at 18 months) and physiological responses among subspecies, cytotype and climate-of-origin. Model assumptions for normality and homogeneity of variance were met unless otherwise noted. F_v/F_{m50} was estimated by least squares regression of the linear relationship of temperatures and F_v/F_m. Freezing-response strategies (avoidance vs. 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's honestly significant difference test for pairwise comparisons (SAS JMP v 12).

Results

SITE CHARACTERISTICS AND SEEDLING SURVIVAL

Seedlings experienced high mortality during unseasonably cold and dry conditions in spring although climate over the whole year was average (see Fig. S2). By June 2013,

there was less than 30·0% survivorship at four of the five blocks, and 78·2% survival at the northernmost block. By May 2014, the most northern block had 71·8% survivorship whereas all other blocks experienced 100% mortality. The most northern block soils had 59·5% clay whereas all other blocks had less than 25% clay content. All other soil properties were similar among blocks (see Table S2).

Seedling survival over the entire duration of the experiment differed among subspecies: for each *A.t. wyomingensis* seedling that died, 1·68 *A.t. vaseyana* ($P < 0.001$) and 1·29 *A.t. tridentata* seedlings perished ($P = 0.072$, diploids and tetraploids combined). Survival of tetraploids was consistently greater than that of diploids (Fig. 1; $\chi^2 = 6.8$, $p = 0.009$ for *A.t. vaseyana* and $\chi^2 = 4.4$, $P = 0.036$ for *A.t. tridentata*). When comparing all five subspecies and cytotypes, no significant differences were detected between survival rates of *A.t. wyomingensis*, 4n-*A.t. tridentata* and 4n-*A.t. vaseyana*; however, both 2n-*A.t. vaseyana* and 2n-*A.t. tridentata* had increased risk of death relative to *A.t. wyomingensis*: for each *A.t. wyomingensis* seedling that died, 2·16 2n-*A.t. vaseyana* ($P < 0.001$) and 1·48 2n-*A.t. tridentata* seedlings perished ($P = 0.011$, data not shown).

According to conditional logistic model selection, mortality patterns were more closely related to minimum temperatures of the soil surface (QIC = 1378) than minimum temperatures of air (QIC = 1473), GDD >10 °C (QIC = 1462) or days of VWC <0·08 (QIC = 1466; the FFD model did not meet data structure requirements). Survival probability of *A.t. wyomingensis* was positively related to minimum temperatures in March, April and June, but not in May (Table 2, Fig. 2). Survival of both cytotypes of *A.t. vaseyana* was not associated with minimum temperatures in March, but was positively correlated in April, May and June (Table 2, Fig. 2). Survival probability of *A.t. tridentata* was positively related to minimum soil temperature in March, particularly for the tetraploid population that exhibited a greater increase in survival per increment of temperature compared to all other subspecies/cytotypes (Table 2, Fig. 2). In April and May, survival of 2n but not 4n *A.t. tridentata* was also positively correlated to minimum soil temperature
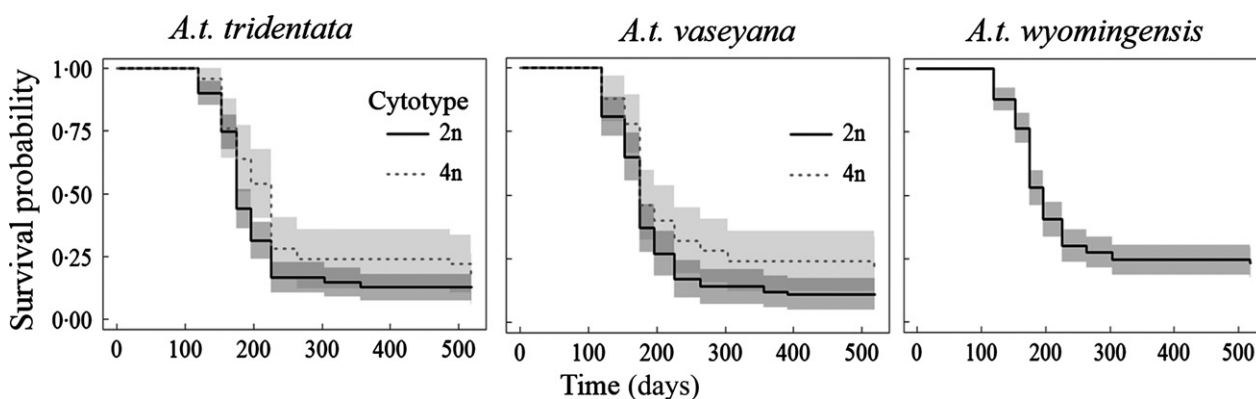

Fig. 1. Survival curves for all three subspecies of big sagebrush seedlings across all blocks from October 2012 to May 2014. Shaded intervals represent 95% confidence intervals.

Table 2. Coefficients for the log-odds of seedling survival, as a function of minimum soil temperature. The coefficients represent the slope and intercept of the fitted probability curve for every one unit change in minimum soil temperature while holding month and cytotype constant. Values after ± symbol represent 95% confidence intervals (CIs) for the slope and intercept of the predicted probabilities (see Fig. 2). Significant differences in both slope and intercept are indicated by different letters among subspecies/cytotypes, as assessed by non-overlapping CIs

Subspecies/Cytotype	March	April	May	June
A.t. tridentata, 2n	$0.27 \pm 0.44(x) + 1.34 \pm 0.12$ A	$0.22 \pm 0.16(x) + -0.38 \pm 0.16$ B	$0.03 \pm 0.06(x) -0.06 \pm 0.23$ A	$0.33 \pm 0.13(x) - 3.60 \pm 1.43$ A,B
A.t. tridentata, 4n	$0.66 \pm 0.12(x) + 2.36 \pm 0.30$ B	$-0.01 \pm 0.13(x) + 0.55 \pm 0.36$ A	$-0.13 \pm 0.07(x) + 1.09 \pm 0.34$ B	$0.32 \pm 0.05(x) - 3.37 \pm 0.56$ A
A.t. vaseyana, 2n	$-0.03 \pm 0.07(x) + 0.62 \pm 0.38$ C	$0.39 \pm 0.16(x) - 0.53 \pm 0.44$ B	$0.20 \pm 0.03(x) - 0.29 \pm 0.21$ A	$0.18 \pm 0.08(x) - 1.43 \pm 0.86$ A
A.t. vaseyana, 4n	$0.06 \pm 0.08(x) + 1.52 \pm 0.20$ C	$-0.26 \pm 0.19(x) - 0.55 \pm 0.50$ B	$0.03 \pm 0.09(x) - 0.10 \pm 0.23$ A	$0.48 \pm 0.03(x) - 5.07 \pm 0.38$ B
A.t. wyomingensis	$0.29 \pm 0.07(x) + 1.52 \pm 0.20$ C	$0.36 \pm 0.09(x) - 0.21 \pm 0.27$ B	$-0.03 \pm 0.07(x) + 0.35 \pm 0.20$ C	$0.34 \pm 0.08(x) - 3.06 \pm 0.96$ A

We assessed seedling survival as a function of climate-of-origin relative to the study site (categorical variable with three levels: 'local', 'cooler/wetter' and 'more warm/dry') by subspecies for the entire observation period only (monthly analyses precluded by small sample size within subspecies resulting from high mortality, data not shown). Climate-of-origin did not relate to seedling survival for *A.t. wyomingensis* ($\chi^2 = 1.5$, $P = 0.463$) or *A.t. tridentata*. ($\chi^2 = 5.9$, $P = 0.117$). *A.t. vaseyana* populations from cooler/wetter climates had 1.6 greater probability of death relative to the most local *A.t. vaseyana* population ($\chi^2 = 6.3$, $P = 0.012$). While climate-of-origin had marginally significant effects on survival, it is notable that only three big sagebrush populations had 100% survivorship at the northern block: one local population of *A.t. wyomingensis*, as well as two populations from relatively cooler/wetter environments than the study site, a 4n-*A.t. vaseyana* and a Montana *A.t. wyomingensis* population. The Montana population was the only group that developed inflorescences by the time the study ended in June 2014.

GROWTH AND PHYSIOLOGY

A.t. wyomingensis was 22–28% taller than both 2n and 4n-*A.t. tridentata* and ssp. *vaseyana* by the end of the 18-month observation period, and no other height differences were observed (seedlings were of the same size initially; $P = 0.05$, Table 3). 2n-*A.t. vaseyana* had -1.5 per mil more negative $\delta^{13}C$ (less WUE) than 2n-*A.t. tridentata* (Table 3). The 4n-*A.t. tridentata* population had 1.4 MPa less negative Ψ_{leaf} than 2n-*A.t. tridentata*. Wood density did not differ among subspecies or cytotypes. Photosynthesis measured in April 2014 (i.e. not in the 2013 cold period), SLA (Table 3) and F_v/F_m (measured in the field in April and November 2013; January and February 2014, data not shown) did not differ among subspecies or cytotypes. Approximately twice the replication would have been necessary for the lower photosynthesis of 4n-*A.t. vaseyana* to be significant (Table 3, according to a power test).

FREEZING RESPONSE

Temperatures required to cause freezing of leaves (measured in spring 2014) were 2–4 °C lower (=greater avoidance) in *A.t. wyomingensis* and 4n-*A.t. tridentata* than *A.t. vaseyana* populations (Table 4, Fig. 3). No other significant differences in freezing point were detected among subspecies/cytotype. Freezing point did not relate to climate-of-origin for any subspecies. Further, freezing point did not differ among 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/cytotypes was much smaller (in °C) than that observed for freezing point (Fig. 3). Freezing resistance (F_v/F_{m50}) varied only
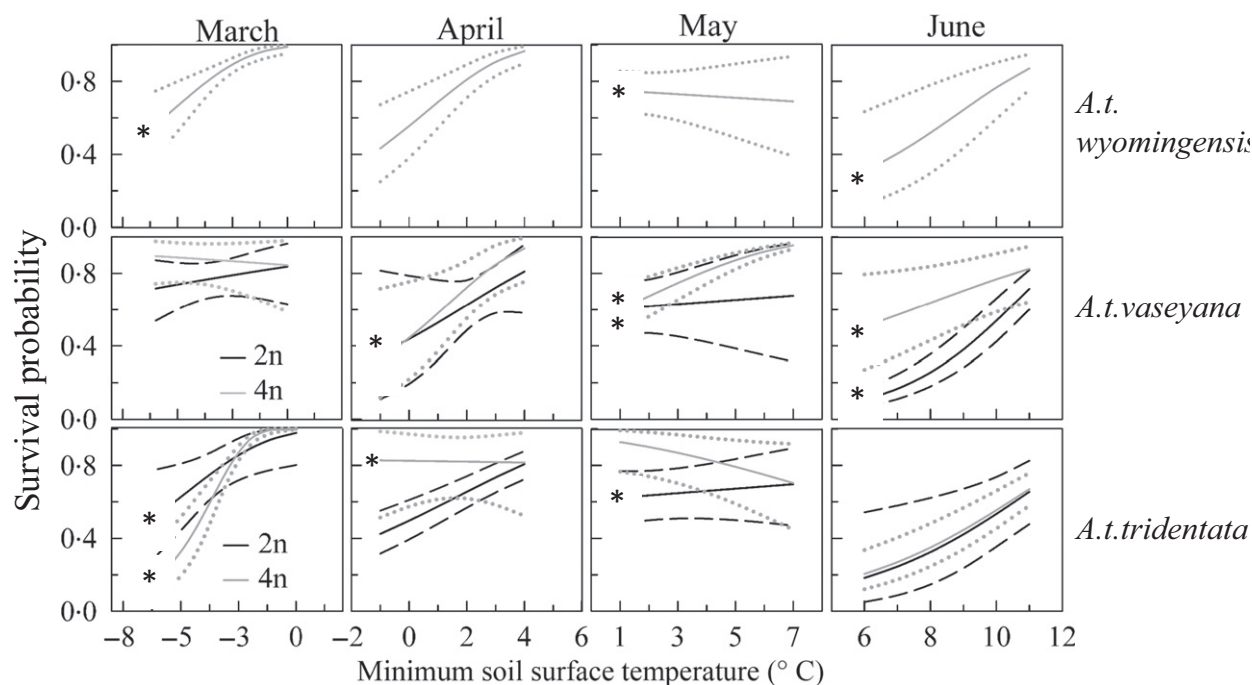
Fig. 2. Relationships of survival probability to minimum soil surface temperatures determined from binomial general linearized model (log-odds of survival where 1 = alive and 0 = dead) for each subspecies/cytotype per degree change in minimum soil temperature per row in March, April, May and June 2013. Dotted or dashed lines show 95% CI, with non-overlap indicating significant differences. Asterisks denote significant relationships between survival and temperature ($P < 0.05$). See Table 2 for additional statistics.

Table 3. Average values (SE ± 1) and statistical results from one-way ANOVAs comparing cytotype/subspecies effects on seedling physiology. Those analyses that were statistically significant at $\alpha = 0.05$ are shown in bold. Unique letters indicate means that are significantly different within in each row using Tukey's honestly significant difference test. WUE is water-use efficiency, SLA is specific leaf area, and Ψleaf is leaf water potential

Variable	*A.t. tridentata*		*A.t. vaseyana*		*A.t. wyomingensis*	F	P
	2n	4n	2n	4n	4n		
WUE (δ^{13})	**−27·20 ± 0·31** [A]	−27·95 ± 0·44	**−28·77 ± 0·31** [B]	−27·93 ± 0·44	−28·03 ± 0·22	**3·20**	**0·03**
SLA (cm^2 mg^{-1})	7·53 ± 0·59	6·90 ± 0·83	7·54 ± 0·59	5·95 ± 0·83	8·02 ± 0·45	1·39	0·26
Wood density (g cm^{-3})	1·75 ± 0·25	1·86 ± 0·25	1·83 ± 0·25	1·99 ± 0·25	2·13 ± 0·11	0·82	0·53
Ψleaf (MPa)	**−2·93 ± 0·22** [A]	**−4·30 ± 0·38** [B]	−3·00 ± 0·296	−3·93 ± 0·38	−3·18 ± 0·19	**3·41**	**0·02**
Photosynthesis (μmol m^{-2} s^{-1})	10·26 ± 1·20	11·70 ± 1·95	10·26 ± 1·51	8·85 ± 1·95	9·12 ± 0·98	0·48	0·75
Height (mm)	**86·14 ± 12·66** [B]	**88·25 ± 16·75** [B]	**77·36 ± 14·28** [B]	**92·80 ± 14·98** [B]	**118·68 ± 8·70** [A]	**2·47**	**0·05**

Table 4. Freezing response of 11 populations of big sagebrush. The Difference column corresponds to the F_v/F_{m50}-freezing point magnitude. Significantly different values notated with asterisks ($P < 0.05$) indicate a freezing-tolerance strategy rather than a freezing avoidance strategy. Data correspond to mean values ± 1SE

| Provenance	Subspecies/cytotype	Freezing Point °C	F_v/F_{m50}	Difference
IDT-2	*tridentata*, 2n	−12·3	−15·52	−3·22
ORT-2	*tridentata*, 2n	−9·7 ± 0·14	−15·06 ± 0·57	−5·22*
UTT-1	*tridentata*, 2n	−8·32 ± 0·46	−14·89 ± 0·77	−6·11*
NMT-2	*tridentata*, 4n	−12·61 ± 0·33	−15·33 ± 0·3	−2·39
IDV-2	*vaseyana*, 2n	−8·12 ± 0·78	−15·5 ± 0·37	−6·60*
IDV-3	*vaseyana*, 2n	−7·96 ± 0·97	−15·08 ± 0·66	−3·97*
ORV-1	*vaseyana*, 4n	−7·44 ± 0·85	−15·8 ± 0·42	−7·51*
BOP-W	*wyomingensis*	−13·13 ± 0·08	−16·75 ± 0·3	−3·55*
IDW-2	*wyomingensis*	−10·7 ± 0·6	−15·86 ± 0·29	−4·56*
IDW-3	*wyomingensis*	−11·4 ± 0·85	−16·65 ± 0·05	−4·40*
MTW-3	*wyomingensis*	−7·58 ± 0·12	−17·21 ± 0·22	−9·51*
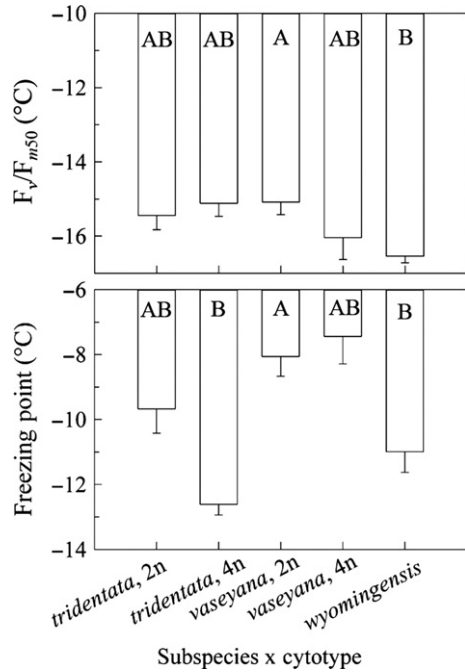
Fig. 3. Variation in F_v/F_{m50} and freezing point (°C) between subspecies and cytotypes. Letters indicate significant difference at $\alpha = 0.05$ between groups using Tukey's HSD.
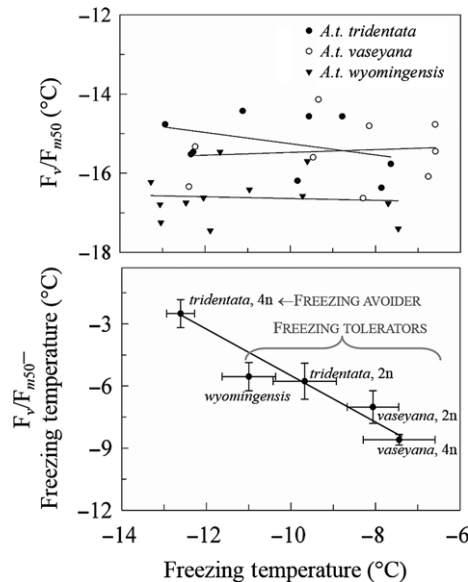

Fig. 4. Relationship of temperature causing 50% loss of F_v/F_m (F_v/F_{m50}) to freezing point (R^2 all three regression lines is $P < 0.05$) and the relationship of the difference between F_v/F_{m50} and freezing point ('F_v/F_{m50} — Freezing Point °C') to freezing point by subspecies and cytotype ($R^2 = 0.776$).

1·1 °C and was greatest in *A.t. wyomingensis* and least in 2n-*A.t. vaseyana* ($P = 0.0017$). There were no differences in F_v/F_{m50} between cytotypes within each subspecies. Freezing resistance did not relate to populations' climate-of-origins.

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 (Fig. 4). The 4n-*A.t. tridentata* population had similar $F_v/$

F_{m50} and freezing point values indicating freezing avoidance while all other populations had tolerance strategies (Fig. 4, Table 4). The relationship between F_v/F_{m50} and freezing point did not reveal a freezing-response trade-off between subspecies and cytotypes (e.g. between freezing tolerance and avoidance; Fig. 4, top). However, plotting the relationship between freezing point and F_v/F_{m50} — freezing point revealed a gradient in the strategies of freezing tolerance and avoidance (Fig. 4, bottom). Variation in F_v/F_{m50} — freezing point among populations was not associated with climate-of-origin.

Discussion

Regarding hypotheses 1 that the local subspecies would have greater survival than 'exotic' subspecies, survival was greater for the native *A.t. wyomingensis* than for the non-native subspecies. Relatively high mortality in *A.t. vaseyana* supported hypothesis 2 that this subspecies, whose native distribution is at higher elevations than the BOP NCA that have considerably different temperature/precipitation regimes, would have less establishment. We did not detect an effect of seed transfer (i.e. 'local' populations vs. those from 'cooler/wetter' and 'more warm/dry' climates) on overall survival (aside from marginally significant effects among populations of *A.t. vaseyana*), but it is notable in the relatively dry spring of our study that populations from warmer/drier climates had no greater survival than populations from local and cooler/wetter climates in *A.t. tridentata* or *A.t. wyomingensis*.

Survival was similar among tetraploids of all subspecies, and their survival and mean height were greater than those of diploids, supporting hypothesis 3 that polyploids would have greater establishment (Fig. 1). While growth and survival were clearly greater for polyploids, physiological differences among cytotypes were evident in only one water deficit parameter and only in *A.t. tridentata* (Ψ_{leaf}). Specifically, low water potential (Ψ_{leaf}) combined with greater survival and similar growth and photosynthesis of 4n- *A.t. tridentata* suggests greater tolerance of water deficit of the tetraploids, adding to their greater freezing avoidance. Tetraploids did not have different WUE, SLA, wood density or photosynthesis, but the differences in survival, growth and freezing responses discussed below suggest cytotype is an important factor in explaining population differences in seedling establishment and survival in sagebrush.

Drought and precipitation patterns are usually thought to drive seedling mortality in desert ecosystems. Although differences in clay content (soil-water retention) among blocks related to their differences in survival, (i) water-related traits (WUE, wood density, water status) did not relate to variation in mortality among subspecies, cytotypes or populations during summer drought, and (ii) survival models that included water-related climate variables (VWC) did not explain as much mortality as minimum temperatures (from model selection). Specifically, the timing of mortality and physiological patterns in freezing responses suggest that

minimum temperatures are a key limiting factor for big sage-brush, even at a relatively low-elevation and warm site for the species (Table 4, Fig. 2). These findings indicate the importance of minimum temperatures and freezing responses in explaining functional diversity in big sagebrush.

EFFECTS OF MINIMUM TEMPERATURE ON SEEDLING SURVIVAL

The ranking of freezing point among subspecies/cytotypes was counterintuitive at first glance, and did not match hypothesis 2 that *A.t. vaseyana* – the subspecies from higher elevations – would be most cold-adapted. 2n-*A.t. vaseyana* seedlings had the least negative freezing points and the least negative F_v/F_{m50} values among all sub-species (Fig. 3) and their considerable mortality was associated with minimum temperatures from March–June 2013 (Fig. 2). We propose that leaves of *A.t. vaseyana* are more likely to freeze in spite of being from higher elevations, per-haps because a greater prevalence of snow cover in their home range normally insulates them against frigid air tem-peratures in winter and requires less physiological invest-ment or allocation into cold adaptation. Snow cover is rare at the BOP NCA (Fig. 5), and increasingly so with recent warming. Therefore, the high mortality in *A.t. vaseyana* may have resulted from less ability to allocate energy to freezing resistance mechanisms, compared to the other sub-species. Adult individuals of *A. tridentata* (presumably *A.t. vaseyana*) growing near their alpine limit had much lower freezing points *in situ* (Loik *et al.* 2004) than the val-ues reported here, and the differences among studies may relate to ontogenic or acclimatizing effects (Loik & Redar 2003). The lower freezing points of *A.t. wyomingensis* and 4n-*A.t. tridentata* (Fig. 3) may relate to their lower-eleva-tion distributions where snow cover is infrequent, and where cold air drains or accumulates and results in low minimum temperatures during winter.

Exposure to winter-like weather in spring 2013 coin-cided with seedling mortality events, but subspecies dif-fered in their response to low temperature. Using the
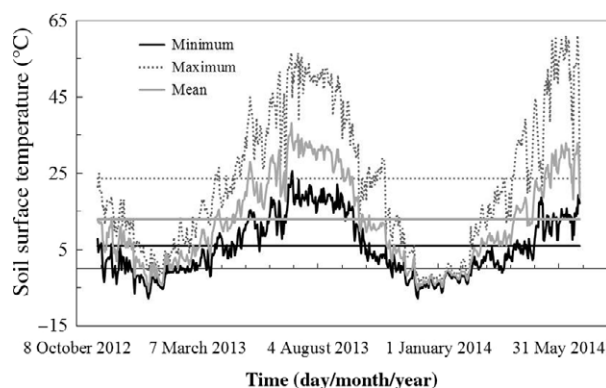

Fig. 5. Mean daily air minimum, maximum and average soil sur-face temperatures °C measured with plots (*n* = 5) over the entire study period. Horizontal lines indicate grand means.

criteria for freezing avoidance vs. tolerance outlined by Sakai & Larcher (1987), our data suggest big sagebrush generally has a freezing-tolerance strategy, except for the 4n-*A.t. tridentata* population, which uses an avoidance strategy owing to its very low freezing point (Fig. 4). Relationships of mortality to minimum temperatures from March to June 2013 corresponded to the variation in freezing-response strategies among subspecies and cytotypes, supporting hypothesis 4 (Fig. 2). Specifically, most mortality of the 4n-*A.t. tridentata* population, an 'avoider', occurred in March, whereas mortality of freez-ing 'tolerators' occurred from April to June. It appears that 4n-*A.t. tridentata* seedlings had less capacity to cope with the winter-like minimum temperatures in March (−10·21 °C, which compares with −10·25 °C in January, respectively), resulting in increased mortality. Freezing avoidance only protects plants for short periods of time during chilling, but the physiological advantage is sub-stantial if ice formation can be avoided (Sakai & Larcher 1987). Freezing events from April to June were less intense and sustained than in March (Fig. 5), and freezing points were not reached for the 4n-*A.t. triden-tata* seedlings, likely resulting in greater probability of survival over these months. It is also likely that freezing avoidance corresponded to high growth rates: in March 2013, height of 4n-*A.t. tridentata* was greatest relative to other groups. Survival of *A.t. wyomingensis,* a 'tolerator', appeared unaffected by the low minimum temperatures in May 2013, unlike other freezing-tolerant subspecies and cytotypes. *A.t. wyomingensis* had low freezing points in addition to greater freezing resistance (indicated by most negative F_v/F_{m50}) and clearly is relatively adapted to cold temperatures compared to the other subspecies.

SUMMARY AND MANAGEMENT IMPLICATIONS

Mortality, growth and response to freezing weather dif-fered among subspecies, cytotypes and populations of big sagebrush during the critical initial establishment phase following fire, when abiotic limitations are pronounced. Physiological differences among the three subspecies were largely driven by cytotype identity, indicating that cyto-type may be as important as subspecies in explaining adaptation and functional diversity in big sagebrush. Additionally, minimum-temperature responses explained more mortality patterns than did water-related responses or traits in big sagebrush, and thus, their consideration in seed-source selection may improve restoration. The impli-cation for management is that matching cytotype of seed source to seeding site in seed selection may enhance restoration, which is problematic considering that cyto-types are not mapped and cannot be readily identified in the field. Cytotype variation occurs in the commonly seeded *P. spicata* and many of the other widespread and dominant species of North American deserts, and the variation can relate to cold adaptation (e.g. *L. tridentata*; Hunter *et al.* 2001). Whether and how cytotype could be

considered seed selection for these other species will require further research, however.

Lastly, we suggest that in spite of tremendous number of seedings of sagebrush and associated species across the Great Basin, not much is known the about the causes of their failures, probably because negative restoration results (i.e. 100% mortality) are usually perceived to yield no insight. The survival modelling procedure we used capitalized on high mortality rates to reveal key factors affecting survival, specifically the effects of weather events. These statistical methods could be applied to actual dryland restoration trials to identify species-specific establishment limitations and enable learning for adaptive management.

Data accessibility

Data are available from https://www.sciencebase.gov/catalog/item/571168 a2e4b0ef3b7ca55572.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Map of study locations and location names at BOP NCA.

Fig. S2. Walter-type climate diagram showing average temperature and precipitation by month for 2013 and the values from 1981 to 2010.

Fig. S3. Mean differences in plant heights among subspecies/cytotypes over the study period at the northern site.

Table S1 Description and climate information for seed sources used in this experiment.

Table S2 Soil properties for the five burned experimental blocks at BOP NCA.