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PLANT REPRODUCTION IN A CHANGING GLOBAL ENVIRONMENT

ORIGINAL ARTICLE

Lethal combination for seedlings: extreme heat drives mortality of drought-exposed high-elevation pine seedlings

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• **Background and Aims** Hotter drought- and biotically driven tree mortality are expected to increase with climate change in much of the western USA, and species persistence will depend upon ongoing establishment in novel conditions or migration to track ecological niche requirements. High-elevation tree species might be particularly vulnerable to increasing water stress as snowpack declines, increasing the potential for adult mortality and simultaneous regeneration failures. Seedling survival will be determined by ecophysiological limitations in response to changing water availability and temperature.
• **Methods** We exposed seedlings from populations of *Pinus longaeva*, *Pinus flexilis* and *Pinus albicaulis* to severe drought and concurrent temperature stress in common gardens, testing the timing of drought onset under two different temperature regimes. We monitored seedling functional traits, physiological function and survival.
• **Key Results** The combined stressors of water limitation and extreme heat led to conservative water-use strategies and declines in physiological function, with these joint stressors ultimately exceeding species tolerances and leading to complete episodic mortality across all species. Growing conditions were the primary determinant of seedling trait expression, with seedlings exhibiting more drought-resistant traits, such as lower specific leaf area, in the hottest, driest treatment conditions. Water stress-induced stomatal closure was also widely apparent. In the presence of adequate soil moisture, seedlings endured prolonged exposure to high air and surface temperatures, suggesting broad margins for survival.
• **Conclusions** The critical interaction between soil moisture and temperature suggests that rising temperatures will exacerbate moisture stress during the growing season. Our results highlight the importance of local conditions over population- and species-level influences in shaping strategies for stress tolerance and resistance to desiccation at this early life stage. By quantifying some of the physiological consequences of drought and heat that lead to seedling mortality, we can gain a better understanding of the future effects of global change on the composition and distribution of high-elevation conifer forests.

Key words: Tree seedling, *Pinus*, heat stress, water stress, functional traits, water balance.

INTRODUCTION

The co-occurrence of drought and heat waves is recognized globally as a leading cause of widespread tree mortality in recent decades (Hammond *et al.*, 2022). High-elevation forests of western North America are experiencing a prolonged mortality event driven by interacting abiotic and biotic stressors (e.g. hotter drought and mountain pine beetle; Dudney *et al.*, 2020) and are projected to face warming of 3–6 °C combined with a potential 80 % reduction in snowpack by the end of the century (Pepin *et al.*, 2015; Schwartz *et al.*, 2017). Warming may be consequential for high-elevation forests owing to its influence on available soil moisture, which is largely determined by snowpack dynamics (Barnett *et al.*, 2005). Furthermore, the position of high-elevation tree species at the upper tree line limits migration potential in response to rapid climate change, with upslope movement limited to where successful dispersal, competitive interactions and suitable substrates align (Davis and Shaw, 2001; Van De Ven *et al.*, 2007). Subsequent reforestation efforts, such as direct seeding, have seen little

success in the harsh, remote rocky conditions characterizing high-elevation environments (Pansing and Tomback, 2019; Hankin *et al.*, 2023). Persistence of snowpack-dependent, high-elevation tree species will probably depend upon continued establishment and survival *in situ*, despite ongoing warming and changing snowpack dynamics in mountainous regions.

Snowpack dynamics (snowpack magnitude and the timing of snowmelt) are strongly linked to high-elevation plant community composition and function, and recent warming-induced changes in snowpack are already impacting these communities (Bader *et al.*, 2017; Andrus *et al.*, 2018; Winkler *et al.*, 2018). Declines in precipitation as snow are likely to accelerate the timing and rate of soil dry-down, lengthening the period of water stress during the growing season (Harpold and Molotch, 2015). As soil moisture declines, plants are susceptible to xylem cavitation (i.e. hydraulic failure), which is a ubiquitous driver of drought-induced tree mortality (Adams *et al.*, 2017; Arend *et al.*, 2021). Associated stomatal closure can also lead to declines in carbon assimilation and carbohydrate transport from the needles to roots, exacerbating drought-induced physiological decline (McDowell *et al.*, 2008). At high elevations, seedlings are readily exposed to desiccation owing to shallow rooting depth, strong winds and high vapour pressure deficits (McIntire *et al.*, 2016); therefore, traits that increase access to water or reduce water loss are particularly advantageous for seedling survival (Kozlowski and Pallardy, 2002; Letts *et al.*, 2014). Drought-adaptive strategies include early investment in roots (Comas *et al.*, 2013), conservative above-ground growth (Kerr *et al.*, 2015), and maintenance of plant water status (e.g. reduced stomatal conductance; Barrios-Masias *et al.*, 2018). Drought acclimatization in seedlings will be critical for surviving the first growing season, when seedling mortality is highest (Castanha *et al.*, 2013) and might ultimately determine tree persistence in high-elevation environments.

Seedling establishment and success in high-elevation forests of the arid Great Basin [dominated by limber (*Pinus flexilis* E. James), bristlecone (*Pinus longaeva* D.K. Bailey) and whitebark (*Pinus albicaulis* Engelm.) pines] are already temperature and water limited, and the early seedling life stage represents a significant bottleneck for population persistence (Hankin *et al.*, 2023). Yet, the primary mechanisms of seedling mortality remain poorly understood (Sala *et al.*, 2010). To address this critical knowledge gap, we examined seedling response to severe drought and extreme temperatures during the first growing season for three high-elevation five-needle pine species. We asked: (1) how do traits related to drought tolerance vary in response to different temperature and moisture conditions within- and among-populations and species?; and (2) what are the ecophysiological limitations to survival during the first growing season for seedlings exposed to severe drought and extreme temperatures, and how do these limitations vary within and among species? Although this was intended as a lethal drought experiment, we expected that populations sourced from warmer, drier conditions would possess adaptive traits that confer greater drought resistance, thus delaying or reducing mortality. This study builds upon prior work in the same populations evaluating variation in emergent seedling performance across different establishment conditions and whether trait and performance variation demonstrates local adaptation and plasticity (Hankin *et al.*, 2023). Evaluating variation in early seedling survival to severe drought in warmer and drier conditions might help in predicting potential shifts in forest composition near the upper tree line and aid in selecting climate-informed seed sources for reforestation if management intervention occurs.

MATERIALS AND METHODS

Study species

Limber, whitebark and bristlecone pines are all members of a group of long-lived, high-elevation pine species. Limber pine (*Pinus flexilis* E. James) is drought tolerant and distributed widely across western North America, from British Columbia to the southwestern USA (Fig. 1A) and across elevations (1830–3540 m). This species exhibits a generalist strategy, with documented broad environmental tolerances (Schoettle, 2004; Hankin and Bisbing, 2021). Whitebark pine (*Pinus albicaulis* Engelm.) likewise has a broad geographical distribution but prefers cooler, moister sites (Tomback *et al.*, 2011) situated at slightly higher elevations (2070–3700 m). Bristlecone pine (*Pinus longaeva* D.K. Bailey) is the most range restricted of the focal species, occurring in limited areas of the Great Basin over a narrow elevational band (2400–3700 m; Fig. 1A).

Study sites

We collected seed from six mountain ranges distributed across the Nevada and California portions of the Great Basin hydrological unit (Fig. 1A). In total, we collected seeds from ten populations, distributed amongst species as follows: two whitebark pine populations (Central and Eastern Sierra), four limber pine populations (Ruby, Snake, Spring and White) and four bristlecone pine populations (Ruby, Snake, Spring and White). For details on site selection, seed collection and preparation methods, see the Supplementary Data (Appendix S2).

Experimental common gardens

To test seedling performance and survival under heat and drought stress, we evaluated the response of each seed source to warmer temperatures and drought in paired indoor and outdoor common gardens located at the Nevada Agricultural Experiment Station (NAES; 1400 m elevation). We planted 100 seeds from each population in each treatment (6 treatments × 10 populations per treatment × 100 seeds per population = 6000 seeds) in a 1:1:3 peat moss:top soil:sand mix by volume. Seeds were sown in pots within the greenhouse in mid-April using a complete randomized design, then transplanted into a raised garden bed either inside the greenhouse or outside in natural conditions 4 weeks after sowing. Seedlings were watered daily following transplant until treatments were implemented.

Treatments included a well-watered control (hereafter, ‘well-watered’) and two watering treatments that simulated two different timings of soil dry-down (i.e. snowmelt timing; Reinhardt *et al.*, 2015), separated by 2 weeks. Snowmelt timing in the source ranges is highly variable year to year depending on

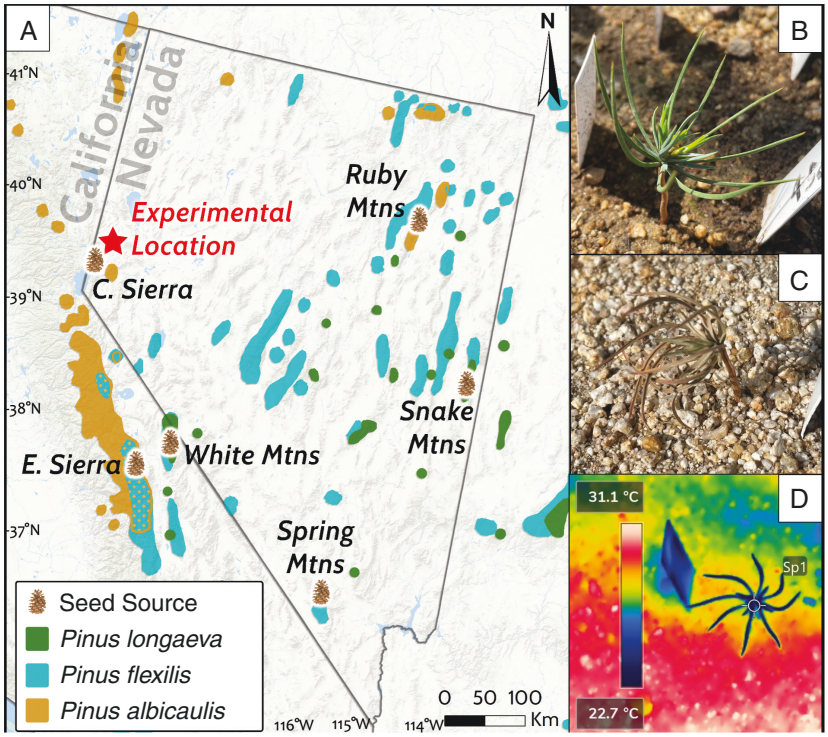

FIG. 1. (A) Seed collection sites in high-elevation, five-needle pine forests across the California and Nevada portions of the Great Basin hydrological unit. Focal species distributions within the study area are shown in varying shades of green. Species distribution polygons are from Data Basin (https://databasin.org). (B) Limber pine seedling grown in the warm early dry-down treatment. (C) Limber pine seedling grown in the hot early dry-down treatment. (D) Thermal image of a bristlecone pine seedling captured with a FLIR infrared camera.

interannual variation in snowpack and temperature; however, within common snowpack years, complete snow-off typically occurs over a 2- to 3-week period (USDA Natural Resources Conservation Service. 2023). The early dry-down treatment represented earlier snowmelt, and the late dry-down treatment extended moisture inputs for 2 weeks. Watering ceased 3 weeks after transplanting in the early dry-down treatments and 5 weeks after transplanting for the late dry-down treatments. Surviving seedlings were grown for the mean length of a growing season averaged across study sites (90 days; Wang *et al.*, 2016). The three watering regimes (well-watered, early dry-down and late dry-down) were duplicated inside (hereafter, 'warm' treatments) and outside (hereafter, 'hot' treatments) the greenhouse, for a total of six treatments. Inside the greenhouse, temperatures were set to the average growing season temperature of the source ranges (daytime air temperature = 18 °C, nighttime air temperature = 8 °C). Owing to extreme outdoor temperatures, we were unable to maintain cool temperature conditions in the greenhouse to match those of the source ranges, hence inside treatments also provided a warming effect (mean daytime air temperature = 21 °C, mean nighttime air temperature = 13 °C). Outside the greenhouse, seedlings were exposed to natural outdoor temperatures (mean daytime air temperature = 26 °C, mean nighttime air temperature = 14.5 °C). NAES is located ~430 m (limber pine) to 1000 m (bristlecone pine) lower than the lowest elevation ranges of the focal species and ~1500 m lower than the average elevation range of the focal species ; therefore, this elevation difference provided a natural warming effect equal to an average of 8 °C during the growing season (Supplementary Data, Table S1) (May–September; PRISM Climate Group, 2017). Of note, limber pine occupies a broad geographical and elevational distribution and therefore might naturally experience some of these more extreme temperatures at the warmest, driest edge of the species distribution. To quantify variation among treatments and growing conditions, we measured soil moisture (in metres cubed per metre cubed, at 10 cm depth) and temperature (in degrees Celsius, 10 cm depth, soil surface, ambient air) hourly over the course of the study period (see Supplementary Data, Appendix S2). We also summarized moisture and temperature conditions for 7 days following treatment implementation and preceding mortality dates.

Seedling traits and performance

After sowing, pots were monitored for emergence (scored as zero or one) every other day to account for the variable timing of emergence expected for conifer species (Bonner and Karrfalt, 2008). Once treatments began after this germination window, we monitored individuals twice a week for visual signs of stress (i.e. wilting, needle discoloration) and mortality (Fig. 1B, C). We then quantified metrics of seedling physiological function on a subset of germinating individuals, including midday stem water potential (Ψ_{stem}; in megapascals) and the difference between midday air and needle temperatures (in degrees Celsius; hereafter, 'delta T'). Delta T was measured weekly on a subset

of five individuals per population within each treatment using a thermal infrared camera (Fig. 1D; FLIR T540, Teledyne FLIR LLC). Whitebark pine sample sizes limited evaluation of needle temperatures, therefore delta T analyses were performed only with data from limber and bristlecone pine seedlings.

To assess water stress, we analysed needles for $\delta^{13}C$ as an indirect measure of intrinsic water-use efficiency (Farquhar *et al.*, 1989). We also measured Ψ_{stem} biweekly using a Scholander pressure chamber (model PMS-1000, PMS Instruments) in three individuals per population within each treatment. The top of each seedling was excised and inserted whole into the pressure chamber. Ultimately, stem water potential was measured only for limber pine owing to extremely limited sample sizes for whitebark pine and consistent stem collapse within the pressure chamber for bristlecone pine.

At the end of the experimental growing season, all surviving seedlings were fully extracted for analysis of above- and below-ground traits. Traits included total growth rate (in grams per day), specific leaf area (SLA; in centimetres squared per gram), specific root length (SRL; in metres per gram), ratio of below-ground to above-ground biomass (hereafter, 'root:shoot') and total biomass (in grams), because these traits reflect strategies for stress tolerance and resource acquisition (Reinhardt *et al.*, 2011; Leger *et al.*, 2019). For methodological details, see Appendix 2.

Statistical analysis

Seedling performance and traits. We first evaluated differences in temperature and soil moisture among treatments across the treatment implementation period using one-way ANOVA to confirm that we achieved the desired drought and warming effects. Owing to the difficulty of applying separate temperature conditions to each of the six watering treatments, all inside treatments were exposed to the same temperature environment, and all outside treatments were exposed to ambient outside temperatures, hence the six treatments were therefore not entirely independent. However, we treated them as such in the fitness and trait comparisons, which could influence our findings.

We used generalized linear models with a binomial distribution (logit link) to test for differences in the binary fitness metrics of emergence and mortality (zero or one), first as a function of species, then population, treatment and their interaction (for mortality only) as species-specific models. Significant interactions, if any, were evaluated for patterns suggesting local adaptation. We also evaluated a continuous measure of daily seedling mortality as a function of daytime soil moisture, maximum air temperature and their interaction using species-specific polynomial regressions.

To test for differences in seedling traits, we used species-specific ANOVA models with each seedling trait as the response variable and with population, treatment and their interaction as explanatory variables. Given that stem water potential and leaf temperature are sensitive to the surrounding weather conditions, we evaluated drivers of Ψ_{stem} and delta T using species-specific polynomial regression models predicting Ψ_{stem} and delta T as a function of maximum air temperature, mean soil moisture during the time of sampling (from ~1000 to 1400 h) and their interaction. We included quadratic terms for maximum air temperature and soil moisture. We selected the final models for each species with the lowest Akaike information criterion (AIC) score if delta AIC > 2 (Burnham and Anderson, 2002). Variables that were not statistically significant were retained if inclusion resulted in a lower AIC with delta AIC > 2. All analyses were performed in R v.4.0.3 (R Core Team, 2020).

RESULTS

Treatment conditions

Treatments varied in both soil moisture and temperature conditions throughout the study period (Fig. 2; Supplementary Data, Table S2). Soil moisture was significantly lower in dry-down treatments and in the hot compared with the warm treatments ($F = 434.5$, $P < 0.001$ for all pairwise hot-warm comparisons). Hot treatments were significantly warmer below ground, on the surface and above ground ($F = 51.6$, 86.8 and 96.2, respectively; $P < 0.001$ for all pairwise hot–warm comparisons) than warm treatments. Surface and soil temperatures were also substantially warmer than ambient air temperatures ($F = 5341$, $P < 0.001$ for all pairwise comparisons).

Seedling performance

Emergence varied significantly among species (Wald $\chi^2 = 69.47$, $P < 0.001$; Supplementary Data, Table S3); total whitebark pine emergence was 19 %, while limber and bristlecone emergence were 86 % and 72 %, respectively. Mortality varied significantly among treatments ($P < 0.001$; Fig. 3; Supplementary Data, Table S3), whereby mortality was 100 % for all species and populations in the hot dry-down treatments, whereas in all other treatments mortality was very low (≤5 %). Within treatments, mortality did not vary among species or populations ($P > 0.05$; Supplementary Data, Table S3).

The timing of mortality showed threshold-like responses to temperature and soil moisture extremes that were conserved regardless of the timing of treatment implementation (Fig. 3). Daily seedling mortality, particularly for limber and bristlecone pine, also varied significantly with soil moisture, maximum temperature and their interaction, and these relationships were non-linear (Fig. 3A; Table 1). The interaction between soil moisture and maximum temperature was not significant for whitebark pine ($P = 0.09$; Table 1). With heat stress (i.e. outside treatments), daily seedling mortality increased below soil moistures of 0.125 m^3/m^3 (Fig. 3A). Most seedlings within the hot dry-down treatments died on 5 July, 3 weeks after implementation of the early treatment and 1 week following implementation of the late treatment (Fig. 3B). All seedlings in the hot, late dry-down treatment died by 7 July. These dates were associated with extreme temperature conditions, with hourly surface temperatures reaching 53 °C and soil temperatures reaching 51 °C in the early dry-down treatment, whereas surface temperatures reached 58.5 °C and soil temperatures reached 52.5 °C in the hot, late dry-down treatment. Soil and surface temperatures in the seed collection zones that were monitored as part of the study by Hankin *et al.* (2023) did not exceed 28 °C.

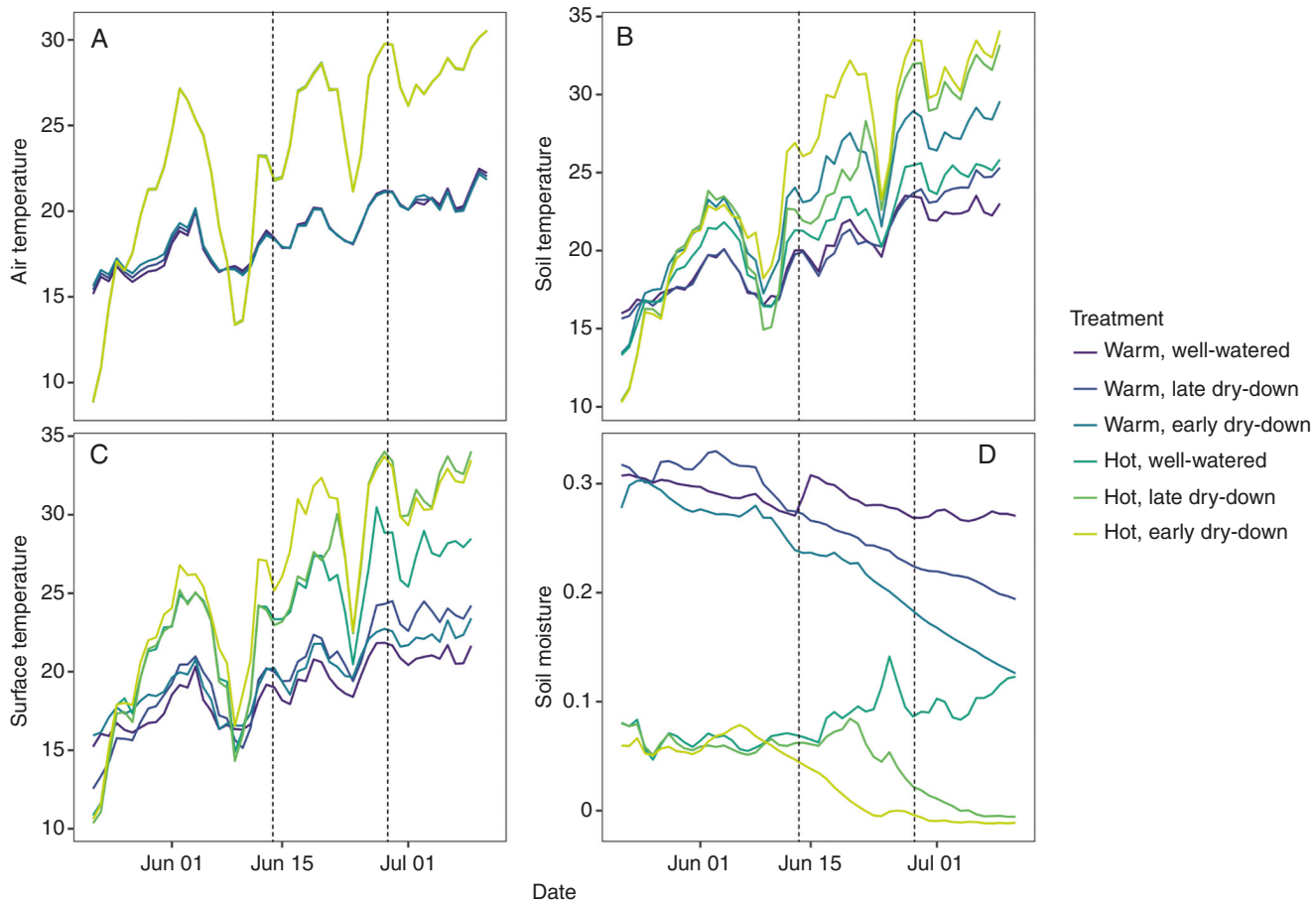

FIG. 2. Mean daily temperature (in degrees Celsius) from sensors located at three positions [(A) ambient air; (B) soil surface; and (C) 10 cm below soil surface] within each treatment, and (D) soil moisture (in metres cubed per metre cubed) recorded 10 cm below the soil surface in the seedling rooting zone.

Seedling traits

Traits differed significantly by species and treatment but did not show consistent or strong differentiation among populations within species (Supplementary Data, Tables S3 and S5; Figs S1–S4). Treatment conditions did, however, drive substantial variation within species, with significant differences in traits between the hot dry-down treatments compared with all other treatments (Figs S1–S3). For example, seedlings in the hot early and late dry-down treatments discriminated against ^{13}C less (mean $\delta^{13}C = -26.3, -26.6$, respectively) than seedlings in the hot, well-watered treatment (mean $\delta^{13}C = -27.4$). The SLA decreased following a gradient of increasing temperature and declining water availability, with the highest SLA in the warm, well-watered treatment (mean = 141.0 cm^2/g) and lowest in the hot, early dry-down treatment (mean = 86.0 cm^2/g). The SRL and total biomass were also highest in well-watered treatments and lowest in hot dry-down treatments.

Seedling physiological function

Leaf temperature relative to air temperature. Delta T and leaf temperature in limber and bristlecone pine seedlings varied among warm and hot treatments (Supplementary Data, Fig. S4). Individual variability in delta T was high within treatments and across a range of maximum temperatures. For both species, delta T increased non-linearly with soil moisture ($P < 0.001$; Table 2), and this relationship was more pronounced in high maximum temperature conditions ($P < 0.001$; Fig. 4). At higher soil moistures (volumetric water content > 0.20 mm^3/mm^3), delta T increased with maximum air temperature, indicating increasing transpirational cooling and lower leaf temperatures relative to air temperatures (Supplementary Data, Fig. S2). These models together explained 17 and 14 % of delta T in limber and bristlecone pine seedlings, respectively (Table 2).

Water relationships. Stem water potential (Ψ_{stem}) varied significantly among limber pine seedlings in different treatments ($F = 16.7$, $P < 0.001$) and between populations within treatments ($F = 2.3$, $P = 0.006$). Water potential was lowest (more negative) in the hottest, driest conditions (Fig. 5). Water potential increased non-linearly with increasing soil moisture; however, this relationship depended on the interaction with maximum temperature (Fig. 5; Table 2). At high temperatures, there was a stronger positive effect of soil moisture on Ψ_{stem} than at low temperatures. Soil moisture, maximum temperature and their interaction explained 29 % of variation in Ψ_{stem} (Table 2).

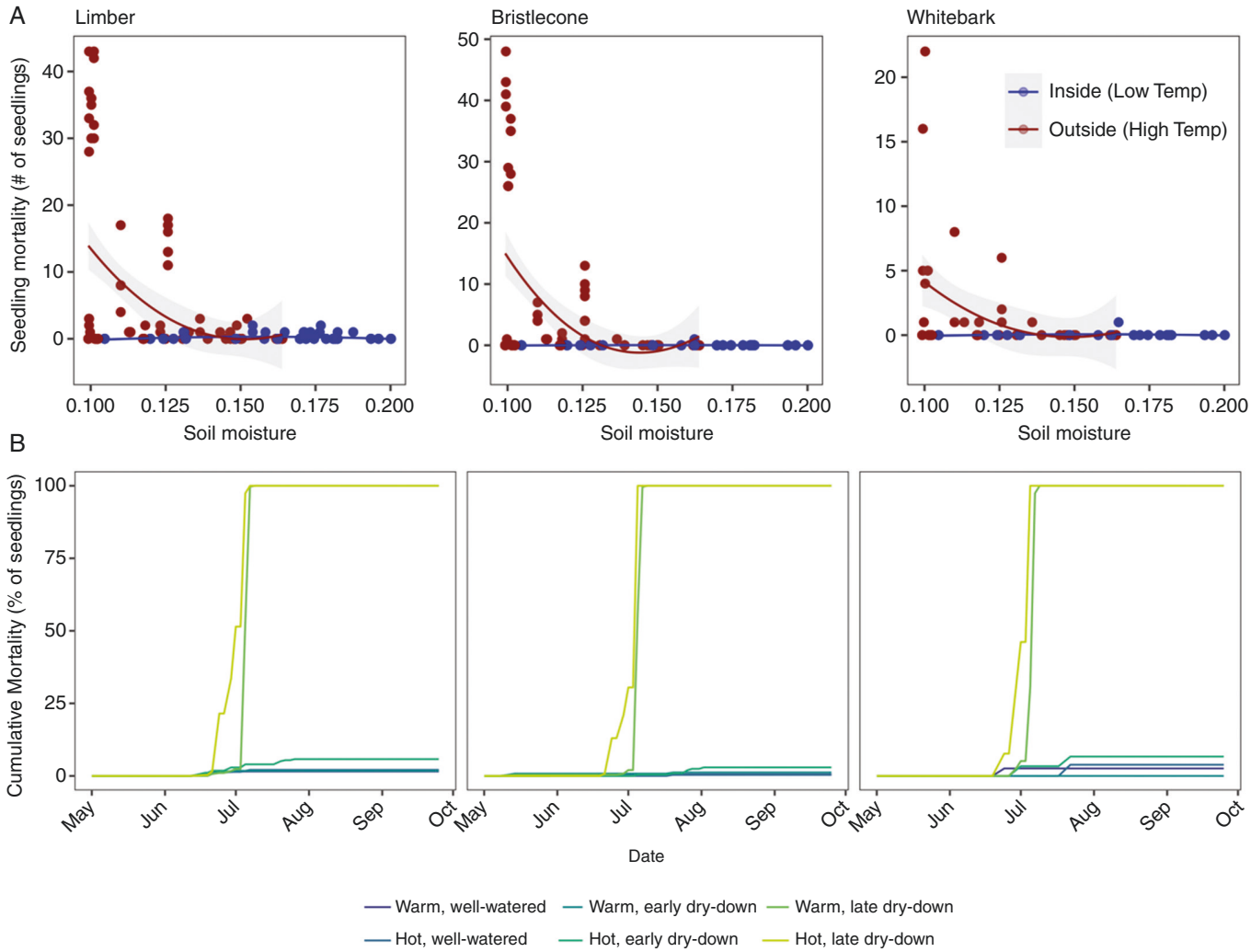

FIG. 3. (A) Seedling mortality (number of seedlings per day) as a function of daytime soil moisture (in millimetres cubed per millimetre cubed), maximum air temperature (in degrees Celsius) and their interaction from polynomial regressions. For visualization, the interaction with maximum temperature is grouped according to warm (blue) and hot (red) treatments. These treatment conditions explained 61, 73 and 63 % of variation in daily limber, bristlecone and whitebark pine seedling mortality, respectively. (B) The timing of mortality for seedlings grown in six treatments varying in temperature and water availability. All seedlings grown in hot early and late dry-down treatments died within 2 weeks of treatment implementation.

DISCUSSION

The fate of high-elevation tree species in the face of ongoing and projected climate change might depend upon their vulnerability to climatic stressors at the seedling life stage, and managing for species persistence requires an understanding of the ecophysiological limitations to early seedling survival (Sala *et al.*, 2010; Brodersen *et al.*, 2019). We tested seedling responses to severe drought and extreme temperatures for populations of three five-needle pines during the first growing season in a common garden below the warm and dry limit of the species ranges. The combined stressors of water limitation and extreme heat ultimately exceeded thresholds for survival. Under adequate soil moisture, however, seedlings endured prolonged exposure to high air and surface temperatures, suggesting much wider margins for survival than conditions characterizing their current extent. Growing conditions, not species or population, were also the primary determinant of seedling trait expression, which might have implications for seedling survival beyond the critical bottleneck of the first growing season, as functional traits influence individual tolerance to resource limitations and stress outside the range of optimal conditions (e.g. temperature). Overall, our study highlights the critical role that the establishment environment, particularly soil moisture, plays for early seedling function and survival. The predicted declines in growing season moisture, exacerbated by rising temperatures and extreme heat events (Harpold and Molotch, 2015; Hammond *et al.*, 2022), will be likely to limit successful recruitment along the warmer, drier edge of five-needle pine species ranges.

Stress-associated trait strategies

Functional trait expression influences the ability of a seedling to cope with the harsh conditions characterizing high-elevation

TABLE 1. *Model estimates from generalized linear models predicting daily seedling mortality (number of seedlings) as a function of daily daytime soil moisture, maximum air temperature and their interaction. Final model structures are shown below, selected by the lowest Akaike information criterion score.*

Species	Variable	Estimate	s.e.	*Z*-value	*P*-value	R^2
Limber	(Intercept)	158.263	22.167	7.139	<0.001	0.61
	Soil moisture	−971.618	80.700	−12.040	<0.001	
	Max temp	−4.812	1.075	−4.474	<0.001	
	Soil moisture^2	1385.583	114.944	12.054	<0.001	
	Max temp^2	0.036	0.013	2.686	0.007	
	Soil moisture: Max temp	14.999	1.955	7.671	<0.001	
Bristlecone	(Intercept)	100.451	18.389	5.463	<0.001	0.73
	Soil moisture	−791.206	209.734	−3.772	<0.001	
	Max temp	−1.617	0.405	−3.997	<0.001	
	Soil moisture^2	6.369	3.602	1.768	0.077	
	Soil moisture: Max temp	100.451	18.389	5.463	<0.001	
Whitebark	(Intercept)	157.356	75.146	2.094	0.036	0.63
	Population E. Sierra	1.089	0.266	4.096	<0.001	
	Soil moisture	−931.525	333.759	−2.791	0.005	
	Max temp	−5.134	3.389	−1.515	0.130	
	Max temp^2	1319.891	456.246	2.893	0.004	
	Soil moisture^2	0.044	0.038	1.173	0.241	
	Soil moisture: Max temp	13.945	8.203	1.700	0.089	

environments and can determine individual stress tolerance and survival (Ambrose *et al.*, 2015; Lazarus *et al.*, 2018). Desiccation-resistance mechanisms, in particular, will be increasingly important for seedling establishment success as declining snowpack and earlier snowmelt combined with warmer temperatures lead to hotter growing season drought (Barnett *et al.*, 2005; Sun *et al.*, 2019). We observed traits conveying drought resistance in the hottest, driest treatment conditions across all species and consistent species-level responses within treatments. Yet, populations within species performed in a similar manner within treatments despite well-documented population-level local adaptation of climatic tolerances in conifers in other study systems (Aitkenand and Bemmels, 2016; Warwell and Shaw, 2019). Our results highlight the importance of local conditions over population-level influences in shaping strategies for stress tolerance and resistance to desiccation at this early life stage, consistent with recent experimental studies of these species (Hankin *et al.*, 2023), although local advantages might emerge after surviving this early population bottleneck.

Stress-associated trait variation within a species might be consequential for persistence potential, because populations exhibiting greater variability or drought-resistant strategies might be more likely to survive in the extreme conditions predicted with ongoing climate change (Razgour *et al.*, 2019; Ulrich *et al.*, 2023). Under temperature and water stress, seedlings of all species expressed more drought-resistant traits, a pattern also observed in seedlings from drier microclimates (Letts *et al.*, 2014) and drier provenances (Reinhardt *et al.*, 2011). For example, low SLA in limber and bristlecone pine seedlings under drought and heat stress might minimize transpiration-driven water loss, reduce heat load and increase structural compounds that resist turgor loss and wilting (Nicotra and Davidson, 2010). Although greater investment in below-ground resources to support water acquisition is a common drought response among tree species (Kozlowski and Pallardy, 2002; Markesteijn and Poorter, 2009), reductions in specific root length could also suggest shedding of fine roots under extreme water stress, increased root suberization and declines in root carbon pools (Olmo *et al.*, 2014; Brunner *et al.*, 2015). Finally, an increase in intrinsic water-use efficiency in limber and bristlecone pine seedlings exposed to our most stressful conditions might be adaptive in drought conditions, because plants maximize carbon gain while minimizing water loss through lower stomatal conductance (Moreno-Gutiérrez *et al.*, 2012). Ultimately, however, conditions exceeded the capacity of seedlings for survival; therefore, we were unable to assess whether these changes in traits would have enhanced seedling performance under more moderate stress environments.

Ecophysiological limitations for survival

Our study highlights early seedling responses to resist heat- and drought-induced mortality, but all species and populations were vulnerable to extreme conditions. Other high-elevation plant species have been shown to regulate stomatal conductance tightly and resist cavitation as water availability declines,

TABLE 2. *Model results from species-specific polynomial regressions evaluating seedling physiological functions as a function of interacting temperature and moisture conditions. Quadratic terms were included based on visual assessments of their potential relationships with each response. Final model structures are shown below, selected by the lowest Akaike information criterion score. Stem water potential was evaluated only on limber pine seedlings.*

Species	Response	Variable	Estimate	*t*-value	*P*-value	R^2
Limber	Stem water potential	Intercept	0.264	1.096	0.275	0.29
		Max temp	−0.035	−4.700	<0.001	
		Soil moisture	−4.940	−2.483	0.014	
		Max temp: Soil moisture	0.203	2.896	0.004	
	Leaf temperature	Intercept	−61.750	−7.674	<0.001	0.72
		Soil moisture	178.500	11.729	<0.001	
		Soil moisture^2	−129.700	−7.100	<0.001	
		Max temp	4.298	9.359	<0.001	
		Max temp^2	−0.046	−7.029	<0.001	
		Soil moisture: Max temp	−5.086	−14.947	<0.001	
	Delta T	Intercept	46.514	6.127	<0.001	0.17
		Soil moisture	−60.901	−7.048	<0.001	
		Max temp	−2.827	−6.355	<0.001	
		Max temp^2	0.039	6.172	<0.001	
		Soil moisture: Max temp	2.390	8.935	<0.001	
Bristlecone	Leaf temperature	Intercept	−62.960	−5.964	<0.001	0.66
		Soil moisture	172.500	7.127	<0.001	
		Soil moisture^2	−102.500	−3.666	<0.001	
		Max temp	4.381	7.237	<0.001	
		Max temp^2	−0.047	−5.383	<0.001	
		Soil moisture: Max temp	−5.137	−9.228	<0.001	
	Delta T	Intercept	54.401	5.647	<0.001	0.14
		Soil moisture	−76.882	−6.425	<0.001	
		Max temp	−3.242	−5.630	<0.001	
		Max temp^2	0.045	5.287	<0.001	
		Soil moisture: Max temp	2.884	7.507	<0.001	

with abundant evidence documenting increases in water-use efficiency late in the growing season when soils are driest (Moreno-Gutiérrez *et al.*, 2012; Winkler *et al.*, 2018). In limber pine, our results are consistent with these patterns of adjustment, in which water balance was maintained until temperature and water thresholds were exceeded. At more extreme water limitation, water potentials were within the documented range of growth inhibition and loss of hydraulic conductivity in several pine species (Reinhardt *et al.*, 2015; Bader *et al.*, 2017). Although we did not measure hydraulic conductivity directly, limber pine seedlings might have increased resistance to water loss in roots (e.g. suberization) and needles (lower stomatal conductance), but probably only towards temperature and drought extremes. Such an ability to maintain water balance under extreme stress (Garcia-Forner *et al.*, 2016; Ulrich *et al.*, 2023) might contribute to the broad geographical distribution of limber pine across heterogeneous landscape conditions and ultimately be advantageous under ongoing and future hotter droughts. Although temperatures in our experiment greatly exceeded current and near-term projected future conditions at high elevations, the seedlings tested here exhibited a remarkable ability to tolerate drought stress until extended stress conditions surpassed the capacity for survival.

Despite maintenance of water balance in limber pine, declining soil moisture conditions significantly impacted the ability of limber and bristlecone pine seedlings to sustain evaporative cooling while exposed to extreme temperatures. At high maximum air temperatures, water-mediated evaporative cooling reduced tissue temperature relative to the surrounding air and increased seedling survival (Kolb and Robberecht, 1996; Urban *et al.*, 2017). Given that leaf water loss is a trade-off for carbon assimilation, seedlings were probably able to maintain photosynthetic activity when water was not limiting. Under the combined stress of drought and heat, however, delta T values

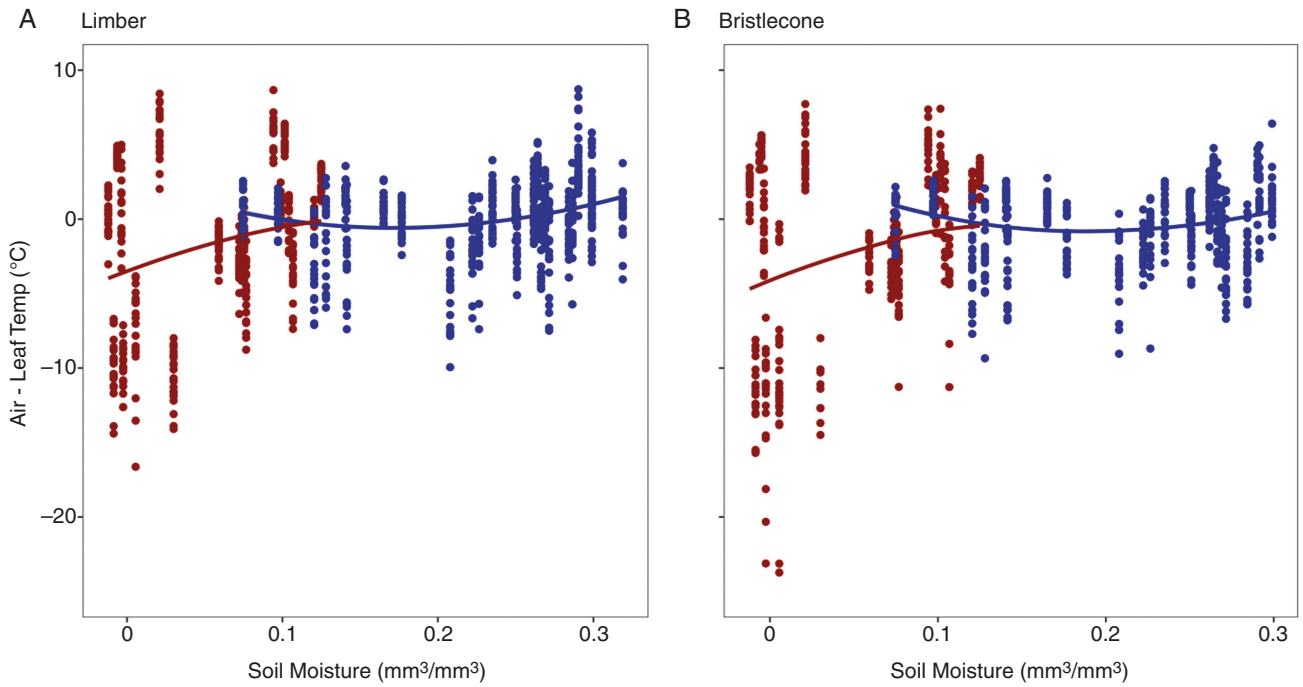

FIG. 4. Delta T of first-year limber pine seedlings (A) and bristlecone pine seedlings (B) as a function of soil moisture (in millimetres cubed per millimetre cubed), maximum air temperature (in degrees Celsius) and their interaction from polynomial regressions. Soil moisture and air temperature were instantaneous measures closest to the time of thermal imaging. For visualization, the interaction with maximum temperature is grouped according to warm (blue) and hot (red) treatments. Maximum temperature, soil moisture and their interaction explained 17 and 14 % of variation in delta T for limber and bristlecone pine seedlings, respectively.

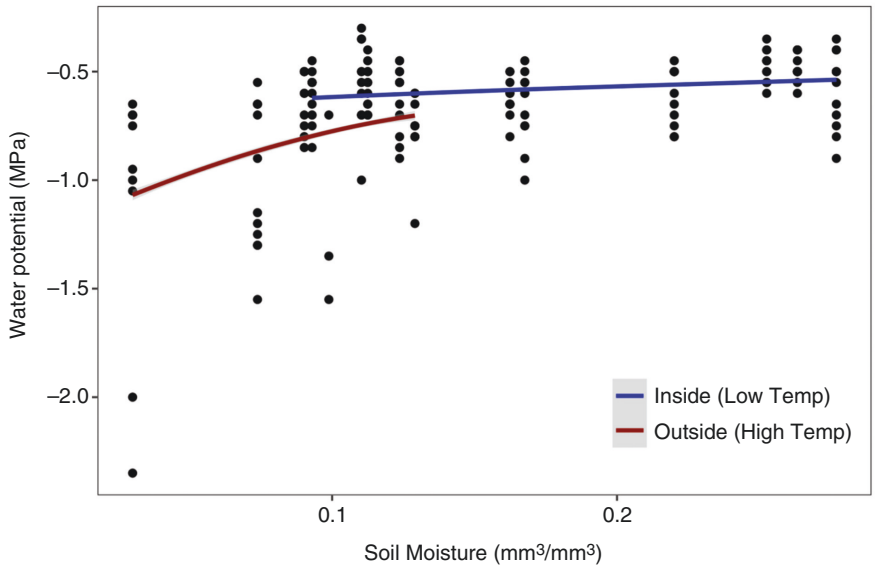

FIG. 5. Relationship between stem water potential (in megapascals) of first-year seedlings of limber pine and soil moisture (in millimetres cubed per millimetre cubed), maximum temperature (in degrees Celsius) and their interaction from a generalized linear model. Soil moisture and temperature conditions were averaged over the time of sampling (from 10.00 to 14.00 h). For visualization, the interaction with maximum temperature is grouped according to warm (blue) and hot (red) treatments. Soil moisture, maximum air temperature and their interaction explained 29 % of variation in stem water potential.

near zero are likely to indicate tight stomatal regulation and associated declines in photosynthesis (Maes and Steppe, 2012; Muller *et al.*, 2021). In these conditions, leaf temperatures can reach damaging levels if exposed for a sufficient amount of time, and seedling desiccation can occur (Rank *et al.*, 2022). Furthermore, prolonged periods of reduced photosynthesis exacerbate seedling carbon deficits and can limit drought tolerance and survival (Miller and Johnson, 2017). Although high-elevation temperatures are unlikely to approach the conditions in our experiment by the end of the 21st century (Dettinger *et*

al., 2018), low water availability, elevated surface temperatures and high vapour pressure deficits might approach the margins of species tolerances under novel climates.

As conditions exceed species tolerances, hydraulic failure is suggested to be the primary driver of drought-induced tree mortality in the context of global climate change, with secondary interactions related to carbon relationships potentially impacting physiological decline (e.g. Sala *et al.*, 2010; Adams *et al.*, 2017). Although we did not test hypotheses of hydraulic failure and carbon starvation directly, our results highlight several potential mechanisms of seedling mortality. The combined stressors of drought and heat appeared jointly to cause complete, episodic mortality, whereas heat stress alone (i.e. hot, well-watered) had very little effect on decreasing seedling survival. Despite expectations of reduced sensitivity to warming for limber pine (Moyes *et al.*, 2013) and local drought adaptation in conifer seedlings (T. E. Kolb *et al.*, 2016), species and populations showed similar vulnerability to mortality under extreme moisture and heat stress. The magnitude of heat stress in the absence of soil moisture probably exceeded thermal tolerances, thus swamping more nuanced species-specific or population-level responses at intermediate levels of temperature stress. Water stress-induced stomatal closure was also widely apparent across our focal species and, in combination with limited root systems, might have impacted transport of water and carbohydrates between above- and below-ground structures (Reinhardt *et al.*, 2015). Furthermore, in the absence of evaporative cooling from plant tissues, we hypothesize that plant tissue damage from lethal surface temperatures compounded with existing water stress was the ultimate cause of mortality (Kolb and Robberecht, 1996; Rank *et al.*, 2022). Notwithstanding vulnerability to extreme conditions, seedlings of all three species were tolerant of warmer temperatures, given adequate soil moisture, suggesting that high-five pine seedlings might have wide margins for survival.

Management implications

Our study highlights morphological and physiological consequences of combined stress from water limitation and extreme heat, particularly the importance of soil moisture in mediating heat impacts. Seedling survival through prolonged exposure to high air and surface temperatures with adequate moisture suggests resilience to climatic warming; however, warming-driven outcomes will rely on interactions with soil moisture dynamics. The soil and surface temperature conditions that seedlings survived with adequate moisture greatly exceeded (>15 °C warmer) temperatures observed in *in situ* common garden studies during the prior 2 years across each of the source mountain ranges (Hankin *et al.*, 2023). Seedling tolerance and ability to adjust water-use strategies suggest that limber and bristlecone pine seedlings might withstand climatic stressors in the near future; however, numerous factors, such as cone production and soil characteristics, will also determine natural regeneration success.

In the face of disturbance-driven mortality, several studies have explored the viability of reforestation strategies for these species within and beyond their current extent (Tomback *et al.*, 2022; Hankin *et al.*, 2023). Our study provides further support for use of these populations as suitable seed sources to increase and/or maintain prevalence of drought-resistant traits should reforestation actions such as replanting or assisted gene flow occur; however, other considerations might limit management intervention. This and previous studies highlight the overwhelming influence of available soil moisture for emergence and survival through the first growing season; therefore, planting success largely depends upon fine-scale planting site selection and inter-annual variability in moisture inputs. Furthermore, soil requirements, seed predation and patterns of local adaptation can further influence successful reforestation outcomes at high elevations (Smithers, 2017; Smithers and North, 2020; Hankin *et al.*, 2023). Although our study did not detect strong population effects in early responses to moisture and temperature conditions in the first growing season, we would expect that additional patterns of population- and/or species-mediated functional trait expression and performance might emerge at later life stages (e.g. Borgman *et al.*, 2015; Kueppers *et al.*, 2017; Lind *et al.*, 2017). Monitoring populations in the face of increased seasonal to annual precipitation variability and declines in precipitation as snow will be crucial for assessing whether management interventions are needed and tracking shifts in species composition and distribution of high-elevation pine forests.

SUPPLEMENTARY DATA

Supplementary data are available at *Annals of Botany* online and consist of the following.

Appendix S1: supplementary tables and figures. Appendix S2: seed collection and processing methods. Table S1: mean, s.d., maximum and minimum climate conditions across the six mountain ranges from which seed was collected. Table S2: mean [s.d.] soil moisture (in metres cubed per metre cubed) and temperature (in degrees Celsius) conditions from May to August 2021 in six treatments. Table S3: analysis of deviance (type II tests) from species-specific generalized linear models, with a binomial distribution predicting seedling emergence and mortality as a function of population, treatment (mortality only) and their interaction (mortality only). Table S4: trait means across species and treatments. Table S5: species-specific analysis of variance models predicting each seedling trait as a function of population, treatment and their interaction. Figure S1: trait variation (mean ± s.d.) in ~2-month-old limber pine seedlings grown in six different treatments varying in temperature and moisture conditions. Figure S2: trait variation (mean ± s.d.) in ~2-month-old bristlecone pine seedlings grown in six different treatments varying in temperature and moisture conditions. Figure S3: trait variation (mean ± s.d.) in ~2-month-old whitebark pine seedlings grown in six different treatments varying in temperature and moisture conditions. Figure S4: Delta T (in degrees Celsius) sampled over the course of the study in limber and Great Basin bristlecone pine seedlings grown in six treatments (shown as panels) varying in temperature and moisture conditions.

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AUTHOR CONTRIBUTIONS

L.H. and S.B. conceived the ideas and designed methodology; L.H. collected and analysed the data; L.H. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY

All data and scripts are available to be shared upon request to the corresponding author.

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