

SUPPLEMENT FOR CHAPTER 2

Table 2.S1: Sample sizes for the four cohorts of the field common garden experiment. We transplanted *B. stricta* seeds into three common gardens (elevations: 2553 m, 2710 m, 2890 m) in the falls of 2013, 2014, 2016, and 2018. This protocol reflects the natural timing of seed dispersal in August - October, and exposes experimental seeds to winter conditions necessary to induce germination. In the subsequent spring, we monitored the seeds 3-4 times per week to record germination phenology, germination success, and seedling survival during the first year of growth.

Garden elevation	2013			2014			2016			2018		
	Seeds sown	Seeds germinated	Seedlings survived	Seeds sown	Seeds germinated	Seedlings survived	Seeds sown	Seeds germinated	Seedlings survived	Seeds sown	Seeds germinated	Seedlings survived
2553 m	2208	37	3	3746	97	37	3988	41	0	1585	7	6
2710 m	2220	703	230	3957	299	134	3973	324	23	1603	145	5
2890 m	2186	340	33	3949	655	371	3967	322	2	1598	65	1

Cohort	Number of accessions	Number of source populations	Range of elevations (m) of source populations
2013	154	45	2694 – 3690
2014	205	56	2519 – 3690
2016	100	50	2499 – 3530
2018	50	50	2499 – 3623

Table 2.S2a: Germination phenology in the field experiment: We analyzed germination phenology as a function of snowmelt timing, garden, and their interactions in a generalized linear mixed model using the glmmTMB function of the R package

glmmTMB ver. 1.1.4 (Brooks et al., 2017), with a fixed effect of source elevation and its interaction with garden. We used a lognormal distribution with a log link, and verified that the residuals were normally distributed and homoscedastic with the simulateResiduals function of the simulateResiduals function of R package DHARMa ver. 0.4.6 (Hartig, 2022). We standardized the timing of snowmelt and source elevation to a mean of 0 and standard deviation of 1 to enable model convergence, but we present exponentiated regression coefficients on the unstandardized scale. Significant effects are in bold typeface.

	χ^2	DF	p-value
Day of snowmelt	8.78	1	0.0030
Garden	15.31	2	0.00047
Source elevation	3.53	1	0.06
Day of snowmelt \times Garden	18.30	2	0.00010
Source elevation \times Garden	1.36	2	0.51
Random Effects			
Accession	6.58	1	0.010
Block	1065	1	<0.0001
Year	0	1	1

Table 2.S2b: Exponentiated regression coefficients for germination timing as a function of snowmelt timing calculated for each of the three sites, extracted using the emtrends function from the emmeans R package (Lenth et al. 2023) Significant beta values are in bold typeface. The formula to calculate the percent change in germination for every day delay in snowmelt is: $100 * (\text{Exponentiated regression coefficient} - 1)$.

Garden Elevation	Exponentiated regression coefficient	95% CI, lower	95% CI, upper	% change in germination phenology for every 1 day delay in snowmelt	95% CI, lower	95% CI, upper
2553 m	0.9934	0.9892	0.9978	-0.66	-1.08	-0.22
2710 m	1.0033	1.0001	1.0066	0.33	0.01	0.66
2890 m	1.0035	1.0017	1.0052	0.35	0.17	0.52

Table 2.S3a: Germination success in the field experiment: We analyzed germination success as a function of snowmelt timing, garden, source elevation, interactions of snowmelt timing and garden, source elevation and garden, with a quadratic term for source elevation using a generalized linear model with a binomial distribution with a logit link (function *glmmTMB*, R package *glmmTMB* ver. 1.1.4, Brooks et al., 2017). We determined significance of fixed effects using Type III Sums of Squares (function *Anova*, R package *car* ver. 3.0-12, Fox & Weisburg, 2019). Significant effects are in bold typeface.

	χ^2	DF	p-value
Day of snowmelt	0.04	1	0.84
Garden	6.08	2	0.048
Source elevation	5.90	1	0.015
Source elevation ²	4.78	1	0.029
Day of snowmelt \times Garden	11.22	2	0.0037
Garden \times Source elevation	0.17	2	0.92
Random Effects			
Accession	481.51	1	<0.0001
Block	1081.6	1	<0.0001
Year	45.09	1	<0.0001

Table 2.S3b: Odds ratios for germination success as a function of snowmelt timing for each of the three sites. We extracted odds ratios and confidence intervals using the `emtrends` function from the *emmeans* R package ver. 1.8.8 (Lenth et al. 2023). Significant odds ratios are in bold typeface. A one-day delay in the timing of snowmelt augments the odds of germination by 5.7% and 1.9%, respectively, at the common gardens at 2710m and 2890m in elevation.

Garden Elevation	Odds Ratio	95% CI, lower	95% CI, upper
2553 m	1.00	0.96	1.04
2710 m	1.057	1.028	1.087
2890 m	1.019	1.0007	1.038

Table 2.S4a: Seedling survival in the field experiment: We analyzed seedling survival amongst germinants as a function of snowmelt timing, garden, source elevation, and two-way interactions between garden and snowmelt timing and garden and source elevation. We used a generalized linear model with a binomial distribution (function `glmmTMB`, R package *glmmTMB* ver. 1.1.4, Brooks et al., 2017). We determined significance of fixed effects using Type III Sums of Squares (R package *car* ver. 3.0-12, Fox & Weisburg, 2019). Significant effects are in bold typeface.

	χ^2	DF	p-value
Day of snowmelt	15.31	1	<0.0001
Garden	14.95	2	0.00057
Source elevation	0.31	1	0.58
Day of snowmelt \times Garden	18.10	2	0.00012
Garden \times Source elevation	0.08	2	0.97
Random Effects			
Accession	3.42	1	0.064
Block	244.99	1	<0.0001
Year	66.32	1	<0.0001

Table 2.4b: Odds ratio for seedling survival calculated for each of the three sites. We extracted odds ratios and confidence intervals using the `emtrends` function from the *emmeans* R package (Lenth et al. 2023). Significant odds ratios are in bold typeface. A one-day delay in the timing of snowmelt augments the odds of seedling survival by 18% and 9%, respectively, at the common gardens at 2553m and 2710m in elevation.

Garden Elevation	Odds Ratio	95% CI, lower	95% CI, upper
2553 m	1.18	1.09	1.28
2710 m	1.09	1.03	1.16
2890 m	1.02	0.98	1.06

Table 2.S5a: Seedling growth in the field experiment: We analyzed plant size (number of leaves) of seedlings alive at the end of the first growing season as a function of snowmelt timing, garden, and their interaction, and source elevation and is interaction with garden in a generalized linear mixed model using a gaussian distribution with an identity link (the glmmTMB function of the R package glmmTMB ver. 1.1.4, Brooks et al., 2017). We included the date of germination as a covariate, as earlier germinants grew over a longer period of the growing season. We determined significance of fixed effects using Type III Sums of Squares (function Anova, R package *car* ver. 3.0-12, Fox & Weisburg, 2019). Significant effects are in bold typeface.

	χ^2	DF	p-value
Day of snowmelt	2.32	1	0.13
Garden	35.75	2	<0.0001
Source elevation	1.43	1	0.23
Day of snowmelt \times Garden	39.02	2	0.0001
Garden \times Source elevation	1.57	2	0.45
Date of germination	74.56	1	<0.0001
Random Effects			
Accession	1.60	1	0.30
Block	95.58	1	<0.0001
Year	0	1	1

Table 2.S5b: Beta values for final plant size calculated for each of the three sites. We extracted odds ratios and confidence intervals using the *emtrends* function from the *emmeans* R package (Lenth et al. 2023) Significant beta values are in bold typeface. These slopes represent the change in final plant size for every one-day delay in the timing of snowmelt.

Garden Elevation	β	95% CI, lower	95% CI, upper
2553 m	-0.14	-0.33	0.04
2710 m	-0.19	-0.26	-0.02
2890 m	0.078	0.028	0.13

Table 2.S6: Sample sizes and experiment schedule for the laboratory experiments evaluating freezing tolerance. We exposed seeds and two size classes of seedlings to 14 hours of cold temperatures in a freezing chamber to simulate a single overnight spring frost event. The minimum temperatures in the four treatments were: (a) -17.2°C , reflecting the coldest night in the week before snowmelt, which represents a spring frost if snowmelt advances by six days; (b) -10.7°C , reflecting the coldest night in the week after snowmelt, which represents a spring frost that can happen under current climates; (c) -5.7°C , which is the average nocturnal temperature in the week after snowmelt; and (d) 4°C , a non-freezing control temperature, which was held constant throughout the 14 hours. We monitored seeds for germination, and seedlings for survival and size (diameter) three times per week for about two months, and then again once at three-months post freezing for the seed experiment.

Treatment minimum temperature ($^{\circ}\text{C}$)	Seed		Seedling: younger		Seedling: older	
	Individuals sown	Individuals germinated	Individuals planted	Individuals survived	Individuals planted	Individuals survived
4	800	304	153	103	147	136
-5.7	800	199	151	138	131	124
-10.7	800	269	152	33	126	33
-17.2	800	93	143	0	139	0

Stage	Number of accessions	Number of source populations	Range of elevations (m) of source populations
Seed	98	72	2498 – 3673
Seedling: younger	20	20	2693 – 3502
Seedling: older	20	20	2693 – 3502

	Treatment minimum temperature	Replicate	Start of seed stratification (2 weeks)	Start of seedling growth	Start of seedling cold acclimation (1 week)	Start of treatment (overnight)	Start of thaw (24 hours)	Start of monitoring period	End of monitoring period
Seed	4	1	9/26/22	NA	NA	10/10/22	10/11/22	10/12/22	1/21/23
	-5.7	1	9/28/22	NA	NA	10/12/22	10/13/22	10/14/22	1/21/23
	-10.7	1	9/29/22	NA	NA	10/13/22	10/14/22	10/15/22	1/21/23
	-17.2	1	9/30/22	NA	NA	10/14/22	10/15/22	10/16/22	1/21/23
	4	2	10/3/22	NA	NA	10/17/22	10/18/22	10/19/22	1/21/23
	-5.7	2	10/4/22	NA	NA	10/18/22	10/19/22	10/20/22	1/22/23
	-10.7	2	10/5/22	NA	NA	10/19/22	10/20/22	10/21/22	1/22/23
	-17.2	2	10/6/22	NA	NA	10/20/22	10/21/22	10/22/22	1/22/23
Seedling	4	1	1/21/22	2/4/22	3/8/22	3/15/22	3/16/22	3/17/22	5/1/22
	-5.7	1	1/22/22	2/5/22	3/9/22	3/16/22	3/17/22	3/18/22	5/1/22
	-10.7	1	1/23/22	2/6/22	3/10/22	3/17/22	3/18/22	3/19/22	5/1/22
	-17.2	1	1/24/22	2/7/22	3/11/22	3/18/22	3/19/22	3/20/22	5/1/22
	4	2	2/4/22	2/18/22	3/15/22	3/22/22	3/23/22	3/24/22	5/8/22
	-5.7	2	2/5/22	2/19/22	3/16/22	3/23/22	3/24/22	3/25/22	5/8/22
	-10.7	2	2/6/22	2/20/22	3/17/22	3/24/22	3/25/22	3/26/22	5/8/22
	-17.2	2	2/7/22	2/21/22	3/18/22	3/25/22	3/26/22	3/27/22	5/8/22

Table 2.S7a: Germination success in the growth chamber study: We analyzed germination success at the accession level (number of individuals germinated/planted for each accession in each treatment) after freezing as a function of source elevation, treatment, and their interaction, using a generalized linear model with a binomial distribution and a logit link (function `glmmTMB`, R package *glmmTMB* ver. 1.1.4, Brooks et al., 2017). We determined significance of fixed effects using Type III Sums of Squares (function `Anova`, R package *car* ver. 3.0-12, Fox & Weisburg, 2019). Significant effects are in bold typeface.

	χ^2	DF	p-value
Source elevation	0.66	1	0.041
Treatment	10.96	3	0.0120
Source elevation \times Treatment	15.08	3	0.0017
Random Effects			
Accession	295.64	1	<0.0001

Table 2.S7b: Odds ratios for germination success as a function of source elevation calculated for each of the three treatments. We extracted odds ratios and confidence intervals using the `emtrends` function from the *emmeans* R package (Lenth et al. 2023). Significant odds ratios are in bold typeface. The odds of germination declined by 12% and 9% for every one-kilometer increase in source elevation in the two most severe freezing treatments.

Treatment Minimum Temperature (°C)	Odds Ratio	95% CI, lower	95% CI, upper
4	1.04	0.95	1.14
-5.7	0.99	0.90	1.09

-10.7	0.91	0.83	0.99
-17.2	0.88	0.79	0.99

Table 2.S8: Seedling survival in the growth chamber study: Results of a logistic regression of seedling survival, which we modeled as a function of source elevation, treatment, and their interactions using a generalized linear model with a binomial distribution (R package *glmmTMB* ver. 1.1.4, Brooks et al., 2017). We included the initial size of the seedlings before the implementation of the treatment as a covariate in this analysis to account for differences in size. We determined significance of fixed effects using Type III Sums of Squares (R package *car* ver. 3.0-12, Fox & Weisburg, 2019). Significant effects are in bold typeface.

	χ^2	DF	p-value
Source elevation	0.85	1	0.35
Treatment	59.65	3	<0.0001
Source elevation \times Treatment	0.90	3	0.83
Initial size	31.78	1	<0.0001
Random Effects			
Accession	21.94	1	<0.0001
Block	15.95	1	<0.0001

Table 2.S9: Seedling relative growth rate in the growth chamber experiment. We analyzed seedling relative growth rate after freezing as a function of source elevation, treatment, and their interaction. (R package *lme4* ver. 1.1-34, Bates et al., 2015). We included stage as a covariate in this analysis to account for differences in age of the seedlings. We determined significance of fixed effects using Type III Sums of Squares (R package *car* ver. 3.0-12, Fox & Weisburg, 2019). Significant effects are in bold typeface.

	χ^2	DF	p-value
Source elevation	1.07	1	0.30
Treatment	10.57	2	0.005
Source elevation \times Treatment	4.81	2	0.09
Life history stage	0.03	1	0.87
Random Effects			
Accession	26.79	1	<0.0001
Block	1.32	1	0.25

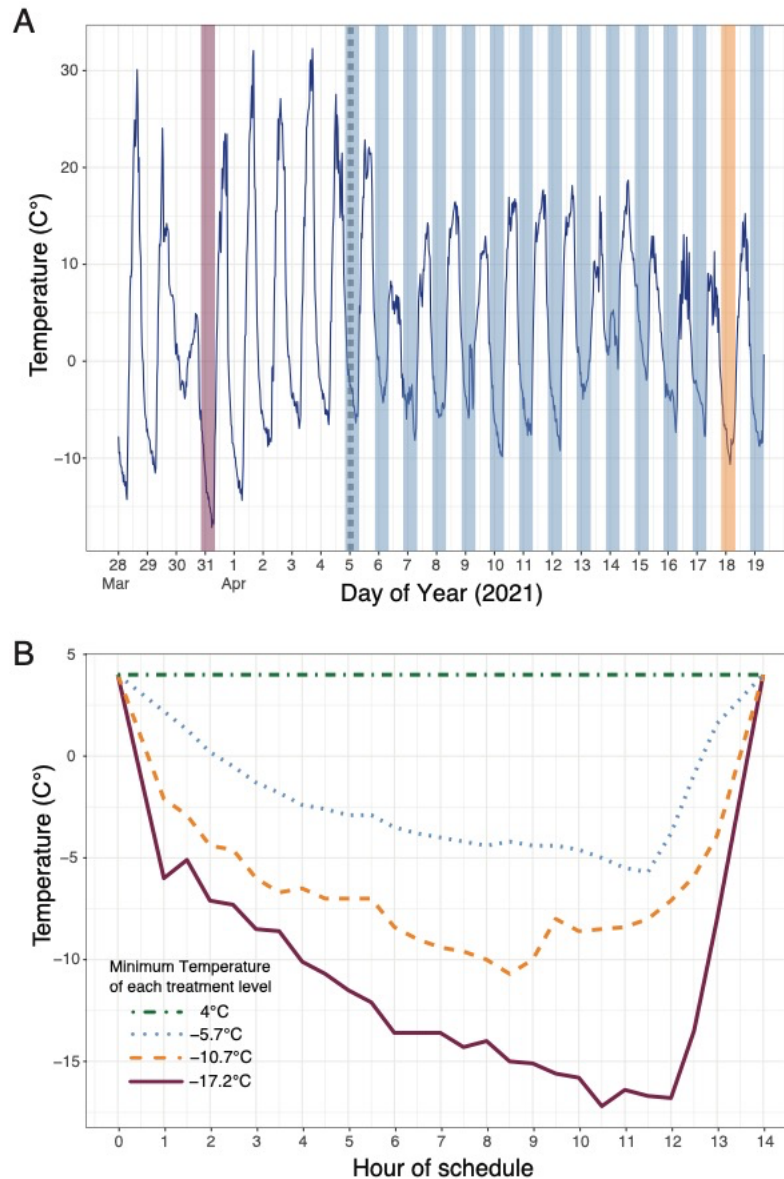


Figure 2.S1: Air temperatures and chamber treatment schedule (a) Air temperatures collected every 30 minutes from March 28 to April 19, 2021 at the lowest elevation garden (2553 m). Snowmelt occurred on April 5th, 2021 indicated by the gray dotted line. The coldest night (8 PM-8 AM) in the week before snowmelt occurred on March 30th, 2021 and is highlighted in purple (minimum temperature: -17.2°C). The coldest night in the two weeks following snowmelt occurred on April 17th, 2021 and is highlighted in orange (minimum temperature: -10.7°C). The average minimum nocturnal temperatures in the two weeks after April 5th (temperatures highlighted in blue and the temperatures highlighted in orange) was -5.7°C . (b) Temperature schedule for the laboratory experiment. Each treatment lasted 12 hours and simulated nighttime air temperatures at lowest elevation garden (2553 m).

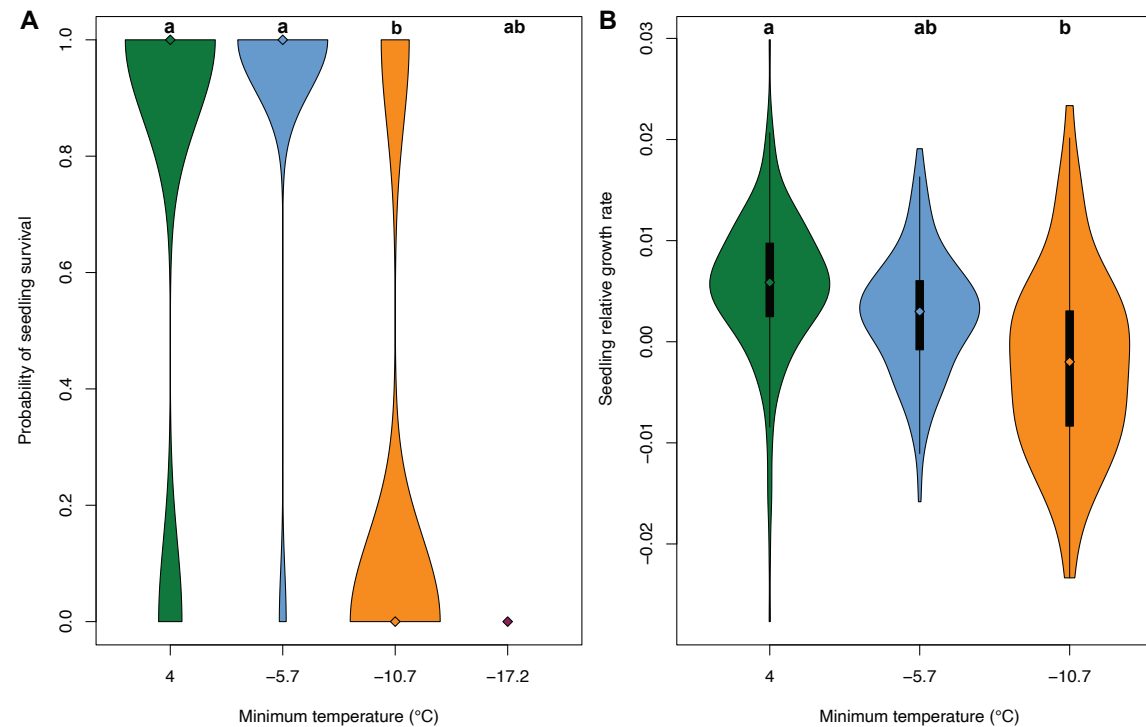


Figure 2.S2: Seedling survival and growth rate in the laboratory experiment. (a) Seedling survival varied with treatment, irrespective of source elevation. No seedling survived under the most severe freezing temperature (minimum $T = -17.2^{\circ}\text{C}$). Seedling survival was significantly lower in the treatment with a minimum temperature of -10.7°C than the other two treatments. (b) Freezing temperatures depressed seedling relative growth rate, which was the lowest under the treatment level with a minimum temperature of -10.7°C . In contrast, we found positive values for relative growth rate under the benign treatment levels (minimum $T = -5.7^{\circ}\text{C}$ and 4°C). We were unable to calculate the relative growth rate of seedlings exposed to the most severe minimum temperature (minimum $T = -17.2^{\circ}\text{C}$) owing to 100% mortality. Letters represent significant differences across treatment levels after Tukey's adjustment for multiple comparisons.

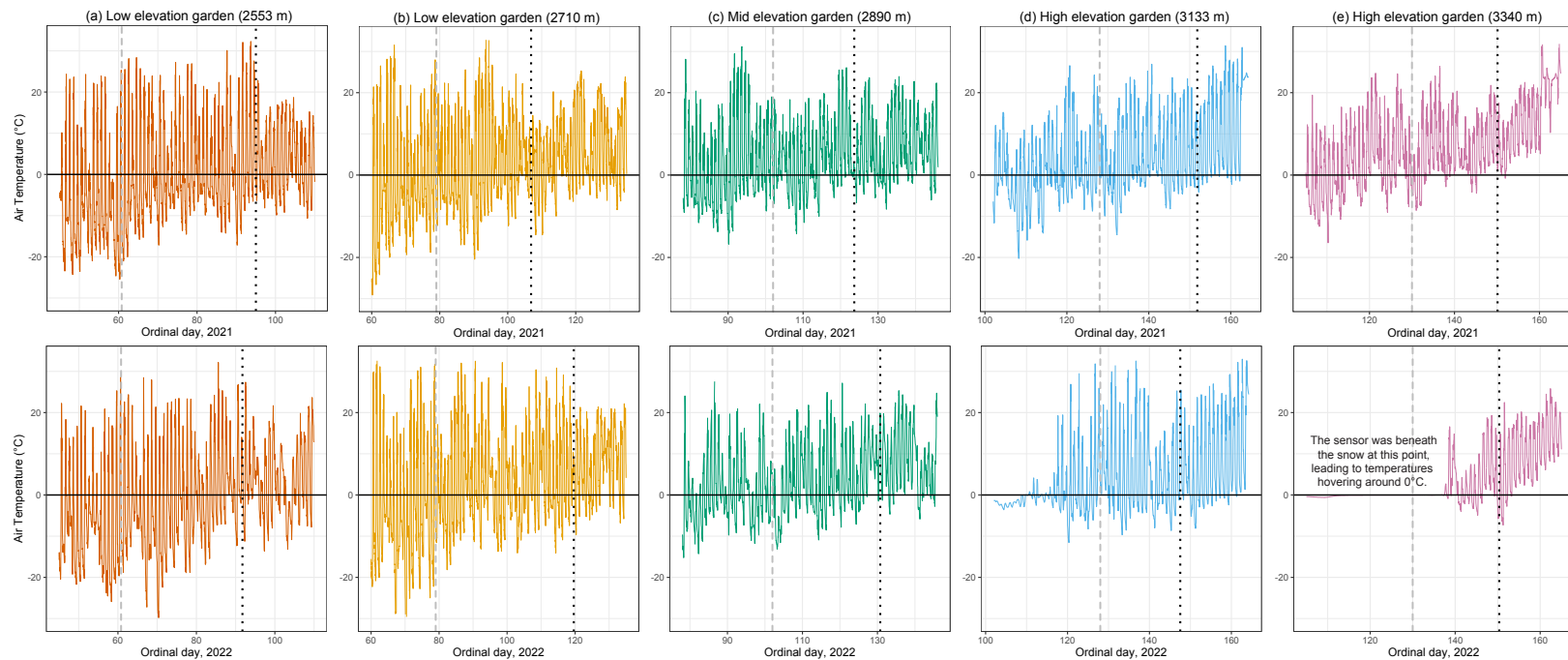


Figure 2.S3: Daily air temperatures collected in the field for each of the five experimental gardens. Air temperature data measured by sensors (HOBO U23 Pro v2, Onset, MA, US) mounted 2 m above the soil for approximately 45 days before snowmelt and 15 days after snowmelt at five experimental gardens in the springs of 2021 and 2022. The dotted black vertical line shows the average snowmelt timing in control blocks in each garden for each year. The dotted grey vertical line depicts projected snowmelt dates for each garden relative to the average snowmelt dates at these sites from 2014-2019. Under the Relative Concentration Pathway 8.5 climate change scenario, models project a 32-day acceleration in snowmelt timing by 2070 in this region (Lute et al., 2022). The horizontal black line indicates 0°C. Please note that the Y axis, showing temperature values, is the same on all panels, but the X axis, showing ordinal day of year, differs across gardens because of divergent snowmelt timings. At the highest elevation garden (3340 m) in 2021, the snowpack was >2 m for much of the winter, which covered the temperature sensor and resulted in values that deviated only moderately from 0°C. Whereas seeds and seedlings at low to mid elevation gardens experience multiple freeze-thaw cycles after snowmelt in contemporary climates, individuals at high elevation gardens rarely experience severe or even moderate frosts owing to delayed snowmelt. Advancing snowmelt timings under climate change could expose mid- to high-elevation populations to novel freezing temperatures and heighten frost damage at low elevation locales. It is possible that increasing temperatures in concert with advancing snowmelt could interact to maintain contemporary exposure to freezing or even reduce it.