Supplementary Material

### Leaf-air temperature decoupling correction

To approximate the leaf-air temperature decoupling in alpine environments while generating climate scenario projected temperatures, we applied a regression between air and plant temperature data, which is presented originally in Salisbury and Spomer (1964) in their Figure 3. The regression formula was *y* = 1.3964*x* with *R*2 = 0.9892.

### Chlorophyll fluorescence assays

After the five-day heatwave treatment, we removed one leaf per plant from both heatwave and non-heatwave plants to measure the maximum efficiency of the photosystem II (Fv/Fm), critical hot temperature (Tcrit), the temperature at 50% fluorescence (T50), and the temperature at maximum fluorescence (Tmax). Harvested leaves were attached with a double-sided tape to filter paper that was then placed on a Peltier plate (CP-121HT; TE-Technology, Inc., Michigan, USA) controlled by a bi-polar proportional-integral-derivative temperature controller (TC-36-25; TE-Technology, Inc.). Double-glazed glass was placed on top to insulate the samples and ensure maximum thermal contact between leaves on the filter paper to the Peltier plate. The Peltier plate was set to a constant ambient temperature (21 ± 1 °C) and leaves were dark-adapted for 30 minutes.

After dark adaptation, we measured minimum chlorophyll fluorescence (F0) (i.e., fluorescence in absence of photosynthetic light) and maximum fluorescence (Fm) (i.e., fluorescence after delivering to a saturating pulse for a short duration). Chlorophyll fluorescence was measured on an area of interest set to the center of the widest section of each leaf, by means of a chlorophyll fluorescence imaging system (MAXI-Imaging-PAM; Heinz Walz GmbH, Effeltrich, Germany). We used the initial F0 and Fm parameters to calculate Fv/Fm as the ratio of (Fm - F0)/ Fm (n = 276). Unfortunately, the Fv/Fm measurements for 43 individuals were not recorded due to software errors. After measuring Fv/Fm we raised the temperature of the Peltier plate from 21 to 60°C at a ramp speed of 60 °C h-1. We recorded F0 values every 10 s during the temperature ramp. The temperature of the leaf samples was measured using two type-T thermocouples that were attached to the underside of two randomly selected leaves, and a thermistor (MP-3193; TE-Technology, Inc.) attached to the Peltier plate. We logged the thermocouple data using a dual-channel data logger (EL-GFX-DTC; Lascar Electronics Ltd., Salisbury, UK). The average temperature of these two thermocouples was then used for all subsequent temperature calculations because, being directly attached to the leaves, we considered it to be a more reliable estimate of leaf temperatures. The difference in temperatures measured by the two thermocouples (average) and the thermistor was on average 2.5 °C across all runs and became larger at higher temperatures.

For 19 individuals the T-F0 curves did not reach a fluorescence maximum and could not measure Tmax and T50 because they would have been underestimated. We included those individuals in the analysis considering them as having Tmax = 61 °C and estimated their T50 as (Tcrit + (61 °C - Tcrit) / 2) + 0.69 °C, where 0.69 °C is the average difference between T50 and (Tcrit + ((Tmax - Tcrit) / 2) between the rest of the 300 individuals. The distribution of curves in which F0 had not reached a maximum, was distributed across elevation and climate scenarios, however 17 out of these 19 individuals did not experience a heatwave. For two individuals we did not obtain canonical T-F0 curves and could not estimate thermal tolerance parameters.

Table S1. Table of the families used during the experiment, the elevation where seeds of the respective mothers and fathers were collected, the elevation classification and the distance as the crow flies of the parental pair.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| F1 Line | Elevation of the mother | Elevation of the father | Elevation | Distance (Km) |
| F1.101 | 1621 | 1590 | Low | 6.98 |
| F1.102 | 1732.9 | 1738.8 | Low | 0.19 |
| F1.103 | 1744 | 1741 | Low | 2.28 |
| F1.104 | 1765 | 1744 | Low | 3.33 |
| F1.106 | 1889.4 | 1852.9 | High | 2.05 |
| F1.107 | 1930.9 | 1926.5 | High | 0.58 |
| F1.108 | 2095.7 | 2094.9 | High | 0.16 |
| F1.109 | 1741 | 1754 | Low | 0.05 |
| F1.110 | 1884.6 | 1837.3 | High | 0.67 |
| F1.111 | 1590 | 1618.3 | Low | 6.92 |
| F1.112 | 1956 | 1977 | High | 1.44 |
| F1.114 | 1733.5 | 1741 | Low | 1.34 |
| F1.115 | 1918.2 | 1902.7 | High | 0.24 |
| F1.117 | 1937 | 1929.5 | High | 1.32 |
| F1.118 | 1754 | 1741 | Low | 0.05 |
| F1.119 | 1765 | 1740 | Low | 5.7 |
| F1.120 | 2096.3 | 2090 | High | 0.36 |
| F1.122 | 1744 | 1721 | Low | 5.97 |
| F1.123 | 2060.7 | 2100 | High | 0.64 |
| F1.125 | 1640 | 1621.3 | Low | 4.18 |
| F1.126 | 1744 | 1741 | Low | 2.27 |
| F1.127 | 1930 | 1956 | High | 0.93 |
| F1.128 | 1956 | 1930 | High | 0.93 |
| F1.129 | 1956 | 1930 | High | 0.93 |
| F1.130 | 1706.4 | 1706.4 | Low | 6.64 |
| F1.137 | 1721 | 1754 | Low | 3.77 |
| F1.138 | 1765 | 1721 | Low | 2.6 |
| F1.142 | 1977 | 1935 | High | 1.48 |
| F1.144 | 1930 | 1892 | High | 4.5 |
| F1.149 | 1977 | 1956 | High | 1.48 |

|  |  |  |  |
| --- | --- | --- | --- |
| Days | Historical scenario temperatures (d/n °C) | Future scenario temperatures (d/n °C) | Photoperiod (d/n hr) |
| 0 | 25/20 | 25/20 | 12/12 |
| 1 | 21/15 | 25/15 | 12/12 |
| 9 | 20/12 | 25/12 | 12/12 |
| 23 | 20/12 | 27/12 | 13/11 |
| 37 | 22/13 | 28/19 | 13/11 |
| 51 | 22/13 | 29/19 | 14/10 |
| 65 | 23/13 | 29/20 | 14/10 |
| 79 | 24/13 | 29/20 | 14/10 |
| 93 | 24/12 | 29/20 | 14.5/9.5 |
| 107 | 23/13 | 28/20 | 14.5/9.5 |
| 121 | 21/13 | 26/20 | 14.5/9.5 |
| 135 | 18/13 | 24/15 | 14.5/9.5 |
| 149 | 16/13 | 21/15 | 13.5/10.5 |
| 163 | 16/13 | 18/13 | 13.5/10.5 |
| 177 | 16/13 | 17/13 | 12.5/11.5 |
| 191 | 16/13 | 17/13 | 12.5/11.5 |

Table S2. Day/night temperatures for historical climate scenario (base period) and future climate scenario (projected period), and photoperiod from the imposition of treatments (day 1) to the end of the experiment (day 191).

**Table S3.** Effects of temperature treatments on number of seeds (LM), seed mass and chlorophyll content: results from linear random intercept models. Main effects model has no interactions; full model has all 2-way interactions. 3-way interactions were never significant and results for them are not presented. Estimates and standard error for seed production and seed mass are in the logarithmic scale. Number of seeds includes only individuals that produced seeds. (no zeros). NA = factor not included in the model for that trait or result not provided by the model. The intercept corresponds to high elevation, historical climate and no heatwave, and other parameter values are relative to this. Significant results, as for p-value < 0.05 from the lmerTest package in R, are presented in bold. e(l) = elevation (low); c(f) = climate (future); hw = heatwave; est. = estimated effect; ML = maximum likelihood; Pr(ML) = p-value of the likelihood ratio test between the models with and without the random effect. Random effects parameters are the variance components associated with that random effect.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Number of seeds (LM) | | | Seed mass (ln) | | | Chlorophyll content | | |
| ***n*** | 233 |  |  | 234 |  |  | 301 |  |  |
| **main effects model:** | est. | (sd) | P-value | est. | (sd) | P-value | est. | (sd) | P-value |
| intercept | 7.182 | 0.275 | NA | -3.498 | 0.268 | NA | 58.226 | 1.193 | NA |
| elevation (low) | -0.720 | 0.308 | 0.027 | **-0.621** | **0.285** | **0.037** | 0.532 | 1.399 | 0.707 |
| climate (future) | **-0.754** | **0.187** | **<0.0001** | **-0.952** | **0.007** | **<0.0001** | 1.009 | 0.817 | 0.218 |
| heatwave(yes) | -0.139 | 0.175 | 0.430 | -0.134 | 0.179 | 0.458 | NA | NA | NA |
| block(B) | 0.092 | 0.218 | 0.674 | 0.050 | 0.224 | 0.825 | -0.892 | 1.013 | 0.379 |
| block(C) | 0.031 | 0.221 | 0.887 | 0.003 | 0.227 | 0.989 | -0.948 | 1.007 | 0.348 |
|  | Marginal R2: 0.103 | | | Marginal R2: 0.117 | | | Marginal R2: 0.008 | | |
|  | Conditional R2: 0.324 | | | Conditional R2: 0.306 | | | Conditional R2: 0.170 | | |
| **full model:** | est. | (sd) | P-value | est. | (sd) | P-value | est. | (sd) | P-value |
| intercept | 7.306 | 0.297 | NA | -3.330 | 0.288 | NA | 57.667 | 1.241 | NA |
| elevation (low) | **-1.391** | **0.378** | **0.001** | **-1.460** | **0.360** | **<<.001** | 1.696 | 1.582 | 0.289 |
| climate (future) | **-0.805** | **0.296** | **0.007** | **-1.103** | **0.297** | **0.000** | 2.161 | 1.103 | 0.051 |
| heatwave(yes) | -0.090 | 0.253 | 0.724 | -0.060 | 0.256 | 0.815 | NA | NA | NA |
| block(B) | 0.080 | 0.205 | 0.697 | 0.029 | 0.207 | 0.888 | -0.851 | 1.009 | 0.400 |
| block(C) | 0.082 | 0.208 | 0.696 | 0.056 | 0.210 | 0.788 | -0.917 | 1.004 | 0.362 |
| e(l)\*C(f) | **1.133** | **0.353** | **0.002** | **1.473** | **0.353** | **<0.001** | -2.520 | 1.628 | 0.123 |
| c(f)\*Hw(y) | **-0.950** | **0.352** | **0.008** | **-1.041** | **0.351** | **0.003** | NA | NA | NA |
| e(l)\*Hw(y) | 0.594 | 0.348 | 0.089 | 0.654 | 0.354 | 0.066 | NA | NA | NA |
|  | Marginal R2: 0.156 | | | Marginal R2: 0.195 | | | Marginal R2: 0.115 | | |
|  | Conditional R2: 0.417 | | | Conditional R2: 0.427 | | | Conditional R2: 0.175 | | |
| **Random effects:** | var.(int.) |  | Pr(ML) | var.(int.) |  | Pr(ML) | var.(int.) |  | Pr(ML) |
| Family | **0.5094** |  | **<<0.0001** | **0.3839** |  | **<0.0001** | **9.5100** |  | **<0.0001** |
| Position | 0.1696 |  | 0.0480 | 0.2394 |  | 0.0111 | NA |  | NA |
| Residual | 1.5184 |  | NA | 1.5355 |  | NA | 48.9600 |  | NA |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Trait | Log transformation | Model | Fixed effects | Random effects | REML | Family |
| Fv/Fm | no | lmer | elev\*clim scn\*hw+block | position | false | \ |
| Tcrit | no | lmer | elev\*clim scn\*hw+block | position | false | \ |
| T50 | yes | lmer | elev\*clim scn\*hw+block | position | false | \ |
| Tmax | yes | lmer | elev\*clim scn\*hw+block | position | false | \ |
| Flowering onset | yes | lmer | elev\*clim scn +block | family | false | \ |
| Number of flowers | no | glmer.nb | elev\*clim scn\*hw+block | family; position | \ | negative binomial |
| Number of seeds | no | glmer.nb | elev\*clim scn\*hw+block | family | \ | negative binomial |
| Individual seed weight | no | lmer | elev\*clim scn\*hw+block | family | false | \ |
| Biomass | no | lmer | elev\*clim scn\*hw+block | clim scn|family; position | false | \ |
| Survival | no | coxme | elev\*clim scn\*hw+block | family; position | \ | \ |
| Probability of seed production | no | glmer | elev\*clim scn\*hw+block | family; position | \ | binomial |
| Number of seeds (LM) | No | lmer | elev\*clim scn\*hw+block | family; position | false | \ |
| Seed mass | yes | lmer | elev\*clim scn\*hw+block | family; position | false | \ |
| Chlorophyll content | no | lmer | elev\*clim scn +block | family | false | \ |

Table S4. Table of the models used to analyze each trait. Removal of the 3-way interaction was obtained using the *update* function in ‘base’ R package. elev = elevation; clim scn = climate scenario; hw = heatwave.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Elevation | | Climate scenario | | Heatwave | | Elevation x Climate scenario | | | | Elevation x Heatwave | | | | Climate scenario x Heatwave | | | | Total |
|  | High | Low | Historical | Future | No | Yes | H x C | H x F | L x C | L x F | H x N | H x Y | L x N | L x Y | C x N | C x Y | F x N | F x Y |  |
| Fv/Fm | 148 | 128 | 147 | 129 | 142 | 134 | 77 | 71 | 70 | 58 | 76 | 72 | 66 | 62 | 74 | 73 | 68 | 61 | 276 |
| Tcrit | 167 | 152 | 172 | 147 | 163 | 156 | 89 | 78 | 83 | 69 | 85 | 82 | 78 | 74 | 87 | 85 | 76 | 71 | 319 |
| T50 | 167 | 152 | 172 | 147 | 163 | 156 | 89 | 78 | 83 | 69 | 85 | 82 | 78 | 74 | 87 | 85 | 76 | 71 | 319 |
| Tmax | 167 | 152 | 172 | 147 | 163 | 156 | 89 | 78 | 83 | 69 | 85 | 82 | 78 | 74 | 87 | 85 | 76 | 71 | 319 |
| Flowering onset | 165 | 148 | 167 | 146 | 160 | 153 | 87 | 78 | 80 | 68 | 84 | 81 | 76 | 72 | 84 | 83 | 76 | 70 | 313 |
| Longevity | 168 | 153 | 173 | 148 | 164 | 157 | 89 | 79 | 84 | 69 | 86 | 82 | 78 | 75 | 87 | 86 | 77 | 71 | 321 |
| Biomass | 89 | 71 | 86 | 74 | 92 | 68 | 47 | 42 | 39 | 32 | 50 | 39 | 42 | 29 | 46 | 40 | 46 | 28 | 160 |
| Number of flowers | 168 | 153 | 173 | 148 | 164 | 157 | 89 | 79 | 84 | 69 | 86 | 82 | 78 | 75 | 87 | 86 | 77 | 71 | 321 |
| Number of seeds | 166 | 152 | 173 | 145 | 163 | 155 | 89 | 77 | 84 | 68 | 86 | 80 | 77 | 75 | 87 | 86 | 76 | 69 | 318 |
| Individual seed weight | 128 | 105 | 153 | 80 | 116 | 117 | 85 | 43 | 68 | 37 | 63 | 65 | 53 | 52 | 75 | 78 | 41 | 39 | 233 |
| Probability to produce seeds | 168 | 153 | 173 | 148 | 164 | 157 | 89 | 79 | 84 | 69 | 86 | 82 | 78 | 75 | 87 | 86 | 77 | 71 | 321 |

Table S5. Sample sizes for each trait and each factor level. All fixed effects and their interactions are presented except for ‘block’. H = ‘high elevation’; C = ‘historical climate’; F = ‘future climate’; L = ‘low elevation’; N = ‘no heatwave’; Y = ‘heatwave (yes)’.

**Table** **S6**. Correlation between longevity and total seed production: results from linear random intercept models. Individuals that did not produce seeds were removed from the analysis. Main effects model has no interactions; full model has all 2-way interactions. 3-way interactions were never significant and results for them are not presented. Estimates and standard error are in the logarithmic scale. NA = factor not included in the model for that trait or result not provided by the model. The intercept corresponds to high elevation, historical climate and no heatwave, and other parameter values are relative to this. Significant results, as for p-value < 0.05 from the lmerTest package in R, are presented in bold. e(l) = elevation (low); c(f) = climate (future); hw = heatwave; est. = estimated effect; ML = maximum likelihood; Pr(ML) = p-value of the likelihood ratio test between the models with and without the random effect. Random effects parameters are the variance components associated with that random effect.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Total seed production (n) | | |
| **main effects model:** | est. | (se) | p-value |
| intercept | 6.169 | 0.381 | NA |
| longevity | **0.012** | **0.003** | **<0.001** |
| elevation (low) | -0.728 | 0.295 | 0.020 |
| climate (future) | **-0.730** | **0.185** | **<0.001** |
| heatwave(yes) | -0.022 | 0.177 | 0.899 |
| block(B) | 0.103 | 0.217 | 0.636 |
| block(C) | -0.013 | 0.219 | 0.952 |
|  | Marginal R2: 0.146 | | |
|  | Conditional R2: 0.313 | | |
| **full model:** | est. | (se) | p-value |
| intercept | 6.349 | 0.400 | NA |
| longevity | **0.011** | **0.003** | **0.001** |
| elevation (low) | **-1.231** | **0.377** | **0.002** |
| climate (future) | -0.752 | 0.306 | 0.015 |
| heatwave(yes) | 0.118 | 0.260 | 0.651 |
| block(B) | 0.081 | 0.213 | 0.705 |
| block(C) | 0.026 | 0.215 | 0.906 |
| e(l)\*C(f) | **1.017** | **0.365** | **0.006** |
| c(f)\*Hw(y) | -0.890 | 0.364 | 0.015 |
| e(l)\*Hw(y) | 0.325 | 0.344 | 0.345 |
|  | Marginal R2: 0.179 | | |
|  | Conditional R2: 0.370 | | |
| **Random effects:** | var.(int.) |  | Pr(ML) |
| Family(int.) | **0.5018** |  | **<0.0001** |
| Position | NA |  | NA |
| Residual | 1.6621 |  | NA |

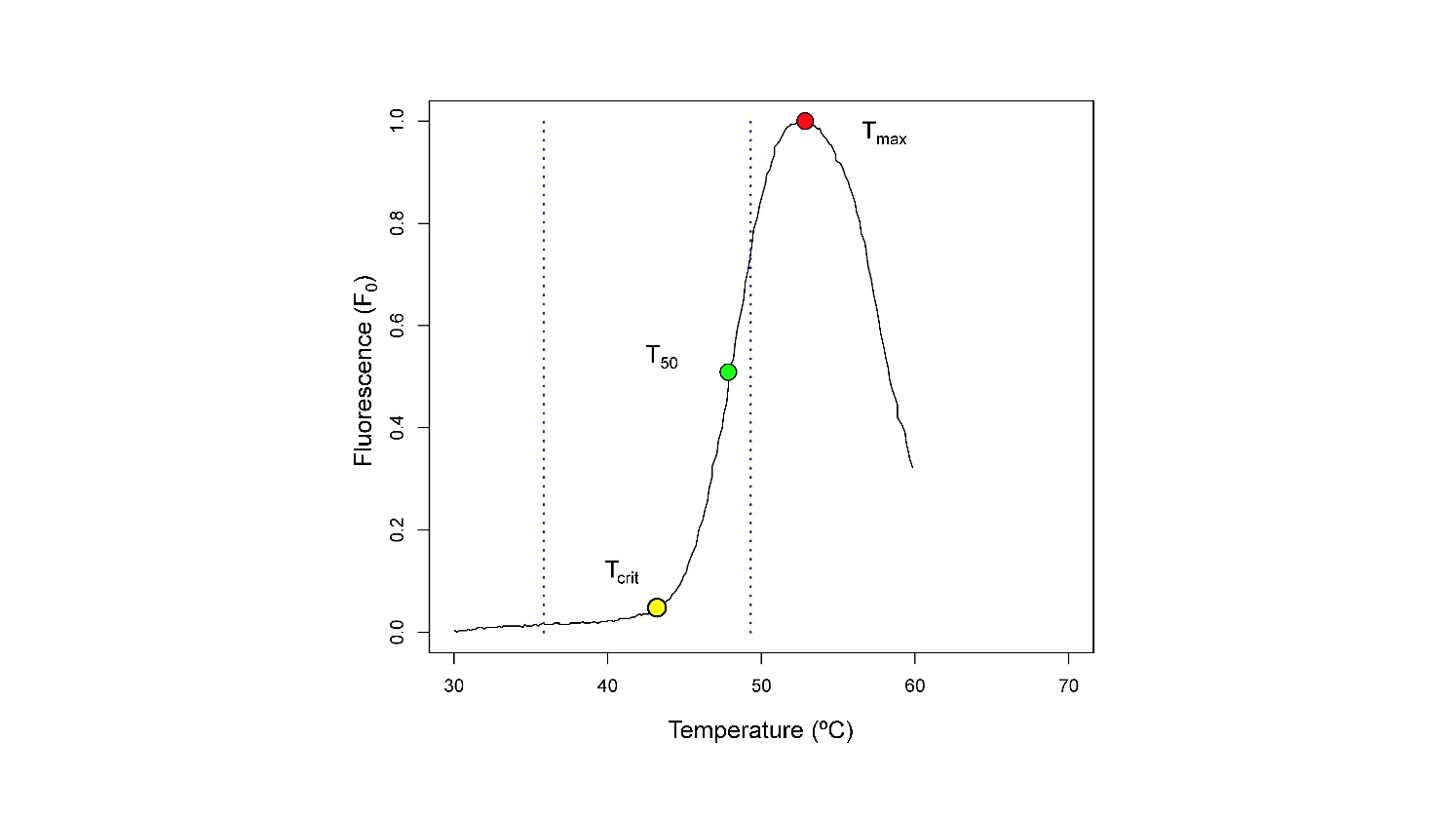


Figure S. Example of a T-F0 curve. This curve was obtained using real data. Tcrit was estimated as the intersection between the two straight lines tangential to the flat and steep part of the curve, using a break-point regression analysis conducted with the ‘segmented’ R package (Muggeo 2017).

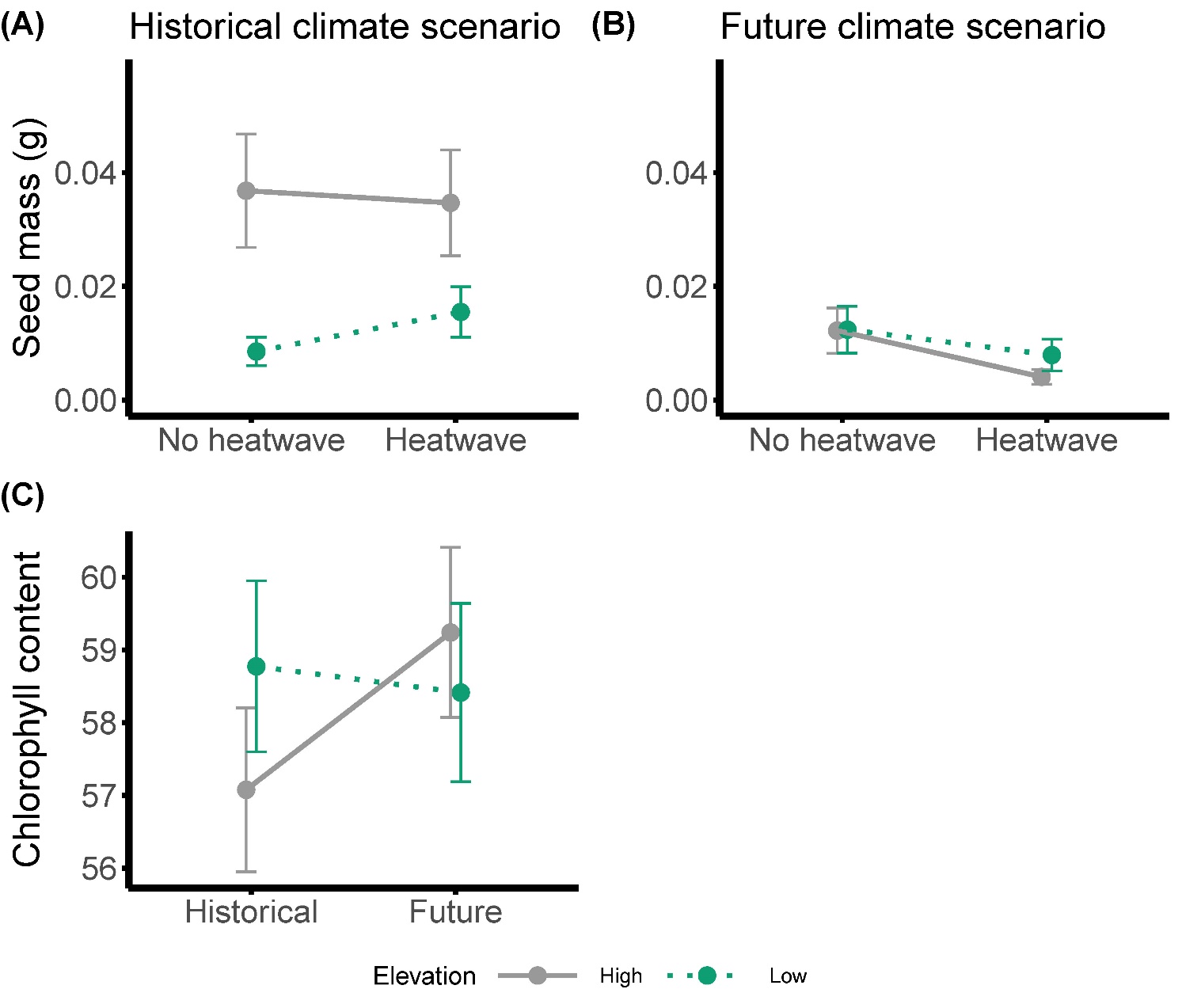


Figure S2. Reaction norms for (A) seed mass in response to the heatwave treatment under a historical climate scenario and (B) under a future scenario and for (C) chlorophyll content in response to climate scenario. Grey and solid lines = high-elevation families; green and dotted lines = low-elevation families. Values were estimated from the models using the *emmeans* function in ‘emmeans’ package, in R; error bars are standard errors.

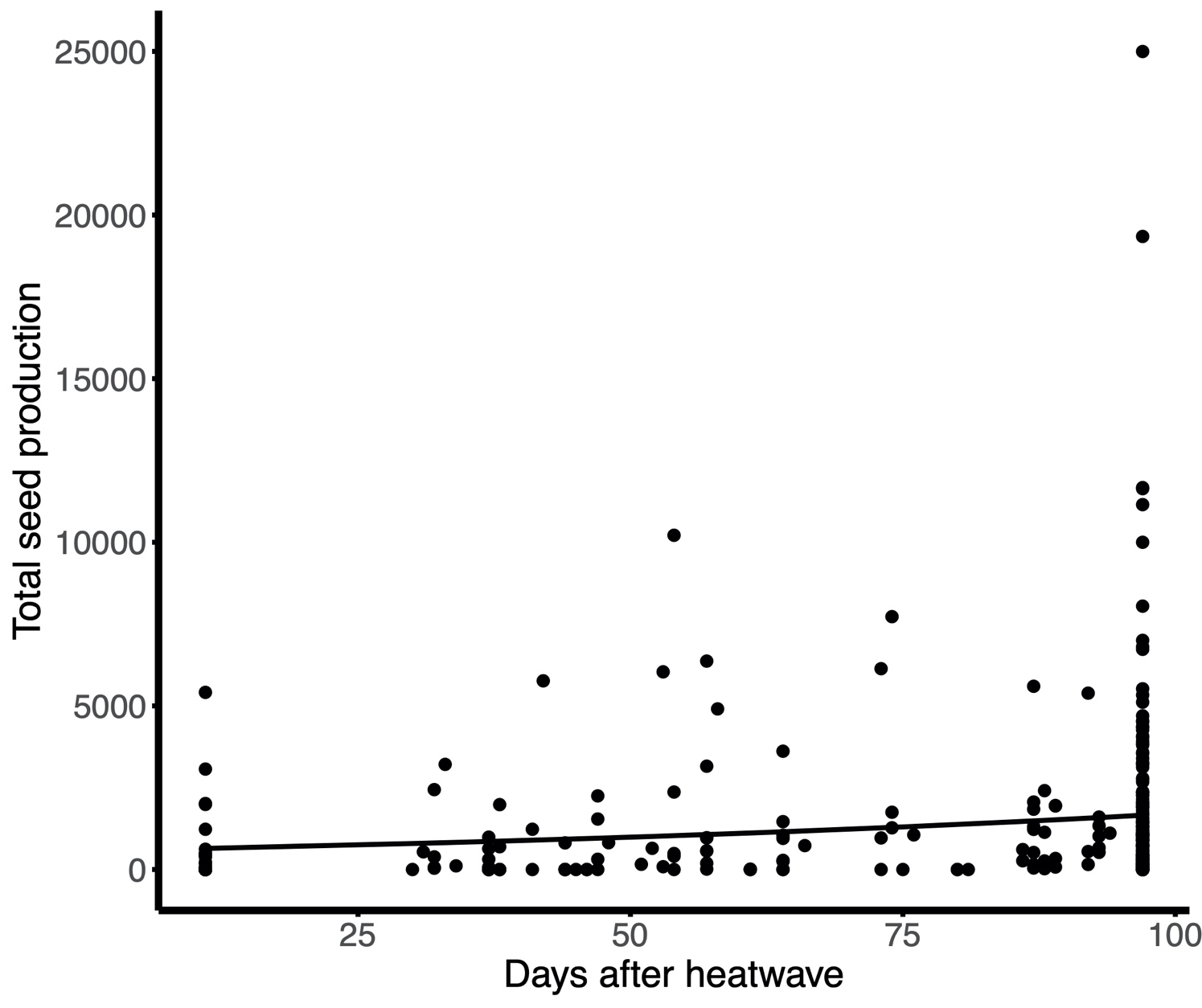


Figure S3. Correlation between longevity (days from the end of the heatwave treatment to death) and total seed production. Only individuals that produced seeds were included in the analysis. The correlation was analyzed using linear mixed effects models (*lmer* function in ‘lme4’ package in R) with total seed production as response variable and longevity, elevation, climate scenario and heatwaves as fixed effects. Random effects were family, block, and position nested in block (block:position). Predictions from the model to plot the graph were obtained using the *ggpredict* function in ‘ggeffects’ package. Black dots = raw data.