

**Original citation:**

Huang, Z., Footitt, Steven, Tang, A. and Finch-Savage, William E. (2018) Predicted global warming scenarios impact on the mother plant to alter seed dormancy and germination behaviour in Arabidopsis. *Plant, Cell & Environment*, 41 (1). pp. 187-197.  
doi:[10.1111/pce.13082](https://doi.org/10.1111/pce.13082).

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1 **Predicted global warming scenarios impact on the mother plant to alter seed dormancy**  
2 **and germination behavior in Arabidopsis.**

3

4 **Running Head:** Global warming and seed production

5

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7

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22 Seed characteristics are key components of plant fitness that are influenced by temperature in  
23 their maternal environment, and temperature will change with global warming. To study the  
24 effect of such temperature changes, *Arabidopsis thaliana* plants were grown to produce seeds  
25 along a uniquely designed polyethylene tunnel having a thermal gradient reflecting local  
26 global warming predictions. Plants therefore experienced the same variations in temperature  
27 and light conditions, but different mean temperatures. A range of seed related plant fitness  
28 estimates were measured. There were dramatic non-linear temperature effects on the  
29 germination behaviour in two contrasting ecotypes. Maternal temperatures lower than 15-16  
30 °C resulted in significantly greater primary dormancy. In addition, the impact of nitrate in the  
31 growing media on dormancy was shown only by seeds produced below 15-16 °C. However,  
32 there were no consistent effects on seed yield, number or size. Effects on germination  
33 behaviour were shown to be a species characteristic responding to temperature and not time  
34 of year. Elevating temperature above this critical value during seed development has the  
35 potential to dramatically alter the timing of subsequent seed germination and the proportion  
36 entering the soil seed bank. This has potential consequences for the whole plant life cycle and  
37 species fitness.

38

39 **Key Words:** Seed germination, seed dormancy, germination timing, life cycle, seed yield,  
40 global warming, temperature, *Arabidopsis*

41

## 42 **Introduction**

43 Evidence for warming of the climate system resulting from anthropomorphic greenhouse gas  
44 emissions is now unequivocal (IPCC, 2014). Such global warming has not only increased  
45 mean temperatures, but reduced the diurnal temperature range as minimum temperature has  
46 increased at twice the rate of maximum temperature (Walther et al. 2002), and impacted on a

47 seasonal scale as biological spring is now earlier and biological winter is later (Penuelas et al.  
48 2009). Temperature is the primary signal determining the timing of the two major transitions  
49 in the plant life cycle; germination (seed to plant transition) and reproduction (plant to seed  
50 transition) that are key components of plant fitness. Temperature also affects depth of  
51 dormancy during seed maturation (Donohue, 2009; Chiang et al. 2011; Kendall et al. 2011;  
52 Kendall and Penfield, 2012; Huang et al. 2014) and during dormancy cycling in the soil  
53 following shedding (Probert, 2000; Finch-Savage and Leubner-Metzger, 2006; Footitt et al.  
54 2011, 2013, 2014). Thermal control both before and after shedding therefore determines  
55 when seeds germinate and the timing of seedling emergence in seasonal climates (Donohue et  
56 al. 2010; Donohue et al. 2015; Burghardt et al. 2016). This control is likely to be disrupted in  
57 the event of future climate change to impact upon plant regeneration from seeds (Walck,  
58 2011). Such potential for compromised seedling emergence and vigour, and shifts in  
59 germination phenology are likely to influence population dynamics, and therefore, species  
60 composition and diversity of communities (Walck et al. 2011). Nevertheless, seedling  
61 emergence timing has until now been largely neglected in global warming studies (Hedhly et  
62 al. 2009).

63

64 Fitness can be considered as the ability of species to survive and reproduce in the  
65 environment in which they find themselves (Orr, 2009) and therefore the probability of  
66 surviving to the next generation. The ability of species to adapt to future climates depends on  
67 the existence of phenotypic plasticity in the life history traits that impact on fitness under  
68 increasing temperatures (Nicotra et al. 2010). In addition to phenotypic plasticity, genetic  
69 variation within populations is also a primary mechanism for adaptation (Jump et al. 2009)  
70 that may 'preadapt' them to future climates. There is considerable genetic evidence of  
71 adaptation, for example, Postma and Ågren (2016) in a reciprocal life cycle experiment using

72 a Recombinant Inbred Line population produced from Swedish and Italian *Arabidopsis*  
73 ecotypes found that fitness selection during seedling establishment was favoured by local  
74 alleles in the establishment Quantitative Trait Loci. Although they have limitations, the  
75 commonly used measures of fitness include seed related variables such as seed number, size  
76 and yield (Primack, 1989). However, the probability for survival to the next generation,  
77 particularly of ruderal annual monocarp species such as *Arabidopsis*, also involves seed  
78 behavioral characteristics such as seed dormancy, germination phenology, longevity and  
79 persistence in the soil seed bank, which can also be influenced indirectly by seed mass  
80 (Fenner and Thompson, 2005; Poschlod et al. 2005; Springthorpe and Penfield, 2015).  
81 Temperature, water stress and nitrate in the maternal environment influence the phenotypic  
82 expression of all these seed characteristics (Fenner, 1991, Case et al. 1996; Meyer & Allen,  
83 1999; Lacey & Herr, 2000; Alboresi et al. 2005; Kochanek et al. 2010; Chiang et al. 2009;  
84 Kendall et al. 2011; He et al. 2014; He et al. 2016). Walck et al. (2011) point out that parental  
85 environments can therefore facilitate the evolutionary divergence of life history patterns  
86 among plant populations. Furthermore, they suggest that as there is substantial variation in  
87 both genetic and phenotypic plasticity for seed dormancy and germination within most  
88 species over elevational and latitudinal gradients (Meyer et al. 1995; Baskin & Baskin, 1998;  
89 Cavieres & Arroyo, 2000; Daws et al. 2006; Vidigal et al. 2016) populations may therefore  
90 be buffered from some of the effects of projected climate change. A degree of environmental  
91 buffering may also occur in the soil seed bank. Fenner and Thompson (2005) concluded that  
92 most evidence suggests that direct effects of global warming on the soil seed bank will be  
93 limited, but there may be large indirect effects of climate change on seed banks. Such indirect  
94 effects may result from changes in the dormancy level and seed mass of newly dispersed  
95 seeds; this may alter the balance between the short-term and persistent seed banks.

96

97 A recent study of phenotypic plasticity in seed dormancy highlights the importance of  
98 considering varying weather conditions rather than just constant average temperatures when  
99 assessing responses to global warming (Fernández-Pascual and Jiménez-Alfaro 2014). In the  
100 present work, we investigate the extent of this phenotypic plasticity in *Arabidopsis* using a  
101 unique thermogradient polyethylene tunnel. The tunnel provides realistic seasonal and diurnal  
102 temperature fluctuations, but with a gradient of simulated global warming depending on the  
103 position that the plant is grown in the tunnel (Wurr et al. 1996). A projected median  
104 emissions scenario for the local experimental area used in this work (West Midlands, UK)  
105 indicates an increase in the summer mean temperature of 3.7 °C by 2080 (UK Climate  
106 Change Projections, 2014; <http://ukclimateprojections.metoffice.gov.uk/>). We therefore  
107 adjusted the tunnel to a gradient from ambient to *c.* + 4 °C. To avoid the confounding effects  
108 of temperature on the timing of flowering and on seed maturation we established the  
109 temperature gradient at the start of seed development in the first three sowings. To compare  
110 with this, at the fourth sowing the gradient was applied throughout plant growth to seed  
111 harvest.

112

113 Other environmental variables not linked to climate change can also impact plant growth, and  
114 seed characteristics (yield, size, dormancy) and may interact with the effect of increases in  
115 mean temperature; a principal one of these is nitrate availability. For example, the nitrate  
116 content in both soil and seed has an impact on dormancy in *Arabidopsis* (Alboresi et al. 2005;  
117 Matakidis et al. 2009). Furthermore, maternal temperature and nitrate availability both alter  
118 dormancy and expression of *CYP707A2* (ABA catabolism) and genes involved in nitrate  
119 metabolism (Matakidis et al. 2009; Kendall et al. 2011; He et al. 2016). Thus temperature  
120 could potentially regulate dormancy by influencing nitrate metabolism during seed  
121 development. We therefore include nitrate availability as a further variable in this study.

122

123 Here, a comparison is made between two *Arabidopsis* ecotypes (Cvi and Bur) that have  
124 adapted to unique environments (Fig. S1), which has resulted in contrasting obligate winter  
125 (Cvi) and summer (Bur) annual behaviours when grown in the local environment used for  
126 this study (Footitt et al. 2013). These two ecotypes were therefore employed to provide  
127 contrasts in key life cycle variables that determine fitness i.e. their flowering time (c. 35 and  
128 46 days at 20°C for Cvi and Bur respectively), seed yield, seed size, and in their dormancy  
129 response to temperature that influences germination time (Huang et al. 2014, 2015). Previous  
130 work has also shown that their ecotypic differences in seed responses to germination  
131 conditions are greatly influenced by seed maturation in different laboratory environments  
132 (Huang et al. 2014, 2015). Comparison of these very different phenotypes within a species  
133 helps to shed light on the potential within-species life cycle plasticity in the face of global  
134 warming; and to determine whether effects are a species characteristic or ecotype specific.  
135 We investigate whether the limited temperature increases in realistic simulated global  
136 warming scenarios could impact significantly upon seed characteristics that can influence  
137 species fitness. We found that these scenarios gave no consistent effects on seed yield,  
138 number or size. However, there were dramatic non-linear temperature effects on the  
139 germination behaviour of the seeds produced in both the contrasting ecotypes. We discuss  
140 how these effects may have long-term consequences for the stability of soil seed banks as  
141 native flora comes under increasing pressure from climate change.

142

### 143 **Materials and methods**

144 Experiments were carried out with *Arabidopsis* over two years (2011, 2012) in a field-based  
145 thermogradient tunnel (Wurr et al. 1996) to investigate the impact of global warming  
146 scenarios on plant growth and development, and seed parameters considered as measures of

147 fitness. The experiments compared two ecotypes, Cape Verde Islands (Cvi) and Burren (Bur)  
148 because they exhibit obligate winter and summer annual behaviour respectively at the  
149 experimental site used. Seeds of both ecotypes were sown to coincide with the time of seed  
150 maturity and shedding of each ecotype grown in the UK (Footitt *et al.* 2013). Key plant  
151 development stages were monitored and seeds were harvested at maturity and their dormancy  
152 characteristics were determined in the laboratory. Furthermore, seed yield (total seed weight)  
153 and seed size (1000-seed weight) were also determined.

154

155 ***Thermogradient tunnel:*** The polyethylene tunnel (32 m long x 9 m wide) structure enabled  
156 plants to be grown at natural day lengths with a high percentage (76%) of natural levels of  
157 irradiance. The ambient air temperature was constantly monitored outside of the tunnel.  
158 Reacting to this an electronic climate control system operated fans that generated opposing  
159 warmed and ambient air flows to establish and maintain a temperature gradient from ambient  
160 at one end of the tunnel to *c.* ambient + 4 °C at the other end (Wurr *et al.* 1996; Fig. 1). Air  
161 and soil temperatures were monitored continuously along the tunnel. Realistic seasonal and  
162 diurnal temperature fluctuations were therefore maintained within the tunnel, but with  
163 varying degrees of simulated climate warming depending on the position that plants are  
164 grown along the tunnel. Four positions along the tunnel were selected to provide *c.* T1,  
165 ambient; T4, ambient + 4 °C and at two equally spaced temperatures (T2 & T3) in between  
166 (Fig. 1).

167

168 ***Seed material for tunnel experiments:*** Bulk seed stocks of *Arabidopsis* ecotypes Bur and  
169 Cvi, were initially produced in a temperature-controlled glasshouse (23/17°C, 16/8 h, light  
170 /dark) and harvested as described below. The glasshouse was vented and heated to control  
171 temperature and had supplementary lighting to maintain light levels and photoperiod. At

172 harvest seeds were dried to an equilibrium relative humidity of 55% above a saturated  
173 calcium nitrate solution at 20 °C for six days (seed moisture content 9.9% on a dry weight  
174 basis). A proportion of the freshly harvested seeds were placed in separate sealed moisture-  
175 proof containers and after-ripened (AR) at 20°C/dark for eight months. This ensured all seeds  
176 were non-dormant before subsequent use to avoid delay in seedling emergence.

177

178 ***Sowing occasions:*** Experiments were set up on 4 occasions (early and late sowings in each of  
179 two years; Table S1): 11 February and 27 July, 2011 at a single nitrogen concentration; 9  
180 February and 1 May 2012 at three nitrate concentrations detailed below. On the first three  
181 occasions seeds were sown in a temperature-controlled glasshouse (23/17 °C, 16/8 h, light  
182 /dark) and then transferred to the tunnel at the initiation of bolting and therefore before  
183 opening of the first flower. Plants were then grown to maturity for seed harvest. On the final  
184 occasion (late sowing 2012) seeds were sown directly into the tunnel to record the full life  
185 cycle: seedling emergence, bolting, flowering, seed maturation and yield components. Across  
186 the four occasions sowing was timed to compare the performance of seeds produced under a  
187 wide range of temperatures experienced during natural winter and summer annual production  
188 times at the experimental site. For example, early sowings in both years were timed for seed  
189 maturation and shedding consistent with a winter annual; the late sowing in 2011 was timed  
190 for seed maturation consistent with a summer annual; the final late sowing in 2012 coincided  
191 with shedding of the winter annual Cvi. At this final sowing, because seeds had been after-  
192 ripened to relieve dormancy and allow rapid germination, seed maturation occurred on these  
193 plants during higher temperatures in summer, and therefore intermediate between summer  
194 and winter annual phenotypes.

195

196 Depending on the experiment, growth media contained three different levels of nitrate:  
197 standard N (SN; Levingtons F1 compost: sand: vermiculite 6:1:1); Low N (LN; 4:1:1); and  
198 very low N (VLN; 4:2:2). They contained 304.3, 263.5 and 127.8 NO<sub>3</sub>-N mg/kg dry weight  
199 respectively. Each experiment had a randomized split-plot design with three replicates at each  
200 tunnel position. Plots were P24 cellular trays (24 cells, each 5 x 5 x 5 cm) containing either  
201 SN, LN or VLN media. Each tray was placed in a second undivided tray lined with capillary  
202 matting to ensure all the plants had an adequate uniform water supply from below. Within  
203 each tray there were two separate subplots of 8 cells sown with seed of Cvi or Bur. Plants  
204 were watered regularly throughout from below to ensure they did not experience differential  
205 water stress along the tunnel. No further nutrient was applied to the trays during the  
206 experiments.

207

208 **Seedling emergence:** For recording seedling emergence twenty-five seeds were sown onto  
209 the surface of pre-watered compost in each of four replicate cells per treatment. The trays  
210 were placed into the four locations along the tunnel. After 24 h exposure to light to remove  
211 the final layer of dormancy the seeds were covered with a uniform layer of clean horticultural  
212 sand (0.5 cm). Seedling emergence through the sand was recorded daily.

213

214 **Bolting time and plant growth to maturity:** For plants grown to maturity, five seeds were  
215 sown into each of the eight cells per treatment and maintained as above; except the trays were  
216 initially covered with transparent propagator lids for at least four days, by which time all the  
217 seedlings had established. One week after sowing, the seedlings were thinned to one per cell.  
218 Plants were visually scored daily for bolting (inflorescence extended 1 cm). At that stage the  
219 rosette diameter and leaf number were also recorded. Aracon bases (Arasystem, Belgium)  
220 were then placed on plants. When the plant had grown through the bases, Aracon tubes

221 (Arasystem, Belgium) were added to the bases to isolate each plant during pollination and to  
222 facilitate collection of all the seeds produced.

223

224 ***Seed harvesting and yield measurement:*** In all cases watering stopped as seeds became fully  
225 mature (i.e. when all the siliques had turned yellow and dry on the plant). Seven days later  
226 the plants were cut just above the rosettes, the height of the inflorescence was measured and  
227 following seed extraction it (minus seeds and siliques) was placed in a paper bag. The bags  
228 were then left to dry at room temperature for 7 days, placed in an oven at 80 °C for 24 h and  
229 the inflorescence dry weight was recorded. Following extraction, seeds were sieved (500 µm)  
230 and then placed at 55% relative humidity/20 °C for six days to equilibrate as above. This  
231 resulted in a seed-moisture content of 8-10% on a dry weight basis. At this point seed yield  
232 (total seed weight) and seed size (1000-seed weight) were determined. Seeds were then  
233 sealed in aluminium foil bags (11×24 cm) (Moore and Buckle, St. Helens, UK) and stored at  
234 -80 °C for germination experiments.

235

236 ***Seed germination:*** Germination tests used seeds directly from -80 °C or seeds AR at 20  
237 °C/dark for 30 days as stated for each experiment. Seeds were surface-sterilized in a 0.125 %  
238 sodium hypochlorite solution (household bleach: 5% sodium hypochlorite, diluted to 2.5%  
239 v/v) for five minutes and then washed three times with distilled water. Germination  
240 experiments were conducted in temperature controlled incubators. Seeds were placed on two  
241 layers of 3MM chromatography paper in clear plastic boxes (8×12×2 cm) (Stewart Plastics  
242 Ltd, Croydon, UK) containing 8ml of distilled water or 1 mM or 10 mM KNO<sub>3</sub>. For each  
243 treatment, there were three replicates of 40 seeds of each ecotype. Germination (radical  
244 emerged through endosperm and testa) was recorded either in the light or dark, for 28 days.  
245 In experiments with dark treatments (germination boxes wrapped in a double layer of

246 aluminum foil) seeds were surface sterilized, sown and germination was recorded in the dark  
247 under a green safe light (Kodak 7B safelight filter/Green, Kodak Limited, London).

248

249 ***Seed nitrate content measurement:*** Triplicate 150 mg samples of fresh dry seeds were  
250 ground using a pestle and mortar, and transferred to a 20 ml scintillation vial that was  
251 weighed before and after drying at 80°C for 16 h. Deionised water (10 ml) was added and the  
252 samples were shaken for one hour and then centrifuged for five minutes at 5000 rpm. The  
253 supernatant was filtered using nitrogen free filter paper, and analysed for NO<sub>3</sub>-N by a steam  
254 distillation method using a FOSS FIAstar 5000 Flow Injection Analyser (Gerber Instruments,  
255 Effretikon, CH) for end point determination (Bremner and Keneney, 1965).

256

257 ***Data analysis:*** Analysis of variance was used to detect the differences and interactions  
258 between variates. Statistical analysis was carried out using the software package GenStat  
259 (VSN International, 2012 or Payne et al. 2003). All percentage germination data were first  
260 angular transformed. The regression analysis function of Sigmaplot (Systat Software Inc,  
261 UK) was used to obtain curves with the best fit in Figs. 4 and 5. Details of fitted curves are  
262 given in Table S2. Mean maturation temperature was calculated for increasing periods of  
263 time prior to harvest to determine best fit to the data (30 days for all occasions and ecotype  
264 except 18 days for Cvi at the early sowing of 2011).

265

## 266 **Results**

267 ***Global warming scenarios:*** A thermal gradient was established along the experimental  
268 polyethylene tunnel and four positions were selected with different air and soil temperature  
269 scenarios (Fig. 1a). The first position (T1) remained at ambient and the fourth (T4) remained  
270 c. 4 °C higher, with two intermediate positions (T2 and 3). Fig. 1b,c shows this gradient was

271 maintained throughout the year as ambient temperature rose and fell. There was a linear  
272 relationship ( $R^2 = 0.936$ ,  $P < 0.001$ ) between air temperature (1m above soil level) and soil  
273 temperature (5 cm below soil surface) measured across all four sites. Therefore, a similar  
274 gradient of soil temperature was also established and maintained in the plant growing  
275 containers along the tunnel.

276

277 ***Effect of temperature and nitrate on the life cycle:*** A full life cycle was recorded at the four  
278 selected positions along the tunnel for both Cvi and Bur following sowing on 1 May 2012  
279 (Fig. 2). In general, at progressively warmer positions along the tunnel the duration of the  
280 plant life cycle decreased in both ecotypes. This reduction resulted largely from a reduced  
281 post-flowering period, which included seed development and subsequent drying to harvest  
282 maturity (siliques sufficiently dry to extract seeds). There was little effect of nitrate content in  
283 the growth media on the length of the life cycle in Bur. The relative delay in seedling  
284 emergence in the high nitrate regime was offset by a reduced period of vegetative growth  
285 (rosette formation) prior to extension of the inflorescence (bolting) (Fig. 2). Cvi seedlings  
286 grown in the low and very low nitrate regimes failed to reach maturity and produce seeds and  
287 therefore post-seedling emergence data is only presented for the standard nitrate regime for  
288 this ecotype.

289

290 ***Effect of temperature on seedling emergence, plant growth and seed yield components:***

291 Final percentage seedling emergence was significantly ( $P < 0.001$ ) higher in Bur than Cvi, but  
292 there was no overall significant effect of tunnel position (temperature regime) or nitrate  
293 regime in either ecotype (Fig. S2) and no interaction between the variates. However, in both  
294 ecotypes there was a significant effect ( $P < 0.001$ ) of nitrate regime on seedling emergence  
295 rate ( $1/T_{50}$ , time to 50% seedling emergence from viable seeds). In both ecotypes the

296 standard nitrate (SN) regime delayed seedling emergence compared to the lower nitrate  
297 regimes (LN, VLN) and seedling emergence rate was fastest in the LN regime.

298

299 There were significant ( $P < 0.001$ ) effects of both temperature regime and ecotype on the time  
300 to bolting time (extension of the inflorescence). This decreased with increasing temperature  
301 along the tunnel in both ecotypes in the SN regime (Table 1). However, there was a  
302 significant ( $P < 0.001$ ) interaction with the effect more marked in Bur (35.6- 30.8 days; T1 to  
303 T4 respectively) than Cvi (34.2-31.3 days; T1 to T4 respectively). In Bur, bolting was  
304 recorded in three nitrate regimes, but as reported above development in Cvi was limited in  
305 the LN and VLN regimes. In Bur, the effect was not significantly different in the three nitrate  
306 regimes, and there was no significant interaction with temperature. In general, Bur tended to  
307 have faster bolting times in the SN regime (Table 1).

308 Table 1 near here

309 There were significant ( $P < 0.001$ ) effects of ecotype on both rosette diameter (measured at  
310 bolting) and leaf number with Bur plants being larger and having much greater vegetative  
311 growth than those of Cvi in all temperature regimes. Where they were compared in the SN  
312 regime, the mean rosette diameters were  $5.58 \pm 0.09$  cm and  $3.67 \pm 0.14$  cm for Bur and Cvi  
313 respectively. Mean leaf number was  $15.1 \pm 0.2$  and  $8.7 \pm 0.3$  for Bur and Cvi respectively. In  
314 Bur, there was a significant ( $P < 0.001$ ) increase in rosette diameter and leaf number with  
315 increasing nitrate and no interaction with temperature (Table S2). In general, Bur rosette  
316 diameter tended to increase with temperature along the tunnel except in the SN regimes,  
317 however, Bur leaf number at bolting was significantly ( $P < 0.001$ ) reduced as temperature  
318 increased (Table S2). However, there was no trend with temperature in these two measures in  
319 Cvi.

320

321 There was no consistent effect of temperature on seed yield, seed size (1000-seed weight) or  
322 seed nitrate content across sowings in either ecotype in the SN regime. However, there was a  
323 significant effect ( $P < 0.001$ ) of nitrate regime in Bur. For example, the VLN regime  
324 significantly ( $P < 0.001$ ) reduced seed yield, and in the LN regime both highest and lowest  
325 temperature regimes reduced seed yield (Fig. 3a) resulting in a significant ( $P < 0.001$ )  
326 temperature x nitrate interaction. In Bur, there was no consistent effect of nitrate regime on  
327 seed size (Fig. 3b), but seed nitrate content was significantly ( $P < 0.001$ ) higher in the SN  
328 regime at the highest temperature compared to any other combination of treatments (Fig. 3c).  
329 There were clear highly significant ( $P < 0.001$ ) positive linear relationships in Bur between  
330 inflorescence dry weight and height, seed yield and seed number (Fig. S3). Data from all  
331 tunnel positions and nitrate regimes could be fitted to the same relationship with a clear  
332 demarcation between low and high values from VLN and SN regimes respectively. In  
333 contrast, these same relationships were not significant in Cvi in the SN regime (Fig. S4).  
334

335 ***Effect of seed production environment (temperature, nitrate) on dormancy and***  
336 ***germination:*** Germination experiments were carried out at 10 and 25 °C in Bur and at 10 and  
337 20 °C in Cvi as their seeds exhibit contrasting germination at lower compared to higher  
338 temperatures. In general, Cvi is more dormant at high temperatures and Bur more dormant at  
339 lower temperatures consistent with their winter and summer annual behaviour respectively  
340 (Huang *et al.*, 2014). In the experiments there were significant ( $P < 0.001$ ) effects of  
341 temperature regime, nitrate regime and ecotype and significant ( $P < 0.001$ ) interactions  
342 between these variables on the percentage germination of seeds produced, which are detailed  
343 below.  
344

345           ***In Bur:*** Mean temperatures during maturation along the tunnel overlapped between  
346 occasions in 2011 but not in 2012 (Fig. 4). The relationship between mean temperature and  
347 percentage seed germination in the light was continuous across both sowings in each year  
348 showing the extent of germination to be a function of maturation temperature not time of year  
349 (Fig. 4a-d). In all combinations of temperature and nitrate regimes germination was lower  
350 when seeds were matured at lower temperatures and higher at higher temperatures;  
351 importantly there was a sharp transition at *c.* 16 °C. These data show dormancy was greater  
352 when seeds were matured at mean temperatures lower than 16 °C. In general, the level of  
353 dormancy displayed was greater when seeds were germinated at 25 compared to that at 10 °C.  
354 In 2012, seeds were produced in three nitrate regimes and this had a highly significant  
355 ( $P < 0.001$ ) effect on depth of dormancy when seeds were produced below 16 °C, but not at  
356 higher temperatures (Fig. 4c, d). This germination response occurred despite there being no  
357 consistent effect on seed nitrate concentration (Fig. 3e). Surprisingly, the relationship  
358 between nitrate level in the growing media and dormancy of seeds produced at lower  
359 temperature was positive; seeds produced in the VLN regime having least dormancy (highest  
360 germination).

361

362 When Bur germination was tested in the dark at 10 or 25 °C, dark-germination was less than  
363 5% in seed produced under the LN and SN regimes. In seeds produced in the VLN regime  
364 dark germination peaked at 30% at 10 °C and 11% at 25 °C, but only below a maturation  
365 temperature of 16 °C (Fig. S6). Nitrate could substitute for the light requirement at 10 °C, but  
366 only in seeds matured at below 16 °C (Fig. 4e) indicating maturation temperature is a  
367 determinant of nitrate sensitivity in Bur. When tested at the higher temperature of 25 °C with  
368 nitrate in the dark the response approached that seen in the light (Fig. 4f).

369

370           ***In Cvi***: Seeds were dormant in all production environments in both years and  
371 consequently there was no germination in the light of freshly harvested seeds at temperatures  
372 from 5 to 25 °C. However, depth of dormancy did differ and this was illustrated by  
373 germination of freshly harvested seeds on Gibberrellin solution at 20 °C (Fig. 5a,c).  
374 Gibberrellin, depending on concentration, can reduce the depth of dormancy allowing  
375 germination in the light. Seeds produced in 2011 under winter annual conditions (flowered in  
376 spring; early sowing) were less dormant at a given production temperature than those grown  
377 as a summer annual (flowered in autumn; late sowing) (Fig. 5a). In 2012, production  
378 temperatures on the two occasions did not overlap and those produced at lower temperature  
379 (early sowing) had greater dormancy than those produced at higher temperature (late  
380 sowing). Interestingly a transition occurred at *c.* 16 °C as it did for Bur. Germination was also  
381 recorded on water at a range of temperatures following dry storage (after-ripening; AR) for  
382 30 days (Fig. 5b,d; Fig. S5). Such storage, depending on temperature and seed moisture,  
383 progressively relieves dormancy. When these AR seeds were placed to germinate at 15 °C  
384 there was a clear relationship between seed maturation temperature and depth of dormancy  
385 (Fig. 5b,d). In general, depth of dormancy was greater when seeds were matured at lower  
386 temperatures than when matured at higher temperatures; again, in both years there was a clear  
387 transition at *c.* 16 °C. However, this relationship differed dependent on the germination  
388 conditions. For example, freshly harvested 2011 seeds on Gibberrellin at 20 °C showed seeds  
389 from early sown plants were less dormant than those from late sown plants (Fig. 5a),  
390 interestingly, the reverse is shown when 30 day AR seeds were germinated at 10 °C on water  
391 (Fig. S5a). At 25 °C dormancy persisted and there was no germination after 30 days AR.  
392 These relationships may change with further AR.

393

394 **Discussion**

395 *Arabidopsis* plants and seeds were produced under realistic global warming scenarios (mean  
396 temperature increase to 2080; UK Climate Change Projections 2014) and under this limited  
397 range of temperature elevation there was no consistent effect on seed yield and size. In  
398 contrast, there were dramatic non-linear temperature effects on the germination behaviour of  
399 the seeds produced in both the contrasting ecotypes studied. We show that maternal  
400 temperatures lower than 15-16 °C resulted in significantly greater primary dormancy than  
401 higher temperatures. In addition, the impact of nitrate availability in the growing media was  
402 shown only by seeds produced below 15-16 °C. A similar dramatic difference in seed  
403 dormancy over a small range of constant temperatures (either side of 14-15 °C) in the  
404 laboratory also occurs in the Col ecotype of *Arabidopsis* (Springthorpe and Penfield, 2015).  
405 Importantly, we show in 2011 these effects occurred along the tunnel in a single experiment  
406 showing they are driven by temperature and not related to the production time of year. These  
407 results therefore illustrate the potentially large impact of small mean temperature increases in  
408 this critical temperature range, and that the impact of global warming in the maternal  
409 environment can dramatically alter subsequent seed performance. This effect is particularly  
410 relevant to temperate regions where seeds are produced in this temperature range. In these  
411 regions, there may be long-term consequences for the stability of soil seed banks as native  
412 flora comes under increasing pressure from climate change. Such differences could greatly  
413 influence phenology expression and future evolution (Burghardt et al. 2016).

414

415 Despite expressing contrasting obligate winter and summer annual behaviours, and  
416 representing the more extreme ends of the dormancy spectrum in *Arabidopsis*, the general  
417 effect of the local UK global warming scenarios used was similar in both ecotypes. A species  
418 characteristic relationship was therefore revealed between maternal temperature (during  
419 maturation) and level of dormancy having a sharp cut off at 15-16 °C. The impact of this

420 species characteristic appears tempered by the ecotypes characteristic higher or lower  
421 reference dormancy levels (Cvi or Bur respectively). For example, the impact of the maternal  
422 temperature was shown to be dependent on conditions in the germination environment that  
423 alter the expression of thermodormancy, and this is greatest in the more dormant Cvi.

424

425 The maternal temperature effect on dormancy is tempered by the availability of nitrate to the  
426 mother plant in Bur, but not Cvi, showing the nitrate effect to be ecotype specific. Bur has a  
427 greater nitrate use efficiency (Chardon et al. 2010) that enabled growth to maturity in the  
428 VLN and LN regimes while Cvi seedling mortality was 100% in the same conditions. In Bur,  
429 there was an interaction between the maternal temperature and nitrate regimes that  
430 manifested itself post maturation exclusively in seeds produced below 16 °C. This resulted in  
431 altered germination in the light and dark at 10 °C (Fig. 4) at the early sowings. Seeds  
432 produced above 16 °C, lost sensitivity to the maternal nitrate regime and light was required  
433 for germination at 10 °C. As maturation temperature determined nitrate sensitivity in Bur this  
434 may negatively impact low temperature spring germination.

435

436 The enhanced nitrate sensitivity of Bur may serve to exploit the impact of temperature and  
437 nitrate availability on dormancy level. Seed maturation under low temperature and low nitrate  
438 conditions both result in increased dormancy and down regulation of genes involved in  
439 nitrogen metabolism (He et al., 2016) while warm temperatures result in reduced dormancy  
440 and increased expression of genes involved in nitrogen metabolism (Kendal et al., 2011).  
441 Expression of the ABA catabolism gene *CYP707A2* is regulated by nitrate signaling via  
442 NITRATE TRANSPORTER1.1 (See discussion in Finch-Savage and Footitt, 2017). As such  
443 in the Bur ecotype increased dormancy induced by seed maturation at low temperature is

444 tempered by increased nitrate sensitivity an adaptation that promotes seedling emergence in  
445 cool spring conditions.

446

447 On the first three occasions the temperature gradient was applied at the start of seed  
448 development so that the effects of temperature during seed development would not be  
449 confounded with the effects of temperature on the timing of flowering and start of seed  
450 development. For comparison, at the fourth sowing the gradient was applied throughout plant  
451 growth to seed harvest. In both ecotypes, the relationship between seed maturation  
452 temperature and the depth of seed dormancy (Figs. 4 and 5) fitted to data from all four  
453 occasions. This occurred even though on the fourth occasion seed development was occurring  
454 earlier at the warm end of the tunnel than at the cooler end. Therefore, temperature during  
455 seed maturation is an important environmental factor influencing depth of dormancy.  
456 However, the temperature history experienced by mother plants during their life cycle before  
457 seed development can also impact on seed characteristics and seed performance in the next  
458 generation (Chen et al. 2014; Auge et al. 2017). It is also important to point out that increased  
459 global warming is likely to be accompanied by other changes to the environment such as  
460 rainfall and the likelihood of drought that may impact on seed dormancy. As winter and  
461 summer annuals the annual life cycle timings of these two ecotypes differ and thus the impact  
462 of these changes may also differ.

463

464 Correlations and trade-offs between traits such as germination and flowering time may limit  
465 the ability of species to adapt to climate change (Etterson & Shaw 2001); this is pertinent in  
466 *Arabidopsis* since flowering time affects seed dormancy under field conditions in this species  
467 (Chiang et al. 2013). Furthermore, Springthorpe and Penfield (2015) suggest that the  
468 temperature control of flowering time may have evolved to constrain when seeds are set (i.e.

469 around 15 °C) to ensure that plants produce seeds with different levels of dormancy. They  
470 predict, low dormant progeny will enter a rapid cycle if the climate permits, to flower and set  
471 seeds later the same summer. This switch therefore represents part of a bet-hedging strategy  
472 where the proportion of the seed population with low dormancy emerge immediately while  
473 the more dormant portion may avoid reproductive failure in variable environments by  
474 entering the persistent seed bank. As the environment varies with global warming so will the  
475 proportion of seeds entering these two strategies. This represents an indirect effect of  
476 predicted warming on the size of the seed bank (Fenner and Thompson, 2005) and will likely  
477 also alter its genetic composition. Seed banks tend to average out the effects of environmental  
478 heterogeneity (Venable and Brown 1998) and therefore the greater the extent of disturbance  
479 and environmental heterogeneity in a habitat the greater the need for seed banks (Long et al.  
480 2014). Thus, any reduction in seed bank size may reduce resilience to the other aspects of  
481 climate change such as the increased likelihood for extreme environmental conditions, which  
482 increases the risk of reproductive failure.

483

484 Genetically identical cohorts of seeds can adapt to contrasting life cycles (Montesinos-  
485 Navarro et al. 2012) and both spring and autumn germination windows have been described  
486 in coastal, but not montane Spanish populations (Montesinos-Navarro et al. 2009). In cold  
487 years, the impact of low temperature will result in increased dormancy (Fernández-Pascual  
488 and Jiménez-Alfaro, 2014) as shown in lab experiments (Chiang et al. 2011; Kendall et al.  
489 2011; Kendall and Penfield, 2012; Huang et al. 2014). This behaviour supports the  
490 predictions of Springthorpe and Penfield (2015) in the Col ecotype at different locations.  
491 However, the strongly contrasting ecotypes used here germinate only in Autumn (Cvi,  
492 obligate winter annual; Footitt et al. 2011) and spring (Bur, obligate summer annual; Footitt  
493 et al. 2013) in the UK so that flowering and seed set occur at different times and therefore

494 temperatures. The species characteristic of a sharp temperature transition in its effect on  
495 depth of dormancy is therefore likely to impact differently in such ecotypes. In contrast those  
496 ecotypes with facultative annual life cycles (e.g. Col-0) are likely to exhibit greater  
497 adaptability.

498

499 A further complicating effect, in addition to the effect of warming on depth of dormancy at  
500 shedding, is that warming during dormancy relief in the soil seed bank could also differ  
501 between these contrasting ecotypes. For example, a greater effect could be expected when  
502 dormancy relief is by low temperature in winter (Bur) rather than by warm temperature in  
503 summer (Cvi; Footitt et al. 2013). Fenner and Thompson (2005) suggest that such potential  
504 side effects of warmer temperatures in winter not relieving dormancy is unlikely since  
505 dormancy relief may occur up to 15 °C. However, this does not take into account that the rate  
506 at which dormancy relief occurs alters with temperature (more rapid at low temperatures).  
507 Furthermore, low and high temperatures in the seed bank can have opposite effects on  
508 dormancy induction and relief in the winter and summer annual ecotypes used here (Huang et  
509 al. 2015; Finch-Savage and Footitt, 2017). Therefore, the consequences of global warming  
510 for seed bank stability (both seed entry and persistence) are currently unclear.

511

## 512 **Acknowledgements**

513 Thanks to William Rimington and Valeriya Taylor and Warwick Crop Centre Horticultural  
514 Services Staff for maintaining experiments and data recording. This work was supported by a  
515 Warwick University Postgraduate Research Scholarship (H.Z.) and the UK BBSRC (W.F.-S.  
516 and S.F., Project BB/I022201/1).

517

## 518 **Author contributions**

519 W.E.F-S and S.F. conceived the experiments; S.F., ZH and AT performed the experiments;  
520 SF, ZH analysed data; W.E.F-S, S.F. and Z.H. wrote the manuscript.

521

## 522 **References**

523

524 Alboresi A., Gestin C., Leydecker M.T., Bedu M., Meyer C. & Truong H.N. (2005) Nitrate, a  
525 signal relieving seed dormancy in *Arabidopsis*. *Plant, Cell and Environment* **28**, 500-512.

526

527 Auge GAA., Leverett LD., Edwards BR. & Donohue K. (2017) Adjusting phenotypes via  
528 within- and across-generational plasticity. *New Phytologist* doi: 10.1111/nph.14495.

529

530 Baskin C.C. & Baskin J.M. (1998) *Seeds: Ecology, Biogeography, and evolution of*  
531 *Dormancy and germination*. Academic Press, San Diego.

532

533 Burghardt L.T., Edwards B.R. & Donohue K. (2016) Multiple paths to similar germination  
534 behavior in *Arabidopsis thaliana*. *New phytologist* **209**, 1301-1312.

535

536 Case A.L., Lacey E.P. & Hopkins R.G. (1996) Parental effects in *Plantago lanceolata* L. II  
537 manipulation of grandparental temperature and parental flowering time. *Heredity* **76**, 287-  
538 295.

539

540 Chen M., MacGregor DA., Dav A., Florance H., Morre K., Paszkiewica., Smirnoff N.,  
541 Graham. & Penfield S. (2014) Maternal temperature history activates flowering locus T in  
542 fruits to control progeny dormancy according to time of year. *Proceedings of the National*  
543 *Academy of Sciences USA* **111**, 18787-18792.

544

545 Chiang G.C., Bartsch M., Barua D., Nakabayashi K., Debieu M., Kronholm I., ..., de Meaux  
546 J. (2011) DOG1 expression is predicted by the seed-maturation environment and contributes  
547 to geographical variation in germination in *Arabidopsis thaliana*. *Molecular Ecology* **20**,  
548 3336-3349.

549

550 Chiang G.C., Barua D., Kramer E.M., Amasino R.M. & Donohue K. (2009) Major flowering  
551 time gene, FLOWERING LOCUS C, regulates seed germination in *Arabidopsis thaliana*.  
552 *Proceedings of the National Academy of Sciences USA* **106**, 11661-11666.

553

554 Chiang G.C., Barua D., Dittmar E., Kramer E.M., de Casas R.R. & Donohue K. (2013)  
555 Pleiotropy in the wild: the dormancy gene DOG1 exerts cascading control on life cycles.  
556 *Evolution* **67**, 883-893.

557

558 Cavieres L.A. & Arroyo M.T.K. (2000) Seed germination response to cold stratification  
559 period and thermal regime in *Phacelia secunda* (Hydrophyllaceae): altitudinal variation in the  
560 Mediterranean Andes of central Chile. *Plant Ecology* **149**, 1-8.

561

562 Chardon F., Barthelemy J., Daniel-Vedele F. & Masclaux-Daubresse C. (2010) Natural  
563 variation of nitrate uptake and nitrogen use efficiency in *Arabidopsis thaliana* cultivated with  
564 limiting and ample nitrogen supply. *Journal of Experimental Botany* **61**, 2293-2302.

565

566 Daws M.I., Cleland H., Chmielarz P., Gorian F., Leprince O., Mullins C.E., ..., Pritchard  
567 H.W. (2006) Variable desiccation tolerance in *Acer pseudoplatanus* seeds in relation to

568 developmental conditions: a case of phenotypic recalcitrance? *Functional Plant Biology* **27**,  
569 59-66.

570

571 Donohue K. (2009) Completing the cycle: maternal effects as the missing link in plant life  
572 histories. *Philosophical Transactions of the Royal Society of London. Series B, Biological*  
573 *Sciences*, **364**, 1059–1074.

574

575 Donohue K., Rubio de Casas R., Burghardt L., Kovach K. & Willis C.G. (2010)  
576 Germination, postgermination adaptation, and species ecological ranges. *Annual Review of*  
577 *Ecology, Evolution, and Systematics* **41**, 293-319.

578

579 Donohue K., Burghardt L.T., Runcie D., Bradford K.J. & Schmitt J. (2015) Applying  
580 developmental threshold models to evolutionary ecology. *Trends in Ecology and Evolution*  
581 **30**, 66-77.

582

583 Etterson J.R. & Shaw R.G. (2001) Constraint to adaptive evolution in response to global  
584 warming. *Science* **294**, 151-154.

585

586 Fenner M. (1991) The effects of the parent environment on seed germinability. *Seed Science*  
587 *Research* **1**, 75-84.

588

589 Fenner M. & Thompson K. (2005) *The ecology of seeds*. Cambridge University Press, New  
590 York.

591

592 Fernández-Pascual E. & Jiménez-Alfaro B. (2014) Phenotypic plasticity in seed germination  
593 relates differentially to overwintering and flowering temperatures. *Seed Science Research* **24**,  
594 273-280.

595

596 Finch Savage W.E. & Leubner-Metzger G. (2006) Seed dormancy and the control of  
597 germination. *New Phytologist* **171**, 501-523.

598

599 Finch-Savage W.E. & Footitt S. (2017) Seed dormancy cycling and the regulation of  
600 dormancy mechanisms to time germination in variable field environments. *Journal of*  
601 *Experimental Botany* **68**, 843-856.

602

603 Footitt S., Douterelo-Soler I., Clay H. & Finch-Savage W.E. (2011) Dormancy cycling in  
604 *Arabidopsis* seeds is controlled by seasonally distinct hormone-signaling pathways.  
605 *Proceedings of the National Academy of Sciences USA* **108**, 20236-20241.

606

607 Footitt S., Huang Z.Y., Clay H.A., Mead A. & Finch-Savage W.E. (2013) Temperature, light  
608 and nitrate sensing coordinate *Arabidopsis* seed dormancy cycling, resulting in winter and  
609 summer annual phenotypes. *Plant Journal* **74**,1003-1015.

610

611 Footitt S., Clay H.A., Dent K. & Finch-Savage W.E. (2014) Environment sensing in spring-  
612 dispersed seeds of a winter annual *Arabidopsis* influences the regulation of dormancy to align  
613 germination potential with seasonal changes. *New Phytologist* **202**, 929-939.

614

615 He H., Vidigal D.S., Snoek L.B., Schnabel S., Nijveen H., Hilhorst H.W.M. & Bentsink L.

616 (2014) Interaction between parental environment and genotype affects plant and seed

617 performance in *Arabidopsis*. *Journal of Experimental Botany* **65**, 6603-6615.

618

619 He, H., Willems, L. A., Batushansky, A., Fait, A., Hanson, J., Nijveen, H., ... & Bentsink, L.

620 (2016). Effects of parental temperature and nitrate on seed performance are reflected by

621 partly overlapping genetic and metabolic pathways. *Plant and Cell Physiology* **57**, 473–487.

622

623 Hedhly A., Hormaza J.I. & Herrero M. (2009) Global Warming and sexual plant

624 reproduction. *Trends in plant Science* **14**, 30-36.

625

626 Huang X., Schmitt J., Dorn L., Griffith C., Effgen S., Takao S., Koorneef M. & Donohue K.

627 (2010) The earliest Stages of adaptation in an experimental plant population: strong selection

628 for QTLS for seed dormancy. *Molecular Ecology* **189**, 1335-51.

629

630 Huang Z., Footitt S. & Finch-Savage W.E. (2014) The effect of temperature on reproduction

631 in the summer and winter annual *Arabidopsis thaliana* ecotypes Bur and Cvi. *Annals of*

632 *Botany* **113**, 921-929.

633

634 Huang Z., Ölçer-Footitt H., Footitt S. & Finch-Savage W.E. (2015) Seed dormancy is a

635 dynamic state: variable responses to pre- and post-shedding environmental signals in seeds of

636 contrasting *Arabidopsis* ecotypes. *Seed Science Research* **25**, 159-169

637

638 Intergovernmental Panel on Climate Change (IPCC). (2014) *Climate Change 2014–Impacts,*  
639 *Adaptation and Vulnerability: Regional Aspects*. Cambridge University Press, Cambridge,  
640 UK & New York.

641

642 Jump A.S., Marchant R. & Penuelas J. (2009) Environmental change and the option value of  
643 genetic diversity. *Trends in Plant Science* **14**, 51-58.

644 Kendall S.L., Hellwege A., Marriot P., Whalley C., Graham I.A. & Penfield S. (2011)  
645 Induction of Dormancy in Arabidopsis Summer Annuals Requires Parallel Regulation of  
646 DOG1 and Hormone Metabolism by Low Temperature and CBF Transcription Factors. *The*  
647 *Plant Cell* **23**, 2568-2580.

648

649 Kendel S. & Penfield S. (2012) Maternal and zygotic temperature signalling in the control of  
650 seed dormancy and germination. *Seed Science Research* **22**, S23–S29.

651

652 Kochanek J., Buckley Y.M., Probert R.J., Adkins S.W. & Steadman K.J. (2010) Pre-zygotic  
653 parental environment modulates seed longevity. *Australian Ecology* **1**, 837-848.

654

655 Lacey E.P. & Herr D. (2000) Parental effects on *Plantago lanceolata* L. III Measuring  
656 parental temperature effects in the field. *Evolution* **54**, 1207-1217.

657

658 Long R., Gorecki M., Renton M., Scott J., Colville L., Goggin D., ..., Finch-Savage W.E.  
659 (2015) The ecophysiology of seed persistence: a mechanistic view of the journey to  
660 germination or demise. *Biological Reviews* **90**, 31-59.

661

662 Matakias T., Alboresi A., Jikumaru Y., Tatematsu K., Pichon O., Renou JP., ..., Truong  
663 H.N. (2009) The Arabidopsis Abscisic Acid Catabolic Gene CYP707A2 Plays a Key Role in  
664 Nitrate Control of Seed Dormancy. *Plant Physiology* **149**, 949-960.  
665  
666 Meyer S.E. & Allen P.S. (1999) Ecological genetics of seed germination regulation in  
667 *Bromus tectorum* L. II Reaction norms in response to a water stress gradient imposed during  
668 seed maturation. *Oecologia* **120**, 35-43.  
669  
670 Montesinos A., Tonsor S.J., Alonso-Blanco C. & Pico F.X. (2009) Demographic and genetic  
671 patterns of variation among populations of *Arabidopsis thaliana* from contrasting native  
672 environments. *PLoS One* **4**, e7213.  
673  
674 Montesinos-Navarro A., Picó FX. & Tonsor S.J. (2012) Clinal variation in seed traits  
675 influencing life cycle timing in *Arabidopsis thaliana*. *Evolution* **66**, 3417-3431.  
676  
677 Nicotra A.B., Atkin O.K., Bonser S.P., Davidson A.M., Finnegan E.J., Mathesius U., ..., van  
678 Kleunen M. (2010) Plant phenotypic plasticity in a changing climate. *Trends in plant science*  
679 **31**, 684-692.  
680  
681 Orr H.A. (2009) Fitness and its role in evolutionary genetics *Nature Reviews Genetics*  
682 **10**, 531-539.  
683  
684 Payne R.W., Lane P.W., Digby P.G.N., Harding S.A., Leech P.K., Morgan G.W., ..., White  
685 R.P. (1993) *Genstat 5 Release 3 Reference Manual*, Oxford University Press, Oxford.  
686

687 Penuelas J., Rutishauser T. & Filella I. (2009) Phenology feedbacks on climate change.  
688 *Science* **324**, 887-888  
689

690 Poschlod P., Tackenberg O. & Bonn S. (2005) Plant dispersal potential and its relation to  
691 species frequency and coexistence. *Vegetation Ecology* (ed. van der Maarel E. ) pp. 147–  
692 171. Blackwell Science, Malden.  
693

694 Postma F.M. & Agren J. (2016) Early life stages contribute strongly to local adaptation in  
695 *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences USA* **113**, 7590-  
696 7595.  
697

698 Primack R.B. (1989) measuring fitness and natural selection in wild plant populations.  
699 *Annual reviews of ecology and systematics* **20**, 367-396  
700

701 Probert R.J. (2000) The role of temperature in the regulation of seed dormancy and  
702 germination. *Seeds: The ecology of regeneration in plant communities* (ed. Fenner M.) pp.  
703 261-292. CABI, Wallingford, UK. 261-292.  
704

705 Springthorpe V. & Penfield S. (2015) Flowering time and seed dormancy control use  
706 external coincidence to generate life history strategy. *Elife* **31**, e05557  
707

708 UK Climate Projections. (2014) UKCP09 User Interface,  
709 <http://ukclimateprojections.metoffice.gov.uk/22340>  
710

711 Venable D.L. & Brown J.S. (1988) The selective interactions of dispersal, dormancy, and  
712 seed size as adaptations for reducing risk in variable environments. *The American Naturalist*  
713 **131**: 360-384.  
714

715 Vidigal D.S., Marques A.C.S.S., Willems L.A.J., Buijs G., Méndez-Vigo B., Hilhorst  
716 H.W.M., Bentsink L., Picó F.X. & Alonso-Blanco C. (2016) Altitudinal and climatic  
717 associations of seed dormancy and flowering traits evidence adaptation of annual life cycle  
718 timing in *Arabidopsis thaliana*. *Plant Cell and Environment* **39**, 1737-1748.  
719

720 VSN International. (2012) GenStat for windows 15<sup>th</sup> edition. Hemel Hempstead, UK: VSN  
721 International.  
722

723 Walck J.L., Hidayati S.N., Dixon K.W., Thompson K. & Poschlod P. (2011) Climate change  
724 and plant regeneration from seed. *Global Change Biology* **17**, 2145-2161.  
725

726 Walther G.R., Post E., Convey P., Menzel A., Parmesan C., Beebee T.J.C., ..., Bairlein F.  
727 (2002) Ecological responses to recent climate change. *Nature* **416**, 389-395.  
728

729 Wurr D.C.E., Fellows J.R. & Phelps K. (1996) Investigating trends in vegetable crop  
730 response to increasing temperature associated with climate change. *Scientia Horticulturae* **66**,  
731 255-263.  
732

733 **Table 1. Bolting time responses of Bur and Cvi sown in May 2012 to different**  
 734 **nitrate compost levels along the thermal gradient tunnel.** Bolting time (days) was  
 735 recorded in response to growth on very low (VLN), low (LN) and standard nitrate  
 736 (SN) compost for Bur and SN for Cvi. Data are mean values of three replicates of  
 737 eight plants  $\pm$  standard error. Differences between the means are compared by the  
 738 L.S.D. at the  $P < 0.05$  level for Bur only (0.604) and Bur and Cvi under SN conditions  
 739 (1.341).

740

Temperature location	Bolting time (days)			
	Bur			Cvi
	VLN	LN	SN	SN
<b>T1</b>	36.79 $\pm$ 0.15	36.56 $\pm$ 0.15	35.63 $\pm$ 0.19	34.15 $\pm$ 0.52
<b>T2</b>	32.72 $\pm$ 0.36	32.67 $\pm$ 0.67	31.29 $\pm$ 0.56	33.68 $\pm$ 0.54
<b>T3</b>	30.29 $\pm$ 0.36	29.71 $\pm$ 0.21	30.58 $\pm$ 0.55	34.36 $\pm$ 0.45
<b>T4</b>	31.08 $\pm$ 0.29	31.3 $\pm$ 0.32	30.83 $\pm$ 0.17	31.29 $\pm$ 0.29

741

742

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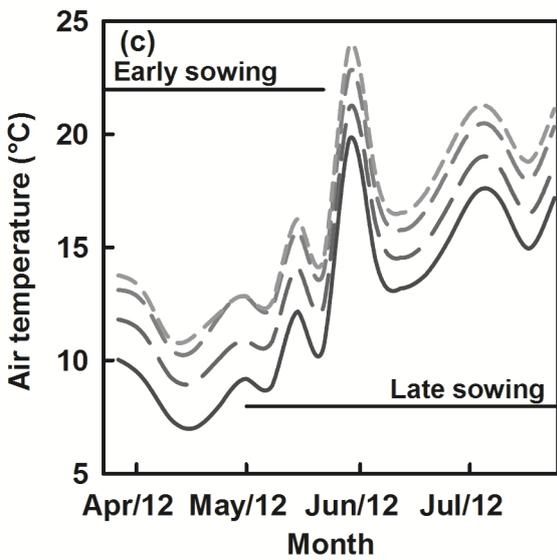
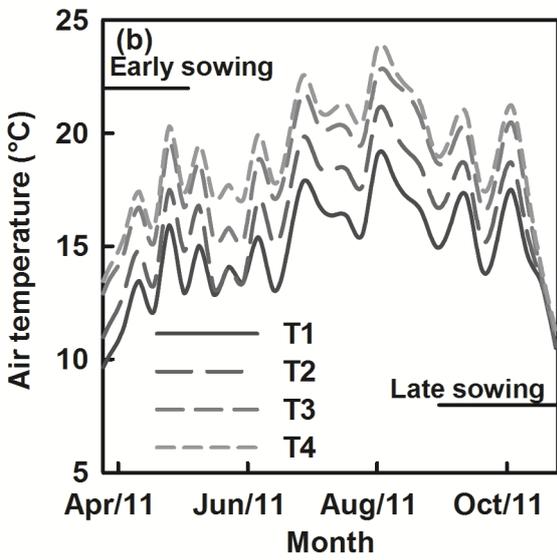
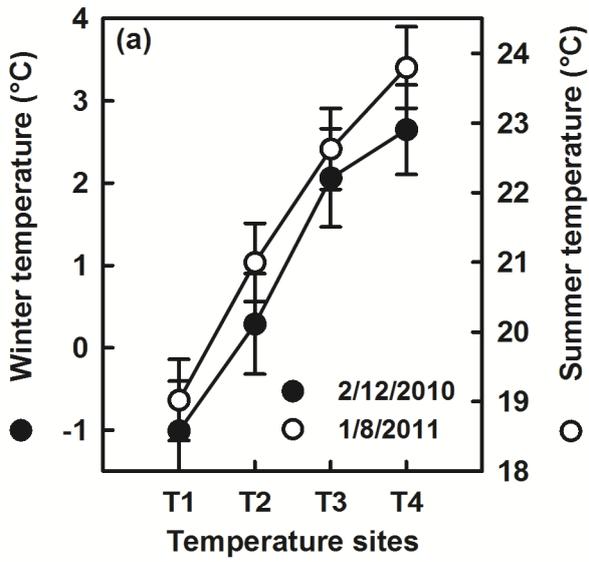
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745 **Figure legends:**

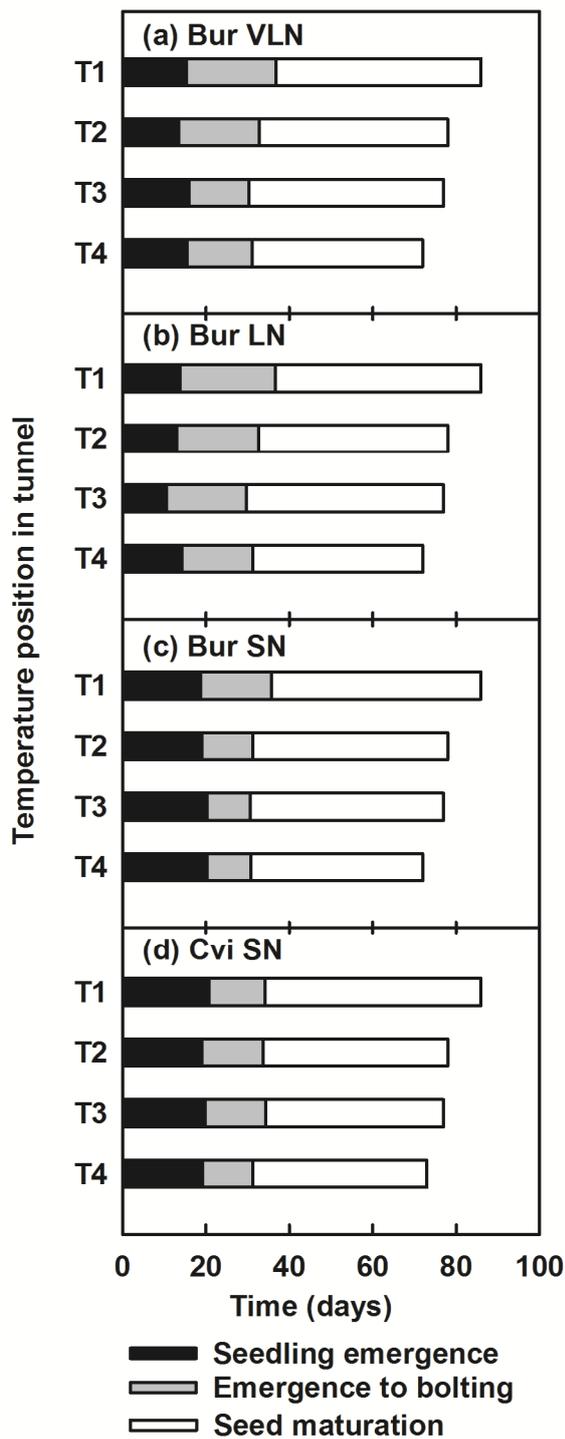
746

747 **Figure 1.** Warming scenarios established along the thermogradient tunnel. (a) Examples of  
748 the linear temperature gradients in the winter (2/12/2010) and summer (1/08/2011). T1 is the  
749 ambient and T4 the warm end of the tunnel. (b) Mean weekly air temperature recorded at  
750 these four positions along the thermogradient tunnel in 2011 and (c) in 2012.

751 The horizontal lines marked early and late sowing denote the time plants spent in the tunnel  
752 from transfer at bolting to seed harvest or in the case of the late sowing in 2012 from sowing  
753 of seeds to seed harvest. Exact dates are to be found in Table S1.



755 **Figure 2.** The impact of temperature (tunnel position) on the life cycle time course of Bur and  
756 Cvi. Seeds were sown on 1 May 2012 and progress was recorded through to seed maturity and  
757 harvest. The seeds were sown at four positions along the thermogradient tunnel (T1 ambient –  
758 T4 warm end). The Bur accession was exposed to three levels of nitrate in the growth media  
759 (SN =standard N, LN = low N, VLN = very low N). Cvi failed to complete its' life cycle at the  
760 two lower levels of nitrate.



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762

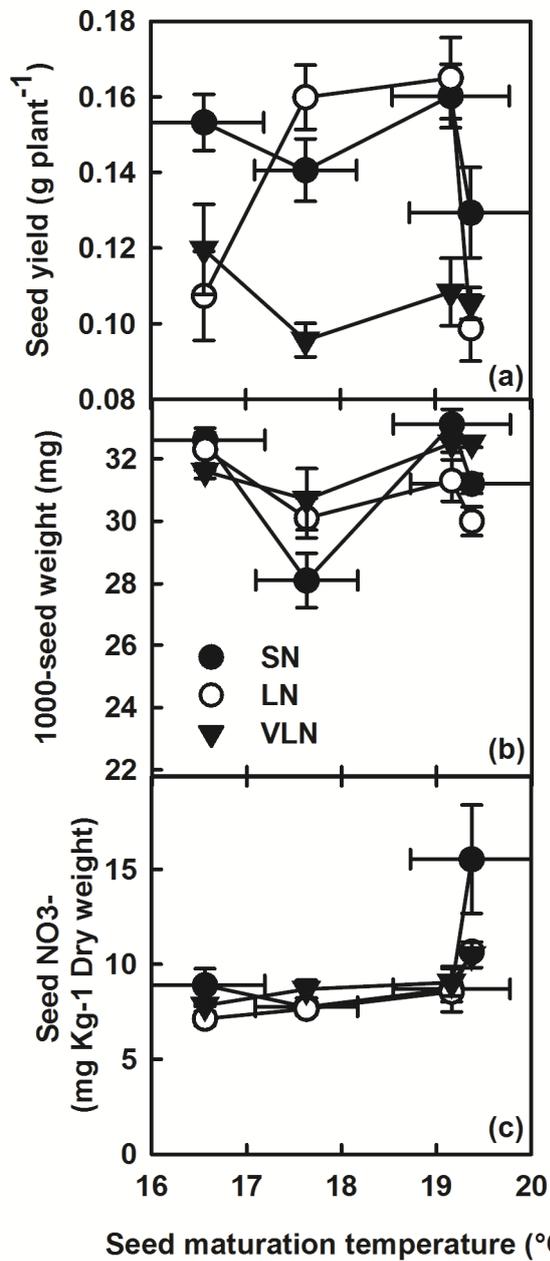
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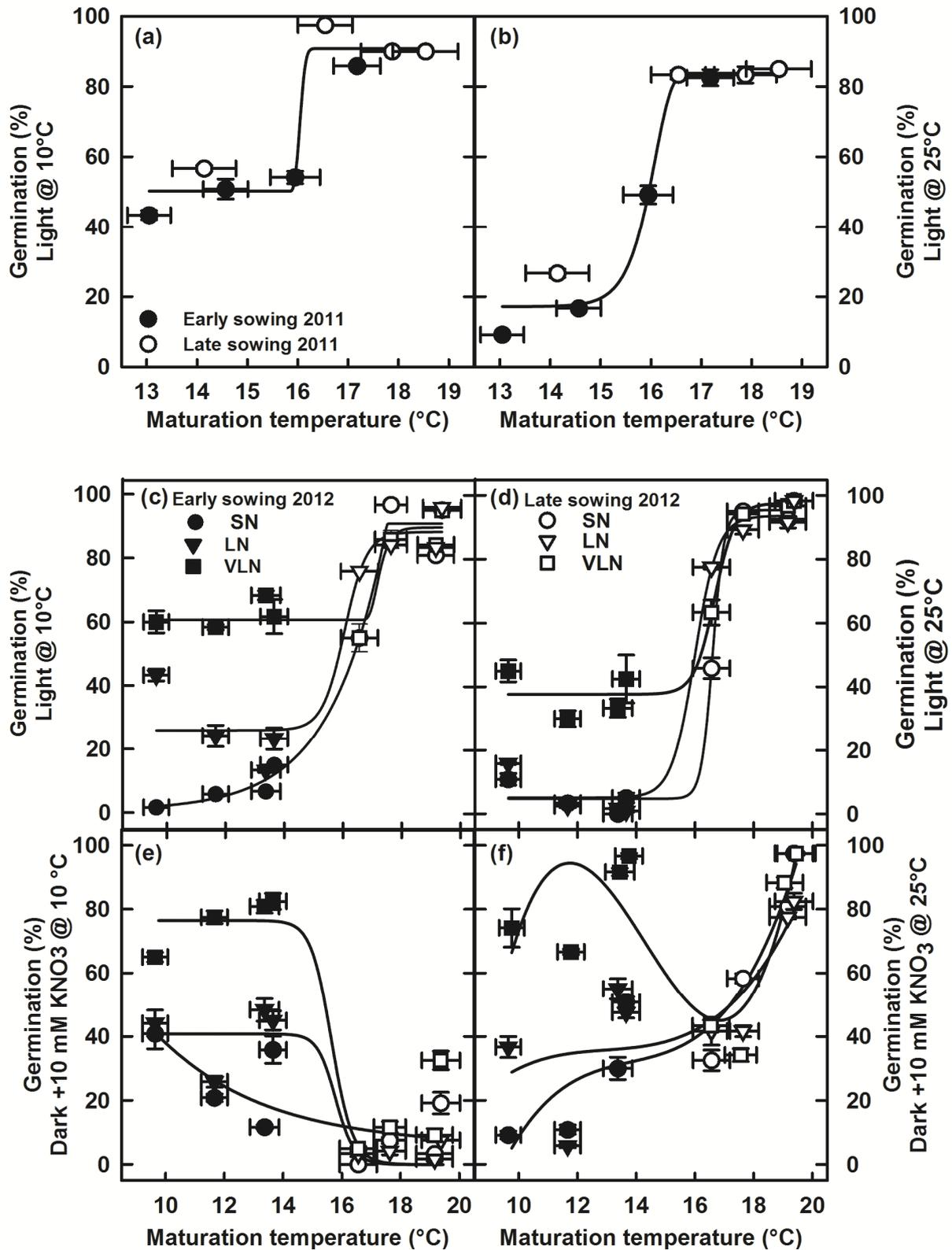
**Figure 3.** The impact of temperature (tunnel position) during seed maturation on seed components in Bur sown May 2012. Seed yield (a), seed size (b; 1000 seed wt) and nitrate content (c) in the seed were recorded following harvest at four positions along the thermogradient tunnel (T1 ambient –T4 warm end). Plants were exposed to three levels of

766 nitrate in the growth media (SN =standard N, LN = low N, VLN = very low N). Data are the  
 767 mean  $\pm$  standard error. No error bar indicates symbol is larger than the error.



769 **Figure 4.** The impact of temperature (tunnel position) during seed maturation on germination  
 770 performance of Bur. Seeds were collected at harvest maturity following early sowing (closed  
 771 symbols) and late sowing (open symbols) in both 2011 ((a), (b)) and 2012 ((c) – (f)) at four  
 772 positions along the thermogradient tunnel (T1 ambient –T4 warm end). The Bur accession was  
 773 exposed to three levels of nitrate in the growth media (SN =standard N, LN = low N, VLN =

774 very low N). Germination was recorded at ((a),(c),(e)) 10 and ((b),(d),(f)) 25 °C, both on ((a)–  
775 (d)) water in the light and ((e),(f)) on a nitrate solution in the dark. Data are the mean  $\pm$  standard  
776 error. No error bar indicates symbol is larger than the error. For details of fitted curves see  
777 Table S3.



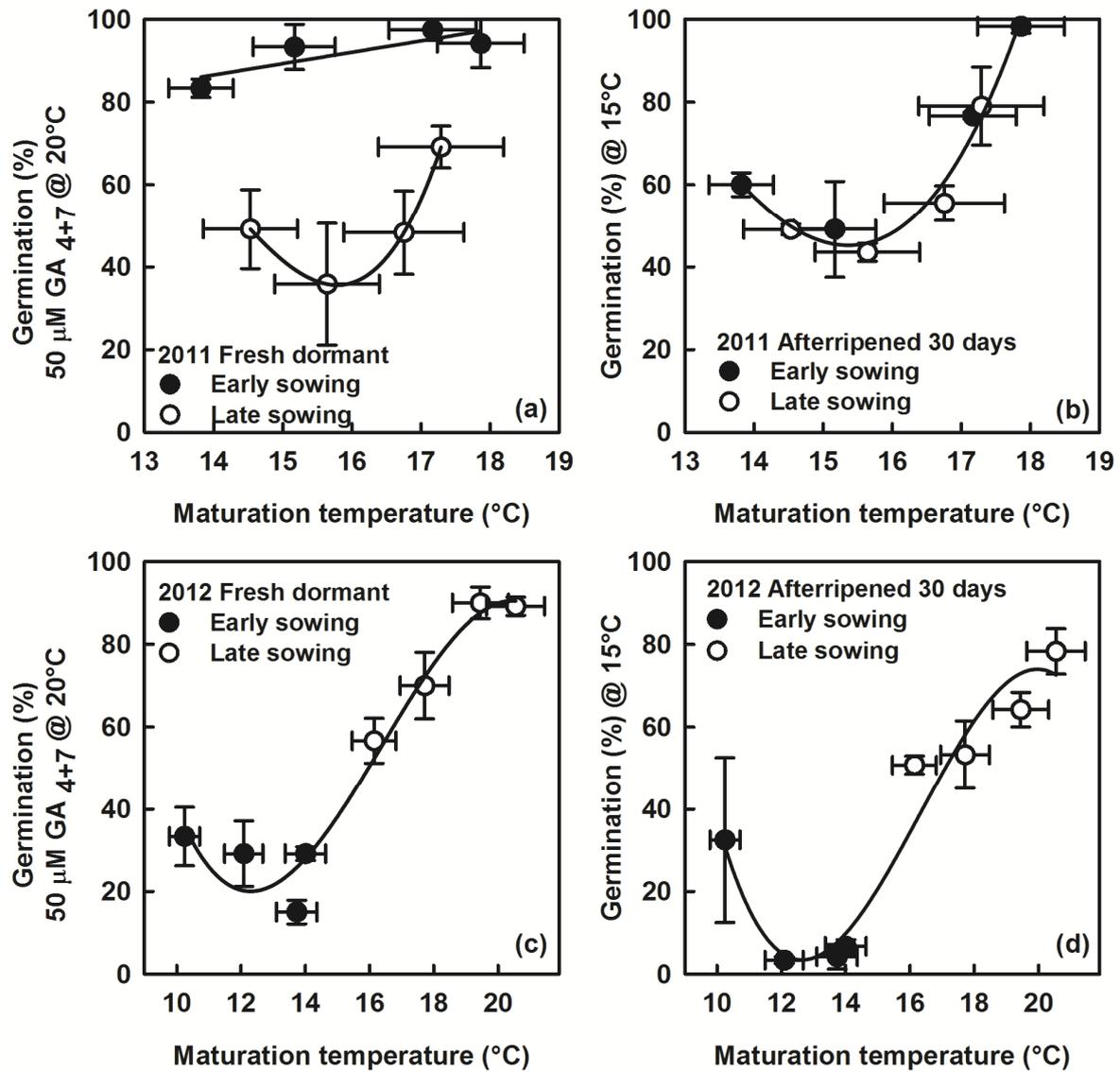
778

779 Figure 5. The impact of temperature (tunnel position) during seed maturation on germination

780 performance of Cvi. Seeds were collected at harvest maturity following early sowing (closed

781 symbols) and late sowing (open symbols) in both 2011 ((a),(b)) and 2012 ((c),(d)) at four

782 positions along the thermogradient tunnel (T1 ambient –T4 warm end). Germination in the  
 783 light was recorded at 20 °C on 50  $\mu$ M GA<sub>4+7</sub> (a,c) and at 15 °C following 30 days AR ((b),(d)).  
 784 Data are the mean  $\pm$  standard error. No error bar indicates symbol is larger than the error. For  
 785 details of fitted curves see Table S3.



786

787 **Supporting information**

788

789 **Fig. S1** Emergence from seeds at four positions in the thermal gradient tunnel.

790

791 **Fig. S2** The relationship between plant size and seed number, seed yield and plant height in  
792 Bur.

793

794 **Fig. S3** The relationship between plant size and seed number, and between seed yield and  
795 plant height in Cvi.

796

797 **Fig. S4** The impact of temperature (tunnel position) during seed maturation on germination  
798 performance of Cvi.

799

800 **Fig. S5** The impact of temperature (tunnel position) on dark germination of Bur seeds  
801 produced in 2012 under VLN conditions.

802

803 **Fig. S6** Mean environmental data in the environment of origin for Bur and Cvi

804

805 **Table S1** Dates of seed production in the thermal gradient tunnel

806

807 **Table S2** Types of curve used to fit data sets for germination of Burren (Fig. 4) and Cape  
808 Verde Islands (Fig. 5) ecotypes. Nitrate level is standard nitrate (SN), low nitrate (LN) and  
809 very low nitrate (VLN). The correlation values for each fitted curve are also given (R and  
810  $R^2$ ).

811

812 **Table S3** Time to bolting, rosette diameter and leaf number at bolting of Bur sown in May  
813 2012 in different nitrate compost levels along the thermal gradient tunnel in Bur

814