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Conflicting selection on the timing of germination in a natural population of
Arabidopsis thaliana

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Running title: Selection on timing of germination

25 **Abstract**

26 The timing of germination is a key life-history trait that may strongly influence plant fitness
27 and that sets the stage for selection on traits expressed later in the life cycle. In seasonal
28 environments, the period favourable for germination and the total length of the growing
29 season are limited. The optimal timing of germination may therefore be governed by
30 conflicting selection through survival and fecundity. We conducted a field experiment to
31 examine the effects of timing of germination on survival, fecundity, and overall fitness in a
32 natural population of the annual herb *Arabidopsis thaliana* in north-central Sweden. Seedlings
33 were transplanted at three different times in late summer and in autumn covering the period of
34 seed germination in the study population. Early germination was associated with low seedling
35 survival, but also with high survival and fecundity among established plants. The advantages
36 of germinating early more than balanced the disadvantage, and selection favoured early
37 germination. The results suggest that low survival among early germinating seeds is the main
38 force opposing the evolution of earlier germination, and that the optimal timing of
39 germination should vary in space and time as a function of the direction and strength of
40 selection acting during different life-history stages.

41

42 *Keywords:* *Arabidopsis thaliana; conflicting selection; fecundity; field experiment; fitness;*
43 *life-history evolution; survival; timing of germination*

44

45 **Introduction**

46 Because of conflicting selection, the adaptive value of a given trait may change during the life
47 cycle. Under conflicting selection, a positive effect of a trait on one fitness component is
48 counteracted by an opposite effect on another component of fitness (e.g. Gómez, 2004) and
49 optimal trait expression is determined by the net effect of selection through different fitness
50 components (Venable, 1984; Schluter *et al.*, 1991; Gómez, 2008). A full understanding of the
51 factors shaping overall selection on such traits therefore requires that the direction and
52 intensity of selection during different life-history stages are quantified (Schluter *et al.*, 1991;
53 Gómez, 2008).

54 In seasonal environments, the timing of seed germination should be subject to conflicting
55 selection. Early germination may increase the risk of mortality during establishment, but
56 provide a competitive advantage and a longer period available for growth and reproduction
57 (Verdú & Traveset, 2005; Donohue *et al.*, 2010). We can thus expect viability selection
58 during establishment and fecundity selection to favour late and early germination,
59 respectively. Although an optimal intermediate germination time can be expected in many
60 situations, most observational studies have indicated selection for early germination (Verdú &
61 Traveset, 2005; Donohue *et al.*, 2010; but see Baskin & Baskin, 1972; Kelly & Levin, 1997).
62 The rarity of documented cases of stabilising selection may be due to incomplete sampling of
63 the true variation in germination timing, but also to limited variation in germination timing
64 within populations, as would be expected if natural selection has removed genotypes with
65 extreme values (Donohue *et al.*, 2010). Phenotypic (Boquet & Clawson, 2009) or genetic
66 (Donohue *et al.*, 2005; Huang *et al.*, 2010) manipulation can increase the variance in
67 germination timing, and thus enable the full characterisation of the fitness function.

68 Here, we conducted a field experiment to examine the effects of germination timing on
69 survival, growth, flowering phenology, and fecundity, and documented the seedling

70 establishment phenology in a natural population of the annual herb *Arabidopsis thaliana* in
71 north-central Sweden. As other *A. thaliana* populations in northern Europe (Koornneef *et al.*,
72 2004), the study population has a winter-annual life cycle. Seeds germinate in late summer
73 and autumn, and established plants overwinter as rosettes, and flower and set seed the
74 following spring and early summer. The study population occurs on thin soil which typically
75 dries out in late June and in July when temperatures are high (Ågren & Schemske, 2012).
76 From August temperatures drop and soil moisture increases, and during winter the
77 temperature stays below 0 °C for extended periods (Ågren & Schemske, 2012). Under such
78 conditions, early germination is likely to be related to high mortality during establishment due
79 to drought, but advantageous because established plants may grow large before winter and
80 thus increase winter survival and fecundity. Few studies have documented germination timing
81 in natural populations of *A. thaliana* (Montesinos *et al.*, 2009; Picó, 2012) and the effects of
82 germination timing on fitness has not been experimentally examined in natural habitats in the
83 native range.

84 In the field experiment, we tested the hypotheses that early germination is associated with
85 (1) low survival during establishment, (2) high survival later in the life cycle, and (3) high
86 fecundity. To explore whether differences in resource acquisition can explain the effect of
87 timing of germination on fitness, we examined whether early germination was associated with
88 large rosette size at the end of autumn, in the beginning of spring and at flowering. To
89 determine whether germination phenology affected flowering time, which is correlated with
90 fecundity in many annual plants (Munguía-Rosas *et al.*, 2011), we documented flowering start
91 in the experiment. Finally, to relate experimental treatments to the phenology of germination
92 in the local population, we monitored seedling establishment in permanent plots.

93

94 **Materials and methods**

95 **Study species and study site**

96 *Arabidopsis thaliana* (L.) Heynh. (*Brassicaceae*) is a highly selfing annual herb, which is
97 native to Eurasia (Al-Shehbaz & O’Kane, 2002; Koornneef *et al.*, 2004). The study was
98 conducted in a natural population at Rödåsen (62°48’N, 18°12’E) in the High Coast area in
99 north-central Sweden. The population is located in dry meadow vegetation on a steep slope
100 facing south-east, ca. 175 m above the sea level (see Ågren & Schemske [2012] for further
101 characterization of the site).

102

103 **Experimental manipulation of the timing of germination**

104 To examine how germination timing affects plant fitness, we transplanted newly-germinated
105 seedlings to the source population in August, September, and October (representing early,
106 peak, and late germination, respectively) in 2008, and recorded survival, growth, flowering
107 time, and fecundity of the three cohorts.

108 We used seeds of eight lines originally collected from the study site. The lines had gone
109 through two generations of selfing in the lab to reduce environmentally induced variation. The
110 seeds were planted in $2.7 \times 2.7 \times 7$ cm plugs (length \times width \times depth) in plug trays filled with
111 equal proportions of unfertilised peat (Weibulls Horto AB), gravel, and sand collected from
112 the field site. The plug trays were placed in a cold room (4°C) for four days of stratification,
113 and then moved to a growth chamber at 18°C with a 16 h light, 8 h dark photoperiod at the
114 Evolutionary Biology Centre, Uppsala University for a week to promote germination. The
115 resulting seedlings were placed outside for three days for acclimation, before being
116 transplanted to the field.

117 In the field, three experimental plots about 40×60 cm large were established prior to the
118 first transplant in August. The vegetation was removed and, to reduce the likelihood of
119 seedling establishment from the seed bank, the top soil was replaced by soil collected locally

120 but outside the population. Plants in the August cohort were germinated in early August and
121 were transferred to the field on 15 August. Plants in the September cohort were germinated in
122 early September and were transferred to the field on 15 September. Plants in the October
123 cohort were germinated in late September and were transferred to the field on 9 October.
124 Within each plot (block), seedlings were planted in a rectangular grid with positions separated
125 by about 3 cm. We planted ten replicates of each of the eight lines per treatment (cohort) per
126 block. Positions within blocks were completely randomized. This design gave a total of 720
127 seedlings ($3 \text{ cohorts} \times 8 \text{ lines} \times 10 \text{ replicates} \times 3 \text{ blocks}$). The August cohort lacked seven
128 seedlings (one individual from each of lines 292 and 323 and five individuals from line 295)
129 because of unexpectedly low establishment. We watered the plants the day after
130 transplantation to support establishment, but except for that the seedlings did not receive any
131 supplemental watering. At transplantation, the seedlings had produced only a pair of
132 cotyledons except for a few plants that were developing their first pair of true leaves.

133 To document survival and growth during the establishment phase, we recorded survival
134 and rosette diameter of transplanted seedlings two weeks after transplantation. Rosette
135 diameter was measured to the nearest mm with a pair of calipers. To monitor the further
136 development of plants, we scored survival of all plants on 23 October 2008 (the end of
137 autumn), on 15 April 2009 (beginning of spring, prior to flowering), and at fruit maturation in
138 late June - early July 2009. From these data we calculated survival through autumn, over
139 winter, and through spring until fruit maturation. On 23 October and 15 April, we in addition
140 recorded the rosette diameter of all plants. Flowering status (flowering or not flowering) was
141 checked once per week after winter, and rosette diameter at flowering was determined.
142 Rosette area was used as an estimate of plant size and was calculated from the diameter using
143 the formula of a circle. At fruit maturation, we recorded the total number of fruits and
144 estimated the mean number of seeds per fruit by counting the number of seeds in up to four

145 fruits per plant. For reproducing plants, fecundity was quantified by multiplying the mean
146 number of seeds per fruit with the total number of fruits. Total fitness was defined as the
147 number of seeds produced per plant (zero for plants that died before reproduction).

148

149 **Phenology of seedling establishment**

150 To relate the experimental treatments to the phenology of germination in the local population,
151 we conducted two studies. In one study, we bi-weekly monitored the number of plants in 12
152 plots ($10 \times 10 \text{ cm}^2$) established across the population. The plots were monitored from mid
153 August to the second half of October 2008, and again after winter once a week from 15 April
154 until fruit maturation. In a second study, we specifically examined whether any seedlings
155 established after the beginning of October corresponding to the latest transplanting. On 11
156 October 2006 and on 2 October 2007, we scored the number of seedlings that had established
157 in twelve $10 \times 10 \text{ cm}^2$ plots and removed all seedlings present. The following spring, the
158 number of plants in these plots was scored early in spring and at or around fruit maturation
159 (13 April and 20 June 2007, and 27 April and 9 June 2008, respectively).

160

161 **Statistical analyses**

162 To reduce the confounding effect of any transplantation shock, all analyses were conducted
163 on a data set excluding plants that died within 24 hours of transplantation (33, three, and one
164 plant from the August, September, and October cohorts, respectively). The exclusion of such
165 plants did not qualitatively affect the outcome of the statistical analyses (data not shown).

166 We used linear mixed models (PROC MIXED in SAS Version 9.2, SAS Institute, Cary,
167 NC, USA) to assess the effects of germination timing (fixed effect), line and block (random
168 effects) on survival (two weeks after transplantation, from transplantation until the end of
169 autumn, over winter, from early spring until fruit maturation, and overall from start to end of

170 experiment), rosette area (two weeks after transplantation, before winter, in the beginning of
171 spring, and at flowering), flowering start, the number of seeds per reproducing plant
172 (fecundity), and the number of seeds per seedling planted (fitness). We used line means for
173 each block and the proportion of plants of a given line that survived in each block as response
174 variables because the number of individuals per treatment \times line combination became strongly
175 unbalanced due to mortality. Survival was arcsine square-root transformed, and all other
176 response variables except flowering start were log-transformed prior to analysis to improve
177 normality of residuals. When the effect of germination cohort was statistically significant,
178 Tukey's HSD test was used to determine which cohorts differed. The statistical significance
179 of random effects was assessed using the log-likelihood ratio test (Littell *et al.*, 1996). The
180 cohort \times line interaction was not statistically significant in any analysis ($P = 0.16-1.00$) and
181 was removed from the final models.

182

183 **Results**

184 **Timing of germination vs. survival**

185 The field experiment demonstrated that the direction of viability selection on germination
186 timing shifted from the establishment phase to later life-history stages (Fig. 1a). Before winter,
187 the August cohort had the lowest survival, followed by the September, and October cohorts,
188 but this order was reversed for survival over winter and in spring. Overall survival from
189 transplantation until reproduction was higher in the August and September cohorts than in the
190 October cohort (Fig. 1a), and did not vary among lines ($P = 1.0$).

191 Survival two weeks after transplantation was 29% ($N = 8$), 44% ($N = 8$), and 78% ($N = 8$)
192 in the August, September, and October cohorts, respectively. This shows that mortality before
193 winter in the August and September cohorts was concentrated to the first two weeks of
194 seedling growth in the field (cf. Fig. 1a) and that differences in survival among cohorts at the

195 end of autumn did not simply reflect differences in time since sowing.

196

197 **Timing of germination vs. fecundity and total fitness**

198 Early germination was associated with high fecundity (number of seeds produced per
199 reproductive plant) and total fitness (number of seeds produced per seedling planted). The
200 August cohort had the highest fecundity and fitness, followed by the September and October
201 cohorts (Figs. 1b and 1c, Table 1). Neither fecundity nor total fitness varied among lines
202 (Table 1).

203

204 **Timing of germination vs. plant size and flowering phenology**

205 Plant size and flowering time varied among cohorts, but not among lines (Table 1). Two
206 weeks after transplantation, before winter, and in early spring, the August cohort had a larger
207 leaf rosette than the September cohort had, and the September cohort in turn had a larger leaf
208 rosette than the October cohort had (Table 1). The August cohort was larger at flowering and
209 began flowering earlier than did the September and October cohorts (Table 1).

210

211 **Phenology of seedling establishment**

212 The monitoring of natural seedling establishment and the experimental removal of seedlings
213 suggested that almost all germination occurred between August and October in the study
214 population. In the observational study, very few seedlings had appeared in mid August and
215 the number of seedlings yet without true leaves peaked before mid September and then
216 decreased (Fig. 2). However, more seedlings with cotyledons only were observed on 23
217 October than on 10 October indicating that some germination occurred also in mid October.
218 No new seedlings were observed the following spring. The seedling removal experiments in
219 2006 and 2007 suggested that fewer than 2% of seedlings germinated after early October

220 (number of seedlings observed per plot in October vs. April the following year in plots from
221 which all seedlings were removed after the October census, mean \pm SE, 2006, 87.8 ± 22.2 vs.
222 1.3 ± 0.4 ; 2007, 102.9 ± 17.8 vs. 1.9 ± 0.7 , $N = 12$). After the census in April, no additional
223 seedling establishment was recorded.

224

225 **Discussion**

226 The present study detected conflicting phenotypic selection on the timing of germination in a
227 natural population of *Arabidopsis thaliana*. The early germinating cohort had low survival
228 during establishment, but high survival later in the life-cycle and high fecundity compared to
229 later cohorts. The advantages of germinating early more than balanced the disadvantage and
230 the earliest cohort had the highest overall fitness. Below, we discuss the results in relation to
231 factors influencing selection on germination timing, the consequences of germination timing
232 for the development of size hierarchies, and the likelihood of detecting conflicting selection
233 on the timing of germination.

234 Early germination was associated with low survival during the establishment phase, but
235 also with large rosette size before winter, high survival later in life and high fecundity.

236 Differences in survival and growth until the end of autumn could to a large extent be
237 attributed to differences in environmental conditions during the first two weeks the seedlings
238 experienced in the field. In the August and September cohorts, most of the mortality before
239 winter occurred during this period. Moreover, two weeks after transplantation, the August
240 cohort had rosettes that were more than twice as large as those produced by the September
241 cohort and more than three times as large as those produced by the October cohort. Drought is
242 likely to be a major challenge to establishing seedlings in August when soil moisture still is
243 low and temperatures relatively high. However, for seedlings that establish in suitable micro-
244 habitats, the relatively high temperatures and long days should be favourable for growth. The

245 density of plants in the experimental arrays increased from when the first to when the last
246 cohort was transplanted, but this is less likely to have affected plant growth and survival. In
247 the arrays, plants were widely spaced (about 3 cm) relative to their size (rosette diameters
248 before winter, median 0.6 cm, range 0.20 - 4.8 cm) and rosettes did not overlap. Moreover,
249 the experimental plots were not colonised or shaded by other plant species.

250 Our results suggest that differences in germination timing contribute to the development of
251 size hierarchies, and thereby to the absence of a phenotypic trade-off between size and age at
252 reproduction. Size before winter varied among cohorts, and the associated differences in
253 winter and spring survival and fecundity are consistent with the common observation of
254 survival and fecundity being positively related to plant size (e.g., Stratton, 1992; Donohue,
255 2002). Moreover, the early cohort flowered earlier than did the two later cohorts. This is
256 consistent with a negative phenotypic correlation between plant size and flowering time in the
257 study population (R. Akiyama & J. Ågren, unpublished) and in natural populations of other
258 annual plants (Rathcke & Lacey, 1985; Munguía-Rosas *et al.*, 2011), but in contrast to the
259 expected trade-off between size and age at reproduction (cf. Mitchell-Olds, 1996).

260 The relative importance of viability and fecundity selection for net selection on
261 germination timing in *A. thaliana* may vary among environments. When locally collected
262 lines of *A. thaliana* were planted as seedlings in a common garden in the introduced range in
263 Kentucky, USA, an early cohort (representing mid-autumn germination) produced larger
264 rosettes before winter, began flowering earlier in spring, and tended to produce more fruits
265 than did a late-autumn cohort (Donohue, 2002). In that experiment, autumn and winter
266 survival was very high and did not differ between cohorts, and all variation in fitness was
267 related to differences in fecundity. The relatively mild winter conditions of Kentucky were
268 apparently associated with reduced importance of viability selection among the cohorts
269 examined, compared to the situation in the Rödåsen population. One caveat is that neither

270 cohort in the experiment in Kentucky represented early germination timing of local
271 populations (Donohue, 2002), and it is thus not clear whether low survival during
272 establishment would reduce the fitness of truly early germinants.

273 Although the direction of selection on timing of germination has been found to differ
274 between life-history stages in several annual species (e.g., Kalisz 1986; Kelly 1992; Stratton
275 1992, González-Astorga & Núñez-Farfán, 2000) suggesting that stabilizing selection on
276 germination time may be common, most observational studies have indicated selection for
277 early germination (Verdú & Traveset, 2005; Donohue *et al.*, 2010; but see Baskin & Baskin,
278 1972; Kelly & Levin, 1997). Also in the present field experiment, where the power to detect
279 stabilizing selection should be increased because of the equal representation of seedlings in
280 different cohorts, the results indicated directional selection for early germination. However,
281 the experiment was conducted in a single year, and among-year variation in the direction of
282 selection could potentially explain the maintenance of an intermediate timing of germination
283 in the study population. In the year of the experimental study, August was relatively cool and
284 the minimum soil temperature in winter was by far the lowest of those observed across 8
285 years (Fig. 3). Both of these aspects of the temperature climate should have favoured the early
286 germinating August cohort. A cool August should reduce the risk of drought-related seedling
287 mortality, whereas low temperatures in winter should increase the advantage of having grown
288 large before winter. Among-year variation in climatic factors likely to influence the direction
289 and strength of selection acting during different life-history stages suggests that repeated
290 experiments across several years would be required to determine whether overall there is
291 selection for an intermediate timing of germination in the study population.

292 In the study population, almost all seedlings established between August and October. In
293 this part of Sweden, the thin soils inhabited by *A. thaliana* hold little water and temperatures
294 are relatively high in July. Germination began in August, i.e., when temperatures decrease

295 (Ågren & Schemske, 2012) and soil moisture increases. The seedling removal experiments
296 demonstrated that some seedlings may establish after early October, but that this represents a
297 very small fraction of all seedlings. Moreover, the experimental transplant suggests that late-
298 establishing seedlings contribute little to seed production in the population. This is in contrast
299 to the situation in some *A. thaliana* populations in north-eastern Spain experiencing mild
300 winter conditions, where a considerable fraction of reproducing plants established during
301 winter (Montesinos *et al.*, 2009; Picó, 2012). Additional quantitative studies of germination
302 schedules in natural populations of *A. thaliana* would help interpret the wide variation in
303 germination characteristics documented among accessions tested under controlled conditions
304 (cf. Alonso-Blanco *et al.*, 2003; Donohue, 2009; Bentsink *et al.*, 2010, Montesinos-Navarro *et*
305 *al.*, 2012).

306 To summarize, our study shows that the direction of selection on germination timing in a
307 natural population of *A. thaliana* shifted from the establishment phase to later life-history
308 stages. Conflicting selection can be expected on a wide range of plant traits including seed
309 size (Alcántara & Rey, 2003; Gómez, 2004), flowering time (Mojica & Kelly, 2010), and
310 floral display (Strauss & Irwin, 2004; Ågren *et al.*, 2013). Identification of the ecological
311 factors governing such conflicting selection is essential for a full understanding of the
312 processes driving adaptive evolution.

313

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321

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Table 1 Effects of germination cohort, maternal line and block on rosette size, flowering time, fecundity, and fitness of *Arabidopsis thaliana* examined with mixed-model ANOVA. F with the degrees of freedom of the numerator and the degrees of freedom of the denominator is given for the effect of cohort, and χ^2 for the random effects line and block. Back-transformed least square means (LSM) and lower and upper confidence intervals (LCI and UCI) are presented for the August, September and October cohorts.

	Cohort	Line	Block	Back-transformed LSM [LCI, UCI]		
	F	χ^2	χ^2	August	September	October
ln (Rosette area two weeks after transplant [mm ²])	69.4 _{2, 61} ***	0.0	3.3 *	44 [39, 49]	18 [16, 20]	12 [11, 14]
ln (Rosette area in autumn [mm ²])	533.1 _{2, 64} ***	0.0	0.0	530 [487, 578]	45 [42, 49]	16 [13, 20]
ln (Rosette area in spring [mm ²])	189.3 _{2, 56.2} ***	0.0	8.4 **	378 [306, 467]	43 [35, 53]	16 [13, 20]
ln (Rosette area at flowering start [mm ²])	32.0 _{2, 37.5} ***	0.0	2.9	346 [276, 434]	80 [64, 101]	63 [48, 82]
Flowering start (day of the year)	20.8 _{2, 38.3} ***	0.0	0.2	129 [125, 132]	140 [137, 144]	141 [137, 146]
ln (Number of seeds per reproductive plant)	38.8 _{2, 33.7} ***	0.0	20.7 ***	2044 [1079, 3871]	391 [206, 745]	84 [43, 164]
ln (Number of seeds per plant)	11.6 _{2, 67} ***	0.0	7.4 **	63 [29, 138]	14 [6, 31]	3 [1, 7]

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Figure legends

Fig. 1 The effects of the timing of germination on survival, fecundity, and total fitness of *Arabidopsis thaliana*. (a) Proportion of plants in the August, September and October cohorts surviving from transplantation until end of autumn, over winter, from early spring until fruit maturation, and across the whole experiment, respectively. *F*-statistic with the degrees of freedom of the numerator and the degrees of freedom of the denominator and associated *P*-value are given for the effect of cohort in mixed-model ANOVA. (b) Number of seeds per reproductive plant. (c) Number of seeds per seedling. In (b) – (c), least-square means \pm S.E are given; different letters indicate statistically significant differences in means based on Tukey's HSD test.

Fig. 2 Phenology of establishment of *Arabidopsis thaliana* in the local population at the experimental site. Total number of plants (filled circle) and number of plants with only cotyledons (open circle) from August 2008 to June 2009 are indicated (mean number per plot \pm S.E., *N* = 12 plots). The arrows indicate the days when seedlings were transplanted.

Fig. 3 Mean air temperatures in August, September, and October, and minimum winter soil temperature in 8 subsequent years at the Rödåsen study site. Means and minima were calculated based on hourly recordings by two sensors placed at ca. 30 cm above ground and two sensors placed at ca. 1 cm below ground (see Ågren & Schemske 2012 for details). The year of the experiment testing the effect of timing of germination on plant performance is indicated.

Fig. 1

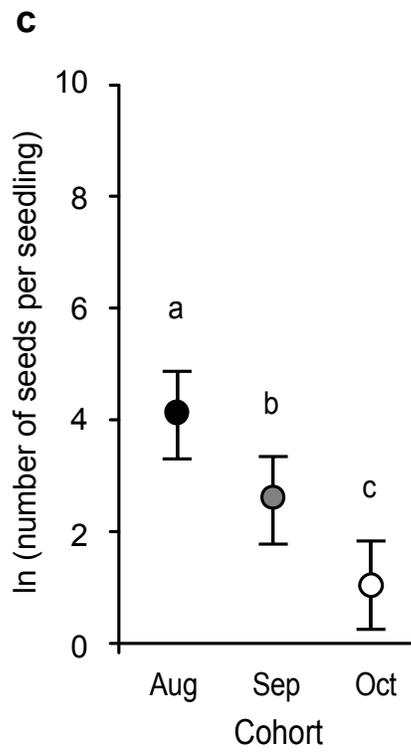
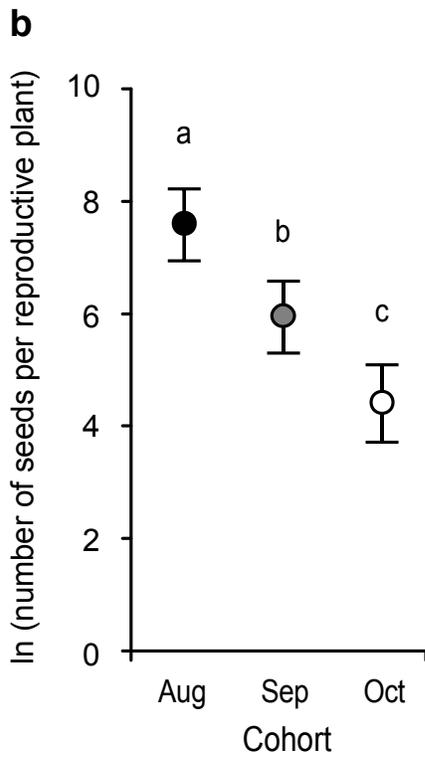
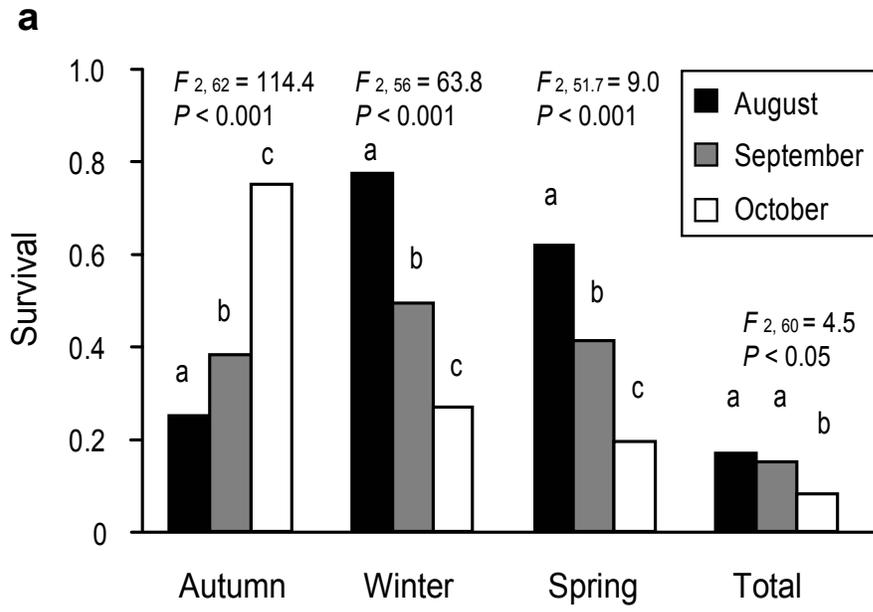


Fig. 2

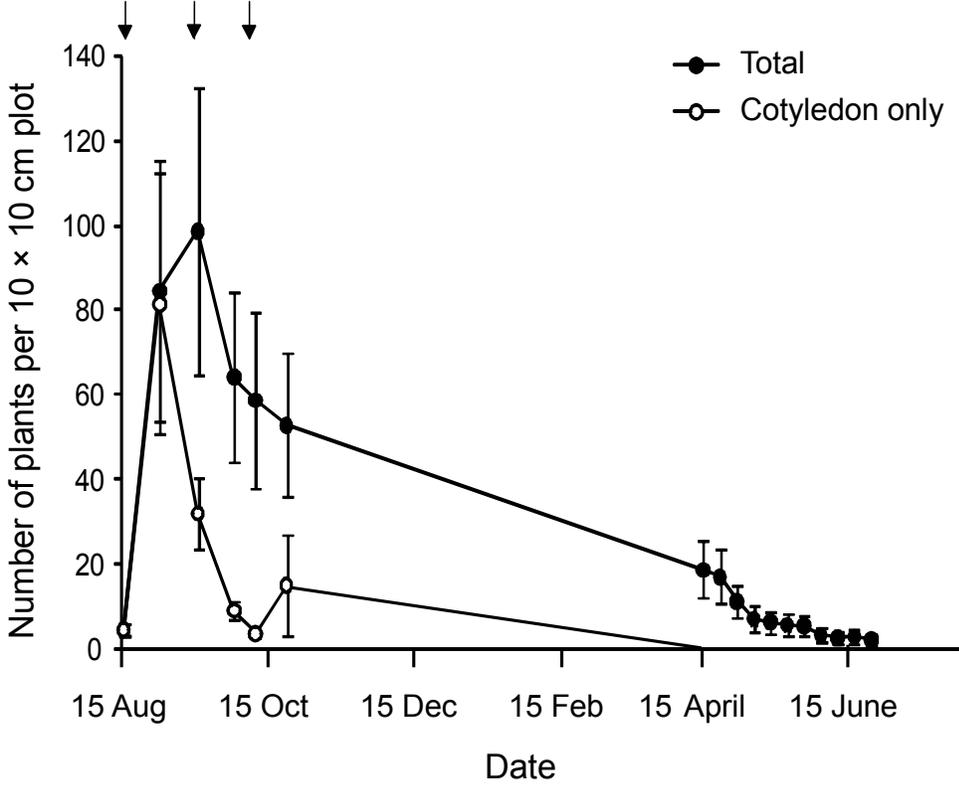


Fig. 3

