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1 **Original article**

2 **The impact of global warming on germination and seedling emergence in *Alliaria***
3 ***petiolata* a woodland species with dormancy loss dependent on low temperature.**

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17 **Key words:** *Alliaria petiolata*, climate change, dormancy, germination, global warming,
18 seedling emergence.

19

20

21 **ABSTRACT**

- 22 • The impact of global warming on seed dormancy loss and germination was
23 investigated in *Alliaria petiolata* (Garlic Mustard), a common woodland/hedge row
24 plant in Eurasia considered invasive in N. America. Increased temperature may have
25 serious implications since seeds of this species germinate and emerge at low
26 temperatures early in spring to establish and grow before canopy development of
27 competing species.
- 28 • Dormancy was evaluated in seeds buried in field soils. Seedling emergence was also
29 investigated in the field, and in a thermogradient tunnel under global warming
30 scenarios representing predicted UK air temperatures through to 2080.
- 31 • Dormancy was simple, and its relief required the accumulation of low temperature
32 chilling time. Under a global warming scenario, dormancy relief and seedling
33 emergence declined and seed mortality increased as soil temperature increased along
34 a thermal gradient. Seedling emergence advanced with soil temperature peaking eight
35 days earlier under 2080 conditions.
- 36 • The results indicate that as mean temperature increases due to global warming the
37 chilling requirement for dormancy relief may not be fully satisfied, but seedling
38 emergence will continue from low dormancy seeds in the population. Adaptation
39 resulting from selection of this low dormancy proportion is likely to reduce the
40 overall population chilling requirement. Seedling emergence is also likely to keep
41 pace with the advancement of biological spring enabling *A. petiolata* to maintain its
42 strategy of establishment before the woodland canopy closes. However, this potential
43 for adaptation may be countered by increased seed mortality in the seed bank as soils
44 warm.

45

46 **INTRODUCTION**

47 Evidence for warming of the climate system resulting from anthropomorphic greenhouse gas
48 emissions is now unequivocal (IPCC 2014). Such global warming has not only increased
49 mean temperatures, but reduced the diurnal temperature range as minimum temperature has
50 increased at twice the rate of maximum temperature (Walther *et al.* 2002). It has also
51 impacted on a seasonal scale as biological spring is now earlier and biological winter is later
52 (Penuelas *et al.* 2009; Parmesan & Hanley 2015). Such a change in climate will alter the
53 environmental cues that drive changes in depth of seed dormancy and therefore germination
54 timing. These shifts in germination phenology and subsequent plant regeneration from seed
55 will influence population dynamics and likely result in changes to the species composition
56 and diversity of communities (Walck *et al.* 2011). In addition to this impact on germination
57 phenology, global warming will also impact upon seed bank dynamics in two ways; firstly
58 increased soil temperature may reduce seed longevity (Ooi *et al.* 2009; Hoyle *et al.* 2013) and
59 secondly increased air temperature may reduce fertility in populations adapted to lower
60 temperatures in the reproductive phase (Huang *et al.* 2014) to reduce the number of seeds
61 entering the seed bank. The combined negative effects outlined will reduce the reservoir of
62 seeds in the seed bank, which may compromise the capacity for future bet hedging (Ooi *et al.*
63 2009).

64 Parmesan and Hanley (2015) report that the impact of global warming on seed and seedling
65 responses have been relatively little studied, yet this critical phase often suffers the highest
66 mortality. Research to date indicates a generally negative impact of global warming. For
67 example, in species adapted to alpine, and mediterranean/arid environments the general
68 response to increased soil temperature in multiple species was negative regardless of the type
69 of seed dormancy (physiological, physical or no dormancy on dispersal) (Ooi *et al.* 2009; Ooi
70 *et al.* 2012; Ooi 2012; Hoyle *et al.* 2013; Cochrane *et al.* 2015). In arid and fire prone areas

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71 the impact of increasing soil temperatures on seed bank dynamics indicates variable
72 responses between species (Ooi *et al.* 2009; 2012; Cochrane *et al.* 2015). Results suggest that
73 in species/ecotypes adapted to specific climates, the contribution of a given species to the
74 seed bank is likely to decline in the face of global warming (Cochrane *et al.* 2015). Further
75 work is required to determine if these generally negative impacts of global warming extend to
76 species in contrasting habitats, which adopt different life cycle strategies. We therefore
77 investigated the impact of global warming on germination phenology in the temperate
78 woodland margin species *Alliaria petiolata*.

79 In Europe, *A. petiolata* (Hedge Garlic, Garlic Mustard) is common in hedgerows and wood
80 margins. It germinates and emerges early in spring to establish and grow before canopy
81 development of competing species. Seeds are dormant at maturity and require cold
82 stratification for seeds to complete germination (Lhotská 1975; Baskin *et al.* 2000). The
83 success of this species is therefore reliant on responding to low temperature exposure during
84 winter to release dormancy for germination in early spring. It can quickly become dominant
85 in understory vegetation and can out compete other species (Weber 2003). This behaviour has
86 allowed *A. petiolata* to spread widely in North America since its introduction by early
87 colonists from Europe (Cavers *et al.* 1979). Indeed, it is now widely considered an important
88 invasive species in woodland and displaces native herbaceous species (Cavers *et al.* 1979;
89 Rodgers *et al.* 2008). However, more recent work suggests this invasive nature is more
90 complex and may be influenced by a number of factors leading to declines in native species
91 diversity (Knight *et al.* 2009; Phillips-Mao *et al.* 2014; Davis *et al.* 2014; Poon & Maherali
92 2015).

93 Baskin and Baskin (1992) show that peak germination time for *A. petiolata* differs between
94 years and occurs from early February in Kentucky, USA. In England, germination and
95 seedling emergence in the field also varied and occurred between January and March

96 (Roberts & Boddrell 1983). Seeds therefore germinate following a period of exposure to low
97 temperature (cold stratification) to break dormancy and 16 weeks between 1 and 5 °C was
98 sufficient (Baskin & Baskin 1992; Raghu & Post 2008). Their data also showed that light and
99 substrate had an influence on germination. The seeds can remain dormant for 18 months and
100 were considered to be moderately persistent in soil (Roberts & Boddrell 1983; Grime *et al.*
101 1988).

102 Climate change predictions in the UK suggest a range of future increases in mean air
103 temperature depending on the scenario adopted for future greenhouse gas emissions. A
104 projected median emissions scenario for the local experimental area used in this work (West
105 Midlands, UK) indicates an increase in the summer mean temperature of 3.7 °C by 2080 (UK
106 Climate Projections 2014). To address the impact of this future scenario on *A. petiolata* we
107 adopted several strategies. Initially we measured germination both beneath the soil surface
108 and following recovery of seeds from field soils. This was followed by an investigation of
109 seedling emergence in natural and elevated winter temperatures in the field and then along
110 the temperature gradient established in a unique thermogradient tunnel apparatus at
111 Wellesbourne, UK (Wurr *et al.* 1996). Using these approaches, we show that germination
112 phenology is likely to be significantly altered by the predicted increases in mean temperature.
113 Thus the clear strategy of this annual species with its early emergence and rapid development
114 relative to canopy development of perennial species could be significantly affected. This is
115 likely to compromise its current competitive advantage within the hedgerow and woodland
116 margin plant communities and therefore its continuing potential as an invasive species.

117 **MATERIAL AND METHODS**

118 **Seed production**

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119 Seeds of *Alliaria petiolata* were obtained from Herbiseed Ltd (UK). Seeds were harvested in
120 2007 from four separate locations within the same field to provide four independent
121 populations (biological replicates). Seeds were dried at ambient temperature before threshing.
122 Seeds were further dried on receipt at 15% RH /15°C for 9 days to a final moisture content of
123 7% on a dry weight basis before sealing in laminated foil bags and storing at -80°C.

124 **Seed burial**

125 **2007 field experiment**

126 To reduce seed mortality, seeds were dressed with Metalaxyl (Hockley International, UK) at
127 1 g active fungicide/ kg seeds (Van Mourik *et al.* 2005). Bags were made up containing 4000
128 seeds (based on a 1000 seed weight of 2.85g) dispersed at a density of 4 seeds/g of sieved
129 sandy loam of the same type found in the experimental area. The soil had previously been
130 sterilised at 80°C for three days to kill weed seeds. The bags containing the soil and seed mix
131 were 30 x 30 cm nylon mesh (200 µm mesh) (Clarcor-UK, UK), sealed with a WeLoc® bag
132 clip (size PA110) (WeLoc - Weland M. AB, Sweden). Each bag was buried separately at a
133 depth of 5 cm on 12 October 2007. The bags were laid out in a randomised block design with
134 four replicates each containing 24 bags. This allowed for harvests of seeds from the four
135 populations on up to 24 occasions.

136 Thermistore temperature probes (Betatherm, Ireland) linked to a data logger (Delta-T Devices
137 Ltd, UK) recorded soil temperature at seed depth in dummy bags (for further details on seed
138 burial experiments see Footitt & Finch-Savage (2011)). Following exhumation of seed bags,
139 seeds were recovered from soil in the light by washing with cold water through a 2 mm sieve
140 into a 1 mm analytical sieve (Endecotts Ltd, UK). Seeds were then placed in a sieve base unit
141 and washed thoroughly to remove remaining soil and plant material, then transferred to 50 ml
142 centrifuge tubes.

143 **2008 field experiment**

144 The 2007 experiment was repeated with the following changes. Soil was replaced with soda
145 lime ballotini balls (0.15 – 0.25 mm diameter) (Potters Ballotini, UK). Bags were buried as
146 before on 15 October 2008. To expose seeds to light at the soil surface duplicate bags were
147 placed on the soil surface; these contained 4000 seeds at a density of 8 seed/g Ballotini balls
148 to disperse seeds in a thin layer on the soil surface.

149 Buried seeds were recovered from the field in the dark. A light proof box with sealed arm
150 holes in the top was placed over the burial site of each bag and the base sealed with soil to
151 exclude light. Each seed bag was exhumed and placed in a laminated foil bag (Moore and
152 Buckle, UK) sealed with a WeLoc® PA150 clip. In the laboratory under a green safe light
153 seeds were immediately separated from the Ballotini balls in cold water using a 1mm gauge
154 sieve. Seeds were transferred to 50 ml centrifuge tubes and maintained in the dark. Seeds
155 from surface bags were treated in the same way, but in the light as above. Both sets of seeds
156 were immediately used for dormancy testing.

157 **Calculation of chilling time.**

158 Chilling degree days was calculated following sowing from the soil temperature at seed depth
159 using equation 1. The temperature of 5°C was taken as the upper value for the cold chilling
160 effect as this provided the best fit to the data; t is the temperature when the soil temperature
161 was between 0 and 5°C, and N is the number days.

162 $\sum_N^1 5 - t$ (Equation 1)

163 If $t = \geq 0^\circ\text{C}$ and $\leq 5^\circ\text{C}$

164 = 5 otherwise

165 N = days

166 t = mean daily temperature (°C)

167 **Analysis of seed dormancy**

168 In the 2007 field experiment the number of germinated seeds was counted in each bag on
169 recovery to determine germination in the soil seed bank. In 2008 after exhumation, seeds
170 were surface-sterilized under a green safe light in a 0.125 % sodium hypochlorite solution
171 (Household bleach (5% sodium hypochlorite) diluted to 2.5%) for 5 min then washed three
172 times in water. Germination experiments consisted of 40 seeds of each biological replicate
173 plated into individual boxes (75 x 75 x 22 mm) (Stewart Plastics Ltd, UK). Each box
174 contained two sheets of Whatman 3MM chromatography paper and 4 mL of the appropriate
175 solution. Boxes were placed in sealable freezer bags, wrapped in two layers of aluminium foil
176 and incubated at the appropriate temperature in the dark. Germination was scored under a
177 green safe light at 2-3 day intervals for up to 28 days. Germination was recorded as
178 emergence of the radical through the seed coat. Thermodormancy (dormant within a specific
179 temperature range) was tested on water at 5, 10, 15, 20, and 25°C. Sensitivity to Gibberellins
180 (GAs) was tested by exposing seeds to 0.1-1.0 mM GA₄₊₇ in 1.7 mM citric acid/ 3.3 mM
181 K₂HPO₄ buffer at pH 5.0 at 20°C. Seed viability was also tested at 20°C using 100 µM GA/
182 50 µM Fluridone (inhibitor of ABA biosynthesis) (Apollo Scientific, UK) in citrate
183 /phosphate buffer (pH 5.0). Preliminary experiments showed this to be an effective method to
184 estimate viability. Seeds from the soil surface were incubated in the light.

185 **Seedling emergence under simulated global warming conditions in the field 2009-2010**

186 The effect of soil temperature on the emergence of *A. petiolata* seedlings in the field under
187 current winter temperatures was compared to warmer winter temperatures resulting from
188 future climate change. To achieve this, four of eight field plots were randomly chosen to be
189 individually covered in small 1.5 m wide semicircular cross-section mini polyethylene

190 tunnels to raise the winter temperature above ambient. Therefore, each of the four
191 populations was represented in an uncovered and a covered plot. Within each plot, seedling
192 emergence was recorded on three replicate pots (described below). Seeds were sown in the
193 pots on 14th September 2009 and polytunnels were put in place on 6th November 2009 and
194 removed on 12th March 2010.

195 Pots were 17.5 cm square rigid black plastic (Fargro, BHGS horticultural, UK) used to
196 provide a standard replicate area for seedling emergence. The pots, with base removed below
197 7 cm, were placed in a shallow trench in each field plot. The trench was then backfilled with
198 field soil leaving the pots 1 cm above ground. Once in position, pots were filled with
199 sterilised soil (see above) to 2 cm from the top. Four hundred *A. petiolata* seeds were then
200 sown on to the surface of the soil in each pot and covered by a further 1 cm of soil, to equal
201 the surrounding soil level. The 1 cm rim at the top of the pot remained above the surface.

202 All uncovered plots were covered in netting for the duration of the experiment to prevent
203 foraging by birds. Surrounding areas were kept weed clear to discourage foraging by birds,
204 insects and gastropods. Thermistore temperature probes (Betatherm, Ireland) linked to a data
205 logger (Delta-T Devices Ltd, UK) recorded soil temperature at seed depth, and air
206 temperature. In the warm-winter plots polyethylene tunnels raised the daily mean soil
207 temperature by $2.69 \pm 0.15^{\circ}\text{C}$. Plots were periodically checked and emerged seedling
208 removed and recorded.

209 **Seedling emergence under simulated global warming conditions in a thermogradient** 210 **tunnel**

211 The polyethylene tunnel (32 m long x 9 m wide) structure enables plants to be grown from
212 seed to seed at natural day lengths with a high percentage (76%) of natural levels of
213 irradiance. The ambient air temperature was constantly monitored outside of the tunnel.
214 Reacting to this an electronic climate control system operated fans that generated opposing

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215 warmed and ambient air flows to establish and maintain an air temperature gradient from
216 ambient at one end of the tunnel to *c.* ambient + 4°C at the other end (Wurr *et al.* 1996). Air
217 and soil temperatures were monitored continuously along the tunnel. Realistic seasonal and
218 diurnal air and soil temperature fluctuations were therefore maintained within the tunnel, but
219 with varying degrees of simulated climate warming depending on the position along the
220 tunnel. Four positions along the tunnel were selected to provide *c.* T1, ambient; T4, ambient
221 + 4°C and at two equally spaced temperatures (T2 & T3) in between.

222 **Seedling emergence in the thermogradient tunnel 2010-2013**

223 To further evaluate the impact of climate change on *A. petiolata* emergence experiments were
224 established on 17 September 2010 within the thermogradient tunnel. A thermal gradient
225 (ambient + 4°C) was maintained throughout the year that simulated predicted UK air
226 temperatures from the present time to approximately 2080 at this location (Wellesbourne,
227 UK: UK Climate Projections (2014)). The soil temperature gradient established along the
228 tunnel over the course of the experiment (2010 – 2013) was $2.51 \pm 0.08^\circ\text{C}$ (T4), and at
229 intermediate positions was $+1.3 \pm 0.02^\circ\text{C}$ at T2 and $+2.39 \pm 0.03^\circ\text{C}$ at T3. This enabled the
230 evaluation of increasing soil temperature on the termination of dormancy and seedling
231 emergence from the soil seed bank. Emergence experiments were set up in free standing 17.5
232 cm square rigid black pots (Fargro, BHGS horticultural, UK). Pots were filled with sterilised
233 soil (see above) to within 2 cm of the top of the pot. Four hundred seeds of a single biological
234 replicate were sown on the soil surface, then covered with 1 cm of soil. Three pots were
235 placed at four positions along the tunnel (see above). Pots were watered weekly to ensure the
236 only variable was soil temperature. Pots were periodically checked and emerged seedlings
237 removed and recorded. At the end of the experiment seeds were recovered from the soil as
238 above and viability tested by assessing the presence of hard seeds. Thermistor temperature

239 probes (Betatherm, Ireland) linked to a data logger (Delta-T Devices Ltd, UK) recorded soil
240 temperature at seed depth along the tunnel.

241 **RESULTS**

242 **Dormancy loss in the soil requires chilling**

243 Following burial in field soils in 2007 and again in 2008 germination within seed bags was
244 observed on exhumation. During this period the pattern of soil temperature at seed depth
245 differed greatly between the years (Fig S1). Comparison of the number of seeds germinated
246 within seed bags buried in 2007 and of germination at 5°C in the dark on recovery of seeds
247 buried in 2008 indicated that dormancy was lost and germination commenced during late
248 winter/early spring of the following year (Fig. 1A). In 2007-08 the germination record ended
249 when seedling growth in the bags prevented accurate recording. In 2008-09, little germination
250 was seen in recovered bags until it began in March when the temperatures rose above 5 °C; at
251 this point the experiment was stopped. Germination time differed between years (Fig. 1A),
252 but this difference could be accounted for by fitting a soil chilling model (Fig. 1B). In this
253 model chilling degree days was calculated following sowing from the soil temperature at seed
254 depth using Equation 1.

255 There was a distribution of chilling degree days ranging from 60+ to greater than 150 when
256 dormancy was lost (Fig 1B). This is indicative of a range of dormancy levels in the seed
257 population as seen in other species. After this point (March) in 2008-9 temperatures rose
258 above 5 °C (Fig. S1) preventing further accumulation of chilling time. Germination following
259 exhumation is shown at 5 °C (Fig. 1A) and it is possible that chilling continued during this
260 period of germination. However, germination was also recorded in the dark at other constant
261 temperatures (10-25°C; Fig. 1B) showing that on a chilling scale germination began at the
262 same time. Nevertheless, percentage germination was reduced at higher temperatures
263 indicating this species has high temperature therm dormancy. Germination was also recorded

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264 under alternating temperature with amplitudes of 5, 10, and 15°C (Fig. S2). The results show
265 maximum germination was observed in the lowest constant temperatures and in alternating
266 temperatures with the lowest mean temperature. Sensitivity to GA₄₊₇ was also low with a
267 maximum germination response of 46% at 0.1 mM GA₄₊₇ and this only occurred following
268 extensive chilling in the field. In the case of seeds held on the soil surface no germinated
269 seeds were found on recovery. When they were subsequently tested in the light, germination
270 did not exceed 5% under any conditions with the exception of the GA/fluoridone viability
271 tests where 93% viability was observed in seed at the final harvest (Fig. 1C). This result
272 shows that seed viability was high and germination was prevented by dormancy in the
273 absence of sufficient chilling; even the GA/ fluoridone combination had difficulty
274 overcoming *A. petiolata* dormancy in the absence of extensive chilling. Interestingly at the
275 first harvest following burial germination declined in the GA/ fluridone treatment and then
276 increased at later harvests indicating the depth of seed dormancy initially increased

277 **The impact of global warming on seedling emergence**

278 Different patterns of temperature in 2007-8 and 2008-9 had an impact on loss of dormancy in
279 buried seeds. To investigate the effect of temperature change due to global warming in 2009-
280 2010 we simulated the impact of higher winter temperatures in the field using mini
281 polyethylene tunnels (warm-winter plots). This raised the mean soil temperature
282 approximately 2.7°C+ above ambient (cold-winter plots) (Fig. 2). Overall there was no
283 difference in seedling emergence between the four replicate populations of seeds on either
284 cold- or warm-winter plots, but comparison of the warm- and cold-winter plots showed there
285 was a large effect of temperature.

286 The first seedling emergence was seen in the warm-winter plots on 16th February 2010 (Fig.
287 2). By this time these seeds had accumulated 91 chilling degree days (°C days). Six days later
288 seedling emergence was seen in the cold-winter plots by which time these seeds had

289 accumulated 157 °C days. Seedling emergence under cold-winter conditions lagged behind
290 the simulated warm-winter conditions until the mean ambient soil temperature increased
291 above 6°C. At this time the covers were removed from the warm-winter plots (12th March
292 2010) and mean soil temperature fell to that of the cold-winter plots (ambient levels). In both
293 cases, emergence finally reached a plateau (89%) at the same time when the chilling
294 requirement to remove dormancy in the majority of the population was satisfied.

295 To further investigate the impact of global warming seedling emergence was then examined
296 in a thermogradient tunnel having temperature-controlled conditions where other variables
297 are minimised. In this scenario, an air temperature gradient of ambient to ambient +4 °C was
298 established to represent the predicted increase in ambient temperature between now and 2080
299 at this location. This established a mean soil temperature gradient of 2.5 ± 0.1 °C along the
300 tunnel (Fig. 3) that followed seasonal changes in temperature during the experiment (Fig.
301 4A). Under these scenarios seedling emergence started on the 9th February 2011 at the
302 ambient end (T1) and five days later at the warm end of the gradient (T4 ambient + 2.5 °C)
303 (Fig. 4B). When thermal chilling time was calculated (Equation 1), seeds along the thermal
304 gradient accumulated 79 (T1), 82 (T2), 62 (T3) and 67 (T4) chilling °C days before 1st
305 February 2011 (Table 1), which was the last day to have chilling temperatures prior to
306 seedling emergence (Fig 4A).

307 In the open field, the onset of dormancy loss occurred after seeds accumulated 60 °C days of
308 chilling time (Fig. 1). In the thermogradient tunnel, chilling time accumulated similarly at all
309 positions up to 60 °C days (*c.* mid January), after which accumulation diverged with more
310 chilling time at the ambient end (Fig.4B). Seedling emergence reached a maximum of 43%
311 under ambient conditions (T1) and declined to 10% (T4) in line with the reduced
312 accumulation of chilling time as the soil temperature gradient increased (Fig. 4B). Plotting
313 the distribution of seedlings that emerged over time revealed the time to peak seedling

314 emergence was positively related to increasing soil temperature above 5°C in 2011 (Fig. 5).
315 Thus, peak emergence was 8 days earlier at temperatures predicted for 2080 (T4) compared
316 to the present day (T1 - ambient).

317 The gradient in the tunnel experiment was maintained until 2013. A small second flush of
318 seedling emergence was seen in spring 2012 followed by a slighter greater flush in 2013
319 (Table 1). Less thermal chilling time was accumulated in 2012 and 2013 at above ambient
320 temperatures (T2-4) than in 2011. The peak in seedling emergence was still earlier at
321 intermediate tempertures (T2 and T3) than under ambient (T1 conditions). When seeds were
322 recovered from the soil in 2013 a small proportion were still viable, but overall seed mortality
323 increased with temperature along the thermal gradient (Table 1).

324 **DISCUSSION**

325 We highlight above that *A. petiolata* is not only a well known European woodland and
326 hedgerow plant, but has also become a serious invasive problem in North America where it
327 displaces the native woodland flora. We examined the behavior of *A. petiolata* seeds during
328 dormancy loss and seedling establishment under ambient field conditions and conditions
329 modified to simulate predicted local global warming scenarios.

330 **Seeds are dormant, but only require adequate exposure to low temperature (chilling) to** 331 **germinate**

332 Buried seeds were deeply dormant unless they had experienced extended periods of chilling
333 in the soil. As seeds accumulated thermal chilling time (°C days) germination increased both
334 within the soil (2008) and on subsequent incubation in the dark at a range of temperatures
335 (2009). Recovered seeds were insensitive to GA₄₊₇ in the light and only responded in the
336 dark following extensive chilling in field soils. These observations with regard to temperature

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337 are consistent with those of Baskin and Baskin (1992); and similar to the low sensitivity to
338 GA₃ seen previously (Sosnoskie & Cardina 2009; Yasin & Andreasen 2015).

339 *A. petiolata* is a summer annual that germinates and emerges exclusively in early spring, but
340 germination timing differed in the two years studied (2008 and 2009). The difference was
341 removed when data were plotted on a “chilling degree days” scale showing dormancy was
342 lost by a simple accumulation of time at low temperature. Therefore, once the seeds have
343 accumulated sufficient chilling time they progress to germination if there is adequate
344 moisture available. Results indicate they have high temperature therm dormancy and so
345 following chilling they germinate best at low temperatures. The seeds do not appear to have a
346 strong requirement for alternating temperature, neither do they have a requirement for light to
347 relieve dormancy. In fact seeds did not germinate on the soil surface (i.e. in the light) despite
348 exposure to a suitable low temperature environment. Thus in *A. petiolata*, light maintains
349 dormancy with seeds being negatively photoblastic. These germination characteristics show
350 they are adapted to germinate and emerge from undisturbed soils or under the leaf litter
351 typical of hedgerows and woodland. Dormancy can also be influenced by low soil moisture
352 and oxygen availability, but over winter this is unlikely to be significant in this environment.
353 In the experiments, very light sandy soils were used in which oxygen availability is not
354 limiting and soil did not dry out significantly at sowing depth (5cm). However, to be
355 competitive in their natural environment the seeds must accumulate sufficient chilling for
356 early germination so that growth of the plant can occur before leaves in the existing perennial
357 plant canopy open. This situation has potential for disruption by global warming.

358 **Seedling emergence is affected by predicted global warming scenarios**

359 We show that dormancy loss, which underlies seedling emergence patterns responds to
360 projected changes in our climate. As soil temperature increased, the percentage of seeds
361 producing seedlings decreased, indicating the proportion of the population that accumulated

362 sufficient chilling time declined. However, it is likely that other factors may contribute
363 positively and negatively to the accumulation of chilling time to break dormancy. For
364 example, the time spent above the chilling temperature ($> 5^{\circ}\text{C}$) may enhance dormancy in
365 those seeds in which the final layer of dormancy has not been removed. This is a normal
366 response to the late spring/summer increase in temperature that reinduces deeper dormancy in
367 seeds that have not previously accumulated sufficient chilling time to allow germination.
368 Germination/seedling emergence is therefore blocked as the woodland/hedgerow canopy is
369 forming. Such behaviour is consistent with the hypothesis that temperature impacts the rate of
370 dormancy induction and relief independently (Totterdell & Roberts 1979; Batlla *et al.* 2009);
371 and the situation seen in *Rumex* species and summer annual *Arabidopsis* ecotypes in which
372 increasing temperature increases the induction of secondary dormancy (Totterdell & Roberts
373 1979; Huang *et al.* 2015; Springthorpe & Penfield 2015; Footitt *et al.* 2017).

374 The results show that *A. petiolata* may be highly sensitive to short-term temperature changes
375 that span the threshold temperature for dormancy relief and induction; and this may
376 contribute to the decreased seedling emergence seen during simulated global warming. The
377 reinduction of deeper dormancy is also consistent with our results that show *A. petiolata*
378 forms a moderately persistent seed bank with emergence predominantly in the first spring
379 after shedding as previously reported (Roberts & Boddrell 1983; Grime *et al.* 1988). Seed
380 mortality increased along the thermal gradient to 68 % at a soil temperature of ambient
381 ambient + 2.5°C . Therefore in *A. petiolata* loss of seed viability may be an additional factor
382 related to global warming that contributes to reduced seedling emergence and seed bank
383 stability. This is in agreement with Ooi and coworkers who showed that increasing soil
384 temperature reduced seed longevity in other species and environments (Ooi *et al.* 2009).

385 **How might global warming impact on the *A. petiolata* life cycle?**

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386 The use of a thermogradient tunnel, rather than a geographical climate or altitudinal gradient,
387 in which the only significant variable is temperature helps to reveal how climate change
388 driven by global warming will impact on the *A. petiolata* life cycle. Under warmer conditions,
389 only that portion of the *A. petiolata* population with low dormancy and therefore a low
390 chilling requirement will emerge and persist in the population. It is therefore likely that
391 progressive adaptation to a lower chilling requirement for dormancy relief will take place in
392 the population allowing it to remain competitive in the woodland/hedgerow environment. In
393 the global warming scenario reported here the peak in seedling emergence was also advanced
394 by eight days in the first year under the predicted 2080 temperature compared to the present
395 day. Seedling emergence therefore advanced at approximately 1 day/decade, the same as the
396 advancement of flowering time reported by Cook and coworkers in an analysis of 20-50 year
397 data sets of multi-species flowering times (Cook *et al.* 2012). This adds to increasing
398 evidence that seedling emergence timing and flowering time are linked (Springthorpe &
399 Penfield 2015). In subsequent years, emergence remained earlier at intermediate temperatures
400 potentially from seeds with low dormancy. While at ambient and ambient + 2.5 °C
401 emergence lagged behind and was potentially influenced by a declining population resulting
402 from (a) previously seedling emergence and (b) increased seed mortality (i.e. compare
403 ambient (T1) and ambient + 2.5 °C (T4) Table 1).

404 The impact of higher temperatures during subsequent vegetative and reproductive growth
405 may also result in reduced dormancy at seed maturity as seen in *Arabidopsis* and wild oats
406 (Sawhney *et al.* 1985; Chen *et al.* 2014; Huang *et al.* 2014; Springthorpe & Penfield 2015).

407 So global warming will influence timing of germination directly (as we show here) and
408 indirectly by its influence during seed production (Walck *et al.* 2011; Chen *et al.* 2014;
409 Springthorpe & Penfield 2015). We demonstrate that as biological spring advances this
410 species has the potential to advance seedling emergence in time to occupy its natural habitat

411 before the woodland canopy closes. However, we do not know the relative effect of global
412 warming on the rates of *A. petiolata* seedling emergence and woodland canopy
413 development. Furthermore, *A. petiolata* has only a moderately persistent seed bank and so the
414 observed increase in seed mortality with soil temperature may restrict the species. A full
415 understanding of the adaptation to global warming will require long-term monitoring along
416 climate gradients to determine the phenological and ecological consequences of adaptation
417 (Walck *et al.* 2011; Parmesan & Hanley 2015).

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425

426 **AUTHOR CONTRIBUTIONS**

427 SF and WEF-S designed research, SF, ZH, HÖ-F and HC performed research, SF analysed
428 data, SF and WEF-S wrote the paper.

429 **SUPPORTING INFORMATION**

430 **Fig. S1.** Temperatures and soil moisture contents recorded during field experiments.

431 **Fig. S2.** Germination of *A. petiolata* seeds in response to alternating temperatures in the dark
432 following recovery from field soils.

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1 **Fig. 1.** Germination of *A. petiolata* in the soil seedbank and following recovery. (A)
2 Germination of seeds within the soil seed bank (2008) following burial on 12 October 2007,
3 and in seeds buried on 15 October 2008, subsequently exhumed at intervals and tested for
4 germination potential at 5°C in the dark under controlled conditions in 2009. (B) The
5 cumulative effect of chilling degree days ($\geq 0^{\circ}\text{C} \leq 5^{\circ}\text{C}$) in the soil before exhumation on
6 germination potential of seeds collected in 2007/8 and 2008/9 . Following recovery of seeds
7 in 2008/9 they were incubated in water at 5 - 25°C in the dark. (C) Percentage germination of
8 seeds on GA/ Fluridone following different harvest dates in 2008/9 of seeds that were either
9 buried or left on the surface. Data are the mean \pm SE (n = 4). No error bar indicates the
10 symbol is bigger than the error.

11 **Fig. 2.** *A. petiolata* seedling emergence and soil temperature in the field following the winter
12 of 2009/2010 under ambient conditions and simulated warm-winter conditions. Seedling
13 emergence from field soils under cold and warm-winter conditions. Soil temperature at seed
14 depth under ambient conditions (cold winter) and simulated warm-winter conditions is
15 shown. Arrow indicates when covers were removed after which soil temperatures were
16 ambient in all plots. Seedling emergence data are the mean \pm SE (n = 3). No error bar
17 indicates the symbol is bigger than the error.

18 **Fig. 3.** Soil temperature gradients along the thermogradient tunnel. Representative soil
19 temperature gradients along the tunnel at seed depth (1 cm) in February and March 2011.

20 **Fig. 4.** *A. petiolata* seedling emergence and soil temperature under simulated global warming
21 conditions along a thermogradient tunnel. (A) Mean daily soil temperature profiles at seed
22 depth from October 2010 to April 2011. For clarity only temperatures at positions T1
23 (ambient) and T4 (ambient + 2.5°C) are shown. (B) The accumulation of thermal chilling
24 time ($^{\circ}\text{C}$ days) ($\geq 0^{\circ}\text{C} \leq 5^{\circ}\text{C}$) at each position along the thermal gradient, and seedling

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1 emergence along the thermal gradient. Seedling emergence data are the mean \pm SE (n = 3).

2 No error bar indicates the symbol is bigger than the error.

3 **Fig 5. *A. petiolata* seedling emergence advances in time with increasing soil temperature**

4 **in the thermogradient tunnel: (A)** Temperature profiles at seed depth along the thermal

5 gradient. **(B)** Normal distribution of seedling emergence shows advancing peak in

6 emergence. Seedling emergence data reanalysed from Fig 4

7

8