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1 **The effect of various after-ripening temperature regimens on the germination**  
2 **behaviour of *Ambrosia artemisiifolia***

3

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14 *Ambrosia artemisiifolia* (common ragweed) is an annual weed known to infest crops and  
15 disturbed areas, and cause human pollinosis. To examine the effect of different thermal  
16 regimens on seed germination, during seed after-ripening, a study was conducted for two  
17 years. In 2012 (year 1) and 2013 (year 2), ragweed seeds collected from a single wheat stubble  
18 field were divided and stored under one of the five temperature regimens: constant -20°C,  
19 +5°C, room temperature (18°C), +25°C, and field conditions. Germination tests were  
20 performed every 15 days of storage from day 17 to day 213. Storage conditions showed a  
21 strong influence on ragweed seed germination duration. At constant low temperatures (-20°C),  
22 seed behaved in an opposite manner depending on the year; in year 1 germination was  
23 inhibited while it was stimulated in year 2. At higher temperatures, seed dormancy was  
24 unbroken due to a lack of exposure to low temperatures. Under field conditions, seeds reached  
25 a high level of germination after a few days of storage due to temperature fluctuations. The  
26 different behaviours of ragweed seeds shown at constant temperatures revealed different  
27 dormancy depths in the two years that might be due to population variability and maternal  
28 environment differences during seed maturation.

29 Key words: Common ragweed, seed dormancy, germination, temperature, *Ambrosia*  
30 *artemisiifolia*, seed after-ripening.

31

## 32 **Introduction**

33 *Ambrosia artemisiifolia* L. (common ragweed) is an annual weed that originated in the Nearctic  
34 ecozone. Its documented European presence dates back to the mid-18th century according to notes  
35 of it having been cultivated since 1772 in writings of the Turin Botanical Garden (northwest Italy)  
36 (Bouvet et al. 2013). Despite its long European presence, *A. artemisiifolia* was only occasionally  
37 reported as spontaneous throughout the 19th century (Bouvet et al. 2013). Its first significant spread  
38 in Europe was reported after World War I and attributed to infested agricultural commodities  
39 imported from the US. A sharp increase after World War II indicated that naturalization and  
40 invasion had occurred (Chauvel et al. 2006; Kazinczi et al. 2008). Only in the last 20 years has *A.*  
41 *artemisiifolia* displayed significant invasion and spread across many areas of Europe (Bouvet et al.  
42 2013). Presently, the species has invaded three main areas: the Rhône Valley (southern France), the  
43 Po Valley (northern Italy), and the Carpathian Basin (southern central Europe) (Storkey et al. 2014).  
44 European diffusion of the species has not been stopped by either recent accidental infestations of  
45 the species by the ragweed leaf beetle (*Ophraella communa*) or by natural unfavourable climatic  
46 conditions (Lommen et al., 2018). In fact, delayed frost in the northern countries may become  
47 suitable for establishment consequent to climate change (Storkey et al. 2014; Rasmussen et al.  
48 2017).

49 *A. artemisiifolia* produces a multitude of tiny pollen grains easily transported by the wind for  
50 as much as hundreds of kilometres (Cecchi et al., 2007). The pioneer species flourishes on disturbed  
51 habitats and readily adapts to different soil textures, pHs, moistures, and abiotic constraints  
52 (DiTommaso 2004; Gentili et al., 2018). In some agricultural contexts, *A. artemisiifolia* is a noxious  
53 weed to summer annual crops, such as sunflower and maize, and it may create dense infestations in  
54 mid-late summer on cereal stubbles (Gerber et al. 2011). The species colonizes disturbed areas in  
55 new environments rapidly for many reasons, one of which is its high plasticity (Fumanal et al.  
56 2007). Others stem from its advantageous biological characteristic: high per plant production of  
57 long-lasting viable seeds; strong competitiveness against other weeds by release of allelopathic

58 compounds, and drought tolerance (Kazinczi et al. 2008; Vidotto et al. 2013; Fenesi et al. 2014).

59         The successful colonization strategy of *A. artemisiifolia* also appears to relate to its complex  
60 germination behaviour. Temperature is considered the most important factor affecting *A.*  
61 *artemisiifolia* germination (Leiblein-Wild et al. 2014); seeds can germinate over a wide range of  
62 temperatures and remain viable for long durations (Fenesi et al. 2014; Ortmans et al., 2016). In  
63 particular, *A. artemisiifolia* germination is affected by temperature fluctuations and light exposure  
64 (Bazzaz 1970; Pickett and Baskin 1973), and it is enhanced in light conditions rather than in the  
65 dark (Willemsen 1975a). *A. artemisiifolia* seeds undergo an innate/primary dormancy following  
66 ripening and dissemination, and then enter a secondary dormancy when primary dormancy is  
67 broken or other factors are unsuitable for germination, such as when the seeds are buried in the soil  
68 (Bazzaz 1970). Then, secondary dormancy is broken when the seeds return to upper soil layers and  
69 are exposed to sunlight (Pickett and Baskin 1973). Each mechanism allows some seeds to germinate  
70 under a broad range of conditions that affords the species an ecological benefit (Fenesi et al. 2014;  
71 Ortmans et al., 2016). The relationship between temperature and *A. artemisiifolia* seed germination  
72 has long been investigated (Bazzaz 1970; Pickett and Baskin 1973; Baskin and Baskin 1998;  
73 Guillemin et al. 2013; Leiblein-Wild et al. 2014). Most studies focused on the effect of temperature  
74 fluctuations, but no information is available on the long-term effect of different thermal regimens.  
75 This study assessed the germination behaviour of an *A. artemisiifolia* population under different  
76 thermal regimens during seed after-ripening, with a focus on simulating temperatures that the seeds  
77 might actually experience between autumn and spring. The seeds were stored under five different  
78 temperature regimens (for different time intervals) until the storage was interrupted and seed  
79 germination was assessed.

## 80 **Materials and Methods**

81 In brief, the study method involved the main following steps: seed collection and preparation,  
82 storage regimen and duration, and germination tests. Seeds of *Ambrosia artemisiifolia* were

83 collected in October 2012 and 2013 from plants growing naturally in a wheat stubble field located  
84 on the campus of the Dipartimento di Scienze Agrarie, Forestali e Alimentari of the Università degli  
85 Studi di Torino in northwest Italy (45°03'59.88''N, 7°35'33.58''E). Each year, the seeds were  
86 collected by hand at peak maturity from the inflorescences of about 30 different ragweed plants and  
87 bulked to build a unique population. All the seeds were dried in open trays for approximately 10  
88 days at room temperature. This identical set of procedures allowed the two years to be considered as  
89 replicates of the same study.

90 After drying, groups of 100 seeds collected in 2012 were placed into paper bags (100 seeds  
91 per bag) in darkness and stored under five different regimens (described below) for varying time  
92 intervals between October 2012 and May 2013 (Year 1). An equal number of seeds collected in  
93 2013 were also counted, bagged, and stored under the same five regimens for varying length of time  
94 between October 2013 and May 2014 (Year 2).

95 Five thermal regimens were employed for seed maintenance: constant -20°C temperature  
96 (seeds kept in freezer), constant +5° (seeds kept in refrigerator), room temperature (average  
97 temperature of about 18±3°C), constant +25°C (seeds stored in growing chamber), and field  
98 temperature conditions (Fogliatto et al., 2010). Each thermal regimen was chosen to simulate actual  
99 temperatures experienced in the region of seed collection between autumn and summer. In the case  
100 of the fixed temperatures, -20°C represents low winter temperatures, +5°C is a typical autumn or  
101 early spring temperature, and 25°C reflects values recorded in summer. The two remaining  
102 regimens characterized by temperature fluctuations were the room temperature regimen (18±3°C)  
103 that simulated late spring temperatures and the field conditions regimen that represented a near-  
104 actual seed experience through seed bag placement in the original collection field beneath an open  
105 shelter to protect the seeds from being wetted by precipitation.

106 Across all storage regimens, 14 storage durations were considered in the study. An identical  
107 17-day first period was imposed on each of durations across all the regimens. Thereafter, an  
108 increasing number of two-week periods of storage were applied until 213 days were attained. This

109 resulted in a total of 70 seed bags being stored per year (14 durations X five regimens). In both  
110 years, at the end of each storage period, a paper bag was taken from each thermal condition and the  
111 seeds were tested for germination.

112 Prior to actual seeding and germination testing, the seeds were soaked in 0.5% sodium  
113 hypochlorite solution for three minutes and then rinsed with deionized water for about 10 minutes  
114 to prevent fungal and bacterial contaminations. The seeds were then lined with two filter papers  
115 (Grade no. 1 filter paper, Whatman) and placed into 9 cm-Petri dishes along with 8 ml of deionized  
116 water. (The tweezers, filter papers, pipette tips, and water had all been sterilized previously in an  
117 autoclave under a pressure of 105 Pa for 10 minutes.) Thirty seeds of *A. artemisiifolia* were  
118 randomly distributed into each of 210 total Petri dishes (3 Petri dishes x 5 regimens x 14 storage  
119 durations), working under a laminar flow hood. The Petri dishes were maintained in a growth  
120 chamber at a constant temperature of +25°C with a 16/8 h alternation in light/dark. Seed  
121 germination was assessed and recorded daily for a 14-day period. Seed viability was determined  
122 visually at the end of the germination test, considering viable and dormant the seeds that were hard  
123 and without discoloration, molds, and signs of seedling deformation (ISTA, 2009).

#### 124 ***Statistical Analyses***

125 Total germination was calculated as the proportion of germinated seeds by the end of the  
126 germination test.

127 For each year and storage condition, a separate regression analysis was performed between  
128 days of storage duration (independent variable) and proportion of germinated seeds (dependent  
129 variable), fitting the following two parameters log-logistic model to binomial response (logit model)  
130 (Equation 1):

$$131 \quad G_T = \frac{1}{1 + \exp(b(\log(x) - \log(e)))} \quad [1]$$

132 where GT is total germination expressed as a proportion of germinated seeds, b and e are the curve  
133 parameters, with b being the relative slope at the point of inflection e, and x is the storage duration  
134 (in days). The regression analysis was performed using the function *drm* of the add-on package *drc*  
135 of the R software (Ritz et al. 2015; RCoreTeam 2017). The time required to obtain 10%, 50%, and  
136 90% germination (ED10, ED50, ED90) was calculated using the function ED of the package *drc*.  
137 As for field conditions, the proportion of germinated seeds was fitted against storage duration only  
138 until the seeds entered secondary dormancy and germination declined (up to 130 days).

139 For each storage condition, the function *EDcomp* of the package *drc* was used to test the  
140 significance of differences of ED10, ED50, and ED90 between the two years. Differences were  
141 considered significant when the confidence interval at  $p \leq 0.05$  did not include zero.

## 142 **Results and Discussion**

143 Study results demonstrated that storage conditions and storage duration strongly influenced *A.*  
144 *artemisiifolia* seed germination. This section reports our results and discusses those results relative  
145 to other studies according to each of the five storage regimens.

146

### 147 *Storage at -20°C*

148 Low temperature storage (-20°C) resulted in variable effects according to the year considered  
149 (Figure 1). In Year 1, seeds did not germinate up to 101 days of storage and the estimated ED10  
150 (storage days needed for 10% germination in germinability test) was 111 days. Starting from 115  
151 days of storage, the germination percentage increased over time, but reaching only 55% by the end  
152 of the study. In contrast, Year 2 germination was highly stimulated by storage at -20°C, as  
153 evidenced by germination in excess of 50% after 17 days of storage. Calculated Year 2 ED10 (1  
154 day) and ED50 (10 days) were quite short days. Furthermore, the proportion of germination  
155 continued to rise with increasing periods of -20°C storage until 100 days when the percentage of  
156 germinated seeds reached almost 100% and then remained until the end of the experiment. A year-

157 to-year comparison of ED values indicated greater than 100 days were required to attain equal  
158 levels of germination (ED10, ED50, and ED90), and Year 2 germination always occurred earlier  
159 than in Year 1 (Table 1).

160 Low temperatures and moist conditions (cold stratification) have been shown to break the  
161 dormancy of *A. artemisiifolia* seeds after shattering (Willemsen 1975b; Guillemin and Chauvel  
162 2011). The requirement of moist stratification to break dormancy has been observed in other  
163 species, such as *Veronica anthelmintica*, in which sustained low moisture content during storage  
164 prevented seed germination (Baskin and Baskin 1998). Moreover, after stratification in dry  
165 conditions, some weed seeds (*Panicum* spp.) have been shown to enter secondary dormancy or  
166 dormancy reversion, which is a return to dormancy after drying (Shen et al. 2001). On the other  
167 hand, seed dormancy has also been demonstrated not to break at temperatures approximating -20°C  
168 in dry conditions because these temperatures sit below the break threshold range (Baskin and  
169 Baskin, 1998). In this study, a series or combination of factors might have been involved to produce  
170 the different behaviours observed between the two years, including different dormancy depths of  
171 the ragweed seeds. More generally, our results could be considered as consistent with the  
172 germination behaviour variability of *A. artemisiifolia* that has been reported in previous studies  
173 (Fumanal et al. 2007; Dinelli et al. 2013; Leiblein-Wild and Tackenberg 2014; Ciappetta et al.  
174 2016).

175

#### 176 *Storage at +5°C*

177 The same year-to-year germination variability was also observed for storage at +5°C (Figure  
178 2). As occurred in low temperature storage, the Year 1 population yielded nil germination until  
179 seeds had undergone about 80 days of storage and ED10 had reached about 83 days. Afterwards,  
180 germination continued to rise until the experiment ended, although total germination failed to  
181 exceed 50%. Specifically, 175 days were required to reach 50% germination; ED90 was never met  
182 (Figure 2). Compared to storage at -20°C, +5°C ED10 values were lower, whilst ED50 values were

183 slightly higher. This seemed to suggest that temperatures just above 0°C probably allowed an earlier  
184 dormancy release, despite attaining an almost identical maximum germination. Conversely, Year 2  
185 ragweed seeds required 5 and 30 days fewer at 5°C storage to attain the same respective ED10 and  
186 ED50 germination levels as in Year 1. At the end of the experiment, germination values  
187 approaching 100% were recorded for the population. Compared to -20°C, storage at +5°C resulted  
188 in a slight delay in reaching a certain level of germination.

189         Previous studies have shown that maintenance of common ragweed seeds at +4°C or +5°C  
190 for a prolonged time not only breaks seed dormancy, but also confers the seeds with the ability to  
191 germinate over a wider range of temperatures (Willemsen 1975b; Dinelli et al. 2013). In our study,  
192 Year 2 seeds showed enhanced germination at +5°C, albeit not as quickly as at lower temperatures.  
193 Year 1 germination, however, seemed less stimulated at this same temperature. The base  
194 temperature for *A. artemisiifolia* was previously studied and estimated to be as low as +3.5°C;  
195 however, other studies reported values of about +5°C (Shrestha et al. 1999; Sartorato and Pignata  
196 2008). The absence of germination during the +5°C study storage period is attributed mainly to the  
197 seeds having been stored dry; however, the possibility exists that some seeds collected in Year 2  
198 could have had a lower base temperature, as it was previously observed that with freezing  
199 temperature storage the germination was stimulated. In the case of Year 1 seeds, they were unable  
200 to germinate for almost 80 days after storage at +5°C.

201         Furthermore, the fact that the dormancy level of a population can influence base temperature  
202 must be considered (Benech-Arnold et al. 2000; Soltani et al., 2017), especially since this influence  
203 has been demonstrated to vary among species or even among populations. In contrast, upon  
204 application of the hydrothermal model (Guillemin et al., 2013), values of *A. artemisiifolia* base  
205 temperature had not varied across a set of different populations in different studies. More recently,  
206 reviews have highlighted that base temperature follows a normal distribution within a seed  
207 population in species that have conditional dormancy (Battla and Benech-Arnold, 2015). In the case  
208 of *A. artemisiifolia*, a constant base temperature within the population cannot be assumed, as it may

209 fall during dormancy loss (Soltani et al., 2017). In our study, the base temperature was not assessed,  
210 but the different degree of dormancy in the population between the two years could have affected  
211 the base temperature. The value of base temperature knowledge, together with base water potential,  
212 is useful for predicting germination onset and end of a population. Determination of the degree of  
213 dormancy can allow supposition as to when germination is possible (Guillemin et al., 2013).

214

#### 215 *Storage at room temperature*

216 At room temperature, differences in germination levels between the two years narrowed slightly  
217 (Figure 3). Specifically, 10% germination was recorded in Year 1 at about 70 days; the level rose to  
218 50% near the end of the experiment. Year 2 ragweed seeds on the other hand, started to germinate  
219 right after the first storage period, reached ED10 and ED50 after almost 15 and 70 days,  
220 respectively, yet failed to reach 90% germination prior to completion of the experiment.

221 Comparison of the germination levels between the two years made evident the significant  
222 differences of about 57 days at ED10 and more than 100 days at ED50 (Table 1). At room  
223 temperature, Year 1 population germination behaviour exhibited only minor differences when  
224 compared to storage at +5°C, despite reaching ED10 approximately 10 days earlier. In contrast, the  
225 Year 2 population was late relative to the germination levels achieved from storage at -20°C and  
226 +5°C. Storage at room temperature surely underwent moderate temperature fluctuations that might  
227 have contributed to stimulate germination. Moreover, Year 1 germination might also have been  
228 enhanced by the higher temperatures relative to previous conditions. The opposite population  
229 response occurred in Year 2, in which dormancy release slowed relative to -20°C and +5°C.

230

#### 231 *Storage at +25°C*

232 Storage at the highest temperature (+25°C) in Year 1 produced an ED10 of only about 50 days  
233 (Figure 4); however, after the initial stimulation, the population failed to reach both ED50 and  
234 ED90 during the study course. Germination of the Year 2 population was also initially triggered by

235 the temperature (ED10 of about 2.5 days), but 50% germination was not recorded until 94 days and  
236 90% germination was never reached. Germination levels between the two years differed widely  
237 (about 50 days) at ED10, yet the difference was not statistically significant (Table 1). Ragweed  
238 seeds in both years at +25°C exhibited the lowest maximum germination values. This might be due  
239 to the fact that these high temperature-stored seeds never experienced the low winter temperatures  
240 that permit dormancy to break. After all, even after-ripened non-dormant seeds have been shown to  
241 germinate at low levels if exposed to high temperatures as they enter into secondary dormancy  
242 (Bazzaz 1970; Dinelli et al. 2013). Indeed, the critical soil temperature at which *A. artemisiifolia*  
243 seeds enter secondary dormancy has been estimated to be about 20°C (Willemsen 1975a).

244

#### 245 *Storage in field conditions*

246 *Ambrosia artemisiifolia* seeds maintained in field conditions showed similar behaviours in both  
247 years and attained high germination levels (Figure 5). Germination differences between the two  
248 years were neither large (about 3 at ED10 and 4 days at ED50) nor significant (Table 1). The Year 1  
249 population was 10% germinated after only two days of storage and about 50% germinated after 30  
250 days. Even though 90% germination was never achieved, the in-field population exhibited the  
251 highest levels and fastest rates of germination compared to all other temperature regimens. In Year  
252 2, the population initially took about 5 days to reach 10%, and 50% germination was reached about  
253 5 days earlier than in Year 1. Year 2 in-field seeds also exhibited a germination trend similar to that  
254 observed at +5°C and at -20°C, with the exception that the -20°C regimen seeds germinated to the  
255 same level a little earlier than in the field. In both years, the highest germination level was reached  
256 in mid-April, after about 130 days of field storage. Thereafter, germination declined until the end of  
257 the experiment, a likely demonstration that the seeds entered secondary dormancy (Figure 5)  
258 (Bazzaz 1970; Dinelli et al. 2013).

259 The high level of germination observed in both years for field-maintained seeds, before  
260 entering in secondary dormancy, might be due to the fluctuating temperatures that occurred (Figure

261 6). As the figure displays, temperature fluctuation at the beginning of the storage period averaged  
262 about 8.0°C and 7.5°C in October 2012 (Year 1) and 2013 (Year 2), respectively, and then averaged  
263 more than 7.0°C in November 2012 and 9.5°C in November 2013, and temperatures peaked at 20°C  
264 of fluctuation on certain days (Figure 6). We attributed the break in dormancy to temperature  
265 fluctuation, as seed hydration was prevented through seed storage in a bag beneath a shelter.  
266 Furthermore, we suggest that if hydration had occurred under the field conditions, then any break in  
267 dormancy would have already germinated seeds in the field. The stimulation of germination caused  
268 by wide temperature variations has been demonstrated in previous studies on many species in which  
269 alternating, as opposed to constant temperatures, led to higher germination (Pickett and Baskin  
270 1973; Rich 1994; Battla and Benech-Arnold, 2015). These studies highlighted the fact that  
271 temperature is a factor that regulates dormancy level by acting over a long time, while fluctuations  
272 in temperature, like those experienced by the seeds in the field, act as a dormancy-terminating  
273 factor. Alternating temperatures terminate dormancy abruptly by altering the processes that prevent  
274 germination (Huarte and Benech-Arnold, 2010). Thus, testing temperature as a factor of seed  
275 germination has a completely different effect compared to that of testing alternating temperatures  
276 (Battla and Benech-Arnold, 2015).

277

#### 278 *Storage at constant temperatures*

279 In our study, only ragweed seeds that germinated under constant temperature regimens permitted to  
280 test the hypothesis of differences in seed degree of dormancy in the two years. Indeed, the  
281 fluctuating temperatures experienced by the seeds in both years of field conditions, even though  
282 they differed a little, were still enough to break dormancy. Moreover, the slightly higher  
283 germination reached in Year 2 could be ascribed to the low winter temperatures and stimulated seed  
284 germination observed in the -20°C and +5°C storage regimens in the same year. The test of seeds at  
285 different constant temperatures was fruitful because it allowed otherwise impossible speculations if  
286 the study had been conducted only with seeds stored in the field. Year 2 ragweed seeds attained

287 higher germinability values after shorter exposure periods at all constant temperature regimens  
288 compared to Year 1. The different germination pattern between the two years might relate to the  
289 fact that the year-specific seed bulks probably comprised seeds of different degrees of dormancy. It  
290 is quite possible that despite having been collected from the same field, the Year 2 bulk contained  
291 naturally less dormant seeds than those contained with the Year 1 bulk, and *vice versa* that seeds  
292 collected in Year 1 were more dormant/less favourable to germination by low temperature  
293 exposure.

294

#### 295 *Dormancy differences*

296 The different degrees of dormancy exhibited in the two study years may also arise from high  
297 individual variability within a ragweed population, and/or from climatic conditions that favour  
298 some biotypes over others. The high diversity among ragweed plants has been documented. One  
299 study of Italian ragweed populations found that the majority of genetic variability lay within  
300 individuals of a population rather than between populations (Ciappetta et al. 2016), and another  
301 found that *A. artemisifolia* produced seeds of wide size variation, that can lead to variation in  
302 germination behaviour. This same variability was detected within the population and even within  
303 the seeds produced by the same mother plant (Ortmans et al., 2016).

304 Another explanation for the different dormancy levels indicated in our study may be the  
305 environmental conditions experienced by the mother plants in the two years, particularly during  
306 seed development, a possibility that aligns with studies that have demonstrated that both parental  
307 genotype and parental environment affect seed morphology (i.e., seed weight) and phenology (i.e.,  
308 seed dormancy) of their offspring (Luzuriaga et al. 2006). Other suggestions of parental  
309 environment influencing dormancy come from a study that puts forth that exert an effect on gene  
310 expression that varies the phenotypic of the offspring, and another has suggested that mother plant  
311 stress during seed development might vary the proportion of dormant seeds in a population (Schmid  
312 and Dolt 1994; Fumanal et al. 2008). Maternal environmental effects that influence seed dormancy

313 most are temperature, water availability, photoperiod, nutrition level, and light quality and quantity  
314 (Fenner 1991; Luzuriaga et al. 2006).

315 In our study, ragweed mother plants were exposed to different climatic conditions in the two  
316 years. Seed development and maturation typically occurs during mid-August and mid-October in  
317 northern Italy, which coincided with average temperatures at the seed collection field of 18.6°C  
318 (Year 1) and 19.7°C (Year 2) (data not shown). The effects of temperature during seed development  
319 have been studied for a number of plant species with the most common finding that higher  
320 temperatures produce less dormant seeds (Fenner 1991; Baskin and Baskin 1998). Applied to this  
321 study, these results would suggest that the higher germination found in the seeds collected in Year 2  
322 might also stem from the higher temperatures experienced by plants during seed formation.

323

324 In general, our study results highlighted that germination behaviour of ragweed seeds  
325 differed between two years when exposed to constant temperatures. These differences probably  
326 were due to variability in the degree of dormancy of the population, as found in previous studies.  
327 The behavioural differences were consistent in all the constant temperature regimens tested and  
328 especially at low temperatures, facts that permitted us to infer a different base temperature of the  
329 seeds constituting the population such that germination was stimulated more in one year than in the  
330 other. Seeds exposed to fluctuating temperatures in the field during the same two years followed a  
331 different pattern. Their germination behaviour was quite similar across the same two study years, as  
332 dormancy seemed to be interrupted, even in seeds considered to have a deeper dormancy. This  
333 phenomenon might explain the invasive nature of the species, wherein different temperature  
334 fluctuations permitted seed germination and gave to *A. artemisiifolia* its ability to succeed in  
335 different environments (Ortmans et al., 2016).

336 Understanding the germination dynamics of a species in different controlled conditions  
337 advances knowledge of its dormancy degree, which consequently can lead to develop more suitable  
338 control strategies. Population control measures can result in variable efficacy when the degree of

339 dormancy varies within a population. For example, more dormant populations can escape early  
340 herbicide treatment by germinating after spray application (Dinelli et al. 2013), while less dormant  
341 populations might emerge earlier in the season and over a wider range of temperatures, possibly  
342 giving the advantage to the weeds rather than to the crops (Guillemin and Chauvel 2011). In  
343 general, the ability of *A. artemisiifolia* to germinate over a wide range of conditions, together with  
344 high variability, has been suggested as one of the main reasons for its success as an invasive weed  
345 (Sang et al. 2011). The alternating temperatures that favour enhancement of ragweed seed  
346 germination generally occur when the seeds are located close to the soil surface. Deep seed burial  
347 reduces temperature fluctuation and, by keeping ragweed seed dormant, may reduce its emergence  
348 in the short term, but may favour seedbank maintenance in the long term (Fumanal et al. 2008).

349 Further studies are necessary to clarify the germination behaviour of the seeds under  
350 different after-ripening conditions, including alternating temperatures and burial at different soil  
351 depths.

352

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356

357 **References**

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455 Willemsen RW. 1975b. Dormancy and germination of common ragweed seeds in the field. Am J  
456 Bot. 62:639–643.

457 Table 1. Differences of ED values between Year 1 and Year 2 of the studied *A. artemisiifolia*  
 458 population in each storage condition. Upper and lower confidence limits of the differences are  
 459 calculated for  $P \leq 0.05$ . Positive values for the differences indicate that Year 1 values were larger  
 460 than those in Year 2.

461

Storage condition	ED10			ED50			ED90		
	difference	lower	upper	difference	lower	upper	difference	lower	upper
-20°C	109.60 *	72.90	146.30	157.25 *	115.10	199.41	174.17 *	20.32	328.03
+5°C	77.13 *	33.73	120.52	143.55 *	71.03	216.07	192.56	-202.52	587.63
room temp	57.35 *	10.63	104.07	104.97 *	10.87	199.08	102.41	-505.08	709.90
+25°C	49.04	-16.91	115.00	>213	-	-	>213	-	-
field	-3.15	-18.67	12.37	4.50	-40.40	49.40	>213	-	-

462 \*: difference is significant at  $P \leq 0.05$ , as the interval between lower and upper confidence limits does not  
 463 include zero.

464

465

466 **Figure captions**

467 Figure 1. *A. artemisiifolia* germination in Year 1 and Year 2 in response to storage at -20°C and the  
468 relative ED10, ED50, ED90. Equation for Year 1:  $GT = 1/(1+\exp(-5.34 \log x - \log 167.36))$ ;  
469 Equation for Year 2:  $GT = 1/(1+\exp(-1.07 \log x - \log 10.10))$ . Solid and dashed lines represent  
470 Year 1 and Year 2, respectively.

471

472 Figure 2. *A. artemisiifolia* germination in Year 1 and Year 2 in response to storage at +5°C and the  
473 relative ED10, ED50, ED90. Equation for Year 1:  $GT = 1/(1+\exp(-2.93 \log x - \log 174.68))$ ;  
474 Equation for Year 2:  $GT = 1/(1+\exp(-1.26 \log x - \log 31.13))$ . Solid and dashed lines represent  
475 Year 1 and Year 2, respectively.

476

477 Figure 3. *A. artemisiifolia* germination in Year 1 and Year 2 in response to storage at room  
478 temperature and the relative ED10, ED50, ED90. Equation for Year 1:  $GT = 1/(1+\exp(-2.51 \log x -$   
479  $\log 173.32))$ ; Equation for Year 2:  $GT = 1/(1+\exp(-1.44 \log x - \log 68.35))$ . Solid and dashed lines  
480 represent Year 1 and Year 2, respectively.

481

482 Figure 4. *A. artemisiifolia* germination in Year 1 and Year 2 in response to storage at +25°C and the  
483 relative ED10, ED50, ED90. Equation for Year 1:  $GT = 1/(1+\exp(-1.22 \log x - \log 312.82))$ ;  
484 Equation for Year 2:  $GT = 1/(1+\exp(-0.60 \log x - \log 94.15))$ . Solid and dashed lines represent  
485 Year 1 and Year 2, respectively.

486

487 Figure 5. *A. artemisiifolia* germination in Year 1 and Year 2 in response to storage at field condition  
488 and the relative ED10, ED50, ED90. Equation for Year 1:  $GT = 1/(1+\exp(-0.81 \log x - \log 31.13))$ ;  
489 Equation for Year 2:  $GT = 1/(1+\exp(-1.35 \log x - \log 26.63))$ . Solid and dashed lines represent  
490 Year 1 and Year 2, respectively.

491

492

493 Figure 6: Field temperatures to which the seeds were exposed from October 2012 to May 2013 for  
494 Year 1 (A) and from October 2013 to May 2014 for Year 2 (B).

495

496

- 20°C

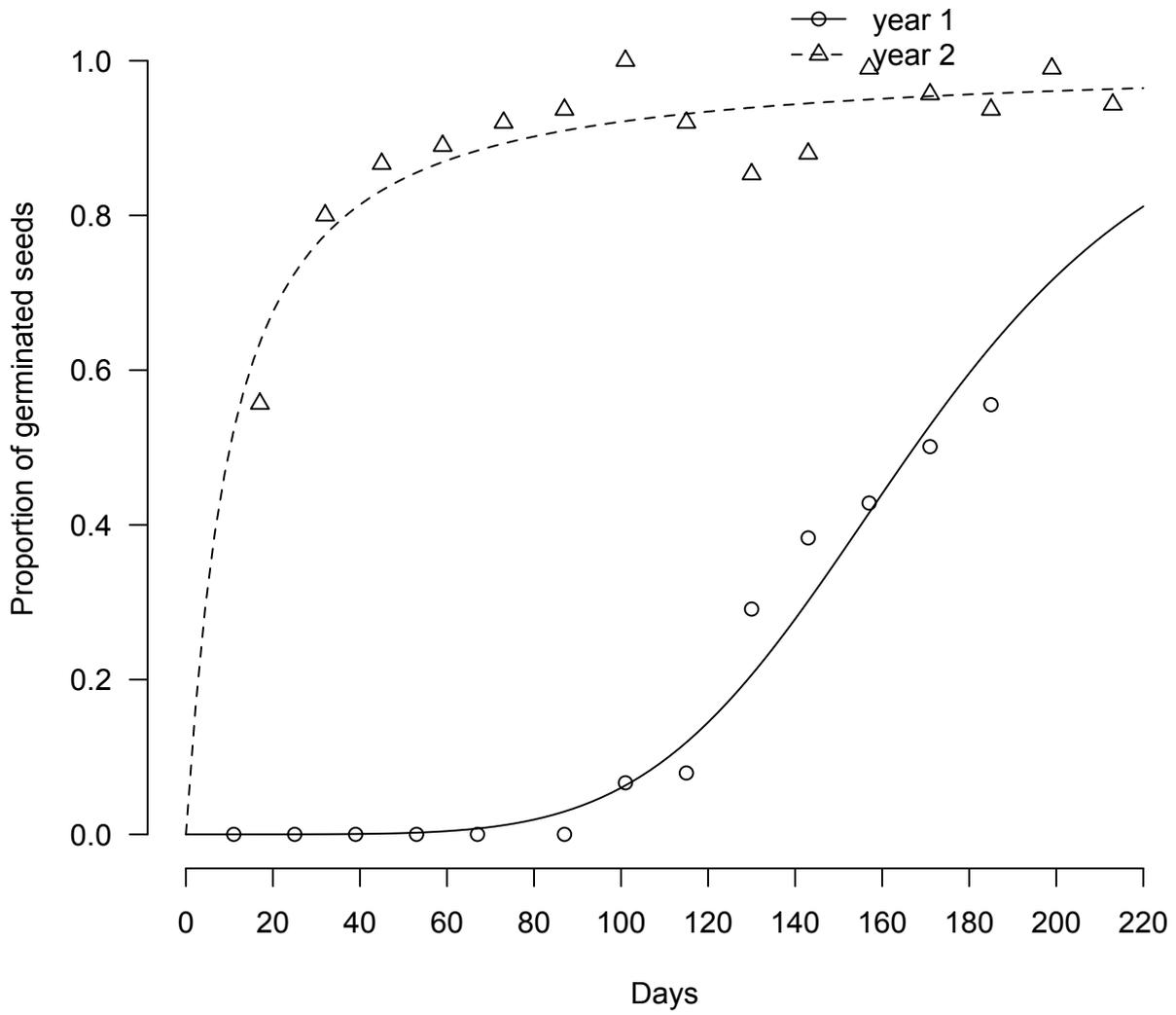


Figure 1

+5°C

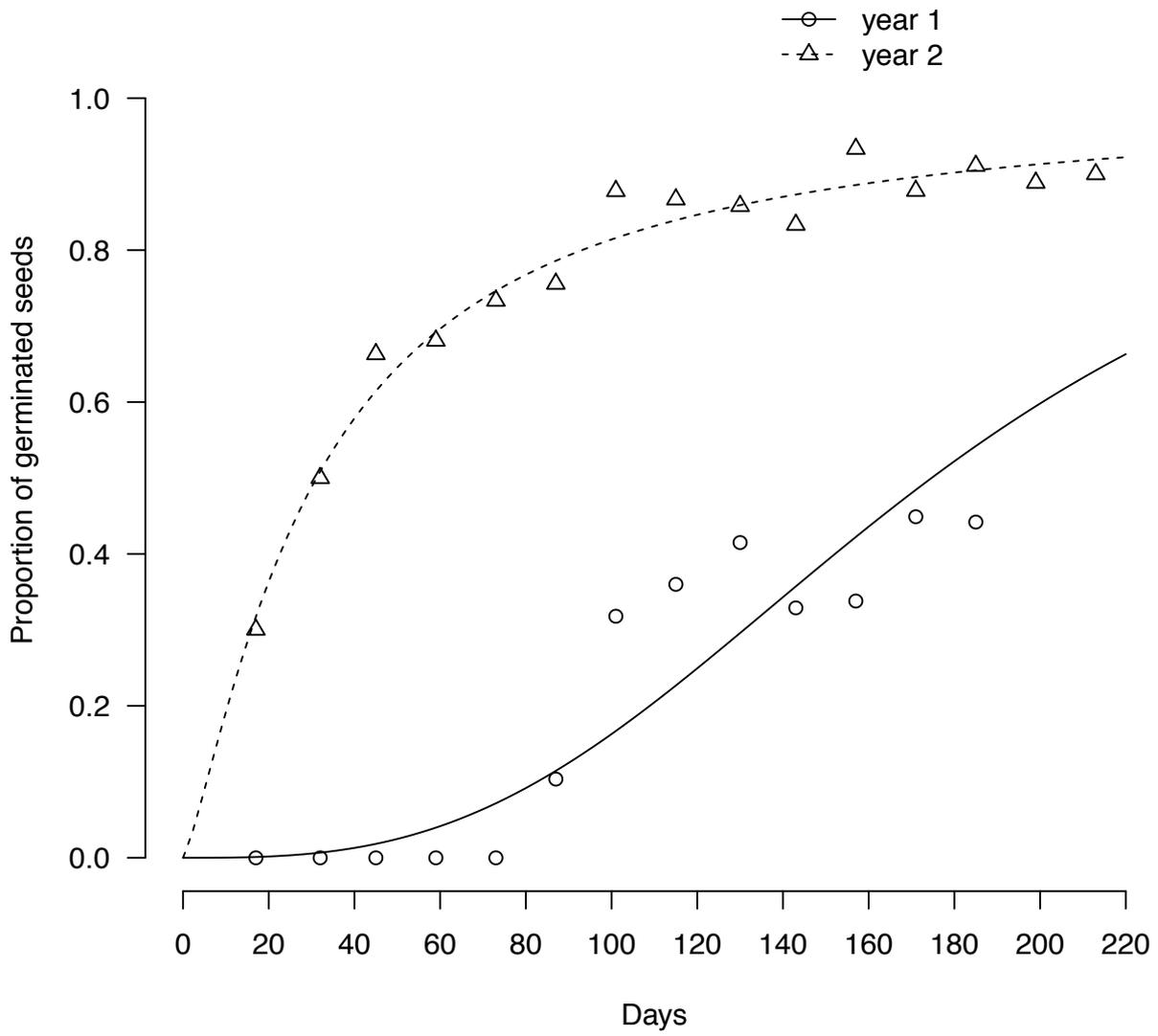


Figure 2

### room temperature

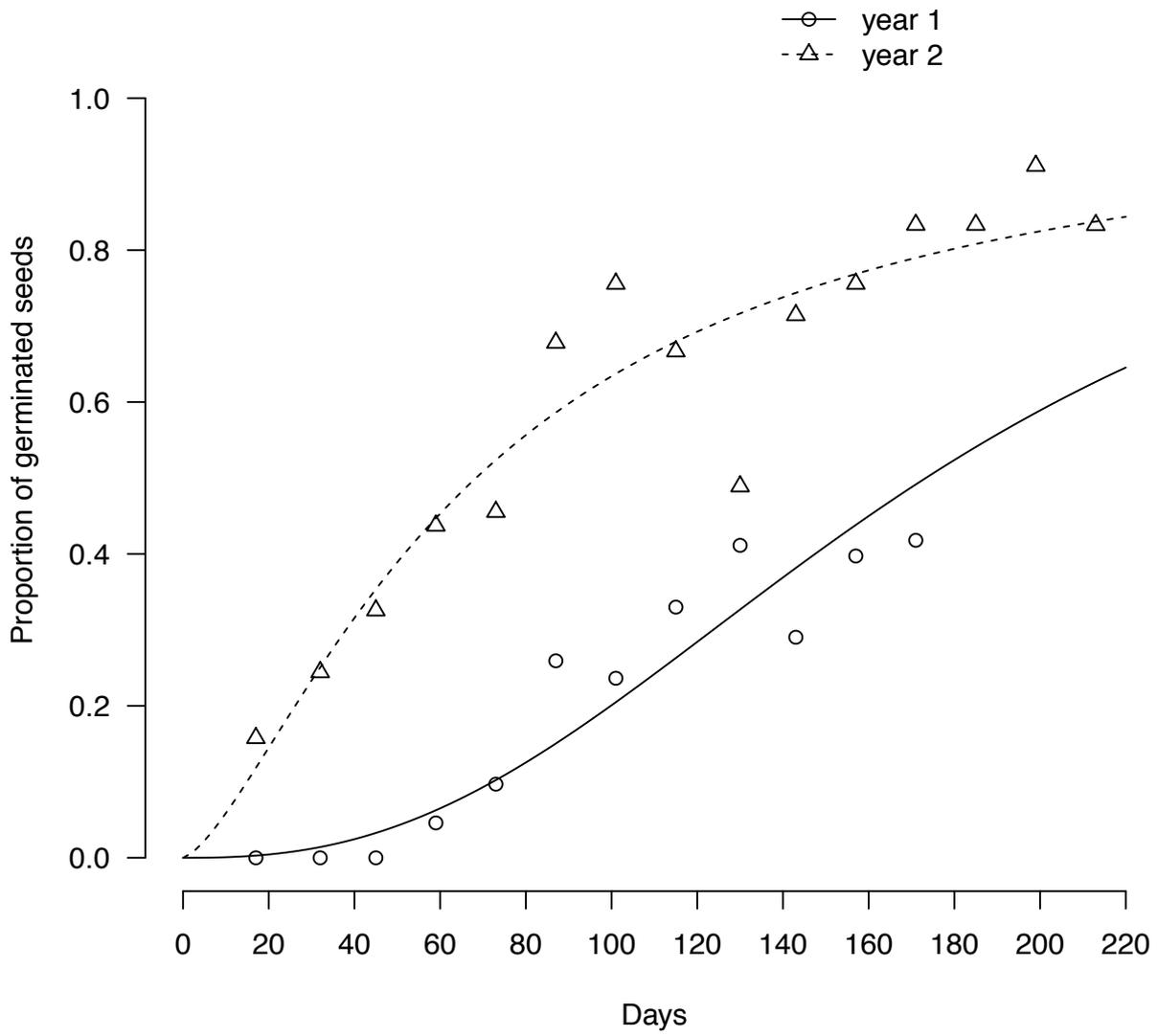


Figure 3

**+25°C**

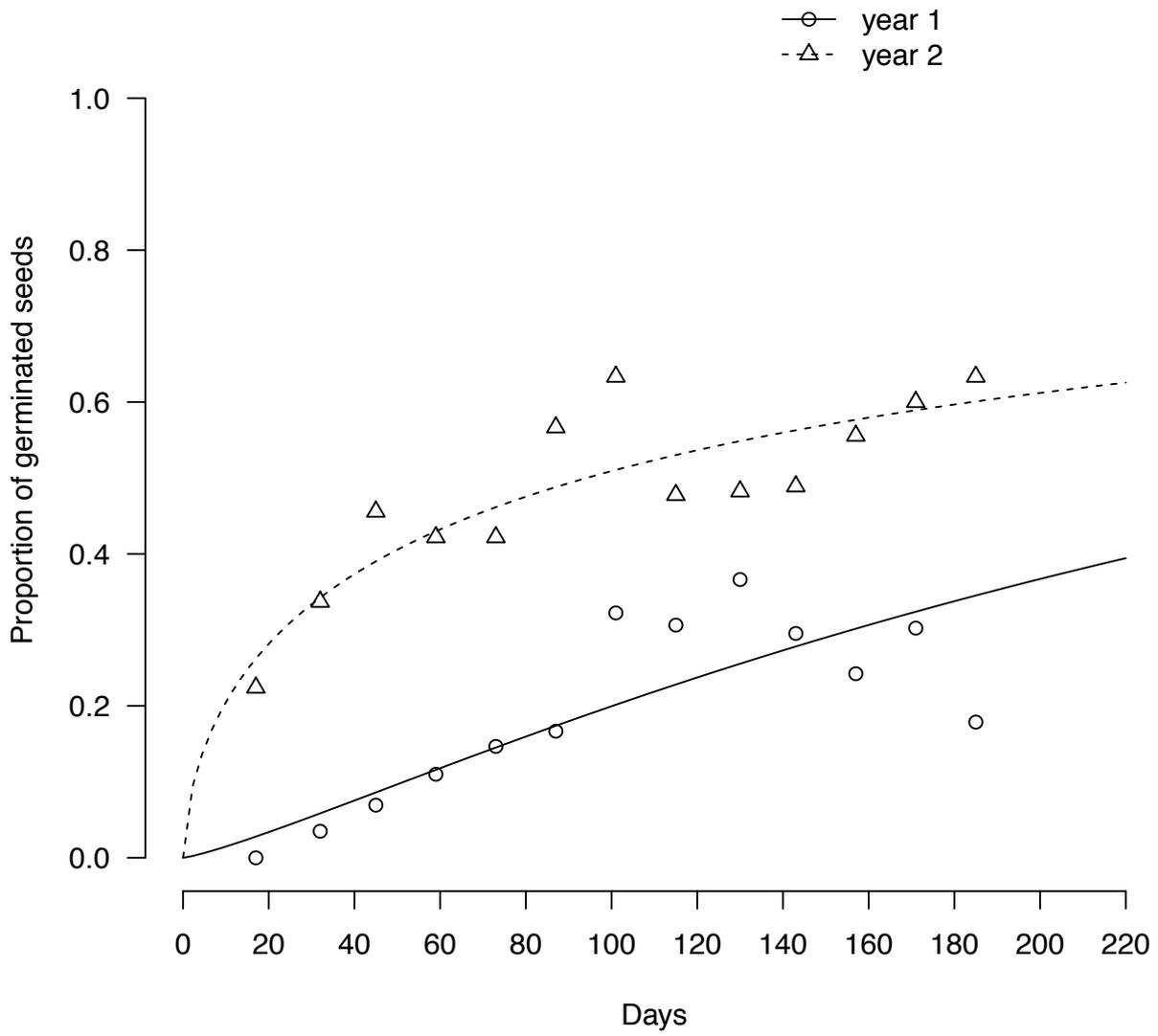


Figure 4

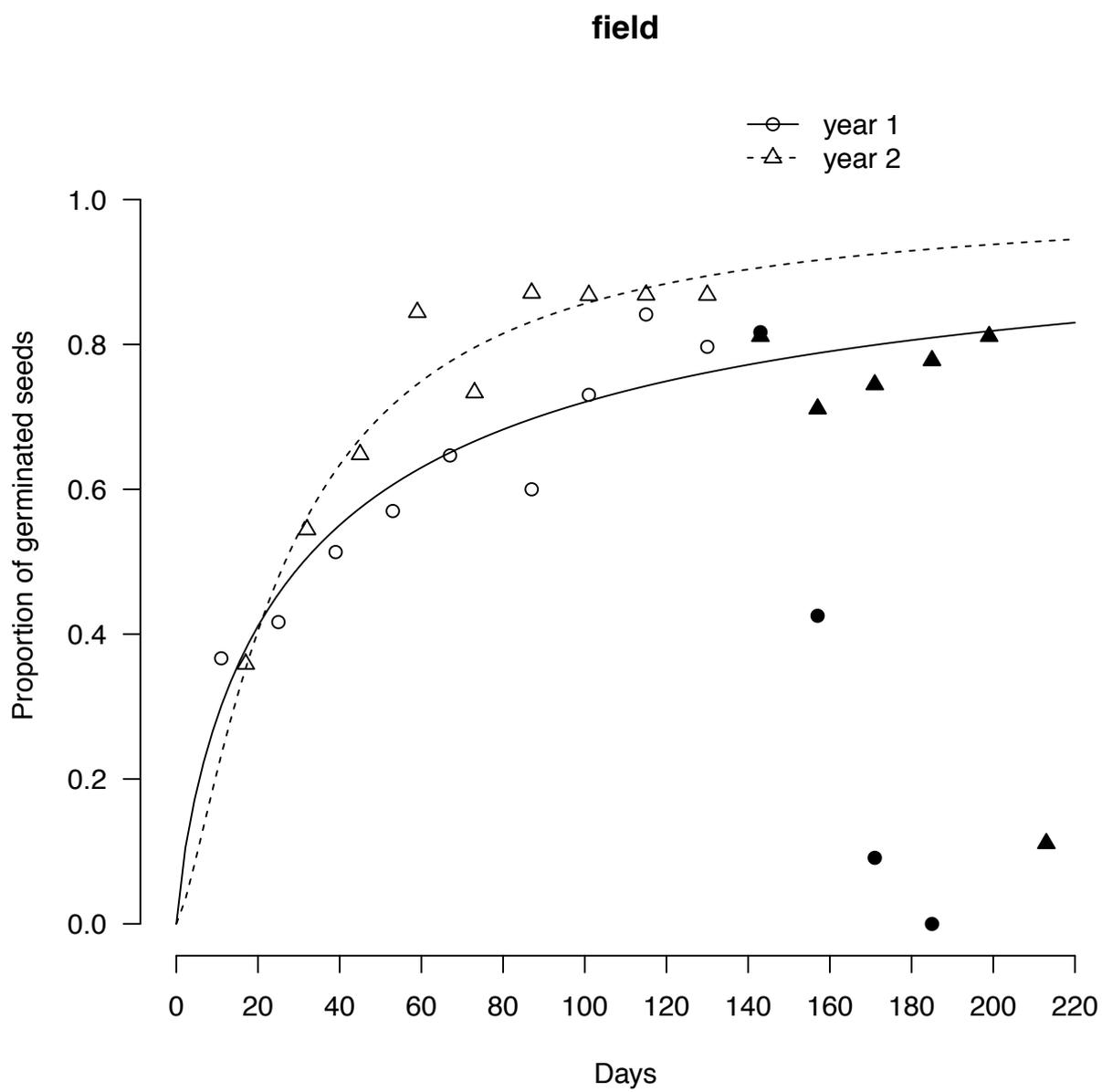
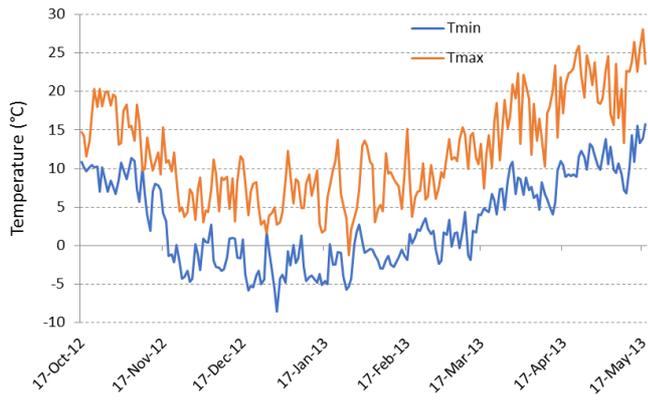
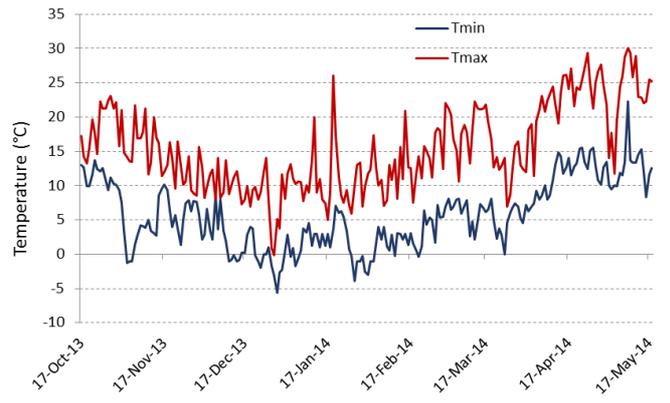


Figure 5



A



B

Figure 6