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

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

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

Introduction

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

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

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

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

Statistical Analyses

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

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

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

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

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

Results and Discussion

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

Storage at -20°C

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

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

Storage at constant temperatures

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

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

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

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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≤0.05, as the interval between lower and upper confidence limits does not
463 include zero.
464
465

Figure captions

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

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

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

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

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

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

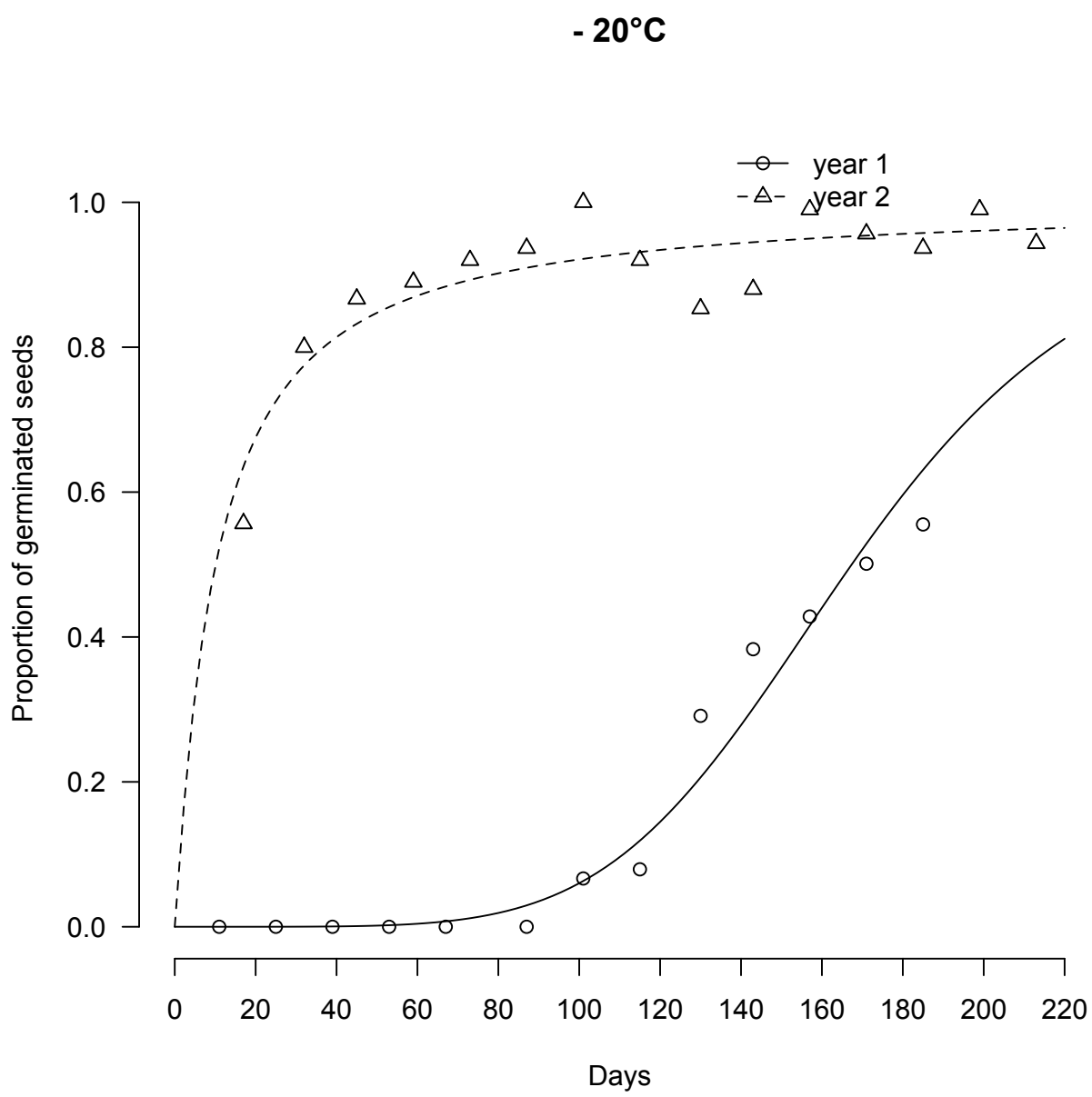

- 20°C
year 1
year 2
Proportion of germinated seeds
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
140
160
180
200
220
Days

Figure 1

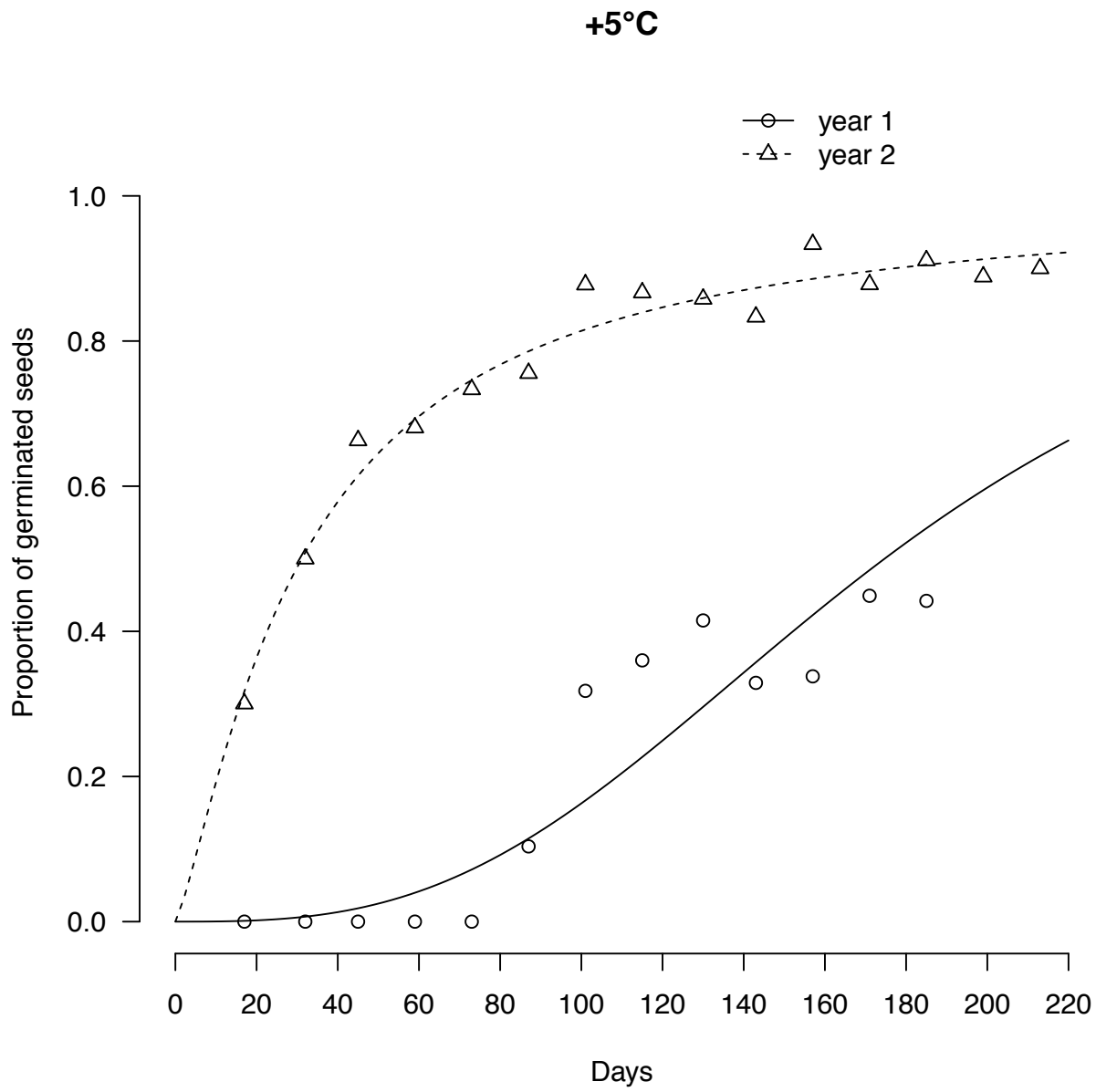

+5°C
year 1
year 2
Proportion of germinated seeds
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
140
160
180
200
220
Days

Figure 2

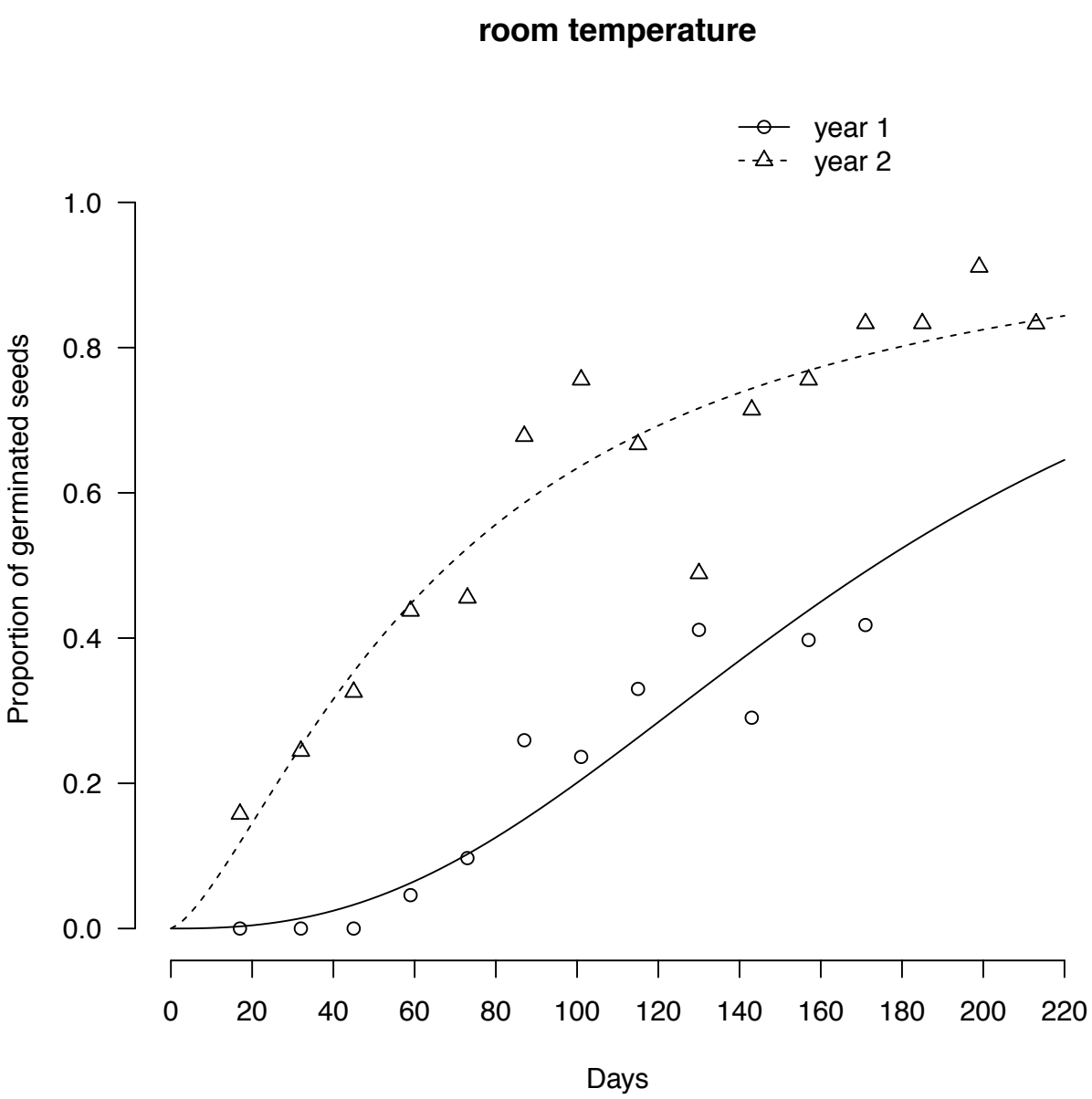

room temperature
year 1
year 2
Proportion of germinated seeds
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
140
160
180
200
220
Days

Figure 3

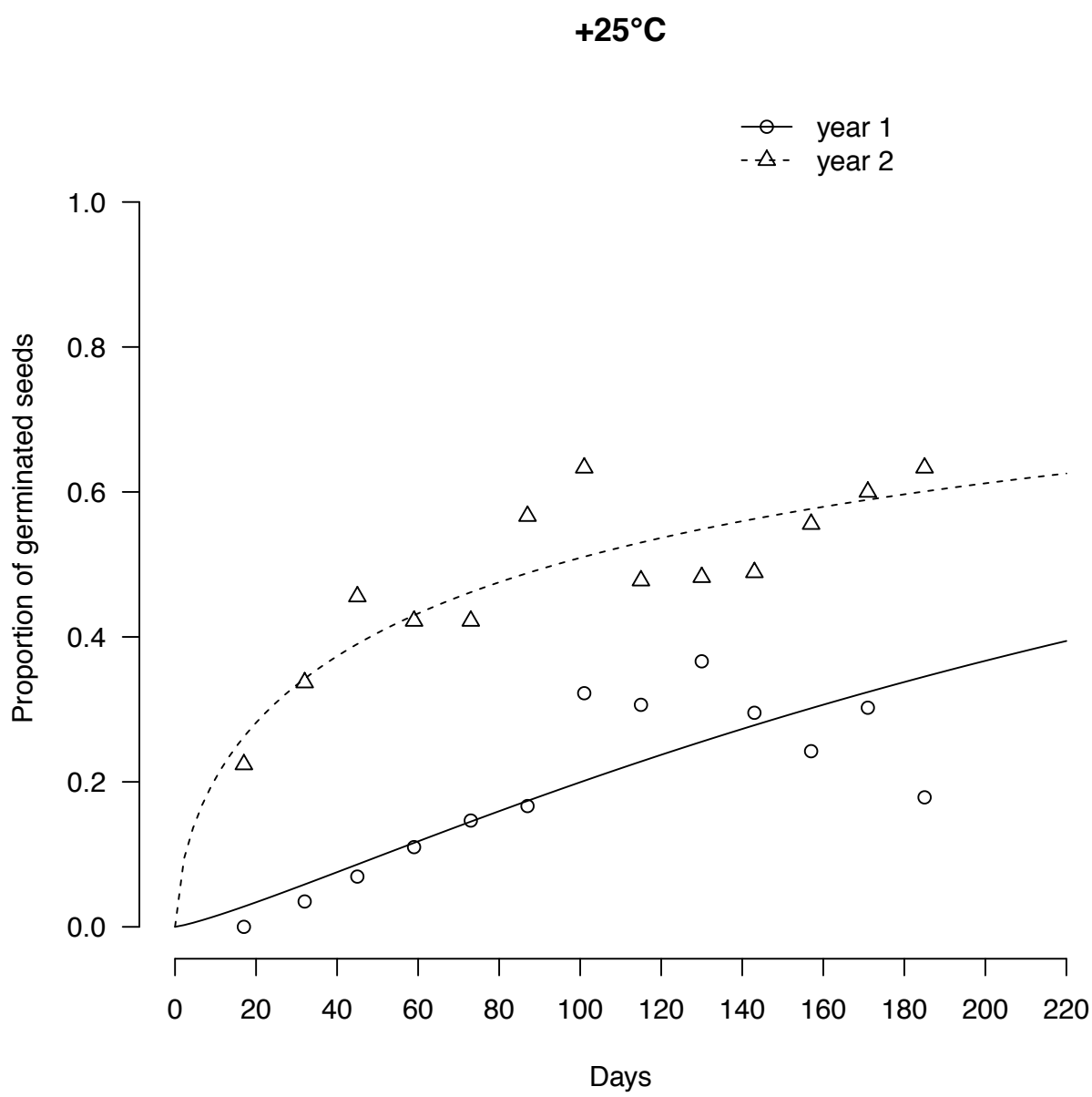

+25°C
year 1
year 2
Proportion of germinated seeds
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
140
160
180
200
220
Days

Figure 4

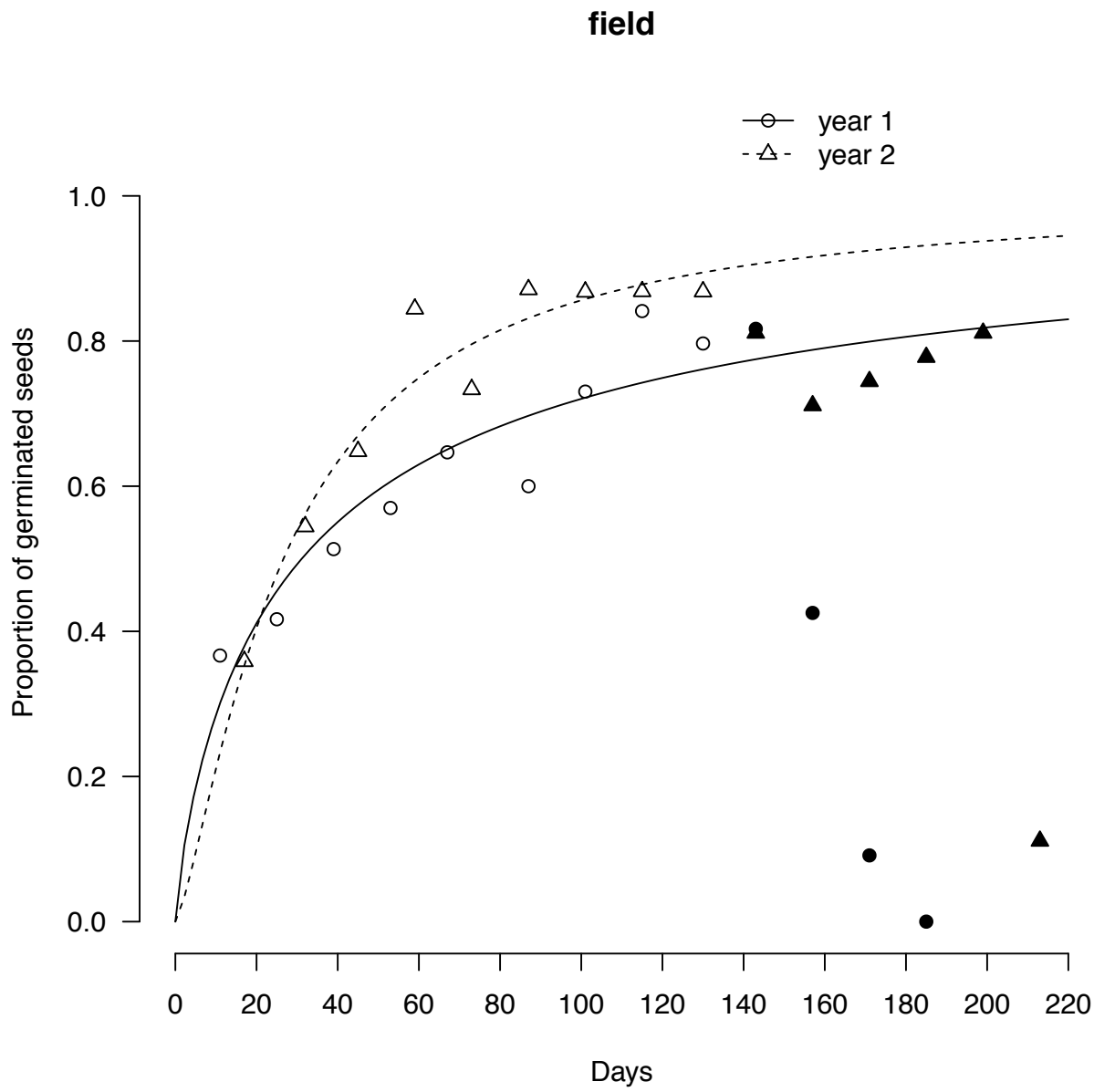
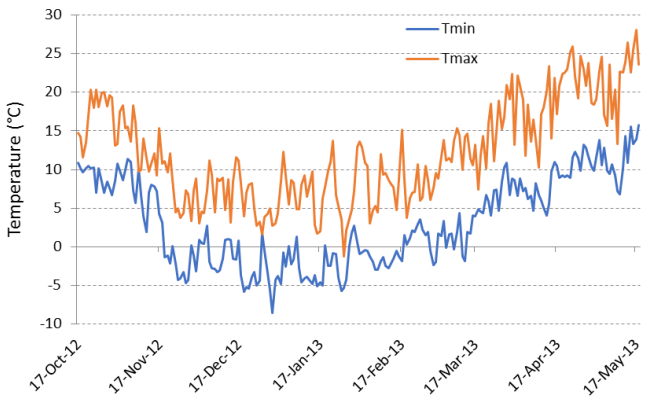
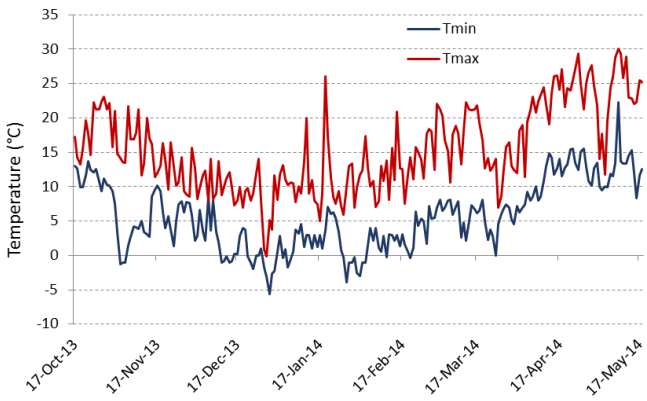

field
year 1
year 2
Proportion of germinated seeds
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
140
160
180
200
220
Days

Figure 5


Tmin
Tmax
Temperature (°C)
30
25
20
15
10
5
0
-5
-10
17-Oct-12
17-Nov-12
17-Dec-12
17-Jan-13
17-Feb-13
17-Mar-13
17-Apr-13
17-May-13

A


Tmin
Tmax
Temperature (°C)
35
30
25
20
15
10
5
0
-5
-10
17-Oct-13
17-Nov-13
17-Dec-13
17-Jan-14
17-Feb-14
17-Mar-14
17-Apr-14
17-May-14

B

Figure 6