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High temperatures affecting weed seeds

Title:

Effect of short-duration high temperatures on weed seed germination

Vidotto F., De Palo F., Ferrero A.

Università di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari. Via Leonardo da Vinci, 44. 10095 Grugliasco - Italy

francesco.vidotto@unito.it
fernando.depalo@unito.it
aldo.ferrero@unito.it

Corresponding author: F. Vidotto. tel +39 011 6708781   fax +39 011 2368781

1 **Summary**

2 Thermal soil disinfestation techniques are effective reducers of weed seedbank and

3 weed emergence. Two experiments (Exp 1 and 2) were conducted to test the effect of

4 brief exposure to varying temperatures on the seed germination of *Amaranthus*

5 *retroflexus*, *Echinochloa crus-galli*, *Galinsoga quadriradiata*, *Portulaca oleracea*,

6 *Setaria viridis*, and *Solanum nigrum*. To this end, species seeds were moistened with

7 loamy-sand soil and placed into test tubes. The tubes were heated rapidly and then

8 cooled by dipping them into a hot water bath until target temperatures were achieved.

9 Exp 1 temperatures ranged between 55 and 85°C at 5°C intervals and Exp 2 ranged

10 between 48 and 86°C at 2°C intervals. Thereafter, the tubes were dipped into a cooling

11 (1°C) water bath. Exposure to target temperatures ranged between 2 and 5 s. Soil

12 temperatures were monitored using embedded thermocouples. A log-logistic dose-

13 response model described the effect of heating on seed germinability; temperatures

14 required for 99% reductions were calculated. Based on the predictive model equation

15 used, weed species' germination sensitivity to high temperature exposure can be ranked

16 as follows: *E. crus-galli* (79.6°C), *S. viridis* (75.8°C), *S. nigrum* (74.6°C), *P. oleracea*

17 (72.2°C), *A. retroflexus* (70.9°C), and *G. quadriradiata* (68.1°C). The interval between

18 no effect to complete seed devitalisation occurred at temperatures varying from 6.5 to

19 15.7°C. Seed size and weight varied directly with heat tolerance. Study results not only

20 inform the timing and optimal adjustment for effective thermal soil treatment, but also

21 demonstrate a relatively simple and generalizable methodology for use in other studies.

22

23 Keywords: dose-response model, heat tolerance, seed germination, thermal weed

24 control, seed devitalisation, soil steaming

25

Introduction

Soil thermal treatments can have strong effects on the survival and harmfulness of several soil-borne organisms, including fungi, nematodes, as well as weed seeds and vegetative propagules. Soil heating has a long agricultural history and has occasionally been utilized. Recently, it has again caught the attention of researchers, especially following the phase-out of methyl bromide, which has long been the most common fumigant for soil disinfestation, particularly in high-value crops (Van Loenen *et al*., 2003; Bàrberi *et al*., 2009).

Many techniques have been developed to transfer thermal energy to soil. Generally, they rely on two concepts—the use of solar energy (Horowitz *et al*., 1983; Linke, 1994) and steam (Kolberg & Wiles, 2002; Melander & Jørgensen, 2005; Bàrberi *et al*., 2009; Peruzzi *et al*., 2012). Solar energy and steam reduce weed emergence from the soil seedbank through exposure to moderate temperatures for long periods (44-55°C for up to 6 weeks) and to high temperatures for short periods (90-100°C for just minutes), respectively (Linke, 1994; Bàrberi *et al*., 2009). Several factors during soil heating are considered key to germination reduction: maximum temperature attained (Thompson *et al*., 1997; Melander & Kristensen, 2011), heat duration (Van Loenen *et al*., 2003), soil moisture and seed water content (Egley, 1990), seed structure, anatomy and morphology (e.g., size, seed coat) (Horowitz & Taylorson, 1984), and seed dormancy dynamics (Thompson *et al*., 1997). The relative importance of any individual factor is difficult to assess, but maximum temperature and heat duration are considered foremost to seed germination reduction.

Overall, much of the literature assumes an inverse relationship between temperature and duration. For example, Dahlquist *et al*. (2007) found that the duration of exposure to heating to obtain complete mortality varied from 0.17 h at 70 °C to 672 h at 39 °C .

Despite these points of general agreement, views differ as to the importance of the temperature × duration of exposure interaction. Thompson *et al*. (1997) found this interaction was often erratic, that maximum temperature was generally more important than duration of exposure, and that temperatures between 50 and 80°C were critical to reaching seed death. Then, in a study that used laboratory-based soil steaming, Melander & Jørgensen (2005) found that in *Lolium perenne* L., *Brassica napus* L., and *Capsella bursa-pastoris* (L.) Medicus seedling emergence after different durations of

58   steaming could be described by a dose-response function, with duration of steaming

59   representing the dose and seedling emergence the response.

60   In all the studies mentioned above, seeds were exposed to target temperatures only after

61   undergoing a heating phase above the target temperature. The duration of that heating

62   phase varied greatly—from as little as 50 s (Melander & Kristensen, 2011) to 30 min

63   (Dahlquist *et al*., 2007), but usually this information is not provided. Similarly, the

64   cooling phase duration between the target temperature and initial temperature is largely

65   variable and it is often not noted in these studies. When it was reported, it ranged

66   between 4 min (Melander & Kristensen, 2011) and 20 min (Melander & Jørgensen,

67   2005).

68   It is known that both seed and soil moisture influence seed susceptibility to heating

69   (Mas & Verdù, 2002; Verdù & Mas, 2004). Soil moisture at levels near field capacity

70   yielded, in general, high heating efficiency values via steaming disinfestation methods

71   (Gay *et al*., 2010*a*).

72   Soil as a seed-heating medium seems to be the method of choice to simulate field

73   conditions in laboratory studies even though non-soil seed-heating mediums are

74   available and have been used (Mas & Verdù, 2002; Verdù & Mas, 2004). In any case,

75   formation of some amount of thermal system inertia is unavoidable, and at times, can

76   result in long heating and cooling phases. These effects have limited the information

77   available on the importance on weed seed devitalisation of the sole effect of high

78   temperatures during soil thermal treatment. This information should also be evaluated

79   considering seed size, which has been reported as one of the traits that may explain

80   differences in sensitivity to thermal treatments among different species.

81   This study has two objectives: (1) to determine the effect of very short exposure of

82   weed seeds to a wide range of temperatures, and (2) to determine the relationship

83   between seed size and species' tolerance to short duration temperature exposure. The

84   study was manly designed to provide information that is relevant for soil treatment with

85   high temperatures for short periods, as in the case of soil steaming. The study was

86   carried out by exposing seeds to different temperatures while dispersed in soil. Ideally,

87   this method would also be suitable for testing the interactive effect between duration of

88   exposure × temperature in further studies.

89

90

91

Materials and methods

Two experiments (Exp1 and Exp 2) were carried out in 2009 and 2010 in a glasshouse at the University of Turin (Italy). The seeds of six weed species were treated at different thermal levels using water baths to determine the effect of maximum temperature on seed viability. During Exp 1, seven target temperatures were tested, ranging from 55 to 85°C at 5°C intervals. In Exp 2, the seeds were exposed to 20 target temperatures between 48 and 86°C at intervals of 2°C. Apart from the target temperatures, the two experiments were executed using the same methodology. Exp 1 was conducted to define the temperature range required to reduce germination percentage to nil. Exp 2 was carried out 120 days after Exp 1.

Six weed species, representing the most common weeds in Italian horticultural fields, were included in the study: *Amaranthus retroflexus* L., *Echinochloa crus-galli* (L.) P. Beauv., *Galinsoga quadriradiata* Cav., *Portulaca oleracea* L., *Setaria viridis* (L.) P. Beauv., and *Solanum nigrum* L. Save for *G. quadriradiata*, whose seeds were collected from NW Italy, all seeds were purchased from Herbiseed Corp. (Berkshire, UK). Exp 1 and Exp 2 utilised the same seed lots, except for *S. nigrum*, which necessitated that a new seed lot be used in Exp 2 due to low germination percentage (<60%) of untreated seeds in Exp 1. Before the initiation of the experiments, all seeds were stored in the dark at 4°C.

Seed preparation

Except for *P. oleracea*, for all species and target temperature 10 ml Pyrex® glass test tubes (16×100 mm) were filled with 3 g of loamy sand soil that had been pre-moistened to 11.2% water content (corresponds to 80% field capacity) and mixed with 30 seeds. The soil used in the study contained 85% sand, 8% silt and 7% clay and it was collected at 0-30 cm depth from a horticultural farm in NW Italy (45.000766° N; 7.720452° E). The amount of seeds included in each tube was defined in order to assure the recovery of at least 20 seeds after the thermal treatment.

Each tube was then fitted with a screw cap to avoid humidity loss. All tubes processed in this manner were prepared 24 h prior to heat treatment to allow seed equilibration with the soil. During this phase, the tubes were stored in the dark at 4°C to prevent seed germination.

As the soil used for treatment testing was naturally rich in *P. oleracea* seeds (pers. observ.), *P. oleracea* seeds were enclosed *sans* soil in bags (2×2 cm) made of

126   nonwoven fabric, and then inserted into tubes and soil was added to evenly coat the

127   bags. Also in this case, four (Exp 1) or three (Exp 2) glass test tubes were prepared for

128   each target temperature. Given the high speed of *P. oleracea* seed germination, these

129   tubes were prepared a mere two hours before treatment.

130   Images were taken of 30 seeds of each species, from the same seed lots as those used in

131   the trial, using a flatbed scanner (Mustek P 3600 A3 Pro) at a resolution of 600 dpi. The

132   images were processed using image analysis software ImageJ (Schneider *et al*., 2012)

133   and measurements were taken and recorded of the length and width of each seed.

134   Finally, three samples of 300 seeds for each species were counted and weighted in order

135   to assess the 1000-seed weight.

136

137   <u>Temperature recording</u>

138   Soil temperatures were monitored using T-type (copper-constantan) thermocouples

139   (probe tubes) connected to a data logger (National Instruments® FP-TC-120) fitted into

140   the test tubes. The thermocouples were inserted into probe tubes through a small hole

141   drilled in the test tube screw-cap, and their tip was placed in the centre of the soil

142   volume by adjusting the connecting wire length. Temperatures were measured and

143   recorded continuously every 2 s from initiation of treatment to end. Temperature

144   readings were also continuously displayed on a portable PC to obtain real-time

145   information of probe tube thermal status. A series of T-type thermocouples were also

146   used to monitor all water bath temperatures. An additional thermocouple connected to

147   the same logging system was immersed simultaneously with the tubes to record the

148   exact time of immersion in all water baths. Before treatment application, all

149   thermocouples were calibrated using a PT100 temperature probe with 0.1°C resolution.

150

151   <u>Thermal treatment</u>

152   Heat treatments were applied using three water baths (REF, HOT, COLD) in which the

153   tubes were sequentially dipped. The tubes were arranged in polypropylene test-tube

154   racks equipped with a handle and moved simultaneously between baths. Temperature

155   was monitored by an average of the values of two probe tubes in each rack. First, the

156   tubes were dipped into the 23 °C REF bath (reference standard for the study) after

157   moisture equilibration at 4 °C and 30 min before thermal treatment. This bath was

158   comprised of a 70-litre plastic tank heated by an immersion circulator (Julabo ED

159   1000 W). Once thermal equilibration was attained in REF, the tubes were dipped into a

160 second water bath (HOT). This bath consisted of a five-litre stainless steel tank set 3 °C

161 above the target temperature to quickly heat the soil and was kept constant during

162 treatment with a laboratory immersion circulator (Julabo ED 2000 W) inserted into the

163 tank. The tank water level was fixed exactly to submerge the tubes up to 2 cm below

164 their caps; extra water was added as needed to compensate for evaporation. Transfer of

165 the tubes to the third water bath (COLD) occurred immediately upon when the target

166 temperature of the soil was reached. This bath was set to approximately 1 °C for quick

167 cooling and to allow the soil to return to temperature of about 23 °C. The tubes were

168 then transferred back to the REF bath.

169 For each species and target temperature, four (Exp 1) or three replications (Exp 2) were

170 considered and a single test tube represented the experimental unit and one replication.

171 Four (Exp 1) or three (Exp 2) untreated tubes for each species were maintained in the

172 REF bath for the entire duration of the treatment as controls. The treatment structure

173 was a two-way factorial, with factors represented by species (6 levels in both Exp 1 and

174 Exp 2) and target temperature (8 levels in Exp 1, 21 levels in Exp 2). The treatments

175 were arranged according to a completely randomized design.

176 Within a few minutes of reaching the reference temperature following the second

177 passage in the REF bath, the mixture of seeds and soil was pulled from the tubes and the

178 seeds manually separated from the soil. From each tube, 20 randomly selected seeds

179 were placed in a Petri dish (9 cm diameter) lined with two No. 1 Whatman filter papers

180 (Whatman International Ltd.) to which 6 ml of deionized water was added. The Petri

181 dishes were incubated in a growth chamber at a constant temperature of 25 °C and

182 16h/8h of light/dark cycles for 20 days. Preliminary tests showed that germination was

183 observed after 10 days for all weed species (data not shown). Germinated seeds were

184 counted daily and water was added as needed to preserve the initial moisture level.

185 The greatest portion (always exceeding 90%) of non-germinated seeds had cracked seed

186 coats after germination test and were assumed dead. For each species, tetrazolium test

187 was performed on a small portion of intact seeds treated in Exp 1 and none were viable

188 (data not shown). The test was not conducted in *P. oleracea* and *A. retroflexus*, as it was

189 not possible to pierce the seed coat without destroying the embryo. In a similar study

190 conducted by Dahlquist *et al.* (2007) percentage of viability in heat-treated seeds was <

191 1% in *E. crus-galli* and *S. nigrum.* Non-germinated, viable seeds were not accounted for

192 in this study and non-germinated seeds were all assumed dead.

193   The germinability, expressed as percentage of germination, refers to the percentage of

194   seeds that produced regular seedlings (ISTA, 2009). Germination data obtained from the

195   untreated tubes maintained in the REF bath during the treatment application represented

196   the initial status of germination of each seed lots at the time the experiment was carried

197   out.

198

199   <u>Data analysis</u>

200   Data were first subjected to ANOVA to test the effect of species, target temperature and

201   its interaction on germination. The analysis was conducted separately for Exp 1 and Exp

202   2 and was performed using the function *lm* of the open source programme and

203   environment R.

204   The germination data for each test species were then fitted to a 3-parameter log-logistic

205   regression model (Streibig *et al*., 1993; Ascard, 1994; Ascard, 1995; Seefeldt *et al*.,

206   1995; Knezevic *et al*., 2007):

207

208
$$Y = \frac{d}{1 + \exp[b(\log x - \log e)]} \tag{1}$$

209

210   where Y is the percentage of germination, d is the upper limit, and b is the relative slope

211   at the point of inflection e. Having recorded the actual temperature of the tubes during

212   the entire thermal treatment, the recorded maximum temperature was set as the

213   independent variable x. In any case, the recorded maximum temperature always differed

214   from the target temperature by less than 0.5 °C.

215   As germination of *G. quadriradiata* at low target temperatures was enhanced in

216   comparison to the control, data of this species were fitted to the following Brain-

217   Cousens hormesis model (Brain & Cousens, 1989; Schabenberger *et al*., 1999):

218

219
$$Y = \frac{d + fx}{1 + \exp[b(\log x - \log e)]} \tag{2}$$

220

221   where the linear term f considers the stimulatory effects at sub-lethal temperatures.

222   Both models do not include an estimate for a parameter representing a lower asymptote

223   of Y, as in this study the percentage of germination fell to zero at high temperatures in

224   all species. In contrast, no constraints were included in the estimate of the higher

225   asymptote *d* (except it had not to be higher than 100 which is equivalent to 100%

226   germination).

227   Model fitting was performed using the function *drm* of the add-on package *drc* of the R

228   software (Ritz & Streibig, 2005; Ritz *et al*., 2006); this package has been developed

229   mainly to perform non-linear regression analysis on bioassay studies. As the initial

230   status of germination was lower than 100% and variable among species, model fitting

231   was performed including the percentage of germination as response variable and the

232   total number of seeds included in the germination test (always 20) as value for the

233   argument *weights* of the function *drm* and specifying the case "binomial" for the

234   argument *type* (Ritz & Streibig, 2012). With this set of instructions, the initial status of

235   germination was considered in the model fitting and the *drm* function gave correct

236   estimations of ET_z values (see below).

237   Data from Exp 1 and Exp 2 were first analysed separately and then pooled to fit into a

238   single model. The *anova* function of R was used to compute a likelihood ratio test to

239   verify if the pooled dataset was significantly better explained by two curves fitting Exp

240   1 and Exp 2 data separately than by a single model fitting all data.

241   With the parameters estimated, the equations allowed to calculate the temperature ET_z

242   (Melander & Jørgensen, 2005) required to obtain a certain level of germination

243   reduction in comparison to untreated seeds. ET_z values and their upper and lower

244   confidence limits (α=0.95) were estimated using the function *ED* of the package *drc*. In

245   this study, ET_z was estimated for $z = 10\%$, 90%, and 99%, which correspond to

246   temperatures that cause 10, 90 and 99% reduction in germination, respectively. For each

247   species and experiment, target temperatures were considered "ineffective" if lower than

248   ET_{10}. A reduction on the percentage of germinated seeds after thermal treatment of 90%

249   (ET_{90}) was considered as a standard reference threshold in previous studies (Hannson &

250   Ascard, 2002; Hannson & Mattsson, 2002). ET_{99} can be regarded as a threshold for

251   complete seed devitalisation.

252   For each species, the function *SI* of the package *drc* was used to test for differences

253   between ET_z calculated from Exp 1 and Exp 2.

254   To evaluate the relationship between seed size and heating tolerance, the values of ET_{99}

255   were plotted as a function of the variables seed length×width and 1000-seed weight.

256   When significant differences in ET_{99} calculated from Exp 1 and Exp 2 were found for

257   some species, only the estimates obtained from Exp 2 were used.

258

259

Results

Temperature dynamics

Thermal treatment can be divided into four phases: a) thermal equilibration at the standard reference temperature (23 °C); b) heating to reach the target temperature; c) cooling, and d) re-stabilization to the standard reference temperature (Fig. 1). Phase b) (heating) began when the temperature recorded by the probe tubes increased by more than 1 °C relative to the standard reference temperature. The time between immersion in the HOT bath and the beginning of phase b) was relatively short in all conditions, as it ranged from 1 to 5 s. Duration in both phase b) and c) varied as a function of target temperature. When exposed to the lower temperatures, only a short time was needed to heat and cool the seeds as opposed to the longer time required at higher target temperatures. Among the species, the average heating phase lasted for 63 s (target temperature 50 °C) to 83 s (target temperature 86 °C) while the cooling phase duration ranged between 33 s (target temperature 50 °C) and 54 s (target temperature 86 °C). The tubes were removed from the COLD bath and transferred to the REF bath exactly when their temperature dropped to 23 °C. Although temperatures continued to fall after immersion in the REF bath for another 30 s and to a low of about 15 °C as recorded by the probe tubes, they eventually rose to the standard reference temperature. This stabilization process (phase d) was a condition of the thermal inertia of the system formed by the tubes and soil.

The methodology used allowed exposure to the target temperature for between 2 s and 5 s, with an average of 2.7 s. Moreover, the difference between the actual and target temperature values was always lower than 0.5 °C.

Effects of thermal treatment on percentage of germination

With the exception of the *S. nigrum* seeds used in Exp 1, the initial percentage of germination of untreated seeds was always at least 60% (Table 1). Results of ANOVA indicated that both species and target temperature had significant effect on the proportion of germinated seeds (data not shown). Also the interaction species × target temperature was significant, indicating that the effect of temperature varied according to the species. This can be explained by the behaviour of *G. quadriradiata*, which germination was enhanced at lower temperatures (see below). For all species, the variation of proportion of germinated seeds as a function of maximum achieved

temperature was well described by the selected regression models in both Exp 1 and Exp 2 (Table 2). The temperature interval gave good coverage of the different responses from no effect to complete seed devitalisation (Fig. 2). The target temperatures gave intermediate responses around the point of inflection of the estimated response curves. This was more evident in Exp 2, where the responses were more evenly distributed between the upper asymptote and zero, which allowed for a more reliable fit.

In general, ET_{10} was very close to 60 °C for the majority of the species. *E. crus-galli* was the only species which deviated strongly from this behaviour, showing an ET_{10} of 68.6 and 73.5 °C in Exp 1 and Exp 2, respectively (Table 3). The transition between ET_{10} and ET_{99} occurred in a temperature range from 6.5 °C (*G. quadriradiata*) to 15.7 °C (*S. viridis*).

G. quadriradiata seeds were the most affected by thermal treatment (Table 3). Even though germination was enhanced by exposure to temperatures between 50 and 56 °C, germination quickly decreased compared to the untreated at temperatures greater than 58 °C. Two separate curves for Exp 1 and Exp 2 provided a significantly better explanation than a single curve fitting all the data from the two experiments (Table 2). This was mainly due to a slightly stronger stimulatory effect at sub-lethal temperatures and a higher sensitivity to high temperatures observed in Exp 2. Consequently, only ET_{10} was similar in the two experiments while ET_{90} and ET_{99} were always significantly higher in Exp 1 (Table 3). Germination dropped to negligible levels after exposure at temperatures above 70.4 °C (Exp 1) and 65.8 °C (Exp 2).

In *A. retroflexus* regression analysis revealed that results from Exp 1 and Exp 2 were significantly different (Table 2). This might be due to a higher initial percentage of germination of untreated seeds in Exp 1 that resulted in a higher upper asymptote and in a higher temperature at the point of inflection between the upper asymptote and zero. This may explain the fact that both ET_{10} and ET_{90} were significantly greater in Exp 1 while ET_{99} was the same between the two experiments, and averaged 70.9 °C (Table 3).

A similar behavior was observed in *P. oleracea*, but in this case the highest percent germination were observed in Exp 2. Significant differences between the two experiments were recorded for ET_{90} only; ET_{99} averaged 72.2 °C.

In the case of *S. nigrum*, Exp 1 and Exp 2 were performed using different seed lots given that the germinability of untreated seeds in Exp 1 was less than 60%. The two curves describing Exp 1 and Exp 2 data differed significantly (Table 2). Nevertheless,

326   differences between ET_z calculated from the two experiments were significant for ET_{10}

327   only. In particular, ET_{99} averaged 74.5 °C (Table 3).

328   In *S. viridis*, the slightly higher germination of Exp 1 untreated seeds resulted in an

329   overall significant difference in the two curves fitting Exp 1 and Exp 2 data (Table 2)

330   even though the computed ET_{10}, ET_{90}, and ET_{99} values never differed significantly

331   between the two experiments and averaged 60.5, 69.95, and 75.7 °C, respectively

332   (Table 3).

333   In *E. crus-galli*, germinability recorded in Exp 2 followed an unexpected course as it

334   initially declined steadily from 75% to 58% in temperatures ranging from about 48 to

335   58 °C. Afterwards, germinability rose to 87% at 66 °C, then finally dropped to values

336   near zero for temperatures above 80 °C (Figure 2). This behavior, coupled with an

337   overall higher tolerance to heating observed in Exp 2, caused the data obtained in the

338   two experiments to not be describable by a single curve (Table 2). Accordingly, ET_z

339   values always differed significantly between the two experiments. In any case, the

340   calculated ET_{10} values indicated that *E. crus-galli* germinability started to be affected at

341   temperatures between 68.6 and 73.5 °C while ET_{99} values indicated that germinability

342   started to be negligible at temperatures ranging from 77.8 to 81.4 °C (Table 3).

343

344   <u>Relationship between seed size and tolerance to thermal treatment</u>

345   The smallest seeds where those of *P.oleracea*, which showed a length×width of 2.49

346   mm^2 and a 1000-seed weight of 0.118 g. At the opposite, *E. crus-galli* showed the

347   biggest seeds, with a length×width of 26.60 mm^2 and a 1000-seed weight of 1.97 g.

348   Seed size and ET_{99} values varied in direct proportion. While the six species considered

349   in this study is insufficient to allow full and evenly distributed coverage of all possible

350   seed sizes, the results indicated that seed size, expressed as length×width or 1000-seed

351   weight, and tolerance to thermal treatment may be described by logarithmic or linear

352   model, respectively (Fig. 3). In particular, the increase of 1000-seed weight by 1 g

353   resulted in an average increase of ET_{99} by about 6.6 °C.

354

355   **Discussion**

356   The methodology adopted in this study tested the effect of short exposure to different

357   temperatures on germination of weed seeds dispersed in a small amount of soil. With

358   the adopted methodology, some amount of thermal inertia was unavoidably introduced

359   into the study. As a consequence, additional time was required to allow the seeds to

360 reach the target temperature and to cool them to the standard reference temperature

361 (23 °C). Both these heating and cooling phases were significantly shorter than those

362 reported in previous studies. Further reduction of the heating and cooling phases could

363 be accomplished by treating the seeds without their dispersal into soil. Although, data

364 acquired under such conditions is limited practically, as real soil thermal treatments are

365 always affected by discrete heating and cooling phases (Gay *et al*., 2010*a*,*b*).

366 Complete seed devitalisation (i.e., the temperature causing at least 99% germination

367 reduction) was achieved in the different species at temperatures spanning 64 °C to

368 80 °C. In particular, *E. crus-galli* showed itself to be the least heat-susceptible, which

369 agrees with results from Melander & Jørgensen (2005) and Bàrberi *et al*. (2009). In

370 contrast, Dahlquist *et al*. (2007) reported that *E. crus-galli* was more susceptible to heat

371 than *S. nigrum* and *P. oleracea*. It should be noted, however, in Dahlquist *et al*. (2007)

372 the seeds that underwent thermal treatment were previously moistened by dipping them

373 in water and then placing between moist paper towels for 24 h. This might have caused

374 the seeds to have higher moisture content which in turn lead to a higher susceptibility to

375 thermal treatment (Egley, 1990) .

376 The higher heat tolerance of *E. crus-galli* found in our study can be partly attributed to

377 seed structure; the caryopsis is protected by its glumellae (adheres to caryopsis), sterile

378 floret, the second glumae, and partially by the first glumae (Maun & Barret, 1986). This

379 structure persists in seeds harvested and stored as was true of those used in this study.

380 However, in field conditions, both the glumae and sterile floret are gradually lost while

381 the seeds stay in the soil. It seems reasonable to hypothesize that the actual average

382 tolerance to soil heating by *E. crus-galli* seeds under field conditions is lower than that

383 observed in our study. It is also possible that the seed structure may have played a role

384 in the erratic behaviour of seed germinability observed after seed exposure to

385 temperatures in the 48 to 66 °C range.

386 Seed size may also play a role in the response to thermal treatments. Among the species

387 in this study, *E. crus-galli* had the biggest seeds and showed the highest tolerance to

388 heating. In general, the model predicted a higher ET_{99} for *P. oleracea* based on its seed

389 size and seed weight. Possible reasons for this lower sensitivity may relate to the

390 appended seed soil permanence before treatment (2 h *versus* 24 h), which may have

391 resulted in a reduced seed moisture content though the nonwoven bag enclosures

392 relative to the other species. Nonetheless, this valuable result highlights the fact that

393 conditions other than the tested temperatures may influence study outcomes. For this

394 reason, study results should be considered carefully, and attention should be paid to

395 methodology.

396 The response of seed germinability to thermal treatment was described using logistic

397 regression models. Similar dose-response relationships were found by others

398 investigating thermal weed control from several directions: laboratory steaming

399 experiments (Melander & Jørgensen, 2005), hot water effects on weed seedling studies

400 (Hansson & Ascard, 2002; Hansson & Mattsson, 2002), and flame-weeding

401 investigations (Ulloa *et al.*, 2010; Ulloa *et al.*, 2012).

402 For all species, results from Exp 1 and Exp 2 were significantly different, likely

403 consequent to the lower initial status of germination of the seeds used in Exp 2. This

404 may be due to the 120-days interval between Exp 2 and Exp 1 during which a certain

405 amount of germinability might have been lost. In the case of *S. nigrum*, the observed

406 behaviour was exactly contrary; however, its variation is attributed to the different seed

407 lots used in Exp 1 and Exp 2.

408 Significant differences in ET_z values reflect Exp 1 and Exp 2 dissimilarities in only

409 some cases. In particular, ET_{99} values between the two experiments were significantly

410 different for *E. crus-galli* and *G. quadriradiata* only. However, even for these species,

411 the ET_{99} values estimated from the two experiments differed by less than 5 °C (3.6 °C

412 and 4.6 °C in *E. crus-galli* and *G. quadriradiata*, respectively). Differences between the

413 two experiments could also be attributed to the higher number of data points in Exp 2

414 and to the different temperature increments tested.

415 *G. quadriradiata* germination data were described using a model that included a

416 parameter that took in account the stimulatory effect at sub-lethal temperatures. This

417 phenomenon is well known for dose-response bioassays, including studies dealing with

418 herbicides (Brain & Cousens, 1989; Cedergreen *et al.*, 2005). Some plant species in

419 natural fire-prone environments exhibit similar behaviour (Read *et al.*, 2000; Delgado *et*

420 *al.*, 2001), however little information exists on annual weeds in agricultural settings

421 (Vidotto *et al.*, 2009).

422 Germination stimulation post heat exposure can result from several cooperating

423 phenomena including increased water and gas permeability of the seed and seed coat

424 inhibitor denaturation (Van Staden *et al.*, 2000; Paula & Pausas, 2008). Considering that

425 different portions of the soil volume can reach sub-lethal temperatures, the overall

426 efficacy of soil thermal treatment could theoretically be lower in species for which

427 germination is stimulated by treatment itself. The size and distribution of soil regions

428   that reach sub-lethal temperatures can vary according to the adopted soil heating

429   methodology and can be largely influenced by soil texture and the presence of soil

430   aggregates, especially in steaming (Melander & Jørgensen, 2005; Vidotto *et al*., 2009).

431   The results of this study can be relevant for soil thermal treatments in general, and may

432   be useful for steaming in particular, as this technique allows the attainment of high soil

433   temperatures for short intervals.

434   For the weeds included in this study, it appears exposure to temperatures of 80 °C for

435   few seconds is sufficient to obtain satisfactory control. This information is relevant for

436   fine-tuning the use of steam in thermal soil treatments and may further reduce the

437   energy requirement of this technique. This can be in particular useful for steam

438   application techniques based on localised injections for short durations, as in the case of

439   band steaming (Ascard *et al.,* 2007) or sub-superficial soil steaming (Gay *et al*., 2010*a*;

440   Gay *et al*., 2010*b*). Caution must be adopted when considering real field treatment and

441   conditions. Both heating and cooling phases are believed to last longer than observed in

442   this study, which suggests that the actual efficacy could be higher than through simple

443   extrapolation. It may even have the potential to compensate for the presence of soil

444   regions reaching sub-lethal temperatures due to the effect of soil aggregates. Moreover,

445   laboratory experiments oftentimes do not accurately reflect the potential effect of soil

446   organisms and chemicals on seed decay (Stapleton & DeVay, 1986; Stapleton *et al*.,

447   2000; Dahlquist *et al*., 2007); such phenomena would suggest this study may

448   overestimate the maximum temperature needed to devitalize the weed seeds.

449   The results of this study are relevant also for solar soil heating, since in this technique

450   the stimulatory effect of sub-lethal temperatures may play an important role. During

451   solar soil heating, in fact, the temperatures attained may be often in a range

452   corresponding to that at which stimulations has been observed in our study. For species

453   behaving similarly to *G. quadriradiata* this may result in increased emergence after

454   treatment. Although, the stimulation may be severely reduced or nullified by the long

455   duration of the exposure, as solar soil heating may require up to several weeks to be

456   effective, depending on the local weather, climate and soil moisture conditions

457   (Stapleton, 2000).

458

459   The methodology described and used here is relatively simple and demands little more

460   than basic laboratory equipment. Thus, it can be easily extended to the study of thermal

461   effects on other species seed viability and/or for media other than soil. Furthermore, this

462 study not only gives insight into the sole effect of temperature, but also it does not
463 exclude the fact that exposure duration impacts loss of seed germinability. Further
464 studies should build upon this information and analyse the effect of time exclusive of
465 temperatures above ET_{99} and focus on the range of temperatures that resulted in only a
466 partial reduction of seed germinability.

467 With this method, it will also be possible to study also the effects of others factors that
468 may affect seed germinability. For instance, the role of soil texture and moisture may
469 deserve to be investigated. The use of soil as medium for dispersing the seeds to be
470 exposed to different thermal conditions may also allow the study on the combined
471 effects of other techniques that may promote the effects of soil heating, such use the use
472 of KOH-activated soil steaming (Bàrberi *et al.*, 2009).

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References

Ascard J. (1994) Dose–response models for flame weeding in relation to plant size and density. *Weed Research,* **34,** 377–385.

Ascard J. (1995) Thermal weed control by flaming: biological and technical aspects. PhD Thesis. Swedish University of Agricultural Sciences.

Ascard J., Hatcher P.E., Melander B., Upadhyaya M.K. (2007) 10 Thermal weed control. In *Non-Chemical Weed Management: Principles, Concepts and Technology*, pp. 155-175. Eds M.K. Upadhyaya and R.E. Blackshaw. Wallingford, UK: CAB International.

Bàrberi P., Moonen A.C., Peruzzi A., Fontanelli M., Raffaelli M. (2009) Weed suppression by soil steaming in combination with activating compounds. *Weed Research,* **49,** 55–66.

Brain P., Cousens R. (1989) An equation to describe dose responses where there is stimulation of growth at low doses. *Weed Research*, **29,** 93–96.

Cedergreen N., Ritz C., Streibig J.C. (2005) Improved empirical models describing hormesis. *Environmental Toxicology and Chemistry*, **24,** 3166–3172.

Dahlquist R.M., Prather T.S., Stapleton J.J. (2007) Time and temperature requirements for weed seed thermal death. *Weed Science*, **55,** 619–625.

Delgado J.A., Serrano J.M., López F., Acosta F.J. (2001) Heat shock, mass-dependent germination, and seed yield as related components of fitness in *Cistus ladanifer*. *Environmental and Experimental Botany*, **46,** 11-20.

Egley G.H. (1990) High-temperature effects on germination and survival of weed seeds in soil. *Weed Science*, **38,** 429–435.

Gay P., Piccarolo P., Ricauda A.D., Tortia C. (2010a) A high efficiency steam soil disinfestation system, part I: physical background and steam supply optimisation. *Biosystems Engineering*, **107,** 74-85.

Gay P., Piccarolo P., Ricauda A.D., Tortia C. (2010b) A high efficacy steam soil disinfestation system, part II: design and testing. *Biosystems Engineering*, **107,** 194-201.

Hansson D., Ascard J. (2002) Influence of developmental stage and time of assessment on hot water weed control. *Weed Research*, **42,** 307–316.

Hansson D., Mattsson J.E. (2002) Effect of drop size, water flow, wetting agent and water temperature on hot-water weed control. *Crop Protection*, **21,** 773–781.

Horowitz M., Regev Y., Herzlinger G. (1983) Solarization for weed control. *Weed Science*, **31,** 170-179.

Horowitz M., Taylorson R.B. (1984) Hardseededness and germinability of Velvetleaf (*Abutilon theophrasti*) as affected by temperature and moisture. *Weed Science*, **32,** 111-115.

ISTA [International Seed Testing Association] (2009) *International Rules for Seed Testing*. 2009/1 edn. Bassersdorf, Switzerland: The International Seed Testing Association.

Knezevic S.Z., Streibig J.C., Ritz C. (2007) Utilizing R software package for dose-response studies: the concept and data analysis. *Weed Technology*, **21,** 840–848.

Kolberg R.L., Wiles L.J. (2002) Effect of Steam Application on Cropland Weeds. *Weed Technology*, **16,** 43–49.

Linke K.H. (1994) Effects of soil solarization on arable weeds under Mediterranean conditions: control, lack of response or stimulation. *Crop Protection*, **13,** 115-120.

Mas M.T., Verdù A.M.C. (2002) Effects of thermal shocks on the germination of *Amaranthus retroflexus*. Use of the EXCEL Solver tool to model cumulative germination. *Seed Science and Technology*, **30,** 299-310.

Maun M.A., Barret S.C.H. (1986) The biology of Canadian weeds. 77. *Echinochloa crus-galli* (L.) Beauv. *Canadian Journal of Plant Sciences*, **66,** 739-759.

Melander B., Jørgensen M.H. (2005) Soil steaming to reduce intrarow weed seedling emergence. *Weed Research*, **45,** 202–211.

Melander B., Kristensen J.K. (2011) Soil steaming effects on weed seedling emergence under the influence of soil type, soil moisture, soil structure and heat duration. *Annals of Applied Biology*, **158,** 194-203.

Paula S., Pausas J.G. (2008) Burning seeds: germinative response to heat treatments in relation to resprouting ability. *Journal of Ecology*, **96,** 543–552.

Peruzzi A., Raffaelli M., Frasconi C., Fontanelli M., Bàrberi P. (2012) Influence of an injection system on the effect of activated soil steaming on *Brassica juncea* and the natural weed seedbank. *Weed Research*, **52,** 140–152.

Read T.R., Bellairs S.M., Mulligan D.R., Lamb D. (2000) Smoke and heat effects on soil seed bank germination for the re-establishment of a native forest community in New South Wales. *Austral Ecology*, **25,** 48–57.

Ritz C., Cedergreen N., Jensen J.E., Streibig J.C. (2006) Relative potency in nonsimilar

dose–response curves. *Weed Science*, **54,** 407–412.

Ritz C., Streibig J.C. (2005) Bioassay analysis using R. *Journal of statistical software*, **12,** 1-22.

Ritz C., Streibig J.C. (2012) Dose response curves and other nonlinear curves in Weed Science and Ecotoxicology with the add-on package drc in R. URL www.bioassay.dk [accessed on 26 March 2013].

Schabenberger O., Tharp B.E., Kells J.J., Penner D. (1999) Statistical tests for hormesis and effective dosages in herbicide dose response. *Agronomy journal*, **91,** 713–721.

Schneider C.A., Rasband W.S., Eliceiri K.W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, **9,** 671-675.

Seefeldt S.S., Jensen J.E., Fuerst P. (1995) Log-Logistic analysis of herbicide dose-response relationship. *Weed Technology*, **9,** 218-227.

Stapleton J.J., DeVay J.E. (1986) Soil solarization: a non-chemical approach for management of plant pathogens and pests. *Crop Protection*, **5,** 190-198.

Stapleton J.J. (2000) Soil solarization in various agricultural production systems. *Crop Protection*, **19,** 837–841.

Stapleton J.J., Prather T.S., Dahlquist R.M. (2000) Implementation and validation of a thermal death database to predict efficacy of solarization for weed management in California. *UC Plant Protect Quarterly*, **10,** 9-10.

Streibig J.C., Rudemo M., Jensen J.E. (1993) Dose-response curves and statistical models. In *Herbicide Bioassays*, pp. 29-55. Eds J.C. Streibig and P. Kudsk. Boca Raton, FL: CRC press.

Tarasoff C.S., Ball D.A., Mallory-Smith C.A. (2007) Extreme ionic and temperature effects on germination of Weeping Alkaligrass (*Puccinellia distans*), Nuttall's Alkaligrass (*Puccinellia nuttalliana*) and Kentucky Bluegrass (*Poa pratensis*). *Weed Science*, **55,** 305–310.

Thompson A.J., Jones N.E., Blair A.M. (1997) The effect of temperature on viability of imbibed weed seeds. *Annals of applied Biology*, **130,** 123-134.

Ulloa S.M., Datta A., Knezevic S.Z. (2010) Tolerance of selected weed species to broadcast flaming at different growth stages. *Crop Protection*, **29,** 1381-1388.

Ulloa S.M., Datta A., Bruening C., Gogos G., Arkebauer T.J., Knezevic S.Z. (2012) Weed control and crop tolerance to propane flaming as influenced by the time of day. *Crop Protection*, **31,** 1-7.

Van Loenen M.C.A., Turbett Y., Mullins C.E., Feilden N.E.H., Wilson M.J., Leifert C., Seel W.E. (2003) Low temperature–short duration steaming of soil kills soil-borne pathogens, nematode pests and weeds. *European Journal of Plant Pathology*, **109,** 993–1002.

Van Staden J., Brown N.A.C., Jäger A.K., Johnson T.A. (2000) Smoke as a germination cue. *Plant Species Biology*, **15,** 167-178.

Verdù A.M.C., Mas M.T. (2004) Modeling of the effects of thermal shocks varying in temperature and duration on cumulative germination of *Portulaca oleracea* L.. *Seed Science and Technology*, **32,** 297-308.

Vidotto F., Letey M., Ricauda A.D. (2009) Effects of soil steaming on weed seed viability. Proceedings of the 8th EWRS Workshop on Physical and Cultural Weed Control, 9-11 March 2009, Zaragoza, Spain, pp. 113.

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Tables

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494   Table 1. Initial status of germination (percent) of seeds used in Exp 1 and Exp 2. Values

495   are average of four (Exp 1) or three (Exp 2) replicates of 20 seeds each.

Species	Germination %	
	Exp 1	**Exp 2**
Amaranthus retroflexus	86.2 (2.39)	80.0 (5.00)
Echinochloa crus-galli	78.1 (1.31)	76.7 (4.41)
Galinsoga quadriradiata	60.0 (7.36)	65.0 (2.89)
Portulaca oleracea	66.2 (8.75)	71.7 (7.26)
Setaria viridis	98.7 (1.25)	95.0 (2.89)
Solanum nigrum[b]	53.7 (5.54)	95.0 (2.89)

[a]SE in parentheses; [b] *different seed lots used in Exp 1 and Exp 2.*

496   Table 2. Parameter estimates for *A. retroflexus*, *E. crus-galli*, *P. oleracea*, *S. viridis*, and

497   *S. nigrum* based on equation (1) and *G. quadriradiata* based on equation (2), R^2 of the

498   regressions, and probability (*P*) of the likelihood ratio test that assumes that data from

499   Exp 1 and Exp 2 can be described by a single model instead of two separated models.

Species	Exp	Estimated model parameters				R^2	P
		b	d	e	f		
A. retroflexus	1	49.148	87.195	65.696	-	0.955	<0.0000
	2	38.278	79.119	61.812	-	0.927	
E. crus-galli	1	53.734	79.409	71.464	-	0.977	<0.0000
	2	66.484	72.160	75.959	-	0.842	
G. quadriradiata	1	40.500	52.467	62.253	0.337	0.954	0.0075
	2	67.417	62.221	61.335	0.189	0.934	
P. oleracea	1	39.807	63.726	64.931	-	0.927	0.0038
	2	33.463	65.698	62.292	-	0.915	
S. viridis	1	31.089	99.145	65.101	-	0.993	0.0043
	2	29.290	93.360	64.980	-	0.970	
S. nigrum	1	40.910	61.626	67.065	-	0.874	<0.0000
	2	34.350	87.539	64.931	-	0.950	

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502 Table 3. Temperatures required to obtain 10%, 90%, and 99% (ET_{10}, ET_{90}, and ET_{99},

503 respectively) germination reduction compared with the untreated seeds and their lower

504 and upper confidence limits estimated from equation (1) for *A. retroflexus, E. crus-galli,*

505 *P. oleracea, S. viridis,* and *S. nigrum* and equation (2) for *G. quadriradiata*[a]. Species are

506 listed for growing values of ET_{99}. *P* values are the probability that ET_z calculated from

507 Exp 1 and Exp 2 are estimates of the same value.

508

Species	Exp	ET_{10}				ET_{90}				ET_{99}			
		Estimate	conf. limits		*P*	Estimate	conf. limits		*P*	Estimate	conf. limits		*P*
			lower	upper			lower	upper			lower	upper	
G. quadriradiata	1	61.3 (0.93)	59.5	63.2	0.372	66.4 (0.78)	64.8	67.9	0.002	70.4 (1.43)	67.6	73.2	0.007
	2	60.3 (0.70)	58.9	61.7		63.5 (0.41)	62.7	64.4		65.8 (0.83)	64.2	67.5	
A. retroflexus	1	62.8 (0.64)	61.6	64.1	<0.001	68.7 (0.54)	67.6	69.7	<0.001	72.1 (1.10)	70.0	74.3	0.152
	2	58.4 (1.04)	56.3	60.4		65.5 (0.49)	64.5	66.4		69.7 (1.25)	67.2	72.1	
P. oleracea	1	61.4 (1.24)	59.0	63.9	0.057	68.6 (0.76)	67.1	70.1	0.027	72.9 (1.71)	69.5	76.2	0.513
	2	58.3 (1.01)	56.4	60.3		66.5 (0.56)	65.4	67.6		71.5 (1.29)	68.9	74.0	
S. nigrum	1	63.6 (0.76)	62.1	65.1	0.015	70.8 (0.73)	69.3	72.2	0.077	75.0 (1.38)	72.3	77.8	0.644
	2	60.9 (0.75)	59.4	62.4		69.2 (0.48)	68.3	70.2		74.2 (1.07)	72.1	76.3	
S. viridis	1	60.7 (0.59)	59.6	61.8	0.654	69.9 (0.59)	68.7	71.0	0.820	75.5 (1.12)	73.2	77.7	0.720
	2	60.3 (0.59)	59.1	61.4		70.0 (0.48)	69.1	71.0		76.0 (1.01)	74.0	78.0	
E. crus-galli	1	68.6 (0.63)	67.4	69.8	<0.001	74.4 (0.58)	73.3	75.6	<0.001	77.8 (1.05)	75.8	79.9	0.008
	2	73.5 (0.59)	72.3	74.6		78.5 (0.42)	77.7	79.3		81.4 (0.86)	79.7	83.1	

[a]SE in parentheses; df are 33 and 60 for Exp 1 and Exp 2, respectively (except for *G. quadriradiata*: 32 and 59 in Exp 1 and Exp 2, respectively).

509

Figure legends

510   Fig. 1. Temperature dynamics recorded during thermal treatment with target

511   temperatures of 50, 60, 70 and 80 °C in Exp 2. (a) Thermal equilibration at standard

512   reference temperature (23 °C); (b) Heating phase to reach the target temperature; (c)

513   Cooling phase; (d) Stabilization to standard reference temperature.

514

515

516   Fig. 2. Relationship between target temperature and germination percentage in Exp 1

517   and Exp 2. Curves of *Amaranthus retroflexus*, *Echinochloa crus-galli*, *Portulaca*

518   *oleracea*, *Setaria viridis* and *Solanum nigrum* are fitted by equation (1); curves of

519   *Galinsoga quadriradiata* are fitted by equation (2). Each data point is the average

520   germination percentage of four (Exp 1) or three (Exp 2) replicates of 20 seeds each.
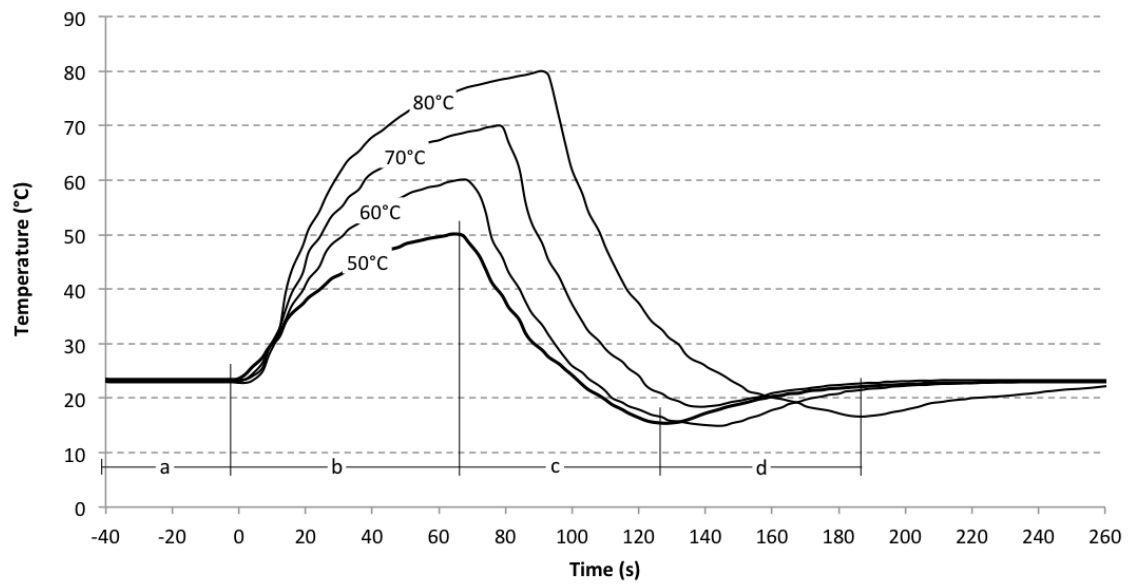
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523   Fig. 3. Temperature required to obtain 99% germination reduction in comparison to

524   untreated seeds (ET_{99}) plotted against length×width of the seed (A) or 1000-seed weight

525   (B). ET_{99} data refer to Exp 2. Regression significance (*P*-value) is 0.01287 and 0.01428
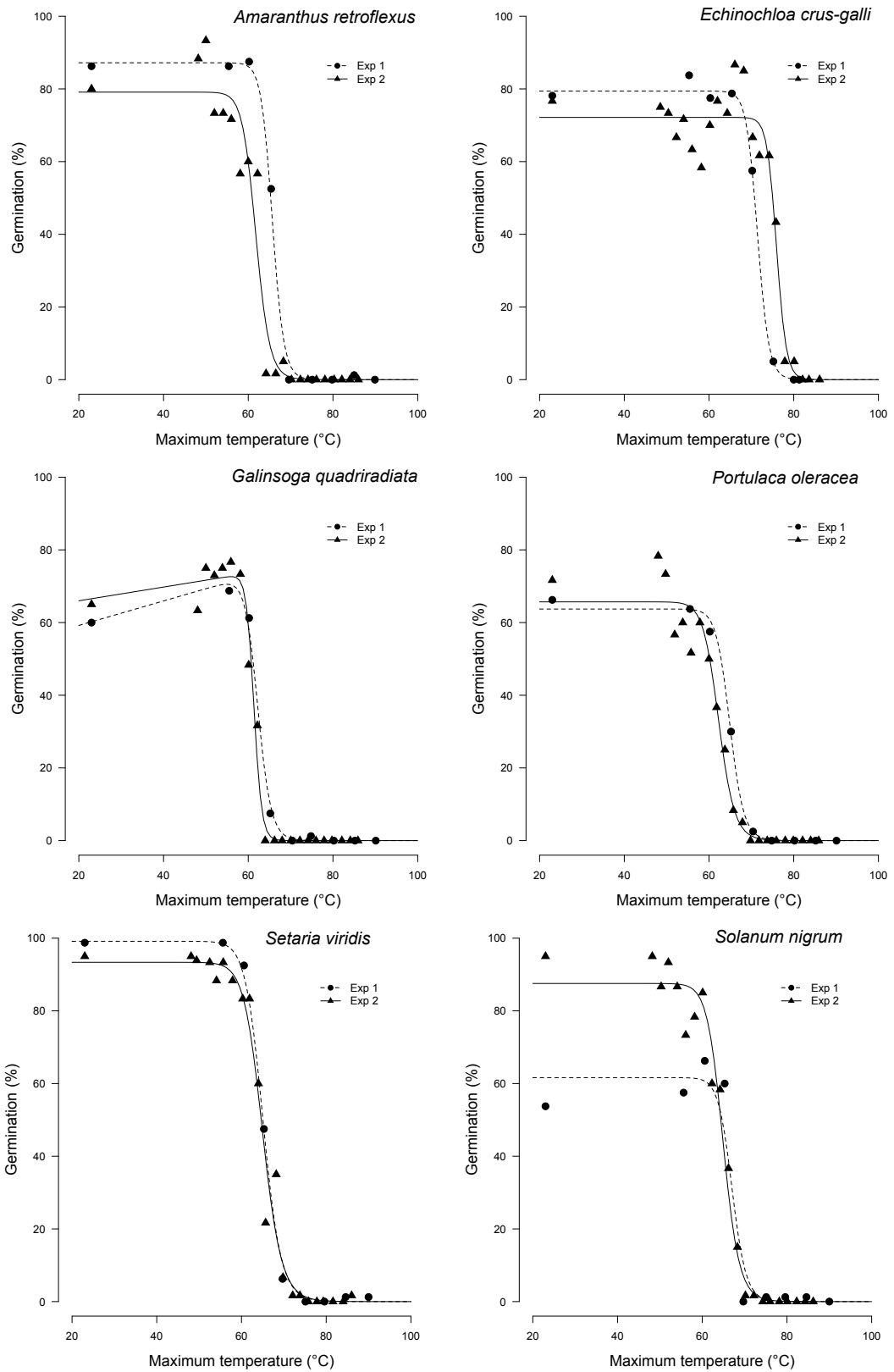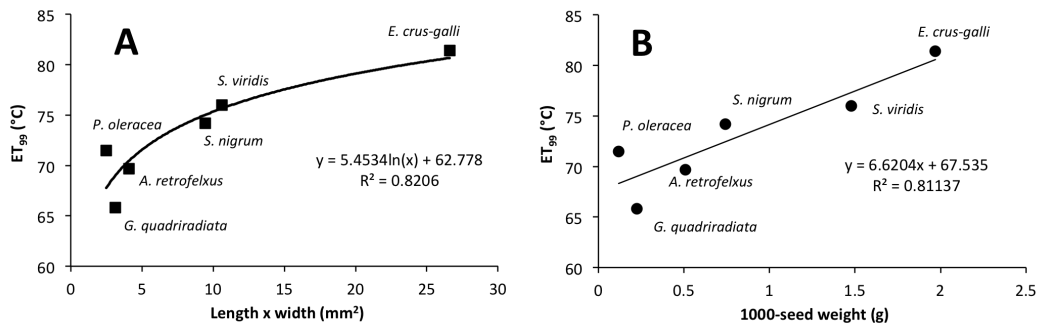
526   for A and B, respectively.

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Figures

Figure 1

530   Figure 2

531   Figure 3.