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Summary

Thermal soil disinfestation techniques are effective reducers of weed seedbank and weed emergence. Two experiments (Exp 1 and 2) were conducted to test the effect of brief exposure to varying temperatures on the seed germination of *Amaranthus retroflexus*, *Echinochloa crus-galli*, *Galinsoga quadriradiata*, *Portulaca oleracea*, *Setaria viridis*, and *Solanum nigrum*. To this end, species seeds were moistened with loamy-sand soil and placed into test tubes. The tubes were heated rapidly and then cooled by dipping them into a hot water bath until target temperatures were achieved. Exp 1 temperatures ranged between 55 and 85°C at 5°C intervals and Exp 2 ranged between 48 and 86°C at 2°C intervals. Thereafter, the tubes were dipped into a cooling (1°C) water bath. Exposure to target temperatures ranged between 2 and 5 s. Soil temperatures were monitored using embedded thermocouples. A log-logistic dose-response model described the effect of heating on seed germinability; temperatures required for 99% reductions were calculated. Based on the predictive model equation used, weed species' germination sensitivity to high temperature exposure can be ranked as follows: *E. crus-galli* (79.6°C), *S. viridis* (75.8°C), *S. nigrum* (74.6°C), *P. oleracea* (72.2°C), *A. retroflexus* (70.9°C), and *G. quadriradiata* (68.1°C). The interval between no effect to complete seed devitalisation occurred at temperatures varying from 6.5 to 15.7°C. Seed size and weight varied directly with heat tolerance. Study results not only inform the timing and optimal adjustment for effective thermal soil treatment, but also demonstrate a relatively simple and generalizable methodology for use in other studies.

Keywords: dose-response model, heat tolerance, seed germination, thermal weed control, seed devitalisation, soil steaming

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

steaming could be described by a dose-response function, with duration of steaming representing the dose and seedling emergence the response.

In all the studies mentioned above, seeds were exposed to target temperatures only after undergoing a heating phase above the target temperature. The duration of that heating phase varied greatly—from as little as 50 s (Melander & Kristensen, 2011) to 30 min (Dahlquist *et al*., 2007), but usually this information is not provided. Similarly, the cooling phase duration between the target temperature and initial temperature is largely variable and it is often not noted in these studies. When it was reported, it ranged between 4 min (Melander & Kristensen, 2011) and 20 min (Melander & Jørgensen, 2005).

It is known that both seed and soil moisture influence seed susceptibility to heating (Mas & Verdù, 2002; Verdù & Mas, 2004). Soil moisture at levels near field capacity yielded, in general, high heating efficiency values via steaming disinfestation methods (Gay *et al*., 2010*a*).

Soil as a seed-heating medium seems to be the method of choice to simulate field conditions in laboratory studies even though non-soil seed-heating mediums are available and have been used (Mas & Verdù, 2002; Verdù & Mas, 2004). In any case, formation of some amount of thermal system inertia is unavoidable, and at times, can result in long heating and cooling phases. These effects have limited the information available on the importance on weed seed devitalisation of the sole effect of high temperatures during soil thermal treatment. This information should also be evaluated considering seed size, which has been reported as one of the traits that may explain differences in sensitivity to thermal treatments among different species.

This study has two objectives: (1) to determine the effect of very short exposure of weed seeds to a wide range of temperatures, and (2) to determine the relationship between seed size and species' tolerance to short duration temperature exposure. The study was manly designed to provide information that is relevant for soil treatment with high temperatures for short periods, as in the case of soil steaming. The study was carried out by exposing seeds to different temperatures while dispersed in soil. Ideally, this method would also be suitable for testing the interactive effect between duration of exposure × temperature in further studies.

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 Temperature recording
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 Thermal treatment
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

second water bath (HOT). This bath consisted of a five-litre stainless steel tank set 3 °C above the target temperature to quickly heat the soil and was kept constant during treatment with a laboratory immersion circulator (Julabo ED 2000 W) inserted into the tank. The tank water level was fixed exactly to submerge the tubes up to 2 cm below their caps; extra water was added as needed to compensate for evaporation. Transfer of the tubes to the third water bath (COLD) occurred immediately upon when the target temperature of the soil was reached. This bath was set to approximately 1 °C for quick cooling and to allow the soil to return to temperature of about 23 °C. The tubes were then transferred back to the REF bath.

For each species and target temperature, four (Exp 1) or three replications (Exp 2) were considered and a single test tube represented the experimental unit and one replication. Four (Exp 1) or three (Exp 2) untreated tubes for each species were maintained in the REF bath for the entire duration of the treatment as controls. The treatment structure was a two-way factorial, with factors represented by species (6 levels in both Exp 1 and Exp 2) and target temperature (8 levels in Exp 1, 21 levels in Exp 2). The treatments were arranged according to a completely randomized design.

Within a few minutes of reaching the reference temperature following the second passage in the REF bath, the mixture of seeds and soil was pulled from the tubes and the seeds manually separated from the soil. From each tube, 20 randomly selected seeds were placed in a Petri dish (9 cm diameter) lined with two No. 1 Whatman filter papers (Whatman International Ltd.) to which 6 ml of deionized water was added. The Petri dishes were incubated in a growth chamber at a constant temperature of 25 °C and 16h/8h of light/dark cycles for 20 days. Preliminary tests showed that germination was observed after 10 days for all weed species (data not shown). Germinated seeds were counted daily and water was added as needed to preserve the initial moisture level.

The greatest portion (always exceeding 90%) of non-germinated seeds had cracked seed coats after germination test and were assumed dead. For each species, tetrazolium test was performed on a small portion of intact seeds treated in Exp 1 and none were viable (data not shown). The test was not conducted in *P. oleracea* and *A. retroflexus*, as it was not possible to pierce the seed coat without destroying the embryo. In a similar study conducted by Dahlquist *et al.* (2007) percentage of viability in heat-treated seeds was < 1% in *E. crus-galli* and *S. nigrum*. Non-germinated, viable seeds were not accounted for 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 Data analysis
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 $$Y = \frac{d}{1 + \exp[b(\log x - \log e)]} \quad (1)$$
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 $$Y = \frac{d + fx}{1 + \exp[b(\log x - \log e)]} \quad (2)$$
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

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

Model fitting was performed using the function *drm* of the add-on package *drc* of the R software (Ritz & Streibig, 2005; Ritz *et al*., 2006); this package has been developed mainly to perform non-linear regression analysis on bioassay studies. As the initial status of germination was lower than 100% and variable among species, model fitting was performed including the percentage of germination as response variable and the total number of seeds included in the germination test (always 20) as value for the argument *weights* of the function *drm* and specifying the case "binomial" for the argument *type* (Ritz & Streibig, 2012). With this set of instructions, the initial status of germination was considered in the model fitting and the *drm* function gave correct estimations of ET_z values (see below).

Data from Exp 1 and Exp 2 were first analysed separately and then pooled to fit into a single model. The *anova* function of R was used to compute a likelihood ratio test to verify if the pooled dataset was significantly better explained by two curves fitting Exp 1 and Exp 2 data separately than by a single model fitting all data.

With the parameters estimated, the equations allowed to calculate the temperature ET_z (Melander & Jørgensen, 2005) required to obtain a certain level of germination reduction in comparison to untreated seeds. ET_z values and their upper and lower confidence limits (α=0.95) were estimated using the function *ED* of the package *drc*. In this study, ET_z was estimated for $z = 10\%$, 90%, and 99%, which correspond to temperatures that cause 10, 90 and 99% reduction in germination, respectively. For each species and experiment, target temperatures were considered "ineffective" if lower than ET_{10}. A reduction on the percentage of germinated seeds after thermal treatment of 90% (ET_{90}) was considered as a standard reference threshold in previous studies (Hannson & Ascard, 2002; Hannson & Mattsson, 2002). ET_{99} can be regarded as a threshold for complete seed devitalisation.

For each species, the function *SI* of the package *drc* was used to test for differences between ET_z calculated from Exp 1 and Exp 2.

To evaluate the relationship between seed size and heating tolerance, the values of ET_{99} were plotted as a function of the variables seed length×width and 1000-seed weight. When significant differences in ET_{99} calculated from Exp 1 and Exp 2 were found for some species, only the estimates obtained from Exp 2 were used.

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

293 temperature was well described by the selected regression models in both Exp 1 and
294 Exp 2 (Table 2). The temperature interval gave good coverage of the different responses
295 from no effect to complete seed devitalisation (Fig. 2). The target temperatures gave
296 intermediate responses around the point of inflection of the estimated response curves.
297 This was more evident in Exp 2, where the responses were more evenly distributed
298 between the upper asymptote and zero, which allowed for a more reliable fit.
299 In general, ET_{10} was very close to 60 °C for the majority of the species. *E. crus-galli*
300 was the only species which deviated strongly from this behaviour, showing an ET_{10} of
301 68.6 and 73.5 °C in Exp 1 and Exp 2, respectively (Table 3). The transition between
302 ET_{10} and ET_{99} occurred in a temperature range from 6.5 °C (*G. quadriradiata*) to
303 15.7 °C (*S. viridis*).
304 *G. quadriradiata* seeds were the most affected by thermal treatment (Table 3). Even
305 though germination was enhanced by exposure to temperatures between 50 and 56 °C,
306 germination quickly decreased compared to the untreated at temperatures greater than
307 58 °C. Two separate curves for Exp 1 and Exp 2 provided a significantly better
308 explanation than a single curve fitting all the data from the two experiments (Table 2).
309 This was mainly due to a slightly stronger stimulatory effect at sub-lethal temperatures
310 and a higher sensitivity to high temperatures observed in Exp 2. Consequently, only
311 ET_{10} was similar in the two experiments while ET_{90} and ET_{99} were always significantly
312 higher in Exp 1 (Table 3). Germination dropped to negligible levels after exposure at
313 temperatures above 70.4 °C (Exp 1) and 65.8 °C (Exp 2).
314 In *A. retroflexus* regression analysis revealed that results from Exp 1 and Exp 2 were
315 significantly different (Table 2). This might be due to a higher initial percentage of
316 germination of untreated seeds in Exp 1 that resulted in a higher upper asymptote and in
317 a higher temperature at the point of inflection between the upper asymptote and zero.
318 This may explain the fact that both ET_{10} and ET_{90} were significantly greater in Exp 1
319 while ET_{99} was the same between the two experiments, and averaged 70.9 °C (Table 3).
320 A similar behavior was observed in *P. oleracea*, but in this case the highest percent
321 germination were observed in Exp 2. Significant differences between the two
322 experiments were recorded for ET_{90} only; ET_{99} averaged 72.2 °C.
323 In the case of *S. nigrum*, Exp 1 and Exp 2 were performed using different seed lots
324 given that the germinability of untreated seeds in Exp 1 was less than 60%. The two
325 curves describing Exp 1 and Exp 2 data differed significantly (Table 2). Nevertheless,

differences between ET_z calculated from the two experiments were significant for ET_{10} only. In particular, ET_{99} averaged 74.5 °C (Table 3).

In *S. viridis*, the slightly higher germination of Exp 1 untreated seeds resulted in an overall significant difference in the two curves fitting Exp 1 and Exp 2 data (Table 2) even though the computed ET_{10}, ET_{90}, and ET_{99} values never differed significantly between the two experiments and averaged 60.5, 69.95, and 75.7 °C, respectively (Table 3).

In *E. crus-galli*, germinability recorded in Exp 2 followed an unexpected course as it initially declined steadily from 75% to 58% in temperatures ranging from about 48 to 58 °C. Afterwards, germinability rose to 87% at 66 °C, then finally dropped to values near zero for temperatures above 80 °C (Figure 2). This behavior, coupled with an overall higher tolerance to heating observed in Exp 2, caused the data obtained in the two experiments to not be describable by a single curve (Table 2). Accordingly, ET_z values always differed significantly between the two experiments. In any case, the calculated ET_{10} values indicated that *E. crus-galli* germinability started to be affected at temperatures between 68.6 and 73.5 °C while ET_{99} values indicated that germinability started to be negligible at temperatures ranging from 77.8 to 81.4 °C (Table 3).

Relationship between seed size and tolerance to thermal treatment

The smallest seeds where those of *P.oleracea*, which showed a length×width of 2.49 mm^2 and a 1000-seed weight of 0.118 g. At the opposite, *E. crus-galli* showed the biggest seeds, with a length×width of 26.60 mm^2 and a 1000-seed weight of 1.97 g. Seed size and ET_{99} values varied in direct proportion. While the six species considered in this study is insufficient to allow full and evenly distributed coverage of all possible seed sizes, the results indicated that seed size, expressed as length×width or 1000-seed weight, and tolerance to thermal treatment may be described by logarithmic or linear model, respectively (Fig. 3). In particular, the increase of 1000-seed weight by 1 g resulted in an average increase of ET_{99} by about 6.6 °C.

Discussion

The methodology adopted in this study tested the effect of short exposure to different temperatures on germination of weed seeds dispersed in a small amount of soil. With the adopted methodology, some amount of thermal inertia was unavoidably introduced into the study. As a consequence, additional time was required to allow the seeds to

reach the target temperature and to cool them to the standard reference temperature (23 °C). Both these heating and cooling phases were significantly shorter than those reported in previous studies. Further reduction of the heating and cooling phases could be accomplished by treating the seeds without their dispersal into soil. Although, data acquired under such conditions is limited practically, as real soil thermal treatments are always affected by discrete heating and cooling phases (Gay *et al*., 2010*a*,*b*).

Complete seed devitalisation (i.e., the temperature causing at least 99% germination reduction) was achieved in the different species at temperatures spanning 64 °C to 80 °C. In particular, *E. crus-galli* showed itself to be the least heat-susceptible, which agrees with results from Melander & Jørgensen (2005) and Bàrberi *et al*. (2009). In contrast, Dahlquist *et al*. (2007) reported that *E. crus-galli* was more susceptible to heat than *S. nigrum* and *P. oleracea*. It should be noted, however, in Dahlquist *et al*. (2007) the seeds that underwent thermal treatment were previously moistened by dipping them in water and then placing between moist paper towels for 24 h. This might have caused the seeds to have higher moisture content which in turn lead to a higher susceptibility to thermal treatment (Egley, 1990) .

The higher heat tolerance of *E. crus-galli* found in our study can be partly attributed to seed structure; the caryopsis is protected by its glumellae (adheres to caryopsis), sterile floret, the second glumae, and partially by the first glumae (Maun & Barret, 1986). This structure persists in seeds harvested and stored as was true of those used in this study. However, in field conditions, both the glumae and sterile floret are gradually lost while the seeds stay in the soil. It seems reasonable to hypothesize that the actual average tolerance to soil heating by *E. crus-galli* seeds under field conditions is lower than that observed in our study. It is also possible that the seed structure may have played a role in the erratic behaviour of seed germinability observed after seed exposure to temperatures in the 48 to 66 °C range.

Seed size may also play a role in the response to thermal treatments. Among the species in this study, *E. crus-galli* had the biggest seeds and showed the highest tolerance to heating. In general, the model predicted a higher ET_{99} for *P. oleracea* based on its seed size and seed weight. Possible reasons for this lower sensitivity may relate to the appended seed soil permanence before treatment (2 h *versus* 24 h), which may have resulted in a reduced seed moisture content though the nonwoven bag enclosures relative to the other species. Nonetheless, this valuable result highlights the fact that 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

that reach sub-lethal temperatures can vary according to the adopted soil heating methodology and can be largely influenced by soil texture and the presence of soil aggregates, especially in steaming (Melander & Jørgensen, 2005; Vidotto *et al*., 2009).

The results of this study can be relevant for soil thermal treatments in general, and may be useful for steaming in particular, as this technique allows the attainment of high soil temperatures for short intervals.

For the weeds included in this study, it appears exposure to temperatures of 80 °C for few seconds is sufficient to obtain satisfactory control. This information is relevant for fine-tuning the use of steam in thermal soil treatments and may further reduce the energy requirement of this technique. This can be in particular useful for steam application techniques based on localised injections for short durations, as in the case of band steaming (Ascard *et al.,* 2007) or sub-superficial soil steaming (Gay *et al*., 2010*a*; Gay *et al*., 2010*b*). Caution must be adopted when considering real field treatment and conditions. Both heating and cooling phases are believed to last longer than observed in this study, which suggests that the actual efficacy could be higher than through simple extrapolation. It may even have the potential to compensate for the presence of soil regions reaching sub-lethal temperatures due to the effect of soil aggregates. Moreover, laboratory experiments oftentimes do not accurately reflect the potential effect of soil organisms and chemicals on seed decay (Stapleton & DeVay, 1986; Stapleton *et al*., 2000; Dahlquist *et al*., 2007); such phenomena would suggest this study may overestimate the maximum temperature needed to devitalize the weed seeds.

The results of this study are relevant also for solar soil heating, since in this technique the stimulatory effect of sub-lethal temperatures may play an important role. During solar soil heating, in fact, the temperatures attained may be often in a range corresponding to that at which stimulations has been observed in our study. For species behaving similarly to *G. quadriradiata* this may result in increased emergence after treatment. Although, the stimulation may be severely reduced or nullified by the long duration of the exposure, as solar soil heating may require up to several weeks to be effective, depending on the local weather, climate and soil moisture conditions (Stapleton, 2000).

The methodology described and used here is relatively simple and demands little more than basic laboratory equipment. Thus, it can be easily extended to the study of thermal effects on other species seed viability and/or for media other than soil. Furthermore, this

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

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

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Tables

Table 1. Initial status of germination (percent) of seeds used in Exp 1 and Exp 2. Values 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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Table 3. Temperatures required to obtain 10%, 90%, and 99% (ET_{10}, ET_{90}, and ET_{99}, respectively) germination reduction compared with the untreated seeds and their lower and upper confidence limits estimated from equation (1) for *A. retroflexus*, *E. crus-galli*, *P. oleracea*, *S. viridis*, and *S. nigrum* and equation (2) for *G. quadriradiata*[a]. Species are listed for growing values of ET_{99}. *P* values are the probability that ET_z calculated from Exp 1 and Exp 2 are estimates of the same value.

Species	Exp	ET_{10}				ET_{90}				ET_{99}			
		Estimate	conf. limits lower	conf. limits upper	*P*	Estimate	conf. limits lower	conf. limits upper	*P*	Estimate	conf. limits lower	conf. limits upper	*P*
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).

Figure legends

Fig. 1. Temperature dynamics recorded during thermal treatment with target temperatures of 50, 60, 70 and 80 °C in Exp 2. (a) Thermal equilibration at standard reference temperature (23 °C); (b) Heating phase to reach the target temperature; (c) Cooling phase; (d) Stabilization to standard reference temperature.

Fig. 2. Relationship between target temperature and germination percentage in Exp 1 and Exp 2. Curves of *Amaranthus retroflexus*, *Echinochloa crus-galli*, *Portulaca oleracea*, *Setaria viridis* and *Solanum nigrum* are fitted by equation (1); curves of *Galinsoga quadriradiata* are fitted by equation (2). Each data point is the average germination percentage of four (Exp 1) or three (Exp 2) replicates of 20 seeds each.

Fig. 3. Temperature required to obtain 99% germination reduction in comparison to untreated seeds (ET_{99}) plotted against length×width of the seed (A) or 1000-seed weight (B). ET_{99} data refer to Exp 2. Regression significance (*P*-value) is 0.01287 and 0.01428 for A and B, respectively.

Figures

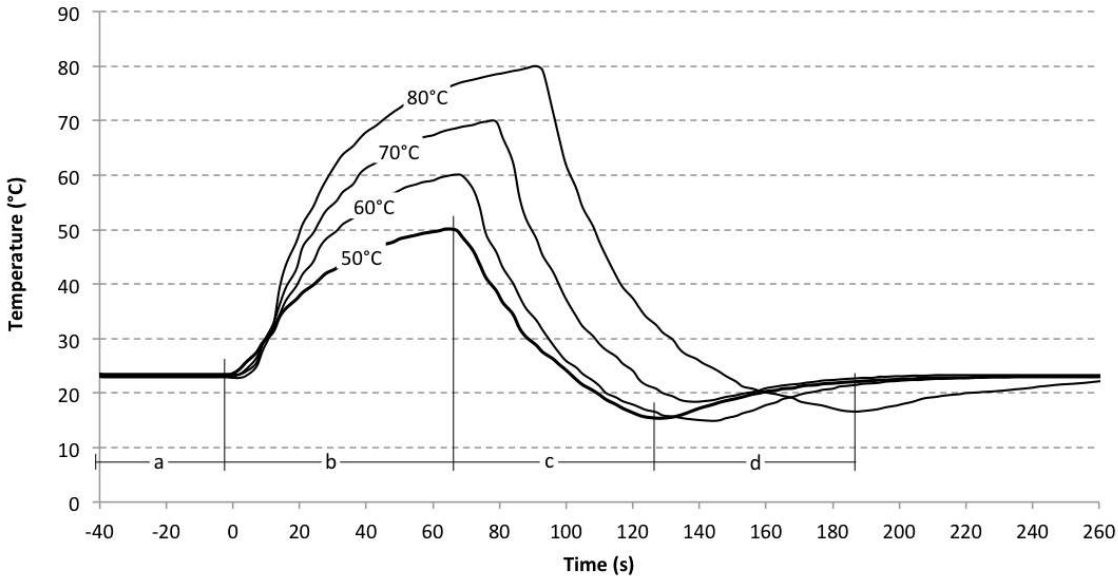

Figure 1

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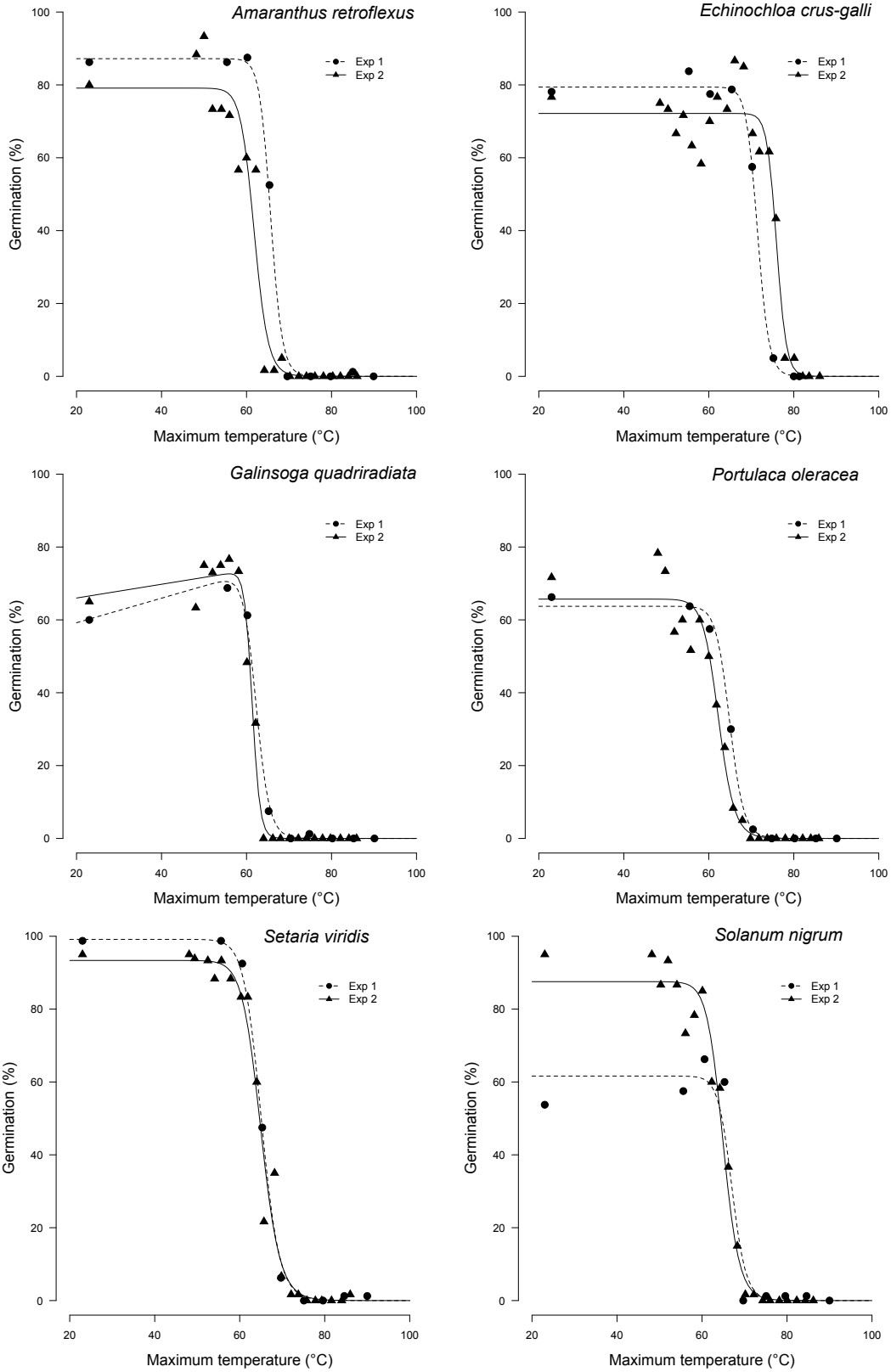
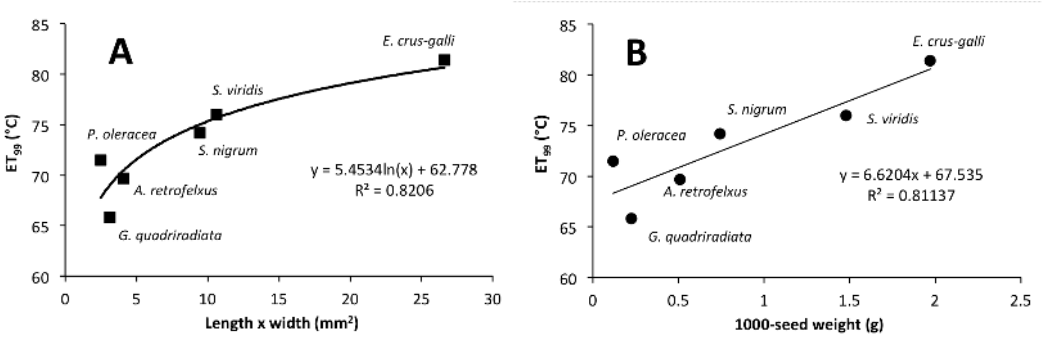

Amaranthus retroflexus
Echinochloa crus-galli
Galinsoga quadriradiata
Portulaca oleracea
Setaria viridis
Solanum nigrum
Exp 1
Exp 2
Germination (%)
Maximum temperature (°C)

530 Figure 2


A
E. crus-galli
S. viridis
P. oleracea
S. nigrum
A. retrofelxus
G. quadriradiata
y = 5.4534ln(x) + 62.778
R² = 0.8206
ET99 (°C)
Length x width (mm²)
B
E. crus-galli
S. nigrum
S. viridis
P. oleracea
A. retrofelxus
G. quadriradiata
y = 6.6204x + 67.535
R² = 0.81137
ET99 (°C)
1000-seed weight (g)

531 Figure 3.