RESEARCH PAPER

Optimization of timing of next-generation emergence in *Amaranthus hybridus* is determined via modulation of seed dormancy by the maternal environment

Rocio Belen Fernández Farnocchia[1,4,*,] , Roberto Luis Benech-Arnold[1,4,] , Anita Mantese[2] and
Diego Batlla[3,4,]

[1] Universidad de Buenos Aires, Facultad de Agronomía, Departamento de Producción Vegetal, Cátedra de Cultivos Industriales, Ciudad de Buenos Aires, Argentina

[2] Universidad de Buenos Aires, Facultad de Agronomía, Departamento de Recursos Naturales y Ambiente, Cátedra de Botánica General, Ciudad de Buenos Aires, Argentina

[3] Universidad de Buenos Aires, Facultad de Agronomía, Departamento de Producción Vegetal, Cátedra de Cerealicultura, Ciudad de Buenos Aires, Argentina

[4] Instituto de Fisiología y Ecología Vinculado a la Agricultura, Consejo Nacional de Investigaciones Científicas y Técnicas (IFEVA-CONICET), Ciudad de Buenos Aires, Argentina

* Correspondence: batlla@agro.uba.ar

Abstract

The timing of emergence of weed species has critical ecological and agronomical implications. In several species, emergence patterns largely depend on the level of dormancy of the seedbank, which is modulated by specific environmental factors. In addition, environmental conditions during seed maturation on the mother plant can have marked effects on the dormancy level at the time of seed dispersal. Hence, the maternal environment has been suggested to affect seedbank dormancy dynamics and subsequent emergence; however, this modulation has not been adequately examined under field conditions, and the mechanisms involved are only partly understood. Combining laboratory and field experiments with population-based models, we investigated how dormancy level and emergence in the field are affected by the sowing date and photoperiod experienced by the mother plant in *Amaranthus hybridus*, a troublesome weed worldwide. The results showed that an earlier sowing date and a longer photoperiod enhanced the level of dormancy by increasing the dormancy imposed by both the embryo and the seed coat. However, this did not affect the timing and extent of emergence in the field; on the contrary, the variations in dormancy level contributed to synchronizing the emergence of the next generation of plants with the time period that maximized population fitness. Our results largely correspond with effects previously observed in other species such as *Polygonum aviculare* and Arabidopsis, suggesting a common effect exists within different species.

Keywords: *Amaranthus hybridus*, embryo, emergence, maternal environmental effect, germination, photoperiod, population-based models, seed coat, seed dormancy, sowing date.

Introduction

The timing of seedling emergence has critical consequences for plant fitness and population dynamics, and hence the ability to predict temporal patterns of emergence is important for understanding how weed populations will respond to current and future ecological and climate scenarios. From an agronomical point of view, weed plants are relatively more vulnerable to control practices during the seedling stage (Batlla and Benech-Arnold, 2007), and hence the possibility of predicting emergence patterns has potential benefits for improving the efficacy of weed control methods (Fenner, 1987; Kruk and Benech-Arnold, 1998).

For seeds that display dormancy (which is common in weed species, particularly from temperate habitats), an accurate prediction of changes in the dormancy level is essential for predicting seedling emergence from natural soil seedbanks. Dormancy can be defined as a seed trait that prevents germination under conditions that would otherwise be suitable for, and result in, germination (Egley 1986; Benech-Arnold *et al.* 2000). The level of dormancy determines the extent of the environmental range in which germination occurs (Vegis, 1964). In general, seeds display a high dormancy level when they are dispersed from the mother plant, and this is progressively lost via a temperature-dependent process. Emergence normally occurs when the dormancy level is low or minimum and the prevailing environment coincides with the range within which germination can proceed. In this way, seed dormancy enables plants to emerge when conditions are most likely to be adequate for seedling survival and plant reproduction.

The dormancy level at the time of dispersal results from the genotype combined with modulation exerted by the environmental conditions that the seeds experience during maturation on the mother plant (Roach and Wulff, 1987; Fenner, 1991). For example, many studies have shown that seeds that mature early in the season frequently show a higher dormancy level than those that mature later (e.g. Barton, 1962; Frost, 1971; Pourrat and Jacques, 1975; Ceccato *et al.*, 2015). These effects are partly mediated by maternal control over seed-coat properties (Kendall *et al.*, 2011; MacGregor *et al.*, 2015), for example as occurs in the genus *Chenopodium*, in which variations in coat thickness have been found (Ceccato *et al.*, 2015). In Arabidopsis, the maternal environment also affects embryo physiology, by modifying the contents of gibberellins (GAs) and abscisic acid (ABA) in mature seeds (Kendall *et al.*, 2011).

On the basis of evidence such as this, the maternal environment has been suggested to affect next-generation emergence patterns in the field, and hence plant population dynamics. However, this hypothesis has only rarely been tested under real field conditions (Donohue, 2009). We recently showed that in the weed species *Polygonum aviculare*, maternal environmental effects allow seeds that have matured and dispersed at different times to synchronize their emergence patterns in the field (Fernández Farnocchia *et al.*, 2019). Such results demonstrate the importance of increasing our understanding of maternal regulation of offspring dormancy levels and its effects on temporal patterns of emergence.

In this current study, we investigated the effect of the maternal environment on seed dormancy in *Amaranthus hybridus*, a summer annual weed that causes significant economic losses in agriculture worldwide. We investigated the effects of mother-plant sowing date and photoperiod on the seed dormancy level at dispersal and during seed post-maturation under different conditions, together with some of the possible mechanisms through which these effects elicit changes in the dormancy level and the subsequent consequences for temporal emergence patterns in the field. To help interpret and consider the results, we developed threshold population-based models and performed simulations under different hypothetical scenarios.

Materials and methods

Maternal environment experiments

All plants were generated from seeds of *Amaranthus hybridus* (ex. *A. quitensis*) collected from fields at Pergamino, Buenos Aires, Argentina (33°53′S, 60°34′W) at their natural dispersal time (in April 2015, 2016, and 2017). Two experiments were performed during three consecutive growing seasons at the Faculty of Agronomy, University of Buenos Aires, Argentina (34°25′S, 58°25′W).

In experiment A, we examined the effects of variations in mother-plant sowing date (Fig. S1). Seeds were sown at the following times: late-winter (12 September), mid-spring (16 November), late-spring (15 December), early-summer (19 January), and mid-summer (16 February) in 2015/16; and late-spring (10 November) and mid-summer (20 February) in 2016/17.

In experiment B, we examined the effects of variations in photoperiod during the mother plant-reproductive phase (Fig. S1). In 2018, seeds were sown in mid-summer (1 February) and were exposed to different daylengths from the time inflorescences became visible through to harvest by artificially extending the photoperiod by either 0 h (control, natural photoperiod), 1.5 h, or 4.0 h. Portable structures were used that combined incandescent and fluorescent lights of low intensity, which provided a photosynthetic photon flux density of 4 μmol m^{-2} s^{-1} (LI-COR 191S quantum sensor) and a light quality similar to natural sunlight [R:FR=1.16±0.21 (±SD); SKR 110 660/730 Skye Instruments Ltd, Wales].

In both these experiments, seeds were sown in trays containing a mixture of soil, sand, and perlite (3:2:1) and kept in a greenhouse (24.56±6.78 °C) for 5–10 d. The seedlings were then transferred to the field and arranged in a randomized complete block design, either planted in the soil (2015/16 and 2018) or in 20-dm^3 plastic pots (2016/17) containing the same soil, sand, and perlite mixture. Four replicates per treatment were used, obtained by pooling seeds from different plants (five plants in 2015/16, four plants in 2016/17, and three plants in 2018). Only plants that began the reproductive phase within the same week were used for the experiments. To hinder cross-pollination, inflorescences were enclosed within fine-mesh bags (similar to those employed to avoid cross-pollination in commercial sunflower hybrid production). At the end of the experiments, plant height was measured. Mature inflorescences of individual plants were collected and gently shaken between paper sheets to obtain the seeds (water content 10.7±0.6%, dry-weight basis), which were placed into paper bags and stored at ~20 °C. The mean seed weight and plant seed production were determined. Environmental data were obtained from a weather station 20 m away from the field.

Seed post-maturation experiments

Experiments 1 (E1) and 2 (E2) began immediately (<2 d) after harvest (i.e. at the dispersal time) of the seeds from experiments A and B (Supplementary Fig. S1). E1 examined dormancy level at different temperatures within the thermal range permissive for seed germination. The seeds were stratified at 4.8±0.07 °C or after-ripened at 25±0.12 °C for 0 d (i.e. at harvest), 5, 10, 25, 60, 75 d, and in some cases 100 d and 400 d, at which times germination was tested at 10, 15, 20, 25, 30, or 35 °C. Seeds from 2016/17 (experiment A) were only stratified at 4.8 °C and incubated at 25 °C and 35 °C.

E2 evaluated seedling emergence in the field using freshly harvested seeds from experiment A (Supplementary Fig. S1). Seeds from plants sown in (i) late-winter and mid-summer (2015/16), and (ii) late-spring and mid-summer (2016/17) were sown in the soil. Emergence was recorded on the following dates: 13 April, 3 July, 19 August, 1 September, 15 October, 10 November, 13 December, and 1 January in (i); and 23 August, 24 October, 20 December, 1 January, and 2 February in (ii).

Seed post-maturation conditions

Seeds (600 per treatment) were placed into nylon mesh bags and buried 5 cm deep in pots containing the soil, sand, and perlite mixture that was either wet (stratification) or dry (after-ripening) in E1, or buried 5 cm deep in the field in E2. To maintain substrate humidity in the wet treatment in experiment E1, the pots were periodically watered and placed on trays with a constant layer of distilled water on their bases. In all cases, temperature and humidity were recorded hourly at 5 cm depth (DAT-10, Temperature and Humidity Dataloggers, China; Logger METEO4, SCH-10, Cavadevices.com, Argentina).

Germination tests (E1)

Bags (4 per treatment) were exhumed from the pots and rinsed with distilled water. Seeds (45–55 per replicate) were placed into 9-cm diameter Petri dishes on two filter paper discs moistened with 5 ml of different media, as follows: distilled water, 100 μM fluridone, 100 μM fluridone + 10 μM ABA, 100 μM fluridone + 50 μM ABA, 10 μM GA₃, or 50 μM GA₃. The seeds were then exposed to a 15-min pulse of red-light (Faccini and Vitta, 2005) with a photon fluence rate of 28 μmol m⁻² s⁻¹ (cabinet temperature ~20 °C), after which the dishes were sealed with a nylon film to prevent water evaporation and incubated for 15 d at 10, 15, 20, 25, 30, or 35 °C according to the experiment. Recently dispersed seeds and dry-stored seeds were first incubated in distilled water for 24 h (at 10, 15, 20, 25, 30, or 35 °C) to allow imbibition, exposed to the red-light, and incubated again at the same temperature. In addition, in some experiments, to evaluate whether maternal treatments affected the embryo dormancy level, the coats of seeds were perforated using a fine needle and a magnifying glass, avoiding damage to the embryo.

Germination (radicle elongation >1 mm) was recorded daily, and germinated seeds were removed. The remaining non-germinated seeds were perforated and incubated at 35 °C to test viability (96.47±1.81%). Fluridone (FLU; Pestanal®, analytical standard, Sigma–Aldrich) was used to prevent *de novo* synthesis of ABA during incubation. Solutions of ABA and GA₃ (both Sigma-Aldrich) were prepared by dissolving them in 1 mol l⁻¹ NaOH and in EtOH, respectively (https://www.sigmaaldrich.com/technical-documents/protocols/biology/growth-regulators.html). The temperature of the incubators was recorded hourly (DAT-10, Temperature and Humidity Dataloggers, China).

Emergence tests under field conditions (E2)

Seed bags were exhumed from the soil, rinsed with distilled water, and seeds (600 per replicate) were placed into Petri dishes with 5 ml distilled water and exposed to natural sunlight for 5 min. The seeds were then dispersed onto the soil in open-bottom PVC cylinders of 15 cm diameter and covered with soil ~1.5 cm deep. Emergence was recorded daily for 1 month. The cylinders were hand-weeded and watered daily. Seed viability during the experiment (97.89±2.67%) was corroborated by exhuming extra bags in October and incubating seeds with perforated coats at 35 °C. The soil temperature and water content at 1.5 cm deep were recorded hourly (Logger METEO4, SCH-10, Cavadevices.com). Field plots were hand-weeded during the experiment.

Seed structure

Seed structure was evaluated using an optical microscopy for recently harvested seeds from experiments A (late-winter and early-summer sowings, 2015/16) and B (photoperiod treatments, 2018). Three portions from the central part of the seeds were embedded in paraffin and serially sectioned at 10–12 μm using a Minot-type rotary microtome. The sections were stained with Safranin–Fast Green and mounted in Canada balsam (Johansen, 1940) to observe seed structure. Sections were imaged using a Zeiss Axioplan optical microscope and analysed using the Zeiss AxioCamERc 5s software.

Model development and validation

Models describing changes in the thermal range permissive for seed germination were developed using final germination data in the range 10–35 °C for seeds collected from the maternal experiment A (2015/16) obtained in E1. The limits of the permissive thermal range for seed germination, expressed as the mean lower ($T_{l(50)}$) and mean higher ($T_{h(50)}$) temperatures for 50% germination, and their standard deviations (σ_{Tl} and σ_{Th}) were quantified by employing a mathematical approach proposed by Batla and Benech-Arnold (2015) based on previous models developed by Washitani (1987) and Covell *et al.* (1986). The model assumes that the temperature limits are normally distributed within the seed population. The fraction of seeds germinating at a given incubation temperature can be calculated using the function:

$$p\left(T\right) \;=\; \Phi\left[\left(T - T_{l(50)}\right)/\sigma_{Tl}\right]\left\{1\Phi\left[\left(T - T_{h(50)}\right)/\sigma_{Th}\right]\right\} \quad (1)$$

where, $p(T)$ is the proportion of seeds germinating at temperature T and Φ is the standard normal cumulative distribution function. Parameters were derived by minimizing the root-mean-square error (RMSE) between simulated and final germination percentages (at 10–35 °C) through a non-linear optimization procedure using the Solver tool of Microsoft Excel.

Results

Environmental conditions, plant development, and reproductive output

The intervals between the first and the last sowing dates determined a wide range of plant growing conditions (Table 1, Supplementary Table S1). During the reproductive phase, delays in the sowing date exposed plants to lower mean daily temperature (from 23.1±3.3 °C to 16.1±4.6 °C), photoperiod (from 14.6 h to 12.1 h), and radiation input (from 18.1±6.4 MJ m⁻² to 11.6±5.2 MJ m⁻²) (all data are ±SD). The delay in sowing resulted in a shorter vegetative phase (from 66±3.4 d for plants sown in late-winter to 21±2.9 d for those sown in mid-summer), and a shorter reproductive phase (the time from visible apical inflorescence to dispersal; from 133±3.4 d

Table 1. Environmental variables measured during the full life cycle, and the vegetative and reproductive phases of mother plants sown on different dates in Experiment A (2015/16)

Sowing date	Period or phase	Mean daily temperature (°C)	Mean minimum daily temperature (°C)	Mean maximum daily temperature (°C)	Mean photoperiod (h)	Mean daily solar radiation (MJ m^{-2})
Late-winter	Full cycle	20.8(±4.7)	16.1(±4.8)	25.7(±5.3)	14.3	18.3(±5.5)
	Vegetative	16.1(±3.4)	11.7(±3.8)	20.6(±4.1)	13.7	17.1(±1.2)
	Reproductive	23.1(±3.3)	18.3(±3.7)	28.2(±3.5)	14.6	18.1(±6.4)
Mid-spring	Full cycle	22.1(±4.1)	17.6(±4.0)	26.9(±4.7)	14.1	18.8(±6.4)
	Vegetative	22.5(±3.3)	17.7(±3.5)	27.4(±3.6)	15.3	21.7(±5.8)
	Reproductive	22.0(±4.3)	17.7(±4.2)	26.8(±5.1)	13.7	17.4(±6.9)
Late-spring	Full cycle	21.8(±4.7)	17.4(±4.7)	26.6(±5.3)	13.8	19.0(±7.1)
	Vegetative	24.7(±2.1)	20.1(±2.6)	29.7(±2.5)	15.4	24.3(±2.0)
	Reproductive	20.9(±4.9)	17.0(±4.7)	25.7(±5.6)	13.3	17.5(±6.9)
Early-summer	Full cycle	19.8(±5.5)	15.6(±5.3)	25.1(±6.2)	13.1	15.4(±7.4)
	Vegetative	25.6(±2.2)	21.0(±2.5)	30.9(±2.6)	14.7	22.5(±0.5)
	Reproductive	18.2(±4.9)	14.0(±4.9)	23.2(±5.7)	12.6	13.5(±7.1)
Mid-summer	Full cycle	17.7(±5.1)	13.5(±5.0)	22.1(±5.7)	12.5	13.5(±7.1)
	Vegetative	22.6(±3.2)	17.7(±3.4)	27.3(±3.7)	13.8	19.4(±3.9)
	Reproductive	16.1(±4.6)	12.2(±4.5)	20.2(±4.9)	12.1	11.6(±5.2)

Errors are ±SD. See Supplementary Fig. S1 for experimental design.
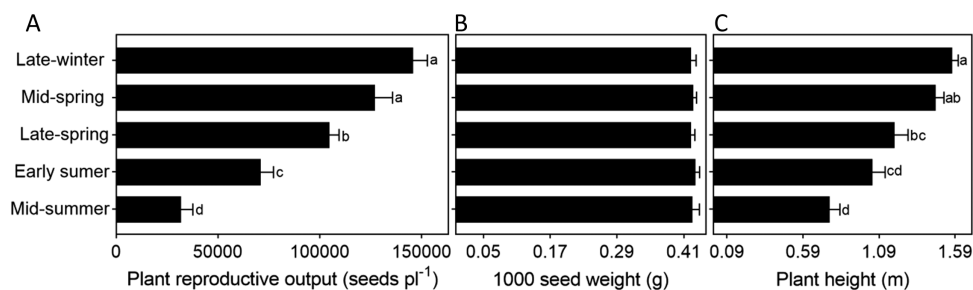
Fig. 1. Reproductive output and growth of *Amaranthus hybridus* mother plants for each sowing date carried out in Experiment A during the 2015/16 growing season (Supplementary Fig. S1), expressed as (A) seeds per plant, (B) 1000 seed weight, and (C) mother plants height. Data are means (±SE) of *n*=4 replicates. Different letters indicate significant differences between as determined using ANOVA followed by Tukey's comparison tests (*P*≤0.05; InfoStat v.2017).

to 75±9 d for plants sown in late–winter and mid–summer, respectively) (Supplementary Fig. S2). As a consequence, the sowing date significantly affected plant size (determined as final plant height) and reproductive output (*P*=0.0001; Fig. 1, Supplementary Fig. S3); however, no differences in individual seed weight were observed.

Effects of sowing date on seed dormancy

Seeds collected from mother plants sown on different dates in Experiment A (Supplementary Fig. S1) showed significant differences in dormancy level at harvest and during post-maturation (Fig. 2, Supplementary Fig. S4). Seeds from plants sown in early–summer and mid–summer showed higher germination than those from plants sown in late–winter, mid–spring, and late–spring. For example, at dispersal (i.e. at 0 d), seeds from the early–summer sowing germinated by ~40% more at 30 °C than those from the late–winter sowing.

Post-maturation conditions (stratification versus dry-storage) only generated subtle differences in the dormancy release rate, which depended on sowing date rather than on the post-maturation method; expression of dormancy depended on the incubation temperature. Notwithstanding post-maturation conditions or time, differences in germination were more evident at incubation temperatures above 25 °C; indeed, at temperatures lower than 15 °C the observed germination percentages were very low.

The effect of photoperiod and the role of the seed coat

We examined the influence of the environmental factors that vary the most with changes in the mother-plant sowing date. To get an indicator of the dormancy level of the seed population, we fitted linear regressions to the final germination data obtained for seeds incubated at 30 °C at the stratification and dry-storage times that were near to 50% germination (see Supplementary
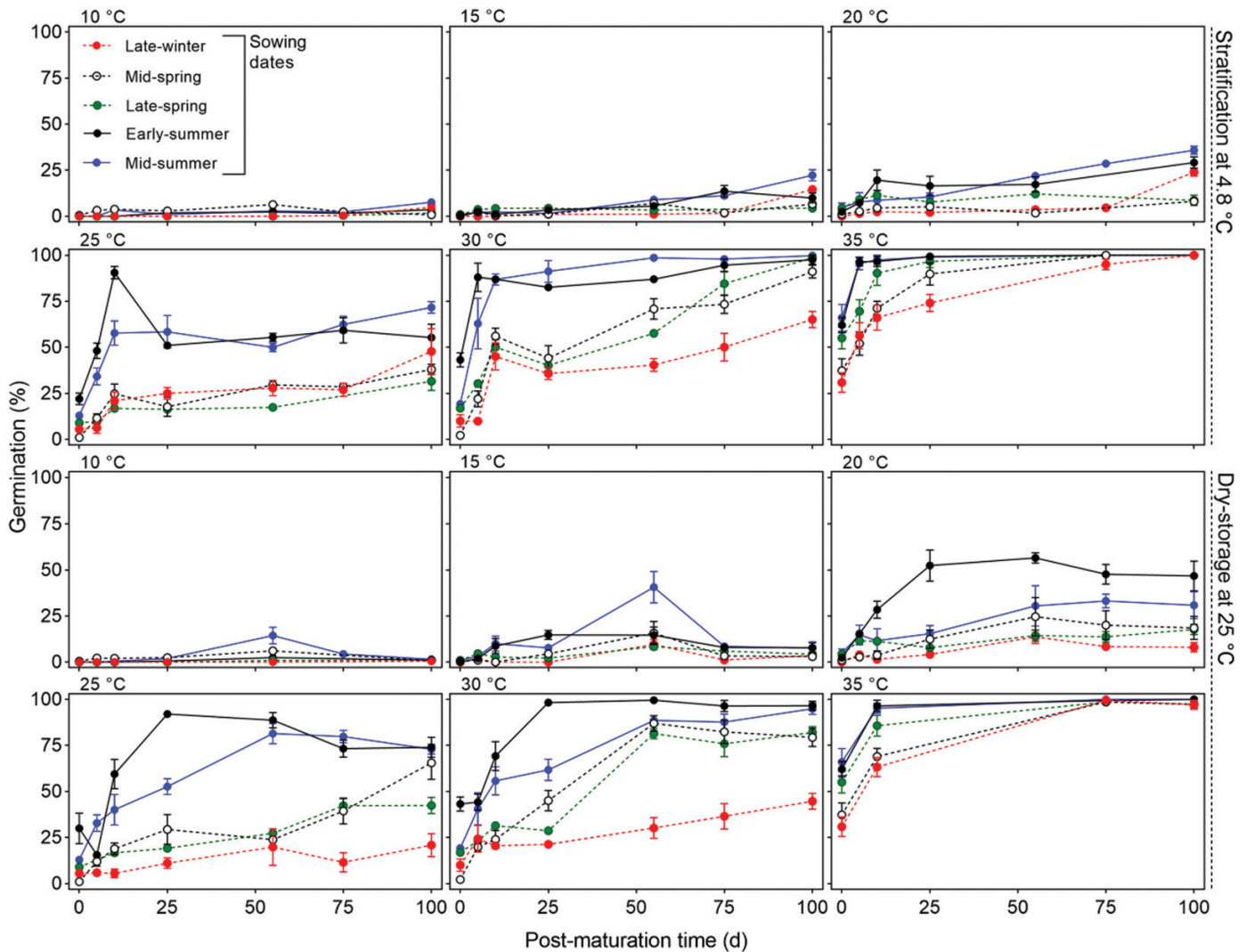
Fig. 2. Final germination percentage of *Amaranthus hybridus* seeds obtained from mother plants sown on different dates and incubated at different temperatures following different periods of post-maturation stratification or dry storage. Seeds were obtained from late-winter, mid-spring, late-spring, early-summer, and mid-summer sowing dates in Experiment A, 2015/16 (Supplementary Fig. S1), and were incubated at the indicated temperatures after stratification at 4.8 °C (upper panels) or dry-storage at 25 °C (lower panels). Data are means (±SE) of *n*=4 replicates.

Fig. S5). This allowed interpolation of the post-maturation time to reach 50% germination. Using the data derived from the linear equations, we analysed correlations between post-maturation times required for 50% germination and the values of mean photoperiod, mean daily temperature, and mean daily solar radiation measured during the vegetative and 'reproductive' phases (Table 1). Correlations were significant only when obtained using the environmental variables measured during the reproductive phase (Table 2), and the significance was greater when the photoperiod was considered. For example, for the combined stratification plus dry–storage treatment, the *P*-value for the correlation with photoperiod was 10-fold lower than the corresponding value for temperature (0.0003 versus 0.0038) and 22-fold lower than for radiation (0.0003 versus 0.0065). In addition, when the germination data from the stratification and

dry-storage treatments were considered individually, the correlations were always significant for photoperiod. We therefore studied the specific role of photoperiod during the reproductive phase on the dormancy level in the offspring.

In agreement with the correlation analysis, we found that a longer photoperiod enhanced dormancy (Figs 3, 4). For example, at 35 °C, freshly harvested intact seeds germinated at a rate of 76.2±2.0% when produced under a natural short photoperiod, but the rate decreased to 45.3±6.2% and 27.4±6.4%, respectively, for +1.5 h and +4 h extensions to the photoperiod (Fig. 3). Differences in germination were observed at high incubation temperatures, while no germination was observed at temperatures below 20 °C (Fig. 3). Differences between photoperiod treatments persisted even after 400 d of dry-storage at 25 °C (Fig. 4).

Table 2. Correlations for the post-maturation time required for 50% germination at 30 °C in relation to mean values of photoperiod, temperature, and solar radiation recorded during the full life cycle, and the vegetative and reproductive phases of mother plants sown at different dates in 2015/16 (Experiment A)

| Environmental factor/Phase	Post-maturation treatment	*r*	*P*-value
Mean photoperiod (h) Vegetative phase	Stratification	–	0.5791
	Dry storage	–	0.9002
	Stratification+Dry storage	–	0.5371
Mean photoperiod (h) Reproductive phase	Stratification	0.91	**0.0307**
	Dry storage	0.94	**0.0186**
	Stratification+Dry storage	0.91	**0.0003**
Mean daily temperature (°C) Vegetative phase	Stratification	–	0.0501
	Dry storage	–	0.1363
	Stratification+Dry storage	−0.82	**0.0038**
Mean daily temperature (°C) Reproductive phase	Stratification	–	0.1040
	Dry storage	0.89	**0.0426**
	Stratification+Dry storage	0.83	**0.0031**
Mean solar radiation (MJ m^{-2}) Vegetative phase	Stratification	–	0.3044
	Dry storage	–	0.5448
	Stratification+Dry storage	–	0.1591
Mean solar radiation (Mj m^{-2}) Reproductive phase	Stratification	–	0.1507
	Dry storage	0.88	**0.0487**
	Stratification+Dry storage	0.79	**0.0065**

Time to 50% germination was estimated by fitting linear regressions to the final germination percentages of seeds incubated at 30 °C at different times during stratification at 4.8 °C and dry-storage at 25 °C (Supplementary Fig. S4). The statistical analysis was performed using the InfoStat software v.2017; significant *P*-values are highlight in bold, and *r*-values are only shown when significant. See Supplementary Fig. S1 for experimental design.
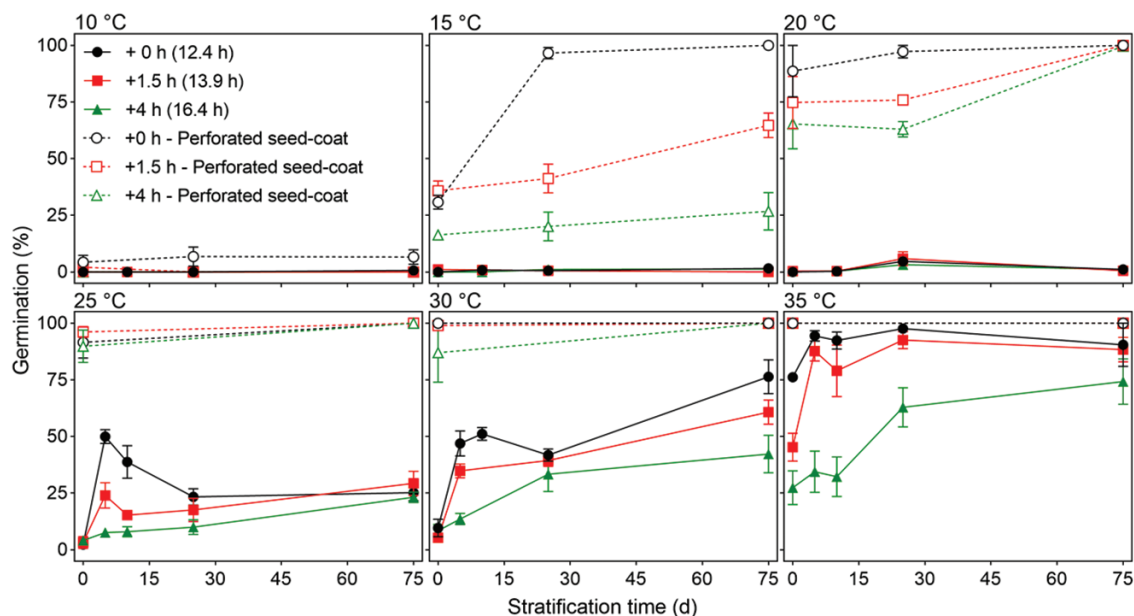
Fig. 3. Final germination percentage of *Amaranthus hybridus* seeds with intact or perforated coats obtained from mother plants that experienced extended photoperiods during the reproductive phases, with the seeds then being incubated at different temperatures following different periods of stratification. Seeds were obtained from plants sown in mid-summer in Experiment B, 2018 (Supplementary Fig. S1), and exposed to extended photoperiods of 0 h (i.e. natural daylight), +1.5 h, or +4 h (Fig. S1). The seeds were incubated at the indicated temperatures after stratification at 4.8 °C for different periods of time. Data are means (±SE) of *n*=4 replicates.

Isolating the embryos and perforating the seed coats increased germination (at similar levels) as compared to that of intact seeds (Figs 4, 5) and, in general, overcame the differences observed for the different sowing dates and photoperiods (Figs 3–5, Supplementary Fig. S4). These increases depended on the maternal treatment and incubation temperature. At 25 °C, perforated seeds from late-winter sowing showed a lower germination at dispersal than
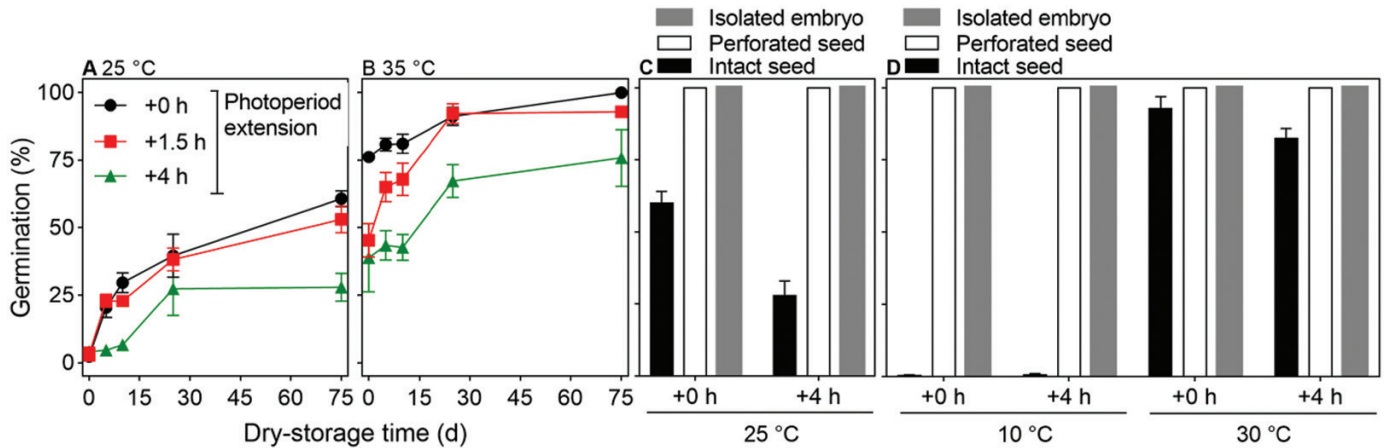
Fig. 4. Effects of extended photoperiod during the mother-plant reproductive phase and perforation or removal of the seed coat on germination of *Amaranthus hybridus* seeds. Seeds were obtained from plants sown in mid-summer in Experiment B (2018), and exposed to extended photoperiods of 0 h (i.e. natural daylight), +1.5 h, or +4 h (Fig. S1). (A, B) Final germination percentage of seeds incubated at (A) 25 °C and (B) 35 °C after different periods of dry-storage after-ripening at 25 °C. (C, D) Final germination percentage of seeds with intact coats, seeds with perforated coats, and isolated embryos incubated at (C) 25 °C following 75 d of dry-storage after-ripening at 25 °C, and (D) at 10 °C or 30 °C following 400 d of dry-storage after-ripening at 25 °C. Data are means (±SE) of *n*=4 replicates.

those from the early-summer (*P*=0.0054) and mid-summer sowings (*P*=0.0002); during dry-storage, germination of perforated seeds was nearly 100% in all cases (Fig. 5). In addition, seeds from the different photoperiod treatments that had perforated coats showed almost no dormancy when incubated above 25 °C (Figs 3, 5). However, below 25 °C, the enhancement of germination was positively related to incubation temperature and negatively related to the extent of the photoperiod (Fig. 3). For example, when incubated at 15 °C, perforated seeds that had matured under a natural photoperiod achieved 100% germination after 25 d of stratification, while those matured under extended photoperiods showed germination values lower than 50% for the same stratification time. After 400 d of dry storage, coat perforation resulted in nearly 100% germination when seeds were incubated at 10 °C, while germination of intact seeds was still almost zero (Fig. 4D).

Differences in the thickness of coats were detected in seeds from late-winter, early-summer, and mid-summer sowings (*P*=0.0017) and in seeds from plants exposed to +0 h and +4 h extensions of the photoperiod (*P*=0.028): the later the sowing and the shorter the photoperiod, the thinner the coats (Fig. 5, Supplementary Fig. S6).

Seed responses to exogenously applied hormones and hormone synthesis inhibitors

Exogenously applied GA$_3$ or FLU alone resulted in only small increases (<12%) in germination of seeds with perforated coats compared with water controls after 0 d or 75 d of stratification when incubated at 30 °C or 10 °C, respectively (Supplementary Figs S7, S8). When ABA and FLU were applied together it reduced the germination of perforated seeds

relative to the water control at 15, 25, 30, and 35 °C at different stratification and storage times (Supplementary Figs S8B, S9, S10). These responses to GA$_3$, FLU, and ABA showed no significant differences between maternal treatments.

Effect of sowing date on emergence patterns under field conditions

In 2016, seeds from late-winter and mid-summer sowings were buried in the field at their corresponding natural dispersal times, respectively on 24 March (dispersal time 'D1', early-autumn) and 21 May 21 ('D2', mid-autumn) (Fig. 6A). In 2017, seeds from late-spring and mid-summer sowings were buried on 8 May ('D1', mid-autumn) and 30 May ('D2', early-winter), respectively. In contrast to the observations of germination under laboratory conditions (Fig. 2), differences in emergence patterns were almost absent (Fig. 6). In all cases, seedling emergence in the field was almost zero up to late-winter and remained low during early-spring. Emergence peaked from mid-spring to early-summer, and tended to decrease thereafter. The maximum recorded emergence values did not exceed 45%.

Analysis of the effects of maternal sowing date on the thermal range permissive for seed germination

The parameters characterizing the thermal range permissive for seed germination were estimated using eqn 1 (Supplementary Table S2). Changes in the mean lower ($T_{l(50)}$) and higher ($T_{h(50)}$) permissive temperatures were adequately described by linear regressions when plotted against stratification and dry-storage time (i.e. post-maturation time) (Fig. 7, top; Supplementary Table S3). The models showed that during dormancy release,
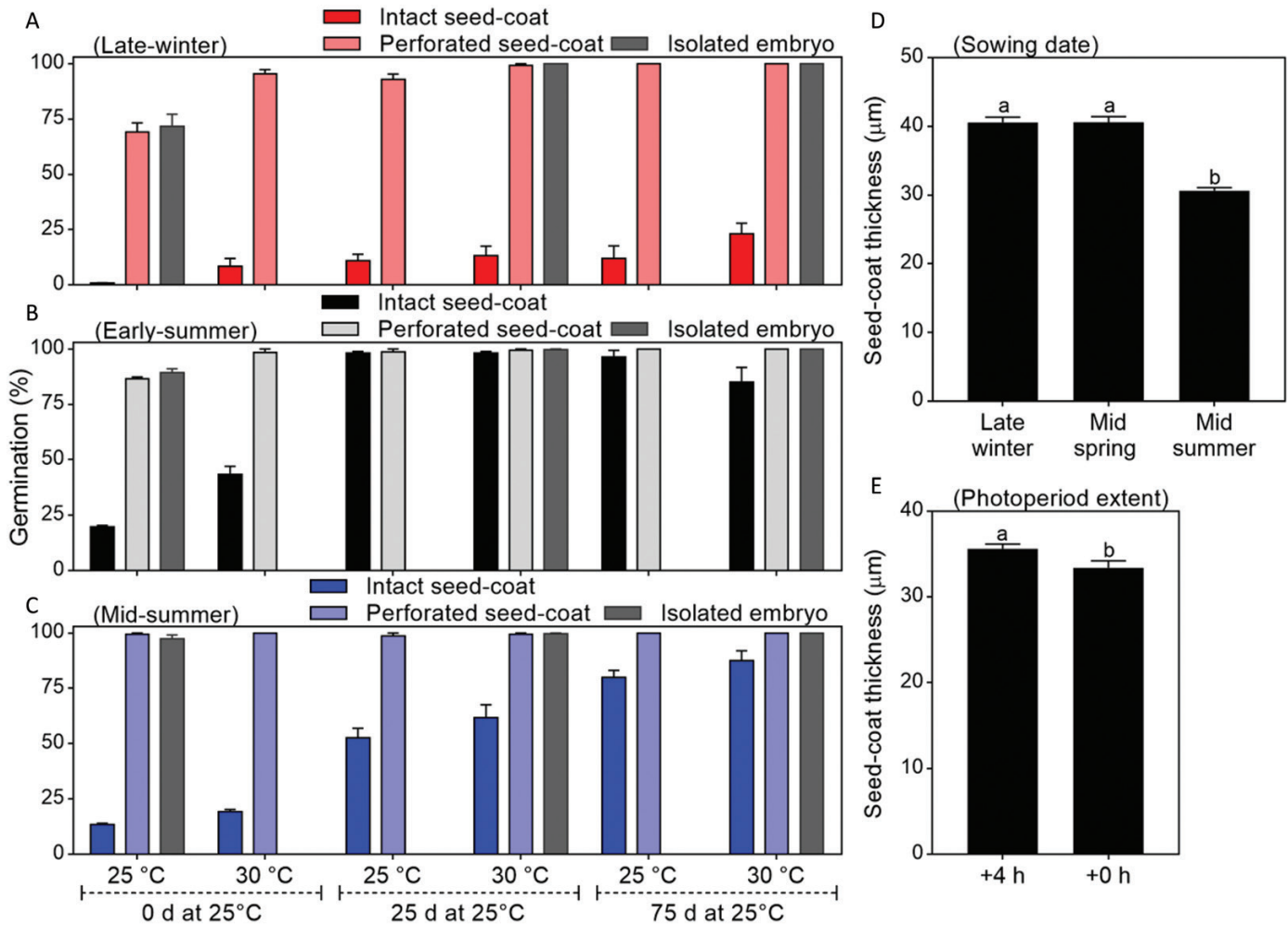
Fig. 5. Effects of the seed coat on final germination percentage of *Amaranthus hybridus* seeds from mother plants sown on different dates in experiment A and subjected to an extended photoperiod in Experiment B (Supplementary Fig. S1). (A–C) Seeds were obtained from plants sown in late-winter, early-summer, and mid-summer in 2015/16. Seeds with either intact or perforated coats, and isolated embryos were incubated at either 25 °C or 30 °C following 0, 25, or 75 d of dry-storage at 25 °C. Germination of isolated embryos was only tested after 0 d and 75 d. Seeds were obtained from (A) late-winter, (B) early-summer, and (C) mid-summer sowing dates. (D) Coat thickness for seeds from different sowing dates in 2015/16. (E) Coat thickness for seeds obtained from plants sown in mid-summer in 2018, and exposed to an extended photoperiod of 0 h (i.e. natural daylight) or +4 h. Data are means (±SE) of *n*=4 replicates. Different letters indicate significant differences between as determined using ANOVA followed by Tukey's comparison tests (*P*≤0.05; InfoStat v.2017).

the thermal range widened as a consequence of changes in both the lower and higher limits; however, the thermal range was wider the later the sowing date –i.e. $T_{l(50)}$ and $T_{h(50)}$ were respectively lower and higher. This effect was explained by significant differences (*P*<0.05) in the initial values of the thermal limits among the sowing dates (i.e. at dispersal), while the slopes of the linear regressions (i.e. the rate of change in seed population dormancy level) were not significantly different (Supplementary Tables S2, S3). To describe the accompanying changes in the standard deviations of the temperature limits, quadratic functions were fitted against post-maturation time (Fig. 7, bottom).

Since the slopes of the regressions describing changes in $T_{l(50)}$ and $T_{h(50)}$ did not differ among the sowing dates

(Supplementary Table S3), a model was developed based on the effect of sowing date on the initial values of $T_{l(50)}$ and $T_{h(50)}$ (Fig. 8). In developing the models, we used only data from the stratification treatment, since this resembled the natural conditions that *A. hybridus* seeds experience in the soil between dispersal (autumn) and the emergence season (spring). Initial mean values for both the thermal limits (i.e. those estimated at dispersal time) and their corresponding standard deviations were plotted against mother–plant sowing date (Fig. 8A, B). The mean thermal limits fitted using linear regressions (Fig. 8A). Standard deviations of $T_{l(50)}$ were averaged (as they did not show a clear pattern of change), and a quadratic regression was fitted for standard deviations of $T_{h(50)}$ (Fig. 8B). To model the rate of dormancy release after dispersal, changes in $T_{l(50)}$
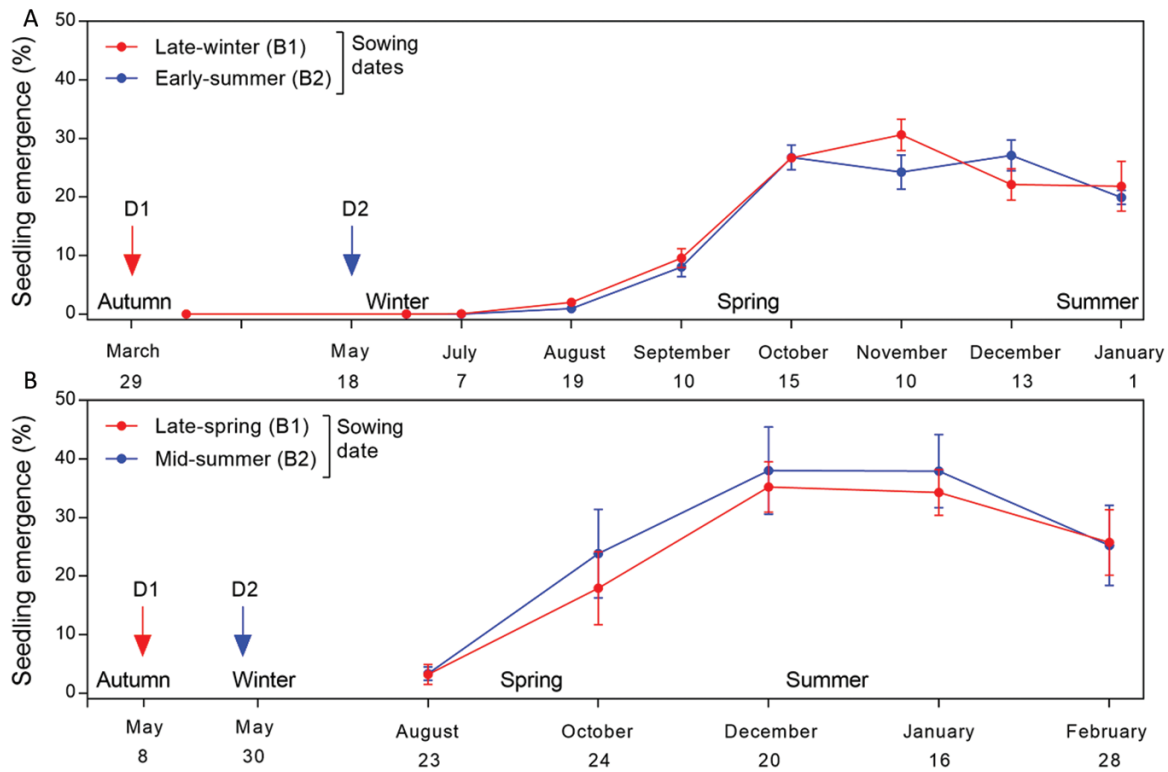
Fig. 6. Effect of sowing date on emergence patterns of *Amaranthus hybridus* seeds under field conditions. Seeds were collected from plants sown in (A) late-winter and mid-summer in 2015/16, and (B) late-spring and mid-summer in 2016/17 (Experiment A, (Supplementary Fig. S1), and they were buried in bags in the field at dispersal times 'D1' for the earlier sowing and 'D2' for the later sowing, as indicated. Seeds were exhumated, removed from the bags, and sown on the dates indicated in each figure panel. Subsequent percentage seedling emergence was then recorded. Data are means (±SE) of *n*=4 replicates.

and $T_{h(50)}$ were expressed in relation to their respective initial values estimated at dispersal time (in Fig. 8A), and linear equations were plotted against stratification time (Fig. 8C). The standard deviations of $T_{l(50)}$ and $T_{h(50)}$ showed no clear pattern of changes during dormancy release, and hence mean values were calculated (Fig. 8D).

Simulating maternal effects on seed dormancy and emergence patterns

Simulations were performed to compare the emergence patterns observed in the field with those predicted by the model under three hypothetical scenarios (Fig. 9). In scenario 1, seeds buried early and late were dispersed with their initial dormancy levels according to Fig. 8(A, B). In scenario 2, seeds that matured and dispersed early displayed the same dormancy level at dispersal as those that matured and dispersed late. In scenario 3, seeds matured and dispersed late displayed the same dormancy level at dispersal as those that matured and dispersed early. The thermal parameters at dispersal were estimated according to the mother–plant sowing date using the equations presented in Fig. 8(A, B). For seeds from the late–winter sowing, the predicted $T_{l(50)}$ was 34.6 °C, $T_{h(50)}$ was 36.3 °C, and their standard

deviations were 3.1 °C and 5.7 °C, respectively. For seeds from plants sown in mid-summer (157 d later), $T_{l(50)}$ was 30.6 °C, $T_{h(50)}$ was 38.2 °C, and standard deviations were 3.1 °C and 5.8 °C, respectively. Changes in the thermal parameters (decrease in $T_{l(50)}$ and increase in $T_{h(50)}$) from their initial values in relation to burial time were simulated using equations and values in Fig. 8(C, D). The lower limit $T_{l(50)}$ was allowed to decrease down to 21 °C, because germination was almost zero at 20 °C (Fig. 2), and emergence in the field of *Amaranthus* species is reduced or almost absent at soil temperatures lower than 20 °C (Keeley *et al.*, 1987; Webb *et al.*, 1987).

According to the simulations performed using the model equations from Fig. 8, germination (hereafter referred to as 'emergence' since this is the measure that we recorded in the field experiment) for scenario 1 was less variable between sowing dates than for scenarios 2 and 3 (Fig. 9B). In scenario 1, the simulation generally matched the temporal emergence pattern observed in the field, although the percentage emergence was overestimated at the later time–points. In scenario 2, the simulation predicted an increase in emergence at early time–points of ~15% in comparison to that simulated in scenario 1. In contrast, scenario 3 showed a marked reduction (~47%) in emergence relative to scenario 1.
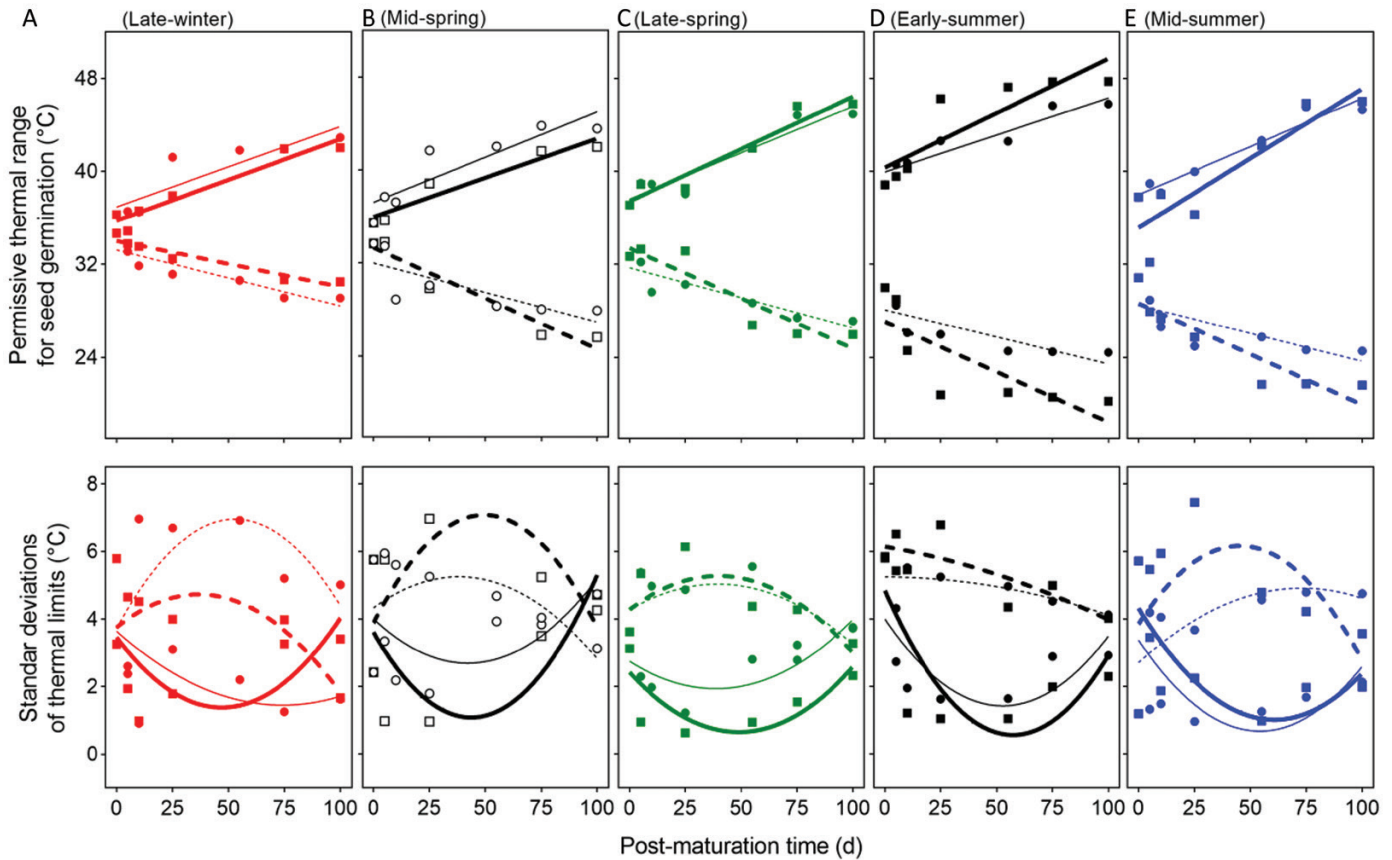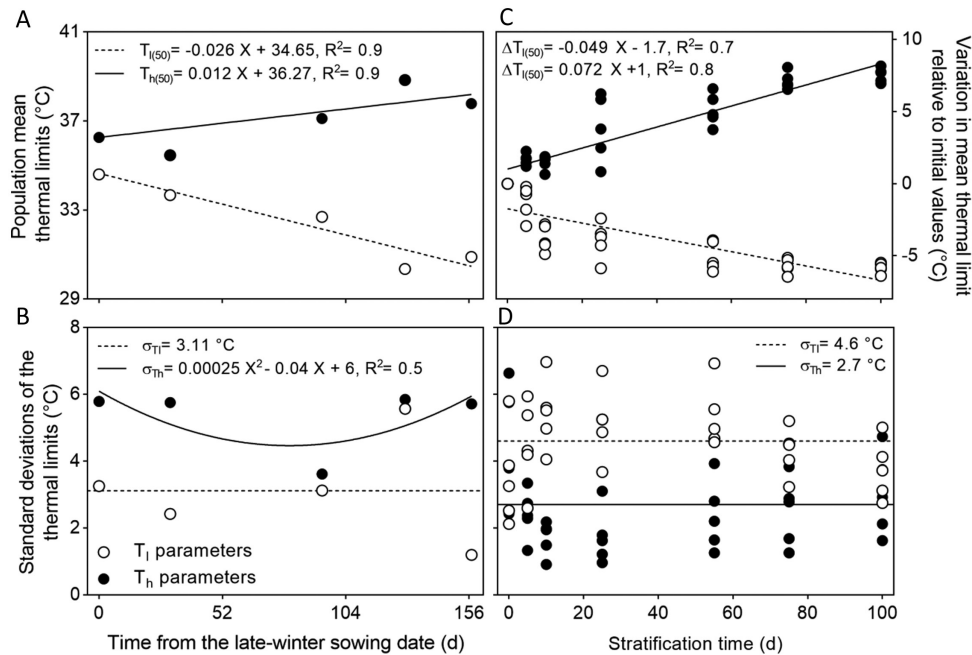
Fig. 7. Modelling of the permissive thermal range for *Amaranthus hybridus* seed germination in relation to post-maturation time. The upper panels show estimated values of the mean lower ($T_{l(50)}$) and mean higher ($T_{h(50)}$) temperatures for 50% germination of the permissive thermal range for germination (estimated based on eqn 1) plotted against post-maturation time, and the lower panels show the corresponding standard deviations. Symbols represent data from laboratory experiments, which were carried out using seeds from maternal experiment A, 2015/16 (see Supplementary Fig. S1). Dotted lines are regressions for $T_{l(50)}$ and T_l standard deviations and solid lines are for $T_{h(50)}$ and T_h standard deviations. Thin lines and circles correspond to values for seeds that were stratified at 4.8 °C; thick lines and squares correspond to dry-storage at 25 °C. Seeds were obtained from plants sown in (A) late-winter, (B) mid-spring, (C) late-spring, (D) early-summer, and (E) mid-summer in Experiment A, 2015/16. The equations for the regressions and R^2 are presented in Supplementary Table S3.

Discussion

The present study examined the influence of maternal environmental conditions on seed dormancy levels in *Amaranthus hybridus* (Figs 2, 3, 4) and the resulting effects on temporal patterns of emergence in the field (Fig. 6). Our results showed that the dormancy level was lower when seeds were produced under late-season maturation environments (Fig. 2, Supplementary Fig. S4) and under short photoperiods (Figs 3, 4). However, despite these effects being observed under laboratory conditions, no differences were found in the timing and extent of emergence in the field. By using population-based threshold models (Figs 7, 8) and performing simulations (Fig. 9), we showed that this synchronization of seedling emergence resulted from the variations in dormancy level that seeds coming from plants sown at different dates presented at harvest time. Our simulations demonstrated that neglecting maternal environmental effects inevitably led to a wrong estimation of emergence patterns under field conditions (Fig. 9B),

thus giving further support to the hypothesis that this effect is crucial for predicting the functioning of seedbanks and hence plant population dynamics.

Similar effects of sowing date on seed dormancy have previously been found or suggested not only for *A. hybridus* (Barton, 1962; Frost, 1971), but also for other species such as *A. retroflexus* (Kigel *et al.*, 1977; Chadoeuf-Hannel and Barralis, 1982), *Chenopodium quinoa* (Ceccato *et al.*, 2015), *C. album* (Karssen, 1970), Arabidopsis (Burghardt *et al.*, 2016; Edwards *et al.*, 2017), and *Polygonum aviculare* (Fernández Farnocchia *et al.*, 2019). Our results indicated that photoperiod is one of the main drivers of changes in dormancy level in *A. hybridus* that result from variations in mother-plant sowing date (Table 2, Figs 3, 4). This has previously been shown to have variable effects on dormancy level (Fenner, 1991); for example, in Arabidopsis, photoperiod has only a subtle effect (Munir *et al.*, 2001; Imaizumi *et al.*, 2017). However, in agreement with our results, a longer

Fig. 8. Development of a model based on the effect of sowing date on initial values of the lower and higher limit temperatures of the permissive thermal range for 50% germination of *Amaranthus hybridus* seeds. Seeds were collected from mother plants sown in late-winter, mid-spring, late-spring, early-summer, and mid-summer in Experiment A, 2015/16 (Supplementary Fig. S1). Only data from the stratification treatment were used, since this resembled the natural conditions that seeds experience in the soil between dispersal (autumn) and the emergence season (spring). (A, B) Initial values of (A) the mean lower ($T_{l(50)}$) and mean higher ($T_{h(50)}$) temperatures of the permissive thermal range for germination and (B) the corresponding standard deviations, σ_{Tl}, and σ_{Th} estimated at dispersal time plotted against the time from the late-winter sowing date of the mother plants. (C) The mean thermal limits expressed relative to the initial values and (D) the corresponding standard deviations plotted against stratification time. Dotted lines and open circles correspond to T_l solid lines and solid circles correspond to T_h. In (D), no clear patterns of variation were observed, so the lines represent the mean values. Analyses were performed using InfoStat v.2017.

photoperiod normally reinforces dormancy, as has been observed in many species of *Amaranthus* (Kigel *et al.*, 1979; Chadoeuf-Hannel and Barralis, 1983). The role of photoperiod in relation to changes in the emergence date of the mother plant has ecological relevance through its acting as a clear seasonal cue, as previously suggested for *P. aviculare* (Fernández Farnocchia *et al.*, 2019). On the other hand, correlations found between dormancy level and environmental factors during seed development and maturation on the mother plant also suggest that other factors varying with photoperiod (temperature and incident solar radiation) could be co-acting, which could at least partly explain why seeds obtained from our early-summer sowing showed higher germination than those from the mid-summer sowing (Fig. 2).

During dry-storage and stratification, germination increased when incubation was performed at high temperatures (i.e. ≥25 °C), but the maternal environment determined differences of up to 40–50% (Figs 2, 3). In contrast, if incubation was performed at low temperatures (i.e. ≤20 °C), germination was reduced or zero. This behavior at high and low temperatures has commonly been observed in *Amaranthus* species, including *A. hybridus*), and in other genera (Schonbeck and Egley, 1980; Weaver and McWilliams, 1980; Ghorbani *et al.*, 1999; Steckel

et al., 2004; Faccini and Vitta, 2005; Cristaudo *et al.*, 2007, 2016; Zhang *et al.*, 2014). Our results showed that differences in the ability of *A. hybridus* seeds to germinate at high temperatures (25-35 °C) mainly resulted from changes in coat-imposed dormancy (Figs 3, 4, 5, Supplementary Fig. S4), and decreased faster for seeds originating from late sowing dates and maturing under short photoperiods (Figs 3, 5, Supplementary Fig. S4). Previous studies have suggested that the seed coat imposes a mechanical restraint on radicle elongation in *A. hybridus* (Faccini and Barat, 1989); however, how the maternal environment influences coat-imposed dormancy is not known in this species. Below 25 °C, differences in both coat- and embryo-dormancy were observed (Figs 3, 4, 5). Embryo dormancy decreased during post-maturation at a faster rate for seeds from shorter photoperiods and later sowing dates, but not enough to overcome the restriction of the coat.

In many species changes in dormancy level are accompanied by changes in the thickness of the seed coat (Karssen, 1970; Pourrat and Jacques, 1975; Dorne, 1981; Lacey *et al.*, 1997; Ceccato *et al.*, 2015). In our study, in agreement with changes in dormancy level, coats were thinner in seeds from late sowings (Fig. 5D, Supplementary Fig. S6) and/or that had matured under short photoperiods (Fig. 5E). We found significant correlations between dormancy level (i.e. the thermal
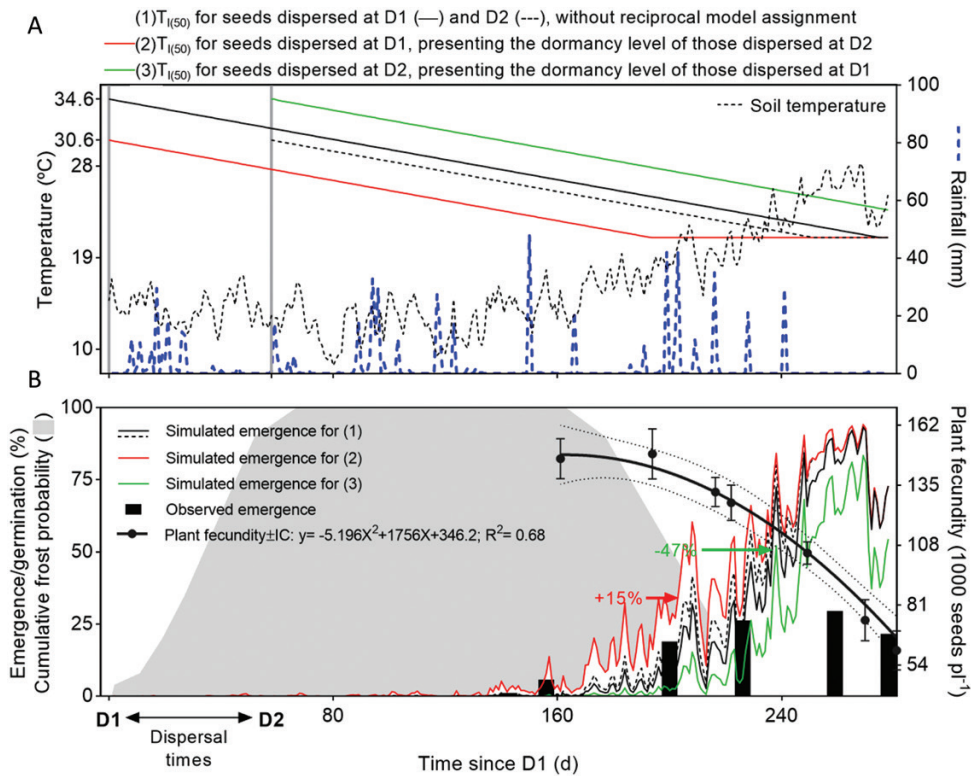
Fig. 9. Environmental conditions and observed versus simulated emergence for *Amaranthus hybridus* seeds obtained from mother plants sown in at different times. (A) Rainfall, mean soil temperature recorded at 5 cm depth, and the modelled lower limits of the permissive thermal range for 50% germination, $T_{l(50)}$, plotted against time from seed dispersal. D1 and D2 are the dispersal dates of seeds obtained from mother plants sown in late-winter and early-summer, respectively (Fig. 6A). (1) Black lines: $T_{l(50)}$ modelled for seeds dispersed at D1 and D2. Seeds dispersed at D1 display a higher initial dormancy level than seeds dispersed at D2. (2) Red lines: $T_{l(50)}$ modelled for seeds dispersed at D1 expressing the initial values of the thermal parameters estimated for those dispersed at D2. (3) Green lines: $T_{l(50)}$ modelled for seeds dispersed at D2 expressing the initial values of the thermal parameters estimated for those dispersed at D1. The simulations were performed using the equations fitted in Fig. 8. (B) Cumulative risk of meteorological frost (i.e. 3 °C) at the field site (https://www.agro.uba.ar/heladas/pergamino_inta_3.htm), mean emergence observed in the field (data from Fig. 6), simulated germination, and seed production per plant (±95% CI) plotted against time from D1. We assumed that the time elapsed from germination to seedling emergence is not significant, and hence we refer only to 'emergence'. The simulated emergences corresponding to the three scenarios presented in (A) are shown, together with the increases (+15%)/decreases (-47%) that they produced relative to scenario 1. Seed production refers to the final reproductive output of plants that had emerged at the time indicated on the *x*-axis (Fig. 1, Supplementary Fig. S3).

limits) and coat thickness (Fig. 10). Although we have not proved any functional relationships, the weight of evidence suggests that examining the association between these variables could lead to uncovering the pathway of maternal regulation of offspring dormancy. When we examined the addition of GA_3 or fluridone (FLU) to the growth media, we found only subtle enhancements of germination at low incubation temperatures (Supplementary Figs S7–S9), whilst ABA+FLU reduced seed germination regardless of the maternal treatment (Figs S8–S10). The role of other GAs and other hormones should be examined in more depth, and determination of the endogenous contents of GAs and ABA should be made in order to establish whether they are involved in regulation of dormancy by the maternal environment in *A. hybridus*, as has been demonstrated for Arabidopsis (Kendall *et al.* 2011; De Giorgi *et al.*, 2015).

As noted above, our models and simulations showed that variations in dormancy level that were determined by the maternal environment were responsible for the synchronization of *A. hybridus* emergence patterns in the field (Figs 6–9). Alternative simulated scenarios where seeds did not show variations in dormancy level at dispersal (scenarios 2 and 3 in Fig. 9) projected either a decrease of 47% in the fraction of seedlings emerging during periods when individuals would maximize reproductive output, or an increase of 15% during earlier periods, when the risks of frost damage would be higher and when a C_4 species such as *A. hybridus* could experience conditions in which it is less competitive (e.g. by having a lower photosynthetic rate than a C_3 species; Pearcy *et al.*, 1981). Very similar results have been reported by Fernández Farnocchia *et al.* (2019) for another spring-emerging weed, *P. aviculare*). Our simulations show the ecological impact that the maternal effect has on next-generation fitness, similar to what has been demonstrated and suggested for other species (Donohue *et al.*, 2005; Burghardt *et al.*, 2016; Edwards *et al.*, 2017).
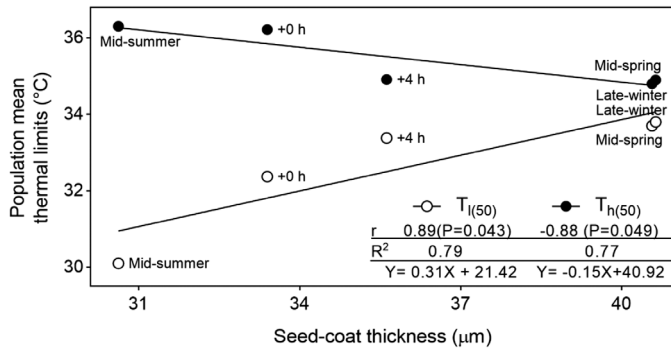
Fig. 10. Estimated values of the lower and higher limit temperatures of the permissive thermal range for 50% germination of *Amaranthus hybridus* seeds, $T_{l(50)}$ and $T_{h(50)}$, at dispersal time as a function of seed-coat thickness for seeds from plants sown in late-winter, mid-spring, and mid-summer in Experiment A, 2015/16, and from plants sown in late-summer and exposed to a natural (+0 h) or extended (+4 h) photoperiod during the reproductive phase in Experiment B, 2018 (Supplementary Fig. S1). The correlations, fitted linear regressions, and R^2 values were determined using InfoStat v.2017.

Although our simulations allow interpretation of the effects of sowing date on the seed dormancy level in *A. hybridus*, they overestimated the percentage emergence compared to what was observed in the field (Fig. 9), mainly later in the summer (December and January). This could be related to the fact that our model only considered temperature as the driver for dormancy release, whereas it could also be affected by soil water content. Alternatively, Faccini and Vitta (2005) have suggested that *A. hybridus* seeds can be induced into secondary dormancy. Although it has not been proven for *A. hybridus*, high summer soil temperatures (Khan and Karssen, 1980; Batlla *et al.*, 2009; Malavert *et al.*, 2017) and seed dehydration for prolonged periods of drought lead to the induction of secondary dormancy in some *Amaranthus* species, and in many other genera (Khan and Karssen, 1980; Auge *et al.* 2015; Edwards *et al.* 2016). In our field experiment, buried seeds experienced both high temperatures and drought during the summer (Fig. 9A) when the model overestimated the percentage emergence (Fig. 9B). This indicates that other environmental variables need to be incorporated to achieve accurate predictions of emergence pattern under field conditions.

Overall, our study highlights the effects of the environmental conditions that seeds experience during their maturation as being a key ecological factor that drives shifts in dormancy levels in the seed bank. Studying this effect in greater detail will allow us to generate a better framework for predicting emergence patterns under natural conditions, which is necessary to improve weed management decisions and to predict plant population dynamics. We consider that our data also indicate that understanding the effects of the maternal environment on seed dormancy will be useful in determining how this seed trait has evolved and how it might change in the future, which is crucial to agricultural management being able to stay ahead of weed evolution and adaptation.

Supplementary data

The following supplementary data are available at JXB online.

Table S1. Environmental variables measured during the full life cycle, and the vegetative and reproductive phases of the mother plants.

Table S2. Thermal parameters estimated for seeds stratified at 4.8 °C or dry-stored at 25 °C.

Table S3. Equations of models fitted to describe the changes in the thermal parameters that characterize the dormancy level.

Fig. S1. Schematic diagram of the experimental design.

Fig. S2. Duration of vegetative and reproductive phases of mother plants for the three growing seasons.

Fig. S3. Reproductive output of plants in 2016/17.

Fig. S4. Final germination of intact seeds, perforated seeds, and isolated embryos.

Fig. S5. Linear regressions fitted to germination data to interpolate the post-maturation time to 50% germination.

Fig. S6. Final germination of perforated seeds under different photoperiods and treated with FLU or GA_3.

Fig. S7. Images of seed coats from plants sown in late-winter, mid-spring, and mid-summer in 2015/16.

Fig. S8. Final germination of intact and perforated seeds at either 10 °C or 15 °C and treated with FLU, GA_3, or ABA+FLU.

Fig. S9. Final germination of perforated seeds at either 25 °C or 35 °C and treated with FLU or ABA+FLU.

Fig. S10. Final germination of perforated seeds after either 50 d or 75 d of dry storage and treated with ABA+FLU.

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Author contributions

DB, RBFF, and RLB-A: conceptualization; RBFF and AM: formal analysis and investigation; RBFF and DB: methodology; RBFF: validation and writing original manuscript draft; RLB-A: visualization and writing—review and editing; DB: investigation, funding acquisition, project administration, resources, supervision, writing—review and editing.

Data availability

All data supporting the findings of this study are available within the paper and within its supplementary data published online.

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