

Research Paper

Cite this article: Holloway TE, Pérez M, Venceslai N, Seville A, Stock D, Nakabayashi K and Leubner-Metzger G (2025) Vernalization enforces seed dormancy in the agricultural weed *Alopecurus myosuroides* (Huds.). *Seed Science Research* **35**, 126–133. <https://doi.org/10.1017/S0960258525100019>

Received: 17 February 2025

Revised: 13 June 2025

Accepted: 17 June 2025

First published online: 23 July 2025

Keywords:

DNA methylation; dormancy; epigenetics; germination; maternal effects; vernalization

Corresponding author:

Thomas Edward Holloway;

Email: Thomas.holloway@syngenta.com

Vernalization enforces seed dormancy in the agricultural weed *Alopecurus myosuroides* (Huds.)

Thomas Edward Holloway^{1,2} , Marta Pérez^{1,3,4} , Nahema Venceslai¹ , Anne Seville² , David Stock², Kazumi Nakabayashi^{1,5}  and Gerhard Leubner-Metzger¹ 

¹Department of Biological Sciences, Royal Holloway University of London, Surrey, UK; ²Syngenta, Jealott's Hill International Research Centre, Warfield, UK; ³Biodiversity Research Institute (IMIB), University of Oviedo–CSIC–Principality of Asturias, Mieres, AS, Spain; ⁴Department of Organismal and Systems Biology, University of Oviedo, Oviedo, AS, Spain and ⁵Department of Agro-Environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, HKD, Japan

Abstract

Seed dormancy is the key factor determining weed emergence patterns in the field. *Alopecurus myosuroides* (black grass) is a serious cereal weed in Europe that experiences two emergence peaks affecting winter and spring cereals, respectively. Seedlings that emerge in autumn encounter a period of cold winter temperatures, whereas those that emerge in spring do not. In this work, we investigated the effects of this overwintering during vegetative growth on the primary seed dormancy of the offspring. *Alopecurus myosuroides* plants were propagated under controlled conditions where a proportion of the population was subjected to a simulated winter period (vernalization) as seedlings. The offspring produced by vernalized plants was significantly more dormant, requiring longer after-ripening and cold stratification treatments to germinate at warm temperatures. However, there was no difference in the range of temperatures under which dormant seeds germinated. We hypothesized that this difference in dormancy was the result of an epigenetic memory of vernalization. Global changes in DNA methylation of seeds were quantified using an ELISA-based approach. Imbibition in dormant seeds produced by vernalized plants was associated with a global demethylation event that was not observed in the offspring of plants that had not been vernalized. Taken together, these results demonstrate the importance of temperature at different stages of the plant lifecycle in determining dormancy levels and consequently weed emergence patterns in the field.

Introduction

Alopecurus myosuroides Huds. (black grass) is often cited as the most destructive agricultural weed in Europe (Lutman et al., 2013) due to its high fecundity (Moss, 1985), multiple herbicide resistance traits (Heap, 2019) and competitive ability against cereal crops (Maréchal and Henriët, 2012). *Alopecurus myosuroides* has a typical winter annual life history (Fig. 1) whereby diaspores (hereafter seeds) are dispersed in the summer with a high degree of primary dormancy that prevents their germination until colder winter temperatures, and consequently, a major peak of emergence occurs in winter cereals. A minor emergence also occurs in spring, which coincides with the planting of spring cereals (Clarke et al., 2015). Previous studies have shown that primary dormancy acts to narrow the range of temperatures permissive to germination, so that dormant *A. myosuroides* will only germinate at lower temperatures (Holloway et al., 2024). In this way, *A. myosuroides* synchronizes its emergence with the cropping cycle to avoid herbicide applications and maximize its competitiveness with the crop (Maréchal and Henriët, 2012), ultimately conferring an evolutionary advantage compared to individuals that emerge outside the crop and are easily controlled by non-selective herbicides.

A long-term trend for earlier sowing of winter wheat has led to increased *A. myosuroides* infestations (Moss, 2017); hence, recent *A. myosuroides* management practices have shifted from winter cereal production to spring cereals and oilseed rape in an attempt to avoid the peak winter *A. myosuroides* emergence (AHDB, 2016, 2017, 2018). This system has been successful in managing *A. myosuroides* infestations (Lutman et al., 2013); however, recent evidence has suggested that *A. myosuroides* is adapting to this strategy by delaying emergence until the spring, leading to similar levels of emergence in the winter and spring (Taylor-Davies, 2017). Models of *A. myosuroides* emergence recognize the importance of seed dormancy in determining the timing and scale of emergence in

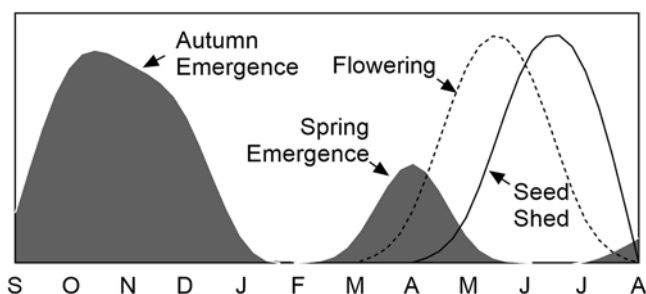


Figure 1. The lifecycle phenology of *A. myosuroides*. Grey shaded areas represent emergence; dotted and solid lines show the period during which flowering and seed dispersal occur, respectively. Letters represent months of the year. Modified from Clarke et al. (2015).

field (Colbach et al., 2006a, 2006b); however, these models focus on the autumn emergence of *A. myosuroides* and do not consider a potential shift to spring emergence.

There is a large body of evidence in crop and model species to suggest that the environment experienced by the mother plant modulates seed dormancy in the offspring (Penfield and MacGregor, 2017). These effects are divided into those that affect the developing zygote whilst it is maturing on the mother plant ('zygotic environment effects') and those effects that are strictly maternal before fertilization occurs, which are mediated by the maternal genome or epigenome ('true maternal effects') (Penfield and MacGregor, 2017). Whilst zygotic environmental effects are well studied even in *A. myosuroides* (Swain et al., 2006), studies on true maternal effects are much less common. For example, in *Avena fatua* (wild oat, another grass weed of European cereal crops), it has been demonstrated that varying the temperature experienced during the vegetative growth can influence the seed dormancy of the progeny (Sawhney et al., 1985).

Interestingly in *A. myosuroides*, although there are two peaks of emergence, flowering and seed dispersal occur only once across the year, suggesting that these two emerged cohorts of *A. myosuroides* have similar zygotic environment; however, the true maternal environmental conditions differ. The autumn-emerged cohort experiences winter and the spring-emerging cohort does not.

In this study, we aim to investigate the relationship between maternal vernalization and the seed production and seed dormancy of the subsequent offspring in *A. myosuroides*. We investigated the different responses of vernalized (V) and non-vernalized (NV) seeds to different stimuli such as varying temperature, cold stratification and germination stimulants to determine in which ways the seeds from the different batches differ in their behaviour. We also aim to understand whether the differences in vernalization or dormancy influenced the global DNA methylation of the seeds.

Materials and methods

Seed material

A field collected parental population of *A. myosuroides* seeds from Germany that was harvested in May 2015 (hereafter LH-170) was used for most experiments. Two additional populations, one harvested in Cambridgeshire (UK) in 2018 (hereafter LH-161) and one sourced from Berkshire (UK) in 2018 (hereafter LH-158) were also included in some experiments. All three populations were incubated at 20°C under constant light (white fluorescent light at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, measured with a LI-250 Light Meter; Li-Cor,

Germany) on moist filter papers for 10 days in an environmental chamber (MLR-352-PE, Panasonic, Osaka, Japan). The resultant seedlings were then transferred to 6 cm diameter pots containing a 10:1 mixture of John Innes Number 1 compost and perlite (Westland Horticulture Ltd, Dungannon, Northern Ireland) and grown for 14 days in a 16/8 h day/night cycle at 18°C. Half of this crop was transferred to a Conviron™ Environmental Test Chamber (Conviron Adaptis® CMP6010) set at the same conditions, where the temperature and photoperiod were gradually reduced over the course of 6 days from 18°C to a 8/16 h day/night cycle at 6°C and 4°C, respectively, whilst the other half of the population was propagated under the original warm conditions. This condition was maintained for 35 days, and then the temperature and day/night cycles were increased up to the initial condition. Five V and five NV plants were then transferred to a glasshouse bay maintained at 18°C with a 16/8 h photoperiod with supplemental lighting from sodium lamps. The onset anthesis was counted over time as the emergence of the tip of the panicle from the culm. All plants from both batches reached maturity at a similar time (data not shown) and were harvested at maturity on the same day by agitating the mature panicles to ensure only mature seeds were collected. These seeds were weighed for each plant separately and the resultant seed batches were pooled into V and NV batches, dry-stored using silica gel (<10% moisture content by weight) and stored at -20°C for the duration of the experiments (<1 year). To determine the 100 seed weight for each batch, triplicates of 100 randomly selected freshly harvested (FH) seeds were weighed using a fine balance.

Germination kinetics

Germination assays were conducted in triplicate using the following standard conditions unless otherwise specified: 30 seeds were incubated in 3 mL distilled water in Petri dishes with two filter papers (Machery-Nagel™ MN-713) and maintained at 20°C under constant light in environmental test chambers (MLR-352-PE). Germination was regularly assessed following Holloway et al. (2024) as the emergence of the coleorhiza (coleorhiza emergence) through the margin of the glumes counted over time until there was no further increase in the number of emerged coleorhizae. This value was reported as the maximum number of germinated seeds within the population (gMAX). For the production of seed batches with different levels of dormancy, dormant FH seeds stored in porous paper bags were incubated in a hermetically sealed chamber containing saturated calcium nitrate, which generated an equilibrium relative humidity of 53%. Incubation under these conditions resulted in a seed moisture content of 19.3% (w/w). Seeds were periodically removed monthly, and germination was tested using standard conditions. For experiments involving the endogenous application of gibberellin A₄₊₇ or fluridone (Duchefa Biochemie, Haarlem, the Netherlands), compounds were first solubilized in absolute dimethyl sulfoxide (DMSO), and consequently, a solvent control of 0.1% (v/v) DMSO was used in these experiments. Separate germination media were made with gibberellin A₄₊₇ applied at 100 μM and fluridone applied at 10 and 100 μM . To investigate the effect of imbibitional temperature on the germination of FH or 196 day after-ripened (AR), V and NV batches were incubated across a gradient of temperatures from 5°C to 28°C under constant light conditions using a two-dimensional thermogradient plate (GRANT GRD1-LH, Grant Instruments Ltd, Cambridge, UK) and germination was scored using the standard conditions procedure. The temperatures across the plate were

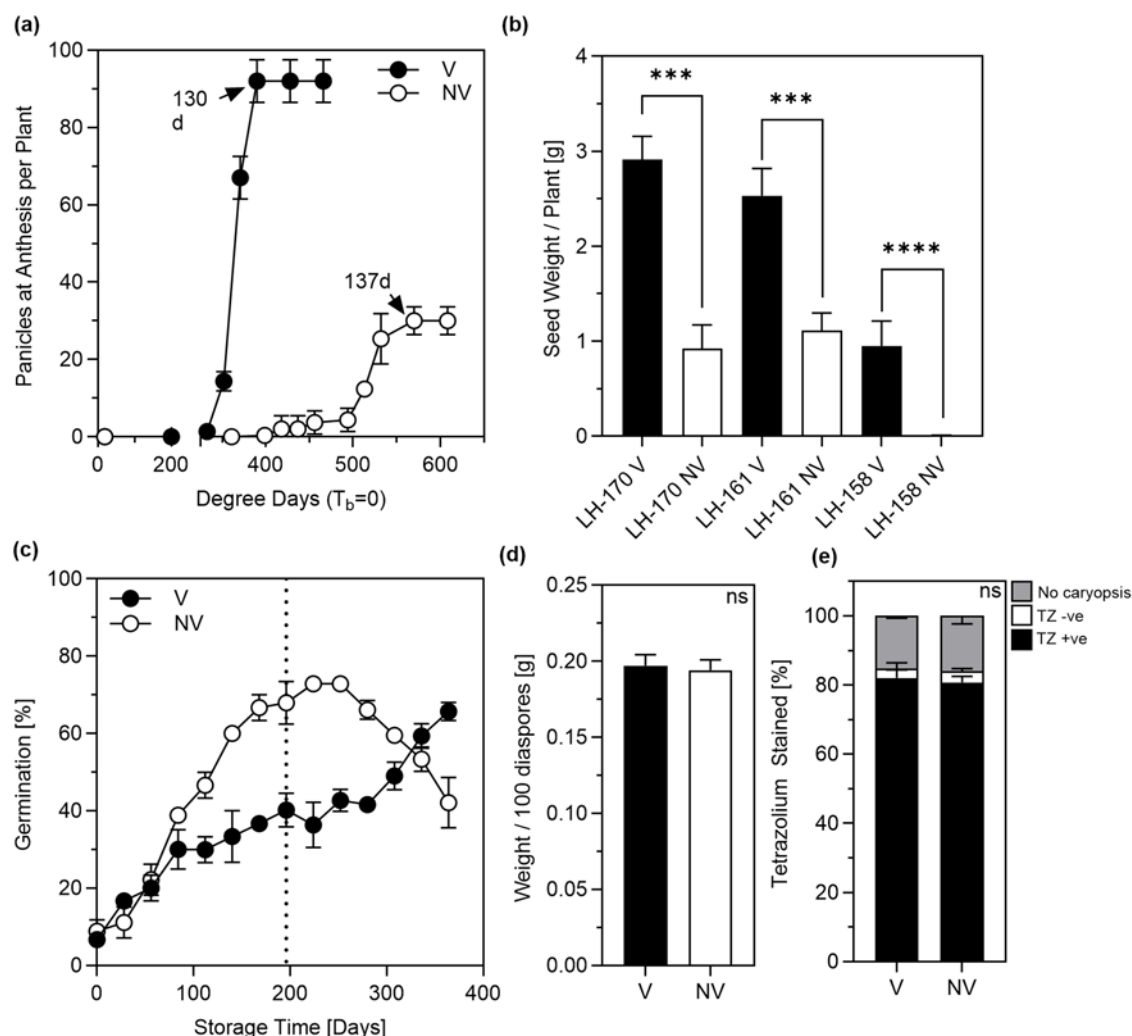


Figure 2. Propagation and description of seed batches. (a) Assessment of flowering time for V and NV *A. myosuroides* plants. Accumulated day degrees were calculated with a base temperature of 0°C. Flowering was counted by the emergence of panicles from the culms. (b) The difference in the seed weight produced by each plant for V and NV treatments. Statistical significance from a Mann-Whitney test is represented by asterisks (***) ($p = 0.0079$). (c) After-ripening timecourse showing the change in maximum germination over storage time at 53% equilibrium relative humidity for V and NV batches. (d) Seed mass for triplicates of 100 seeds in V and NV batches. (e) Viability testing of freshly harvested V and NV batches showing the proportion of diaspores containing no caryopsis (grey) and that had embryos stained (black) or non-stained (white) by incubation in 2,3,5-triphenyltetrazolium chloride (TTC) solution. Error bars show standard error of the mean.

recorded using a handheld infrared thermometer (Robert Bosch GmbH, Germany).

Seed viability testing

V and NV batches that had been AR for 196 days were imbibed for 24 h at 20°C under constant light as previously described, using the same set of LH-170 seeds as used in other assays. One hundred diaspores in triplicate were dissected and the number of diaspores that contained a fully formed caryopsis were recorded. Intact caryopses were then transferred to a Petri dish containing filter papers as described before, with 3 mL of a medium containing 1% (w/v) 2,3,5-triphenyltetrazolium chloride (TTC). As negative (dead) control, another set of caryopses were incubated at 90°C in ultrapure water for an hour and then transferred to the TTC medium. These plates were then incubated for a further 24 h at 20°C in the dark. Staining was assessed using a stereomicroscope. Embryos were considered stained when the entirety of the visible external portion of the embryo (underneath the testa and pericarp)

was fully stained dark red. Caryopses with weak or patchy staining were considered unstained.

Cold stratification

Triplicates of 30 FH V and NV LH-170 diaspores were imbibed in Petri dishes as previously described with 1 mL of autoclaved distilled water. These plates were incubated in the dark at 4°C using a laboratory refrigerator for 3, 7, 14 and 21 days. No germination occurred during the stratification. After this time, an additional 2 mL of autoclaved distilled water was added and the plates were transferred to an incubator at 20°C under constant light and germination was scored regularly as previously described.

ELISA-based quantification of DNA methylation

Forty milligrams (dry weight) of LH-170 V or NV seeds, which were either dormant or AR and were either dry or had been imbibed for 90 h under standard germination conditions, were

homogenized by pestle and mortar in liquid nitrogen. DNA was extracted using a DNeasyTM Plant Mini Kit (Qiagen, Venlo, the Netherlands) following the manufacturer's recommendations with modifications (5% (v/v) β -mercaptoethanol in lysis buffer). DNA yield and quality were quantified using a NanoQuant PlateTM (Tecan, Männedorf, Switzerland). Quantification of 5-methylcytosine (5-mC) was performed using a fluorometric enzyme-linked immunosorbent assay (ELISA) kit (MethylFlashTM Methylated DNA Quantification Kit, EpigenTek, New York, USA) following the manufacturer's instructions for 10 ng of genomic DNA. Fluorescence proportional to 5-mC concentration was measured for three biological and two technical replicates using a microplate reader (Tecan SPARKTM, Männedorf, Switzerland) at 530_{EX}/590_{EM} nm and %5-mC was calculated from a standard curve of synthetic 5-mC, as a relative quantification following the manufacturer's recommendations.

Statistical analysis

Statistical analysis of the data was conducted using GraphPad Prism (version 10.4.2, GraphPad Software LLC, San Diego, CA, USA). The specific statistical tests used are described in the Results. Degree days were used to represent flowering time phenological data (Fig. 2a) using the formula $DD = T - T_b$, where DD is degree days, T is the mean daily temperature and T_b is a base temperature. For our calculations, we used 0°C as a base temperature.

Results

Vernalization of plants had a strong impact on *A. myosuroides* plant morphology, flowering and seed production. V plants were shorter than NV plants with more dense culms (data not shown). The initiation of flowering in both V and NV plants occurred simultaneously (130–140 days after germination); however, when expressed as degree days there was a delay in the flowering of NV plants (Fig. 2a) yet V plants produced approximately 3-fold more panicles (Fig. 2a) which equated to a 3-fold increase in seed mass per plant (Mann–Whitney $U(40,15)$, $p = 0.0079$) (Fig. 2b). There was no significant difference in the 100 seed weight produced by V and NV plants (Mann–Whitney $U(12,9)$, $p = 0.7$) (Fig. 2d) and there was no difference in the viability of seeds produced by either treatment as measured by presence of a caryopsis or by tetrazolium staining (two-way ANOVA, $F(2,12) = 0.225$, $p = 0.8018$). The absence of a caryopsis contributed more to the non-viable population than a lack of staining by incubation in TTC solution (Fig. 2e).

The seeds produced by NV plants AR more rapidly than seeds produced by V plants, reaching a fully AR state after approximately 200 days. In the fully AR state, seeds from NV plants reached ~75% maximum germination (Fig. 2c), reflecting the number of tetrazolium-stained embryos (~80%) (Fig. 2e). The V seed batch did not reach a fully AR state even after a year of after-ripening treatment (Fig. 2c). Interestingly, after 250 days of after-ripening, the maximum germination of seeds produced by NV plants was steadily reduced, indicating that seeds were losing germinability during after-ripening (Fig. 2c).

In the dormant state, seeds produced by V and NV plants germinated over a similar range of temperatures between 5°C and 15°C (Fig. 3a). A low proportion of seeds (~20%) from the V batch germinated under this temperature range, whereas a larger proportion

of seeds (~50%) from NV plants germinated under these conditions (Fig. 3a). This effect was most pronounced at 7°C (Fig. 3c). After 196 days of after-ripening, the temperature range permissive to germination was greater in seeds produced by both V and NV plants (Fig. 3b). Seeds from NV plants reached high germination percentages (60–75%) over a broad range of temperatures between 5°C and 22°C, whereas this level of germination was only achieved in AR seeds produced by V plants at a single temperature of 12°C (Fig. 3e). AR seeds from NV plants also germinated to a higher maximum proportion at 5°C, the coldest temperature tested (Fig. 3d).

Seeds produced by V and NV plants also differed in their response to cold stratification. Whilst in the dormant state there was low germination of both batches when imbibed at 20°C under constant light (<5%), cold stratification at 4°C in the dark increased the germination of both batches when transferred to 20°C under constant light in a dose-dependent manner (Fig. 4). However the effect of cold stratification was consistently ~2-fold greater in seeds from NV plants (Fig. 4). After 21 days of cold stratification, the NV treatment reached similar final germination proportions (mean = 68%) as their respective fully AR seed batch (mean = 73%). Cold stratification of seeds produced by V plants for 21 days reached lower germination proportions (mean = 27%), particularly when compared to their respective 196 day AR batch (mean = 40%) (Fig. 4e).

Incubation of V and NV batches of LH-170 in gibberellin A₄₊₇ (GA₄₊₇) did not show a strong response, with only the NV batch showing a slight increase in germination in response to GA₄₊₇ (Fig. 5a,b). However, the both the V and NV batches of LH-170 showed increased germination in response to 10 μ M and 100 μ M fluridone.

Quantification of global DNA methylation levels in both dormant and AR batches of dry and imbibed seeds produced from V or NV plants identified a pattern of differential methylation only in the V condition. There was no significant difference between the dry dormant and AR seeds of V and NV batches. Whilst there was no change in methylation between 0 and 90 h in the AR batch, a significant demethylation was observed in the dormant batch during comparing the dry and 90 day imbibed seeds (two-way ANOVA, $F(1,15) = 5.12$, $p = 0.0377$) (Fig. 6a). No significant changes in methylation were observed in seeds from the NV batch associated with after-ripening or imbibition (Fig. 6b).

Discussion

Vernalization has a clear effect on *A. myosuroides* morphology and seed production as previously discussed by other authors (Chauvel et al., 2002). The original result from this study is that vernalization modulates the dormancy of the offspring of *A. myosuroides* plants, as demonstrated by an increase in after-ripening and cold stratification requirement. The implication of this finding is that winter emerging *A. myosuroides* may produce offspring that have higher dormancy levels than spring emerging populations. We might therefore hypothesize that spring emerging populations are likely to produce low dormancy seeds which emerge early (i.e. during winter) and autumn emerging plants might produce high-dormancy seeds with delayed germination that are more likely to germinate in the spring of the following year – further studies in the field would be required to confirm this. This would be in contrast to some previous examples, where maternal effects have been reported to synchronize the emergence timing of seeds produced

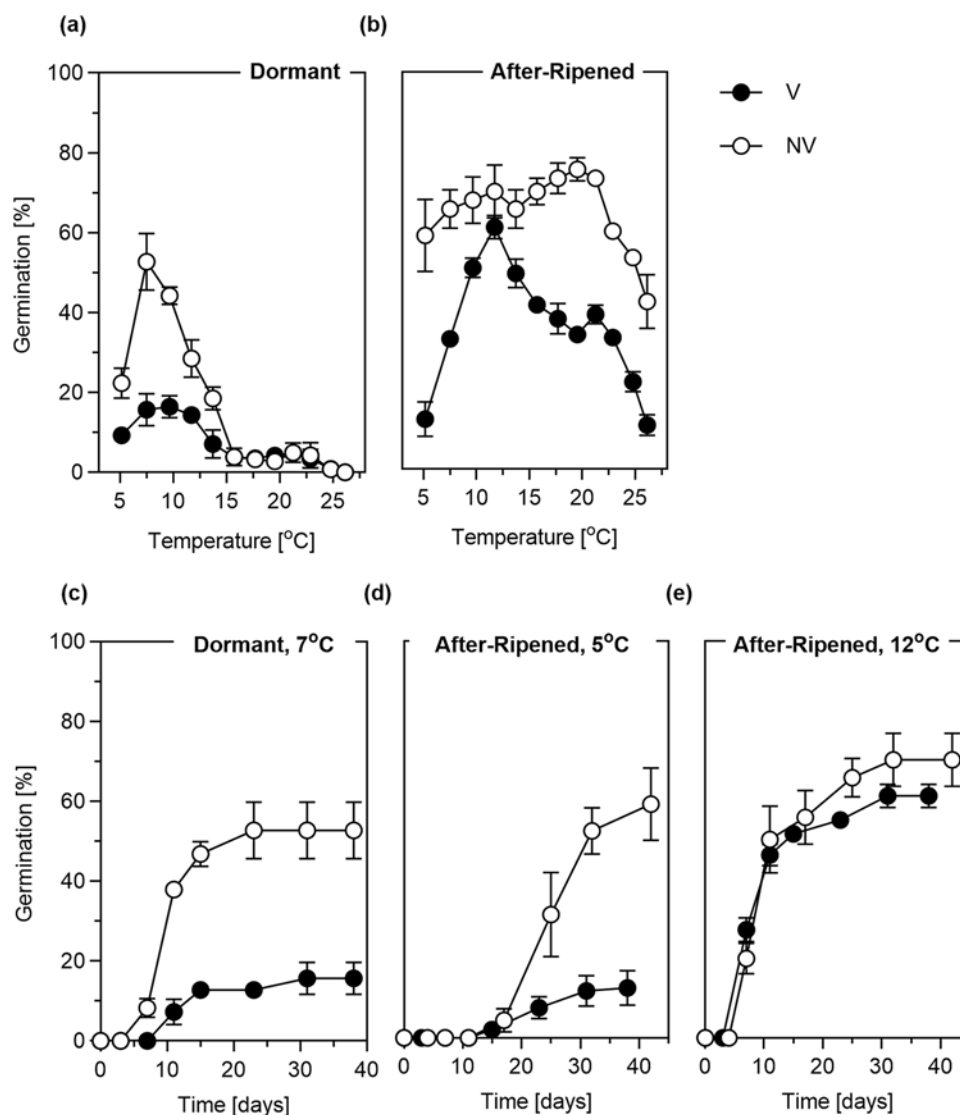


Figure 3. The effect of dormancy and vernalization on the response of *A. myosuroides* seeds to temperature. (a,b) The effect of imbibition of dormant (a) and 196 day after-ripened (b) LH170-V and NV seeds across a gradient of temperatures. (c–e) Germination curves for LH-170 V and NV batches at selected temperatures: (c) dormant seeds incubated at 7°C, (d) after-ripened seeds incubated at 5°C and (e) after-ripened seeds incubated at 12°C. Germination was scored as coleorhiza emergence for triplicates of >30 seeds. Error bars show standard error of the mean.

by plants shedding seeds at differing times of year (Guterman, 1992). Both of the *A. myosuroides* cohorts flower and set seed at the same time in the field (Clarke et al., 2015), yet the dormancy of the seed returned to the seedbank is likely to be heterogeneous. This could serve as a bet-hedging strategy in the constantly changing environment of an agricultural field where herbicide application, cultivation and crop rotation could drastically change year on year. Such seed progeny bet-hedging strategies have recently been reviewed by Bezodis and Penfield (2024), who propose that bet hedging maximizes the fitness of the mother plant when seeds are dispersed into a risky environment for plant mortality. This strategy has also been described for heteromorphic seeds, where the fitness benefit of heteromorphy correlates with the unpredictability of the environment (Arshad et al., 2019). It is also interesting to note that total seed production in NV plants was much lower than V plants, indicating a fitness disadvantage for spring germinating *A. myosuroides* mother plants. We might therefore hypothesize that the fitness advantages of a bet-hedging strategy might outweigh

the reduction in fitness of the parents due to low seed production caused by a lack of vernalization. Further research in the field over multiple generations would be required to test this hypothesis, for example tracking the seed production of autumn and spring emerging *A. myosuroides* over several years.

Although seeds produced from V and NV plants differed in their dormancy levels, the range of temperatures under which these batches germinate in the dormant state did not differ; in contrast to established physiologically dormant species, where differences in dormancy level are typically associated with changes in the temperature window permissive to germination (Baskin and Baskin, 2004). This indicates that it is unlikely that the two emergence peaks described by previous authors (Fig. 1) are driven by differential temperature preferences of the offspring of previous autumn or spring emerging *A. myosuroides*. Additionally, these two dormant batches did not differ greatly in their responses to the classical dormancy breaking treatments of exogenous gibberellin and fluridone application, although the NV batch was more sensitive to

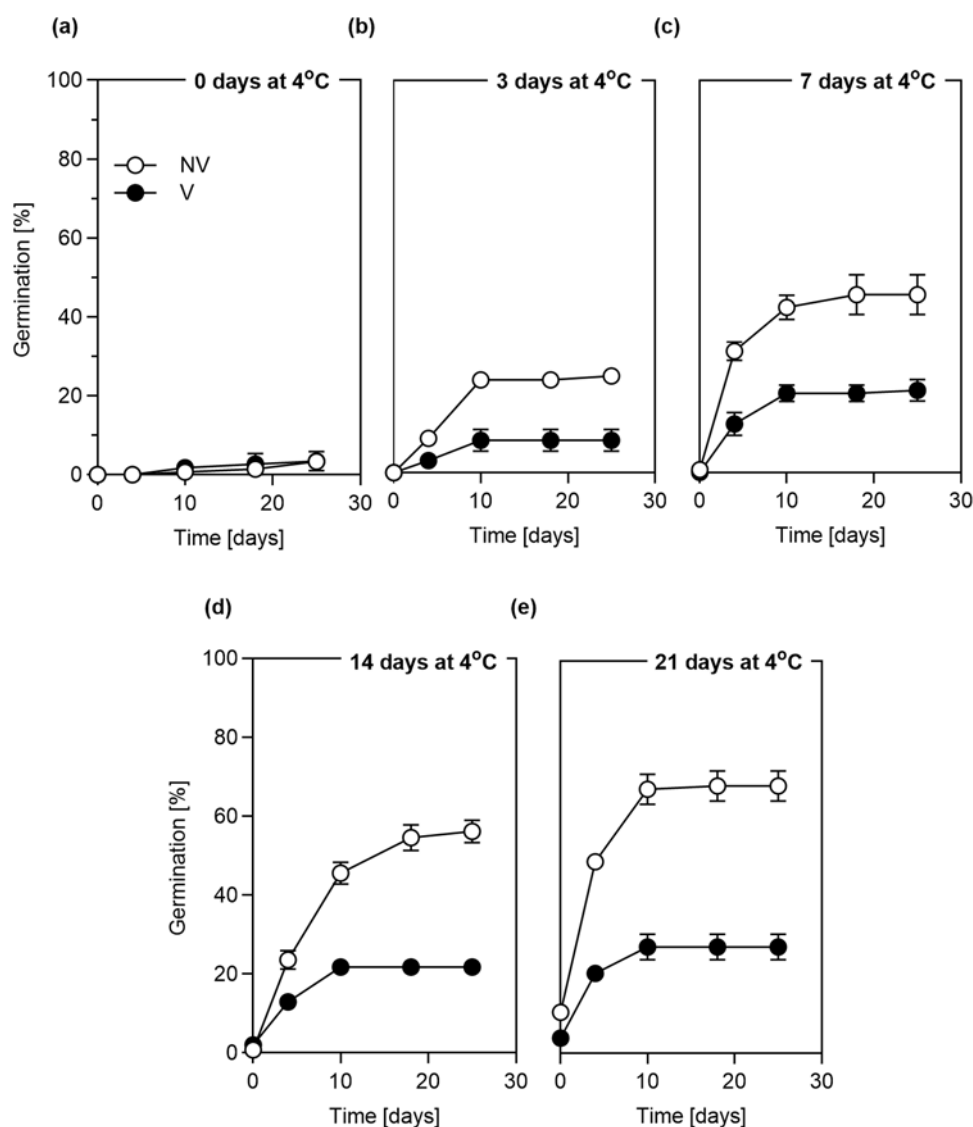


Figure 4. Cold stratification of dormant *A. myosuroides* seeds produced by LH-170 V or NV plants. The effect of imbibition of seeds produced by LH-170 V and NV plants at 4°C in constant darkness prior to transfer to 20°C in constant light where seeds were incubated in the cold for (a) 0 days, (b) 3 days, (c) 7 days, (d) 14 days and (e) 21 days. Germination was scored as cumulative coleorhiza emergence. Error bars show standard error of the mean.

gibberellin application. Fluridone is an inhibitor of phytoene desaturase, an enzyme involved in the biosynthesis of the carotenoid precursors of abscisic acid (ABA), the phytohormone associated with the enforcement of dormancy (Bartels and Watson, 1978; Nambara et al., 2010). These results indicate some involvement of *de novo* ABA biosynthesis in the enforcement of dormancy in *A. myosuroides*; however, this process cannot explain the difference we see in the dormant seeds from V and NV plants. These results agree with recent transcriptomic studies, which indicate a role for both gibberellin and abscisic acid in the regulation of seed dormancy and germination in *A. myosuroides* (Holloway et al., 2024).

True maternal effects on seed dormancy are rare in literature, typically involving temperature changes during plant growth (Auge et al., 2017). These effects are linked to genes controlling flowering timing, such as *FLOWERING LOCUS C* (*FLC*), circadian clock genes, *FLOWERING LOCUS T* (*FT*) and *MOTHER OF FT AND TFL1* (*MFT*), which also regulate vernalization and day length responses in *Arabidopsis thaliana* (Chen and Penfield,

2018). Disrupting these genes alters seed dormancy, hormone metabolism and seed coat pigmentation (Penfield et al., 2005; Chiang et al., 2009; Macgregor et al., 2015). Signals during vegetative development are 'remembered' through epigenetic gene silencing, with the *Arabidopsis* vernalization response being a well-understood example (Song et al., 2012). DNA methylation plays a crucial role in vernalization-induced flowering, embryo maturation and cold-stratification induced bud dormancy breaking in forestry, with global DNA demethylation patterns preceding developmental transitions (Santamaría et al., 2009; Viejo et al., 2010; Pérez et al., 2015).

The analysis of patterns of histone modifications and DNA methylation at the gene level that are performed in model species are challenging for agricultural weed species such as *A. myosuroides*. What we sought to achieve with the quantification of 5-mC was to investigate the general trends in genomic methylation that are associated with the interaction between vernalization, dormancy level and imbibition. Our results for the V batch show

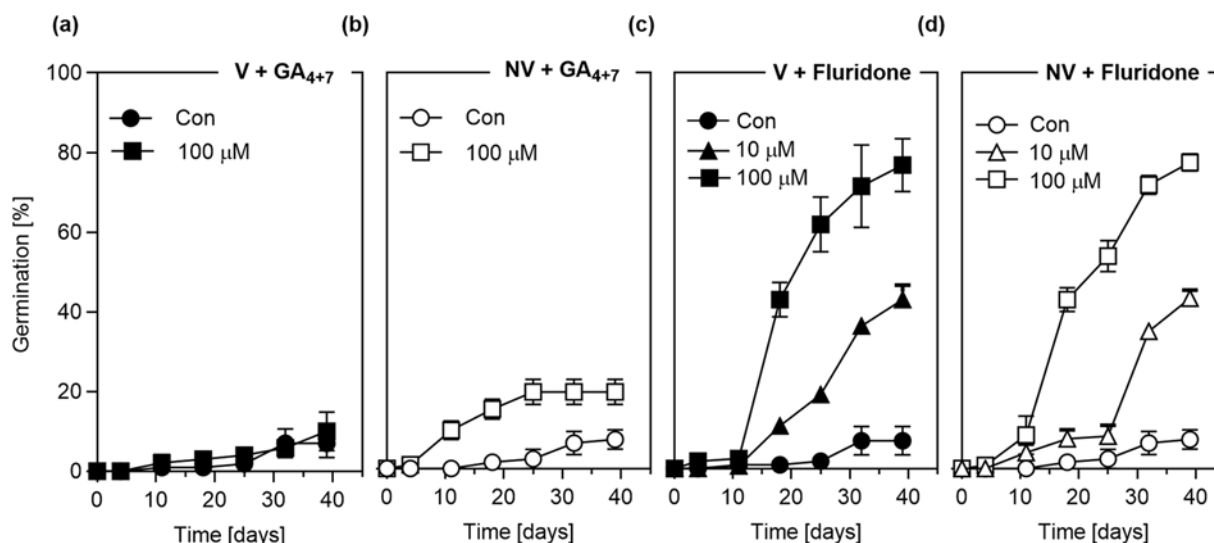


Figure 5. The effect of gibberellin and fluridone application on dormant seeds from LH-170 V and NV plants. (a,b) The effect of 100 μM gibberellin A₄₊₇ on dormant seeds from (a) V and (b) NV plants. (c,d) The effect of application of 10 or 100 μM fluridone on dormant seeds from (c) V and (d) NV plants. Germination was counted as cumulative coleorhiza emergence for triplicates of >30 seeds incubated at 20°C under constant light. Error bars show standard error of the mean.

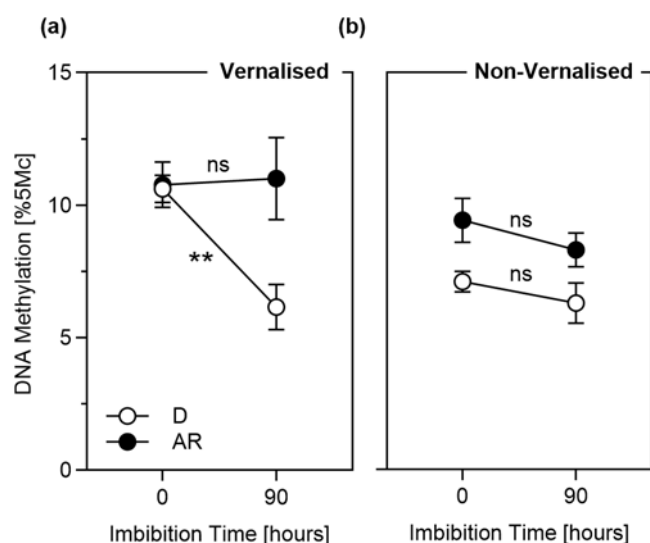


Figure 6. Changes in DNA methylation associated with the enforcement of dormancy. Results from ELISA-based quantification of global DNA methylation from dormant (D) and after-ripened (AR) LH-170 batches of seeds produced by (a) vernalized and (b) non-vernalized plants either dry or after 90 h of imbibition at 20°C under constant light. Error bars show standard error of the mean for >3 biological replicates of 30 seeds. Asterisks show significance at $p < 0.01$ from a two-way ANOVA where 'ns' shows no significant effect.

demethylation in response to imbibition only in the D state and no changes in the NV batch. This indicates that, rather than seed dormancy being a 'quiescent state' in *A. myosuroides*, a developmental program may be induced to prevent germination and that this program may be associated with environmental cues throughout the plant lifecycle. Transcriptomic analysis of *A. myosuroides* seeds have observed large numbers of up- and down-regulated genes after 90 h of imbibition (Holloway et al., 2024). In *Capsella bursa-pastoris* (Shepherd's purse), differential patterns of global methylation have also been observed between dry and imbibed dormant seeds (Gomez-Cabellos et al., 2022). These patterns were

observed to be dynamic over time and tissue specific, indicating that further work would be required to better understand how global methylation influences the seed dormancy program in *A. myosuroides*.

Our results clearly demonstrate that vernalization modulates offspring seed dormancy in *A. myosuroides*. This is a strong example of phenotypic plasticity in agricultural weeds and a mechanism by which *A. myosuroides* can increase the heterogeneity of its soil seedbank as a potential bet-hedging strategy against the constantly changing environment of the agricultural field. Models for predicting weed emergence should consider the plasticity of weed seed dormancy, especially in the context of changing weed management practice and climate change.

Conclusions

Our results show the first demonstration that vernalization of *A. myosuroides* plants increased the offspring seed dormancy. This was evidenced by an increased after-ripening and cold stratification requirement for germination but had no effect on the window of temperatures permissive to germination in the dormant state. After-ripening widened the temperature window more slowly in seeds produced from V plants. The enforcement of dormancy in seeds from V plants was associated with a global DNA demethylation event that was not observed in seeds produced by NV plants. In the context of autumn and spring emergence peaks in the lifecycle of *A. myosuroides*, these results indicate that vernalization acts to increase the heterogeneity of dormancy in the weed seedbank. Consequently, models should consider the effect of temperature throughout the lifecycle of weeds to more effectively predict weed emergence in the field. Better models for predicting the emergence of weeds in a changing climate will have an important role in improving future integrated weed management strategies for farmers.

Acknowledgements. We thank Mark Levy, Diane Grant and Sarah Rabjohn for assistance with seed processing.

Funding statement. This work was supported by Biotechnology and Biological Sciences Research Council research grants (BB/R505730/1; BB/M02203X/1) to G.L.-M.

Author contributions. T.H., M.P., K.N., D.S., A.S. and G.L.-M. planned and designed the research; T.H., M.P. and N.V. performed experiments; A.S. provided access to materials; T.H., K.N. and G.L.-M. analysed and interpreted the data; and T.H. and G.L.-M wrote the manuscript with contributions from all authors.

References

- AHDB (2016) Cereals and oilseeds report: Fall in winter cropping across England and Wales.
- AHDB (2017) Cereals and oilseeds report: Planting survey results: regional split for 2017 OSR area.
- AHDB (2018) Cereals and oilseeds report: Oilseed rape area bounces back in England and Scotland.
- Arshad W, Sperber K, Steinbrecher T, Nichols B, Jansen VAA, Leubner-Metzger G and Mummenhoff K (2019) Dispersal biophysics and adaptive significance of dimorphic diaspores in the annual *Aethionema arabicum* (Brassicaceae). *New Phytologist* **221**, 1434–1446.
- Auge GA, Blair LK, Neville H and Donohue K (2017) Maternal vernalization and vernalization-pathway genes influence progeny seed germination. *New Phytologist* **216**, 1–13.
- Bartels P and Watson C (1978) Inhibition of carotenoid synthesis by fluridone and norflurazon. *Weed Science* **26**, 198–203.
- Baskin JM and Baskin CC (2004) A classification system for seed dormancy. *Seed Science Research* **14**, 1–16.
- Bezodis W and Penfield S (2024) Maternal environmental control of progeny seed physiology: a review of concepts, evidence and mechanism. *Seed Science Research* **34**(2), 48–55.
- Chauvel B, Munier-Jolain NM, Grandgirard D and Gueritane G (2002) Effect of vernalization on the development and growth of *Alopecurus myosuroides*. *Weed Research* **42**, 166–175.
- Chen M and Penfield S (2018) Feedback regulation of COOLAIR expression controls seed dormancy and flowering time. *Science* **360**, 1014–1017.
- Chiang G, Barua D, Kärmer E, Amasino R, Donohue K and Koornneef M (2009) Major flowering time gene, *FLOWERING LOCUS C*, regulates seed germination in *Arabidopsis thaliana*. *PNAS* **106**, 11661–11666.
- Clarke J, Ginsburg D, Clare K, and Tonguc L (2015) *The Encyclopaedia of Arable Weeds*. Stoneleigh Park, Kenilworth, UK: AHDB.
- Colbach N, Busset H, Yamada O, Dürr C and Caneill J (2006a) AlomySys: modelling black-grass (*Alopecurus myosuroides* Huds.) germination and emergence, in interaction with seed characteristics, tillage and soil climate: II. Evaluation. *European Journal of Agronomy* **24**, 113–128.
- Colbach N, Dürr C, Roger-Estrade J, Chauvel B and Caneill J (2006b) AlomySys: modelling black-grass (*Alopecurus myosuroides* Huds.) germination and emergence, in interaction with seed characteristics, tillage and soil climate: I. Construction. *European Journal of Agronomy* **24**, 113–128.
- Gomez-Cabellós S, Toorop PE, Cañal MJ, Iannetta PPM, Fernández-Pascual E, Pritchard HW and Visscher AM (2022) Global DNA methylation and cellular 5-methylcytosine and H4 acetylated patterns in primary and secondary dormant seeds of *Capsella bursa-pastoris* (L.) Medik. (shepherd's purse). *Protoplasma* **259**(3), 595–614.
- Gutterman Y (1992) Maturation dates affecting the germinability of *Lactuca serriola* L. Achenes collected from a natural population in the Negev desert highlands. Germination under constant temperatures. *Journal of Arid Environments* **22**, 353–362.
- Heap I (2019). International Survey of Herbicide Resistance. URL: www.weedscience.org (last accessed 14, October 2019).
- Holloway T, Perez M, Chandler JO, Venceslai N, Garcia L, Cohn J, Schilling K, Seville A, Leubner-Metzger G and Nakabayashi K (2024) Mechanisms of seed persistence in blackgrass (*Alopecurus myosuroides* Huds. *Weed Research* **64**(3), 237–250.
- Lutman PJW, Moss SR, Cook S and Welham SJ (2013) A review of the effects of crop agronomy on the management of *Alopecurus myosuroides*. *Weed Research* **53**, 299–313.
- Macgregor DR, Kendall SL, Florance H, Fedi F, Moore K, Paszkiewicz K, Smirnov N and Penfield S (2015) Seed production temperature regulation of primary dormancy occurs through control of seed coat phenylpropanoid metabolism. *New Phytologist* **205**, 642–652.
- Maréchal P and Henriot F (2012) Ecological review of black-grass (*Alopecurus myosuroides* Huds.) propagation abilities in relationship with herbicide resistance. *Biotechnology, Agronomy, Society and Environment* **16**, 103–113.
- Moss S (2017) Black-grass (*Alopecurus myosuroides*): why has this weed become such a problem in Western Europe and what are the solutions? *Outlooks on Pest Management* **28**(5), 207–12. doi:10.1564/v28_oct_04
- Moss SR (1985) The survival of *Alopecurus myosuroides* Huds. seeds in soil. *Weed Research* **25**, 201–211.
- Nambara E, Okamoto M, Tatematsu K, Yano R, Seo M and Kamiya Y (2010) Absciscic acid and the control of seed dormancy and germination. *Seed Science Research* **20**, 55–67.
- Penfield S, Josse EM, Kannangara R, Gilday AD, Halliday KJ and Graham IA (2005) Cold and light control seed germination through the bHLH transcription factor SPATULA. *Current Biology* **15**, 1998–2006.
- Penfield S and MacGregor DR (2017) Effects of environmental variation during seed production on seed dormancy and germination. *Journal of Experimental Botany* **68**, 1–7.
- Pérez M, Viejo M, LaCuesta M, Toorop P and Cañal MJ (2015) Epigenetic and hormonal profile during maturation of *Quercus Suber* L. somatic embryos. *Journal of Plant Physiology* **173**, 51–61.
- Santamaría ME, Hasbún R, Valera MJ, Meijón M, Valledor L, Rodríguez JL, Toorop PE, Cañal MJ and Rodríguez R (2009) Acetylated H4 histone and genomic DNA methylation patterns during bud set and bud burst in *Castanea sativa*. *Journal of Plant Physiology* **166**, 1360–1369.
- Sawhney R, Quick WA and Hsiaof AI (1985) The Effect of Temperature during Parental Vegetative Growth on Seed Germination of Wild Oats (*Avena fatua* L.). *Annals of Botany* **55**, 25–28.
- Song J, Angel A, Howard M and Dean C (2012) Vernalization - a cold-induced epigenetic switch. *Journal of Cell Science*. **125**, 3723–3731.
- Swain AJ, Hughes ZS, Cook SK and Moss SR (2006) Quantifying the dormancy of *Alopecurus myosuroides* seeds produced by plants exposed to different soil moisture and temperature regimes. *Weed Research* **46**, 470–479.
- Taylor-Davies B (2017) Nuffield Report: system BEN offers alternative solutions to resistance management. URL: <https://www.nuffieldscholar.org/news/ben-taylor-davies-report-published> (last accessed 14, October 2019).
- Viejo M, Rodríguez R, Valledor L, Pérez M, Cañal MJ and Hasbún R (2010) DNA methylation during sexual embryogenesis and implications on the induction of somatic embryogenesis in *Castanea sativa* Miller. *Sexual Plant Reproduction* **23**, 315–323.