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Inheritance of deep seed dormancy and stratification-mediated dormancy alleviation in *Amaranthus tuberculatus*

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Abstract

Amaranthus tuberculatus is a weed species that has shifted emergence patterns over the past few years, presumably due to changes in seed dormancy in response to selection in agricultural fields. Although it is recognized that the seed dormancy phenotype is greatly affected by the environment, it is also acknowledged that the genotype plays a significant role. However, the importance of the genotype in determining intra-population seed dormancy variability, and the effect on emergence patterns, is not well understood. The objective of the present study was to determine the importance of the genotype on deep dormancy and the stratification-mediated dormancy alleviation in *A. tuberculatus*. Wild populations differing in seed dormancy were crossed and F_2 families were generated. These families were used to determine narrow sense heritability of dormancy and stratification-mediated dormancy alleviation at the individual (h_i^2) and family (h_f^2) levels. h_i^2 ranged from 0.13 to 0.4 and 0.04 to 0.06 for the dormancy and stratification response, respectively. In the case of h_f^2, the values ranged from 0.76 to 0.91 for deep dormancy and from 0.33 to 0.58 for the stratification response. The genetic correlation between these two traits was below 0.075, indicating that different genes control them. High temperature strengthened the dormancy of deeply dormant seeds, making them less sensitive to stratification. However, high temperature promoted the germination of non-deeply dormant seeds. It is proposed that delayed weed emergence can be generated by selecting genes that control stratification response, and not necessarily only the genes that are directly responsible for deep dormancy.

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Introduction

Seed dormancy is an important adaptive trait for wild and weedy plant species (Baskin and Baskin, 1998; Silvertown and Charlesworth, 2001). Variability of this trait within a population favours variability in the timing and magnitude of seedling emergence by preventing germination, even when the conditions are favourable (Bewley, 1997). Historically, dormancy has been inappropriately considered a developmental stage during which the seed is physiologically inactive. However, evidence indicates that during this stage, weed seeds are active and highly responsive to environmental cues (Vleeshouwers *et al.*, 1995). The depth of seed dormancy, as well as the rate of dormancy loss, are greatly influenced by the environment (Foley, 1994; Fennimore *et al.*, 1998). Temperature and moisture are especially important in determining seed dormancy intensity (Lunn *et al.*, 2002; Nyachiro *et al.*, 2002). For example, *Polygonum aviculare* L. seeds reduce their dormancy proportionally to the time they are exposed to low temperatures, and this reduces the requirement for temperature fluctuation cycles to trigger germination (Batlla *et al.*, 2003). In spite of the great effect that the environment has on seed dormancy, the importance of the genotype on seed dormancy variability is also substantial, and heritability values ranging from 0.50 to 0.99 have been reported for species such as *Avena fatua* L., *Bromus tectorum* L. and *Oryza sativa* L. (Fennimore *et al.*, 1998; Meyer and Allen, 1999; Gu *et al.*, 2003). It seems contradictory that the phenotype of a trait that has a high heritability can be strongly influenced by the environment. A possible explanation for this apparent contradiction is that a significant proportion of the genes that

control seed dormancy are involved in environmental signal sensing. However, there is limited information about the existence and involvement of this type of 'environment-sensing' gene in seed dormancy. Most of the information available is related to genes involved in the biosynthesis of gibberellic acid for germination in response to light quality or cold stratification (Vleeshouwers *et al.*, 1995; Yamaguchi *et al.*, 1998; Yamauchi *et al.*, 2004).

It has been proposed that the selection pressure imposed by herbicides and tillage can change the dormancy level of weed seeds, altering emergence patterns (Mortimer, 1997). Ghersa *et al.* (1994) demonstrated that a *Lolium multiflorum* Lam. biotype, resistant to diclofop-methyl, emerges later than a susceptible biotype. In addition, they observed that in the seed bank, seeds showing greater dormancy were more likely to be herbicide resistant than less dormant seeds. *Amaranthus tuberculatus* (Moq.) J.D. Sauer (syn. *A. rudis*) is a dioecious species considered to be an important weed in the Midwestern United States. Several *A. tuberculatus* populations from agricultural fields show irregular and delayed emergence patterns, which helps avoid high mortality early during the growing season due to cultivation and herbicide applications (Hartzler *et al.*, 1999, 2004). These emergence patterns are presumably due to changes in seed dormancy, because seeds with deep dormancy require longer periods of cold stratification and/or temperatures closer to the optimum temperature for germination (Leon and Owen, 2004; Leon *et al.*, 2004). However, the importance of the genetic component of the seed dormancy phenotype, and whether the genetic variability within a population could explain the irregular and delayed emergence patterns shown by this species, are not known. The fact that seed dormancy variability is largely controlled by the environment raises the question of how natural and human selection influences seed dormancy. One possibility is that selection acts upon genes that are involved in the sensing and transduction of environmental signals that ultimately modify seed dormancy, and not necessarily upon genes that are directly responsible for seed dormancy.

We hypothesized that seed dormancy in *A. tuberculatus* is controlled both by genes that impose deep dormancy and genes that are responsible for modifying seed dormancy level in response to the environment. The objectives of the study were: (1) to estimate the heritability of the deep dormancy trait; (2) to estimate the heritability of the seed dormancy alleviation response to stratification; (3) to estimate if these two traits are controlled by different genes; and (4) to evaluate how segregation for these genes might contribute to germination variability at the population level.

Materials and methods

Plant materials

Seeds of *A. tuberculatus* were collected from wild populations from an agricultural field in Iowa (Ames biotype) and from a pristine area in Ohio (Ohio biotype), USA. The Ames biotype was selected because it exhibits deeper dormancy than the Ohio biotype. The former requires stratification to alleviate dormancy, while the latter can germinate without it (Leon *et al.*, 2006). We considered that both biotypes show dormancy because they require temperature alternation to induce germination (Leon *et al.*, unpublished data). Here, we use the stratification requirement for dormancy alleviation to distinguish between two different depths of dormancy on the dormant–non-dormant continuum. The seeds were stratified for 16 weeks at 4°C under wet conditions in the dark and then germinated. The plants produced were grown in 1-litre pots in growth chambers at 25°C and 16 h photoperiod to produce seeds for the experiments. Plants from the two biotypes were grown in different chambers to prevent cross-pollination. All the plants were watered and fertilized to favour rapid growth. Seeds were harvested manually and dried at room temperature (RT) for 3 weeks until reaching 5% moisture content (dry weight basis). After drying, the seeds were cleaned and processed with an air-column seed cleaner to remove plant residues and non-viable seeds. Cleaned seeds were stored at 4°C and 40% relative humidity (RH) in the dark until used.

Generation of recombinant F_2 families

Plants were grown in growth chambers under a long photoperiod (as described above). Eight weeks after planting, the plants were kept under a short photoperiod (8 h light and 16 h darkness) for 48 h to induce flowering. Ames and Ohio plants were reciprocally crossed by pairing male and female individuals, and covering them with transparent plastic bags, generating a total of 80 crosses. Four weeks later, the plants were uncovered, and the males were removed from the chamber. The seeds were harvested 5 months after planting, and dried and cleaned as above. F_1 seeds were stratified at 4°C for 4 weeks and then germinated for 7 d. From each F_1 family, 10–20 seedlings were randomly selected and grown in growth chambers as described previously and, after flowering, plants were randomly chosen from within each F_1 family to be crossed to generate the recombinant F_2 progeny.

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Seed dormancy and stratification response tests

Fifty seeds from each F_2 family were placed in a 9-cm diameter Petri dish with a blotter paper and 7.5 ml of deionized water, and the dish was sealed with Parafilm. Two different treatments were applied to evaluate seed dormancy and stratification responses (Fig. 1). One treatment consisted of exposing seeds to germination conditions (200 $\mu E\,s^{-1}\,m^{-2}$ continuous white light and alternating temperatures of 31.3°C for 16 h and 21.5°C for 8 h) for 14 d, stratifying the seeds for 21 d, and exposing them again to germination conditions for 14 d. The second treatment consisted of stratifying the seeds for 21 d, and then germinating them for 28 d under the previously mentioned germination conditions. Germination (radicle protrusion) was recorded every 7 d during the germination periods (Fig. 1). Germinated seeds were removed from the dish. Seeds that had not germinated by the end of the experiment were air-dried and subjected to a seed crush test to determine viability (Sawma and Mohler, 2002). This technique showed a high correlation ($r^2 > 0.81$) with the tetrazolium (TZ) test, as described by Moore (1985). There were no significant differences among treatments for seed viability, which was 96–100%. The seed dormancy and stratification tests were replicated three times, and were conducted twice as completely randomized designs. The first time, experiments were conducted using 57 recombinant F_2 families (Experiment 1), and the second time using 62 recombinant F_2 families (Experiment 2). Differences in family number between experiments were due to seed availability. Bartlett's test was conducted, and homogeneity of variances was rejected. The data were transformed using square root, log and arcsine transformations, but no transformation improved homogeneity of variances. Therefore, the results were analysed using untransformed data. Germination percentage data were used to conduct analysis of variance (ANOVA) using the General Linear Model of SAS (PROC GLM, SAS, Version 8.0, Cary, North Carolina, USA) to determine the existence of differences between families and experiment repetitions. Also, Tukey's Studentized Range test ($P \leq 0.05$) was used to compare families.

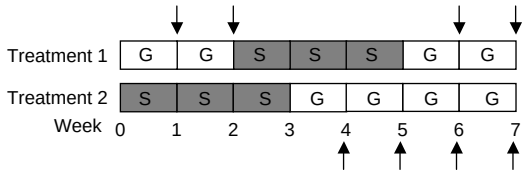

Figure 1. Diagram representing the two treatments to which parental and F_2 family *Amaranthus tuberculatus* seeds were subjected to study deep dormancy and stratification-mediated dormancy alleviation. Germination and stratification periods are indicated with G and S, respectively. The arrows indicate the times at which germination was evaluated.

Heritability estimation

ANOVA was used to estimate the phenotypic variance of the traits studied, and partition this variance into its different components to estimate the *narrow sense heritability* (h^2) of the traits. The variance for the average seed germination per replication per family [$\mathrm{Var}(\overline{X}_f)$] was estimated as follows:

$$\mathrm{Var}(\overline{X}_f) = [t + (1 - t)/n]\, V_P \quad (1)$$

where t is the correlation between two seeds for each replication for each family, and V_P is the variance for an individual seed. Because within each family, the seeds were full sibs, then,

$$t = 1/2h^2. \quad (2)$$

Assuming that the dominance was negligible (Lynch and Walsh, 1998, pp. 570–574), then

$$V_T = [(1/2 + 1/2n)V_A] + V_E/n \quad (3)$$

where n represents the number of seeds tested per replication and family, and V_T, V_A and V_E represent the total, genetic and environmental variances, respectively. Thus,

$$\sigma_e^2 = V_T - V_A/2 \quad (4)$$

where σ_e^2 represents the residual variance. Therefore, by combining equations (3) and (4), σ_e^2 can be estimated as:

$$\sigma_e^2 = (V_A/2 + V_E)/n. \quad (5)$$

The expected mean square from the ANOVA was used to estimate the family genetic variance (V_f). Considering that the covariance between two families [$\mathrm{cov}\,(X_{i1}, X_{i2})$] is equivalent to the V_A, then:

$$\mathrm{cov}\,(X_{i1}, X_{i2}) = V_A/2 = V_f. \quad (6)$$

Equations (5) and (6) were used to estimate V_A and V_E, respectively. Thus, the heritability by individual seed (h_i^2) and its approximated standard error (Falconer and Mackay, 1996, pp. 177–181), and by family (h_f^2) were estimated as:

$$h_i^2 = V_A/(V_A + V_E) \quad (7)$$

and

$$h_f^2 = V_f/\left(V_f + \sigma_e^2\right). \quad (8)$$

The heritability estimates were determined for seed dormancy (X) and for stratification (Y) response. The genetic correlation between these two traits (r_A), and its estimated standard error, were calculated as described by Falconer and Mackay (1996, p. 316). MANOVA and the General Linear Model of SAS

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(PROC GLM, SAS, Version 8.0, Cary, North Carolina, USA) were used to obtain the covariance between *X* and *Y* from the matrix of the sums of squares for *X* and *Y* and the cross products of the same variables.

Results

Germination distribution and stratification response

The germination of F_2 families indicated that deep seed dormancy in *A. tuberculatus* behaved as a quantitative trait (Fig. 2). Unstratified Ames parent seeds showed 3 and 10% germination at 7 and 14 d after imbibition (DAI), respectively, whereas Ohio seeds showed 80 and 85% germination for the same evaluations. At 7 DAI (Fig. 2A, B), F_2 families showed a skewed distribution towards zero germination (Ames phenotype). However, at least two families showed higher germination than the Ohio phenotype, which might be due to a reduction in non-deep dormancy. At 14 DAI (Fig. 2C, D), the F_2 germination distribution was closer to normal, with a mean germination around 50%. After the first period of germination, the non-germinated seeds were stratified

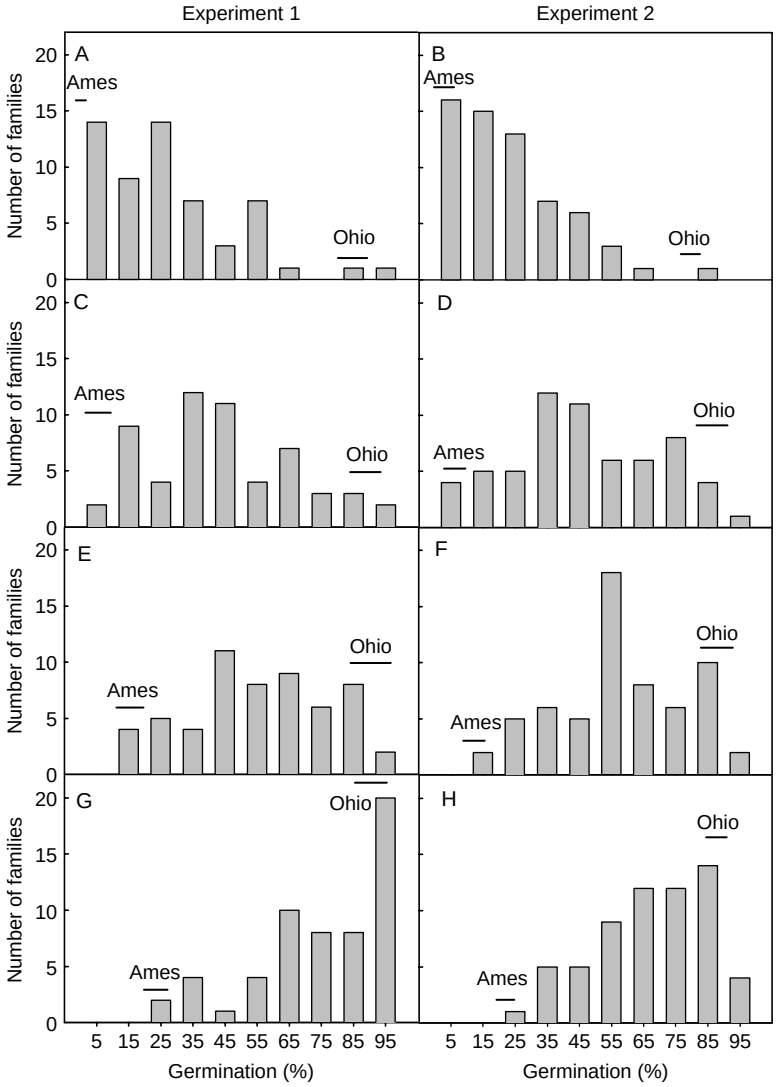

Figure 2. Frequency distribution of F_2 *Amaranthus tuberculatus* families based on germination means. Seeds were placed under germination conditions for 14 d then stratified for 3 weeks followed by another germination period of 14 d (Treatment 1). Germination of unstratified seeds at 7 d after imbibition (DAI) (A and B) and 14 DAI (C and D), stratified seeds at 7 d after stratification (DAS) (E and F) and 14 DAS (G and H). The germination ranges of the parental lines (Ames and Ohio) are indicated as horizontal bars. Results are presented for germination at 7 and 14 d for two independent experiments.

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for 3 weeks and then placed under germination conditions (Fig. 1, Treatment 1). The stratification did not reduce the germination variability but changed the germination distribution (Fig. 2E–H).

When the seeds were stratified before the germination experiment (Fig. 1, Treatment 2), the F_2 germination mean was 40–50% at 7 d after stratification (DAS). However, as germination time increased, the germination mean increased to values similar to that of the Ohio phenotype (Fig. 3). Similarly, stratification did not decrease the germination variability (Figs 2, 3). In this case, it was evident that the F_2 families differed in their sensitivity to stratification. In fact, after stratification, 9 and 12 families in Experiments 1 and 2, respectively, showed lower germination than the Ames parent at 14 DAS (Fig. 3).

Deep dormancy heritability

Deep dormancy heritability was not particularly high and was variable at the individual seed level (h_i^2) ranging from 0.13 to 0.4 (Table 1). On the other hand, the heritability at the family level (h_f^2) was considerably higher, ranging from 0.76 to 0.91. Comparing

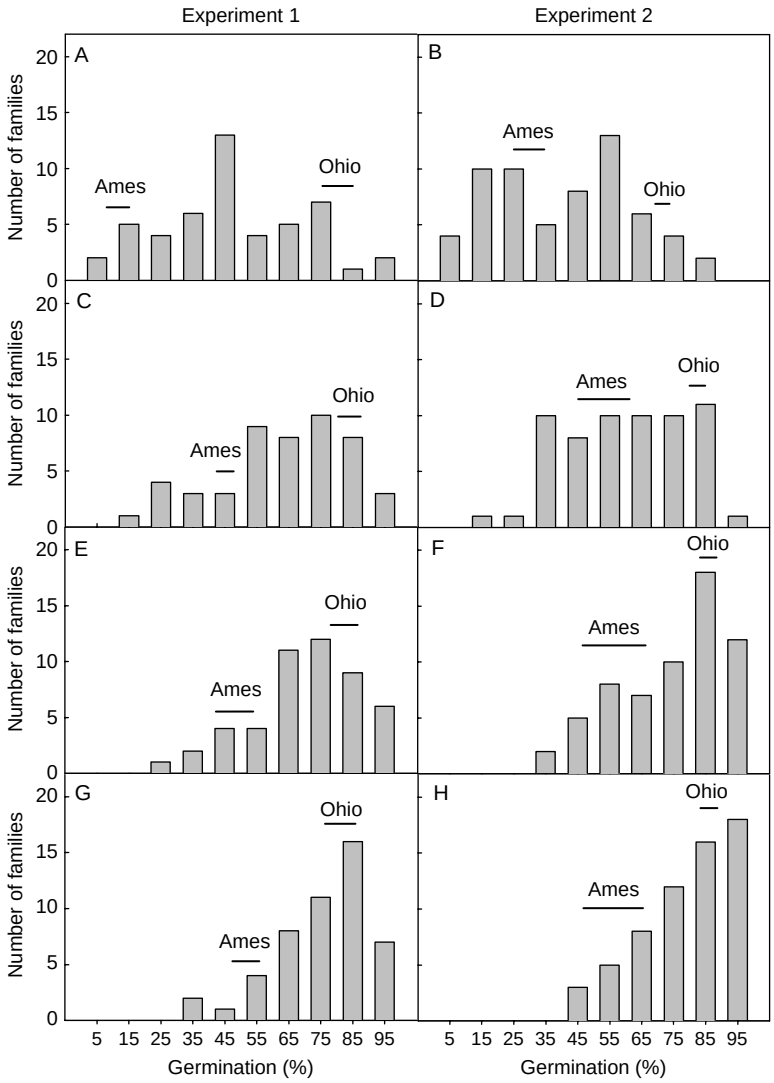

Figure 3. Frequency distribution of F_2 *Amaranthus tuberculatus* families based on germination means at 7 (A and B), 14 (C and D), 21 (E and F) and 28 d after stratification (DAS) (G and H) using seeds stratified for 3 weeks (Treatment 2, Fig. 1). The germination ranges of the parental lines (Ames and Ohio) are indicated as horizontal bars. Results are presented for two independent experiments.

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Table 1. Deep dormancy heritability estimates of individual *Amaranthus tuberculatus* seeds (h_i^2) and families (h_f^2). The seeds were germinated for 14 d, then stratified for 21 d, and then germinated for 14 d (Treatment 1, Fig. 1). The h_i^2 and h_f^2 estimates were based on the germination at 7 and 14 d after imbibition (DAI) before and after stratification. Results are presented for two independent experiments

Experiment	Stratification	DAI	h_i^2	h_f^2
1	Before	7	0.40 ± 0.05[a]	0.91
		14	0.36 ± 0.04	0.90
	After	7	0.29 ± 0.04	0.89
		14	0.14 ± 0.03	0.77
2	Before	7	0.35 ± 0.04	0.90
		14	0.22 ± 0.03	0.85
	After	7	0.20 ± 0.03	0.83
		14	0.13 ± 0.03	0.76

[a] h_i^2 ± estimated standard error.

heritability estimates before and after stratification (Table 1) or with and without stratification (Tables 1, 2), indicated that stratification reduced the deep dormancy heritability estimate, and this was more evident for h_i^2 than for h_f^2. However, the reduction in the heritability estimates was not marked, and the h_i^2 and h_f^2 estimates were constant across experiments and over time. These results indicated that the differences in seed dormancy between F_2 families had an important genetic component, and this could be detected even after 3 weeks of stratification. The heritability estimates after stratification are a combination of the heritability of the deep dormancy and stratification response. However, the fact that the reduction in deep dormancy heritability after stratification was relatively small indicated that most of the variability across seeds and families was due to the deep dormancy trait, and that the response to stratification depended upon its presence.

Table 2. Deep dormancy heritability of individual *Amaranthus tuberculatus* seeds (h_i^2) and families (h_f^2) of seeds stratified for 21 d. The h_i^2 and h_f^2 estimates were based on the germination at 7, 14, 21 and 28 d after stratification (DAS) (Treatment 2, Fig. 1). Results are presented for two independent experiments

Experiment	DAI	h_i^2	h_f^2
1	7	0.19 ± 0.03[a]	0.84
	14	0.20 ± 0.03	0.83
	21	0.13 ± 0.03	0.76
	28	0.12 ± 0.03	0.76
2	7	0.19 ± 0.03	0.84
	14	0.16 ± 0.03	0.80
	21	0.12 ± 0.02	0.76
	28	0.14 ± 0.03	0.77

[a] h_i^2 ± estimated standard error.

Deeply dormant *A. tuberculatus* seeds require stratification to become sensitive to temperature alternation for induction of germination, while non-deeply dormant seeds do not (Leon *et al.*, unpublished data). The germination curves of most families levelled off at approximately 8–12 DAI. Therefore, seeds that germinated 14 DAI without stratification were not considered to be deeply dormant, whereas seeds that did not germinate after the same period were. The number of seeds that were deeply dormant and responded to stratification was determined by subtracting the germination percentage at 14 DAI before stratification from that at 14 d after stratification (Fig. 1, Treatment 1). The heritability of the stratification response was estimated using two variables: the number of deeply dormant seeds that germinated after stratification, and the proportion between this number and the total number of deeply dormant seeds. For the stratification response, the h_i^2 was significantly lower than for deep dormancy, but still the h_f^2 showed relatively high values ranging from 0.33 to 0.58 (Table 3). The lower heritability values shown by the response to stratification, compared with those shown by the deep dormancy trait, are because expression of the first trait depends directly on the environment. Thus, it is understandable that a higher proportion of the phenotypic variance is caused by environmental variability.

Genetic relationship between germination rate and stratification response

The possibility that the deep dormancy and stratification responses were the same trait was considered. The phenotypic correlation was determined between non-deeply dormant seeds and the proportion of deeply dormant seeds that responded to stratification based on the total number of deeply dormant seeds. Only 8% and 1% of the variation could be explained by this correlation in Experiments 1 and 2, respectively (Fig. 4). This suggests that deep dormancy and stratification responses were traits controlled by different genes, and that these genes segregated independently. To confirm these results, the genetic correlation coefficients (r_A) between these two traits were estimated (Table 4). The r_A estimates were lower than 0.075, confirming that deep dormancy and the response to stratification are traits controlled by different genes.

Important interactions between incubation conditions and seed dormancy

The sequence in which the seeds are exposed to stratification and germination conditions determines the changes in depth of seed dormancy in response to

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Table 3. Stratification response heritability of individual *Amaranthus tuberculatus* seeds (h_i^2) and families (h_f^2). Results are presented for two independent experiments

Estimate	Experiment	h_i^2	h_f^2
DPS[a]	1	0.04 ± 0.01[b]	0.52
	2	0.05 ± 0.02	0.58
DPS/DP[c]	1	0.06 ± 0.02	0.45
	2	0.06 ± 0.02	0.33

[a] DPS, number of deeply dormant seeds in which dormancy was alleviated in response to stratification.
[b] h_i^2 ± estimated standard error.
[c] DPS/DP, proportion of deeply dormant seeds in which dormancy was alleviated in response to stratification (DPS) based on the total number of deeply dormant seeds (DP).

these conditions. When the seeds were stratified between germination periods (Fig. 1, Treatment 1), the parental line phenotypes were always at the extremes of the distribution of the F_2 families (Fig. 2). In this case, Ames seeds were not particularly responsive to stratification. Conversely, when the seeds were stratified before being placed in germination conditions (Fig. 1, Treatment 2), a significant number of F_2 families showed phenotypes more extreme than the parental lines (Fig. 3). Thus, in this case, Ames seeds were more responsive to stratification than the seeds of at least 8–12 F_2 families. Similarly, several families did not reduce their dormancy under Treatment 1 (Fig. 2). However, when the same families were maintained under Treatment 2, these families showed germination percentages higher than the Ohio parental line (Fig. 3). Therefore, it seems that germination conditions such as high and fluctuating temperatures and moisture strengthen the dormancy of deeply dormant seeds.

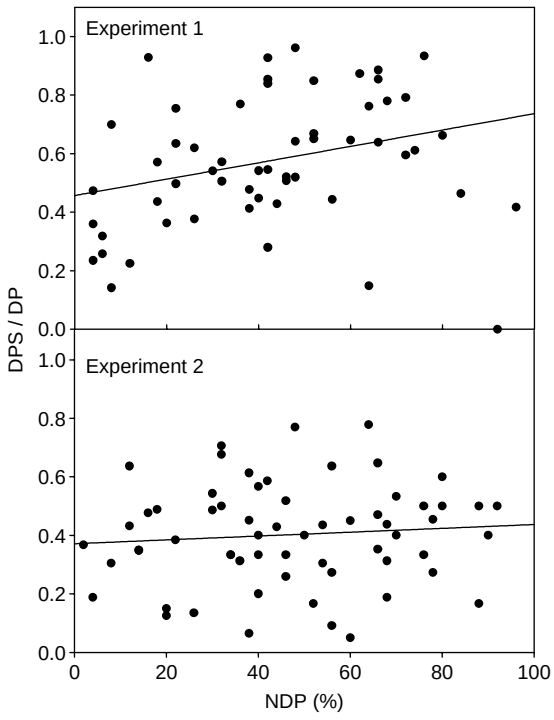

Figure 4. Phenotypic correlation between non-deeply dormant (NDP) and the proportion of deeply dormant *Amaranthus tuberculatus* seeds that responded to stratification (DPS) based on the total number of deeply dormant seeds (DP). Results are presented for two different experiments. The lines represent the best-fitted equation where $y = 0.003x + 0.457$ with $r^2 = 0.08$ for Experiment 1 and $y = 0.001x + 0.371$ with $r^2 = 0.01$ for Experiment 2.

Table 4. Genetic correlation coefficient (r_A) between deep dormancy and stratification dormancy alleviation response for *Amaranthus tuberculatus*. The stratification response was estimated using two variables. Results are presented for two independent experiments

	r_A	
Variables	Experiment 1	Experiment 2
DPS[a]	−0.057 ± 0.001[b]	−0.071 ± 0.004
DPS/DP[c]	0.070 ± 0.004	0.047 ± 0.004

[a] DPS, number of deeply dormant seeds in which dormancy was alleviated in response to stratification.
[b] r_A ± estimated standard error.
[c] DPS/DP, proportion of deeply dormant seeds in which dormancy was alleviated in response to stratification (DPS) based on the total number of deeply dormant seeds (DP).

Discussion

Variability is important in the role that seed dormancy plays as an adaptive trait in weedy and wild plant species (Allen and Meyer, 1998). Differences in seed dormancy can be observed between different species, different populations of the same species, and seeds produced by the same plant (Meyer and Kitchen, 1994). Inter-specific differences in seed dormancy can be commonly explained by the presence or absence of seed dormancy genes (Baskin and Baskin, 1998; Silvertown and Charlesworth, 2001). However, intra-specific variation for dormancy depends on both the presence of seed dormancy genes and phenotypic plasticity (Silvertown and Charlesworth, 2001; Allen and Meyer, 2002; Lacerda *et al.*, 2004). The importance of the genetic component of the seed dormancy phenotype will determine how effective selective forces will be in modifying seed dormancy

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and consequently affecting the adaptability of the species (Allen and Meyer, 1998; Silvertown and Charlesworth, 2001). The quantitative nature of seed dormancy has been documented widely (Foley, 2001; Koornneef *et al.*, 2002). In many studies, this has been demonstrated by crossing wild, weedy or domesticated plants, which have high levels of seed dormancy, with domesticated plants selected for low or no dormancy (Wan *et al.*, 1997, 2005; Fennimore *et al.*, 1999; Alonso-Blanco *et al.*, 2003; Gu *et al.*, 2003). An important biological question is whether intra-population phenotypic variation for seed dormancy in the field relies more on the genetic or environmental component. Many studies have shown that environmental factors, such as temperature and moisture, can dramatically interact with the seed dormancy genotype, thus generating phenotypic variation (Fennimore *et al.*, 1998; Lunn *et al.*, 2002; Nyachiro *et al.*, 2002; Torada and Amano, 2002; Schütz and Rave, 2003). However, intra-population genetic variation for seed dormancy can be advantageous because it can enable the population to respond to environmental changes (Lacerda *et al.*, 2004). Meyer and Allen (1999) observed that *Bromus tectorum* L. populations evolved different dormancy levels, as well as different levels of phenotypic plasticity for seed dormancy, and they determined that these differences were better explained by the genetic component of the phenotype. One possible explanation for variation in seed dormancy phenotypic plasticity is the presence of genes involved in sensing environmental cues that will ultimately modify the depth of dormancy. In the present study, crossing two naturally occurring biotypes that differed in the extent of deep dormancy produced a wide variety of germination phenotypes, which would be manifested as a more variable emergence pattern in the field. Considering that *A. tuberculatus* is a dioecious species, it is likely that outcrossing and the resulting genetic recombination are important mechanisms for generating intra-population genetic variation affecting seed dormancy phenotypic variability.

The heritability of deep dormancy in *A. tuberculatus* at the family level is high, ranging from 0.76 to 0.91. At the individual level, the heritability was not as high, and ranged between 0.12 and 0.4. Similar values were observed in wild oat (*Avena fatua* L.) (Naylor and Jana, 1976; Jana and Naylor, 1980; Fennimore *et al.*, 1998). The genetic component of the stratification response was an important component of the phenotypic variability at the family level, although it was not as high as that for deep dormancy. The observation that these two traits are important for determining the seed dormancy phenotype, and that they are likely controlled by different genes, suggests a mechanism for causing variable seedling emergence. An important limitation in the present studies is that the stratification response might be present in seeds that are not deeply dormant (i.e. lack deep dormancy genes), but because they do not show deep dormancy (i.e. they germinate without stratification), the ability to detect the stratification response is limited. Thus, estimates of heritability for the stratification response are likely to be underestimations.

One of the most rapid changes observed in weed populations is the evolution of herbicide resistance. Other changes, such as altered emergence patterns, have occurred slowly (Mortimer, 1997). In the past 10 years, *A. tuberculatus* has become a more difficult weed to control, and this has been attributed, in part, to its late and irregular emergence patterns (Hartzler *et al.*, 1999, 2004; Leon and Owen, 2004). *A. tuberculatus* seeds with high dormancy germinate later during the growing season than seeds with low dormancy, because the former require higher temperatures to induce germination than the latter (Leon and Owen, 2004). Seed dormancy is a quantitative trait. Thus, it is difficult to envision that a population, such as the Ames biotype, with a combination of genes that promote very deep dormancy, could increase their dormancy in order to generate a delayed emergence phenotype. On the other hand, if the deep dormancy trait and the response to the stratification trait are controlled by different genes, early and late emergence phenotypes could be expected. Early emergence will occur in seeds with no deep dormancy, regardless of their stratification response. Also, seeds that have the deep dormancy trait, but are responsive to stratification, will show early emergence because the low temperatures of the winter and spring can remove the deep dormancy. Finally, deeply dormant seeds that lack the cold stratification response will emerge late. It is possible that high mortality during the beginning of the growing season, attributable to herbicides and/or tillage, has selected for individuals that have deep dormancy and a reduced response to cold stratification (Mortimer, 1997).

Another interesting result of the present study was that germination conditions reinforced the dormancy of deeply dormant seeds, making them less sensitive to stratification. Two aspects of this observation are noteworthy. One is that the same environmental signal that triggers germination of non-deeply dormant seeds also prevents the germination of deeply dormant seeds. The dual role of temperature is important for ensuring that seeds do not germinate when favourable conditions occur, but the chances for seedling survival are low (Vleeshouwers *et al.*, 1995). For example, during the fall, soil moisture and temperature can be adequate for the germination of summer annuals, but if germination occurs, seedlings will die during the winter before completing their life cycle. This type of genotype by environment interaction has been proposed as a mechanism to increase species adaptability

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by reducing the probability of germination in unfavourable conditions (Fennimore *et al.*, 1998). The other aspect is that seed dormancy is a dynamic phenomenon that could move in both directions along the dormant–non-dormant continuum.

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