

Environmental maternal effects on seed morphology and germination in *Sinapis arvensis* (Cruciferae)

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Summary

Pioneer plants are adapted to grow in unpredictable environments. These plants have evolved several traits related to seed morphology and germination to cope with this unpredictability. The main aim of this study was to investigate the effect of plant identity and maternal environment on seed mass and germination of *Sinapis arvensis*. This study was undertaken with *S. arvensis* plants grown in four experimentally manipulated resource availability scenarios: (i) nitrogen addition, (ii) water addition, (iii) nitrogen and water simultaneously added, and (iv) control. Plants grew under field conditions in an emerging plant community. Germination response was mainly affected by maternal plant identity. This reflects not only genotype quality but also phenotypic plasticity, which allows adaptation to habitat conditions. Seed coat colour together with

mean seed mass per plant explained significant fractions of the variability in germination rate. Changes in the germination response because of maternal environmental effects were also detected. Germination rate decreased in seeds from nitrogen-enriched conditions; this may be due to the induction of dormancy caused by high concentration of nitrogen in the seeds. Addition of water to maternal environment caused a decrease in total germination percentage and germination rate of seeds. Thus, our results provide evidence for maternal environmental effects on germination under field conditions. Furthermore, changes in germination timing, as detected in our study, may determine plant development and fitness in unpredictable conditions.

Keywords: dormancy, maternal effect, phenotypic plasticity, resource addition, seed mass.

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Introduction

Plants growing in unpredictable environments have evolved adaptations related to seed morphology and physiology, such as dormancy, seed size variability and the presence of special structures for dispersal to cope with uncertain conditions (Venable & Brown, 1988). Seed traits are determined both by seed genotype and parental environment (Stanton, 1984; Schmitt *et al.*, 1992; Platenkamp & Shaw, 1993; Donohue & Schmitt, 1998; Galloway, 2001a,b). Parental environment can influence the proportion of seeds that enter dormancy and become part of the seedbank (Baskin & Baskin,

1998; Munir *et al.*, 2001), the frequency distribution of seed weights produced by a plant (Fenner, 1991a; Sultan, 1996), as well as seed germinability (Fenner, 1991b; Paolini *et al.*, 1999).

Parental effects may be due to two main causes: first, direct transmission of genetic material from the parent to the offspring through three possible routes: (i) the transmission of cytoplasmic organelles during the cell partition process (mitochondria and chloroplast DNA), (ii) the endosperm or (iii) heritable chromosome mutations. Secondly, transmission of non-genetic information: environmental conditions induce changes in gene expression. Direct transmission constitutes the *sensu lato*

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parental effect, whereas indirect transmission is the *sensu stricto* parental effect (Lacey, 1998). Parental effects may as well be the result of interaction between genotype and maternal environment. In any case, parental effects are, at least partially, genetically determined and thus potentially evolvable (Schmid & Dolt, 1994; Lacey, 1998).

It is widely accepted that post-zygotic effects on seed development, i.e. those occurring in the mother plant after fertilization, become dominant over pre-zygotic ones (Lacey, 1996). Thus, parental effects detected in early stages of plant development, such as seed mass, probability and rate of germination, are primarily the contribution of the mother plant environment (Stanton, 1984; Crawley & Nachapong, 1985; Schmid & Dolt, 1994). Such maternal effects in the earliest stages of plant life can persist or even be enlarged in the mature plant (Stanton, 1984; Houssard & Escarré, 1991; Weiner *et al.*, 1997; El-Keblawy & Lovett-Doust, 1998) and eventually lead to differences in reproductive success.

Among the seed features that have high adaptive implications for survival are seed mass, dormancy level and germination rate. These seed traits vary with environmental conditions like temperature (Lacey, 1996), photoperiod (Gutterman, 1992; Munir *et al.*, 2001), nutrient availability (Parrish & Bazzaz, 1985) or soil moisture conditions (Gutterman, 1993). Germination is a crucial process in the life cycle of a plant, because time of germination determines the environment in which the plant will develop, and eventually the fitness of the plant. The moment of germination can determine when reproduction and fruit ripening will occur (Kalisz, 1986; Biere, 1991; Stratton, 1992; Galloway, 2001a; Luzuriaga, 2004). Environmental control of germination acts through the seed coat, the endosperm, and resource and hormone supply (Biere, 1991; Platenkamp & Shaw, 1993; Lacey *et al.*, 1997; Baskin & Baskin, 1998; El-Keblawy & Lovett-Doust, 1998). Parental effects are the expression of trans-generation phenotypic plasticity that may probably be adaptive, in the sense that it could increase offspring reproductive success (Schmitt *et al.*, 1992; Schmitt, 1995; Donohue & Schmitt, 1998; Mousseau & Fox, 1998; Munir *et al.*, 2001).

The main aim of this study was to assess the effects of maternal plant identity and maternal environment on the germination response of a weed species that grows in highly unpredictable environments – *Sinapis arvensis* L. (Cruciferae). More specifically, we analysed the effect of nitrogen and water availability during seed ripening, on seed mass and germination rate and percentage. We assessed the integrated effect of resource addition in field conditions, because plants grew in a natural pioneer weed community. We explored the extent to which germination response and seed size were influenced by maternal plant identity, maternal environmental variab-

ility or any morphological feature of the mother plant (height and total number of fruits). Germination features were analysed in the laboratory in two different years in order to test temporal changes in germination response. Specific questions of this study were: (i) which fraction of variability in seed mass and germination rate and percentage can be attributed to plant characteristics, which to the maternal environment, and which to their interaction and (ii) to what extent seed mass and germination response depended on the mother plant's environment or on its identity?

Materials and methods

The study area and the plant

The study site was located 3 km east of Vitoria-Gasteiz (Basque Country) in northern Spain (42°51'N; 2°37'W and 510 m asl), very close to the boundary between the Eurosiberian and Mediterranean regions (Rivas-Martínez, 1987). Climatic conditions in this region include annual average rainfall of 843 mm and mean temperatures around 11.7°C, ranging between 1.3°C in the coldest and 26.1°C in the warmest months. Rainfall is highly unpredictable in terms of time and quantity and pronounced summer droughts are common. The study was undertaken in a perennial grassland that had been cultivated 5 years before and then developed under sheep-grazing conditions. Dominant species included native perennials such as *Elytrigia repens* (L.) Desv. ex Nevski, *Arrhenatherum elatius* var. *bulbosum* (Willd.) St-Amans and *Rumex crispus* L. Soils were vertisols with a slight hydromorphy. The soil seedbank mainly consisted of annual forbs, among which *S. arvensis* was well represented (Luzuriaga *et al.*, 2005).

Sinapis arvensis (wild mustard) is an annual cruciferous (Brassicaceae) plant widely distributed in Europe. Adult size ranges from a few centimetres to around 80 cm height depending on environmental conditions. Yellow flowers are placed in long bunches and siliqua-type fruits contain four to eight nearly spherical seeds each (Castroviejo *et al.*, 1989–2004). Flowering intensity ranges from a few flowers (three to four) to several hundred flowers per individual (400–500). This species grows in unpredictable environments such as old abandoned fields and in disturbed and nitrified habitats (Fogg, 1950).

Experimental design

In February 1999, the study area (1 ha) was thoroughly ploughed to a depth of 30 cm and hand-raked to smooth the surface and remove any remaining clumps of vegetation. Then, 20 quadrats (each 4.75 m × 1.75 m) were regularly distributed with a 1.75-m-wide buffer.

Levels of water (watered: W^+ ; not watered: W) and nitrogen availability (nitrogen addition: N^+ ; no nitrogen addition: N) were factorially manipulated resulting in four treatments (control, N^+ , W^+ and N^+W^+). We used a randomized design, with five replicates per treatment. On N^+ quadrats 6 g N m^{-2} was added by manually scattering urea in granular form on the surface from the walkways in the last week of March. On W^+ quadrats we added $8.65 \text{ l water m}^{-2}$ fortnightly during June and July (five irrigations). During this period, water demand reaches a maximum and summer drought usually begins, so it is a critical time for plant development. Total increase in water supply above mean rainfall during this period was 21.5%. Resource addition experiments are named maternal treatments in order to distinguish them from germination treatments.

Data collection

Four *S. arvensis* plants were harvested at random in each quadrat among those spontaneously developed from the local seedbank. In mid-July 1999, plant height and number of fruits were measured. Afterwards, plants were harvested when most fruits had already developed but prior to dispersal. Five replicates of 10 seeds per plant were weighed to obtain mean fresh seed mass per plant (COBOS AX-120 balance, accuracy of 0.1 mg; Eurolab, Barcelona, Spain). Seed colour in *S. arvensis* reflects the ripening level of seeds, black seeds being more developed than red ones (Durán & Retamal, 1983a). Thus, in order to analyse the effect of seed colour, plants were sorted according to the dominant seed colour into three categories: red-seeded (>90% red), black-seeded (>90% black), and plants with seeds of mixed colour. To estimate seed moisture content per plant, 100 seeds were weighed prior to and after a heating treatment of 21 h at 100°C . Seed moisture was obtained using the formula:

$$S_m = \frac{F_m - D_m}{D_m} \times 100, \quad (1)$$

where S_m is seed moisture percentage, F_m the seed fresh mass (100 seeds) and D_m the seed dry mass (100 seeds).

The plant set grown in each quadrat was clipped and subsequently dry mass was calculated in the laboratory (see Luzuriaga *et al.*, 2002). This measure was introduced as a covariate in the models, in order to control for differences in productivity at the plot level among maternal environments.

Germination experiments

Germination experiments were carried out in two different years: first, 4 months after seed collection

(1999 experiment). This experiment simulated germination following natural dispersal; secondly, after 2 years of storage in darkness at laboratory room temperature (2001 experiment). Seeds from all plants were submitted to the first germination experiment, but as some plants did not have enough seeds to reproduce the experiment two years later, only 36 plants were used for the second germination experiment. Durán and Retamal (1983a,b,c) showed that most seeds of *S. arvensis* do not germinate for a long time even in optimal germination conditions and that gibberellic acid (GA_3) effectively broke seed endogenous dormancy in *S. arvensis*. Thus, in order to discern seed dormancy and seed viability, two germination experiments per storage treatment were undertaken: (i) in the GA_3 addition treatment seeds were watered with a GA_3 solution of 250 mg L^{-1} , and (ii) in the control treatment seeds were watered with distilled water. Thus, four germination treatments were undertaken (Control_99, GA_3 _99, Control_01 and GA_3 _01) with four replicates per plant. Each replicate consisted of 25 seeds in 1999 and of 20 seeds in 2001, due to seed scarcity for the latter experiment.

Seeds were placed on two sheets of filter paper in 7-cm Petri dishes (Whatman no. 40) soaked with the corresponding solution. Petri dishes were located at random in the germination chambers (Hotcold-GL; ICT, Madrid, Spain), at 25°C . Incubation of seeds took place under 16 h light/8 h dark photoperiod and an irradiance of $35 \mu\text{mol m}^{-2} \text{ s}^{-1}$ provided by cool white fluorescent tubes (OS-RAM L 58W/20; ICT).

Filter papers were kept soaked during the whole experimental period (45 days) and every 3 or 4 days seeds showing radicle emergence were counted and thereafter removed from the Petri dishes. Dish location in the chamber was periodically changed. Dormancy level for each plant was calculated as follows:

$$D = \frac{\text{GA}_3 - \text{Control}}{\text{GA}_3} \times 100, \quad (2)$$

where D is seed dormancy percentage, GA_3 the percentage of seeds that germinated in the GA_3 addition treatment and Control is the percentage of seeds that germinated in control treatments.

Statistical analysis

The effect of manipulating resource availability (nitrogen and water) of the maternal environment on germination percentage was tested by fitting generalized linear mixed models (GLMM). Conventional generalized linear models allow the analysis of germination proportions that are generally non-normally distributed, but this estimation treats all effects in a model as fixed and

our experimental design required the variables 'plant identity' and 'quadrat' to be considered as random effects. GLMMs combine the relaxing normality assumptions and allow for a distinction between fixed and random effects in the model (see Littell *et al.*, 1996). This statistical analysis was performed using the SAS statistical package (SAS Institute, 1996). A SAS Macro program GLIMMIX (version 19 May 1997; available at <http://support.sas.com/ctx/samples/index.jsp?sid=536&tab=downloads>), was used for this analysis. The MIXED procedure implements a generalization of the standard linear model that allows for proper incorporation of random effects (Littell *et al.*, 1996; SAS Institute, 1996). The distributions of the response variables were fitted depending on the nature of the data. Germination was a probability ranging from 0 to 1; hence we applied a binomial estimation of the model using a logit link function. Plant height, moisture content and seed mass showed a normal distribution of the data and a Gaussian link function was used. Finally, total fruit number corresponded with a Poisson distribution and consequently we applied a log link function (Venables & Ripley, 1997). The variable plant identity was only considered for germination proportions and seed mass, as we had several data per plant. Otherwise, we had unique data per plant for plant height, fruit number and moisture content.

The model was built with both resource addition treatments and with the interaction between both as fixed variables, and plant and quadrat as random variables. Total plant biomass in each plot was introduced in the model in order to control the effect of different productivity.

As a result of right-censored data, the Kaplan–Meier method was adopted to estimate germination curves in different maternal conditions. The pair-wise shape differences in the modelled germination curves were tested by non-parametric log-rank tests (Pyke & Thompson, 1986). In order to test the effect of several covariates on germination curves, Cox regression models were used. These analyses are also suitable for censored data (Fox, 2001). Cox function for the accumulative germination is:

$$S(t) = [S_o(t)]^p \quad (3)$$

This formula represents germination rate, where accumulative germination $S(t)$ is a function of two independent variables: $S_o(t)$ is the time-dependent germination function, and p a linear combination of covariates and independent variables. A partial likelihood approach was used to calculate the covariate coefficients and consequently the effect of several covariates on the model. Model significance was tested using a likelihood ratio. The statistic for this test consisted in the comparison by means of a chi-squared test of the $-2LL$ value (-2

logarithm of the maximum likelihood) of a model built with all coefficients being 0 and of the $-2LL$ value of the model built with the coefficients of interest. Wald's forward stepwise selection process was used for the selection of the covariates that better explained the model and testing each partial model in the above-mentioned manner. The selection procedure ends when the addition of a new variable to the model does not increase the variability explained. The R -statistic was calculated to evaluate the partial correlation of each independent variable with the dependent one (germination time). In this study, plant height, number of fruits, seed colour (as a dummy variable), seed mass and seed moisture content were analysed as covariates.

Non-parametric Spearman correlations were undertaken to evaluate the association between some plant morphological features vs. seed coat colour, and seed morphological characters vs. germination response.

Results

Effect of maternal resource availability on seed morphology and germination features

None of the resource addition treatments exerted any significant effect on seed mass (Table 1) or on seed coat colour (Fisher's exact test: $\chi^2 = 3.28$; d.f. = 3; $P = 0.35$). However, germination percentage showed a meaningful effect of the maternal environment. Germination of *S. arvensis* seeds in control treatments ranged from 0% to 10%, similar to other studies (Andersson & Milberg, 1998) and in GA_3 addition treatments germination increased to 40–100%. Although no effect of any maternal resource availability scenario was detected on plant or seed morphological features (Table 1), water addition in the mother plant environment showed a negative effect on seed viability (i.e. germination percentage in GA_3 treatments), especially after the 2-year storage period (Table 2 and Fig. 1). Furthermore, in GA_3 treatments, seeds from experimentally watered plants showed a significant delay in germination rate, while those from maternal control treatments showed the highest germination rates (Table 3 and Fig. 2). Conversely, even nitrogen addition in the maternal environment showed no effect on germination percentage; it significantly reduced germination rate. Seeds stored for two years showed a significant decrease in germination rate (log rank = 4309.86; $P < 0.001$; Fig. 2C).

Effect of plant and seed morphological features on germination

The main source of variation in seed morphology and germination percentage was plant identity in all experi-

Table 1 GLMM evaluated the effect of the sampling quadrat and maternal environmental treatments on plant height, fruit number and moisture content

Source of variation	Height (Gauss)			Fruit number (Poisson)		
	Coef (SE)	Z	P	Coef (SE)	Z	P
Quadrat (R)	144.5 (60.6)	2.38	0.009	0.83 (0.35)	2.35	0.009
	Coef (SE)	t	P	Coef (SE)	t	P
(A) Plant traits						
Nitrogen	17.4 (9.8)	1.76	0.32	1.14 (0.76)	1.5	0.4
Water	-0.4 (8.9)	-0.04	0.97	0.5 (0.7)	0.7	0.6
Nitrogen * Water	-10.5 (11.7)	-0.89	0.53	-0.9 (0.9)	-1.05	0.5
Total biomass	-0.01 (0.15)	-0.07	0.9	-0.009 (0.01)	-0.8	0.4
Source of variation	Moisture content (Gauss)			Seed mass (Gauss)		
	Coef (SE)	Z	P	Coef (SE)	Z	P
Quadrat (R)	0.44 (0.31)	1.4	0.08	120.7 (84.5)	1.43	0.077
	Coef (SE)	t	P	Coef (SE)	t	P
(B) Seed traits						
Nitrogen	-0.8 (0.79)	-0.99	0.5	12.5 (12.6)	0.99	0.5
Water	-0.4 (0.7)	-0.61	0.6	10.8 (10.9)	0.99	0.5
Nitrogen * Water	1.35 (0.9)	1.53	0.4	-28.1 (14.3)	-1.96	0.3
Total biomass	-0.001 (0.01)	-0.12	0.9	-0.1 (0.18)	-0.62	0.5

Coef, coefficient value; SE, standard error.

The effect of plant identity was introduced into the model as a random factor for seed mass. Total biomass was introduced as a covariate in order to remove their effect from the model. We applied a Gaussian estimation of the modeled coefficients for plant height, moisture content and seed mass and a poisson estimation for fruit number.

Table 2 GLMM evaluated the effect of plant identity and maternal environmental treatments on germination percentages

Source of variation	Control			GA ₃		
	Coef (SE)	Z	P	Coef (SE)	Z	P
(A) Germination percentage in 1999						
Plant (RANDOM)	2.57 (0.55)	4.67	<0.0001	3.78 (0.74)	5.13	<0.0001
	Coef (SE)	t	P	Coef (SE)	t	P
Nitrogen	1.01 (1.5)	0.67	0.5	-2.19 (1.79)	-1.23	0.22
Water	1.5 (1.5)	1.0	0.3	-3.15 (1.7)	-1.80	0.07
Nitrogen * Water	-0.8 (0.9)	-0.87	0.39	1.64 (1.14)	1.44	0.15
(B) Germination percentage in 2001						
Plant (RANDOM)	1.3 (0.5)	2.7	0.003	2.06 (0.6)	3.5	0.0003
	Coef (SE)	t	P	Coef (SE)	t	P
Nitrogen	-0.31 (0.7)	-0.4	0.7	-1.75 (0.8)	-2.06	0.04
Water	0.7 (0.8)	0.88	0.4	-2.45 (0.8)	-2.9	0.004
Nitrogen * Water	0.82 (1.1)	0.7	0.5	3.39 (1.25)	2.7	0.008

Coef, coefficient value; SE, standard error.

Quadrat identity and total biomass were introduced as covariates in order to remove their effect from the model. Plant identity was introduced into the model as a random factor. We applied a binomial model using a logit link function. Significant terms are shown in italics. Germination treatments consisted in the addition (GA₃) and absence (Control) of gibberellic acid for seeds recently collected (1999) and stored for 2 years (2001).

ments (Tables 1 and 2). Plant height did not show any significant correlation with germination percentage in any of the germination treatments ($P > 0.05$). Conversely, fruit number was positively correlated to germination percentage in 1999 in absence of GA₃ (Spearman's $R = 0.3$; $P = 0.008$). Thus, plants that produced a larger number of fruits developed seeds with

lower levels of dormancy (Spearman's $R = -0.4$; $P < 0.0001$). Seed colour was correlated to a large extent with plant morphological characters: small plants with few fruits mainly produced black seeds, while larger plants with many fruits produced red seeds (Table 4 and Fig. 3A). Black seeds germinated in smaller proportions than the rest in control treatments, but viability was

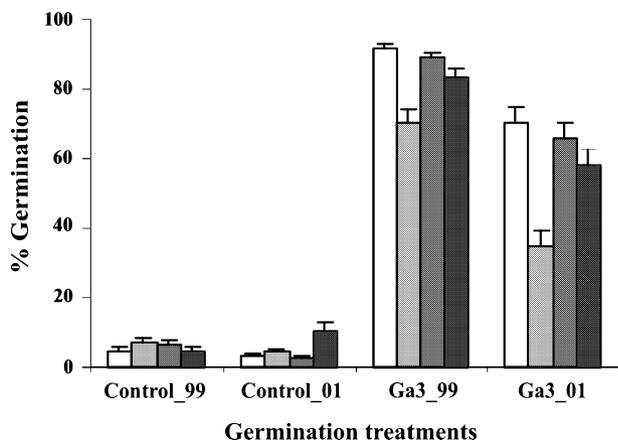


Fig. 1 (A) Germination percentage of seeds coming from four maternal resource availability conditions in both experimental years (1999 and 2001). Control (white bars), water addition (W^+ , light grey bars), nitrogen addition (N^+ , dark grey bars) and simultaneous addition of both resources (N^+W^+ , black bars). On the horizontal axis germination treatments are represented, control: no gibberellic acid addition, GA_3 : addition of gibberellic acid. Vertical bars indicate the standard error.

Table 3 Log-rank statistic for the pair-wise comparisons between germination curves in the GA_3 germination treatment for *Sinapis arvensis* seeds from four maternal treatments (Control, W^+ , N^+ and N^+W^+) – recently collected (1999) and after a 2-year storage period (2001)

	Control	W^+	N^+
(A) 1999			
W^+	479.26**		
N^+	47.78**	243.83**	
N^+W^+	98.46**	160.8**	NS
(B) 2001			
W^+	369.68**		
N^+	NS	433.27**	
N^+W^+	37.46**	193.1**	61.11**

W^+ , water addition; N^+ , nitrogen addition; N^+W^+ , both resources addition.

Bonferroni correction method was used in order to account for multiple comparisons. ** $P < 0.001$; NS, non-significant.

higher for black and mixed seeds (Table 5 and Fig. 3D). In the 1999 germination experiment, black seeds showed stronger dormancy, while in 2001 no difference in dormancy was observed among different-coloured seeds. Seed mass and colour were tightly related, black seeds being significantly heavier than the red ones (Table 5 and Fig. 3C). Seed mass and moisture content did not affect germination percentage in any experiment ($P > 0.05$).

Seed colour explained the largest fraction of variability in germination curves; while in the 1999 experiment, black seeds germinated faster than the red ones, in the 2001 experiment, black seeds germinated slower than

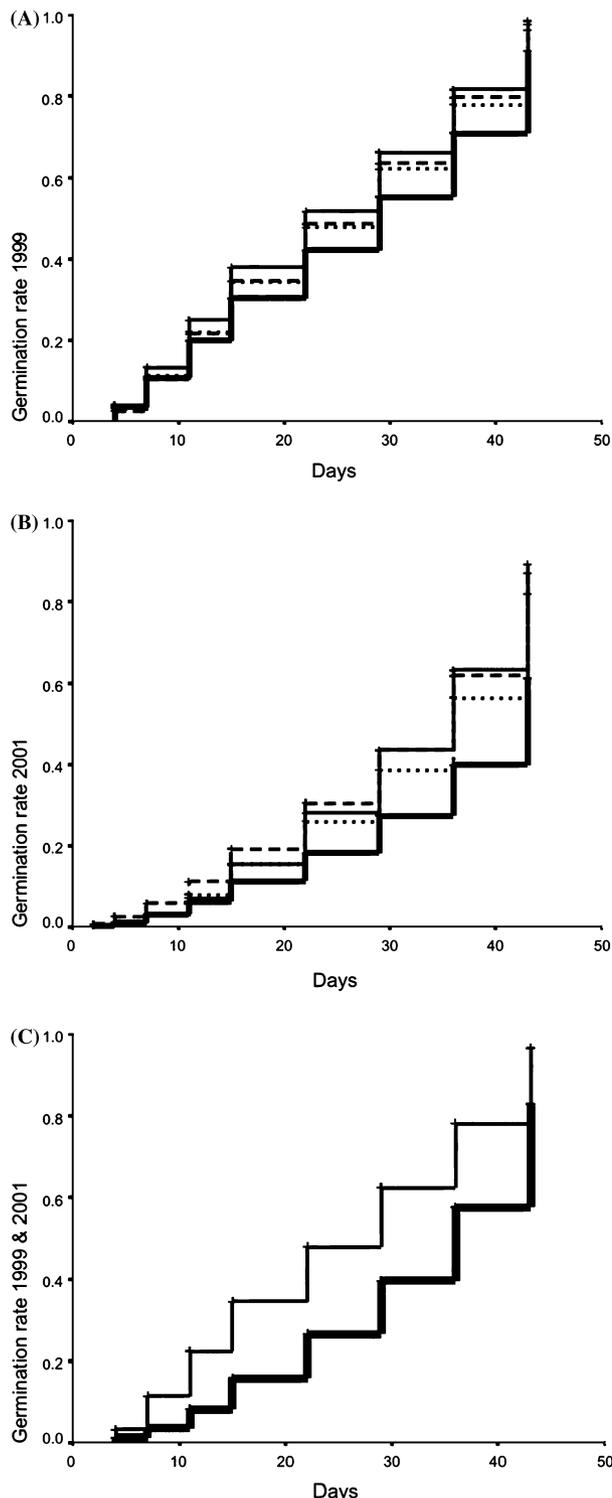


Fig. 2 Germination patterns for the GA_3 germination treatment by means of Kaplan–Meier models: (A) GA_3 germination treatment in 1999, (B) GA_3 germination treatment in 2001. Maternal environmental treatments are: water addition (W^+ , thick solid lines), nitrogen addition (N^+ , dashed line), both resources simultaneously (N^+W^+ , dotted line), and control (thin solid line). (C) Seed germination patterns before storage (1999, thin line) and after the 2-year storage (2001, thick line).

the red ones (Table 6 and Fig. 4). Other morphological features such as plant height, fruit number, seed mass and moisture content were also introduced in the Cox analyses in order to model germination patterns. Each variable explained significant portions of the germination rate after the 2-year storage period; conversely, for

Table 4 Kruskal–Wallis analyses with seed colour as the grouping factor for plant height, fruit number and mean seed fresh mass

Dependent variables	χ^2 -value	d.f.	Seed colour		
			Black	Mixed	Red
Plant height	13.99***	75	a	b	b
Fruit number	22.08***	75	a	b	b
Mean seed mass/10 seeds	23.86***	299	b	b	a

The same letter expresses no significant differences 'b': mean value is greater than 'a'. *** $P < 0.001$.

Multiple comparisons analysis with the non-parametric Dunn's test (Zar, 1999).

the recently collected seeds, plant height and seed moisture content were not significant (likelihood ratio test 1999: $\chi^2 = 786.4$; $P < 0.0001$; 2001: $\chi^2 = 1183.6$;

Table 5 Kruskal–Wallis analysis (2 degrees of freedom) and non-parametric multiple comparisons analysis (Dunn's test) for seed colour as the grouping variable and using as the dependent variables the percentage germination in control and in GA_3 germination treatments in recently collected seeds (1999) and after a storage period of 2 years (2001)

Dependent variables	χ^2 -value	Seed colour		
		Black	Mixed	Red
Germination %				
Control_99	13.72***	a	b	b
Seed viability (GA_3 _99)	10.27**	b	b	a
Dormancy_99	15.9***	b	a	a
Control_01	3.56 NS	a	b	b
Seed viability (GA_3 _01)	7.87*	ab	b	a
Dormancy_01	4.07 NS	a	a	a

The same letter expresses no significant differences. 'b': mean value is greater than 'a'. * $P < 0.05$; ** $P < 0.01$ *** $P < 0.001$.

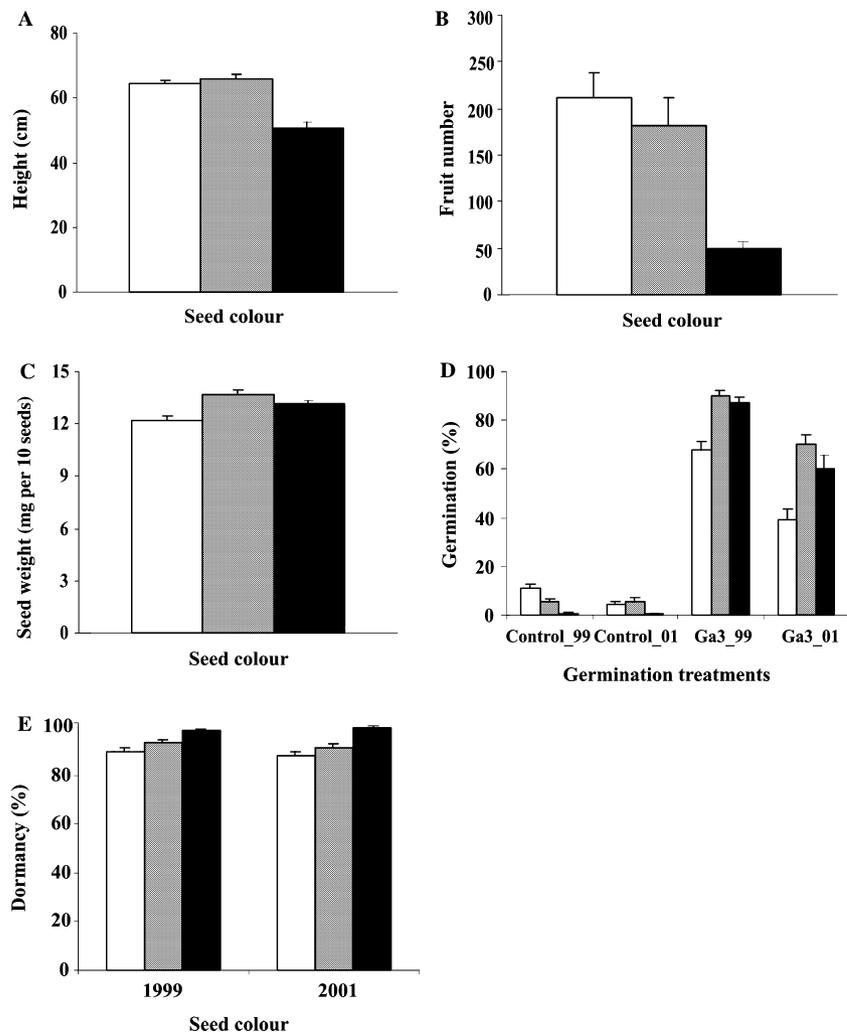


Fig. 3 (A) Height, (B) fruit number of the mother plants that mainly produced each seed colour type (C) mean seed mass of each seed colour type (D) germination percentage and (E) dormancy percentage depending on seed colour and year of experiment. Horizontal axis in (D) represents germination treatments. Control: no gibberellic acid addition, GA_3 : gibberellic acid addition. White bars: red seeds, grey bars: mixed coloured seeds, black bars: black seeds. Vertical bars represent standard error.

Table 6 Cox regression models for the germination curves of the GA₃ germination treatment introducing plant height number of fruits, seed colour, mass and moisture content as covariates; (A) Germination curves for 1999, (B) germination curves for 2001

Source of variation	Wald	d.f.	R-value	P-value
(A)				
Height	0.854	1	0.0	NS
Fruit number	190.08	1	-0.018	0.0000
Colour	347.07	2	0.025	0.0000
Black (vs. Red)	346.64	1	0.025	0.0000
Mixed (vs. Red)	67.61	1	0.011	0.0000
Seed mass	133.26	1	-0.015	0.0000
Moisture content	0.3036	1	0.0	NS
(B)				
Height	178.92	1	0.0295	0.0000
Fruit number	55.3131	1	-0.0162	0.0000
Colour	724.73	2	0.0596	0.0000
Black (vs. Red)	465.673	1	0.0478	0.0000
Mixed (vs. Red)	85.735	1	-0.0203	0.0000
Seed mass	6.3194	1	0.0046	0.0119
Moisture content	45.7	1	0.0147	0.0000

$P < 0.0001$; Table 6). Interestingly, plants that produced a larger number of fruits developed seeds with slower germination rates, and seed mass was negatively related to germination rate just after seed collection but positively related after the 2-year storage period.

Discussion

High morphological heterogeneity (colour and mass of seeds) and variability in the germination response of *S. arvensis* seeds from different mother plants was detected in this study (see also Durán & Retamal, 1983a,b,c; Andersson & Milberg, 1998). Variability in seed dormancy within a plant favours spread of germination in space and time. Thus, it reduces the risk that all seedlings will simultaneously suffer unfavourable conditions and sibling competition. Several studies relate seed dormancy level to mother plant identity, seed morphology, maternal environment and to some population features (Crawley & Nachapong, 1985; Schmid & Dolt, 1994; Andersson & Milberg, 1998).

Effect of resource availability on germination features

Independently of environmental conditions, germination percentage was primarily determined by the identity of the mother plant. Prevalence of the individual plant effect over environmental effects may guarantee the persistence of this population in the field of study, because this allows a wider range of conditions in which germination can occur in that specific population. Indeed, this may explain the wide distribution of this

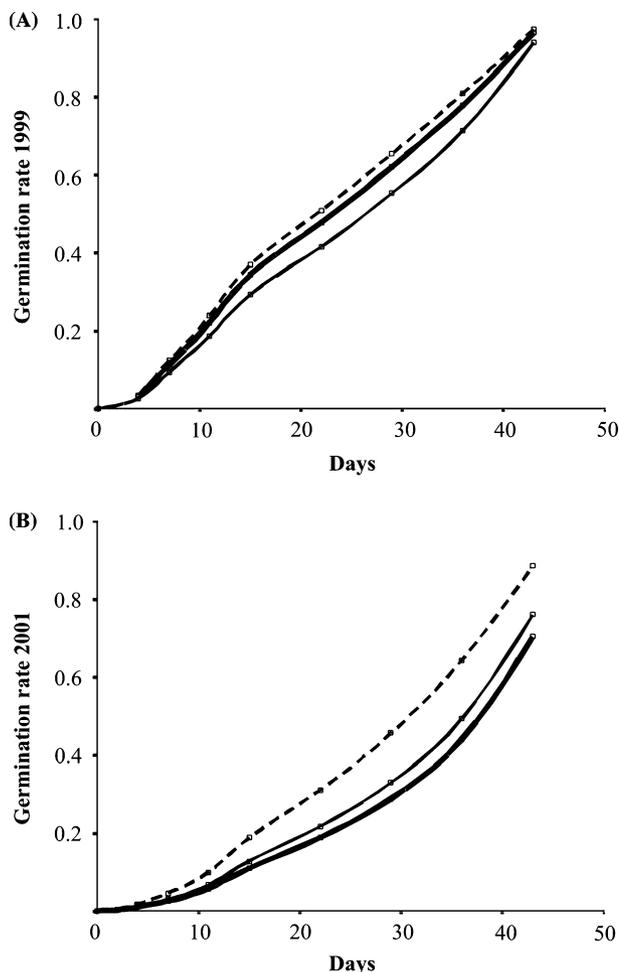


Fig. 4 Germination curves for the GA₃ germination treatment by means of a Cox regression method using seed colour as covariate. (A) Germination before the storage period (1999); (B) germination after the 2-year storage period (2001). Thick solid line: black seeds, dotted line: mixed colour seeds, thin solid line: red seeds.

species (Fogg, 1950) and its presence in highly variable environments (see also Andersson & Milberg, 1998; Huang *et al.*, 2001).

However, water addition in the maternal environment caused a significant decrease in germination percentage and rate, similar to the results obtained by Wright *et al.* (1999) for *S. arvensis* (see Tables 2 and 3). The decrease in germination rate is usually related to higher dormancy levels of seeds. Consequently, our results support the hypothesis of Wright *et al.* (1999) that adequate moisture during seed formation is expected to result in the production of more dormant seeds than in drier conditions, probably because better developed seeds are produced. The effect of maternal environment is usually reflected in the early life cycle of the following generation, such as in the dormancy level, as well as in germination rate and time (Miao *et al.*, 1991a;

Wulff & Bazzaz, 1992; Schmid & Dolt, 1994; Galloway, 2001a,b). In the literature four possible transmission mechanisms of the environmental effect are proposed: (i) by affecting the quantity and/or quality of the resources supplied (Stratton, 1989; Miao *et al.*, 1991b; Mazer & Wolfe, 1992; Weiner *et al.*, 1997), (ii) by changing the structure and thickness of the seed coat (Lacey *et al.*, 1997), (iii) by determining abortion patterns of seeds (Marshall & Ellstrand, 1988) and (iv) affecting the levels of hormones, enzymes, etc. We could reject the quantity or quality of the resources supplied and the thickness or structure of the seed coat as transmission mechanisms, as maternal environmental conditions did not affect either mean mass or seed coat colour. The main transmission mechanisms would probably be related to the effect of water addition on changes in hormone or enzyme activity, which would decrease germination rate and would cause a slight reduction in germination percentage as well.

Although nitrogen addition in the maternal environment caused a decrease in germination rate in the 1999 germination experiment, total germination percentage was not affected (see Tables 2 and 3). The decrease in germination rate was probably not related to ripening level (and consequently to dormancy level) of seeds developed in nitrogen enriched conditions, as our results show that nitrogen availability did not determine either mean mass or seed coat colour, and both traits are indicative of seed ripening level (Durán & Retamal, 1983a,b,c). More likely, the decrease in germination rate of seeds coming from nitrogen-enriched conditions could be due to the induction of dormancy caused by high concentrations of nitrogen in the seeds (Peterson & Bazzaz, 1978; Goudey *et al.*, 1986, 1988). This hypothesis is reinforced by the fact that, after the 2-year storage period, no difference was observed between the germination rate of seeds from nitrified and non-nitrified maternal environments; probably after the 2-year storage period the nitrogenous compounds would disappear from the seed tissues (Goudey *et al.*, 1988).

Changes in germination rates because of environmental maternal treatments denote changes in germination time. Germination timing has a crucial contribution to life-history traits and reproduction of the plant (Kalisz, 1986; Biere, 1991; Stratton, 1992; Galloway, 2001a; Donohue, 2002; Luzuriaga, 2004), and eventually on the establishment of competitive hierarchies in the plant community. Although maternal environment had a slight effect on total germination percentage, its significant effect on germination rate may largely determine not only the development and fitness of the individual, but also the constitution of the plant community.

Effect of seed mass and seed coat colour on germination

Several studies show that the effect of resource availability in the maternal environment is mainly directed to the next generation through seed size (Stanton, 1984; Crawley & Nachapong, 1985; Schmid & Dolt, 1994; El-Keblawy & Lovett-Doust, 1998). Seed size variability is considered a maternal effect, because the mother plant tissues in contact with the embryo are responsible for nutrient transference to the seed. Seed size is determined to a large extent by maternal environmental conditions (Winn, 1991; Fenner, 1992; Wolfe, 1995), such as nutrient availability (Wulff, 1986a), soil moisture content (Wright *et al.*, 1999), temperature (Alexander & Wulff, 1985), herbivory (Crawley & Nachapong, 1985; Sills & Nienhuis, 1995) and pollination (Quesada *et al.*, 1993, 1996). In this study, no resource availability scenario affected any plant or seed morphological trait, probably because in our experiment the control treatment was likely above moisture and nitrogen thresholds for plant growth.

No significant effect of seed mass on total germination percentage was found in this study, as in other studies based on mean seed mass values per plant (Pérez-García *et al.*, 1995; Wulff *et al.*, 1999). However, mean seed mass per plant accounted for a significant fraction of the variability in germination rate (see Table 6), and consequently seed mass may affect plant fitness by means of its effect on the timing of germination. Seed size is considered an early indicator of the offspring quality (Wulff *et al.*, 1999); a positive seed size effect on seed germination and seedling establishment has been repeatedly found (Stanton, 1984; Wulff, 1986a,b; El-Keblawy & Lovett-Doust, 1998), although this effect usually disappears when plants reach maturity (Stratton, 1989; Houssard & Escarré, 1991; Weiner *et al.*, 1997; El-Keblawy & Lovett-Doust, 1998). Other studies considering individual seed mass variability detected that the effect of seed size on germination response depended on population and species identity (Escudero *et al.*, 2000).

Our study found a strong association between red-coated seeds with low mass and black seeds with high mass. If the strong association of seed mass and seed coat thickness observed for other species (e.g. for *Plantago lanceolata* L.; Lacey, 1996), is fulfilled for *S. arvensis*, our results would suggest that black seeds are provided with thicker coats than red seeds. Seed coat is considered one of the main ways for transmission of information to the following generation, as it comes entirely from maternal tissues. Seed coat can affect germination response through three mechanisms: (i) by imposing a mechanical constriction to germination (Biere, 1991; Platenkamp & Shaw, 1993), (ii) by

determining seed coat permeability and thus enzymatic activation in the embryo (Baskin & Baskin, 1998), and (iii) by changing the light conditions experienced by the embryo (Botto *et al.*, 1995). Thus, the greater dormancy of black seeds observed in this study may be induced: (i) by their thick coat, either mechanically or physiologically because of changes of the environmental conditions inside the seed or (ii) by the low concentration of gibberellic acid in totally ripe seed tissues. Conversely, immature seeds usually contain higher concentrations of GA₃ than ripe ones (Pharis & King, 1985) because this hormone controls the transport of assimilates during the seed-filling process (Gray & Thomas, 1982). This agrees with our results that germination percentage of red seeds was higher in control germination experiments, i.e. no gibberellic acid addition, and that black seeds showed higher viability than red ones.

Seed coat colour explained the largest fraction of the variability in germination rate. It may indicate past selection in a heterogeneous environment, as the production of different coloured seeds by an individual plant seems to enlarge the range of germination timing, which guarantees the possibilities for establishment in unpredictable environments. Otherwise, our results may also be consequence of a strong association of seed coat colour to other germination traits not measured in our study that would turn out in this spurious result.

Conclusions

In this study, we detected that the largest fraction of germination response of *S. arvensis* was mainly determined by the mother plant's identity. However, after removing the effect of plant identity, we also observed a significant effect of water addition in the maternal environment on germination response. Furthermore, slight changes in germination response due to maternal environmental effects, as the changes in timing of germination detected in this experiment, may affect the development and fitness of the following generation (Platenkamp & Shaw, 1993; Schmid & Dolt, 1994; Galloway, 2001a; Donohue, 2002). Nevertheless, many other variables not explored in this study such as the environment where the offspring is established (Schmitt *et al.*, 1992; Wulff & Bazzaz, 1992; Wulff *et al.*, 1994) and competitive conditions (Parrish & Bazzaz, 1985; Stratton, 1989; Schmitt & Ehrhardt, 1990; Houssard & Escarré, 1991) may have eventually determined the phenotypic expression of the trans-generation effects.

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