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Germination ecology of eleven species of *Geraniaceae* and *Malvaceae*, with special reference to the effects of drying seeds

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Abstract

Germination and survival of water-impermeable seeds of 11 species of *Geraniaceae* and *Malvaceae* were monitored during dry storage and during burial in soil for up to 2.5 years. During dry storage, seeds of annual *Geraniaceae* became permeable and also lost their physiological dormancy. However, during burial in natural conditions, most seeds remained impermeable and viable, with no seasonal change in germination capacity. Germination in only one species (*Geranium robertianum*) was enhanced by daily alternating temperatures when seeds were exhumed in spring. Drying of exhumed seeds broke physical dormancy. Seeds of the perennial *Geranium pratense* gradually became permeable in a prolonged germination test of 31 weeks. Most seeds of *Malva* remained impermeable during dry storage. Buried seeds gradually germinated *in situ*, and exhumed seeds had a low germination capacity in all seasons. We concluded that dormancy of hard seeds in natural conditions may be broken by drying during summer, by specific temperature regimes or by gradual softening of the seed coat, ensuring the spread of germination over many seasons.

Keywords: activated carbon, after-ripening, dormancy, *Erodium*, *Geranium*, *Malva*, winter annuals

Introduction

Most species of *Geraniaceae* and *Malvaceae* produce seeds with a water-impermeable seed coat. These seeds remain in a state of physical dormancy until the coat is made permeable by scarification or some unknown factor in natural conditions. The anatomy, evolution and ecology of physical dormancy were recently reviewed by Baskin and Baskin (1998) and by Baskin *et al.* (2000). Many water-impermeable seeds survive for long periods in the soil. *Erodium* is known to form a persistent seed bank (Roberts, 1986), and *Malva rotundifolia* survived for 120 years in Beal's experiment (Telewski and Zeevaart, 2002).

During dry storage at room temperature, seeds of many species of *Geraniaceae* and *Malvaceae* lose their physical dormancy because their seed coat becomes permeable (Egley and Paul, 1981; Grime *et al.*, 1981; Meisert, 2002). It is not known whether this phenomenon also occurs when the seeds are buried in soil. In addition to physical dormancy, other mechanisms of dormancy may be involved. In at least one species, *Geranium carolinianum*, the embryos in fresh seeds were physiologically dormant (Baskin and Baskin, 1974).

The phenology of seedling emergence in species of *Geraniaceae* and *Malvaceae* was studied by Roberts and Boddrell (1984, 1985); for most of these, a small fraction germinated soon after dispersal, but some seeds were incorporated into the seed bank and germinated during summer in subsequent years. *Geranium pratense* was the only species for which all seeds emerged in the first spring after sowing.

Hard seeds can be softened artificially by chemical or mechanical scarification. However, little is known about the natural factors that render the seed coat permeable. Large daily fluctuating temperatures can stimulate germination of annual legumes (Taylor, 1981) and *Erodium* (Young *et al.*, 1975; Rice, 1985). Other factors such as fire, freezing and thawing, and passage through the digestive tracts of animals can break physical dormancy (Baskin and Baskin, 1998). There is little evidence for the role of mechanical abrasion by soil particles or decomposition of the seed coat by microbial action (Baskin and Baskin, 2000).

Two hypotheses can be proposed concerning germination of hard seeds in nature without fires or

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scarification. First, the seeds could remain dormant in the soil for a long time, and sporadically germinate in different seasons after the coat becomes permeable by some unknown process. Secondly, specific environmental signals could 'prime' seeds for germination, as we showed for some legumes that reacted to seasonal temperature differences (Van Assche *et al.*, 2003).

To test these hypotheses, seeds were buried in natural soil conditions. At regular time intervals, seeds were exhumed and their germination tested. According to the first hypothesis a low germination percentage is expected, regardless of the season. If temperature acts as a signal for germination, according to the second hypothesis, seasonal patterns in germination would be expected.

In several of the above-mentioned papers, germination was reported to be enhanced by dry storage. Since drying of the top soil regularly occurs during summer, the effect of drying exhumed seeds was tested as a possible environmental cue for initiating the germination process.

In a parallel series of experiments, seeds were stored in dry conditions, and changes in physical and physiological dormancy were analysed. If only physical dormancy was involved, scarified seeds were expected to germinate immediately. A delay in germination, and/or a requirement of low temperatures in scarified seeds, would indicate that the embryo also had a physiological dormancy, i.e. combinational dormancy (Baskin and Baskin, 2004).

For our study we collected all available native species in Belgium of *Geraniaceae* and *Malvaceae*. Most of the *Geraniaceae* are winter annuals and grow in open, disturbed places. The biennial *G. robertianum* was growing in woodlands, the perennial *G. pratense* was found in meadows and the three *Malva* species in grassy places. A comparison of the germination ecology of related species might show adaptations of germination requirements to specific habitat conditions or correlations with life history.

Materials and methods

Eleven species were used in this study (Table 1). Most seeds were collected from populations growing along roadsides or in grasslands. *Geranium lucidum* and *G. pratense* were grown in a garden to obtain a sufficient number of seeds. Ripe fruits were harvested during May to August, 2002–2005. *Geranium* fruits were collected daily, since their ripe fruits or seeds are released from the parent plant. Fruits of *Malva* were manually cleaned, and individual mericarps were separated before germination experiments.

Mean mass of dispersal units was determined by weighing 100 air-dry seeds or fruits. To estimate the standard deviation, this sample was visually divided into 10 size classes, and the total weight of the 10 dispersal units in each class was determined (Table 1).

Fresh seeds were used for germination tests, and other samples were buried within 7 d after harvest. A sample of the same harvest was stored at room temperature (about 20°C) for 3 months to 2 years, to determine the effects of dry storage on germination.

Germination tests

In standard germination tests, 100 dispersal units (hereafter seeds) were placed in Petri dishes on filter paper (Schleicher and Schuell No. 2282) moistened with distilled water. The seeds were incubated under a 12-h photoperiod at 23°C, and also at daily alternating temperatures of 12 h at 20°C and 12 h at 10°C. Light was provided by fluorescent tubes (Philips TLD 80) with a photon irradiance of $36\,\mu mol\,m^{-2}\,s^{-1}$ (400–700 nm). Preliminary experiments showed that seeds of all 11 species germinated equally well in light and darkness. Germinated seeds were counted after 10 d. All tests were performed in triplicate. Since germination percentages at alternating temperatures were generally similar to those at constant

Table 1. A list of the 11 studied species and their life form, mean mass (mg), type of dispersal unit and place of collection. Values are means ± SD. A, annual; WA, winter annual; P, perennial; B, biennial; (f), fruit; (m), mericarp; (s), seed

	Life form	Mean mass (mg)	Place of collection
Erodium cicutarium (L.) L'Hérit.	WA	2.77 ± 0.28 (f)	Leuven
Geranium columbinum L.	WA	4.22 ± 0.53 (s)	Viroinval
Geranium dissectum L.	WA	2.38 ± 0.23 (s)	Kortrijk
Geranium lucidum L.	WA	0.90 ± 0.07 (f)	Viroinval
Geranium molle L.	WA	1.11 ± 0.13 (f)	Leuven
Geranium pratense L.	P	7.40 ± 0.91 (s)	Yvoir
Geranium pusillum L.	WA	0.96 ± 0.14 (f)	Leuven
Geranium robertianum L.	B	1.30 ± 0.22 (f)	Leuven
Malva moschata L.	P	2.10 ± 0.38 (m)	Diest
Malva neglecta Wallr.	A/P	2.06 ± 0.34 (m)	Diest
Malva sylvestris L.	P	3.73 ± 0.63 (m)	Leuven

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temperature, only the results at constant temperature of 23°C are given, except in one case (see Results).

Germination of *G. pratense* was monitored for 31 weeks. During the first month, a low concentration (3 p.p.m.) of merthiolate (sodium ethylmercury-thiosalicylate) was added to the Petri dishes to avoid microbial infections. No inhibition of germination was observed. After 1 month the filter paper was replaced every month and no merthiolate was added. Germinated seedlings were counted weekly and discarded.

Scarification and germination rate at different temperatures

The germination rate of scarified seeds was determined for fresh seeds and for seeds dry-stored for 3 months at room temperature. A low germination rate of scarified seeds would indicate physiological dormancy of the embryo. Seeds were scarified by cutting a small hole in the seed coat with a surgical knife. When fruits were the dispersal units, seeds were extracted from the pericarp with a needle before scarification. Three replicates of 50 seeds each were used for germination tests at constant temperatures of 23°C, 10°C and 5°C with a 12-h light period. Germinated seeds were counted daily until germination was complete, or for a maximal period of 30 d. Germination rate is expressed as the number of days required to attain 50% germination of the total number of seeds tested (T_{50}).

Burial experiments

To detect possible seasonal patterns, fresh seeds were buried in nylon bags within 7 d after harvest. For each species, 500 seeds mixed with sand were put into 8 or 14 nylon bags, which were buried at a depth of 7 cm in pots filled with soil and placed in an open, sunny position. Every 2 months, bags were exhumed, and their contents spread on six Petri dishes. Any seedlings present in the bags were counted and removed. After 10 d, the germinated seeds, as well as the intact non-germinated seeds, were counted. The latter were regarded as viable since occasional tests showed that they germinated after scarification. Germination percentage was calculated on the basis of number of surviving seeds.

Effects of drying

After a standard germination test, non-germinated seeds in each Petri dish were transferred to a dry filter paper. The open Petri dishes were put in a desiccator over silica gel at room temperature (about 20°C). After 1 week, the filter papers were wetted, and germination at 23°C was tested again, as described above.

Statistical analysis

The effects of temperature and storage on germination rate after scarification were analysed using a Scheirer–Ray–Hare extension of the Kruskal–Wallis test (Sokal and Rohlf, 1997). In the burial experiment, the results were analysed by analysis of variance (ANOVA), followed by the Tukey multiple comparison test. Germination percentages were arcsin transformed before analysis to stabilize variances. The effects of drying on seeds exhumed after different periods of burial were analysed using a Mann–Whitney U test.

Results

Effects of dry storage and scarification

All of the 11 species have seeds with impermeable seed coats. In preliminary experiments, we found that scarified fresh seeds swelled in water within 6 h at 23°C, while non-scarified fresh seeds did not swell (data not shown).

Fresh, non-scarified seeds germinated to a very low percentage (Table 2). During storage at room temperature, most species of *Geranium* became permeable within 3 months and *Erodium* after 6 months to 1 year, while *G. pratense* remained impermeable during the 2-year storage period. In *Malva* species, only a small proportion became permeable.

Although impermeability of the seed coat was the main factor preventing germination, embryos were also partially or conditionally dormant. Scarified fresh seeds of most species germinated very slowly at 23°C, but *Erodium* and most *Geranium* species germinated somewhat faster at lower temperatures (Table 3). After storage for 3 months, scarified seeds germinated rapidly and nearly completely after 2 d at 23°C. Germination rates at lower temperatures were also higher after storage.

Burial experiments

Table 4 shows the results of germination experiments of seeds buried for up to 30 months. After exhumation, *Erodium* and most species of *Geranium* germinated poorly, regardless of the season. Germination of *Malva* species was slightly higher in spring, but no marked seasonal phases in germination were observed. *G. robertianum* may be an exception; after exhumation in the first spring, seeds germinated to a high percentage at daily alternating temperatures of 20/10°C, but to a low percentage at constant temperature. The same

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Table 2. Germination percentages of fresh seeds and seeds after different periods of dry storage. –, no observation; ±SE; $n = 3$

	Period of dry storage			
	0	3 months	1 year	2 years
Erodium cicutarium	0	2.7 ± 0.7	62.5 ± 3.6	87.8 ± 1.2
Geranium columbinum	3.3 ± 1.8	80.0 ± 4.6	84.7 ± 7.9	–
G. dissectum	0	80.8 ± 1.7	90.2 ± 5.0	–
G. lucidum	0.6 ± 0.6	74.2 ± 1.4	99.9 ± 0.07	–
G. molle	0	87.7 ± 1.0	97.3 ± 0.7	–
G. pratense	4.3 ± 2.2	4.7 ± 0.8	3.2 ± 1.6	2.8 ± 1.4
G. pusillum	0	66.2 ± 1.0	98.8 ± 0.7	–
G. robertianum	6.7 ± 2.7	13.3 ± 1.7	36.0 ± 4.6	–
Malva moschata	5.3 ± 2.4	–	13.8 ± 0.4	15.8 ± 2.6
M. neglecta	2.0 ± 1.1	–	6.1 ± 3.0	4.2 ± 0.3
M. sylvestris	2.0 ± 1.0	–	3.3 ± 0.7	3.7 ± 2.1

results were obtained in two preliminary experiments in different years. The other species were also tested at alternating temperatures, but germination in all months was similar to that at 23°C (data not shown).

The persistence of seeds in the soil was estimated by counting the intact exhumed seeds after different periods of burial (Table 5). *Erodium* and most *Geranium* species retained high viability in the soil for 1.5 or 2.5 years, but survival of *G. robertianum* may be lower. Many seeds of *Malva* germinated in the soil at a depth of 7 cm, and survival after 2.5 years was rather low, especially for *M. neglecta* (18%).

The behaviour of seeds of *G. pratense* during burial was totally different from that of the other species (Table 6). During autumn and winter, many seeds germinated in the soil, and thus very few intact seeds were recovered the next spring. The non-germinated seeds were swollen, and after exhumation, nearly all germinated in 1 or 2 d at 23°C. Obviously, these seeds became permeable during autumn and winter, but emergence of the radicle was delayed by low soil temperatures. Mean soil temperatures during 2001–2004 were 7.1°C in November, 2.9°C in January and 7.9°C in March. In laboratory conditions, the few scarified seeds that germinated did so very slowly at 10°C and no seeds germinated at 5°C (Table 3).

Germination of unscarified *G. pratense* seeds during 9 months' burial in natural conditions (Table 6) was compared to germination during nearly 8 months at a constant 23°C in Petri dishes and germination during and after a cold treatment at 5°C for 8 weeks (Fig. 1). During this long incubation period, seeds gradually became permeable, as indicated by visible swelling, several days before germination. During incubation at 5°C, a few seeds swelled and subsequently germinated during the first week at 23°C. However, there was no typical stimulatory effect of low-temperature stratification.

Effect of drying

After the standard germination test on fresh seeds and exhumed seeds, non-germinated seeds were dried for

Table 3. Germination rate, expressed as time in days to attain 50% germination. Seeds were either freshly matured or stored for 3 months and scarified before the germination test. *x*, germination less than 50% in 30 d. The number between brackets is the total germination percentage after 30 d, if the final germination was less than 95%. T, temperature; S, storage; T × S, interaction. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant; – not determined; $n = 3$

	Fresh seeds			Stored seeds					
	23°C	10°C	5°C	23°C	10°C	5°C	T	S	T × S
Erodium cicutarium	20.5 (75)	12.8 (74)	28.3 (52)	2.2	2.5	3.5	NS	***	NS
Geranium dissectum	22.5 (72)	7.2	10.5	1.5	3.0	6.0	NS	***	NS
G. lucidum	3.5	4.0	7.7	1.5	3.5	9.0	***	NS	NS
G. molle	17.7 (70)	4.5	13.5	1.5	3.0	7.0	*	**	*
G. pratense	6.0 (89)	*x* (0.6)	*x* (0)	2.7	*x* (14)	*x* (1.3)	–	–	–
G. pusillum	22.0 (72)	6.0	14.3	1.5	3.0	5.5	NS	***	NS
G. robertianum	14.2 (72)	*x* (35)	*x* (0)	3.7	*x* (49)	*x* (5.4)	–	–	–
Malva moschata	1.5	4.0	*x* (16)	1.0	3.7	27.7 (58)	***	NS	NS
M. neglecta	20.0 (58)	*x* (14)	*x* (1.3)	2.0	*x* (33)	*x* (0)	–	–	–
M. sylvestris	9.7 (82)	15.2 (60)	24.3 (60)	10.3 (92)	12.0 (61)	*x* (38)	***	NS	NS

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Table 4. Germination percentages of seeds following 2–30 months of burial in soil. *Means are significantly different within a species at the $P < 0.05$ level (Tukey's multiple comparison test); $n = 3$; – no observation

		Month of exhumation													
	Month of burial	Oct.	Dec.	Feb.	Apr.	Jun.	Aug.	Oct.	Dec.	Feb.	Apr.	Jun.	Aug.	Oct.	Dec.
Erodium cicutarium	Jun.	0.6	0	0	0.5	0	0	0	0	0	0.3	0	0	0.4	1.2
Geranium columbinum	Aug.	1.7	2.4	1.2	0	0.7	0	0.8	1	–	–	–	–	–	–
G. dissectum	Jun.	0	1.0	0	0	0	0.3	0.7	0	–	–	–	–	–	–
G. lucidum	Jun.	0	0	0	0	0	0	1.3	0.3	–	–	–	–	–	–
G. molle	Jun.	0.5	1.0	0	0	1.0	2.4	5.5*	1.6	–	–	–	–	–	–
G. pusillum	Jun.	0.5	0.9	1.1	0	0	0.6	0.8	0	0	1.4	0	1.6	1.1	0
G. robertianum tested at 23°C	Jun.	7.1	0.0	3.7	2.2	0.8	–	0.7	2.7	–	–	–	–	–	–
G. robertianum tested at 20/10°C	Jun.	1.3	4.1	66.0*	5.8	9.8	–	1.6	2.7	–	–	–	–	–	–
Malva moschata	Aug.	0.3	1.9	6.9*	4.2	1.2	0.2	1.7	2.6	3.6	0.9	0.7	0.9	0.1	1.6
M. neglecta	Aug.	0.2	1.9	9.4	5.0	0.8	7.0	2.3	0.4	7.5	6.0	0	2.7	1.4	4.7
M. sylvestris	Aug.	0.5	0.5	4.5*	1.2	0.6	1.6	1.8	0	0.8	0.5	0.7	0.9	0	0

1 week over silica gel in a desiccator and tested again at 23°C (Table 7).

Drying of exhumed seeds had a marked stimulatory effect on germination of *Erodium* and most *Geranium* species. Nearly all seeds germinated in 1–2 d, which is similar to the germination rate of scarified seeds after storage (Table 3). However, drying of fresh seeds had no effect on subsequent germination, except for *G. robertianum*, where even fresh seeds germinated to a higher percentage after drying. Drying also had very slight effects on germination of *Malva* species.

Discussion

Freshly matured seeds of all 11 species have water-impermeable seed coats (physical dormancy). In addition, physiological dormancy occurs in most *Geraniaceae* and in *Malva neglecta*. Combinational dormancy has been shown previously for *Geranium carolinianum* (Baskin and Baskin, 1974).

Table 5. Percentages of intact seeds recovered after burial for different periods. Values are means ± SE; $n = 2$; – no observation (initial number of seeds was 500)

	Period of burial		
	0.5 years	1.5 years	2.5 years
Erodium cicutarium	95.0 ± 2.0	92.5 ± 0.7	83.5 ± 2.5
Geranium columbinum	95.5 ± 0.5	61.5 ± 14.5	–
G. dissectum	95.0 ± 0.5	94.5 ± 0.5	–
G. lucidum	98.5 ± 0.5	79.5 ± 6.5	–
G. molle	93.5 ± 1.5	84.0 ± 0.0	–
G. pusillum	91.5 ± 1.5	91.5 ± 3.5	67.0 ± 2.0
G. robertianum	80.5 ± 2.5	44.5 ± 12.5	–
Malva moschata	79.0 ± 8.0	51.0 ± 0.0	44.5 ± 1.5
M. neglecta	95.5 ± 0.5	28.0 ± 0.5	18.0 ± 2.0
M. sylvestris	97.0 ± 0.5	55.0 ± 2.0	55.0 ± 2.0

During the first months of dry storage, both dormancy states can change. The seed coat of many species becomes permeable during dry storage (Baskin and Baskin, 1974; Egley and Paul, 1981; Grime *et al*., 1981; Meisert, 2002). We confirmed that the seed coats of *Erodium* and most *Geranium* species became permeable within 3–12 months, but most seeds of *Malva* remained impermeable during the 2-year storage period. However, the dormant state of the embryo also changed during storage. Freshly matured seeds were most dormant, since the germination rates of scarified seeds were very low at 23°C. It is possible that inhibitors are present in the embryo, since in our preliminary experiments we found that covering fresh, scarified seeds with activated carbon induced nearly complete germination in a few days. After 3 months of dry storage, this conditional dormancy was relieved, since nearly all scarified seeds germinated promptly. Consequently, embryo dormancy in these species could be classified as non-deep physiological.

A similar post-harvest dormancy has been described for several varieties of *Trifolium subterraneum* (Ballard, 1958). Germination percentages and germination rates of fresh, scarified seeds were low, but both increased after storage for several weeks. Dormancy could be broken by carbon dioxide (0.5–5%) or by covering scarified seeds with activated carbon. The effects of activated carbon are usually ascribed to removal of inhibitors, but Ballard suggested that activated carbon may increase the carbon dioxide concentration.

Grime *et al*. (1981) mentioned the ecological advantages of dry-storage effects. The need for after-ripening prevents premature germination in dry summer months. From their large-scale experiments, Grime and co-workers concluded that beneficial effects of dry storage upon germination percentages

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Table 6. Percentages of intact seeds recovered and germination percentages of *Geranium pratense* after burial for 3–9 months. Seeds were buried on 1 August. Values are means ± SE

	Exhumation date			
	Nov. 1	Jan. 1	Mar. 1	May 1
Percentage intact seeds ($n = 2$)	62.5 ± 2.5	48.5 ± 1.5	34.0 ± 7.0	1.1 ± 0.8
Germination percentage ($n = 4$)	93.1 ± 3.0	93.0 ± 3.0	96.7 ± 1.2	(100)[a]

[a] All of the few surviving seeds germinated in the standard test (initial number of seeds was 500).

are typical among winter annuals. From our results, it is obvious that after-ripening of *Erodium* and annual *Geranium* species involves two different processes. The seed coat becomes permeable to water and the physiological dormancy of the embryo is broken. High germination rates at low temperature are typical for these winter annuals.

In sharp contrast to the loss of permeability during the first months of dry storage, buried seeds of most species remained impermeable during 1.5–2.5 years. There was no effect of season on the germination capacity of exhumed seeds. Germination was absent or very low, indicating that the seed coat remained impermeable in the humid conditions of the soil, and showing that these seeds can survive for years in the soil.

These results could be obtained only when fresh seeds were buried immediately. In a preliminary experiment, we buried seeds of *G. molle* and *G. dissectum* after 3 months dry storage and found very few surviving seeds in the following months (data not shown). These seeds already had permeable seed coats after 3 months, and thus they germinated in the soil.

Probert (2000) drew attention to the fact that dry storage reduces dormancy and changes germination behaviour. He suggested that these findings have important practical implications for understanding the environmental control of germination. In their practical guidelines for ecologically focused germination studies, Baskin and Baskin (1998) also stressed that freshly matured dispersal units should be used.

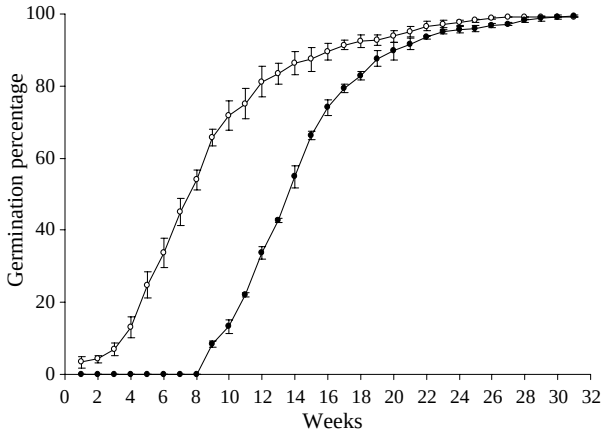

Figure 1. Cumulative germination percentages of unscarified *Geranium pratense* seeds at 23°C (open circles) and during stratification at 5°C for 8 weeks, followed by transfer to 23°C (closed circles). $n = 3$; vertical bars are standard errors.

After drying exhumed seeds of *Erodium* and several *Geranium* species, nearly all of them germinated in 1 or 2 d. Alternative methods of drying, such as in a desiccator over saturated lithium chloride (15% relative humidity), or storage in an incubator at 30°C (40% relative humidity) for 1 week, gave similar results (data not shown). Breaking of dormancy in buried seeds by drying may be a widespread environmental cue. It is described for other winter annuals with non-deep physiological dormancy: *Sisymbrium officinale* (Bouwmeester and Karssen, 1993), *Spergula arvensis* (Karssen *et al.*, 1988) and *Cardamine hirsuta* (unpublished personal observations). Drying is presumably an environmental cue, ensuring that seeds from the upper soil layers germinate after dry summer periods, which is crucial for the timing of germination of winter annuals. Seeds in deeper layers of the soil may remain dormant for several years. Our results are also in agreement with observations of Roberts and Boddrell (1984, 1985), showing that some seeds of ruderal *Geranium* species germinated shortly after sowing. However, some seeds were incorporated in the seed bank and germinated in the following years during summer months.

Dry storage and drying of exhumed seeds that have physical dormancy may act through the same mechanism, both rendering the seed coat permeable. Although the mechanism is unknown, La Croix and Staniforth (1964) suggested that drying the seed of *Abutilon theophrasti* (*Malvaceae*) induced stress and fracturing of tissues near the chalazal slit, resulting in a water-permeable seed coat. Several histological investigations showed that entry of water into seeds of *Malvaceae* was controlled by the chalazal cap (Christiansen and Moore, 1959; Winter, 1960; Egley *et al.*, 1986). Meisert *et al.* (1999) showed that the chalazal slit is the main pathway for entry of water in soft seeds of *Geraniaceae*. However, it is not known whether uptake of water also occurs through the chalazal region after breaking of physical dormancy.

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Table 7. Germination percentages of fresh and exhumed seeds without drying (C) and after drying (AD), following different periods of burial. ± SE; * means are significantly different at the $P < 0.05$ level (Mann–Whitney U test); $n = 3$; – no observation

	Period of burial							
	0		0.5 years		1.5 years		2.5 years	
	C	AD	C	AD	C	AD	C	AD
Erodium cicutarium	0	0	0	88.1 ± 2.1*	0	100*	1.2 ± 0.2	100*
Geranium columbinum	3.3 ± 1.8	2.0 ± 1.1	1.2 ± 1.2	2.0 ± 1.2	1.0 ± 1.0	28.7 ± 4.0*	–	–
G. dissectum	0	0	0	82.0 ± 2.8*	0	97.5 ± 1.4*	–	–
G. lucidum	0.6 ± 0.6	0	0	14.1 ± 2.3*	0.3 ± 0.2	77.8 ± 5.1*	–	–
G. molle	0	0	0	74.4 ± 4.4*	1.6 ± 0.3	62.5 ± 1.7*	–	–
G. pusillum	0	0	1.1 ± 0.6	85.4 ± 0.9*	0	93.7 ± 2.3*	0	93.7 ± 1.7*
G. robertianum	6.8 ± 2.4	53.2 ± 4.8*	0	37.9 ± 4.8*	2.8 ± 2.8	25.6 ± 3.4*	–	–
Malva moschata	5.3 ± 2.4	7.7 ± 2.7	6.9 ± 1.7	6.8 ± 1.0	3.6 ± 0.6	7.2 ± 1.1*	1.6 ± 1.2	13.5 ± 3.4*
M. neglecta	2.0 ± 1.1	16.0 ± 4.2*	9.4 ± 3.1	5.0 ± 1.2	7.5 ± 4.6	9.6 ± 3.8	4.7 ± 2.7	13.2 ± 4.3
M. sylvestris	2.0 ± 1.0	4.0 ± 0.6	4.5 ± 2.2	5.4 ± 1.4	0.8 ± 0.8	0.5 ± 0.3	0	3.0 ± 1.1*

It is possible that drying is a cue for germination of *Erodium botrys* and *E. brachycarpum*. Rice (1985) found that temperature fluctuation during dry storage enhanced the germination of these species. However, this author did not mention a control experiment with drying at a constant higher temperature, which might be sufficient to increase germination.

Among the 11 studied species, *G. pratense* was the only one of which nearly all seeds germinated in one season, the first spring after sowing (Roberts and Boddrell, 1985). Consequently, a positive effect of low temperature stratification on germination percentage could be expected. However, there was no effect of chilling on subsequent germination in laboratory conditions. All seeds gradually became permeable in the course of incubation for 31 weeks at 23°C. The mechanism of this continuous germination is unknown. It is unlikely that microbial infections soften the seed coats, since all seeds in one Petri dish would be infected at the same time, and germination would occur simultaneously. There seems to be an ageing effect with endogenous breakdown of the seed coat or disruption of the chalazal region.

Buried seeds of *G. pratense* underwent the same process of gradual dormancy loss during autumn and winter, resulting in swelling of the seeds and radicle emergence, although this last phase was delayed by low temperature. Normally, seedlings emerged in late winter or early spring, and very few remained ungerminated.

The gradual softening of the seed coat may be a widespread phenomenon in hard seeds. Garrard (1955) found that the seeds of the legume *Adenanthera pavonina* gradually germinated over a period of 436 d. Baskin and Baskin mentioned in their book (Baskin and Baskin, 1998, p. 101) an ongoing experiment with hard legumes, where germination is spread over many years. In our experiments with *Malva* species, a small number of germinated seeds were regularly found in the exhumed bags during spring and summer. This showed again a gradual loss of impermeability, resulting in a rather low survival percentage after 2.5 years.

G. robertianum may be one case where seasonal temperature change might induce germination; seeds exhumed in spring were stimulated to germinate by alternating temperatures prevailing in that season. However, there are indications that lower temperatures are responsible for germination. Further investigations are needed to confirm these results.

We can conclude that different mechanisms regulate the timing of germination of hard seeds in natural environments in a temperate climate without fires. Even within the family *Geraniaceae*, striking differences are found in germination ecology, which are related to life history and phenology. *Erodium* and several ruderal *Geranium* species are typical winter annuals. Their freshly matured seeds may lie near the soil surface and become permeable during dry summer months. These seeds germinate a few months later when the soil is moistened. Seeds buried in deeper layers can survive in a dormant stage for several years. After they are brought to the surface, they lose physical dormancy by drying in summer.

Seeds of *G. robertianum*, a biennial species of woodlands, may be induced to germinate by specific seasonal temperatures, as described for some legumes in our previous work (Van Assche *et al.*, 2003). Seeds of the perennial *G. pratense* gradually lose water impermeability during autumn and winter, thus ensuring germination in spring.

For some other hard seeds, such as those of *Malva*, no environmental cue for dormancy break is known. It seems possible that the seeds soften gradually, and germination is spread over several years in different seasons, provided temperature is above a threshold and they can imbibe water.

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