Dormancy, germination and mortality of seeds in heathland and inland sand dunes

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SUMMARY

Seeds of seven dominant species from heathland and inland sand dunes were buried in their natural habitat and exhumed twice a year, in March and September, during a 2- or 3-year period. The number of dead, germinated and surviving seeds was recorded and the latter were subjected to a germination test in a fluctuating temperature regime of 12/22°C in light. All the seeds of *Deschampsia flexuosa* had germinated *in situ* within 6 months of burial. This was also the case for almost all seeds of *Agrostis vinealis* and the majority of the seeds of *Corynephorus canescens*; the remaining seeds survived until the end of the experiment (2 years). About 35% of the seeds of *Molinia caerulea* died or germinated *in situ*; the remaining seeds survived until the end of the experiment (3 years) and showed a seasonal pattern of changing dormancy that has been described for many summer annuals. Dormancy in the soil is enforced by a requirement for temperature fluctuation. The winter annual *Spergula morisonii* showed a dormancy pattern which was typical for that life form, i.e. alleviation of dormancy in summer and induction of dormancy during winter. The two dwarf shrubs *Calluna vulgaris* and *Erica tetralix* had an absolute light requirement for germination and no seeds germinated in the soil during the 3 years; the mortality of *C. vulgaris* was low, whereas 40% of the *E. tetralix* seeds died. No seasonal change in dormancy was detected in either species.

Key-words: heathland, inland sand dunes, seasonal changes in dormancy, seed germination, survival.

INTRODUCTION

Seeds of many species can survive long periods in the soil and form persistent seed banks, while other species cannot (Chippendale & Milton 1934; Thompson & Grime 1979; Kivilaan & Bandurski 1981). Species that form a persistent seed bank can postpone germination until more favourable conditions for establishment and growth exist (Angevine & Chabot 1979). Survival of the seeds in the soil depends on tolerance of abiotic factors in the soil, resistance against microbial attack, avoidance of predation, and avoidance of germination.

Various dormancy mechanisms have evolved that postpone germination (Bewley & Black 1982). The most important one that enforces dormancy upon buried seeds is light requirement but temperature fluctuations may also be involved (Thompson & Grime 1983).

Dormancy may change with the seasons in temperate regions (Karssen 1982; Baskin & Baskin 1985a). Dormancy of many winter annuals appears to be alleviated in summer and is induced in the remaining seeds during the following winter, which makes them least dormant in autumn, the period of emergence in the field. The reverse is true for many summer annuals; their seeds loose primary dormancy during winter and develop secondary dormancy in summer. Hence, these seeds are least dormant in spring when they germinate in the field.

Weeds and species with a short life span have predominantly been studied; fewer studies are available on species from other habitats with different life cycles (Baskin & Baskin 1981; 1985b; Granström 1987).

In the present study the survival of seeds underground and seasonal changes in dormancy of the surviving seeds was investigated in seven species from heathland and inland sand dune habitats on acid sandy soil in The Netherlands. Dispersal units were buried in the natural habitat and the mortality of the seeds, their germination in the soil and their germination in a standard test after they were exhumed twice a year were recorded. Potential constraints for establishment from seed in the natural habitat are discussed.

MATERIALS AND METHODS

The experiments were carried out on three locations where seeds were collected and buried: a wet heathland area located in the nature reserve 'Kruishaarse heide' near Nijkerk, The Netherlands; a dry heathland ('Oud Reemster veld') and a stabilized sand dune area ('Oud Reemster zand') located in the national park 'Hoge Veluwe'. The Kruishaarse heide is partly dominated by *Erica tetralix* L. and partly by *Molinia caerulea* (L.) Moench; the experiment was carried out in an area dominated by *E. tetralix* (*Ericetum tetralicis* Schwick. 1933). The soil water level rises to the soil surface during winter but lowers to *c*. 1·7 m below during summer (Berendse & Aerts 1984). The Oud Reemster veld was, until recently, dominated by *Calluna vulgaris* (L.) Hull but is now largely dominated by the grasses *M. caerulea* and *Deschampsia flexuosa* (L.) Trin. (Heil, 1984). The experiment was carried out on a place dominated by *C. vulgaris* (Genisto pilosae Callunetum R. Tx. 1933 em. Preising 1953). The Oud Reemster zand is an inland sand dune area that is relatively stable. Frequently occurring species are, apart from lichens, *Corynephorus canescens* (L.) Beauv., *Agrostis vinealis* Schreber, *Festuca ovina* L. and *Spergula morisonii* Bor. (Spergulo-Corynephoretum Libbert 1932 em. Passarge 1960). *C. vulgaris* was encroaching on the area where the experiment was carried out.

The following species were investigated: *Calluna vulgaris, Molinia caerulea* and *Deschampsia flexuosa*, a dwarf shrub and two perennial grasses on dry heathland; the dwarf shrub *Erica tetralix* and *M. caerulea*, on wet heathland; *Agrostis vinealis, Corynephorus canescens* and *Spergula morisonii*, two perennial grasses and a winter annual on stabilized inland sand dunes.

Seeds, and in the case of grass species, caryopses (further referred to as seeds) were collected where the experiments were carried out: *E. tetralix* and *M. caerulea* in October 1983, *C. vulgaris* in November 1983, *D. flexuosa, A. vinealis* and *C. canescens* in August 1984 and *S. morisonii* in June 1985. As soon as possible after collection and drying, seeds

were enclosed in polyamide bags (mesh 0·1 mm), 50 seeds per bag for the grasses and
S. morisonii and *c.* 100 for *C. vulgaris* and *E. tetralix.* The bags (25 per species) were
buried at a depth of 10 cm with a rope connected to the bag protruding from the soil. In
September and March each year, three bags of each species were exhumed. These times
were selected because if there are changes in dormancy, the differences can be expected to
be maximal (Baskin & Baskin, 1985a). The number of dead, germinated and apparently
viable seeds was recorded. Seeds that had recently germinated *in situ* could easily be
recognized by the remains of the seedlings; after decay of the seedlings, germinated seeds
could be identified by the typical slit in the seed coat. The apparently viable seeds were
subjected to a germination test. Seeds that germinated during the test were recorded as
non-dormant, seeds that did not germinate and were apparently viable were considered
dormant, and seeds that died during the test were considered non-viable at the time of
sampling. The death of a seed was confirmed by crushing at the end of the germination
test. Seeds were recorded as dead when they had a soft brownish content as opposed to the
firm white embryo and endosperm of apparently viable seeds.

Exposure to light during excavation for germination tests in darkness was prevented by
taking a soil core with the bag with the seeds inside. The seeds were taken out of the core
and subjected to the test in darkness; no 'green safe light' was used.

The germination tests were carried out on the cloth that was used for the burial of the
seeds; it was spread over wet cotton wool in a Petri dish. The dishes were placed in a
temperature-controlled cabinet with a day and night temperature of 22°C and 12°C,
respectively and light and thermoperiods of 12 h. Photon flux densities (PFD) of
fluorescent light were *c.* 100 μmol m^{-2} s^{-1} for *C. vulgaris* and *E. tetralix* and *c.*
20 μmol m^{-2} s^{-1} for the other species. Seeds of the heather species may require a rather
high PFD for germination (Pons 1988) while the other species do not. The germination
test had a maximum duration of 8 weeks. Germination of the grass species was generally
completed in 3 weeks, but *C. vulgaris, E. tetralix* and *S. morisonii* sometimes required
longer periods. Before burial a germination test was carried out in the light and in
darkness under the same conditions as the tests during the burial experiment.

An experiment on the influence of various constant and fluctuating temperature
regimes on germination of stratified seeds of *M. caerulea* was carried out; light conditions
were the same as in the standard test, only the temperature was varied. Stratification was
carried out at 4°C in darkness during an 8-week period.

The data of the burial experiment were analysed with a one-way ANOVA after angular
transformation of germination percentages.

RESULTS

Viable seeds of *D. flexuosa* and *A. vinealis* were not dormant at the beginning of the burial
experiment; they all germinated in light as well as in darkness (Table 1). In the following
spring the majority of the seeds had germinated; this had probably already occurred in
autumn. The remaining seeds of *D. flexuosa* had died, but a few *A. vinealis* seeds were still
alive and remained viable until the end of the 2-year duration of the experiment.

Most of the seeds of *C. canescens* germinated readily in both light and dark before the
seeds were buried on 8th October 1984 (Table 1). The majority of these seeds had germi-
nated underground by the following spring, but the remaining *c.* 30% of the seeds did not
germinate *in situ* and remained viable until the end of the experiment. The remaining seeds
showed a very low germinability after the winter of 1984/85, but all germinated at the end

Table 1. Mortality: germination during burial in the soil and germination in the test after exhumation (% ± SE of total number of seeds) of *Deschampsia flexuosa*, *Agrostis vinealis* and *Corynephorus canescens* in the seed burial experiment. Seeds not accounted for were dormant. The first test was carried out in light (l) and darkness (d) just before burial at 8 October 1984, after 2 months of dry storage at 4°C; there were hardly any differences with a test carried out directly after collection of the seeds. The tests after exhumation were carried out in light only

Date	Deschampsia flexuosa			Agrostis vinealis			Corynephorus canescens		
	Dead	Germination		Dead	Germination		Dead	Germination	
		In situ	In test		In situ	In test		In situ	In test
8 October 1984									
l	16±1·6	—	83±2·3	4±1·6	—	94±2·3	8±2·5	—	71±1·4
d	13±2·7	—	84±3·5	9±1·3	—	91±0·7	7±2·7	—	75±1·3
12 March 1985									
l	10±2·0	90±2·0	0	3±0·5	93±1·3	4±1·2	4±1·6	58±2·3	3±0·7
29 August 1986									
l	—	—	—	6±0·0	86±0·0	8±0·0		75±4·1*	23±4·8

*Germination *in situ* and dead seeds combined.

of 1986. Germination in the tests in September 1985 and March 1986 was intermediate and there were no significant changes in the time required for 50% germination (data not shown).

The seeds of *C. vulgaris* and *E. tetralix* had an absolute light requirement before burial; and no germination occurred in seeds exhumed in darkness on 10th May 1985. The percentage and rate of germination of viable seeds in the test before burial were low, particularly in *C. vulgaris* (Fig. 1); these were higher after a period in the soil. The rate of germination tended to decrease during residence in the soil, as can be seen from the time required for 50% germination. The mortality of *C. vulgaris* seeds did not change during the experiment and was low. The mortality of *E. tetralix* had increased considerably after 6 months of burial, but did not increase further during the experiment (Table 2). The high mortality in 1984 was mainly due to mortality during the test, while dead seeds were identified, as such, directly after the seeds were exhumed in 1985 and 1986.

Two populations of *M. caerulea* were used in the experiment; one from a dry heath-land and one from a wet heathland. Both populations responded qualitatively simi-larly; only quantitative differences were found (Fig. 1). Most seeds were dormant before burial. After the first winter, 20–25% of the seeds had died, which is higher than the mortality during the test before burial. Almost all of the remaining seeds germi-nated, indicating that dormancy had been alleviated. In September 1984 the germi-nation percentage and rate had decreased, indicating that dormancy had been induced. Alleviation and induction of dormancy occurred in the winter and summer, respect-ively, of 1985 and 1986 again. Germination *in situ* did not occur until after 2 years, probably in the spring of 1985, and was not observed thereafter. In 1986 dead and germinated seeds could not be separated any more because the remains of the latter had deteriorated too much. Alleviation and induction of dormancy were very regular in the population on the dry heathland, but dormancy on the wet heathland was much less in September 1984 than in 1985. It must be noted here that the vegetation and the upper 2–3 cm of the litter layer had been removed in March 1985 on the wet heathland site where the seeds were buried, leaving bare soil for the rest of the experimental period. This cutting of sods was carried out in an attempt to restore the dominance of *E. tetralix*. The different course of the dormancy pattern in the 2 years could be related to this change in conditions.

Seeds of *M. caerulea* that were exhumed in darkness on the wet heathland on 10th May 1985, i.e. after alleviation of dormancy during winter, germinated readily in darkness (71% of viable seeds, standard temperature regime of 12/22°C). Hence, dormancy *in situ* was not enforced by darkness. A fluctuating temperature appeared to stimulate germi-nation of stratified *M. caerulea* seeds (wet heathland collection 1984) in both the light and dark (Table 3). A particularly large amplitude of fluctuation was required in the dark. Hence, dormancy of buried seeds was most probably enforced by the constant temperature that prevails at a depth of 10 cm.

Germination percentages of *S. morisonii* were low at 12/22°C and at 4°C directly after collection in June 1985, but after 3 months of storage at room temperature germi-nation had increased appreciably (Table 4). At the end of the first summer, in the field, germinability had also increased but not to the same degree (Fig. 1). In the winter of 1985/86 dormancy was induced which was alleviated again during the subsequent sum-mer; this was evident both from the higher percentage and the rate of germination. Mortality was low throughout the period of the experiment and germination *in situ* did not occur.
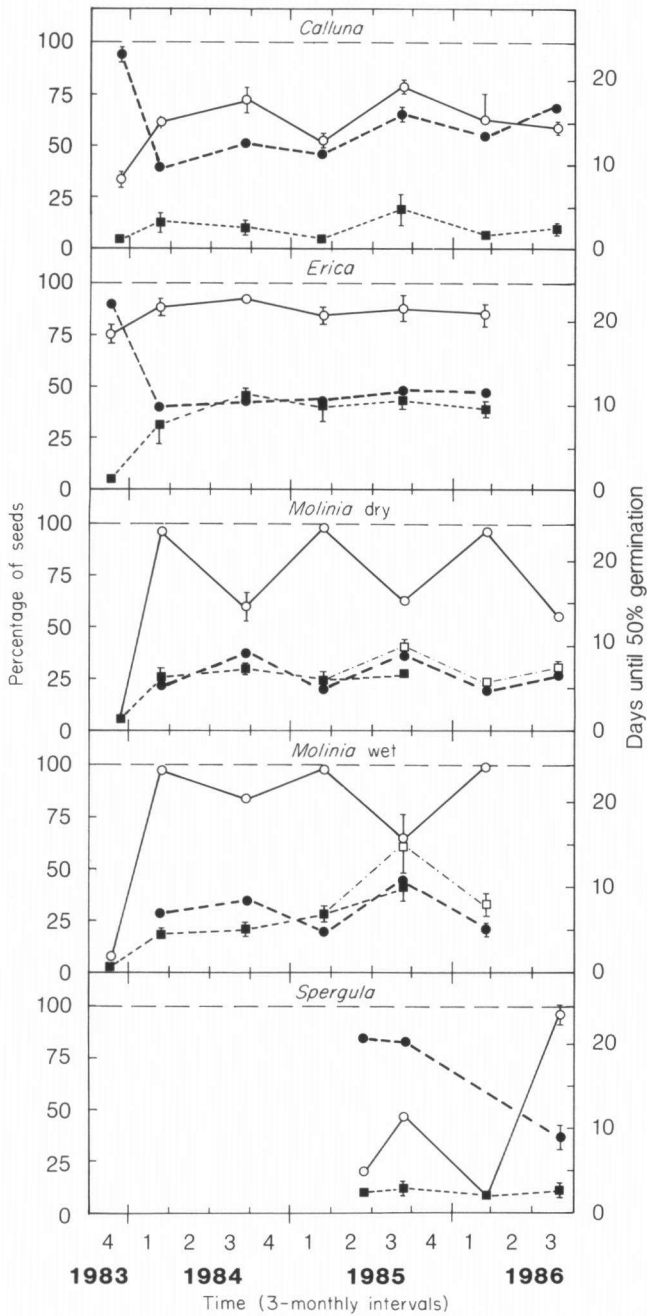
Fig. 1. Fate and germination characteristics of the seeds of *Calluna vulgaris, Erica tetralix, Molinia caerulea* buried in the dry heathland (*Molinia* dry), *M. caerulea* buried in the wet heathland (*Molinia* wet) and *Spergula morisonii* in the seed burial experiment. (■−−−■) Percentage of dead seeds; (□−·−□) percentage of dead + germinated *in situ*; (O———O) percentage of dead + germinated *in situ* and in test; (●−−−●) days until 50% of final germination percentage of apparently viable seeds.

Table 2. ANOVA on percentage mortality (plus germination *in situ* in the case of *Molinia caerulea*) (percentage of total number of seeds), germination in the test (percentage of viable seeds) and time until 50% of final germination. The analysis was carried out including and excluding the germination test before burial of the seeds

Species	Mortality		Germination in test		Time until 50% germination	
	Inclusion	Exclusion	Inclusion	Exclusion	Inclusion	Exclusion
Calluna vulgaris	NS	NS	*	NS	***	***
Erica tetralix	**	NS	NS	NS	***	*
Molinia caerulea						
(dry)	***	NS	***	***	—	***
(wet)	**	*	***	***	—	***
Spergula morisonii	NS	NS	***	***	***	**

NS = not significant at $P < 0.05$.
*, **, *** = $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

Table 3. Germination (% $\pm$ SE) of *Molinia caerulea* in various constant and fluctuating temperature regimes in light and dark. The seeds were collected in the wet heathland in 1984 and stratified at 4°C in darkness for 8 weeks

	Temperature regime (°C)				
	constant			fluctuating	
	22	18	12	22/18	22/12
Light	23±4·9	8±1·4	2±1·3	67±1·1	98±1·2
Dark	11±1·4	4±2·2	0	4±1·9	38±4·1

Table 4. Effect of storage at room temperature (*c.* 20°C) on germination (% $\pm$ SE) of *Spergula morisonii* at 4°C and 22/12°C in light and dark. The first test was carried out within 1 week after collection of the seeds

Date	4°C		22/12°C	
	Light	Dark	Light	Dark
12 June 1985	9±1·0	1±0·6	11±3·5	0
12 September 1985	58±4·1	5±0·8	78±2·0	14±3·3

DISCUSSION

Seeds were buried in fine mesh bags at a depth of 10 cm. Hence, this discussion refers to seeds in deeper soil layers in the absence of predation. Such seeds of different species in one habitat showed large differences in dormancy characteristics.

C. vulgaris can remain viable for long periods in the soil (Hill & Stevens 1981; Granström 1988; Willems 1988), often in large numbers (Chippendale & Milton 1934; Mallik *et al.* 1984). Records are also available on persistent seed banks of *E. tetralix* (Hill & Stevens 1981; Stieperaere & Timmerman 1983; Heil 1984). No seasonal changes in dormancy of the heather seeds were found, as described for many annual species with a persistent seed bank (Karssen 1982; Baskin & Baskin 1985a). Heather seeds of the populations used for the present experiments have an almost absolute light requirement (Pons 1988). Hence, avoidance of germination of buried heather seeds relies mainly on dormancy enforced by the lack of light. The higher degree of dormancy of freshly ripened seeds (Pons 1988) may be important for avoiding germination before the seeds are incorporated into the soil. The lack of dormancy of the heather seeds after burial enables them to germinate at any time as soon as they are exposed to light, such as after soil disturbance, provided that temperature and moisture conditions are favourable.

E. tetralix had a rather high mortality, although this did not increase after the first exhumation. Nevertheless, the remaining *c.* 60% of the seeds can make a substantial contribution to the seed bank when the production of large numbers of small seeds (*c.* 0·02 mg) is taken into account (Berendse *et al.* 1987).

M. caerulea showed a similar seasonal change in dormancy as described for many summer annual weed species (Courtney 1968; Baskin & Baskin 1980; Karssen 1980/81; Roberts & Boddrell 1985). This experiment shows that this is not restricted to annuals and biennials (Baskin & Baskin 1981) but can be true for perennials as well. The lack of dormancy in spring coincides with the period of emergence of *M. caerulea* seedlings in the field. The dormancy in autumn prevents germination in an unsuitable season for seedling growth, at least to some extent. The results indicate that *M. caerulea* seeds can survive for long periods in the soil, but the author knows of no records of persistent seed banks of this species. The requirement for temperature fluctuation in darkness, as found for several grass species, will be involved in the avoidance of fatal germination in deeper soil layers and under a vegetation cover (Thompson & Grime 1983).

The results for *D. flexuosa* are an indication that this species cannot form a persistent seed bank because the seeds had no dormancy and they all germinated. In addition, Hill & Stevens (1981) found no evidence for survival of *D. flexuosa* seeds for longer periods. Granström (1987), however, found a few surviving seeds that had been buried for 5 years in a Swedish coniferous forest. Hence, there may be conditions where some seeds survive for longer periods, but generally *D. flexuosa* seeds cannot survive beyond the year in which they are produced.

A similar argument can be found for *A. vinealis* and *C. canescens*, but the evidence is stronger that some seeds can survive for longer periods, particularly for *C. canescens*. This is indicated by the results of the present experiment and by the seed bank study of Symonides (1978), in Poland, in a similar habitat.

S. morisonii showed the typical seasonal change in dormancy as described for other winter annuals (Roberts & Neilson 1982; Baskin & Baskin 1983). However, the often reported high germination at low temperatures was not found in this species (Ernst 1981; Roberts & Neilson 1982; Baskin & Baskin 1983). The seeds did not germinate

underground, probably due to a light requirement. The results indicate that the species can form a persistent seed bank, as found by Symonides (1978), for a related species (*Spergula verna*) in a similar habitat in Poland.

ACKNOWLEDGEMENTS

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