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The seed bank in the soil, that great unknown in rare plant population studies

Abstract

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Studies on the regulation of dormancy and germination may be valuable for rare plant population studies. Results of such studies on *Spergula arvensis* are described. Seeds were buried in the field and exhumed at regular intervals. Germination tests showed clear seasonal changes in dormancy, but test conditions strongly influenced the expression of the dormancy pattern. Seeds germinated during a longer period of the year at 15°C than at 2°C and 30°C. Irradiation with red light, addition of nitrate and desiccation of the seeds prior to the germination test stimulated germination and lengthened the germination period. The seasonal germination pattern was modelled with a multiple linear regression model which was used to calculate the seasonal changes in the temperature range within which germination can proceed, and the effect of nitrate on this range. Similar calculations were made with models developed for *Chenopodium album, Polygonum persicaria* and *Sisymbrium officinale.* Graphs thus produced showed conspicuous differences in the ways in which dormancy in the four species is regulated and expressed. For rare plant population studies, knowledge of the temperature requirements for dormancy breaking and of the effect of temperature, light, nitrate and desiccation on germination may be valuable.

Introduction

Survival of seeds in soil

For many species production of seeds is the most important way to pass on their genes. Seeds not only allow for genetic diversity, they are also stress-resistant organs that can survive for many years. Most soils contain vast numbers of seeds. In arable soils, for example, up to 67,000 seeds per m^2 in the upper 15 cm were reported by Roberts & Chancellor (1986). These seed banks are sometimes transient: at the end of the growing season seeds have all germinated or died (e.g. in grasses); but most annual dicotyledons form persistent seed banks and are able to survive for considerable periods of time (Thompson & Grime 1979). Ødum (1978), for example, recovered viable seeds

of *Chenopodium album* L. and *Spergula arvensis* L. from soil samples that were presumably 1600 years old.

The seed bank can also act as a reservoir in which seeds are preserved. Walters (1974) reported reappearance of *Senecio paludosus* L. after 70 years of absence, when buried seeds came to the surface during the digging of ditches. Rowell & al. (1982) reported germination of seeds of *Viola persicifolia* Schreb. in soil samples from a swamp area in East Anglia. The species had been registered as extinct in that area since 1916. These studies show that the absence of plants of a species in a certain area does not necessarily indicate that the species is extinct there. The seed bank may still contain its seeds. The question then is, why do these seeds not germinate.

Dormancy

Studies on both artificially buried and natural seed populations have shown that the emergence of many species occurs in a seasonal pattern (Stoller & Wax 1973, Ogg & Dawson 1984). Emergence may be restricted to one or two months in spring for some species, others may only germinate in autumn. These seasonal variations in emergence reflect seasonal changes in dormancy. Usually seeds are dormant when they are shed. This is called primary dormancy (Fig. 1). Primary dormancy may be relieved and when suitable conditions are present germination may occur. If germination does not occur, secondary dormancy may develop. Secondary dormancy can be relieved and re-induced during many successive years (Karssen 1982).

Under normal environmental conditions, dormancy prevents germination occurring in the seasons of least favourable conditions for plant survival. Thus, summer annuals are dormant in summer and autumn, dormancy is relieved during winter and, if suitable conditions prevail, they germinate in spring. If germination is prevented, because suitable conditions are lacking, secondary dormancy is induced (Fig. 1). For winter annuals it is just the opposite. Their seeds are dormant in winter and spring. Dormancy is relieved during summer and, provided that suitable germination conditions are present,

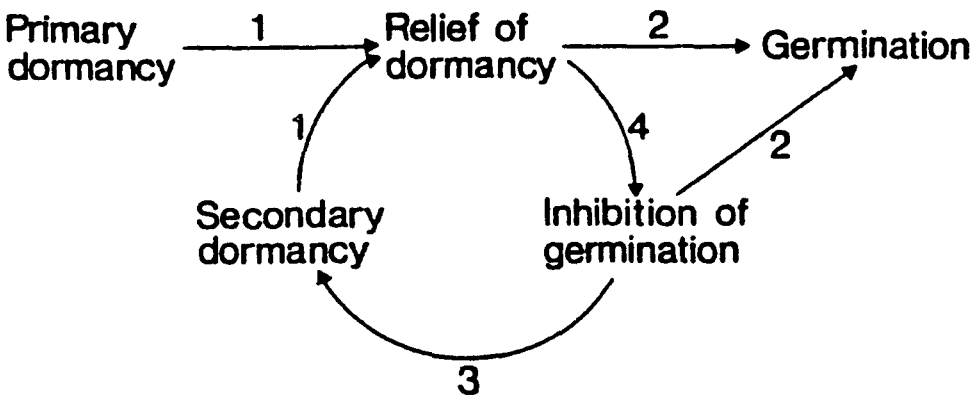


Fig. 1. Dormancy in seeds. (**1**) conditions suitable for breakage of primary or secondary dormancy, (**2**) germination requirements are met and the seed germinates, (**3**) conditions prevail that lead to induction of dormancy, (**4**) germination requirements are not met, germination is inhibited (from Karssen, 1982).

they can germinate in autumn. If germination is prevented secondary dormancy develops during winter. Often, dormancy induction during winter is slow, so that germination may also occur in (early) spring.

Dormancy patterns are studied by burying seeds in soil, usually in nylon gauze to aid retrieval. At regular intervals, packages of seeds are exhumed and germination is tested. These tests were often carried out at one test condition, but Baskin & Baskin (e.g. 1984) have shown that the test temperature strongly affects germination and therefore influences the observation and interpretation of the dormancy pattern.

Tests at non-optimal temperatures can give the false impression that the seeds are dormant, whereas at the same time up to 100 % germination may be achieved at an optimal temperature. Therefore, germination tests of exhumed seeds should be performed over a range of temperatures. Such tests have shown that relief of dormancy is characterized by a widening of the range of temperatures over which germination can proceed, whereas during induction of dormancy this range becomes narrower (Karssen 1982).

In this paper we present results of a study on dormancy and germination of the annual *Spergula arvensis*. Seeds of this species were buried in December 1986 and exhumed at regular intervals during three successive years. The effects of environmental factors such as temperature, light, nitrate and desiccation on dormancy and germination were studied. The results of this experiment will be described with a regression model. Results of calculations with these models are presented and compared with similar experiments with *Chenopodium album*, *Polygonum persicaria* L., and *Sisymbrium officinale* (L.) Scop. The implications of the outcome of these calculations for rare plant population studies are discussed.

Methods

Burial in the field

Seeds of *Chenopodium album*, *Polygonum persicaria*, *Sisymbrium officinale* and *Spergula arvensis* were collected in the vicinity of Wageningen and buried in December 1986. Thirty lots of 1000 to 2000 seeds for each species were packed separately in envelopes made of fine-mesh nylon gauze. Each envelope of seeds was placed in a plastic net pot (10 cm diameter) on a thin layer of sandy loam with which the pots were subsequently filled. To prevent loss of soil during handling, the pots were lined with gauze. The pots were buried in the field so that the seeds were at 10 cm below the surface. The open structure of the pots allowed good contact with the surrounding soil. Because the seeds were surrounded by soil, light could not reach them during exhumation.

Germination tests

At regular intervals, one lot of seeds of each species was exhumed to test germination. During transport to the laboratory the pots were covered with black polyethylene. In the laboratory, exhumed seeds were handled in dim green safelight. The seeds from each envelope were divided into smaller lots of approximately 50 seeds. The lots were

incubated in 50 mm glass Petri dishes on one layer of filter paper (N° 595, Schleicher & Schüll, Dassel, Germany). Germination was tested at several temperatures, depending on species, in water or 50 mM KNO_3, with (from May 1987) or without desiccation, with or without a 15 min red light irradiation.

Desiccation of imbibed seeds followed by re-imbibition stimulates germination of quite a few species. To investigate the effect of desiccation on germination of our species, half of the lots were desiccated before imbibition. Desiccation occurred in a closed box with a small fan over a saturated solution of LiCl for 24 h, giving a seed moisture content of about 9 % of the dry weight.

If appropriate, seeds were irradiated for 15 min with red light, the photon fluence rate at seed level being 11 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Irradiation occurred before the desiccation treatment.

Vincent & Cavers (1978) and Bouwmeester (1990) showed that the light stimulus is preserved during desiccation of the seeds.

Germination tests were made in cooled incubators (Gallenkamp, Crawley, UK, T ± 1°C) and, from March 1987 onwards, also outdoors at a height of 1.5 m in the shade. The temperature during the outdoor germination tests was obtained from the meteorological station at Wageningen. Petri dishes were placed in closed plastic boxes to prevent loss of moisture. When germination was tested outdoors, boxes were covered with black polyethylene to exclude light. In preliminary experiments (data not shown) it was determined that no additional germination occurred after 3 days at 30°C and after 25 days at 2°C. Therefore, both germinated and non-germinated seeds were counted between 3 and 25 days after incubation, depending on the test temperature, to determine germination percentage.

Statistical procedures

Statistical procedures were performed with the statistical package SAS (SAS Institute Inc., Cary, NC, USA). The germinated fraction (G) was transformed with an arcsin

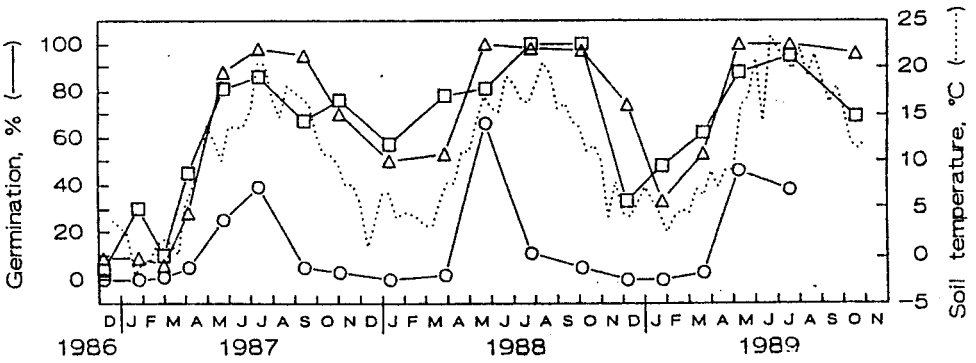


Fig. 2. Seasonal variation in germination of exhumed seeds of *Spergula arvensis* at different test temperatures. Seeds were buried in December 1986 at 10 cm in sandy loam, and at regular intervals seeds were exhumed. Germination was tested in water in Petri dishes after a 15 min red light irradiation with test samples of c. 50 seeds at 2°C (O), 15°C (Δ) or 30°C (□). The dotted line indicates the soil temperature at 10 cm in bare soil.

transformation, 2·arcsin$\sqrt{G}$, to get approximately normally distributed data with an equal variance. Results from calculations were transformed back to germination percentages to facilitate comparison with other data. Multiple linear regression models were made to describe the germination data obtained from germination tests in incubators as described by Bouwmeester & Karssen (1992, 1993a-c). With these models we aimed to explain results from germination tests made outdoors.

Results

In the present paper we focus on experiments performed with *Spergula arvensis*, and for the other species we only present data of model calculations.

Seasonal germination pattern

Fig. 2 shows germination of exhumed seeds of *Spergula arvensis* in water at 2°C, 15°C and 30°C. In all three years dormancy was relieved in April-June. Seeds germinated to higher percentages at 15°C than at 2°C and 30°C. The range of temperatures over which germination could proceed varied. In summer, when dormancy was low, seeds could germinate at all temperatures tested. In winter, when seeds were dormant, they did not germinate at any of the temperatures tested.

If germination was tested in nitrate instead of water, it reached much higher percentages (Fig. 3). At 30°C, nitrate enhanced germination more than at 2°C. Dormancy breaking was visible earlier in the year (March-May) and dormancy induction later (November-December) than when tested in water. As a consequence, seeds could germinate during a longer period of the year. In contrast to tests in water, in nitrate germination during winter did not entirely cease.

In the germination tests described above, after exhumation seeds were always irradiated with red light and then imbibed in either water or nitrate. When seeds were not irradiated with red light they did not germinate at 15°C in either water or nitrate (Fig. 4B). A desiccation treatment before imbibition in water or nitrate could restore germi-

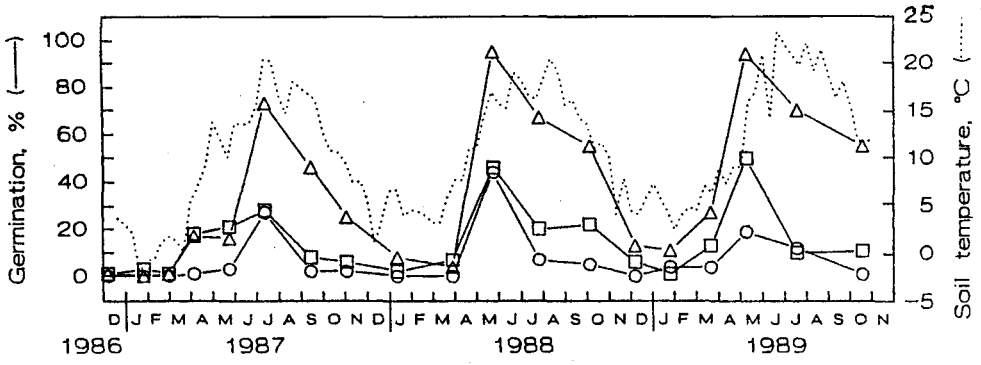


Fig. 3. As Fig. 2, but germination was tested in 50 mM KNO_3 instead of water.

nation of non-irradiated seeds to about 50-90 % (Fig. 4B). Fig. 4A shows again germination of light-irradiated seeds at 15°C in water and nitrate (data from Fig. 2-3). When irradiated seeds were desiccated, they germinated almost invariably to 100 % in both water and nitrate.

Dormancy model

From these experiments, and other experiments under controlled conditions (Bouwmeester & Karssen, 1992, 1993a-c), it can be concluded that field temperature is the driving force behind changes in dormancy. The dormancy status then determines the range of temperatures over which germination can proceed. Environmental conditions at the moment germination is tested, such as light, nitrate and desiccation, affect the size of the temperature range.

Based on this concept, we developed a multiple linear regression model for *Spergula arvensis* . The model relates germination (G_t) during the 3-year experimental period to field temperature, temperature after exhumation, and the absence or presence of nitrate (Equation 1) :

$$G_t = (-0.020 \cdot T_{p,40} - 0.063 \cdot M_g) \cdot T_g^2 + (0.697 \cdot T_{p,40} + 4.135 \cdot M_g) \cdot T_g + 3.046 \cdot C - 0.146 \cdot H + 1.237 \cdot T_{p,40} - 22.562$$

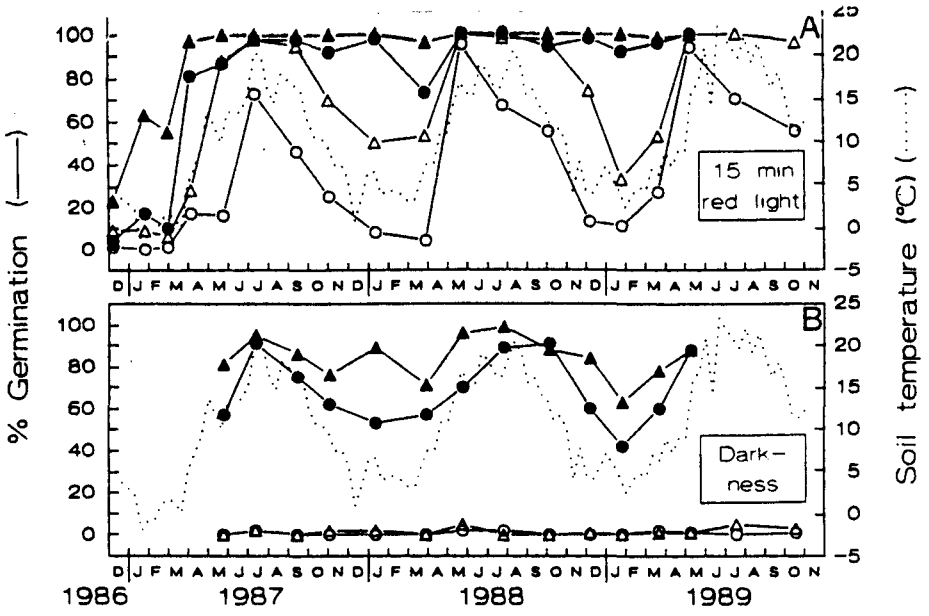


Fig. 4. Effect of light, nitrate and desiccation on the seasonal variation of germination of exhumed seeds of *Spergula arvensis*. Burial and germination tests as in Fig. 2. Germination was tested at 15°C with a 15 min red light irradiation (**A**) or in darkness (**B**), in water (O) or 50 mM KNO_3 (Δ), with (closed symbols) or without (open symbols) a preceding desiccation treatment.

where $T_{p,40}$ is the mean temperature during 40 days before exhumation, M_g is the absence ($M_g = 0$) or presence of nitrate ($M_g = 1$), Tg is the germination temperature (the temperature after exhumation) and C and H are the cold sum and heat sum until exhumation (see Bouwmeester & Karssen 1992 for comprehensive explanation).

Fixing G_t at 50 % and filling in $T_{p,40}$, M_g, C and H gives two values for T_g, the highest value being the maximum temperature at which seeds can germinate to 50 % ($T_{g,max}$), the lowest value being the minimum temperature ($T_{g,min}$). When the test or field temperature after exhumation is between $T_{g,min}$ and $T_{g,max}$ seeds will germinate to 50 % or more. When $T_{g,max}$ equals $T_{g,min}$ germination can not proceed at any temperature.

For *Polygonum persicaria, Sisymbrium officinale,* and *Chenopodium album* we developed similar regression models (Bouwmeester & Karssen 1992, 1993b, c). We will not explain these models in detail here, but only present some results of calculations with these models. Fig. 5 shows results of such calculations for *P. persicaria, S. officinale, Spergula arvensis* and *C. album.* For *P. persicaria* only $T_{g,max}$ and $T_{g,min}$ for germination in water are shown, because nitrate only slightly stimulated germination of this species. For *C. album* only germination in nitrate is shown, because in water this species did not germinate to more than 50 %.

The graphs show that germination in the field depends on overlap of the germination-temperature range and the actual field temperature (hatched areas). Nitrate increased the germination-temperature range, which resulted in a longer overlap with the field temperature (Fig. 5B-C) and in an increased period in which germination could occur. The predicted periods of germination (hatched areas) agreed fairly well with results of germination tests made in Petri dishes outdoors (arrows).

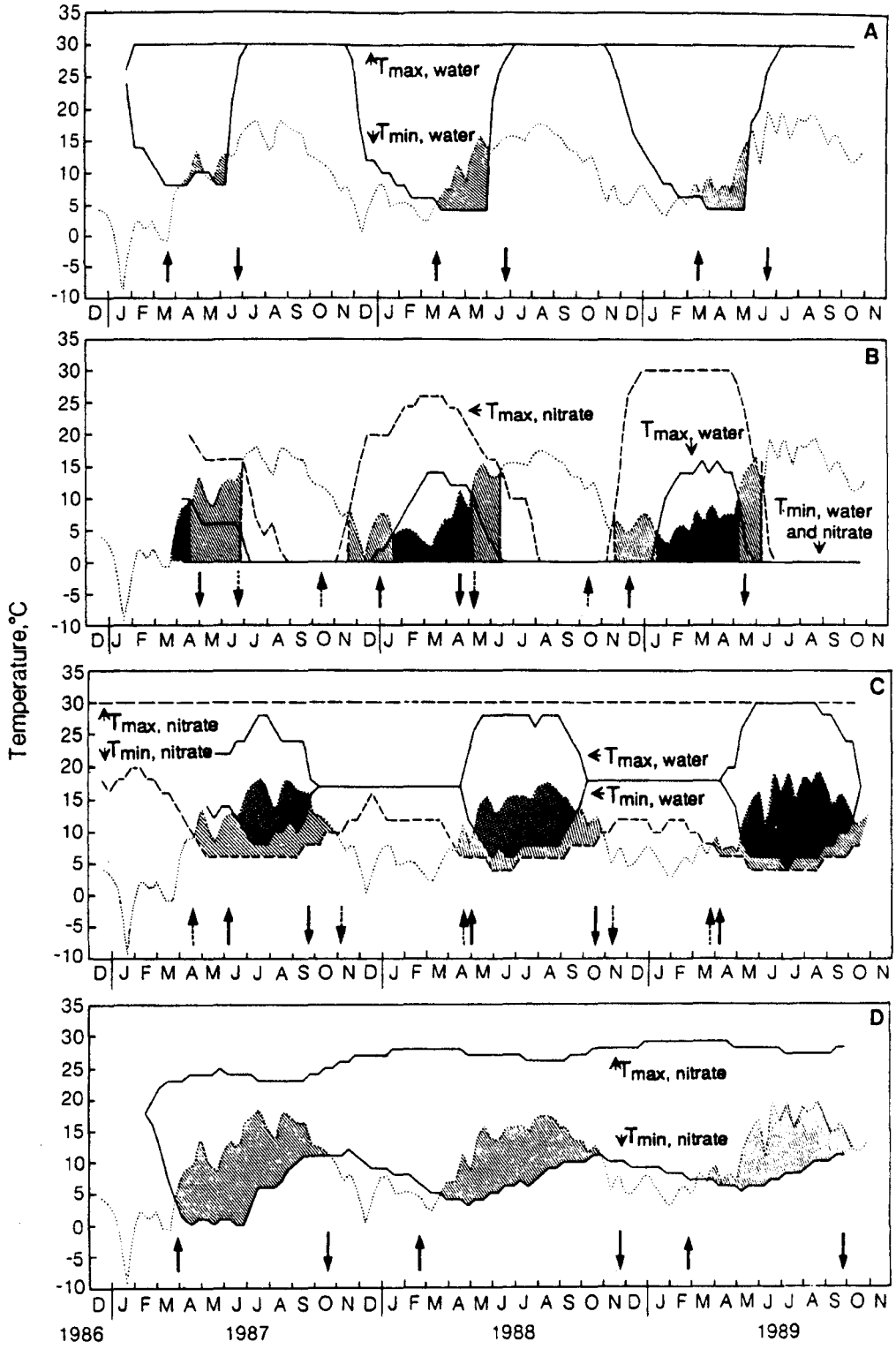
Polygonum persicaria showed the characteristic features of a summer annual. Dormancy was relieved best at low temperatures and the width of the germination-temperature range varied through $T_{g,min}$ (Fig. 5A). Germination could occur in spring when the field temperature increased above $T_{g,min}$. In summer, $T_{g,min}$ rapidly increased above the field temperature (dormancy induction). Therefore, germination was restricted to spring.

Sisymbrium officinale showed the germination-temperature range of a winter annual. Changes in dormancy resulted in changes in $T_{g,max}$ (Fig. 5B). In this case the field temperature was usually higher than $T_{g,min}$.

Chenopodium album and *Spergula arvensis* did not show the characteristic features of either winter or summer annuals. Their germination-temperature range seemed to be a combination of the germination-temperature range of a summer and a winter annual. That is, the range became narrower through both a decrease of $T_{g,max}$ and an increase of $T_{g,min}$. Widening of the range occurred inversely (Fig. 5C-D).

Discussion

Germination of seeds in the field during the course of the year depends on the one hand on the actual conditions that affect germination (temperature, light, nitrate, desiccation) and on the other hand on the conditions that preceded and determined dormancy (temperature). Both aspects may be important when studying rare plant populations, and will therefore be discussed.


A
Tmax, water
Tmin, water
B
Tmax, nitrate
Tmax, water
Tmin, water and nitrate
C
Tmax, nitrate
Tmin, nitrate
Tmax, water
Tmin, water
D
Tmax, nitrate
Tmin, nitrate
Temperature, °C
35
30
25
20
15
10
5
0
-5
-10
D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N
1986
1987
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Control of dormancy

Our studies have shown that the seasonal changes in temperature are the main regulator of the dormancy pattern. Changes in soil moisture or soil nitrate content had no effect on the changes in dormancy (Bouwmeester 1990; Bouwmeester & Karssen 1992, 1993b).

Fig. 5 shows that dormancy of *Polygonum persicaria*, *Sisymbrium officinale* and *Chenopodium album* were relieved during winter at low temperatures. For *P. persicaria* and *C. album* this is in accordance with results of studies on other typical summer annuals (Baskin & Baskin 1977, 1985; Karssen 1982). However, *S. officinale* clearly is a winter annual, germinating in autumn and early spring and particularly at low temperatures (Fig. 5B). According to Baskin & Baskin (1976, 1986) and Roberts & Neilson (1982), winter annuals require high temperatures to lose dormancy. Breaking of dormancy during summer then would enable germination in autumn. Although dormancy of *S. officinale* is relieved by low temperatures, relieve is so quick that germination may still occur in autumn. Dormancy relief for *Spergula arvensis* did not start until spring. It seems that not the low temperatures during winter, but rather the increasing temperature in spring is required for dormancy relief of this species (Karssen & al. 1988).

Control of germination

The dominant role of temperature in the regulation of seasonal fluctuations of dormancy is evident. It is also clear that the dormancy state of a population of seeds is characterized by the range of temperatures over which germination can proceed, the germination-temperature range. Accordingly, temperature has a dual effect. On the one hand, it regulates the changes in dormancy. On the other hand, germination can only occur when the actual temperature is within the germination-temperature range.

The expression of the dormancy status was strongly influenced by other environmental factors. Germination of the four species was stimulated by light, nitrate and desiccation, although the effects strongly varied depending on species (Bouwmeester & Karssen 1992, 1993a-c). The range of temperatures over which seeds of *Ambrosia artemisiifolia* L. could germinate was much wider in light than in darkness (Baskin & Baskin 1980). Nitrate had a similar effect on the germination-temperature range of *Sisymbrium officinale* and *Spergula arvensis*. The range became much wider when

⇐ Fig. 5. Seasonal changes in the minimum and maximum temperature required for 50 % germination of exhumed seeds of (**A**) *Polygonum persicaria*, germination in water; (**B**) *Sisymbrium officinale* and (**C**) *Spergula arvensis*, germination in water (solid lines) and 50 mM KNO_3 (broken lines); (**D**) *Chenopodium album*, germination in 50 mM KNO_3. Data were calculated from regression models such as Equation 1. The dotted line indicates air temperature at 1.5 m. Hatched areas indicate overlap of field temperature and germination-temperature range (**B, C**: cross-hatched for germination in water, hatched in nitrate). Arrows (**B, C**: solid for water, broken for nitrate) indicate the moment germination in Petri dishes placed outdoors at 1.5 m increased above (↑) or decreased below 50 % (↓) (data from Bouwmeester & Karssen 1992, 1993a-c).

germination was tested in nitrate instead of water (Fig. 5). The germination-temperature range for desiccated seeds was even wider. Following desiccation, the germination-temperature range of *Sisymbrium officinale* and *Spergula arvensis* was so wide that germination could occur throughout the year (Bouwmeester & Karssen 1993a, b).

The strong effect of light (Fig. 4) explains the large effect of soil disturbance on seedling emergence. During disturbance, seeds are exposed to light and will germinate over a much broader range of temperatures than without exposure to light.

Because nitrate concentrations in the field are of the same order of magnitude as the concentrations that stimulate germination in the laboratory it seems that nitrate can also play an important ecological role (Bouwmeester 1990). Due to the large effect on the germination-temperature range, germination and emergence on nitrate-rich soils may occur during a longer period of time. The same may hold for desiccation (Bouwmeester 1990). It seems that occasionally conditions in the field can occur that are similar to the desiccation conditions that were used in our experiments, for example after soil disturbance. Since desiccation had a large stimulatory effect on the germination of some species, it seems valuable to consider this factor when studying germination of seeds in the field.

The wide range of conditions used in the germination tests in the present experiments ensured a large variation in germination. This enabled a proper determination of the changes in dormancy. Optimal test conditions best showed the changes in dormancy when seeds were deeply dormant, whereas sub-optimal conditions best showed these changes when seeds were not or hardly dormant.

Implications for rare plant population studies

The absence of plants of a species in a certain area does not necessarily imply that the species is extinct there, nor does occurrence of just a few plants indicate that the species is rare, because the seed bank may still contain a vast amount of dormant seeds. For the restoration of rare plant populations, seeds may be suitable. However, for both freshly harvested seeds and seeds incorporated in soil samples germination may not always be easy to obtain, due to dormancy. If it is known whether the species is a summer or a winter annual this may ease the development of a suitable temperature treatment to relieve dormancy. However, one may also get misled when the species requires another temperature than expected, to break dormancy. The summer annual *Spergula arvensis*, for example, requires a high temperature to break dormancy, whereas usually summer annuals require a low temperature. A study of the temperature requirements for dormancy breaking and the effect of temperature and other factors such as light, nitrate, and desiccation may greatly improve the chances of getting seeds to germinate.

For seeds in soil samples the situation is more complicated, because several species may be mingled. Each species may have its own requirements for optimal germination. Nevertheless, manipulation of soil samples may greatly increase the germination of seeds incorporated (Post 1984). It is concluded that a study of the seed bank may be a valuable addition to rare plant population studies.

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