THE EFFECT OF ENVIRONMENTAL CONDITIONS ON THE SEASONAL DORMANCY PATTERN AND GERMINATION OF WEED SEEDS

Promotoren:

dr. C.M. Karssen
persoonlijk hoogleraar in de plantenfysiologie

dr. P. Zonderwijk
oud hoogleraar in de vegetatiekunde, plantenoecologie en onkruidkunde

Harro J. Bouwmeester

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This thesis contains results of a research project of the Agricultural University in Wageningen, Department of Plant Physiology, Arboretumlaan 4, NL-6703 BD Wageningen, The Netherlands and Department of Vegetation Science, Plant Ecology and Weed Science, Bornsesteeg 69, NL-6708 PD Wageningen, The Netherlands.

The author's present address is:
Centre for Agrobiological Research (CABO), Postbox 14, NL-6700 AA Wageningen, The Netherlands.

STELLINGEN

I De afwezigheid van sterke seizoensgebonden fluctuaties in de omvang van het temperatuurtraject van kieming sluit een duidelijk seizoensgebonden opkomstpatroon niet uit, omdat, althans bij een smal temperatuurtraject, ook de wisselingen in de veldtemperatuur zelf een regulerende rol kunnen spelen.
Dit proefschrift.

II Het is van het grootste belang in het kiemrustonderzoek de kieming te toetsen bij een reeks van temperaturen en eventueel andere kiemomstandigheden. Het grotere inzicht dat hierdoor verkregen wordt weegt ruimschoots op tegen het extra werk.
Dit proefschrift.

III Het onvermogen om de lichtpenetratie en de schommelingen in temperatuur en vocht-en nitraatgehalte op het microniveau van het zaaibed te voorspellen vormt op het moment het grootste struikelblok voor de voorspelling van opkomst in het veld.
Dit proefschrift.

IV Bij hun verklaring voor de relatie tussen de temperatuur tijdens de voorbehandeling en de benodigde kiemtemperatuur bij *Rumex obtusifolius* gaan Van Assche en Vanlerberghe ten onrechte voorbij aan de veranderingen in het kiemtemperatuur-traject.
J.A. van Assche and K.A. Vanlerberghe, 1989. The role of temperature on the dormancy cycle of seeds of *Rumex obtusifolius* L.. Functional Ecology 3(1): 107-115.

V Aangezien het merendeel van de problemen met de zaadkwaliteit bij land- en tuinbouwgewassen door omstandigheden vóór de oogst wordt bepaald is het noodzakelijk een groter gedeelte van de onderzoeksinspanningen op deze fase te richten.

VI Er is geen aanleiding om het 'cowpea aphid-borne mosaic virus' en het 'blackeye cowpea mosaic virus' als twee aparte virussen te beschouwen.
J. Dijkstra, L. Bos, H.J. Bouwmeester, Tutung Hadiastono and H. Lohuis, 1987. Identification of blackeye cowpea mosaic virus from germplasm of yard-long bean and from soybean, and the relationship between blackeye cowpea mosaic virus and cowpea aphid-borne mosaic virus. Netherlands Journal of Plant Pathology 93: 115-133.

VII Het ombuigen dan wel verwijderen van de spruit bij rozen versnelt de grondscheutvorming. Het is echter niet aannemelijk dat hierbij naast het wegvallen van de apicale dominantie ook de omzetting van het actieve cis in het niet actieve trans-ABA door het dan op de basis van de plant vallende licht, zoals beweerd door Zieslin en Khayat, een grote rol speelt.
N. Zieslin and E. Khayat, 1982/83. Involvement of cytokinin, ABA and endogenous inhibitors in sprouting of basal buds in rose plants. Plant Growth Regulation 1: 279-288.

VIII Het streven naar een hoger carvongehalte in de etherische olie van karwij is economisch wellicht niet verantwoord gezien de vermoedelijke rol van dit monoterpeen bij de waardplantselectie door de bladluis *Cavariella aegopodii.*

R.F. Chapman, E.A. Bernays and S.J. Simpson, 1981. Attraction and repulsion of the aphid, *Cavariella aegopodii*, by plant odors.

Anonymus, 1990. Onderzoeksprogramma ter verbetering van karwij als akkerbouwgewas en ter introductie van nieuwe afzetmogelijkheden.

IX Een groot gedeelte van de huidige argumenten om, in het kader van de ontwikkeling van industriële produkten uit agrarische grondstoffen (agrificatie), onderzoek aan karwij te doen is al minstens 75 jaar oud. Agrificatie onderzoek kan dus beter als een inhaalrace dan als innovatie worden gekarakteriseerd.

K. Zijlstra, 1915. Over karwij en de aetheriese karwijolie. Mededeelingen van de Rijks Hoogere Land-, Tuin- en Boschbouwschool 8(1,2): 1-128.

X Oecofysiologisch onderzoek profiteert van de meerwaarde die het werken op het grensvlak van plantenoecologie en plantenfysiologie oplevert, maar lijdt onder het onbegrip dat het kan opwekken bij de bedrijvers van dat monodisciplinaire onderzoek.

XI In tegenstelling tot de eerste mens op de maan kan een proefschrift worden beschouwd als 'one giant leap for man, one small step for mankind'.

XII Analoog aan kool en geit zal het in de toekomst niet langer mogelijk zijn bos en heilige koe te sparen.

Stellingen behorende bij het proefschrift: "The effect of environmental conditions on the seasonal dormancy pattern and germination of weed seeds" door Harro J. Bouwmeester.

Wageningen, 21 september 1990

Abstract

Weeds cause considerable losses in horticultural and agricultural crops. Weeds are still predominantly controlled with herbicides. To reduce the use of chemicals, a better understanding of the biology of weeds is required. In this thesis the effect of environmental conditions on dormancy and germination of *Chenopodium album* L., *Polygonum persicaria* L., *P. lapathifolium* L. subsp. *lapathifolium*, *Sisymbrium officinale* (L.) Scop. and *Spergula arvensis* L. was investigated.

It was shown that changes in dormancy of these species were regulated by temperature. Soil moisture and nitrate content did not affect these changes. The dormancy status of the seeds was visualized by the range of temperatures over which germination of exhumed seeds was possible. During relief of dormancy, seeds could germinate over a progressively wider range of temperatures. During induction of dormancy, this range became narrower.

Germination of *C. album*, *S. officinale* and *S. arvensis* was stimulated by light, nitrate and desiccation. These factors all increased the width of the range of temperatures over which germination could proceed and therefore affected the expression of dormancy. That is, seeds seemed less dormant and they could germinate during a longer period of the year. Endogenous nitrate, that entered the seeds via the mother plant during seed development, only temporarily stimulated germination. After burial the effect disappeared because of equalization of the nitrate content. The effect of desiccation was stronger, the more seeds were desiccated.

With descriptive models the changes in the range of germination temperatures of the investigated species and the effect of nitrate upon these changes were simulated for a period of three years as a function of soil temperature during burial. When the field temperature after exhumation and the germination-temperature range overlapped, germination was possible. Accordingly, temperature had a dual effect. Germination depended on the one hand on the **actual field temperature after exhumation**, on the other hand on the width of the germination-temperature range, which was determined by the dormancy status of the seeds and was regulated by **soil temperature during burial**. When nitrate was added during the test, the germination-temperature range became wider and germination could occur during a longer period of the year.

additional index words: *Chenopodium album* L., *Polygonum persicaria* L., *P. lapathifolium* L. subsp. *lapathifolium*, *Sisymbrium officinale* (L.) Scop., *Spergula arvensis* L., temperature, light, (endogenous) nitrate, desiccation, model, fertilization, seed development.

Voorwoord

Bij het terugkijken op de afgelopen vier jaren realiseer je je hoeveel mensen eigenlijk een bijdrage hebben geleverd aan het tot stand komen van het proefschrift. Op de eerste plaats is dat mijn dagelijkse begeleider en eerste promotor prof. dr. C.M. Karssen. Kees, je hebt me ingewerkt in de zaad(oeco)fysiologie en je hebt me geholpen het proefschrift te maken tot wat het nu is. Bedankt daarvoor. De onkruidkundige poot van het werk is ondersteund vanuit de vakgroep Vegetatiekunde, Plantenoecologie en Onkruidkunde door prof. P. Zonderwijk, mijn tweede promotor, die waardevolle suggesties heeft geleverd aangaande de oecologische en onkruidkundige aspecten en zeker ook door Ben Post, door zijn bereidheid altijd te discussiëren over de relaties tussen mijn experimenten en zijn veldwerk.

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Verder wil ik mijn ouders bedanken, omdat ze me de gelegenheid hebben gegeven te gaan studeren en natuurlijk Marianne die me in die vier jaar gesteund heeft, door ups en downs heeft heengeloodst en mijn enthousiaste maar ook minder enthousiaste verhalen heeft aangehoord.

Contents

Chapter 1

General introduction

In recent years there has been an increasing interest in the quality of our environment. Mankind is more aware of potential threats to the world's ecosystems and is becoming increasingly intolerant of the current levels of air, water and soil pollution. It is recognized that apart from industry and traffic, agriculture is also a serious source of pollution. In countries like The Netherlands the production of ammonia by intensive animal husbandry is one of the major causes of acid rain. But also the use of pesticides in agriculture contributes to the pollution of soil and water.

Weeds cause considerable losses in horticultural and agricultural crops. In the early seventies the losses in tropical crops due to weeds were as large as 50% or more. World wide approx. 11.5% of the total potential production was lost due to weeds (approx. 300 million tons a year) (Parker and Fryer, 1975). Moreover, weeds (and ruderals) are often the host for pest organisms and therefore contribute to the damage done by these organisms. The economic loss caused by weeds is about as important as losses caused by other pests. At the end of the sixties worldwide losses due to weeds were approx. 10% of the total potential production, compared to 12% for both insects and fungi (Cramer, 1967). Nevertheless, the biology of weeds is rather poorly studied compared to the extensive studies on other pests. For insects the first successful systems for biological control have been developed, but weeds are still almost entirely controlled by herbicides. Detailed knowledge of the life cycle of pest organisms is the basis for the development of any system of biological or integrated control, the best way to limit the use of pesticides.

Therefore, the development of systems for integrated weed control should be encouraged. In such systems, decisions are made on a cost-benefit basis with a minimum use of chemicals. At some point, a certain level of weed infestation is acceptable.

To decide whether control measures are necessary, a prediction of the competition between weeds and crop is needed (Spitters *et al.*, 1987; Kropf, 1988*a*). Models that predict crop losses on the basis of established weed and crop populations are now available (Kropf, 1988*a*). Although it has been shown that the timing of weed emergence largely influences yield losses (Kropf, 1988*b*), these models neglect the emergence phase and start when a weed and crop vegetation are already present. It would improve the efficacy of these models in supporting decision making if they would also predict the time of emergence and the quantity and composition of the emerging weed populations.

In addition to perennials, annuals are important weeds. The prediction of

emergence of these annuals requires qualitative and quantitative information about the presence of weed seeds in soil, the seed bank. The accurate determination of size and composition of the seed bank has been the subject of many studies (Thorsen and Crabtree, 1977; Fay and Olsen, 1978; Post, 1984). Recently, the European Weed Research Society started a project to develop a reliable and practical method for the estimation of the seed bank volume and composition.

However, in addition to precise knowledge of the seed bank composition, knowledge about the physiology of the seeds is required for the prediction of emergence. Studies with both artificially buried and natural seed populations have shown that the emergence of many weed species occurs in a seasonal pattern. Often emergence occurs at specific intervals during the year. For instance, emergence of many species is restricted to one or two months in spring, *e.g. Setaria lutescens*, *Abutilon theophrasti*, *Ambrosia trifida*, *A. artemisiifolia* and *Polygonum pensylvanicum* (Stoller and Wax, 1973), *Polygonum convolvulus* (Roberts and Feast, 1973; Håkansson, 1983; Van den Brand, 1986, 1987), *Solanum ptycanthum* and *S. triflorum* (Ogg and Dawson, 1984), *Chenopodium album*, *Polygonum persicaria*, *P. aviculare* and *Solanum nigrum* (Van den Brand, 1986, 1987). Germination of others mainly occurs in autumn and/or winter, *e.g. Aphanes arvensis* (Roberts and Neilson, 1982*b*), *Veronica hederifolia* (Roberts and Feast, 1973; Roberts and Lockett, 1978*a*), *Papaver rhoeas*, *Alopecurus myosuroides* and *Veronica persica* (Van den Brand, 1986, 1987). However, many of these species also show (some) emergence in spring.

For some species, the first large flush of emergence in spring is followed by several smaller flushes throughout summer, particularly when the soil is frequently cultivated, *e.g. Capsella bursa-pastoris* (Popay and Roberts, 1970; Roberts and Feast, 1973), *Senecio vulgaris* (Popay and Roberts, 1970), *Spergula arvensis* (Håkansson, 1983), *Chenopodium album*, *Solanum nigrum* and *Amaranthus retroflexus* (Ogg and Dawson, 1984). Frequent cultivation of the soil increases the number of emerging seedlings for most species, but it does not essentially influence the periodicity of emergence (Roberts and Feast, 1973; Roberts and Lockett, 1978*b*).

Emergence can also be restricted to a fixed interval of the year because the number of seeds in the soil is limiting. This is particularly true for grasses that form transient seed banks that exist only for a limited period of the year. However, most dicotyledonous annuals form persistent seed banks that are present during all seasons but vary seasonally in volume (Thompson and Grime, 1979).

Dormancy

Terminology. An excessive terminology has evolved in botanical literature on dormancy (Lang *et al.*, 1987). Also in seed physiology many terms are in use to describe a seed's disability to germinate (for review, see Roberts, 1972). Widely used is the terminology adapted by Roberts (1972) from Harper (1957). Three types of

dormancy are recognized: **innate, enforced** and **induced. Innate dormancy** may be exhibited at the time of seed dispersal. Innately dormant seeds will not germinate under any set of normal environmental conditions until dormancy is relieved. Prevention of germination by an unfavourable environment is called **enforced dormancy** and when dormancy is induced in seeds that have first lost innate dormancy this is called **induced dormancy**.

The use of the term enforced dormancy was rightly questioned by Karssen (1982). Actually enforced dormancy is just the lack of suitable germination conditions. Therefore, the use of the terms primary and secondary dormancy as suggested by Karssen (1982) is preferred. Primary dormancy (comparable to innate dormancy) is the dormancy state of the freshly shed seed. Primary dormant seeds are the input of the seed bank. When primary dormancy is relieved and suitable conditions are present, germination may occur. If germination does not occur, secondary dormancy (comparable to induced dormancy) may develop. Secondary dormancy can be relieved and re-induced during many successive years. In this definition, dormancy is relative. During burial seeds go through continuous changes in dormancy (Baskin and Baskin, 1985). The dormancy status of the seeds can be estimated from their response to a set of conditions (*e.g.* a range of temperatures). When seeds do not germinate under any set of environmental conditions they are truly dormant (Karssen, 1980/81*a*).

Seasonal changes in dormancy. Seeds in persistent seed banks are often subject to seasonal fluctuations in dormancy. That is, both relief and induction of dormancy occur in fixed seasons only. As a consequence, low levels of dormancy and emergence are restricted to a species-specific interval of the year (Karssen, 1982; Baskin and Baskin, 1985). Dormancy patterns are studied by burying seeds in soil, usually in nylon sachets to aid retrieval. At regular intervals, portions of the seeds are exhumed and germination is tested.

Germination tests were often carried out at one test condition only, but Baskin and Baskin (1980, 1981, 1983*a*,*b*, 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).

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.

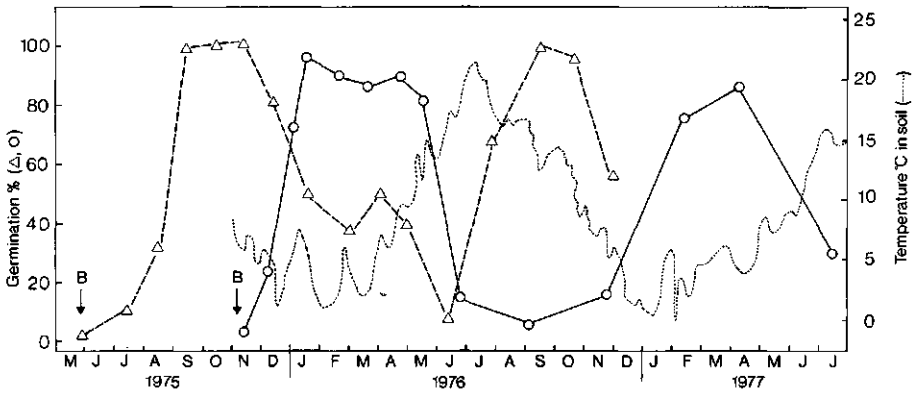

Fig. 1.1 Changes in the germination capacity of seeds of *Veronica hederifolia* (△) and *Polygonum persicaria* (○) buried in the field. Seeds of *V. hederifolia* were buried (B) outdoors in June 1975 at 5 cm in sandy loam in Wellesbourne, U.K.. Seeds of *P. persicaria* were buried in November 1975 at 10 cm in sandy loam in Wageningen, The Netherlands. Soil temperature in the field at a depth of 10 cm at 9.00 a.m. was recorded in Wageningen (dotted line). Germination was tested in 9 cm Petri dishes containing filter paper and distilled water at alternating temperatures: 16 h 4°C/8 h 10°C for *V. hederifolia* and 12 h 12°C/12 h 22°C for *P. persicaria*. The seeds were exposed to natural daylight for short periods in non-illuminated incubators (*V. hederifolia*) or illuminated for 12 h at 22°C with white fluorescent light (*P. persicaria*) (data for *V. hederifolia* redrawn from Roberts and Lockett, 1978*a*, for *P. persicaria* from Karssen, 1980/81*b*). From Karssen (1982).

If germination is prevented, because suitable conditions are lacking, secondary dormancy is induced (Fig. 1.1, *Polygonum persicaria*). The experiments of Baskin and Baskin (*e.g.* Baskin and Baskin, 1980) have clearly shown that the induction and relief of dormancy of summer annuals is characterized by an increase and a decrease, respectively, of the minimum temperature at which germination can proceed. The more dormancy is relieved, the wider the range of temperatures over which germination can proceed. Germination in the field can occur when the field temperature overlaps with this germination-temperature range (Fig. 1.2A) (Karssen, 1982).

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, they can germinate in autumn. If germination is prevented secondary dormancy develops during winter (Fig. 1.1, *Veronica hederifolia*). Often, dormancy induction during winter is slow, such that germination may also occur in (early) spring (Fig. 1.2B). In winter annuals, changes in dormancy are characterized by fluctuations of the maximum temperature at which germination can proceed (Fig. 1.2B). Again, germination can only occur when the field temperature reaches values between the minimum and maximum temperature (Karssen, 1982).

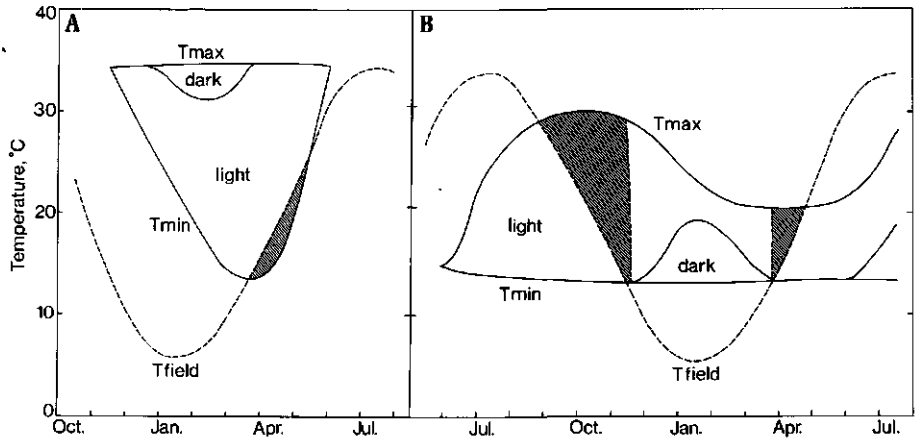

Fig. 1.2 Relationship between the field temperature and the changes in the range of temperatures over which germination can proceed. Solid lines represent the maximum and minimum temperature at which germination is possible. The broken line indicates the mean daily maximum temperature in the field. In the hatched area the actual and the required temperature overlap. (A) Summer annual (data obtained from a study with *Ambrosia artemisiifolia* by Baskin and Baskin, 1980); (B) Winter annual (data obtained from a study with *Lamium amplexicaule* by Baskin and Baskin, 1981*a*). Partly redrawn from Karssen (1982).

In addition to annuals that only germinate in autumn or spring, there are also species with a less definite germination period. Nonstrict or facultative winter annuals can germinate in autumn and spring. Seeds of other species can germinate during both spring and summer (Baskin and Baskin, 1985, 1987).

The seasonal character of the changes in dormancy suggests that the patterns are mainly regulated by the field temperature. Low winter temperatures break dormancy of summer annuals whereas high summer temperatures induce it (Baskin and Baskin, 1977, 1985; Totterdell and Roberts, 1979; Karssen, 1982, Fig. 1.1). On the other hand, low temperatures induce dormancy in winter annuals (Baskin and Baskin, 1984), whereas high temperatures are required for breaking of dormancy (Baskin and Baskin, 1976, 1986; Roberts and Lockett, 1978; Roberts and Neilson, 1982*a*, Fig. 1.1).

Thus field temperature has a dual effect on germination and emergence. In the long term it is the driving force behind the changes in dormancy and therefore determines the **width** of the germination-temperature range. In the short term its actual value and therefore its **overlap** with the germination-temperature range determines whether germination will occur (Fig. 1.2).

Germination often depends on the presence of light. Although germination can sometimes occur in darkness as well, the range of temperatures over which it can proceed is much wider in light than in darkness (Baskin and Baskin, 1980, 1981,

1983*a,b*, 1984, Fig. 1.2).

Other ecological factors such as moisture, nitrate and wetting and drying cycles are also frequently reported to influence germination. The role of nitrate is extensively studied. Germination of several species is stimulated by nitrate (*e.g.* Henson, 1970; Vincent and Roberts, 1977, 1979; Hilhorst *et al.*, 1986). Desiccation (Kiviliaan, 1975; Karssen, 1980/81; Karssen *et al.*, 1988) or wetting and drying cycles (Stoller and Wax, 1973; Baskin and Baskin, 1974, 1984; Thomas and Allison, 1975) do also stimulate germination of some species. Nitrate and desiccation may have similar influences on the expression of the dormancy pattern as has been shown for temperature and light.

Control of dormancy

There is some evidence that apart from temperature also other environmental factors may influence the changes in dormancy. Karssen (1980/81*a*) showed that in seeds of *Sisymbrium officinale* induction of secondary dormancy at elevated temperatures is prevented by incubation in a nitrogen atmosphere. Baskin and Baskin (1987) mention several other species were oxygen is required for changes in dormancy. However, also the reverse occurs. In some aquatic species, afterripening is promoted by low oxygen concentrations (for references see Baskin and Baskin, 1987).

Evidence of an effect of CO_2 levels in soil on changes in dormancy is limited (Baskin and Baskin, 1987; Karssen, 1980/81*b*). Measurements in the course of one year showed no great fluctuations in O_2 and CO_2 concentrations in sandy loam at a depth of 10 cm (Karssen, 1980/81*b*).

Induction of secondary dormancy in *Sisymbrium officinale* occurs faster in saturated than in low humidity soil (Karssen, 1980/81*a*). Seeds of *S. officinale* that have been buried in sandy loam germinate much better after exhumation than seeds that have been buried in sand. The difference disappears when nitrate is added to the sand (Karssen, 1980/81*b*). In experiments under controlled conditions it was shown that nitrate is not required during burial, but only has to be present during actual germination (Karssen and De Vries, 1983).

Environmental conditions during seed development

There is some evidence that dormancy and germination of crop seeds also depend on the environmental conditions during seed development. Warm and dry conditions often improve the quality of the produced seed. Therefore, commercial seed production is often located in areas of the world with hot, dry weather at the time seeds ripen (Austin, 1972; Gray and Thomas, 1982).

Although mineral **deficiencies** have relatively minor effects on seed composition,

application of mineral fertilizers can cause manifold changes in the elemental composition of seeds (Austin, 1972). Although increased levels of elements sometimes enhance seedling vigour, the effects on germination are inconsistent (Austin, 1972; Gray and Thomas, 1982).

Studies on the effect of environmental conditions on seed development in weedy species are very scarce, with *Chenopodium album* being an exception. Application of nitrate fertilizer to *C. album* plants increases the nitrate content and the germination percentages of the produced seeds (Fawcett and Slife, 1978; Saini *et al.*, 1985*b*).

Photoperiod also has a profound effect on dormancy of *Chenopodium* spp.. Long days induce the formation of seeds with a thick seed coat and deep dormancy (Karssen, 1970). With other species contrasting results were obtained. In some species dormancy is induced by short days; in others, by long days. Even on one plant, differences in dormancy can arise from differences in day length during ripening (Gutterman, 1982).

Also the temperature during seed ripening can effect the degree of dormancy. Again species show different responses. High temperatures during ripening can decrease or increase the dormancy of seeds (Austin, 1972). High temperatures and moisture stress during ripening of *Avena fatua* decreased dormancy of the produced seeds (Peters, 1982).

To enable prediction of germination and emergence it is necessary to take into account the effects of environmental factors during seed ripening. This thesis concentrates on the effect of nitrate fertilization of the mother plant. It is investigated whether in addition to *C. album*, in other species nitrate can accumulate in the seeds and in this way stimulate germination and, more importantly, whether an effect of increased endogenous nitrate levels persists when seeds are buried in the seed bank.

Outline of the thesis

This thesis discusses the analyses of dormancy and germination of weedy species. The experiments are carried out with seeds of four species:

1. *Chenopodium album* L. (lamb's quarters, fat-hen, pigweed, white goosefoot) is one of the most widely distributed weeds in the world. It is a troublesome weed in sugar beets, potatoes, corn and (summer) cereals, wherever they are grown in the world (Holm *et al.*, 1972). It is frequently found in autumn-sown crops in The Netherlands, but is not a weed then, because it remains small and dies after the first frost (P. Zonderwijk, pers. comm.).
2. *Polygonum persicaria* L. (red shank) is a principal weed in potatoes in Belgium, Chile and New Zealand (Holm *et al.*, 1972). Some experiments are also performed with *P. lapathifolium* L. subsp. *lapathifolium*. In general *Polygonum* spp. are weeds in grain crops in many parts of Canada (Staniforth and Cavers, 1979) and in sugar beets in The Netherlands (P. Zonderwijk, pers. comm.).

3. *Spergula arvensis* L. (corn spurrey) is reported to be a weed in 25 crops in 33 countries throughout the world. It is most troublesome in grain crops but also occurs in potatoes, sugar beets and vegetables (Holm *et al.*, 1972).
4. *Sisymbrium officinale* (L.) Scop. (hedge mustard) is a ruderal species confined to the Eurasian continent. It is not a weed in agriculture. It was included in this thesis because of its suitability as a model system.

The thesis examines the results of the following experiments:
Burial experiments. Seeds of the four species are buried under field conditions or under conditions of controlled soil moisture content. At regular intervals, part of the seeds is exhumed. Germination of these seeds is tested in light and - in some of the experiments and treatments - in darkness, at various temperatures, in water or in nitrate, with or without a preceding desiccation treatment.
Incubator experiments. Changes in field temperature are simulated in incubators to study the effect of temperature on changes in dormancy under controlled conditions. The results are compared to experiments where seeds are pretreated at constant temperatures.
Simulation. Changes in dormancy and germination are simulated on the basis of temperature during burial and temperature and nitrate during the germination test (after exhumation).

The results and the implications of the experiments are discussed for each of the four species separately in Chapters 2, 3, 4 and 5.
Desiccation. The effects of desiccation on dormancy and germination are also investigated in more detail. The results of these experiments and a discussion about the possible mechanisms involved in the effects of desiccation are presented in Chapter 6.
Seed development. In Chapter 7 the effects of nitrate fertilization of weeds on the nitrate content and on dormancy and germination of the produced seeds are investigated in more detail. These effects are studied both in freshly harvested and in buried seeds. The ecological implications of an increased seed nitrate content are discussed.

In Chapter 8 the similarities and differences between dormancy pattern and germination and the simulation models of the investigated species are discussed.

Objectives

The objectives of this study are to improve the knowledge of the seed biology of weeds. A better understanding of the effects of environmental factors on changes in dormancy and on actual germination will provide, on the one hand, the basis for a physiological analysis of mechanisms involved in these processes. On the other hand, it may lead eventually to models that can predict emergence in the field, which may reduce the use of herbicides in the control of weeds.

Chapter 2

Seasonal dormancy patterns in buried weed seeds. I. Polygonum persicaria L. and P. lapathifolium L. subsp. lapathifolium.

Abstract. The effect of environmental factors on changes in dormancy was studied in seeds of *Polygonum persicaria* L. and *P. lapathifolium* L. subsp. *lapathifolium*. Seeds were buried in the field and under controlled conditions. Portions of seeds were exhumed at regular intervals and germination was tested over a range of conditions. Both *Polygonum* spp. showed seasonal dormancy patterns that had the clear features of summer annuals, *i.e.* dormancy was relieved at low winter temperatures, the germination peak occurred in spring and dormancy was re-induced in summer. The expression of the dormancy pattern was strongly influenced by the temperature at which germination was tested. At 30°C exhumed seeds germinated over a much longer period of the year than at 20 or 10°C. Nitrate added during the germination test and a desiccation treatment prior to the test occasionally stimulated germination. The seasonal changes in dormancy of buried seeds were regulated by the field temperature. Soil moisture and nitrate content did not influence the changes in dormancy. The fact that, on the one hand, field temperature determined the changes in dormancy and, on the other hand, germination itself was influenced by temperature, was used to simulate the seasonal germination pattern of *P. persicaria*. Germination of exhumed seeds in Petri dishes at field temperature was accurately simulated with this model. Germination in the field was restricted to the period where the range of temperatures over which germination could proceed (computed with the model), and field temperature overlapped.

2.1 Introduction

Polygonum spp. are weeds in many crops all over the world. *Polygonum persicaria* L. (redshank, persicaria) is a weed in potatoes in Belgium, Chile, England, New Zealand, Germany and the United States, in wheat in several countries (Holm *et al.*, 1972) and in several horticultural crops in The Netherlands (P. Zonderwijk, pers. comm.). *P. lapathifolium* subsp. *lapathifolium* is an important weed in sugar beets in The Netherlands (P. Zonderwijk, pers. comm.). Several *Polygonum* spp. are weeds in grain crops in many parts of Canada (Staniforth and Cavers, 1979).

On small experimental fields where only the investigated species were allowed to

grow and disperse seeds, Van den Brand (1986, 1987) observed that emergence of *P. lapathifolium* subsp. *lapathifolium* and *P. persicaria* in The Netherlands occurred in three successive years mainly in April-May. Occasionally some seedlings of *P. persicaria* emerged in June. Roberts and Neilson (1980) made similar observations in Great Britain. In Japan and in Sweden, emergence of *P. lapathifolium* subsp. *lapathifolium* occurred in May and May-June, respectively (Watanabe, 1982; Håkansson, 1983).

The restriction of emergence to spring is a typical feature of summer annuals. Karssen (1982) hypothesized that the restriction of germination to a fixed interval of the year is determined by the overlap of field temperatures with the range of temperatures suitable for germination (Chapter 1). This is illustrated by results from burial experiments of Baskin and Baskin (1980) with seeds of *Ambrosia artemisiifolia*, a species closely resembling *P. persicaria* in its (summer annual) behaviour. Although exhumed seeds of *A. artemisiifolia* can germinate during a much longer period of the year at high temperatures, germination in the field is restricted to spring because only in that period is there an overlap between the field temperature and the range of temperatures over which germination can proceed (Chapter 1).

Dormancy of *P. persicaria* seeds fluctuates in a seasonal pattern (Karssen, 1980/81*b*). However, no information is available about the germination-temperature range since germination was tested at one temperature only in the experiment of Karssen.

The seasonal character of the changes in dormancy suggests that dormancy is mainly regulated by the field temperature. This implies that temperature in the field has a dual effect. On the one hand, it is the driving force for the changes in dormancy and therefore determines the **width** of the germination-temperature range. On the other hand, germination itself depends on whether the temperature is **within** this range. In the present study this dual role of temperature was investigated both in burial experiments in the field and under controlled conditions in incubators.

Germination of *P. persicaria* is strongly stimulated by nitrate (Vincent and Roberts, 1977, 1979; Karssen, 1980/81*b*). Data from studies with several other species have shown that dormancy and germination are sometimes also influenced by desiccation (Kiviliaan, 1975; Karssen, 1980/81*b*, Karssen *et al.*, 1988) and soil moisture content (Karssen, 1980/81*a*; Lonchamp *et al.*, 1984). Therefore the effects of these three factors on changes in dormancy and germination of seeds of *P. persicaria* and *P. lapathifolium* subsp. *lapathifolium* were also investigated in the present study. The influence of several of these environmental conditions on the seasonal emergence pattern was simulated with a preliminar descriptive model.

2.2 Materials and methods

Seeds

Several seed lots of *P. persicaria* and *P. lapathifolium* subsp. *lapathifolium* were collected in 1985 and 1986 in the vicinity of Wageningen. After collection seeds were air dried, rubbed mechanically to remove perianth segments, sieved and winnowed to remove small and light seeds and then stored dry at 2 °C.

Burial in the field

In December 1986 seed lots of *P. lapathifolium* subsp. *lapathifolium* and *P. persicaria*, both harvested that year, were divided into 24 and 48 portions, respectively, that were packed separately in envelopes made of fine mesh nylon gauze. Each envelope of seeds was buried in sandy loam in a plastic net pot (ø 10 cm), that permitted good contact with the surrounding soil. To prevent loss of soil during handling, the pots were lined with gauze. The soil that surrounded the seeds prevented light reaching the seeds during exhumation.

The pots with the seeds of *P. persicaria* were either buried in the field in sandy loam or, at the same location under a transparent plastic roof in a 100 l polyethylene container, sunk in the ground and holding the same soil as in the field. Seeds of *P. lapathifolium* subsp. *lapathifolium* were only buried in the container. All seeds were buried at a depth of approx. 10 cm below the surface.

Using a tube attached to the bottom of the container and connected to a reservoir, the container could be supplied with water from underneath. The moisture content of the soil was regulated at 10 to 20% (dwt). Seeds buried in the field were exposed to seasonal fluctuations in soil moisture content. At regular intervals the moisture content of the soil from the different treatments was determined in sub samples of approx. 70 g (dwt) taken from a depth of 10 cm, by weighing before and after drying in an oven for 8 h at 130°C. The nitrate content of the soil from the different treatments was determined at regular intervals according to the method of Houba *et al.* (1986). This method is described in detail in Chapter 7.

Soil temperatures measured at 10 cm in the container showed minor discrepancies from temperatures measured in the field. The latter temperatures were identical to those recorded at a meteorological station at Wageningen. Therefore the recordings of the meteorological station were used.

Germination tests

At regular intervals, from all treatments one portion of each species was exhumed to test germination. During transport to the laboratory the pots were covered with black polyethylene. The seeds from each envelope were divided into smaller portions. These portions were incubated in 50 mm Petri dishes on 1 layer of filter paper (Schleicher and Schüll no. 595). Before imbibition in Milli-Q water or 50 mM KNO_3, half of the portions were desiccated. Desiccation occurred in a hygrostat (Weges and Karssen, 1987) over a saturated solution of LiCl (r.h. approx. 16%) for 24 h, the time required for equilibrium between seed moisture content and the r.h. in the hygrostat. The moisture content of the seeds was determined by weighing before and after drying for 1.5 h in an oven at 130°C.

Seeds were irradiated for 15 min with red light. Red light was obtained by filtering light from 6 red fluorescent tubes (Philips TL 20W/15) through one layer of 3 mm plexiglas (red 501, Röhm & Haas, Darmstadt, GFR), the light intensity at seed level being 250 $\mu W.cm^{-2}$. If appropriate, irradiation occurred before the desiccation treatment.

Petri dishes were placed in closed plastic boxes to prevent loss of moisture. Germination tests occurred in cooled incubators (Gallenkamp, Crawley, U.K., T ± 1°C) or outdoors at a height of 1.50 m in the shade in the plastic boxes, covered with black polyethylene.

Handling of exhumed seeds occurred in dim green safelight, obtained by filtering light from one green fluorescent tube (Philips TL 40W/17) through two layers yellow no. 46 and two layers blue no. 62 Cinemoid filters (Strand Electric, London, U.K.).

Between 6 and 14 days after incubation, depending on the test temperature, when no additional germination occurred, both germinated and non-germinated seeds were counted to determine germination percentage. Protrusion of the radicle was the criterion for germination. The temperature during the outdoor germination tests was obtained from the meteorological station at Wageningen.

Burial under controlled conditions

Seeds of *P. persicaria* (Wageningen, 1987) were buried in sandy loam in black 9 cm plastic Petri dishes between two layers of fine mesh gauze and pretreated at various temperature regimes. At regular intervals, and at each change of temperature one Petri dish was removed from the incubators to test the germination capacity of the seeds.

At the start of the experiment soil moisture content was 18%. Because Petri dishes were kept in plastic boxes lined with moist filter paper, soil moisture content only changed slightly.

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 transformation, 2*arcsin√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.

2.3 Results

Germination of exhumed seeds

P. persicaria

The results of the germination tests with the exhumed seeds of *P. persicaria* are presented in Figs 2.1 and 2.2. Fig. 2.1 depicts the results of germination tests in water at 10, 20 and 30°C. In Fig. 2.2 a comparison is shown for the three test temperatures between germination of the exhumed seeds in water and KNO_3 with or without a preceding desiccation treatment. The desiccation treatment was included until May 1989.

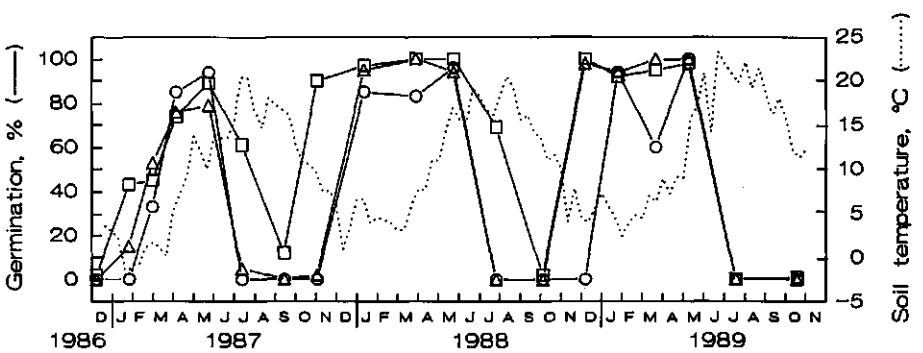

Fig. 2.1 Seasonal variation in germination of exhumed seeds of *Polygonum persicaria* at different test temperatures. Seeds were buried in portions of 1.8 g in December 1986 in sandy loam under field conditions and exhumed at regular intervals. Germination was tested in Petri dishes with test samples of approx. 35 seeds, at 10 (○), 20 (△) or 30°C (□) in Milli-Q water after a 15 min red light irradiation. The dotted line indicates the soil temperature at 10 cm in bare soil.

All data show a seasonal pattern of dormancy that started directly after burial of the seeds in December 1986 with an alleviation of dormancy. Re-induction of dormancy occurred in spring-early summer 1987. The pattern was repeated in the following 2 years.

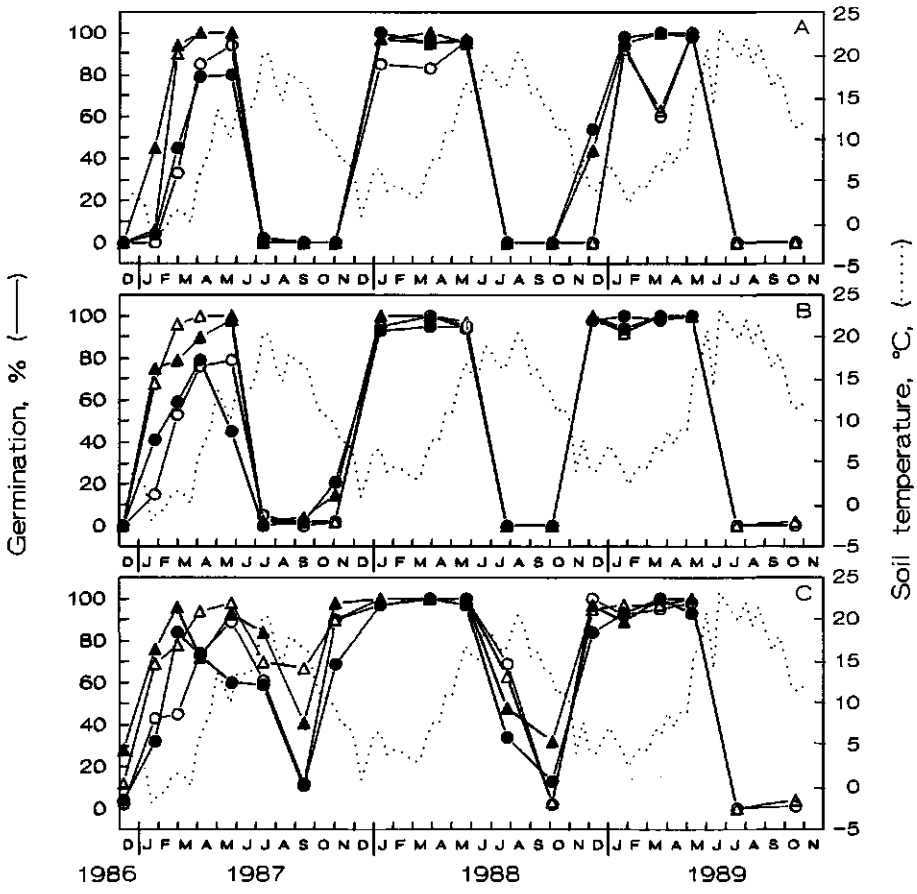

Fig. 2.2 Effect of nitrate and desiccation on the seasonal variation of germination of exhumed seeds of *Polygonum persicaria*. Burial as in Fig. 2.1. Germination was tested in Petri dishes with test samples of approx. 35 seeds after a 15 min red light irradiation, at 10 (A), 20 (B) or 30°C (C) in water (circles) or 50 mM KNO_3 (triangles) with (closed symbols) or without (open symbols) a preceding desiccation treatment. Desiccation occurred for 24 h above a saturated solution of LiCl (r.h. approx. 16%), which gave a seed moisture content of approx. 9%. The dotted line indicates the soil temperature at 10 cm in bare soil.

Test temperature. In particular the germination tests in summer-autumn 1987 and 1988 showed that more *P. persicaria* seeds germinated at 30°C than at 20 and 10°C (Fig. 2.1). At 30°C, seeds germinated during a longer period of the year than at 20 and particularly at 10°C.

Nitrate and desiccation. Nitrate only stimulated germination of *P. persicaria* during the first annual cycle of breaking and induction of dormancy in 1987 (Fig. 2.2). At 30°C, nitrate still stimulated germination in September 1987 when germination had already ceased in water (Fig. 2.2C). The stimulatory effect of nitrate did not return in the next two annual cycles at any test temperature.

Desiccation of *P. persicaria* seeds prior to germination tests at 10, 20 and 30°C, only had a small stimulating effect on germination, *e.g.* at 10°C in December 1988 and May 1989 (Fig. 2.2A).

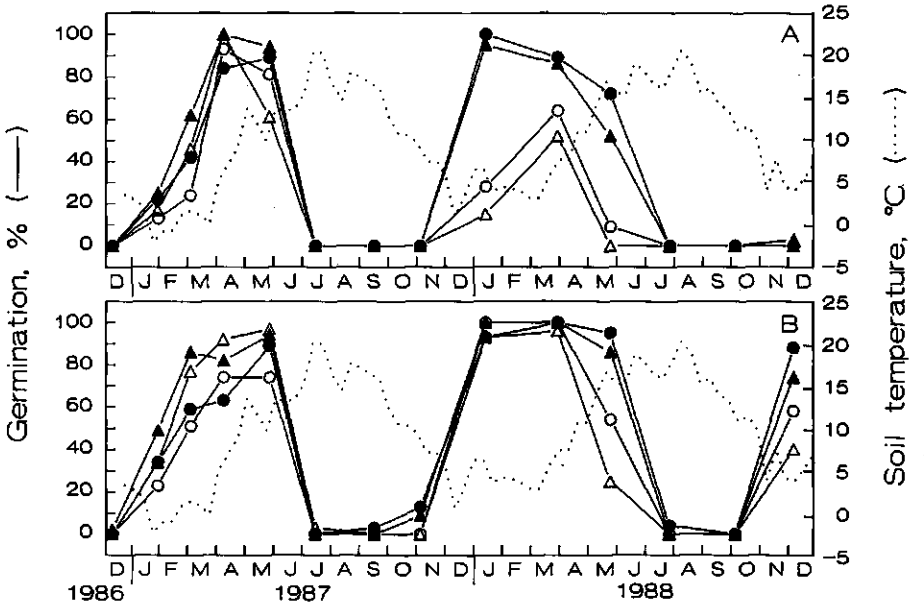

Fig. 2.3 As Fig. 2.2 for seeds of *Polygonum lapathifolium* subsp. *lapathifolium*. Seeds were buried in December 1986 in portions of 1.7 g in sandy loam in a 100 l plastic container, sunk in the ground and covered with a transparent plastic roof. Soil moisture content was regulated at 10 to 22% (dwt). Only results of germination tests (with test samples of approx. 30 seeds) at 10 (A) and 20°C (B) are shown. The dotted line indicates the soil temperature at 10 cm in bare soil.

P. lapathifolium subsp. lapathifolium

For *P. lapathifolium* subsp. *lapathifolium*, tests were performed for two years. Only results of tests at 10 and 20°C are shown (Fig. 2.3). The dormancy pattern of *P. lapathifolium* subsp. *lapathifolium* was very similar to that of *P. persicaria*.
Only in the second year was germination of *P. lapathifolium* subsp. *lapathifolium* at 10°C in water and nitrate somewhat lower than germination of *P. persicaria* (Fig. 2.3A versus Fig. 2.2A). In the second year, germination of *P. lapathifolium* subsp. *lapathifolium* was clearly stimulated by desiccation, particularly at 10°C (Fig. 2.3A) or when seeds were germinated outdoors (data not shown). Nitrate only stimulated germination of *P. lapathifolium* subsp. *lapathifolium* during the first winter when germination was tested at 20°C (Fig. 2.3B).

When seeds of *P. lapathifolium* subsp. *lapathifolium* were pretreated in Petri dishes in water, instead of under field conditions in soil, application of nitrate during the test enhanced germination to a large extent at all test temperatures (Table 2.1).

Table 2.1 Effect of nitrate on germination of *Polygonum lapathifolium* subsp. *lapathifolium*. Seeds were pretreated in darkness at 2°C in Milli-Q water. After the pretreatment seeds were surface dried on a Büchner funnel and incubated in Milli-Q water or 50 mM KNO_3 at 15, 22 or 30°C after a 10 min red light irradiation. Dark germination was also tested but rarely occurred (data not shown). Results are means of triplicates of 50 seeds.

	germination temperature (°C)					
pretreatment at 2°C	15		22		30	
(weeks)	H_2O	KNO_3	H_2O	KNO_3	H_2O	KNO_3
0	0	0	0	0	4	7
1	0	0	1	9	57	69
2	0	2	3	21	66	96
3	3	12	4	62	71	93
6	4	55	19	95	95	99

Control of dormancy pattern

The above results show that the conditions during the germination test, particularly temperature, influenced the **expression of dormancy**. A question that has to be answered is which factor(s) control(s) the **changes in dormancy**.

Soil moisture and nitrate content. To study the effect of soil moisture on changes in dormancy, seeds were buried either outdoors in sandy loam with fluctuating moisture content or, under a transparent roof, in a container with sandy loam with a more or less constant soil water content. In the uncovered situation, the soil moisture content showed a seasonal fluctuation: Moisture content was lowest in May in both years and highest during winter. In the covered treatment the soil moisture content also varied, but did not show the seasonal fluctuation that occurred in the uncovered location (Fig. 2.4A).

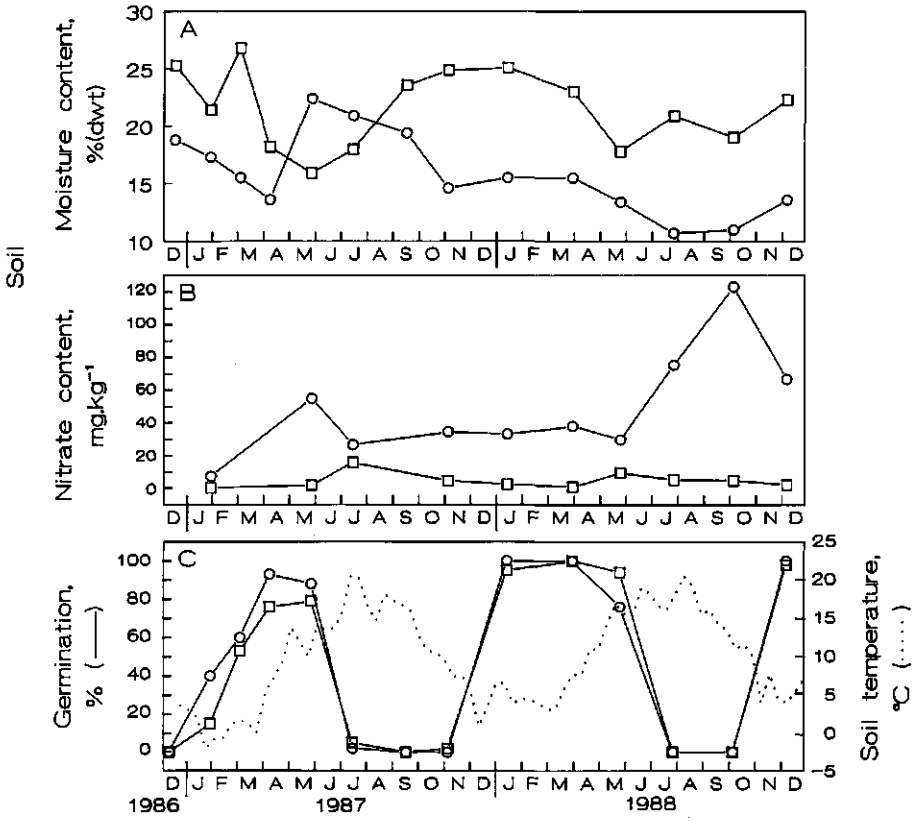

Fig. 2.4 Changes in soil moisture (A), soil nitrate content (B) and soil temperature (C) during burial of seeds of *Polygonum persicaria* as compared to changes in germination of exhumed seeds (C). Seeds were buried under field conditions as in Fig. 2.1 (fluctuating soil moisture and nitrate content) (□) or in a 100 l plastic container as in Fig. 2.3 (○). At regular intervals, soil nitrate and moisture content were determined and germination tested as in Fig. 2.1 at 20°C in water (C).

The two treatments showed large differences in soil nitrate content (Fig. 2.4B). The nitrate content of the covered treatment was always higher than that of the uncovered location. It increased during summer, particularly in the second year.

The differences in soil moisture and nitrate content were not reflected in the changes in dormancy (Fig. 2.4C). Here results of germination tests at 20°C in water are shown. Also for other test conditions there were no differences between the two treatments (data not shown).

Temperature. The seasonal character of the changes in dormancy suggests that the pattern might be controlled by field temperature. Comparison of the germination data with the mean soil temperature in the field suggests that dormancy was induced when temperature rose above approx. 10-15°C, whereas dormancy relief started when temperature dropped below these values (Figs 2.1, 2.2). This hypothesis was tested both experimentally and statistically.

Experimental test. In Fig. 2.5A the results of germination tests in nitrate at 10, 20 or 30°C from the first 8 months of Fig. 5.2 are shown again to compare them with the results of similar tests of seeds that had been pre-incubated in Petri dishes with soil at 2°C (Fig. 2.5B) or at a sequence of temperatures starting at 2°C and rising stepwise to 10 (Fig. 2.5C) or 15°C (Fig. 2.5D).

During the first three months (Fig. 2.5A) or 75 days (Fig. 2.5B-D), relief of dormancy was very similar for the four treatments. Induction of dormancy had started at the end of the experiment for all treatments except the 2°C → 6°C → 10°C treatment (Fig. 2.5C). However, when seeds were pretreated at 2°C, dormancy induction was only seen in germination tests at 20 and 30°C (Fig. 2.5B), whereas after pretreatment in the field (Fig 2.5A) and at a temperature program rising to 15°C (Fig. 2.5D), dormancy induction was most evident in germination tests at 10 and 20°C. Dormancy was not induced after 150 days when the rise in temperature stopped at 10°C (Fig. 2.5C). Apparently, dormancy is either re-induced at constant 2°C or at a rising temperature when it is increased to 15°C. There is a striking similarity between the results of the latter experiment and the pattern observed in the field experiment (Fig. 2.5A). In the field, a rise above approx. 10-15°C seems critical for dormancy induction.

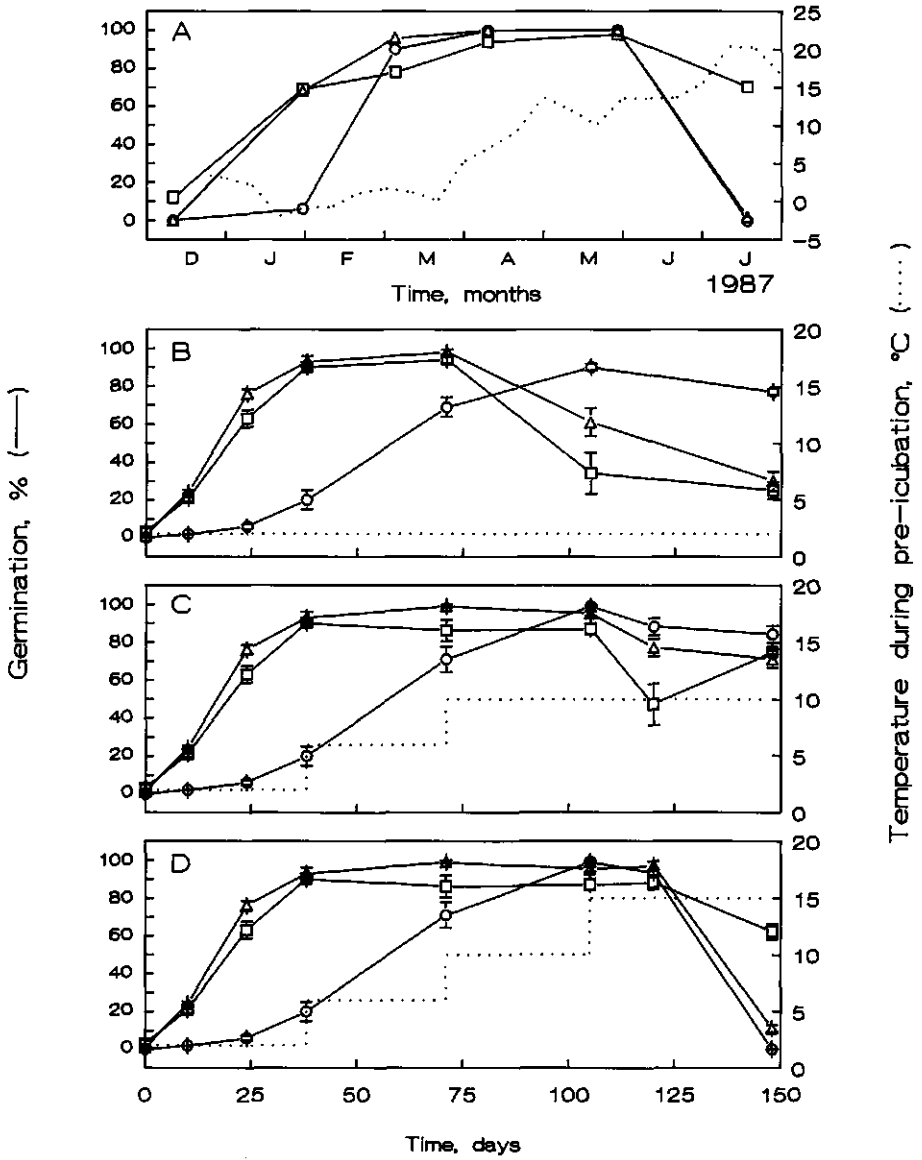

Fig. 2.5 Effect of pretreatment temperature on dormancy of *Polygonum persicaria*. Seeds were buried in the field, exposed to natural fluctuating temperatures (A) or seeds were pretreated in sandy loam in black 9 cm plastic Petri dishes between two layers of fine mesh gauze at 2°C (B) or at temperatures rising stepwise from 2 to 6 and 10 (C) or to 15°C (D). Seeds were "exhumed" at regular intervals. Germination was tested with single test samples of approx. 35 seeds (A) or triplicates of approx. 55 seeds (B,C,D) in 50 mM KNO_3 in Petri dishes at 10 (○), 20 (△) or 30°C (□) after a red light irradiation for 15 min. Vertical bars indicate standard error. The dotted line indicates the temperature during "burial".

Statistical test. To test the hypothesis statistically the theories that were developed by Totterdell and Roberts (1979) were followed. They hypothesized that stratification, the loss of dormancy at low temperatures, of seeds of *Rumex obtusifolius* and *R. crispus* was the result of two sub processes: **Relief** of primary dormancy and **induction** of secondary dormancy.

1) **Relief** of primary dormancy only occurred at temperatures **below** a certain value. This border temperature, was estimated to be 15°C for the *Rumex* spp. Totterdell and Roberts investigated. **Relief** of primary dormancy was independent of the actual temperature so long as it was below 15°C.

2) **Induction** of secondary dormancy occurred at **all** temperatures but the rate of induction increased with increase in temperature.

Totterdell and Roberts hypothesized that the effect of a temperature pretreatment on dormancy of the *Rumex* spp. depended on these two sub processes. This implies that, although **relief** of primary dormancy occurred equally at all temperatures below 15°C, temperatures just above zero caused the most effective net relief of dormancy, because the rate of **induction** of secondary dormancy was lowest at these low temperatures. In the present study the theory of Totterdell and Roberts was confirmed for *P. persicaria* in a parallel experiment. Stratification of *P. persicaria* occurred faster in a pretreatment at 2 than at 6 and 10°C. Dormancy was not relieved at 15°C (data not shown). Dormancy of *P. lapathifolium* subsp. *lapathifolium* was also relieved better at 1°C than at 5°C (Espeby, 1989).

From the soil temperature data, cumulative dormancy breaking and dormancy inducing factors were calculated (Fig. 2.6). They are indicated as cold and heat sum (C and H), respectively. For each period of 10 days that the mean soil temperature (at 10 cm) was below a border temperature, the value of C was raised with an arbitrary value 1. Different border temperatures were used to calculate C to determine which border temperature was suitable for *P. persicaria*. H was calculated by summing the value of the mean soil temperature of every 10 day period. When the mean soil temperature in a 10 day period was below 0°C, which occurred only three times during the experiment, C nor H were increased. C and H were never reset to zero, because the determination of the moment this should occur would have been unreliable. Therefore, both factors increased throughout the experiment, C only in some parts of the year (when the field temperature was below the border temperature), H continuously (Fig. 2.6). This implies that time was necessarily involved in both parameters.

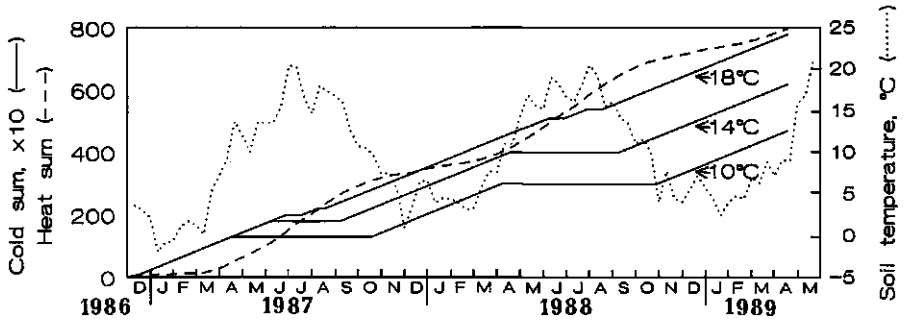

Fig. 2.6 Changes in soil temperature at 10 cm (dotted line) and in cold sum (C) (solid line) and heat sum (H) (broken line) during two and a half years of burial. For each period of 10 days that the mean soil temperature was below a border temperature the value of C was raised with an arbitrary value 1. To illustrate the effect of the value of the border temperature on C, it was calculated using border temperatures of 10, 14 and 18°C. H was calculated by summing the value of the mean soil temperature of every 10 day period.

It was assumed that the sub processes of dormancy relief and induction were regulated by C and H, respectively and that dormancy (D) therefore was a function of both parameters:

$$D = f\,(C, H) \qquad (2.1)$$

Although Totterdell and Roberts (1979) used the term stratification for the net result of the two sub processes, in this thesis the use of dormancy relief or dormancy induction as the **net** result of a temperature pretreatment is preferred. Where these terms are further used the net result of the two sub processes is meant. When germination increases in time, due to a pretreatment at a certain temperature, dormancy is said to be relieved, although both sub processes as defined by Totterdell and Roberts occur simultaneously.

Induction of dormancy in *P. persicaria* seeds also occurred after 100 days at 2°C (Fig. 2.5B). Although this result can not be explained by the involvement of just C and H, the observation was ignored since such long periods of low temperatures do not occur in the temperate zone.

In Fig. 2.7 some transformed germination data from Fig. 2.1 are expressed as a function of the germination temperature. It is clear that the expected transformed germination (G_t) at a certain moment can be described by a quadratic function of the germination temperature (T_g).

$$G_t = a.T_g^2 + b.T_g + c \qquad (2.2)$$

where a, b and c are the coefficients of the quadratic function.

Since germination of exhumed seeds fluctuates throughout the year as a function of dormancy (Figs 2.1, 2.2), it was assumed that one or more of the coefficients a, b and c are a function of dormancy, and also of the factors that influence the result in the germination test, such as the composition of the germination medium (M_g), in the current study the presence or absence of KNO_3.

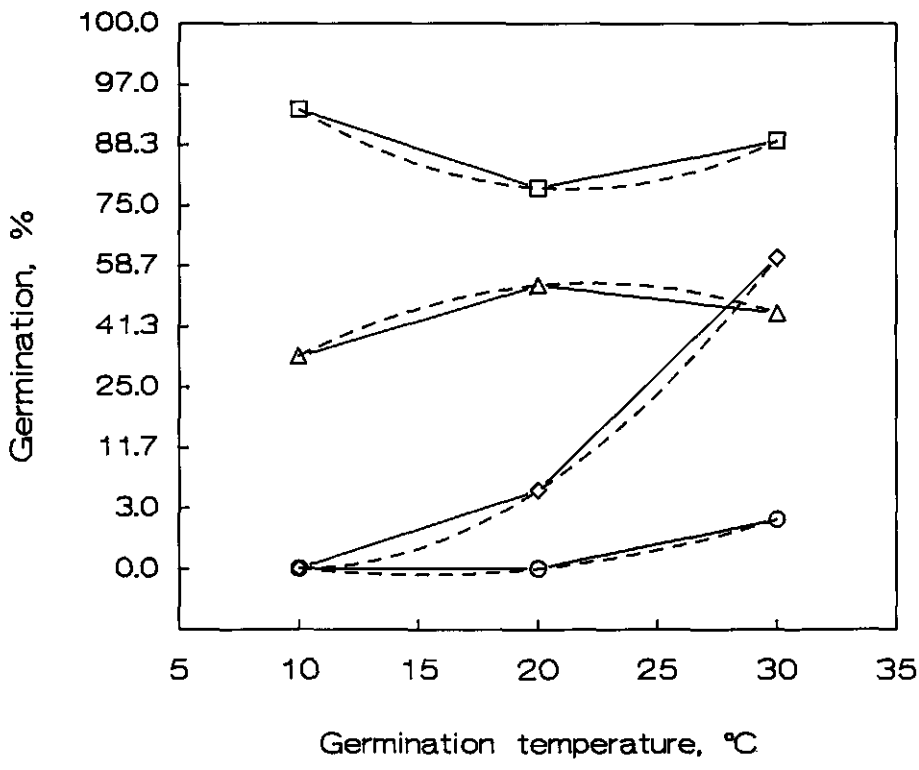

Fig. 2.7 Germination data of *Polygonum persicaria* from Fig. 2.1 as a function of germination temperature (intact line). Quadratic curves (dotted line) are shown to indicate the quadratic character of the relationship between germination temperature and germination. (○) December 1986, (△) March 1987, (□) May 1987, (◇) July 1987.

The observation that dormancy induction at 2°C was primarily seen in germination tests at 20 and 30°C (Fig. 2.5B) and induction of dormancy at rising temperatures in tests at 10 and 20°C, suggests that the temperatures shortly before exhumation influenced germination directly and not through C and H alone. Therefore also the mean pretreatment temperature (T_p) during a period (δt) prior to exhumation ($T_{p,\delta t}$) was introduced as possibly influencing a, b and c. The effect of light was not simulated because seeds were always irradiated with 15 min red light between exhumation and germination test. Neither was desiccation used as a parameter. In summary:

$$a, b, c = f\ (C, H, M_g, T_{p,\delta t}) \qquad (2.3)$$

Substituting Equation 2.3 in Equation 2.2 gives:

$$G_t = f\ (C.T_g^2, H.T_g^2, M_g.T_g^2, T_{p,\delta t}.T_g^2, T_g^2, C.T_g, H.T_g, M_g.T_g, T_{p,\delta t}.T_g, T_g, C, H, M_g, T_{p,\delta t}) \qquad (2.4)$$

With forward and backward stepwise regression (procedure Stepwise, SAS; Anonymous, 1985) the parameters that maximized the fit of the data were selected from Equation 2.4. The significance level for entry into the model was 0.15. Data from the germination tests in July and October 1989 were not used anymore to develop the model.

The best value of the border temperature for the calculation of C and the best period δt (20, 30, 40, 50 or 60 days) of $T_{p,\delta t}$ were determined by fitting equations with the selected parameters (procedure Generalized Linear Models, SAS; Anonymous, 1985) using different values for the border temperature and δt. A border temperature of 15°C gave the highest correlation (R^2) and the lowest estimated variance ($\hat{\sigma}^2$) (Table 2.2). For δt in $T_{p,\delta t}$ a period of 30 days gave the best fit (data not shown). Therefore C, computed with a border temperature of 15°C and $T_{p,30}$ (the mean soil temperature in the 30 days before exhumation) were used in the model. The expected transformed germination (G_t) could be estimated by:

$$G_t = (-0.050*C + 0.003*H + 0.040*T_{p,30} + 0.065)*T_g^2 + (1.785*C - 0.113*H - 1.479*T_{p,30} + 0.658*M_g)*T_g + 7.366*T_{p,30} - 10.081 \qquad (2.5)$$

When a model was developed with the parameter time (weeks of burial) instead of C and H, R^2 decreased from 0.76 to 0.33 and $\hat{\sigma}^2$ increased from 1348 to 3421.

Table 2.2 Estimated variance ($\hat{\sigma}^2$) and squared multiple correlation (R^2) of models simulating germination of *Polygonum persicaria* on the basis of cold and heat sum, germination temperature, germination medium and the mean temperature in 30 days before exhumation. Different border temperatures were used to calculate cold sum. See text for explanation.

	Border temperature (°C)								
	10	11	12	13	14	15	16	17	18
$\hat{\sigma}^2$	1731	1557	1630	1510	1386	1348	1709	1574	1491
R^2	0.687	0.719	0.705	0.727	0.749	0.756	0.691	0.715	0.730

Germination under field conditions

Germination data calculated with Equation 2.5 and transformed back to germination percentages were compared to germination data obtained with exhumed seeds that were tested in Petri dishes placed outdoors. In these tests, germination also fluctuated in a seasonal pattern (Fig. 2.8). Particularly in water and nitrate, germination occurred during a much shorter time than at constant temperatures (Fig. 2.2). Germination in water showed in all years a peak in May. Nitrate but particularly desiccation advanced the moment germination started to February-March or even December-January, respectively.

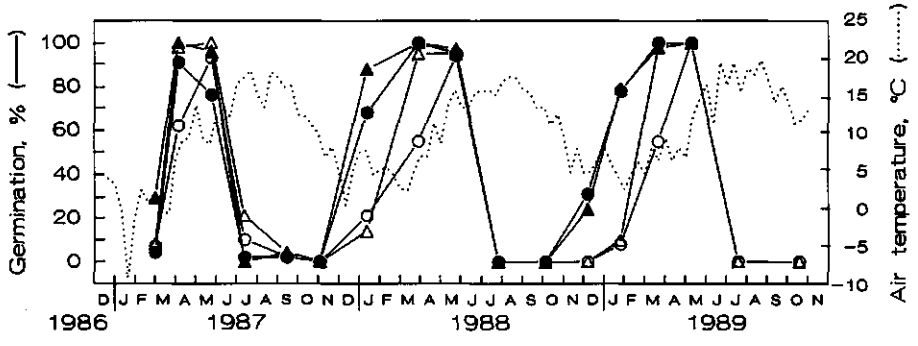

Fig. 2.8 Germination of exhumed seeds of *Polygonum persicaria* outdoors as a function of exhumation date. As Fig. 2.2 except the Petri dishes with seeds were incubated in airtight plastic boxes covered with black polyethylene, placed outdoors at a height of 1.50 m in the shade. The dotted line indicates the air temperature at 1.50 m.

The model (Equation 2.5) was developed with results of germination tests with exhumed seeds, in incubators. The intriguing question now is whether the tests of exhumed seeds at the different constant temperatures in incubators do indeed explain the seasonal emergence pattern in the field as shown in Fig. 2.8.

Fig. 2.9 shows the changes in the minimum and maximum temperature required for 50% germination ($T_{g,min}$ and $T_{g,max}$) in water, calculated with Equation 2.5. These calculations were restricted to the range of 0°C to 30°C to maintain ecological significance. $T_{g,min}$ and $T_{g,max}$ were only calculated for germination in water, since germination in water and nitrate were fairly similar.

The periods of predicted field germination/emergence are the periods where the field temperature overlapped with the germination-temperature range (hatched areas). The arrows indicate the moments that germination in Petri dishes placed outdoors actually increased (↑) or decreased (↓) to 50% (data from Fig. 2.8).

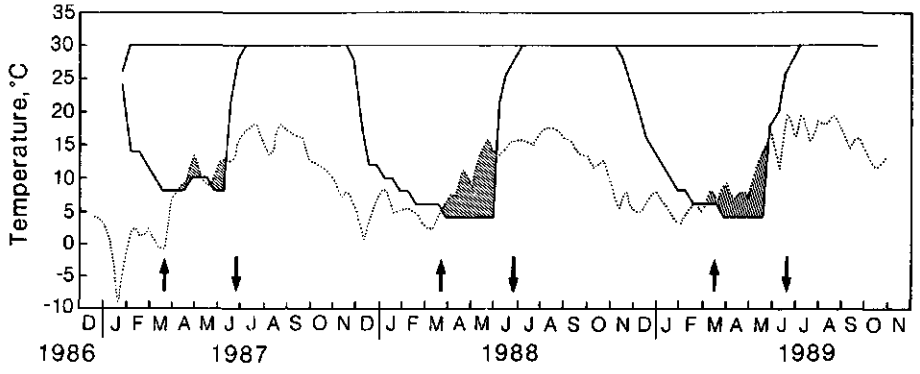

Fig. 2.9 Seasonal changes in the range of temperatures over which at least 50% of exhumed *Polygonum persicaria* seeds germinate. Solid lines represent maximum and minimum temperature required for 50% germination in water, calculated with Equation 2.5. The dotted line indicates air temperature at 1.50 m (see text for explanation). Hatched areas indicate overlap of field temperature and germination-temperature range. Arrows indicate the moment germination in Petri dishes outdoors actually increased above (↑) or decreased below 50% (↓) (data from Fig. 2.8).

There was a good agreement between the calculated periods of germination and the actual results of outdoor germination tests.

When air temperature at 1.50 m (the height seeds were placed for germination tests outdoors) was used as T_g in Equation 2.5 also the germination pattern outdoors in water and nitrate was simulated fairly accurately with the model (Fig. 2.10). Although data from May 1989 onwards were not used to make the model, outdoor germination in July and October 1989 was also closely simulated/predicted.

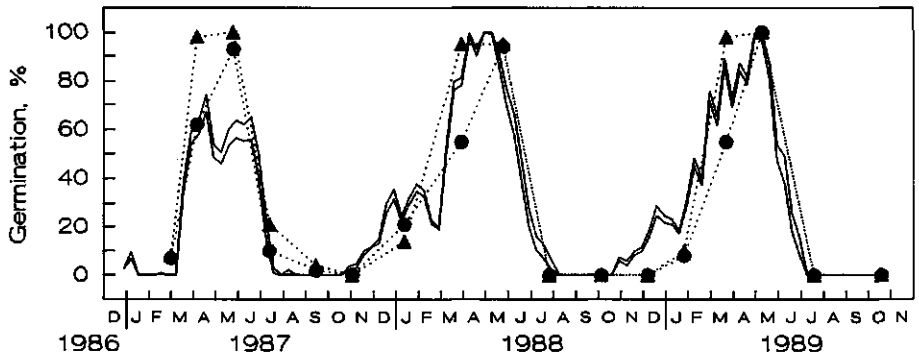

Fig. 2.10 Simulated (intact line) and observed (dotted line) germination of *Polygonum persicaria* in Petri dishes outdoors as a function of exhumation date. Germination outdoors as in Fig. 2.8, in water (●) or 50 mM KNO_3 (▲). Simulation was carried out with Equation 2.5. See text for explanation.

2.4 Discussion

Germination of exhumed seeds

Germination tests of exhumed seeds of *P. persicaria* during the first year of burial supported most of the emergence data reported in literature (Roberts and Neilson, 1980; Watanabe, 1982; Håkansson, 1983; Van den Brand, 1986, 1987). Germination and emergence occurred in April-May (Fig. 2.8). In the second and third year, germination in water and nitrate occurred already in tests in March and when seeds were desiccated prior to the germination test as early as January. In 1988 and 1989, temperatures in The Netherlands were quite high in January (Fig 2.8). However, also in the studies of Roberts and Neilson (1980) and Van den Brand (1986) in some years emergence occurred already in March. Moreover, it has to be considered that particularly at low temperatures there is a lag time of several weeks between germination and emergence.

Control of germination

Test temperature. The importance of the effect of the test temperature on the expression of dormancy is seen clearly in Fig. 2.1. At a test temperature of 30°C germination of exhumed seeds occurred during a much longer period of the year than at 20 or 10°C. It is obvious that information about the range of temperatures over which germination can proceed is essential for the prediction of germination (Figs 2.9, 2.10). The germination temperature range calculated using Equation 2.5 predicted

fairly well the period of at least 50% germination (Fig. 2.9).

The germination-temperature range of *P. persicaria* and *P. lapathifolium* subsp. *lapathifolium* showed the typical features of a summer annual. Germination was usually best at the higher temperatures and changes in dormancy were obtained by changes in the minimum temperature suitable for germination (Figs 2.1, 2.9). These features closely resembled those of other typical summer annuals *e.g. Ambrosia artemisiifolia* (Baskin and Baskin, 1977*b*, 1980) (Chapter 1), *Verbascum blattaria* and *V. thapsus* (Baskin and Baskin, 1981*b*) and *Rumex crispus* (Baskin and Baskin, 1978) and *R. obtusifolius* (Van Assche and Vanlerberghe, 1989). In Chapters 4 and 5 it will be shown that germination of *Spergula arvensis* and *Chenopodium album* in spring could also be obtained with a germination-temperature range that differed strongly from that of these typical summer annuals.

Nitrate. The stimulatory effect of nitrate on germination of *P. persicaria* has been reported before. Stimulation occurred particularly after chilling in light (Vincent and Roberts, 1977, 1979) but also in darkness (Karssen, 1980/81*b*). Germination of seeds of *P. lapathifolium* subsp. *lapathifolium* pretreated in water in Petri dishes, was also stimulated by nitrate (Table 2.1). However, during the first year, germination tests of exhumed seeds showed only a small effect of applied nitrate and no effect at all during the second year (Figs 2.2, 2.3B).

The different reaction of fresh and buried seeds to applied nitrate might be a function of differences in their endogenous nitrate content. Fresh seeds of *Polygonum* spp. contain very low levels of nitrate (approx. 0.2 μmol.g^{-1} seed (dwt), data not shown) and therefore a reaction to exogenous nitrate is to be expected.

Seeds of *Chenopodium album* (Chapter 7) and *Sinapis arvensis* (Goudey *et al.*, 1988) can take up nitrate from the soil during burial. If this also occurs in *Polygonum* seeds, the uptake must be a slow process because nitrate applied during germination tests in the first few months still stimulated germination of exhumed seeds (Figs 2.2, 2.3B). Furthermore, nitrate concentrations in soil are generally low during winter, which may also slow down uptake by the seeds (Fig. 2.4B; Chapter 7; Popay and Roberts, 1970; Young and Aldag, 1982). It seems, that after the first few months of burial the amount of nitrate taken up by the seeds is saturating, because applied nitrate can not further enhance germination. This in contrast to *Sisymbrium officinale*, *Spergula arvensis* and *Chenopodium album* that apparently have a higher requirement for nitrate. Germination of these species was enhanced by nitrate throughout the three years of burial (Chapters 3, 4 and 5).

Desiccation. Desiccation had a small stimulating effect on germination of *Polygonum* spp. (Figs 2.2, 2.8). The effect of desiccation was only visible under conditions that limited germination, for instance, at low temperatures (Figs 2.3A, 2.8) or without a

red light irradiation (Chapter 6). This may indicate that the primary depth-sensing mechanism for seeds of *P. lapathifolium* subsp. *lapathifolium* and *P. persicaria* is light. If seeds are buried too deep to perceive light, then desiccation may act as a depth-sensing mechanism. It may cause germination when a dry spell is followed by rain although the seeds were not exposed to light.

Control of dormancy

Soil moisture content. The results on the effect of soil moisture and nitrate content indicate that a (seasonal) fluctuation in soil moisture or nitrate content is not a prerequisite for changes in dormancy of *P. lapathifolium* subsp. *lapathifolium* and *P. persicaria* (Fig. 2.4). Also during pretreatment in Petri dishes changes in dormancy occurred, despite the absence of changes in moisture content (Fig. 2.5). In Chapters 3, 4 and 5 similar results are described for *Sisymbrium officinale*, *Spergula arvensis* and *Chenopodium album*.

Our data do not exclude an influence of extreme situations such as water logging or drying of the soil.

Temperature. Dormancy of summer annuals is usually relieved by low temperatures (Karssen, 1982; Baskin and Baskin, 1977). Vincent and Roberts (1977, 1979) and Staniforth and Cavers (1979) showed that dormancy of *P. persicaria* was indeed relieved by a chilling pretreatment for 4 weeks at 1°C and 15 weeks at 4°C, respectively. The latter authors showed that there was no difference between a 15 weeks dormancy breaking treatment during winter in the field or on moist filter paper at 4°C. However, extending the pretreatment at 4°C up to 19 and 25 weeks caused induction of secondary dormancy. This is in agreement with the present results (Fig. 2.5). Apparently dormancy induction is inevitable, even at low chilling temperatures.

In addition to an effect on dormancy, the pretreatment temperature also seems to influence the changes in the germination-temperature range directly. With a constant low temperature pretreatment dormancy induction was first seen in germination tests at 20 and 30°C (Fig. 2.5B). However, following a pretreatment at rising temperatures or in the field germination first decreased in tests at 10 and 20°C (Fig. 2.5A,C). This was confirmed by results with *Spergula arvensis* (Chapter 4). Germination of seeds of this species at 10, 20 and 30°C was not stimulated equally by different pretreatment temperatures, although they all relieved dormancy.

Both the present burial experiment with *P. persicaria* and the experiments of Totterdell and Roberts (1979) with *Rumex obtusifolius* and *R. crispus* seem to suggest that 15°C is the crucial temperature for dormancy relief and induction for some summer annuals.

Simulation

The descriptive model fairly accurately simmulated and even predicted germination under field conditions. The large decrease of R^2 and increase of $\hat{\sigma}^2$ when C and H were replaced by the parameter time, indicates the validity of the use of cold and heat sum as a basis for the simulation of the dormancy pattern of *P. persicaria*.

Knowledge of the physiological processes responsible for the changes in dormancy should lead to a more mechanistic approach of the simulation of dormancy patterns. In addition, detailed knowledge about processes influencing temperature, nitrate and desiccation in the soil are a prerequisite for an accurate prediction of emergence in the field.

Chapter 3

Seasonal dormancy patterns in buried weed seeds. II. Sisymbrium officinale (L.) Scop.

Abstract. This study examined the effect of environmental conditions on germination and changes in dormancy of seeds of *Sisymbrium officinale* (L.) Scop.. Seeds were buried in soil under field and controlled conditions. After exhumation at regular intervals, germination was tested over a range of conditions. Seeds buried under field conditions showed clear seasonal changes in dormancy. Unburied seeds and seeds buried for only a few months germinated best at elevated temperatures. After induction of secondary dormancy had started, exhumed seeds germinated better at low temperatures, during the remaining two years of the experiment. Dormancy was relieved in periods of low temperatures (autumn-winter), whereas high temperatures in summer induced it. Light, nitrate added during the germination test and desiccation prior to the test stimulated germination. As a consequence, germination occurred during a much longer period of the year. Seasonal changes in soil moisture and nitrate content were not required for the seasonal changes in dormancy of buried seeds. The seasonal dormancy pattern of *S. officinale* was simulated on the basis of the dual role of temperature, on the one hand regulating dormancy and, on the other hand, affecting germination. This model fairly accurately simulated germination of exhumed seeds in Petri dishes at field temperature.

3.1 Introduction

Buried seeds of many weeds and ruderals annually pass through a cycle of changes in dormancy. Therefore, germination and emergence of these species are often restricted to certain periods of the year. Such dormancy patterns prevent germination in the seasons unfavourable for growth and development of the species (Karssen, 1982; Baskin and Baskin, 1985).

Temperature seems to have a dual role in the control of the seasonal emergence patterns. During burial, the field temperature seems to be the main driving force behind the changes in dormancy. When germination of exhumed seeds is tested, the main characteristic of the dormancy pattern appears to be the changes in the range of temperatures over which germination can proceed (Karssen, 1982). Baskin and Baskin (1981*a*,*b*, 1983*a*,*b*, 1984) have shown, that during dormancy relief, the range

of suitable germination temperatures widens and that during dormancy induction it narrows again. It was shown for seeds of two *Polygonum* spp. that as a consequence, the interpretation of the dormancy pattern depends on the temperature at which germination is tested after exhumation of the seeds (Chapter 2).

Several authors have also suggested a role for the soil moisture content in the control of changes in dormancy (Karssen, 1980/81*a*, Lonchamp *et al.*, 1984; Baskin and Baskin, 1987). The results were not unequivocal, however.

Sisymbrium officinale (L.) Scop. (hedge mustard) is a member of the *Brassicaceae* that is endemic in the Eurasian continent. It commonly grows on ruderal places and disturbed sites such as roadsides, waste land and field margins (P. Zonderwijk, pers. comm.).

In a preliminary study, Karssen (1980/81*b*) showed that the germination response of buried seeds of *S. officinale* undergoes seasonal changes. Other studies have shown that germination of seeds of *S. officinale* was highly stimulated by nitrate. Often germination depended on the combined action of light and nitrate (Karssen, 1980/81*b*, Karssen and De Vries, 1983, Hilhorst *et al.*, 1986, Hilhorst and Karssen, 1988, 1989, 1990). Desiccation of pre-incubated seeds followed by re-imbibition also increased germination of *S. officinale* (Karssen, 1980/81*b*).

In this thesis the dormancy pattern of buried *S. officinale* seeds was studied in more detail. The factors that control the changes in dormancy were investigated and the character of the pattern was analyzed by testing germination over a range of temperatures, in light or darkness, in water or nitrate and following desiccation treatments. Changes in dormancy were also studied under controlled conditions. The influence of several of these environmental factors on the seasonal emergence pattern was simulated with a preliminar descriptive model.

3.2 Materials and methods

Ripe seeds of *S. officinale* were collected on waste land in the vicinity of Wageningen. After collection, seeds were allowed to dry and then they were sieved and winnowed.

In addition to sandy loam, seeds of *S. officinale* were also buried in sand with a constant soil moisture content. Germination percentages were determined between three and 25 days after incubation. Further methods are the same as in Chapter 2.

3.3 Results

Germination of exhumed seeds

Temperature, nitrate and desiccation. Seeds of *S. officinale* were buried in December 1986 and at regular intervals samples were exhumed. Germination tests were performed at 2, 15, 24 and 30°C in light, in water (Fig. 3.1A,C) or 50 mM KNO_3 (Fig. 3.1B,D) with or without a preceding desiccation treatment (Fig. 3.1C,D versus Fig. 3.1A,B). The desiccation treatment was included until May 1989.

At burial, seeds of *S. officinale* were not deeply dormant (Fig. 3.1). Depending on the test conditions, seeds germinated to 10-75% (Fig. 3.1A,B). Germination increased in the range of 2 to 30°C, it was enhanced by nitrate (Fig. 3.1A,B). Desiccation did not affect germination of unburied seeds (Fig. 3.1C,D).

Primary dormancy was relieved rapidly after burial. Seeds that were exhumed at the end of February 1987, germinated to 100% over a temperature range of 2 to 30°C both in water and nitrate (Fig. 3.1A,B). From April 1987 onwards, dormancy induction started. From that moment the results of the tests in water and nitrate began to differ and desiccation started to stimulate germination of the exhumed seeds. Tests in water showed that within one to two months the germination capacity was lost at all temperatures except 2°C (Fig. 3.1A). Tests in nitrate revealed a more gradual closure of the temperature range that evidently began at the higher temperatures and gradually proceeded to 2°C (Fig. 3.1B). In contrast to unburied seeds at the beginning of the experiment, seeds now germinated best at low temperatures.

In September 1987, germination did not occur at any temperature in water or nitrate (Fig. 1A,B). However, desiccated seeds germinated throughout summer. A drop in germination capacity was seen only in nitrate at 30°C and in water also at 24°C (Fig. 3.1C,D).

From September 1987 onwards dormancy was relieved again. The widening of the germination-temperature range commenced at low values. As the test temperature increased germination started to rise later. In the second cycle, germination in both water and nitrate reached lower maxima than during the first cycle (Fig. 3.1A,B). Particularly in water at 24 and 30°C hardly any seeds germinated. Re-induction of dormancy started again around April 1988. Dormancy was not as absolute as the previous year. In water at 2°C some seeds still germinated; in nitrate, also at 15 and 30°C. After desiccation, no induction of dormancy was seen during summer 1988.

The start of the third cycle was again around September. Unlike the previous cycle, germination at 2°C in nitrate showed little difference with other test temperatures. In water, germination was still optimal at 2°C. Induction of dormancy started aroun April-May.

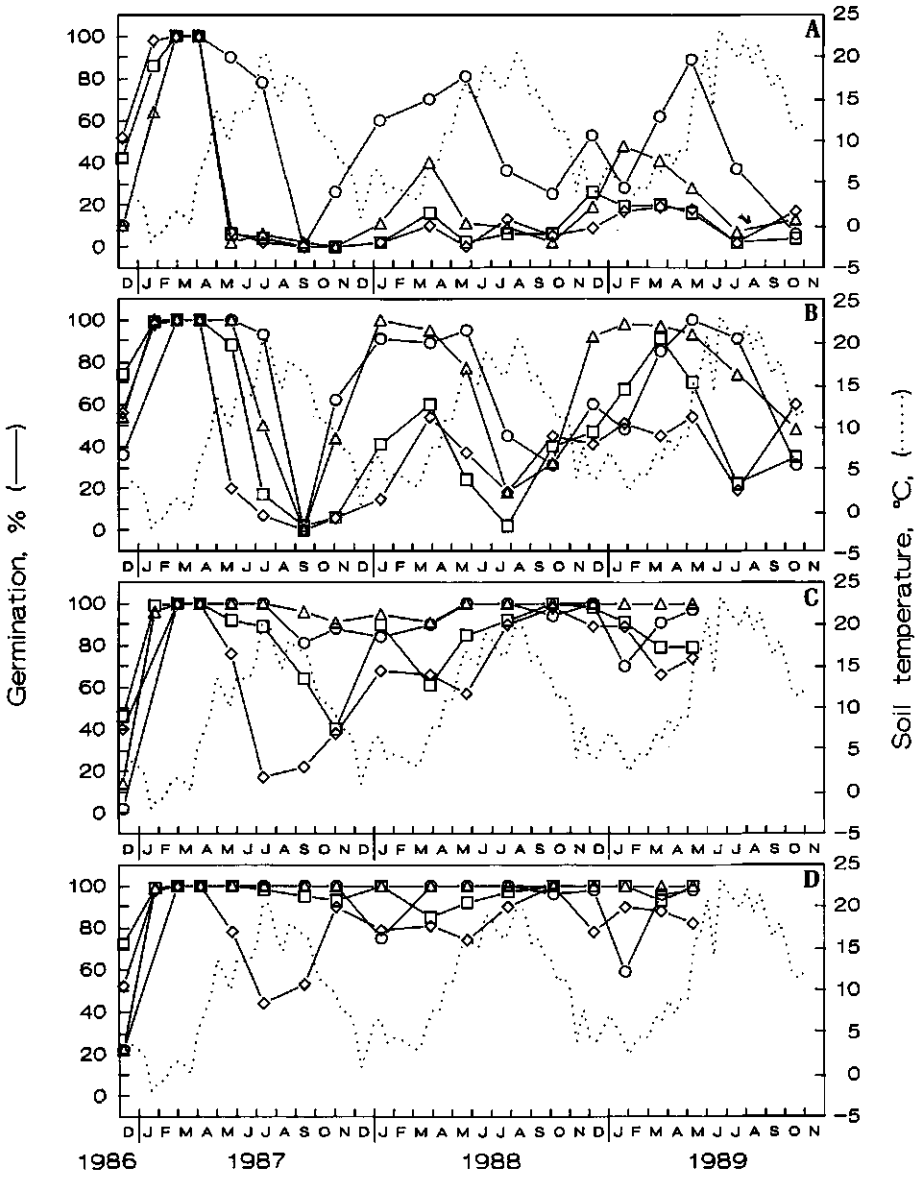

Fig. 3.1 Seasonal variation in germination of exhumed seeds of *Sisymbrium officinale* at different test temperatures. Seeds were buried in portions of 0.7 g in December 1986 in sandy loam under field conditions and exhumed at regular intervals. Germination was tested in Petri dishes after a 15 min red light irradiation with test samples of approx. 55 seeds at 2 (○), 15 (△), 24 (□) or 30°C (◇) in water (A,C) or in 50 mM KNO_3 (B,D), with (C,D) or without (A,B) a preceding desiccation treatment. Desiccation occurred for 24 h above a saturated solution of LiCl (r.h. approx. 16%) which gave a seed moisture content of approx. 6%. The dotted line indicates the soil temperature at 10 cm in bare soil.

Light. Germination of exhumed seeds at 2, 15 and 24°C was also tested in darkness. Only results of tests at 24°C are shown (Fig. 3.2B). They are compared with results of tests at 24°C in light (Fig. 3.2A). Before burial, seeds did not germinate in darkness (Fig. 3.2B). However, during burial seeds lost their light requirement. In April 1987 all seeds germinated in darkness both in water and in nitrate. After the re-induction of dormancy in spring 1987, germination capacity in darkness did not return during tests in water. In nitrate, seeds germinated to approx. 30% in darkness during spring 1988 and to approx. 50% in spring 1989. Also in light, germination in the second year was not as high as in the first (Fig. 3.2A). Desiccation also had a strong stimulatory effect on germination in darkness. Apart from a temporary reduction in autumn 1987, desiccated seeds germinated to 90-100% in darkness from March 1987 onwards in both water and nitrate. There were no differences in germination of desiccated seeds in light and darkness.

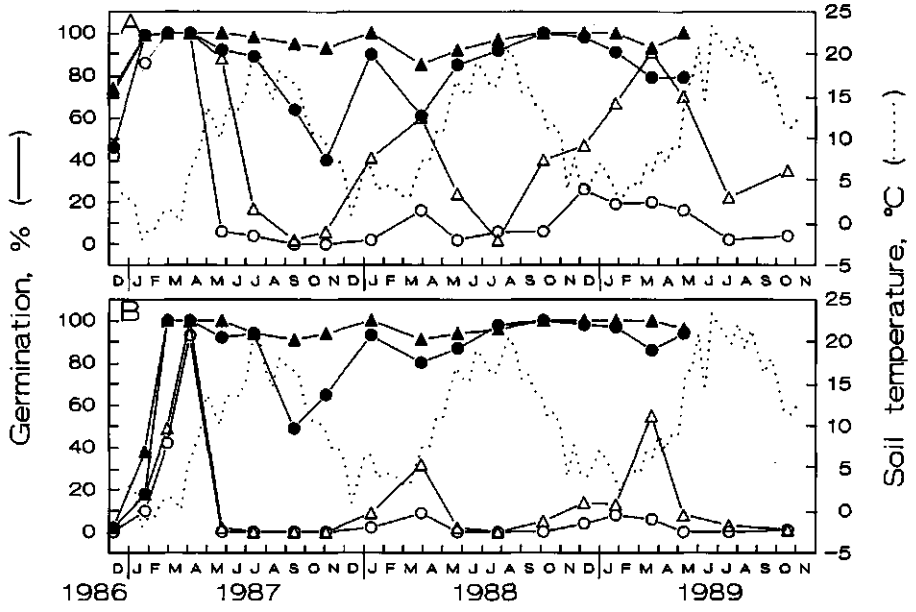

Fig 3.2 Effect of light, nitrate and desiccation on the seasonal variation of germination of exhumed seeds of *Sisymbrium officinale*. Burial and desiccation as in Fig. 3.1. Germination was tested in Petri dishes with test samples of approx. 55 seeds at 24°C with a 15 min red light irradiation (A) or in darkness (B) in water (circles) or 50 mM KNO_3 (triangles), with (closed symbols) or without (open symbols) a preceding desiccation treatment. The dotted line indicates the soil temperature at 10 cm in bare soil.

Control of dormancy pattern

The present results clearly showed the large effect the conditions of the germination test have on the **expression** of the dormancy pattern. These factors were only present during the test and could therefore not **cause** the seasonal changes in dormancy. The question is, which factors are responsible for these changes?

Soil moisture content. Soil moisture may be involved in the control of changes in dormancy. To investigate this, seeds were either buried outdoors in sandy loam with fluctuating moisture content or in containers under a roof in either sandy loam or quartz sand. The soil moisture content of the sand varied little and was approx. 3% (dwt) (Fig. 3.3A). The moisture content of the sandy loam in the field varied between approx. 17 and 27% (dwt) and showed seasonal fluctuations. Moisture content increased during winter and decreased during summer. In the covered treatment, the moisture content of the sandy loam varied between 12 and 19% (dwt) without the seasonal fluctuations that occurred in the uncovered treatment.

The nitrate content of sand was nearly always lower than the nitrate content of the sandy loam (Fig. 3.3B). However, because of the low water content, the estimated nitrate concentration in the soil solution was usually higher in sand than in the uncovered sandy loam (data not shown). The nitrate content of the covered sandy loam treatment increased during the course of the experiment and was always higher than in the other two treatments (Fig. 3.3B).

The differences in soil nitrate content, particularly the estimated nitrate concentration in the soil solution, were, from September 1987 onwards, reflected in the germination results of exhumed seeds (Fig. 3.3C). In water, seeds from the two covered treatments, always germinated much better than seeds that had been buried outdoors. These differences largely disappeared when germination was tested in nitrate except at the end of the experiment, when the nitrate content of the covered treatment had increased to a very high level (Fig. 3.3D). Although nitrate levels during burial influenced germination of exhumed seeds, the seasonal character of the dormancy pattern was not affected by the soil nitrate content. Although the moisture and nitrate content of the sand were stable at low values, the dormancy pattern showed the same seasonal changes as in the other treatments.

Temperature. The previous experiments suggested that the dormancy pattern is mainly controlled by the seasonal fluctuations in field temperature. After burial in December 1986 dormancy was relieved by the low winter temperature (Figs 3.1, 3.2). In the next cycles dormancy relief started when temperature dropped to 15-10°C. Induction of dormancy occurred when temperature increased above this value. The validity of this hypothesis was tested experimentally as well as statistically.

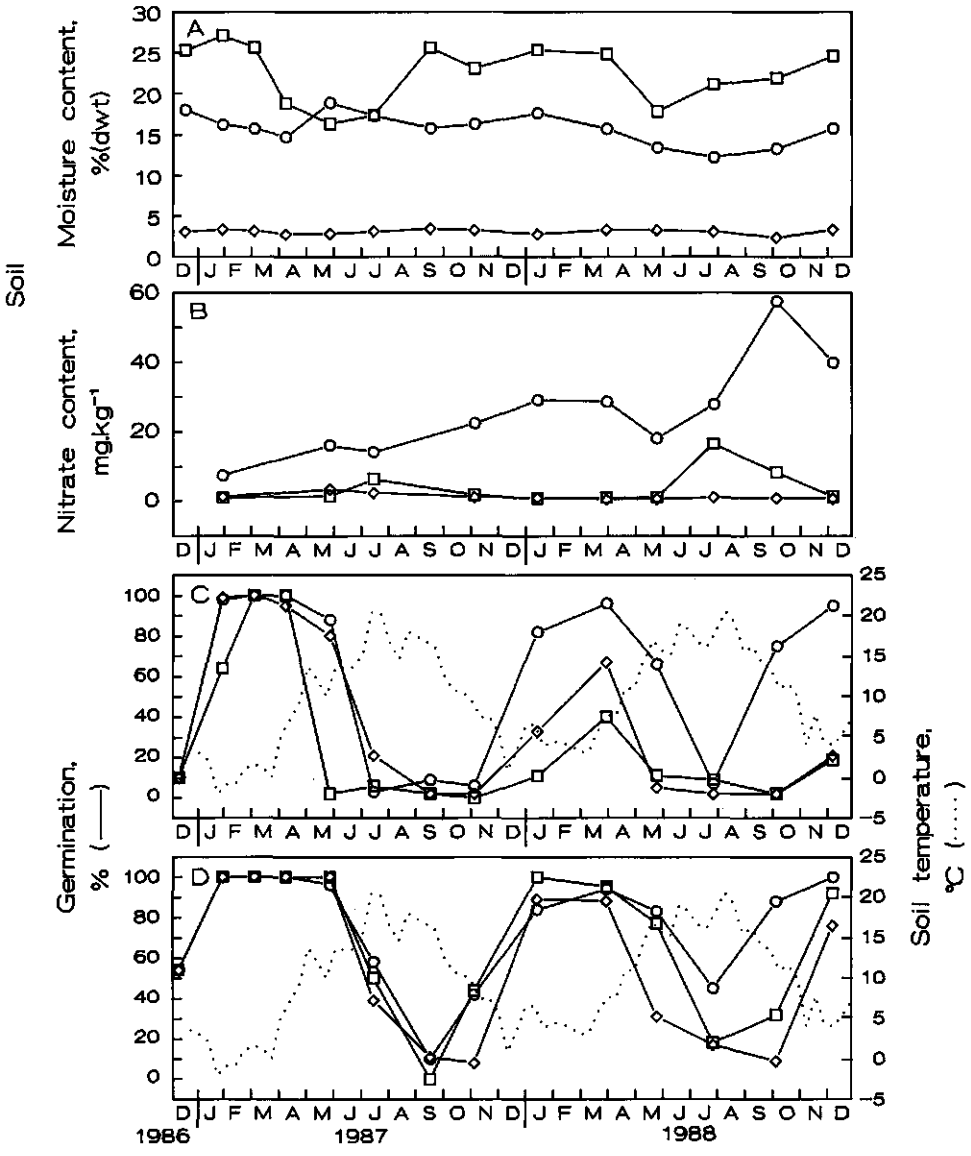

Fig. 3.3 Changes in soil moisture (A) soil nitrate content (B) and soil temperature (C,D) during burial of seeds of *Sisymbrium officinale* as compared to changes in germination of exhumed seeds (C,D). Seeds were buried in sandy loam under field conditions as in Fig. 3.1 (□) or in sandy loam (○) or quartz sand (◇) in a 100 l plastic container, sunk in the ground and covered with a plastic roof. In the containers soil moisture content was regulated at 3% (dwt) (quartz sand) or at 12 to 19% (dwt) (sandy loam) (A). At regular intervals, soil moisture and nitrate content were determined and germination tested as in Fig. 3.1 at 15°C in water (C) or 50 mM KNO_3 (D). The dotted line indicates the soil temperature at 10 cm in bare soil (C,D).

Experimental test. In Fig. 3.4A the results of germination tests in nitrate at 2, 15, 24 and 30°C in light and 24°C in darkness during the first eight months of the burial experiment are shown again (data from Figs 3.1B, 3.2). The results in Fig. 3.4A were compared with results from experiments with seeds that had been pretreated in Petri dishes with soil at a sequence of temperatures: 2°C → 6°C → 10°C → 15°C (Fig. 3.4B) or at constant temperatures (Fig. 3.4C,D).

When seeds were pretreated at rising temperatures, primary dormancy was released rapidly. However, when the temperature was increased from 10 to 15°C, secondary dormancy was induced within several weeks (Fig. 3.4B). The decrease in germination first occurred in tests at 24°C in darkness and 30°C in light, followed later by germination in tests at 24, 15 and 2°C in light. This pattern which was typical for seeds that had been buried in the field (Fig. 3.4A), was closely simulated by the pretreatment in Petri dishes (Fig. 3.4B).

In a first set of experiments where pretreatment occurred at constant temperatures, seeds were used with very little primary dormancy, which was rapidly lost during pretreatment at 2 and 6°C (Fig. 3.4C). At 10 and, especially at 15°C, secondary dormancy was induced immediately after the start of the experiment, followed subsequently by a loss of dormancy at the same pretreatment temperature (Fig. 3.4C). At the end of the experiment, some induction of secondary dormancy had occurred at 6 and maybe 10°C.

In a second experiment, seeds were first pretreated for 8 days at 24°C to induce secondary dormancy. Subsequently, the seeds were placed at 2, 6, 10 or 15°C. Alleviation of dormancy occurred significantly faster at 6 than at 2°C, differences between 10°C and 2 and 6°C were not significant. Dormancy relief at 15°C was a lot slower.

Statistical test. The role of temperature in the control of dormancy was also investigated statistically. Just as for *Polygonum persicaria* (Chapter 2), it was assumed that dormancy of *S. officinale* was a function of a cold and heat sum (C and H, respectively) as hypothesized by Totterdell and Roberts (1979) for *Rumex obtusifolius* and *R. crispus*. According to their theory, changes in dormancy were the result of two sub-processes: relief and induction of dormancy. The first sub-process - relief of dormancy - was independent of the actual temperature as long as it was below a critical temperature, in the present paper called the border temperature. The latter sub-process - dormancy induction - occurred at all temperatures and increased with increase of temperature. Because the rate of the latter process was lowest at low temperatures, **net** relief of dormancy was optimal at a temperature just above zero. This theory is explained in full detail in Chapter 2.

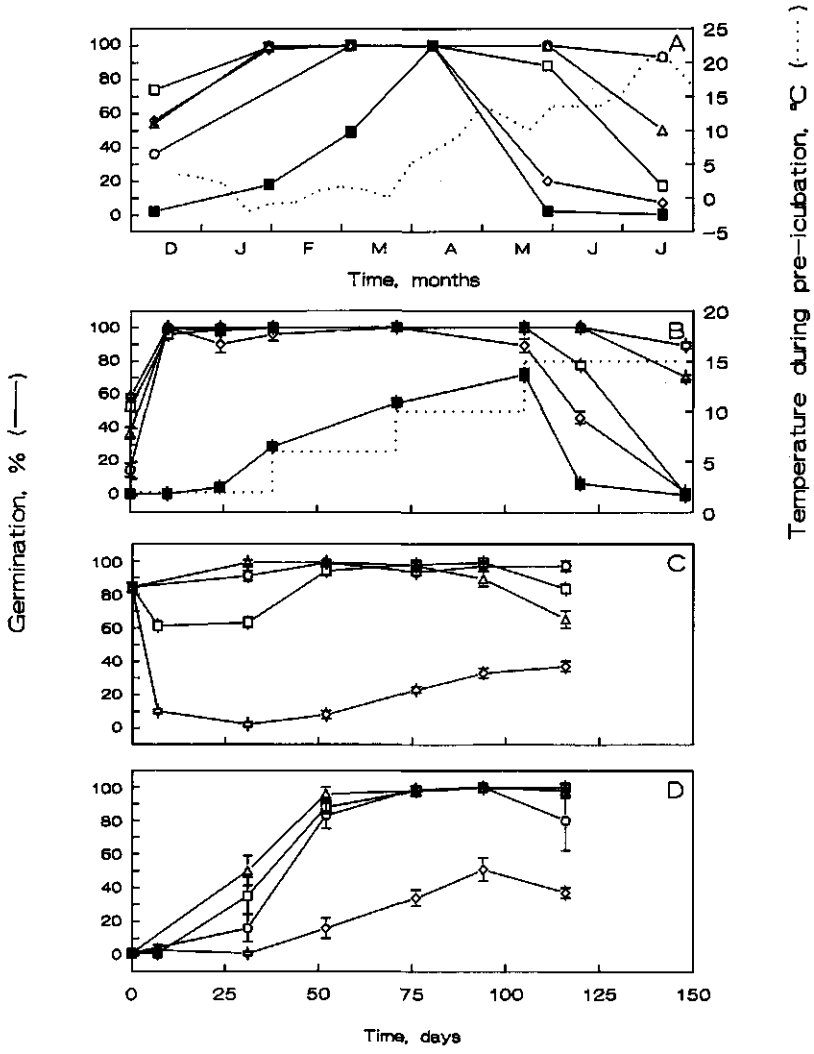

Fig. 3.4 Effect of temperature on dormancy and germination of *Sisymbrium officinale*. A. Seeds were buried in the field as in Fig. 3.1.

B. Seeds were pretreated in sandy loam in black 9 cm plastic Petri dishes between two layers of fine mesh gauze at a rising temperature: 2°C → 6°C → 10°C → 15°C.

For both A and B, portions of seeds were "exhumed" at regular intervals. They were divided into smaller portions and germination was tested in 50 mM KNO_3 after a 15 min red light irradiation at 2 (○), 15 (△), 24 (□) or 30°C (◇) or in constant darkness at 24°C (■). Each treatment consisted of one test sample of approx. 55 seeds (A) or of triplicates of approx. 70 seeds (B). The dotted line indicates the temperature at 10 cm in bare soil (A) or the temperature during pretreatment (B).

C,D. Seeds were pretreated in sandy loam at constant 2 (○), 6 (△), 10 (□) or 15°C (◇), with (D) or without (C) a preceding dormancy inducing pre-incubation of 8 days at 24°C. At regular intervals portions of seeds were "exhumed". Germination was tested with triplicates of approx. 50 seeds after a 15 min red light irradiation in 25 mM KNO_3 at 20°C. Vertical bars indicate standard error.

For *S. officinale* the theory of Totterdell and Roberts (1979), as described in Chapter 2, was applied as an approximation, because it is obvious that for this species temperatures somewhat higher than just above zero (approx. 6°C) were optimal for dormancy relief (Fig. 3.4D). The (pretreatment) temperature (T_p) in a period δt before exhumation ($T_{p,\delta t}$) seemed to influence germination of *Polygonum persicaria* and *Spergula arvensis* directly in addition to its influence on dormancy (Chapters 2 and 4). It was assumed, that this may also be true for *S. officinale*. Therefore, also $T_{p,\delta t}$ was tested for its influence on the seasonal germination pattern of *S. officinale*. Furthermore, nitrate strongly stimulated germination (Figs 3.1, 3.2) and therefore influenced the expression of the dormancy pattern. Accordingly, it was also tested whether the composition of the germination medium (M_g), *i.e.* the absence or presence of nitrate also influenced the seasonal changes in germination.

With the same methods as described for *Polygonum persicaria* (Chapter 2), the parameters that significantly maximized the fit of the data were selected. Only data from July 1987 onwards were used because germination in the months before July was a-typical for the rest of the dormancy pattern and would therefore decrease the fit. Data obtained after July 1989 were not used to make the model.

For the calculation of C different border temperatures were used. The calculation of C with a border temperature of 16°C gave the best fit: the highest correlation (R^2) and the lowest estimated variance ($\hat{\sigma}^2$) (Table 3.1). For the period δt (20, 30, 40, 50 or 60 days) in $T_{p,\delta t}$ a period of 30 days gave the best fit (data not shown). The expected transformed germination (G_t) could be estimated by:

$$G_t = (-0.257*C + 0.022*H - 1.797)*T_g + 9.036*C - 0.693*H - 2.916*T_{p,30} + 42.992*M_g + 62.467 \qquad (3.1)$$

where T_g is the germination temperature.

When germination was simulated with the parameter time (weeks of burial) instead of C and H, R^2 decreased from 0.77 to 0.68 and $\hat{\sigma}^2$ increased from 569 to 769.

Table 3.1 Estimated variance ($\hat{\sigma}^2$) and squared multiple correlation (R^2) of models that simulate germination of *Sisymbrium officinale* on the basis of cold and heat sum, germination temperature, germination medium and the mean temperature in 30 days before exhumation. Different border temperatures were used to calculate cold sum. See text for explanation.

	Border temperature (°C)								
	10	11	12	13	14	15	16	17	18
$\hat{\sigma}^2$	610	609	596	585	576	581	569	573	594
R^2	0.758	0.759	0.764	0.768	0.771	0.770	0.774	0.773	0.765

Germination under field conditions

From March 1987, germination of exhumed seeds was also tested in Petri dishes outdoors. Data calculated with Equation 3.1 and subsequently transformed back to germination percentages were compared to the results obtained in these tests.

In the second and third cycle, germination in water in Petri dishes outdoors occurred from approx. December to April-May (Fig. 3.5). The period in which germination occurred was extended by several months when germination of exhumed seeds was tested in nitrate. Unlike the previous years, germination in nitrate did not stop in summer 1989. After desiccation, seeds germinated to 100% throughout the experiment. Germination in Petri dishes outdoors occurred in a clear seasonal pattern that was the reverse of the changes in temperature. Germination occurred in the seasons with low temperatures and was inhibited in seasons with high temperatures.

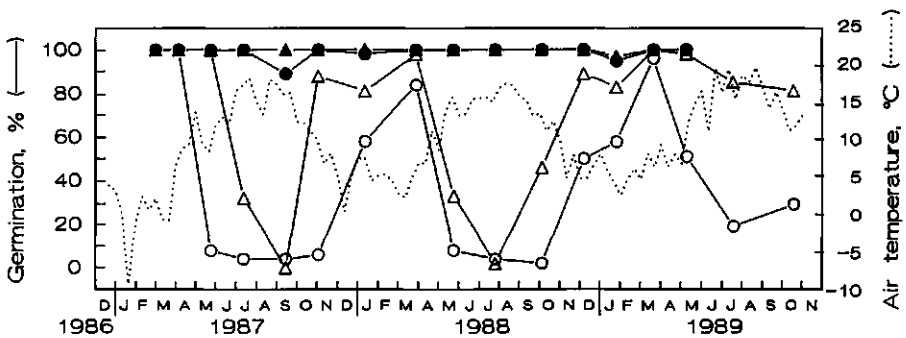

Fig. 3.5 Seasonal variation in germination of exhumed seeds of *Sisymbrium officinale* tested outdoors. As Fig. 3.2 except the Petri dishes with seeds were incubated in airtight plastic boxes covered with black polyethylene, placed outdoors at a height of 1.50 m in the shade. The broken line indicates the air temperature at 1.50 m.

Karssen (1982) hypothesized that germination in the field is restricted to the period where field temperature and germination-temperature range overlap. To illustrate this for *S. officinale*, Equation 3.1 was used to compute the minimum and maximum temperature required for 50% germination ($T_{g,min}$ and $T_{g,max}$, respectively) in water or nitrate (Fig. 3.6). $T_{g,max}$ and $T_{g,min}$ were calculated for the period of April 1987 to October 1989, whereas the model was made with data from July 1987 to May 1989. To maintain ecological significance, calculations were restricted to the range of 0°C to 30°C. Over the range of temperatures between $T_{g,min}$ and $T_{g,max}$ 50% germination or more would be possible. If $T_{g,min}$ equals $T_{g,max}$ germination can not occur.

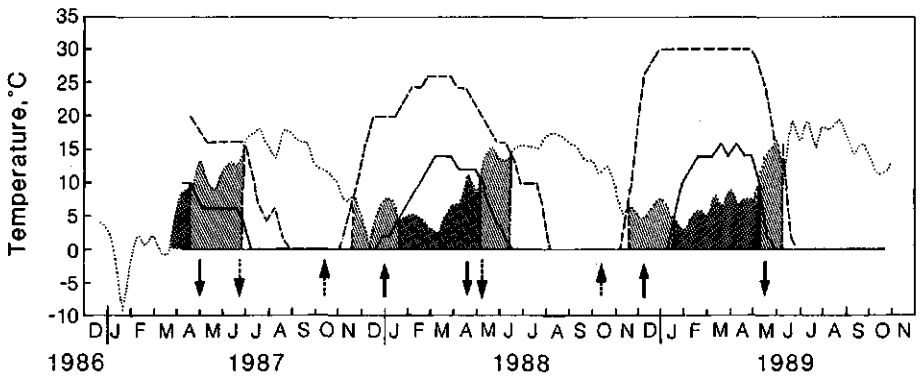

Fig. 3.6 Seasonal changes in the range of temperatures over which at least 50% of exhumed *Sisymbrium officinale* seeds germinate. Solid and broken lines represent maximum and minimum temperature required for 50% germination in water and KNO_3, respectively, calculated with Equation 3.1. Dotted line indicates air temperature at 1.50 m (see text for explanation). Hatched areas indicate overlap of field temperature and germination-temperature range in water (▩) or 50 mM KNO_3 (▨). Arrows (solid for water, broken for KNO_3) indicate the moment germination in Petri dishes outdoors actually increased above (↑) or decreased below 50% (↓) (data from Fig. 3.5).

The hatched areas, where field temperature and germination-temperature range overlap are the periods of predicted field germination (50% or more in Petri dishes outdoors). The arrows indicate the moment that actual germination in Petri dishes outdoors increased (↑) or decreased (↓) to 50% (data from Fig. 3.5). The periods of predicted germination to 50% or more (hatched areas) agreed in outline with the data from Fig. 3.5 (see arrows). Differences of one to two months between actual and simulated beginning and end of these periods did however occur.

With air temperature at 1.50 m (the height seeds were placed outdoors) as T_g in Equation 3.1, the germination pattern outdoors in water and nitrate as depicted in Fig. 3.5 was simulated fairly accurately (Fig. 3.7). The unexpected high germination in nitrate in July 1989 was not predicted.

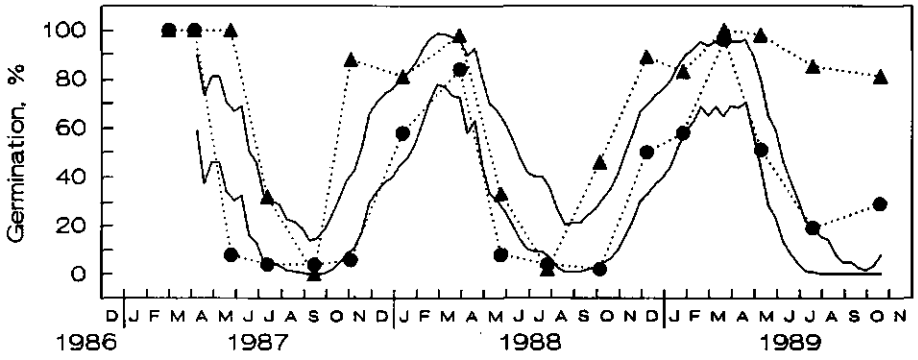

Fig. 3.7 Simulated (intact line) and observed (dotted line) germination of exhumed seeds of *Sisymbrium officinale* in Petri dishes outdoors as a function of exhumation date. Germination outdoors as in Fig. 3.5 in water (●) or 50 mM KNO_3 (▲). Simulation was carried out with Equation 3.1. See text for explanation.

3.4 Discussion

Dormancy pattern

The present study clearly showed the large influence of germination-test conditions on the expression of the seasonal dormancy pattern. Seasonal changes in dormancy were most apparent when germination was tested in nitrate at low temperatures (Fig. 3.1B). In water, particularly the second and third cycle were less clear (Fig. 3.1A). After desiccation, the changes in dormancy were no longer visible because seeds almost invariably germinated to 100% (Fig. 3.1C,D).

The three successive cycles in the dormancy pattern of *S. officinale* differed considerably. This was most evident in tests in water (Fig. 3.1A), and in nitrate at 24°C, both in light and darkness (Fig. 3.2). The most striking difference was, that when induction of secondary dormancy had started in April 1987, exhumed seeds germinated better at low than at high temperatures, whereas unburied seeds or seeds buried for only limited time behaved in the opposite way. In the next cycles low temperatures remained optimal for germination, particularly in water (Fig. 3.1A).

The results of the burial experiment showed, that dormancy relief occurred at temperatures below approx. 10-15°C and dormancy induction at temperatures above this value (Fig. 3.1). The experiment with seeds buried under controlled temperature conditions in incubators showed, that when temperature was increased from 10 to 15°C secondary dormancy was induced (Fig. 3.4B).

When seeds were incubated at constant temperatures, dormancy relief occurred at 2, 6 and 10°C, but also at 15°C (Fig. 3.4C,D). Apparently, the effect of temperature also depends on the physiological state of the seeds. When they are non-dormant, high temperatures lead to induction of dormancy (Fig. 3.4A,B,C). When they are dormant, the same temperature may also alleviate dormancy (Fig. 3.4C,D).

Summer or winter annual

The criterion to characterize a species as a winter or summer annual, is the period of germination and emergence. Summer annuals germinate and emerge in early spring-early summer, (facultative) winter annuals in autumn (and early spring) (Baskin and Baskin, 1987). Additionally, winter annuals can survive winter as a seedling or young plant, whereas summer annuals are killed by frost.

In summer annuals such as *Ambrosia artemisiifolia* (Baskin and Baskin, 1980; Chapter 1) and *Polygonum persicaria* (Chapter 2), the induction and relief of dormancy is characterized by respectively an increase and a decrease of the **minimum** temperature at which germination can proceed (Karssen, 1982; Baskin and Baskin, 1985; Chapter 1). Low winter temperatures break dormancy, *i.e.* they increase the width of the germination-temperature range, whereas dormancy-inducing summer temperatures narrow the range.

In winter annuals the **maximum** temperature required for germination shows seasonal fluctuations. This was clearly shown for *Lamium amplexicaule* by Baskin and Baskin (1981*a*) (Karssen, 1982; Chapter 1). Both primary and secondary dormancy of seeds of the winter annual *Veronica hederifolia* were relieved during summer (Roberts and Lockett, 1978). Baskin and Baskin (1976, 1986) showed that several other winter annuals required wet or dry storage at elevated temperatures to loose dormancy. Low temperatures were ineffective.

The characterization of *S. officinale* is rather complicated because the fluctuations in the germination-temperature range during the first period of dormancy breaking were typical for summer annuals, whereas later on germination of exhumed seeds was better at low temperatures, a characteristic feature of winter annuals.

An argument in favour of classification as a summer annual, is the relief of dormancy, both in field experiments and under controlled conditions, by low temperatures and the re-induction of dormancy at high temperatures.

Also statistically the changes in dormancy of *S. officinale* showed the characteristics of a summer annual. Similarly to *Polygonum persicaria* changes in dormancy were described fairly well with the concept of a cold and heat sum. Moreover, the border temperatures used to compute the cold sum were almost identical (16°C for *S. officinale*, 15°C for *P. persicaria*).

However, dormancy of *P. persicaria* was relieved slowly and was expressed by an increase of the number of seeds that germinated at elevated temperatures. Therefore

germination of *P. persicaria* could not occur in autumn. Although also dormancy relief of *S. officinale* did not start until autumn, the seeds were able to germinate before winter because dormancy relief was quick and the widening of the germination-temperature range started (except in the first year) at the lower end of the range (Figs 3.5, 3.6).

In conclusion, the emergence in autumn-early spring and the changes in the maximum temperature of the germination-temperature range favours the characterization of *S. officinale* as a winter annual, whereas the effects of temperature on the dormancy pattern (relief of dormancy at low, induction at high temperatures) are typical for summer annuals. Nevertheless, it is suggested to name *S. officinale* a facultative winter annual. The interesting conclusion is that such behaviour can be reached either in the classical way (high temperatures break and low temperatures induce dormancy) or in the way of a summer annual, but with a quick relief of dormancy and a winter-annual like germination-temperature range.

Ecological consequences. The ability to lose dormancy also at more elevated temperatures as shown in Fig. 3.4C,D could be of ecological importance. Although mature *S. officinale* seeds were always harvested in autumn, in the field seeds often remain in closed silicles on the mother plant during winter (P. Zonderwijk, pers. comm.). If the seeds are shed at first in spring, a requirement for a prolonged period of cool temperatures for the loss of dormancy could prevent germination that same spring. The rapid loss of dormancy at elevated temperatures, enables the seeds of *S. officinale* to germinate almost immediately after they are shed. Germination under unfavourable conditions and/or too late in spring will be prevented by the subsequent rapid induction of secondary dormancy at the same high temperatures.

The summer-annual like temperature range of fresh seeds might be an adaptation to this late dispersal. To enable germination in spring the range should be open at the high, rather than at the lower end of the temperature spectrum. This hypothesis is somewhat strengthened by the fact that fresh seeds of the closely related winter annual *Arabidopsis thaliana*, which are dispersed in summer and do not remain on the mother plant, exhibit a winter-annual like germination-temperature range directly after maturation (M.P.M. Derkx, pers. comm.).

Control of germination

Light. The present observation, that exhumed *S. officinale* seeds were able to germinate in darkness is in accordance with burial experiments with seeds of *Verbascum blattaria*, *V. thapsus* and *Panicum dichotimiflorum* (Baskin and Baskin, 1981*b*, 1983). In April 1987, exhumed seeds germinated to more than 50% in darkness at 24°C (Fig. 3.2B) and 15 and 2°C (data not shown). Surprisingly, seeds

of *S. officinale* did not germinate in soil during burial, in spite of their capacity to germinate in darkness over such a wide range of temperatures.

Soil factors other than the lack of light have to be responsible for the prevention of germination during burial. Improper aeration or the presence of volatile inhibitors have been suggested as inhibitory conditions (Wesson and Wareing, 1969*b*, Baskin and Baskin, 1981*b*, Karssen, 1982).

Dark germination of exhumed seeds of *S. officinale* was much lower in the second spring (1988), just like the results of Baskin and Baskin (1981*b*) with *V. blattaria* and *V. thapsus*. Probably, dark germination is due to the presence of pre-existing Pfr, the active, far-red absorbing form of phytochrome. High summer temperatures may either stimulate destruction of Pfr or its reversion to Pr, the inactive red absorbing form of phytochrome, which would decrease the capacity to germinate in darkness. It is unlikely that the rise of dark germination in spring 1987, 1988 and 1989 was due to an increase of Pfr. Changes in sensitivity to Pfr or the products of its action are more likely explanations. Germination in **light** was also strongly reduced in the second and third cycle. A general decreased responsiveness to germination stimulants may also explain the decreased dark germination in the second and third cycle. Nevertheless, in the long run buried seeds of *S. officinale* developed a nearly absolute light requirement as described by Wesson and Wareing (1969*a*,*b*) for several species. It is an important feature for species that form persistent seed banks.

Nitrate. Hilhorst *et al.* (1986) showed that the stimulating action of nitrate in *S. officinale* seeds depended on the simultaneous presence of Pfr. At first view, their conclusion seems to disagree with the present observation of dark germination in water and of the stimulation of dark germination by nitrate (Fig. 3.2B).

However, it has to be realized that in the present experiments seeds of *S. officinale* were exposed during burial to low temperatures for prolonged periods of time (3 to 4 months), whereas in the experiments of Hilhorst *et al.* (1986) seeds were pre-incubated for only 40 h at 15°C. Prolonged pretreatment at low temperatures, may have increased the sensitivity of the seeds to Pfr to such an extent that seeds could react to the dark level of Pfr. The apparent lack of requirement for nitrate, as suggested by the dark germination in water, is probably accounted for by the uptake of nitrate from the soil solution during burial (Chapter 7; Goudey *et al.*, 1988).

The stimulation of germination by nitrate changed the form of the dormancy pattern of *S. officinale* (Figs 3.1, 3.2A, 3.5, 3.7). Nitrate increased the width of the germination peaks by several months. In the present experiments, a rather high, saturating concentration of 50 mM KNO_3 was used. Stimulation of germination of *S. officinale* occurs already in the range of 1 to 10 $mmol.l^{-1}$ (Hilhorst and Karssen, 1989). Small differences in nitrate content that occur between soil types and within soils (Chapter 7) may therefore strongly influence germination and emergence of *S. officinale*.

Desiccation. A desiccation treatment prior to the germination test was very stimulatory to germination irrespective of the other test conditions such as light, temperature and nitrate (Figs 3.1C,D, 3.2A,B, 3.5). The effect of desiccation was determined by the rate of drying and/or the seed moisture content after desiccation (Chapter 6).

A stimulating effect of desiccation has been reported before for *Verbascum blattaria* (Kiviliaan, 1975), *S. officinale* (Karssen (1980/81*b*; Chapter 6), *Spergula arvensis* (Karssen *et al.*, 1988; Chapters 4 and 6) and *Chenopodium album* (Chapters 5 and 6).

Karssen *et al.* (1988) were the first to show that the stimulatory effect of desiccation on germination of *Spergula arvensis* seeds was not an incidental effect but occurred throughout a simulated compressed "year" of burial in an incubator. In the present study it was shown that germination of exhumed *S. officinale* seeds was stimulated by desiccation throughout two and a half years of burial in the field (Figs 3.1C,D, 3.2A,B, 3.5).

It is concluded that sensitivity to desiccation may serve as an additional depth and gap sensing mechanism to nitrate (Pons, 1989) and light and alternating temperatures (Thompson and Grime, 1983; Chapter 6). In Chapter 6 it is shown that desiccation to moisture contents that can stimulate germination, may occur in the field.

Control of dormancy

Soil moisture content. The present data showed that seasonal fluctuations in soil moisture and nitrate content were not required for the changes in dormancy of buried *S. officinale* seeds (Fig. 3.3). The fact that changes in dormancy also occurred in Petri dishes in soil with a constant soil moisture content (Fig. 3.4) supports this conclusion.

Induction of secondary dormancy in *S. officinale* occurred faster in saturated than in low humidity soil (Karssen, 1980/81*a*). In the wet treatment the nitrate concentration is likely to be lower and/or possibly an accelerated leakage of endogenous nitrate from the seeds may have occurred. The lower nitrate availability may have suggested that induction of secondary dormancy was faster, because germination was inhibited by a lack of nitrate.

The effect of burial of seeds of *S. officinale* in sand was also investigated by Karssen (1980/81*b*). Upon exhumation, seeds that had been buried in sandy loam germinated much better than seeds that had been buried in sand. This difference disappeared when nitrate was added to the sand. In experiments under controlled conditions it was shown that nitrate was not required during the changes in dormancy, but only had to be present during actual germination (Karssen and De Vries, 1983). It appears therefore, that the absence or presence of nitrate during burial does not influence changes in dormancy. The amount of nitrate available at the moment seeds

are irradiated determines the expression of the actual dormancy state of the seed. If nitrate is available, seeds germinate better than when little or no nitrate is present, regardless of whether the nitrate was added momentarily or was already there during pretreatment (see *e.g.* Figs 3.2A, 3.3).

Simulation

Equation 3.1 fairly accurately simulated changes in dormancy and germination outdoors. However, at the end of the three year experiment there was already a large discrepancy between simulated/predicted and actual germination in nitrate (Fig. 3.7). For *Chenopodium album*, *Polygonum persicaria* and *Spergula arvensis* there were no large differences between the three successive cycles of the dormancy pattern (Chapters 2, 4 and 5). Nevertheless, it seems that studies extending over even longer periods than three years are required to elucidate the effect of time on the seasonal dormancy pattern of *S. officinale*.

Environmental factors such as temperature, light, nitrate and desiccation had a large effect on the expression of the dormancy pattern (Figs 3.1, 3.2, 3.5). Much research will be needed to predict the effect of meteorological parameters such as air temperature, sunshine, precipitation, and clouds on all factors that influence the expression of the dormancy pattern.

Although the concept of prediction of changes in dormancy based on a cold and heat sum is promising, more research is needed to explain the phenomena that were found under controlled conditions such as changes in dormancy at a constant temperature (Fig. 3.4). Further knowledge of these phenomena may lead to a more mechanistic approach of seasonal dormancy and emergence patterns.

Chapter 4

Seasonal dormancy patterns in buried weed seeds. III. Spergula arvensis L.

Abstract. The effect of environmental factors on germination and changes in dormancy of seeds of *Spergula arvensis* L. was investigated. Seeds were buried in the field and under controlled conditions. After exhumation at regular intervals, germination was tested over a range of conditions. Seeds buried under field conditions, showed clear seasonal changes in dormancy during three successive years. Dormancy was relieved in spring and re-induced in autumn at a rising and falling temperature, respectively. In experiments in incubators, dormancy was relieved better at 15°C than at 2, 6 and 10°C. Like in the field, induction of dormancy occurred when the pre-incubation temperature was lowered. The expression of the dormancy pattern was strongly influenced by the germination test conditions. At 15°C, seeds germinated during a longer period of the year than at 2 or 30°C. Irradiation with red light, addition of nitrate and desiccation of the seeds prior to the germination test strongly stimulated germination. All three factors enabled germination of exhumed seeds during a longer period of the year. When light, nitrate and desiccation were combined, exhumed seeds germinated in all seasons. The changes in dormancy of buried seeds were regulated by the seasonal fluctuations in temperature and were not influenced by soil moisture and/or nitrate content. The seasonal germination pattern was simulated with a descriptive model, based on the dual effect of temperature on both dormancy and germination. With this model, germination at field temperature was closely simulated. Germination of exhumed seeds in the field was restricted to the period of overlap between the germination-temperature range (computed with the model) and field temperature.

4.1 Introduction

Spergula arvensis L. (corn spurrey) has been characterized as one of the world's most serious weeds (Holm *et al.*, 1977). It is particularly a weed of cereals in almost all areas of the world, but has also been reported to be a weed in at least 20 other crops. *S. arvensis* is a cosmopolitan species. It is not only a weed in the most northerly agricultural areas of Finland and Alaska but is also found as far south as Tasmania. It is found north of the arctic cycle, but also in high places on the equator. *S. arvensis* prefers to grow in open places and on arable land on light, acid soils (Holm *et al.*,

1977).

In New-Zealand, *S. arvensis* is one of the species that has become tolerant to the use of phenoxy herbicides in cereal fields and pastures (Holm *et al.*, 1977). This particular group of herbicides hardly endangers the environment because they are easily broken down (Zonderwijk, pers. comm.). Nevertheless, the persistent use of (other) herbicides is, apart from the risk of tolerance or resistance, unacceptable from an environmental point of view. Therefore, the development of systems for integrated weed control should be encouraged. A prerequisite for such integrated control is the ability to predict emergence of weeds in the field. Seeds of many weeds and ruderals pass through seasonal changes in dormancy that restrict germination to a more or less fixed period of the year (Karssen, 1982; Baskin and Baskin, 1985). These changes in dormancy prevent germination in the season that is unfavourable for growth and reproduction. The dormancy patterns are studied with buried seeds. Part of these seeds is exhumed at regular intervals for germination tests and changes in germination percentages indicate changes in dormancy. The temperature during these germination tests strongly effects what is seen of the dormancy pattern (Baskin and Baskin, 1981*a,b*, 1983*a,b*, 1984). As dormancy is relieved, the range of suitable germination temperatures widens and it narrows again when dormancy is induced (Karssen, 1982).

The field temperature is most likely to be the driving force behind the changes in dormancy. Low temperatures break dormancy of summer annuals such as *Polygonum persicaria* (Chapter 2), whereas in most winter annuals these temperatures induce dormancy (Baskin and Baskin, 1984). The latter species require high temperatures for breaking of dormancy (Baskin and Baskin, 1976, 1986; Roberts and Lockett, 1978; Roberts and Neilson, 1982*a*).

In a previous study, it was demonstrated that *S. arvensis* can not easily be classified as a summer or a winter annual. On the one hand, its seeds germinate in spring and summer, a characteristic feature of a summer annual, on the other hand, dormancy seems to be relieved by high temperatures, which is typical for winter annuals (Karssen *et al.*, 1988).

Germination of *S. arvensis* is stimulated by light (Olatoye and Hall, 1972; Vincent and Roberts, 1979; Karssen *et al.*, 1988), but also by nitrate and desiccation (Vincent and Roberts, 1979; Post, 1984; Karssen *et al.*, 1988). Germination of *S. arvensis* is also stimulated by ethylene and CO_2 (Olatoye and Hall, 1972; Jones and Hall, 1979). Ethylene may accumulate in water saturated soil and act as an indicator for the presence of sufficient water for successful germination and emergence (Olatoye and Hall, 1972).

Previously, the dormancy pattern of *S. arvensis* was studied using an incubator programmed with a condensed annual temperature cycle where each day represented the mean temperature of five successive days of an average year (Karssen *et al.*, 1988). The present paper shows results of experiments where the dormancy pattern was studied during burial of seeds under field conditions for three years. Germination

was tested at different temperatures and the effects of light, nitrate and desiccation on the germination of the exhumed seeds were investigated. The effect of several of these environmental factors on the seasonal emergence pattern was simulated.

4.2 Materials and methods

Ripe seeds of *S. arvensis* were collected in 1986 and 1987 from a non-fertilized experimental field in the vicinity of Wageningen. After collection, seeds were allowed to dry and then they were sieved and winnowed.

Seeds collected in July 1986 were buried in December of the same year. The next year, seeds collected in July were buried in August. Germination percentages were determined between five and 25 days after incubation. Other methods are described in Chapter 2.

4.3 Results

Germination of exhumed seeds

Seeds of two different seed lots of *S. arvensis* were buried under field conditions, either in December 1986 or August 1987. Portions of seeds were exhumed at regular intervals, and germination of exhumed seeds was tested over a range of temperatures in water or KNO_3 (Fig. 4.1). The effect of an irradiation with red light at the beginning of the germination test was compared to germination in darkness (Fig. 4.2). The effect of desiccation of the seeds prior to the germination test was also studied (Fig. 4.2).

Temperature and nitrate. Before burial in December 1986 seeds of *S. arvensis* were deeply dormant. No seeds germinated in water (Fig. 4.1A) and not more than 10% germinated in nitrate (Fig. 4.1B). In tests with exhumed seeds, germination rose in spring 1987 and decreased in late summer-early autumn, a pattern that was repeated during the next two years (Fig. 4.1A,B). The expression of the dormancy pattern strongly depended on the conditions of the test. During germination in water, changes in dormancy were most evident at 15°C. At more extreme temperatures such as 2 and 30°C, the germination tests showed the pattern of changes less clearly (Fig. 4.1A). Addition of KNO_3 to the germination medium, strongly stimulated germination, particularly at the higher temperatures. Data of tests in KNO_3 at 30°C now resembled germination at 15°C, germination at 2°C was less stimulated (Fig. 4.1B).

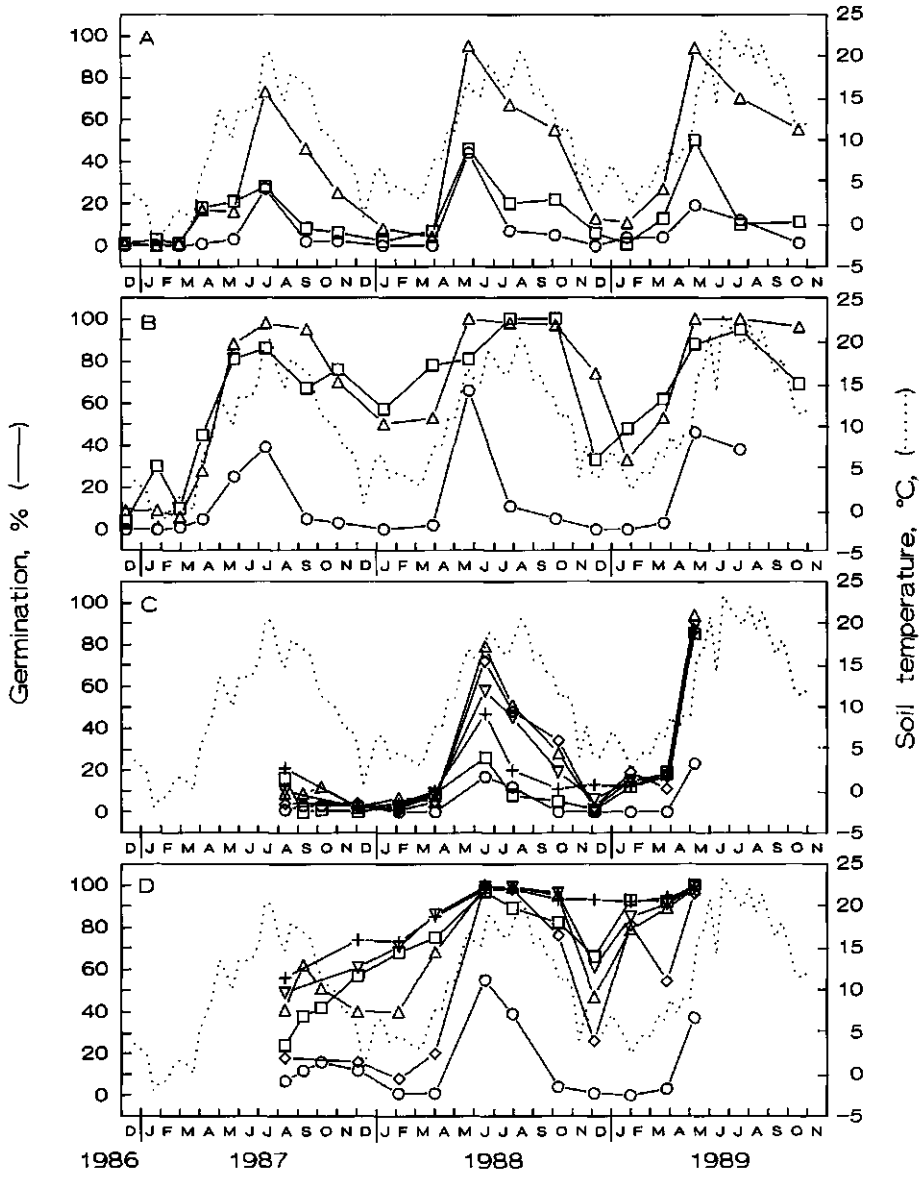

Fig. 4.1 Seasonal variation in germination of exhumed seeds of *Spergula arvensis* at different test temperatures. Seeds collected in July 1986 were buried in portions of 0.6 g in December 1986 (A,B), seeds collected in July 1987 in portions of 1.2 g in August 1987 (C,D). Burial occurred in sandy loam under field conditions and at regular intervals seeds were exhumed. Germination was tested in Petri dishes after a 15 min red light irradiation with test samples of approx. 50 seeds (A,B) or triplicates of approx. 60 seeds (C,D), at 2 (○), 10 (◇), 15 (△), 20 (▽), 25 (+) or 30°C (□) in water (A,C) or in 25 (D) or 50 (B) mM KNO_3. The dotted line indicates the soil temperature at 10 cm in bare soil.

The seeds that were collected and buried in summer 1987 were less dormant than the 1986 seeds. This was best seen in a germination test in nitrate at 15°C (Fig. 4.1B,D). After burial in August, the seeds immediately showed the same changes in dormancy as seeds that had been buried for almost one year. Tests in water showed a slight but clear induction of dormancy. These changes were similar to those observed in the same period for seeds buried in December 1986. Also further tests both in water and in nitrate showed that the dormancy patterns of December and August buried seeds kept pace with each other. In spite of the use of 25 instead of 50 mM KNO_3 in the tests with the August-buried seeds, these seeds germinated to higher percentages during winter 1988/89 than December-buried seeds. Particularly at 25°C, but also at 30°C, germination was barely reduced during this winter (Fig. 4.1B,D).

Light and desiccation. From May 1987 onwards, tests at 15°C were also performed without a preceding red light irradiation (Fig. 4.2B). Germination in these tests was compared with germination of seeds that were irradiated with red light (Fig. 4.2A). Germination of *S. arvensis* entirely depended on light during tests in water as well as nitrate. Until May 1989, tests also included a desiccation treatment, which strongly stimulated germination (Fig. 4.2). When desiccation and red light were combined, germination was constantly at or close to 100% from March 1987 onwards (Fig. 4.2A). In darkness, desiccation also strongly increased germination (Fig. 4.2B). In tests at 30°C in nitrate, particularly following desiccation, occasionally incomplete germination occurred. This incomplete germination was characterized by a splitting of the seed coat opposing the radicle, without protrusion of the radicle through endosperm and testa. It closely resembled incomplete germination of *Chenopodium album* (Karssen, 1976*a*; Chapter 5).

Incompletely germinated seeds rapidly completed germination after exposure to diffuse laboratory light, also at high temperatures. Incompletely germinated seeds were desiccation tolerant and could be stored dry for several weeks. Upon re-imbibition at all temperatures, germination was rapidly completed.

Control of dormancy pattern

The present results clearly showed that temperature, light, nitrate and desiccation through their effect on germination, strongly influenced the expression of the changes in dormancy. These factors were only present during the germination test and could therefore not **cause** the changes in dormancy. Considering the seasonal character of the changes, they are likely to be regulated by (a) factor(s) closely associated with the seasons.

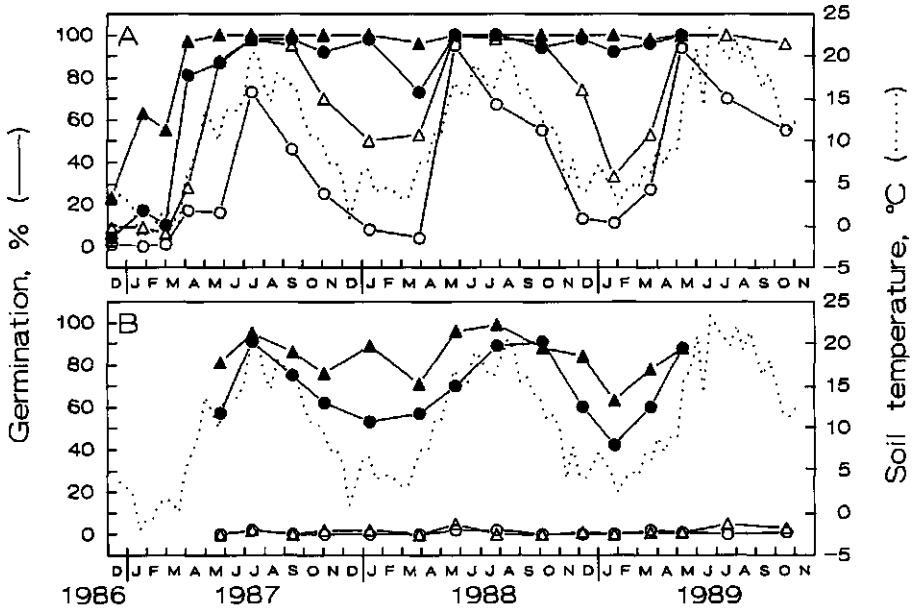

Fig 4.2 Effect of nitrate and desiccation on the seasonal variation of germination of exhumed seeds of *Spergula arvensis*. Burial as in Fig. 4.1 for 1986 seeds. Germination of exhumed seeds was tested in Petri dishes with test samples of approx. 50 seeds, at 15°C with a 15 min red light irradiation (A) or in darkness (B) in water (circles) or 50 mM KNO_3 (triangles) with (closed symbols) or without (open symbols) a preceding desiccation treatment. If appropriate, seeds were desiccated for 24 h above a saturated solution of LiCl (r.h. approx. 16%), which gave a seed moisture content of approx. 6%.

Soil moisture content. Lonchamp *et al.* (1984) suggested that the seasonal changes in soil moisture content could play a role in the regulation of the dormancy patterns of *Aethusa cynapium*, *Euphorbia exigua* and *Alopecurus myosuroides*.

To test this hypothesis for *S. arvensis*, seeds of this species were buried in sandy loam with either a naturally fluctuating or a more or less constant moisture content. The latter condition was achieved at the same location under a transparent roof. The moisture content of the uncovered soil varied between approx. 17 and 27% (dwt) and showed seasonal fluctuations, as shown in Chapter 3, Fig. 3.3A. In the covered treatment soil moisture content varied between 12 and 19% (dwt) without seasonal fluctuations. The soil nitrate content in the covered treatment was always higher than under field conditions, and increased during the course of the two years experiment (Chapter 3, Fig. 3.3B). In the field, the nitrate content fluctuated, it was highest in summer and lowest during winter.

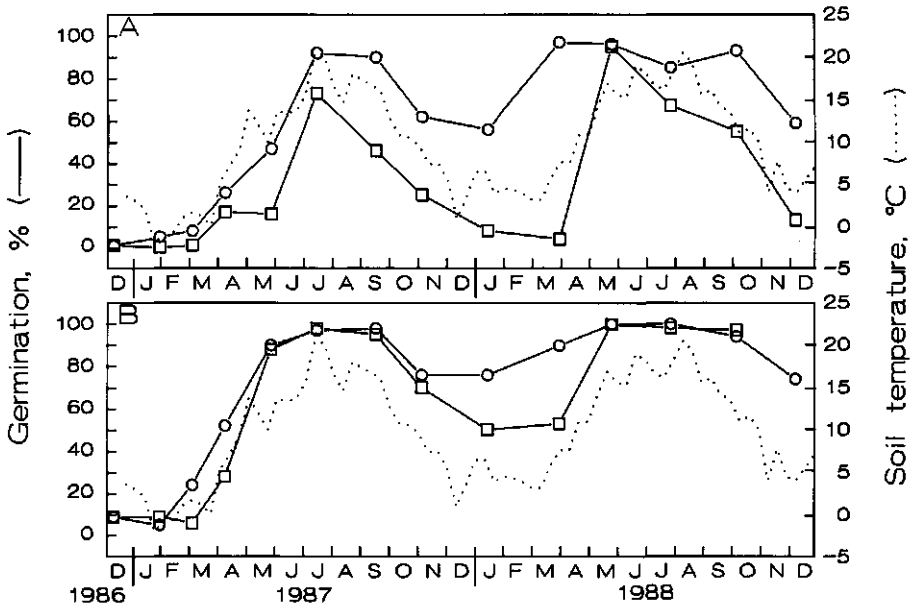

Fig. 4.3 Changes in soil moisture, soil nitrate content and soil temperature (A,B) during burial of seeds of *Spergula arvensis* as compared to changes in germination of exhumed seeds (A,B). Seeds harvested in 1986 were buried under field conditions with fluctuating soil moisture and nitrate content (□) (see Fig. 3.3 for data) as described in Fig. 4.1 or in sandy loam in a 100 l plastic container, sunk in the ground and covered with a plastic roof (○). In the latter situation soil moisture content was regulated at approx. 12 to 19% (dwt) (Fig. 3.3A). The soil nitrate content of the covered treatment was higher than of the uncovered one (Fig. 3.3B). Seeds were exhumed at regular intervals. Germination was tested after a 15 min red light irradiation with test samples of approx. 50 seeds in Petri dishes at 15°C in either water (A) or 50 mM KNO_3 (B).

Germination of exhumed seeds in water was positively related to the soil nitrate content. Seeds from the covered treatment always germinated to higher percentages (Fig. 4.3A). When nitrate was added during the test, these differences largely disappeared (Fig. 4.3B). The absence of a seasonal fluctuation in soil moisture content in the covered treatment did not effect the changes in dormancy.

Temperature. It is more likely that the field temperature regulates the changes in dormancy. In the burial experiment, dormancy was not relieved until April-May when temperature in the field increased (Fig. 4.1A,B). Also in the next two years, dormancy relief did not start until April-May. Dormancy induction occurred simultaneously with the decrease in field temperature. Also after burial in August, changes in dormancy

coincided with changes in temperature (Fig. 4.1C,D). The effect of temperature on dormancy was investigated experimentally as well as statistically.

Experimental test. To investigate the effect of temperature on changes in dormancy more carefully, seeds were pre-incubated at four constant temperatures: 2, 6, 10 and 15°C (Fig. 4.4A-D) and at various temperature regimes (Fig. 4.5). At regular intervals, germination was tested at 10, 20 and 30°C (Fig. 4.4) or 10 and 20°C (Fig. 4.5) in 25 mM KNO_3 after a 15 min red light irradiation.

Relief of primary dormancy of *S. arvensis* seeds occurred on a similar time scale at all four pre-incubation temperatures (Fig. 4.4). However, after 147 days at 15°C, germination was at all three test temperatures higher than after a pre-incubation at 2, 6 and 10°C. Only at a test temperature of 20°C there was no difference between a pre-incubation at 10 or 15°C. After 85 days at 2 and 6°C, induction of secondary dormancy started but not in the pretreatments at 10 and 15°C. Espeby (1989) also reported relief and induction of dormancy in seeds of *S. arvensis* during incubation at constant 1°C or 5°C.

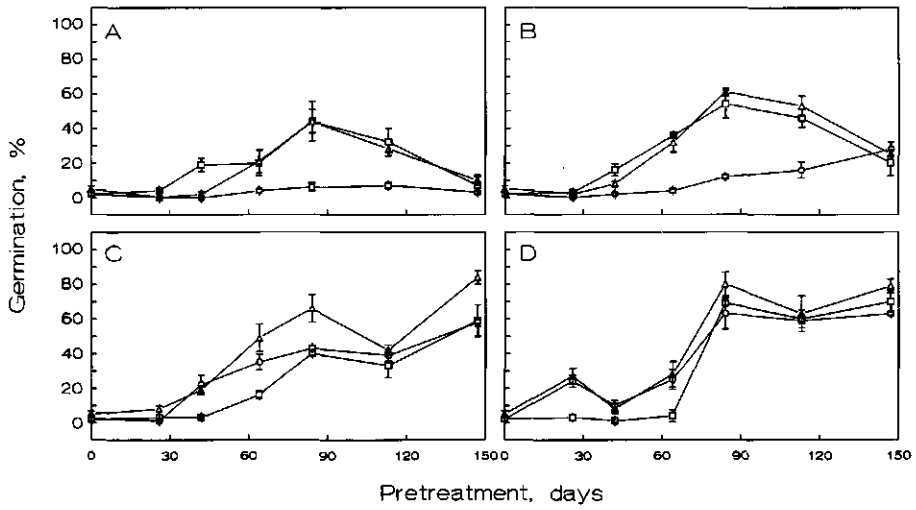

Fig. 4.4 Effect of pre-incubation temperature on dormancy of *Spergula arvensis*. Seeds were pretreated in sandy loam in black 9 cm plastic Petri dishes between two layers of fine mesh gauze at 2, 6, 10 and 15°C (A, B, C and D, respectively). At regular intervals, germination was tested in Petri dishes with triplicates of approx. 50 seeds at 10 (○), 20 (△) or 30°C (□) in 50 mM KNO_3 after an irradiation for 15 min with red light.

The changes in the range of suitable germination temperatures were influenced by the pre-incubation temperature. This was shown particularly by germination tests at 10°C. Seeds that were pretreated at 2 and 6°C barely germinated at 10°C (Fig. 4.4A,B), but a pre-incubation at 10 and 15°C increased germination at 10°C to 40-60% (Fig. 4.4C,D). Moreover, dormancy induction during pre-incubation at 6°C was not seen in germination tests at 10°C (Fig. 4.4B). The differences between the four pre-incubation temperatures were smaller in germination tests at 20 and 30°C.

An increase of the pre-incubation temperature from 2 to 15°C via 6 and 10°C did not cause induction of dormancy (Fig. 4.5). Seeds that were exposed to a further increase in temperature to 20°C, germinated better than seeds that were held at 15°C. However, germination of seeds moved from 15 to 10°C was significantly lower than germination of seeds kept at 15°C or moved to 20°C. A shift from 10 to 6°C instead of 15°C also triggered dormancy induction. Results were similar for the two germination test temperatures 10 and 20°C (Fig. 4.5A,B).

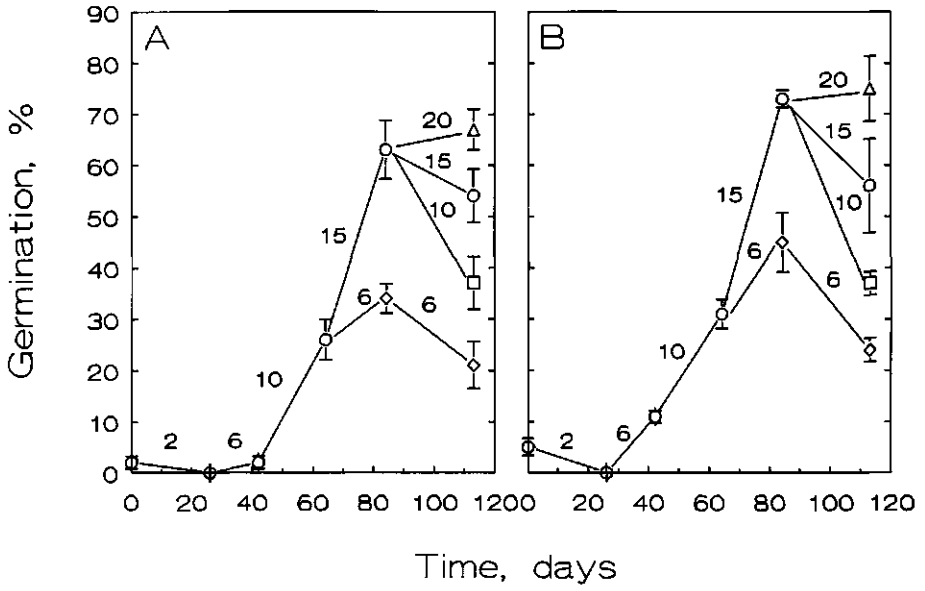

Fig. 4.5 Effect of changes in the pre-incubation temperature on dormancy of *Spergula arvensis*. Seeds were pretreated as in Fig. 4.4 at the temperatures indicated in the figure. At every temperature shift germination was tested in Petri dishes with triplicates of approx. 50 seeds at 10 (A) and 20°C (B) after an irradiation for 15 min with red light.

Statistical test. The relationship between temperature and the dormancy pattern was also tested statistically. It was assumed that dormancy of *S. arvensis* was a function of a cold and heat sum (C and H, respectively) as was shown for *Polygonum persicaria* (Chapter 2). According to the theory that was developed by Totterdell and Roberts (1979) for *Rumex obtusifolius* and *R. crispus*, changes in dormancy were the result of two sub-processes: relief and induction of dormancy. The first sub-process - relief of dormancy - was independent of the actual temperature as long as it was below a critical temperature, in the present paper called the border temperature. The latter sub-process - dormancy induction - occurred at all temperatures and increased with increase of temperature. Because the rate of the latter process was lowest at low temperatures, **net** relief of dormancy was optimal at a temperature just above zero, This theory was explained in full detail in Chapter 2.

The effect of temperature on changes in dormancy of *S. arvensis* seeds seemed to be opposite to the effect on *Polygonum persicaria* seeds. Dormancy relief was optimal at 15°C instead of 2°C (Fig. 4.4). Nevertheless, the concept of a cold and heat sum (C and H, respectively) as regulatory mechanism was applied also for this species. This will be discussed later on.

The (pretreatment) temperature (T_p) in a period δt before exhumation ($T_{p,\delta t}$) influenced the shape of the germination-temperature range of *S. arvensis* directly in addition to its influence on dormancy (Fig. 4.4). Therefore, also $T_{p,\delta t}$ was tested for its influence on the seasonal germination pattern of *S. arvensis*. Furthermore, nitrate strongly stimulated germination (Figs 4.1, 4.2) and therefore influenced the expression of the dormancy pattern. Accordingly, it was also tested whether the seasonal changes in germination were related to the composition of the germination medium (M_g), *i.e.* the absence or presence of nitrate.

With the methods that were described for *Polygonum persicaria* in Chapter 2, the parameters that maximized the fit of the data were selected. Data of the germination tests from December 1986 until May 1989 were used for simulation.

For the calculation of C, different border temperatures were used. The calculation of C with a border temperature of 12°C gave the highest correlation (R^2) and the lowest estimated variance ($\hat{\sigma}^2$) (Table 4.1). For $T_{p,\delta t}$ a period of 40 days gave the best fit (data not shown). The expected transformed germination (G_t) could be estimated by:

$$G_t = (-0.020*T_{p,40} - 0.063*M_g)*T_g^2 + (0.697*T_{p,40} + 4.135*M_g)*T_g + 3.046*C - 0.146*H + 1.237*T_{p,40} - 22.562 \quad (4.1)$$

where T_g is the germination temperature.

When a model was developed with the parameter time (weeks of burial) instead of C and H, R^2 decreased from 0.85 to 0.81 and $\hat{\sigma}^2$ increased from 447 to 545.

Table 4.1 Estimated variance ($\hat{\sigma}^2$) and squared multiple correlation (R^2) of models simulating germination of *Spergula arvensis* on the basis of cold and heat sum, germination temperature, germination medium and the mean temperature in 40 days before exhumation. Different border temperatures were used to calculate cold sum. For explanation see text.

	Border temperature (°C)								
	10	11	12	13	14	15	16	17	18
$\hat{\sigma}^2$	449	458	447	451	470	465	468	466	472
R^2	0.846	0.843	0.846	0.845	0.839	0.840	0.839	0.840	0.838

Germination under field conditions

Germination of exhumed seeds that were buried in December 1986 and August 1987 was also tested in Petri dishes outdoors. Just like in Fig. 4.1, seeds of different burial dates and seed lots, showed similar germination patterns (Fig. 4.6A).

Germination in Petri dishes outdoors clearly fluctuated in a seasonal pattern. The fluctuations in germination coincided with the changes in (air) temperature. In general, germination occurred in periods of high temperatures. The germination percentages and the period during which germination occurred were increased by nitrate. When seeds were desiccated and then re-imbibed in nitrate, germination even occurred in January (Fig. 4.6B).

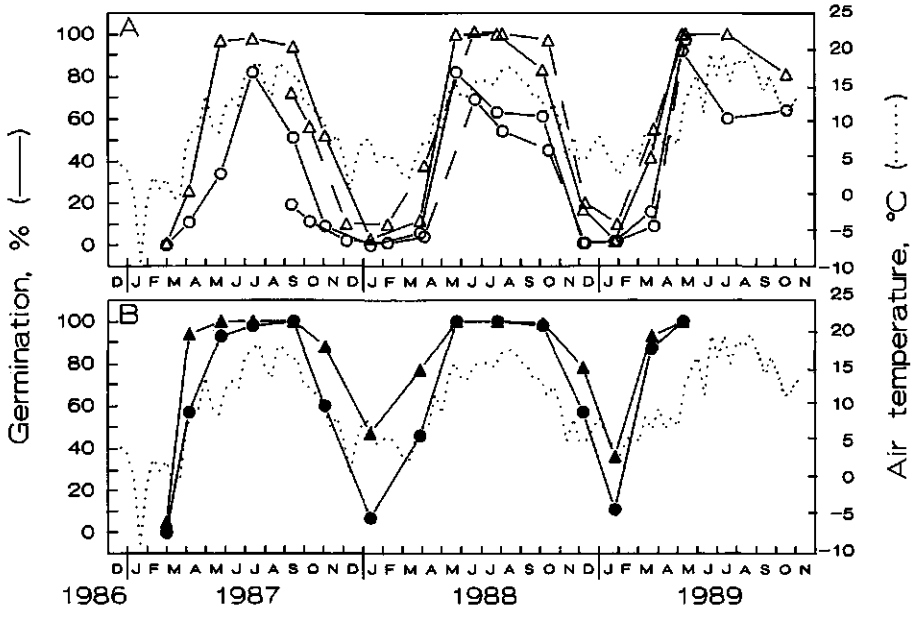

Fig. 4.6 Seasonal variation in germination of exhumed seeds of *Spergula arvensis* tested outdoors. Burial as in Fig. 4.1. Germination tests as in Figs 4.1 and 4.2 in airtight plastic boxes covered with black polyethylene outdoors at a height of 1.50 m in the shade. Germination was tested in water (circles) and KNO_3 (triangles) (A: 1986 seeds (solid line) and 1987 seeds (broken line)), with (B: 1986 seeds, closed symbols) or without (A, open symbols) a preceding desiccation treatment. The dotted line indicates the air temperature at 1.50 m.

Karssen (1982) hypothesized that germination in the field is restricted to the period where field temperature and germination-temperature range overlap. To visualize this interaction for *S. arvensis* the minimum and maximum temperatures required for at least 50% germination ($T_{g,min}$ and $T_{g,max}$) were calculated with Equation 4.1 (Fig. 4.7). To maintain the ecological meaning, these calculations were restricted to the range of zero to 30°C. The periods of predicted germination in Petri dishes outdoors are the periods where the field temperature overlapped with this germination-temperature range (hatched areas). The arrows indicate the moment that germination in Petri dishes outdoors actually increased (↑) or decreased (↓) to 50% (data from Fig. 4.6A).

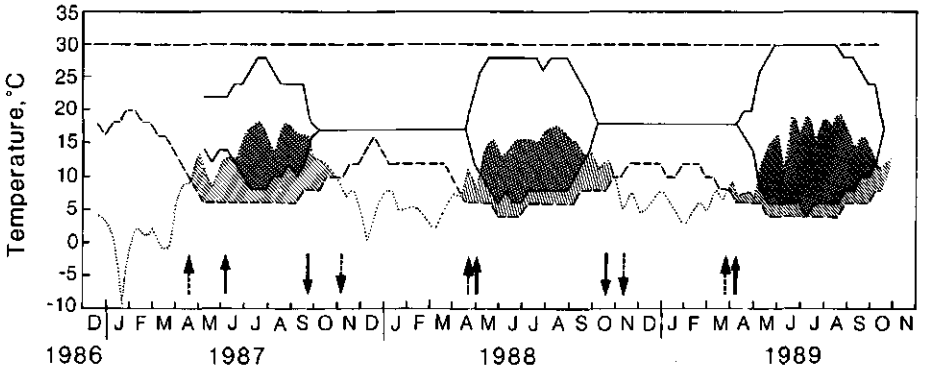

Fig. 4.7 Seasonal changes in the range of temperatures over which at least 50% of exhumed *Spergula arvensis* seeds germinate. Solid and broken lines represent maximum and minimum temperature required for 50% germination in water and nitrate, respectively (calculated with Equation 4.1). Dotted line indicates air temperature at 1.50 m (see text for explanation). Arrows (solid for water, broken for nitrate) indicate the moment germination in Petri dishes outdoors actually increased above (↑) or decreased below 50% (↓) (data from Fig. 4.6). Hatched area indicates overlap of field temperature and germination-temperature range (▩, water; ▨, nitrate).

The seasonal germination pattern outdoors was simulated by using air temperature at 1.50 m (the height seeds were placed outdoors) as T_g in Equation 4.1. The results, transformed back to germination percentages, are depicted in Fig. 4.8. The changes in dormancy and the effect of nitrate were closely simulated. Although data from May 1989 onwards were not used to make the model, outdoor germination in July and October 1989 was also simulated fairly well.

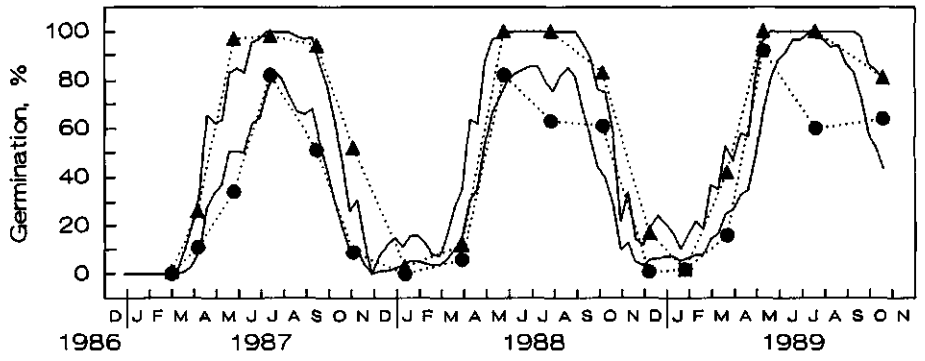

Fig. 4.8 Simulated (intact line) and observed (dotted line) germination of exhumed seeds of *Spergula arvensis* in Petri dishes outdoors as a function of exhumation date. Germination outdoors as in Fig. 4.6A for seeds buried in December 1986, in water (●) or 50 mM KNO_3 (▲). Simulation was carried out with Equation 4.1. See text for explanation.

4.4 Discussion

Dormancy pattern and emergence

The data from the germination tests outdoors showed that germination in water was possible from May to October with maximal germination in May-July (Fig. 4.6A). Environmental conditions strongly influenced the germination pattern. When nitrate was present and/or when seeds had been desiccated, germination could occur almost the whole year (Fig. 4.6A,B).

Several studies do not unequivocally show when emergence of *S. arvensis* occurs in the field. In Canada, emergence occurred from the end of April to the end of July (Chepil, 1946). In Great Britain, peak emergence occurred in April. However, upon soil disturbance, emergence could occur during the whole year (New, 1961). Håkansson (1983) showed, that in Sweden emergence occurred from May to July and after cultivation also in August-September.

The large effect of environmental conditions on germination seems a reasonable explanation for these differences. In emergence studies environmental conditions are not under control. The large effect of cultivation found by New (1961) and Håkansson (1983), is probably mediated by the strong effect of light on germination of *S. arvensis* (Fig. 4.2). Moreover, seeds may have been desiccated after cultivation, which also stimulates germination (Figs 4.2, 4.6B).

Germination-temperature range. The study of the changes in germination capacity over a range of temperatures provided a good basis for the prediction of germination of *S. arvensis* at field temperatures (Fig. 4.8).

It is shown in Fig. 4.7 that the seasonal changes in germination outdoors (Fig. 4.6) are the result of the overlap between the changing range of suitable germination temperatures (Figs 4.1, 4.7) and the changing field (air) temperature. When germination was tested in water, the range of suitable germination temperatures was entirely closed in winter, no seeds germinated over the whole range of temperatures tested (Figs 4.1A,C, 4.7). And indeed, during winter no seeds germinated outdoors in water in Petri dishes (Figs 4.6, 4.8).

Although the germination-temperature range of seeds tested in nitrate did not entirely close during winter, outdoor germination in nitrate ceased in winter, because temperature in the field decreased below the lower limit of the germination-temperature range (Figs 4.1, 4.6, 4.7).

The effect of desiccation at a test temperature of 15°C is shown in Fig. 4.2A. Data on other temperatures are not shown, but were similar. Therefore, the temperature range for desiccated seeds was even wider than for germination in nitrate. As a consequence, germination outdoors was possible during a much longer time (Fig. 4.6). In winter, germination of desiccated seeds in water ceased but in nitrate, seeds still germinated to approx. 30%.

The changes in the germination-temperature range were very remarkable. The widening and narrowing of the germination-temperature range indicates relief and induction of dormancy (Karssen, 1982). For summer annuals, the changes in the width of the range usually occurs by changes in the minimum temperature at which germination can proceed. For winter annuals, usually the maximum germination temperature changes (Karssen, 1982; Chapters 2 and 3). For germination in water the temperature range of *S. arvensis* widened by both an increase of the maximum temperature and a decrease of the minimum temperature for germination (Figs 4.1, 4.7).

Light. The data reported on the light requirement of *S. arvensis* seeds are rather contradictory. It has been reported that freshly harvested *S. arvensis* seeds do not require light for germination (New, 1961; Wesson and Wareing, 1969*a*), but that a light requirement was induced during burial (Wesson and Wareing, 1969*b*). Vincent and Roberts (1979) reported on seeds of *S. arvensis* that did have a light requirement without burial. The present experiments showed that differences between seed lots are common (Fig. 4.1). Possibly, these differences are caused by differences in weather conditions during seed development and maturation (Austin, 1972), which may explain the contradictory results in literature. In the present study both unburied and buried seeds had an absolute requirement for light when germination was tested in water or nitrate (Fig. 4.2B). After desiccation exhumed seeds germinated up to

90% in darkness.

Desiccation. The stimulating action of desiccation was also demonstrated by Karssen *et al.* (1988) and by Post (1984) who found considerably higher emergence of *S. arvensis* from soil samples that were air dried and subsequently re-moistened. The possible mechanisms of the desiccation effect are discussed in Chapter 6.

Light apparently is the best indicator for a position at or near the soil surface. When light is absent because seeds are buried to deeply, but they are still close enough to the surface to enable successful emergence, desiccation might be an additional indicator for a suitable position. Alternating temperatures, for several species another indicator for a position at or near the soil surface (Thompson and Grime, 1983) are reported not to stimulate germination of *S. arvensis* (Vincent and Roberts, 1977).

Control of dormancy pattern

The present study shows that the expression of the dormancy pattern of *S. arvensis* was strongly influenced by the conditions of the germination test. The **changes in dormancy** were, obviously, not influenced by the test conditions. The two seasonally fluctuating variables soil moisture content and temperature were investigated for an effect on changes in dormancy.

Soil moisture content. Seasonal changes in soil moisture content were not required for the occurrence of changes in dormancy (Fig. 4.3). In Petri dishes with a constant soil moisture content, changes in dormancy also occurred (Figs 4.4, 4.5).

Because no nitrate leakage occurred in the covered treatment, the soil nitrate content was higher than in the uncovered treatment. This difference was reflected in the dormancy patterns when germination was tested in water. Germination of seeds from the covered treatment was always better (Fig. 4.3A). This was not an effect of the different nitrate levels on changes in dormancy, since after application of nitrate to the germination medium the differences had completely disappeared (Fig. 4.3B). This indicates that high levels of nitrate in soils do not effect the changes in dormancy. When these high levels are present at the moment the seed is irradiated (soil cultivation), germination is stimulated and can occur during a longer period of the year just as with nitrate applied in the germination test (Figs 4.1, 4.2A, 4.6, 4.8).

These results agree with those obtained for *Polygonum persicaria*, *Sisymbrium officinale* and *Chenopodium album* (Chapters 2, 3 and 5).

Temperature. Dormancy patterns of seeds from two seed lots, buried in December and August were similar, almost immediately after burial of the second seed lot (Figs. 4.1, 4.6). Karssen *et al.* (1988) also showed that dormancy relief of *S. arvensis* seeds incubated in 'October' or 'April' of a condensed annual temperature cycle, occurred exactly at the same time in spring. Their conclusion was that rather than low winter temperatures, the rising temperature in spring was responsible for the relief of dormancy of *S. arvensis*. The present incubator experiments showed that dormancy was relieved by increasing and induced by decreasing temperatures (Fig. 4.5). It seems that *S. arvensis* seeds are able to register whether soil temperatures are increasing or decreasing. When temperatures are increasing dormancy is relieved, when temperatures are decreasing dormancy is induced.

Summer or winter annual

S. arvensis did not show the clear features of a typical winter or summer annual. Dormancy was not relieved during summer or winter, but in spring. Induction of dormancy seemed to occur in periods of decreasing soil temperatures (Fig. 4.1). *S. arvensis* did not require the low temperatures needed for induction of dormancy in winter annuals such as *Veronica hederifolia* (Roberts and Lockett, 1978; Roberts and Neilson, 1982*a*), nor the high temperatures required by summer annuals such as *Polygonum persicaria* and *Sisymbrium officinale* (Chapters 2 and 3). Moreover, dormancy induction occurred in autumn at the same temperatures at which dormancy was relieved in spring (Fig. 4.1).

Not only the unusual temperature requirement for relief and induction of dormancy indicated that *S. arvensis* has the characteristic features of both winter and summer annuals, also the germination-temperature range supported this conclusion. Changes in dormancy were visible by changes in the maximum as well as in the minimum temperature required for germination in water (Fig. 4.7). When germination was tested in nitrate the germination-temperature range more or less resembled that of summer annuals such as *Polygonum persicaria* (Chapter 2).

Germination and emergence of *S. arvensis* started in spring. Therefore, the name summer annual would probably be most appropriate. Since germination continued during summer, the prefix facultative, used for winter annuals that germinate in autumn and spring, could be used. A facultative summer annual can germinate in both spring and summer-autumn.

Simulation

Germination was simulated on the basis of a cold and heat sum (C and H). The calculation of C and H was based on the assumption that dormancy relief was optimal at a temperature just above zero. This was true for *Rumex obtusifolius* and *R. crispus* (Totterdell and Roberts, 1979, who developed this theory) and *Polygonum persicaria* (Chapter 2). For *S. arvensis*, the optimal temperature for dormancy relief was 15°C (or higher). This implies, that C and H can not give an accurate description of dormancy relief for this species. When temperatures just above zero are optimal for dormancy relief, the relationship between temperature and dormancy relief is more or less linear. The **lower** the temperature, the better dormancy **relief** occurs and the **higher** temperature, the faster dormancy **induction** occurs. When the optimal temperature for dormancy relief is higher, the relationship becomes hyperbolic. The higher the optimum temperature for dormancy relief, the worse the estimation of the hyperbolic relationship between temperature and dormancy when it is carried out with a linear equation and the lower the descriptive value of C and H. Consequently, removal of C and H from the simulation model hardly decreased the correlation for *S. arvensis*.

Dormancy of *S. arvensis* was relieved rather late in winter-spring (because of the high optimum temperature for dormancy relief). The later dormancy relief occurs, the higher the correlation of the changes in dormancy with the actual field temperature will be (Fig. 4.1). This implies that when C and H are excluded from the model and are replaced with the parameter "time", the pretreatment temperature (T_p) in a period δt before exhumation ($T_{p,\delta t}$) will still assure a high correlation for the model.

The minor decrease of R^2 and increase of $\hat{\sigma}^2$ of the simulation model, when C and H were replaced by the parameter time, supported the hypothesis that dormancy of *S. arvensis* is regulated by the actual temperature changes rather than a cold and heat sum. Nevertheless, the descriptive model closely simulated and even predicted germination under field conditions (Fig. 4.8). The seasonal changes in the germination-temperature range that were calculated with the model, visualized the mechanism by which germination of *S. arvensis* is restricted to fixed periods of the year (Fig. 4.7).

Further research on the nature of the regulation of dormancy of *S. arvensis* in particular and weed seeds in general should lead to a more mechanistic approach of the simulation of dormancy patterns.

Chapter 5

Seasonal dormancy patterns in buried weed seeds. IV. Chenopodium album L.

Abstract. The present paper describes the effects of environmental conditions on dormancy of buried seeds of *Chenopodium album* L.. Seeds were buried under field and controlled conditions and at regular intervals, the germination capacity of exhumed seeds was tested over a range of conditions. Seeds buried in the field, showed seasonal changes in dormancy that were less evident than reported before for other species (Chapters 2, 3 and 4). Dormancy relief occurred in winter-early spring, dormancy induction in summer. The seasonal changes in germination were more evident when germination was tested at field temperatures than at constant temperatures in incubators. Tests in these incubators showed that exhumed seeds germinated best at 10-20°C and less good at 30°C. Other conditions of the germination test strongly influenced what was seen of the changes in dormancy. Of the naturally occurring factors that influence germination, nitrate and light always promoted germination. There was a strong positive interaction between the effects of the two factors on germination. Desiccation only stimulated germination under particular conditions. The effect was most evident when germination was tested in nitrate in darkness. When nitrate and light where combined, exhumed seeds germinated over a much longer period of the year than in water and/or darkness. Burial experiments under controlled conditions showed that changes in dormancy were mainly regulated by the field temperature and not by seasonal fluctuations in soil moisture or nitrate content. The seasonal changes in dormancy were simulated on the basis of the field temperature. The model closely simulated germination at field temperatures throughout the year. An analysis of the germination-temperature range (computed with the model) showed that the restriction of field emergence to spring-late summer could be explained by the overlap of the actual field temperature and the range of temperatures suitable for germination. Despite the absence of apparent seasonal changes in the germination-temperature range, germination in the field did show seasonal periodicity, because the field temperature and the germination-temperature range only overlapped in restricted periods.

5.1 Introduction

Chenopodium album L. (lamb's quarters, fat-hen, pigweed, white goosefoot) is a successful colonizing species, and is one of the most widely distributed weeds in the world (Holm *et al.*, 1972). It is found on all continents, but is most competitive in the cool regions. It is a short day plant that grows vegetatively during the long photoperiod in the temperate zone and therefore is fully grown when flowering is induced by short days (Holm *et al.*, 1972).

The seeds of *C. album* show considerable polymorphism. Some seeds are brown, most are black and they can be either smooth or reticulate. All these different seed forms can be found on one plant (Williams and Harper, 1965). The seeds also differ in dormancy and/or in their need for dormancy relieving conditions (Williams and Harper, 1965).

Seed polymorphism will undoubtedly be related to the genetically heterogeneous composition of populations of this species, but the variance in seed morphology also depends on environmental factors. The formation of brown seeds with a high seed weight and a thin seed coat is promoted by short days during seed formation (Karssen, 1970). Long days promote the formation of small, black seeds with thick seed coats. Black seeds are dormant, brown seeds germinate readily directly after harvest. Dormancy increases with the thickness of the testa. Also nitrate fertilization during seed formation influences seed dormancy of *C. album*. Seeds formed on plants fertilized with nitrate, contain higher nitrate levels and germinate better (Fawcett and Slife, 1978; Saini *et al.*, 1985*a,b*; Chapter 7).

Apart from environmentally induced differences in dormancy and germination, *C. album* also shows profound ecotypic variation. The optimum temperature for germination varies from 10°C in India to 25°C in Canada (Holm *et al.*, 1972). In India, the existence of a winter and summer population with different temperature optima for germination has been reported (Kapoor and Ramakrishnan, 1973).

Germination of *C. album* strongly depends on light (Cumming, 1963; Karssen, 1967; Henson, 1970; Taylorson, 1970; Vincent and Roberts, 1977, 1979; Roberts and Benjamin, 1979). Also nitrate is highly stimulatory to germination of *C. album* (Williams and Harper, 1965; Henson, 1970; Vincent and Roberts, 1977; Roberts and Benjamin, 1979) and sometimes an interaction between light and nitrate is found (Henson, 1970; Roberts and Benjamin, 1979).

Emergence of *C. album* has been widely studied. Some variation in emergence is caused by local differences in environmental conditions, but in the temperate zone emergence of *C. album* usually starts in spring, frequently in March, is highest in April-May and often continues during summer (Roberts, 1964; Roberts and Ricketts, 1979; Håkansson, 1983; Ogg and Dawson, 1984; Van den Brand, 1986, 1987). Several authors report some late-season emergence in August-September (Williams, 1963; Williams and Harper, 1965; Roberts and Ricketts, 1979; Håkansson, 1983).

Why is germination of C. album restricted to this period? Seeds of many weeds and ruderals pass through seasonal cycles of dormancy (Karssen, 1982). These changes in dormancy see to it that germination occurs in seasons with conditions favourable for growth and reproduction. Changes in dormancy are characterized by changes in the range of temperatures over which germination can proceed. During relief of dormancy this range becomes progressively wider, during induction of dormancy it becomes narrower (Baskin and Baskin, 1981*a,b*, 1983*a,b*, 1984). It has been hypothesized that the restriction of germination to a more or less fixed period of the year is determined by the overlap of this germination-temperature range and the actual field temperature (Karssen, 1982).

Dormancy of *C. album* also fluctuates in a seasonal pattern (Baskin and Baskin, 1977; Karssen, 1980/81*b*). In the present paper the changes in dormancy of buried *C. album* seeds were studied in more detail. In a burial experiment of three years the repeatability of the pattern was studied, the changes in the range of temperatures suitable for germination were determined and the effect of light, nitrate and desiccation on germination and changes in dormancy were investigated.

5.2 Materials and methods

After collection at an arable field in the vicinity of Wageningen in 1986, seeds were allowed to dry and then they were cleaned by gentle rubbing. Seeds were winnowed and sieved to remove perianth segments and small and light seeds. See for conditions of burial and germination tests Chapters 2 and 3.

5.3 Results

Germination of exhumed seeds

Seeds of *C. album* were buried in December 1986. At regular intervals, part of the seeds were exhumed and germination tested. Half of the exhumed seeds, except the dark controls, were irradiated for 15 min with red light and then incubated in water or 50 mM KNO_3. The other half of the seeds were first desiccated and then incubated in water or 50 mM KNO_3. These seeds were irradiated after 24 h of re-imbibition. The desiccation treatment was included until May 1989. For all light treatments the red light irradiation was repeated 24 h after the first one. Tests occurred at 10, 20 and 30°C (Fig. 5.1). Germination with the two red light irradiations was compared to dark germination in Fig. 5.2.

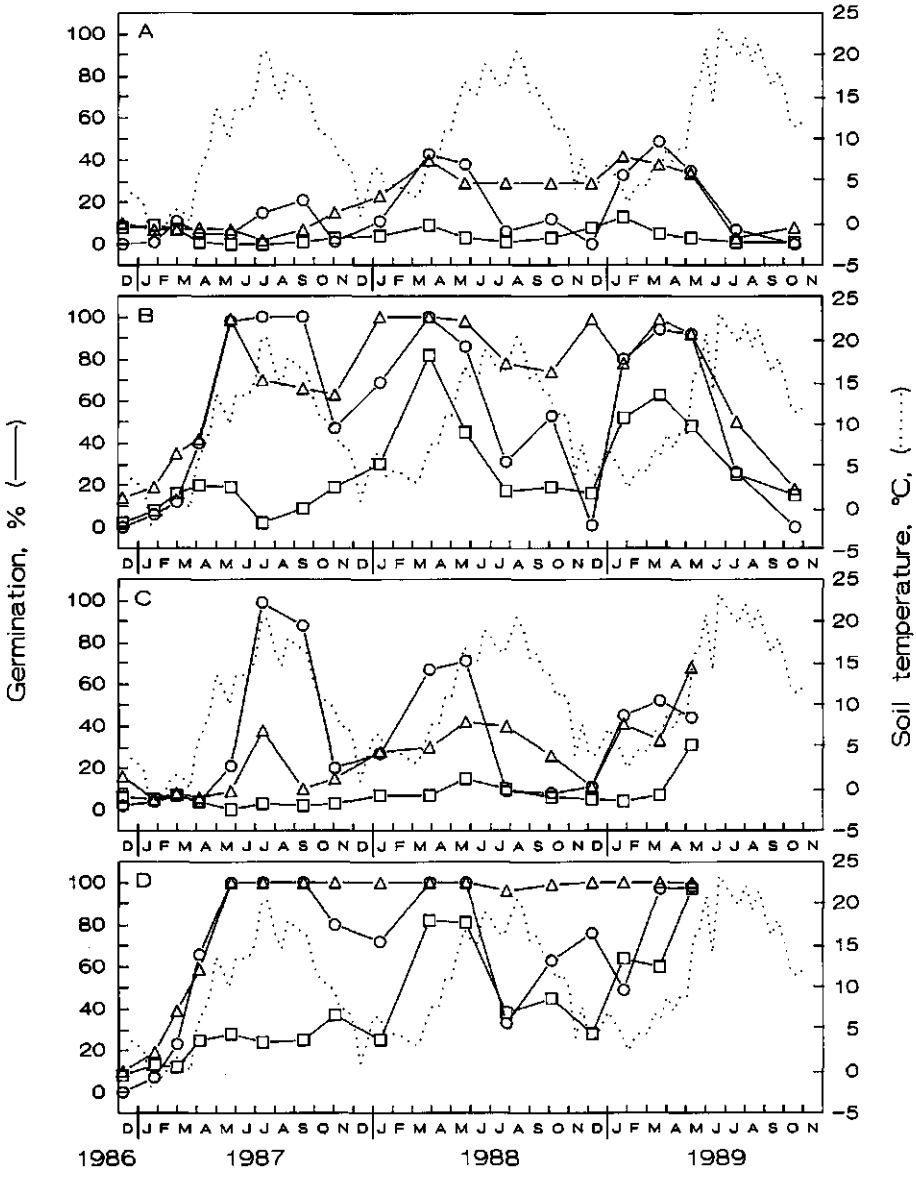

Fig. 5.1 Seasonal variation in the germination of exhumed seeds of *Chenopodium album* under different test conditions. Seeds were buried in portions of 2.0 g in December 1986 in sandy loam under field conditions and exhumed at regular intervals. After exhumation, germination was tested in Petri dishes with test samples of approx. 70 seeds at 10 (○), 20 (△) or 30°C (□) in water (A,C) or in 50 mM KNO_3 (B,C) either with (C,D) or without (A,B) a preceding desiccation treatment. Seeds were desiccated for 24 h above a saturated solution of LiCl (r.h. approx. 16%) which gave a seed moisture content of approx. 9%. Seeds were irradiated twice with 15 min red light at 24 h intervals. When seeds were desiccated, they received their first irradiation after 24 h re-imbibition. The dotted line indicates the soil temperature at 10 cm in bare soil.

Temperature, nitrate and desiccation. Before burial, *C. album* seeds were deeply dormant. They germinated to only 0-15% at all test temperatures (Fig. 5.1).
During the three years of the burial experiment seasonal changes in dormancy became apparent in tests with exhumed seeds, but the expression of these changes strongly depended on the test conditions. In water (Fig. 5.1A), some seasonal fluctuation was only seen in 1988 and 1989, particularly when germination was tested at 10°C. Addition of nitrate to the test medium enhanced germination (Fig. 5.1B). Now, seasonal fluctuations in dormancy were more evident. Desiccation of exhumed seeds prior to the germination test, stimulated germination both in water and in nitrate (Fig. 5.1C,D). As a result, tests in water showed a seasonal germination pattern. In nitrate germination at 20°C was constantly close to 100%, whereas at 30°C and to a lesser extent at 10°C some seasonal fluctuation could be seen. In general germination was best at approx. 10 to 20°C.

The pattern differed during the three successive years. Germination of desiccated seeds in water at 10°C started to increase in 1987 in May-June, but in 1988 and 1989 already around January. In nitrate, the increase in germination of desiccated seeds in 1987 became visible as early as February-March (Fig. 5.1C,D). In 1988 and 1989, the moment germination of desiccated seeds increased was similar for tests in water and nitrate. Induction of dormancy also occurred earlier in 1988 and 1989 than in 1987.

Light. At 20°C, germination of exhumed seeds was also tested in darkness (Fig. 5.2B). Results from germination tests at 20°C with two red light irradiations from Fig. 5.1 are shown again to illustrate the effect of light (Fig. 5.2A). In darkness, seeds did not germinate in water. After desiccation or with application of nitrate, seeds occasionally germinated to approx. 20%. The combination of desiccation and re-imbibition in nitrate gave the best results. Seeds germinated up to 80-90% in May 1988 and 1989. With the two red light irradiations, again the large stimulatory effect of nitrate was seen (Fig. 5.2A). There were only small differences in germination of desiccated and non-desiccated seeds.

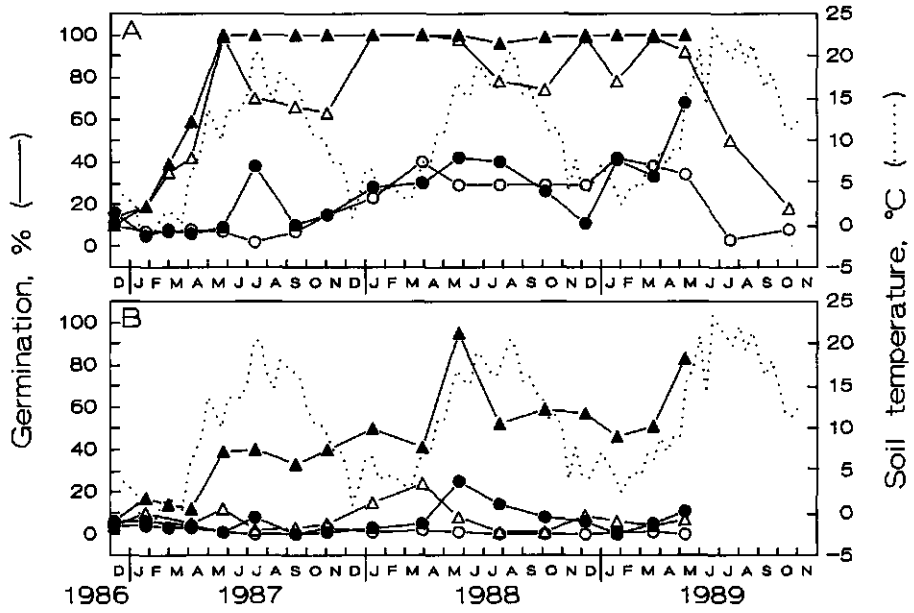

Fig 5.2 Effect of test conditions on the seasonal variation of germination of exhumed seeds of *Chenopodium album*. Burial as in Fig. 5.1. Germination of exhumed seeds was tested at 20°C after two red light irradiations as in Fig. 5.1 (A) or in darkness (B) in water (circles) or 50 mM KNO_3 (triangles) with (closed symbols) or without (open symbols) a preceding desiccation treatment as described in Fig. 5.1. The dotted line indicates the soil temperature at 10 cm in bare soil.

Incomplete germination. During the entire experiment, incomplete germination was observed under certain test conditions. In incompletely germinated seeds the outer testa layer was ruptured and the radicle was often extended from within that layer, but the radicle did not protrude the endosperm and inner testa layer (Karssen, 1976*a*). Incomplete germination occurred mostly in tests at 10°C in water and nitrate, with or without a preceding desiccation treatment. At other test temperatures, incomplete germination also occurred but to a much smaller extent. It was verified that incomplete germination did not occur until after exhumation.

Fig. 5.3 shows that in the period from October 1988 to May 1989 incomplete germination gave a different impression of dormancy of exhumed seeds than complete germination. That is, total (complete and incomplete) germination decreased (Fig. 5.3A,C), indicating induction of dormancy, whereas complete germination increased (Fig. 5.3A-D), which is indicative of dormancy relief. Incompletely germinated seeds completed germination upon transfer to higher temperatures. They were desiccation tolerant and immediately completed germination upon imbibition after dry storage for several weeks.

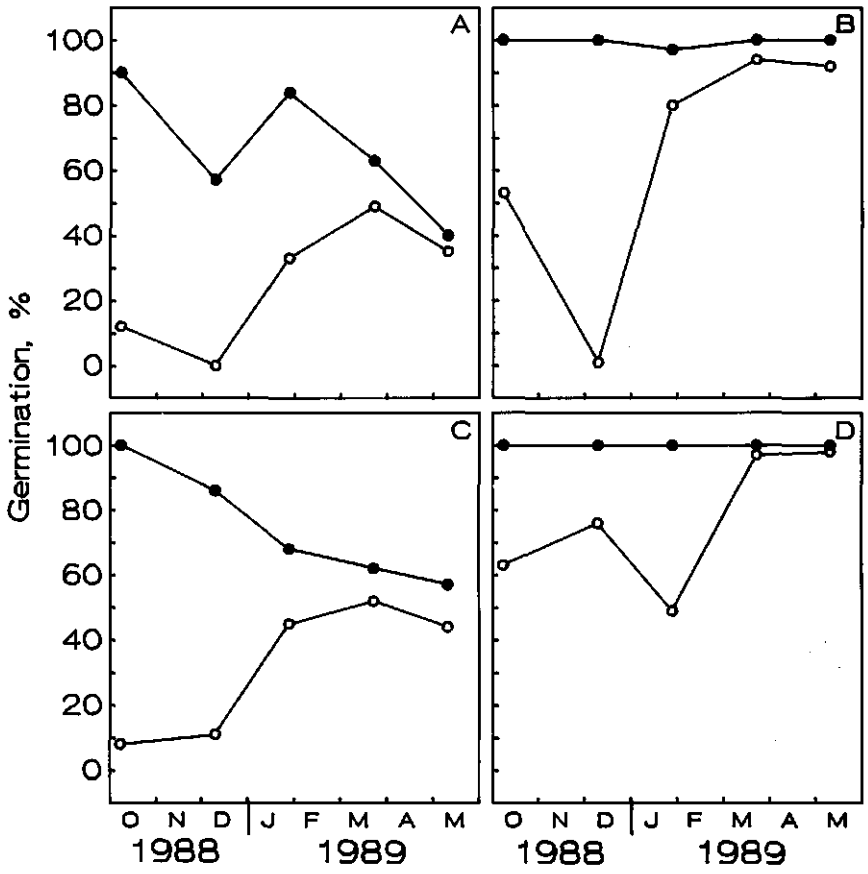

Fig. 5.3 Effect of test conditions and exhumation date on the seasonal variation of normal germination (○) and total germination (normal plus incomplete germination, (●)) of *Chenopodium album*. Germination was tested after two red light irradiations, at 10°C in water (A,C) or in 50 mM KNO_3 (B,C) either with (C,D) or without (A,B) a preceding desiccation treatment as described in Fig. 5.1. After 12 days, germination percentages were determined. Seeds were considered incompletely germinated when the outer testa layer was cracked and/or protruded by the radicle, but the inner testa layer and endosperm still surrounded the radicle.

Control of dormancy pattern

The seasonal character of the changes in dormancy as shown in Fig. 5.1 suggests that a factor closely associated with the seasons is responsible for the changes in dormancy.

Soil moisture content. In most areas of the world soil moisture content shows seasonal changes (Lonchamp *et al.*, 1984). To study whether seasonal fluctuations in soil moisture content played a role in the regulation of the dormancy pattern of *C. album*, seeds were either buried outdoors in sandy loam with fluctuating moisture content or in containers under a transparent roof in either sandy loam or quartz sand.

It has been reported in Chapter 3 (Fig. 3.3A) that the soil moisture content of the quartz sand was approx. 3% without seasonal variability. The moisture content of the uncovered sandy loam varied between approx. 17 and 27% and showed seasonal fluctuations. Moisture content increased during winter and decreased during summer. In the covered sandy loam treatment the moisture content varied between 12 and 19% (dwt) without the seasonal fluctuations that occurred in the field. The nitrate content of the sand was always lower than the nitrate content of the sandy loam outdoors (Fig. 3.3B). The nitrate content of the covered sandy loam treatment increased during the course of the experiment and was always higher than in the other two treatments. The nitrate content of the uncovered sandy loam fluctuated seasonally. (Chapter 3, Fig. 3.3B).

The dormancy patterns of seeds buried under the three different conditions, although exposed to varying conditions of moisture and nitrate were very similar (Fig. 5.4). Here data of germination at 10°C in water after desiccation are shown. For other test conditions dormancy patterns were similar (data not shown).

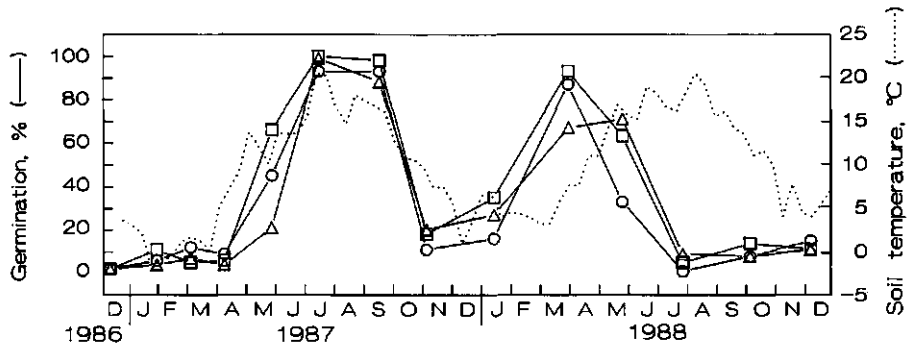

Fig. 5.4 Changes in soil moisture, soil nitrate and soil temperature during burial of seeds of *Chenopodium album* as compared to changes in germination of exhumed seeds. Seeds were buried under field conditions as in Fig. 5.1 (□) with fluctuating soil moisture and nitrate content (see Fig. 3.3 for data) or in quartz sand (◇) or sandy loam (○) in two 100 l plastic containers, sunk in the ground and covered with a transparent plastic roof. In the latter situation soil moisture content was regulated between approx. 12 and 19% (dwt) (sandy loam) or at approx. 3% (dwt) (quartz sand) (Fig. 3.3A). The nitrate content of the sand was lower than of the sandy loam, of the covered sandy loam treatment it was higher than in the uncovered one (Fig. 3.3B). Seeds were exhumed at regular intervals. Germination was tested in Petri dishes with test samples of approx. 70 seeds in water at 10°C, after a preceding desiccation treatment. Desiccation and red light irradiation as in Fig. 5.1. The dotted line indicates the soil temperature at 10 cm in bare soil.

Temperature. In studies with buried seeds of *Polygonum persicaria*, *Sisymbrium officinale* and *Spergula arvensis*, it was concluded that field temperature regulated the changes in dormancy (Chapters 2, 3 and 4). This hypothesis was also tested for *C. album*. The seasons of dormancy relief and induction are best seen in Fig. 5.4. In 1987, germination increased from March onwards. In 1988, the rise occurred already in winter. Dormancy induction occurred in autumn and summer, respectively. Data in Fig. 5.1 confirm these observations. Apparently, dormancy relief and dormancy induction occurred when the field temperature was respectively lower or higher than a critical temperature, as was also reported for *Polygonum persicaria* and *Sisymbrium officinale* (Chapters 2 and 3). This critical temperature for relief and induction of dormancy was investigated experimentally.

Experimental test. Fig. 5.5A shows germination of seeds exhumed from the field in nitrate at 10 and 20°C, during the first 10 months of the experiment (enlarged section of Fig. 5.1B). These results were compared with results from experiments with seeds which were pretreated in soil in Petri dishes at 2°C → 6°C → 10°C → 15°C (Fig. 5.5B).

In the field, relief of dormancy started during winter. In the first months germination was best at 20°C, thereafter it also rose at 10°C (Fig. 5.5A). When the temperature increased to approx. 13-20°C, dormancy induction began. This was first visible in a germination test at 20°C and later at 10°C. These changes in dormancy were simulated very well by a pretreatment in soil in incubators at a rising temperature (Fig. 5.5B). Again, in the beginning of the experiment seeds germinated best at 20°C. When the pretreatment temperature was raised to 15°C, dormancy induction was visible in a germination test at 20°C, whereas germination at 10°C still increased (compare to Fig. 5.5A). Seeds that were not transferred to 15°C, but were kept at 10°C showed no decrease in germination at 20°C (Fig. 5.5B).

Statistical test. Testing of the involvement of temperature in the regulation of changes in dormancy also occurred statistically. Just as for *Polygonum persicaria*, it was assumed that dormancy of *C. album* was a function of a cold and heat sum (C and H, respectively) as hypothesized by Totterdell and Roberts (1979) for *Rumex obtusifolius* and *R. crispus*. According to their theory, changes in dormancy were the result of two sub-processes: relief and induction of dormancy. The first sub-process was independent of the actual temperature as long as it was below a critical temperature, in the present paper called the border temperature. The latter sub-process occurred at all temperatures and increased with increase of temperature. Because the rate of the latter process was lowest at low temperatures, net relief of dormancy was optimal at a temperature just above zero. This theory is explained in full detail in Chapter 2.

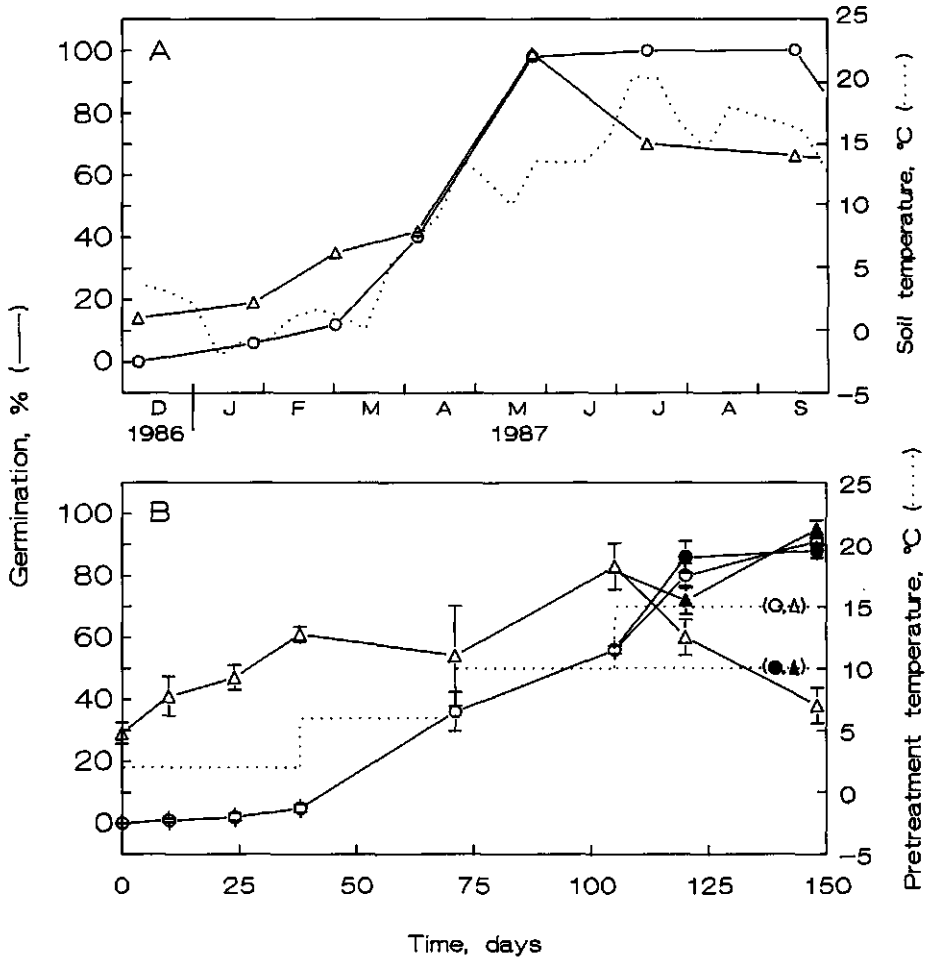

Fig. 5.5 Effect of pretreatment temperature on germination of *Chenopodium album*. Seeds were buried in the field, exposed to naturally occurring fluctuating temperatures as in Fig. 5.1 (A) or they were pretreated in sandy loam in black 9 cm plastic Petri dishes between two layers of fine mesh gauze at a rising temperature: 2°C → 6°C → 10°C → 15°C (open symbols) or 2°C → 6°C → 10°C → 10°C (closed symbols) (B). Seeds were "exhumed" at regular intervals. Germination was tested at 10 (○) or 20°C (△) in 50 mM KNO_3. Each treatment consisted of triplicates (B) or of one test sample (A) of approx. 70 seeds. Irradiation as in Fig. 5.1. Vertical bars indicate standard error (B). The dotted line indicates the soil temperature at 10 cm in bare soil (A) or the temperature during pretreatment (B).

Just as for *Sisymbrium officinale* (Chapter 3), the theory of Totterdell and Roberts was applied for *C. album* as an approximation, since dormancy of the latter species was also relieved better at temperatures somewhat higher than just above zero (10°C in stead of 2°C, data not shown). The (pretreatment) temperature (T_p) in a period δt before exhumation ($T_{p,\delta t}$) seemed to influence germination of *Polygonum persicaria* and *Spergula arvensis* directly in addition to its influence on dormancy. Parallel experiments suggested that this was also true for *C. album* (data not shown). Therefore, also $T_{p,\delta t}$ was tested for its influence on the seasonal germination pattern of *C. album*. Furthermore, nitrate strongly stimulated germination (Figs 5.1, 5.2) and therefore influenced the expression of the dormancy pattern. Accordingly, it was also tested whether the seasonal changes in germination were related to the composition of the germination medium (M_g), *i.e.* the absence or presence of nitrate.

With the methods that were described for *Polygonum persicaria* (Chapter 2), the parameters that maximized the fit of the data were selected. Only data until May 1989 were used. The calculation of C with 14°C as the border temperature, gave the highest correlation (R^2) and the lowest estimated variance ($\hat{\sigma}^2$) (Table 5.1). Of the time intervals (δt) of 20, 30, 40, 50 and 60 days that were tested, a period of 20 days gave the best fit for $T_{p,\delta t}$ (data not shown). The expected transformed germination (G_t) could be estimated by:

$$G_t = (-0.001*H - 0.238*M_g + 0.052)*T_g^2 + (-0.157*C + 0.036*H - 0.227*T_{p,20} + 8.391*M_g)*T_g + 6.461*C - 0.561*H + 5.124*T_{p,20} - 35.013 \quad (5.1)$$

where T_g is the germination temperature.

When a model was developed with the parameter time (weeks of burial) instead of C and H, R^2 decreased from 0.76 to 0.70 and $\hat{\sigma}^2$ increased from 754 to 932.

Table 5.1 Estimated variance ($\hat{\sigma}^2$) and squared multiple correlation (R^2) of models simulating germination of *Chenopodium album* on the basis of cold and heat sum, germination temperature, germination medium and the mean temperature in 20 days before exhumation. Different border temperatures were used to calculate cold sum. For explanation see text.

	Border temperature (°C)								
	10	11	12	13	14	15	16	17	18
$\hat{\sigma}^2$	821	791	828	802	754	758	824	811	795
R^2	0.742	0.752	0.740	0.748	0.763	0.762	0.741	0.745	0.751

Germination under field conditions

From March 1987 onwards, germination of exhumed seeds was also tested in Petri dishes outdoors. Results from these tests were compared to data calculated with Equation 5.1.

Germination in Petri dishes outdoors (Fig. 5.6) showed a much clearer seasonal pattern than germination at constant temperatures (Fig. 5.1). Germination occurred in all three years first in March and continued until September (1987) or October-December (1988 and 1989). Germination outdoors was strongly promoted by nitrate but only incidentally by desiccation. In the second and third spring, seeds germinated to higher percentages in water than in the first. Usually germination only occurred at temperatures above approx. 5°C.

It is striking that for *C. album* germination tests at constant temperatures did not show the clear seasonal pattern that was observed for *Polygonum persicaria*, *Sisymbrium officinale* and *Spergula arvensis* (Chapters 2, 3 and 4). Nevertheless, germination outdoors, at field temperature, clearly fluctuated in a seasonal pattern.

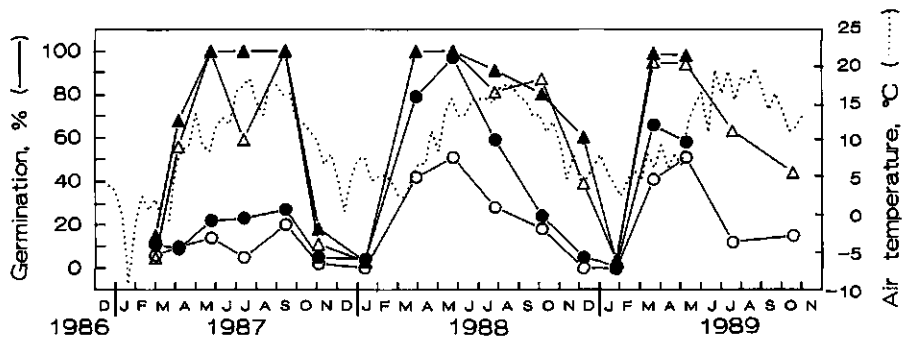

Fig. 5.6 Germination of *Chenopodium album* seeds outdoors. As Fig. 5.2, except germination was tested in Petri dishes in airtight plastic boxes covered with black polyethylene, outdoors at a height of 1.50 m in the shade. The dotted line indicates the air temperature at 1.50 m.

The intriguing question is whether these seasonal changes can still be explained by changes in the germination-temperature range. With Equation 5.1 the minimum ($T_{g,min}$) and maximum ($T_{g,max}$) temperature required for at least 50% germination were computed for the whole experimental period (Fig. 5.7). To maintain ecological significance, the calculations were restricted to the range of zero to 30°C. Because germination in water seldom increased above 50%, $T_{g,min}$ and $T_{g,max}$ were only computed for germination in nitrate.

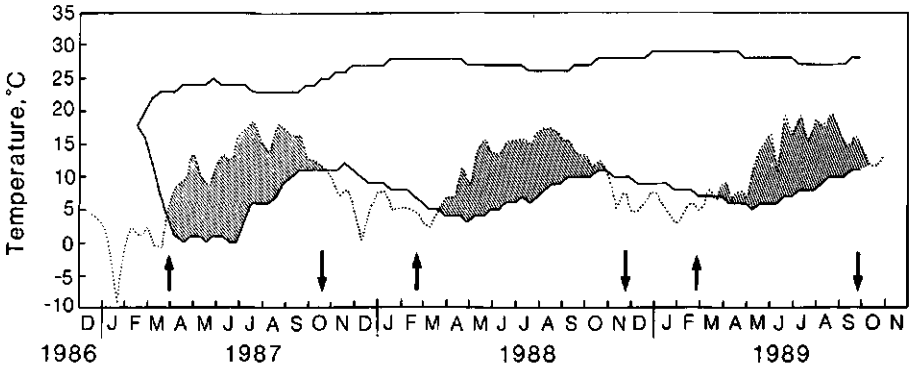

Fig. 5.7 Seasonal changes in the range of temperatures over which at least 50% of exhumed *Chenopodium album* seeds germinate. Solid lines represent maximum and minimum temperature required for 50% germination in 50 mM KNO_3, calculated with Equation 5.1. Dotted line indicates air temperature at 1.50 m (see text for explanation). Hatched area indicates overlap of field temperature and germination-temperature range. Arrows indicate the moment germination in Petri dishes outdoors actually increased above (↑) or decreased below 50% (↓) (data from Fig. 5.6).

As was already clear from Fig. 5.1 the seasonal changes in the germination-temperature range were not very marked. Nevertheless, the seasonal germination/emergence pattern was perfectly explained by Fig. 5.7. Only in restricted periods did the field (air) temperature and the germination-temperature range overlap (hatched areas). These are the periods of predicted field germination. The predicted periods agreed fairly well with the periods in which germination in Petri dishes outdoors actually increased above (↑) or decreased below (↓) 50% (data from Fig. 5.6).

The seasonal germination pattern outdoors was also closely simulated when air temperature at 1.50 m (the height seeds were placed outdoors) was used as T_g in Equation 5.1 (Fig. 5.8). However, in July and October 1989 germination was over-predicted.

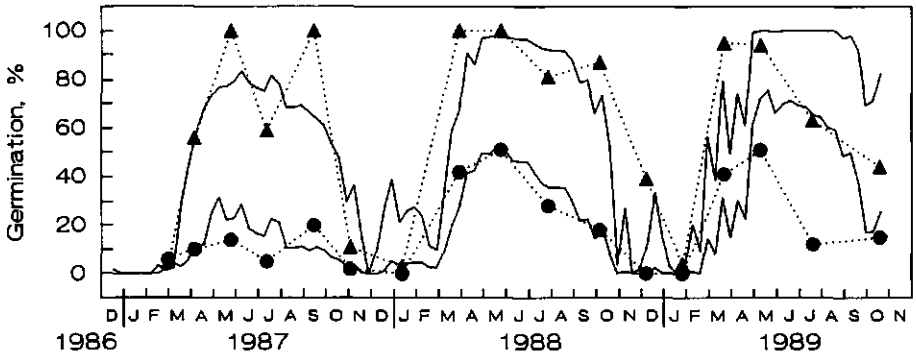

Fig. 5.8 Simulated (intact line) and observed (dotted line) germination of exhumed seeds of *Chenopodium album* in Petri dishes outdoors as a function of exhumation date. Observed germination as in Fig. 5.6 in water (●) or 50 mM KNO_3 (▲). Simulation was carried out with Equation 5.1. See text for explanation.

5.4 Discussion

Dormancy pattern and emergence

The clear seasonal pattern of germination that was observed when exhumed seeds of *C. album* were tested under field conditions (Fig. 5.6) corresponds with the results of studies on the emergence of this species (Roberts, 1964; Roberts and Ricketts, 1979; Håkansson, 1983; Ogg and Dawson, 1984; Van den Brand, 1986, 1987). Interestingly, Håkansson (1983) also found a temporary decrease in emergence during summer as shown in the present study when germination was tested in nitrate (Fig. 5.6). In emergence studies, moisture may be the primary limiting factor for germination during summer. It is, however, clear from the present results that because of the relatively low $T_{g,max}$ in summer, germination may also be inhibited when the field temperature increases above $T_{g,max}$ (Fig. 5.7). This seems a valuable ecological adaptation since it prevents germination in the warm season. This objective is reached by other species such as *Polygonum persicaria*, by an early induction of secondary dormancy (Chapter 2). This excludes germination in summer, but also in autumn, whereas *C. album* seeds can still germinate in autumn. Since *C. album* is a short day plant flower formation in autumn is induced within several weeks. Probably, seeds can still be produced, but observations to confirm this hypothesis are lacking.

The germination pattern outdoors, particularly in nitrate (Fig. 5.6) and the changes in the germination-temperature range (although small, Fig. 5.7) were very similar to *Spergula arvensis* (Chapter 4). Both $T_{g,max}$ and $T_{g,min}$ fluctuated (Fig. 5.7), in contrast to *Polygonum persicaria* and *Sisymbrium officinale*, where changes in dormancy

occurred through changes in $T_{g,min}$ or $T_{g,max}$ only (Chapters 2 and 3).

Just as with *Spergula arvensis*, it is suggested that *C. album* has to be named a facultative summer annual because it can germinate both in spring and summer-autumn.

Control of germination

The present study shows that just as for *Sisymbrium officinale* and *Spergula arvensis* (Chapters 3 and 4) the expression of the dormancy pattern of *C. album* strongly depended on the conditions during the germination test. In addition to temperature (Fig. 5.1), the three factors light, nitrate and desiccation that also may be of ecological importance, were investigated. As was reported by Henson (1970), Vincent and Roberts (1977) and Roberts and Benjamin (1979), there was a strong positive interaction between the effects of light and nitrate on germination of *C. album*. Alone, both factors only slightly stimulated germination (compare germination in water to germination in KNO_3 in Fig. 5.2B, and germination in water in Fig. 5.2B to germination in water at 20°C in Fig. 5.1A). When both factors were combined, germination sometimes increased from zero to 100% (compare germination in water in Fig. 5.2B to germination in KNO_3 at 20°C in Fig. 5.1B). Consequently, germination could occur during a much longer period of the year than when germination was tested in either water with a red light irradiation or in nitrate in darkness (Figs 5.1A, 5.2B).

The absence of germination in darkness in water or KNO_3 (Fig. 5.2B) agrees with results of Baskin and Baskin (1977). During one year of burial, exhumed seeds did not germinate in darkness at any of the constant temperature they tested. However, considerable germination occurred in darkness, when seeds were incubated at alternating temperatures (30/15°C and 35/20°C, 12/12h). Also in the experiment of Karssen (1980/81*b*) germination occurred in darkness at 22/12°C (12/12h). In the present experiment, seeds germinated fairly well in darkness at 20°C when they were incubated in nitrate after a desiccation treatment (Fig. 5.2B). Under these conditions the stimulatory effect of desiccation was most evident. The effect of desiccation became apparent only after approx. half a year of burial (Fig. 5.2). This is discussed in more detail in Chapter 6.

For *C. album* seeds, light seems to be the best indicator for a position at or near the soil surface. When other indicators for a position close to the surface such as desiccation (Fig. 5.2) or alternating temperatures (Baskin and Baskin, 1977; Karssen, 1980/81*b*) are present, light is no longer absolutely required.

Incomplete germination. Incomplete germination particularly occurred when exhumed seeds were tested at 10°C and also showed periodicity (Fig. 5.3). These results agree with Cumming (1963) who found incomplete germination of several *Chenopodium* spp. at 10°C. Karssen (1968) found that abscisic acid (ABA) blocked germination of *C. album* in the incomplete stage. He distinguished two sites of hormonal action in the germination process of *C. album* (Karssen, 1976*b*). In imbibed seeds, Pfr, gibberellins 4 and 7 (GA_{4+7}) and ethylene *induced* growth, which caused splitting of the outer testa layer. ABA did not influence the induction of growth but *inhibited* the subsequent extension of the radicle, hence preventing complete germination. The ABA action was antagonized by GA_{4+7}, GA_3, zeatin, kinetin and ethylene.

The present data show that induction and completion of growth had a different temperature requirement. At 10°C induction of growth often occurred, whereas extension of the radicle was inhibited at that temperature. At first sight, this suggests that the changes in dormancy of *C. album* were regulated by ABA. Induction of growth by GA always occurred and ABA regulated visible germination. Changes in the sensitivity to ABA could then regulate the seasonal germination/emergence pattern. However, both parts of the overall germination process were influenced during changes in dormancy in *C. album* seeds. The simultaneous **decrease of growth induction**, as seen by a decrease in total germination in Fig. 5.3A,C, and an **increase in visible germination** (Fig. 5.3A-D), suggest that changes in dormancy of *C. album* seeds were regulated at more than one site. The seasonal changes in germination/emergence may be the result of a combination of changes in sensitivity to ABA and GA.

Karssen *et al.* (1989) suggested that changes in sensitivity to GA could well be the mechanism responsible for changes in dormancy in weed seeds. It seems that changes in sensitivity to ABA (present results) may also be involved in these changes. For *Arabidopsis thaliana* (M.P.M. Derkx, pers. comm.) and *Sisymbrium officinale* (H.W.M. Hilhorst, pers. comm., Chapter 7) there is evidence that also sensitivity to factors, such as light and nitrate varies during changes in dormancy. Probert *et al.* (1989) and VanDerWoude and Toole (1980) showed that sensitivity to light increased due to stratification in *Ranunculus sceleratus* and *Lactuca sativa*, respectively.

Cumming (1963) suggested that incomplete germination of *Chenopodium* spp. is of survival and adaptive value, particularly under arid conditions to which some of the species are adapted. In the incompletely germinated stage, *C. album* seeds can survive long dry periods, whereas seedlings would undoubtedly die. The present study shows that incomplete germination of *C. album* also occurs in The Netherlands and particularly in late autumn-winter (Fig. 5.3). It is hypothesized that incomplete germination is also of adaptive value in the temperate zone. On the one hand, it may prevent emergence of non-frost-resistant seedlings before and during winter. On the other hand, the presence of incompletely germinated seeds in the seed bank would enable a rapid emergence and establishment of the species in the following spring.

Incomplete germination of *Spergula arvensis*, also a member of the *Centrospermae*,

mainly occurred when exhumed seeds were desiccated and germination was tested at 30°C (Chapter 4). It may be of survival and adaptive value in the drier habitats of *Spergula arvensis*.

Control of dormancy pattern

What is striking about *C. album* is that there were only some minor seasonal changes in the range of temperatures over which germination could proceed (Fig. 5.7). The clear seasonal germination pattern when germination was tested outdoors (Fig. 5.6) almost entirely depended on the fluctuations in the field temperature (Fig. 5.7). The small changes in the germination temperature range in Fig. 5.7 were reflected in the absence of clear seasonal patterns in Fig. 5.1. It may also explain the absence of a clear seasonal pattern for example in the experiment of Karssen (1980/81*b*) where germination was tested at only one test temperature. The cosmopolitan character of C. album may also be related to the broadness and small variability of the germination-temperature range. Regardless of the season, germination can occur whenever the field temperature is between approx. 5 and 25°C and light and sufficient nitrate are available.

Despite fairly large differences in moisture and nitrate content, there were no differences in the dormancy patterns of seeds buried outdoors or in sand or sandy loam under the transparent roof (Fig. 5.4). Also in Petri dishes changes in dormancy occurred although changes in soil moisture content were absent (Fig. 5.5). Vanlerberghe and Van Assche (1986) could mimic the changes in dormancy of *Verbascum thapsus* even in Petri dishes in distilled water by exposing the seeds to a stepwise rising and subsequently decreasing temperature. Their results also indicate that besides temperature, other environmental factors are probably insignificant for the regulation of changes in dormancy.

Dormancy of *C. album* seeds was in the first year relieved particularly during spring (Fig 5.4). In the second and third year, relief of secondary dormancy started during winter (Figs 5.1, 5.4, 5.5). Data from literature on relief of dormancy of *C. album* seeds are rather confusing. Williams and Harper (1965) relieved dormancy partially by an incubation for 21 days at 5°C. Extending this period to three months did not break dormancy any further. Roberts and Benjamin (1979) found that 4 days at 4°C were optimal to relieve dormancy. After 21 days at that temperature seeds had started to enter secondary dormancy. These variable results are typically related to the profound ecotypic variation of *C. album*.

In Fig. 5.5B dormancy induction occurred when the pretreatment temperature was raised to 15°C. This was, however, only visible in a germination test at 20°C. Nevertheless, it seems that induction of dormancy can occur in two ways: (i) if temperatures in the field rise above a certain value (Fig. 5.5A,B) or (ii) if

pretreatment occurs for a prolonged time at low temperatures (Roberts and Benjamin, 1979). Both these mechanisms also occurred in seeds of *Polygonum persicaria* and *Sisymbrium officinale* (Chapters 2 and 3).

Simulation

Temperature, light, nitrate and desiccation all influenced germination to a large extent. Apart from temperature, which had an effect on both changes in dormancy and on germination, it is assumed that the other environmental factors in soil did not influence the **changes in dormancy**. The development of models to predict emergence should therefore concentrate on the effect of environmental factors on germination, that is, on the **expression of dormancy**, rather than on the effect on dormancy itself.

The descriptive model, developed on this basis fairly accurately simulated outdoor germination. In general, such models can increase the understanding of the dormancy patterns, as is shown by Fig. 5.7.

Part of the discrepancy between calculated and actual germination (Fig. 5.7) may be explained by the fact that the field temperature which was used for the calculation of germination was the **mean** air temperature. Obviously, fluctuations in temperature occurred and it is well documented that alternating temperatures stimulate germination of *C. album* (Murdoch *et al.*, 1989). This may enable germination during a longer period than expected or predicted when the mean temperature is used for simulation. The over-prediction of germination in July and October 1989, indicates that the model over-estimated the effect of burial time (which was necessarily involved in C and H, see Chapter 2). The increase in germination in the second year in comparison to the first year, was probably an effect of the longer low-temperature period in the second year and not of burial time. Simulation based also on data of July and October 1989 may improve the model.

Detailed knowledge of the physiological processes leading to changes in dormancy and the effect of environmental conditions on these processes and on germination, should lead to a more mechanistic approach of the simulation of dormancy and emergence patterns.

Chapter 6

Stimulatory effect of desiccation on weed seed germination

Abstract. The effect of desiccation of pre-incubated or exhumed seeds on germination was studied in the weedy species: *Chenopodium album* L., *Polygonum persicaria* L., *P. lapathifolium* L. subsp. *lapathifolium*, *Sisymbrium officinale* (L.) Scop. and *Spergula arvensis* L.. Desiccation stimulated germination of *S. officinale* and *S. arvensis* after a pretreatment of a few days in water. Germination of *C. album* and the two *Polygonum* spp. was only stimulated when the seeds had been buried for a prolonged period of time before they were exhumed and the effect of desiccation was tested. Moreover, desiccation barely stimulated germination of *Polygonum* spp.. For the other species a linear relationship was found between the reduction of seed moisture content and the stimulation of germination. The stimulatory effect of an irradiation with red light on germination of *S. officinale* was fully preserved when seeds were desiccated to low seed moisture contents but only partly when desiccation was less complete. For *S. officinale*, a cycle of induction of dormancy by incubation of imbibed seeds at a warm temperature, followed by dormancy breaking by desiccation could be repeated four times sequentially. Potassium leaked from imbibing seeds. From pre-incubated and subsequently desiccated seeds, much less potassium leaked during re-imbibition. The physiological mechanisms that might be involved in the stimulatory effect of desiccation are discussed. Drying conditions that stimulate germination in the laboratory were also found in the field. It is concluded that the effect of desiccation on germination may act as an additional gap and depth sensing mechanism.

6.1 Introduction

Dehydration of imbibed weed seeds or exposure to wet-dry cycles improved germination of many species, such as *Brassica alba* (Kidd, 1914; Kidd and West, 1917), *Lepidium densiflorum*, *Rumex mexicanus*, *Artemisia incompta* and *Rudbeckia occidentalis* (Griswold, 1936), *Abutilon theophrasti* (Lacroix and Staniforth, 1964), *Geranium carolinianum* and *Sida spinosa* (Baskin and Baskin, 1974, 1984), *Rottboellia exaltata* (Thomas and Allison, 1975), *Verbascum blattaria* (Kiviliaan, 1975), *Sisymbrium officinale* (Karssen, 1980/81*b*) and *Spergula arvensis* (Karssen *et al.*, 1988). However, desiccation did not effect germination of many other species, such as *Bromus anomalus*, *Chenopodium album*, *Lupinus parviflorus*, *Plantago tweedyi* and *Stipa*

columbiana (Griswold, 1936).

In previous studies it was shown that desiccation of exhumed seeds of *Spergula arvensis* L. (corn spurrey), *Sisymbrium officinale* (L.) Scop. (hedge mustard) and *Chenopodium album* L. (lamb's quarters) stimulated germination over a range of test conditions (Chapters 3, 4 and 5), but hardly affected germination of exhumed seeds of *Polygonum persicaria* L. (redshank) and *P. lapathifolium* subsp. *lapathifolium* L. (Chapter 2).

Drying may have ecological significance, because it softened the seed coat of some hard-coated species (*e.g.* Baskin and Baskin, 1974) or broke secondary dormancy in others (Kidd and West, 1917). Karssen (1982) hypothesized that the effect of desiccation may be responsible for the smaller flushes of emerging seedlings in summer that follow upon the major flush in spring, as was shown for example by Popay and Roberts (1970*b*) and Stoller and Wax (1973). The mechanism of the desiccation effect is still unknown and therefore it can not be explained why some species do and others do not respond to this treatment.

In the present paper the effect of desiccation on the germination of *C. album*, *P. lapathifolium* subsp. *lapathifolium*, *S. officinale* and *S. arvensis* was investigated in more detail. The physiological mechanisms that may be involved in the stimulatory effect of desiccation and its ecological significance are discussed.

6.2 Materials and methods

Seeds

Ripe seeds of *C. album*, *P. lapathifolium* subsp. *lapathifolium*, *P. persicaria*, *S. officinale* and *S. arvensis* were collected on arable fields and waste lands in the vicinity of Wageningen. After collection, seeds were allowed to dry. Perianths were removed by rubbing the seeds. Seeds were sieved and winnowed to remove perianth segments and small and light seeds. They were stored dry at 2°C.

Desiccation treatments and germination tests

Seeds were incubated in 50 mm Petri dishes on 1 layer of filter paper (Schleicher and Schüll no. 595) moistened with the appropriate solution (Milli-Q water or KNO_3). Pretreatment and germination tests were carried out in temperature-controlled incubators (Gallenkamp, Crawley, U.K., T ± 1°C). In one experiment seeds were used that had been buried. See Chapter 2 for method of burial.

After pretreatments in water, seeds were surface dried on a Büchner funnel and transferred to a dry filter paper. Undessicated controls were incubated under germination conditions immediately.

Seeds were desiccated for 24 h in hygrostats (Weges and Karssen, 1987) containing silica gel, a saturated salt solution or water. Following this, the seed moisture content was in equilibrium with the r.h. in the hygrostat. Seed moisture content was determined in a sub sample by weighing before and after drying for 1.5 h in an oven at 130°C and is expressed on a dry weight basis.

If appropriate, seeds were irradiated with red light. Red light was obtained by filtering light from 6 red fluorescent tubes (Philips TL 20W/15) through one layer of 3 mm plexiglas (red 501, Röhm & Haas, Darmstadt, GFR). Handling of seeds occurred in dim green safelight, obtained by filtering light from one green fluorescent tube (Philips TL 40W/17) through two layers yellow no. 46 and two layers blue no. 62 Cinemoid filters (Strand Electric, London, U.K.).

To determine germination percentages, both germinated and non germinated seeds were counted between 3 and 14 days after the start of the germination test, depending on test temperature and species, when no further germination occurred. Protrusion of the radicle was the criterion for germination.

Potassium leakage

Potassium was measured with a flame photometer (Elex 6361, Eppendorf Gerätebau GmbH, Hamburg, GFR). Potassium content of seeds was measured by extraction of dry seeds in a mixture of 0.02 M HCl, 0.03 M CsCl and 0.14 M oxalic acid (Weges, 1987).

6.3 Results

Factors influencing the effect of desiccation

Extent of desiccation. In the studies of the seasonal dormancy patterns of *C. album*, *P. lapathifolium* subsp. *lapathifolium*, *P. persicaria*, *S. officinale* and *S. arvensis*, exhumed seeds were desiccated in a standard procedure above a saturated solution of LiCl (Chapters 2, 3, 4 and 5). It was investigated which seed moisture content was critical for the observed stimulation of germination by desiccation. Of the two *Polygonum* spp. only *P. lapathifolium* subsp. *lapathifolium* was tested.

The experiments with *C. album*, *P. lapathifolium* subsp. *lapathifolium* and *S. arvensis* were performed with samples of exhumed seeds. Such seeds reacted much more strongly to desiccation than seeds that had not been buried (Chapters 2, 4 and 5). In parallel experiments it was shown that seeds of *S. officinale* could also be made sensitive to desiccation by a pretreatment in water. The pretreatment time in soil or water has been indicated in the legends of Figs 6.1 to 6.3 and Table 6.1 for each species separately.

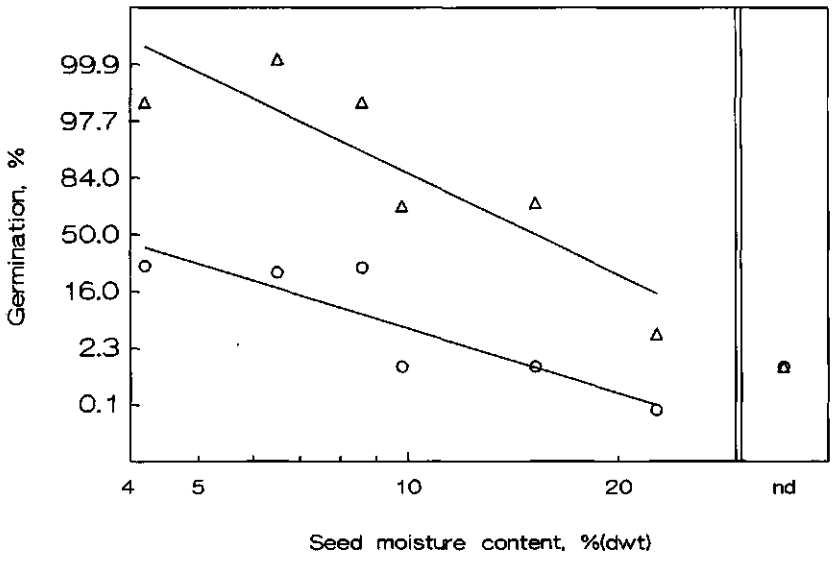

Fig. 6.1 Effect of seed moisture content after desiccation (log scale) on germination (probit scale) of *Sisymbrium officinale*. Seeds were pretreated for 7 days at 24°C in Milli-Q water in darkness. Seed moisture content after the pretreatment was 89% (dwt). Subsequently, seeds were irradiated for 15 min with red light. Seeds were desiccated in hygrostats containing water, various saturated salt solutions or silica gel. After desiccation, seed moisture content was determined in duplicate sub samples. Triplicates of 50 seeds were then incubated in Milli-Q water (O) or 25 mM KNO_3 (Δ) at 24°C to test germination. Control seeds (nd = not desiccated) were incubated under test conditions immediately after irradiation.

After exhumation or at the end of the pretreatment, seeds were desiccated over a range of r.h.'s. After desiccation seed moisture content was determined and germination tested.

A linear relationship existed between the seed moisture content after desiccation and probit germination of *S. officinale*, *S. arvensis* and *C. album* (Figs 6.1, 6.2, 6.3). Germination of these three species increased with a decrease in the seed moisture content. Desiccation only stimulated germination of *P. lapathifolium* subsp. *lapathifolium* when it was tested in darkness (Table 6.1). There was no difference in the effect of desiccation to a seed moisture content of 8.6 and 7.7% (dwt). With a red light irradiation, desiccation did not stimulate germination of this species at 20°C or even inhibited it at 30°C.

The slopes of the regression equations show that the impact of desiccation on germination was stronger for germination tests in KNO_3 than for tests in water (Figs 6.1, 6.2).

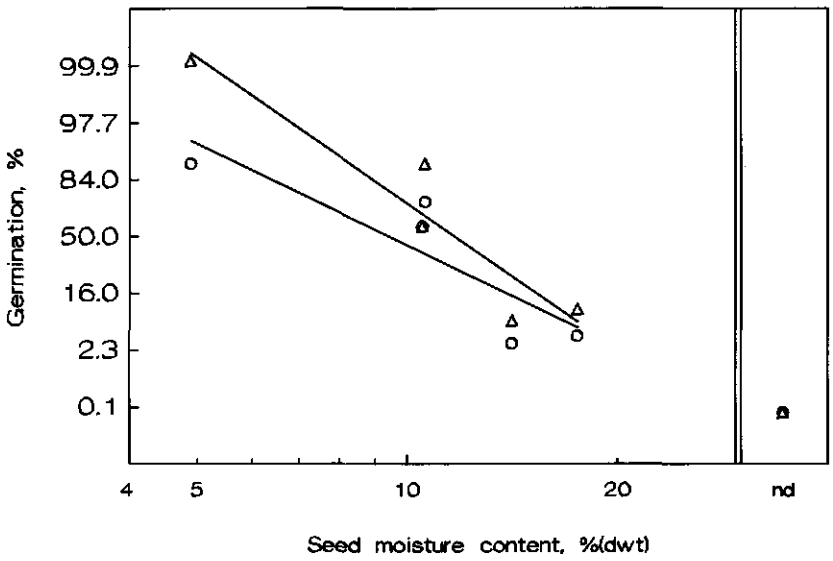

Fig. 6.2 As Fig. 6.1 for seeds of *Spergula arvensis* that had been buried for 14 months in sandy loam at a depth of 10 cm, and were exhumed in February 1988. Seed moisture content after exhumation was 58% (dwt). Germination was tested with duplicates of approx. 30 seeds at 15°C in darkness in Milli-Q water (O) or 50 mM KNO_3 (Δ). Control seeds (nd = not desiccated) were incubated under test conditions immediately after exhumation.

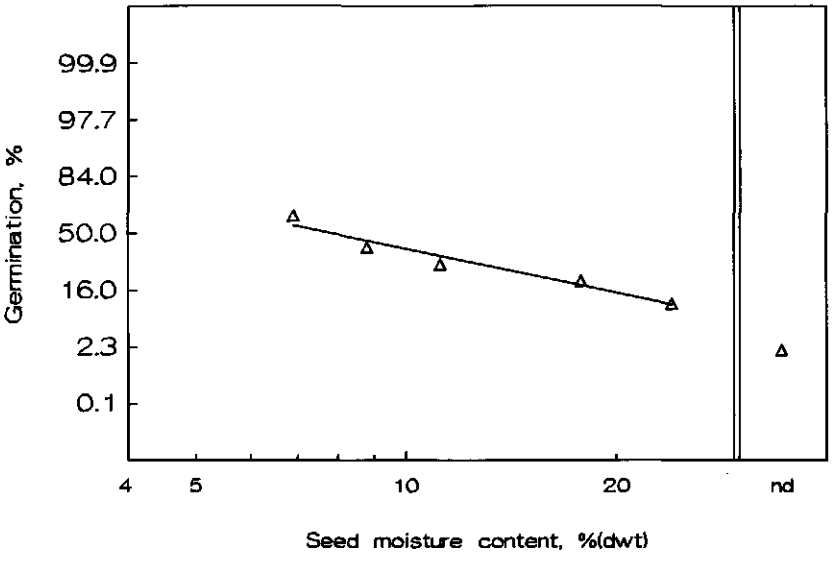

Fig. 6.3 As Fig. 6.2 for seeds of *Chenopodium album*. Seed moisture content after exhumation was 54% (dwt). Germination was tested with triplicates of approx. 45 seeds in darkness in 50 mM KNO_3. Control seeds (nd = not desiccated) were incubated under test conditions immediately after exhumation.

Table 6.1 Effect of seed moisture content after desiccation on germination percentage of *P. lapathifolium* subsp. *lapathifolium*. Seeds had been buried for 2 years in sandy loam at a depth of 10 cm and were exhumed in December 1988. Seed moisture content after exhumation was 52% (dwt). After exhumation the seed sample was divided into smaller portions. Half of the portions were irradiated for 15 min with red light. Subsequently seeds were not dried (nd) or desiccated above silica gel or a saturated solution of LiCl which gave a seed moisture content of 7.7 and 8.6% (dwt), respectively. Seed moisture content was determined in single sub samples. To test germination, duplicates of approx. 30 seeds were incubated in 50 mM KNO_3 at 6, 20 or 30°C.

	darkness			light		
	seed moisture content after desiccation, %(dwt)					
test temperature, °C	7.7	8.6	nd	7.7	8.6	nd
6	2	0	0	0	0	0
20	37	37	3	68	69	74
30	19	24	0	52	56	75

Irradiation. All species that were studied required light for germination. Several authors have reported the persistence of the stimulatory effect of light during desiccation and subsequent storage for periods even up to one year (Cumming, 1963; Vidaver and Hsiao, 1972; Bartley and Frankland, 1985). However, it is necessary that the water content is reduced quickly to prevent destruction of the active far-red absorbing form of phytochrome (Pfr) or reversion of Pfr to Pr, the inactive, red absorbing form (Hsiao and Vidaver, 1973; Bartley and Frankland, 1985). It was investigated whether with the present desiccation methods, the red light effect on germination was preserved during desiccation. The experiments were performed with seeds of *S. officinale*. After the initial dormancy breaking pretreatment in water seeds were irradiated before or after a desiccation treatment. Subsequently, germination was tested (Fig. 6.4).

Also in this experiment germination increased when the seed moisture content decreased due to desiccation (Fig. 6.4). When seeds were desiccated to low moisture contents there were no differences in germination of seeds irradiated before or after the desiccation treatment. When seeds were desiccated to a lesser extent, they germinated better when the irradiation was given after the desiccation treatment.

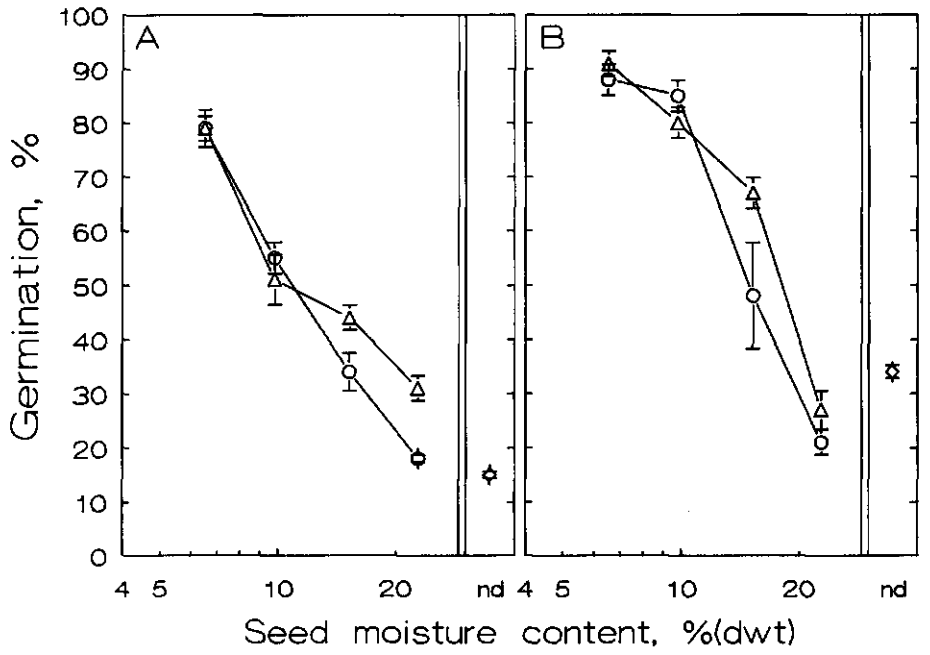

Fig. 6.4 Effect of irradiation before or after desiccation to various seed moisture contents on germination of *Sisymbrium officinale*. Triplicates of 50 seeds were pretreated during 40 h at 15°C in Milli-Q water, desiccated at various r.h.'s and subsequently incubated in 50 mM KNO_3 at 10 (A) or 15°C (B). Irradiation with 5 min red light occurred either just before (○) or after desiccation (△). In the latter case seeds were re-imbibed during 1.5 h at room temperature before irradiation occurred. Control seeds (◇) were irradiated after pretreatment and not desiccated (nd) before incubation at 10 and 15°C. Vertical bars indicate standard error.

Pretreatment. In Chapters 2, 3, 4 and 5 it was demonstrated for all the species that are studied that desiccation did not stimulate germination directly after burial. Therefore, it was investigated whether a relationship existed between the duration of the pretreatment and the effect of desiccation. Seeds of *C. album*, *P. persicaria*, *S. officinale* and *S. arvensis* were pretreated in water in darkness. At regular intervals, germination of a sample of the pretreated seeds was tested after irradiation with red light, in water or KNO_3, with or without a preceding desiccation treatment.

In seeds of *S. officinale*, relief of primary dormancy occurred at 24°C within half a day (Fig. 6.5). It was followed rapidly by induction of secondary dormancy. During the period of dormancy relief, desiccation had no effect on germination. However, when induction of secondary dormancy had started, desiccation strongly stimulated germination and therefore masked the induction of dormancy. When the pretreatment was continued for periods over 5 days, germination of desiccated seeds decreased (Fig. 6.5). Dormancy breaking of *S. officinale* seeds occurred parallel to the increase in seed moisture content. Seeds were fully imbibed within half a day.

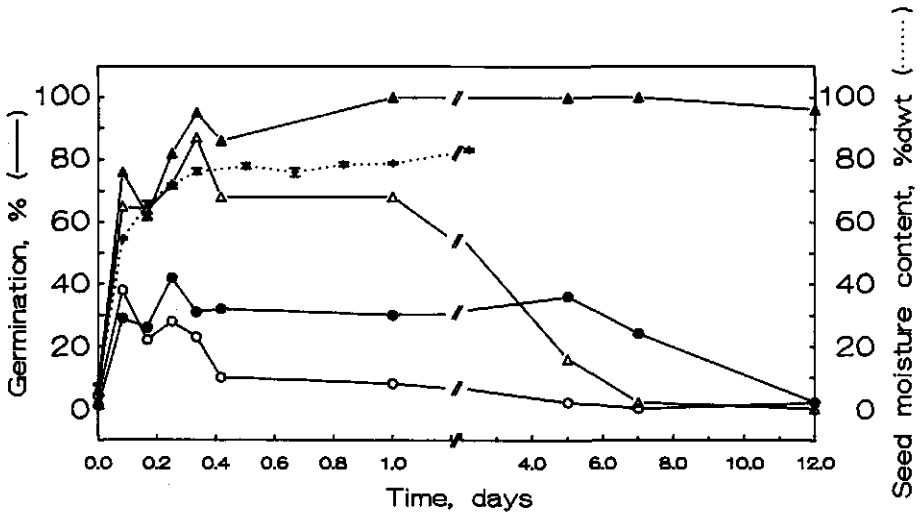

Fig. 6.5 Effect of the duration of the pretreatment on the effect of desiccation on germination of *Sisymbrium officinale*. Seeds were pretreated in water at 24°C in darkness. At regular intervals, germination was tested with one test sample of 50 seeds. After a 15 min red light irradiation, seeds were incubated in Milli-Q water (circles) or 25 mM KNO_3 (triangles) at 24°C, with (closed symbols) or without (open symbols) a preceding desiccation treatment which gave a seed moisture content of approx. 6% (dwt). At regular intervals during the pretreatment, the seed moisture content was determined in triplicate sub samples (dotted line). Vertical bars indicate standard error.

The relief of primary dormancy of *S. arvensis* at 15°C occurred at a slower rate. It was within 30 days not followed by induction of secondary dormancy (Fig. 6.6). In this species, desiccation stimulated germination both in water and nitrate already during relief of primary dormancy.

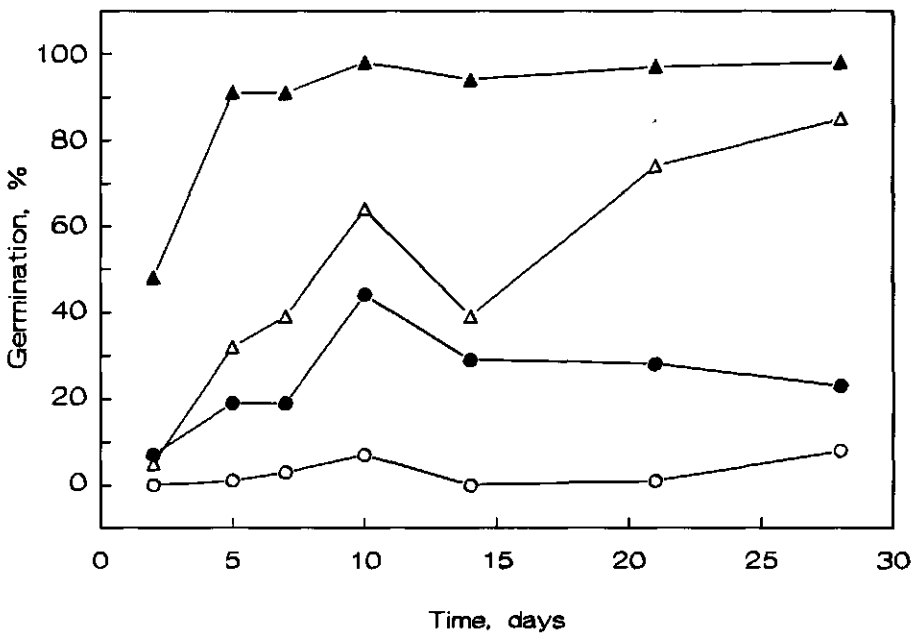

Fig. 6.6 As Fig. 6.5 for seeds of *Spergula arvensis*. Seeds were pretreated at 15°C in water in darkness. Germination was tested with triplicates of 50 seeds at 22°C in Milli-Q water or 50 mM KNO_3. Seed moisture content during the pretreatment was not determined.

Also primary dormancy of *P. persicaria* and *C. album* were relieved normally at 2 and 10°C, respectively. However, desiccation did not stimulate germination of these species even after 6 or 8 weeks of pretreatment, respectively (data not shown). A possible explanation for the differences in the stimulatory effect of desiccation could be differences in the rate of imbibition. The imbibition rate of *P. persicaria* and *C. album* (Fig. 6.7) were somewhat slower than of *S. officinale* (Fig. 6.5). However, seeds of both species were fully imbibed after approx. 4 days, whereas desiccation did not stimulate germination even after 6 or 8 weeks of pretreatment.

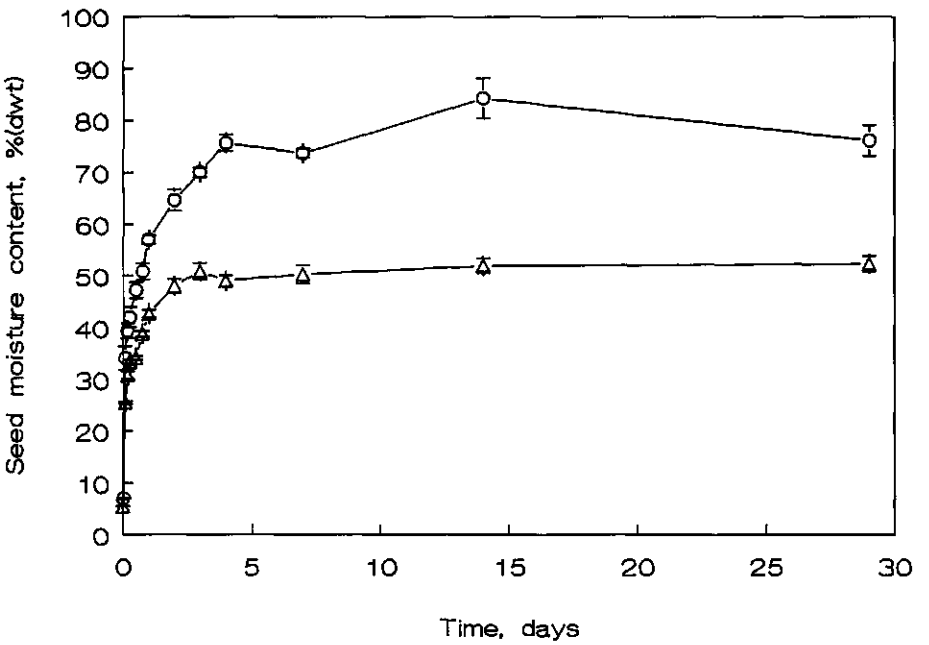

Fig. 6.7 Imbibition of *Chenopodium album* at 10°C (○) and *Polygonum persicaria* at 2°C (△). At regular intervals seed moisture content was determined in triplicates of approx. 100-200 mg (dwt). Vertical bars indicate standard error.

Reversibility

The previous experiments have shown that desiccation is often highly stimulatory to germination. The intriguing question is, whether the dormancy breaking effect of desiccation could be reversed by a dormancy inducing treatment.

In *S. officinale* seeds, relief of dormancy by desiccation was indeed followed by re-induction of dormancy during a 4 days incubation at 24°C in water (Fig. 6.8). The cycle of dormancy relief by desiccation and dormancy induction by incubation at 24°C could be repeated an additional three times.

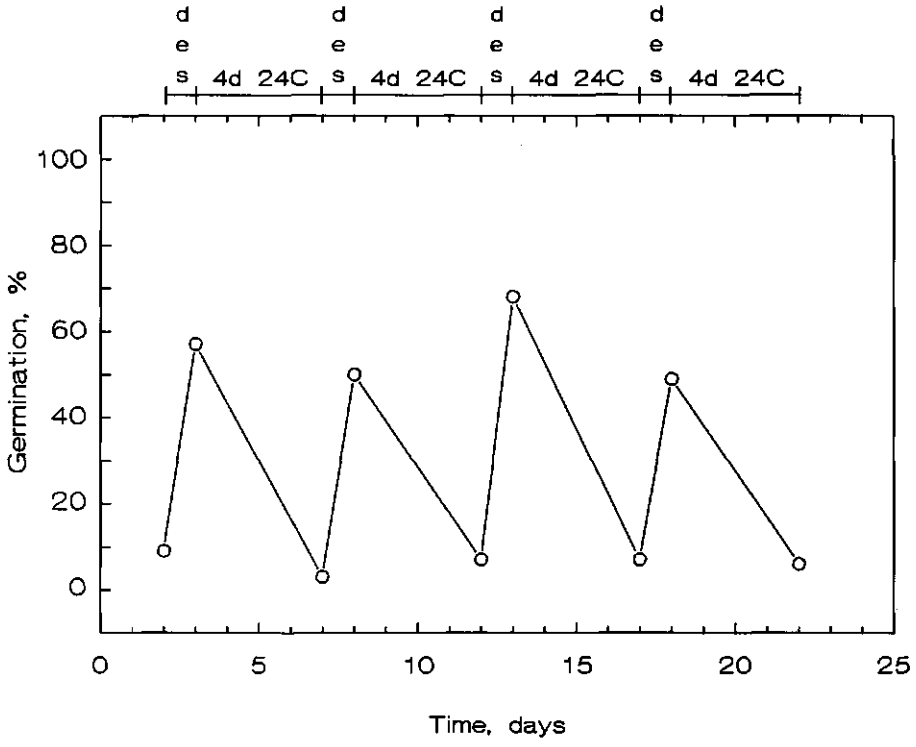

Fig. 6.8 Effect of repeated desiccation and dormancy induction treatments on germination of *Sisymbrium officinale*. Secondary dormancy was induced in *S. officinale* seeds by a pretreatment for 2 days at 24°C in water. Subsequently, dormancy was relieved by a desiccation treatment (des) that reduced the seed moisture content to approx. 8.5% (dwt). Several cycles of dormancy induction (4 days at 24°C) and dormancy relief (desiccation) were given. After each treatment, germination was tested in 50 mM KNO_3 at 24°C with triplicates of 50 seeds following a 15 min red light irradiation.

Potassium leakage

Desiccation often causes membrane damage in seeds (Hegarty, 1978; Simon, 1984). A common parameter to measure this damage is the leakage of potassium ions into the incubation medium. Potassium leakage was measured during imbibition of control seeds of *S. officinale* and of seeds that had been pre-incubated for 2 days at 24°C in water followed by a desiccation treatment (Fig. 6.9). From fresh seeds 0.7 mg $K^+.g^{-1}$ seed (fwt) leaked out in 3 h, which is approx. 15% of the total potassium content (4.79 mg $K^+.g^{-1}$ seed (fwt)). Leakage from pre-incubated and desiccated seeds stopped after about 20 min at 0.2 mg $K^+.g^{-1}$ seed (fwt), which is approx. 5% of the potassium remaining after the leakage from fresh seeds.

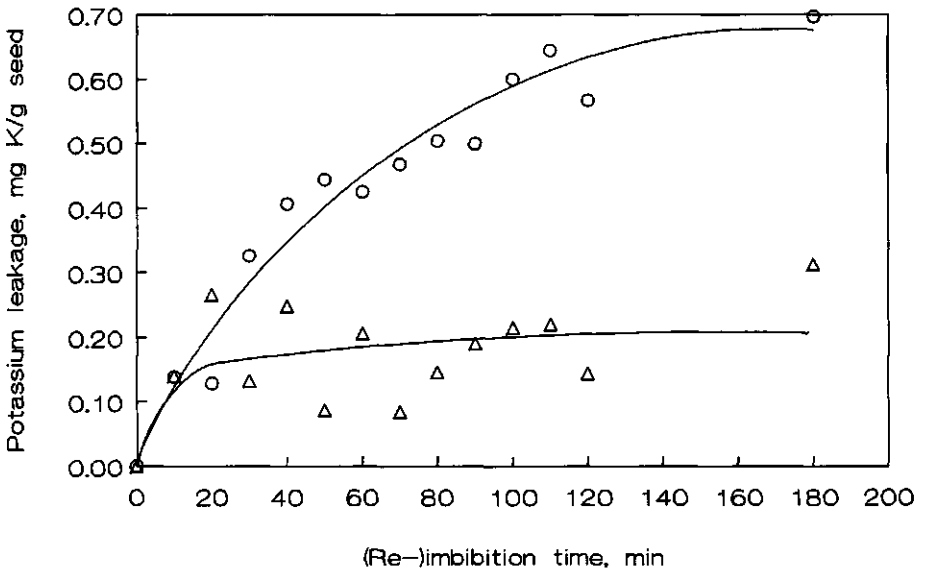

Fig. 6.9 Potassium leakage from imbibing fresh (○) and re-imbibing desiccated (△) *Sisymbrium officinale* seeds. Hundred and fifty *S. officinale* seeds were imbibed either directly in 5 ml Milli-Q water or they were first pretreated for 2 days at 24°C in Milli-Q water, surface dried on a Büchner funnel, desiccated to a seed moisture content of approx. 8.5% (dwt) and then incubated in 5 ml Milli-Q water. At regular intervals, from two dishes of both treatments samples of the incubation medium were taken, in which potassium was measured with a flame photometer.

6.8 Discussion

Effect of desiccation

Desiccation stimulated germination of *C. album*, *S. officinale*, *S. arvensis* and *P. lapathifolium* subsp. *lapathifolium* (Figs 6.1 to 6.3, Table 6.1). A stimulatory effect of desiccation was reported before by Griswold (1936), Kiviliaan (1975), Thomas and Allison (1975), Karssen (1980/81*b*) and Karssen *et al.* (1988). For the first three species the stimulation rose parallel to the decrease of the seed moisture content. A further reduction of the seed moisture content stimulated germination of *S. officinale* and *S. arvensis* more than germination of *C. album* (Figs 3.1 to 3.3). This will doubtlessly be related to differences in the composition of reserve food material, the relative size of the embryo etc., which may lead to a different distribution of the remaining water within the seeds of the various species.

The results are in contrast to data from studies on some crop seeds. Dehydration treatments following (osmotic) priming of seeds, caused a partial loss of the beneficial effects of the pretreatment in certain species (Heydecker and Coolbear, 1977; Weges and Karssen, 1987). It seems that dehydration leads to a loss of information. Depending on the kind of information the result may be either positive (some weed seeds) or negative (some crop seeds). In weed seeds the information of "being dormant" is removed, whereas in crop seeds the information of "being non-dormant" is lost.

The effect of a red light irradiation was preserved when *S. officinale* seeds were desiccated to low moisture contents (Fig. 6.4). Similar results have been described for other species, such as lettuce (Vidaver and Hsiao, 1972), *Plantago major*, *Sinapis arvensis* and *Bromus sterilis* (Bartley and Frankland, 1985) and incompletely germinated seeds of *Chenopodium* spp. (Cumming, 1963). Apparently, destruction of Pfr or reversion of Pfr to Pr was prevented when *S. officinale* seeds were desiccated to a moisture content of approx. 15% (dwt) or lower (Fig. 6.4). The fact that desiccation only stimulated germination of *P. lapathifolium* subsp. *lapathifolium* when it was tested without a red light irradiation, could indicate that in seeds of this species some destruction of Pfr or reversion of Pfr to Pr occurred during desiccation. The positive effect of desiccation on germination may then be counteracted by the negative effect of a loss of Pfr. The latter negative effect will not (or to a lesser extent when pre-existing Pfr is present) occur in seeds that were not irradiated. It seems, that particularly for large-seeded species, such as *P. lapathifolium* subsp. *lapathifolium*, rapid desiccation is needed to preserve the red light effect.

Factors influencing the effect of desiccation

Apparently, the induction of sensitivity to desiccation was a slow process in *C. album*, *P. lapathifolium* subsp. *lapathifolium* and *P. persicaria*. Desiccation did not stimulate germination of *P. persicaria* and *C. album* even after imbibition for 6 or 8 weeks, respectively (data not shown). When seeds of *C. album*, *P. lapathifolium* subsp. *lapathifolium* and *P. persicaria* were buried in soil, desiccation of exhumed seeds became stimulatory to germination after approx. 6 months (*C. album*) and 1 year (*Polygonum* spp.) of burial. Additionally , the effect of desiccation was apparent only under specific test conditions such as low temperatures (Figs 2.3, 2.8) or the absence of a red light irradiation (Fig. 5.2). The slow induction of sensitivity to desiccation and the need for specific test conditions may well be the reason that several authors failed to demonstrate an effect of desiccation on germination of *C. album*.

At first sight, the results with *S. officinale* indicated that the effect of desiccation of imbibed seeds was related to the dormancy status of the seeds (Fig. 6.5). During relief of primary dormancy desiccation had no effect. When induction of secondary dormancy had started, desiccation could stimulate germination. Also during experiments with buried seeds when germination of exhumed seeds was tested in light, desiccation only seemed to stimulate germination after primary dormancy was relieved (Chapter 3, Fig. 3.2A). However, when tested in darkness, germination was also stimulated by desiccation when primary dormancy was not completely relieved (Chapter 3, Fig. 3.2B). Also germination of *S. arvensis* was stimulated by desiccation already during relief of primary dormancy (Fig. 6.6, Chapter 4).

Although in general the effect of desiccation seemed not to depend on the dormancy status, the sensitivity to desiccation did increase in most species with burial or pretreatment time. Consequently, for all species the stimulatory effect of desiccation was largest when induction of secondary dormancy had started for the first time (Chapters 2 to 5).

Solute leakage

The leakage from imbibing and re-imbibing seeds (Fig. 6.9) may be caused by damage to membranes. This damage may occur in two ways:
(i) Desiccation may cause damage if seeds are desiccated when cell division and enlargement has commenced and, consequently, the seeds have lost their desiccation tolerance (Berrie and Drennan, 1971; Hegarty, 1978; A.M. Haigh, pers. comm.). This is also likely in weed seeds, when germination has proceeded beyond this point. However, in the present experiments the germination process was either not induced before desiccation, because seeds received no red light irradiation, or seeds were desiccated immediately after the germination process was started by a red light irradiation and before radicle growth had commenced. Therefore, this type of damage

probably did not occur.
(ii) Damage may occur during imbibition because of membrane rupture (Simon, 1984). For many crop seeds, rapid imbibition leads to imbibition damage (Simon, 1984; Powell, 1989). The faster the imbibition occurs, the more solutes leak out. The origin of the leaking solutes seems to be cytoplasmic rather than apoplastic, which indicates damage to cell membranes during imbibition (Simon, 1984).

During imbibition, the volume of seeds increases. This was shown for species such as pea, rapeseed, wheat and corn (Shaykewich, 1973; Spurny, 1973). This causes a change in the physical structure of the seed or the seed coat and therefore a faster water uptake by pre-imbibed and desiccated seeds than when fresh seeds are imbibed (Kidd and West, 1919; Berrie and Drennan, 1971; Heydecker and Coolbear, 1977). Also re-imbibition of *S. officinale* seeds occurred faster than imbibition of fresh seeds (data not shown).

From *S. officinale* (Fig. 6.9), pea (Simon, 1984) and lettuce seeds (Weges, 1987) 10-15% of the total potassium content leaked upon imbibition. From *S. officinale* approx. 5% of the remaining potassium leaked during re-imbibition after desiccation (Fig. 6.9). Nevertheless, repeated cycles of wetting and drying seemed unharmful (Fig. 6.8).

Mechanism of desiccation effect.

It is possible that due to the rapid uptake of water during re-imbibition some specific conformational changes in cell membranes occurred. As a consequence, the orientation of proteinaceous receptors may have altered such that sensitivity to germination stimulants was increased. The positive interaction between the effects of nitrate and desiccation on germination of *S. officinale* and *S. arvensis* (Figs 6.1, 6.2) may point to an effect of desiccation on the nitrate receptor. This is confirmed by H.W.M. Hilhorst (pers. comm.), who also found a positive interaction between the effects of desiccation and nitrate on germination of *S. officinale*.

In maturing wheat grains both high temperatures (Norman *et al.*, 1982) and drying (Armstrong *et al.*, 1982) induced sensitivity to GA. Possibly the effects of drying and high temperatures were mediated through changes in membrane structure such that GA binding sites became available (Norman *et al.*, 1982). The dormancy breaking action of desiccation of weed seeds could well be in agreement with the hypothesis of Karssen *et al.* (1989) that changes in dormancy are regulated by changes in GA sensitivity. The reversibility of the dormancy breaking effect of desiccation on *S. officinale* seeds by a dormancy inducing treatment of 4 days in water at 24°C (Fig. 6.8), may indicate that both treatments indeed influenced the same mechanism, possibly the conformation of membranes and hence the availability of the GA receptor. The possibilities that receptors other than just for GA are involved in the seasonal changes in dormancy were discussed in Chapter 5.

Ecological importance

Wetting and drying cycles occur in the field, particularly in the upper layers of the soil. Measurements of soil moisture content at 1 cm in the field showed that values below 2% (dwt) did occasionally occur (B.J. Post, pers. comm.). Probably, these values will be reached even sooner closer to the surface. Measurements on *S. officinale* seeds in samples of sandy loam with different moisture contents, showed that below a soil moisture content of approx. 2.3% (dwt) the seed moisture content dropped below 10% (dwt) (data not shown). In the laboratory, a clear stimulation of germination already occurred at this seed moisture content (Fig. 6.1).

Several authors have suggested a role for wetting and drying cycles in the regulation of changes in dormancy of weed seeds (Stoller and Wax, 1973; Baskin and Baskin, 1974; Thomas and Allison, 1975; Mott, 1978; Vincent and Cavers, 1978). In parallel experiments it was shown that germination of *C. album*, *S. officinale* and *S. arvensis* was stimulated by desiccation throughout a burial experiment of two and a half years. Seeds could germinate during a much longer period of the year or even during the whole year when they were desiccated before the germination test (Chapters 3, 4 and 5).

These three species and *P. lapathifolium* subsp. *lapathifolium* normally did not germinate in the absence of light. However, when they were desiccated, germination could also occur in darkness (Figs 6.2, 6.3, Table 6.1; Chapters 3, 4 and 5). Apparently, for most species light is the strongest indicator for a position at or (very) close to the surface. It is hypothesized that desiccation, in addition to nitrate (Pons, 1989) and light and alternating temperatures (Thompson and Grime, 1983) may act as an additional gap and depth sensing mechanism.

Chapter 7

Effect of nitrate fertilization of weeds on nitrate content, germination and dormancy pattern of the produced seeds.

Abstract. The nitrate content of seeds of *Chenopodium album* L. and *Sisymbrium officinale* (L.) Scop. was raised by weekly nitrate fertilizations of the mother plants. Seeds with an increased nitrate content germinated to higher percentages. *S. officinale* seeds showed a clear linear relationship between germination and nitrate content. Seeds of the two species with different endogenous nitrate contents were buried at 10 cm in sandy loam. At regular intervals seeds were exhumed and germination was tested. Differences in germination and nitrate content rapidly disappeared during burial. Differences in seed nitrate content were also found in seed lots collected in the field. However, it is concluded that the increased nitrate content will stimulate germination only temporarily, since nitrate leaks out quickly after imbibition.

7.1 Introduction

There have been many attempts to influence seed dormancy by treating the parent plants. Such attempts involved spraying of the plants with growth regulators and other chemicals (Austin, 1972) or application of fertilizers (for reviews see e.g. Barton, 1965; Austin, 1972; Pollock and Roos, 1972; Gray and Thomas, 1982).

Usually, mineral **deficiencies** in the parent plant only affected the number of seeds and had relatively minor effects on the chemical composition of the seeds (Austin, 1972). However, **application** of fertilizers not only changed the number of seeds, it also altered the elemental composition of the seeds (Gray and Thomas, 1982).

An increased nitrogen and/or protein content of seeds has been found as a result of nitrogen fertilization of species such as carrot, wheat, long bean and snap bean. Sometimes this increased N-content resulted in improved **seedling vigour** (Gray and Thomas, 1982). The effects of N-fertilization on **germination** however, were inconsistent (Austin, 1972; Pollock and Roos, 1972; Gray and Thomas, 1982).

Fenner (1986*a*,*b*) tested the effects of variations in all major elements of a nutrient solution on which he grew plants of *Senecio vulgaris*. He found only small differences in the mineral composition of the produced seeds (Fenner, 1986*a*). In some cases the differences in the mineral composition of seeds had an effect on

growth of the seedlings (Fenner, 1986*b*). Effects on germination were not tested.

Nitrate strongly promoted germination of *Chenopodium album* L. (lamb's quarters, fat-hen) (Williams and Harper, 1965; Henson, 1970; Vincent and Roberts, 1977). Nitrate application to the mother plant raised the nitrate content of *C. album* seeds, which correlated with higher germination percentages (Fawcett and Slife, 1978; Saini *et al.*, 1985*a,b*)

In the present study the experiments with *C. album* are repeated and compared to similar attempts to raise the endogenous nitrate content of *Sisymbrium officinale* (L.) Scop. (hedge mustard) seeds. Germination of this species is also strongly stimulated by nitrate (Hilhorst *et al.*, 1986). Effects of variation in endogenous nitrate will be compared to that of applied nitrate.

In Chapters 3, 4 and 5 it was shown that nitrate influenced the expression of the dormancy pattern of buried seeds of various weed species. In nitrate, exhumed seeds germinated over a much longer period of the year compared with germination in water. This effect was especially clear for *C. album* (Chapter 5). In the present study, seeds with different endogenous nitrate levels are buried to test whether the dormancy pattern is also influenced by **endogenous** nitrate.

7.2 Materials and methods

Plant cultivation

Seedlings of *C. album* and *S. officinale* were collected in the vicinity of Wageningen from a uniform group of plants for each species. Plants were grown outdoors in summer 1987 in pots (10 l) containing sandy loam, one plant per pot. From the beginning of flowering onwards plants were irrigated weekly with 250 ml of a KNO_3 solution of 0, 0.5, 1, 3, 6 or 12 g/l. Four plants were used per treatment. Seeds were harvested when mature. The seeds of the four plants were combined.

In 1988 the experiment with *S. officinale* was repeated. Now seeds were harvested separately for each plant.

Seeds were dried on air, cleaned and sieved and stored dry at 2°C.

Burial

From the 1987 experiment three seed lots of each species with different nitrate contents were packed in each of 12 bags of fine mesh gauze. The bags were buried in sandy loam in a plastic net pot (ø 10 cm), that provided good contact with the surrounding soil. To prevent loss of soil during handling the pots were lined with gauze. The soil surrounding the seeds prevented light reaching the seeds during exhumation.

The pots with the seeds were buried in the field in sandy loam at a depth of 10 cm. At burial on December 4, 1987 (week 0) and 8, 16, 29 and 53 weeks after burial the germination capacity of the seed lots was tested. Germination at week 0 was tested with triplicates of 50 seeds. After exhumation the seeds were equally divided into portions of varying number but close to 50 in each Petri dish. Germination of exhumed *C. album* seeds was tested with triplicates. For *S. officinale* not enough seeds were available. Therefore, germination of exhumed seeds of this species was tested with duplicates.

Germination

Germination tests were carried out in cooled incubators (Gallenkamp, Crawley, U.K., T± 1°C). Seeds were incubated in 50 mm Petri dishes on 1 layer of filter paper (Schleicher and Schüll no. 595) moistened with the appropriate solution (Milli-Q water or 25 mM KNO_3). Seeds were irradiated with a saturating dose of red light (15 min). For seeds of *C. album* this irradiation was repeated after 24 h. Red light was obtained by filtering light from 6 red fluorescent tubes (Philips TL 20W/15) through one layer of 3 mm plexiglas (red 501, Röhm and Haas, Darmstadt, FRG), the light intensity at seed level being 250 $\mu W.cm^{-2}$. Handling of exhumed seeds, also of dark controls, occurred in dim green safelight, obtained by filtering light from one green fluorescent tube (Philips TL 40W/17) through two layers yellow no. 46 and two layers blue no. 62 Cinemoid filters (Strand Electric, London, U.K.).

Between 3 and 24 days after incubation, according to species and test temperature, when no additional germination occurred, both germinated and non germinated seeds were recorded to determine germination percentage. Protrusion of the radicle was the criterion for germination.

Germination of *C. album* was tested in Milli-Q water and 25 mM KNO_3 at 10, 15, 20, 25 and 30°C. Germination of *S. officinale* was tested in Milli-Q water or (on week 0 and 8) 25 mM KNO_3 at 2, 10, 15, 20, 25 and 30°C.

Nitrate measurements

Whenever seeds were exhumed samples were stored in 1.5 ml Eppendorf tubes at -20°C until measurement of nitrate. Seed moisture content was determined in a separate sample by weighing before and after drying for 1.5 h at 130°C. For measurement of nitrate in fresh seeds, 100 mg of seeds were weighed in the 1.5 ml Eppendorf tubes.

The seeds were homogenized in 1.5 ml of Milli-Q water with a tissue grinder (IKA-RW15, Janke und Kunkel KG, Staufen im Breisgau, FRG) for 1 min, after addition of some purified sea sand (Merck, Darmstadt, FRG). The homogenate was

shaken for 15 min. After centrifugation for 15 min at 16000 g, 750 µl of the supernatant was transferred to another Eppendorf tube containing 5 mg of activated charcoal CN-1 (Norit Clydesdale, Glasgow, UK). The Eppendorf tube was shaken for 15 min, centrifuged for 15 min at 16000 g and the supernatant was passed through a MA 25 prefilter (Millipore, Etten-Leur, The Netherlands) (Hilhorst and Karssen, 1989).

Nitrate of seeds exhumed in October and December 1988 was extracted similarly but now purification was performed by filtration over a small column containing a few mg of Lichroprep RP-8 (particle size 25-40 µm, Merck, Darmstadt, FRG) on a MA 25 prefilter moistened twice with 500 µl of methanol. This purification step made the measurement more sensitive.

Samples of 20 µl were injected into a HPLC system (model 3500 B, Spectra Physics, Santa Clara, California, USA) equipped with a spectrophotometric detector (model 770, Spectra Physics) set at 210 nm and an integrator (model C-R1B, Shimadzu, Kyoto, Japan). The column was a Lichrosorb 10NH2 column (Chrompack, Middelburg, The Netherlands). The mobile phase consisted of 25 mM KH_2PO_4 pH 3.7 (Hilhorst and Karssen, 1989). Nitrate levels were calculated on the basis of a linear relationship between concentration and peak height of nitrate standards. Nitrate was measured in duplicates and expressed on a dry weight basis for the exhumed seeds and on a fresh weight basis for fresh seeds.

Whenever seeds were exhumed samples of the soil surrounding the seeds were dried on filter paper at room temperature and stored dry until measurement of nitrate. Soil moisture content was determined in a separate sub sample by weighing before and after drying in an oven for 8 h at 130°C. Samples were sieved over a 2 mm sieve and 3.0 g of soil was weighed out in a 40 ml polyethylene bottle. After addition of 30 ml 0.01 M $CaCl_2$ the bottles were shaken mechanically for 2 h. Subsequently the bottles were centrifuged for 10 min at 2500 rpm (Houba *et al.*, 1986). The supernatant was diluted 5 times and nitrate was determined after reduction to nitrite on a Cu-Cd column on an autoanalyser (Technicon Ltd., Swords Co., Dublin, Ireland) equipped with a sample controller (model 222, Gilson, France) at 550 nm. Nitrate content was expressed on a dry weight basis.

Total N measurement

Total N content was determined after wet digestion of dried samples. The digestion mixture was made by dissolving 3.5 g selenium powder in 1 l concentrated H_2SO_4. Subsequently 72 g of salicylic acid was dissolved in 1 l of the sulphuric acid-selenium mixture. Two and a half ml of the digestion mixture was added to 300 mg of dried seeds and placed in a digestion tube. After 2 h the digestion tubes were placed in a heating block for another 2 h at 100°C. After cooling, three 1 ml aliquots of H_2O_2 were added at 10 sec intervals, with careful but thorough mixing after each addition.

Subsequently, the tubes were heated for 2 h at 330°C. The clear digest was made up to a 50 ml volume with demineralized water. Total N was determined colorimetrically at 660 nm by a standard autoanalyser method (Technicon Ltd., Swords Co., Dublin, Ireland) equipped with a sample controller (model 222, Gilson, France) (Anonymous, 1978).

Additional laboratory experiments

Germination of two seed lots of *S. officinale* from the 1987 experiment was also tested after pretreatment in Petri dishes in an incubator instead of outdoors in soil. Seeds of two seed lots were pretreated for 41 days at 2°C in Milli-Q water or 0.1, 0.5, 1, 10 or 25 mM KNO_3 . After the pretreatment, the seed nitrate content was determined after rinsing the seeds three times with Milli-Q water in a duplicate sub sample and germination was tested at 24°C with triplicates of 50 seeds.

Nitrate leakage of *C. album* seeds was determined in Petri dishes. One hundred mg (fwt) of seeds were imbibed in 1.5 ml Milli-Q water in 50 mm Petri dishes and incubated at 2°C. At regular intervals nitrate was determined in the incubation medium and the seeds from two Petri dishes. The seeds were rinsed three times and surface dried on a Büchner funnel. Samples of seeds and incubation medium were frozen and stored at -20°C until measurement of nitrate.

In the 1988 experiment seed nitrate content was determined for each plant separately. After a pretreatment of 40 h at 15°C in Milli-Q water in darkness, seeds were irradiated with red light for 15 min and incubated at 24°C in water to test germination.

7.3 Results

In Fig. 7.1 the effect of the nitrate fertilization on the nitrate and total N content of seeds of *C. album* and *S. officinale* in the 1987 experiment is shown. In both species, application of KNO_3 clearly increased the endogenous nitrate content of the seeds. The total N content did also increase but the rise was relatively much smaller than that of nitrate (Fig. 7.1).

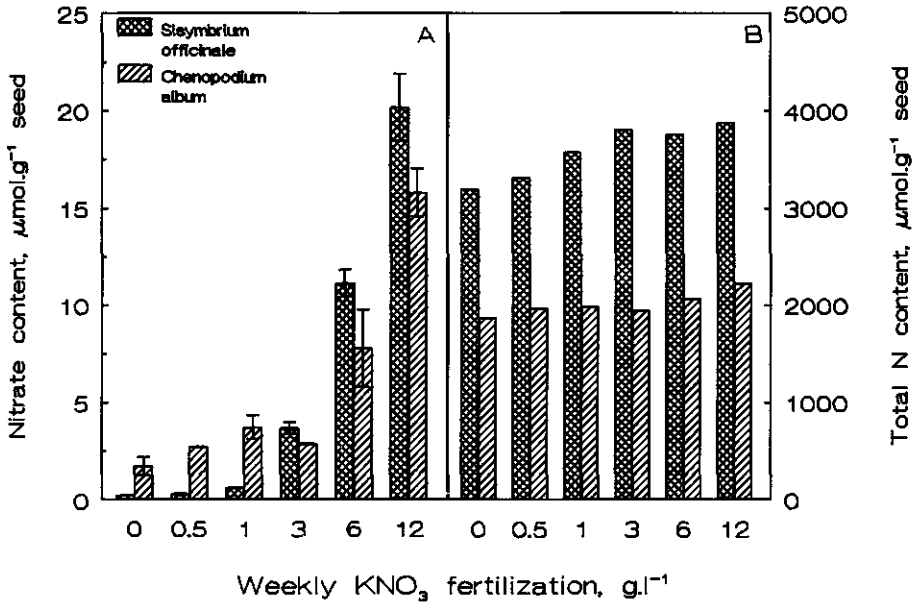

Fig. 7.1 The effect of KNO_3 fertilization of *Chenopodium album* and *Sisymbrium officinale* plants on nitrate (A) and total N content (B) of produced seeds. The plants were cultivated in pots in the open field. Once a week 250 ml KNO_3 solution was applied per plant starting from the moment of flowering onwards. Per treatment, four plants were used. After harvest the seeds were combined. *C. album* seed lots of plants that were given 1, 6 and 12 g/l KNO_3 were named C_{low}, C_{mid} and C_{high} respectively. *S. officinale* seed lots of plants that were given 0, 0.5, 3 and 12 g/l were named S_{low}, S_{low}' (used in the Petri dish pretreatment experiment), S_{mid} and S_{high} respectively. Nitrate contents are means of duplicates ± standard error. Total N was measured in one sample only.

The red light induced germination of the *S. officinale* seeds from the 1988 experiment, expressed in probits, showed a linear relationship to the logarithm of the endogenous nitrate content (Fig. 7.2). Germination percentage approached zero when endogenous nitrate decreased. For *C. album* the positive relationship between nitrate content and germination was less apparent. I will return to this later.

The seed lots that were selected for the further experiments were C_{low}, C_{mid} and C_{high} from *C. album* plants that were fertilized with 1, 6 and 12 g/l KNO_3, respectively and S_{low}, S_{mid} and S_{high} from *S. officinale* plants that were fertilized with 0, 3 and 12 g/l KNO_3, respectively (Fig. 7.1).

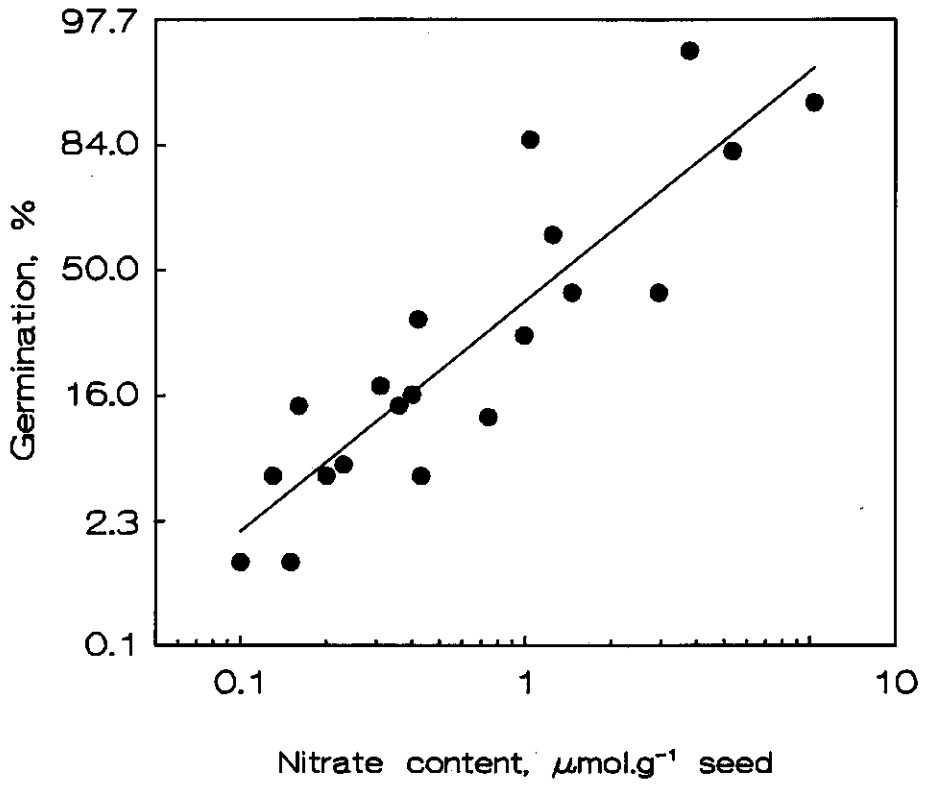

Fig. 7.2 Relationship between germination (probit scale) and endogenous nitrate content (log scale) of *Sisymbrium officinale* seeds. Seeds were pretreated for 40 h at 15°C in water, irradiated with 15 min red light and subsequently incubated at 24°C. Percentage germination was determined after 3 days.

Chenopodium album

At the start of the burial experiment on December 4, 1987 seeds of *C. album* germinated best at 20°C both in water and KNO_3 (Fig 7.3C). During burial in winter, spring and summer dormancy was relieved which was best seen in tests at 15 and 20°C. Nitrate in the germination medium always increased the percentage of germinated seeds.

The influence of the different endogenous nitrate contents was only seen before burial, particularly at 20°C and to a smaller extend at 25 and 30°C (Fig. 7.3C,D,E). The germination was proportional to the endogenous nitrate content. From 8 weeks of burial onwards, the seeds of the different seed lots only incidentally showed differences in germination, for example, in January and March at 15°C and in March at 20°C (Fig. 7.3B,C).

Measurements of the endogenous nitrate content of the seeds after burial provided an explanation for the results of the germination tests (Fig. 7.4A). Evidently, the large differences between C_{low}, C_{mid} and C_{high} disappeared rapidly during burial (data of C_{high} on 29/01/88 are missing). The nitrate content of the seeds slightly fluctuated throughout the year relative to the changes in soil nitrate content. It was low in January, increased in March and June and decreased again in autumn.

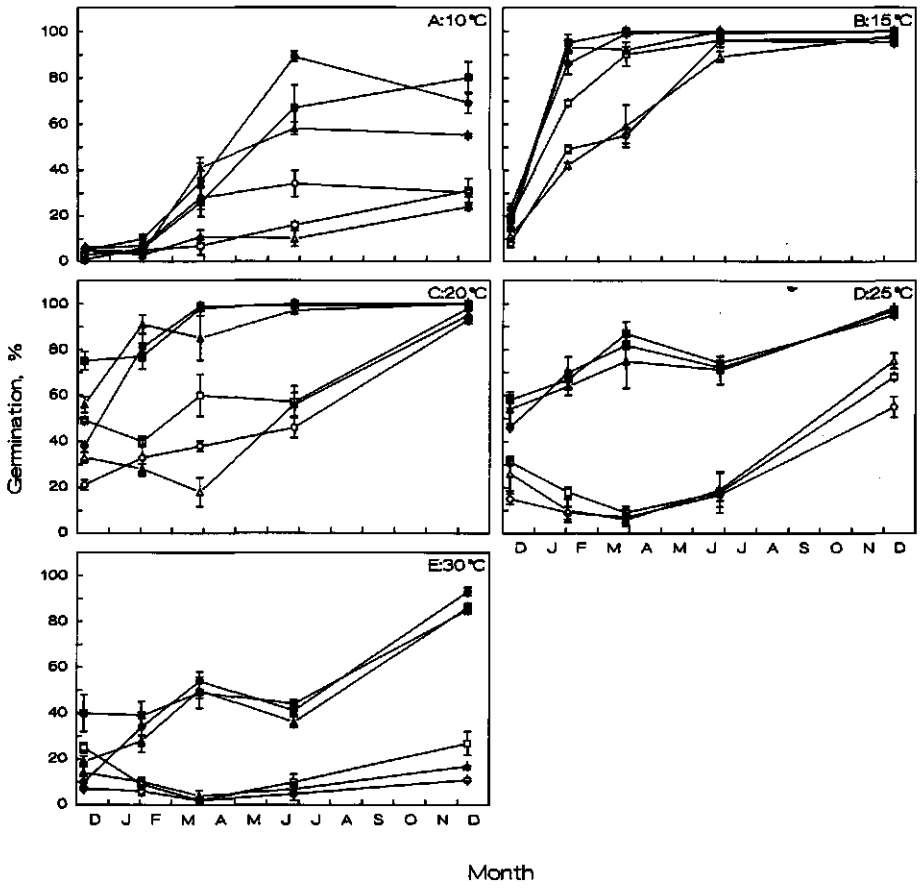

Fig. 7.3 Changes in the germination capacity of three seed lots of *Chenopodium album* with different endogenous nitrate content during burial in soil C_{low} (○), C_{mid} (△) and C_{high} (□) (see Fig. 7.1 for nitrate contents on moment of burial). Seeds were buried in December 1987 at a depth of 10 cm and exhumed on the indicated dates. Germination was tested in water (open symbols) and 25 mM KNO_3 (closed symbols) at 10 (A), 15 (B), 20 (C), 25 (D) and 30°C (E). Before the test seeds were irradiated twice with 15 min red light at a 24 h interval. Results are means of triplicates of 50 (December 1987) or approx. 50 seeds (at other dates). Final germination was recorded when no further germination was observed between 5 and 14 days after incubation, according to test temperature. Vertical bars indicate standard error.

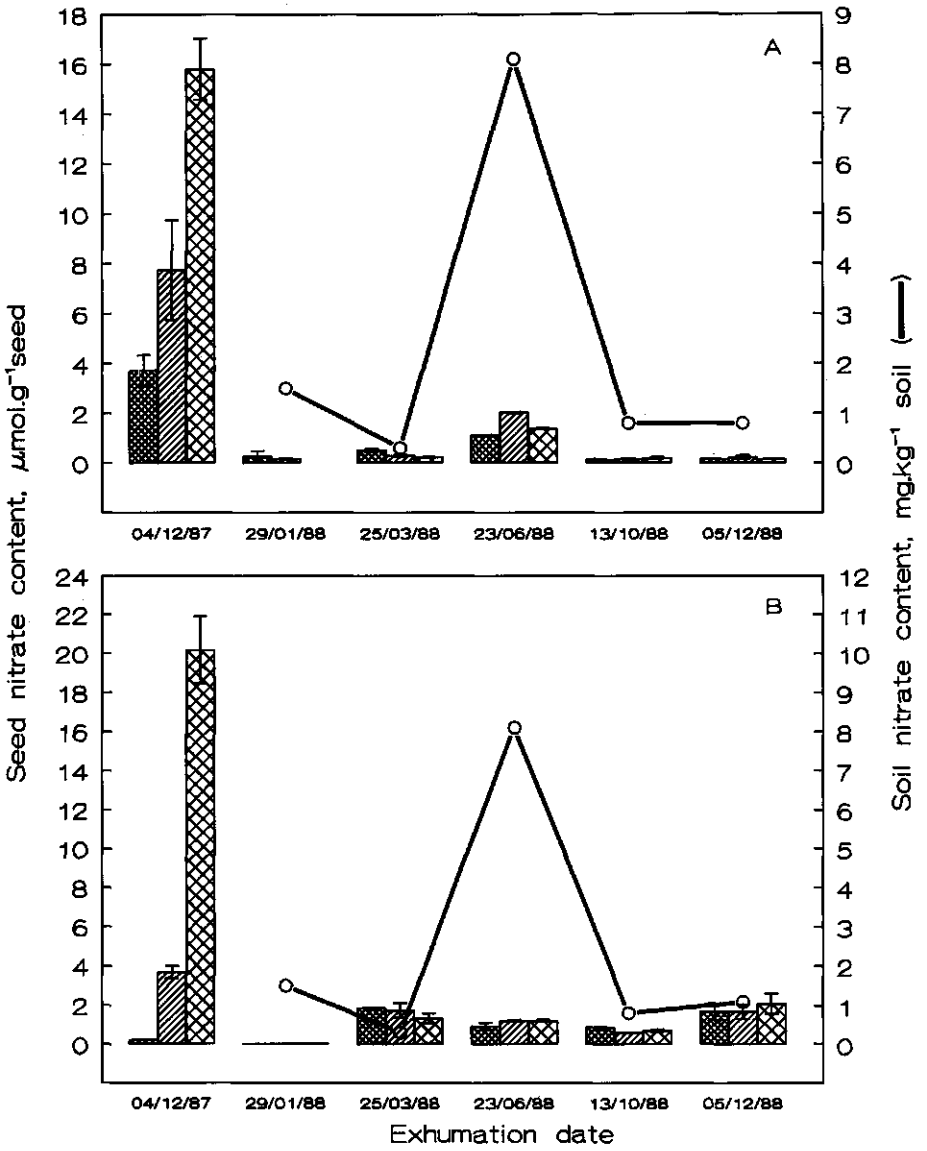

Fig. 7.4 Changes in endogenous nitrate content during burial in soil of seeds of *Chenopodium album* (A) and *Sisymbrium officinale* (B) with low (▩), middle (▨) and high initial nitrate content (▩) (see Fig. 7.1). Also shown is the nitrate content of the soil. Seed nitrate contents are means of duplicates. Vertical bars indicate standard error.

Pretreatment of *C. album* seeds in Petri dishes showed that at 10°C most endogenous nitrate leaked out within one day (Fig. 7.5). After seven days approx. 97% of the nitrate had leaked out. The leaked nitrate was entirely recovered in the incubation medium (Fig. 7.5).

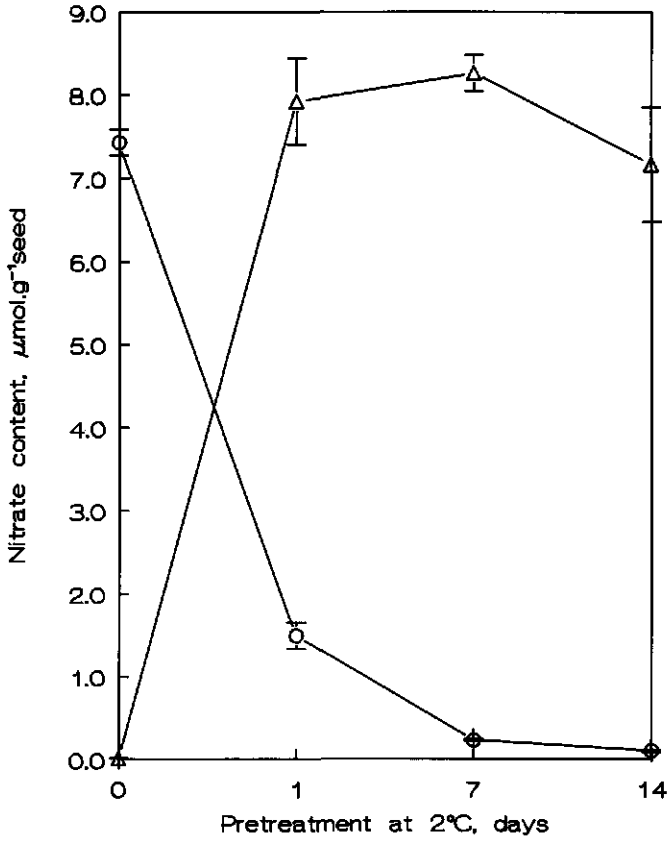

Fig. 7.5 Nitrate leakage from *Chenopodium album* seeds. Hundred mg (fwt) of *C. album* seeds with an initial nitrate content of 7.4 $\mu mol.g^{-1}$ was imbibed in 1.5 ml Milli-Q water in 50 mm Petri dishes and incubated at 10°C. At regular intervals from two Petri dishes samples of the seeds (O) and imbibition medium (Δ) were taken. The seeds were rinsed three times and surface dried on a Büchner funnel. Samples were frozen and stored at -20°C until measurement of nitrate. Nitrate concentration in the incubation medium was expressed on the basis of the weight of the imbibed seeds to ease comparison with seed nitrate content. Vertical bars indicate standard error.

Sisymbrium officinale

The results of the burial and germination experiments with seeds of *S. officinale* that contained different levels of endogenous nitrate are depicted in Figs 7.6 and 7.7. In Fig. 7.6 the germination data are expressed against burial time for the different germination temperatures and in Fig. 7.7 the data for 0 and 8 weeks of burial are plotted against the germination temperature. Most data represent germination in water (Fig. 7.6, 7.7A,C), but Figs 7.7B and D show the effects of applied KNO_3.

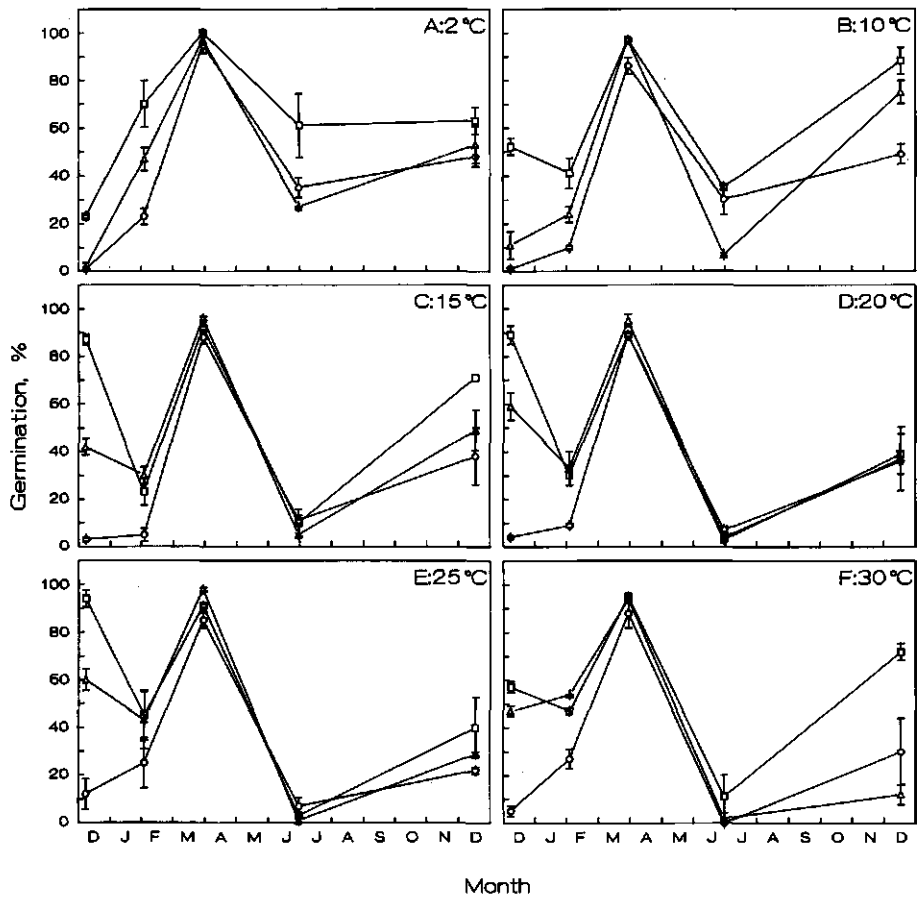

Fig. 7.6 Changes in the germination capacity of three seed lots of *Sisymbrium officinale* with different endogenous nitrate content: S_{low} (○), S_{mid} (△) and S_{high} (□) during burial in soil. As Fig. 7.3 except test temperatures were 2 (A), 10 (B), 15 (C), 20 (D), 25 (E) and 30°C (F). Before the test seeds were irradiated once with 15 min red light. Results are means of triplicates of 50 (December 1987) or duplicates of approx. 50 seeds (at other dates). Vertical bars indicate standard error.

Before burial, germination of the three seed lots in water clearly depended on endogenous nitrate levels (Fig. 7.6, 7.7A). The optimum temperature for germination was approx. 20 to 25°C (Fig. 7.7A). Application of KNO_3 increased germination of the S_{low} and S_{mid} seeds but had very little effect on S_{high} seeds (Fig. 7.7B). Evidently, the endogenous nitrate of S_{high} was saturating. As shown previously (Karssen, 1980/81*b*; Chapter 3), it is seen in Fig. 7.6 that dormancy of *S. officinale* seeds was relieved during winter, re-induced in spring and again relieved in the next autumn.

The expression of the dormancy pattern depended on the temperature during the germination test. After 8 weeks of burial, germination at 2°C in water of all three seed lots had increased, whereas at most other temperatures, the germination of S_{mid} and S_{high} after 8 weeks was lower than before burial (compare Fig. 7.6A to 7.6B-F and Fig. 7.7A to 7.7C). However, the sensitivity to (exogenous) nitrate had increased dramatically during winter (compare Fig. 7.7B to D).

At the end of March (16 weeks of burial), all seeds germinated to nearly 100% over the whole temperature range. In June, dormancy was induced and very few seeds germinated. Differences between seed lots were only observed at 2 and 10°C (Fig. 7.6A,B). However, early December differences were observed again at some other temperatures.

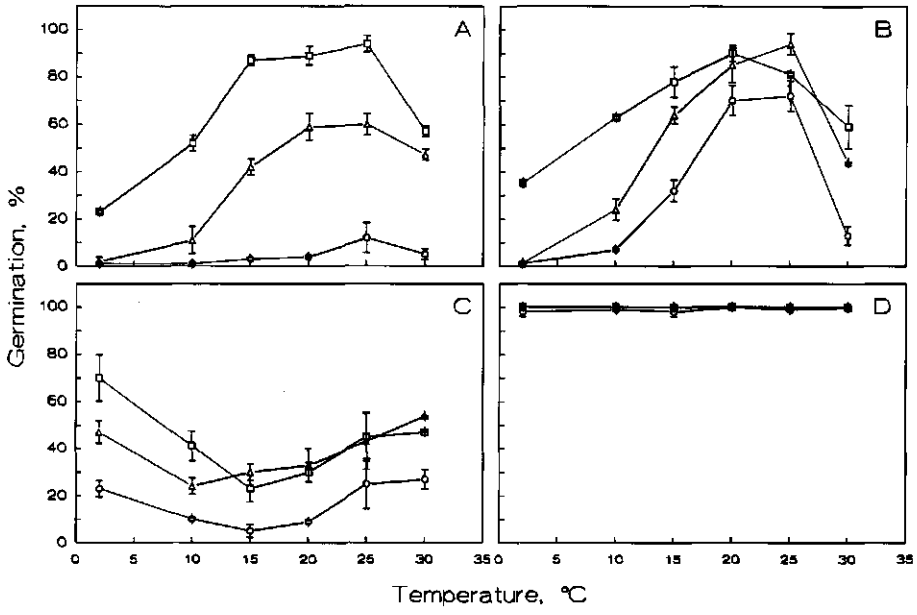

Fig. 7.7 Results from Fig. 7.6 plotted against germination temperature instead of burial time for 0 (A,B) and 8 weeks of burial (C,D). Germination was in addition to water (A,C) also tested in 25 mM KNO_3 (B,D). Symbols as in Fig. 7.6. Vertical bars indicate standard error.

Fig. 7.4B shows the endogenous nitrate content of *S. officinale* seeds during burial. Similar to *C. album* seeds, endogenous nitrate was completely lost during the first weeks of burial. On later exhumation dates some endogenous nitrate could be detected again in the seeds. However, consistent differences between the seed lots did not occur, nor was there a correlation between nitrate levels in seeds and soil.

After a pretreatment for 41 days at 2°C in Petri dishes in water or 0.1, 0.5 or 1 mM KNO_3, the nitrate content of the seed lots S_{low}' and S_{high} from plants fertilized with 0.5 and 12 g/l KNO_3, respectively (Fig. 7.1), was below or close to the detection level (Fig. 7.8). When pretreatment occurred in 10 or 25 mM KNO_3 detectable amounts of nitrate were present in the seed. Despite the high KNO_3 concentration in the imbibition medium, S_{high} seeds still contained a slightly higher nitrate level than the S_{low}' seeds. Although after pretreatment in the low KNO_3 concentrations differences in seed nitrate content between the two seed lots were not detectable, germination in these nitrate concentrations of the S_{high} seeds was much better than of the S_{low}' seeds.

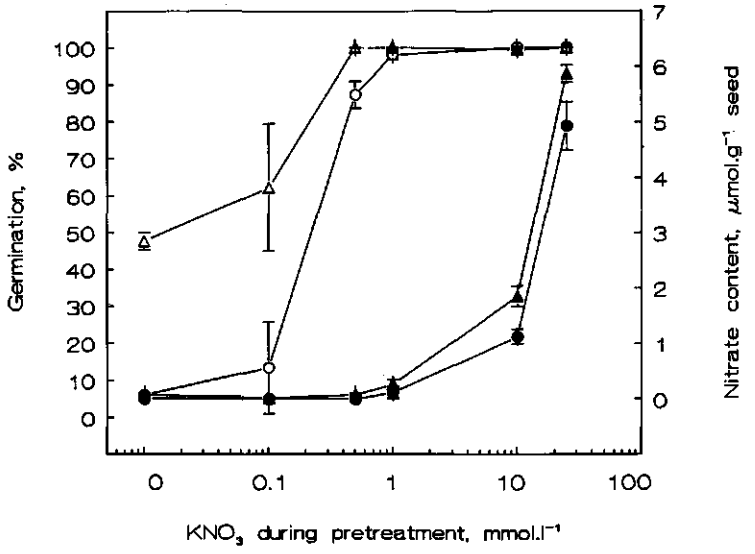

Fig. 7.8 Germination (open symbols) and nitrate content (closed symbols) of seeds of S_{low}'(○) and S_{high} (△) after a pretreatment for 41 days at 2°C in Petri dishes in a range of KNO_3 concentrations. See Fig. 7.1 for nitrate levels of S_{low}' and S_{high} before pretreatment. After 41 days seeds from 2 Petri dishes were rinsed three times with Milli-Q water and surface dried on a Büchner funnel. Samples were frozen and stored at -20°C until nitrate measurement. Germination of seeds from 3 other dishes (50 seeds per dish) was tested at 24°C after 15 min red light, in their incubation medium. Vertical bars indicate standard error.

7.4 Discussion

Endogenous nitrate in seeds

Fertilization of *C. album* and *S. officinale* plants with KNO_3 increased the nitrate content of seeds of both species. For *C. album* these results agree with Fawcett and Slife (1978) and Saini *et al.* (1985*b*). They also found a positive relation between application of nitrate to *C. album* plants and seed nitrate content in both a field trial and an experiment with plants grown in pots. Also quantitatively, the present data agree with their results. For *C. album*, Saini *et al.* (1985*a,b*) observed similar differences in nitrate levels in seeds collected in the field as in seeds collected in their experiments.

Differences in nitrate accumulation in *S. officinale* seeds were much larger than in *C. album* seeds (Fig. 7.1). In seed lots of *S. officinale* collected in the vicinity of Wageningen at various places and in several years, nitrate levels varied between 0.1 and 2 $\mu mol.g^{-1}$ seed, which is approx. a factor 10 lower than the differences found in the present experiments. Nevertheless, these field-collected seeds showed a similar linear relationship between the logarithm of the endogenous nitrate content and the probit germination as shown in Fig. 7.2 (H.W.M. Hilhorst, pers. comm.).

It is concluded that differences in nitrate content of seeds of both species occur in the field. The experiments strongly suggest that they depend on differences in nitrate levels in soil during seed development. Not all species, however, accumulate nitrate in their seeds. In the same field trial where Fawcett and Slife (1978) obtained seeds of *C. album* with different endogenous nitrate contents they found no differences in nitrate content of *Abutilon theophrasti* seeds. In parallel experiments, similar negative results were obtained with *Polygonum persicaria* L.. Not only was any relationship between applied KNO_3 and seed nitrate level absent, but nitrate levels in seeds collected both in trials and in the field were also approx. 10 times lower than in *C. album* seeds (data not shown).

Differences between species in the accumulation of nitrate in seeds might be caused by differences in uptake or metabolism of nitrate. Van Beusichem (1987) found rather large differences in the partitioning of nitrate reductase activity (NRA) over root and shoot between pea, maize and sunflower. In pea, 45% of the NRA occurred in the roots in contrast to 37% and 20% in maize and sunflower, respectively. In a preliminary experiment no large differences were observed in nitrate uptake nor in the partition of nitrate reductase activity over root and shoot between *C. album* and *P. persicaria*.

Endogenous nitrate and germination

For both species, a positive relationship was demonstrated between endogenous nitrate levels and germination of freshly harvested seeds (Figs 7.2, 7.3, 7.6, 7.7). Fawcett and Slife (1978) and Saini *et al.* (1985*a,b*) also found such a positive relationship for *C. album* but it is difficult to compare their results quantitatively with the present data because of differences in test conditions. The strong influence of test conditions, in particular temperature, was clearly shown in the present experiments *e.g.* Fig. 7.3.

For *S. officinale*, the relationship between probit germination and the logarithm of the endogenous nitrate content was linear (Fig. 7.2). When the nitrate content approached zero, germination did not occur any more, indicating an absolute requirement for nitrate.

The larger differences in germination between the three seed lots of *S. officinale* than of *C. album* (Fig. 7.2, 7.3, 7.6, 7.7). might be due to the larger differences in nitrate content of *S. officinale* seeds. The seeds of S_{low} and S_{high} differed by a factor of approx. 100, whereas seeds of C_{low} and C_{high} differed only by a factor of 5 (Fig. 7.1). Besides the larger differences in nitrate level, *S. officinale* seeds also seemed to be more sensitive to nitrate than seeds of *C. album* (compare Fig. 7.7C,D to Fig. 7.3).

Effects during burial

Exhumed seeds of *C. album* showed no distinct seasonal dormancy pattern (Fig. 7.3). Previous results showed that the major dormancy changes of *C. album* seeds occurred in the period June-November (Chapter 5). Unfortunately that period was missing in the present observations.

S. officinale seeds showed a much more obvious dormancy pattern (Fig. 7.6) that was very similar to the results reported in Chapter 3. Changing levels of dormancy were accompanied by a shift in the optimum temperature from 25°C at the start of the experiment to 2-10°C from January onwards (Figs 7.6, 7.7).

At first, it seemed that dormancy was induced in S_{high} and, less obviously, in S_{mid} seeds between burial and the first exhumation. The apparent dormancy induction was visible at all test temperatures except 2°C (Fig. 7.6). This observation was in contrast to previous burial experiments that showed that dormancy relief of *S. officinale* seeds occurred at low temperatures (Karssen, 1980/81*b*; Chapter 3). The difference is probably caused by the leakage of nitrate directly after burial, which was largest from S_{high} seeds (Fig. 7.4).

The idea of decreasing nitrate levels as a cause for dormancy induction was supported by the results in Fig. 7.7. When germination was tested in nitrate, dormancy was indeed relieved for all seed lots at all test temperatures (compare Fig. 7.7A,C to 7.7B,D). The fact that germination of S_{high} in January at 2°C was higher

than at the beginning of the experiment in spite of nitrate leakage (Fig. 7.6A) may be caused by the shift in optimum germination temperature to lower values and/or by an increased sensitivity to nitrate at 2°C.

For both species germination and therefore the expression of the dormancy pattern were strongly influenced by the test temperature. This effect is discussed in more detail in Chapters 3 and 5.

The differences in germination capacity that existed between seed lots at the beginning of the experiment disappeared during burial (Figs 7.3, 7.6). The seed lots of *S. officinale* still showed some minor differences in germination after one year of burial (Fig. 7.6). The differences might be caused by the slightly increased nitrate content of S_{high} seeds at the end of the experiment (Fig. 7.4B). It is not known whether this increased level was a consequence of the variation in soil nitrate content or still an effect of the high nitrate content in the freshly harvested seeds.

For *S. officinale* nitrate levels in soil and in buried seeds did not correlate (Fig. 7.4B). For *C. album* they did (Fig. 7.4A). Since the nitrate content of *S. officinale* seeds clearly corresponded to the nitrate concentration of a solution (Fig. 7.8), seed-soil contact seems to have prevented optimal exchange of nitrate, at least under the conditions in the present burial experiment. According to Goudey *et al.* (1988), nitrate levels in *Sinapis arvensis* also respond in a passive fashion to the external available nitrate.

Sites of nitrate action

Germination of freshly harvested *S. officinale* seeds in water positively correlated to the endogenous nitrate content (Fig. 7.2). As discussed above, such a relationship was missing for seeds that had been buried and also after a pretreatment for 41 days in water at 2°C (Fig. 7.8). These results give rise to the assumption that nitrate is only active at a specific site in the seed, the active site, that requires very low concentrations (that might be lower than the detection level). Maternal nitrate seems to be located at or near this active site or there is at least a direct correlation between total seed nitrate content and the amount of nitrate at the active site. Exogenously applied nitrate can be taken up by the seed and thus forms a pool of nitrate. Nitrate from this storage pool can leak readily from the seed but it can also be replenished from the imbibition medium. Between this pool and the active site an exchange of nitrate exists. High nitrate concentrations in the imbibition medium can increase the concentration of nitrate at the active site by diffusion or active transport first to the storage pool and then to the active site.

The bulk nitrate in the storage pool may mask the amount of nitrate on the active site and therefore potential differences between seed lots in the amount of nitrate on the active site. This could explain why differences in germination were still found (Fig. 7.6 January and June) although there were no differences in nitrate

content (Fig. 7.4B). On the other hand, if nitrate levels in the seeds are below the detection level, differences in nitrate at the active site may still exist. The pretreatment for 41 days at 2°C may have increased the sensitivity to nitrate to such an extent that it enabled an effect of these low nitrate levels on germination (Fig. 7.8).

Ecological importance of endogenous nitrate

In spite of the present data and those reported by Fawcett and Slife (1978) and Saini *et al.* (1985*a,b*) it still has to be questioned whether the maternal effect on nitrate levels in seeds plays an important ecological role in the regulation of dormancy and germination.

The nitrate concentration in the soil solution fluctuates and can vary from virtually zero to 50 mmol.l^{-1} (Adams, 1971; Nye and Tinker, 1977; Young and Aldag, 1982). This fluctuation is caused by the natural variation between soil types and the variation within a soil, due to seasonal effects, agricultural practices and differences in moisture content and mineralization (Popay & Roberts, 1970; Adams, 1971; Nye and Tinker, 1977; Young & Aldag, 1982; Chapters 2, 3, 4, 5).

Although differences in nitrate content between seeds, resulting from variation in nitrate availability to the mother plant may occur, they can only have a temporary effect on germination. Immediately after seed shedding there may be an enhanced germination, but upon imbibition, endogenous nitrate leaks out and possible differences are equalized by the surrounding soil nitrate.

The ecological importance of this leakage would be that rather than reacting to the fertilizer status of the soil at the moment the seeds develop they react to the actual presence of nitrate.

Chapter 8

General discussion

In the experiments described in Chapters 2 to 5 seeds of *Chenopodium album* L., *Polygonum lapathifolium* L. subsp. *lapathifolium*, *P. persicaria* L., *Sisymbrium officinale* (L.) Scop. and *Spergula arvensis* L. were buried in soil. All five species showed seasonal changes in dormancy, that were characterized by changes in the range of temperatures over which germination could proceed. During relief of dormancy the range widened, during induction of dormancy the range narrowed.

As was also shown by Karssen *et al.*, 1988, the changes in dormancy as they occur in the field, could be simulated in incubators at any moment of the year. This indicates that the changes were not endogenously determined, but were regulated by environmental signals only.

Ample evidence was presented that the seasonal changes in dormancy were regulated by field temperature. Other factors, such as the moisture and nitrate content of the soil, did not affect the changes in dormancy of any of the investigated species.

Emergence

Although emergence of all investigated species could occur in spring, considerable differences existed in the mechanisms that achieved spring emergence. The species also differed in the total duration of the emergence period.

The *Polygonum spp.* (Chapter 2) showed the characteristic features of summer annuals. Dormancy was relieved best at low temperatures and the width of the germination-temperature range varied through the minimum temperature required for germination ($T_{g,min}$) (Fig. 8.1A). 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.

S. officinale (Chapter 3) showed the germination-temperature range of a winter annual. Changes in dormancy resulted in changes in $T_{g,max}$ (Fig. 8.1B). In this case the field temperature was usually higher than $T_{g,min}$.

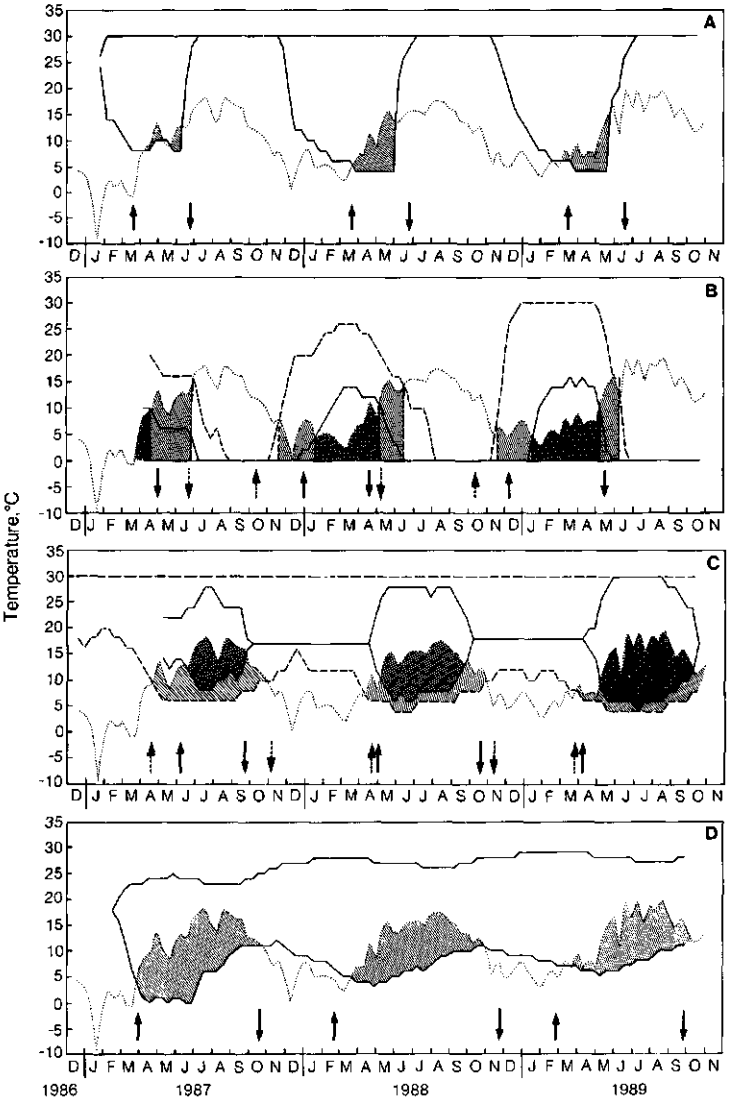

Fig. 8.1 Changes in the minimum and maximum temperature required for 50% germination of exhumed seeds of A: *Polygonum persicaria*, germination in water; B: *Sisymbrium officinale* and C: *Spergula arvensis*, germination in water (solid lines) and 50 mM KNO_3 (broken lines); D: *Chenopodium album*, germination in 50 mM KNO_3. Data were calculated with simulation models that were described in Chapters 2 to 5. Dotted line indicates air temperature at 1.50 m, where seeds were placed for outdoor germination tests (see for example Chapter 2 for explanation). Hatched areas in A-D indicate overlap of field temperature and germination-temperature range. For B and C: germination in water (▩) or 50 mM KNO_3 (▨). Arrows (B,C: solid for water, broken for KNO_3) indicate the moment germination in Petri dishes outdoors actually increased above (↑) or decreased below 50% (↓) (data from Chapters 2 to 5).

Germination was therefore mainly determined by the maximum temperature required for germination ($T_{g,max}$). During winter and spring, the field temperature was lower than $T_{g,max}$ (and usually higher than $T_{g,min}$) and therefore germination could occur in this period. In summer, the field temperature was higher than $T_{g,max}$. Consequently, germination was inhibited.

In contrast to most winter annuals, dormancy relief did not occur in summer at high temperatures (Baskin and Baskin, 1976; Roberts and Lockett, 1978), but in autumn-winter when the field temperature had decreased. This was confirmed by the incubator experiments. Relief of dormancy occurred best at approx. 6°C.

Freshly harvested *S. officinale* seeds temporarily behaved like a summer annual. Germination was best at elevated temperatures. This seems of ecological importance since seeds of *S. officinale* often remain on the mother plant when mature (P. Zonderwijk, pers. comm.). The rapid relief of primary dormancy over a range of temperatures and the summer annual-like germination-temperature range that is open at the high temperature side, may enable germination under optimal conditions even after seed dispersal in late winter and early spring.

Considering both the germination-temperature range of seeds that have been buried for prolonged periods of time and the fact that germination and emergence occur from autumn to early spring, it is suggested to name *S. officinale* a facultative winter annual.

C. album and S. arvensis (Chapters 4 and 5) 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 the maximum and an increase of the minimum temperature. Widening of the range occurred inversely (Fig. 8.1C,D).

Karssen (1982) partly adapted the ideas of Vegis (1964) about dormancy in plants. The former author suggested that changes in dormancy in seeds of summer and winter annuals occur through changes in respectively the minimum **or** the maximum temperature required for germination. Evidently, also the third mechanism that was proposed by Vegis (1964) should be considered. Changes in dormancy can also occur through changes in the minimum **and** maximum temperature that is required for germination (Fig. 8.1C,D).

The fluctuations in the germination-temperature range of *S. arvensis* were much larger than those in the range of *C. album*. Particularly in summer, germination of the former species could occur over a much wider range of temperatures than germination of *C. album*. Despite these differences germination of both species in Petri dishes outdoors was fairly similar. It occurred from spring to autumn.

Also emergence studies showed such similarities. After soil cultivation *C. album* emerged in spring and often in August-September (Williams, 1963; Williams and

Harper, 1965; Roberts and Ricketts, 1979; Håkansson, 1983). The same was reported for *S. arvensis* (Håkansson, 1983). Germination of the two species in hot dry periods may be prevented by a lack of moisture or, in the case of *C. album*, because field temperature temporarily rises above $T_{g,max}$.

It is concluded that the ability to germinate throughout the growing season can be assured in two different ways. For *S. arvensis* the width of the germination-temperature range seemed to be regulated by the actual field temperature only. When the field temperature increased the range widened, when it decreased it became narrower. The optimum temperature for dormancy relief of *S. arvensis* was at least 15°C (higher temperatures were not tested). This is the highest optimum temperature for dormancy relief of all four investigated species.

For *C. album* the changes in dormancy were not as large as for the other species. Dormancy relief occurred in winter-spring and was optimal at approx. 10°C. In summer, dormancy induction started, but before the germination-temperature range was closed entirely, field temperature had already dropped, such that the widening of the range started again.

It is suggested that, analogous to the use of the prefix facultative for winter annuals that germinate in autumn **and** spring, *C. album* and *S. arvensis* should be named facultative summer annuals because they germinate in spring **and** summer-autumn.

Regulation of dormancy by temperature

The experiments have clearly shown that the seasonal changes in temperature are the main regulator of the dormancy pattern. This is supported by the fact that the changes in dormancy could be simulated closely on the basis of temperature derived parameters. In *P. persicaria* the entire sequence of winter and spring temperatures was required to give the pattern of changes in the range of germination temperatures as it occurs in the field. The low temperature in winter was required to increase germination capacity over the entire temperature range. The increasing temperature in early spring prevented a premature loss of the capacity to germinate at warm temperatures. The high temperature of early summer reduced the capacity to germinate at the lower temperatures (Chapter 2), which is, as previously mentioned, characteristic for (obligate) summer annuals (Karssen, 1982; Baskin and Baskin, 1985).

This shows the relative value of laboratory experiments, especially when germination is tested at only one condition. These experiments can lead to conclusions that are not relevant for the analysis of field data. An exceptional example of this is *S. officinale*. In laboratory experiments germination proceeded best at 24 and 30°C, which is typical for summer annuals (Baskin and Baskin, 1985). However, after a few months of burial the optimum temperature for germination had

changed to temperatures as low as 2°C (Chapter 3). Restriction of the experiments to the laboratory or burial experiments with only one test temperature, would not have revealed this conversion of a confined summer annual to winter annual behaviour.

Germination

The dominant role of temperature in the regulation of the 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 requirement for light to initiate germination, enables distinction between the two effects of temperature. (i) Temperature before the irradiation affects dormancy (=pre-incubation or pretreatment temperature), (ii) temperature after irradiation affects germination (germination temperature). It is suggested that at the moment a seed is irradiated, the range of temperatures over which the seed can germinate is fixed.

This distinction between pretreatment and germination is illustrated by the response of *P. persicaria* seeds to temperature (Chapter 2). **Low** temperatures broke dormancy of this species, whereas germination was usually best at **high** temperatures (Fig. 2.9). If, following a cold pretreatment to break dormancy and a red light irradiation to initiate germination, seeds were transferred to 30°C they usually germinated to high percentages. Without the red light irradiation to trigger germination, a transfer to a high(er) temperature immediately induced dormancy, visualized by the narrowing of the germination-temperature range (Fig. 2.5A,B). It is concluded that a transfer to a higher temperature did not increase germination because it relieved dormancy but because it stimulated germination (which was initiated by the red light irradiation).

The expression of the dormancy status was also strongly influenced by other environmental factors. Germination of the five species was stimulated by light, nitrate and desiccation, although the effects strongly varied according to the species (Chapters 2, 3, 4 and 5). The range of temperatures over which seeds of *Ambrosia artemisiifolia* could germinate was much wider in light than in darkness (Baskin and Baskin, 1980), which was schematically depicted by Karssen (1982) (Fig. 1.2). Nitrate had a similar effect on the germination-temperature range of *C. album*, *S. officinale* and *S. arvensis*. The range became much wider when germination was tested in nitrate instead of water (Chapters 3, 4 and 5; Fig. 8.1). The germination-temperature range for desiccated seeds was even wider (not shown in Fig. 8.1). Following desiccation, the germination-temperature range of *S. officinale* and *S. arvensis* was so wide that

germination could occur throughout the year (Chapters 3 and 4).

The nitrate concentration in the soil solution fluctuates due to natural variation between soil types or variation within a soil, because of seasonal variation, agricultural practices and differences in moisture content and mineralization (Popay and Roberts, 1970; Adams, 1971; Nye and Tinker, 1977; Young and Aldag, 1982, Chapters 2 and 3). It can range from virtually zero to 30 mmol.l^{-1} (Nye and Tinker, 1977) or even 50 mmol.l^{-1} (Adams, 1971). Stimulation of germination occurred in the range of 1 to 10 mmol.l^{-1} KNO_3 for *S. officinale* (Hilhorst and Karssen, 1989*a*) and 1 to 30 mmol.l^{-1} KNO_3 for *S. arvensis* (M.P.M. Derkx, pers. comm.). It seems that nitrate can play an important ecological role, since nitrate concentrations in the field are of the same order of magnitude as the concentrations that stimulate germination in the laboratory. Because of the large effect on the germination-temperature range, germination and emergence on nitrate-rich soils may occur during a longer period of time.

As was discussed in Chapter 6, the same holds for desiccation. Measurements in the field showed that at a depth of 1 cm, soil moisture content occasionally dropped below 2% (dwt) (B.J. Post, pers. comm.). The moisture content of *S. officinale* seeds that were buried in soil with a moisture content of approx. 2.3% (dwt) was lower than 10% (dwt). In laboratory experiments, desiccation of imbibed seeds to a moisture content of 10% (dwt) or lower, strongly stimulated germination of *S. officinale*. It seems that occasionally conditions in the field can occur that are similar to the desiccation conditions that were used in the laboratory experiments. Since desiccation had a large stimulatory effect on germination of some species, it seems valuable to consider this factor when the behaviour of weed seeds in the field is studied.

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. For *e.g. S. officinale* this is illustrated in Figs 3.1A,B and 3.2 (Chapter 3). When seeds were deeply dormant (autumn 1987) changes in dormancy were best seen when germination was tested in light instead of darkness (compare Fig. 3.1A,B to Fig. 3.2B). When seeds were not dormant in early spring, changes in dormancy were most clear when germination was tested in darkness (spring 1987, Fig. 3.2B) or water with light (Fig. 3.1A) instead of in nitrate with light (Fig. 3.1B).

Simulation

Using data of germination tests with exhumed seeds in incubators, the seasonal pattern of germination of such seeds in Petri dishes outdoors could fairly accurately be simulated (Chapters 2, 3, 4 and 5). These data show in the first place that

germination outdoors at a certain temperature was approx. the same as germination at that temperature in an incubator, because all other conditions determining germination such as burial time, red light irradiation and moisture and nitrate availability were equal. However, the most important conclusion is that the seasonal changes in dormancy could be described accurately using only temperature-derived parameters, which is additional support for the key role of temperature in the regulation of the seasonal changes in dormancy.

Germination was simulated on the basis of a cold and heat sum (C and H). The calculation of C and H was based on the assumption that dormancy relief was optimal at a temperature just above zero. This was true for *Rumex obtusifolius* and *R. crispus* (Totterdell and Roberts, 1979, who developed this theory) and *P. persicaria* (Chapter 2) (Fig. 8.2A).

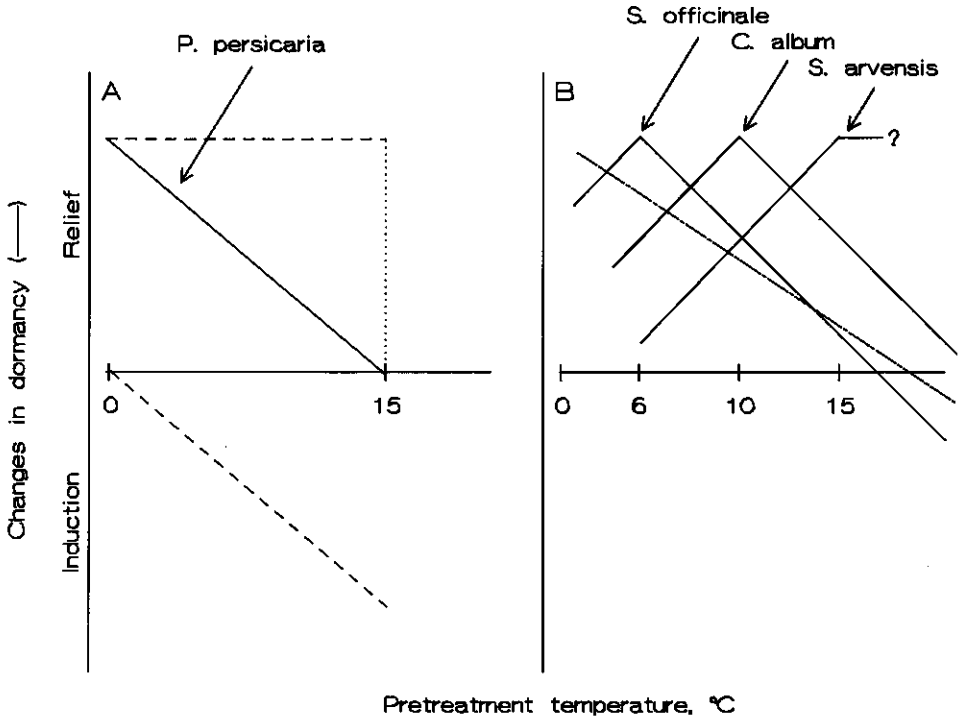

Fig. 8.2 Schematic representation of the effect of temperature on the two sub processes relief and induction of dormancy (broken lines) (A) and on the net (resulting) changes in dormancy (solid lines) for *Polygonum persicaria* (A) and *Chenopodium album*, *Sisymbrium officinale* and *Spergula arvensis* (B). For *P. persicaria* the relationship between temperature and changes in dormancy is linear. With a higher optimum temperature for dormancy relief, the relationship between temperature and changes in dormancy is poorly estimated with a linear equation which is shown for *S. officinale* (B, ⋯⟶). (A) adapted from theory of Totterdell and Roberts, 1979.

For *S. officinale*, *C. album* and *S. arvensis*, the optimal temperature for dormancy relief was not just above zero, but 6°C, 10°C and 15°C (or higher), respectively (Chapters 3, 4 and 5). This implies, that C and H can not give an accurate description of dormancy relief for these three species. When temperatures just above zero are optimal for dormancy relief, the relationship between temperature and dormancy relief is more or less linear (Fig. 8.2A). When the optimal temperature for dormancy relief is higher, the relationship becomes hyperbolic (Fig. 8.2B). The higher the optimum temperature for dormancy relief, the worse the estimation of the hyperbolic relationship between temperature and dormancy when it is carried out with a linear equation and the lower the descriptive value of C and H. Consequently, removal of C and H from the models most strongly decreased the correlation for *P. persicaria* and the least for *S. arvensis* (Table 8.1).

In a review about vernalization, Wiebe (1989) stated that it was essentially incorrect to predict the effect of temperature on vernalization with a cold sum, because that would imply that temperatures just above zero are most effective. Just as with the relief of dormancy of *S. officinale* and *C. album*, the optimum temperature for vernalization of many biennials is higher, approx. 7 to 15°C (Wiebe, 1989). The effect of temperature on vernalization can be predicted rather precise with a cold sum in areas of the world where temperature does not decrease too much below the optimum temperature for vernalization (Wiebe, 1989). Temperatures in The Netherlands were rather high during the course of the present experiment and did indeed only occasionally decrease below 5°C. This may explain why the dormancy patterns of *S. officinale* and *C. album* were nevertheless simulated closely with C and H.

Table 8.1 Squared multiple correlation (R^2), estimated variance ($\hat{\sigma}^2$) and percentage change of these parameters of models simulating the dormancy pattern of *Polygonum persicaria*, *Sisymbrium officinale*, *Chenopodium album* and *Spergula arvensis* on the basis of either cold and heat sum (C+H) or time (weeks of burial). For both options also germination temperature, germination medium and the mean temperature in a period before exhumation (that varied according to species) were used in the models. See Chapters 2 to 5 for explanation.

	P. persicaria		*S. officinale*		*C. album*		*S. arvensis*	
	R^2	$\hat{\sigma}^2$	R^2	$\hat{\sigma}^2$	R^2	$\hat{\sigma}^2$	R^2	$\hat{\sigma}^2$
C+H	0.76	1348	0.77	569	0.76	754	0.85	447
time	0.33	3421	0.68	769	0.70	932	0.81	545
change, %	-57	+154	-12	+35	-8	+24	-5	+22

Dormancy of *C. album* and particularly *S. arvensis* were relieved rather late in winter-spring (because of the high optimum temperature for dormancy relief). The later dormancy relief occurs, the higher the correlation of the changes in dormancy with the actual field temperature will be. This implies that when C and H are excluded from the model and are replaced with the parameter "time", the pretreatment temperature (T_p) in a period δt before exhumation ($T_{p,\delta t}$) will still assure a high correlation for the model (Table 8.1).

The fact that the regulation of the changes in dormancy was not so simple as described by Totterdell and Roberts (1979), suggests that dormancy is regulated by more than just the two opposite sub processes relief and induction of dormancy (see Chapter 2 for explanation). The present data suggest that changes in dormancy are caused by a range of physiological and/or biochemical processes, for example changes in sensitivity to germination stimulants such as gibberellins, nitrate and light. The optimum temperature for dormancy relief or induction depends on the optima of the required processes. These processes and therefore the optimum temperature for dormancy relief and induction can vary according to species (Fig. 8.2). A complete loss of dormancy can only be reached when all the essential processes take place.

This may explain why a pretreatment at a constant temperature affected dormancy differently than a pretreatment in the field or at a rising temperature in incubators. For all species, a prolonged pretreatment at a low temperature first broke dormancy but subsequently led to the induction of dormancy at that same temperature. The opposite also occurred. Dormancy of *S. officinale* seeds was first induced at 15°C, but subsequently it was also relieved at that same temperature (Chapter 3). Loss and induction of dormancy at one temperature have been reported before for *C. album* (Roberts and Benjamin, 1979), *P. persicaria* (Staniforth and Cavers, 1979) and *S. arvensis* (Espeby, 1989). Apparently, dormancy induction of summer annuals usually occurs at warm temperatures but when only part of the dormancy relieving processes, *e.g.* at 2°C, occur, some sort of feedback mechanism may also lead to induction of dormancy. However, as was shown for *S. officinale*, if after dormancy **induction** at a certain temperature this temperature pretreatment is continued, dormancy relief may also occur at that same temperature (Chapter 3). This indicates that the effect of temperature on dormancy of a seed also depends on the physiological status of the seed and not only on the temperature. This may be of ecological importance under conditions such as unusual warm or cold summers or winters, to synchronize the dormancy pattern.

Nevertheless, since there is no exact knowledge of the processes that do regulate dormancy and their temperature dependency, the theory of Totterdell and Roberts (1979) was used as an approximate basis for the development of the models.

temperatures. Germination of this species in the field, occurred from autumn to early spring. Accordingly, the species was called a **facultative winter annual**. *S. arvensis* and *C. album* did not obey the definition of either summer or winter annual. Germination was usually best at an intermediate temperature of approx. 15°C. When dormancy of these species was relieved, germination could occur at increasingly lower but also higher temperatures. Germination of these species in the field occurred from spring to early autumn. Accordingly, they were named **facultative summer annuals**.

The timing of germination in the field strongly depended on temperature. On the one hand, temperature regulated dormancy, expressed by the **width** of the range of temperatures suitable for germination, the germination-temperature range. On the other hand, germination could only occur when the field temperature was **within** this range. Additionally, the width of the germination-temperature range of *C. album*, *S. officinale* and *S. arvensis* was influenced by the environmental conditions light, nitrate and desiccation. The germination-temperature range was much wider when germination was tested in light and/or nitrate than in darkness and/or water. When seeds were desiccated prior to the germination test, germination could also proceed over a much wider range of temperatures. Not only was the germination-temperature range much wider as a consequence of these three environmental conditions, it was also open much longer. Consequently, germination could occur during a longer period of the year. After desiccation, *S. officinale* and *S. arvensis* could at certain temperatures even germinate during the entire year.

With the data from the burial experiments of *P. persicaria*, *S. officinale*, *S. arvensis* and *C. album*, models were developed that simulated the changes in the germination-temperature range as a function of the field temperature. Additionally, the effect of nitrate on the width of the germination-temperature range was simulated. The models were developed with data from germination tests in incubators, but when for the germination temperature in the model the field temperature at the moment of exhumation was used, germination under field conditions could be simulated. For all four species, there was a good similarity between these simulated data and data of germination tests that occurred in Petri dishes outdoors. It was concluded that the results with these descriptive models were promising. They could be the starting point for a more mechanistic approach in the future.

The effects of desiccation on germination were investigated in more detail in Chapter 6. It was shown that the more seeds were desiccated, the more germination was stimulated in the subsequent test. Before desiccation could stimulate germination, freshly harvested seeds needed a period of imbibition that varied from several hours for *S. officinale* and *S. arvensis* to several months for *C. album* or even more than a year for *P. persicaria* and *P. lapathifolium* subsp. *lapathifolium*. A discussion about the mechanisms, possibly involved in the effect of desiccation was presented.

In Chapter 7 the nitrate content of seeds of *C. album* and *S. officinale* was raised by

weekly nitrate fertilizations of the flowering mother plants. Seeds of both species germinated better with higher endogenous nitrate contents. However, when the seeds were buried the differences in endogenous nitrate between seed lots rapidly disappeared because nitrate leaked from the seeds. It was concluded that high endogenous nitrate contents may temporarily stimulate germination of seeds of these species. However, a prolonged effect on germination characteristics can not be expected because of equalization of the nitrate contents.

In Chapter 8 the results from Chapters 2 to 5 and the value of the models were discussed in more detail with emphasize on the similarities and differences between the investigated species.

Although the models closely simulated germination under controlled conditions, the practical application, to predict emergence in the field, is probably still far away. Particularly, knowledge of the field behaviour of the environmental factors light, temperature, nitrate and desiccation that control dormancy and germination, is lacking. Nevertheless the approach of the relationship between dormancy and germination seems promising. It shows that germination depends on the overlap of the field temperature and the germination-temperature range. The shape of the germination-temperature range is on the one hand determined by the dormancy status of the seed, that is regulated by the temperature during burial, on the other hand, by environmental conditions such as light, nitrate and desiccation, experienced shortly before or at the moment germination is triggered *e.g.* by soil cultivation.

Samenvatting

Onkruiden veroorzaken grote opbrengstverliezen in land- en tuinbouw. Doordat de mens zich bewust wordt van de bedreiging van het milieu wordt het gebruik van chemische middelen, om onkruidpopulaties onder controle te houden, steeds minder acceptabel. Een manier om het gebruik van pesticiden terug te dringen is het gebruik maken van geïntegreerde of biologische teeltsystemen. Of het nu gaat om insekten of onkruiden, om gebruik te kunnen maken van dergelijke systemen is het van belang kennis te hebben van het gedrag van de schadeverwekker.

Voorspelling van de opkomst van onkruiden zou een doelmatiger bestrijding met gebruik van minder chemische middelen mogelijk kunnen maken. Om dit te bereiken is het, naast kennis van de omvang en samenstelling van de zaadvoorraad in de akker, van belang te weten welke factoren kieming en opkomst beïnvloeden. Uit de literatuur blijkt dat zaden van onkruiden vaak niet gedurende het hele jaar kunnen kiemen, maar dat ze in en uit rust kunnen gaan. Door dit zogenaamde kiemrustritme wordt voorkomen dat zaden kiemen op een voor de groei en reproduktie van de plant ongunstig tijdstip.

In de hoofdstukken 2 tot en met 5 is het onderzoek naar de kiemrustritmes van *Polygonum persicaria* L. (perzikkruid), *P. lapathifolium* L. subsp. *lapathifolium* (knopige duizendknoop), *Sisymbrium officinale* (L.) Scop. (gewone raket), *Spergula arvensis* L. (spurrie) en *Chenopodium album* L. (melganzevoet) beschreven. Zaden van de vijf soorten werden begraven en op geregelde tijden werd telkens een deel van het zaad opgegraven. Van de opgegraven zaden werd de helft gedroogd. Vervolgens werden alle zaden verdeeld over petrischaaltjes met water of kaliumnitraat en geplaatst bij verschillende temperaturen. Voor de meeste tests werden de zaden gedurende enige tijd belicht, maar in enkele tests werd dit achterwege gelaten, om het effect van licht te onderzoeken. Behalve bij vastgestelde temperaturen in incubatoren werd de kieming van opgegraven zaden ook altijd in petrischaaltjes buiten getest, de zogenaamde veldomstandigheden. Naast de begraafexperimenten werden ook experimenten uitgevoerd in incubatoren om effecten ook onder gecontroleerde omstandigheden te kunnen toetsen.

Alle onderzochte soorten vertoonden seizoensgebonden wisselingen in kiemrust. Deze veranderingen bleken geheel door de temperatuur, waarbij de zaden zich in de grond bevonden, veroorzaakt te worden. Vocht- en nitraatgehalte van de grond speelden daarbij geen rol. Tussen de soorten onderling bestonden echter grote verschillen in de reactie op de temperatuur. De kiemrust van *P. persicaria* werd het beste bij 2°C gebroken, terwijl bij *S. officinale*, *C. album* en *S. arvensis* dit het beste bij respectievelijk 6, 10 en 15°C gebeurde. De veranderingen in kiemrust kwamen tot uiting in het traject van temperaturen waarbij kieming op kon treden. Zaden die

in rust waren kiemden bij geen of slechts een enkele temperatuur, terwijl zaden die niet in rust waren kiemden bij een hele reeks temperaturen. Ook hierin bestonden weer grote verschillen tussen de soorten. Kieming van de twee *Polygonum* soorten was gedurende bijna het hele jaar mogelijk bij een hoge temperatuur en alleen in bepaalde vastliggende periodes - als de zaden uit rust waren - ook bij lage temperaturen. Onder veldomstandigheden kiemde deze soort in het voorjaar. Dit alles zijn duidelijk kenmerken van een **zomerannuel**. Zaden van *S. officinale* kiemden juist beter bij lage temperaturen, wat een kenmerk is van winterannuellen. Omdat kieming onder veldomstandigheden naast het najaar (typisch voor echte winterannuellen) óók in het vroege voorjaar optrad werd deze soort **facultatieve winterannuel** genoemd. *S. arvensis* en *C. album* gedroegen zich niet als een echte winter- of zomerannuel: Ze kiemden beter bij gemiddelde dan bij hoge of lage temperaturen. Omdat ze onder veldomstandigheden van voor- tot najaar kiemden werden ze **facultatieve zomerannuellen** genoemd.

Kieming werd eigenlijk op twee manieren door de temperatuur gestuurd. Aan de ene kant, zoals hierboven reeds werd gemeld, stuurde de temperatuur de **kiemrust**, waarbij kiemrust zich uitte in de reeks temperaturen waarbij kieming kon optreden (het kiemtemperatuurtraject). Aan de andere kant kon **kieming** alleen optreden wanneer de temperatuur van de kiemtest binnen dit traject viel. Daarnaast werd de kieming van opgegraven zaden van *C. album*, *S. officinale* en *S. arvensis* sterk beïnvloed door omgevingsfactoren als licht, nitraat en drogen. Het kiemtemperatuurtraject was breder wanneer kieming werd getest in licht en/of nitraat dan in het donker en/of water. Als zaden werden gedroogd voor de eigenlijke kiemtest konden ze ook over een veel breder temperatuurtraject kiemen. Behalve dat deze factoren het kiemtemperatuurtraject breder maakten, was het traject ook gedurende een langere periode open. Daardoor konden de zaden gedurende een langere periode van het jaar kiemen. Na drogen konden zaden van *S. officinale* en *S. arvensis* bij bepaalde temperaturen zelfs gedurende het hele jaar kiemen.

Met behulp van de gegevens uit de begraafexperimenten werden voor *C. album*, *P. persicaria*, *S. officinale* en *S. arvensis* beschrijvende modellen ontwikkeld, die de veranderingen in kiemrust simuleerden als een funktie van de veldtemperatuur, waarbij de zaden begraven lagen. Ook het effect van nitraat op de kieming werd met behulp van deze modellen gesimuleerd. De modellen werden ontwikkeld met resultaten van kiemtesten in incubatoren. Wanneer voor de parameter kiemtemperatuur in het model de buitentemperatuur op het moment van opgraven van een portie zaden werd gebruikt kon de kieming onder veldomstandigheden worden gesimuleerd. Voor alle vier de soorten bestond er een goede tot zeer goede overeenkomst tussen deze gesimuleerde veldkieming en de resultaten van de kiemtesten die in petrischaaltjes buiten waren uitgevoerd. De resultaten met deze beschrijvende modellen lijken veelbelovend. Mogelijk kunnen ze het uitgangspunt vormen voor een meer mechanistische benadering.

Het effect van drogen op de kieming is nader onderzocht in hoofdstuk 6. Hoe lager het vochtgehalte van de zaden na het drogen, hoe hoger het kiempercentage in de erop volgende kiemtest. Echter voordat drogen de kieming kon stimuleren moesten zaden gedurende een bepaalde periode geïmbibeerd zijn. Deze periode varieerde van enkele uren voor *S. officinale* en *S. arvensis* tot enkele maanden (*C. album*) of zelfs meer dan een jaar (*P. persicaria* en *P. lapathifolium* subsp. *lapathifolium*). In hoofdstuk 6 zijn ook de mogelijke mechanismen, verantwoordelijk voor het effect van drogen, bediscussieerd.

In hoofdstuk 7 is beschreven hoe het nitraatgehalte van zaden van *C. album* en *S. officinale* verhoogd kon worden door de bloeiende moederplanten wekelijks met nitraat te bemesten. Zaden met een hoger nitraatgehalte kiemden beter. Wanneer de zaden echter begraven werden, verdwenen de verschillen in zowel nitraatgehalte als kieming, omdat het nitraat uit de zaden lekte. Hoge nitraatgehaltes kunnen blijkbaar de kieming van deze soorten slechts tijdelijk verbeteren. Een langdurig effect op het kiemgedrag valt niet te verwachten doordat de nitraatgehaltes van alle zaden in evenwicht komen met het nitraatgehalte van het omgevende medium.

In hoofdstuk 8 zijn de resultaten van de hoofdstukken 2 tot en met 5 en de waarde van de ontwikkelde modellen nader bediscussieerd met de nadruk op overeenkomsten en verschillen tussen de onderzochte soorten.

Hoewel de gepresenteerde modellen de kieming onder gecontroleerde omstandigheden behoorlijk goed simuleerden, is het gebruik van dit soort modellen voor de voorspelling van opkomst in het veld waarschijnlijk nog ver weg. Vooral de kennis van het gedrag van factoren als temperatuur, licht, nitraat en uitdroging in het zaaibed schiet nog tekort, terwijl deze factoren bepalend zijn voor kiemrust en kieming. Desalniettemin lijkt de benadering van kiemrust en kieming zoals gebruikt in de modellen veelbelovend. Kieming hangt af van overlapping van de veldtemperatuur en het kiemtemperatuurtraject. De breedte van het kiemtemperatuurtraject wordt op de eerste plaats bepaald door de rusttoestand van het zaad, die gereguleerd wordt door de temperatuur tijdens het verblijf in de grond. Daarnaast wordt de breedte van het kiemtemperatuurtraject beïnvloed door omgevingsfactoren zoals licht, nitraat en drogen die een rol gaan spelen op het moment dat het zaad door bijvoorbeeld grondbewerking aan de oppervlakte komt.

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Curriculum vitae

Op 30 november 1960 ben ik geboren in Zeist. Na het doorlopen van het Johan van Oldenbarnevelt gymnasium in Amersfoort ben ik in 1979 in Wageningen aan de Landbouwhogeschool Tuinbouwplantenteelt gaan studeren. Tijdens mijn studie heb ik me vooral met de gewaskundige/plantenfysiologische kant van de tuinbouw bezig gehouden. Nadat ik in september 1985 (met lof) ben afgestudeerd kon ik in februari 1986 beginnen met een promotieonderzoek naar de regulering van kiemrustwisselingen in onkruidzaden in een samenwerkingsverband tussen de vakgroepen Plantenfysiologie en Vegetatiekunde, Plantenoecologie en Onkruidkunde van de Landbouwuniversiteit. Sinds 1 december 1989 verricht ik als wetenschappelijk onderzoeker op het Centrum voor Agrobiologisch Onderzoek (CABO) te Wageningen (gewas)fysiologisch onderzoek aan karwij.