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Evaluation of dormancy and germination responses to temperature in *Carduus acanthoides* and *Anagallis arvensis* using a screening system, and relationship with field-observed emergence patterns

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Abstract

Experiments on the facultative winter annuals *Carduus acanthoides* and *Anagallis arvensis* were performed: (i) to determine thermal conditions that induce or release dormancy, (ii) to investigate to what extent changes in dormancy level resulting from those thermal conditions explain the seasonal pattern of emergence of these species, and (iii) to estimate required thermal time and base temperature for the germination of non-dormant seeds. *Carduus acanthoides* required high temperatures followed by decreasing temperatures for dormancy release; however, low winter temperatures did not induce secondary dormancy as expected for a winter annual. To the contrary, low temperatures stimulated dormancy release in the long term. In *A. arvensis*, dormancy relief was enhanced by dry storage at 25°C, and the response to low temperature was different depending on moisture conditions. Prolonged exposure to moist-chilling increased the dormancy level of the population, while dry storage at 4°C relieved dormancy. For both species, changes in the thermal range permissive for germination as a result of dormancy modifications explained to a large extent the timing of the emergence periods observed in the field. In neither species did base temperature for germination change with the dormancy level of the population. Thermal time required for germination of *C. acanthoides* varied with dormancy, while for *A. arvensis* seeds it was constant.

Keywords: after-ripening, *Anagallis arvensis*, *Carduus acanthoides*, dormancy, facultative winter annuals, germination, temperature, thermal time

Introduction

Soil temperature and soil moisture are the most important factors regulating seed behaviour under natural conditions (Bewley and Black, 1982). Temperature is known to have a dual effect: first, it regulates changes in dormancy level of seed populations and/or terminates dormancy in those seeds requiring fluctuating temperatures (Benech-Arnold *et al.*, 1990a,b; Benech-Arnold and Sánchez, 1995; Bouwmeester and Karssen, 1992). Second, temperature determines the rate with which seeds germinate after they have been released from dormancy (Bewley and Black, 1982; Benech-Arnold *et al.*, 1990a,b; Bouwmeester and Karssen, 1992). In relation to the first effect, the thermal environment may either increase or decrease the dormancy level of a seed population (Roberts, 1988). Dormancy cycles observed in some species are known to be regulated mainly by temperature (Baskin and Baskin, 1977, 1984; Bouwmeester and Karssen, 1992, 1993; Kruk and Benech-Arnold, 1998). For example, some summer annuals undergo dormancy relief by low temperatures experienced during winter, and their dormancy level is enhanced by high temperatures experienced during summer (Baskin and Baskin, 1977, 1984; Bouwmeester and Karssen, 1992, 1993; Kruk and Benech-Arnold, 1998). Several winter annuals species show the reverse dormancy pattern. Hence, high temperatures during summer results in dormancy relief, and low temperatures during winter can induce secondary dormancy (Thompson, 1970; Baskin and Baskin, 1976; Karssen, 1982; Bouwmeester

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Abbreviations: *IT regime*, increasing temperature regime within the range 4–36°C in steps of 4°C; *DT regime*, decreasing temperature regime within the range 36–4°C in steps of 4°C; *Tl*, mean lower limit temperature; *Th*, mean higher limit temperature; σ_{Tl}, standard deviation of the lower limit temperature; σ_{Th}, standard deviation of the higher limit temperature; *Tb*, base temperature; Θ_{50}, thermal time required for 50% germination of the germinable fraction.

and Karssen, 1992; Probert, 1992). These changes in dormancy level are generally expressed through narrowing and widening of the temperature range permissive for germination (Vegis, 1964). Thus, when the dormancy level of a population is at its minimum, the temperature range permissive for germination is at its widest. When soil temperature enters that permissive range, germination in the field occurs, and the role of temperature is limited to modulating the rate at which germination proceeds (the second above-mentioned effect).

Thus, a comprehensive knowledge of temperature effects on dormancy and germination rate is essential to understand the functioning of seed banks. Further, if seed behaviour in the field is to be predicted by modelling, then seed thermal responses should be analysed functionally and quantitatively. A complete characterisation of seed thermal responses that clearly distinguishes temperature effects on dormancy from those on germination rate is complex, and thus information is incomplete on the behaviour of many species. Recently, we have shown that the screening method developed by Washitani (1987) is a useful means for studying seed thermal responses both functionally and quantitatively (Kruk and Benech-Arnold, 1998). This method basically consists of (1) a test system for screening seed responses to temperature under controlled conditions, and (2) a mathematical model that describes (a) parameters to characterize the dormancy level of the population, the latter in relation to the width of the thermal range in which germination can occur, and (b) parameters to characterize the thermal-germination behaviour of non-dormant seeds (*i.e.* required thermal time and base temperature above which thermal time is accumulated). We are involved in a comparative study in which the main objective is to understand and to model seed bank functioning of weeds in winter crops of the humid Pampa in Argentina in relation to temperature. In this article, we present results from experiments carried out with the winter annuals *Carduus acanthoides* L. and *Anagallis arvensis* L., two world-wide weeds that are particularly noxious in winter crops grown in this and other regions. These experiments were conducted under controlled conditions following the procedure of Washitani (1987), and results were validated against field-collected data. Our objectives were: i) to determine thermal conditions that induce or release dormancy, ii) to investigate to what extent changes in dormancy level resulting from these thermal conditions can explain the seasonal pattern of emergence of the two species, and iii) to estimate required thermal time and base temperature for germination of non-dormant seeds, with the final aim of predicting seedling emergence dynamics within the emergence periods.

Materials and methods

Seeds

Fully ripe seeds from *Carduus acanthoides* and *Anagallis arvensis* were hand collected from many plants growing in a wheat field at Balcarce (lat. 37°45′S, long. 58°15′W), Argentina, during the 1993 dispersal season, at mid-summer (February). After collection, seeds were bulked and stored in containers at room temperature (20°C) in the laboratory for 2 weeks until they were used for the experiments.

Storage pre-treatments

To subject seeds to contrasting thermal and moisture conditions during their after-ripening, they were stored under different conditions before germination tests were carried out. Storage conditions were: (1) dry storage at 25°C in paper bags; (2) dry storage at 4°C in paper bags; (3) moist-chilling at 4°C on filter paper in Petri dishes; (4) burial at 0.5 cm depth in soil with a natural vegetation (grassland) cover, under prevailing soil temperature and moisture conditions and (5) immediately after seed dispersal without any pretreatment ('initial test'). Seeds were stored under the above-mentioned conditions for 2, 6 and 11 months. They were maintained in the dark inside incubators but exposed to white light during the set up of the experiments carried out with the test system. For burial in the soil, the seeds were placed inside transparent polyester 3 cm × 5 cm mesh envelopes with 0.5 mm × 0.5 mm openings, resulting in a microenvironment within the envelopment similar to that of their soil surroundings. The percentage of seeds from both species found germinated '*in situ*' after each exhumation was never higher than 1%.

Germination test system

Briefly, the germination test system (Washitani, 1987) used in this work consists of exposing one batch of seeds to gradually increasing temperatures from 4 to 36°C in steps of 4°C (*IT regime*) and a second batch to gradually decreasing temperatures from 36 to 4°C (*DT regime*). Since germination rate is generally higher at higher temperatures in the physiological range, duration of exposure differed depending on prevailing temperature: 7 days at 4°C, 6 days at 8°C, 5 days at 12°C, 4 days at 16, 20 and 24°C, 3 days at 28°C, 2 days at 32°C and 1 day at 36°C (Kruk and Benech-Arnold, 1998). The number of germinated seeds was recorded immediately before a temperature change and at the end of a test. Germinated seeds were removed after they were counted. The criterion for seed germination was radicle protrusion. Three replications of 50 seeds were allocated to each regime (*IT* and *DT*). Seeds were placed on three disks of Whatman No. 3 filter paper with 5 ml of distilled water in 9-cm diameter Petri dishes. They were exposed to white light during counting but otherwise were maintained in the dark inside incubators.

Remaining ungerminated seeds were later exposed to 25°C and daily alternating temperatures of 25°C (12h) / 12°C (12h) for 7 days at the end of the *DT* and *IT regimes,* respectively (Kruk and Benech-Arnold, 1998). Percentage germination attained immediately before seeds were subjected to either 25°C or 25°C/12°C was defined as 'final percentage germination' at that regime, while that attained after incubation at 25°C or 25°C/12°C was defined as 'maximum percentage germination'.

Quantification of thermal germination parameters

The thermal parameters were quantified using a mathematical model described in detail by Washitani (1987). The model predicts germination dynamics of a seed population as a function of time and temperature. Thus, germination curves obtained with the above-mentioned screening system were reproduced by simulation for recently shed seeds and for those buried in the soil under prevailing soil temperature conditions at 0.5-cm depth for 2, 6 and 11 months. With the test system and simulation model, two kinds of parameters were estimated: (1) those describing dormancy level of populations, and (2) those describing the relationship between germination rate and temperature of non-dormant seeds.

Briefly, model assumptions are as follows (Washitani, 1987):

(1) a given non-dormant seed can germinate only within the temperature range between two limits, *i.e.* a lower limit temperature (*Tl*) and a higher limit temperature (*Th*), and both limit temperatures vary among seeds within a seed population according to normal distributions. Therefore, the fraction (*Fl*) of seeds with lower limit temperatures below a given temperature (*T*) can be given by the following distribution function:

$$Fl(T) = \int_{-\infty}^{(T-\mu_{Tl})/\sigma_{Tl}} (1/\sqrt{2\pi}) \bullet \exp(-x^2/2)\mathrm{d}x \qquad (1)$$

where μ_{Tl} and σ_{Tl} are the mean and standard deviation of *Tl*. Similarly, the fraction (*Fh*) with a higher limit temperature higher than *T*, can be given by the following distribution function:

$$Fh(T) = \int_{(T-\mu_{Th})/\sigma_{Th}}^{\infty} (1/\sqrt{2\pi}) \bullet \exp(-x^2/2)\mathrm{d}x \qquad (2)$$

where μ_{Th} and σ_{Th} are the mean and standard deviation of *Th*.

(2) The temperature dependency of the rate of germination of individual seeds can be approximated by two linear equations with four parameters, *i.e.*, base temperature (*Tb*), optimal temperature (*To*), maximal temperature (*Tm*), and thermal time required for germination (Θ_{50}):

For the suboptimal range,

$$r = 1/\Theta_{50} \bullet (T-Tb), \qquad (3)$$

while for the supraoptimal range,

$$r = 1/\Theta_{50} \bullet (To-Tb) \bullet [(Tm-T) / (Tm-To)], \qquad (4)$$

(3) The distribution of Θ_{50} within a seed population can be described by the following distribution function:

$$Ft(\Theta_{50}) = 1 - [3D^{-3}(\Theta_{50} - m + D)^3 + 1]^{-\frac{1}{2}}, \qquad (5)$$

where *m* is the median of the distribution, *i.e.*, the required thermal time for the seeds which germinate at the cumulative percentage of 50%, and *D* is the differential thermal time between 0 and 50% germination.

(4) The distribution of *Tl*, *Th* and Θ_{50} are independent of each other.

Therefore, the cumulative percentage of germination (G_i) at the time of temperature change from the *ith* to the *i*+1*th* temperature in the test system can be approximated by the following expression:

$$G_0 = 0\text{, for both regimes,} \qquad (6)$$

$$Gi = G_{i-1} + [Fl(T_i) \bullet Ft(h_i) - G_{i-1}] \bullet Fh(T_i) \qquad (7)$$

for the *IT regime*, and

$$Gi = G_{i-1} + [Fh(T_i) \bullet Ft(h_i) - G_{i-1}] \bullet Fl(T_i) \qquad (8)$$

for the *DT regime*, where h_i is the accumulated thermal time at the time of temperature change from the *ith to i*+1*th* temperature.

Tl and *Tb* should be clearly distinguished from each other in the suboptimal range. *Tl* is a parameter for characterizing the dormancy level of the populations and indicates the lower limit of the thermal range permissive for germination, while *Tb* is the theoretical temperature above which thermal time is accumulated after temperature enters that permissive range. Similarly, in the supraoptimal range *Th* (a parameter characterizing dormancy) should be distinguished from *Tm.*

Values for *Tl*, *Th*, *Tb*, *To*, *Tm*, *m*, *D* and Θ_{50} were given *a priori* to the computer program (custom-written in Basic) to run the model. Therefore, the parameters were obtained by systematically modifying their values until the germination curve resulting from simulation fitted the germination curve obtained for each of the conditions of the screening system.

Field monitoring of seedling emergence and seed dispersal patterns

To investigate the thermal conditions to which seeds are exposed after dispersal and to determine to what extent the characterization carried out with the screening system can be used to predict seedling emergence periods, we monitored phenology of seed dispersal and seedling emergence of *C. acanthoides* and *A. arvensis* within a wheat crop cycle and during the fallow period for two consecutive years.

Twenty quadrats (100 cm by 20 cm) were used to study seedling emergence. After the wheat crop was sown, the quadrats were distributed at 2 m intervals along three north-south transects spaced 2 m apart. They remained in the field throughout the entire crop cycle and also during the fallow period. Each quadrat was divided into 80 5 cm by 5 cm subquadrats. Number of emerged seedlings from each species was counted every 20 days. Newly emerged seedlings were left rooted in the plots so as not to modify the environment, but they were marked with a coloured wire ring to differentiate them from the previously emerged ones. Seed dispersal was assessed by counting seeds captured in five 20 cm × 7 cm × 3 cm adhesive trays located beside each quadrat. Trays were replaced about every 20 days. Soil temperature was recorded hourly at 0.5 cm depth in the wheat crop with a DataLogger (LI-COR model 1000) and soil temperature sensors (LI-COR model 1015). Rainfall data were obtained from a meteorological station 100 m from the study site.

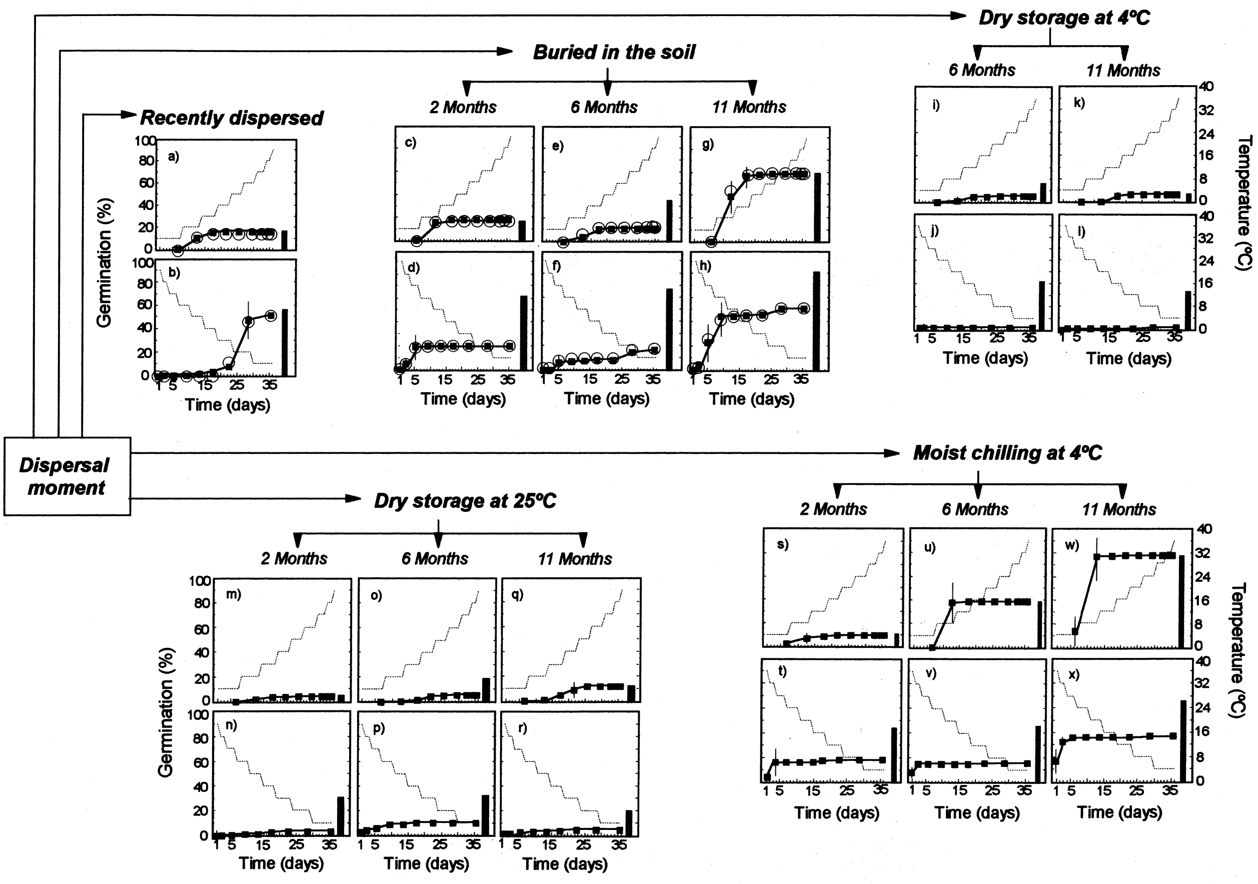

Figure 1. Germination behaviour of the seed population of *Carduus acanthoides* in the test system without (a–b) or with various thermal pretreatments: buried in the soil (c–h); dry storage at 4°C (i–l); dry storage at 25°C (m–r) and moist-chilling at 4°C (s–x). Dotted lines show the courses of temperature change. In the germination curve, mean cumulative percentage germination of three replications of 50 seeds is plotted (■) with a vertical bar showing standard deviations; SD's within data points are not shown. The vertical black bar at the right shows the maximum germination percentage in the *IT regime* after the seeds had been subjected to daily alternating temperatures (25°C[12h]/12°C [12h]) for 7 days or that in the *DT regime* after the seeds had been subjected to 25 °C for 7 days. The circles (○) indicate germination values obtained by simulation (see Materials and Methods for details).

Results and Discussion

Carduus acanthoides and *A. arvensis* are facultative winter annual species since they emerge in autumn and in spring. Other facultative winter annuals include *Capsella bursa-pastoris* (Baskin and Baskin, 1989) and *Sisymbrium officinale* (Bouwmeester, 1990). Winter annuals undergo the active part of the their life cycles during the colder part of the year. Exposure to high temperatures is considered to result in dormancy release via processes that occur during dry or moist after-ripening, and Probert (1992) proposed that low temperature results in complete or partial induction of secondary dormancy. A seasonal pattern of changes in dormancy would be expected as a result of changes in the maximum temperature permissive for germination (Karssen, 1982).

Seed behaviour in germination test system

Carduus acanthoides. Recently shed seeds of this species attained a final germination of 52% under the DT regime (Fig. 1b), but only 17% under the *IT regime* (Fig. 1a). Germination occurred at 4–12°C under both regimes (Figs 1a and 1b). This difference might have resulted from a stimulating effect of gradually decreasing temperatures (though no examples of this kind of effect are given in the literature).

Seeds that had been buried for two months attained a similar germination percentage in the *IT regime* to that observed for fresh seeds immediately after dispersal (Figs 1a and 1c), while final germination percentage under the *DT regime* was lower than that observed immediately after dispersal (Figs 1b and 1d). The temperature range permissive for germination of 20% of the population appeared to have been widened, since seeds germinated as much in the 32–28°C range in the *DT regime* as they did in the 8–12°C range in the *IT regime*. The possibility that the 20% of seeds that germinated in the 28–32°C range in the *DT regime* were a different group from the 20% that germinated in the 8–12°C range in the *IT regime* cannot be ruled out. However, since no additional germination was observed in the 28–32°C range in the *IT regime*, a widening of the thermal range permissive for germination appears to be the most plausible explanation. Another 30% of the seeds were released from dormancy after they were subjected to decreasing temperatures (*DT regime*), but their germination was only possible after incubating them at 25°C (Fig. 1d). Moist-chilling storage for two months (Figs 1s and 1t) also resulted in the lowering of the dormancy level of about 20% of the seed population (*i.e.* germination proceeded over a wider thermal range). Also in this case, the effect of gradually decreasing temperatures (*DT regime*) might have been instrumental for dormancy release in a small fraction of the population (Fig. 1t). Dry storage at 25°C was not as effective as burial or moist-chilling for lowering dormancy of the populations (Figs 1m and 1n).

Six months after dispersal, no major changes were observed in the responses of seeds in the *DT regime* given any pretreatment (Figs 1f, 1j, 1p, and 1v). In contrast, differences in the behaviour of seeds transferred either from the soil or from the moist-chilling pretreatment were evident under the *IT regime* (Figs 1e and 1u). Prolonged exposure to moist-chilling was the most effective treatment for dormancy relief. Thus, moist chilled seeds no longer required *DT regime* to attain increased germination percentages (Fig. 1u). In contrast, buried seeds still required the *DT regime* to release a further proportion of the population from dormancy. This difference was maintained even after 11 months of pretreatment, although in both cases a further proportion of the population was released from dormancy. In contrast, no major changes were observed in seeds stored dry at 4°C or at 25°C (Figs 1k–1l and 1q–1r). Seeds exhumed after 11 months of burial had been exposed to the higher soil temperatures of spring and also to cycles of desiccation – hydration, which probably decreased the dormancy level of the population (Baskin and Baskin, 1984).

These results suggest that recently shed seeds of *Carduus acanthoides* are released from dormancy when exposed to gradually decreasing temperatures. This requirement is maintained even after 6 months of burial, but it appears to be eliminated by prolonged exposure to moist-chilling (Figs 1e and 1f; 1u and 1v). Nevertheless, two months of after-ripening either in soil or under moist-chilling conditions decreased the dormancy level of about 20% of the population, thus allowing germination under a wider thermal range (8–32°C) (Figs 1c and 1d; 1s and 1t). These results are not in agreement with the findings of McCarty *et al.*, (1969), who not only reported a very low dormancy level after dispersal in this species, but also showed that moist-chilling at 4°C induced dormancy, as expected for a winter annual. Seed hydration conditions during after-ripening appear to be very important for dormancy release in this species, since seeds moist-chilled at 4°C were released from dormancy (Figs 1s–1x), but those stored dry at the same temperature did not exhibit a similar behaviour (Figs 1i–1l). Baskin and Baskin (1976) reported that after-ripening in seeds of the winter annuals *Stellaria media*, *Valerianella umbilicata* and *Phacelia purshii* occurred during storage at high temperatures (20, 25, 30/15°C) and that lower temperatures were ineffective or inhibitory; moisture conditions of the seeds were less relevant. In *C. acanthoides*, dry storage storage at 25°C did not release seeds from dormancy (Figs 1m–1r) .

Therefore, in contrast to what is reported for other winter annuals, seeds of *C. acanthoides* require high temperatures followed by decreasing ones to come out of dormancy, and low winter temperatures do not induce secondary dormancy. To the contrary, low temperatures stimulated dormancy release in the long term, and dry storage at 25°C was not effective in relieving dormancy (Figs 1m–1r).

Anagallis arvensis. Recently dispersed seeds of this species had a moderately low level of dormancy. Germination of seeds incubated in the *IT regime* was 40% (Fig. 2a), while in the *DT regime* it was only 7% (Fig. 2b). This difference might have resulted from induction of secondary dormancy by high temperatures (30–36°C) at the beginning of the *DT regime*. However, a brief exposure to the lowest temperatures of the gradient appeared to have partially overcome the secondary dormancy. This was evident when the seeds were placed at 25°C at the end of the *DT regime* (Fig. 2b). Under both regimes, germination occurred at 12°C and at 16°C (Figs 2a and 2b).

Two months after dispersal, germination occurred between 12°C and 16°C (Figs 2c, d, m, n, s, t). Burial in soil was the most efficient pretreatment for releasing seeds from dormancy, at least in terms of the germination percentage attained (Figs 2c and 2d). Dry storage at 25°C did not produce pronounced modifications with respect to the initial test (Figs 2a and 2b; 2m and 2n), while moist-chilling at 4°C increased the dormancy level of the population (Figs 2a and 2b; 2s and 2t).

After both burial in soil and dry storage at 25°C,

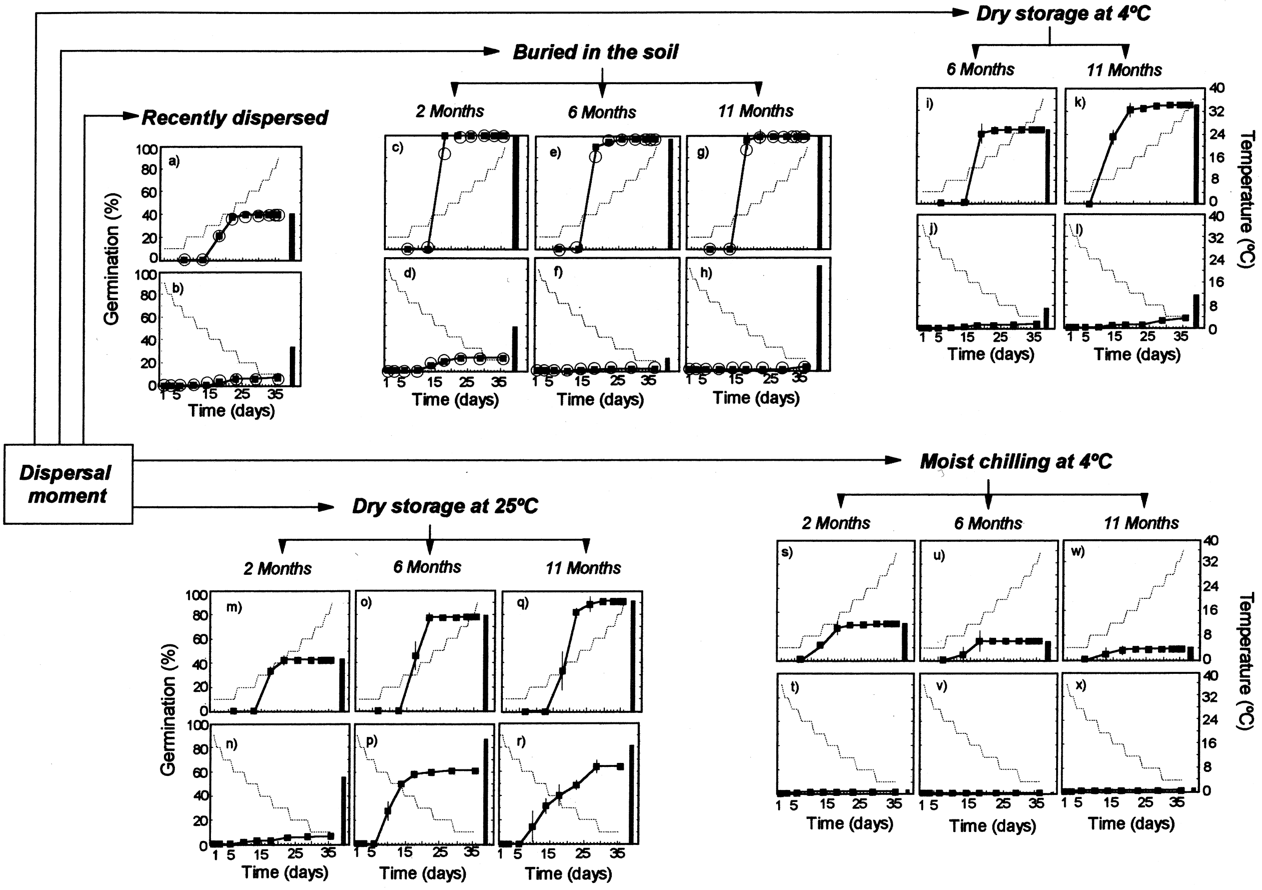

Figure 2. Germination behaviour of the seed population of *Anagallis arvensis* in the test system without (a, b) or with various thermal pretreatments: buried in the soil (c–h); dry storage at 4°C (i–l); dry storage at 25°C (m–r) and moist-chilling at 4°C (s–x). Dotted lines show the courses of temperature change. In the germination curve, mean cumulative percentage germination of three replications of 50 seeds is plotted (■) with a vertical bar showing standard deviations; SD's within data points are not shown. The vertical black bar at the right shows the maximum germination percentage in the *IT regime* after the seeds had been subjected to daily alternating temperatures (25°C[12h]/12°C[12h]) for 7 days or that in the *DT regime* after the seeds had been subjected to 25 °C for 7 days. The circles (○) indicate germination values obtained by simulation (see Materials and Methods for details).

dormancy imposed by high temperatures at the beginning of the *DT regime* (36–28°C) was partially overcome by brief exposures to low temperatures at the end of the gradient (Figs 2d and 2n). This was not the case with moist-chilled seeds, where dormancy imposed by high temperatures was not overcome by brief exposure to low temperatures. It appears that long exposures to low temperatures under moist-chilling conditions increased the dormancy level of the population (Fig. 2t), while a similar condition given for a brief period of time decreased it (Figs 2b, d and n).

Seed behaviour was not modified markedly after either six or eleven months of burial (Figs 2e–2h). The most remarkable feature of seeds buried for eleven months was their ability to come totally out from dormancy imposed by high temperatures after brief exposures to low temperatures (Fig. 2h), which was not observed in seeds that had been buried for six months (Fig. 2f). Seeds dry-stored at 4°C responded similarly to buried seeds (Figs 2e–2h and 2i–2l), although final germination percentage was lower and dormancy imposed by exposure to high temperatures (*DT regime*) was no longer overcome by brief exposures to low temperatures (Fig. 2l). Seeds dry-stored at 25°C were gradually released from dormancy, but at the same time they lost almost all sensitivity to high temperatures, suggesting an effect of ageing under this storage condition (Figs 2o–2r). Remarkably, moist-chilling at 4°C gradually induced a higher dormancy level in the population (Figs 2u–2x). Regardless of pretreatment, the temperature range permissive for germination remained between 12 and 16°C throughout the whole experimental period (Fig. 2).

These results show that 50% of the *Anagallis arvensis* seed population had a low dormancy level immediately after dispersal. As soon as seeds entered the soil seed bank, dormancy level decreased rapidly, but short exposures to high temperatures induced secondary dormancy if the seeds were imbibed. In contrast, dry storage at either 25°C or 4°C promoted dormancy relief. High temperatures have been reported to stimulate dormancy relief in other winter annuals (Baskin and Baskin, 1983, 1984). However, our study suggests that the effect of high temperatures strongly depends on the seeds' moisture condition because, while high temperatures stimulate dormancy relief in dry seeds (Figs 2m–2r), they appear to induce dormancy in imbibed ones (*DT regime*). This is in agreement with results obtained by Bauer *et al*. (1998) with the winter annual *Bromus tectorum*. However, the dormancy releasing effect of dry storage at 25°C does not seem to be equivalent to the process that buried seeds undergo. Indeed, while sensitivity to high temperatures, which upon imbibition induced secondary dormancy, was retained in buried seeds until after 6 months after pretreatment (Fig. 2f), it was lost after six months of dry storage at 25°C (Fig. 2p).

Prolonged exposure to moist-chilling increased the dormancy level, as has been found in seeds of other winter annuals like *Torilis japonica*, *Sedum pulchellum*, *Phacelia dubia* var. *dubia* and *Thlaspi perfoliatum* (Baskin and Baskin, 1975, 1977, 1978, 1979). However, dry storage at 4°C relieved dormancy (Figs 2i–2l).

Quantification of thermal germination parameters

Parameters were obtained by systematically modifying their values until the germination curve resulting from simulation fitted the germination curve obtained with each of the conditions of the screening system for recently shed seeds and for those buried in the soil under prevailing soil temperature conditions at 0.5 cm depth, for 2, 6 and 11 months (see Figs 1a–1h and 2a–2h, square and circle symbols, observed and simulated curves, respectively).

Carduus acanthoides. The temperature range within which germination can occur for recently shed seeds was established within 4 ± 0.5°C (*Tl*, mean lower limit temperature) and 10.5 ± 2°C (*Th*, mean higher limit temperature) (Fig. 3). Estimated required thermal time for germination of 50% of the non-dormant fraction (Θ_{50}) was 68°C-days, accumulated above an estimated base temperature (*Tb*) of 0°C.

After 2 months of burial (summer and onset of autumn), dormancy level of 20% of the population decreased. Thus, the temperature range within which germination could occur widened, and values of *Tl* and *Th* were 7 ± 0.5°C and 34 ± 0.5°C, respectively (Fig. 3). The estimated required thermal time for germination of 50% of the non-dormant fraction (Θ_{50}) was lower than that estimated for recently shed seeds (21°C-days, accumulated above an estimated base temperature (*Tb*) of 0°C). The 30% of the population that would germinate, if gradually decreasing temperatures are experienced, is assumed to germinate under the same thermal range.

The temperature range for germination after six and eleven months of burial changed slightly with respect to that estimated for seeds buried for two months (Fig. 3). However, the value of Θ_{50} after 6 and 11 months of burial was higher than that for seeds buried only two months (Θ_{50} = 32°C-days for six months and Θ_{50} = 41°C-days for eleven months of burial, accumulated above a base temperature (*Tb*) of 0°C). Maximum and optimum temperatures (45°C and 15°C, respectively) remained constant and independent of time after dispersion. Optimum temperature estimated with the test system and the model for *C. acanthoides* are within the range observed by McCarty *et al*. (1969).

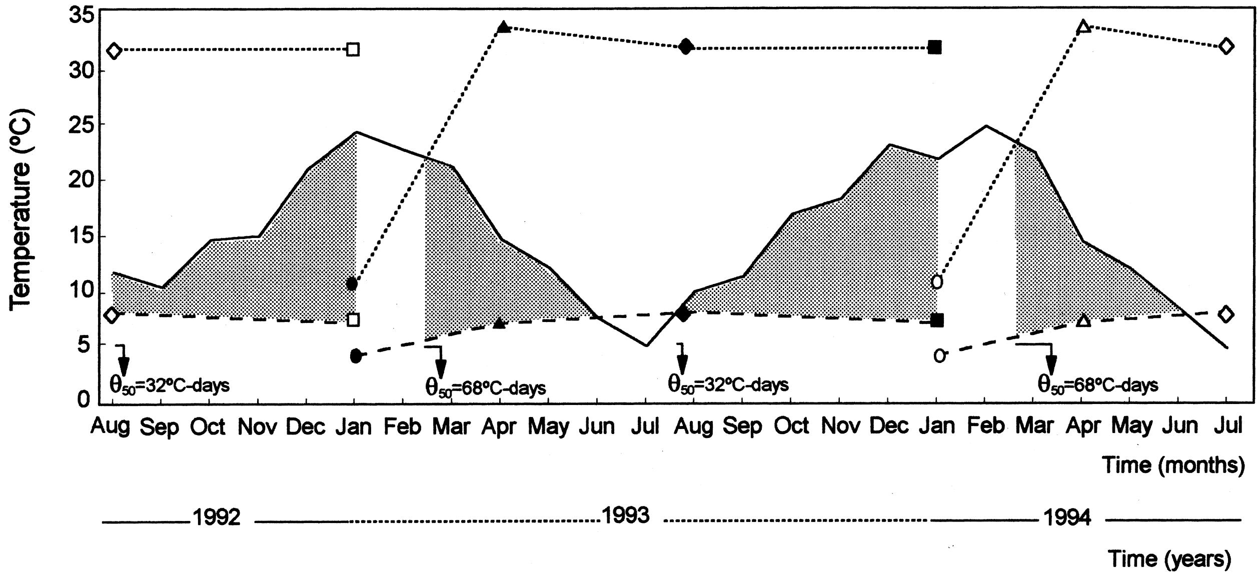

Figure 3. Relationship between field temperature (recorded at Balcarce) and changes in the range of temperature over which germination can proceed for *Carduus acanthoides*. Dotted (· · · ·) and dashed (- - - -) lines represent the mean higher limit (Th) and mean lower limit (Tl) temperature, respectively, at which germination can proceed according to results of the experiments under controlled conditions with the simulation model. These temperatures were estimated from recently shed seeds (●) and after 2 (▲), 6 (◆) and 11(■) months of burial and extrapolated for the second year of field experiments. Open and closed symbols correspond to different experimental years. Continuous lines indicate the monthly mean temperature in field. The hatched area indicates the moment when the actual and required temperatures overlap. Arrows (⅂) show the moment at which the required thermal time (Θ_{50}), for germination of 50% of the fraction of the population with thermal requirements within *Th* and *Tl*, should have been accumulated according to the soil temperatures recorded in the field starting from the moment at which soil temperature entered the permissive thermal range (see text for details).

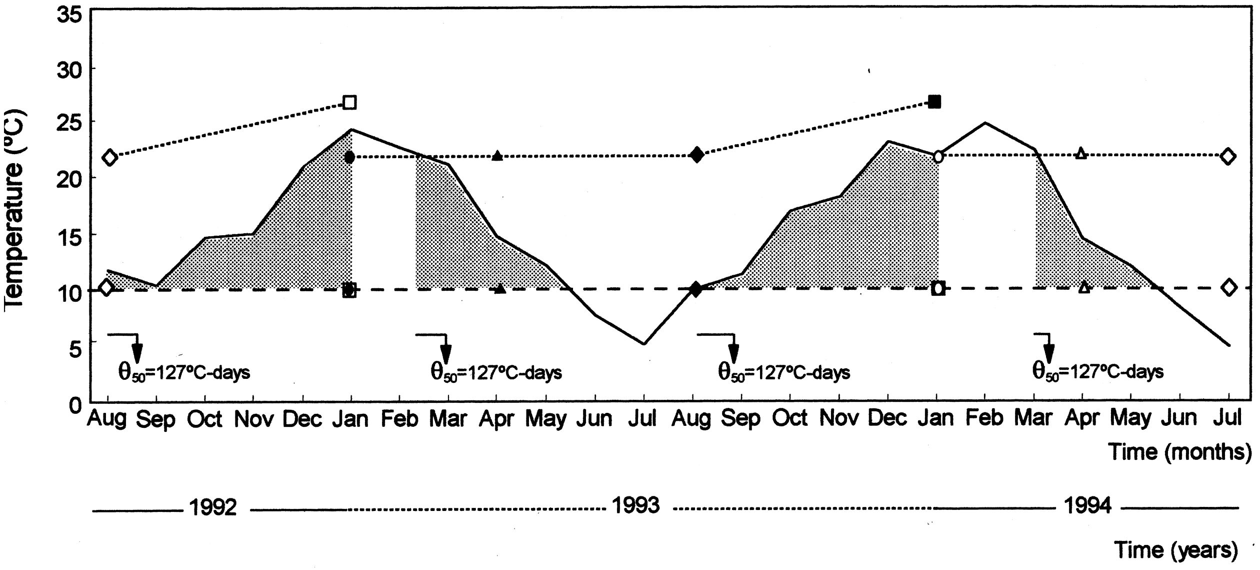

Figure 4. Relationship between field temperature (recorded at Balcarce) and changes in the range of temperature over which germination can proceed for *Anagallis arvensis*. Dotted (· · · ·) and dashed (- - - -) lines represent the mean higher limit (Th) and mean lower limit (Tl) temperature, respectively, at which germination can proceed according to results of the experiments under controlled conditions with the simulation model. These temperatures were estimated from recently shed seeds (●) and after 2 (▲), 6 (◆) and 11(■) months of burial and extrapolated for the second year of field experiments. Open and closed symbols correspond to different experimental years. Continuous lines indicate the monthly mean temperature in field. The hatched area indicates the moment when the actual and required temperatures overlap. Arrows (⅂) show the moment at which the required thermal time (Θ_{50}), for germination of 50% of the fraction of the population with thermal requirements within *Th* and *Tl*, should have been accumulated according to the soil temperatures recorded in the field starting from the moment at which soil temperature entered the permissive thermal range (see text for details).

Anagallis arvensis. Germination curves in the test system could be reproduced by simulation with the model (Figs 2a–2h). The temperature range within which germination can occur for fresh seeds was 10 ± 2°C (*Tl)* to 22 ± 6°C (*Th*) (Fig. 4).

The dormancy level of buried seeds remained low and unchanged regardless of time of burial; consequently, seed population parameters remained almost constant during burial throughout the year. Two and six months after dispersal, *Tl* had the same value as that estimated for a recently dispersed population, but σ_{Tl} (0.5°C) was different. After eleven months of burial, *Th* was 27 ± 1°C (Fig. 4). The estimated required thermal time for germination of 50% of the non-dormant fraction once it has entered the permissive thermal range for germination (Θ_{50}) was constant throughout the year (127°C-days accumulated above a base temperature (*Tb*) of 0°C). Maximum and optimum temperatures (60 and 16°C, respectively) remained constant and independent of the time after dispersion. The optimum temperature estimated with the test system and the model are within the range observed by other researchers (Lauer, 1953; Grant Lipp and Ballard, 1963).

Predicting seedling emergence and monitoring seedling emergence and seed dispersal patterns in a wheat field

Karssen (1982) emphasised that the seasonal periodicity in field-emergence of annual species is the result of seasonal periodicity in the temperatures and in the range of temperatures suitable for germination. Thus, germination in the field would be restricted to the period when soil temperature and the thermal range for germination overlap. From those parameters related to the dormancy level of the population (*Tl* and *Th*), we predicted periods of emergence in the field. Hence, we plotted the values of those parameters as functions of time after dispersal, together with values of daily mean soil temperature recorded during the two consecutive years of the field experiment. Each time soil temperature values were within those at which germination could occur, seedling emergence was considered to proceed in the field. Once soil temperature entered this range, accumulation of thermal time (Θ_{50}) above the estimated base temperature (*Tb*) was assumed to start; from this, the moment for germination of 50% of the non-dormant fraction was inferred (Figs 3 and 4).

Carduus acanthoides. Two periods of seedling emergence in the field were observed during the year: (1) September to October, and (2) February to April (Fig. 5a). This is typical behaviour of a facultative winter annual (Probert, 1992) and is in agreement with what is shown in Fig. 3, where one emergence flush also was predicted for May and June. The fact that no flushes were observed in the field during these two months probably was related to depletion of the fraction of the population with a low dormancy level. Results obtained with the test system gave a good explanation for emergence in the field (Fig. 3 and Fig. 5a). Seeds with a low dormancy level that had been buried for a year could have produced the flush in February (Figs 1g and 5a). The emergence flush in April probably resulted from the 20% of the population with a low dormancy level as occurred in the test system. The source of these seedlings may have been seeds dispersed in February and March that were stimulated to germinate by gradually decreasing temperatures (Figs 1d and 5a). Moreover, the number of seedlings that emerged during the period that followed dispersal in 1994 (February, March, April and May) accounted for approximately 20% of the total number of seeds dispersed in January (Fig. 5a). During September and November, the emergence flush probably originated from seeds dispersed in the summer that had been exposed to the low winter temperatures for several months. The number and the timing of seedling emergence within the crop cycle were similar in 1992 and 1993, but the number of seeds produced prior to crop harvest was higher in 1993 than in 1992 (266 seeds m^{-2} *vs*. 10 seeds m^{-2}).

Although changes in the dormancy level of seeds of *C. acanthoides* were determined throughout the year, a cyclic dormancy pattern was not observed in this species (Fig. 3). The absence of a cyclic dormancy pattern reported in this study is in agreement with previous work showing that this species has a transient seed bank (Roberts and Chancellor, 1979; Leguizamón *et al*., 1981). Nevertheless, it should be noticed that most of the seed population is expected to germinate during the second year after dispersal, when the fraction of the population with high dormancy has been released from it.

Anagallis arvensis. In contrast to what was found for *C. acanthoides*, *A. arvensis* had two dispersal periods: summer (January to February) and winter (May to August) (Figs 5a–5b). Nevertheless, seedling emergence periods in the field for this species were similar to those for *C. acanthoides*; however, during the second year the flush extended into winter (Figs 5a–5b). This is in agreement with what is shown in Fig. 4, where two emergence flushes were predicted: (1) February to May, and (2) August to January. No seedling emergence was observed in December or January (Fig. 5b).

The emergence flushes then could be explained by results and parameters derived from the test system (Figs 2, 4 and 5b). Immediately after dispersal

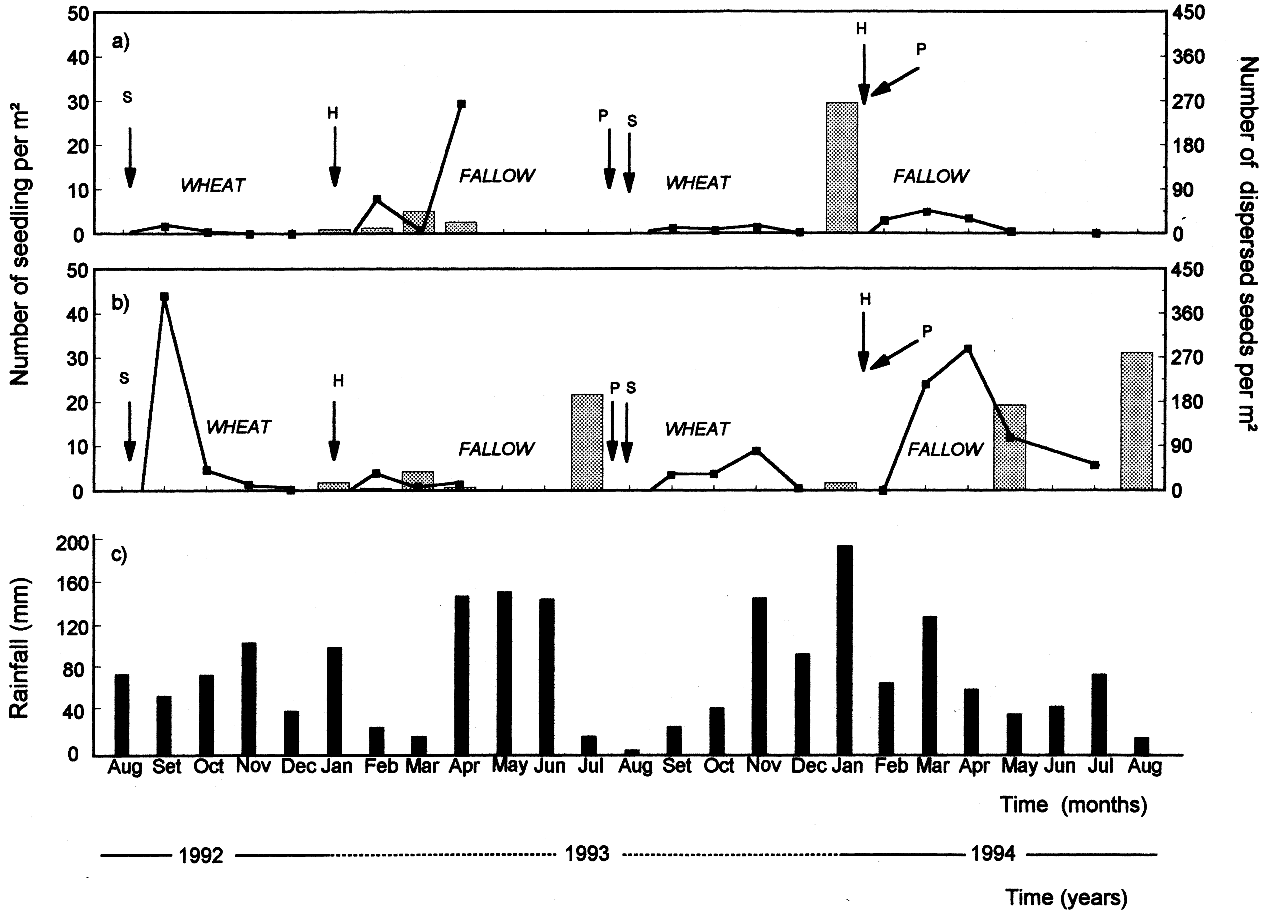

Figure 5. Seasonal pattern of seedling emergence (line) and seed dispersal (dotted bars) for *Carduus acanthoides* (a) and *Anagallis arvensis* (b). S: sowing; H: harvest; P: plowing. (c) Monthly mean precipitation recorded during the two-year field experiments.

(January) (Fig. 5b), seeds had a low level of dormancy, but soil temperature was not within the range for germination (Figs 2a and 4), thus explaining lack of emergence in the field (Fig. 5b). As long as soil temperature was within the permissive range for germination, emergence began. The spring emergence flush probably was from seeds dispersed in July (Fig. 5b); low winter temperatures combined with low water availability might have been instrumental for dormancy release (Figs 2i, 2k and 5c). Emergence flushes in March, April and May occurred with temperatures close to the optimum for germination (16°C). Interestingly, emergence during 1992 was concentrated at the beginning of September, while during 1993 it occurred throughout spring. This might have resulted from the difference in distribution of rainfall in the two years. Rainfall accumulated during August and September was approximately 120 mm in 1992, but only approx. 20 mm during these two months in 1993 (Fig. 5c).

According to results obtained with other winter annuals (Baskin and Baskin 1975, 1977, 1978 and 1979), entrance into secondary dormancy in buried seeds during the winter, which leads to development of a cyclic dormancy pattern, might have been expected. However, this did not occur, possibly because soil temperature was as low as 5°C during only one month (July), when seeds probably were not imbibed because of the very low amount of rainfall during this period (Figs 4 and 5c). In other words, entrance in secondary dormancy might be prevented in dry winters, thus preventing dormancy cycling.

Conclusions

Carduus acanthoides and *A. arvensis* are facultative winter annual species since they emerge in autumn and in spring. In *C. acanthoides*, the most effective thermal conditions for dormancy release were high temperatures followed by gradually decreasing ones.

In *A. arvensis*, dry storage at either 25 or 4°C produced dormancy relief; however, prolonged exposure to moist-chilling increased the dormancy level of the population. Although changes in the dormancy level of seeds of *C. acanthoides* and *A. arvensis* were determined throughout the year, a cyclic dormancy pattern was not observed in either species.

Some seed populations of both *C. acanthoides* and *A. arvensis* are known to have a light requirement for termination of dormancy (Grant Lipp and Ballard, 1962; McCarty *et al.*, 1969). In our work we 'by–passed' this requirement by exposing the seeds to a number of saturating pulses of light each time germination was counted in the test system before transferring the seeds from one temperature to the following one in the gradient. This was done to assess seed thermal responses without the interference of a light requirement. Therefore, the lack of '*in situ*' germination in seeds buried in mesh envelopes in the soil might have been related to the fact that those seeds were not exposed to light until their exhumation, thus suggesting a light requirement in our populations.

The interpretation of the seed bank functionality in terms of seedling production dynamics could be an important step towards elaboration of predictive models that would increase the efficacy with which control methods are used.

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