The germination ecology of the clonal grassland herb *Knautia arvensis* (Dipsacaceae): regeneration strategy, geographic variation and ecological consequences

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Abstract

It can be argued that the overall importance of the seed and seedling stages should be low for clonal species. However, clonals differ in their rate of seedling recruitment, and this has consequences for their life histories, genetic and spatial structure, and population dynamics. We investigate germination strategies in the clonal grassland herb *Knautia arvensis* (Dipsacaceae) using phytotron germination experiments, field experiments, and observational data. Phytotron responses predict that gap or depth-sensing strategies are absent in *K. arvensis,* and that germination is seasonally synchronised. These predictions are corroborated by the field results. Seed carry-over across years was demonstrated in both laboratory and field experiments. Seed batches from four different geographical regions in Norway responded differentially to laboratory treatments, with cold stratification, temperature responses, and seed carry-over increasing with the unpredictability of winter climate rather than with the severity of winter climate.

Keywords: competition, field experiment, gap, germination cueing, phytotron experiments, regeneration niche, repeated measures ANOVA, seasonal timing.

Introduction

From a life-history perspective, the overall importance of the seed and seedling stages of plants are expected to decrease from annual to perennial species, and further for species with the capacity for clonal growth (Harper 1977). Regeneration from seed in clonal species has generally been considered a rare event, primarily linked to the initial establishment at new sites. Eriksson (1989) argues that clonal species differ considerably in their rate of seedling recruitment, and that this has consequences for their life histories, genetic diversity, spatial structure, and population dynamics. Seed regeneration strategies of clonal species have, however, been relatively little studied (but see Eriksson and Fröborg 1996, Eriksson 1999, Amiaud et al. 2000).

Seed germination and seedling establishment and growth are vulnerable stages in the life histories of plants. Seedlings are smaller, less competitive, and less protected against predators, and more vulnerable to adverse environmental conditions such as freezing or deficiency of water or nutrients than mature plants. Consequently, the seedlings are often restricted in their ecological tolerances, an observation that has been formalised in concepts such as 'safe sites' (Harper 1977) or 'regeneration niches' (Grubb 1977). The probability of seedling survival is not constant, however, but varies through space and time, and survival may be greatly increased if germination can be avoided at times and places where mortality risk is comparatively high. This may occur if seed germination is sensitive to the environment (environmental cueing). It has been shown that seed germination can be initiated or inhibited by minute changes in environmental factors such as temperature regime (Grime et al. 1981, Rice 1985, Schütz 1997, Cavieres and Arroyo 2000), light intensity (Bell et al. 1995) or spectral quality (Batlla et al. 2000, Mandák and Pyšek 2001), soil nutrients (Karssen and Hilhorst 1992, Bell et al. 1995), moisture (Pérez-Fernández et al. 2000), and cold-stratification prior to germination (Murdoch and Ellis 1992, Milberg and Andersson 1998), as well as to interactions between such factors. Different temporal or spatial germination patterns may result from environmental cueing. Examples are the seasonal germination commonly observed in temperate species (e.g. spring, autumn, or winter germination, Thompson and Grime 1979,

Olff et al. 1994), and cueing to ephemeral low-risk microsites such as vegetation gaps (Rice 1985, Kotorová and Lepš, 1999), temporarily favourable hydrological conditions (Pérez-Fernández et al. 2000), or local resource pulses.

If good and bad times occur stochastically rather than predictably, however, the effectiveness of environmental cueing breaks down. Under stochastic environmental variation bet-hedging strategies (Venable and Brown 1988, Philippi 1993a), that leave a fraction of the seeds dormant through periods of good germination conditions, ensure a carry-over of seeds across years and the build-up of persistent seedbanks that may buffer populations against years of high stochastic mortality. It has been shown that the optimal dormancy fraction increases with the frequency of bad years (Philippi 1993a).

For species with large ecological amplitudes, or with wide geographical or altitudinal distributions, the factors and processes controlling seedling mortality risk may not be constant throughout the range. If there are predictable differences in the timing and location of safe germination sites, then populations may optimise local survival probability by adjusting their responses to environmental cues. Under such circumstances, germination responses to environmental cues should vary throughout the range (Meyer and Monsen 1991, Meyer et al. 1995, Schütz and Milberg 1997, Pérez-Fernández et al. 2000, Cavieres and Arroyo 2001). If habitats differ in their degree of stochasticity, populations should differ in their annual germination fractions, seed dormancy levels, and seedbank build-up (Venable and Brown 1988, Philippi 1993b).

In this paper we use a combination of field and laboratory experimental and observational studies to investigate the germination ecology of the clonal grassland herb *K. arvensis* throughout its distributional range in Norway. We attempt to predict the regeneration niche of *K. arvensis* by investigating its responses to a number of potential environmental cues for seasonal and micro-site variability, and we test some of these predictions in the field. At the population-scale we investigate if four populations in different geographical regions show differential responses to environmental cueing and in their potential for seed carry-over.

Material and methods

The species

Knautia arvensis (L.) Coult. (Dipsacaceae) is a clonal herb widely distributed in Europe and adjacent parts of Africa and Asia (Hultén and Fries 1986). In Norway, it is common in the lowlands and valleys of the southern and central parts of the country (Nemoral to Middle Boreal vegetation zones), and occasional in the northern parts and in the mountains (North Boreal, Lid and Lid 1994). *Knautia arvensis* grows in dry meadows, pastures, dry hills, open woods, and roadsides. It has a sympodial, branched stock, a taproot, and lateral underground stolons. The flowers are arranged in one to several dense capitula, each containing 55-100 flowers. The fruits are 1-seeded, and have an elaiosome, promoting ant dispersal, as well as hairs, promoting dispersal by animals (Tutin et al. 1976, Lid and Lid 1994). As the fruits are one-seeded they are denoted as seeds hereafter.

Plant material used

Mature seeds of *K. arvensis* were collected from five populations in different geographical regions in Norway during the summer of 1998 (Fig. 1, Table 1). The seeds were combined into bulk samples from each population; each containing seeds from one to four different sites, and from more than 50 individuals. For the field germination experiment, 20 batches of 100 seeds from the Mountain population were sown within two weeks from collection (see below). The remaining seeds were stored in paper bags at 4ºC until the phytotron experiments were initiated. Then batches of 50 apparently ripe and undamaged seeds were prepared. These seed batches were the experimental units in all experiments.

Selection of potential environmental cues

Treatments for the phytotron experiments were selected to represent potential environmental cues that correlate with seasonality and vegetation gaps within the vegetation types and regions studied. First, germination at four different temperatures and in light and darkness was compared. Daily thermal amplitude is greatest on bare soil, and decreases under a leaf canopy and with soil depth. In order to test for gap- or depth-sensing strategies (Thompson and Grime 1979) we included a daily fluctuating temperature regime. A cold-stratification requirement will delay germination of newly shed seeds until the following spring (Baskin and Baskin 1998, Grime et al. 1981), and germinability of stratified vs. unstratified seeds was therefore investigated. Gibberellins serve vital physiological functions in seed germination (Vleeshouwers et al. 1995, Toyomasu et al. 1998), and may initiate germination in dormant seeds, or under suboptimal conditions (e.g. Bell et al. 1995, 1999). If the addition of gibberellic acid increases germination, then this indicates that the environmental cues that break dormancy and initiate germination in *K. arvensis* were not included in our experimental protocol.

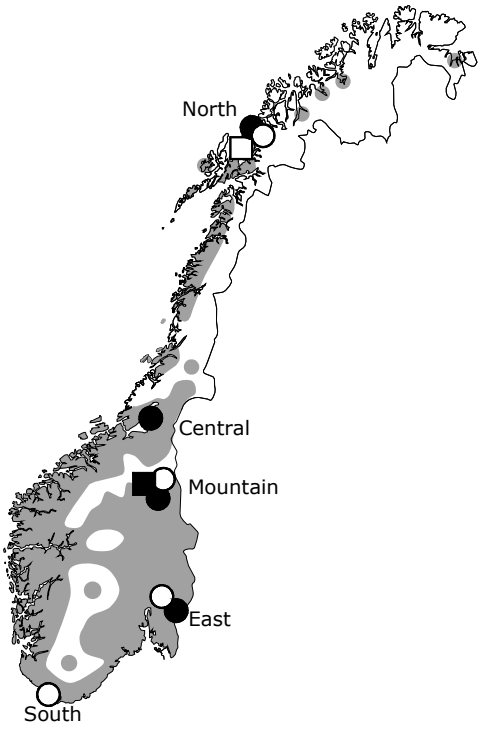

Figure 1. The geographical location of the populations used for the phytotron germination experiment (●) and the viability after cold storage experiment (❍), and sites of field regeneration studies (■ = experiment and ❑ = observational study). The geographical distribution of *Knautia arvensis* in Norway is shaded (Mossberg et al. 1992, Lid & Lid 1994).

Phytotron germination experiments

Seed germination requirements and dormancy levels were tested for four populations (Fig. 1) in a series of phytotron experiments. Experiments were set up to investigate the effect of (a) light and temperature, (b) fluctuating temperatures, (c) cold-stratification, and (d) dormancy breaking by means of gibberellic acid. In the temperature and light experiments (a, b) four constant temperatures (10ºC, 15ºC, 20ºC and 25ºC) and a diurnal cycle (25ºC for 16H and 10ºC for 8H) were compared in light and darkness. The 24H temperature sums of the 20ºC and 25/10ºC treatments are identical; by testing germination responses to fluctuating temperature against germination at 20ºC we investigate the effect of diurnal variation *per se*. In the cold stratification experiment (c) unstratified seeds were set to germinate at 20ºC in light and darkness, using germination of stratified seeds at 20ºC as control. In the gibberellic acid experiment (d), the petri dishes were watered with 0.8% gibberellic acid, GA_3, and set to germinate in light at 20ºC, using germination of stratified seeds at 20ºC and light as the control. The factorial combinations of treatments were replicated two (a, b, c) or four (d) times for each population, for a total of 112 petri dishes and 5600 seeds. Germination was recorded and seedlings removed after 2, 4, 6, 10, 16, 24, and 32 days. In these experiments the seed batches were placed on three layers of moist Whatman # 3 filter paper in 100 mm seal-tight petri dishes. For all except the immediate germination treatment (c), moist seeds were stratified in darkness at 4ºC for two months prior to the onset of the experiments. Temperatures were regulated in specially constructed growth chambers. Light treatments were full light, using standard artificial greenhouse light for a photoperiod of 16H per day, or darkness. Petri dishes receiving dark treatment were wrapped individually in two layers of aluminium foil, and these were opened and seeds counted under a safe green light (<0.05µmol/ m^2 s). The experiments were carried out at the Centre for Plant Research in Controlled Climate at the Agricultural University of Norway. Repeated measures analysis of variance (ANOVA) on arcsine-transformed data was used to test for differences in germination responses among treatments and populations (Sokal and Rohlf 1995). All experimental treatments and days since onset were treated as class variables, and a first-order autoregressive covariance structure assumed. Least square means were computed for populations, and statistical differences between means were evaluated by Tukey multiple comparisons tests (Sokal and Rohlf 1995). All models were fitted using the procedure Proc mixed in SAS version 8.0 (SAS institute 1999).

Viability after cold storage experiment

Seed germination after 8, 12, 16, 20, 24, 28, and 32 months of storage at 4ºC in darkness was tested for four populations (Fig. 1) in a phytotron experiment. Storage times were replicated two times for each population, for a total of 56 petri dishes and 2800 seeds. The seed batches were placed on two layers of moist Munktell no. 1700 filter paper in covered petri dishes. Germinated seeds were counted and removed weekly for 6 weeks. Phytotron conditions were constant artificial greenhouse light and 21ºC for 5 weeks, then 18ºC onwards. The experiment was carried out at the phytotron at the University of Tromsø. Because of problems with moisture regulation, the 12 and 16 months germination trials were not included in the analyses. Two-way ANOVA on arcsine-transformed data was used to test for differences in germinability between storage times and populations. The significances of pairwise differences among populations were adjusted for multiple comparisons (Bonferroni correction). To assess loss of germinability through time, a continuous exponential survival function was fitted to the data: $N = N_0 e^{-bt}$, where N is the number of seeds germinating at time t, N_0 is the number of seeds germinating at the first trial, t is time in months between N_0 and N, and b is the seed loss rate (Cousens and Mortimer 1995). For

Region	Geographical localisation	Elevation	Precipitation	Temperatures (°C)			
				Mean		Number of months	
		(m.a.s.l.)	(mm/year)	Annual	January	T<-2 °C	-2°C<T<2 °C
Mountain	Os (62 °34´N, 11 °05´E)	700	504	0.3	-11.2	5	2.0
North	Bø, Andøy, Evenes (ca. 68 °50´N, 15 °50´E)	5	1032	3.9	-2.5	2	4.3
East	Nes (60 °01´N, 11 °45´E)	200	665	4.0	-6.9	3	2.0
Central	Levanger (63 °47´N, 11 °20´E)	50	855	4.6	-4.2	3	2.5
South	Lista (58 °05´N, 06 °47´E)	20	1147	7.4	1.0	0	2.0

Table 1. Geographic location, altitude and climatic characteristics of the five populations used in the different experimental and observational studies (see Figure 1). Data are from the Norwegian Meteorological Institute, and are based on the nearest meteorological station, or mean values of several stations if relevant.

comparative purposes the results were expressed as 'half-lives', which is the time, in months, until half of the originally germinable seeds has died: $t_{1/2} = (\log_e 2)/b$. Analyses were performed with SPSS 10.0 (SPSS Inc., 1999).

Seed regeneration in perennial grassland

Data on the seed regeneration of *K. arvensis* in perennial grassland were obtained in a field germination experiment at the Mountain site (Vigdis Vandvik unpublished) and a study of clonal structure at the North site (V. Vange unpublished). The field germination experiment was initiated in October 1998. Batches of 100 seeds were sown into 20 0.040 cm^2 plots in a randomised block design with four treatments (turf-stripped, vegetation cut at ground level, vegetation cut at 5 cm above ground, and standing vegetation) in five replicate blocks. Individual seedlings were mapped early and late in the growing season for the next two years. In the clonal structure study, permanent plots were set up in three grazed and three abandoned grassland sites in September 1998. Individual seedlings were mapped late in the growing season the first year, and early and late the following year. In the field experiment, the numbers of new seedlings per plot per season, and the number of deaths between seasons, were $\log_e (n+1)$ transformed. The effect of experimental treatments was analysed using mixed model ANOVA (Proc mixed, SAS 8.0, SAS institute).

Results

Seed germination and regeneration of Knautia arvensis

Knautia arvensis germinated under all experimental conditions in the phytotron, but the final percentage germinated, as well as the speed of germination, differed greatly between treatments (Figs. 2 and 3, Table 2). There was an overall effect of temperature on germination ($F=82.1$, $p<0.0001$) and on the shape of the germination curves through time ($F=13.6$, $p<0.0001$). Mean germination was only 5.4% at 10ºC, and increased with temperature to a maximum of 48% at 20ºC. Light regime (light or darkness) was of much less overall importance than mean temperature, but there were statistically significant effects both on the final percentage ($F=6.8$, $p=0.0128$) and timing ($F=8.3$, $p<0.0001$) of germination (Table 2a). The ecological significance of this is unclear, however, as germination was actually lower and slower in light than in darkness (Fig. 2). There were no significant interactions between light and the experimental factors in (b) and (c) (preliminary analyses, not presented). Diurnally fluctuating temperature (25/10ºC) did not increase germination beyond the 20ºC levels (Table 2b). Cold stratification increased germination speed ($F=12.1$, $p=<0.0001$), so that final germination was 1.8-fold higher in stratified (48%) as compared to unstratified (27%) seeds ($F=66.6$, $p=<0.0001$, Fig. 3, Table 2c). This demonstrates that the seeds were weakly dormant when shed, and that cold-stratification contributed to breaking this dormancy. The addition of gibberellic acid (GA_3) to unstratified seeds did not increase germination beyond the stratified seed levels (Fig. 3), suggesting that there are no major unidentified additional sources of dormancy in *K. arvensis.*

The germinability of *K. arvensis* decreased substantially during cold storage (Fig. 4, $F=15.44$, $p=<0.0005$), but still 16.5% of the seeds germinated after 32 months. The estimated seed half-life for *K. arvensis*, based on three populations (North, East, and South), was 17.2 months. The Mountain population was not included in these calculations, as the data did not meet the assumption of a negative exponential response through time (Fig. 4). The data reflect survival under near optimal storage conditions in the laboratory (4ºC and darkness), and the results should therefore not be interpreted directly as potential field survivals. Still the estimated seed half-life implies that seeds of *K. arvensis* may remain viable for more than one year.

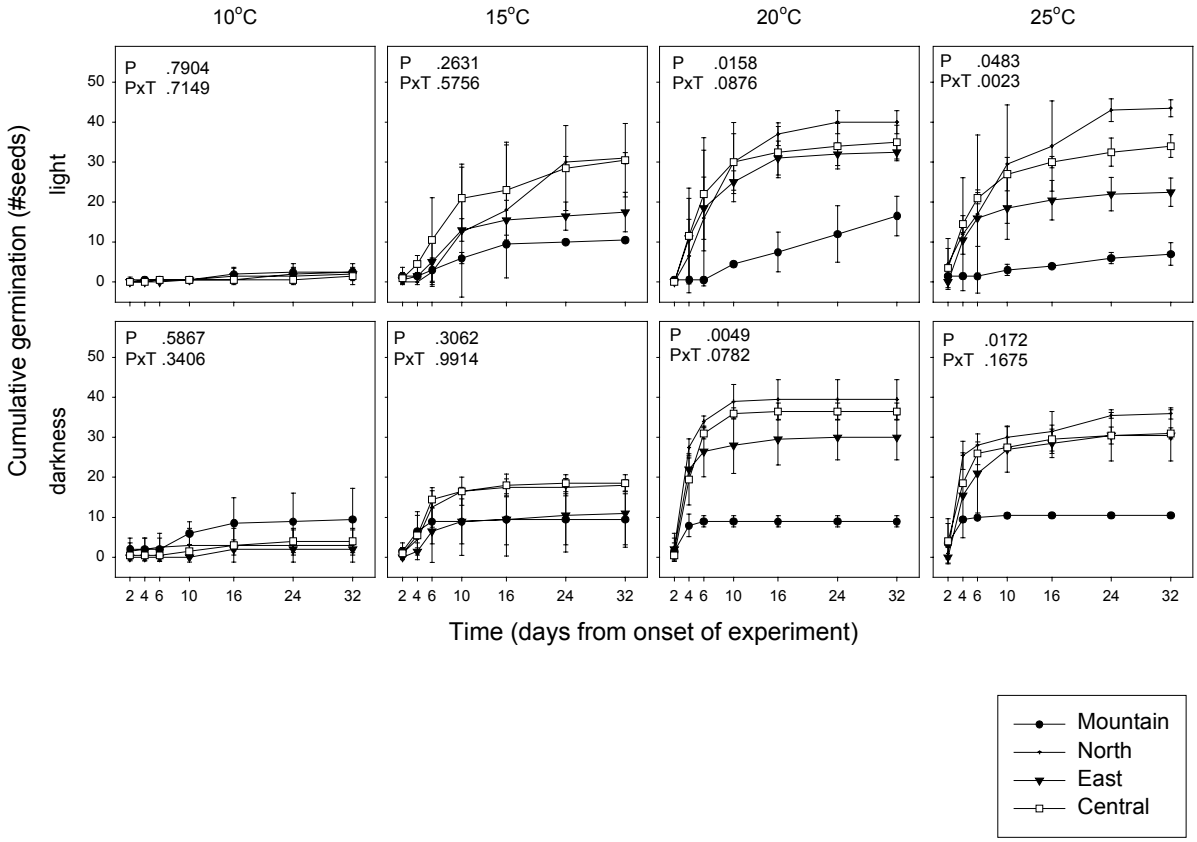

Figure 2. The effects of light and temperature on the germination of *Knautia arvensis*. The mean numbers of seeds germinated in two replicate dishes ± 1 SD are shown. Repeated measures ANOVAs were performed to test for differences between populations within treatments. P-values for the overall effects of population (P), and the population x time interaction (PxT), are given on each plot (Tukey's test).

Germination in the field experiment was low, as only 73 seedlings, i.e. 3.7% of the seeds sown, germinated over the first two years. The experimental microsite treatments (bare ground, vegetation cut 0 cm and 5 cm above ground, and control) did not affect germination (p=0.9767) or seedling mortality (p=0.9304). New seedlings were recorded both in the spring and autumn censuses (1.1, 1.0, and 1.6% in autumn 1999, spring 2000 and autumn 2000, respectively). Seedling mortality was 45% between the first and second census, and 11% between the second and third census. In the clonal structure study, 1687 seedlings were recorded. Out of these 26.8%, 38.1% and 35.1% germinated in autumn 1998, spring 1999 and autumn 1999, respectively.

Between-population variation

Within the overall germination responses of *K. arvensis* described above, there were highly significant differences between populations (Figs. 2 and 3, Table 2a, c, d, population and population x treatment effects). Across all treatments 20%, 54%, 44%, and 53% of the seeds germinated for the Mountain, North, East, and Central populations, respectively. The four populations were differentially affected by temperature, both in timing of germination (F=1.4, p=0.0430) and overall germinability (F=5.7, p<0.0001) (Table 2a). When these differences were broken down for the different temperatures, we found that all populations germinated relatively poorly at 10ºC, and best at 20ºC, but that populations differed considerably in the magnitude of their temperature response (Fig. 2). In the North, Central, and East populations temperature effects were strong (6-80%, 6-72%, and 4-63% from 10ºC to 20ºC, respectively), and the rank of these populations was consistent across treatments and times (Fig. 2, Table 2a). The Mountain population differed from the other three, having seeds that germinated better at low temperatures, and a much weaker temperature response (12 – 26% from 10ºC to 20ºC). Diurnally fluctuating temperature (Table 2b) or light (Table 2a) did not affect populations differentially. The effect of cold-stratification differed weakly between populations (F=2.8, p=0.0534). The North population had a very strong response, with germination increasing 2.7-fold (29 – 80%) after stratification. The effect was weaker, but still positive, in the other three populations, as germination increased 1.8-fold (15 – 26%) in the Mountain population, 1.6-fold (45 – 72%) in the

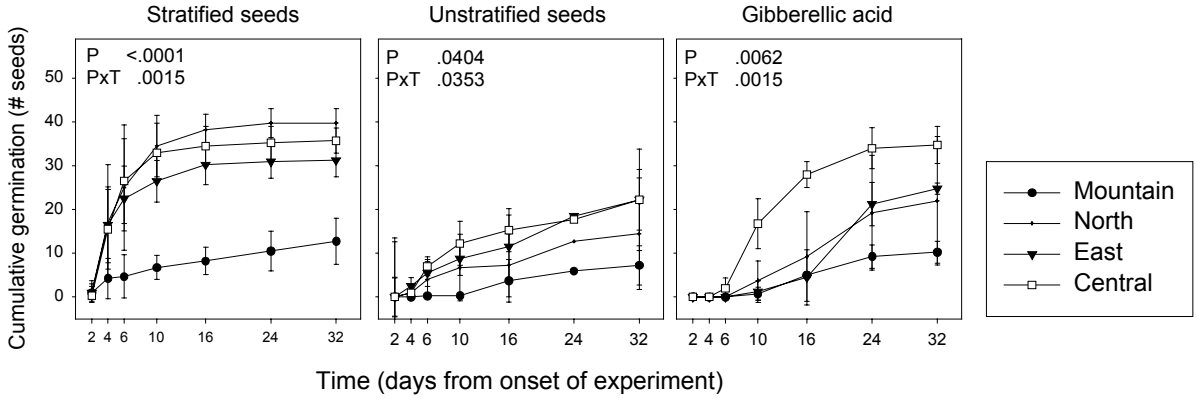

Figure 3. Germination of *Knautia arvensis* at 20ºC, showing differences between seeds that are stratified (4ºC for two months), unstratified, and treated with gibberellic acid. The mean number of seeds germinated in four replicate dishes ± 1 SD is shown. Repeated measures ANOVAs were performed to test for differences between populations within treatments. P-values for the overall effects of population (P), and the population x time interaction (PxT), are given on each plot.

Central population, and 1.4-fold (45 – 63%) in the East population (Fig. 3, Table 2c).

Loss of germinability during cold storage varied significantly among populations (F=22.25, p=<0.0005, Fig. 4), and pairwise comparisons showed that the Mountain and East populations had distinct responses, while the North and South populations did not differ from each other. Seed half-lives were estimated to 19.6 months for the North, 18.7 for the South, and 15.1 months for the East population. Seed half-lives could not be estimated for the Mountain population (se above), but germinability declined most dramatically in this population, as only one seed germinated after 28 months, and no seeds after 32 months (Fig. 4).

Discussion

Based on the results from the phytotron experiments, we predict that *Knautia arvensis* has a wide germination niche; the seeds germinated both with and without cold-stratification, under a wide range of constant as well as fluctuating temperatures, and in both light and darkness. The field experiment and observational study confirmed these predictions, seedlings are common within natural populations, germinate both in gaps and in a closed grassland sward throughout the growing season. In relation to Eriksson's (1989) dichotomy of recruitment patterns into 'initial' and 'repeated', our results demonstrate that *K. arvensis* is a typical example of the latter. For clonal species with 'repeated' germination, Eriksson (1989) predicts high seedling competitiveness, absence of dormancy, and high probability for evolution of locally adapted populations. Below we discuss the germination ecology of *K. arvensis* and differences between the four populations in light of these predictions.

The regeneration ecology of Knautia arvensis

Seed recruitment in space. Seed germination requirements for light or diurnally fluctuating temperatures may function as mechanisms for detecting ephemeral bare-ground gaps in grassland vegetation (Rice 1985, Olff et al. 1994, Kotorová and Lepš 1999). In our phytotron experiments, germination in *K. arvensis* was not affected by either of these cues, suggesting that such gap-detecting mechanisms are absent (Figs. 2 and 3, Table 2). The germination ecology of *K. arvensis* may therefore be interpreted as an optimisation of seedling competitiveness at the expense of micro-site selectivity. The field experiment and population study supports this interpretation, as germination was observed throughout the grasslands studied, with no detrimental effect due to the presence, absence, or height of the grassland sward. Furthermore, the subsequent survival of individual seedlings was also little affected by sward characteristics, giving further strength to the interpretation that *K. arvensis* seedlings survive by tolerating, rather than avoiding, grassland sward competition.

Seasonal timing of recruitment. Cold stratification effects are frequent in species of the temperate region worldwide (Grime et al. 1981, Baskin and Baskin 1988, Washitani and Masuda 1990, Olff et al. 1994), and have generally been interpreted as strategies to inhibit germination in freshly shed seed, postponing seedling emergence to the following spring. The cold-stratification response in *K. arvensis* was a quantitative rather than a qualitative trait; however, and in accordance with this the field results showed that timing of germination was not precise. A conspicuous feature of the phytotron germination responses of *K. arvensis* was the high temperature threshold (Fig. 2, Table 2). Germination was high and fast at 20ºC, slow at 15ºC, and very few seeds germinated at

Scale	(a) Temperature and light experiment Source	model df	error df	F-ratio	p
Species	temperature	3	41.5	82.1	<0.0001
	light	1	41.5	6.8	0.0128
	temperature x light	3	41.5	1.1	0.3425
	time	6	175.0	128.4	<0.0001
	time x temperature	18	175.0	13.6	<0.0001
	time x light	6	175.0	8.3	<0.0001
	time x temperature x light	18	175.0	2.0	0.0149
Population	population	3	41.5	18.0	<0.0001
	population x temperature	9	41.5	5.7	<0.0001
	population x light	3	41.5	0.7	0.5426
	population x temperature x light	9	41.5	0.4	0.9161
	population x time	18	175.0	4.2	<0.0001
	population x time x temperature	54	175.0	1.4	0.0430
	population x time x light	18	175.0	1.2	0.2313
	population x time x temperature x light	54	175.0	0.4	0.9999
	(b) Fluctuating temperature experiment Source	model df	error df	F-ratio	p
Species	fluctuation	1	24.0	0.1	0.8345
	time	6	144.0	190.3	<0.0001
	time x fluctuation	6	144.0	0.8	0.5614
Population	population	3	24.0	22.1	<0.0001
	population x fluctuation	3	24.0	0.2	0.8845
	population x time	18	144.0	6.8	<0.0001
	population x time x fluctuation	18	144.0	0.6	0.8671
	(c) Cold-stratification experiment Source	model df	error df	F-ratio	p
Species	stratification	1	32.7	66.6	<0.0001
	time	6	134.0	73.4	<0.0001
	time x stratification	6	134.0	12.1	<0.0001
Population	population	3	32.7	14.7	<0.0001
	population x stratification	3	32.7	2.8	0.0534
	population x time	18	134.0	3.5	<0.0001
	population x time x stratification	18	134.0	1.1	0.3762
	(d) Dormancy breaking experiment Source	model df	error df	F-ratio	p
Species	gibberellic acid	1	21.8	35.7	<0.0001
	time	6	86.1	58.1	<0.0001
	time x gibberellic acid	6	86.1	8.9	<0.0001
Population	population	3	21.8	10.5	0.0002
	population x gibberellic acid	3	21.8	2.9	0.0609
	population x time	18	86.1	2.3	0.0062
	population x time x gibberellic acid	18	86.1	2.1	0.0139

Table 2. Repeated measures ANOVAs on the germination of *Knautia arvensis* from four populations (Mountain, North, East, and Central) in Norway. The effects of (a) temperature and light, (b) diurnally fluctuating temperatures, (c) cold-stratification, and (d) dormancy breaking by means of gibberellic acid were tested. For experiment (b) and (c) controls were germinated at 20ºC in light and darkness, for experiment (d) controls were germinated at 20ºC and light. Significant treatment and interactions between treatments effects show the overall effects of treatments on *Knautia arvensis* germination, treatment x time effects signify an influence on the timing of germination, population and population x time effects signify differences between populations, and population x treatment and population x time x treatment signify differential responses of populations to treatments. The effect of time does not merit interpretation, as it simply reflects that germination of seed batches accumulates through time.

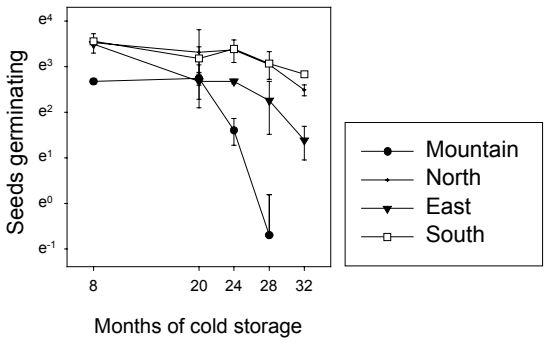

Figure 4. Seed germination of *Knautia arvensis* from four populations (Mountain, North, East, and South) after 6 – 32 months of storage at 4ºC and darkness. The mean number of seeds germinated in two replicate dishes ± 1 SD is shown. Significant differences ($p<0.05$; Tukey's test) between populations are denoted with different letters.

10ºC. High temperature thresholds for germination have previously been reported for northern as compared to southern species in Britain (Grime et al. 1981), and for mountain as compared to lowland populations in the Andes (Cavieres and Arroyo 2001). Schütz and Milberg (1997) suggest that as favourable germination conditions (temperature, light, moisture, and nutrient status) would not be likely to persist for extended periods of time during, for example, winter thaws, very slow germination may be almost as effective as dormancy in preventing precocious emergence under temporarily benign conditions. A consequence of this argument is that *germination rate* under different experimental conditions in the phytotron may give more information about field behaviour than *germination percentages* achieved at the end of the sometimes prolonged laboratory trials. This should be taken into account both in the experimental design and in the statistical testing of seed germination studies.

Potential for seed carry-over across years. Although little is known about the long-term survival of seeds of *K. arvensis* in the soil, the relatively large seeds and wide regeneration niche suggest that it does not have a persistent seed bank (Eriksson 1989). *Knautia arvensis* seed banks have been classified as transient, although viable seeds have been found after 35 years in the soil (Thompson et al. 1997). Additional evidence for the possibility of across-year persistence was found in a seed bank study at the Mountain population, where *K. arvensis* was recorded once in the upper five cm soil layer (V.Vandvik, unpublished). The estimated half-years based on germination after cold storage suggest that *K. arvensis* seeds may potentially survive across seasons. This was supported in our two-year field experiment where a fraction of the seeds remained ungerminated across the first year, and germinated in the second year.

Combining field and laboratory experimental data we have shown that *K. arvensis* has a potential for a carry-over of seeds across years, and that this actually occurs in the field. Based on these results we suggest that *K. arvensis* has a short-term persistent seed bank (*sensu* Thompson et al. 1997).

Differences between populations

Between-population variation in germination traits has previously been shown for a variety of species over a range of spatial scales (e.g. Billings and Mooney 1968, Meyer and Monsen 1991, Schütz and Milberg 1997, Cavieres and Arroyo 2000). While such differences may be interpreted in terms of adaptations to different environmental conditions (Meyer et al. 1989), there has recently been considerable debate regarding sources of variation, genetic vs. environmental and seed vs. maternal (e.g. Roach and Wulff 1987, Andersson and Milberg 1998), and the potential consequences of this for the ecological interpretability of variation at larger scales (e.g. Schütz and Milberg 1997). Within-population variance in the germination characteristics of *K. arvensis* is discussed later (V. Vange unpublished); here we focus on variation at the between-population scale.

The four investigated populations differ in overall germinability, in germination timing, and in responses to environmental cues. As we have sampled one population within each region only, these results are interpreted as a set of case studies, rather than as data that represent general regional patterns.

Dormancy and germination along climatic gradients. An area that has received particular attention is the relationship between the cold-stratification requirements of populations and local climate. A number of studies have found that the overall effect of cold-stratification on germination, as well as the number of months of cold-stratification required, increases with the adversity of the winter conditions (Meyer et al. 1989, Meyer and Monsen 1991, Meyer et al. 1995, Cavieres and Arroyo 2001). It is argued that untimely germination during the cold season is more detrimental in harsh climates, and that mountain or northern populations therefore put more effort, in terms of adaptation, into avoiding it (Meyer et al. 1989). In our data, the North population had a cold stratification response more than two times stronger than the Mountain, East, and Central populations (2.7-fold increase vs. 1.4 to 1.8-fold, $p= 0.0534$, Fig. 3, Table 2c). Additionally, the Mountain population germinated relatively well at low temperatures, both in absolute numbers (12% germination vs. 4-6%) and relative to the germination at 20ºC (2.1-fold increase vs. 13 – 15.6-fold, Fig. 2, Table 2a). Overall, the germination responses that relate to seasonal timing

were strong in the North, weak in the Mountain, and intermediate in the East and Central populations. Dormancy levels and germination thresholds were thus highest under mild winter climates and lowest under cold winter climates (Table 1), opposing the trend described above. Previously, a few studies have reported similar results (Fowler and Dwight 1964, ter Borg 1987), as well as indifferent responses (Thompson 1975, Schütz and Milberg 1997), along winter climatic gradients.

These seemingly contradictory results can be reconciled if the germination strategies in seasonal climates are interpreted as responses to the predictability, rather than the severity, of the adverse season (cf. Vleeshouwers et al. 1995). In continental boreal regions, in our study represented by the Mountain population (Table 1, Fig. 1), winters are cold and snow cover is stable. Under such conditions the very good insulation and light reflection capacity of the snow pack leave the seeds facing a winter of relatively constant temperatures (typically ranging from -5 to 0ºC), low light, and 100% relative humidity (Marchand 1996). These are actually very good conditions for seed storage, and as long as germination at low temperature is prevented, very little effort, in terms of adaptation, is needed to carry the seeds through to the spring (Vleeshouwers et al. 1995). Predictably mild and moist winters (temperatures typically above 0ºC), as often are the case in temperate climates, may also be seen as relatively unproblematic. A number of species, with winter annuals as the prime example, even take advantage of the benign environmental conditions and low competition levels and stay active throughout the winter (e.g. Grime et al. 1981). Where winters are less predictable, such as is the case in oceanic parts of the boreal region (e.g. North population, Table 1, Fig. 1); different sets of seed traits may be adaptive. Here snow and ice are frequent, but are not constant throughout the cold season. Consequently, temperature, light, and moisture conditions at the ground are much more variable, with temperatures typically spanning more than 10 degrees within days or weeks. At the same time mean differences between summer and winter temperatures are small, making precise cueing of germination difficult. A cold stratification requirement and/or very slow germination at low temperature may safeguard seedlings against germination during winter warm spells, followed by certain mortality during later frosts.

The net effect of dormancy and high germination thresholds may then be to avoid germination under temporarily benign conditions during the adverse season (Vleeshouwers et al. 1995). Such germination syndromes could be expected to behave as bet-hedging strategies (Philippi 1993a). Bet-hedging theory predicts that dormancy fractions should increase as the environment becomes less predictable (Venable and Brown 1988, Philippi 1993a). This was observed in the cold-stratification and temperature responses of *K. arvensis*. Further, the results from the storage experiment are also in accordance with this prediction, as seed survival was lower in the populations with the coldest and most stable winter climates (Mountain and East populations).

Conclusions

Concerning Eriksson's (1989) predictions for clonal species with 'repeated' germination, the first prediction is confirmed for *Knautia arvensis*: Seedlings are competitive, as seed germination and seedling survival in the field were not affected by the presence or height of the established vegetation. Further, Eriksson (1989) predicts that dormancy should be absent in these species. Although germination occurs under all experimental treatments in the phytotron; the quantitative differences in germination between stratified and unstratified seeds, and between high and low temperatures, are considerable. Environmental cueing and dormancy (i.e. temperature and cold-stratification effects) have additive effects in germination in *K. arvensis,* and their combined effect will be near zero germination during autumn and early spring. This is a functional equivalence to dormancy. The field experiment confirms this interpretation, as seed carry-over across years is demonstrated in *K. arvensis*. Finally, Eriksson (1989) predicts that clonal species with 'repeated' germination should also have high prospects for evolution of local adaptions. This was demonstrated for *K. arvensis*, as we found substantial between-population variation in germination characteristics. These differences were interpreted as adaptions to gradients in winter climate.

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References

Amiaud, B., Bonis, A. and Bouzillé, J-B. 2000. Conditions de germination et rôle des herbivores dans la dispersion et le recrutement d'une espèce clonale: *Juncus gerardi* Lois. Can. J. Bot. 78: 1430-1439.

Andersson, L. and Milberg, P. 1998. Variation in seed dormancy among mother plants, population,

and year of seed collection. Seed Sci. Res. 8: 29-38.

Baskin, C. C. and Baskin, J. M. 1988. Germination ecophysiology of herbaceous plant species in a temperate region. Am. J. Bot. 75: 286-305.

Baskin, C. C. and Baskin, J. M. 1998. Seeds. Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, California.

Batlla, B. C., Kruk, C. and Benech-Arnold, R. L. 2000. Very early detection of canopy presence by seeds through perception of subtle modifications in red:far red signals. Funct. Ecol. 14: 195-200.

Bell, D. T., King, L. A. and Plummer, J. A. 1999. Ecophysiological effects of light quality and nitrate on seed germination in species from Western Australia. Austr. J. Ecol. 24: 2-10.

Bell, D. T., Rokich, D. P., McChesney, C. J. and Plummer, J. A. 1995. Effects of temperature, light, and gibberellic acid on the germination of seeds of 43 species native to Western Australia. J. Veg. Sci. 6: 797-806.

Billings, W. D. and Mooney, H. A. 1968. The ecology of arctic and alpine plants. Biol. Rev. 43: 481-529.

ter Borg, S. J. 1987. Qualitative and quantitative aspects of the interaction between *Rhinanthus* and *Orobanche* species and their hosts. In: Parasitic Flowering Plants, ed. Weber, H. C. and Forstreuter, W. Philipps-Universität, Marburg, pp. 109-120.

Cavieres, L. A. and Arroyo, M. T. 2000. Seed germination response to cold stratification period and thermal regime in *Phacelia secunda* (Hydrophyllaceae). Plant Ecol. 149: 1-8.

Cavieres, L. A. and Arroyo, M. T. 2001. Persistent soil seed banks in *Phacelia secunda* (Hydrophyllaceae): experimental detection of variation along an altitudinal gradient in the Andes of central Chile (33ºS). J. Ecol. 89: 31-39.

Cousens, R. and Mortimer, M. 1995. Dynamics of weed populations. Cambridge University Press, Cambridge.

Eriksson, O. 1989. Seedling dynamics and life histories in clonal plants. Oikos 55: 231-238.

Eriksson, O. 1999. Seed size variation and its effect on germination and seedling performance in the clonal herb *Convallaria majalis*. Acta Oecol. 20: 61-66.

Eriksson, O. and Fröborg, H. 1996. 'Windows of opportunity' for recruitment in long-lived clonal plants: experimental studies of seedling establishment in *Vaccinium* shrubs. Can. J. Bot. 74: 1369-1374.

Fowler, D. P. and Dwight, T. W. 1964. Provenance differences in the stratification requirements of white pine. Can. J. Bot. 42: 669-675.

Grime, J. P., Mason, G., Curtis, A. V., Rodman, J., Band, S. R., Mowforth, M. A. G., Neal, A. M. and Shaw, S. 1981. A comparative study of germination characteristics in a local flora. J. Ecol. 69: 1017-1059.

Grubb, P. J. 1977. The maintenance of species-richness in plant communities: the importance of the regeneration niche. Biol. Rev. 52: 107-145.

Harper, J. L. 1977. Population biology of plants. Academic press, London.

Hultén, E. and Fries, M. 1986. Atlas of North European vascular plants. Koeltz Scientific Books, Königstein.

Karssen, C. M. and Hilhorst, H. W. M. 1992. Effect of Chemical Environment on Seed Germination. In: Seeds – The ecology of regeneration in plant communities, ed. Fenner, M. CAB International, Wallingford, pp. 327-348.

Kotorová, I. and Lepš, J. 1999. Comparative ecology of seedling recruitment in an oligotrophic wet meadow. J. Veg. Sci. 10: 175-186.

Lid, J. and Lid, D. T. 1994. Norsk flora, 6th edn. by R. Elven. Det norske samlaget, Oslo.

Mandák, B. and Pyšek, P. 2001. The effects of light quality, nitrate concentration and presence of bracteoles on germination of different fruit types in the heterocarpous *Atriplex sagittata*. J. Ecol. 89: 149-158.

Marchand, P. J. 1996. Life in the cold. An introduction to Winter Ecology, 3rd ed. University Press of New England. Hanover and London.

Masuda, M. and Washitani, I. 1990. A comparative ecology of the seasonal schedules for 'reproduction by seeds' in a moist tall grassland community. Funct. Ecol. 4: 169-182.

Meyer, S. E. and Monsen, S. B. 1991. Habitat-correlated variation in mountain big sagebush (*Artemisia tridentata* ssp. *vaseyana*) seed germination patterns. Ecology 72: 739-742.

Meyer, S. E., Kitchen, S. G. and Carlson, S. L. 1995. Seed germination timing patterns in intermountain *Penstemon* (Scrophulariaceae). Am. J. Bot. 82: 377-389.

Meyer, S. E., McArthur, E. D. and Jorgensen, G. L. 1989. Variation in germination response to temperature in rubber rabbitbush (*Chrysothamnus nauseosus*: Asteraceae) and its ecological implications. Am. J. Bot. 76: 981-991.

Milberg, P. and Andersson, L. 1998. Does cold stratification level out differences in seed germinability between populations? Plant Ecol. 134: 225-234.

Mossberg, B., Stenberg, L. and Ericsson, S. 1992. Den Nordiska Floran. Wahlström and Widstrand, Stockholm.

Murdoch, A. J. and Ellis, R. H. 1992. Longevity, Viability and Dormancy. In: Seeds – The ecology of regeneration in plant communities. ed. Fenner, M. CAB International, Wallingford, pp. 193-229.

Olff, H., Pegtel, D. M., van Groenendael, J. M. and Bakker, J. P. 1994. Germination strategies during grassland succession. J. Ecol. 82: 69-77.

Pérez-Fernández, M. A., Lamont, B. B., Marwick, A. L. and Lamont, W. W. 2000. Germination of seven exotic and seven native species in south-western Australia under steady and fluctuating water supply. Acta Oecol. 21: 323-336.

Philippi, T. 1993a. Bet-hedging germination of desert annuals: beyond the first year. Am. Nat. 142: 474-487.

Philippi, T. 1993b. Bet-hedging germination of desert annuals: variation among populations and maternal effects in *Lepidum lasiocarpum*. Am. Nat. 142: 488-507.

Rice, K. J. 1985. Responses of *Erodium* to varying microsites: the role of germination cueing. Ecology 66: 1651-1657.

Roach, D. A. and Wulff, R. D. 1987. Maternal effects in plants. Ann. Rev. Ecol. Syst. 18: 209-235.

SAS Institute. 1999. The SAS System Version 8. SAS Institute Inc., Cary, NY.

Schütz, W. 1997. Are germination strategies important for the ability of cespitose wetland sedges (*Carex*) to grow in forests? Can. J. Bot. 75: 1692-1699.

Schütz, W. and Milberg, P. 1997. Seed dormancy in *Carex canescens*: regional differences and ecological consequences. Oikos 78: 420-428.

Sokal, R. R. and Rohlf, F. J. 1995. Biometry, 3rd edn. Freeman, New York.

SPSS Inc. 1999. SPSS for Windows Version 10.0. SPSS Inc., Chicago.

Thompson, K. and Grime, J. P. 1979. Seasonal variation in the seed bank of herbaceous species in ten contrasting habitats. J. Ecol. 67: 893-921.

Thompson, K., Bakker, J. and Bekker, R. 1997. The soil seed banks of North West Europe: methodology, density, and longevity. Cambridge University Press, Cambridge.

Thompson, P. A. 1975. Characterisation of the germination response of *Silene dioica* (L.) Clairv. populations from Europe. Ann. Bot. 39: 1-19.

Toyomasu, T., Kawaide, H., Mitsuhashi, W., Inoue, Y. and Kamiya, Y. 1998. Phytochrome Regulates Gibberellin Biosynthesis during Germination of Photoblastic Lettuce seeds. Plant Physiol. 118: 1517-1523.

Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M. and Webb, D.A. 1976. Flora Europaea, vol. 4. Cambridge University Press, Cambridge.

Venable, D. L. and Brown, J. S. 1988. The selective interactions for dispersal, dormancy and seed size as adaptations for reducing risk in variable environments. Am. Nat. 131: 360-384.

Vleeshouwers, L. M., Bouwmeester, H. J. and Karssen, C. M. 1995. Redefining seed dormancy: an attempt to integrate physiology and ecology. J. Ecol. 83: 1031-1037.

Washitani, I. and Masuda, M. 1990. A comparative study of the germination characteristics of seeds from a moist tall grassland community. Funct. Ecol. 4: 543-557.