Germination ecology of the grassland biennial *Linum catharticum*

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SUMMARY

The germination ecology of the short-lived grassland biennial *Linum catharticum* L. was studied. Its seeds had a primary dormancy that was overcome by cold-treatment. Seeds buried in the soil became germinative in midwinter (December), but since the seeds germinate only at relatively high temperatures, autumn-sown seeds did not germinate in the field until late spring, at which time the average soil temperature was 20°C. During the first half of the summer, buried seeds re-entered dormancy, but by August this secondary dormancy had already been broken. In the following summer some of the seeds became dormant again. Hence, buried *L. catharticum* seeds exhibited an annual dormancy cycle. Most exhumed seeds needed light to germinate, which is probably why *L. catharticum* accumulates a persistent soil seed bank. After 25 months, 96% of the seeds buried were still ungerminated and alive.

Key-words: biennial, germination, grassland, *Linum catharticum,* seed dormancy cycle, short-lived, Sweden.

INTRODUCTION

Species differ in their ability to accumulate seeds in a persistent soil seed bank, and the germination behaviour and shape and size of the seeds are likely to be the main determinants of these differences (Baskin & Baskin 1989; Thompson *et al.* 1993). Short-lived plants have to establish from seeds regularly to maintain populations and, for such species, knowledge of seed bank and germination is important to understand the biology and to predict population changes under different management.

The European, short-lived forb *Linum catharticum* L. (Linaceae) appears to be a strict biennial (Kelly 1985) in Sweden and occurs in a variety of habitats, e.g. species-rich grasslands, fens and road verges. The seeds are small (0·15–0·19 mg) and have no structures to enhance seed dispersal by wind or animals.

Seeds of *L. catharticum* possess a strict primary dormancy (van Tooren & Pons 1988; Pons 1991) and can accumulate in persistent soil seed banks (Donelan & Thompson 1980; Ryser & Gigon 1985; Roberts 1986; Jefferson & Usher 1987; Kelly 1989; Pons 1991; Milberg & Hansson 1994). Further, seedling emergence is almost completely restricted to early spring (Bradshaw & Doody 1978; Keizer *et al.* 1985; Roberts 1986; van Tooren & Pons 1988; Kelly 1989; Pons 1991; Ryser 1993). By conducting a number of germination experiments, I wanted to (i) describe how seed dormancy changes under

field conditions, (ii) explain why seeds accumulate in the soil, and (iii) explain why seedling emergence is so restricted in time.

I did germination experiments in light and darkness with exhumed seeds that had been buried in soil for up to 25 months. Experiments in an incubator (temperature-gradient bar) established optimum temperature regimes for germination. I also sowed seeds to record the emergence of seedlings in the spring and related this event to soil temperatures at seed depth.

METHODS

Seeds were collected from a road verge grassland at Örnslid on Omberg in Östergötland, southern Sweden, on 5 August 1991. Seeds collected were used in a burial experiment, two incubator experiments and a sowing experiment.

Burial experiment

After 1 month dry storage (20°C), 200 seeds were placed in each of 50 fine-mesh polyester bags (5 cm × 5 cm). Two bags were put in each of 25 ceramic pots (12 cm diameter) with peat soil (70% *Sphagnum*-peat, 30% sand). The pots were buried at *c.* 15 cm depth in a wooden box (120 cm × 80 cm and 30 cm high) filled with peat soil. The box was placed on a pallet on the ground in a net-enclosure, outdoors at the Swedish University of Agricultural Sciences campus, south of Uppsala. The box was sheltered from heavy snowfall during winter and watered occasionally during dry, summer periods.

Once a month, from 1 October 1991 to 1 October 1993, one pot (two bags) was exhumed. In complete darkness, the contents of one bag were distributed in a dish which was then wrapped in aluminium foil. Before being placed to germinate in light, the contents of the second bag were inspected in search of seeds that had germinated while buried.

Germination experiments with the exhumed seeds, as well as with fresh seeds and seeds dry-stored (20°C) for 4 months, were conducted in a room at 20°C for 16 h of light (minimum of photosynthetically active light was 4 µmol m^{-2} s^{-1}; ratio 660/730 nm was *c.* 12) and 8°C for 8 h of darkness. The seeds were put on filter paper (Munktell 1701) wetted with distilled water and placed in plastic dishes (18 cm diameter). Each dish was covered with a glass lid. In light, germination had ceased after *c.* 15 days, and seed germination recording was terminated after 30 days. Seeds that were not overgrown with fungi and were not deformed by light pressure from a pair of tweezers were considered alive.

Incubator experiments

Two experiments were made in a temperature-gradient apparatus (Ekstam & Bengtsson 1993) with seeds that had been buried in peat soil at 15 cm depth (as described above). The intention was to establish the optimum, constant temperature for germination, and to test if daily temperature fluctuations stimulated germination.

In the first experiment, seeds buried for 4 months were subjected to one of 10 constant temperatures (5·0, 7·8, 10·6, 13·3, 16·1, 18·9, 21·7, 24·4, 27·2 & 30·0°C). In the second experiment, the influence of diurnal fluctuations in temperature on germination was investigated with seeds from the same seed batch (during the course of the first experiment they had been stored for 3 weeks on wet filter paper in darkness at 2°C). The

daily mean temperature was kept at 15·0°C while the fluctuation amplitude was varied, resulting in 10 different amplitudes (0·0, 3·3, 6·7, 10·0, 13·3, 16·7, 20·0, 23·3, 26·7 & 30·0°C). The highest and lowest temperature was maintained for 2 h, respectively, within 24 h, while the temperature slowly increased or decreased there between. A photoperiod of 12 h light (photosynthetically active light: 5–7 μmol m^{-2} s^{-1}; ratio 660/730 nm was 7) and 12 h darkness was maintained throughout the experiments. Light was coincided with high temperature. In both experiments 3 × 100 seeds were subjected to each temperature regime.

Germinated seeds (radicle emergence) were counted and removed daily for 21 days, by which time germination had ceased. For each temperature regime, the final germination percentage and median germination time, i.e. number of days to reach 50% of final germination, were calculated.

Sowing experiment

In November 1991, 4 months after seed collection, 10 batches of 50 dry-stored (20°C) seeds were sown at 2–3 mm depth in a wooden box (120 cm × 80 cm and 30 cm high) filled with sandy soil. The box was placed on the ground in a net-enclosure outdoors at the University campus south of Uppsala. The soil surface was kept moist and was watered up to once a day during warm, dry periods in the summer. Emergence was registered every week during the first and second growing season (until October 1993), and the seedlings were removed after each count.

Soil temperature at seed depth (2–3 mm) was monitored from 16 March to 18 August 1992, and the daily average temperature was calculated from hourly registrations.

RESULTS

Fresh seeds did not germinate in light, nor did seeds subjected to 4 months of dry storage at 20°C.

Burial experiment

The exhumed seeds were germinative in their first winter from December to May but became dormant again in the summer. In August this dormancy was partly broken and complete germination was recorded in the second winter from November to April. In the second summer germination decreased, but complete dormancy was not achieved (Fig. 1).

The proportion of living seeds recovered did not decrease during the experiment and varied between 91 and 100% of those buried. After 25 months in the soil, in October 1993, 96% of the seeds were still alive.

Few seeds germinated in darkness (61 out of 4168 tested), and this happened when germination in light was at maximum (Fig. 1).

Incubator experiments

The final germination percentages in seeds kept at a constant temperature, were highest at 16–27°C (Fig. 2). The seeds germinated fastest at temperatures of 18·9°C and higher.

Diurnal temperature fluctuations of amplitudes less than 15°C increased the germination percentage (Fig. 3). The time required to reach 50% of final germination was not affected by amplitude.
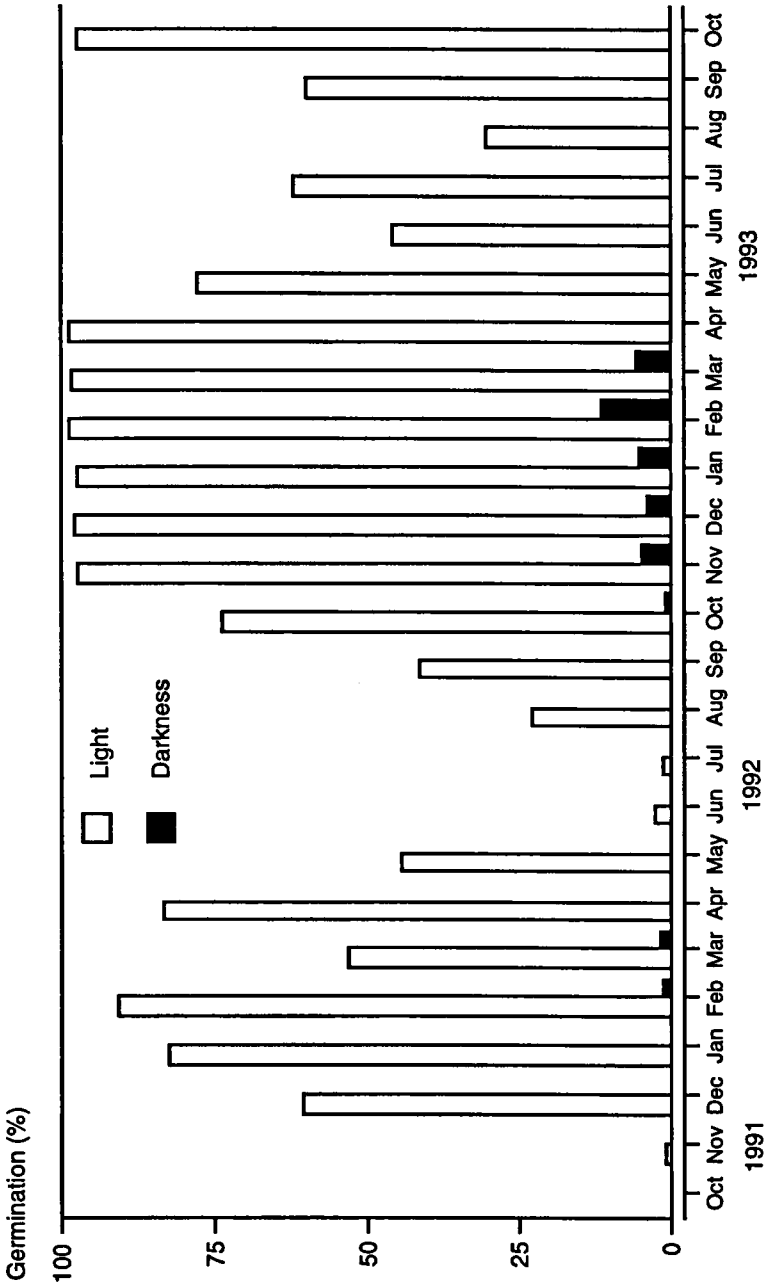
Fig. 1. Temporal variation in percentage germination for exhumed seeds of *Linum catharticum* buried in August 1991: *c.* 200 seeds were used in each germination experiment in light and darkness.
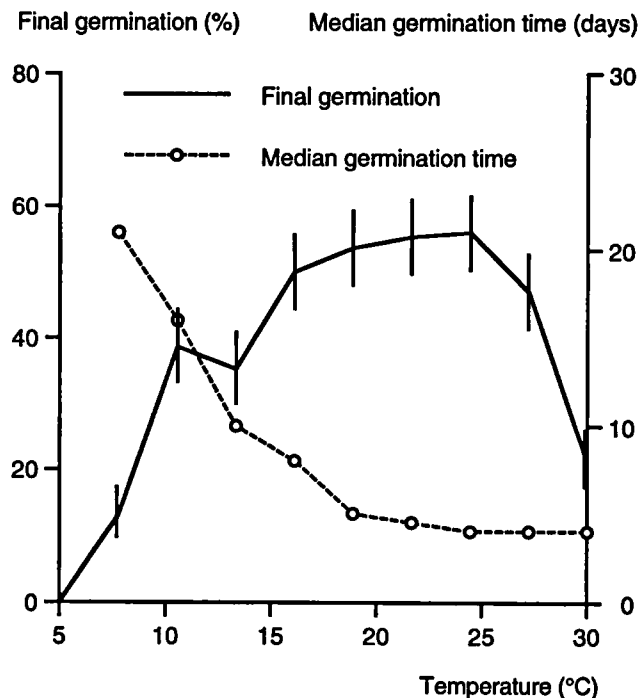
Fig. 2. Final germination and median germination time of *Linum catharticum* at different constant temperatures. Median germination time measured as days to reach 50% of final germination. Bars indicate 95% confidence intervals, assuming a binomial distribution ($n=300$).

Sowing experiment

Seedling emergence peaked in late May, at which time the daily mean soil temperature was 20°C (Fig. 4). In the second growing season after sowing, no seedlings were recorded. Of the seeds sown, 6·6% produced seedlings.

DISCUSSION

Seed dormancy

This study has shown that seeds of *Linum catharticum* exhibit an annual dormancy cycle. Primary and secondary dormancy is probably broken by a cold-treatment (in the winter and autumn, respectively) while secondary dormancy probably is induced by high temperatures (in the early summer). The existence of an annual dormancy cycle can explain why seeds of *L. catharticum* germinated almost exclusively after periods of cold (winter) storage in a number of seed bank studies (Ryser & Gigon 1985; Kelly 1989; Milberg 1993). Similar dormancy patterns in spring-germinating species have been demonstrated for several annuals (e.g. Courtney 1968; Baskin & Baskin 1980; Håkansson 1983) and perennials (e.g. Baskin & Baskin 1981; Baskin *et al.* 1989; Pons 1991; Meyer & Kitchen 1992; Milberg 1994) and seem to be widespread among species in temperate regions (Baskin & Baskin 1985, 1988, 1989). Thus, if soil samples are taken in the summer for a seed bank assessment, there is a risk that spring-germinating species with a seed dormancy cycle will not be detected

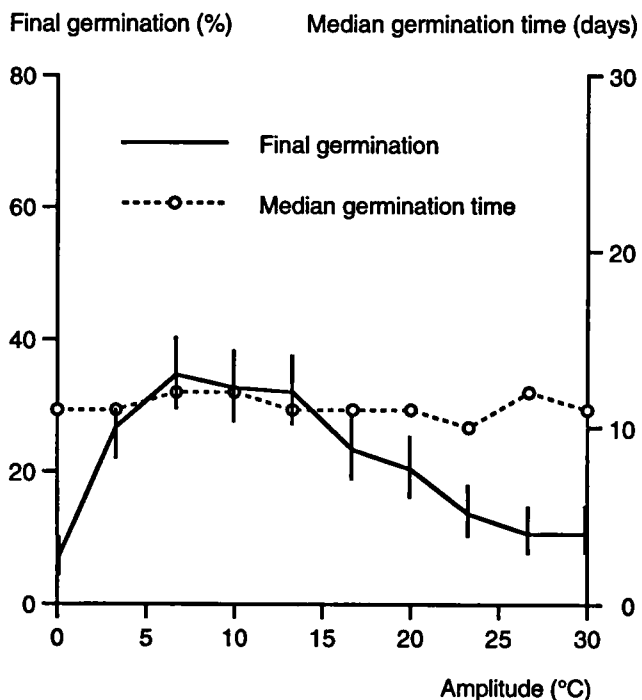Final germination (%)   Median germination time (days)

Fig. 3. Final germination and median germination time of *Linum catharticum* at diurnally alternating temperatures of different amplitude. Daily mean temperature was 15°C at all amplitudes. Median germination time measured as days to reach 50% of final germination. Bars indicate 95% confidence intervals, assuming a binomial distribution ($n = 300$).

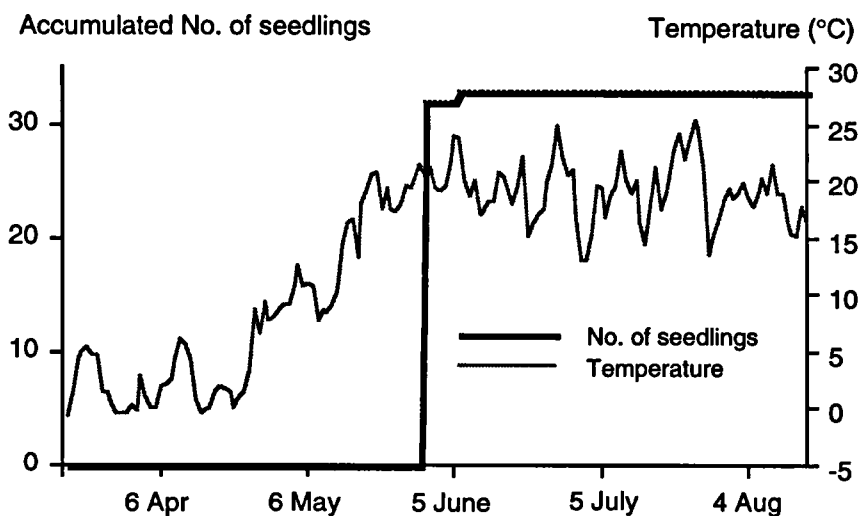Accumulated No. of seedlings   Temperature (°C)

Fig. 4. Seasonal variation in the emergence of autumn-sown *Linum catharticum* and daily mean soil temperature at sowing depth. A total of 500 seeds were sown in November. No seedlings were recorded in the second growing period after sowing.

in a single germination test. Hence, to determine the total species content of the seed bank by counting emerging seedlings, it is vital that the seeds in the soil be subjected to a cold-treatment.

It is noteworthy that the period of almost complete secondary dormancy in the second summer was only 2 months long. Pons (1991) showed that in *L. catharticum* the period of cold-treatment required to break secondary dormancy was much shorter than that needed to break primary dormancy. Hence, seeds differ in their autumn germinability depending on their age.

This study documented differences in dormancy between years: secondary dormancy was less pronounced in the second summer than in the first and seeds germinated slightly better in darkness in the second winter than in the first (Fig. 1). However, it is not possible to clarify if these changes are due to difference in temperature regime between years or if it is an inherent levelling-out of seed dormancy with increasing seed age.

Since *Linum catharticum* seeds require a rather high temperature for germination and are not particularly stimulated by temperature fluctuations, the soil temperature may already be low enough to prevent autumn germination by the end of August. *L. catharticum* generally germinates only in the spring (Fig. 4, Bradshaw & Doody 1978; Keizer *et al.* 1985; van Tooren & Pons 1988; Kelly 1989; Ryser 1993), but Roberts (1986) recorded a few seedlings emerging after soil disturbance in September and October in a British study. The findings that the buried seeds in the present study had become germinative again by August and that secondary dormancy was not complete in the summer, suggest that some emergence in summer or autumn could be expected in Sweden as well. However, seedlings will be recruited only from seeds on or close to the soil surface and the dormancy changes at 15 cm soil depth, described here, might not be representative of those of seeds near the surface. Soil temperatures are higher near the surface (Rodskjer *et al.* 1989) and, since secondary dormancy is probably induced by high temperatures, seeds there are more likely to be dormant in the summer.

Seed bank

Linum catharticum has been found to form a persistent seed bank in several studies (Donelan & Thompson 1980; Ryser & Gigon 1985; Roberts 1986; Jefferson & Usher 1987; Kelly 1989; Milberg & Hansson 1994). However, Schenkeveld & Verkaar (1984) reported a transient seed bank for *L. catharticum*, but this could result from seeds being strictly dormant in soil samples taken in the summer, as discussed above. The build-up of a persistent soil seed bank is probably facilitated by the requirement for light for the germination of buried seeds (Fig. 1, van Tooren & Pons 1988). Hence, once the seeds have entered the soil, very little germination will occur until a soil disturbance allows light to reach the seeds, or the seeds are brought back to the surface by, e.g. earthworms.

Field emergence

Even though seeds of *Linum catharticum* are germinative during a large part of the year, seedling emergence in the field was restricted to a short period in the end of May (Fig. 4). Similar abrupt emergence of *L. catharticum* seedlings has previously been noted by Bradshaw & Doody (1978) and Kelly (1989). In the present study, seedling emergence coincided with a rise in the soil temperature to about 20°C. Although seeds germinated at lower temperatures in the incubator, germination proceeded slowly. Therefore,

low-temperature-germination would be difficult to detect in the field in May, when soil temperatures rise steadily from 5 to 20°C.

It is surprising that field emergence of *L. catharticum* started at a time when buried seeds were beginning to acquire secondary dormancy (cf. Fig. 1). The high temperature requirement for germination probably prevents earlier emergence, and the increasing soil temperature in the spring induces secondary dormancy. In combination, this would restrict seedling emergence in the field to a very short period.

Kelly (1989) suggested, from British and Dutch data showing consistency in seedling emergence date between years, that the germination of *Linum catharticum* was governed by daylength. However, the daylength during seedling emergence in Sweden (end of May) is very much longer than during seedling emergence in Britain or The Netherlands (March/April). If photoperiod is involved in the timing of seedling emergence, populations from different latitudes would have to react differently to the same photoperiod.

Few seedlings emerged compared to the number of seeds sown. Since *L. catharticum* require light to germinate, it is possible that light levels at the sowing depth used (2–3 mm) were too low to stimulate germination (Bliss & Smith 1985; Kasperbauer & Hunt 1988).

In conclusion, soil temperature has a dual role in regulating the germination of *Linum catharticum*. The seed dormancy cycle is regulated by temperature and hence, germination during the summer is indirectly inhibited. The actual *timing* of germination is probably directly determined by the soil temperature in the spring.

ACKNOWLEDGEMENTS

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