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Seed dormancy and germination in the summer annual *Galeopsis speciosa*

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Running title: Dormancy and germination in *Galeopsis speciosa*

4 Figures
3 Tables
Ca. 5400 Words
Summary

This study examined germination and dormancy in *Galeopsis speciosa* (Lamiaceae), a common summer annual weed in cold-temperate areas. Seeds collected in southern Sweden were subjected to several experiments. The seeds were dormant at maturity. Seeds sown outdoors after collection produced a small number of seedlings, that emerged early in the spring. After long cold stratification or stratification outdoors over two winters, the maximum germination was 40 to 50%; germination occurring over a wide range of temperatures. Warm stratification preceding cold stratification had no effect on germination, but repeated warm and cold periods seemed to promote germination. Gibberellic acid (GA) stimulated germination but full germination was only achieved after more than two months of incubation at the most suitable temperature regime tested. Excised embryos grew and developed into normal seedlings. With these results, the species does not fit into the currently used system for seed dormancy classifications. The response to GA and the growth of excised embryos indicate non-deep or intermediate physiological dormancy, but dormancy alleviation by stratification was not in line with the guiding principles for these classifications. *Galeopsis speciosa* has a strong dormancy that is sufficiently alleviated during the winter to allow germination of only part of a seed batch each year, hence a stepwise germination pattern occurs over a period of several years.

Keywords: Lamiaceae, large-flowered hemp-nettle, physiological dormancy, Sweden, weed.

Introduction

Three common summer annual weeds of the genus *Galeopsis* (Lamiaceae) occur in crops in Scandinavia: *G. tetrahit* L., *G. bifida* Boenn. and *G. speciosa* Miller. They occur mainly in spring-sown crops, but may some years be abundant also in autumn-sown crops (Milberg et al., 2000). The three species are difficult to distinguish when not flowering and have therefore rarely been treated separately in Scandinavian weed research. Nevertheless, *Galeopsis speciosa* is widespread on arable land in Sweden and is locally the most abundant of the three (personal observation). The three *Galeopsis* species are competitive weeds (Simpson & Carnegie, 1989; Weaver & Ivany, 1998; Boström et al., 2003; Milberg & Hallgren, 2004), and are especially abundant on organogenic and sandy soils (Erviö et al., 1994; Hallgren et
al., 1999). At least one of them, *Galeopsis tetrahit*, has been introduced to, and become an important weed in, cold-temperate parts of North America (O’Donovan & Sharma, 1987).

The germination rate from fresh *G. speciosa* seed is often low or negligible but increases after cold stratification (Milberg & Andersson, 1998). The emergence of *G. speciosa* in fields occurs between March and May in Sweden (Håkansson, 1983; 1992). Taken together, this would suggest that *G. speciosa* is a typical summer annual species, and that is is likely to have non-deep physiological dormancy where the seasonal pattern of emergence is determined mainly by dormancy cycles (Baskin & Baskin, 1985). However, unpublished observations (Per Milberg & Lars Andersson) indicate that *G. speciosa* germinates at low soil temperatures, as well as in darkness, and that the species seems to require a long stratification treatment. These observations contradict the proposed dormancy classification, and would suggest intermediate or deep physiological dormancy as being more likely.

The purpose of this study was to examine the germination biology of *Galeopsis speciosa* and more specifically to evaluate its dormancy type, under what conditions dormancy is broken and the temporal pattern of seedling emergence.

**Materials and methods**

**Seed collection**

All sites used for seed collection were in Östergötland, southern Sweden, and the collections were done in 2001 and 2003. Seeds were collected at Dagsmosse (N58°19' E14°42') on the 9th August, 2001 on the fringe of an arable field, along a small gravelled road and on a fallow field. On the 14th August, 2001 seeds were collected 10 km northeast of Kisa (N58°2' E15°47') along a small road with fallow fields on both sides. On the 10th August, 2003, seeds were collected at Gävbo (N58°17' E15°20') on the edge of a wheat field and at Älvan (N58°28' E15°17') in a corner of a barley field. Seeds were collected when natural dispersal occurred and only seeds that fell off carefully-shaken plants were used. Thus, the seeds collected were fully matured, and representative for newly dispersed seeds of the species. The seeds were then kept indoors at ca 20°C for about one week before experiments commenced. Each seed batch was mixed several times before the start of experiments.
**Common procedures**

Temperature regimes used were 15/5, 20/10, 25/15 and 30/20°C (10 h at each temperature with two 2 h transitions between them) in germination incubators (Rubarth Apparatebau, Laatzen, Germany). The higher temperature coincided with light (12 h). Constant temperatures used were 0, 4.5 and 5°C; 0 and 5°C with light for 12 h d⁻¹. Light was provided by fluorescent tubes (22-42 µmol m⁻² s⁻¹, R/FR ratio ≥ 2.6); this treatment is hereafter referred to as “light”. Petri dishes (diameter 52 mm) sealed with parafilm were used, and dishes subjected to continuous darkness ("darkness") were wrapped in aluminium foil.

All experiments were started about one week after seed collection. During tests, seeds were regarded as germinated at radicle protrusion. Apparently dead seeds (i.e. soft and/or overgrown with mould) were excluded from germination calculations. Data were subjected to angular transformation before analysis of variance was performed.

**Seeds collected in 2001**

In all experiments, expect sowing outdoors, involving seeds collected in 2001 (Table 1), 30 and 40 seeds per dish were used of the Dagsmosse and Kisa seeds batches, respectively. Each test was done with three petri dishes prepared with two pieces of filter paper (Munktell 1003, 52 mm), moistened with 2.25 ml of de-ionized water. For experiments involving a pre-treatment followed by a test (experiment B, C, D; Table 1), final counting of seedlings were done after three weeks of incubation in the test environment.

**Seeds collected in 2003**

In all experiments, expect sowing outdoors, involving seeds collected in 2003 (Table 1) 10 mL quartz sand (Baskarpsand 35, AB Baskarpsand, Habo, Sweden) moistened with 4 mL de-ionized water (or GA solution) was used per petri dish. This was preferred to filter paper as it allows longer periods of incubation by lessening the risk for the substrate drying out. To each dish, 40 seeds were placed on the surface of the sand, and three dishes were used in all experiment except storage outdoors (C) when two dishes were used. For experiment C, storage outdoors, that involved a pre-treatment followed by a test, final counting of seedlings were done after six weeks of incubation in the test temperature regime.
Table 1 Overview of the seven experiments conducted and the seed batches used in them.

<table>
<thead>
<tr>
<th></th>
<th>2001</th>
<th>2001</th>
<th>2003</th>
<th>2003</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dagsmosse</td>
<td>Kisa</td>
<td>Gävbo</td>
<td>Älvan</td>
</tr>
<tr>
<td>A: Continuous incubation</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>B: Cold stratification in a refrigerator</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C: Storage outdoors</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>D: Effect of alternating cold and warm periods</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>E: Sowing on the soil surface of pots outdoors</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>F: Tetrazolium tests</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>G: Gibberellic acid experiment</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>H: Growth of excised embryos</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

The experiments

We conducted eight different experiments (Table 1).

Continuous incubation (A)

Seeds from the 2001 seed batches were incubated at 15/5, 20/10 and 25/15°C, in light and in darkness, for eight months. The dishes in light were checked regularly for germinated seeds and the ones in darkness were opened when the experiment was terminated after eight months. Seeds from the 2003 seed batches were placed in light at a constant 5°C and under 15/5, 20/10, 25/15 and 30/20°C regimes for 24 months. Seeds were checked every second to fourth week for germination, and apparently dead seeds were removed.

Cold stratification in refrigerator (B)

Seeds from the 2001 seed batches were stratified in a refrigerator (4.5 ± 0.6°C), in darkness. At three to four-week intervals, dishes were distributed among the incubators. The experiment continued for 34 weeks. Temperatures used for incubation were 15/5, 20/10 or 25/15°C, and both light and darkness treatments were included.
Storage outdoors (C)

The Kisa seed batch and the two seed batches collected in 2003 were involved in this treatment. Dishes with imbibed seeds (as described above) were put in darkness outside (7 km northwest of Linköping), but were protected from rain and direct sunshine. Temperature in the environment was recorded five times per day (Tinytag12 temp logger, Intab, Sweden). Seeds were tested for germination in light and in darkness at 15/5, 20/10 and 25/15°C for the Kisa seed batch and in light at 5, 15/5 and 20/10°C for the 2003 seed batches. Germination was tested 12 times, including one winter for the Kisa seeds and two winters for the 2003 seeds. With the Kisa seeds, germination tests in the incubators began in the early autumn (beginning of September), three weeks after the experiment commenced. Test were then made every third week for 33 weeks, i.e. throughout the winter and until spring (middle of April). For the two batches collected 2003, the first germination tests in the incubators were made in the first winter (middle of January), then in the early spring (middle of March) and then in the middle of each month until July. The tests in the second year were done five times; from the middle of winter until the end of spring (middle of January and February, end of Mars, April and May).

Incubation in alternating cold and warm temperatures (D)

To evaluate the effect of warm-preceding-cold stratification, seeds from the Kisa seed batch (2001) were stratified in darkness at 23.6 ± 1.2°C for 0, 3, 6 or 9 weeks, transferred to a refrigerator for 12 weeks at 4.5 ± 0.6°C, and tested for germination in light and in darkness at 15/5, 20/10 and 25/15°C.

The 2003 seed batches were used to test for a possible effect of repeated cold and warm periods. The treatments involved cold (C; 0°C) temperatures for 8 or 16 weeks, with 8 weeks of warm (W; 20/10°C) temperature between; all incubation was in light. The experiment was done with three temperature patterns: CWCWC.... WCWCW.... and CCWCCW...., each letter representing a period of eight weeks. A fourth treatment included 0°C for 88 weeks and a final eight-week period at 20/10°C. All treatments continued for 96 weeks.

Sowing on a soil surface of pots outdoors (E)

Seeds from each seed batch were sown outdoors, on the surface of commercial peat soil, in pots (diameter 90 mm, height 70 mm), about one week after seed collection. Fifty seeds were
sown in each pot; three and five pots were used in the 2001 and 2003 seed batches, respectively. The pots were stored under a table on the north side of a greenhouse (sheltered from direct sun and precipitation), 7 km northwest of Linköping. The pots were checked weekly or biweekly and emerged seedlings were counted and removed. All pots were kept until the end of May 2005.

Tetrazolium tests (F)

At the end of experiments, the seeds collected in 2003 were tested for survival with 1% 2,3,5-Triphenyltetrazoliumchlorid (Merck, Germany) solution. Tests were performed by scarification of seeds followed by incubation in the tetrazolium solution at 30°C for 8h (following Ellis et al. 1985). From all treatments, with the exception of GA, about one third of the seeds that remained ungerminated and were judged to be viable when terminating an experiment, were tested.

Gibberellic acid (G)

Fresh seeds from the seed batches collected in 2003 were incubated in gibberellic acid solution, GA, (1000 mg L⁻¹ GA₃, Sigma Chemical, USA) in light at 5 and 20/10°C. Both sand (10 ml sand, 4 ml GA) and filter paper (two pieces of paper, 2.25 ml GA) was used as substrate. Controls of this test were handled in the same way, but de-ionized water was used instead of the GA solution. The experiment continued for 96 weeks. If needed, de-ionized water was added to the dishes during experiment.

Growth of excised embryos (H)

Five embryos each of the Gävbo and Älvan seed batches were carefully excised, taking extra care to remove all the endosperm. The embryos were placed on moist, quartz sand (Baskarpsand 35, AB Baskarpsand, Habo, Sweden) at 20/10°C. After 14 days, seedlings were planted in individual pots (12 cm diameter) with a 50/50 volume mix of the sand and sieved (6 mm) commercial peat soil. The pots were placed in a temperature controlled greenhouse (day/night 20/10°C) provided with supplemental light a sodium lamp (Osram Vialox 400 W Nav-T Super (Son-T Plus) 100 cm over the pots) during the daytime.
Results

**Continuous incubation (A)**

Two of the 540 tested seeds from the Dagsmosse batch germinated during 34 weeks whereas none germinated from 720 seeds from the Kisa batch. The seeds from 2003 were kept for a longer time (96 weeks). After 40 weeks, germination began at 30/20°C, ending up with 24 and 7% germination for Ålvan and Gävbo, respectively, by the end of the experiment. Otherwise there was less than 5% germination, regardless of treatment and seed batch.

**Cold stratification in refrigerator (B)**

The initial germination rate was negligible but increased with the length of cold stratification (Fig. 1, Table 2). For the Kisa seed batch, incubation temperatures and light vs darkness significantly affected germination (Table 2). The seeds from Kisa showed a higher germinability in light than in darkness, and germinated most at the lower incubation temperature (Fig. 1). One seed germinated during cold stratification. The maximum germination after 34 weeks of cold stratification was 35-40% for the two seed batches (Fig. 1).

**Outdoor storage (C)**

The 2001 seeds subjected to outdoor temperatures (Kisa seed batch only) germinated to a maximum of 30% (Fig. 2). A few seeds had germinated during storage outdoors (detected when dishes were transferred to incubation in light): four seeds (1.1%) had germinated before the middle of March and five (1.4%) before early April.

A small number of seeds from the 2003 seed batches germinated during incubation after stratification in January, the first winter (Fig. 2). Germination of the same magnitude, but with large variation between dishes and test occasions, occurred outside in dishes during the following spring, and only little further germination occurred during incubation at these test occasions (Fig. 2). Over the second winter, the pattern was repeated; additional germination in dishes occurred during incubation in the winter and early spring, but later on nearly all germination occurred in dishes outdoors (Fig. 2). Ålvan and Gävbo samples reached a maximum of 43 and 46% germination, respectively, during the experiment.
**Fig. 1** Percentage of germinated seeds of *Galeopsis speciosa* incubated in different temperature regimes in light and darkness after cold stratification in refrigerator. Seeds were collected from the two sites in Sweden, Dagsmosse and Kisa.

**Effect of alternating cold and warm periods (D)**

Between 5 and 30% of the seeds (Kisa, 2001) subjected to warm-preceding-cold stratification germinated when tested in the incubators. There was no detectable effect of a warm period preceding cold stratification compared to only cold stratification (data not shown).

The seed subjected to repeated warm and cold periods (2003) began to germinate after about 40 weeks for all treatments (Fig. 3). Repeated 8 or 16-week cold periods, alternating with warm periods, had a positive effect on germination, compared to one long cold period (Fig. 3).
Table 2 General Linear Model of germination data of seeds of Galeopsis speciosa collected at two different sites in Sweden, after cold stratification (5 °C). “Length of cold stratification” was considered as a continuous variable. Seeds were dispersed among three dishes, considered as replicates, per treatment.

<table>
<thead>
<tr>
<th>Factor</th>
<th>DF</th>
<th>MSS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dagsmosse</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A Length of cold stratification</td>
<td>1</td>
<td>3.33</td>
<td>183.9</td>
<td>0.0000</td>
</tr>
<tr>
<td>B Incubation temperature</td>
<td>2</td>
<td>0.0125</td>
<td>0.689</td>
<td>0.5039</td>
</tr>
<tr>
<td>C Light/darkness</td>
<td>1</td>
<td>0.0138</td>
<td>0.764</td>
<td>0.3837</td>
</tr>
<tr>
<td>B * C</td>
<td>2</td>
<td>0.00262</td>
<td>0.144</td>
<td>0.8658</td>
</tr>
<tr>
<td>Error</td>
<td>137</td>
<td>0.0181</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Kisa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A Length of cold stratification</td>
<td>1</td>
<td>4.68</td>
<td>229.3</td>
<td>0.0000</td>
</tr>
<tr>
<td>B Incubation temperature</td>
<td>2</td>
<td>0.115</td>
<td>5.64</td>
<td>0.0041</td>
</tr>
<tr>
<td>C Light/darkness</td>
<td>1</td>
<td>0.143</td>
<td>6.97</td>
<td>0.0089</td>
</tr>
<tr>
<td>B * C</td>
<td>2</td>
<td>0.00972</td>
<td>0.475</td>
<td>0.6224</td>
</tr>
<tr>
<td>Error</td>
<td>209</td>
<td>0.0204</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sowing on soil surface of pots outdoors (E)

No seedlings emerged in the autumn or winter. In the spring, emergence started in the middle of March, with a peak in the latter half of March (Table 3). The seeds from the Dagsmosse collection germinated to a lesser extent than those from the Kisa collection (Table 3).

Tetrazolium tests (F)

Only one seed (of 688 tested) did not stain, and was consequently scored as dead. In addition to this estimate of mortality, 0.63% of the seeds used had been discarded during the experiment as they had been scored dead.

Gibberellic acid (G)

Between 90 and 100% of the seeds from both seed batches collected in 2003 germinated in GA, albeit at a very slow rate (Fig. 4). At 20/10°C, the first seedlings appeared after 2 weeks,
and after 10 weeks 60–90% had germinated (Fig. 4). At 5°C, germination began eight weeks after the start of the experiment, and reached 60% after 30 weeks (Fig. 4). Full germination was achieved after 18 weeks at 20/10°C and after 96 weeks at 5°C (Fig. 4). Without GA only a few seeds germinated at 5°C regardless of substrate and seed batch.

**Fig. 2** Germination of Galeopsis speciosa incubated at different temperature regimes after storage, imbibed, outdoor in darkness. Seeds were collected from three sites in Sweden, Kisa, Ålvan and Gävbo. The Kisa seed batch was subjected to light during daytime as well as continuous darkness during incubation, while the other two seed batches were subjected to only the light treatment. Temperature outdoors is daily mean on a thirty year basis.

**Growth of excised embryos (H)**

By one week after excision of embryos, several cotyledons had become greenish. After two weeks, when the seedlings were planted in pots, all cotyledons were green, all embryos had elongated radicles and some had developed root hairs. The embryos developed normally: cotyledons grew, and hypocotyl and true leaf appeared without delay. The plants were kept until they had 6-10 leaves, two months after excision of the embryos.
**Fig. 3** Cumulative germination of seeds of *Galeopsis speciosa*, collected at two sites in Sweden, Ålvan and Gävbo, incubated in different combinations of 20/10°C and 0°C, and provided with light during daytime. No germination occurred at continuous 20/10°C.

**Fig. 4** Cumulative germination of seeds of *Galeopsis speciosa* from two different sites in Sweden, Ålvan and Gävbo. Seeds were incubated in gibberellic acid solution (1000 mg L⁻¹) at two different temperature regimes, on two different substrates, and provided with light during daytime.
Table 3 Timing of emergence of seedlings of Galeopsis speciosa sown outdoors in Sweden at seed maturity in late summer (August). Numbers are percentages based on the number of seeds sown.

<table>
<thead>
<tr>
<th></th>
<th>Aug.-Feb.</th>
<th>Early March</th>
<th>Late March</th>
<th>Early April</th>
<th>Late April</th>
<th>May-July</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dagsmosse</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st year</td>
<td>0</td>
<td>0</td>
<td>2.2</td>
<td>2.2</td>
<td>6.7</td>
<td>0</td>
</tr>
<tr>
<td>2nd year</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.2</td>
<td>3.3</td>
<td>2.2</td>
</tr>
<tr>
<td>3rd year</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4th year</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Kisa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st year</td>
<td>0</td>
<td>0</td>
<td>27.3</td>
<td>5.3</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>2nd year</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.0</td>
<td>3.3</td>
<td>1.3</td>
</tr>
<tr>
<td>3rd year</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4th year</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td><strong>Älvan</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st year</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2nd year</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Gävbo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st year</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2nd year</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Discussion

Germination ecology

Fresh seeds of *Galeopsis speciosa* exhibited negligible germination, irrespective of incubation temperature and the presence of light, as shown by four seed batches collected on different sites and in two different years (Figs 1, 2). Cold stratification enabled an increasing proportion of the seeds to germinate but, even after 33 weeks of stratification 70-80% of the seeds collected in 2001 remained ungerminated (Fig. 1), despite being tested at different temperatures in light and darkness, and after two different stratification environments. There
were slight differences between germination in light and darkness and at different temperatures (Table 2). However, overall, these differences were small or inconsistent (Fig. 1, 2). Hence, light was not a particularly important germination cue, confirming a previous report on this species (Milberg & Andersson, 1998); nor were temperature differences, within the range tested, important (Fig. 1, 2). After repeated cold stratification, germination increased compared with a single long cold stratification period (Fig. 3).

Together, these findings would suggest that: (i) only some seeds become non-dormant during winter while most remain dormant; (ii) the non-dormant fraction will germinate, irrespective of light availability, at some point in time when the soil temperature increases beyond some lower limit. This was confirmed when only 35% of the seeds sown outdoors in 2001 produced a seedling within 12 months (Table 3), and almost exclusively early in the spring, when the temperature was still low (0-5 °C; Fig. 2). The 2003 seed batches produced nearly no seedlings in pots during 2004 and 2005 (Table 3). In contrast, 25-40% of seeds stratified outdoors had germinated in dishes outdoors by the end of the spring 2005 (Fig. 2).

As continuous incubation at 15/5 °C for 34 or 96 weeks resulted in negligible germination, it seems that effective stratification temperatures have to be quite low. Long cold stratification in the refrigerator (Fig. 1) resulted in more germination when compared with seeds kept moist outdoors during one winter (Fig. 2). This is to be expected since the first two months outdoors (September & October) involved temperatures exceeding 15/5 °C (Fig. 2).

**Dormancy classification**

Seeds of *Galeopsis speciosa* are strongly dormant at dispersal (Figs 1, 2; also Milberg & Andersson, 1998). *Galeopsis speciosa* does not exhibit physical dormancy (impermeable to water) as its seeds quickly absorbed water.

Of the endogenous dormancy types, none of the morphological types are relevant in the case of *G. speciosa*. Morphological dormancy involves small embryos (Baskin & Baskin, 2004), but *Galeopsis* seeds are large with a well-developed embryo and a thin layer of endosperm (Martin, 1946). *Galeopsis speciosa* therefore has some kind of physiological dormancy. Since *G. speciosa* requires a long cold stratification (Fig. 1), the non-deep type of physiological dormancy is unlikely. Also, the seeds did not show a clear light requirement (Tables 1 and 2; cf. Milberg & Andersson, 1998) as seeds with non-deep physiological dormancy often do (Baskin & Baskin, 1998).
In seeds with deep physiological dormancy, gibberellic acid does not break the dormancy, but in intermediate dormancy it may substitute for cold stratification (Baskin & Baskin, 1998). Gibberellic acid did have an effect on germination given sufficient time (Fig. 4), suggesting intermediate physiological dormancy. Furthermore, excised embryos grew and produced normal seedlings which should not be the case for species with deep physiological dormancy. Nevertheless, species with intermediate physiological dormancy should, according to the current classification scheme (Baskin & Baskin, 2004), germinate to a high degree after one single stratification period, which is not the case with *G. speciosa*. *Galeopsis speciosa* seems to be an example of a species which dormancy characteristics prevent its classification according to the currently prevailing classification system (Baskin & Baskin, 2004). Without doubt the species’ dormancy is alleviated by cold periods (Fig. 1), but responsiveness of individual seeds varies within seed batches. There was no single treatment, including alternating cold and warm periods, which gave germination over 50%. Regardless of treatment, germination occurred after or during cold periods. Our interpretation is that fresh seeds of *G. speciosa* exhibit a strong dormancy that is alleviated by cold periods (Figs 1, 2), and the variation in dormancy level within seed batches gives distribution over time, with a small part of each cohort emerging each spring for several years after dispersal.

Our results highlight a general issue: a negative publication bias for species with strong kinds of dormancy (Karlsson *et al.*, 2003), especially when combined with large intra-batch seed variability (Milberg *et al.*, 1996; Andersson & Milberg, 1998). Studies of such species might never be published because researchers often work with the underlying assumption that it should be possible to get nearly all seeds to germinate when subjected to a suitable environment or combinations of environments. This might not be a valid assumption, especially not for annual weeds where strong selection might have favoured large intra-batch variability.

**Weed characteristics and implications**

Up to now, the general consensus seems to have been that many, or even most, annual weeds in temperate climates have seeds with non-deep physiological dormancy (Baskin & Baskin, 1985). The present study indicates that at least one important annual weed has a different kind of physiological dormancy. It is possible that other weedy, summer annual *Galeopsis* species, like *G. tetrahit*, *G. bifida* and *G. ladanum*, also have the same kind of physiological dormancy.
The type of dormancy might have implications for weed management. The combination of (i) a long stratification requirement, (ii) germination in darkness, and (iii) unspecific temperature requirements once the dormancy level has been alleviated, can explain both the relatively early emergence date often noted for *Galeopsis* spp. (Erviö, 1981; Håkansson, 1983; Legere & Deschenes, 1989), and the relatively short time-span over which emergence occurs (Legere & Deschenes, 1989). Hence, this kind of strong physiological dormancy might, at first sight, seem promising from the point of view of control because it is likely to result in a more predictable timing of seedling emergence than for summer annuals with non-deep physiological dormancy. Furthermore, soil cultivation should have less stimulatory effect on species with the kind of dormancy we observed for *G. speciosa* than on those exhibiting non-deep physiological dormancy, for which light is often an important germination cue. Whether this could be exploited in weed control, e.g. by manipulating sowing dates, is less clear (Legere, 1997). The predictability might be restricted to within a seed cohort, since there were interpopulation variations in dormancy in *Galeopsis speciosa* (Fig. 2, Table 3; see also Milberg & Andersson, 1998). This prediction needs to be validated under field conditions.

It has been shown that in another Lamiaceae summer annual (*Isanthus brachiatus*), a long continuous cold stratification is not sufficient to reduce the dormancy level in all seeds. Instead, an interrupting warm period is needed (Baskin & Baskin, 1975). In *G. speciosa* the alternating warm and cold periods increased germination a little compared to a single long period (Fig. 3), but the difference was not substantial and even several repetitions did not result in more than 40% germination.

The proportion of germinated seeds was low in all experiments, and did not exceed 40% (in the absence of gibberellic acid), suggesting a substantial carry-over of seeds from the first to the second spring. As far as we know, there is no information available on the longevity of seeds of *Galeopsis speciosa* in soil but there are some reports on closely related species. Seeds of *Galeopsis bifida* survive at least ten years in forest soil (Hintikka, 1987) and those of *G. tetrahit*, 3-5 years (Roberts, 1986; Conn & Deck, 1995; Lutman et al., 2002). Seedlings of *Galeopsis speciosa* emerged from a seed bank after 40 years at a site in Östergötland (a forest plantation on former arable land; Nyström et al. MS). It remains to be established how large the carry-over is from year to year, and for seed survival in *G. speciosa* to be monitored. There might be differences in survival and emergence between species with non-deep and those with the kind of strong dormancy we observed for *G. speciosa*. In the case of non-deep physiological dormancy, the likelihood of germination is determined by germination cues
during periods of low dormancy levels of seeds, and not by the dormancy level *per se*. Hence, their seedling numbers might vary greatly between years and their maximum longevity in soil might be longer than for species with stronger physiological dormancy and without light requirement for germination. In the latter case one can assume that as soon as dormancy is sufficiently alleviated germination will occur over a wide range of temperatures, as for *G. speciosa* (Fig 1, 2). This should lead to a stepwise dormancy alleviation and germination of each cohort; some of the seeds germinate soon after each dormancy alleviation period during several consecutive years.

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