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Laila M. Karlsson and Per Milberg

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Variation within species and inter-species comparison of seed dormancy and germination of four annual *Lamium* species

Laila M. Karlsson, Per Milberg

IFM Division of Ecology, Linköping University, SE-581 83 Linköping, Sweden

Correspondence: Per Milberg

Email: permi@ifm.liu.se

Running title: Dormancy and germination in *Lamium*

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Abstract

In an ecological context, knowledge of intra-species variation in dormancy and germination is necessary both for practical and theoretical reasons. We used four or five seed batches (replicates) of four closely related annuals co-occurring in arable fields in Sweden: *Lamium amplexicaule*, *L. confertum*, *L. hybridum* and *L. purpureum*. Seeds used for experiments stemmed from plants cultivated on two sites, each site harbouring one population of each species, thereby ensuring similar environmental history of seeds. Seeds were tested for germination when fresh and after three different pre-treatments (cold or warm stratification, or dry storage) for up to 24 weeks. Seeds were also sown outdoors. Despite substantial intra-species variation, there were clear differences between species. The general seed dormancy pattern, i.e. which environmental circumstances that affect dormancy, was similar for all species; dormancy reduction occurred during warm stratification or dry storage. Even though the response to warm stratification indicate a winter annual pattern, successful plants in Sweden were mostly spring emerged. Germination in autumn occurred, but plants survived winters poorly. Consequently, as cold stratification did not reduce dormancy, strong dormancy in combination with dormancy reduction during dry periods might explain spring germination. It is hypothesized that local adaptations occur through changes mainly in dormancy strength, i.e. how much effort that is needed to reduce dormancy; strong dormancy restrict the part of each seed batch that germinate during autumn, and thus risk winter mortality, in Sweden.

Keywords: Deadnettle; Lamiaceae; phenology; physiological dormancy; summer annual; winter annual

Introduction

Studies of germination ecology frequently involve the characteristic "seed dormancy", which is used with various definitions (e.g. Harper, 1957, 1977; Lang, 1987; Baskin and Baskin, 2004). It can refer to either those circumstances that directly promote/prevent germination in combination with those circumstances (including seed morphology) that make seeds resist germinating even when subjected to suitable germination environments, or only to the latter. Without doubt, both these characteristics are ecologically important. Studying dormancy and germination from an ecological perspective, we define seed dormancy as a seed characteristic that prevents germination, even if suitable germination conditions prevail, not involving embryo or seed morphology, or germination mechanisms.

Closely related and co-occurring species, with similar emergence patterns, can be used to study species-specific dormancy and germination responses: by replicating each species at the population level, differences between species can be detected through common statistical methods. If the phylogenetic relationship between species is known, results from such studies can also allow interpretation of evolutionary conservatism and possible divergences of seed dormancy and germination characters.

There are four annual *Lamium* species that occur on arable land or in ruderal situations in Sweden: *L. amplexicaule* L., *L. confertum* Fr., *L. hybridum* Vill. and *L. purpureum* L. According to Mennema (1989), *L. amplexicaule* (section *Amplexicaule*) is least related to the other three species that all belong to the section *Lamium*. Within the latter section, *L. hybridum* and *L. purpureum* are more closely related to each other than to *L. confertum*. Intra-species variation in germination is known to be substantial for *L. amplexicaule*, *L. hybridum* and *L. purpureum* (Roberts and Boddrell, 1983;

Milberg et al., 1996; Milberg and Andersson, 1998). *Lamium amplexicaule* occurs as an important weed worldwide, except around the equator and in Africa (Holm et al., 1997), and is frequently present today despite modern methods of weed control (e.g. Harker et al., 2005; Topal et al., 2006). *Lamium purpureum* is widely spread in Europe and North America (Holm et al., 1979; Hultén and Fries, 1986), is present in Australia (Adkins and Peters, 2001) and is frequently reported as an agricultural weed (e.g. Holm et al., 1979; Walter et al., 2002; Mitchell, 2003). *Lamium confertum* and *L. hybridum* mainly occur in the cold temperate climate in Europe (Hultén and Fries, 1986): *L. confertum* on the Scandinavian Peninsula, on the British Isles, on Iceland and on southern Greenland, and *L. hybridum* in Northern and Central Europe (Hultén and Fries, 1986). There are also a few reports of *L. hybridum* from North America (Jones and Jones, 1965; Taylor, 1991), and of both *L. confertum* and *L. hybridum* from southern Europe (Mennema, 1989).

In order to compare these closely related and co-occurring species we decided to use two populations of each species, and cultivate one of each species at each of two sites. Therefore, the environmental circumstances the mother plants were subjected to were the same for all species, even though there were environmental differences between sites and years. Our aim was to describe dormancy and germination responses of the species in the Swedish environment. If species-specific differences are detected, a tentative evaluation of the extent of which dormancy characteristics are evolutionarily conservative could be done, using ecologically important and species-specific differences.

Material and methods

Notes on taxonomy

There are several synonyms for *L. confertum* [e.g. *L. moluccellifolium* Fr., *L. intermedium* Fr.] and for *L. hybridum* [e.g. *L. dissectum* With., *L. moluccellifolium* Schumach.]. In the seemingly only existing taxonomic revision of *Lamium*, based on phenotypic characters, the taxon here referred to as "*L. hybridum*" was regarded as three varieties of *L. purpureum*: *L. purpureum* var. *incisum*, var. *hybridum* and var. *moluccellifolium* (Mennema, 1989). However, in this paper, as by IPNI (2005), *L. hybridum* is considered a species. Attempts to artificially produce hybrids of any combination of *L. amplexicaule*, *L. confertum*, *L. hybridum* and *L. purpureum* only succeeded for *L. hybridum* × *L. amplexicaule* as long as plants with natural chromosome numbers were used (Bernström, 1953), and Mennema (1989) did not find evidence for natural hybridisation of any pair of these four species. Hence, plants found in the field are unlikely to be hybrids, and phenotype of each plant used and observed during our study could easily be classified to one of the mentioned four species following Krok et al. (2001).

Seed collection

Seeds used in the experiments stemmed from plants cultivated for seed production. The latter originated from large populations of *Lamium* that had been found in arable fields in southern Sweden during 2002 and 2003. Seeds from two populations each of *L. amplexicaule*, *L. confertum*, and *L. purpureum* were collected, at time for mayor dispersal (15 July-15 August), in 2003, and of *L. hybridum*, one seed batch was collected in 2002 and one in 2003 from different sites. There were at least 20 km between sites used for seed collection of each species. Of these, one population of each

species was sown for cultivation at one of two sites (Ledberg, 58°26'N 15°28'E, and Råby, 58°32'N 15°25'E), with 12 km between them. All sites were located in arable areas in southern Sweden. Seeds from the two cultivation sites were collected in 2004 and 2005 for germination experiments (Table 1). During cultivation, the areas were watered when needed and supplied with nutrients (*ca* 60 g m⁻², Substral nutrient grains, Scotts, Marysville, Ohio) each spring. The sites were hand-weeded when needed, and when there were no or few *Lamium* plants present, the soil surface was slightly disturbed and un-wanted weeds were removed. The cultivated sites were visited regularly until the summer of 2006, and thereby provided an opportunity to take notes on phenology of emergence, flowering and survival.

At seed collection for experiments, plants were harvested and spread out in a non-heated building for one night. Seeds that fell off the plants were used, and were stored at room temperature (*ca* 20°C, *ca* 35% RH) for six days before experiments commenced. During 2005, harvests on the Ledberg site were done to include seeds from early, intermediate and late-ripening parts of the cohorts of *L. amplexicaule*, *L. confertum* and *L. hybridum* (Table 1). Therefore, two sixths of the cultivated areas were harvested each time. Times for collection were chosen subjectively: 1) early – as soon we regarded it possible to get enough seeds for the experimental setup, 2) intermediate – after seeds in the lower whorl of calyxes had dispersed, at the time we considered the maximal harvest per plant to occur, and 3) late – when most seeds had dispersed, but before we regarded it as too late to get a sufficient harvest. In contrast, *Lamium purpureum*, which to some extent consisted of autumn-germinated, over-wintered plants, was harvested at the intermediate time for both autumn and spring germinated plants (Table 1).

Table 1. Collection dates for *Lamium* seeds from two cultivation sites, Ledberg and Råby, in southern Sweden. There were 12 km between the cultivation sites, and each harboured one population of each species. Experiments commenced one week after collection.

Species	Part of cohort ¹	Cultivation site	Collection date
<i>L. amplexicaule</i>	Intermediate	Ledberg	29 June 2004
	Intermediate	Råby	29 June 2004
	Early	Ledberg	20 June 2005
	Intermediate	Ledberg	30 June 2005
	Intermediate ²	Råby	4 July 2005
	Late	Ledberg	7 July 2005
<i>L. confertum</i>	Intermediate	Ledberg	7 July 2004
	Intermediate	Råby	7 July 2004
	Early	Ledberg	30 June 2005
	Intermediate	Ledberg	7 July 2005
	Intermediate ²	Råby	4 July 2005
	Late	Ledberg	17 July 2005
<i>L. hybridum</i>	Intermediate	Ledberg	1 July 2004
	Intermediate	Råby	1 July 2004
	Early	Ledberg	30 June 2005
	Intermediate	Ledberg	7 July 2005
	Intermediate ²	Råby	4 July 2005
	Late	Ledberg	17 July 2005
<i>L. purpureum</i>	Intermediate	Ledberg	1 July 2004
	Intermediate	Råby	7 July 2004
	Intermediate ³	Ledberg	3 June 2005
	Intermediate	Ledberg	30 June 2005
	Intermediate ²	Råby	4 July 2005
	Late ²	Ledberg	7 July 2005

¹⁾ Ripe seeds of early, intermediate or late ripening parts of a cohort.

²⁾ Used only for phenology study.

³⁾ Seeds from over-wintered plants.

Phenology of seedling emergence

Seeds of all batches were sown on top of soil in pots outdoors at the Ledberg site (Table 1). A non-woven fibreglass sheet was placed in the bottom and about 300 mL of sieved (6 mm mesh size), sterilized (autoclaved 20 minutes) soil from the Ledberg site that was put in each pot (120 mm diameter). Two pots, with 100-200 seeds each (depending on seed availability), were used for each seed batch.

A hollow in the ground, 0.15 m deep, was filled with 2-6 mm diameter ceramic clay pellets (AB Svenska Leca, Sweden) and the pots were buried to such a depth that the surface of the soil was at the same level as the surface of the surroundings. The pots were protected from direct wind, but otherwise subjected to natural weather conditions. Animals were excluded from the area by net (20 mm mesh size). Temperature was measured every hour (TinytagPlus, Intab°, Sweden) at the soil surface without protection from the sun, and precipitation was measured daily. Pots were checked every week (if snow-free) and emerged seedlings were counted and removed. All pots were kept until July 25th 2007, i.e. until *ca* two or three years after sowing.

During the autumn of 2005, mosses became established in several pots. Gametophytes were occasionally removed with tweezers, trying to not disturb the soil or to remove seeds, during the remaining study period.

Germination experiments

For germination tests, two petri dishes (50 mm diameter), with 30-50 (depending on seed availability) seeds each, were used. Seeds were placed on two sheets of filter paper (Munktell 1003, 52 mm diameter) wetted with 2.1 mL de-ionized water. Petri dishes were sealed with parafilm.

Germination tests were performed in incubators (Rubarth Apparatebau, Laatzen, Germany) at 15/5, 20/10, 25/15 or 30/20°C day/night, with two hours of linear transference between. There was a photoperiod of 12 h/day, coinciding with the higher daily temperature. The photon flux density (400-700 nm) was 33-68 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (photometer: SKP 200, sensor SKP 215, Skye Instruments Ltd, Wales, measure precision 1 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and the 660/730 nm photon fluence ratio was 3.4-3.5 (photometer: SKR 100, sensor SKR 110, Skye Instruments Ltd, Wales).

Seeds were tested for germination when fresh and after three different pre-treatments: warm (*ca* 20°C) or cold (*ca* 0°C) stratification, or dry storage (*ca* 20°C, *ca* 35% RH). All pre-treatments were performed in continuous darkness. For germination tests, seeds were incubated for four weeks at the four temperatures and in both light (i.e. light during daytime) or in darkness (i.e. continuous darkness achieved by wrapping dishes in aluminium foil). Root protrusion, which was clearly visible in a dissection microscope, was used as limit for regarding a seed as having germinated, but when dishes were checked after four weeks of incubation, there were seldom any newly germinated seeds. Germination tests were done after 3, 6, 12 and 24 weeks of pre-treatment, with the exception of the seeds from 2004 for which dry storage was tested only after 12 and 24 weeks. The fresh seeds collected in 2004 and used for germination tests in light were left in the incubators for an additional period of 20 weeks, until the end of the last test after pre-treatments. The dishes were checked for seedlings at least every fourth week.

The four *Lamium* species' response to gibberellic acid solution (GA₃, 1000 mg L⁻¹, BDH Electran[®], VWR International Ltd, England) was tested with seeds from the 2005 intermediate collection occasion. At the same time as fresh seeds were tested for germination, an analogous set of dishes, with GA₃ solution instead of de-ionized water, was incubated in light at the four test temperatures.

Calculations and analyses

Results from the two pots or dishes used for each treatment were merged; thus, the two repetitions were not considered replicates. In the phenology study, emergence was calculated as the fraction of sown seeds. In germination tests, apparently dead seeds

(soft and/or overgrown with mould) were excluded from calculation of the germinated fractions.

Analyses of variance were done, with Statistica (StatSoft, Inc. 2004), both on arcsine-transformed germination data and on the absolute difference between germination after pre-treatments and for fresh seeds. The different seed batches of each species were considered replicates of the species in both analyses: in total four (*L. purpureum*) or five batches. Categorical predictors were species, pre-treatment, germination test temperature and light condition. Time in pre-treatment was treated as a continuous variable. For interpretation, special attention was paid to the factor "species" and interactions including "species", to evaluate possible differences between species.

Results

Observations from cultivation

Emergence occurred mostly during spring and autumn, but also during summer. Plants possible to use for seed harvest were mainly spring-germinated, while seedlings that emerged in autumn seldom survived winter. In the late autumn of 2004, there were newly emerged *L. amplexicaule* at both sites, newly emerged and plenty of large, flowering *L. confertum* at Ledberg and some newly emerged and some flowering plants of remaining species at both sites. Winter survival, for more than a few plants, occurred only for *L. purpureum* at Ledberg. At the end of November of 2005, there were large, flowering plants of all species at the Ledberg site. However, an attempt to collect seeds for experiments failed because only very few seeds were ripe before the plants were killed by frost and snow. The unripe seeds of dead plants did not fall off the plants, even when dried at room temperature for several days.

Phenology of seedling emergence

Emergence did not exceed 50% for any of the seed batches during the time span considered (Fig. 1). Variations, in amount of emergence, between seed batches were considerable for all species, but emergence time was well correlated for all seed batches and species (Fig. 1).

Part of all seed batches; except one *L. amplexicaule*, emerged in the late summer or autumn following harvest (Fig. 1). Emergence occurred the spring following sowing for seeds sown 2004, i.e. in the spring of 2005. In the autumn of 2005, seedlings emerged from all seed batches. There was no emergence, except a few *L. purpureum*, during the spring of 2006, regardless of year of sowing (Fig. 1). During both the last autumn, 2006, and the last spring, 2007, additional seedlings of all seed batches emerged (Fig. 1).

The weather differed between the years. The first (2004/05) and third (2006/07) winter had little snow, and therefore, the daily soil surface mean temperature was frequently below 0°C (Fig. 1), and the environment seeds encountered on soil surface can be assumed to have been relatively dry. During the second winter (2005/06) there was an unusually long period with continuous snow cover (from the middle of December until the end of March); soil surface temperature was therefore around zero without much variation with time (Fig. 1), and the environment seeds encountered on soil surface can be assumed to have been relatively humid. The summer of 2004 was, for southern Swedish conditions, relatively cool, 2005 normal and 2006 was warm until August. The summer of 2006 had unusually little precipitation until August (Fig. 1).

Figure 1 Seedling emergence of seeds from plants of two populations of four *Lamium* species that had been grown at two sites (Ledberg and Råby) in southern Sweden; each site harbouring one population of each species. Seeds were collected in 2004 and 2005. During 2005, seeds were collected from early, intermediate or late ripening parts of the cohort, or, in the case of *L. purpureum*, from over-wintered plants at the Ledberg site. Seeds were sown, one week after collection, on sterile local soil in pots outdoors in Ledberg, and subjected to natural weather conditions. Temperature was measured at the soil surface, without covering from the sun.

Germination experiments

Germination of fresh seeds

Fresh seeds of *L. amplexicaule* did not germinate at any condition tested (Fig. 2). For *L. confertum* and *L. purpureum* there was some germination at all temperatures tested (Fig. 2), with the *L. purpureum* seed batch from Ledberg 2004 (Table 1) germinating more than the other (Fig. 2). Germination of fresh seeds of *L. hybridum* was higher than for the other species but varied considerably between batches, with Råby 2004 (Table 1) germinating the most and the three batches from Ledberg 2005 (Table 1) the least (Fig. 2). For the two species exhibiting reasonable germination, temperatures at 20/10-30/20°C and light promoted germination of fresh seeds (Fig. 2).

Figure 2 Germination of fresh seeds from plants of *Lamium amplexicaule* (amp), *L. confertum* (con), *L. hybridum* (hyb) and *L. purpureum* (pur) that had been grown at two sites, Ledberg (L) and Råby (R), in southern Sweden; each site harboured one population of each species. Seeds were collected in 2004 and 2005. During 2005 seeds were collected from early (e), intermediate (i) or late (l) ripening parts of the cohort, or, in the case of *L. purpureum*, from over-wintered (o) plants. One week after collection, seeds were tested for germination at 15/5, 20/10, 25/15 or 30/20°C day/night, both with light during daytime (open symbols) and in continuous darkness (filled symbols).

Germination during continuous incubation

Some germination, up to 10-14% for the different species, occurred during the 24 weeks of continued incubation of fresh seeds in light treatment (percentages were, in this case, calculated from the numbers of fresh seeds tested per batch and treatment).

For *L. amplexicaule* and *L. purpureum* there was germination mainly at 25/15 and 30/20°C and for *L. confertum* at 30/20°C, but for *L. hybridum* at all temperatures. Germination occurred mostly during 12-20 weeks after terminating the test of fresh seeds, except for *L. hybridum*, for which germination occurred more evenly over the 24 weeks.

Germination after pre-treatments

Within species, there were between-batch differences in germination after pre-treatment (Fig. 3). The *L. hybridum* batches that germinated when fresh (Fig. 2), germinated also after cold stratification, but germination at 15/5°C, and in darkness at all temperatures, decreased (Fig. 3). For the other species, there was no germination after cold stratification (Fig. 3). When subjected to warm stratification, all seed batches of all species increased germination in light, at least after 24 weeks (Fig. 3). For all species, at all temperatures and in both light and darkness, the largest germinated fractions per species occurred in response to dry storage (Fig. 4), but some batches of *L. purpureum* germinated more in light after 12 and/or 24 weeks of warm stratification than after dry storage (Fig. 3).

Despite variations between seed batches (Fig. 3), the factor "species" was the most important for variation in germination, and the third most important for effect of pre-treatments in the ANOVAs (Table 2). Overall, *L. hybridum* germinated the most, *L. amplexicaule* the least and *L. confertum* and *L. purpureum* in between and about the same (Fig. 4). Regarding difference in germination between fresh seeds and after pre-treatment, the ranking, from the most to the least, was: *L. confertum*, *L. amplexicaule*, *L. purpureum* and *L. hybridum* (Fig. 4).

Figure 3 Germination of seeds from *Lamium* plants of four species that had been grown at two sites, Ledberg (L) and Råby (R), in southern Sweden; each site harboured one population of each species. Seeds were collected in 2004 and 2005. During 2005 seeds were collected from early (e), intermediate (i) or late (l) ripening parts of the cohort, or, in the case of *L. purpureum*, from over-wintered (o) plants. Seeds were subjected to cold or warm stratification, or dry storage for 3, 6, 12 or 24 weeks before germination tests at 15/5-30/20°C day/night, either with light during daytime (open symbols) or in continuous darkness (filled symbols).

The most important second order interaction was "species*pre-treatment", both for germination and effect of pre-treatments (Table 2, Fig. 4). Also "species*temperature" was significant in both analyses (Table 2). In the analysis of germination, "species*light" was of importance (Table 2). Regarding three-factor interactions, "species*pre-treatment*light" was significant in ANOVA in both analyses (Table 2, Fig. 3). In the analysis of germination, also "species*temperature*light" was significant (Table 2).

Of 175,460 seeds included in germination tests, 0.45% were considered dead. When incubated with GA₃, all species germinated to 90-100% at 15/5, 20/10 and

25/15°C. At 30/20°C, however, germination with GA₃ was 50, 99, 94, and 15% for *L. amplexicaule*, *L. confertum*, *L. hybridum* and *L. purpureum*, respectively.

Figure 4 Average germination of four to five seeds batches of each of four *Lamium* species, collected from plants in southern Sweden. Seeds were subjected to three different pre-treatments: cold (ca 0°C) or warm (ca 20°C) stratification, or dry storage (ca 20°C) for 3, 6, 12 or 24 weeks, followed by germination tests at 15/5-30/20°C day/night, either with light (—) during daytime or in continuous darkness (---).

Discussion

Intra-species variation

For all species there were large, frequently 25-50 percent units, differences in response between seed batches of the same species that were treated in the same way (Figs. 1, 3). Substantial intra-species differences of *L. amplexicaule* and *L. purpureum*

batches collected in different years were also observed by Roberts and Boddrell (1983), e.g. 40-70 percent units difference in distribution of yearly emergence between spring and autumn emergence (June and July excluded) between batches of the same species. Also, there were up to 20, 40 and 50 percent units difference between populations of *L. amplexicaule*, *L. purpureum* and *L. hybridum*, respectively, in laboratory tests (Milberg et al., 1996; Milberg and Andersson, 1998).

Table 2. ANOVA of germination results of four *Lamium* species. "Germination" was analyzed as arcsine transformed germination fraction, and "difference" (i.e. effect of pre-treatment) as absolute difference between germination results after pre-treatments and for fresh seeds. Time was treated as a continuous factor. Five or four (*L. purpureum*) seed batches were used as replicates of each species.

Factor	df	Germination		Difference	
		F	p	F	p
Intercept	1	155.3	0.000	53.6	0.000
Time ¹	1	174.7	0.000	167.5	0.000
{S} Species ²	3	215.2	0.000	42.2	0.000
{P} Pre-treatment ³	2	210.6	0.000	351.8	0.000
{T} Test temperature ⁴	3	39.8	0.000	2.4	0.062
{L} Light condition ⁵	1	81.0	0.000	32.1	0.000
S*P	6	31.4	0.000	50.1	0.000
S*T	9	11.8	0.000	2.6	0.006
S*L	3	10.8	0.000	1.8	0.141
P*T	6	4.3	0.000	6.4	0.000
P*L	2	4.1	0.016	6.1	0.002
T*L	3	16.7	0.000	8.0	0.000
S*P*T	18	1.0	0.414	1.5	0.081
S*P*L	6	3.6	0.002	5.8	0.000
S*T*L	9	5.6	0.000	1.6	0.119
P*T*L	6	0.6	0.752	0.8	0.545
S*P*T*L	18	0.4	0.989	0.6	0.899
Error (germination)	2055				
Error (difference)	1599				

1) Tested after 0, 3, 6, 12 or 24 weeks in pre-treatment.

2) *Lamium amplexicaule*, *L. confertum*, *L. hybridum* or *L. purpureum*

3) Cold or warm stratification, or dry storage.

4) Germination tested at 15/5, 20/10, 25/15 or 30/20°C day/night.

5) Germination tested in light during daytime or in continuous darkness.

The seed batches used here originated from plants within the same area, and the ones from 2005 had been cultivated on the same site. The relatively small differences present in the growing environment for the various seed batches are then such differences that will always occur in nature and will influence the characteristics of seeds. Intuitively, an inspection of Fig. 1 and Fig. 3 suggests that late-ripened parts (downwards triangles) of the cohorts of *L. amplexicaule*, *L. confertum* and *L. hybridum* had weaker dormancy, and therefore germinated more easily, than the early parts (upwards triangles). Without doubt, statistical comparisons of points in time would confirm such a conclusion, but because only one seed batch from each collection occasion and species was included in this part of the study, the basis for general conclusions is too limited. However, the results imply that a substantial variation within each seed cohort may occur depending on time for dispersal and/or age of the mother plant.

By cultivating plants for seed production, we minimised differences between species depending on different growing environments. The ANOVA results showed that there were species-specific responses, with the factor "species" and interactions including "species" making up a large part of the total variation in both analyses (Table 2), despite the intra-species variation (Fig. 3). Consequently, we regard it as meaningful to describe and compare the dormancy and germination patterns of the species at a general level.

Responses to pre-treatments

Cold stratification did not decrease dormancy for any of the included *Lamium* species collected in Sweden (Fig. 4). For seed batches of all species that germinated when fresh, it was observable that warm stratification did initially increase dormancy

(c.f. Fig. 2 & Fig. 3). However, warm stratification led to decreased dormancy over time for all species (Fig. 4). In the present study, we used 20°C, which is "warm" for a Swedish summer temperature (Fig. 1), while Baskin and Baskin (1984a; 1984b) found 35/15°C to be most efficient for dormancy reduction in *L. amplexicaule* and *L. purpureum*. For all seed batches tested here, no, or nearly no, germination occurred in darkness after cold or warm stratification (Fig. 3).

The pre-treatment with dry storage was the most efficient to reduce dormancy for all four annual *Lamium* occurring in Sweden (Fig. 4). Especially, in contrast to cold or warm stratification, dry storage led to germination in darkness for all species (Fig. 4). There is a report of *L. amplexicaule*, probably collected in Arkansas, which did not germinate when fresh but germinated in darkness after dry storage for an un-specified time period (Jones and Bailey, 1956), but we are not aware of such studies for the other species. Further, germination at the lowest temperature tested, 15/5°C, was much higher after dry storage than after cold or warm stratification (Fig. 4). Thus, dry periods are important not only to reduce dormancy, but also to increase the range of environmental conditions which allows germination.

Comparison of species

Comparisons of species with similar responses require a way to detect and describe differences in dormancy and germination patterns, even if these are not fundamentally different. Following the classification system suggested by Baskin and Baskin (2004), the *Lamium* species studied here have non-deep physiological dormancy (i.e. they have fully developed embryos, imbibe easily, and respond to cold or warm stratification and to GA₃). Thus, that classification system cannot be used to detect differences between these species. The method of using ANOVA on both actual

germination over time and response in relation to germination when fresh (Table 2) revealed differences between species. Such a conclusion requires that the populations of the compared species be replicated, i.e. a number of dishes with seeds from one batch cannot be used as replicates of a species. In a similar way, Pezzani and Montaña (2006) detected differences between four co-occurring Poaceae species.

To describe dormancy and germination patterns of species, and identify when, how and between which species differences occur, we think it is practical to structure the description of the process leading to germination. For this purpose, we use (i) "dormancy pattern" – described by the kinds of environmental events that reduce and, if applicable, induce dormancy, (ii) "germination preferences" – described by the kinds of environments that are (or become during dormancy reduction) suitable for germination, and (iii) "dormancy strength" – described by how much effort is needed to reduce dormancy (cf. Karlsson & Milberg, 2007a; 2007b). To be ecologically meaningful, seed dormancy should be regarded as a continuous property of a seed batch (even though it is not known whether or not it is a continuum or an on-off property for a single seed). Dormancy strength, referring to the general pattern, is described as strong to weak, and the extent of dormancy present at any specific moment is referred to as "degree of dormancy".

The dormancy pattern was similar for the four species, as judged from the currently used experimental settings: warm stratification, for a sufficient time, or dry storage reduced dormancy level (Fig. 4). Also germination preferences were similar for the species: after warm stratification nearly no germination occurred at 15/5°C or in darkness at any temperature, but after dry storage, germination occurred at all temperatures tested and in both light and darkness, even though 15/5°C was the least

preferred temperature also after dry storage (Fig. 4). The characteristic dormancy strength differed between species: *L. amplexicaule* had the strongest, with degree of dormancy only slightly reduced during warm stratification, and substantial germination did not occur until 24 weeks of dry storage for any of the seed batches (Fig. 3). *Lamium hybridum* had the weakest dormancy; fresh seeds of several batches germinated to some extent (Fig. 2) and after dry storage for 12 or 24 weeks, all seed batches germinated to at least 50% in both light and darkness at 25/15°C, which did not occur during any circumstances for the other species (Fig. 3). In response to warm pre-treatment, *L. purpureum* responded positively after a shorter time period than all the other species (Fig. 3). Otherwise, *L. confertum* and *L. purpureum* had about the same dormancy strength; responding in the same magnitude to all treatments (Fig. 4).

Field implications

Our results imply that dry periods (Fig. 4) are important to take into consideration to understand emergence patterns of all these *Lamium* species. In England, both *L. amplexicaule* and *L. purpureum*, collected in three different years, germinated in both spring and autumn during all three years they were observed when mixed with soil that was disturbed three times a year (Roberts and Boddrell, 1983). The proportions that emerge in autumn or spring varied substantially between years (Fig. 1; Roberts and Boddrell, 1983) and soil humidity may be an important factor to explain these observations. Spring emergence does obviously occur (Fig. 1; Roberts and Boddrell, 1983 [*L. amplexicaule* and *L. purpureum*]; Baskin et al., 1986 [*L. amplexicaule*]), even though cold stratification does not reduce dormancy level, but on the contrary increases it (Baskin and Baskin, 1984a [*L. amplexicaule*]; 1984b [*L. purpureum*]; Milberg and Andersson, 1998 [*L. hybridum*]).

Germination in spring may occur in response to dormancy reduction during such winters as 2004/05 and 2006/07 in southern Sweden (Fig. 1), i.e. similar to dry, cold, storage. Another explanation for spring emergence is germination requirements: albeit dormancy is reduced enough to allowing germination during late summer, there will be a delay of germination until spring, if temperatures are too low during autumn and winter. That explanation presupposes that dormancy is not induced during winter. Dormancy is probably not induced when conditions are too cold or too dry, as during the winters of 2004/05 and 2006/07 in Sweden (Fig. 1). However, dormancy was probably induced during the winter of 2005/06 (Fig. 1), when soil surface was covered with snow for several months, i.e. an environment similar to cold stratification. Therefore, there are alternative explanations for spring emergence, in the absence of soil cultivation, to the one proposed by Baskin et al. (1986), i.e. that all *L. amplexicaule* seeds that germinated during spring must have been transferred from burial to surface, by natural soil movements or animals, during winter.

Also, emergence during autumns differed between years (Fig. 1). The emergence during the second autumn in pots, for the different collection years, was, overall, 1% of the seeds sown in pots in 2004 that remained ungerminated after the summer of 2005, and the corresponding number for seeds sown in 2005 was 13% for the autumn of 2006 (Fig. 1). This difference can be explained by seeds being imbibed, because of moist soil, during the summer of 2005 but dry during much of the summer of 2006 (Fig. 1). The summer of 2005 could therefore have been similar to circa eight weeks of warm stratification, and the summer of 2006 as periodic dry storage (Fig. 1).

Even though the four *Lamium* have been well established for a long time in the area used for this study (Kindberg, 1880; Genberg, 1992), the autumn emergence (Fig.

1) does not seem to be a performance suitable for the environment, with *L. purpureum* partly being an exception. Plants of different ages of *L. amplexicaule*, *L. confertum* and *L. hybridum* that were present during autumn at the cultivation sites died during the observed winters, which were quite different when considering temperature and protective snow cover. Seeds from these plants were not ripe before winter, and did not fall off the plants even when completely dry. Thus, the possibility for successful reproduction is low for autumn-germinated plants of these three species. In contrast, *Lamium purpureum* survived winter better, yet far from completely, and could therefore grow, flower and disperse earlier in spring and summer than the other species (Table 1). Nevertheless, also *L. purpureum* failed to produce ripe seeds from the plants that flowered during autumn. Thus, autumn germination is a drawback for all four species under conditions prevailing in southern Sweden. Despite this, the species performs well enough to be weeds; to a high extent through spring emergence.

Annual *Lamium* species have been shown to be able to survive in the soil for long periods. Ødum (1965) used archeologically dated soil samples from Denmark and Sweden and found *L. amplexicaule* and *L. purpureum* that germinated after circa 450 and 650 years, respectively, and reported evidence, based on germination in excavation sites, of *L. hybridum* germinating after at least 160 years in soil. When a mowed lawn was established covering a natural weed seed flora on former arable soil in Oxford, emergence of *L. amplexicaule* varied between soil samples taken over years, but did not decline substantially over 20 years (Chancellor, 1986). Germination of fresh seeds in the field is low (Fig. 1) because of high degree of dormancy (Fig. 2) that is not easily reduced (Fig. 4). Further, germination preferences do not allow germination at so low a temperature as 15/5°C or in darkness at any temperature if not first subjected to a dry

period (Fig. 4). Thus, germination is restricted to a limited part of each cohort during limited periods of each year, even if seeds are not buried in soil (where germination is not favoured because of lack of light). This explains how soil seed banks of the four *Lamium* investigated here are easily formed, persist over long time, and are not easily reduced even by soil cultivation in the Swedish climate.

Dormancy characteristics in an evolutionary perspective

The common way to evaluate dormancy and germination evolution is to base comparisons on embryo/seed morphology (Baskin and Baskin, 1998; Verdú, 2006; Finch-Savage and Leubner-Metzger, 2006); this coarse method seldom allows separation of dormancy and germination patterns for relatively closely related species, e.g. within a smaller clade like family or genus. Therefore, another approach is needed to investigate evolutionary changes in dormancy and germination ecology, especially to study smaller and small-scale changes and differences, as when investigating possible differences between species of one genus and/or populations from different provinces (e.g. Keller and Kollman, 1999; Copete et al., 2005; Bischoff et al., 2006). We hypothesise that the dormancy and germination characteristics described here can be used to compare responses in such cases (c.f. Karlsson and Milberg, 2007a; 2007b).

The annual Lamiaceae *Galeopsis speciosa* occurs in the same environment as, and sometimes co-occurs with, the *Lamium* studied here. It decreases its degree of dormancy during cold stratification, but not during warm stratification or dry storage, and germinates at low temperatures (Karlsson et al., 2006). Also two other Lamiaceae, not co-occurring with the studied *Lamium* but occurring in cold temperate climate, decrease dormancy during cold periods (*Collinsonia canadensis* [Albrecht and

McCarthy, 2006], *Lycopus europaeus* [Brändel, 2006]). Thus, within the Lamiaceae family, different dormancy patterns and germination preferences occur.

Dormancy pattern and germination preferences seem to be more evolutionary conservative than dormancy strength (Karlsson and Milberg, 2007a; 2007b). Dormancy pattern is similar for closely related taxa, but may differ within a family, even between co-occurring species with similar life history (c.f. Fig. 4 and Karlsson et al., 2006).

Dormancy strength, differing between closely related species (Fig. 4, e.g. large differences between *L. hybridum* and *L. purpureum*, the closest related of the species included here), is probably the least conservative factor that influences dormancy and germination for these species. Following this hypothesis, dormancy strength makes possible local adaptations and divergence of closely related species, and also contributes to differences between seed batches (Fig. 3).

The dormancy pattern of the Swedish annual *Lamium* species, with reduction during warm stratification (Fig. 4), is similar to one population of each *L. amplexicaule* and *L. purpureum* from Kentucky (Baskin and Baskin, 1984a; 1984b). However, regarding the characteristic "dormancy strength", there is a difference as the Swedish populations had stronger dormancy. This could be a result of adaptation to environments; both summers and winters are colder in Sweden than in Kentucky (e.g. Weather Channel, 2007). In Sweden, populations with dormancy that is easily reduced during warm periods will germinate in autumn and increase the risk of mortality during winter. It is notable that *L. purpureum*, which was the only species with substantial winter survival for autumn germinated plants on our cultivation sites in Sweden, was the species that most quickly reduced dormancy in response to warm pre-treatment among the populations from Sweden (Fig. 3), thus indicating that a pressure to avoid autumn

germination may have led to slower response to warm stratification for the other species.

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