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Germination ecology of five arable *Ranunculaceae* species

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Short title: Germination of arable *Ranunculaceae* plants

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Summary

Germination and emergence are critical life stages for annual plants and so their full understanding is essential for managing arable plant populations. This study investigated the most important species-specific environmental cues that regulate seed germination and emergence (temperature and light) of the arable *Ranunculaceae* species *Consolida orientalis*, *Consolida pubescens*, *Delphinium gracile*, *Delphinium halteratum* ssp. *verdunense* and *Nigella gallica*, in order to propose management strategies for their preservation in agro-ecosystems. Growth chamber and outdoor pot experiments were conducted for two consecutive seasons to analyse light (complete darkness or 12 h light) and temperature (5/10, 5/15 and 10/20°C) requirements and emergence patterns. The relative light germination requirement (ΔG_{light}), which extends from -100 (complete darkness) to 100 (light), was estimated. Weibull functions were fitted to observed emergence (%) in pots. For all species, germination was higher in complete darkness than with a light regime ($-60 < \Delta G_{light} < -95$). This dark requirement indicates better germination for buried seeds. A tillage operation just after seed shed is therefore recommended. *Consolida* spp. germinate and emerge almost exclusively in autumn-winter, while *Delphinium* spp. and *N. gallica* can also germinate in spring. These arable plants would be able to adapt to delayed sowings, an important strategy for avoiding early-emerging competitive weeds. Facultative winter-germinating species could face early herbicide treatments if sufficient emergence occurs in winter-spring. These results bring new information to develop conservation strategies for these species in agro-ecosystems.

Keywords: emergence, temperature, light, *Consolida orientalis*, *Consolida pubescens*, *Delphinium halteratum* ssp. *verdunense*, *Delphinium gracile*, *Nigella gallica*

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Introduction

In the last 50 years, agricultural intensification, habitat loss and landscape simplification have reduced plant diversity in many agricultural areas of Europe (Tschardtke *et al.*, 2005). A number of management changes have been implicated in the decline of their populations, including alteration to tillage and sowing times, increased crop density and shading by the crop canopy, decreased crop diversity, increased fertiliser and herbicide use, and more efficient seed cleaning (Fried *et al.*, 2009; Storkey *et al.*, 2012; Meyer *et al.*, 2013). Various studies indicate that many arable species across Europe, which are adapted to low input systems, are threatened (Storkey *et al.*, 2012; Richner *et al.*, 2015). Arable plants have potential conservation value, for example because of their importance in supporting biodiversity (Marshall *et al.*, 2003; Storkey *et al.*, 2012). These plants are particularly sensitive to changes in land use or management practices that reduce seedbank germination, seedling survival or fecundity.

A better understanding of germination and emergence is very important for conservation purposes and for predicting the long-term effects of management strategies on these rare arable plants (Gardarin *et al.*, 2011). Information on germination requirements and emergence period (extension and magnitude) should help in choosing sowing dates, timing tillage and selecting crops, so as to preserve or re-introduce these species in arable fields or suitable places for *ex situ* conservation efforts. *Ranunculaceae* is an important family contributing to arable flora, and represents around 20% of the total rare arable species in Europe (Aboucaya *et al.*, 2000). *Consolida orientalis* (J.Gay) Schrödinger, *Consolida pubescens* (DC.) Soó, *Delphinium gracile* DC., *Delphinium halteratum* ssp. *verdunense* (Balb.) Graebn. & P. Graebn. and *Nigella gallica* Jord. are considered rare across Europe (Aboucaya *et al.*, 2000) for the above mentioned reasons (Solé-Senan *et al.*, 2014). These Mediterranean species are annual herbs distributed in Southern Europe (mainly France and

1 Spain) and North Africa. Owing to habitat loss in most of their natural distribution area, these
2 species have found a refuge in winter cereal fields.

3 Detailed information on the different life stages of threatened species will help to fill
4 the existing gap in scientific knowledge, improve understanding of the phenomenon of rarity,
5 and assist conservation management decisions for the species under study (Schemske *et al.*,
6 1994). Rare arable plants show specific trait syndromes, often related to plant height,
7 competitive abilities, flowering time, seed size and dispersal, and germination and emergence
8 (Peters & Gerowitt, 2014). Therefore, to conserve threatened plant species, it is necessary to
9 have basic information on dormancy release, germination and emergence (Navarro & Guitian,
10 2003). Seed dormancy and germination are two of the most important processes affecting
11 seedling emergence, a critical life stage of annual plants (Forcella *et al.*, 2000). These
12 processes may have a major influence on the distribution of rare species (Herranz *et al.*,
13 2010). Temperature and light are among the most important species-specific environmental
14 cues that regulate germination and emergence (Baskin & Baskin, 1998; Schutte *et al.*, 2014).
15 The response of seed germination to temperature has been studied for many species, but data
16 is lacking for species specific to Southern Europe (Guillemin *et al.*, 2012; Gardarin *et al.*,
17 2011), especially rare arable plants. Light regulates germination depending on its spectral
18 composition and irradiance, the physiological status of the seeds and other environmental
19 factors such as temperature (Batlla & Benech-Arnold *et al.*, 2014). Light allows seeds to sense
20 both their position in the soil profile and the occurrence of soil disturbance, and can influence
21 weed seed response to cultivation (Batlla & Benech-Arnold *et al.*, 2014). Understanding these
22 factors is essential for establishing adequate measures, such as optimising the timing of
23 sowing or soil management techniques that can help conserve or re-introduce these species
24 into arable crops.

25 In this work, the objectives were to study the germination ecology of the five
26 *Ranunculaceae* species listed above, focusing on: (1) temperature and light requirements for
27 germination and (2) the emergence patterns of seeds sown in pots and exposed to natural
28 temperatures in intervals.

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1 **Material and Methods**

2

3 *Plant material*

4 Seeds of *C. orientalis*, *C. pubescens*, *D. gracile*, *D. halteratum* ssp. *verdunense* and *N. gallica*
5 were harvested at maturity from arable fields in Spain from late June to September in two
6 seasons: 2010-11 (Y1) and 2011-12 (Y2). Collection sites were located in areas that host a
7 high diversity of arable weeds, in the provinces of Teruel and Lleida. Both years, the same
8 populations were sampled, with the exception of *C. orientalis*, as no plants were found at the
9 collection sites in 2010. *Consolida orientalis* seeds were collected in the towns of Calamocha
10 (7 July in Y1) and Camarillas (13 July in Y2), in Teruel; *C. pubescens* seeds in the town of
11 Àger in Lleida (22 July in Y1, 21 July in Y2); *D. gracile* seeds in Bellmunt in Lleida (3
12 August in Y1, 9 August in Y2); *D. halteratum* ssp. *verdunense* and *N. gallica* seeds in Àger (2
13 September in Y1, 14 September in Y2). Seeds were collected from at least 20 plants
14 throughout the field and mixed in paper bags.

15 Seeds were air-dried for one week and dry stored (with silica gel) at room temperature
16 (approximately 21°C) until experiments started. Seeds that were visibly deficient were
17 excluded based on seed firmness (determined by squeezing them with tweezers) and the
18 presence of mould (Borza *et al.*, 2007), thus we assume that only viable seeds were used.
19 Each year in November, seeds were further assessed for viability by longitudinally slicing 100
20 seeds to expose the endosperm in four Petri dishes (25 seeds/dish). They were then incubated
21 in 1% triphenyltetrazolium chloride solution for 48 h in the dark at room temperature. An
22 embryo that was entirely pink was scored as viable. The percentage of viable seeds per
23 species per year was calculated.

24

25 *Experiment 1: Temperature and light requirements for germination (E1)*

26 A series of growth chamber experiments were carried out in two seasons (Y1 and Y2). Seeds
27 were exposed to two light regimes (12 hours of photoperiod or complete darkness) in growth
28 chambers that were set at one of three temperature regimes (5/10°C, 5/15°C, and 10/20°C
29 day/night temperatures), with four replicates per species and light treatment. Each replicate
30 consisted of 25 seeds in a Petri dish with agar medium. For *D. gracile*, only 10 seeds were
31 added per Petri dish in Y2 because of limited seed availability. Half of the Petri dishes were
32 wrapped in aluminium foil to achieve complete darkness. Experiments were initiated at
33 intervals throughout the season with room temperature-stored seeds: in Y1, experiments were

1 done on 19 November, 21 January and 6 April; in Y2, on 16 September, 22 November, 17
2 January and 20 March (hereinafter called April). In Y2, an additional experiment was
3 conducted immediately after collecting freshly harvested seeds to determine the dormancy
4 status: in July for *C. pubescens*, August for *C. orientalis* and *D. gracile* and in September for
5 *D. halteratum* ssp. *verdunense* and *N. gallica*. The percentage of germination was assessed as
6 seeds with a radicle protruding through the seed coat after 21 days.

7 The relative light germination requirement (ΔG_{light}) was estimated for each species
8 according to Saatkamp *et al.* (2011). Positive values (up to +100) indicate better germination
9 in light, and negative values (down to -100) better germination in darkness:

$$\Delta G_{light} = [(G_{light} - G_{dark}) / (G_{light} + G_{dark})] \times 100 \quad (\text{Eq. 1})$$

10

11

12

13 where G_{light} is number of seeds germinating in light, and G_{dark} is the number of seeds
14 germinating in darkness, at the day/night regime with highest germination across species
15 (5/15°C). The means across seasons and sowing months are presented.

16

17 *Experiment 2: Emergence (E2)*

18 Outdoor pot experiments were conducted in two consecutive seasons, using the same batches
19 of seeds as used in E1. There were four 20 cm diameter pots per species, each containing 100
20 seeds sown to a depth of 1 cm in a mixture of commercial potting peat and sand (1:1). Pots
21 were placed in an experimental field in Lleida (University of Lleida). In the case of *D.*
22 *gracile*, only 45 seeds were sown per pot in Y2 because of limited seed availability. From the
23 beginning of the experiment until 31 May when emergence stopped, the soil was watered
24 regularly as needed to keep it moist. Every 2 or 3 days, the number of emerged seedlings was
25 counted in each pot and removed. Experiments were initiated at intervals throughout the
26 season: in Y1, experiments started on 16 November, 20 January and 4 April; in Y2, on 16
27 September, 23 November, 21 January and 22 March (hereinafter called April). Percentages of
28 emergence and total cumulative emergence per pot were calculated. Daily minimum and
29 maximum temperatures were recorded at a nearby local weather station (4.5 km).

30 The relationship between the percentage of cumulative emergence (based on
31 maximum total emergence) and days after sowing (DAS) was described by a four parameter
32 Weibull model:

33

1
$$y = a \left[1 - e^{-\left((x - x_{50} + b \ln 2^{1/c}) / b \right)^c} \right] \quad (\text{Eq. 2})$$

2
3 where y is the percentage of emergence, x is time expressed as DAS, and a , b , c and x_{50} are
4 empirically derived constants: a , is the maximum percentage of emergence recorded, b is the
5 rate of increase, c is a shape parameter and x_{50} is the DAS required to obtain 50% of
6 emergence. To simplify this Weibull model, a was assumed to be 100%. Fitting of the
7 Weibull function was performed using SigmaPlot 11.0. To describe the emergence pattern, x_{10}
8 and x_{90} were calculated as the time that 10% and 90% emergence occurred.

9
10 *Statistical analysis*

11 For each species, an analysis of variance (ANOVA) was used to analyse data from E1 and E2,
12 namely the percentage of germination and the total percentage of cumulative emergence. If
13 required, percentages were arcsine ($\sqrt{(x/100)}$) transformed to reduce non-normality of the
14 dataset distribution. In preliminary analyses, differences between years (data not shown) were
15 found in both experiments. Therefore, the data is analysed for Y1 and Y2 separately.
16 Explanatory variables were month (M), temperature regime (T), light regime (L) and their
17 interactions in E1 and sowing month in E2. Tukey ($P=0.05$) was used as a *post hoc* test.
18 Analyses were done with the program SPSS 15.0 for Windows (SPSS Inc., Chicago IL,
19 USA).

20
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1 **Results**

2

3 The percentage of viable seeds in November were as follows: for *C. orientalis*, 46% ± 8 and
4 92% ± 5 in Y1 and Y2, respectively, for *C. pubescens*, 76% ± 10 and 89% ± 7, for *D. gracile*,
5 46% ± 11 and 55% ± 9, for *D. halteratum ssp. verdunense*, 20% ± 4 and 84% ± 7 and for *N.*
6 *gallica*, 41% ± 6 and 73% ± 10.

7

8 *Temperature and light requirements for germination (E1)*

9 All two way interactions were significant in all cases, except month (M) x light (L) in Y1 for
10 *C. orientalis*, *C. pubescens*, *D. gracile* and *N. gallica*, except for M x temperature (T) for *D.*
11 *gracile* in Y2 and *D. halteratum ssp. verdunense* in Y1 and except for L x T for *N. gallica*.
12 None of the three way interactions was significant, except for *D. gracile* in Y2 (Table 1).

13

14 *Table 1 near here*

15

16 In Y2, the seed dormancy status at the time of seed shed differed between species
17 when seeds germinated in darkness, but not in the 12 h photoperiod where percentages were
18 low (Tables 2, 3, 4). *Consolida orientalis*, *D. halteratum ssp. verdunense* and *N. gallica* had
19 high dormancy levels, while *C. pubescens* and *D. gracile* were partially non-dormant
20 (approximately 50%).

21

22 *Tables 2, 3 & 4 near here*

23

24 For all species in both seasons, germination was significantly higher in complete
25 darkness than with a light regime, usually differing by a factor two (Tables 1, 2, 3, 4). When
26 germination was too low (<10%), no differences were detected. This occurred in most cases
27 for *D. halteratum ssp. verdunense* and at the highest temperature regime for *C. orientalis*
28 (Table 2, 3). ΔG_{light} values were all negative and lower than -50, so all species germinated
29 better in complete darkness. Estimated values were -65 ± 5 for *C. orientalis*, -60 ± 9 for *C.*
30 *pubescens*, -76 ± 8 for *D. gracile*, -95 ± 6 for *D. halteratum ssp. verdunense*, and -79 ± 5 for
31 *N. gallica*.

32 Germination was significantly lower at 10/20°C than at 5/10°C and 5/15°C for *C.*
33 *orientalis* and *C. pubescens* (Table 2). Germination in *D. gracile* increased gradually from

1 5/10°C to 10/20°C (Table 3). No clear patterns were found for *D. halteratum* ssp. *verdunense*
2 and *N. gallica*, with no differences between temperature regimes in Y1 and Y2, respectively
3 (Table 3, 4).

4 Depending on the species, year, light and temperature regime, germination period and
5 dormancy release could differ significantly between months (Tables 1, 2, 3, 4). *Consolida*
6 *orientalis* had lower germination in darkness in April in Y1 and at seed shed (both light
7 regimes) in Y2. *Consolida pubescens* germination decreased in April in Y1 (at the two highest
8 temperature regimes), but increased until January in Y2 (Table 2). For *D. halteratum* ssp.
9 *verdunense*, there was no clear pattern because this species had the lowest percentage of
10 germination across sowing months (from 0% to 8%). Germination in *D. gracile* was similar
11 among months in both light regimes in Y1 (but higher in April at 5/10°C), while in Y2 it
12 tended to be higher in September and April. In darkness, *N. gallica* tended to have an increase
13 in germinability until April of Y2, but with a light regime, differences were lost because of
14 low germination percentages (Table 4).

15 16 *Emergence (E2)*

17 From the beginning of the experiments in September until the end in May, minimum and
18 maximum temperatures recorded were -9.1°C and 34.0°C in Y1 and -8.0°C and 33.3°C in Y2.
19 Autumn was much cooler in Y1 (4.6°C) than in Y2 (8.1°C). Spring was also slightly cooler in
20 Y1 (4.3°C compared with 5.2°C).

21 Overall, for all species, recorded emergence was greater in Y2 than in Y1 (Fig. 1).
22 Lower temperatures in autumn-winter in Y1 could be the main reason for lower observed
23 emergence (Fig. 1). Emergence response to environmental conditions at each sowing time and
24 dormancy release patterns were variable, depending on the species. In both years, seed
25 emergence increased for *Consolida* species in seeds sown from autumn until those sown in
26 January. For *D. gracile*, seed emergence increased until April in Y1 but decreased from
27 November to April in Y2. For *D. halteratum* ssp. *verdunense*, emergence increased until
28 January in Y1, but was constant in Y2. *Nigella gallica* did not have differing emergence
29 patterns between the two years.

30
31 *Fig. 1 near here*
32

1 In all cases, the cumulative emergence patterns as a function of DAS were well
2 described by the Weibull function ($P < 0.001$ and $R^2 > 0.91$, Table 5). Emergence was
3 characterised by an initial flush, followed by more gradual emergence. With the exception of
4 the pots sown in April, x_{10} , x_{50} and x_{90} were higher in Y1 than in Y2, indicating a faster
5 emergence in Y2 (Table 5). Emergence in pots sown in April started earlier ($x_{10} < 30$) than the
6 rest (usually $x_{10} > 40$). For *N. gallica* seeds sown in September in Y2, two marked flushes of
7 emergence were observed (autumn and spring), and the Weibull model did not converge.
8 Therefore, each flush was modelled separately ($P < 0.001$ and $R^2 > 0.98$), namely September Y2
9 (autumn) and September Y2 (spring) (Table 5, Fig. 2). In the case of *C. orientalis*, no plants
10 emerged in pots sown in April of both years and in September of Y2.

11
12 *Table 5, Fig. 2 near here*
13
14

15 Discussion

16
17 The germination and emergence patterns indicated that the five species can be separated into
18 two groups: (1) those with obligate germination mainly in autumn-winter (*C. orientalis* and *C.*
19 *pubescens*) and (2) facultative autumn-winter annuals (*D. halteratum* ssp. *verdunense*, *D.*
20 *gracile* and *N. gallica*), which can germinate in spring as well as in autumn (Baskin & Baskin,
21 1998). A lower thermal requirement was recorded for *Consolida* species, which is in
22 accordance with an emergence pattern in autumn and winter. In contrast, the other species had
23 a higher thermal requirement for germination (highest in *D. gracile*).

24 The observed requirements for germination and emergence patterns of seeds sown in
25 different months are important for establishing conservation strategies. All species had a dark
26 requirement for germination; without light they germinated to a greater extent (Baskin &
27 Baskin, 1998). Therefore, a superficial tillage operation soon after seed shed or crop harvest,
28 burying seeds in the soil but close to the soil surface, should facilitate emergence. Since
29 optimal tillage depths for these species are unknown, more research is required in this
30 direction.

31 In no-till fields, too low numbers of seeds may be buried in the soil to ensure sufficient
32 emergence during the next period for emergence. This could be especially true for the late
33 maturing species, *D. halteratum* ssp. *verdunense* and *N. gallica*, which produce seeds in mid-

1 to late September from plants re-grown after harvest in the cereal stubble (pers. obs. J Torra).
2 Therefore, it is feasible that no-till is not the most suitable soil management option for
3 preserving these species. Unfortunately, the plant densities that are necessary to ensure seed
4 production and seed bank replenishment, thus guaranteeing population viability, are unknown.
5 In the case of *D. halteratum* ssp. *verdunense* and *N. gallica*, not all seeds on the mother plant
6 had fully ripened yet. Delayed tillage of the stubble could therefore give all flowers time to
7 produce mature seeds (Epperlein *et al.*, 2014).

8 The second important decision with regard to the conservation of these species
9 concerns the optimal time of sowing in relation to the sowing time of the crop and the
10 emergence peak of competitive weeds. Results indicate that the rare species could
11 successfully be re-introduced with an early sowing in autumn, because seeds did not
12 germinate until at least one month after sowing (x_{10} , Table 5), and in most cases they required
13 at least 50 days to achieve 90% emergence (x_{90} , Table 5). However, a word of caution is
14 required. In our experiments, seeds were not “conditioned” by natural weather and soil
15 conditions, as they had been stored at room temperature. Seeds that are naturally released
16 could germinate outside the range of months tested in this study. Repeated seeding in multiple
17 years is therefore advisable. Delayed sowing of the crop for preservation could be another
18 management option for two reasons. Firstly, the target species seem to be well-adapted to
19 delayed sowing of the crop in late autumn or early winter. Flexibility with regard to the
20 sowing date of the crop indicates that these species could do well in diverse crop rotations, for
21 example, in rotations that include leguminous crops that would not outcompete these rare
22 species (Ianucci *et al.*, 2008). Secondly, later sowing of the crop could reduce competition
23 between the rare arable plants and dominant weeds, which have an emergence peak in early
24 autumn (García *et al.*, 2014). The success of re-introduction efforts with seeds will depend
25 heavily on competition with dominant species in the local seed bank (Tschardtke *et al.*, 2011),
26 and delayed sowing may alleviate this problem.

27 Information about weed emergence patterns is also critical for deciding when and
28 which herbicides can be applied. Chemical management can be important for controlling
29 pernicious weeds, while conserving desired arable species (Storkey & Westbury, 2007).
30 Facultative winter germinating species (*Delphinium* and *Nigella* species) would better survive
31 pre-emergence herbicides with a short residual effect, or early-post-emergence herbicides,
32 because a large part of the population could avoid these chemical treatments by emerging
33 later, in winter or spring.

1 The knowledge acquired in this study on the germination ecology of these
2 *Ranunculaceae* species is important. This information should, however, be broadened with
3 trials verifying its usefulness in the development of conservation and management strategies
4 in arable fields in terms of sowing dates, crop rotations, herbicides or tillage. This study
5 focuses on the first stages of plant life. More studies are required to better understand how
6 plant growth, reproductive fitness and seed bank dynamics are affected by agricultural
7 practices, as this information is crucial for designing successful conservation strategies.

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Table 1 *P* values for main effects and interactions (NS: non-significant) in E1 germination experiment on seed germination of five arable *Ranunculaceae* plants in two seasons (Y1, 2010/11; Y2 2011/12) analysed by ANOVA

| Season | <i>Consolida orientalis</i> | | <i>Consolida pubescens</i> | | <i>Delphinium gracile</i> | | <i>Delphinium halteratum</i> ssp. <i>verdunense</i> | | <i>Nigella gallica</i> | |
|-----------|-----------------------------|--------|----------------------------|--------|---------------------------|--------|---|--------|------------------------|--------|
| | Y1 | Y2 | Y1 | Y2 | Y1 | Y2 | Y1 | Y2 | Y1 | Y2 |
| Month (M) | NS | <0.001 | <0.001 | <0.001 | 0.003 | <0.001 | 0.002 | <0.001 | <0.001 | <0.001 |
| Light (L) | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| T°C (T) | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | NS | 0.001 | <0.001 | 0.162 |
| M x L | NS | <0.001 | NS | <0.001 | NS | <0.001 | 0.01 | <0.001 | NS | 0.006 |
| M x T | 0.001 | <0.001 | 0.008 | <0.001 | <0.001 | NS | NS | <0.001 | NS | NS |
| L x T | 0.004 | <0.001 | <0.001 | <0.001 | <0.001 | 0.003 | 0.007 | <0.001 | NS | NS |
| M x L x T | NS | NS | NS | NS | NS | <0.001 | NS | NS | NS | NS |

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2 Table 2 Seed germination (% \pm SEM) of *Consolida orientalis* and *Consolida pubescens* in Petri dishes (n=4) in growth chambers in two light regimes (12 h
3 photoperiod, Light; complete darkness, Dark), and three different temperature regimes (5/10°C, 5/15°C, and 10/20°C day/night temperatures). Experiments
4 were done in two consecutive seasons, 2010/11 (Y1) and 2011/12 (Y2) after seed shed, and in September, November, January and April (late March in Y1).
5 Different letters denote statistical differences (Tukey test, $P < 0.05$) between months within a temperature and light regime. Columns between years: * ($P <$
6 0.05), ** ($P < 0.01$) or *** ($P < 0.001$) if germination was significantly different (Tukey test) between light regimes; NS, there were no differences. Last row
7 denotes statistical differences (Tukey test) between temperature regimes within a year and light regime irrespective of the month

| | | <i>Consolida orientalis</i> | | | | | <i>Consolida pubescens</i> | | | | | | |
|---------|-----------|-----------------------------|--------------|--------------|---------------|--------------|----------------------------|----------------|--------------|---------------|--------------|--------------|-----|
| | | Y1 | | Y2 | | | Y1 | | Y2 | | | | |
| | | Light | Dark | Light | Dark | | Light | Dark | Light | Dark | | | |
| 5/10°C | At shed | - | - | 0 \pm 0 a | 11 \pm 2 a | *** | - | - | 12 \pm 3 a | 60 \pm 2 a | *** | | |
| | September | - | - | 22 \pm 5 c | 40 \pm 6 b | * | - | - | 33 \pm 9 b | 64 \pm 4 a | * | | |
| | November | 16 \pm 7 a | 36 \pm 6 b | * | 6 \pm 2 b | 54 \pm 9 b | *** | 14 \pm 3 a | 72 \pm 2 a | *** | 1 \pm 1 a | 55 \pm 4 a | *** |
| | January | 12 \pm 8 a | 34 \pm 9 b | NS | 12 \pm 3 bc | 51 \pm 6 b | *** | 17 \pm 10 a | 71 \pm 8 a | *** | 23 \pm 2 b | 81 \pm 3 b | *** |
| | April | 4 \pm 3 a | 12 \pm 5 a | NS | 21 \pm 8 bc | 55 \pm 7 b | * | 12 \pm 7 a | 70 \pm 5 a | *** | 8 \pm 3 a | 55 \pm 6 a | *** |
| 5/15°C | At shed | - | - | 0 \pm 0 a | 0 \pm 0 a | NS | - | - | 7 \pm 4 a | 50 \pm 1 a | *** | | |
| | September | - | - | 8 \pm 4 b | 20 \pm 5 b | NS | - | - | 33 \pm 6 c | 62 \pm 10 b | * | | |
| | November | 6 \pm 3 a | 22 \pm 2 a | * | 4 \pm 2 b | 27 \pm 1 b | *** | 31 \pm 9 ab | 67 \pm 8 b | * | 0 \pm 0 a | 75 \pm 2 b | *** |
| | January | 5 \pm 3 a | 29 \pm 7 a | * | 5 \pm 3 b | 26 \pm 4 b | ** | 67 \pm 7 ab | 71 \pm 7 b | *** | 23 \pm 2 b | 81 \pm 3 b | *** |
| | April | 3 \pm 2 a | 16 \pm 7 a | ** | 5 \pm 3 b | 36 \pm 2 b | * | 15 \pm 3 a | 51 \pm 3 a | *** | 10 \pm 1 b | 53 \pm 8 a | *** |
| 10/20°C | At shed | - | - | 0 \pm 0 a | 0 \pm 0 a | NS | - | - | 0 \pm 0 a | 2 \pm 1 a | NS | | |
| | September | - | - | 0 \pm 0 a | 0 \pm 0 a | NS | - | - | 25 \pm 4 c | 33 \pm 6 b | NS | | |
| | November | 0 \pm 0 a | 0 \pm 0 a | NS | 0 \pm 0 a | 0 \pm 0 a | NS | 28 \pm 3 b | 37 \pm 9 a | NS | 0 \pm 0 a | 39 \pm 5 b | *** |
| | January | 0 \pm 0 a | 4 \pm 3 a | NS | 0 \pm 0 a | 2 \pm 1 a | NS | 11 \pm 10 ab | 31 \pm 5 a | NS | 4 \pm 2 b | 47 \pm 8 b | *** |
| | April | 0 \pm 0 a | 2 \pm 1 a | NS | 0 \pm 0 a | 6 \pm 4 a | NS | 3 \pm 1 a | 27 \pm 5 a | ** | 0 \pm 0 a | 8 \pm 4 a | * |
| | | <0.001 | <0.001 | <0.001 | <0.001 | | <0.001 | <0.001 | <0.001 | <0.001 | | | |

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2 **Table 3** Seed germination (% \pm SEM) of *Delphinium gracile* and *Delphinium halteratum* ssp. *verdunense* in growth chambers in two consecutive seasons
 3 after seed shed at intervals (\dagger seed shed for *D. halteratum* ssp. *verdunense*). See Table 2 for details

| | | <i>Delphinium gracile</i> | | | | | <i>Delphinium halteratum</i> ssp. <i>verdunense</i> | | | | | |
|---------|---------------------|---------------------------|---------------|---------------|---------------|---------------|---|-------------|--------------|-------------|-------------|-------------|
| | | Y1 | | Y2 | | | Y1 | | Y2 | | | |
| | | Light | Dark | Light | Dark | | Light | Dark | Light | Dark | | |
| 5/10°C | At shed | - | - | 5 \pm 4 a | 27 \pm 7 b | * | - | - | - | - | NS | |
| | September \dagger | - | - | 0 \pm 0 a | 33 \pm 10 b | ** | - | - | 0 \pm 0 a | 1 \pm 1 a | NS | |
| | November | 0 \pm 0 a | 33 \pm 7 a | *** | 0 \pm 0 a | 0 \pm 0 a | NS | 0 \pm 0 a | 0 \pm 0 a | 0 \pm 0 a | NS | |
| | January | 0 \pm 0 a | 14 \pm 4 a | *** | 0 \pm 0 a | 25 \pm 7 b | ** | 0 \pm 0 a | 3 \pm 2 ab | 0 \pm 0 a | 3 \pm 2 a | NS |
| | April | 9 \pm 2 b | 44 \pm 10 b | ** | 15 \pm 3 b | 8 \pm 6 a | NS | 0 \pm 0 a | 9 \pm 3 b | ** | 0 \pm 0 a | 0 \pm 0 a |
| 5/15°C | At shed | - | - | 2 \pm 2 a | 32 \pm 4 b | ** | - | - | - | - | *** | |
| | September* | - | - | 5 \pm 3 a | 43 \pm 10 b | ** | - | - | 0 \pm 0 a | 8 \pm 3 a | *** | |
| | November | 5 \pm 2 a | 38 \pm 2 a | *** | 0 \pm 0 a | 5 \pm 6 a | NS | 0 \pm 0 a | 2 \pm 1 a | 0 \pm 0 a | 5 \pm 1 a | *** |
| | January | 15 \pm 2 a | 46 \pm 4 a | *** | 0 \pm 0 a | 45 \pm 7 b | *** | 2 \pm 1 a | 9 \pm 5 a | 0 \pm 0 a | 2 \pm 1 a | NS |
| | April | 14 \pm 5 a | 38 \pm 4 a | ** | 5 \pm 3 a | 25 \pm 7 b | * | 0 \pm 0 a | 5 \pm 1 a | * | 0 \pm 0 a | 5 \pm 4 a |
| 10/20°C | At shed | - | - | 15 \pm 2 ab | 47 \pm 5 a | * | - | - | - | - | NS | |
| | September* | - | - | 35 \pm 7 b | 50 \pm 5 a | NS | - | - | 2 \pm 2 a | 2 \pm 2 a | NS | |
| | November | 39 \pm 3 a | 43 \pm 5 a | NS | 8 \pm 6 a | 35 \pm 10 a | * | 0 \pm 0 a | 0 \pm 0 a | 0 \pm 0 a | 4 \pm 2 a | * |
| | January | 37 \pm 3 a | 36 \pm 7 a | NS | 0 \pm 0 a | 55 \pm 7 a | *** | 5 \pm 3 a | 2 \pm 2 a | 0 \pm 0 a | 1 \pm 1 a | NS |
| | April | 28 \pm 6 a | 46 \pm 6 a | * | 10 \pm 5 ab | 53 \pm 6 a | ** | 0 \pm 0 a | 2 \pm 1 a | NS | 3 \pm 2 a | 1 \pm 1 a |
| | | <0.001 | <0.001 | <0.001 | <0.001 | | NS | NS | <0.001 | <0.001 | | |

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Table 4 Seed germination (% \pm SEM) of *Nigella gallica* in growth chambers in two consecutive seasons after seed shed at intervals. See Table 2 for details

| | | Y1 | | | Y2 | | |
|---------|----------|--------------|--------------|-----|-------------|---------------|-----|
| | | Light | Dark | | Light | Dark | |
| 5/10°C | At shed | - | - | | 2 \pm 1 a | 11 \pm 1 a | ** |
| | November | 0 \pm 0 a | 26 \pm 3 b | *** | 0 \pm 0 a | 12 \pm 2 ab | *** |
| | January | 0 \pm 0 a | 12 \pm 2 a | *** | 0 \pm 0 a | 14 \pm 7 a | * |
| | April | - | - | | 3 \pm 2 a | 35 \pm 6 b | ** |
| 5/15°C | At shed | - | - | | 3 \pm 2 a | 23 \pm 5 ab | ** |
| | November | 8 \pm 0 a | 41 \pm 5 a | *** | 2 \pm 1 a | 14 \pm 4 a | * |
| | January | 5 \pm 4 a | 31 \pm 5 a | ** | 0 \pm 0 a | 13 \pm 4 a | *** |
| | April | - | - | | 3 \pm 2 a | 32 \pm 4 b | ** |
| 10/20°C | At shed | - | - | | 3 \pm 2 a | 15 \pm 3 a | * |
| | November | 7 \pm 3 a | 4 \pm 3 a | ** | 3 \pm 1 a | 11 \pm 2 a | * |
| | January | 35 \pm 3 b | 21 \pm 3 a | * | 0 \pm 0 a | 10 \pm 4 a | * |
| | April | - | - | | 0 \pm 0 a | 21 \pm 5 a | *** |
| | | <0.001 | <0.001 | | NS | NS | |

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2 **Table 5** Parameter estimates of cumulative emergence as a function of days after sowing
 3 estimated with the Weibull model for five arable *Ranunculaceae* plants sown in outdoor pots
 4 at intervals during two seasons (2010/11, Y1; 2011/12, Y2). Parameter a was assumed to be
 5 100%. X_{10} and x_{90} , when 10% and 90% of emergences occurred according to the model

| | | | Model parameters | | | | | |
|--|---------|--------------------|------------------|-------|----------|-------|----------|----------|
| | | | b | c | x_{50} | R^2 | x_{10} | x_{90} |
| <i>Consolida orientalis</i> | Y1 | November | 7.52 | 0.62 | 58.90 | 0.99 | 54.94 | 83.69 |
| | | January | 29.07 | 1.99 | 64.32 | 0.99 | 49.53 | 84.35 |
| | | April | - | - | - | - | - | - |
| | Y2 | September | - | - | - | - | - | - |
| | | November | 10.50 | 0.94 | 34.65 | 0.92 | 28.50 | 52.99 |
| | | January | 10.67 | 1.14 | 53.04 | 1.00 | 46.79 | 67.45 |
| | April | - | - | - | - | - | - | |
| <i>Consolida pubescens</i> | Y1 | November | 13.52 | 1.07 | 64.01 | 1.00 | 56.06 | 83.95 |
| | | January | 42.60 | 3.56 | 69.11 | 1.00 | 53.30 | 84.54 |
| | | April | 3.41 | 0.43 | 8.27 | 0.98 | 6.85 | 31.10 |
| | Y2 | September | 1.14 | 0.28 | 52.30 | 1.00 | 52.00 | 75.40 |
| | | November | 2.50 | 0.53 | 35.23 | 1.00 | 32.04 | 46.00 |
| | | January | 8.31 | 1.08 | 55.66 | 1.00 | 50.77 | 67.71 |
| | April | 16.32 | 1.26 | 33.95 | 0.98 | 24.49 | 53.30 | |
| <i>Delphinium gracile</i> | Y1 | November | 2.76 | 0.29 | 57.40 | 0.91 | 56.61 | 104.30 |
| | | January | 35.59 | 2.89 | 72.28 | 1.00 | 57.27 | 88.42 |
| | | April | 0.17 | 0.24 | 3.99 | 1.00 | 3.00 | 9.22 |
| | Y2 | September | 43.61 | 6.15 | 42.17 | 1.00 | 31.33 | 51.03 |
| | | November | 4.04 | 0.63 | 35.92 | 1.00 | 33.55 | 48.96 |
| | | January | 6.48 | 0.86 | 56.48 | 1.00 | 52.73 | 69.36 |
| | April | 10.99 | 2.39 | 22.35 | 1.00 | 17.21 | 28.50 | |
| <i>Delphinium halteratum ssp. verdunense</i> | Y1 | November | 11.40 | 0.98 | 66.79 | 0.99 | 60.10 | 85.68 |
| | | January | 11.08 | 2.44 | 80.38 | 0.99 | 75.26 | 86.44 |
| | | April | 0.12 | 0.24 | 7.01 | 1.00 | 6.98 | 10.62 |
| | Y2 | September | 1.62 | 0.29 | 55.46 | 0.99 | 55.00 | 83.80 |
| | | November | 5.11 | 0.93 | 45.90 | 1.00 | 42.00 | 55.02 |
| | | January | 15.22 | 1.20 | 59.93 | 1.00 | 51.04 | 79.25 |
| | April | 16.09 | 3.00 | 22.49 | 1.00 | 15.86 | 29.50 | |
| <i>Nigella gallica</i> | Y1 | November | 17.49 | 1.05 | 94.02 | 0.99 | 83.74 | 120.36 |
| | | January | 27.04 | 94.02 | 72.52 | 0.99 | 59.90 | 87.58 |
| | | April | - | - | - | - | - | - |
| | Y2 | September (autumn) | 8.75 | 0.74 | 47.80 | 0.99 | 42.89 | 69.47 |
| | | September (spring) | 2.44 | 0.62 | 5.34 | 0.98 | 4.05 | 13.48 |
| | | November | 12.27 | 0.90 | 50.23 | 1.00 | 43.08 | 73.22 |
| | January | 3.40 | 0.61 | 54.42 | 1.00 | 52.64 | 65.76 | |
| | April | 1.67 | 0.50 | 23.47 | 1.00 | 22.69 | 31.56 | |

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