Rate of Change in Dormancy Level and Light Requirement in Weed Seeds During Stratification

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The rate of change in dormancy level and light requirement, induced during stratification at 3-2 °C, was investigated in seeds of Stellaria media, Cerastium fontanum, Veronica agrestis and Taraxacum officinale. Two stratification environments, soil and wet filter paper in petri-dishes, were used. On nine occasions during a 6 week stratification period, germination was tested under three light conditions at 3:5/18:5 °C: (1) darkness; (2) light; and (3) short-term light exposure followed by darkness. Prior to stratification, germination in all species was ≥ 89% in light and ≥ 53% in darkness. Within 2 weeks of stratification, germinability in V. agrestis and S. media seeds decreased in all treatments. In C. fontanum and T. officinale seeds, germinability also decreased after 2 weeks but only in the dark treatment, indicating induction of a light requirement. After two more weeks of stratification, the induced dormancy in S. media seeds stratified in soil became weaker and the light requirement in T. officinale seeds was lost. Differences between the two stratification environments and/or interactions between light conditions, stratification environments and time were found for all species. These results suggest that: (1) the dormancy level and the light requirement of seeds may change dramatically over relatively short-time periods during stratification; and (2) germinability depends on the stratification environment experienced by the seeds. Predicting the dormancy level or light requirement in a seed batch is difficult and requires a thorough knowledge of the effect of the stratification conditions used.

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Key words: Burial, Cerastium fontanum Baumg., chickweed, cold stratification, common mouse-ear, dandelion, dormancy, light requirement, seed, Stellaria media (L.) Vill., speedwell, Taraxacum officinale group, Veronica agrestis L., weed.

INTRODUCTION

One of the most important means by which weeds survive is their ability to accumulate seeds in the soil. Persistence of seeds in the soil depends on mechanisms to prevent germination of buried seeds. Consequently, many weed seeds become strongly dormant, and some gain a light requirement for germination after burial.

The effect of burial on the requirement for light to germinate is well documented. Wesson and Wareing (1969) demonstrated the induction of a light requirement by burial in previously non-light-requiring weed seeds. Scopel, Ballaré and Sánchez (1991) showed that an enormous increase in light sensitivity was induced, within 2 months of burial, in seeds of Datura ferox. In addition, there are reports on loss and regain of a light requirement for germination during burial (e.g. Taylorsson, 1972), as well as during artificial dark-incubation outdoors (Bouwmeester and Karssen, 1989). Patterns of seasonal changes in the dormancy level over years have also been demonstrated for several species (e.g. Baskin and Baskin, 1985; Pons, 1991a; Milberg, 1994a, b).

Temperature also has an impact on the germinability of imbibed seeds. Pons (1991b), noted that temperature has a great effect on the rate of induction of light requirement; at low temperatures the process slows down and it can take over 2 months. However, in Plantago major seeds incubated in polyethylene glycol at 4 °C, the process took only 7 d (Pons, 1991b). Most available information on how low temperatures affect dormancy involves spring germinating species, mainly summer annuals. In such species, cold is one of the main factors that relieves dormancy (Roberts, 1981; Baskin and Baskin, 1985; Milberg, 1994b), and this often happens over a time span of a month or more (Baskin, Baskin and Chester, 1995; Vleeshouvers, Bouwmeester and Karssen, 1995). Fewer studies have been done on autumn germinating species, thus, knowledge about the effect of cold on these species is scant. Generally, low temperatures are considered to induce dormancy in winter annual species (e.g. Baskin and Baskin, 1986), but the rate at which dormancy can be induced is not well known, and changes in dormancy have been considered only during seasonal cycles.

A remaining question regards the seed’s environment during stratification. Under laboratory conditions stratification is usually conducted on filter paper in petri-dishes; it is assumed that this simulates burial in soil. However, the soil environment contains important chemical and biological factors that can affect germination (Roberts, 1981), and whose absence in tests on filter paper might give misleading results. Thus, uncertainties remain as to whether seed stratification in petri-dishes on filter paper resembles that in soil.

This study was designed to examine the effect of the length of stratification on (a) induction of dormancy and (b)
induction of a light requirement for germination, in seeds of
four autumn-germinating weed species lacking or with a
weak primary dormancy. Germination tests were conducted
in light and darkness, and after a short-term light exposure,
to document possible changes in light requirement during
stratification. Two stratification environments, soil and
petri-dishes, were compared to verify whether or not seed
stratification in petri-dishes resembles stratification in soil.
The rate of dormancy release in summer annuals and in
other species with primary seed dormancy is, generally, a
slow process, occurring over several weeks (Baskin et al.,
1995; Vleeshouwers et al., 1995). Thus, the rate of dormancy
induction in species lacking primary dormancy might be
expected to be similar. However, for a light requirement to
exert a meaningful function it should be induced quickly;
over a period of days or a few weeks. A slower process
would not help to prevent germination in shed seeds that
have become buried.

**MATERIALS AND METHODS**

The species chosen showed an increase in dormancy level or
in light requirement after stratification in previous experi-
ments (Milberg, Andersson and Noronha, 1996; Andersson,
Milberg and Noronha, 1997).

Seeds were collected in the first half of August 1994 in
southern Sweden and initially dried (approx. 10 d) at room
temperature (approx. 22°C). Then they were dry-stored in
a room at 22 ± 1°C and 30% relative humidity for 5
weeks. On 23 Sep. 1994, the seeds were transferred to a cold
room (3.2°C) until May 1995 when this study commenced.
The germination percentage in light and darkness of these
seed batches did not change during storage (data not
shown).

**Pre-treatments**

**Stratification in soil.** Fifty seeds of *Stellaria media* (L.) Vill.
(chickweed), *Cerastium fontanum* Baumg. (common mouse-
ear) and *Taraxacum officinale* group (dandelion), and 40
seeds of *Veronica agrestis* L. (speedwell) were placed in fine-
mesh polyester bags (6 x 6 cm). For each species, 81 bags
were buried in a moistened peat soil (70% *Sphagnum*-peat,
30% sand), in plastic pots (10 x 10 x 7 cm). Three groups of
four bags, one bag for each species, were placed in each pot
and the pots transferred to a dark room at 3.2 ± 0.5°C. Due
to a power failure for 6 h, the air temperature in the room
rose to 8.1°C for 1 h after 15 d of stratification.

**Stratification in petri-dishes.** For each species the same
number of seeds described above were placed in each of 81
petri-dishes (9 cm diameter) with moistened (40 ml de-
ionized water) filter paper (two Munktell 1003, 9 cm
diameter). The dishes were wrapped in aluminium foil in
groups of four, one for each species, and stored parallel to
the pots.

**Germination tests**

Germination tests were started after 1, 2, 4, 8, 15, 22, 29,
36 and 43 d of stratification. The seeds were subjected to
one of three treatments: (1) light; (2) darkness; or (3) short-
term (5 s) light exposure followed by darkness (STLE),
described below. During the germination tests, all the seeds
were in petri-dishes with filter paper as described above. All
handling of dishes in the dark and short-term exposure
treatments was conducted in complete darkness.

On each sampling day, three pots and nine groups of
wrapped dishes were taken from the cold room. From each
pot two groups of bags, one for the dark and the other for
the STLE treatment were exhumed, and the seeds transferred
to petri-dishes. The seeds in the remaining group of bags in
each pot were transferred to petri-dishes in light and
germination during stratification was recorded. These dishes
were then sealed with parafilm.

In the dark treatment, the four dishes (one dish of each
species) were immediately and individually wrapped in a
layer of aluminium foil and then wrapped, all four together,
with a second layer of foil. In the short-term light exposure
treatment, each dish (without lid) was exposed for 5 s to a
photon flux density (PAR) of 210 ± 10 μmol m⁻² s⁻¹ (red
light: 18 ± 1 μmol m⁻² s⁻¹; far-red light: 23 ± 1 μmol m⁻² s⁻¹;
ratio R/FR = 0.85). The light source was a xenon bulb
(OSRAM XBO 150 W/1) with a Bausch & Lomb armature.
The light bulb was in a light-proof box that was air-cooled.
Seeds were exposed by letting light pass through a hole and
the length of the exposure was measured with a stopwatch.
Immediately after being exposed, each dish was individually
wrapped in a layer of aluminium foil and then all four
wrapped together in a second layer of foil.

From the nine groups of wrapped dishes, three were
assigned to the light, three to the dark and three to the
STLE treatment. The treatments were conducted as de-
scribed above. In the dark treatment, the lids of the dishes
were taken off to allow a short aeration (approx. 5 s) before
being wrapped again. In the dishes assigned to the light
treatment germination during stratification was recorded.

All germination tests were conducted in a room with 14 h
of light (PAR 105 ± 1.5 μmol m⁻² s⁻¹; ratio R/FR = 8)
provided by cool white fluorescent tubes (OSRAM L65W/
20R) and 10 h of darkness. The temperature was 3.5 ± 1°C
for 9 h, during the dark period, and 18 ± 2°C for 11 h,
coinciding with the light period. Transitions between these
temperatures accounted for the other 4 h.

During the germination period, 3 to 4 weeks, the
germinated seeds in the light treatment were recorded and
removed regularly. The emergence of the radicle was used as
the criterion for germination. At the end of the germination
period, the dishes wrapped in aluminium foil were opened
and the germinated seeds were counted. Ungerminated
seeds were categorized as ‘alive’ or ‘dead’. The distinction
between categories was based on seed firmness (firm vs. soft)
and endosperm properties (presence *vs.* absence of a white
and hard endosperm), determined by squeezing the seeds
with a pair of tweezers.

**Control tests**

During the 6 weeks of stratification, the germinability of
dry-stored seeds, stored in the cold (3°C) parallel to the
seeds under stratification, was tested on three occasions (0,
29 and 43 d), in light and darkness. Batches of 50 seeds of *Stellaria media*, *Cerastium fontanum* and *Taraxacum officinale*, and 40 seeds of *Veronica agrestis*, were placed in petri-dishes as described above and transferred to the germination room. For each species there were six dishes: three were sealed with parafilm and assigned to test germination in light, the other three were wrapped in aluminium foil in groups of four (one dish of each species) and used to test germination in darkness.

**Statistics**

Data for each species were analysed separately by logistic regression, using the LOGISTIC procedure of SAS (1989). For each petri-dish and date, the proportion of germinated seeds, of those viable, was modelled as a function of light conditions, stratification environment, length of stratification and the following interactions: light conditions × length of stratification; stratification environment × length of stratification; and light conditions × stratification conditions × length of stratification.

**RESULTS**

**Germination tests**

*Stellaria media*. At the beginning of the experiment almost 100% of viable seeds germinated in all conditions tested (Table 1, Fig. 1). After 2 weeks of stratification, germination was reduced in all treatments (by 60–65% in light, approx. 70% in STLE and approx. 75% in darkness), indicating the induction of a high dormancy level (Fig. 1). In addition, the seeds became light sensitive as indicated by reduced germination in darkness compared to light or STLE treatments, especially in the seeds stratified in soil (Fig. 1B).

In the seeds subjected to stratification in petri-dishes, the high dormancy level induced showed no significant changes during the rest of the experimental period (Fig. 1A). On the contrary, seeds subjected to stratification in soil showed weaker dormancy after 1 month of stratification, but the seeds remained light-requiring throughout the experimental period (Fig. 1B).

Differences in seed germination were found between stratification conducted in soil and in petri-dishes (Table 2), and interactions were found between the stratification environment, light conditions and time (Table 2). This suggests that the changes in germinability over time detected in the different light treatments depended on the stratification environment.

After 2 and 3 weeks of stratification in petri-dishes and in soil, respectively, a proportion of seeds started to germinate while under stratification (≤18%). This proportion was significantly higher (t-test, $P < 0.05$) in the petri-dish pre-treatment (average 14%) than in the soil pre-treatment (average 8%). During the experimental period there was no significant increase in the proportion of seeds that germinated during stratification (Spearman rank correlation, $P > 0.05$).

| Table 1. Germination percentage (± s.e.) of the dry-stored seeds used in the control tests |
|---------------------------------------------|-------------|-------------|-------------|-------------|
| Light                                      | Stellaria media | Veronica agrestis | Cerastium fontanum | Taraxacum officinale |
| Initial                                   | 97 ± 1       | 89 ± 2       | 89 ± 4       | 99 ± 1      |
| 29 d                                      | 99 ± 1       | 81 ± 2       | 71 ± 4       | 100         |
| 43 d                                      | 98 ± 2       | 79 ± 4       | 44 ± 3       | 100         |
| Darkness                                  | 98 ± 1       | 53 ± 13      | 58 ± 5       | 78 ± 3      |
| Initial                                   | 98 ± 1       | 50 ± 6       | 46 ± 8       | 84 ± 2      |
| 29 d                                      | 98 ± 1       | 48 ± 3       | 33 ± 1       | 78 ± 2      |

Values are the average of three replicates with 50 seeds each (40 for *Veronica agrestis*).
Veronica agrestis. Within 2 weeks of stratification, germination of viable seeds decreased by >60% in seeds stratified in petri-dishes and by >80% in soil-stratified seeds (Fig. 2). This suggests the induction of a high dormancy level, which persisted throughout the experimental period.

The initial responsiveness to light (germination in light was almost double that in darkness) manifested by the *Veronica agrestis* seeds (Table 1, Fig. 2), practically disappeared within the first 8 d of stratification in the seeds stratified in petri-dishes (Fig. 2A). In soil-stratified seeds it remained detectable until the end of the experiment (Fig. 2B).

There were differences in germination between the light treatments but not between the two stratification environments (Table 2). However, the changes in germinability occurring over time were dependent on the stratification environment and the treatments, shown by significant interactions between seed environment, light conditions and time (Table 2).

During stratification, some germination started to occur after 1 month in the petri-dishes (average 14%). In the soil, however, no germination occurred during stratification.

*Cerastium fontanum*. During the 6 weeks of stratification, no substantial changes in germinability were detected in the light or STLE treatments although germination did increase slightly with the length of stratification when the seeds were subjected to the soil pre-treatment (Fig. 3B), and decreased slightly when stratification was conducted in petri-dishes (Fig. 3A). However, after 2 weeks of stratification, germination in the dark treatment was strongly reduced (Fig. 3) to almost 0%, indicating that an almost complete dependence on light for germination was induced. Only approx. 60% of the seeds tested were sensitive to short-term light exposure, whereas germination in the light treatment was approx. 80%.

The different light treatments, as well as the stratification environments, affected seed germination (Table 2). The interaction between light condition and time, as well as that between the stratification environment and time, was significant (Table 2). Thus, alterations in germinability over the stratification period differed between treatments and also between stratification environments. However, no interactions were found between light condition, stratification environment and time (Table 2), suggesting that, over time, the pattern of germination response to the different treatments was similar in both stratification environments.

No germination of *C. fontanum* occurred during stratification.

*Taraxacum officinale*. At the beginning of the experiment, approx. 25% of the viable seeds showed a light requirement for germination (Table 1, Fig. 4). The stratification induced a slight increase in this requirement which occurred within 1 week in the soil, where approx. 50% of the seeds failed to germinate in darkness (Fig. 4B) and within 2 weeks in the
petri-dishes, where approx. 40% of the seeds failed to germinate in darkness (Fig. 4A). After these periods, the light requirement gradually decreased during the next 2 weeks of stratification, and germination in darkness became almost equal to that in the light and STLE treatments.

During the first 2 d of stratification, germination in STLE was similar to germination in darkness, after which the seeds became more sensitive to the short-term light exposure until germination in the STLE treatment equalled germination in light (Fig. 4).

Differences were found in the germination response to the different light treatments and to the different pre-treatments (Table 2). There were also interactions between the pre-treatments and time, indicating that changes in germinability over time depended on the pre-treatment.

In the fifth and sixth weeks of stratification, some seeds germinated while under stratification in both pre-treatments (average 5%). For *T. officinale*, the proportion of dead seeds was significantly higher (paired t-test, \( P < 0.01 \)) after stratification in soil (average 30.5%) than after stratification in petri-dishes (average 17.0%). This proportion did not increase during the experimental period (Spearman rank correlation). Furthermore, a reduction in vitality was observed in seedlings resulting from seeds stratified in soil compared to those stratified in petri-dishes (pers. obs.).

**Control tests**

During the 6 weeks of stratification, germinability of dry-stored seeds did not change (Table 1), with the exception of
seeds of *C. fontanum*. For this species the germinability decreased in both light and darkness (*t*-test, *P* < 0.001 and *P* < 0.01, respectively).

**DISCUSSION**

**Induction of dormancy and a light-requirement**

Seeds of most of the species that are strict or facultative autumn germinative become dormant or conditionally dormant during cold stratification (Baskin and Baskin, 1988, 1989). Therefore, the high level of dormancy induced in seeds of *S. media* and *V. agrestis* (Figs 1 and 2), was expected after stratification at 3 °C. However, the rate of dormancy induction brings new information on this phenomenon. It shows that the mechanisms involved in the process of induction can act within days and not months as is often assumed in connection with induction or alleviation of dormancy (e.g. Vleeshouwer et al., 1995). Also the induced light requirement in seeds of *C. fontanum* and *T. officinale* (Figs 3 and 4) was expected, since a light requirement can be induced by burial (e.g. Wesson and Wareing, 1969) or by dark incubation (Bouwmeester and Karssen, 1989).

The ecological importance of the induction of a high dormancy level and a light requirement is the prevention of germination in conditions unfavourable for seedling development. The fact that this induction occurred within days is important as a slower process would probably mean more seeds germinating before they acquired a light requirement or became strongly dormant. In the field, this would lead to more seeds germinating when the conditions are unfavourable for seedling development. Rapid establishment of a light requirement also facilitates the build up of a persistent soil seed bank (e.g. Pons, 1991b).

Rapid induction of a light requirement can be particularly vital for autumn germinating species. When dormancy is induced in seeds of these species, they normally lose the ability to germinate under high temperatures first (Baskin and Baskin, 1988). Thus, they might still retain the potential to germinate at low temperatures which means that germination is possible even during late autumn. Consequently, the induction of a light requirement is an important and pivotal mechanism for preventing germination in situations where the chances of seedling emergence are small (e.g. deep burial) or where future conditions for plant growth and survival are bad.

**Reversibility over the stratification period**

During the experimental period (43 d), *S. media* and *T. officinale* showed a reversibility in the induced level of dormancy and in the light requirement, respectively (Figs 1B and 4). This means that not only the induction, but also the weakening of dormancy, and the loss of the light requirement can occur within days i.e. changes in dormancy and alterations in light requirement are not only seasonal phenomena but can also occur within very short time periods.

Under what situations is the quick release from dormancy an advantage for the species? It certainly increases the risk of germination occurring when temporarily favourable conditions interrupt a longer period of unfavourable conditions. In the case of *S. media*, this risk is diminished by retaining the light requirement. Although these short-term reversible changes could have functional consequences, with the first seedlings to emerge in the field facing less competition, we find it difficult to interpret them as an adaptive trait.

An interesting comparison may be drawn between these results and those obtained by Totterdell and Roberts (1979) with seeds of *Rumex obtusifolius* L. and *Rumex crispus* L. In these species, the stratification treatments (in the range 1.5–15 °C) lead to a rapid loss of primary dormancy. In addition to the loss of primary dormancy, an induction of secondary dormancy also occurred after a longer period of stratification and the two processes overlapped. This is an example of other species showing reversibility of dormancy during stratification. Hence, a possible explanation for the reversibility showed by *S. media* and *T. officinale*, is that the loss of dormancy and light requirement, respectively, overlaps with the induction of dormancy and light requirement that occurred during stratification. In this case, seeds that had lost their ability to germinate could regain it after a longer period of treatment.

**Stratification environment: soil vs. petri-dish**

Differences in germinability between seeds subjected to stratification in soil and in petri-dishes were observed in the species studied, with the exception of *V. agrestis*. The differences were particularly pronounced in *S. media* and *T. officinale*. In *S. media* seeds that were stratified in soil, the strong dormancy started to get weaker just 2 weeks after it had been induced, whereas in petri-dishes no such change was detected. In *T. officinale* the loss of the induced light requirement, observed in both pre-treatments, occurred more quickly in the soil pre-treatment. This indicates an interaction with some factor(s) (abiotic and/or biotic) present in the soil, which induced a weaker seed dormancy.

These differences confirm, as Roberts (1981) points out, that the interpretation of field behaviour, based on laboratory tests carried out in petri-dishes, can be misleading. There are obviously some factors in the soil which interact with seeds, affecting their germinability. The nature of these factors may be chemical and/or biological. One such factor could be the presence of nitrate, which has been shown to alleviate dormancy and stimulate germination in several species, e.g. *S. media*, *Arabidopsis thaliana* and *C. fontanum* (Roberts and Locket, 1975; Derkx and Karssen, 1994; Milberg, 1997). Nitrate has also been shown to sensitize seeds to light (Cone and Kendrick, 1985; Milberg, 1997). Hence, these studies suggest that if nitrate was the dominant factor to explain soil and petri-dish differences, seeds would be less dormant and more sensitive in the former treatment. Indeed, in our study seeds of *S. media* and *T. officinale* stratified in soil eventually, and after 20 to 30 d became, respectively, less dormant and more responsive to light compared to seeds stratified in petri-dishes. For the third species which showed differences between soil and petri-dishes, *C. fontanum*, the pattern was less clear.
CONCLUSIONS

In all four species included in the present experiment, germination after short-term light exposure was consistently higher than in the dark treatment. Since the difference is caused by just a few seconds of exposure to light (1000 µmol m⁻² s⁻¹), this shows a high light sensitivity in the seeds of these species.

The results of this study show that the induction of a high dormancy level and a light requirement for germination occur rapidly—within days—under stratification. They also indicate that these inductions can be reversed while seeds remain under stratification. This suggests that the processes of induction and release of dormancy and light requirement may overlap.

Another conclusion is that the stratification environment can affect the dormancy status or the expression of light requirement. Therefore, caution is needed when extrapolating laboratory results to dormancy levels or light requirement after stratification in a field situation.

Finally, the differences between stratification environments, coupled with the rapidity at which dormancy changes can occur, as well as their reversible character, points to the necessity of a much better understanding of the mechanism determining dormancy and light responses before it is possible to predict dormancy levels of seeds in a field seed bank.

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LITERATURE CITED


