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Weed seed germination after short-term light exposure: germination rate, photon fluence response and interaction with nitrate

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Summary

Three laboratory experiments were carried out to answer certain important questions related to the use of 'photo control' as a weed-control strategy. The first experiment documented that seeds of *Rumex obtusifolius* L. and *Silene noctiflora* L. germinated more slowly in total darkness than after a short exposure to light, whereas there were no significant differences for *Cerastium fontanum* Baumg. This suggests that seedling emergence in total darkness would not only result in fewer seedlings, but would also be slower; hence the crop might be given a competitive advantage. The second experiment demonstrated that germination of *C. fontanum* and *S. noctiflora* showed a linear response to the logarithm of photon fluence, with levels >1 μmol m^{-2} being stimulatory. This suggests that a near-complete elimination of light during dark harrowing would give the best result. *R. obtusifolius*, however, had a sigmoid dose–response curve with a lower threshold for germination at 500 μmol m^{-2}. Hence, this species had a clear threshold under which unnecessary germination was prevented. The third experiment tested for interaction between light and nitrate in their stimulatory effect on germination percentages. For *Descurainia sophia* (L.) Webb ex Prantl, *R. obtusifolius* and *Thlaspi arvense* L., but not for *C. fontanum*, such interactions were significant. This stresses the fact that light response will vary substantially depending on the seed's environment.

Introduction

The emergence of weed seedlings can be reduced if soil-disturbing activities are conducted in darkness (e.g. Hartman & Nezadal, 1990; Ascard, 1994; Scopel *et al.*, 1994; Jensen, 1995). In addition, it has been observed that seedling emergence in field experiments with dark harrowing can be delayed in the dark treatment (Jensen, 1992, 1995; Ascard, 1994). Such a delay would enhance the effect of dark harrowing by reducing the weed biomass even further because the crop would be given a competitive advantage.

One issue in relation to dark harrowing is how dark it has to be during soil disturbance to attain maximum reduction in weed emergence, i.e. when should the work be done in relation to sunset, and can tractor headlights be used? Threshold values would also be useful when developing covered implements for use during the day (Ascard, 1994; Scopel *et al.,* 1994).

Light is one of the main cues initiating germination in weeds but its effect can interact with other factors in the seed's environment. Nitrate is one of the main agents that can weaken the dormancy level of a seed batch and stimulate germination (Bouwmeester & Karssen, 1989; Pons, 1989; Carmona & Murdoch, 1995).

Here, three experiments are reported, each with three or four weed species. The first tested whether germination rate in darkness differed from that after short exposure to light. The

second experiment aimed at establishing the minimum amount of light, i.e. photon fluence, needed to induce germination. The third experiment was designed to reveal any interaction between short-term light exposure and nitrate in their stimulatory effect on germination.

Materials and methods

Seeds

Seeds of *Descurainia sophia* (L.) Webb ex Prantl, *Silene noctiflora* L. and *Thlaspi arvense* L. were collected in August 1994 and seeds of *Rumex obtusifolius* L. and *Cerastium fontanum* Baumg. in August 1995, in southern Sweden. Seeds were collected from natural populations on arable land or on waste land. They were then dried and stored at *c*. 22 °C for about 6 weeks. The 1994 collections were thereafter stored in paper bags at 3 °C for 16–17 months, while the others were kept in open plastic containers at *c*. 18–21 °C and <30% RH for 4–5 months before pretreatment was started.

The seeds of *D. sophia* and *T. arvense* were dormant (<5% germination in light and appropriate temperature) whereas the other species had non-dormant seeds (>90% germination).

All experiments included three replicate batches of *c*. 100 seeds except for *S. noctiflora,* for which 45–50 seeds were used owing to seed shortage.

Pretreatment

Before subjecting seeds to different treatments, they were sensitized to light by a pretreatment. Previous trials with four species had shown that 8–16 days at 3 °C resulted in a substantial light sensitivity and that this temperature was too low for germination to occur during the pretreatment (Noronha, Andersson & Milberg unpubl. obs). The seed batches were placed on filter paper (two Munktell 1003, 90 mm diameter) in Petri dishes (90 mm diameter). In two experiments the papers were wetted with 4.0 mL of deionized water and in the third experiment with distilled water with or without potassium nitrate. The dishes were immediately wrapped in two layers of aluminium foil and stored at 3±0.5 °C before use in an experiment.

Short-term light exposures

All experiments included some treatments in which pretreated seeds were exposed to light for short periods. The source was a xenon bulb (Osram XBO 150 W/1 or General Electric G-E XE500EAR46) placed in an air-cooled, light-proof box. The ratio R/FR was 0.8. Seeds were exposed by letting light through a hole. The length of each exposure longer than 1 s was measured with a stopwatch. For the shorter exposures, exposure time was regulated by letting the light through a camera. After the light exposure, each dish was wrapped in aluminium foil. All opening and wrapping of dishes was conducted in complete darkness.

Germination rate

After 7 days of cold stratification, dishes with *Silene noctiflora, Rumex obtusifolius* and *Cerastium fontanum* were transferred to 16±1 °C for 24 h. The dishes were then subjected to one of four treatments: (i) darkness; (ii) 1 s exposure to light followed by darkness; (iii) 15 s exposure to light followed by darkness; (iv) a 'full-light' treatment that included 12 h of light per day. The two short-term light exposures were of a photon fluence rate (PAR) of 325±20 μmol m^{-2} s^{-1}. Treatment (i) included a short aeration (*c*. 3 s) of the dishes, which were then wrapped in aluminium foil again. In the full-light treatment, dishes were sealed with parafilm. All dishes were then placed in stacks of four (one dish for each treatment with full-light treatment on top) in a room providing a daily, fluctuating temperature: 18±2 and 6.5±1.5 °C for 14 and 9 h respectively, with transitions between these temperatures for 1 h. The full-light treatment received 70±10 μmol m^{-2} s^{-1} [PAR; R/FR ratio was *c*. 8 for 12 h per day from cool white fluorescent tubes (Osram L65 W/20R)].

Germination (radicle emergence) in three randomly chosen stacks of dishes per species was registered and terminated at the same time (± 30 min) each day, starting from the first observed germination in the full-light treatment. This procedure continued for 7 or 8 days.

Response to different photon fluence

After 10 days of cold stratification, dishes with

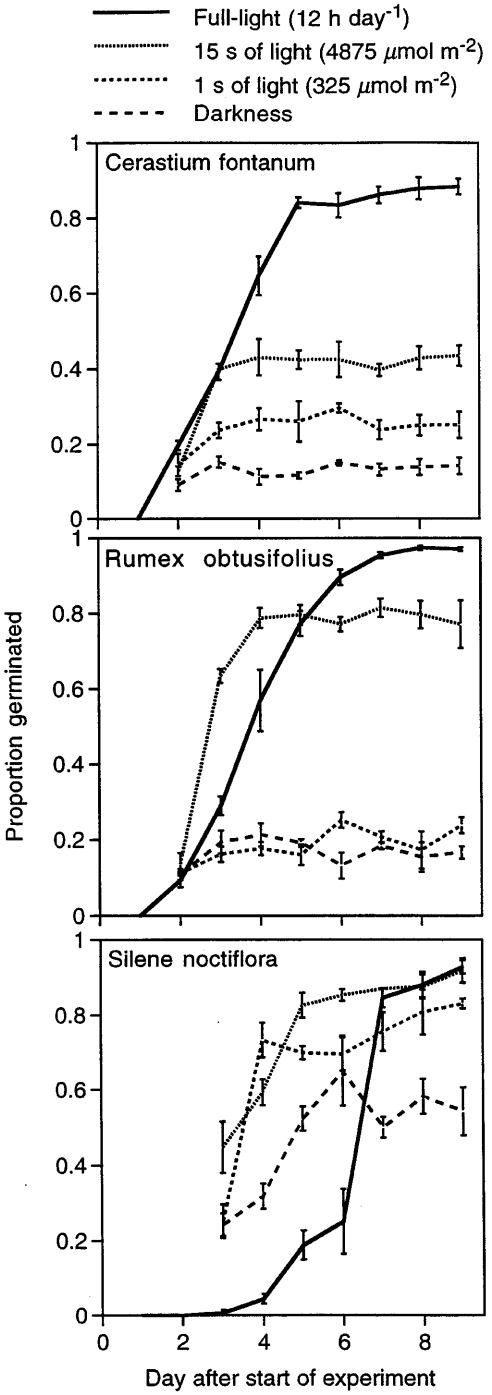

Fig. 1. Temporal patterns in the average germination in different light treatments. Bars indicate SE (n = 3).

seed of *Cerastium fontanum*, *Rumex obtusifolius* and *Silene noctiflora* were transferred to 16±1 °C for *c.* 24 h. Three dishes of each of the species were then subjected to a short-term exposure of light. Fluences from 1.3 to 78000 μmol m^{-2} were given by varying the time of exposure to 325±20 μmol m^{-2} s^{-1} from 1/250 s to 240 s. Five dishes kept as dark controls were subjected to a short aeration (as described above).

All dishes were placed in an incubator with a daily, fluctuating temperature: 16 and 7 °C for 9 h each with transitions between these temperatures for 6 h. The experiment was terminated, and germinated seeds were counted, 19–22 days after the light exposure.

Light and nitrate

This experiment included two species with primary seed dormancy, *Descurainia sophia* and *Thlaspi arvense*, and two lacking such dormancy, *Cerastium fontanum* and *Rumex obtusifolius*. Seeds were placed on filter paper wetted with (i) distilled water, or with (ii) 0.01 mol L^{-1} or (iii) 0.1 mol L^{-1} potassium nitrate. The seeds were cold-stratified for 10 days before storage at 16±1 °C for 24–29 h. Seeds were then subjected to one of five different light treatments: (i) darkness; (ii) 0.4 μmol m^{-2} (1/500 s of 200 μmol m^{-2} s^{-1}); (iii) 400 μmol m^{-2} (2 s of 200 μmol m^{-2} s^{-1}); (iv) 4000 μmol m^{-2} (2 s of 2000 μmol m^{-2} s^{-1}); (v) 40000 μmol m^{-2} (20 s of 2000 μmol m^{-2} s^{-1}).

All dishes were placed in an incubator with a daily, fluctuating temperature: 16 and 7°C for 9 h each, with transitions between these temperatures for 6 h. The experiment was terminated, and germinated seeds were counted, 9–10 days after the light exposure.

Statistics

As proportions of dead seeds were very low in the seed batches used, no corrections were made for viability.

To check for significant differences in the rate of germination, the recorded values were transformed, for each light environment and species, to proportions of the maximum germination value recorded. Effects of light and time elapsed (in days) since the start of experiments were evaluated in an ANOVA containing the following factors: 'light' (with three levels, excluding the full-light treatment); 'elapsed time' (number of days elapsed since start); and the interaction factor 'light' × 'elapsed time'. Differences between

Table 1. Statistical analyses (ANOVA) of data on germination rate in three different light treatments (0, 1, 15 s) over a period of 7 or 8 days ('elapsed time')

	Cerastium fontanum	*Rumex obtusifolius*	*Silene noctiflora*
Light treament	NS (0.19)	0.0004	<0.0001
Light treatment×elapsed time	NS (0.44)	0.0010	0.0050
Post hoc tests			
0 vs. 1 s		NS (0.20)	<0.0001
0 vs. 15 s		<0.0001	<0.0001
1 vs. 15 s		0.0055	NS (0.85)

Before analyses, the data had been transformed to eliminate the effect of differences in final germination. The factor 'elapsed time' significantly affected germination in all species ($P<0.0001$). Numbers are *P*-values.

the three light treatments were evaluated in *post hoc* tests (Fisher's PLSD).

Effect of nitrate and light environment were analysed using ANOVA, after transformation of the germination data (arcsine of the square root of the proportion of germinated seeds).

Results and discussion

Germination rate

For all three species, germination proceeded most slowly in the full-light treatment (Fig. 1). For *Silene noctiflora*, this treatment substantially delayed germination but final germination eventually reached levels similar to that after 15 s of light (Fig. 1). For *Rumex obtusifolius* also, exposure to full light slowed down the germination, but final germination eventually superseded that of the 15-s treatment. It is surprising that germination was slowest in the full-light treatment, but it should be kept in mind that this treatment is not directly comparable with the short-term light exposures as the type of light source differed (fluorescent tube vs. xenon bulb). Thus, it remains to be determined whether the main factor responsible for the delayed germination in the full-light treatment was photo-inhibition or light quality. The fact that *S. noctiflora* actually germinated more slowly in full light than in darkness implies that inhibition was involved.

As the full-light treatment had a different light source, it was excluded from the statistical evaluation of the data transformed to eliminate differences in final germination. *Silene noctiflora* germinated more quickly after both 1 s and 15 s of light exposure than in darkness, whereas for *Rumex obtusifolius* only the 15-s treatment resulted in significantly quicker germination than darkness (Table 1, Fig. 1). These results are in accordance with the field observations that seedling emergence can be delayed in dark-harrowed plots (Ascard, 1994; Jensen, 1995), but the practical importance of this delay in a field situation remains to be evaluated.

The present results suggest the following scenario in a field situation: for *Rumex obtusifolius* and *Silene noctiflora,* soil cultivation in the light would result in seedlings emerging quickly and in a concentrated flush. In a dark treatment, fewer seeds would germinate and emergence would be somewhat delayed. For *Cerastium fontanum,* treatments would differ in the number of seedlings emerging but not in their rate of emergence. However, the accuracy of these predictions will be reduced if a large number of the seedlings are recruited from seeds on or near the soil surface, because such seeds might germinate considerably more slowly and over an extended period.

Response to different photon fluence

Rumex obtusifolius had a sigmoid dose–response curve to the logarithm of the photon fluence, with maximum germination at >5000 μmol m^{-2} and minimal at <500 μmol m^{-2} (Fig. 2). In contrast, the other two species displayed graded response curves to the logarithm of the photon fluence (Fig. 2). Regression analyses suggest that no germination was induced by light at a photon fluence <1.3 and <0.77 μmol m^{-2} (*Cerastium fontanum* and *Silene noctiflora* respectively).

Minimum threshold values for the amount of light needed to stimulate germination are useful for determining light elimination requirements

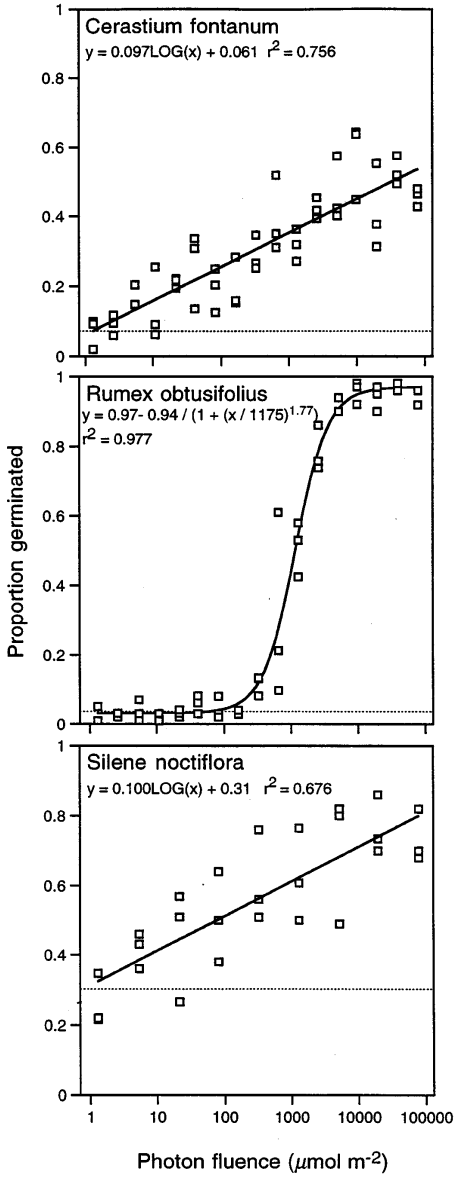

Fig. 2. Germination after exposure to a photon fluence rate of 325 μmol m^{-2} s^{-1} for different lengths of time. The dotted line indicates mean germination in darkness based on counts made on five replicate dishes.

during dark harrowing. However, in the present study only *Rumex obtusifolius* had a clear minimum threshold whereas the curves for the other two species suggest that a near-complete elimination of light during dark harrowing is required to prevent unnecessary germination (Fig. 2). How then do the results presented here relate to a field situation? Daylight can be around 2000 μmol m^{-2} s^{-1} on a clear Swedish summer day, but photon fluence rates are likely to be lower than this under an implement, say 1000 μmol m^{-2} s^{-1}. This would mean that 1 ms or more of daylight would be needed to induce some germination in *C. fontanum* and *S. noctiflora* whereas more than 0.5 s would be needed for *R. obtusifolius*. Another way of relating the data in Fig. 2 to a field situation is to calculate the photon fluence rate needed to stimulate germination at a fixed exposure time. Assuming that seeds are exposed to light for 1 s during the passage of an implement, this would mean that a photon fluence >1 μmol m^{-2} s^{-1} could stimulate germination in *C. fontanum* and *S. noctiflora*. For *R. obtusifolius,* the corresponding value would be 500 μmol m^{-2} s^{-1}. It should be noted that the minimum threshold estimates are conservative. First, the calculations assume seeds to be lying on the soil surface when exposed. However, soil particles and dust adhere to seeds, reducing the photon fluence reaching them. Second, most dark harrowing is likely to be conducted early or late in the day, rather than at noon, and in spring or autumn when the sun has a lower elevation, resulting in lower photon fluence rates (Olesen, 1992). Nevertheless, the minimum threshold estimates above suggest that the actual timing of dark harrowing in the night and the use of tractor headlights should have little influence on the results. Photon fluence rate drops quickly below 1 μmol m^{-2} s–1 *c.* 15 min after sunset (early September in southern Sweden; Börjesdotter, 1994) and the light from headlights reaching the soil behind a tractor is negligible (Jensen, 1991).

The minimum photon fluences to stimulate germination of *Cerastium fontanum* and *Silene noctiflora* suggest that the very low fluence response (VLFR) of the phytochrome was activated in some seeds (Kronenberg & Kendrick, 1986). However, there was no indication of a biphasic response curve of the type previously reported for some species (Cone & Kendrick, 1985; Cone *et al.,* 1985; Rethy *et al.*, 1987; Scopel *et al.,* 1991). As in all germination experiments, the response is very much a consequence of the pretreatments and the conditions under which the tests are performed. Hence, it is possible that after other pretreatments, and possibly under other germination conditions as well, the response curve would have looked different. On the other hand, most of the germination in *C. fontanum* and *S. noctiflora* and all in *Rumex obtusifolius* occurred after longer light exposures,

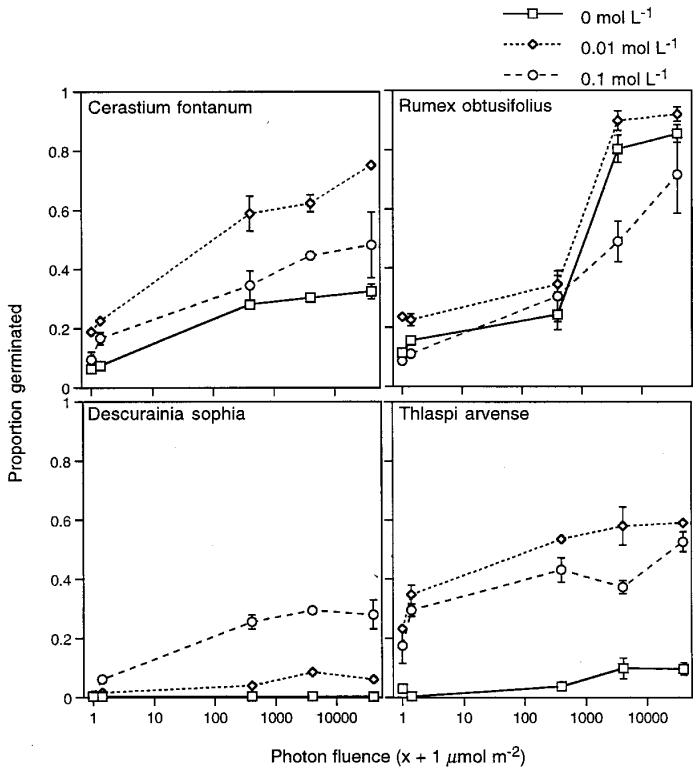

Fig. 3. Average proportion germination in environments, differing in nitrate concentration, after different short exposures to light. Bars indicate SE when larger than 0.03 (n=3).

suggesting a low fluence response (LFR). It remains to be clarified how important the VLFR is for 'photo control' in the field.

It should be kept in mind that the light sensitivity of a seed batch is probably related to the degree of dormancy, and that this can vary from season to season (e.g. Baskin & Baskin, 1989; van Assche & Vanlerbergh, 1989). Thus, the light response can undergo seasonal changes in many species (Baskin & Baskin, 1981; Froud-Williams *et al.*, 1984; Derkx & Karssen, 1993; Milberg, 1994; Andersson & Milberg, 1996). Hence, seasonal changes in the effect of dark harrowing could be expected.

Nitrate and light

Both light and nitrate stimulated germination (Fig. 3; Table 2), but the response to nitrate levels varied between species. For three of the four species, maximum germination was achieved at the lower nitrate concentration and, with the ex-

Table 2. Results from statistical analyses (ANOVA) on data from experiments with three different levels of nitrate and five different light treatments

	A: Light	B: Nitrate	A×B
Cerastium fontanum	***	***	NS
Rumex obtusifolius	***	***	*
Descurainia sophia	***	***	***
Thlaspi arvense	***	***	**

*P<0.05; **P<0.01; ***P<0.001.

ception of *Rumex obtusifolius,* seeds germinated least in the treatment without nitrate. That nitrate broke primary seed dormancy in both *Descurainia sophia* and *Thlapsi arvense* is indicated by the findings that germination percentages were higher in both nitrate treatments, especially in combination with light (Fig. 3; cf. Hartmann *et al.*, 1996).

In the absence of nitrate, germination after the shortest exposure (0.4 μmol m^{-2}) was not any better than that in darkness (cf. Fig 2). In the presence of nitrate, however, this light treatment stimulated germination in several cases, especially in *T. arvense* (Fig. 3). This germination response should be mediated by the VLFR and shows that nitrate can sensitize seeds of these species (cf. Cone *et al.,* 1985; Cone & Kendrick, 1985). This could mean that seeds in the field are more responsive to short-term light exposures than under, e.g. the laboratory conditions in the two first experiments reported here.

Nitrate and light in combination increased germination percentages and seeds were sensitizised to very short-term light exposures by nitrate (Fig. 3). Therefore, one would expect that a fertilizer application before soil disturbance would result in more weed seedlings compared with a later application.

Interactions between light and nitrate in their effects on germination were significant for three of the four species (Table 2), which means that the relative germination cannot be predicted from fluence rate alone. Previous studies have documented significant interactions between light and nitrate for many species after chilling (e.g. Vincent & Roberts, 1977; Roberts & Benjamin, 1979). In another study, nitrate was unable to stimulate the germination in darkness of seeds that had been buried in soil (Bouwmeester & Karssen, 1989). Together, these results and those presented here clearly show that the light sensitivity recorded in an experiment can be strongly influenced by the chemical environment that seeds have been subjected to before and after illumination. This, together with the variability in dormancy levels between seed populations (Milberg *et al*., 1996) and the seasonal changes in light sensitivity (Andersson & Milberg, 1996), is probably the major cause of the observed variability in weed reduction in field trials comparing dark- and light-harrowed plots (e.g. Niemann, 1996).

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