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Sensitivity to green safelight of 12 Mediterranean species

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Summary

In most germination experiments under dark conditions, green safelight is used to check the course of germination. However, there is evidence that even short exposures to such light can stimulate seeds to germinate. The extent of this effect is needed to determine if it is a generalization. The effect of green safelight was studied in a set of 12 Western Mediterranean species by testing germination responses to continuous darkness or to periodic short exposures to green safelight. Seeds were incubated at 15°C for 6 weeks. Half of the species showed significant effects of green safelight, the majority increasing as a result of safelight exposure. *Cistaceae* species seemed to be less sensitive to safelight than other families, like *Compositae* or *Labiatae*. Our results confirm that sensitivity to green safelight might be rather general and thus must be avoided when checking germination under darkness.

Introduction

Light is one of the many environmental factors that regulates seed germination. The response to light may be due to changes in both the quality (the wavelength distribution) and irradiance (the magnitude) of light radiation (Smith, 1982). Numerous papers compare germination differences of seeds under light versus dark conditions from which ecological interpretations can be derived. The light response can control the timing of germination in the field, a crucial factor for the survival of the resulting seedlings. For instance, light-promoted seed germination has been interpreted as the most important mechanism by which seeds can detect gaps in vegetation (Pons, 1992), or as a mechanism for avoiding germination from too deep into the soil to reach the surface (Shipley and Parent, 1991). Light-inhibited seed germination has been interpreted as an adaptive mechanism for plants inhabiting sandy habitats (Bell, 1993), for desert plants (Koller, 1956) and for Mediterranean maritime plants (Thanos *et al.*, 1991).

In numerous papers that test the germination response under dark conditions, green safelight is usually utilized for periodically checking the course of germination. However, exposure to green light could affect the germination of some species (Valio *et al.*, 1972; Baskin and Baskin, 1979; Doussi and Thanos, 1997; Amaral and Takaki, 2001). Nevertheless, the generality of this phenomenon is unclear. This is important since many germination experiments evaluating the role of light continue to use green safelight, assuming that it has no effect on seed germination. Thus, results obtained could modify the interpretation of factors regulating germination.

In the present study, the effect of green safelight with respect to absolute darkness on the germination response of a set of 12 Mediterranean species was determined in order to evaluate how general the sensitivity to green safelight might be.

Material and methods

Seeds of a set of 12 species, representing six plant families were collected from Serranía Baja de Cuenca (central-eastern Spain) (province of Cuenca; 1°20'-1°35' W and 39°45'-39°54' N) (table 1). Altitude is 900-1400 m, annual mean temperature 12-12.5°C and annual mean rainfall 500-550 mm.

Seeds were collected from ripe fruits in the field during summer and fall. They were cleaned by removing fruit tissues that would normally be lost in dispersal and stored in paper bags in the dark at room temperature until spring of the following year, when germination tests were started. In many cases, the "seed" that was sown included the entire fruit, such as achenes in the *Compositae* (table 1).

No seed pre-treatments were carried out before germination tests. Seeds were incubated in 5.5-cm-diameter plastic Petri dishes on 2 layers of Whatman No. 1 filter paper and moistened with 1.2 ml of distilled water at the beginning of the experiment. Four replicates of 25 seeds each were used per treatment. All Petri dishes were sealed with parafilm to prevent desiccation. Immediately after wetting the filter paper, Petri dishes were wrapped in 2 sheets of aluminum foil. The seeds were stratified for 4 weeks at 5°C and then incubated for 6 weeks at 15°C in the dark. Petri dishes were placed randomly in a temperature and humidity controlled chamber (Model G-21, Ibercex).

Table 1. List of the species studied.

Species	Growth form*	Family	Seed mass (mg)	Germinule type
<i>Achillea ageratum</i> L.	Hc	Compositae	0.1	Achene
<i>Chondrilla juncea</i> L.	Hc	Compositae	0.3	Achene
<i>Helichrysum stoechas</i> (L.) Moench.	Cham	Compositae	0.1	Achene
<i>Santolina chamaecyparissus</i> L.	Hc	Compositae	0.2	Achene
<i>Fumana ericoides</i> (Cav.) Gand.	Cham	Cistaceae	2.4	Seed
<i>Halimium viscosum</i> (Willk.) P. Silva	Cham	Cistaceae	1.4	Seed
<i>Helianthemum apenninum</i> (L.) Miller	Cham	Cistaceae	1.0	Seed
<i>Antirrhinum litigiosum</i> Pau	Hc	Scrophulariaceae	0.1	Seed
<i>Linum narbonense</i> L.	Hc	Linaceae	3.2	Seed
<i>Plantago sempervirens</i> Crantz.	Hc	Plantaginaceae	2.0	Seed
<i>Marrubium supinum</i> L.	Cham	Labiatae	1.3	Nutlet
<i>Salvia verbenaca</i> L.	Cham	Labiatae	1.1	Nutlet

* Growth form: Hc, hemicryptophyte; Cham, chamaephyte.

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-21, Ibercex).

Seed mass (mg)	Germinule type
	Achene
	Achene
	Achene
	Achene
	Seed
	Seed
	Seed
	Seed
	Seed
	Seed
	Nutlet
	Nutlet

For seeds incubated in the dark, germination was recorded only once, at the end of the incubation period. In the safelight treatment, the seeds were exposed to green safelight for 1 to 2 minutes once a week during the 6 weeks incubation-period. Criterion of germination was the visible protrusion of the radicle. Green, safelight (0.05 $\mu\text{mol m}^{-2} \text{s}^{-1}$; showing between 500 and 750 nm a unique major emission band centered at 546 nm; emission between 600 and 750 nm was nearly undetectable) was produced by one green fluorescent tube (F 15T8.G6, 15 W Green-Photo, General Electric, USA), two Plexiglas sheets, one red-orange, 478, and one green, 700.

Seed viability was checked in seeds that did not germinate using the tetrazolium test, and germination percentages were corrected accordingly. Germination percentages were arcsine transformed and analyzed statistically by analysis of variance for each species separately.

Results and discussion

Half of the species (*Chondrilla juncea*, *Helichrysum stoechas*, *Fumana ericoides*, *Linum narbonense*, *Marrubium supinum* and *Salvia verbenaca*) had a statistically significant response in germination when exposed to the safelight (figure 1). Except for *Chondrilla juncea*, seeds of these species had a germination increase when periodically exposed to green safelight (figure 1). Green safelight was enough to overcome the inhibition of germination by darkness. This means that seeds of these species are very sensitive to light, requiring only a short exposure to germinate. The fact that one species (*Chondrilla juncea*) was negatively affected by green safelight indicates that inhibition of germination may also be caused by exposure to light.

A. ageratum, *M. supinum* and *S. verbenaca* did not germinate in dark but did when exposed to safelight. In general, it appears that species of the *Compositae* were rather sensitive to light. Safelight effects on *H. stoechas* germination had been previously reported by Doussi and Thanos (1997), who found a significant germination increase when exposed to green safelight. Likewise, Walck *et al.* (2001) found an increment in germination for three species of *Solidago* (*Compositae*). On the contrary, in our study *Cistaceae* species hardly showed a response to safelight, and had very low germination percentages. Only *F. ericoides* showed a significant difference between treatments. It appears that light is not a decisive factor in promoting *Cistaceae* seed germination. Probably it is due to its hard and impermeable seed coat, characteristic of the *Cistaceae* family. Scarification treatment seems to be necessary for breaking dormancy. The two *Labiatae* species germinated only under safelight treatment. Thanos and Doussi (1995) found for seeds of several endemic *Labiatae* an increase in germination with white and red light. Blom (1978) found germination differences between complete dark and green light conditions for *Plantago major*. However, in the present study, no differences were observed for *P. sempervirens*.

In conclusion, we present evidence that the germination of many species might be sensitive to short-duration exposures to safelight. Therefore, if safelight effects are unknown it seems clear that safelight should not be used when evaluating the effect of

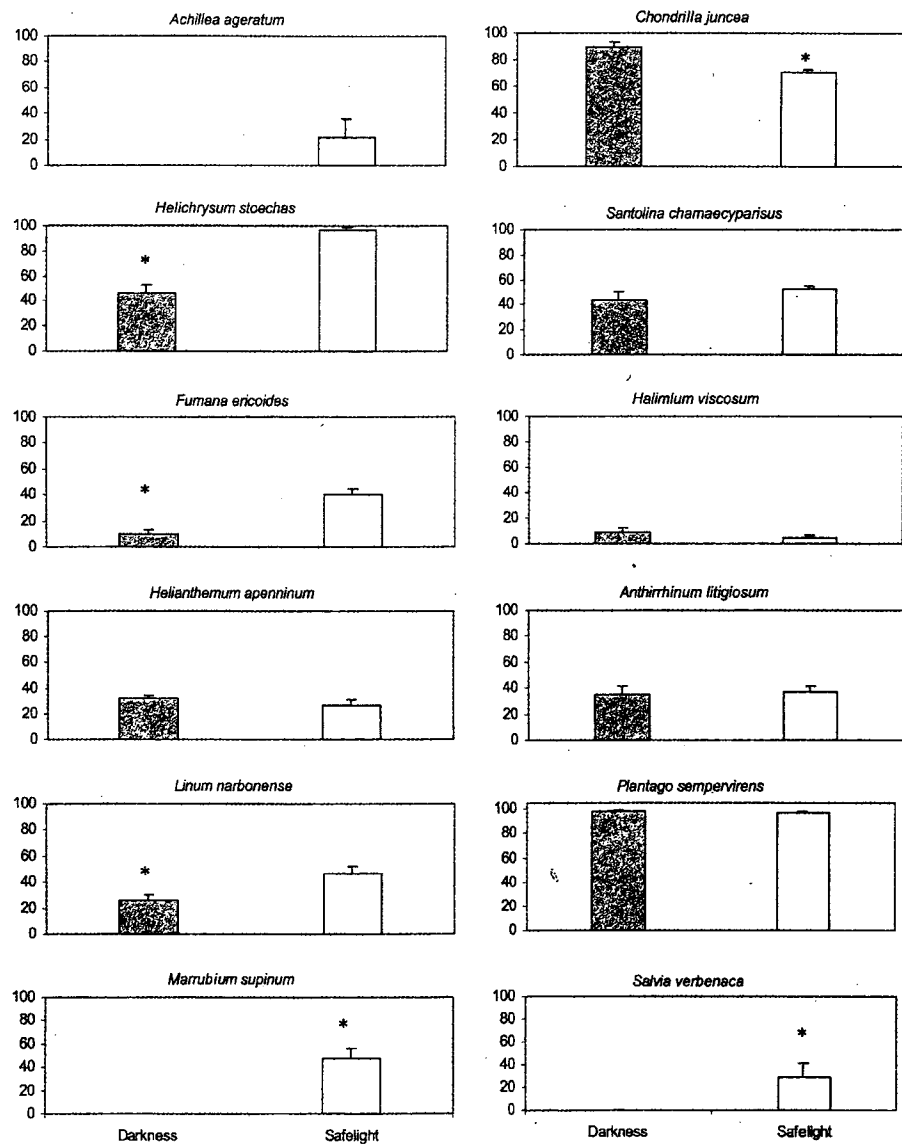
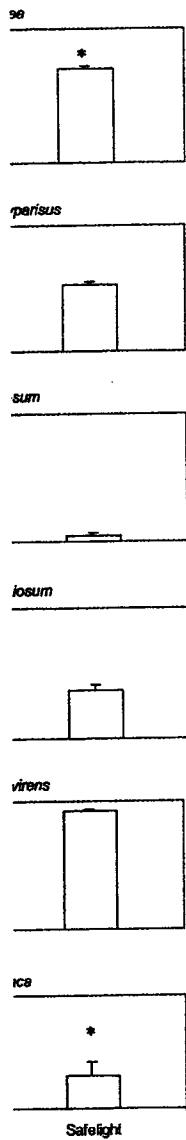


Figure 1. Germination percentages after 6 weeks incubation at 15°C and in complete darkness or after exposed to green safelight. (*) indicate statistically significant differences ($p \leq 0.05$) among light treatments.



rkness or after exposed treatments.

light and darkness on germination. Our results agree with the recommendations of Baskin and Baskin (1998). Seeds tested in the dark should not be exposed to any light until the end of the germination experiment. Some seeds may require such small amounts of light to change their germination response that it is impossible to check them for germination (even under safelight) without altering them. Thus, reports in the literature of seeds germinating in darkness probably should be reevaluated in cases where the investigators exposed them to any light at all during the incubation period.

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