

71. Ecophysiology of Seed Germination in Composites Inhabiting Fire-prone Mediterranean Ecosystems

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Abstract

Within the framework of a research project concerning adaptive mechanisms of postfire regeneration in Mediterranean ecosystems, the ecophysiology of seed germination was studied in the following composites: *Dittrichia viscosa* (L.) Greuter, *Helichrysum stoechas* (L.) Moench ssp. *barrelieri* (Ten.) Nyman and *Phagnalon graecum* Boiss. & Heldr. All three species are common in the Mediterranean region; the former is a colonizer while the latter two are usually present in phrygana (low-shrub, fire-prone Mediterranean vegetation). As the result of their numerous, anemochorous dispersal units the three species are endowed with the potential to invade disturbed areas, in general, and burned ones, in particular.

Germination in *Dittrichia* was generally very low in the dark (0–10%). Moreover, an absolute light requirement was revealed and germination could be promoted even by green safelight. In *Helichrysum*, final dark germination reached its highest value (ca. 50%) at relatively low temperatures (10°C, 15°C). Light and nitrates (optimal concentration 20 mM) promoted germination dramatically above dark control. Nevertheless, full induction of germination required the presence of both these factors. *Phagnalon* germinated optimally (70–100%) over a broad temperature range (10–25°C), in the dark. However, light proved beneficial at suboptimal temperatures. In the three species studied, light and, in the case of *Helichrysum*, nitrate availability are particularly important for seed germination and eventual seedling recruitment in the postfire environment.

Introduction

Wildfires are an integral component of ecosystem structure and function in all three major vegetation types (pine forests, maquis and phrygana) which dominate the Mediterranean-type environment of Greece. Postfire regeneration is characterized by the remarkable resilience of established plants through resprouting from both epicormic meristems and below-ground organs and also through germination from a soil- or plant-stored seed bank. A potentially important additional contribution to postfire regeneration is the long-distance dispersal of seeds into recently burned areas.

One characteristic that allows a plant species to exploit newly disturbed sites is the production of large numbers of seeds which may be dispersed long distances. Apparent adaptations that would allow long-distance dispersal, i.e. small, light seeds with structures such as a pappus, are certainly prominent

among pioneers in newly burned sites (Whelan, 1986). Many references are given by Kozłowski and Ahlgren (1974) to the well dispersed seeds that germinate soon after the fire. *Haplopappus tenuisectus* and *Gutierrezia sarothrae* (both composites) are postfire pioneer shrubs, disseminated by wind in the Sonoran Desert. In the Mediterranean maquis, several annuals may appear in large numbers during the first year after fire; these annuals are apparently opportunistic invaders and are not stimulated by fire *per se*. It has been proposed that the long-term presence of disturbed habitats around the Mediterranean produced pioneer, weedy genotypes that became highly successful invaders (Cody and Mooney, 1978).

Dittrichia viscosa is a densely glandular, viscid perennial, up to 130 cm tall with stems woody at the base. It is widely distributed in South Europe and grows on pine woods, dry stream-beds, ditches, cliffs, fallow fields, roadsides, waste places and along hillside paths. *Helichrysum stoechas* ssp. *barrelieri* is a woody-based perennial, 10–50 cm tall, growing on edges of pine forests and in phrygana or maquis on stony hillsides from Sicily to Turkey. *Phagnalon graecum* is a dwarf shrub up to 30 cm tall, growing in phrygana on dry, rocky ground throughout South-eastern Europe (Tutin *et al.*, 1964–1980; Burnie, 1995).

All three plant species mentioned above belong to the Compositae. Apart from certain general rules for laboratory germination (e.g. Ellis *et al.*, 1985; ISTA, 1993), there is practically no information concerning the taxa under study. In the broad context of studying the adaptive mechanisms of postfire regeneration in Mediterranean ecosystems, the ecophysiology of their seed germination was investigated.

Materials and Methods

Plant Material

Achenes of *Dittrichia viscosa* (L.) Greuter (formerly *Inula viscosa* (L.) Aiton) were collected in December 1993 from plants growing in the University Campus. Achenes of *Helichrysum stoechas* (L.) Moench ssp. *barrelieri* (Ten.) Nyman and *Phagnalon graecum* Boiss. & Heldr. were collected in June 1994 from a burned pine forest at Mt Parnes, Attica, five years after the fire. Nomenclature follows Tutin *et al.* (1964–1980). Achenes, hereafter called seeds for simplicity, were stored in moisture- and light-proof containers at room conditions. The average, air dry seed weight for the three species was 0.240, 0.030 and 0.046 mg, respectively. The percentage of unsound (empty and non-viable) seeds (revealed by dissection under a stereomicroscope) was considerable: 20, 50 and 30%, respectively. At the end of germination tests, non-viable seeds were found disintegrated, while those considered viable were still hard, with a firm embryo. Seeds were used after an initial storage of 6 months at room conditions; thereafter, no changes in germination characteristics were observed throughout the experimentation period.

Germination Conditions

Germination experiments were conducted in glass Petri dishes (7 cm in diameter) lined with two filter paper discs and moistened with 3 ml of distilled water or other imbibition medium. Criterion of germination was the visible protrusion of the radicle through the fruit wall. In one set of experiments, measurements were taken regularly (and germinated seeds were discarded after each count) while in another, germination was recorded only once, at the end of the incubation period. The former tests were considered finished when no additional seeds germinated. Germination was expressed as a percentage from five samples of 25 seeds each (except for the experiments on *H. stoechas* where 50 seeds were sown per dish). At the end of each experiment ungerminated seeds were dissected and inspected under a stereomicroscope; unsound seeds were counted out and all the results and graphs presented are consequently corrected for germinable seeds only. For germination experiments in darkness, seeds were incubated within light-proof, metal containers in controlled temperature cabinets (Model BK 5060 EL, W.C. Heraeus GmbH, Germany) where temperature was kept constant within $\pm 0.5^\circ\text{C}$ of the value set.

Light Sources

The red and far-red broad-band irradiations were produced by a bank of tubes and the light was filtered through an appropriate combination of coloured Plexiglas sheets (each 3 mm thick, Röhm GmbH, Germany). Red (R) light ($11.0 \mu\text{mol m}^{-2} \text{s}^{-1}$) was produced by ten red fluorescent tubes (TL 20W/15, Philips) and one layer of red Plexiglas, 501. Far-red (FR) light ($18.2 \mu\text{mol m}^{-2} \text{s}^{-1}$) was obtained by twelve white incandescent tubes (Philinea 6276X60 W, Philips), filtered through three layers of Plexiglas (two blue, 627, and one red, 501). All manipulations of imbibed seeds were carried out under a dim green safelight ($0.05 \mu\text{mol m}^{-2} \text{s}^{-1}$; one green fluorescent tube F 15T8.G.6, 15 W Green-Photo, General Electric, USA, two Plexiglas sheets, one red-orange, 478, and one green, 700). Total flux density values refer to light in the visible range (400–800 nm) at the seed surface, calculated from the measurements taken with a spectroradiometer (ISCO SR, USA).

Statistical Analysis

Statistically significant differences were assessed using the two-sample *t*-test (for two means) and the Newman-Keuls multiple range test (for more than two means); percentage values were arcsine transformed prior to statistical tests (Zar, 1974).

Results

The germinability of *Dittrichia* seeds was very low (0–8%) throughout the temperature range 5–30°C when seeds remained in total darkness and germination was scored only once, at the end of the incubation period. When seeds were exposed to safelight (for a few min each time) during the routine inspection for germination, a dramatic induction of germination took place (Fig. 1). At 5 and 10°C no effect of safelight was obtained. Further study of this light-facilitated

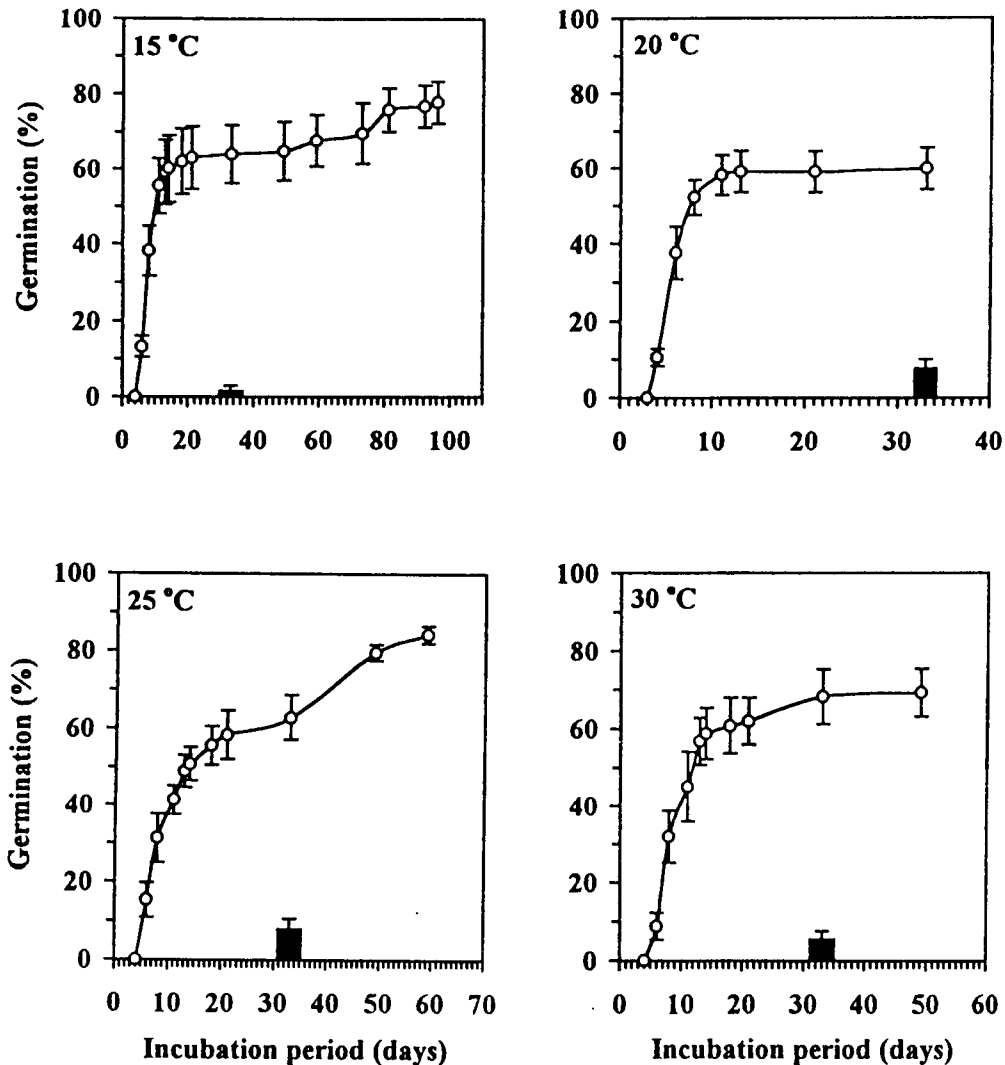


Figure 1. Time-courses of dark germination of *Dittrichia viscosa* seeds at various constant temperatures. Seeds were exposed for a few minutes to a dim green safelight while being inspected for germination. Bars represent final dark germination values when seeds were examined only once, at the end of a 33-day-long incubation period. Vertical lines indicate \pm s.e.

Table 1. Mean (\pm s.e.) germinability of *Dittrichia viscosa* seeds at 20°C, in the dark. Light pulses were given 1 d after onset of imbibition. With the exception of safelight (seeds inspected twice weekly under dim green safelight), in all other cases germination was recorded only at the end of the incubation period (30 d). Statistically different ($p < 0.05$) means are followed by different letters

Treatment	Germination (%)
Darkness	8.3 \pm 1.8 ^a
Safelight	60.0 \pm 5.6 ^c
5 min R	69.6 \pm 3.2 ^c
10 min FR	19.8 \pm 3.4 ^b
5 min R + 10 min FR	26.0 \pm 4.3 ^b
5 min R (in 3 consecutive d)	90.5 \pm 1.7 ^d

promotion of germination revealed phytochrome mediation (Table 1). Maximum germinability (90%) was obtained with 5 min R per day (d) given for 3 consecutive days. Prechilling at 5°C induced only a slight enhancement of subsequent dark germination at 20°C; 2 and 4 weeks of chilling resulted in 25.9 \pm 6.6 and 19.4 \pm 3.2%, respectively. Nitrates were ineffective and showed germination similar to that in water.

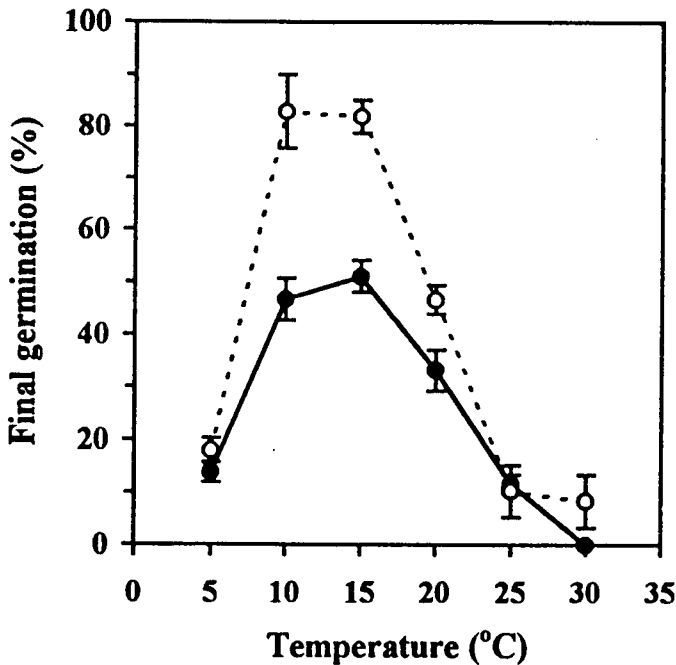


Figure 2. The germinability of *Helichrysum stoechas* ssp. *barrelieri* seeds as a function of temperature. Seeds were inspected for germination only once, at the end of the incubation period (●) or weekly under a dim green safelight (○). Vertical lines indicate \pm s.e.

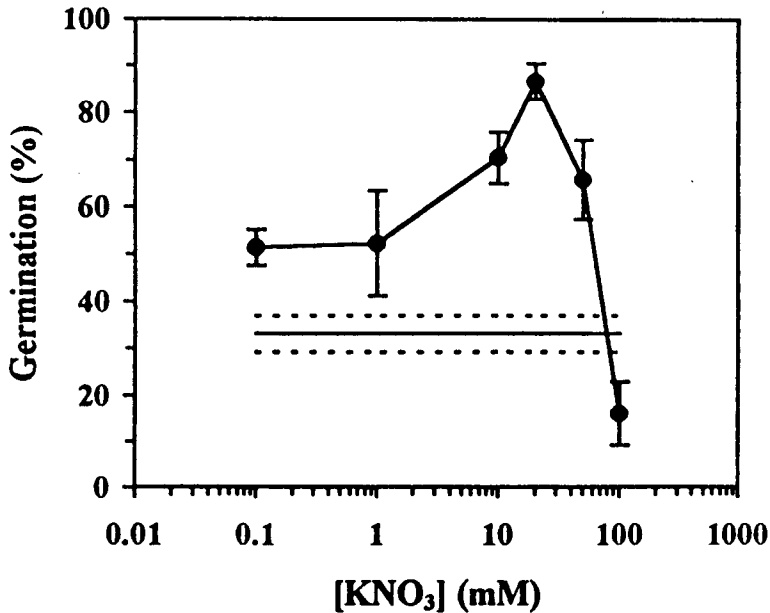


Figure 3. Final germination (at 20°C, in the dark) of *Helichrysum stoechas* ssp. *barrelieri* seeds as a function of potassium nitrate concentration. The horizontal line represents final germination in water (stippled lines: \pm s.e.). Vertical lines: \pm s.e.

Dark germination of *Helichrysum* was optimal at a rather narrow range of cool temperatures. A maximum final germination level of about 50% was obtained at 15 and 10°C while at 20°C the germination scored was 33%. A sharp decline of germinability was observed at more extreme temperatures (5, 25 and 30°C). However, the presence of green safelight, routinely used for recording germination in darkness, resulted in significant (10°C: 0.001

Table 2. Mean (\pm s.e.) germinability of *Helichrysum stoechas* ssp. *barrelieri* seeds at 20°C, in the dark. Light pulses were given 1 d after onset of imbibition. With the exception of safelight (seeds inspected twice weekly under dim green safelight), in all other cases germination was recorded only at the end of the incubation period (30 d). Statistically different ($p < 0.05$) means are followed by different letters

Treatment	Germination (%)
Darkness	33.1 \pm 3.9 ^a
Safelight	46.5 \pm 2.7 ^b
5 min R	89.6 \pm 2.7 ^c
20 mM KNO ₃	86.5 \pm 3.8 ^c
20 mM KNO ₃ + 5 min R	96.4 \pm 1.3 ^d

$<p < 0.002$, 15°C : $p < 0.001$, 20°C : $0.02 < p < 0.05$) enhancement of germinability over the dark control (Fig. 2). Moreover, dark germination was greatly enhanced in the presence of nitrates (Fig. 3). Maximum promotion of germination (87%) was obtained with a concentration of 20 mM KNO_3 . A statistically significant enhancement of germinability was clearly observed at 10–50 mM; moreover, a considerable stimulation of germination was observed even at 0.1 mM although this result would need further confirmation. Finally, the concentration of 100 mM proved inhibitory. As shown in Table 2, although both light and nitrates resulted, individually, in a statistically significant promotion of germination, the combined presence of these factors was most inductive (final germination: 96%).

Dark germination of *Phagnalon* was optimal (73–96%) over the whole temperature range 10– 25°C (maximum percentages at 20 and 15°C) with little or no germination at more extreme temperatures (30 and 5°C). Pulses of green safelight, given during the inspection of seeds for germinated ones, resulted in a significant (5°C : $0.005 < p < 0.01$, 10, 25 and 30°C : $p < 0.001$) enhancement of germination over samples which had remained in total darkness throughout the incubation period (Fig. 4). No significant effect of nitrates was detected when tested at the various constant temperatures in the dark.

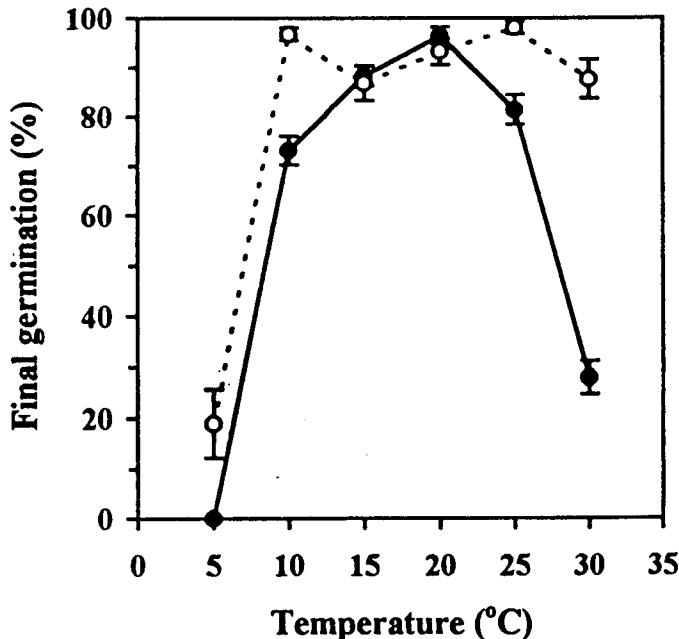


Figure 4. The germinability of *Phagnalon graecum* seeds as a function of temperature. Seeds were inspected for germination only once, at the end of the incubation period (●) or weekly under a dim green safelight (○). Vertical lines indicate \pm s.e.

Discussion

In the present work seeds of *Dittrichia* were found to be deeply dormant. Very low germination (up to 8%) was scored in the dark; however, 90% germination was obtained with 5 min R per d given for 3 consecutive days. The small seeds of *Dittrichia* were clearly light-requiring. Even a few short exposures to a dim, green safelight strongly promoted germination, suggesting a very low threshold level of photosensitivity. On the other hand, prechilling at 5°C resulted in only a slight enhancement of subsequent dark germination, thus emphasizing the role of light as the principal agent of germination induction.

Seeds of *Helichrysum* exhibited a partial dormancy since only a portion (30–50%) germinated in the dark under the relatively cool, 'Mediterranean' temperature range, 10–20°C. Nitrates (optimal concentration 20 mM) resulted in a dramatic enhancement of germinability at 20°C, in the dark, thus establishing *Helichrysum* as the first instance of nitrate-promoted species in the postfire Mediterranean flora. The stimulatory effect of nitrates on seed germination of numerous plant species is, nowadays, well documented. In addition, both the American and the International Associations for seed testing have, since 1954, adopted officially the systematic use of 0.2% (about 20 mM) KNO₃ in their suggested germination protocols for many species (AOSA, 1981; ISTA, 1993). A curve of seed germination induction as a function of nitrate concentration similar to that obtained with *Helichrysum* has been observed in numerous cases (e.g. in the chaparral fire annual *Emmenanthe penduliflora*; Thanos and Rundel, 1995). Fire leads to a massive volatilisation of simple nitrogenous compounds, mainly nitrate and ammonium (e.g. DeBano *et al.*, 1979). On the other hand, several measurements in various ecosystems have shown a depletion of available nitrates in the presence of actively growing plants (e.g. Pons, 1989). Thus, a dormancy relief mechanism triggered by an appropriate level of nitrates would serve as a competition-avoiding, gap detector. As far as role of light is concerned, regular pulses of safelight were able to increase the final germination level in the range of optimal temperatures. The effect of a short exposure to R was of the same magnitude as that of the nitrates; moreover, a significantly increased effect (full germination induction, 96%) was obtained when both treatments were applied together.

In *Phagnalon*, dark germination was maximal (73–96%) in the range of 10–25°C (optimal temperatures: 20 and 15°C) while a considerable level (nearly 30%) was scored also at the warm 30°C. Although light was not required at the optimal temperatures it was found to significantly enhance germinability at the marginal ones. The 'aggressive' germination behaviour exhibited by *Phagnalon* is typical of an opportunist colonist with a capacity for expansion into disturbed land (not necessarily a burned one).

The results presented show that illumination of all three species was an important (to a different extent for each species) inducing agent of germination. This conclusion is in agreement with the well established fact that most light-requiring seeds are small-sized. In a survey of 69 species, many of the small-

seeded species (<0.1mg) showed a light requirement while the majority of species with seed weights 1 mg or more were light-indifferent (Grime *et al.*, (1981). Several authors have already reported light stimulation within the Compositae (e.g. Mott, 1972; Atwater, 1980; Willis and Groves, 1991; Plummer and Bell, 1995). Light requirement of seed germination is considered as a potentially significant mechanism for gap detection and this suggestion could well also extend to burned areas (as shown previously with Mediterranean plants; Thanos and Skordilis, 1987; Thanos *et al.*, 1995). Changes of light quantity and, more importantly, quality can be encountered in the postfire environment as a result of soil disturbance and/or removal of the 'far-red-enrichment filters' of the canopy and the litter. The prolific seed production and effective dispersal coupled with the inductive role of light place the three species in the category of 'widely dispersed' colonizers of disturbed habitats (e.g. recently burned sites).

Acknowledgements

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