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Photoinhibition of Seed Germination in the Maritime Plant *Matthiola tricuspidata*

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The three-horned stock, *Matthiola tricuspidata* (L.) R. Br. is a widespread annual plant of the Mediterranean sandy shores. Its seeds are dark germinating and negatively photosensitive, in accordance with our previous findings for a number of other maritime plants. Full germination was obtained at a wide range of temperatures (5–25 °C) in the dark. Inhibition of germination under light of various spectral qualities could be generally correlated, negatively and positively, respectively, with phytochrome photostationary state (ϕ) and relative cycling rate of phytochrome (H). The inhibition of germination by white (fluorescent), blue and far-red light, applied either continuously or intermittently, consistently showed a linear dependence upon the logarithm of the flux density of the irradiation. The resulting photoinhibition curves had parallel slopes and, compared to those of other maritime plants, they were shifted to higher flux densities. Continuous blue or far-red irradiations, both establishing a similar ϕ value (0·26), resulted in statistically similar regression curves, thus favouring the hypothesis that phytochrome is the single photoreceptor in the photoinhibition of seed germination.

Key words: *Matthiola tricuspidata* (L.) R. Br., three-horned stock, seed germination, light, photoinhibition, phytochrome.

INTRODUCTION

Photoinhibition of seed germination for maritime plants has been reported in *Spinifex hirsutus* (Harty and McDonald, 1972), *Pancratium maritimum* (Keren and Evenari, 1974), *Glaucium flavum* (Thanos, Georghiou and Skarou, 1989) and *Allium staticiforme*, *Brassica tournefortii*, *Cakile maritima* and *Otanthus maritimus* (Thanos *et al.*, 1991). The light-mediated inhibition of germination in these plants has been suggested to be a surface-avoiding mechanism of seedling establishment in the harsh sandy or shingle coastal habitats (Thanos *et al.*, 1989, 1991).

The control of the photoinhibition is attributed to phytochrome through the high irradiance response (HIR), which in seed germination is antagonistic to the low fluence response (LFR) and strongly dependent on the flux density (Thanos, 1993). It is postulated that germination is regulated by two antagonistically interacting factors: cycling rate of phytochrome (determined by both light intensity and quality) and active phytochrome (P_{fr}) level (expressed by ϕ, the $[P_{fr}]$ fraction at phytochrome photostationary state; Bartley and Frankland, 1982). The models explaining the HIR, in general, have not yet fully resolved the role of the blue/UV region and therefore the possible interference of the blue/UV-A light-absorbing photoreceptor (cryptochrome) is still under investigation.

Matthiola tricuspidata is a small annual crucifer, widespread in the sandy and shingle Mediterranean shores (Ball, 1964; Meikle, 1977; Greuter, Burdet and Long, 1986). The aim of the present study was to investigate the germination response of *Matthiola tricuspidata* seeds towards light, with special emphasis on flux density dependence and on the role of each spectral region.

MATERIALS AND METHODS

Seeds of the three-horned stock [*Matthiola tricuspidata* (L.) R. Br.] were collected from the ripe siliquae of numerous plants growing in the sand-gravel seashores of K. Diminio (northern Peloponnesus, Greece), Mati (Pylos, western Peloponnesus, Greece) and Agia Marina (Chania, Crete), in the summers of 1989 (for the former lot) and 1990 (for the latter two collections, used only in Fig. 1). The average seed weight was found to be approximately 0·8 mg. The seeds were stored in moisture- and light-proof plastic containers, at room temperature (20 ± 5 °C); no change of germination behaviour was observed during the experimentation period which lasted for 1 year after harvest.

Germination tests were performed in Petri dishes (7 cm diameter) lined with two discs of filter paper and moistened with 3·5 ml deionised water. The criterion of germination was visible radicle protrusion and measurements were usually taken daily. Germinated seeds were discarded and final counts were scored 7 d after onset of sowing, at the latest. Each value is the mean from five samples of either 25 or 50 seeds; ± numbers (in the Table) and vertical bars (in the Figures) represent standard error (s.e.).

All manipulations of imbibed seeds, including sowing, were carried out under a dim green safelight described elsewhere (Thanos *et al.*, 1989). The experiments were performed in controlled temperature and light conditions provided by plant growth cabinets (Model BK 5060 EL, W.C. Heraeus GmbH, Germany), a plant growth chamber (Model EY8VH, Conviron, Canada) and a programmable Growth Bench unit (model GB48, Conviron, Canada).

Two comparable, white fluorescent (W), light sources were used in both the growth bench (Table 1, Fig. 2) and the

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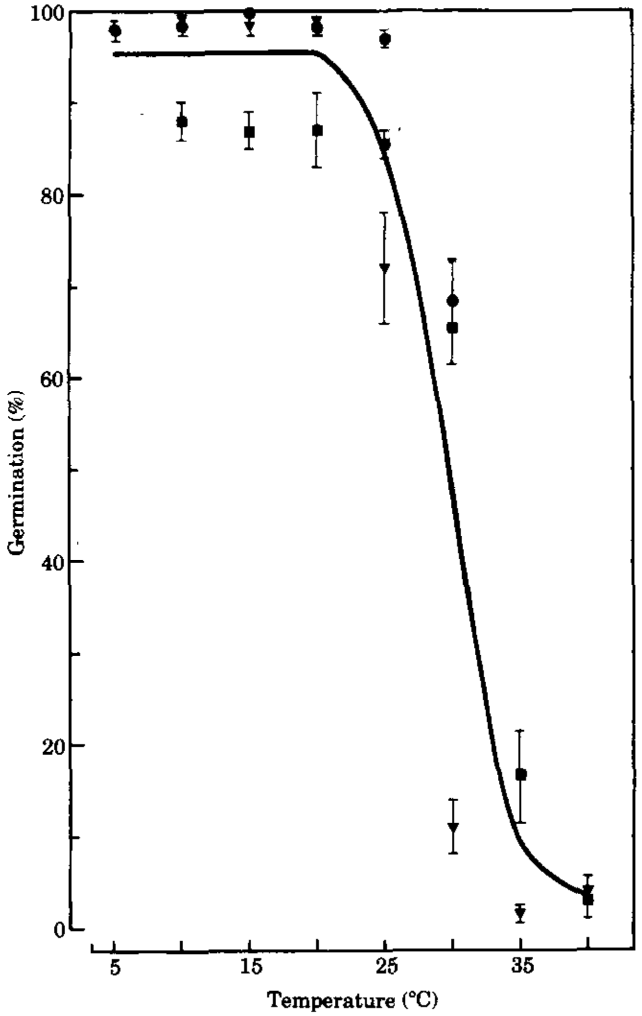

FIG. 1. Final dark germination of *Matthiola tricuspidata* seeds as a function of constant temperature. The solid line was plotted by hand through the pooled average of the three seed provenances [K. Diminio (●), Mati (▼), Agia Marina (■)]. Vertical bars represent ± s.e.

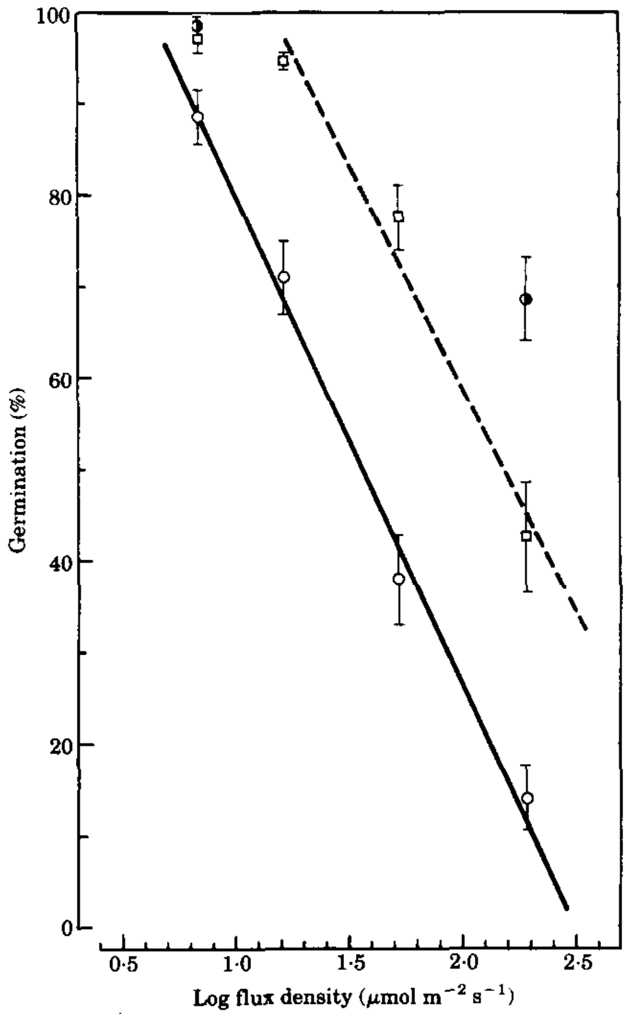

FIG. 2. Final germination of *Matthiola tricuspidata* seeds under continuous white (fluorescent) light as a function of the logarithm of photon flux density, at 15 °C (○, solid line) and 20 °C (☐, stippled line). The regression curves are given by the equations:

$$Y = 133{\cdot}2 - 53{\cdot}2X\ (r^2 = 0{\cdot}99,\ \text{d.f. } 2,\ 0{\cdot}002 < P < 0{\cdot}005),\ \text{at } 15\ °\text{C}$$

$$Y = 156{\cdot}5 - 49{\cdot}0X\ (r^2 = 0{\cdot}97,\ \text{d.f. } 1,\ 0{\cdot}10 < P < 0{\cdot}20),\ \text{at } 20\ °\text{C}.$$

◑: germination under alternating light and dark periods, 12 h each, at 15 °C. Vertical bars represent ± s.e.

cabinets (Figs 3 and 4). The two sources consisted of 12 Sylvania Cool White FR96T12/CW/VHO-235/1 tubes and 10 Philips TLD 18W/33 tubes, respectively. Illuminations of various colour qualities (Table 1 and Fig. 4) were obtained by filtering white fluorescent light through 3 mm thick plexiglas (Röhm GmbH, Germany) or coloured glass (Schott, Mainz, Germany) filters. The plexiglas filters used were: red (R) no. 501, blue (B) no. 627, green (G) no. 700, orange (O) no. 478; for far-red (FR) light, two blue and one red were combined. Two coloured glass filters with sharp cut-off towards lower wavelengths (50 % of transmittance at 515 and 665 nm, respectively) were used: yellow (Y_{515}) OG 515 and dark red (R_{665}) RG 665. Decreased flux densities were obtained using the appropriate number of either neutral density (diffuse white) plexiglas filter sheets (each 3 mm thick) or black voile layers. The broad band far-red light, used in the experiment presented in Fig. 5, was obtained in a plant growth chamber by filtering the white light of eight incandescent bulbs (Philips, 60 W) through a 3 mm thick, sky blue plexiglas filter (locally manufactured) and one dark red (no. 224) gelatine sheet (Gelatine Products Co., USA). Both blue and far-red light irradiations (Figs 4 and 5, respectively) yielded a similar value of ϕ (0·26) and their spectral composition at the four wavebands 400–500, 500–600, 600–700, 700–800 nm was 68, 6, 2, 24 % and 0, 0, 16, 84 %, respectively.

Spectral flux density measurements at the visible range (400–800 nm) were carried out with a spectroradiometer (ISCO SR, USA). For the sake of comparison, in addition to continuous illuminations, total flux densities were expressed in μmol m^{-2} s^{-1} in intermittent illuminations as well, by distributing the total irradiation dose over the entire treatment period (including dark intervals). Calculations of total and partial flux densities, phytochrome photostationary state (ϕ) and relative cycling rate (H) were made using an upgraded (by the present authors) version of a

TABLE 1. *The effect of various continuous irradiations on final germination of* Matthiola tricuspidata *seeds*

Irradiation	Flux density (μmol m^{-2} s^{-1})	ϕ	H (10^{-3} s^{-1})	% content of each waveband B	G	R	FR	Germination (% ± s.e.) 15 °C	20 °C
W	189·7	0·75	40·3	16	54	27	3	13·7 ± 3·7	42·4 ± 3·2
W+	54·0	0·75	11·7	13	53	30	4	37·8 ± 5·0	
W++	16·3	0·75	3·8	12	52	32	4	71·2 ± 4·0	
W+++	6·8	0·74	1·7	11	49	36	4	88·6 ± 3·2	96·8 ± 1·8
B	11·0	0·20	1·0	58	4	3	35	3·6 ± 1.8	8·1 ± 1·8
B+	1·7	0·20	0·2	48	3	3	46	24·5 ± 3·8	26·6 ± 6·4
G	13·6	0·39	1·9	11	52	7	30	35·2 ± 3·4	
Y_{515}	142·2	0·77	34·4	0	64	33	3	63·4 ± 4·4	
O	100·3	0·76	31·9	0	48	47	5	67·0 ± 5·0	
R	47·8	0·75	21·8	0	14	77	9	61·2 ± 3·8	
R_{665}	9·1	0·46	4·0	0	0	45	55	68·7 ± 3·5	96·8 ± 2·3
FR	2·6	0·09	0·2	0	0	4	96	18·9 ± 5·8	38·7 ± 4·1
Darkness								100·0 ± 0·0	99·2 ± 0·8

W, white; B, blue; G, green; Y, yellow; O, orange; R, red; FR, far-red.
+ Denotes the addition of neutral filters.
Flux density refers only to the visible range (400–800 nm).
ϕ is the ratio of 660 nm/730 nm photons; H is phytochrome cycling rate.
B, G, R and FR wavebands correspond to 400–500, 500–600, 600–700 and 700–800 nm, respectively.

software application developed by Andrew J. MacLellan and Barry Frankland (Queen Mary and Westfield College, London, UK). The calculations were based on phytochrome photoconversion constants estimated from modified data of Butler, Hendricks and Siegelman (1964); ϕ was assumed to be 0·80 at 660 nm and 0·025 at 730 nm.

RESULTS

The germinability of *Matthiola tricuspidata* seeds at various constant temperatures, in the dark, is shown in Fig. 1. In addition to the seed provenance (K. Diminio) used throughout this work, two supplementary seed lots were examined and, in comparison to the former lot, showed only a minor shift towards higher values of temperature. It is evident that the seeds of the three-horned stock are non-dormant with a notably high germination potential over a wide range of constant temperatures. Moreover, maximum germination was attained within 3 d at 15 and 20 °C (the optimum temperatures for all three lots) while the rate of germination decreased at either higher or lower temperatures.

Seed germination of *M. tricuspidata* was inhibited under continuous, white fluorescent light. The extent of inhibition was found to depend upon the intensity of light and final germination was linearly correlated with the logarithm of flux density (Fig. 2). The two regression curves have identical slopes but photoinhibition is higher at 15 °C; at 20 °C, seeds were less sensitive than at 15 °C, requiring a six- to seven-fold greater flux density for similar levels of inhibition. When irradiations were given at 12-h light/dark cycles a significantly decreased effect was obtained.

The photoinhibition effect of several continuous light regimes, showing diverse spectral properties, was examined at 15 and 20 °C (Table 1). Inhibition was observed in virtually all treatments and germinability was found to be, in general, directly proportional to phytochrome photostationary state (ϕ) and inversely proportional to the relative cycling rate of phytochrome (H). Nevertheless, taking into account the entire sample of the irradiations given, no simple relationship could be found to predict germination according to certain combinations of ϕ and H. However, it was evident that blue and far-red irradiations reduced germination most effectively, followed by green (rich in far-red) and white light. Yellow, orange and red illuminations (particularly rich in the green and red wavebands), all establishing a ϕ approx. 0·75, were least inhibitory, especially in comparison to white light of similar flux density or cycling rate.

Since inhibition of germination was greatly diminished when the irradiation was given in light/dark cycles (Fig. 2), it should be investigated whether this obvious alleviation of photoinhibition brought about by the long dark interval would still be obtained in shorter cycles. Consequently, the effect of intermittent white and blue irradiations was examined at 15 °C. The intermittent irradiations were administered for a 6-d period (though germination was virtually completed within 4 d) and included either 2 min light pulses separated by various dark intervals (2, 4, 8, 15 and 30 min) or a 15 min light irradiation followed by 15 min darkness. The irradiations were expressed as total dose per second (flux density, μmol m^{-2} s^{-1}) and germination percentage was plotted against the logarithm of the flux density, resulting again in linear regression curves (Figs 3 and 4). The lines obtained for white and blue light had statistically similar slopes, although the latter were shifted to lower intensities by nearly 1·5 order of magnitude (Figs 3 and 4). Moreover, the regression line for continuous blue light coincided with that obtained with intermittent blue light, and the overall effect of blue light could be expressed as one linear regression line (Fig. 4).

Subsequently, in order to investigate the probable mediation of the elusive blue photoreceptor, the inhibitory effects of broad band, blue and far-red irradiations were compared to each other. The far-red source was made to

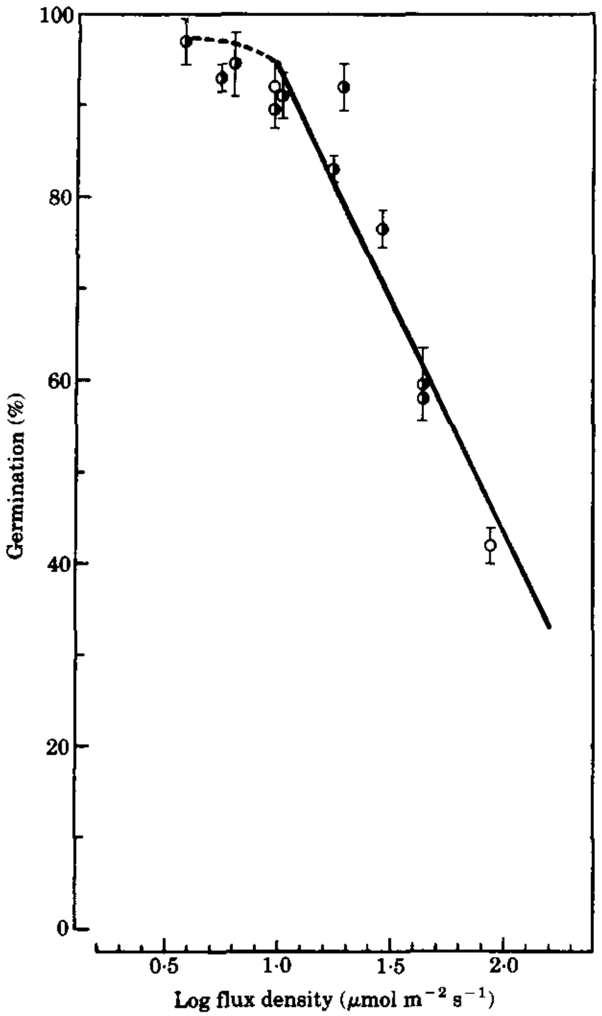

FIG. 3. Final germination of *Matthiola tricuspidata* seeds at 15 °C under intermittent (◑) and continuous (○) white (fluorescent) light as a function of the logarithm of photon flux density. The resulting regression curve of the pooled data is a straight line:

$$Y = 142{\cdot}1 - 51{\cdot}0X\,(r^2 = 0{\cdot}90,\ \text{d.f. } 7,\ P < 0{\cdot}001).$$

Vertical bars represent ± s.e.

establish a similar ϕ value to that of blue light (0·26); once more, the resulting regression curve of photoinhibition was linear (Fig. 5). Although this line is less steep than the one obtained with continuous blue irradiation, the slopes of the two lines are not statistically different ($t = 1{\cdot}33$, d.f. = 8, $0{\cdot}20 < P < 0{\cdot}50$).

DISCUSSION

The seeds of *Matthiola tricuspidata* germinated fairly quickly over a wide range of constant temperatures (5–30 °C), in darkness. Although full germination was obtained from 5 to nearly 25 °C, the highest rate of germination (100% in 3 d) was scored at 15–20 °C. Nevertheless, 20 °C should be considered the best temperature for germination, on the basis of the data of Table 1 and Fig. 2, where germination was suppressed by light to a clearly lower extent in comparison to 15 °C.

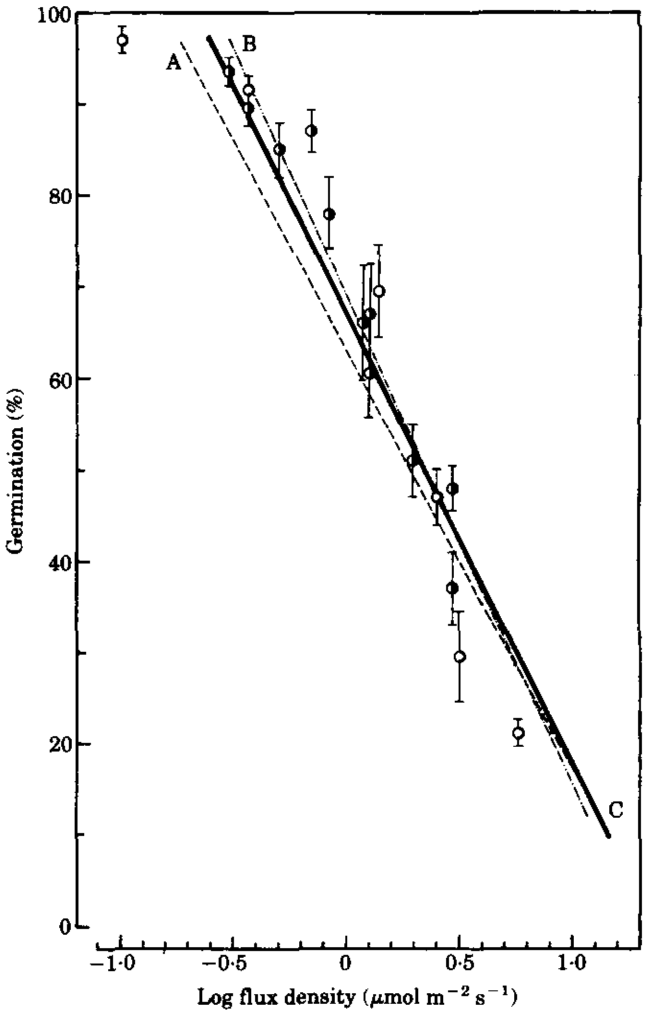

FIG. 4. Final germination of *Matthiola tricuspidata* seeds at 15 °C as a function of the logarithm of photon flux density of blue light (ϕ = 0·26): (– – – –) applied continuously (○); (-·-·-·) applied intermittently (◑). The solid line corresponds to pooled data.

A, $Y = 60{\cdot}3 - 45{\cdot}9X\,(r^2 = 0{\cdot}88,\ \text{d.f. } 4,\ 0{\cdot}005 < P < 0{\cdot}01)$

B, $Y = 66{\cdot}1 - 54{\cdot}2X\,(r^2 = 0{\cdot}94,\ \text{d.f. } 9,\ P < 0{\cdot}001)$

C, $Y = 64{\cdot}3 - 49{\cdot}4X\,(r^2 = 0{\cdot}89,\ \text{d.f. } 15,\ P < 0{\cdot}001)$.

Vertical bars represent ± s.e.

M. tricuspidata did not show any of the germination requirements displayed by its close relatives *M. incana* and *M. longipetala*, i.e. pre-chilling, potassium nitrate or light (Ellis, Hong and Roberts, 1985). On the contrary, the seeds of the present species not only germinated promptly in darkness but, moreover, light was strongly inhibitory. The inhibition of germination by various light regimes depended primarily upon the flux density and the spectral composition of the irradiation, consistent with the typical characteristics of the high irradiance response, HIR, in seeds (Frankland, 1986; Cone and Kendrick, 1986). In particular, germination was inhibited by continuous white light, even though the irradiation applied was characterized by a high ζ (R/FR photon ratio, 6·7) and established a high value of phytochrome photostationary state (ϕ = 0·75). The inhibition by white light showed a dependence upon the logarithm of the

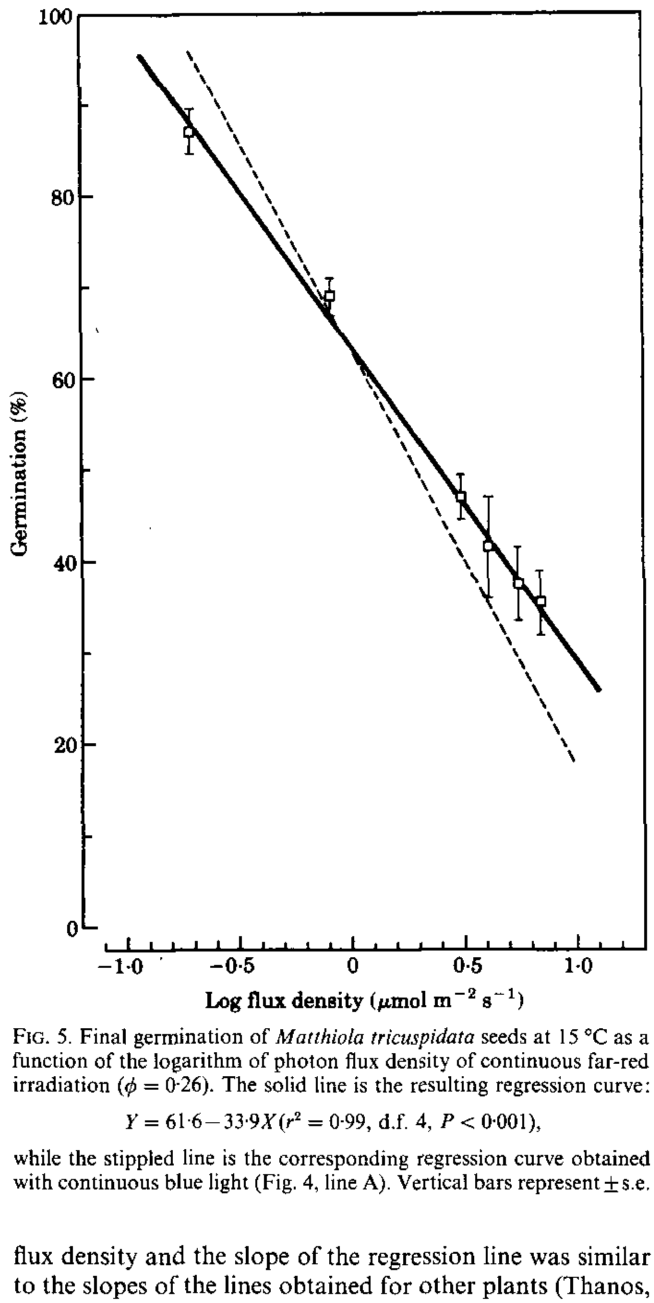

FIG. 5. Final germination of *Matthiola tricuspidata* seeds at 15 °C as a function of the logarithm of photon flux density of continuous far-red irradiation ($\phi = 0\cdot26$). The solid line is the resulting regression curve:

$$Y = 61\cdot6 - 33\cdot9X \, (r^2 = 0\cdot99, \text{ d.f. } 4, \ P < 0\cdot001),$$

while the stippled line is the corresponding regression curve obtained with continuous blue light (Fig. 4, line A). Vertical bars represent ± s.e.

flux density and the slope of the regression line was similar to the slopes of the lines obtained for other plants (Thanos, 1993), though shifted towards higher flux densities.

The second parameter that determined the inhibition was the duration of the irradiation. Using intermittent white or blue irradiations with dark intervals up to 15 min long, a log–linear 'dose/response' dependence of photoinhibition was observed. If the dose was 'distributed' over the entire treatment period, identical regression lines were obtained irrespective of the method of the attenuation of flux density (i.e. by means of either lower intensities or intermittent irradiations). Although the considerably decreased action of diurnal illumination cycles may be attributed to an escape of germination (induced during the long dark period), the dose/response effect displayed with short dark intervals cannot be explained in terms of phytochrome cycling.

Predicting germination inhibition by the high irradiance response, as observed at *M. tricuspidata* seeds, according to certain combinations of H and ϕ would have been possible if not for the discrepancy of the results of the red, orange and yellow irradiations, which did not cause the inhibition expected by their estimated H and ϕ values, compared to white light. However, it can be safely concluded that blue and far-red illuminations are obviously the most effective in inducing photoinhibition; green and white light come next while yellow, orange and red wavebands are the least inhibitory. It is also concluded that seed germination in *M. tricuspidata* shows the typical characteristics of HIR: induction of germination inhibition depends on both the duration and the flux density of irradiation while the threshold of the latter is reduced at lower ϕ values.

Although H and ϕ values of the applied irradiations may be reasonably calculated, the light conditions within the seed remain virtually unknown because of a possible selective modification of light through the filtering action of the seed coat (Widell and Vogelmann, 1985). In the case of *M. tricuspidata*, the seed coat is tinted lightly brown, probably due to a certain chlorophyll degradation product. In the vicinity of the radicle end, the presumed site of photosensitivity, the seed coat is only a few cell layers thin; however, subsequent research on the subject should include an investigation of the seed coat filtering properties. Nevertheless, despite the reservation of the possible filtering action of the seed coat, the present finding that the regression lines of the photoinhibition, induced by blue and far-red irradiations of similar ϕ value, are statistically coincident supports the concept of a sole photoreceptor (phytochrome) and makes the mediation of a blue photoreceptor unnecessary. However, the coincidence of the photoinhibition curves requires confirmation both with more numerous experimental data and in other plant species.

Matthiola tricuspidata is a common herb, endemic to the Mediterranean rim (Greuter *et al.*, 1986), growing under typical Mediterranean climates, in shingle and sandy beaches, often in large populations, and sometimes also by the roads near a beach. It was clearly shown that *M. tricuspidata* is another example of a photoinhibited maritime plant; for germination to take place, a considerable attenuation of natural light is required, a condition satisfied when seeds are adequately buried. Compared to the other plants tested, photoinhibition of seed germination in *M. tricuspidata* was the least sensitive. Nevertheless, it should be noted that the flux density of direct sunlight measured on an early autumn day was 2350 μmol m^{-2} s^{-1}, which is at least one order of magnitude higher than the estimated white light flux density resulting in full inhibition. A notable characteristic of the three-horned stock arises from both the very wide (significantly extended towards higher values) temperature range and the fast pace of germination, features which do not conform with the germination physiology syndrome of a typical Mediterranean plant (Thompson, 1973; Thanos *et al.*, 1989, 1991). This inconsistency may be partly attributed to the annual life habit of the species as well as its 'weedy' strategy. The opportunistic strategy of *M. tricuspidata* is additionally verified by the very long flowering period (Meikle, 1977; Polunin and Huxley, 1981), illustrated by an interesting incident that took place in December 1989. After an early, partly wet autumn and an exceptionally dry winter, dense stands of *M. tricuspidata* in full bloom were observed at Varkiza beach near Athens. It is assumed that the seeds had been able to germinate immediately after the first rains, which occurred during September. Relatively high temperatures and short duration of moisture availability (limiting factors of germination in typical Mediterranean plants) did not obstruct seed germination and subsequent seedling establishment, while the untimely drought of December may have forced flowering.

In conclusion, the *M. tricuspidata* seed ecology syndrome includes dispersal by sea drift, a wide temperature range of dark germination with relatively high rates, and full suppression of germination by light intensities encountered naturally on or slightly below the beach surface.

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