

# METHODS IN COMPARATIVE PLANT ECOLOGY

*A laboratory manual*

---

EDITED BY G.A.F. HENDRY AND J.P. GRIME

NERC Unit of Comparative Plant Ecology  
Department of Animal and Plant Sciences  
University of Sheffield  
UK

1993



CHAPMAN & HALL

London · Glasgow · New York · Tokyo · Melbourne · Madras

## 3.56 Germination and the high irradiance reaction

C.A. Thanos

### *Rationale*

According to the definition of the seed dormancy concept shared by plant physiologists, for a seed to be considered non-dormant germination has to be capable of occurring in darkness [1]. In a number of plants, light pulses during imbibition have been adopted, among numerous treatments, to break dormancy and promote germination [1]. To make this procedure simpler, the protocol for routine seed testing of many species includes a prolonged, diurnal photoperiod (at least 8 hours of rather dim, white fluorescent light) [2]. In contrast to the mild illumination regimes used in laboratories, harsh conditions over lengthy periods with very high light irradiance are experienced in

the field, particularly at or close to the soil surface. Numerous investigations of the high irradiance reaction (HIR) in many morphogenetic events during plant development have shown that, in contrast to the LER (or LFR, low energy – or fluence–reaction), an HIR requires long-term irradiation; it is both wavelength and fluence rate dependent and does not show red/far-red reversibility, neither does it obey the reciprocity law [3]. Despite the scarcity of relevant data on seed germination, it is evident that an HIR suppresses germination while an LER is promotory. This is in striking contrast to other photomorphogenetic actions where both HIR and LER run in parallel [4]. Therefore the importance of the present test lies in the possible detection of germination inhibition by ‘natural daylight’ among dark-germinating species. In an ecological context, such a finding might be of further value by demonstrating an adaptative trait in native plant populations.

### Methods

The light source used should be, both qualitatively and quantitatively, as close to daylight as possible. Thus  $\zeta$  (R/FR photon ratio) should be 1.0–1.2 and, as a better indicator, the estimate of  $\phi$  (the concentration of the active phytochrome form, [Pfr], as a fraction of total phytochrome, [Ptot]) should be 0.50–0.65 [5]. Xenon lamps, in addition to being a good match to daylight, provide high values of white light irradiance (flux density, fluence rate). Nevertheless, for reasons of safety, cheapness and simplicity, and despite the lower intensities achieved, the construction of a bank of both fluorescent and incandescent filament tubes is strongly suggested [6]. The rated wattage supplied by the incandescent lamps will vary in the range 50–70% of the total, according to the lamp types used. Attention should be paid to uniformity of light output, the

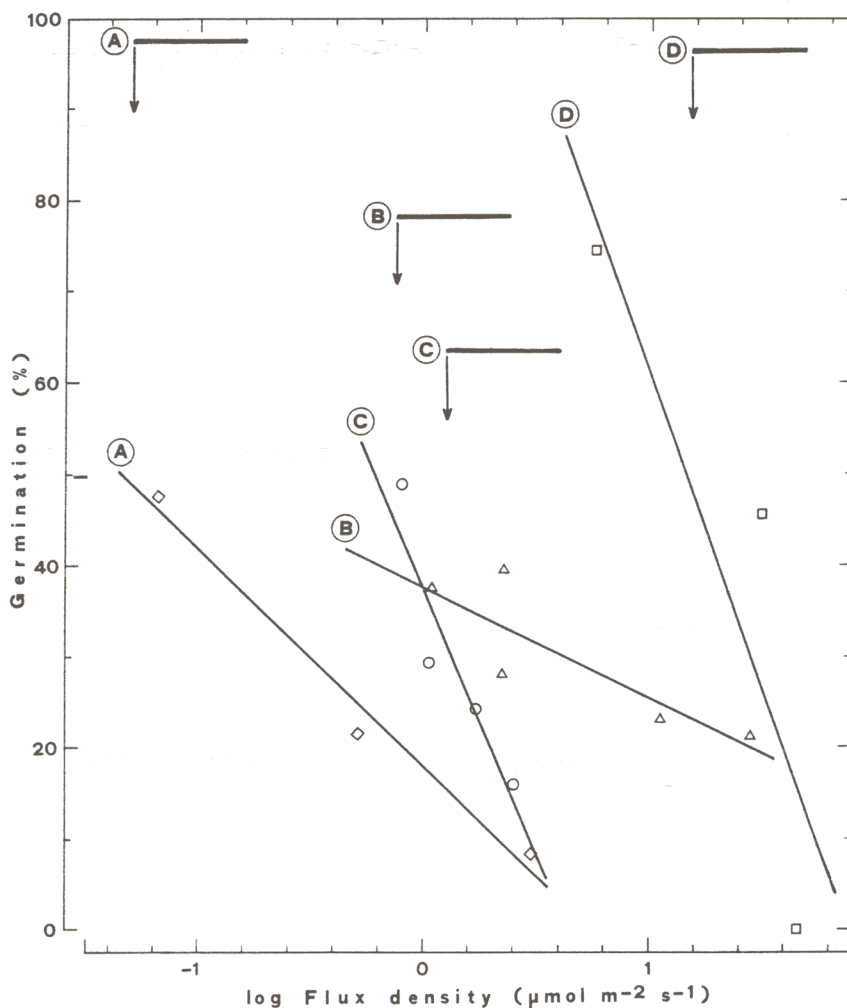
intensity of which on the seed surface should be at least  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$  at the visible range (400–800 nm). Decreased values of irradiance can be obtained by numerous methods, preferably by neutral filters (glass or plastic). Germination is usually carried out in Petri dishes, on filter paper [2], using at least five replicates of at least 20 seeds each. The test should be performed at the optimum temperature, constant or alternating (see germination at constant and fluctuating temperatures). An alternative would be a multi-step simulation of diurnal temperature fluctuations, resembling those prevailing in nature at the period when field germination of a particular plant is being observed. Where a constant temperature is adopted for the test, illumination should be continuous, or supplied during the simulated ‘day’.

### Results

Germination data should be drawn as a function of log photon flux density (Figure 3.75). A typical photoinhibition curve is a straight line (obtained by regression analysis) with a particular slope and an  $I_{50}$  value (the log irradiance that suppressed germination by 50%). An important, yet largely unresolved, matter concerning photosensitivity may be the involvement of the optical properties of the seed coat and other tissues [10] surrounding the radicle (presumably the site of light perception).

### Relevance

Photoinhibition of seed germination may be considered as a mechanism based on a light quantity measuring ability. Thus it may be viewed as a soil depth sensing device which enables seeds (and eventually seedlings) to avoid germinating (and establishing) at the harsh conditions of the surface (e.g. in the water-stressed, sandy or shingle, Mediterranean beaches [7,8,9]).



**Figure 3.75** Final seed germination as a function of white light flux density in *Glaucium flavum* Crantz (A), *Cakile maritima* Scop. (B), *Brassica tournefortii* Gouan (C) and *Allium staticiforme* Sibth. & Sm. (D). White light, mixed fluorescent and incandescent ( $\phi$  ca. 0.6;  $\zeta$  ca. 1.2) is applied either continuously, at 20°C (B) or during the warm period of a diurnally alternating temperature regime: 10.5 h (15°C)/13.5 h (10°C) for A; 11 h (20°C)/13 h (13°C) for C and D. In the case of *C. maritima* only the seeds from the lower fruit segments are tested following an initial 3 week chilling pretreatment. The resulting regression curves for the cases presented are:

A:  $Y = 18.03 - 23.76X$  ( $r^2 = 0.98$ ,  $0.025 < P < 0.05$ )

B:  $Y = 37.66 - 12.14X$  ( $r^2 = 0.74$ ,  $0.025 < P < 0.05$ )

C:  $Y = 37.54 - 58.32X$  ( $r^2 = 0.86$ ,  $0.025 < P < 0.05$ )

D:  $Y = 130.28 - 69.096X$  ( $r^2 = 0.78$ ,  $0.10 < P < 0.25$ )

The horizontal bars represent corresponding dark controls; the vertical arrows indicate  $I_{50}$ , the values of flux density resulting in 50% suppression of dark germination. (Redrawn data after [7] for A, [9] for B, and [8] for C and D).

**Caution.** a) Phytochrome is considered to be involved in this mechanism although the additional mediation of a blue-absorbing photoreceptor cannot be excluded [4,7,8]. b) Use of incandescent white light (or broad band far-red light) may lead to confusion with the LER of seed germination while, on the other hand, fluorescent light may fail to reveal the photoinhibition response.

Induction of a light requirement during seed development and its ecological consequences *Nature*, **291**, 583–585.

## References

1. Bewley, J.D. and Black, M. (1982) *Viability, Dormancy, and Environmental Control (Physiology and Biochemistry of Seeds in Relation to Germination, Volume 2)*, Springer-Verlag, Berlin, Heidelberg and New York.
2. International Seed Testing Association (1985) International rules for seed testing. Annexes 1985. *Seed Science and Technology*, **13**, 356–513.
3. Smith, H. and Whitelam, G.C. (1990) Phytochrome, a family of photoreceptors with multiple physiological roles. *Plant, Cell and Environment*, **13**, 695–707.
4. Frankland, B. (1986) Perception of light quantity, in *Photomorphogenesis in Plants* (eds R.E. Kendrick and G.H.M. Kronenberg), Martinus Nijhoff Publishers, Dordrecht, Boston and Lancaster, pp 219–235.
5. Smith, H. (1986) The perception of light quality, in *Photomorphogenesis in Plants* (eds R.E. Kendrick and G.H.M. Kronenberg), Martinus Nijhoff Publishers, Dordrecht, Boston and Lancaster, pp 187–217.
6. Holmes, M.G. (1984) Light sources, in *Techniques in Photomorphogenesis* (eds H. Smith and M.G. Holmes), Academic Press, London, pp 43–79.
7. Thanos, C.A., Georgiou, K. and Skarou, F. (1989) *Glaucium flavum* seed germination – an ecophysiological approach. *Annals of Botany*, **63**, 121–130.
8. Thanos, C.A., Georgiou, K., Douma, D.G. and Marangaki, C.J. (1991). Photo-inhibition of seed germination in Mediterranean maritime plants. *Annals of Botany*, (in press).
9. Delipetrou, P. (unpublished data).
10. Cresswell, E.G. and Grime, J.P. (1981)