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27 Dormancy and Germination in *Eucalyptus globulus* Seeds

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Abstract

Under laboratory test conditions commercial *Eucalyptus globulus* Labill. seed lots generally have a high level of viability and rapid, uniform germination. However, commercial nurseries occasionally report seed lots in which germination is spread over a period of up to 10 weeks. The present study examined apparent dormancy induction in seeds that were initially imbibed for periods ranging from 0 to 48 h in darkness followed by air drying and open storage for 1 week at 25°C ± 1°C. Germination tests were carried out in a 12 h light/12 h dark (12/12) photoperiod or in complete darkness. Longer periods of imbibition prior to drying reduced the percentage of seeds that germinated and slowed the rate of germination in seeds germinated under a 12/12 photoperiod. Seeds with the same pretreatment germinated normally in continuous darkness. These data suggest a previously unreported inducible, light-regulated dormancy in *E. globulus* seeds.

Introduction

Eucalyptus globulus Labill. is widely used in plantations in southern Australia. Generally seedlings are raised in Lannen® type trays and grown under nursery conditions for ~6 months before planting out in winter. Under most circumstances *E. globulus* seeds used in commercial nurseries have high viability (95%) and usually complete germination in 5–6 days. Occasionally, germination is erratic. Anecdotal evidence suggests that this problem occurs following a brief period of desiccation or high temperatures between sowing and germination.

Germination characteristics of *Eucalyptus* have not been widely studied but Clifford (1953) noted that *E. globulus* might germinate better in the dark. Wilson *et al.* (2005) found that *E. ovata* Labill. seed, hydrated and subsequently dehydrated during pelleting, exhibited a type of secondary dormancy broken by germination in complete darkness. A hydration–dehydration treatment has been

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used to promote germination in the seeds of several species, including tomato (*Lycopersicon esculentum* Mill.), oats (*Avena sativa* L.) (Berrie and Drennan, 1971), wheat (*Triticum aestivum* L.) (Hanson, 1973) and *Aster kantoensis* Kitam. (Kagaya *et al.*, 2005). The main purposes of this study were to investigate the effects of hydration–dehydration treatments under laboratory conditions, and light conditions during germination, on the induction and expression of secondary dormancy in *E. globulus* seeds.

Materials and Methods

Plant material and standard germination trials

Four commercial, size-graded seed lots used in experiments were from *E. globulus* orchards located near Mount Gambier in South Australia. The orchard consists of trees originating from the geographical range of *E. globulus* Labill. species and populations intergrading with other *E. globulus* subspecies. The majority (~90%) of trees present in seed orchards are from Western Otways, Strezelecki ranges, Furneaux and south-eastern Tasmanian races. For further details on the racial classification, refer to Dutkowski and Potts (1999). Seed lot 'h' (1000–1200 μm) and 'Li' (1588–1936 μm) were from orchard mixtures, whereas seed lots 'KI' (1693–1954 μm) and 'Ki' (1954–2183 μm) were from maternal parents recorded as King Island within the seed orchard.

All experiments were randomized complete block designs, with blocks arranged according to position in the germination cabinet. There were four replicates of 50 seeds in each trial. An analysis of variance (ANOVA) was conducted on the results using the general linear models package of SPSS (version 12.0.1, 2003) and means were compared using Fisher's least significant difference (LSD) test (Steel and Torrie, 1981). In all cases, individual seed lots were subjected to separate ANOVAs. Error bars shown on graphs are standard errors (SEs) of the mean. Laboratory experiments were carried out according to the International Seed Testing Association (ISTA, 1999) guidelines for the species. Germination was carried out at 25°C ± 1°C in a Lindner and May model LMRIL-1-SD germination cabinet with 12/12 photoperiod where lighting was provided by two GE fluorescent tubes (F30W/33). Seeds were germinated on Petri dishes with two layers of Type 2 Advantec 80 mm filter paper moistened with 7 ml of deionized water. Additional deionized water was added as required during the germination test to avoid drying. Seeds were considered to be germinated when the radicle was longer than 4 mm. At each count, germinated seeds were removed from the Petri dishes after scoring. A squash test was performed at the end of the germination period to determine the viability of the ungerminated seeds (Yates *et al.*, 1996).

Presoaking

Seeds from the 'h' and 'Li' seed lots were surface-sterilized with 10 ml of 12% commercial bleach for 10 min before being rinsed thoroughly in distilled water

4–5 times and blotted dry with a paper towel. Four replicates of 50 seeds were then soaked in separate vials of distilled water for 0 (control), 6, 12, 24 and 48 h at room temperature of 18–20°C in natural light and dark conditions (~16/8 h light/dark period). The distilled water was changed every 6 h to prevent hypoxia. Seeds were then placed on filter paper in Petri dishes and transferred immediately to the germination cabinet with a 12/12 photoperiod. Germination was scored on days 5, 8 and 10.

Presoaking and drying back

Seeds from the 'h' and 'Li' seed lots were soaked as above for 0, 18, 24 and 30 h in complete darkness at 25°C, dried back and then stored at room temperature (18–20°C) under the same natural light conditions for 7 days before germination in 12/12 photoperiod. Germination was scored on days 5 and 11.

Responses to light during germination

Seeds from the 'KI' and 'h' seed lots were pre-imbibed for 0 (control), 7, 14, 24, 30 and 35 h in the dark on wet filter paper in Petri dishes at 25°C ± 1°C, surface-dried with a paper towel before being dried back and stored. Germination was carried out in complete darkness or in a 12/12 photoperiod. Covering Petri dishes with two layers of aluminium foil created complete darkness and the dishes were unwrapped under a green safe light for germination counts. Dishes were left unwrapped for the 12/12 treatments. Germination counts were conducted on days 6, 10, 13 and 19. The experiment was analysed as a factorial design with six times of imbibition by two light treatments (i.e. 12/12 photoperiod and complete darkness).

Glasshouse trial

Seeds from the 'KI' seed lot were imbibed for 30 h in the dark at 25°C ± 1°C, dried with a paper towel and stored at room temperature (18–20°C in 16/8 h light/dark period) for 7 days. After storage, seeds were germinated in six Lannen® trays filled with Richgro® seed-raising mixture. Each treatment contained 60 seeds, with 5 seeds per cell. After sowing, trays were irrigated before half of each tray was covered with an opaque reflective insulation sheet. The remaining half of each tray was covered with a transparent polythene sheet. Sensors attached to 'Tiny Talk' temperature loggers (Gemini Data Logger, Chichester, West Sussex, UK) were inserted just below the surface of the potting mix in both treatments to record temperatures near the germinating seeds. The trial was a factorial design with two light and two imbibition (i.e. 0 and 30 h) treatments with four replicates. Germinated seeds were scored between days 13 and 20 in natural light conditions.

Results and Discussion

Presoaking

Soaking seeds in laboratory conditions for >24 h significantly reduced germination on day 5 ($P < 0.05$) when compared with the control in seed lot 'h' (Fig. 27.1), but in seed lot 'Li' soaking did not adversely affect germination (data not shown). Battaglia (1993) reported a similar result in *E. delegatensis* seeds, where a longer period of imbibition decreased germination capacity, but significant interprovenance differences in germination capacity were observed.

Presoaking and drying back

Dark soaking for 18 h or more significantly reduced germination on day 5 and 11 at $P < 0.05$ (Fig. 27.2). In addition, this pretreatment contributed to a greater spread in germination through the test period (data not shown). There was a consistent reduction in initial germination with increased soak time up to 25 h, which suggests that the response is related to changes in the seed during the very early stages of germination.

Responses to light during germination

Studies carried out by Bell *et al.* (1995, 1999) with *E. marginata* and by Wilson *et al.* (2005) with *E. ovata* demonstrated that darkness may increase germination in some *Eucalyptus* species. In both seed lots used in the present trial, there was a significant ($P < 0.05$) interaction between germination conditions and imbibition time, with a marked decrease in day 6 germination when time of imbibition increased for seed germinated in alternating light and dark conditions (Fig. 27.3a and c). In contrast,

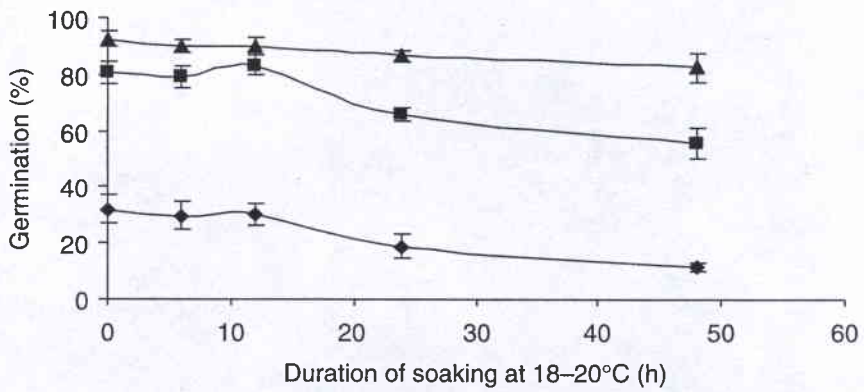

Fig. 27.1. Mean germination percentage on days 5 (◆), 8 (■) and 10 (▲) for seed lot 'h', germinated after soaking in laboratory conditions at 18–20°C for 0–48 h. Data points are results of four replicates of 50 seeds ± standard error (SE) of the mean.

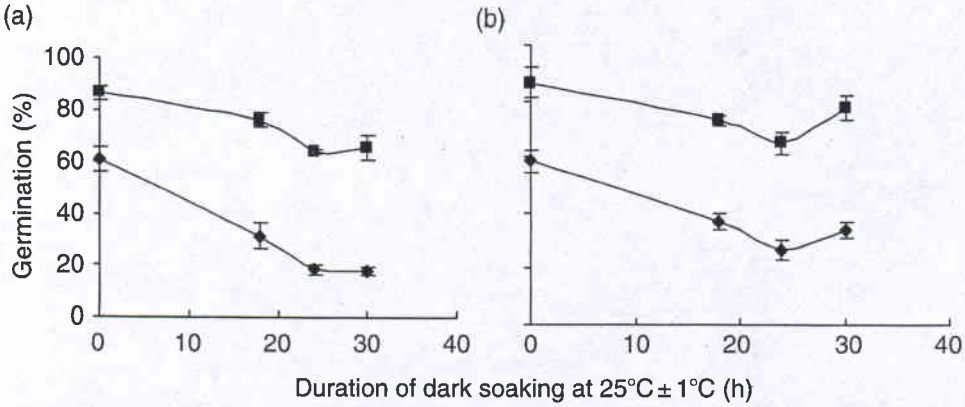

Fig. 27.2. Mean germination percentage on days 5 (◆) and 11 (■) for seed lot (a) 'h' and (b) 'Li' germinated after different periods of dark soaking at 25°C ± 1°C, followed by 7 days drying back at room temperature (18–20°C) in natural light conditions. Data points are results of four replicates of 50 seeds ± standard error (SE) of the mean.

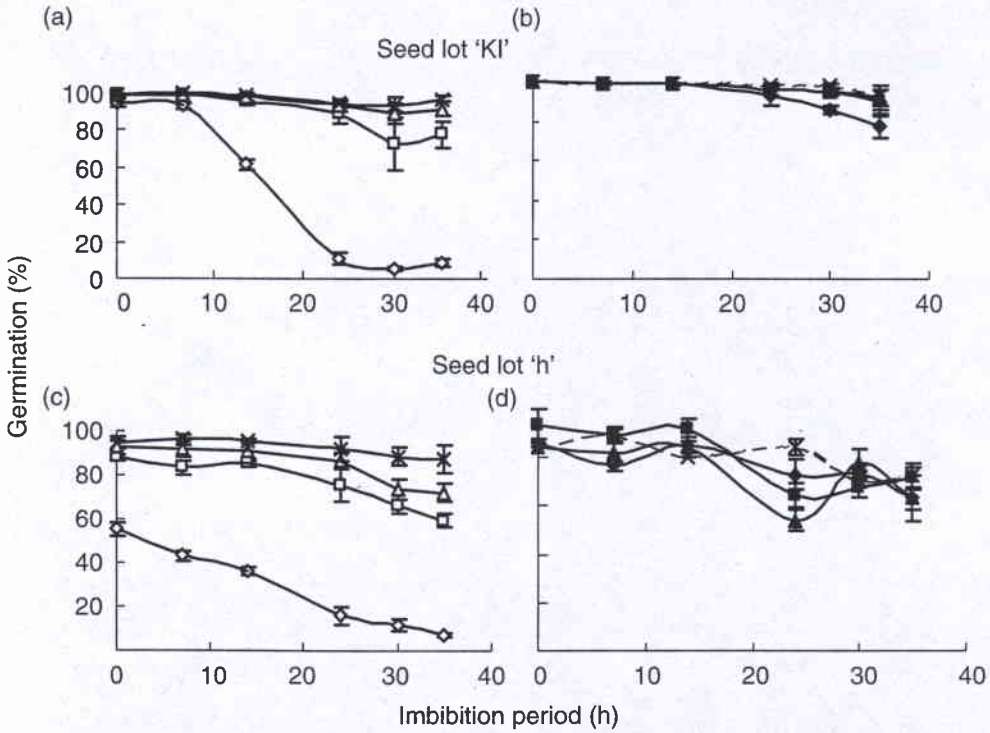

Fig. 27.3. Mean germination (%) in alternating light and dark (open symbols) and darkness (solid symbols) on days 6 (◇, ◆), 10 (□, ■), 13 (△, ▲) and 19 (×, –×–) for seeds imbibed for different time periods, followed by drying back for 7 days at 18–20°C in natural light conditions. Data points are results of four replicates of 50 seeds ± standard error (SE) of the mean.

seed germinated in the dark showed no significant response to pretreatment (Fig. 27.3b and d). There were no significant effects of either pretreatment or germination conditions on days 11–19 of germination.

The results clearly indicate delayed germination or possibly a secondary dormancy induced by pretreatment, and an effect of continuous darkness during germination in overcoming this imposed inhibition of germination.

Glasshouse trial

Under transparent plastic covering, temperature ranged from daytime maxima of 47–50°C to night-time minima of 9–10°C. In trays under the opaque cover, temperatures ranged from maxima of 38–40°C to minima of 9–10°C. There was a marked difference in germination between pretreated and non-treated seed, with pretreatment causing a significant reduction ($P < 0.05$) in germination (Fig. 27.4). Further, germination was significantly higher ($P < 0.05$) in darkness compared with natural light, but there was no significant interaction ($P > 0.05$) between pretreatment and light during germination.

The experiments described show that pretreatment, by allowing the seed to imbibe water in darkness for around 20–30 h, followed by drying back, leads to reduced germination or possibly secondary dormancy in this species. From the results obtained, especially the significant reduction in germination with soaking without drying back in the first experiment, it is difficult to separate soaking (or perhaps soaking conditions) from the drying back treatment. The response to light during germination indicates that the induced dormancy is broken by germination in darkness. Dark-promoted germination of dormant seeds is relatively uncommon, but the induction of dark-responsive dormancy reported here suggests some re-examination of the possible influences of environmental conditions, particularly during early germination, in seed testing and dormancy studies. From a practical

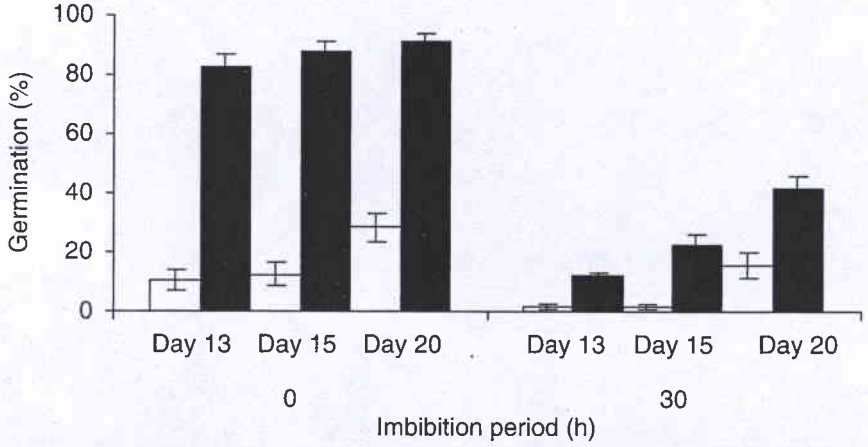

Fig. 27.4. Glasshouse germination on days 13, 15 and 20 for seeds imbibed in darkness for 0 or 30 h at 25°C ± 1°C and dried back and stored for 7 days at room temperature (18–20°C) and germinated in alternating natural light and dark (□) or continuous dark (■) conditions. Bars are standard errors (SE) of the mean ($n = 6$).

point of view, appropriate management of light during the early stages of germination appears to be critical in commercial nurseries. The implications of such a dormancy response system in natural ecosystems remain to be explored.

Acknowledgements

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