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Original article

The effect of temperature and water stress on laboratory germination of *Eucalyptus globulus* Labill. seeds of different sizes

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Abstract – Germination rate and germination capacity of *Eucalyptus globulus* Labill. increased significantly with increasing temperature (13º to 33 ºC) for all seed sizes to an optimum at 28 ºC, then decreased. Biggest seeds generally germinated best at all temperatures. Germination was also very sensitive to water potential (0 to –0.75 MPa), with no germination occuring at potentials below –0.25 MPa.

***Eucalyptus globulus* / germination / polyethylene glycol / seed size / temperature / water potential**

Résumé – Effet de la température et du stress hydrique sur la germination en laboratoire de graines d'*Eucalyptus globulus* Labill de différentes tailles. On a étudié l'influence sur la germination des graines d'*Eucalyptus globulus* Labill de températures constantes comprises entre 13º et 33 ºC et de potentiels hydriques compris entre 0 et –0,75 MPa. La germination était significativement influencée par la température et la taille des graines. La vitesse et le taux de germination augmentaient avec la température pour atteindre un optimum à 28 ºC et ensuite diminuaient. Quand la germination était effectuée en conditions de stress on observait une diminution du taux de germination entre –0,01 et –0,75 MPa. Plus aucune graine ne germait à –0,25 MPa et au-delà.

***Eucalyptus globulus* / germination / dimension de la semence / température / potentiel hydrique**

1. INTRODUCTION

Eucalypt pulp has excellent properties for paper making and is in high demand. The development of new pulping technologies and the potential to provide a low cost, uniform resource through silviculture, selection and breeding, suggest a continuing bright future for eucalypt plantations [26]. However, the cellulose pulp market in the European Union (EU) shows a supply shortage that is being compensated by imports from South American countries or New Zealand. Productivity of plantations, particularly in Spain, through breeding and better management practices will result in a smaller area being required to produce the same amount of wood. This is especially important in the EU because regions where *E. globulus*, the most common eucalypt species in Europe, grows naturally are confined to southern warm and humid environments.

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Seed handling in the nursery is one factor that determines the time required for seed germination. Poor emergence of *Eucalyptus* spp. and delayed full emergence are serious limitations, not only in achieving efficient seed usage, but also in avoiding the additional production costs of pricking in. These problems are specially important when using seedlots from different provenances because seedling crops tend to be uneven. They are difficult to manage because larger plants from one seed source may shade smaller ones from another seed source, and also because watering regimens may have to be tailored to different sources. Consequently, the need for producing uniform seedling crops is increasing. Since germination synchrony partly determines seedling size, grade and overall quality, several practices including stratification, seed sizing, sowing by family and seed priming are used to enhance crop homogeneity and reduce cull percentages [22]. In spite of this, the response of eucalypt seeds in the nursery is normally quite low.

Eucalypt seed research has focussed mainly on germination responses of one particular species to only one or two environmental stimuli [1–4, 12, 14]. A more holistic approach to determine the effects of other environmental factors and their interactions in *Eucalyptus occidentalis* germination was described by Zohar and co-workers [28]. Likewise, Battaglia [2] demonstrated that sub- and supra-optimal temperatures and water stress interacted in their effect on cumulative germination and the germination rate of *Eucalyptus delegatensis*, revealing significant inter-provenance variations in germination traits. However, the main objective of these articles was to predict sowing times to optimise reforestation efforts, because regeneration following clear-felling of native overstorey trees is usually done by direct seeding.

The purpose of this report was to determine how temperature, water potential and seed size in *E. globulus* might be exploited to improve germination efficiency and seedling uniformity.

2. MATERIALS AND METHODS

E. globulus seeds of Flinders Island (Australia) provenance, obtained from a commercial supplier, were stored with silicagel in darkness at 4 ºC before use. To study the effect of seed size on germination, seeds were sized using screens of different square mesh apertures: 1.2, 1.5, 1.7, 2 and 2.2 mm, and divided into 5 different groups (sizes 1 to 5, respectively).

Germination tests were carried out in controlled environment chambers using cool-white fluorescent tubes (16 h, photosynthetic photon flux of 90 μmol m^{-2} s^{-1} at the germination surface). Seeds for different experiments were placed in clear-plastic boxes (600 × 650 × 60 mm) on cellulose paper (Fanoia 1516/400) moistened with water through an absorbent wick except as indicated, then covered with 80 mm diameter Petri dishes to maintain the relative humidity close to 100%. In the boxes the same volume of water or polyethylene glycol solutions was maintained.

To determine initial moisture content four replications of 100 seeds each of the two main sizes in a seedlot (3 and 4) and of an unsorted samples, were dried at 103º–105 ºC for 17 hours [18]. Afterwards, seeds were removed and chilled for 5–10 minutes in a dessicator at room temperature, then weighed again to determine the loss of water suffered by the seeds. Seed imbibition rate was monitored at 10º and 23 ºC by measuring the increases in seed weight at intervals after being placed on the moist cellulose medium.

Five replications of 100 seeds each from the five size classes were randomly placed in germination boxes, and tested over a range of sub- to supra-optimal constant temperatures of 13º, 18º, 24º, 28º and 33 ºC (Å 2 ºC) that were based on data from Spanish nurseries that grow eucalypt seedlings.

For the purpose of this study, germination was considered as being complete when the radicle emerged from the seed. Germinated seeds were counted and removed every 24 h until germination stopped.

The rate of germination was estimated from the reciprocal of the time taken to reach 50% of the final cumulative germination, T_{50}, under the test conditions following the beginning of imbibition.

Germination was observed in a series of polyethylene glycol (PEG 8000, Sigma) solutions ranging from 0.01 to 0.75 MPa. PEG solutions were prepared according to Michel [20], and the 1 was verified using a vapour pressure osmometer (Wescor model 5500) calibrated against NaCl standards.

Four replications of 100 seeds each from seed size 3 were randomly placed in germination boxes. The cellulose paper was moistened with the PEG solutions except for a control that was moistened with distilled water. Based on results from the temperature experiments conducted previously, and because *E. delegatensis* seeds are less affected by moisture stress when germinated near the optimal temperature [2], the soil water potential experiments were conducted at 25 ºC (± 2 ºC).

Differences in germination (capacity and rate) were subjected to analyses of variance [24]. Data transformations were used conducting an ad-hoc procedure for finding appropriate transformations to normalize the variables and achieve homogeneity of variances.

Germination parameters were treated as dependent variables, temperature, seed size and time to germination as independent variables.

To examine the influence of temperature, size and water potential on germination, sigmoidal or Weibull models were used for determination of T_{50} ($r \geq 0.85$) [9]. Germination rate and germination capacity were the dependent variables, whereas temperature, seed sizes and number of days until germination were the independent variables.

3. RESULTS

Germination of unsized *E. globulus* seeds was significantly affected by temperature *(figure 1a)*. Visible signs of germination occured between 24 and 36 hours after sowing, being earlier at higher temperatures. Fastest and most complete germination occured at 28 ºC *(figure 1b)*. Germination capacity declined at 33 ºC, revealing 28 ºC as the optimum germination temperature for this unsorted seedlot.

Germination rate increased with temperature to an optimum of 28 ºC and then declined *(figure 1b)*. The lower and upper temperature thresholds for germination of *E. globulus* were not encountered in this study, but were observed to be lower thatn 10 ºC and above 33 ºC, respectively.

All size classes showed the same pattern of increasing germination rate with increasing temperature to a maximum at 28 ºC, then a decrease *(figure 1c)*. Maximum germination capacities for sizes 1 and 2 occurred between 13 and 33 ºC; for seed sizes 3 and 4 the maximum occurred between 18º and 24 ºC. While a significant interaction was found between temperature and seed size *(table I)*, all seed sizes appeared to germinate well over a range of constant temperatures between 18º and 28 ºC. Although differences were small, seed sizes 4 and 5 appeared to be the least sensitive to temperature within this range. Maximal differences in germination capacity among seed sizes were found at 13 ºC.

Germination rate was highest in all seed sizes at 28 ºC and above 28 ºC, germination rate declined sharply for all seed sizes *(figure 1d)*. A significant interaction between temperature and seed size on germination rate was observed *(table Ib)*.

Seed sizes 3 and 4 imbibed at 23 ºC began germinating after approximately 36 h. At this temperature, moisture levels increased quickly during the first 24 h, then leveled off at around 63–75%. This was followed by a period of relative slow water uptake, until RWC once again increased rapidly as radicle emergence commenced. Imbibition speed and moisture content increased as temperature increased: after 48 hours at 10 ºC, moisture content was 60%, but was 65% after 24 hours at 23 ºC. Rate of imbibition and moisture level was higher in larger seeds: after 48 hours, size class 2 had a moisture content of 63%, while size class 3 had reached 75%.

Germination capacity and germination rate in size 3 seeds decreased with decreasing water potential (*figures 1e* and *1f*). Although osmotic potentials of –0.01 MPa had little effect on germination capacity, potentials greater than –0.05 greately reduced germination and no seeds germinated under water potentials of –0.25 MPa or lower *(figure 1e)*, despite the high relative humidities maintained during the tests. The response of germination rate to water potential was similar *(figure 1f)*.

Table I. Analysis of variance table for temperature and seed size effects.

Source	d.f.	Sum of squares	Mean square	*F* value	*P*
(a) Germination capacity					
Temperature	4	1.296	0.324	34.810	0.001
Size	4	1.671	0.418	44.906	0.001
Interaction	16	0.485	0.03033	3.26	0.001
(b) Germination rate ($1/T_{50}$)					
Temperature	4	1.214	0.303	273.39	0.001
Size	4	0.290	0.072	65.40	0.001
Interaction	16	0.088	0.0055	4.99	0.001

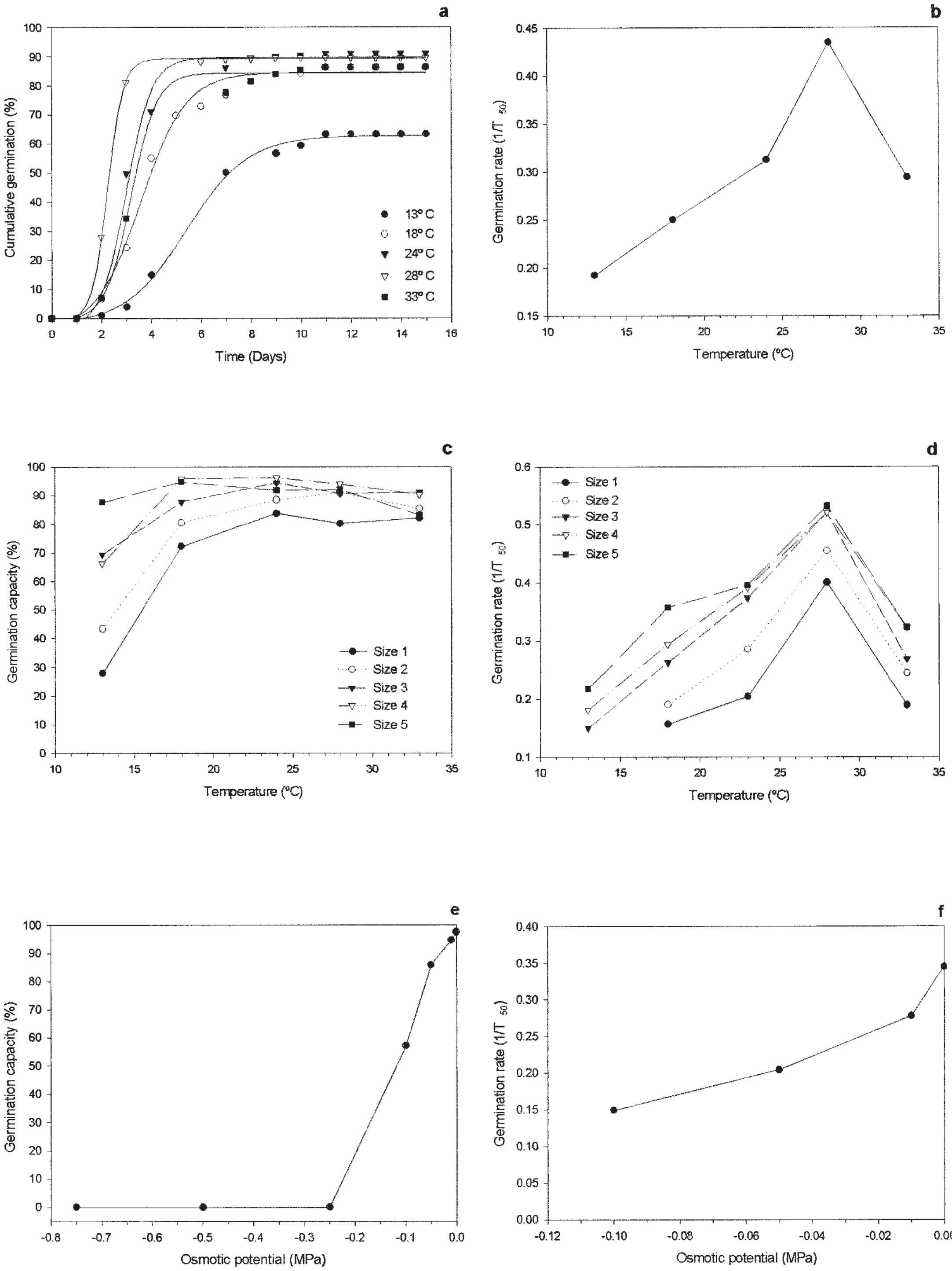

Figure 1. The effect of temperature, water stress and seed size on germination of *E. globulus.* **a)** Effect of temperature on germination capacity of an unsorted lot. **b)** Effect of temperature on germination rate of an unsorted lot. **c)** Effect of temperature and seed size on germination capacity. **d)** Effect of temperature and seed size on germination rate. **e)** Effect of water potential on germination capacity of seed size class 3 at 25 ºC. **f)** Effect of water potential on germination rate of seed size class 3 at 25 ºC.

4. DISCUSSION

The results demonstrated that the supra-optimal temperature became lower as *E. globulus* seed size increased. An optimum temperature for germination rate was determined (28 °C), which is supported by the findings of Battaglia [2]. The difficulty encountered by other authors to clearly recognize an optimum temperature might partly result from the graphical representation of the data used by different authors, whether they prefer to use the germination energy index (GEI) or the reciprocal of time to reach 50% of germination (T_{50}). When GEI was calculated in our work, only a slight decline in rate above the optimum was observed. The GEI effectively integrates the area under the germination curve and takes it as a proportion of the area as defined by the product of the time to maximum germination and the germination capacity. According to Battaglia [2], increasing the ratio of these areas, long-tailed or positively skewed distributions reduce the sensitivity of the GEI to changes in germination rate. By contrast, the T_{50} measure, which takes into account the average slope of what is normally the steepest part of the cumulative germination curve, is reasonably robust in this regard, facilitating the identification of an optimum temperature for the seedlot studied which, as previously detailed, was 28 ºC for all sizes of *E. globulus* tested in this study.

Earlier work on *E. globulus* recommended an optimal temperature of 25 ºC [6], whereas Eucalyptus species growing in South Africa did best at 17 – 22 ºC [11]. An optimum of 15º and 20 ºC has been reported for *E. Delegatensis*, and while short periods of higher temperature did not seriously affect germination [2], other researchers have shown adverse effects of high temperature on germination capacity of this species [16].

The presence of an optimum temperature above and below which the rate of germination declines has been noted in several reviews [5, 7]. The decline in rate of germination with decreasing ambient temperature partly results from the decline in the imbibition rate observed with a reduction in temperature. Moreover, according to Bewley and Black [5], the rate of water penetration into seeds is critical to the success of germination. A higher speed in imbibition was recorded for higher temperatures and larger sizes, what led to a faster protrusion of the radicle. A decrease in temperature is related to an increase in the time necessary to reach RWCs similar to those for seeds imbibed at higher temperatures. It can be concluded that under the experimental conditions tested here, *E. globulus* seeds begin their radicle emergence when their RWC is close to 70 ± 5%.

Reports on the effect of seed size on germination in eucalypts are contradictory [23, 27]. In this study seed-size effects were significant for several temperatures, demonstrating that sorting is essential to achieve germination uniformity in *E. globulus*, and that seed size has operational importance. When seedlot size varies widely, as in *E. globulus*, larger within- lot variability in germination parameters can be expected. The results reported here are supported by studies on other species [21], although the use of only two or tree size fractions may have masked some of the variation as was demonstrated for Sitka spruce [10].

Water deficits below –0.01 MPa were required to affect germination of *E. globulus* seeds, results that agree substantially for a range of other eucalypt species some of which showed decreases in germination at deficits of only –0.003 MPa [1, 14, 15]. Whereas Battaglia [2] found *E. delegatensis* was unaffected by matric potentials as high as –0.1 MPa, he pointed out that most experiments on water stress are done directly on a sintered plate. This provides a medium on which seed contact is poor and, consequently, seeds could be highly susceptible to any decline in moisture level. In the study reported here, seeds were placed directly on and in good contact with the germination medium and were kept under 98% relative humidity.

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