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Paternal and maternal effects on the response of seed germination to high temperatures in *Eucalyptus globulus*

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Abstract

• **Context** High temperature stress in nurseries germinating *Eucalyptus globulus* seed is an important problem affecting germination synchrony and rate. Where there is a risk of high-temperature stress, then the choice of female parent may be important. This issue is particularly relevant to the production of full-sib families from mass-supplementary pollination where there may be opportunities for seed producers to manipulate the directionality of the crossing done in seed orchards.

• **Aims** This study aimed to quantify the maternal versus paternal influence of seed sensitivity to high temperature stress during germination.
• **Methods** A diallel crossing scheme involving four genotypes was used to test the relative importance of male and female genetic influences on the germination and development of *E. globulus* seed and their response to high temperature stress. Seed was germinated at optimum (25°C) and supra-optimal (32°C and 37°C) temperatures, and six traits

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Cameron J. Spurr: Helped with designing experiments and interpreting results.

Peter L. Gore: Provided industry support (financial and in-kind) to the PhD project from which this work was derived.

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describing the proportion and rate of seed germination and early seedling development were assessed.

- **Results** Both paternal and maternal effects affected the germination response, arguing for at least some influence of the nuclear genotype of the embryo. However, the response to high temperature stress was more influenced by the maternal than paternal parent.

- **Conclusion** Both the male and female genotype may affect various aspects of seed germination and early seedling development independent of seed size; however, some facets of the germination response will be mainly affected by the female parent.

Keywords *Eucalyptus globulus* · Maternal · Paternal · Seed germination · Temperature stress · Genetic · Forest tree

1 Introduction

In plants, the genotype of the maternal parent may influence early life cycle traits through both Mendelian contributions to the embryo nuclear genotype as well as ‘maternal genetic effects’ (Donohue 2009; Roach and Wulff 1987). A ‘maternal genetic effect’ arises when there is an additional genetic contribution of the maternal genotype beyond the expected nuclear contribution (Roach and Wulff 1987). In angiosperms, maternal genetic effects may arise from (1) maternal inheritance of plastids; (2) an additional genetic contribution to the endosperm, which is triploid and two thirds of its genotype is of maternal origin; (3) the seed coat and other maternal tissue immediately surrounding the embryo; (4) maternal provisioning during seed development, with hormones, proteins, transcripts and nutrients all provisioned to seeds by the maternal parent; and (5) the maternal determination of the progeny environment via dispersal (Donohue 2009; Linkies et al. 2010). These influences mean that in most angiosperms, the maternal plants have a greater effect on offspring phenotype and fitness than paternal plants (Donohue 2009; Galloway 2001; Roach and Wulff 1987; Schmid and Dolt 1994). Indeed, many studies suggest that the primary control of seed dormancy and germination is through the maternal tissues surrounding the embryo (Baskin and Baskin 1998; Bischoff and Muller-Scharer 2010).

Maternal effects on seed mass have been previously reported in the forest tree *Eucalyptus globulus* (López et al. 2003), which appear to have carry-over effects on germinative capacity and early seedling growth (López et al. 2003; Lopez et al. 2000; Martins-Corder et al. 1998), however no direct maternal effect was detected on seed germination traits in the study of López et al. (2003).

Maternal effects can influence the sensitivity of seeds to environmental conditions (Roach and Wulff 1987). A study by Sung et al. (1998) showed that the sensitivity of lettuce

seeds to high temperature stress during germination was at least partly related to maternal environment. The present study aimed to determine the maternal and paternal genotype influence on seed germination traits in *E. globulus* Labill. (Tasmanian Blue Gum), particularly the response to high temperature stress. *E. globulus* is a hardwood forest tree, native to south-eastern Australia (Dutkowski and Potts 1999). The high cost of elite seed production in this species has increased the focus on efficiency in seedling production nurseries where seeds are mechanically sown into fixed cell containers. Whether a selected genotype is used as the male or female in manual pollination systems may have a significant effect on the cost of seed production as there are large genetically based differences in the many traits affecting sexual reproduction in this species, including flowering time (Jones 2009; Potts and Gore 1995), seed set (McGowen et al. 2004) and seed size (McGowen et al. 2004).

The female genotype may also affect seed germination responses; however, it is unknown whether this is due to a nuclear genetic effect or a maternal genetic effect. If maternal genetic effects are important, the choice of whether to use a selected genotype as a male or female for mass manual pollination will also be influenced by their effects on seed germination as well as the above factors. Extranuclear paternal genetic or environmental effects on progeny phenotype are generally considered to be of less importance than extranuclear maternal genetic effects because of the absence of plastids in the generative or sperm cells of many taxa (Corriveau and Coleman 1988), lower volume of pollen cytoplasm relative to egg cells, and the relatively low dose of paternally derived genes present in endosperm cells (Mazer and Gorchov 1996). Several studies have reported significant effects of the paternal environment on offspring traits but they are smaller than maternal environmental effects (Galloway 2001; Roach and Wulff 1987; Schmid and Dolt 1994).

We are not aware of any published information on the paternal influences on seed germination in eucalypts and the relative importance of the embryonic nuclear genes compared with maternal genetic effects. The present study therefore specifically aimed to determine if there is an effect of the embryonic nuclear genotype and a maternal genetic effect on the seed germination response of *E. globulus* and its sensitivity to high temperature stress.

2 Materials and methods

2.1 Study system

E. globulus trees used in this study were located in a seed orchard in Cambridge, south-eastern Tasmania (42°48'27.23" S 147°25'58.48"E), encompassing genotypes from different races as defined by Dutkowski and Potts (1999). The

genotypes in the orchard were selections from the Southern Tree Breeding Association National *E. globulus* Breeding Program. Genotypes were arranged in multiple lines of varying length which were distributed at random throughout the orchard.

2.2 Maternal versus paternal influence

Four genotypes (7558, 7335, 4886, and 7537) from the Western Otway race (hereafter referred to as Otway) exhibiting a range of germination responses were selected as parents for a diallel crossing scheme. Each genotype was represented by two ramets from different plots in the seed orchard, giving a total of eight trees used as females in the diallel crossing which included reciprocal crosses but not self pollinations. Pollen from each genotype was applied to each tree resulting in 24 crosses (four genotypes $\times$ two ramets per genotype $\times$ three crosses per ramet). For each cross, 15 flowers were pollinated from around the canopy of each tree, giving a total of 360 flowers pollinated. The pollen from each genotype was derived from flowers collected from multiple ramets of each genotype, except for 7558 where pollen was collected from a single ramet. The ramets used for flower collection were from different plots in the seed orchard for two genotypes (7335 and 4886) but not for the other two genotypes (7558 and 7537). For each genotype, pollen from the different ramets was then combined to give a single pollen for each genotype, used in the diallel crossing design.

Pollen viability was tested by in vitro pollen germination prior to pollination as detailed in Potts and Marsden-Smedley (1989). Controlled pollinations were undertaken using the single visit pollination procedure outlined by Williams et al. (1999). Capsules were harvested 12 months after pollination. They were placed in an oven set at 40°C in darkness for 24 h and extracted seeds were then stored at 5°C in darkness until germination testing commenced. Chaff was removed from samples using a vertical Dakota air column and by hand picking to 100% purity. The number of seeds per capsule and weight of 100 seeds were recorded from each cross on each ramet.

2.3 Seed germination

Seeds were germinated 17 days after harvest in controlled environment incubators set at 25°C (optimal), 32°C and 37°C (high temperatures) under continuous white fluorescent light. Within each incubator, seeds were arranged in custom-built perspex germination cabinets with eight separate sections per cabinet, each able to accommodate 50 evenly spaced seeds. Seeds were placed on 5 $\times$ 12.5 cm lengths of moistened Advantec filter paper (number 1 type). Fifty seeds from a single cross from each tree (seedlot) were

placed on each length of filter paper and this comprised the experimental unit. One such replicate of each seedlot was germinated at each temperature. The allocation of seedlots was random within and between boxes at each temperature, and the boxes re-randomised within the incubators after each assessment. Of the distilled water, 75 mL was added to each section of the germination cabinets and wicked up to the seeds to maintain a consistent level of moisture for the duration of the experiment.

Germination was scored daily or every second day during periods of peak germination and every 3–7 days later on. At each assessment date, a seed was recorded as germinated when there was visible protrusion of the radicle through the seed coat. Seedlings with a radicle at least one third the length of the seedling, a hypocotyl and two expanded cotyledons were classified as normal, in accordance with International Seed Testing Association (ISTA) guidelines (ISTA 1999) and removed. On the final day of scoring, remaining seeds were scored as fresh-ungermated or dead, in accordance with ISTA (1999). Tweezers were used to squash remaining ungerminated seeds to separate the fresh-ungermated (firm, intact embryo inside seed coat) from dead seeds (embryo soft and degraded). Remaining seedlings on the final day of scoring were scored as normal or abnormal. Abnormalities included stunted radicles, failure of radicles to emerge but emergence of cotyledons and failure of seedlings to “push-off” seed coats, in accordance with ISTA guidelines for seedling evaluation.

2.4 Statistical analysis

The data were summarised into six response traits for each experimental unit of 50 seeds, three measuring proportions and three measuring rates. The proportional data included: the proportion of seeds sown which germinated (germ/sown); the proportion of seeds which germinated that developed into normal seedlings (normals/germ); and the proportion of seeds sown which developed into normal seedlings (normals/sown). The rate data included: the time taken to reach 50% (T50) of the maximum germination attained (T50germ); the T50 for maximum normal seedlings developed (T50normals); and the difference between the T50 for germination and T50 to develop a normal seedling (T50normals–T50germ).

For analysis of seed characteristics including seed weight and the number of seeds per capsule, female genotype, male genotype and female genotype by male genotype were treated as fixed effects and tree within female genotype was treated as a random effect and used as the error term to test the female fixed effect. All other fixed effects were tested with the residual term.

For analysis of germination traits, a linear mixed model was fitted to the data with male genotype, female genotype, temperature and their two- and three-way interactions treated as fixed effects. Tree within female genotype was the

Fig. 1 Least square means ($\pm$ SE) for female (*solid*) and male (*open*) effects on **a** proportion of germinated seeds **b** proportion germinated seeds which developed into normal seedlings **c** proportion of normal seedlings developed **d** rate (T50) of germination (days) **e** rate (T50) of development from germination into normal seedlings (days) and **f** rate (T50) of normal seedling development (days). Results are averaged across the three temperatures (25°C, 32°C and 37°C) tested. Where the female or male effect was significant (Table 1), common letters represent least *squares* means within a male or female series which were not significantly ($P>0.05$) different using the Tukey–Kramer multiple range test

Fig. 2 Least square means ($\pm$ SE) for four genotypes (*empty circle* 4886, *empty triangle* 7537, *empty square* 7335, *multiplication symbol* 7558) as females for **a** proportion of germinated seeds **b** proportion of germinated seeds which developed into normal seedlings **c** proportion of normal seedlings developed **d** rate (T50) of germination (days). Results are averaged across the three temperatures tested (25°C, 32°C and 37°C). Significance of the genotype by temperature interaction for each trait is indicated next to each figure heading (*n.s.* not significant; * $P<0.05$, ** $P<0.01$, *** $P<0.001$)

4 Discussion

By employing a diallel crossing design, the present study provides a direct comparison of maternal and paternal genetic contributions to germination in *E. globulus*. Non-nuclear maternal effects are likely to result from asymmetries in genetic contributions via plastid DNA or to differences in provisioning by the parental genotype, rather than from differences in environment among parents (Burgess and Husband 2004; Husband and Gurney 1998). The present study indicates that the nuclear genes transmitted by the pollen of distinct paternal genotypes caused phenotypic differences among the progeny (Mazer and Gorchoff 1996). These paternal effects were consistent with trends in the female effects, suggesting that embryonic nuclear genetic effects dominate these germination traits and that specific maternal genetic effects are small.

The present study provides strong evidence that embryo nuclear genes do affect the overall germination response in *E. globulus* as the paternal genetic influence was significant and correlated with female influence. This finding differs from trends reported in previous reviews which argue that characters measured during early growth are strongly influenced by maternal effects that overshadow any nuclear contribution (Donohue 2009; Helenurm and Schaal 1996).

There is evidence to suggest that the differential response to high temperature may be more under maternal genetic control than the nuclear control of the embryo as there were significant interaction effects with temperature for the maternal but not the paternal parent. One of the primary controls of germination and dormancy is through the maternal tissues surrounding the embryo (Mayer and Poljakoff-Mayber 1982). It is possible that the maternal genetic effects observed are due at least in part to differences in the maternal tissue. The maternally-derived seed coat is perhaps the most direct manner whereby the maternal genotype exerts control over germination (Donohue 2009) and it is possible that it regulates the response to temperature in *E. globulus*. The *Eucalyptus* seed consist of an embryo inside a seed coat derived from the ovule integuments. Initial growth of the seedlings depends on the embryo reserve and then on the cotyledons through photosynthesis (Boland et al. 1980). A clear seed coat-related maternal effect on germination was shown by Leubner-Metzger (2002), showing that GA signalling and testa characteristics appear to be a common feature in the after-ripening-mediated release of coat-imposed dormancy of endospermic and non-endospermic seeds.

Alternatively, the maternal genetic effect dominating the differential response to temperature could be due to maternally transmitted plastids and their genomes (Helenurm and Schaal 1996). In the case of *E. globulus*, both the chloroplast (McKinnon et al. 2001) and mitochondrial DNA (Vaillancourt et al. 2004) are maternally inherited. These plastid genomes contain genes which are directly involved

in photosynthesis and respiration (Steane 2005; Vaillancourt et al. 2004). The observed female by temperature effects could have been due to differences between females in the sensitivity of plastid gene translation or metabolic processes in the plastid to high temperature stress.

The finding that both the male and female genotype may affect various aspects of seed germination and early seedling development independent of seed size, has implications for nursery operations. The traits reported to exhibit significant genetic control are likely to affect the percentage and rates of seed germination and seedling development in production nurseries. Mechanical sowing is the norm in such nurseries and there are significant economic losses associated with unfilled positions in seedling trays and asynchronous germination of seedlots and seedling growth. With the genetic-based differences demonstrated, the present study suggests that knowledge of genetic pedigrees and composition of seedlots will provide an opportunity to reduce these losses, by for example keeping single tree seedlots separate or combining like genotypes through the nursery operations. Both the male and female pedigree will potentially impact on many traits, but some facets of the germination response will be mainly affected by the female parent. In production facilities where there is a risk of high temperature stress, then the choice of female may be important. This issue is particularly relevant to the production of full-sib families from mass-supplementary pollination where there may be opportunities for seed producers to manipulate the directionality of the crossing done in seed orchards. However, nursery responses will be only one of several factors which impact on decisions affecting the directionality of a mass-produced cross across the whole production system. Both maternal effects on breeding objective traits, such as growth rate, e.g., (López et al. 2003) and reproductive traits such as flower abundance e.g. (McGowen et al. 2004) and abortion (Suitor et al. 2009) will also impact this decision but at different stages of the production system. It must be considered that this study was based on only four *E. globulus* genotypes and the results can therefore not necessarily be generalised to the species. Further research which expanded the study to a broader range of genotypes could be used to assess if these results can be generalised to the species.

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