Genetic Variances and Covariances for Frost Tolerance in Eucalyptus globulus and E. nitens

By P. W. Volker¹, J. V. Owen² and N. M. G. Borraini³

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Summary

Controlled- and open-pollinated families of Eucalyptus globulus subsp. globulus (referred to here as E. globulus) and E. nitens, were tested for frost resistance at 3 test temperatures (−5.5 °C, −7.0 °C and −8.5 °C) using the electric conductivity method. Additive, dominance and error variance and covariances were estimated using a derivative-free restricted maximum likelihood (DFREML) procedure andheritabilities, genetic and phenotypic correlations were calculated. E. nitens was more frost tolerant than E. globulus, the former being largely undamaged at −6.3 °C. E. globulus control-pollinated material (GCP) was tolerant to −7.5 °C but was severely damaged at −8.5 °C. Open-pollinated E. globulus from both seed orchards (GSOP) and natural stands (GOP) was severely damaged at −7.0 °C with the 50 % damage criteria probably met at about −6.0 °C. Heritage were moderate to high for both species, ranging from 0.29 to 0.50 for GCP and 0.23 to 0.44 for E. nitens control-pollinated material (NCP). It is suggested that estimates of heritability from E. globulus open-pollinated families from native stands (GCP), with values ranging from 0.53 to 0.61, are over-estimated due to the effects of selfing and neighbourhood inbreeding. Estimates of heritability for open-pollinated families from seed orchards in both species (GSOP and N SOP) were similar to corresponding control-pollinated families (GCP and NCP, respectively) suggesting that removal of selfing and other inbreeding effects have occurred. Genetic correlations between relative conductivity at different temperatures were high, suggesting the same genes are involved despite differing levels of damage.

Key words: Relative conductivity, REML, provenances, heritability, genetic correlation.

FDC: 165.2; 181.221.6; 422.1; 176.1 Eucalyptus globulus; 176.1 Eucalyptus nitens.

Introduction

Temperate eucalypts are widely used as a source of pulp and paper manufacture in the cool-temperate regions of the world but further extension of their planting is generally limited by seasonal low temperatures and occasional frosts. Temperate eucalypt species which have been shown to be relatively frost hardy such as Eucalyptus nitens, E. gunnii, E. delegatensis, E. viminalis and E. dalrympleana tolerate temperatures as low as −12 °C provided acclimation has taken place before the onset of frost (Paton, 1981; Davidson and Reid, 1987; Tibbits and Reid, 1987a). More sensitive temperate species include E. globulus and E. regnans, with critical temperatures around −7 °C (Turnbull and Paton, 1978; Hallam et al., 1988; Eldridge et al., 1993).

E. globulus subsp. globulus (hereafter referred to as E. globulus) and E. nitens are 2 of the most important temperate eucalypt species, due to their excellent productivity, fibre characteristics and pulp yield. They are currently being established in large areas in Australia, South America and south-western Europe. E. nitens, the more frost tolerant of the 2, has become the preferred species for eucalypt plantation forestry in higher altitude areas, subject to frequent winter frosts, whereas E. globulus has been confined to milder sites.

Improvements in frost tolerance have the advantage if significantly increasing the area of land suitable for eucalypt plantations by reducing the risk of losses due to frost. Evidence of provenance differences in frost tolerance have been reported for E. nitens (Tibbits and Reid, 1987b; Hallam and Tibbits, 1988; Raymond et al., 1989), and for E. globulus (Almeda, 1993) but studies on the magnitude of additive genetic variation have been limited. Frost damage in leaves and branches of field grown trees of open-pollinated origin has been shown to be highly Heritable in E. nitens (Tibbits and Reid, 1987b) with h² above 0.6 but low to moderate in E. grandis (Van Wrix, 1976) and E. gunnii (Cauvin et al., 1993) with h² between 0.10 and 0.29. More recently, indirect methods for frost tolerance assessment, based on electric resistance (or conductivity) of leaf extracts have been developed. They have the advantage of being non-destructive and provide a continuous variable, more suitable to statistical treatment and interpretation (see Raymond et al., 1992a for a review). Inheritance of electric conductivity measurements were carried out in E. nitens (Tibbits and Reid, 1987; Raymond et al., 1992b) and E. regnans (C.A. Raymond and J.V. Owen, unpublished) and have shown a high heritability, with values usually above 0.6.

Although the economic importance of tolerance to frost damage is obvious, basic information on the genetic control of this trait in eucalypts, and in particular in E. nitens and
E. globulus is required. This study reports genetic parameters of frost damage at 3 test temperatures, based on electric conductivity measurements in both open-pollinated and control-pollinated material.

Materials and Methods

Mating designs in Eucalyptus globulus

The E. globulus plants tested for frost tolerance were derived from an incomplete factorial crossing involving 19 unselected parents from 3 provenances (Table 1), namely Taranna, SE Tasmania (with 11 parents), King Island (7) and Flinders Island (1) (see Volker and Osmek 1986) for provenance information. In total, 580 seedlings from 58 full-sib families (10 per family) from the incomplete 8 x 11 factorial were assessed. Open-pollinated progeny came from the same 11 parents used as males in the factorial mating, plus 3 extra Taranna parents. All were located in native stands. Open-pollinated progeny from the remaining eight parents (those used as females in the factorial mating) were derived from seed collected in a multi-provenance seedling seed orchard as described in Volker et al. (1990).

Mating design in Eucalyptus nitens

The control-pollinated E. nitens plants were derived from an almost complete half diallel mating (see Table 2) of 10 first generation parents from Toorongo, Victoria (see Pedrick 1979) for provenance information. In total, 350 seedlings from 35 full-sib families (10 seedlings per family) were assessed. Open-pollinated progeny was also obtained from all the 10 parents used in the half diallel. Four of the parents were located in Huntsman seedling seed orchard (Tasmania, lat. 41° 45’ S, long. 146° 31’ E), 3 in Hampshire seedlings seed orchard (Tasmania, lat. 41° 20’ S, long. 149° 50’ E) and the remaining were located in plantations near Hampshire.

Plant material

The seedlings of both E. nitens and E. globulus were raised in North Forest Products’ East Ridgley nursery in northern Tasmania. In January 1990 seed was germinated in flat trays filled with alluvial soil under controlled light and temperature conditions. Approximately 3 weeks after germination when seedlings were still in the cotyledonary stage they were pricked out into paper pots of 4 cm diameter and 10 cm depth. The seedlings were then moved outdoors, under shade for a further 10 weeks. In April the seedlings were transplanted into a field nursery with formed beds. Families were planted as lines with four families across each bed. Spacing within the beds was approximately 5 cm within lines and 10 cm between lines. Families were randomly allocated in the nursery as unreplicated line plots. A control seedlot of E. globulus was placed in 10 locations throughout the nursery to test for environmental variation.

Sampling strategy and frosting techniques

Measurements of frost damage were taken at 3 test temperatures: -5.5 °C, -7.0 °C and -8.5 °C. Four leaf discs per test temperature were sampled from each of 10 seedlings per family following guidelines set by Raymond et al. (1992a). Two leaves were collected from each plant, 1 from the youngest fully expanded leaf pair and the other from the leaf pair directly below (following Raymond et al., 1986). Two discs from each of the 2 leaves per seedling were frosted at each temperature. The testing was carried out over a 2 week period in July 1990. The control seedlot was sampled every 4 days throughout the testing period and no changes in relative conductivity were detected.

The screening for frost tolerance was based on electrical conductivity methods. The equipment consisted of plexiglass baths containing aqueous ethylene glycol solution in which racks of test tubes were suspended (see Owen and Raymond (1987) for technical details). Cooling to -5.5 °C, -7.0 °C, and -8.5 °C was achieved by pumping liquid at -30 °C from a refrigerated bath through a pressure regulated copper coil in the test baths with temperature regulated by a thermomix.

Single 8 mm diameter leaf discs were placed in separate test tubes and racks of tubes were placed in test baths at 2.0 °C. Bath temperature was lowered at 4 °C per hour

<table>
<thead>
<tr>
<th>Female parents</th>
<th>Male parents</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>X</td>
</tr>
<tr>
<td>N2</td>
<td>X</td>
</tr>
<tr>
<td>N3</td>
<td>X X X</td>
</tr>
<tr>
<td>N4</td>
<td>X X</td>
</tr>
<tr>
<td>N5</td>
<td>X X X</td>
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<tr>
<td>N6</td>
<td>X X X</td>
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<tr>
<td>N7</td>
<td>X X X X X</td>
</tr>
<tr>
<td>N8</td>
<td>X X X</td>
</tr>
<tr>
<td>N9</td>
<td>X X X</td>
</tr>
<tr>
<td>N10</td>
<td>X X X</td>
</tr>
</tbody>
</table>

Table 2. — Open-pollinated and control-pollinated families from an half-diallel mating for E. nitens plants used in the frost damage experiment (the X indicates family tested). NSOP is open-pollinated seed collected from parents located in seedling seed orchards or plantations.

<table>
<thead>
<tr>
<th>Female parents</th>
<th>Male parents</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>X</td>
</tr>
<tr>
<td>N2</td>
<td>X</td>
</tr>
<tr>
<td>N3</td>
<td>X X X</td>
</tr>
<tr>
<td>N4</td>
<td>X X</td>
</tr>
<tr>
<td>N5</td>
<td>X X X</td>
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<tr>
<td>N6</td>
<td>X X X</td>
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<tr>
<td>N7</td>
<td>X X X X X</td>
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<tr>
<td>N8</td>
<td>X X X</td>
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<tr>
<td>N9</td>
<td>X X X</td>
</tr>
<tr>
<td>N10</td>
<td>X X X</td>
</tr>
<tr>
<td>NSOP</td>
<td>X</td>
</tr>
</tbody>
</table>
to $-2.0\, ^\circ C$ when 0.1 g of finely crushed ice was added such that it was in contact with the leaf disc to prevent super cooling. The bath temperature was then lowered to the required minimum ($-5.5\, ^\circ C$, $-7.0\, ^\circ C$ or $-8.0\, ^\circ C$) and held at this temperature for 1 hour. The racks of tubes were then removed and placed in a refrigerator at 3 $^\circ C$ for a 24 hour post-frost recovery period. Two millilitres of deionised distilled water were then added to each test tube and the racks were allowed to stand for a further 24 hours at room temperature. Electrical conductivity of leachate from each sample (denoted ct) was then measured. Racks of tubes were then immersed in a hot water bath at 80 $^\circ C$ for 10 minutes. After standing for a further 24 hours at room temperature, conductivity of leachate (denoted ck) was remeasured and this was assumed to be the absolute maximum conductivity. The 3 test temperatures were conducted simultaneously as there were 3 baths available. The degree of damage sustained by the leaf tissue was assessed using a relative conductivity value calculated as (RAYMOND et al., 1986):

$$\text{RC}^* = \sqrt{(ck - ct)/ck}$$

(1)

**Statistical analyses**

An initial analysis of residuals (not presented) was carried out to locate and remove outlier observations after checking each test temperature. These outliers were generally where the ct value exceeded the ck value or they were so close together as to indicate that the freezing methodology was not effective. In the most extreme case 4 % of observations were removed. Analysis of the *E. nitens* half-diallel and the *E. globulus* factorial were carried out according to the following model (in matrix notation):

$$y = Xb + Za + Ws + e$$

(2)

where $y$ is a $n \times 1$ vector of observations for relative conductivity (RC$^*$) taken on the individuals, $b$ is a $p \times 1$ vector of the overall mean in the case of *E. nitens* or the cross type mean in the case of *E. globulus*, $a$ is a $q \times 1$ vector of unobservable additive genetic effects of the seedlings and parents, $s$ is a $q \times 1$ vector of genetic effects common to all sib family (i.e. specific combining ability effects equivalent to one quarter of the dominance effects (FALCONE, 1986)), assumed random and $e$ is a $n \times 1$ vector of residuals which includes three quarters of the dominance and environmental error. $X$, $Z$ and $W$ are known incidence matrices relating observations $y$ to effects in $b$, $a$ and $s$ respectively. In the open-pollinated progeny analysis the $Ws$ term was not included. The different measurements of RC$^*$ from the same seedlings were treated as repeated measurements, with environmental covariances between traits set to zero. The model assumes all random terms are multivariate normally distributed random variables with zero means and (co)variances as follows:

$$\text{Var} = \begin{bmatrix}
\sigma_{a}^2 & \sigma_{a} \sigma_{s} & \sigma_{a} \sigma_{e} \\
\sigma_{a} \sigma_{s} & \sigma_{s}^2 & \sigma_{s} \sigma_{e} \\
\sigma_{a} \sigma_{e} & \sigma_{s} \sigma_{e} & \sigma_{e}^2
\end{bmatrix}$$

(3)

where $G = \text{Diag}(A \times T_2)$; $1 \times T_2$ is the genetic variance-covariance matrix, $A$ the numerator relationship matrix between trees, $T_2$ the matrix of additive genetic covariance of traits, and $T_3$ the matrix of covariances for the additional random effects (METER, 1989), $R$ is the matrix of variance-covariance of the residuals and $S$ the matrix of variance-covariance of the SCA effects.

Estimates of variance components were obtained by restricted maximum likelihood (REML) procedures (PATTERSON and THOMPSON, 1971). (REML) is an iterative procedure where each iteration comprises an evaluation of the likelihood for a given set of variance components. The process is repeated until the likelihood is maximized. Assuming normality, an expression for this likelihood (L), or equivalently, $-2 \times$ its logarithm, is:

$$-2 \log L = \text{const} + \log |R| + \log |G| + \log |C| + y'Py$$

(4)

where $R$ and $G$ are as defined above, and $C$ is the coefficient matrix in HENDERSON'S Mixed Model Equations (HENDERSON, 1975). The matrix $P$ is called the projection matrix and is given by:

$$P = V^{-1} - V^{-1}X(X'V^{-1}X)^{-1}X'V^{-1}$$

(5)

where $V = (R + ZGZ' + WSW')$ is the total variance. A quick solution for the terms in Equation 4, based on derivative-free methods, has been formulated by GRASER et al. (1987) for the univariate analysis, and extended to multivariate case by METER (1989), JUGA and THOMPSON (1990) and METER (1991a).

The maximum of $L$ (or the minimum of $-2 \log L$) and the derivation of variances and covariances were carried out using DFREML program written by METER (1991b). Estimators of $\sigma_a^2$, $\sigma_s^2$ and $\sigma_e^2$ were used to estimate heritabilities (h$^2$) and the dominance ratio (d$^2$) as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_s^2 + \sigma_e^2}$$

(6)

$$d^2 = \frac{\sigma_s^2}{\sigma_a^2 + \sigma_s^2 + \sigma_e^2}$$

(7)

where $\sigma_s^2 = 4\sigma_a^2$ (FALCONE, 1986). Sampling errors of these estimates are usually approximated by the inverse of the matrix of second derivatives (also called the Hessian matrix) of the log likelihood function with respect to the parameters to be estimated. However a Hessian matrix is not available with DFREML algorithms and an approximation was obtained by numerical differentiation as described by NELDER and MEAD (1965).

**Results and Discussion**

**Means**

Frost tolerance, as measured by the relative conductivity (RC$^*$), was clearly superior in *E. nitens* compared with *E. globulus* (Table 3, Figure 1). Relative conductivity in *E. nitens* control-pollinated progeny (NCP) decreased slightly, but significantly (P<0.01) from around 0.88 ± 0.01 at $-5.5\, ^\circ C$ to around 0.83 ± 0.01 at $-8.5\, ^\circ C$ (Table 3). In *E. globulus* control-pollinated progeny (GCP) the drop in RC$^*$ with decreasing temperatures was more marked, from
around 0.86 ± 0.01 at −5.5 °C to 0.61 ± 0.04 at −8.5 °C (Table 3). The differences in RC* between *E. nitens* and *E. globulus* were not significant at −5.5 °C, but became larger and statistically significant at −8.5 °C (Table 3).

Differences among *E. globulus* provenances (TT and KK) and interprovenance crosses (TK, KT, FK, FT) were also apparent but they were generally within one standard error (Table 3) at all temperatures. The Taranna provenance (TT) seems to be more tolerant than King Island (KI), with interprovenance crosses (TK and KT) being intermediate. Crosses between Flinders Island and either Taranna (FT) or King Island (FK) were also intermediate between Taranna and King Island.

In *E. globulus*, open-pollinated progeny (GSOP and GOP) were consistently less tolerant to frost than control-pollinated progeny (GCP). Differences in RC* between the 2 crossing types were small at −5.5 °C but became much larger at lower temperatures. Differences were likely to be significant between GCP and GSOP (open-pollinated progeny from a seed orchard) and between GCP and GOP (open-pollinated progeny from native stands) at all temperatures. There was no significant difference between GSOP and GOP at any test temperature. In *E. nitens* there was no difference in RC* between the control-pollinated (NCP) and the open-pollinated (NSOP) progeny at any temperature (Table 3), although at −8.5 °C, RC* values for NCP were marginally higher than for NSOP. This result might be misleading since the levels of damage (even at −8.5 °C) were not as severe in *E. nitens* as they were in *E. globulus*. The apparent lower performance of open-pollinated material to frost found in *E. globulus* has also been found in other traits (Hodge, Volker, Owen and Potts, unpublished) and suggests that some level of inbreeding depression occurs in the progeny as a result of selfing and related mating. Such neighbourhood inbreeding is likely to be more frequent in native stands (Elbridge et al., 1993, p. 193), which agrees with the poorer performance of GOP seedlots.

Raymond et al. (1992a) found the value of RC* = 0.8 as the limit between reversible and irreversible damage in *E. nitens* and *E. regnans* leaf tissue with values lower than 0.8 indicating irreversible damage. Furthermore, the mean RC* value for discs taken from leaves was shown to be strongly correlated with the amount of leaf damage in seedlings, with 0.8 corresponding to 50% leaf damage (Raymond et al., 1992a). Although the relationship has not been established for *E. globulus* it is reasonable to assume that it would be similar. The relationship between RC* values and the survival of seedlings is sigmoidal, therefore the straight lines in Figure 1 are only approximations of the real trends. The RC* values for *E. nitens* suggest that the species would suffer little damage even at the lowest temperature of −8.5 °C and much lower temperatures could have been tolerated before a 50% damage (or RC* = 0.8) is reached (Figure 1). In *E. globulus* RC* = 0.8 is reached at about −7.0 °C in GCP progeny and around −6 °C in GOP and GSOP. Although the correspondence between bath temperatures and natural frost temperatures are not well established, these critical temperatures agree well with direct estimates of frost tolerance in hardened *E. globulus* seedlings in Portugal (Almeida, 1993).

### Table 3 — Number of discs tested (N) and generalised least squares means (and standard errors) of RC* for different cross types of *E. globulus* and *E. nitens* at 3 test temperatures. Crosses within and between provenances of *E. globulus* are: Taranna x Taranna (TT), King Island x Taranna (KT), Taranna x King Island (TK), King Island x King Island (KK), Flinders Island x Taranna (FT), Flinders Island x King Island (FK). Overall means for control-pollinated (GCP), seed orchard open-pollinated (GSOP), natural stand open-pollinated (GOP) in *E. globulus* and control-pollinated (NCP), open-pollinated (NSOP) in *E. nitens* are also presented.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cross type</th>
<th>N</th>
<th>t.e.</th>
<th>mean ± t.e.</th>
<th>mean ± t.e.</th>
<th>mean ± t.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. globulus</td>
<td>TT</td>
<td>737</td>
<td>.871 (.008)</td>
<td>.832 (.017)</td>
<td>.642 (.038)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KT and TK</td>
<td>777</td>
<td>.864 (.006)</td>
<td>.805 (.014)</td>
<td>.600 (.031)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KK</td>
<td>430</td>
<td>.836 (.009)</td>
<td>.795 (.021)</td>
<td>.588 (.046)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FT</td>
<td>157</td>
<td>.855 (.013)</td>
<td>.831 (.029)</td>
<td>.601 (.065)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FK</td>
<td>199</td>
<td>.819 (.014)</td>
<td>.819 (.030)</td>
<td>.621 (.067)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GCP</td>
<td>208</td>
<td>.864 (.007)</td>
<td>.813 (.016)</td>
<td>.613 (.055)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GSOP</td>
<td>320</td>
<td>.839 (.006)</td>
<td>.741 (.013)</td>
<td>.507 (.021)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GOP</td>
<td>500</td>
<td>.814 (.013)</td>
<td>.700 (.017)</td>
<td>.478 (.032)</td>
<td></td>
</tr>
<tr>
<td>E. nitens</td>
<td>NCP</td>
<td>1400</td>
<td>.800 (.008)</td>
<td>.809 (.006)</td>
<td>.811 (.014)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NSOP</td>
<td>320</td>
<td>.900 (.003)</td>
<td>.884 (.004)</td>
<td>.812 (.012)</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. — Additive ($\sigma_a^2$), SCA ($\sigma_{sa}^2$) and error variance ($\sigma_e^2$), narrow sense heritability ($h^2$) and approximate standard errors, proportion of dominance ($d^2$) for each cross type within species and test temperature, for E. globulus and E. nitens.

<table>
<thead>
<tr>
<th>Cross type</th>
<th>Test temp.</th>
<th>$\sigma_a^2$ (x10^-4)</th>
<th>$\sigma_{sa}^2$ (x10^-4)</th>
<th>$\sigma_e^2$ (x10^-4)</th>
<th>$h^2$</th>
<th>s.e.</th>
<th>$d^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. globulus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCP</td>
<td>-5.5</td>
<td>0.39</td>
<td>0.14</td>
<td>0.52</td>
<td>0.29</td>
<td>0.03</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>-7.0</td>
<td>2.82</td>
<td>0.48</td>
<td>2.51</td>
<td>0.42</td>
<td>0.03</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>-8.5</td>
<td>10.21</td>
<td>2.40</td>
<td>7.97</td>
<td>0.50</td>
<td>0.03</td>
<td>0.48</td>
</tr>
<tr>
<td>GSOP</td>
<td>-5.5</td>
<td>0.80</td>
<td>-</td>
<td>1.86</td>
<td>0.30</td>
<td>0.07</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-7.0</td>
<td>5.22</td>
<td>-</td>
<td>6.71</td>
<td>0.44</td>
<td>0.06</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-8.5</td>
<td>9.60</td>
<td>-</td>
<td>9.38</td>
<td>0.51</td>
<td>0.06</td>
<td>-</td>
</tr>
<tr>
<td>GOP</td>
<td>-5.5</td>
<td>6.52</td>
<td>-</td>
<td>4.19</td>
<td>0.61</td>
<td>0.04</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-7.0</td>
<td>11.69</td>
<td>-</td>
<td>6.46</td>
<td>0.64</td>
<td>0.04</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-8.5</td>
<td>5.61</td>
<td>-</td>
<td>4.98</td>
<td>0.53</td>
<td>0.04</td>
<td>-</td>
</tr>
<tr>
<td>E. nitens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NCP</td>
<td>-5.5</td>
<td>0.49</td>
<td>0.24</td>
<td>0.60</td>
<td>0.37</td>
<td>0.04</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>-7.0</td>
<td>0.34</td>
<td>0.22</td>
<td>0.93</td>
<td>0.23</td>
<td>0.04</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>-8.5</td>
<td>1.75</td>
<td>0.43</td>
<td>1.83</td>
<td>0.44</td>
<td>0.04</td>
<td>0.44</td>
</tr>
<tr>
<td>NSOP</td>
<td>-5.5</td>
<td>0.22</td>
<td>-</td>
<td>0.57</td>
<td>0.28</td>
<td>0.06</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-7.0</td>
<td>0.34</td>
<td>-</td>
<td>1.25</td>
<td>0.21</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-8.5</td>
<td>2.99</td>
<td>-</td>
<td>6.03</td>
<td>0.33</td>
<td>0.06</td>
<td>-</td>
</tr>
</tbody>
</table>

Variance and covariances

In E. globulus the GCP additive ($\sigma_a^2$), SCA ($\sigma_{sa}^2$) and error ($\sigma_e^2$) variances generally increase with decreasing freezing temperature (Table 4). This was due to an overall increase in the level of damage with lower temperatures. However, $\sigma_a^2$ seems to increase more compared with $\sigma_{sa}^2$, resulting in an increase in heritabilities, with $h^2 = 0.29$ at $-5.5 \, ^\circ C$ to $h^2 = 0.50$ at $-8.5 \, ^\circ C$ (Table 4).

Variance components also change with temperatures in open-pollinated progeny. In GSOP, variances increased from $-5.5 \, ^\circ C$ to $-8.5 \, ^\circ C$, with estimates of heritability being very similar to those found for GCP (Table 4). In GOP, however, the variances peaked at $-7.0 \, ^\circ C$. The most likely explanation for the drop in variance at $-8.5 \, ^\circ C$ is that most of the material was killed. Extrapolating again from the RAYMOND et al. (1992a) relationship, the RC* value of 0.478 ± 0.012 (Table 3) would correspond to 90% to 100% mortality for the GOP progeny at $-8.5 \, ^\circ C$. Levels of additive and phenotypic variance at $-8.5 \, ^\circ C$ in GOP were of the same order of magnitude as those at $-7.0 \, ^\circ C$ in GSOP, and almost as high as those at $-8.5 \, ^\circ C$ in GCP. Heritability estimates in GOP were consistently high (above 0.53) and except for the lowest temperature, estimates of $h^2$ for GOP were larger than those for GSOP and GCP (Table 4). These heritabilities might be considerably underestimated because of unaccounted relatedness due to full sibs, parent relatedness and selfing among progeny (SQUILLACE, 1974; BORRALHO, 1994). To make GOP estimates comparable to GCP and GSOP, the intraclass coefficient of relationship would have to be 2 (for $-5.5 \, ^\circ C$ and 2.6 (for $-7 \, ^\circ C$), i.e. GOP progeny would be, say, as related as full-sibs or about half of the progeny being selfed. In both cases these results indicate that levels of selfing and relatedness in open-pollinated progeny of E. globulus from native stands may be very high.

The similar variances and resulting heritability estimates between GSOP and GCP, on the other hand, suggests that the assumption of half-sib relationship among the GSOP progeny would be reasonably valid. In fact, relatedness between male and female parents in seed orchard material would only occur through selfing, and given the lack of success of control-pollinated selfing on these parents (data not presented here), it is unlikely there was selfing in the GSOP progeny examined here.
Table 5. — Genetic correlations (r_g, above diagonal) and pheno-
typic correlations (r_p, below diagonal) between relative conduc-
tivity (RC) measured at different temperatures, for each species
and cross type.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cross type</th>
<th>Temp.</th>
<th>-5.5</th>
<th>-7.0</th>
<th>-8.5</th>
</tr>
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<tr>
<td>E. globulus</td>
<td>GCP</td>
<td>-5.5</td>
<td>0.81</td>
<td>0.53</td>
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<tr>
<td></td>
<td></td>
<td>-7.0</td>
<td>0.38</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-8.5</td>
<td>0.27</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GSOP</td>
<td>-5.5</td>
<td>1.00</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-7.0</td>
<td>0.31</td>
<td>0.87</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td>-8.5</td>
<td>0.31</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GOP</td>
<td>-5.5</td>
<td>0.77</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-7.0</td>
<td>0.49</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-8.5</td>
<td>0.35</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>E. nitens</td>
<td>NCP</td>
<td>-5.5</td>
<td>1.00</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-7.0</td>
<td>0.48</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-8.5</td>
<td>0.34</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NSOP</td>
<td>-5.5</td>
<td>0.73</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-7.0</td>
<td>0.20</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-8.5</td>
<td>0.17</td>
<td>0.33</td>
<td></td>
</tr>
</tbody>
</table>

In E. nitens, there was little change in the magnitude of
variance components between —5.5 °C and —7.0 °C (Table
4), reflecting the lack of damage caused by these test tem-
peratures. However, heritabilities were significantly higher
at —5.5 °C (h² = 0.37 ± 0.04 and 0.28 ± 0.06 for NCP and
NSOP respectively; Table 4) than at —7.0 °C (h² = 0.23 ±
0.04 and 0.21 ± 0.05 for NCP and NSOP respectively; Table
4). At —8.5 °C, o²_a and, to a lesser extent, o²_a, were
larger in magnitude, resulting in higher heritabilities
compared with higher temperatures (h² = 0.44 ± 0.04 and
0.33 ± 0.06 for NCP and NSOP respectively; Table 4).
Although the analyses of NSOP progeny gave larger varia-
dences between control-pollinated and open-
pollinated progeny were less marked than in E. globulus.

Dominance effects were large for both E. globulus and E.
nitens, accounting for 36 % to 72 % of the total variance
(Table 4). This is consistent with the apparent large in-
breeding depression of open-pollinated progeny in E. glo-
bulus, but not in E. nitens. More evidence for dominance
effects is the small but apparent heterotic effect found
when comparing interprovenance RC* values (KT and TK)
with the mean of both their control-pollinated parental
provenances (KK and TT, Table 3). High levels of domi-
nance variance compared with additive variance were
reported in a study by Van Wyk (1976) in E. grandis.

Despite the clear differences in means and heritabil-
ities of RC* at different temperatures, genetic correlations
between pairs of temperatures were always positive and
high (Table 5). In both species, correlations between the
2 extreme temperatures (—5.5 °C and —8.5 °C) were above
0.52. Correlations between pairs of close temperatures
(—5.5 °C and —7.0 °C or —7.0 °C and —8.5 °C) were usually
above 0.7, except in the case of the NSOP, where genetic
correlation between RC* at —7.0 °C and —8.5 °C was 0.43.
Phenotypic correlations were considerably lower. The
strong genetic correspondence between levels of frost
damage at different temperatures suggests that the same
genes should be operating across a range of temperatures
and levels of tolerance.

Conclusion

Reliable estimates of genetic parameters of economically
important traits are essential to the success of a breeding
program. Risk of significant frost damage in Eucalyptus
is known to vary due to environmental and physiological
conditions pertaining at a particular locality and time
(Eldridge, 1968; Raymond et al., 1986; Tsiatis and Reid,
1978b; Tsiatis and Hallam, 1988). While it may not be
possible to account for all these factors when determining
the absolute levels of resistance, results suggest that prove-
nances and individual trees can be accurately ranked when
grown in a common environment and tested at the same
time. This and previous studies with E. nitens (Tsiatis and
Reid, 1978b; Raymond et al., 1992b) show a significant
variation in frost tolerance, with the trait being under
strong additive genetic control. Comparable levels of
heritability were found for E. globulus. The good genetic
correlation between RC* values at different temperatures
also indicates that the actual testing temperature chosen
is not critical. However heritabilities were higher at tem-
peratures corresponding to RC* values around 0.7 to 0.8,
I.e. at intermediate levels of leaf damage, a known sta-

tistical property of the trait used (Raymond et al., 1992a).
In the E. globulus control-pollinated families, heritability
improved from 0.29 at —5.5 °C (with RC* = 0.86), to 0.50
at —8.5 °C (with RC* = 0.61). However, in the open-poll-
nated progeny from the native stands, heritability peaked
at —7.0 °C, (where RC* = 0.7), decreasing subsequently as
RC* dropped to 0.48, a level which would correspond to
around 90 % mortality. In E. nitens, heritability increased
with decreasing temperatures, but differences were small.
All levels of damage were always below the 50 % mark
(RC* above 0.82). From a selection point of view, RC* val-
ues based on temperatures corresponding to around 50
% damage threshold of the species (e.g. —7.0 °C for E.
globulus) seem to be a better selection procedure, as they
are more heritable whilst they are strongly correlated with
less extreme, but probably more meaningful, testing tem-
peratures. It seems well established that the relationship
between frost tolerance in the field and in vitro measure-
ments of relative conductivity at extremely low tempera-

tures, is strong enough to warrant such a method (Ray-
mond et al., 1992a). It is less clear whether specific testing
conditions are of importance. RC* breeding values are
well correlated across different temperatures, but heritabil-
ities vary considerably so careful decisions on test tem-
peratures do have an important impact on selection effi-
ciencies.

Earlier frost studies of eucalypt have been largely con-
fined to seedlings grown from open-pollinated seed collect-
ed in native stands (the exceptions being the study of
Van Wyk, 1978 in E. grandis and a few control-pollinated
families of E. regnans in Raymond et al., 1986). It was clear
from the results that the use of this material can result in
significantly lower frost tolerance levels and inflated estimates of additive genetic variance when compared with control-pollinated crosses from the same parent. This is possibly caused by higher levels of selfing, full-sibling and neighbour inbreeding in open-pollinated progeny. In *E. globulus* open-pollinated material was less tolerant to frost, with RC* values significantly lower than corresponding values from control-pollinated families and variances were much larger, which resulted in larger heritabilities. In *E. nitens*, these differences in variance components and heritability between control and open-pollinated material were not apparent, but the result is not conclusive since the range of damage levels was not large.

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**Literature Cited**


