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

By P. W. VOLKER¹⁾), J. V. OWEN³⁾ and N. M. G. BORRALHO²⁾

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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 electrical conductivity method. Additive, dominance and error variance and covariances were estimated using a derivative-free restricted maximum likelihood (DFREML) procedure and heritabilities, genetic and phenotypic correlations were calculated. *E. nitens* was more frost tolerant than *E. globulus*, the former being largely undamaged at –8.5 °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. Heritabilities 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 (GOP), 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 NSOP) 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: 165.3; 165.53; 181.221.1; 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 PRYOR, 1978; HALLAM et al., 1989; 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* (ALMEIDA, 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 h^2 above 0.6 but low to moderate in *E. grandis* (VAN WYK, 1976) and *E. gunnii* (CAUVIN et al., 1993) with h^2 between 0.10 and 0.29. More recently, indirect methods for frost tolerance assessment, based on electric resistance (or conductivity) of leaf exudates 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

¹⁾ ANM Forest Management, 59 Hamilton Road, New Norfolk, Tasmania 7140, Australia.

²⁾ Cooperative Research Centre for Temperate Hardwood Forestry, Locked Bag No 2, Post Office, Sandy Bay, Tasmania 7005, Australia.

³⁾ CSIRO, Division of Forestry, P.O. Box 4008, Queen Victoria Terrace, Canberra, ACT 2601, Australia.

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 ORME (1988) for provenance information). In total, 580 seedlings from 58 full-sib families (10 per family) from the incomplete 8×11 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 Toorong, Victoria (see PEDERICK (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. 145° 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

Table 1. — Open-pollinated and full sib families from a factorial mating of *E. globulus* tested for frost damage (the X indicates family tested). Provenances are Taranna (T), King Island (K), and Flinders Islands (F). Shaded areas indicate between provenance crosses. GSOP is open-pollinated seed collected from trees in a seedling seed orchard which were used as females, GOP is open-pollinated seed collected from trees in natural stands which were used as males.

Female parents	Male parents														
	T5	T8	T9	T10	T11	T14	T15	T16	T17	T19	K8	K10	K13	K14	GSOP
T1				X		X	X	X							X
T2		X		X		X	X	X		X					X
T3		X		X		X	X	X							X
T4				X			X	X		X					X
F1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
K1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
K2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
K3	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
GOP	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

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.

Female parents	Male parents									
	N1	N2	N3	N4	N5	N6	N7	N8	NSOP	
N1										X
N2										X
N3	X	X								X
N4	X	X				X				X
N5	X	X		X						X
N6	X	X	X		X					X
N7	X	X	X	X	X	X				X
N8	X	X		X		X	X			X
N9	X	X	X		X	X	X			X
N10	X		X		X	X		X	X	X

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 plexi-glass 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

to -2.0 °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 °C, -7.0 °C or -8.0 °C) and held at this temperature for 1 hour. The racks of tubes were then removed and placed in a refrigerator at 3 °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 °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):

$$RC^* = \sqrt{(ck - ct)/ck} \quad (1)$$

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):

$$y = Xb + Za + Ws + e \quad (2)$$

where y is a $n \times 1$ vector of observations for relative conductivity (RC^*) taken on the individuals disc, 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 a full-sib family (i.e. specific combining ability effects equivalent to one quarter of the dominance effects (FALCONER, 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 W s 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:

(3)

$$\text{Var} \begin{bmatrix} y \\ a \\ s \\ e \end{bmatrix} = \begin{bmatrix} R + ZGZ' + WSW' & ZG' & WS' & R \\ & GZ' & G & 0 & 0 \\ & SW' & 0 & S & 0 \\ & R & 0 & 0 & R \end{bmatrix}$$

where $G = \text{Diag}\{A \times T_A; I \times T_S\}$ is the genetic variance-covariance matrix, A the numerator relationship matrix between trees, T_A the matrix of additive genetic covariance of traits, and T_S the matrix of covariances for the

additional random effects (MEYER, 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:

(4)

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

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:

$$P = V^{-1} - V^{-1}X(X'V^{-1}X)^{-1}X'V^{-1} \quad (5)$$

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 MEYER (1989), JUGA and THOMPSON (1990) and MEYER (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 MEYER (1991b). Estimates of σ_a^2 , σ_s^2 and σ_e^2 were used to estimate heritabilities (h^2) and the dominance ratio (d^2) as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_s^2 + \sigma_e^2} \quad (6)$$

$$d^2 = \frac{\sigma_d^2}{\sigma_a^2 + \sigma_s^2 + \sigma_e^2} \quad (7)$$

where $\sigma_d^2 = 4\sigma_s^2$ (FALCONER, 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 °C to around 0.83 ± 0.01 at -8.5 °C (Table 3). In *E. globulus* control-pollinated progeny (GCP) the drop in RC^* with decreasing temperatures was more marked, from

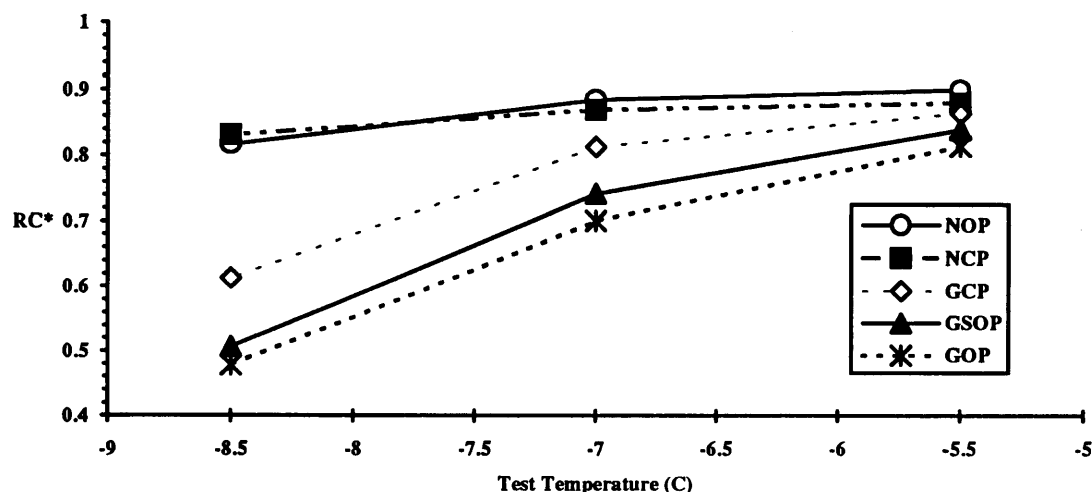


Figure 1. — Mean relative conductivity (RC*) of *E. nitens* open-pollinated (NSOP) and control-pollinated (NCP) families, *E. globulus* open-pollinated from natural stands (GOP), open pollinated from seed orchards (GSOP) and control-pollinated (GCP) families.

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 PORTS, 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 (ELDRIDGE et al., 1993, p. 195), 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.

Species	Cross type	N	-5.5°C		-7.0°C		-8.5°C	
			mean	s.e.	mean	s.e.	mean	s.e.
<i>E. globulus</i>	TT	757	.872	(.008)	.832	(.017)	.642	(.038)
	KT and TK	777	.864	(.006)	.805	(.014)	.600	(.031)
	KK	430	.856	(.009)	.795	(.021)	.588	(.046)
	FT	157	.855	(.013)	.821	(.029)	.601	(.065)
	FK	159	.859	(.014)	.810	(.030)	.621	(.067)
	GCP	2308	.864	(.007)	.813	(.016)	.613	(.035)
	GSOP	320	.839	(.006)	.741	(.015)	.507	(.021)
	GOP	560	.814	(.013)	.700	(.017)	.478	(.012)
<i>E. nitens</i>	NCP	1400	.880	(.008)	.869	(.006)	.831	(.014)
	NSOP	320	.900	(.003)	.884	(.004)	.812	(.012)

Table 4. — Additive (σ_a^2), SCA (σ_s^2) and error variance (σ_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*.

Cross type	Test temp.	σ_a^2 ($\times 10^{-3}$)	σ_s^2 ($\times 10^{-3}$)	σ_e^2 ($\times 10^{-3}$)	h^2	s.e.	d^2
<i>E. globulus</i>							
GCP	-5.5	0.39	0.14	0.82	0.29	(0.03)	0.40
	-7.0	2.82	0.48	2.51	0.42	(0.03)	0.36
	-8.5	10.21	2.40	7.97	0.50	(0.03)	0.48
GSOP	-5.5	0.80	-	1.86	0.30	(0.07)	-
	-7.0	5.22	-	6.71	0.44	(0.06)	-
	-8.5	9.60	-	9.38	0.51	(0.06)	-
GOP	-5.5	6.52	-	4.19	0.61	(0.04)	-
	-7.0	11.69	-	6.46	0.64	(0.04)	-
	-8.5	5.61	-	4.98	0.53	(0.04)	-
<i>E. nitens</i>							
NCP	-5.5	0.49	0.24	0.60	0.37	(0.04)	0.72
	-7.0	0.34	0.22	0.93	0.23	(0.04)	0.60
	-8.5	1.75	0.43	1.83	0.44	(0.04)	0.44
NSOP	-5.5	0.22	-	0.57	0.28	(0.06)	-
	-7.0	0.34	-	1.25	0.21	(0.05)	-
	-8.5	2.99	-	6.03	0.33	(0.06)	-

Variations and covariances

In *E. globulus* the GCP additive (σ_a^2), SCA (σ_s^2) and error (σ_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, σ_a^2 seems to increase more compared with σ_e^2 , resulting in an increase in heritabilities, with $h^2 = 0.29$ at -5.5 °C to $h^2 = 0.50$ at -8.5 °C (Table 4).

Variance components also change with temperatures in open-pollinated progeny. In GSOP, variances increased from -5.5 °C to -8.5 °C, with estimates of heritability being very similar to those found for GCP (Table 4). In GOP, however, the variances peaked at -7.0 °C. The most likely explanation for the drop in variance at -8.5 °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 °C. Levels of additive and phenotypic variance at -5.5 °C in GOP were of the same order of magnitude as those at -7.0 °C in GSOP, and almost as high as those at -8.5 °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 overestimated 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 °C) and 2.6 (for -7 °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_a , above diagonal) and phenotypic correlations (r_p , below diagonal) between relative conductivity (RC*) measured at different temperatures, for each species and cross type.

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

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 temperatures. However, heritabilities were significantly higher at -5.5 °C ($h^2 = 0.37 \pm 0.04$ and 0.28 ± 0.06 for NCP and NSOP respectively; Table 4) than at -7.0 °C ($h^2 = 0.23 \pm 0.04$ and 0.21 ± 0.05 for NCP and NSOP respectively; Table 4). At -8.5 °C, σ_a^2 and, to a lesser extent, σ_e^2 , were larger in magnitude, resulting in higher heritabilities compared with higher temperatures ($h^2 = 0.44 \pm 0.04$ and 0.33 ± 0.06 for NCP and NSOP respectively; Table 4). Although the analyses of NSOP progeny gave larger variances, differences 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 inbreeding depression of open-pollinated progeny in *E. globulus*, 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 dominance variance compared with additive variance were reported in a study by VAN WYK (1976) in *E. grandis*.

Despite the clear differences in means and heritabilities 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; TIBBITS and REID, 1987b; TIBBITS 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 provenances 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* (TIBBITS and REID, 1987b; 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 temperatures corresponding to RC* values around 0.7 to 0.8, i.e. at intermediate levels of leaf damage, a known statistical 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-pollinated 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 as levels of damage were always below the 50 % mark (RC* above 0.82). From a selection point of view, RC* values 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 temperatures. It seems well established that the relationship between frost tolerance in the field and *in vitro* measurements of relative conductivity at extremely low temperatures, is strong enough to warrant such a method (RAYMOND et al., 1992a). It is less clear whether specific testing conditions are of importance. RC* breeding values are well correlated across different temperatures, but heritabilities vary considerably so careful decisions on test temperatures do have an important impact on selection efficiencies.

Earlier frost studies of eucalypts have been largely confined to seedlings grown from open-pollinated seed collected in native stands (the exceptions being the study of VAN WYK, 1976 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-sibbing and neighbourhood 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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