# Genetic Parameters for Cold Hardiness in *Eucalyptus nitens* (Deane & Maiden) Maiden

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(Received 22nd July 2002)

**Abstract**

Provenance effects and genetic parameters for survival, growth, and frost damage aspects of cold hardiness in *Eucalyptus nitens* (Deane & Maiden) Maiden were estimated from almost 5,000 trees in two field tests established in 1984 and 1986. Significant and consistent differences amongst the six provenances were found for all cold hardiness traits. Macalister, Rubicon and Toorongo provenances from the central highlands of Victoria performed similarly and were always the best (overall survival 71%). Northern New South Wales (NSW) and Southern NSW were ranked next (overall survival 34%) and were often not significantly different from each other. Errinundra was the poorest (overall survival 13%). Northern NSW, whilst best on the basis of artificial freeze testing (T50, –8.4 °C c.f –7.5 °C overall average), appeared poorly adapted to survive and grow on either test site. Only the Macalister, Rubicon and Toorongo provenances were used for genetic parameter calculation (188 families and 3,500 trees). Individual tree narrow sense heritability ($h^2$) for basal area (BA) at about 6 years of age was lowest at the coldest site ($h^2$ range 0.10–0.27), whilst for frost damage in the field (F88) at about 2–4 years of age it was highest at the coldest site, ($h^2$ range 0.14–0.44). Separate T50 assessments appeared to measure a heritable trait ($h^2$ = 0.29 and 0.96) and to be repeatable ($r_{Bg}$ = 0.86). However, there was apparently only a moderate relationship between T50 and F88 (average $r_{Bg}$ = 0.39 and 0.29 for the two sites respectively). At the two sites F88 from the same autumn freeze event, was moderately correlated ($r_{Bg}$ = 0.45). Strong negative genetic correlations existed between F88 and BA, indicating that high levels of frost damage are associated with poor growth and survival, as one might expect. Breeding values predicted using best linear prediction, indicated that the best families were about 1.4 °C (19%) better for T50, 20 cm$^2$ (20%) better for BA and had 11% less foliage damage (22% better) for F88. The findings are discussed in terms of the interaction between frost tolerance and growth conferring ability to survive and grow on very cold sites.

*Key words:* heritability, genetic correlation, survival, frost, Macalister, Rubicon, Toorongo, Errinundra.

## Introduction

The ability to tolerate cold conditions is important in cool-temperate eucalypts. Tolerance may be required in any season to frosts (PATON, 1981), and to cold winds, ice and snow (CREMER, 1983). Most of the research into cold hardiness of eucalypts has focused on frost tolerance, since this seems the aspect that is of most relevance to applied, intensive farming of trees and forestry interests. However, the impact of cold conditions on growing *Eucalyptus* is at times unclear in as much as cold hardiness is an issue that appears to vary over time in its perceived relevance to growing trees. A year of particularly cold and damaging conditions may tend to focus attention on the need for cold hardiness. For instance, a very cold period in winter of 1983 was experienced throughout Tasmania, leading to publications like that of DAVIDSON and REID (1985) highlighting its intensity and damage. At that same time there was noticeable damage to planted *Eucalyptus*, in an area called Surrey Hills on the north-west of Tasmania, owned by a private forestry company. Consequently, the effect of this one winter event was to cause some people to reconsider the relative importance of cold hardiness.

In temperate climatic regions of the world, and particularly in the southern hemisphere, there is considerable interest in growing species of *Eucalyptus* in plantations on short rotations and using the wood fibre from these trees for pulp, paper and possibly solid wood products. *Eucalyptus nitens* (Deane & Maiden) Maiden is an important species in those regions where the climate is too cold for the often preferred species, *E. globulus* Labill., which is renowned for its fast growth and high pulp yield (TIBBITS *et al.,* 1997). It has a discontinuous distribution throughout the mountain ranges of southeastern Australia, and PEDERICK (1979) identified six distinct provenances: Northern NSW, Southern NSW, Macalister, Rubicon, Toorongo and Errinundra. In addition, PEDERICK (1979) and PEDERICK and LENNOX (1979) described two forms of *E. nitens.* The first was known as the "juvenile-persistent" form, so called because of its retention of juvenile foliage after the first year of growth. The other form was known as "early-adult", came from the Errinundra provenance and parts of the Toorongo provenance, being characterised by slower growth, finer branching and straighter stems. It has also been found to have poorer frost tolerance (TIBBITS and REID, 1987a) and to be characterised by lower growth rate and pulp yield (TIBBITS and HODGE, 1998). The Errinundra provenance, has since been described as a separate species (COOK and LADIGES, 1991).

Much of the land carrying *E. nitens* tree-farms throughout the world is subject to a variety of cold conditions including frost, winds, and snow. In fact, *E. nitens* is often planted because environmental conditions are considered too cold for other species (TIBBITS *et al.,* 1997). Some information has been published on the genetic (TIBBITS and REID, 1987a, RAYMOND *et al.,* 1992, VOLKER *et al.,* 1994), physiological (TIBBITS and REID, 1987b) and seasonal (TIBBITS and REID, 1987a) aspects of variation in freezing tolerance. There have also been reports on the potential of hybrids (TIBBITS, POTTS and SAVVA, 1991) and cellular aspects of variation (TIBBITS, 1995). Almost all of this research has focused on seedlings either in the nursery or in the early stages of growth in the field. However, there is little information to indicate the relative effects of cold environmental conditions on the longer-term survival and growth of *E. nitens* trees. This appears to be an important issue facing those planting this species.

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This study assessed the level of genetic control of cold hardiness of young trees in an open-pollinated breeding population of *E. nitens.* Specific objectives included: estimates of genetic parameters (individual tree narrow-sense heritabilities, genetic correlations amongst traits), comparisons of field and laboratory measures of tolerance to cold, prediction of breeding values and determination of potential genetic gains.

## Materials and Methods

*Genetic Base and Tests*

The genetic base comprised material collected from natural forests largely covering the full range of *E. nitens,* including all known provenances (see PEDERICK, 1979) and altitudinal ranges. In addition, there were some families from Toorongo, which upon visual inspection of leaf morphology were identified as exhibiting an "early-adult" form. Initial analyses included the genetic material from Errinundra. However, all Errinundra families and families from Toorongo which exhibited an "early-adult" form, that is characteristic of the Errinundra provenance, were removed from the data before analyses for genetic parameters and selection, in consideration of their poor performance for traits of interest for many tree-farm programs (TIBBITS and HODGE, 1998). All families were of open-pollinated (OP) origin from single mother trees in native forest stands. Details of the populations and numbers of families represented are in *Table 1.* Locations of populations in all provenances except Northern NSW are mapped in *Figure 1.*

*Table 1.* – Details of the genetic base of *E. nitens*, including populations within provenances, numbers of families in Test 3 and Test 4, and the mean altitude above sea level (m). Provenance abbreviations are: northern New South Wales (NN), southern New South Wales (SN), Macalister (MA), Rubicon (RU), Toorongo (TO), "early-adult" form in Toorongo (TO/EA) and Errinundra (ER).

| Population | Provenance | Population | Altitude | Test 3 | Test 4 |
|---|---|---|---|---|---|
| 1 | NN | Ebor - Barren Mountain | 1300 | 0 | 5 |
| 2 | NN | Ebor - Majors Point | 1420 | 1 | 6 |
| 3 | NN | Ebor - Point Lookout | 1530 | 0 | 8 |
| 4 | NN | Barrington Tops - Khowlha Trail | 1450 | 2 | 13 |
| 5 | NN | Barrington Tops - Mt Carson | 1425 | 10 | 6 |
| 1-5 | | All NN Populations | - | 13 | 38 |
| 6 | SN | Tallaganda State Forest | 1310 | 8 | 26 |
| 7 | SN | Badja State Forest | 1090 | 6 | 7 |
| 8 | SN | Glenbog State Forest | 1045 | 6 | 10 |
| 6-8 | | All SN Populations | - | 20 | 43 |
| 9 | MA | Mount Wellington | 1280 | 3 | 4 |
| 10 | MA | Mount Skene | 1120 | 4 | 7 |
| 11 | MA | Connors Plain | 1240 | 4 | 5 |
| 12 | MA | Mt Useful | 1200 | 4 | 1 |
| 6-12 | | All MA Populations | - | 15 | 17 |
| 13 | RU | Barnawall Plain - Mount Torbrek | 1170 | 5 | 2 |
| 14 | RU | Snobs Creek | 990 | 4 | 14 |
| 15 | RU | Royston River | 990 | 3 | 4 |
| 16 | RU | Tweed Spur | 1000 | 3 | 6 |
| 17 | RU | Bullfight Creek | 1000 | 0 | 1 |
| 13-17 | | All RU Populations | - | 15 | 27 |
| 18 | TO | Penny's Saddle | 805 | 2 | 0 |
| 19 | TO | Marshall Spur/ Little Boy's Creek | 1000 | 4 | 6 |
| 20 | TO | Link Road | 900 | 0 | 5 |
| 21 | TO | Mount Saint Gwinear | 1150 | 3 | 26 |
| 22 | TO | Mount Erica | 1150 | 2 | 1 |
| 23 | TO | Newlands Road | 1070 | 0 | 1 |
| 24 | TO (TO/EA) | Loch Valley Road - general | 900 | 0 | 14 (5) |
| 25 | TO (TO/EA) | Loch Valley Road - site 1 | 900 | 0 | 16 (7) |
| 26 | TO (TO/EA) | Loch Valley Road - site 2 | 900 | 0 | 7 (3) |
| 27 | TO (TO/EA) | Loch Valley Road - site 3 | 900 | 0 | 6 (6) |
| 28 | TO (TO/EA) | Mount Toorongo | 900 | 0 (2) | 15 (5) |
| 29 | TO | Mount Toorongo - MMBW Road | 1000 | 0 | 18 |
| 30 | TO | Toorongo Plateau | 900 | 0 | 6 |
| 31 | TO | Powelltown | 820 | 3 | 2 |
| 18-31 | | All TO (TO/EA) Populations | - | 14 (2) | 123 (26) |
| 32 | ER | Kelly's Creek Road | 770 | 3 | 0 |
| 33 | ER | Cottonwood Road | 950 | 4 | 0 |
| 34 | ER | Errinundra Road, Gunmark Range | 1080 | 4 | 2 |
| 35 | ER | Goongerah | 760 | 2 | 2 |
| 36 | ER | Rooty Breach Creek | 1020 | 0 | 1 |
| 37 | ER | Sassafras Creek | 1000 | 0 | 2 |
| 38 | ER | Clarkeville Road | 920 | 1 | 0 |
| 39 | ER | Splitters Creek | 1070 | 1 | 0 |
| 32-39 | | All ER Populations | - | 15 | 7 |
| 1-39 | All | All Populations in all provenances | - | 94 | 281 |

Four major tests were undertaken (*Table 2*). The first and second assessed the cold hardiness of nursery seedlings in terms of tolerance to artificial freezing. The third and fourth assessed the cold hardiness of trees in two separate field tests, where the trees established in the field trials were related to the seedlings in the earlier two tests. More information is detailed for Tests 1 and 3 by TIBBITS and REID (1987a) and for Test 2 by RAYMOND *et al.* (1992).

*Artificial Freezing Assessments of Seedlings – Tests 1 and 2*

Two separate and independent testings were carried out on artificial freezing of leaf disks excised from nursery-grown seedlings. The first test (Test 1) was previously reported in TIBBITS and REID (1987a), and data were used here for genetic parameter estimation, including paired comparisons for genetic correlations. Of the 36 families of *E. nitens* that were grown as potted seedlings and hardened under natural conditions in Hobart, Tasmania (see TIBBITS and REID, 1987a), seven were not used here, as they were either from the Errinundra provenance or the Toorongo provenance with the "early-adult form". There were seven seedlings per family and six families per provenance for the Rubicon, Macalister, Northern NSW and Southern NSW provenances and five families for the Toorongo provenance.

The second artificial freezing test (Test 2) took place in the winter of 1986 and involved the seedlings being grown for one of the field trials (Test 4 below). The main scientific findings were published as relative electrolyte conductivities at 3 temperatures (RAYMOND *et al.,* 1992). A new trait was derived for use in this analysis, which was the predicted freeze temperature, which leads to 50% leakage of cellular electrolytes (T50). RAYMOND *et al.* (1992) did not use this trait. The advantage of this approach is that all trees are compared on the magnitude of a stress (°C) resulting in a given strain (percentage loss of electrolytes). The method of deriving T50 is given in TIBBITS *et al.* (1991). Only a few seedlings had T50 values extrapolated beyond the freezing temperatures used. There were 10 seedlings per family. Each seedling assessed was uniquely identified and subsequently planted in a field trial (Test 4).

*Field Trials – Tests 3 and 4*

Two field trials were used. Both were at about 650 m in elevation and with deep red ferrosol soils where mean annual rainfall was about 1,500 mm. Experimental designs were randomised complete blocks with single tree plots and 3 m or 4 m spacing between planting rows and trees within rows. Test 3 (Racecourse Plains field trial) was established in December 1984 and January 1985 for the specific purpose of assessing genetic variation in cold hardiness of *E. nitens.* This site was likely to be at the limits of environmental extremes, in terms of cold, for *E. nitens,* as it was exposed to substantial cold elements (see TIBBITS and REID, 1987a). Test 4 (Painter Road field trial) was established in October 1986 as part of the main planting for the 1.0-generation base population of *E. nitens.* At that time, the site was considered to be typical high elevation, high quality land where productivity of *E. nitens* could be adequately tested. The site fortuitously proved to be particularly harsh in terms of exposure to cold.

Trees were assessed at a number of times for a number of growth, frost damage and frost tolerance traits (*Table 2*), since

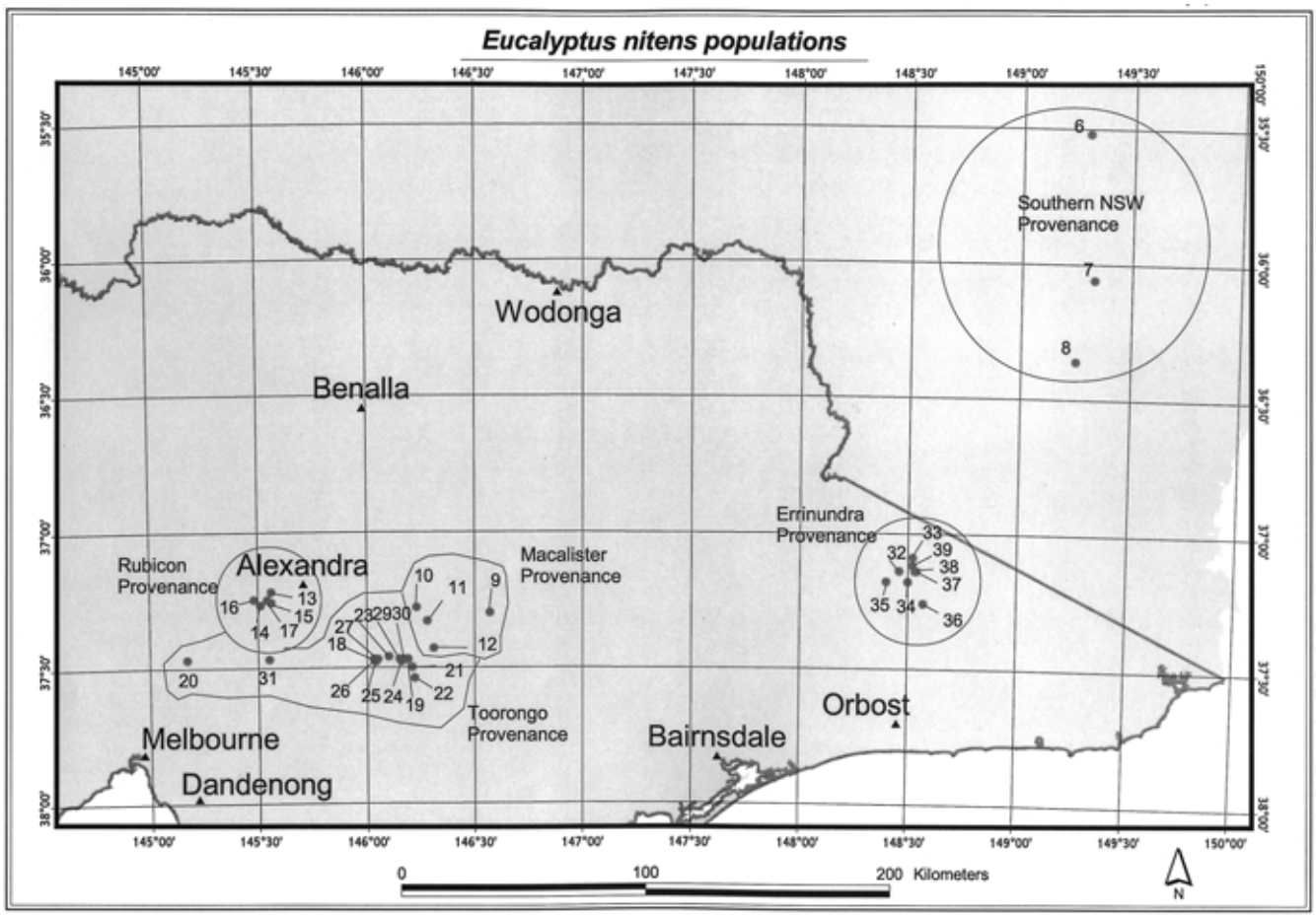


*Figure 1.* – Location of populations and provenances within Victoria and southern NSW.

very little was known about the relative genetic, practical and economic importance of each trait. Growth was measured, at age 3- and 6-years at Test 3 (Racecourse) and at 6-years at Test 4 (Painter Road), as girth at 1.3 m height using fibreglass tapes and then diameter and sectional area were estimated assuming a circular cross section. By convention, stem sectional area at breast height (1.3 m) height is basal area (BA). The decision to use BA as the growth trait was made on the basis that in *E. nitens* BA has been found to be highly correlated with volume and/or height and over bark measurements are well correlated with under bark measurements (TIBBITS and HODGE, 1998). Trees in Tests 3 and 4 were assessed for leaf damage some weeks after a particularly damaging frost in early April (autumn) 1988, which occurred after some very mild weather. Frost damage as assessed at age 1-year by TIBBITS and REID (1987a) in Test 3 was also included in the analyses. Survival was as recorded in the last growth assessment.

*Table 2.* – Variables assessed in the four tests.

| Variable (Test) | Description of variable including when assessed |
|---|---|
| T50 (Test 1) | cellular freezing damage (°C ) on nursery seedlings in winter 1985 |
| T50 (Test 2) | cellular freezing damage (°C) on nursery seedlings in winter 1986 |
| F85 (Test 3) | frost damage in winter 1985 on scale 0 to 5 (Tibbits and Reid 1987a) |
| F88 (Test 3) | frost damage following April 1988 frost on scale 0 to 100% (3 years) |
| BA88 (Test 3) | basal area over bark at 1.3m height in $cm^2$ at time of F88 (3 years) |
| BA91 (Test 3) | basal area over bark at 1.3m height in $cm^2$ in 1991 (6 years) |
| SURV (Test 3) | Survival in 1991, a 0 or 1 trait (6 years), means expressed as % |
| P (Test 3) | productivity, being BA91*SURV (6 years) |
| F88 (Test 4) | frost damage following April 1988 frost on scale 0 to 100% (1 year) |
| BA92 (Test 4) | basal area over bark at 1.3m height in $cm^2$ in 1992 (6 years) |
| SURV (Test 4) | survival, a 0 or 1 trait (6 years), means expressed as % |
| P (Test 4) | productivity, being BA92*SURV (6 years) |

We derived another trait for analysis, productivity, which is BA if the tree is alive, and 0 if the tree is dead.

*Statistical and Genetic Analyses*

The ultimate goal of all the genetic analyses in this study was to use artificial freezing and field trial data to predict breeding values for the traits of interest, which relate to survival and growth productivity on cold sites. In order to accomplish the breeding value prediction, it was necessary to 1) estimate provenance effects, and 2) estimate genetic parameters such as heritability, genotype x environment interaction and genetic correlation among traits.

*Estimating Provenance Effects*

The *E. nitens* population consisted of families from all six distinct provenances according to PEDERICK (1976). Initial analyses for provenance effects included all provenances, but subsequent analyses excluded the Errinundra provenance and some families from Toorongo, which exhibited phenotypes characteristic of the Errinundra provenance. These families were deleted from the data for genetic parameter estimation, as they were not considered as possible candidates for selection for advanced-generation orchards or breeding populations. These phenotypes have been described as a separate species (LADIGES and COOK, 1992), and have low potential for traits of interest in tree-farming (see TIBBITS and HODGE, 1998). Hence, all Errinundra families were removed from the data before more detailed analyses. Analyses of variance for all traits were initially conducted separately for test, and F-statistics were calculated to examine if significant differences among provenances

existed. Provenance means were estimated as the mean of family means, since data were reasonably well balanced.

*Single-Site Genetic Analyses*

Data for each site were analysed separately, principally to understand and partition the sources of variation for the traits of interest and their covariance with one another. Analyses were conducted using the Generalised Linear Model Procedure of SAS, where variance components were estimated using the Type I output. Similar linear models were used for the two field trials, since both had similar experimental designs (randomised complete blocks with single tree plots, and families nested within provenances).

The following linear model was used for the individual site analyses:

$$y_{hijk} = \mu_i + r_{ij} + P_h + F(P)_{hk} + w_{hijk} \quad [1]$$

where:

$\mu_i$ = the test mean,

$P_h$ = fixed effect of the $h^{th}$ provenance

$r_{ij}$ = random effect of $j^{th}$ replication within the $i^{th}$ test, $E(r_{ij}) = 0$, $Var(r_{ij}) = \sigma^2_r$,

$F(P)_{hk}$ = random effect of $k^{th}$ female within its provenance, $E(F(P)_{hk}) = 0$, $Var(F(P)_{hk}) = \sigma^2_F$,

$w_{hijk}$ = random tree error of the $hijk^{th}$ tree, $E(w_{hijk}) = 0$, $Var(w_{ijk}) = \sigma^2_w$.

Single-site (biased) heritabilities were calculated as:

$$h^2 = 2.5(\sigma^2_F)/(\sigma^2_P) \quad [2]$$

where $\sigma^2_P = \sigma^2_F + \sigma^2_w$. The choice of the coefficient of 2.5 in the calculation of heritability is commonly used by many authors with *Eucalyptus* (VOLKER *et al.,* 1994). The single-site heritabilities are referred to as "biased" because they are inflated by the presence of family x environment interaction variance (COMSTOCK and MOLL, 1963, HODGE and WHITE, 1992).

Single-site genetic correlations between traits (Type A, BURDON, 1977) were calculated using a dummy variable x+y for each pair of traits x and y. Since:

$$Var(x+y) = Var(x) + Var(y) + 2\,Cov(x,y) \quad [3]$$

The variance components associated with these dummy variables can be decomposed into variances due to x, variance due to y, and the covariance of x and y, and whilst the method is old it does work (SEARLE *et al.,* 1992). The family covariance component ($\sigma_{F:x,y}$) was used to estimate the single-site genetic correlation ($r_{Ag}$) as follows:

$$r_{Ag} = \sigma_{F:x,y} / [\sigma^2_{F:x} * \sigma^2_{F:y}]^{1/2} \quad [4]$$

*Paired-Site Genetic Analyses*

Family means were calculated for each trait in all four tests. Using the covariance of family means ($\sigma_{F1F2}$) for each pair of tests, BURDON's (1977) Type B genetic correlations ($r_{Bg}$) were then calculated as

$$r_{Bg} = \sigma_{F1F2} / (\sigma_{F1} * \sigma_{F2}) \quad [5]$$

where $\sigma_{F1}$ and $\sigma_{F2}$ are the single-site family variances, and $\sigma_{F1F2}$ = covariance of family means for traits 1 and 2.

*Prediction of Breeding Values*

The purpose of this project was to predict breeding values for *E. nitens* families for cold hardiness. This was done using best linear prediction (see WHITE and HODGE, 1989). Parental breeding values were predicted using an approach similar to that detailed by TIBBITS and HODGE (1998). Since "cold hardiness" was difficult to define, we decided to predict three traits, which might measure different aspects of cold hardiness that could be of interest to those planting *E. nitens.*

The three traits predicted were:

BA, which is basal area at sites similar to Painter Road (Test 4),

F88, which is damage from a frost in autumn on sites similar to the average of the two field trials, and

T50, which is artificial freezing temperature resulting in 50% loss of cellular electrolytes from disks frozen as per the CSIRO assessment (RAYMOND *et al.,* 1992).

## Results

*Provenance Effects*

TIBBITS and REID (1987a) previously reported highly significant effects for provenance in Test 1. In Test 2, there were large and significant ($P<0.01$) differences among the provenances in tolerance of artificial freezing as measured by T50. Similarly, RAYMOND *et al.* (1992) found significant provenance effects at individual frost temperatures. Provenance mean values for T50 covered a range of 1.5°C, from –6.8 to –8.4°C, with an overall mean of –7.5°C (*Table 3*). The least tolerant provenance was Toorongo with the "early-adult" form. This was 0.5°C less tolerant than both the remainder of the Toorongo provenance, with the "juvenile-persistent" form, and the Southern NSW provenance. The most tolerant provenances were Macalister, Rubicon and Northern NSW, with mean values of T50 of –8.0 to –8.4°C. The Errinundra provenance was not assessed for artificial freezing tolerance.

*Table 3.* – Population means for seven traits for cold hardiness in *E. nitens* (abbreviations and units of measure as in *Table 2*). Locations of numbered populations are as in *Table 1*. For populations 24–28 the numbers in brackets relate to families producing trees of the "early-adult" form, as distinct to families producing trees of the "juvenile-persistent" form.

| Population | T50 (Test 2) | F88 (Test 3) | F88 (Test 4) | SURV (Test 3) | SURV (Test 4) | BA 91 | BA92 |
|---|---|---|---|---|---|---|---|
| 1 | -7.7 | - | 66 | - | 45 | - | 45 |
| 2 | -8.5 | 100 | 54 | 0 | 73 | - | 59 |
| 3 | -8.5 | - | 54 | - | 69 | - | 69 |
| 4 | -8.1 | 92 | 58 | 2 | 62 | 74 | 74 |
| 5 | -8.4 | 85 | 55 | 11 | 75 | 61 | 77 |
| 1-5 | -8.4 | 87 | 57 | 9 | 65 | 62 | 67 |
| 6 | -7.7 | 89 | 59 | 7 | 70 | 53 | 60 |
| 7 | -7.1 | 93 | 72 | 4 | 41 | 33 | 47 |
| 8 | -7.4 | 91 | 68 | 1 | 60 | 115 | 59 |
| 6-8 | -7.5 | 91 | 63 | 4 | 63 | 53 | 58 |
| 9 | -8.1 | 37 | 48 | 78 | 81 | 134 | 73 |
| 10 | -7.6 | 75 | 49 | 36 | 79 | 79 | 98 |
| 11 | -8.6 | 36 | 39 | 84 | 89 | 129 | 105 |
| 12 | -7.8 | 52 | 46 | 63 | 81 | 115 | 88 |
| 6-12 | -8.0 | 51 | 46 | 65 | 82 | 113 | 94 |
| 13 | -8.4 | 49 | 43 | 78 | 91 | 106 | 112 |
| 14 | -8.3 | 62 | 50 | 58 | 86 | 110 | 97 |
| 15 | -7.8 | 62 | 49 | 54 | 76 | 88 | 91 |
| 16 | -8.0 | 42 | 47 | 78 | 80 | 112 | 108 |
| 17 | -7.1 | - | 52 | - | 87 | - | 81 |
| 13-17 | -8.1 | 54 | 49 | 68 | 84 | 105 | 98 |
| 18 | - | 84 | - | 18 | - | 111 | - |
| 19 | -7.1 | 62 | 50 | 47 | 78 | 93 | 112 |
| 20 | -6.7 | - | 56 | - | 78 | - | 78 |
| 21 | -7.8 | 66 | 50 | 43 | 83 | 94 | 105 |
| 22 | -7.1 | 43 | 47 | 91 | 100 | 109 | 94 |
| 23 | -6.7 | - | 38 | - | 94 | - | 117 |
| 24 | -7.0 (-6.7) | - | 57 (71) | - | 73 (51) | - | 89 (59) |
| 25 | -7.3 (-6.6) | - | 53 (70) | - | 80 (53) | - | 109 (61) |
| 26 | -7.1 (-7.1) | - | 60 (59) | - | 71 (63) | - | 71 (58) |
| 27 | -6.9 (-7.0) | - | 61 (67) | - | 68 (58) | - | 93 (37) |
| 28 | -7.2 (-7.2) | - (87) | 52 (67) | - (20) | 75 (61) | - (43) | 80 (33) |
| 29 | -7.4 | - | 58 | - | 76 | - | 96 |
| 30 | -7.0 | - | 57 | - | 78 | - | 100 |
| 31 | -7.5 | 64 | 42 | 52 | 89 | 83 | 135 |
| 18-31 | -7.3 (-6.8) | 67 (87) | 54 (68) | 46 (20) | 78 (57) | 91 (43) | 97 (46) |
| 32 | - | 94 | - | 5 | - | 143* | - |
| 33 | - | 92 | - | 0 | - | - | - |
| 34 | - | 97 | 68 | 2 | 60 | 25 | 32 |
| 35 | - | 100 | 69 | 0 | 0 | - | - |
| 36 | - | - | 79 | - | 20 | - | 62 |
| 37 | - | - | 93 | - | 19 | - | 10 |
| 38 | - | 96 | - | 5 | - | 58 | - |
| 39 | - | 86 | - | 0 | - | - | - |
| 32-39 | - | 95 | 77 | 1 | 25 | 75 | 34 |
| 1-39 | -7.5 | 75 | 57 | 31 | 71 | 89 | 81 |

* only one tree alive

Overall, in the field trails, *E. nitens* performed better at Test 4 (Painter Road) than at Test 3 (Racecourse Plains). The same frost in autumn of 1988 resulted in 56.7% leaf damage at Painter Road and 74.6% at Racecourse Plains. Average survivals at 6 years were 71.4 and 31.3% respectively. Growth was very similar at both sites, with BA being 80.9 and 88.9 $cm^2$ respectively for trees about 6 years old.

There were significant provenance effects at the 1% level in both field trials for all traits (*Table 3* and *Figure 2*). Generally at both sites, the early-adult form whether from Errinundra or Toorongo provenances, was on average characterised by the most frost damage (*Figure 2a*), the least survival (*Figure 2b*) and the lowest basal area (F*igure 2c*). The only slight exception was at Test 3 (Racecourse Plains), where its basal area was slightly better than the two NSW provenances, but the standard error was very large.

The Errinundra and early-adult form in Toorongo provenances were not to be considered for more detailed genetic analyses because of their known poor performance in other studies (see above). However, the two provenances from NSW appeared to be so poorly adapted to either test site that there was consequently little reason to include them in the analyses since they would not be contributing to selections. For the purposes of this study, further analysis and discussion focused on the parameters estimated using the data only from three provenances Macalister, Rubicon and Toorongo (juvenile-persistent form only), and these parameters were used in the prediction of breeding values. The significance of provenance effects altered upon reducing the number of provenances. For all traits provenance effects were not significant at Test 3 (Racecourse Plains). At Test 4 (Painter Road) highly significant provenance effects existed for T50 and F88, and SURV was significant at 5%. No particular provenance was quite distinct from the others, although Toorongo was poorest in terms of T50 in Test 2 (–7.3 *c.f.* –8.0 and –8.1°C for Rubicon and Macalister respectively) and frost damage (53.8 *c.f.* 49.4 and 45.8% respectively) and both frost damage scores, F85 and F88. Toorongo was also poorest for the T50 trait in Test 1.

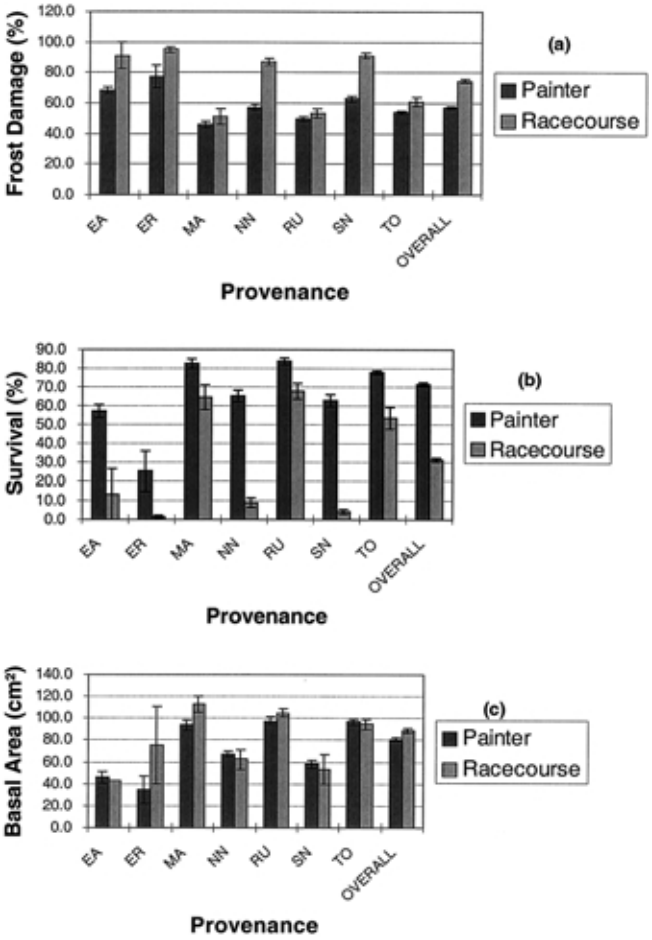


*Figure 2.* – Provenance means and standard errors for frost damage, survival and basal area at Test 3 (Racecourse Plains) and Test 4 (Painter Road). Provenance abbreviations are: northern New South Wales (NN), southern New South Wales (SN), Macalister (MA), Rubicon (RU), Toorongo (TO), "early-adult" form in Toorongo (EA) and Errinundra (ER).

*Single-Site Genetic Analyses – Heritability*

The only trait assessed in Test 1 was T50, and variance components for it are indicated in *Table 5*. Individual narrow-sense heritability estimates were 0.29 when using data only from the three central Victorian provenances and 0.37 including provenances from New South Wales, indicating that about 30–40% of variation is under additive genetic control. Family mean heritability was 0.61 (*Table 7*). As in Test 1, T50 was the only trait assessed in Test 2. Its narrow-sense heritability was very high at 0.96 and family mean heritability was 0.86 (*Table 7*).

Variance components for traits assessed at Tests 3 and 4 are indicated in *Table 6*.

In Test 3 (Racecourse Plains field trial) heritabilities were moderate, at around 0.4, for traits associated with tolerance to frost, such as F85 and F88 (*Table 6*). Growth traits had lower heritabilities, with that for basal area at the last assessment, BA91, being very low at 0.10. Survival (SURV) and productivity (P) had similarly moderate heritabilities. It appears that P is more influenced by the contribution of SURV rather than BA91, since it more closely matches the former. Family mean heritability ranged from around 0.8 for P, SURV, F85 and F88 to 0.35 for BA91 (*Table 7*).

Heritabilities in Test 4 (Painter Road) ranged from very low to moderate for traits associated with cold hardiness (*Table 6*). For example, $h^2$ was 0.14 for F88, the sole growth trait BA92 had a heritability of 0.27, fairly typical for growth traits, survival (SURV) showed no genetic variance ($h^2 = 0.01$), and thus,

*Table 4.* – Four cold hardiness traits (Tests 2 & 4) within five distinct *E. nitens* populations in the Toorongo provenance, comparing families producing trees of the "juvenile-persistent" form with those families producing trees of the "early-adult" form. Shown are the differences in family means of the "juvenile-persistent" form and the "early-adult" form. Numbers in brackets relate to population codes used in *Table 1*.

| Trait | Lock Valley general (24) | Lock Valley site 1 (25) | Lock Valley site 2 (26) | Lock Valley site 3 (27) | Mt Toorongo (28) | Overall |
|---|---|---|---|---|---|---|
| T50 (°C) | -0.3 | -0.7 | 0.0 | 0.0 | -0.1 | -0.2 |
| F88 (%) | -14 | -18 | 1 | -5 | -15 | -10 |
| SURV (%) | 22 | 28 | 9 | 10 | 15 | 17 |
| BA92 ($cm^2$) | 30 | 48 | 13 | 56 | 48 | 39 |

*Table 5.* – Statistical parameters for the T50 trait assessed in Tests 1 and 2 for (a) the three main Victorian provenances (Macalister, Rubicon, and Toorongo) and (b) all five provenances (Macalister, Rubicon, Toorongo, northern New South Wales, and southern New South Wales). Shown are variance components for reps ($\sigma^2_r$), provenances ($\sigma^2_p$), families within provenance ($\sigma^2_f$) and error ($\sigma^2_e$), as well as number of trees (n), narrow-sense heritabilities ($h^2$) and the F statistic for the provenance.

| Test and Provenances | $\sigma^2_r$ | $\sigma^2_p$ | $\sigma^2_f$ | $\sigma^2_e$ | n | $h^2$ | F |
|---|---|---|---|---|---|---|---|
| Test 1 (a) three provenances | 0 | 0.02 | 0.04 | 0.27 | 119 | 0.29 | 0.117 |
| Test 1 (b) all five provenances | 0 | 0.07 | 0.05 | 0.22 | 192 | 0.37 | 0.002 |
| Test 2 (a) three provenances | 0 | 0.26 | 0.37 | 0.59 | 1067 | 0.96 | 0.001 |
| Test 2 (b) all five provenances | 0 | 0.29 | 0.39 | 0.59 | 1216 | 0.99 | 0.001 |

*Table 6.* – Statistical parameters for traits assessed at (a) Test 3 (Racecourse Plains field trial) and (b) Test 4 (Painter Road field trial), for the three main Victorian provenances (Macalister, Rubicon, and Toorongo). Shown are variance components for provenances ($\sigma^2_p$), reps ($\sigma^2_r$,), families within provenance ($\sigma^2_f$,) and error ($\sigma^2_e$), as well as number of trees (n), narrrow-sense heritabilities ($h^2$) and the F statistic for the provenance. Significance levels are ns (P>0.05), * (P<0.05), ** (P<0.01).

| Variable | Mean | $\sigma^2_r$ | $\sigma^2_p$ | $\sigma^2_f$ | $\sigma^2_e$ | n | $h^2$ | F |
|---|---|---|---|---|---|---|---|---|
| (a) Test 3 (Racecourse Plains) | | | | | | | | |
| F85 | 1.8 | .0251 | 0 | .1728 | .8660 | 890 | .42 | .95 ns |
| F88 | 74.6 | 86.74 | 17.4 | 165.0 | 769.1 | 749 | .44 | 1.70 ns |
| BA88 | 5.6 | 6.947 | 0 | 12.21 | 88.18 | 749 | .30 | .135 ns |
| BA91 | 88.9 | 466.1 | 126.8 | 127.4 | 3089 | 557 | .10 | 1.94 ns |
| SURV | 31.3 | .0123 | .0017 | .0369 | .1855 | 890 | .41 | 1.75 ns |
| P | | 4143 | 116.8 | 814.5 | 3850 | 890 | . 44 | 2.74 ns |
| (b) Test 4 (Painter Road) | | | | | | | | |
| F88 | 56.7 | 225 | 15.58 | 38.71 | 659.3 | 2329 | .14 | 7.0 ** |
| BA92 | 80.9 | 67.04 | -12.75 | 276.3 | 2283 | 1856 | .27 | 26.0 ns |
| SURV | 71.4 | .0227 | .0010 | .0007 | .1403 | 2329 | .01 | 3.84 * |
| P | | 236.1 | -5.085 | 286.5 | 3123 | 2329 | .21 | .78 ns |

*Table 7.* – Type-B genetic and family mean correlations for cold hardiness and growth traits. Pearson correlations of family means ($r_{Bfm}$) are shown above the diagonal, whilst Type-B genetic correlations ($r_{Bg}$) are shown below the diagonal. Family heritabilities ($h^2_f$) are shown in brackets. Values for F88 (Test 4) with BA92 are actually a Type A family mean correlation (above the diagonal) and a Type A genetic correlation (below the diagonal) since they are both measurements from the same site.

| | $T50_{Test2}$ (.86) | $T50_{Test1}$ (.61) | BA92 (.56) | $F88_{Test4}$ (.44) | $F88_{Test3}$ (.79) |
|---|---|---|---|---|---|
| $T50_{Test2}$ | | 0.62 | -0.05 | 0.18 | 0.32 |
| $T50_{Test1}$ | 0.86 | | -0.03 | 0.46 | 0.24 |
| BA92 | -0.07 | -0.06 | | -0.45 | -0.19 |
| $F88_{Test4}$ | 0.29 | 0.88 | -0.58 | | 0.26 |
| $F88_{Test3}$ | 0.39 | 0.34 | -0.29 | 0.45 | |

productivity (P) was essentially the same trait as BA92. Family mean heritabilities ranged around 0.4 to 0.5.

*Single-Site Genetic Analyses – Correlations*

Genetic correlations between traits assessed on trees at the same site are indicated in *Table 8*. In Test 3, F85 and F88, the two frost damage/tolerance assessments had a large and positive genetic correlation of 0.69, indicating that resistance of one-year-old plants to winter cold and of four-year-old trees to an autumn freeze are linked. Other strong positive genetic correlations existed between BA88 and SURV (ultimate survival), between BA88 and P, and between SURV and its derived trait P. Strong negative genetic correlations existed between F85 and growth or survival, and between F88 and final growth and survival. These indicate that high levels of frost damage are associated with poor growth and survival, as one might expect. Interestingly, there was a strong negative correlation between BA88 and F88, and a moderately strong positive correlation between the two growth assessments, BA88 and BA91. These indicate that large trees were better able to tolerate the 1988 frost, and subsequently remained bigger trees in the 1991 measurement.

In the other field trial (Test 4), strong negative genetic correlations existed between F88 and BA92. Disappointingly, the two frost damage/tolerance assessments (T50 and F88) had a genetic correlation of only 0.37, indicating that resistance of seedlings assessed by artificial freezing of leaf disks, and resistance of two-year-old trees to an autumn freeze are only weakly linked. T50 was also very weakly correlated to BA92 ($r_{Ag}$ = –0.04), in contrast to the correlation between F88 and BA92 ($r_{Ag}$ = –0.58). This is another indication that the laboratory freezing test may have only limited use to assess cold hardiness and growth in the field, or else cold hardiness assessment in field tests is less repeatable due to damage caused by unexpected frost events.

*Table 8.* – Type A genetic correlations ($r_{Ag}$) between traits measured at (a) Test 3 (Racecourse Plains field trial) and (b) Test 4 (Painter Road field trial). Correlations are based on data from three provenances (Macalister, Rubicon, Toorongo). NA signifies not available because of negative covariance or variance estimates. Figures with asterisk (*) are Type A family mean correlations: Type A genetic correlations for some pairs were not calculated due to oversight.

| Genetic Correlation ($r_{Ag}$) | F88 | BA88 | BA91 BA92 | SUR | P |
|---|---|---|---|---|---|
| (a) Test 3 (Racecourse Plains) | | | | | |
| F85 | 0.69 | -0.63 | -0.88 | -0.78 | -0.81 |
| F88 | | -0.88 | -0.91 | -0.97 | -0.95 |
| BA88 | | | 0.42 | 0.91 | 0.81 |
| BA91 | | | | 0.52* | 0.79* |
| SUR | | | | | 0.98 |
| (b) Test 4 (Painter Road) | | | | | |
| T50 | 0.37 | | -0.04 | NA | -0.30 |
| F88 | | | -0.58 | - | -0.78 |
| BA92 | | | | NA | 1.00 |

*Paired-Site Genetic Analyses*

Genetic correlations across sites are presented in *Table 7* for some traits. There is a good relationship between the two laboratory freezing assessments T50 in Test 1 and Test 2 ($r_{Bg}$ = 0.86), thus the techniques are repeatable and measure a heritable trait. However, there is apparently only a moderate relationship between T50 measurements and frost damage in the field. Average Type B genetic correlation between T50 and F88 at Painter Road was 0.58, while at Racecourse it was 0.37. Further support indicating that laboratory screening may be of limited utility is the low correlations between T50 and basal area growth at Painter Road ($r_{Bg}$ = –0.07 and –0.06).

Frost damage at the two sites was moderately correlated ($r_{Bg}$ = 0.45). This correlation applies to damage from the same autumn freeze event, but on 2-year-old trees at Painter Road and 4-year-old trees at Racecourse Plains. In addition, both field frost damage assessments were better correlated with growth (BA92) at Test 4 ($r_{Bg}$ = –.58 and –.29) than the T50 assessments were with the same growth trait ($r_{Bg}$ = –.07 and –.06).

*Population effects and the "early-adult" form*

Population within provenance was not included in the model to keep it simple. However, some examination of populations within provenance may be helpful for selection purposes. When populations within provenances were considered (*Table 3; Figure 1*), there appeared to be some populations, which had relatively low levels of frost damage (F88), high levels of survival (SURV) and high average basal area growth (BA) across both test sites. Two populations had levels of performance much better than average for all these traits (*Table 3*), including Connors Plain of the Macalister provenance (population 11) and Barnwall Plains from the Rubicon provenance (population 13). They were characterised by frost damage of less than 50%, survival of 78% or higher, and basal area of 105 $cm^2$ or above.

These populations also had the best artificial freezing tolerance within provenance.

For the two NSW provenances, frost damage was so severe and survival and growth so low in Test 3 at Racecourse, that it was difficult to identify better populations across both sites. However, population differences were more pronounced when examining performance only in Test 4 at Painter Road. In this test, the two populations in northern NSW from Mount Carson in the Barrington Tops National Park and Point Lookout in the New England National Park near Ebor, appeared to be slightly better than either of the other two populations. Of the three populations from the southern NSW provenance, the Tallaganda population was the best for all three traits in Test 4. Due to high levels of frost damage and low levels of survival and growth for the Errinundra provenance in both tests, it was not possible to make meaningful comparisons among populations.

In the Toorongo provenance there were five separate populations collected where trees produced progeny of distinctly either the "juvenile-persistent" or the "early-adult" form (*Table 1*). Hence, within five populations it was possible to classify families as being distinctly either "juvenile-persistent" or "early-adult" form. Four of these populations were listed as coming from the Lock Valley Road area and the fifth from Mount Toorongo. This enabled paired comparisons of the "juvenile-persistent" and "early-adult" forms from within the same general population area. These were included in Tests 2 and 4. In all five cases, the "juvenile-persistent" families on average performed better than the "early-adult" families for survival and growth (SURV and BA92 respectively), with average differences of 17% (range 9 to 28%) and 39 $cm^2$ (range 13 to 56 $cm^2$) respectively (*Table 4*). In terms of frost damage (F88), the "juvenile-persistent" families had 5 to 18% less damage than the "early-adult" families in four of the five pairs and equal damage in the fifth pair. For artificial freeze testing (T50), differences were generally small: in three of the five cases the "juvenile-persistent" and "early-adult" forms from the same population were within 0.1°C of each other.

*Prediction of Breeding Values*

The family with the best overall cold hardiness in terms of all three traits combined with equal emphasis was a family from Macalister provenance. This family was ranked best overall for F88 and its breeding value was about –11% damage, which represents about a 22% improvement from average. It was also in the 97 percentile for BA and its breeding value was about 13.7 $cm^2$, which represents about a 14% improvement in growth on cold sites. It was in the 95 percentile for T50 and its breeding value was about –0.8°C, which represents about a 10% improvement. Not surprising, this family and two others in the top four families on combined breeding values were from the Connors Plain population that as mentioned above performed very well (population 11, *Tables 1* and *3*, *Figure 1*). Understandably, due to the lack of strong correlations between certain traits (*Tables 7* and *8*), some families ranked well in breeding values for one trait but not for others, whilst some ranked well for two but not three traits.

## Discussion

Artificial freeze testing using the electrical conductivity method is a useful way of investigating the genetic control of frost tolerance in *E. nitens* (TIBBITS and REID, 1987a, RAYMOND *et al.* 1992). The high genetic correlation ($r_{Bg}$ = 0.86) between T50 values in two separate artificial freezing experiments is evidence that such assessments are repeatable. In the present study, heritability estimates for T50 were 0.29 and 0.96, for Test 1 and Test 2 respectively. This indicates a moderate to very high level of genetic control. VOLKER *et al.* (1994) found moderate estimates of heritability in *E. nitens* (0.21 to 0.33 for open-pollinated and 0.23 to 0.44 for control-pollinated material), using exactly the same equipment and essentially the same methodology as used by RAYMOND *et al.* (1992) to generate data at individual frost temperatures for Test 2 in this study. It is possible that the higher heritability reported in Test 2 compared to Test 1 (*Table 5*) may be accounted for by a reduced error variance because seedlings were in family blocks and differences among families may be confounded by nursery microsite and family genetic differences. A similar explanation has been discussed for high heritabilities reported with similar experimental testing of *E. globulus* (TIBBITS e*t al.,* 2003).

However, this study produced some findings which indicate that one should exercise caution when relying on artificial freeze testing using the electrical conductivity method to identify and select superior plants that will be hardy for deployment in field conditions. These include, firstly, changes in provenance frost tolerance ranking when comparing artificial freeze testing and performance in the field, and secondly, lack of high genetic correlations between artificial freeze testing and the level of damage from natural frosts.

The first point of concern with respect to cold hardiness testing is the major changes in provenance ranking between assessment by artificial freeze testing and field performance. The most frost tolerant provenance identified here during the artificial freeze testing was Northern NSW (RAYMOND *et al.,* 1992; this study, *Table 3*). In separate artificial freezing experiments on whole seedlings and leaf disks, TIBBITS and REID (1987a) found that the Northern NSW provenance was equal top in hardiness to the three provenances from the central highlands of Victoria, whether plants were unhardened, partially hardened or at winter hardiness. However, in this study, the three provenances from the central highlands of Victoria, Macalister, Rubicon and Toorongo, were always better than the other provenances, in terms of F88, survival and basal area. At Test 4 (Painter Road), the Northern and Southern NSW provenances generally were intermediate in performance, between the better three provenances and Errinundra, whilst at Test 3 (Racecourse Plains) they were generally nearly as poor as Errinundra. Whilst Northern NSW was always marginally better than Southern NSW, it appeared to be poorly adapted to either test site, particularly Test 3 at Racecourse.

One likely explanation for the relatively poor performance of the Northern NSW provenance has to do with the interaction between frost tolerance and growth conferring ability to survive and grow on very cold sites. Strong negative genetic correlations existed between F88 and BA ($r_{Ag}$ = –0.91 and –0.58, *Table 8*), indicating that high levels of frost damage in the field are associated with poor growth and survival, as one might expect. Of the provenances with a high level of artificial frost tolerance (Macalister, Rubicon and Northern NSW), the slower-growing Northern NSW provenance was likely to be more exposed to cold air layers close to the ground, and consequently to be more affected by freezing conditions. In other words, cold hardiness is an interaction between cellular frost tolerance and growth potential. This is more likely to be the case on sites such as those in this study, which are at the extremes that a particular species can tolerate. Indeed, MESKIMEN (1983) documents the case in Southwest Florida where inversion freezes have the coldest temperatures at ground level yet moderating quickly in the next couple of metres, and *E. grandis* "can reduce risk simply by growing fast, elevating tender crown tissue above lethal temperatures." ROCKWOOD *et al.* (1989)

describe this as "frost resilience". Additional evidence for this at Test 3 (Racecourse field trial) comes from *E. coccifera,* a slow-growing species known for its superior frost tolerance to *E. nitens,* which suffered much higher mortality than the *E. nitens* (TIBBITS and HODGE, 1995).

The second point of concern for identifying hardy plants is that whilst the high genetic correlation ($r_{Bg}$ = 0.86) between T50 values in two separate artificial freezing experiments is evidence that such assessments are repeatable, the genetic correlations between T50 and the level of damage from natural frosts (F88) was not nearly so high (average $r_{Bg}$ = 0.41 and 0.37 for the two sites respectively). This indicates that artificial freeze testing is not necessarily a strong indicator of ability to tolerate frosts of the kind experienced at the field trials in this study.

One explanation for this is as mentioned above for the Northern NSW provenance. In other words, for all trees the variable F88 may be a mixture between damage from natural frosts and growth effects, as different size trees were not exposed to the same frost temperatures. If this is so, then adjusting F88 using growth as a covariate may improve the correlations between T50 and F88. Yet, there were also strong negative correlations between frost damage at one-year, when all plants were very small, and growth at three and six years (*Table 8*). This could imply that frost susceptible trees may each season be getting more damaged than resistant trees, and therefore grow less because they need to recover from damage. Should this be so, then using growth as a covariate may obscure correlations that breeders are seeking to estimate. However, the main point in this study was to identify provenances, families and trees that are hardy in the field and not those that are hardy in the laboratory. Hence, if growth rates contribute to cold hardiness or frost resistance, even through means other than physiological cellular freezing processes, then breeders are likely to be more interested in the combined hardiness trait than in frost tolerance *per se.*

Another explanation for the apparent poor correlations between laboratory and field frost assessments may relate to how precisely a critical frost temperature can be identified from artificial freeze testing. RAYMOND *et al.* (1992) who produced the raw data for test two, investigated analysing a critical temperature for each seedling, similar to T50, but did not follow this through since the top ranked family survived and the bottom ranked family was killed by all three freeze temperatures. The relative level of electrolyte loss following freezing which RAYMOND *et al.* (1992) considered critical is 36%, whilst that used here is 50% (T50). It is possible that what constitute critical frost temperatures were not accurately identified. Another explanation is that there is a poor correlation between winter and autumn frost tolerance, essentially measured by T50 and F88 respectively. It is also possible that the correlations around 0.4 here are at least in part due to the difficulty in reliably estimating genetic correlations.

Heritability estimates varied substantially between the two sites for all traits (*Table 6*), and this is not surprising. This variance may be due simply to sampling error (genetic parameters are difficult to estimate precisely), but may also represent real differences among the sites (one site may be more cold or uniform than another, or may allow for greater expression of genetic variance). If BA91 and BA92 are considered similar traits, $h^2$ was 0.10 for Test 3 (Racecourse Plains), and 0.27 for Test 4 (Painter Road). For F88, $h^2$ was 0.44 at Test 3 (Racecourse Plains) and 0.14 at Test 4 (Painter Road). Racecourse Plains is much colder than Painter Road (for example, mean survival was 31 and 71%, respectively, see *Table 3* and *Figure 2b*), so this is not surprising. If the site is too cold for many trees to grow, heritability for growth will necessarily be low. On the other hand, this same environment allows good expression of cold hardiness. The heritability estimates for survival of between 0.01 and 0.42 compare reasonably favourable with that of 0.092 for *E. grandis* by ROCKWOOD *et al.* (1989), yet they need to be cautiously treated as they are for a binary trait where the variances and hence heritability depend on the mean.

This work has confirmed previous findings (TIBBITS and REID, 1987a, RAYMOND *et al.,* 1992) concerning the poor level of cold hardiness in the Southern New South Wales provenance (*Figure 2*) and the two provenances with the "early-adult" form (*Table 4*), including Errinundra and some families from Toorongo. They were characterised by high levels of frost damage, low survival and low growth. Consequently, there would be little reason to include them in selections for breeding improvement, where cold hardiness was required.

The challenge in estimating parameters and predicting cold hardiness is to reliably identify genotypes with sufficient cold hardiness for use in the kinds of environments where it will be needed. This study identifies the complexities, which arise when artificial testing and field evaluation are used. In order to reliably identify genotypes with a sufficient level of cold hardiness, it would appear that thorough testing and evaluation is required, including field-testing across a range of sites using a range of genetic material. Nonetheless, if the breeding values predicted in this present study are reliable, then there is reason to be confident that using such genotypes should confer improvements in the performance of the species on cold sites, in terms of reduced risk of frost damage and faster growth.


## Acknowledgements

North Forest Products (now Gunns Limited) greatly assisted this work in many practical, financial and supportive ways, and Dr DAVID DE LITTLE and Mr IAN RAVENWOOD are particularly acknowledged. Dr. LEON PEDERICK and the Tree Seed Centre of CSIRO Canberra are thanked for assisting WAYNE TIBBITS undertake seed collections. Prof JIM REID of the School of Plant Science, University of Tasmania is thanked for his guidance during the work in establishing Tests 1 and 3. Ms CAROLYN RAYMOND of CSIRO is thanked for providing data and useful discussions. The work was undertaken with assistance from a Commonwealth Postgraduate Research Award to WAYNE TIBBITS, and a grant from the Visiting Scientist Scheme of the Cooperative Research Centre for Temperate Hardwood Forestry to GARY HODGE.


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# Mapping the Gene Encoding Cry j 1: a Major *Cryptomeria japonica* Pollen Allergen

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(Received 2nd September 2002)

**Abstract**

*Cryptomeria japonica* D. Don (Sugi, Japanese cedar) causes a serious allergic disease in Japan. In this report, we describe cDNA polymorphisms of Cry j 1, one of the major allergens involved in *C. japonica* pollinosis. We detected two cDNA sequences encoding Cry j 1, one of which was different from all sequences previously reported. A cleaved amplified polymorphic sequence (CAPS) marker that distinguishes a nucleotide difference between the detected cDNA sequences was developed and designated CRYJ1-352 because it has a polymorphic site at position 352 of mature Cry j 1. In an inheritance analysis based on 65 progeny trees from a controlled cross between Iwao-sugi x Boka-sugi, this CAPS marker showed a Mendelian segregation pattern. In addition, the location of CRYJ1-352 was determined on the linkage map of Iwao-sugi.

*Key words:* Cry j 1, *Cryptomeria japonica,* pollinosis, CAPS, linkage map.

## Introduction

*Cryptomeria japonica* D. Don (Sugi, Japanese cedar) is a coniferous tree species that covers 4.53 million ha and comprises about 45% of the man-made forests in Japan. An allergic disease caused by pollen released from *C. japonica* has become a serious problem. More than 10% of Japanese people currently suffer from *C. japonica*-associated pollinosis, and the number of affected individuals is increasing. Reports have been published on two major allergens responsible for *C. japonica* pollinosis, Cry j 1 and Cry j 2, that include details of their physiological functions (OHTSUKI *et al.,* 1995; TANIGUCHI *et al.,* 1995), partial amino acid sequences (YASUEDA *et al.,* 1983; TANIAI *et al.,* 1988; SAKAGUCHI *et al.,* 1990), and cDNA sequences (GRIFFITH *et al.,* 1993; KOMIYAMA *et al.,* 1994; NAMBA *et al.,* 1994; SONE *et al.,* 1994; WANG *et al.,* 1998). The Cry j 1 and Cry j 2 contents in pollen (w/w) are known to vary widely among individual trees (SASAKI *et al.,* 1996; GOTO *et al.,* 1999; SAITO and TERANISHI, 2002). The Cry j 1 content, however, shows minor variations among pollen samples from the same clones, indicating that the Cry j 1 content in pollen is under strong genetic control. This suggests that planting trees that produce relatively small amounts of the two allergens could be a useful tool for reducing pollinosis. However, the selection of such trees requires a marker that would indicate trees of reduced pollen allergenicity. We report now on a molecular marker for the Cry j 1 gene, which would enable the mapping of the corresponding locus within the genome of *C. japonica.*

## Materials and Methods

### *Plant materials*

Pollen samples for RNA isolation were collected from a single ramet of Chichibusyo 3, a plus tree clone, in March 1999. In addition, young leaf samples of eight plus trees (Chichibusyo 3,

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