haviour of coniferous seed. IV. Stratification period and incubation temperature, *Pseudotsuga menziesii* (Mirb.) Franco. For. Chron. **36**: 18—29 (1960). — Allen, G. S.: Testing Douglas-fir seed for provenance. *Proc. Int. Seed Test. Assoc.* **26**: 388—403 (1961). — Andersson, E.: Cone and seed studies in Norway spruce. *Stud. For. Suec.* **23**: 1—214 (1965). — Association of Official Seed Analysts: Rules for testing seeds. *Proc. Assoc. Office. Seed Anal.* **60**: 1—116 (1970). — Bagchi, S. K., Joshi, D. N. and Rawat, D. S.: Variation in seed size of *Acacia spp.* Silvae Genet. **39**: 107—110 (1990). — Baker, H. G.: Seed weight in relation to environmental conditions in California. *Ecology* **53**: 997—1010 (1972). — Bramlett, D. L., Dell, T. R. and Pepper, W. D.: Genetic and maternal influences on Virginia pine seed germination. Silvae Genet. **32**: 1—4 (1983). — Burgar, R. J.: The effect of seed size on germination, survival and initial growth in white spruce. *For. Chron.* **41**: 93—97 (1964). — Cavers, P. B., and Harper, J. L.: Germination polymorphism in *Rumex crispus* and *Rumex obtusifolius.* *J. Ecol.* **54**: 367—382 (1966). — Choi, S. K. and Kim, K. C.: Studies on the characteristics of selected plus trees. II. The difference in seed germination capacity between plus-tree clones. *Res. Rep. Inst. For. Genet.* Korea **7**: 81—90 (1969). — Czabator, F. J.: Germination value: An index combining speed and completeness of pine seed germination. *For. Sci.* **8**: 386—396 (1962). — Datta, S. C., Evenari, M. and Gutterman, Y.: The heteroplasty of *Aegilopsis ovata* L. Israel *J. Bot.* **19**: 463—483 (1970). — De Matos Malavasi, M., Stafford, S. G. and Lavender, D. P.: Stratifying, partially redrying and storing Douglas-fir seeds: effects on growth and physiology during germination. *Ann. Sci. For.* **42**: 371—384 (1985). — Dunlap, J. R., and Barnett, J. P.: Influence of seed size on germination and early development of loblolly pine (*Pinus taeda* L.) germinants. *Can. J. For. Res.* **13**: 40—44 (1983). — Edwards, D. G. W.: Maturity and quality of tree seeds — a state-of-the-art review. *Seed Sci. & Technol.* **8**: 625—657 (1980). — Edwards, D. G. W. and El-Kassaby, Y. A.: Effect of flowering phenology, date of cone collection, cone-storage treatment and seed pretreatment on yield and germination of seeds from a Douglas-fir seed orchard. *For. Ecol. Manage.* **25**: 17—29 (1988). — El-Kassaby, Y. A., Edwards, D. G. W. and Taylor, D. W.: Genetic control of germination parameters in Douglas-fir and its importance for domestication. *Silvae Genet.* **41**: 48—54 (1992). — Ellner, S.: Germination dimorphisms and parent-offspring conflict in seed germination. *J. Theor. Biol.* **123**: 173—185 (1986). — Falconer, D. S.: Introduction to quantitative genetics. 2nd ed. John Wiley & Sons, Inc., New York (1986). — Heit, C. E.: Laboratory germination and recommended testing methods for 16 spruce *Picea* species. *Proc. Assoc. Off. Seed Anal.* **51**: 165—171 (1961). — Hellum, A. K.: Grading seed by weight in white spruce. *Tree Planters' Notes* **27** (1): 16, 17, 23 (1976). — Hellum, A. K.: Seed ecology in a popula-

tion of *Acacia holoserica.* *Can. J. For. Res.* **20**: 927—934 (1990). — Hoff, R. J.: Dormancy in *Pinus monticola* seed related to stratification time, seed coat, and genetics. *Can. J. For. Res.* **17**: 294—298 (1987). — International Seed Testing Association: International rules for seed testing 1985. *Seed Sci. & Technol.* **13**: 299—513 1985). — Jain, S. K.: Variation and adaptive role of seed dormancy in some annual grassland species. *Bot. Gaz.* **143**: 101—106 (1982). — Koller, D.: Preconditioning of germination in lettuce at time of fruit ripening. *Amer. J. Bot.* **49**: 841—844 (1962). — Kozlowski, T. T. and Gentle, A. C.: Influence of the seed coat on germination, water absorbtion, and oxygen uptake of Eastern white pine seed. *For Sci.* **5**: 389—395 (1959). — Larson, M. M.: Initial root development of ponderosa pine seedlings as related to germination data and size of seed. *For. Sci.* **9**: 456—460 (1963). — Linck, A. J.: The morphological development of the fruit of *Pisum sativum* var. *Alaska. Phytomorphology* **11**: 79—84 (1961). — Lindgren, D.: Fractionation of seed orchard seeds by weight does have genetic implications. *Silva Fenn.* **16**: 156—160 (1982). — Naylor, J. M.: Studies on the genetic control of some physiological processes in seeds. *Can. J. Bot.* **61**: 3561—3567 (1983). — Perry, T. O.: Maternal effects on the early performance of tree progenies. In: *Tree physiology and yield improvement.* Ed. M. G. R. Cannell and F. T. Last. pp. 473—481. Academic Press, New York (1976). — Roach, D. A. and Wulff, R. D.: Maternal effects in plants. *Ann. Rev. Ecol. Syst* **18**: 209—235 (1987). — Sawhney, R. and Naylor, J. M.: Dormancy studies in seed of *Avena fatua.* 9. Demonstration of genetic variability affecting the response to temperature during seed development. *Can. J. Bot.* **57**: 59—63 (1979). — Sawhney, R. and Naylor, J. M.: Dormancy studies in seed of *Avena fatua.* 12. Influence of temperature on germination behaviour of nondormant families. *Can. J. Bot.* **58**: 578—581 (1980). — Schaal, B. A.: Reproductive capacity and seed size in *Lupinus texensis.* *Amer. J. Bot.* **67**: 703—709 (1980). — Shoulders, E.: Effect of seed size on germination, growth, and survival of slash pine. *J. For.* **59**: 363—365 (1961). — Silen, R. and Osterhaus, C.: Reduction of genetic base by sizing of bulked Douglas-fir seed lots. *Tree Planters' Notes* **30**: 24—30 (1979). — Sorensen, F. C. and Franklin, J. F.: Influence of year of cone collection on seed weight and cotyledon number in *Abies procera.* Silvae Genet. **26**: 41—43 (1977). — Spurr, S. H.: Effect of seed weight and seed origin on the early development of eastern white pine. *J. Arn. Arbor.* **25**: 467—481 (1944). — Tyson, H.: Genetic control of seed weight in flax (*Linum usitatissimum*) and possible implications. *Theor. Appl. Genet.* **77**: 260—270 (1989). — Wulff, R.: Intrapopulational variation in the germination of seeds in *Hyptis suaveolens.* *Ecology* **54**: 646—649 (1972). — Wulff, R. D.: Seed size variation in *Desmodium paniculatum.* I. Factors affecting seed size. *J. Ecol.* **74**: 87—97 (1986).

Genetic Variation for Frost Tolerance in A Breeding Population of Eucalyptus nitens

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Abstract

Patterns of genetic variation for frost tolerance were studied in a first-generation breeding population of *Eucalyptus nitens* (Deane and Maiden) Maiden. Winter hardened seedlings from 198 families, representing 5 provenances were found to differ significantly in their frost tolerance at all three tested temperatures (—5.0° C, —6.5° C and —8.0° C). Provenance effects accounted for between 11% (at —5.0° C) and 29% (at —8.0° C) of the total variation. The most frost tolerant provenance was Northern NSW, with Toorongo being the least tolerant. Significant differences were also found between families within provenances and between seedlings within families. Family effects accounted for 14% to 22% of total variation and seedling effects accounted for between 22% and 26% of the total variation. All five provenances were represented in the top seven ranked families. When each provenance was analysed separately, there were significant differences between families but no strong relationship was found with altitude of origin.

Key words: Frost tolerance, genetic variation, *Eucalyptus nitens.*

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Introduction

With increasing areas of eucalypt plantation currently being established in temperate regions of the world, there is a need for detailed examination of frost tolerance in

the more cold hardy species to allow extension of plantations into somewhat colder conditions. *Eucalyptus nitens* is one such cold-hardy, fast-growing species suited to plantation establishment (PEDERICK, 1979). Although this species has a limited natural distribution in mainland Australia, it is planted quite extensively in northern Tasmania to provide pulpwood as a short rotation crop.

The natural distribution and morphological variation within *E. nitens* has been described by PEDERICK (1979). *E. nitens* is restricted to the central highlands of Victoria (provenances designated Rubicon, Macalister and Toorongo), east Gippsland area of Victoria (Errinundra provenance and small isolated populations in both southern and northern New South Wales (designated SNSW and NNSW provenances respectively). Two distinct forms of the species occur. Trees from Errinundra and a small area of the Toorongo provenance are characterised as having slower growth rates, narrow, weakly glaucous juvenile leaves which change to adult foliage at about 12 months of age, and adult leaves which have slightly denticulate margins with gland-like structures (PEDERICK, 1979). In contrast to these 'early adult' forms the remaining provenances (designated 'juvenile persistent') generally have a relatively fast growth rate, broad glaucous juvenile leaves which remain for up to 4 years after planting, and adult foliage with smooth leaf margins and no gland-like structures.

E. nitens is currently planted on higher altitude, frost prone sites in Tasmania. Breeding programs, based on the juvenile persistent form of the species, have been established. A detailed examination of patterns of genetic variation for frost tolerance is required to ensure success of breeding programs aiming to produce more frost tolerant planting material. The magnitude of provenance differences, together with the degree of variation within each provenance, is of interest. This study reports the screening of a first-generation breeding population for this character. One hundred and ninety eight families from five provenances were screened during the winter of 1986 using the electrical conductivity technique developed by RAYMOND et al. (1986), OWEN and RAYMOND (1987) and RAYMOND et al. (1992). Hardened seedlings were used as the damaging frosts predominantly occur as advective frosts in winter and the discrimination of provenances and families is greatest in hardened material (TIBBITS and REID, 1987b; HALLAM and REID, 1989).

Materials and Methods

A.) Plant Material Used

One hundred and ninety eight open pollinated families collected in native stands from five provenances were screened. These families represented a subset of a first generation breeding population for this species being raised at the APPM Forest Products research centre at Ridgley in Tasmania (latitude 41⁰ 10'S, longitude 145⁰ 50'E, altitude 300m). All seedlings were subsequently planted in progeny trials with their identity retained.

Provenances were designated according to PEDERICK (1979). The distribution of families across provenances is given in *table 1*. For the Toorongo provenance 27 families were subsequently identified in field trials as being of the 'early adult' form. This distinction was noted and the effects of the two foliage types examined. The Errinundra provenance was not included in this breeding population as it had already been found to be much inferior in growth

Table 1. — Origin and distribution of *E. nitens* seedlots, mean relative conductivity values (RC*) and standard deviations (SD) for each test temperature. (Mean RC* values for provenances at the same temperature followed by the same letter are not significantly different at $p < 0.05$).

	Provenance				
	Toorongo	Rubicon	Macalister	NNSW	SNSW
No. families	127(27)[1]	29	17	18	7
Altitude (m)	760-1165	856-1180	915-1280	1290-1530	935-1310
-5.0°C RC*	0.888 d	0.896 c	0.902 b	0.911 a	0.899 bc
SD	0.023	0.020	0.014	0.019	0.026
-6.5°C RC*	0.748 c	0.812 b	0.814 b	0.835 a	0.816 b
SD	0.077	0.055	0.056	0.096	0.057
-8.0°C RC*	0.577 d	0.674 b	0.670 b	0.699 a	0.632 c
SD	0.093	0.082	0.083	0.080	0.086

[1]) No. of early adult families in brackets.

rate to the Toorongo, Macalister and Rubicon provenances (TIBBITS, 1986).

Seed was sown onto sieved granitic sand in punnets and placed in a germination room. Approximately two weeks after germination seedlings were pricked out into paper pots filled with red-brown krasnozem soil with 22.7 kg of MAGAMP fertilizer added per cubic metre of soil. Pot packs were held in wire baskets and families were arranged in randomised unreplicated plots. Additional seedlings of one seedlot were grown to act as a control. All seedlings were raised on shadecloth covered frames one metre above ground level. Covers were generally rolled up once seedlings had recovered from transplanting shock, allowing full exposure to the prevailing environment. Fertilizer, insecticide and fungicide sprays were applied as necessary. Spraying was terminated in early May to avoid chemical contamination of leaves which were to be selected for frost screening.

Seedlings were screened in mid winter when hardening effects caused by low temperatures were at a maximum (TIBBITS and REID, 1987b). With the large number of families to be tested it was necessary to conduct the screening over a 30 day period. It was essential to try to ensure that the seedlings did not deharden or receive extra hardening during this period. Night temperatures below 5⁰ C have been established by ELDRIDGE (1968), HARWOOD (1980) and TIBBITS and REID (1987a) to be necessary to induce substantial hardening in eucalypt seedlings. Maximum and minimum temperatures were recorded daily on the nursery bed where the seedlings were raised. Temperature records were examined both before and during the frost screening period.

Prior to commencing the main sampling, leaf material from the control family was screened at regular intervals to follow the progress of the hardening of the seedlings. Once the seedlings had stabilised, having reached maximum hardness under these conditions, the main sampling program commenced.

For each family 10 seedlings were screened together on a single day. As only 90 seedlings could be tested each day in the laboratory the assessment of the 198 families was spread over a period of 30 days with the control family being sampled at regular intervals to indicate any changes in relative hardiness over time. As far as possible the families tested each day contained a mixture of provenances to avoid confounding provenance effects with sample time.

B.) Sampling Strategy

Following guidelines established by RAYMOND et al. (1992) for assessing differences between families four leaf discs per test temperature were sampled from each of 10 seedlings per family. As RAYMOND et al. (1986) indicated that differences may exist between leaf pairs within an individual seedling, a stratified sampling technique was used. Two leaves were collected from each plant, one from the youngest fully expanded leaf pair and the other from the leaf pair directly below. Two discs from each of the two leaves per seedling were frosted at each temperature.

After collection the leaves were placed between layers of moist paper towelling and their position noted. The bundle of paper towelling and leaves were then sandwiched between stiffener boards, sealed in bubble plastic and airfreighted overnight to Canberra, Australian Capital Territory, for screening. The removal and storage of leaves had previously been shown not to affect RC* determination (RAYMOND et al., 1986).

C.) Frosting Technique

The equipment consisted of three plexiglass baths containing aqueous ethylene glycol solution in which racks of test tubes were suspended (see OWEN and RAYMOND (1987) for technical details). Cooling was achieved by pumping liquid at —30° C from a refrigerated tank through a pressure regulated copper coil in the test baths with bath temperature positively controlled with a Braun thermomix.

Single 8mm leaf discs were placed in separate test tubes and racks of tubes were placed in test baths at 2° C. Bath temperature was lowered at 4° C per hour to —2° C when 0.1g of finely crushed ice (made from deionised distilled water) was added to each tube to prevent supercooling. The bath temperature was then lowered to the required minimum 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 was then added to each test tube and the racks were covered and allowed to stand for a further 24 hours at room temperature. Electrical conductivity of leachate from each sample was measured. Racks of tubes were then immersed in a hot water bath at 80° C for 10 minutes. After standing for 24 hours at room temperature, conductivity was remeasured. Two measures of conductivity were obtained: conductivity for the test temperature (ct) and an absolute conductivity (ck) after the hot water treatment.

The degree of damage sustained by the leaf tissue was assessed using a relative conductivity value calculated as (RAYMOND et al., 1986):

$$RC* = [(ck — ct)/ck]^{0.5}$$

A relative conductivity value of 0.8 had previously been found to discriminate potential survival or death of *E. nitens* leaf tissue (RAYMOND et al., 1992). An RC* greater than 0.8 for a particular disc indicates that the leaf tissue has survived the treatment. The mean RC* value for a seedling is directly and strongly correlated with the amount of leaf damage sustained (RAYMOND et al., 1992). Decreasing values of RC* reflect increasing degrees of leaf damage with a seedling mean RC* value of 0.8 indicating 50% leaf damage.

D.) Choice of Test Temperatures

Three test temperatures were tested simultaneously, one in each of the baths. The material under test originated from a range of provenances which may be expected to have different levels of frost tolerance. As the aim was to discriminate genotypes it was essential that the test temperatures be chosen so as to damage but not kill most genotypes. If all genotypes remain undamaged or are killed little information is gained. The aim was for the middle temperature to provide the best discrimination of genotypes with approximately half the samples being killed. The highest temperature serves to identify the least frost tolerant genotypes whilst the lowest temperature will identify the most tolerant genotypes (ROOK et al., 1980). The actual test temperatures (—5.0° C, —6.5° C and —8.0° C) were established by regularly sampling material for one month prior to the main frost screening.

E.) Adjustment of Data for Changes in Hardening

As the 198 families were assessed over a 30 day period it was necessary to evaluate any changes in relative hardiness of seedlings during this period. Prior to commencing the main sampling minimum night temperature had been monitored to ensure they had remained below 5° C for several weeks. Regular screening of the control family indicated that the seedlings had reached maximum hardiness. Examination of both temperature data and behaviour of the control family (*Figure 1*) during the 30 day main sampling period indicated only small changes in these factors and no consistent trends were detected in relative hardiness for any of the test temperatures. The small changes in relative conductivity observed were consistent with different seedlings having been sampled on each occasion. Night minimum temperatures were below 5° C from the second day of screening through to day 25. The minimum temperature on day two of 6° C was preceeded by 3 nights of temperatures below 5° C; this should not have led to a dehardening of seedlings. From day 25 onwards the minimum temperature was between 4° C and
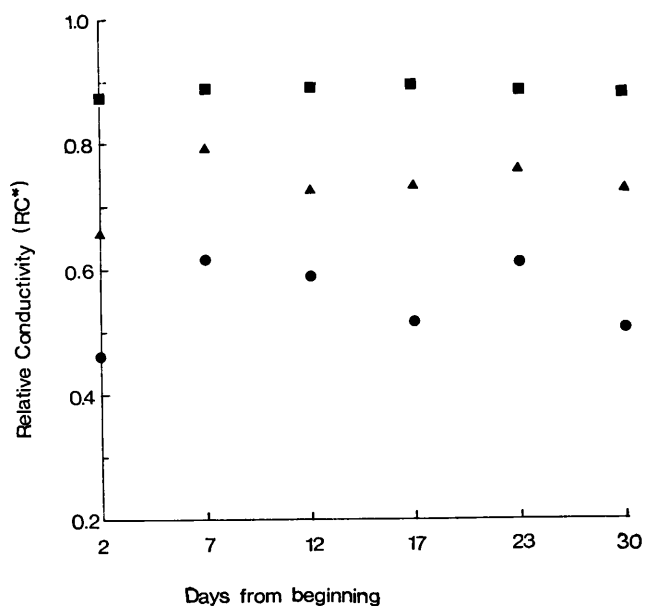

Figure 1. — Mean relative conductivity (RC*) values for the control family sampled at different times during the frost screening period (■ —5.0° C, ▲ —6.5° C, ● —8.0° C).
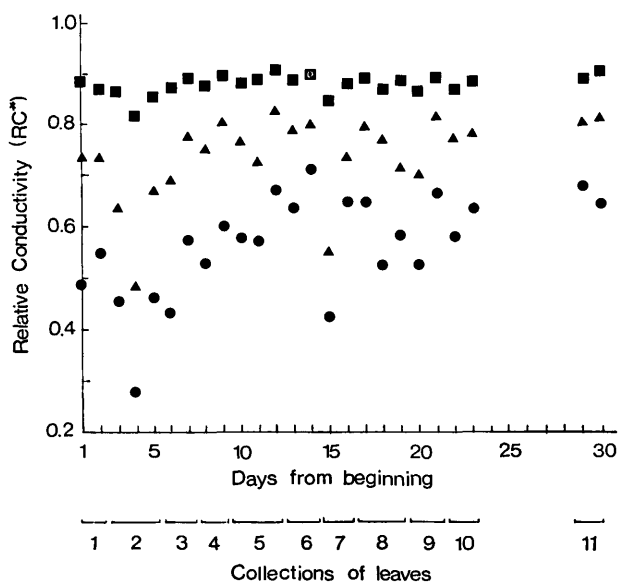
Figure 2. — Mean relative conductivity (RC*) values calculated for each day during the frost screening period. Each collection of leaves was processed over several days (■ —5.0° C, ▲ —6.5° C, ● —8.0° C).

6^0 C which may have lead to a slight dehardening of the seedlings. However, this was not reflected in the results for the control family (*Figure 1*) or in the mean RC*s for each day (*Figure 2*). It is reasonable to assume that the seedlings had not altered from their maximum hardiness state during the 30 days.

Examination of the mean RC* values (*Figure 2*) and their associated variances for the seedlings screened in the laboratory each day did not indicate a consistent trend. These mean values and their variances were influenced by the combination of families screened each day and were not as reliable an indicator of trends in hardiness as were the minimum temperature and control family data. With the exception of two days (days 4 and 15) the mean RC* values were reasonably constant. For these two days an examination of the families screened indicated a predominance of Toorongo families with half of these families being of the 'early adult' form. As Toorongo is the least frost tolerant provenance (see Table 1) and the 'early adult' form is the least frost tolerant material screened, there is a reasonable explanation for these two drops in RC*.

Leaves were sampled from the nursery on 11 occasions with either 18 or 27 families being sampled each time, giving sufficient individuals to keep the laboratory fully operational for two (18 families) or three (27 families) days. In general, all provenances were represented at each sampling time. Each collection of leaves thus contained a broad range of material and was a better indicator than the control family (which was tested every five days) of possible trends in seedling hardiness. The means and variances for each collection of leaves were relatively constant over time but still displayed some changes over the 30 day period. Thus, it was decided to adjust the data to stabilise the means and variances for each collection of leaves prior to analysis. All data for each collection of leaves was adjusted such that the mean and variance equalled that for the fifth collection of leaves (representing days 10 to 12 and chosen as it was in the middle of the main sampling period and the mean for this collection was similar to the overall mean for all collections).

F.) Data Analysis

As the mean RC* values for each seedling have been shown by RAYMOND et al. (1992) to be directly and strongly correlated with the degree of leaf damage sustained the RC* data was analysed directly. Two sets of analyses were conducted. The first set of analyses examined the distribution of variation across the provenance, family, seedling and within seedling levels. For these analyses the full data set containing data from each sample disc was used. Analyses of variance were conducted for each test temperature fitting a model containing terms for provenance, family within provenance and trees within family. Variance components for the provenance, family, tree and within tree levels were calculated by equating the expected and observed values.

The second set of analyses examined the performance of the families in more detail. For these analyses the data were converted to mean values for each seedling at each temperature. All families were ranked on their RC* values for the middle test temperature (—6.5° C) to provide the best discrimination of families.

Heritability values were not determined for the following reason. The sampling strategy used here was defined by RAYMOND et al. (1992) who estimated the sample sizes required to give predetermined levels of accuracy for estimates of seedling and family means. This sampling strategy ensures good discrimination of families but may lead to inflated heritability values. However, as correlations between characters should not be affected, phenotypic and genetic correlations were calculated.

For the purpose of the above analyses, the Toorongo provenance was not subdivided into foliage types. The reason for treating it as a single group is that generally in a seedling study the foliage type would not be known a priori. Thus, the results obtained for that provenance would include both foliage types. However, for the families included in this study the foliage type was ascertained from field trials. To examine the variation within the provenance, data were coded as being of either the 'juvenile persistent' or 'early adult' type. Data were then analysed fitting a model containing terms for type and family within type.

Each provenance was also analysed separately using data from all sample discs to determine whether there was significant variation between and within families for each provenance group. Variance components were estimated for family, trees within family and within tree levels. Phenotypic and genetic correlations were also calculated for each provenance.

Altitudinal trends in frost tolerance within each provenance were also examined. Although the families had not been selected to specifically cover a transect of altitude there was a range in altitude of origin of between 240 m and 405 m present in each provenance (*Table 1*). As the middle test temperature (—6.5° C) resulted in the best discrimination of families, these data were used. Correlations and regressions of family mean RC* values with altitude of origin were estimated.

Results

Provenance effects were significant at all test temperatures as were differences between families within prove-

Table 2. — Mean squares and levels of significance from analyses of variance and variance components (as percentage of total) calculated across all provenances for each test temperature. (* p<0.05 minimum).

	d.f.	TEST TEMPERATURE -5.0°C	-6.5°C	-8.0°C
MEAN SQUARES				
Provenance	4	0.108 *	2.377 *	4.646 *
Family within provenance	193	0.0097 *	0.150 *	0.209 *
Between trees within family	1782	0.0019 *	0.0189 *	0.0299 *
Within trees	6131	0.0007	0.0050	0.0060
VARIANCE COMPONENTS (%)				
Provenance		11	22	29
Family within provenance		14	22	19
Between trees within family		22	23	26
Within trees		53	33	26

Table 4. — Mean squares and levels of significance from analyses of variance and variance components (as percentage of total) calculated for each provenance at each test temperature. Level refers to the level of variation with F being among families, T being among trees within families and W being within trees. (* p<0.05 minimum).

	LEVEL	D.F.	TEST TEMPERATURE -5.0°C	-6.5°C	-8.0°C
MEAN SQUARES					
Toorongo	F	126	0.0111 *	0.1758 *	0.2300 *
	T	1143	0.0021 *	0.0223 *	0.0321 *
	W	4006	0.0007	0.0055	0.0062
Rubicon	F	28	0.0086 *	0.1036 *	0.1927 *
	T	261	0.0016 *	0.0120 *	0.0258 *
	W	869	0.0007	0.0036	0.0049
Macalister	F	16	0.0041 *	0.1259 *	0.1344 *
	T	153	0.0008 *	0.0126 *	0.0275 *
	W	510	0.0004	0.0031	0.0068
NNSW	F	17	0.0066 *	0.0939 *	0.1827 *
	T	162	0.0014 *	0.0139 *	0.0235 *
	W	536	0.0006	0.0055	0.0062
SNSW	F	6	0.0066 *	0.0439 *	0.1331 *
	T	63	0.0026 *	0.0131 *	0.0287 *
	W	210	0.0014	0.0032	0.0042
VARIANCE COMPONENTS (%)					
Toorongo	F		17	28	28
	T		27	31	37
	W		56	41	35
Rubicon	F		15	29	29
	T		20	26	36
	W		65	45	35
Macalister	F		15	34	18
	T		19	28	35
	W		66	38	47
NNSW	F		14	21	27
	T		19	22	30
	W		67	57	43
SNSW	F		6	12	20
	T		17	39	47
	W		77	49	33

nances (Table 2). Variance components (Table 2) indicated an increase in the percentage of total variation accounted for by provenance with decreasing test temperature, from 11% at —5.0° C to 29% at —8.0° C. This greater discrimination of provenances at the lower test temperature is reflected in the range of mean RC* values for —8.0° C (Table 1) which vary from 0.699 for NNSW to 0.577 for Toorongo.

At all test temperatures the NNSW provenance was the most frost tolerant (highest RC* values) and the Toorongo provenance was the least tolerant (Table 1). Rubicon, Macalister and SNSW provenances displayed intermediate frost tolerance and were not significantly different from each other at the two highest test temperatures. Ranking of provenances across temperatures was consistent with the exception of SNSW which showed a large drop in mean RC* between —6.5° C and —8.0° C.

Discrimination of genotypes was greatest at the middle temperature, with provenance, family and seedling effects accounting for 22%, 22% and 23% of total variation respectively. When families were ranked for mean RC* values at —6.5° C (Table 3) the provenance effects become less relevant. Families from all 5 provenances appear in the top seven, indicating that frost tolerant families may be found in each provenance.

At each test temperature variance components for provenance and family levels were relatively similar in

Table 3. — Ranking of families based on mean RC* at —6.5° C. Provenance identity and mean relative conductivity (RC*) values for the ten top families out of 198.

Rank	Provenance	Mean RC*
1	NNSW	0.921
2	Toorongo	0.901
3	Macalister	0.897
4	NNSW	0.882
5	Macalister	0.882
6	Rubicon	0.880
7	SNSW	0.880
8	Rubicon	0.879
9	Rubicon	0.878
10	NNSW	0.876

magnitude but slightly smaller than components for the seedling within family level. These results indicate scope for selection for improving frost tolerance at all three levels. The variation within families is of interest, with many individual seedlings surviving undamaged at —8.0° C, having a mean RC* for the four discs greater than 0.8.

For Toorongo provenance the 'early adult' foliage type was found to be significantly less frost tolerant than the 'juvenile persistent' type (mean RC* values at —6.5° C of 0.711 and 0.757 respectively). However, when compared with other provenances both types of Toorongo seedlots were significantly less frost tolerant than all other provenances at each test temperature.

When each provenance was analysed separately (Table 4), significant differences were found both between families

Table 5. — Phenotypic and genetic (± standard error) correlations between RC* values for different test temperatures calculated across provenances (overall) and for each provenance.

	Phenotypic		Genetic correlations	
	-6.5°C	-8.0°C	-6.5°C	-8.0°C
OVERALL				
-5.0°C	.536	.438	.671±.050	.628±.056
-6.5°C		.704		.801±.031
TOORONGO				
-5.0°C	.545	.448	.673±.061	.651±.066
-6.5°C		.699		.792±.040
RUBICON				
-5.0°C	.513	.440	.749±.107	.571±.155
-6.5°C		.760		.879±.055
MACALISTER				
-5.0°C	.511	.407	.483±.227	.372±.272
-6.5°C		.750		.869±.083
NNSW				
-5.0°C	.612	.344	.796±.123	.650±.187
-6.5°C		.747		.971±.034
SNSW				
-5.0°C	.339	.518	.291±.576	.721±.332
-6.5°C		.507		.257±.510

Table 6. — Correlation coefficients and percentage of variation removed by fitting a linear regression of family mean relative conductivity (RC*) for the middle test temperature (—6.5°C) with altitude of origin of each seedlot for each provenance.

Provenance	Correlation coefficient	Variance removed by regression
Toorongo	0.438 *	18.6
Rubicon	0.109	#
Macalister	0.606 *	32.5
NNSW	0.132	#
SNSW	-0.027	#

* p<0.05 minimum.
# Residual Variance exceeds variance of Y variate.

and between seedlings within families for all provenances. Examination of variance components indicates that the best discrimination of families occurred at the middle test temperature (—6.5° C) for Victorian provenances (Toorongo, Rubicon and Macalister). For NNSW and SNSW the best discrimination of families occurred at the lowest test temperature.

Phenotypic and genetic correlations between RC* values for different temperatures are presented in table 5. Across provenances the genetic correlations were high, ranging from 0.628 to 0.810, indicating that families are behaving in a similar manner across all tested temperatures. When the genetic correlations were examined within provenances there was a trend of decreasing accuracy of estimates (increasing standard errors) with decreasing number of families in the provenance. However, genetic correlations between RC* values remained high, particularily for adjacent test temperatures, for Toorongo, Rubicon and NNSW provenances. For Macalister and SNSW the correlations are lower. For Macalister the correlations with the highest test temperature (—5.0° C) were low but the correlation between —6.5° C and —8.0° C was high. For SNSW correlations were low and errors large, reflecting the small number of families included (7 families) and the low proportion of variance accounted for by family differences (10% to 23%, Table 4).

Correlations of family mean RC* values (for —6.5° C) with altitude are presented in table 6. Significant correlations were found for two provenances, Toorongo and Macalister. However, the amount of variation removed by fitting a linear regression of RC* on altitude was relatively small with 18.6% removed for Toorongo and 32.5% removed for Macalister. Detailed examination of a plot of RC* against altitude for Macalister indicated that a single family occuring at the lowest altitude had a large influence on the regression analysis (having a high leverage value). Deleting this point reduced the amount of variation removed by regression analysis to 18%.

Discussion

For a breeding program to be successful it is necessary to determine patterns of genetic variation in the trait of interest. Information on the relative degree of variation between provenances, between families and within families indicates where selection pressure should be applied to gain greatest benefit. Superior seed sources may also be identified and genetic parameters estimated to develop a breeding strategy.

Significant levels of variation for frost tolerance were found between provenances and between families within provenances in this study. The proportion of total variance accounted for by provenance ranged from 11% at the highest test temperature (—5.0° C) to 29% at the lowest test temperature (—8.0° C). The levels of variation for family within provenance were similar ranging from 14% at —5.0° C to 22% at —6.5° C. These results contrast with those of TIBBITS and REID (1987b) who found that provenance accounted for 75% of the total variation whilst families accounted for 11% of the variation. There are several important differences between these two studies.

Different sets of genetic material and different numbers of families per provenance were used in these studies so results should be compared with caution. TIBBITS and REID (1987b) examined a stratified sample of families. The families were grouped into altitudinal populations within each provenance. In addition, the Errinundra provenance was included in their study.

The least frost tolerant material in both studies was of the 'early adult' foliage type. In the current study the 'early adult' Toorongo families were significantly less frost tolerant than the 'juvenile persistent' families. Similarily, TIBBITS and REID (1987b) found the 'early adult' Errinundra provenance to be the least frost tolerant. The large provenance component of variance found by these authors appears to be due to this provenance.

Comparison of frost tolerance of the 5 provenances common to the two studies reveals similarities. Rubicon and Macalister were similar in both studies. The current study found NNSW to be most frost tolerant provenance and Toorongo to be the least tolerant. In contrast, TIBBITS and REID (1987b) found no significant difference between these two provenances and Rubicon and Macalister provenances. SNSW was found by TIBBITS and REID (1987b) to be the least frost tolerant of these provenances, whereas in

the current study it was not significantly different from Rubicon or Macalister. However, the degree of variation found within the SNSW provenance differed in the two studies. In the current study it had the smallest degree of within provenance variation whereas in TIBBITS and REID's study it was the only provenance showing differences between altitudinal populations. Given the different set of families used, this difference in results is not of concern.

Family effects were found to be significant in both studies. In the current study the percentage of variance accounted for by family effects was similar to that for provenance effects indicating scope for selection at both levels. When families were ranked for frost tolerance all five provenances were represented in the 7 top ranked families. All provenances thus contain equally frost tolerant families. These results are supported by TIBBITS and REID (1987b) who found no significant differences between the most-tolerant families in each provenance.

Given the degree of genetic variation found in this species, selection for improving frost tolerance would be effective at both provenance and within provenance levels. The degree of variation within each provenance is also of interest. When provenances were analysed separately, significant differences were found both between and within families for all provenances. Significant family differences were also found by TIBBITS and REID (1987b) with the exception of the NNSW provenance. Examination of the percentage of variation accounted for by families in the current study indicates scope for selection within each provenance. For the four provenances represented by 17 or more families the family variance component ranged from a minimum of 14% for NNSW at -5.0^0 C to 34% for Macalister at -6.5^0 C (*Table 4*).

This study revealed no strong relationship between frost tolerance and the altitude of origin of each family. There were no significant correlations found for Rubicon, NNSW and SNSW provenances. For Toorongo and Macalister a significant correlation was found with altitude but fitting a linear regression on altitude removed less than 20% of the variation for Toorongo. For Macalister the linear regression removed 32.5% of the variation but a single point exerted a large effect on the regression (by having a high leverage value). When this point was removed the percentage of variance removed dropped to 18% indicating that there was generally no strong relationship with altitude.

In contrast, TIBBITS and REID (1987b) found significant differences between high and low altitude populations for SNSW but not for other provenances. When frost damage was scored in a field trial containing families from four altitudinal populations of each provenance they found a general trend for decreasing damage (or increasing frost tolerance) with increasing altitude. As stated above the two studies used different sets of genetic material and different numbers of families to represent provenances.

In other eucalypts the relationship between frost tolerance and altitude is not always clear and appears to depend upon the range of altitude covered by the species and the intensity with which this range is sampled. When a single mountain side was studied intensively a strong trend of increasing frost tolerance with increasing altitude was found in *E. regnans* by ELDRIDGE (1969), in *E. urnigera* by THOMAS and BARBER (1974), and in *E. pauciflora* by PRYOR (1956) and GREEN (1969). In a provenance study of

E. regnans ROOK et al. (1980) found a significant correlation between frost tolerance and altitude of origin. However, PATON (1972) found no strong correlation with altitude in *E. viminalis*, with a seedlot from sea level being as frost tolerant as one from 1100m altitude. HARWOOD (1980) found an inverted relationship with *E. pauciflora* growing at the edge of frost hollows, where frost tolerance was found to increase with decreasing altitude. This was caused by cold air drainage causing temperatures to be lower at the bottom of hollows.

This study used the electrical conductivity method developed by RAYMOND et al. (1986, 1992) to screen seedlings for frost tolerance at three separate temperatures. When developing the screening method RAYMOND et al. (1992) specified that 3 test temperatures be used to cover the range of expected levels of frost tolerance in the material. The aim was to establish a temperature range such that approximately 50% of the seedlings will be killed by the middle test temperature (RAYMOND et al., 1992). At -6.5^0 C, 81 out of 198 families (or 40%) survived, having mean RC* values greater than 0.8. At the highest test temperature almost all families survived whilst at the lowest temperature the majority of families were killed.

The 3^0 C range of temperatures used here corresponded to the range of frost tolerance exhibited by the families indicating that the temperatures selected were appropriate. To define the temperature range in terms of LD_{50} values a probit analysis was run for the top and bottom ranking families. These analyses were unsuccessful as the probit model could not be fitted as the top ranked family survived all test temperatures undamaged and the bottom ranked family was severely damaged or killed by each temperature. These results lead to the conclusion that the range of LD_{50} values is somewhat greater than from -5.0^0 C to -8.0^0 C.

The high genetic correlations found indicate that families behaved in a consistent manner across the three test temperatures. This indicates the absence of an interaction between family and test temperature. Thus, it is valid to rank families at the test temperature giving the best discrimination of families.

The high levels of genetic variation found at all three levels here indicate that selection for increasing frost tolerance would be efficient at the provenance level and both between and within families.

Conclusion

Significant levels of genetic variation for frost tolerance were found within a first-generation breeding population of *E. nitens*. The seedlots screened represented all provenances currently being used commercially in Tasmania. The patterns of genetic variation indicate that selection pressure for increasing frost tolerance may be exerted at all three levels: provenance, family and within family. Selection for frost tolerance may be incorporated into breeding programs resulting in more frost tolerant seedlings being available to increase the area of plantation established on harsher sites.

References

Eldridge, K. G.: Physiological studies of altitudinal variation in *Eucalyptus regnans*. Proc. Ecol. Soc. Aust. **3**: 70—76 (1968). —— Eldridge, K. G.: Altitudinal variation in *Eucalyptus regnans* F. Muell. Ph. D. Thesis, Australian National University (1969) —— Green, J. W.: Temperature responses of altitudinal populations of *Eucalyptus pauciflora* Sieb ex Spreng. New Phytol. **68**: 399—410 (1969). —— Hallam, P. M. and Reid, J. B.: Seasonal and genetic variation in frost hardiness of *Eucalyptus delegatensis*. Can. J. For. Res. **19**: 480—488 (1989). —— Harwood, C. E.: Frost resistance of subalpine *Eucalyptus species*. I. Experiments using a radiation frost room. Aust. J. Bot. **28**: 587—599 (1980). —— Owen, J. V. and Raymond, C. A.: Laboratory techniques for frost experiments with eucalypts. DFR User Series Report, Number 11. CSIRO Division of Forest Research, Canberra. 11pp. (1987). —— Paton, D. M.: Frost resistance in *Eucalyptus*: A new method for assessment of frost injury in altitudinal provenances of *E. viminalis*. Aust. J. Bot. **20**: 127—139 (1972). —— Pederick, L. A.: Natural variation in shining gum (*Eucalyptus nitens*). Aust. For. Res. **9**: 41—63 (1979). —— Pryor, L. D.: Variation in snow gum (*Eucalyptus pauciflora* Sieb.). Proc. Linn. Soc. NSW **81**: 299—305 (1956). —— Raymond, C. A., Harwood, C. E. and Owen, J. V.: A conductivity method for screening populations of eucalypts for frost damage and frost tolerance. Aust. J. Bot.. **34**: 377—393 (1986). —— Raymond, C. A., Owen, J. V., Eldridge, K. G. and Harwood, C. E.: Screening eucalyptus for frost tolerance in breeding programs. Can. J. For. Res. **22**, 1271—1277 (1992). —— Rook, D. A., Wilcox, M. D., Holden, D. G. and Warrington, I. J.: Provenance variation in frost tolerance of *Eucalyptus regnans*. Aust. For. Res. **10**: 213—238 (1980). —— Thomas, D. A. and Barber, H. N.: Studies on leaf characteristics of a cline of *Eucalyptus urnigera* from Mount Wellington, Tasmania. I. Water repellency and the freezing of leaves. Aust. J. Bot. **22**: 501—512 (1974). —— Tibbits, W. N.: Eucalypt plantations in Tasmania. Aust. For. **49**: 219—225 (1986). Tibbits, W. N. and Reid, J. B.: Frost resistance in *Eucalyptus nitens* (Deane and Maiden) Maiden: Physiological aspects of hardiness. Aust. J. Bot. **35**: 235—250 (1987a). —— Tibbits, W. N. and Reid, J. B.: Frost resistance in *Eucalyptus nitens* (Deane and Maiden) Maiden: Genetic and seasonal aspects of variation. Aust. For. Res. **17**: 29—47 (1987b).

Selection of Crown Form Traits in Controlled Crosses of Coastal Douglas-fir

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Summary

Genetic parameters for crown-form traits and bole taper were investigated in full-sib progeny of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Significant amounts of additive genetic variance were shown for all traits except branch thickness. Non-additive genetic variance was statistically significant (p < .05) for three of the eight crown from traits investigated. High individual-tree heritability was expressed for branch angle, indicating that this trait could be improved through phenotypic selection. Progeny testing would be more effective for most other form traits. Branch thickness and branch length indicate stronger genetic differences when they are expressed as a proportion of stem size.

A positive association existed between yield and a fine branching form type. Path-analysis showed that branch number was associated with 42% of family differences for stem volume. Multi-trait index selection can be used to improve both yield and fine branching in Douglas-fir seed orchard progeny.

Key words: Douglas-fir, crown form, branching traits, bole taper, index selection.

Introduction

Mature Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) trees are characterized by a long, branch-free, cylindrical stem and a short, columnar, flat-topped crown, and young open-grown trees have narrow conical crowns that extend to the ground (Hosie, 1973). In a closed stand, lower limbs die rapidly with increasing overhead shade,

however, natural pruning of these limbs can take a long time (Isaac and Dimock, 1965). Branching and crown-form traits are important for the effect they have on the quality of the clearwood resource; compression wood is increased with acute angled branching, and heavy branches produce persistent knots and retard the ability to produce clear stem wood (von Wedel et al., 1968). Clear stem wood is desirable not only for clearwood products and peeler logs (von Wedel et al., 1968; Shelfourne, 1970), but also in the production of uniform pulp products (Blair et al., 1974; Zobel and Kellison, 1978). Large knot size is also one of the key factors in reducing structural strength in sawn pieces. Crown characteristics are likely to be important not only because of their effect on wood quality, but because biomass potential is more economically valuable when partitioned into stem wood than into undesirable branch wood.

This study characterizes the genetics of crown form traits and their interrelationships in juvenile Douglas-fir, and investigates ways in which selection can improve both crown form and volume.

Extreme phenotypic variability for limb and crown traits in natural populations of Douglas-fir was found by Campbell (1961, 1963) who measured crown characteristics (branch numbers, angles, thicknesses and lengths) in 15- to 35-year-old Douglas-fir in each of 10 locations. Most of the variation of these traits could be explained by variation in stem volume, except in the case of branch angle. The relationships between stem volume and branch number, length, and thickness were all strong and positive. Age of the tree did not affect branch characteristics,

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