

Family variation and potential for genetic gain in frost resistance of *Pinus radiata*

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Application. A controlled-environment study showed that there were significant differences in frost resistance between radiata pine families. Thus there is potential within New Zealand breeding populations to produce frost-resistant populations.

Abstract. Twenty-three *Pinus radiata* seed orchard clones of the New Zealand “850” series were each control-pollinated with a mix of 10 pollens to produce polycross families. The seed was sown in open nursery beds. Seedlings were potted up at six times of the year and subjected to white advective frosts in controlled-environment rooms, with up to seven frosting runs of variable severities at each time. There were significant differences ($p < 0.001$) between families in frost injury, such that, although there was statistically significant family \times time interaction, some families were consistently more frost resistant (tolerant) than others. No association between frost resistance and growth rate was evident.

Individual-tree heritability estimates for frost damage scores were around 0.3 at given times, and 0.20 over all times. Prospective gain in resistance from progeny testing over several seasons of the year, with 2:23 selection, is about 1°C. An additional gain of 0.4°C resistance appears obtainable from 1:90 selection of individuals within pair-crosses in a single frosting run. This additional (within-cross) gain might be increased to around 0.75°C, with the same culling rate but testing one ramet per seedling at each of four times of the year.

Introduction

Frost can be a problem when establishing certain tree species in temperate regions. A conservative solution has been to choose a more frost-resistant but less productive species. For example in New Zealand, sites with a high frost risk were often planted with *Pinus nigra* Arnold or *P. ponderosa* Lawson rather than the more productive *P. radiata* D. Don (radiata pine) or *Pseudotsuga menziesii* (Mirbel) Franco (Kirkland 1969). Another solution is to risk planting a more frost-susceptible species but take extra precautions in site preparation and weed control to ameliorate the site (Menzies & Chavasse

1982; Washbourn 1978). However, these latter measures are insufficient to ensure an established crop of radiata pine on all desired sites. It would be advantageous if more frost-resistant radiata pine families or clones could be indentified and planted on very frosty sites.

For several reasons, frost resistance has been omitted from the selection criteria in New Zealand's radiata pine breeding programme. Frost only causes problems in the first few years of the rotation, and on a national scale it is not a severe problem. The field occurrence of frost damage is seldom conducive to good resolution of any genetic differences in resistance. Moreover, selection for frost resistance would be at the expense of genetic improvement in other traits. However, special seed orchards are now being established to allow production of control-pollinated seed of superior families which might allow breeding for much more specific sites or end products (New Zealand Forest Service 1985). It would be possible to produce crosses that were more frost resistant if the appropriate parents were present and indentified in these orchards; so it is desirable to ascertain if there is a range of frost resistance within the general breeding population and, more specifically, between the candidate parents for these orchards.

There is now the capability to screen for frost resistance in controlled environments (Robotham et al. 1978). This paper describes a study involving frosting of seedling families of a sample of the Forest Research Institute's "850" series of selected clones, which for nearly 20 years have been the basis of New Zealand's seed orchards. The frost resistance of radiata pine seedlings clearly amounts to tolerance rather than avoidance. The resistance of field-grown radiata pine seedlings changes almost throughout the year (Menzies & Holden 1981). There are considerable variations from year to year, and the figures can vary by a month either way. Moreover, maximum frost levels can vary at any given time of year by up to 2°C between years. Therefore it is necessary to screen seedlings at different times of the year and also to use a range of frost levels at each time.

Experimental procedures

Twenty-three seed orchard clones of the "850" series had been control-pollinated with a mix of 10 unrelated "850"-series pollens to produce seed of twenty-three polycross families for field progeny trials (C.J.A. Shelbourne unpubl.). The parents had grown in various parts of New Zealand.

For this study, seed was sown at Rotorua in the Forest Research Institute's nursery in November, for frosting during the next 6-15 months. The design was randomised complete blocks, with four block replicates extending over three nursery beds, each family being randomly allocated to a row-plot in one

Table 1. Frost levels °C and numbers of seedlings used at each frosting time.

	Time					
	May	June	Aug.	Early Oct.	Late Oct.	Feb.
Dates involved	(25-28)	(8-17)	(20/8-1/9)	(11-13)	(25-27)	(1-3)
No. of runs	7	7	7	7	6	3
Lightest frost	- 8	-10	-12.5	- 8	-6	-6
Heaviest frost	-10	-12.5	-15	-10	-8.5	-6.5
Mean seedlings/family	27.7	27.7	27.7	27.2	23.8	9.8

of the centre four drills of each replicate, with the two outer rows as buffers. The seedlings were conditioned by undercutting, and repeated wrenching and lateral root pruning (van Dorsser & Rook 1972).

The seedlings were frosted at six different times of the year, from May to February (see Table 1). The seedlings were lifted four weeks before each frosting time, immediately air-freighted to Palmerston North, and potted up into 1.2-l pots containing a 40:40:20 volume-for-volume mixture of soil:peat:pumice. At each frosting time, each family was randomly arranged in rows within a sheltered outdoor area until frosting. Seedlings were randomly chosen from each family block for each frost run as required. Frosting was done using controlled frost rooms at the DSIR Climate Laboratory, Palmerston North (Robotham et al. 1978). A range of frosts, representing 2-4 different temperatures, was used at each time to differentiate between the families with up to seven frost runs at each frosting time (Table 1). The frost temperatures were initially chosen from results of a pilot run, which was made the week before each frosting time, with initial guidance set from prior results (Menzies & Holden 1981). The temperature for each subsequent run was set in the light of overall damage from its immediate predecessor. There were normally four seedlings from each family in each run, with one seedling being randomly located on each of the four trollies in the frost room. There were minor shortages in some families because of poor seed germination, or failure of seedlings to recover from potting; such shortfalls were spread as far as possible over all frosting times apart from being concentrated in February. Only healthy seedlings were frosted.

White advective frosts were provided, where a moving saturated air mass was reduced to freezing temperatures (Robotham et al. 1978), starting from a +10°C day temperature, and reducing during a night period to the selected frost temperature. Seedlings were in the frost room for up to an hour at 10°C before the temperature decreased. The rate of temperature change and duration of the frosts were programmed to simulate those recorded previously

at central North Island field sites (Menzies & Holden 1981). A programme using a 6-hour temperature decline, 6 h at the minimum temperature, and a 4-hour rise back to day temperature (6-6-4) was used for frost temperatures down to -8.5°C in May, late October and February; and an 8-8-4 programme was used at all other times. Soil temperatures were kept above freezing (about 5°C) by holding the pots in heated, insulated trays.

After being frosted, the seedlings were held in a sheltered outdoor site for a month for visual evaluation of induced damage, particularly death of needles and buds. Final damage was rated on a scale of 0-5 (Menzies & Holden 1981) as follows:

- 0 no damage
- 1 some needle reddening
- 2 10-30% needles killed
- 3 40-60% needles killed and/or bud death
- 4 70-90% needles killed; upper stem dead
- 5 All needles killed; stem dead

At time of frosting each seedling was measured for height and root collar diameter.

Statistical analyses

At each frosting time

Two-way analyses of variance including families and frost runs were used to test for differences in frost resistance of the seedlings from different families

Table 2. Basic analysis of variance showing approximate expectations of mean squares used for the estimation of variance components and individual heritability at a single time.

Source of variation	Degrees of freedom	Expected composition of mean squares ¹
Families (f)	22	$\sigma_{w_t}^2 + k\sigma_{f_t}^2 + rk\sigma_t^2$
Runs (r)	r-1	$\sigma_{w_t}^2 + k\sigma_{f_t}^2 + 23k\Theta_{f_t}^2$
Interaction (f × r)	22(r-1)	$\sigma_{w_t}^2 + k\sigma_{f_t}^2$
Within subclasses (w)	N-23r	$\sigma_{w_t}^2$

Where k = average number of seedlings per family per run; r = number of frost runs; N = total seedlings for the time; $\sigma_{w_t}^2$ = within-subclasses variance, for the particular time, etc.; $\Theta_{f_t}^2$ = fixed-effect "variance" among runs.

¹ With the pattern of imbalance encountered in subclass numbers approximations involved in the coefficients shown above for the variance components were minor.

² Including this term in the families mean square entails the conservative assumption that the interaction represents a random effect.

at each frosting time. Sums of squares were computed by the least-squares method of Harvey (1960). Family variance components were estimated using the model implied by Table 2.

Individual heritability at time t (h_t^2) was estimated as:

$$\hat{h}_t^2 = 4 \hat{\sigma}_t^2 / [(1 + 1/10)(\hat{\sigma}_{ft}^2 + \hat{\sigma}_{frt}^2 + \hat{\sigma}_{wt}^2)] \quad (1)$$

the coefficient term of 1/10 reflecting the use of 10 instead of infinite pollen parents.

Repeatability (heritability) of family means (h_f^2), in terms of Table 3, conforms to:

$$\hat{h}_f^2 = \hat{\sigma}_{ft}^2 / (\hat{\sigma}_{wt}^2 / rk + \hat{\sigma}_{frt}^2 / r + \hat{\sigma}_{ft}^2) \quad (2)$$

This is equal to $(F - 1)/F$, where F equals the F-ratio for the families mean square. The simpler expression holds whatever the expected composition of the families mean square, provided there is a satisfactory F-test against a single other mean square.

The statistical significance of differences among families within frosting times was determined from F-tests and Least Significant Differences.

Variations of the above analyses were carried out using seedling size variables as covariates, to check for associations between seedling size measurements and frost damage scores.

Over all frosting times

A combined analysis of variance over all six times (Table 3) was conducted to test for families and family \times time interaction, and estimate variance

Table 3. Analysis of variance over all times, showing approximate expectations of mean squares which indicate appropriate F tests.

Source of variation	Degrees of freedom	Expected composition of mean squares
Times (t)	5	$\sigma_w^2 + 3.82 \sigma_{fr(t)}^2 + 551.30\theta_t^2$
Runs within t (r(t))	31	$\sigma_w^2 + 3.82 \sigma_{fr(t)}^2 + 89.40\theta_r^2$
Families (f)	22	$\sigma_w^2 + 3.82 \sigma_{fr(t)}^2 + 143.8 \sigma_f^2$
f \times t	110	$\sigma_w^2 + 3.82 \sigma_{fr(t)}^2 + 23.97\sigma_{fr}^2$
[f within t (f(t))	22 + 110	$\sigma_w^2 + 3.82 \sigma_{fr(t)}^2 + 143.8 \sigma_{fr(t)}^2$
f \times r(t)	676	$\sigma_w^2 + 3.82 \sigma_{fr(t)}^2$
Within subclasses (w)	2464	σ_w^2

Conventions for designating variance components as indicated in Table 2.

Table 4. Average frost damage ratings for the 23 polycross families at the six frosting times and tests for significance.

Family	All times			May			June			August			Early Oct.			Late Oct.			February			
	Rank	Average frost damage		Rank	Av. frost damage		Rank	Av. frost damage		Rank	Av. frost damage		Rank	Av. frost damage		Rank	Av. frost damage		Rank	Av. frost damage		
422	1	2.18		4=	0.9		1	1.4		1	2.7		1	2.7		1	1.7		1	1.7		21
373	2	2.22		3	0.8		4	1.5		2	3.7		5	3.5		2	1.8		2	1.8		2
96	3	2.29		1	0.7		2	1.5		5	3.8		8	3.6		12=	2.4		12=	0.8		1
245	4	2.33		2	0.8		3	1.5		9	3.9		7	3.6		8	2.2		8	1.8		5
364	5	2.35		4=	0.9		8=	1.8		6	3.9		2	3.0		6	2.1		6	2.6		12
314	6	2.45		7	1.0		8=	1.8		4	3.8		6	3.5		10	2.3		10	1.8		4
83	7	2.48		9	1.1		6	1.7		8	3.9		9	3.6		9	2.2		9	1.8		3
363	8	2.52		15	1.3		8=	1.8		3	3.7		21=	4.0		3	2.0		3	1.9		6=
231	9	2.60		19	1.4		13	1.8		19	4.1		3	3.2		11	2.4		11	2.8		15
92	10	2.61		14	1.3		11	1.8		11=	4.0		12	3.7		7	2.2		7	2.4		11
395	11	2.63		17	1.3		19	2.0		20	4.3		11	3.7		15	2.5		15	1.9		6=
38	12	2.64		21	1.4		14	1.9		11=	4.0		23	4.1		4	2.0		4	2.0		8
181	13	2.64		18	1.4		21	2.1		15=	4.1		4	3.3		5	2.1		5	2.7		13
396	14	2.74		12	1.2		15	2.0		7	3.9		20	4.0		17	2.6		17	2.8		16=
534	15	2.76		22	1.5		5	1.6		11=	4.0		10	3.7		21	2.9		21	2.7		14
372	16	2.76		11	1.1		16	2.0		18	4.1		15	3.9		12=	2.4		12=	3.5		22
226	17	2.77		13	1.2		18	2.0		14	4.1		19	4.0		14	2.5		14	2.9		18
239	18	2.83		6	1.0		20	2.1		22	4.5		16	3.9		16	2.5		16	3.3		20
228	19=	2.83		20	1.4		22	2.2		15=	4.1		13	3.8		19	2.7		19	2.3		10
377	19=	2.83		10=	1.1		17	2.0		15=	4.1		17	3.9		18	2.7		18	4.1		23
87	21	2.85		16	1.3		12	1.8		10	4.0		18	4.0		22	2.9		22	2.8		16=
276	22	2.88		8	1.1		7	1.7		23	4.5		21=	4.0		20	2.9		20	3.3		19
397	23	3.01		23	1.7		23	2.3		21	4.4		14	3.8		23	3.1		23	2.3		9

Table 4. Average frost damage ratings for the 23 polycross families at the six frosting times and tests for significance.

Family	All times			May			June			August			Early Oct.			Late Oct.			February			
	Rank	Average frost damage		Av. frost damage	Rank		Av. frost damage	Rank		Av. frost damage	Rank		Av. frost damage	Rank		Av. frost damage	Rank		Av. frost damage	Rank		
Mean		2.612		1.2		1.9		4.0		3.7		2.4		2.5		2.4		2.5		2.5		(2.9)
LSD (0.05)		—		0.8		0.8		0.8		1.0		1.1		—		—		—		—		—
F-tests —																						
Families		8.6***		3.4***		2.7***		2.8***		3.7***		3.3***		1.4 NS		—		—		—		—
Runs (r(t))		66.1***		27.7***		215.4***		105.6***		85.8***		91.2***		<1 NS		—		—		—		—
(f × r(t))		1.08 NS		1.07 NS		1.29*		1.25 NS		1.34*		<1 NS		<1 NS		—		—		—		—
f × times (f × t)		2.2***		—		—		—		—		—		—		—		—		—		—

NS = not significant ($p > 0.05$)* = significant ($p < 0.05$)*** = very highly significant ($p < 0.001$)

components and heritabilities across times. Because of the size of the classification, the sums of squares were arrived at by a rather elaborate approximation. However, given the pattern of the imbalance and the fact that alternative approximations yielded very similar figures the solution was deemed acceptable. The overall individual heritability (h^2) was estimated as:

$$\hat{h}^2 = 4 \hat{\sigma}_f^2 / [(1 + 1/10)(\hat{\sigma}_f^2 + \hat{\sigma}_{fr(t)}^2 + \hat{\sigma}_w^2)] \quad (3)$$

The corresponding repeatability of family means was estimated as $(F - 1)/F$ (see above).

Correlations were calculated between average family frost damage scores at the six frosting times, and the genetic correlations (r_g) were calculated from:

$$r_g = r_{12} / (\hat{h}_{t_1}^2 \hat{h}_{t_2}^2)^{1/2} \quad (4)$$

where r_{12} = correlation between family means at two frosting times; $\hat{h}_{t_i}^2$ = repeatability of family means at frosting time t . This cross-checks the treatment of family \times time interaction in the analysis of variance (Burdon 1977) – any interaction which only reflects between-time heterogeneity of variances will not contribute to departures from $r_g = 1$.

Correlations were calculated between average frost damage at all frosting times and height growth to age 6 years at each of the six field progeny tests.

The quantitative impact of frost level on damage score was studied by analysis of covariance of average damage score on the frost level of a run.

Results

Family means and genetic statistics

Family mean damage ratings for the six frosting times are given in Table 4. The families are ranked overall from highest to lowest in frost resistance for their average damage over all frosts.

There was some variation in the size of the seedlings, but covariance analyses showed that neither height, basal diameter, nor sturdiness (height: diameter ratio) were significantly associated with frost damage. Similarly, the position of the seedling in the frost rooms did not affect its frost damage.

There were highly significant differences ($p < 0.001$) between families at all frosting times except February (when $p = 0.1$). Family \times run interactions were sometimes statistically significant ($p < 0.05$). However, they were not important enough to affect inferences concerning family differences, depending on whether they were either viewed as a fixed effect or even pooled

with within-subclass variation. Family differences over all times were marked, despite statistically significant family \times time interaction. The overall differences are evidenced in: repeatability of family means ($\hat{h}_F^2=0.88$), estimated individual heritability ($\hat{h}^2=0.20$) (Table 5), and consistently positive correlations between times (Table 6).

Table 5. Estimates of variance components (σ^2), repeatabilities of family means (\hat{h}_F^2) and heritabilities (\hat{h}^2) for frost damage scores.

Statistic	Frosting time(s)						
	Overall	May	June	Aug.	Early Oct.	Late Oct.	Feb.
$\hat{\sigma}_F^2$	0.047	0.049	0.034	0.038	0.093	0.097	0.149
$\hat{\sigma}_{F(t)}^2$	0.083	—	—	—	—	—	—
$\hat{\sigma}_F^2$	0.017	0.009	0.028	0.029	0.061	0	0
$\hat{\sigma}_w^2$	0.802	0.526	0.437	0.463	0.702	1.004	3.308
\hat{h}_F^2	0.88	0.71	0.63	0.64	0.73	0.70	0.31
\hat{h}^2	0.20	0.31	0.25	0.26	0.40	0.32	0.16

$\hat{\sigma}_F^2$ denotes either σ_F^2 or $\sigma_{F(t)}^2$, according to whether it applies across times or at a single time respectively, etc.

Table 6. Correlation matrices between frosting times for levels of frost damage in the families (21 d.f.). Phenotypic correlations of family means ($r_{\bar{F}}$) above diagonal, estimates of genetic correlations below diagonal.

	1	2	3	4	5	6
1 May	—	0.63	0.43	0.32	0.48	0.17
2 June	0.94	—	0.64	0.43	0.47	0.31
3 August	0.63	1.00	—	0.42	0.67	0.41
4 Early Oct.	0.45	0.63	0.62	—	0.52	0.05
5 Late Oct.	0.68	0.71	1.01	0.73	—	0.28
6 February	0.37	0.70	0.89	0.11	0.61	—

$p = 0.05, 0.01, 0.001$: $r_{\bar{F}} = 0.41, 0.52, 0.66$ respectively

The three most frost-resistant families overall (422, 373, and 96) remained near to the top of the rankings for all frosting times (except for 422 in summer). Similarly, the least frost-resistant family (397) was consistently at the bottom of the rankings, except in February. With the smaller sample sizes and greater non-genetic variation in February, there were no significant differences between families at that time, when some of the rankings differed widely from those at the other five times.

The significant overall family \times frosting time interaction must in part reflect

seasonal differences in the expression of variation. However, some of this interaction was evidently due to families changing ranking depending on the season. For instance, Family 276 had a high frost resistance in autumn and early winter, but low resistance the rest of the year; 239 was high in autumn only; 181 was high in spring only, and 87 and 396 were high in winter, but low in autumn and spring. However, overall the ranking did not change greatly.

There was no evidence of a trade-off between frost resistance and subsequent growth rate. Correlations between family means for frost damage and year-6 height growth at six sites throughout New Zealand (C.J.A. Shelbourne unpubl.) were never statistically significant, and clustered around zero. There was also no sign of any pattern in frost resistance of families in relation to their New Zealand origins.

There were significant differences between frosting runs within times (Table 4, F-tests). Analysis of covariance showed a strong average within-time regression of mean damage on minimum temperature °C ($\hat{b}_{\text{damage.frost}} = -0.68 \pm 0.09$). Heterogeneity of within-month regressions was not significant ($p = 0.2$), and was therefore assumed to be non-existent. There was no obvious tendency for regression slopes to flatten as damage scores approached zero or 5.

Mean damage levels for individual times were adjusted, on the basis of the pooled within-time regression, to the overall mean temperature. The adjusted means (Y') are shown in Table 7. These figures indicate a strong and coherent pattern of seasonal variation in frost resistance, even though overlaps between times were poor for both variables concerned. Estimates of temperatures expected to give mean damage levels of 2.5 (°C_{2.5}) are also shown in Table 7, and were calculated as:

$$\hat{\circ}C_{2.5} = \bar{X} - (Y' - 2.5)b \quad (5)$$

Table 7. Results of analysis of covariance of damage level on designated frost level. Adjusted means are for a frost level of -9.786°C . Estimates of temperatures that would cause average damage levels of 2.5 (°C_{2.5}) are obtained from Eq. 4.

Time	Adjusted mean damage level \pm s.e. (Y')	$\hat{\circ}C_{2.5}$
May	1.40 \pm 0.18	- 11.4
June	0.98 \pm 0.21	- 12.0
August	1.44 \pm 0.39	- 11.4
Early October	4.08 \pm 0.20	- 7.5
Late October	5.18* \pm 0.40	- 5.9
February	4.95 \pm 0.43	- 6.2

* This figure implies that the threshold of outright killing would be exceeded, on the average, by a frost level of -9.786°C .

where \bar{X} = overall mean minimum temperature ($= -9.786^\circ\text{C}$); $b = (\hat{b}_{\text{damage.frost}})$. The extreme values of $\hat{b}_{C_{2.5}}$ estimated from Eq. 5 coincided exactly with those inferred independently in an earlier study (Menzies & Holden 1981).

Estimation of prospective genetic gains

Genetic gain (ΔG) is given by:

$$\Delta G = Sh^2 \quad (6)$$

where S = observed selection differential between the selections and the population mean or, assuming normal distributions, by:

$$\Delta G = ih^2\sigma_P = i\sigma_A^2/\sigma_P \quad (7)$$

where i = selection intensity in standard deviations; σ_P^2 = the appropriate phenotypic variance; σ_A^2 = additive genetic variance between candidates; and h^2 and σ_P may apply either to family means or individual values.

Between-family selection. Applying Eq. 6 to the overall means of the two top families (Table 4) and to the overall heritability (Table 5), and multiplying by 2 because the pollens were non-select for the trait, we predict a gain of 0.74 damage score points. Multiplying in turn by 1.47 (i.e. $(\hat{b}_{\text{damage.frost}})^{-1}$) predicts a gain of 1.08°C in frost resistance.

Applying Eq. 7 using expected i for selecting two out of 23 families ($i_{2,23}$) (Becker 1967; Lindgren & Nilsson 1985), and σ_P implied by the variance component estimates, we expect:

$$\begin{aligned} \Delta G &= i_{2,23} \times 2 \times \hat{h}_F^2 \times 1.47 [\hat{\sigma}_F^2 + \hat{\sigma}_{f(t)}^2/3.82 + \hat{\sigma}_w^2/143.8]^{\frac{1}{2}} \quad (8) \\ &= 1.05^\circ\text{C extra resistance} \end{aligned}$$

Within-family selection. Further gains will be available from selecting within pair-cross families. Applying Eq. 7 for selecting unreplicated seedlings (ortets), and assuming an additive genetic model the prediction of further gain (ΔG_w) from selecting one seedling per roomfull is given by:

$$\Delta G_w = i_{1,92} (2/1.1) \hat{\sigma}_{f(t)}^2 r_g \times 1.47 / [\hat{\sigma}_w^2 - (1/1.1) \hat{\sigma}_{f(t)}^2]^{\frac{1}{2}} \quad (9)$$

where r_g = estimated genetic correlation between damage at a single time and

damage overall, which reflects that a seedling ortet might be assessable at only one time of the year, and is inferred from Table 6 to be approximately 0.7; and $\hat{\sigma}_f^2$ and $\hat{\sigma}_w^2$ are statistics for all times combined. This gives $\Delta G_w = 0.45^\circ\text{C}$ gain in resistance.

An alternative expectation is given by:

$$\begin{aligned} \Delta G_w &= i_{1,92} (2/1.1) \hat{\sigma}_f^2 \times 1.47 / [\hat{\sigma}_w^2 - (1/1.1) \hat{\sigma}_{f(t)}^2 + (2/1.1) \hat{\sigma}_{f(t)}^2]^{\frac{1}{2}} \quad (10) \\ &= 0.35^\circ\text{C gain in resistance} \end{aligned}$$

If four runs were made, at different times of the year, each using one clonal replicate (ramet) per seedling per run, we would expect:

$$\begin{aligned} \Delta G_w &= i_{1,92} (2/1.1) \hat{\sigma}_f^2 \times 1.47 / [(\hat{\sigma}_w^2 - (3/1.1) \hat{\sigma}_{f(t)}^2)/4 + (2/1.1) \hat{\sigma}_f^2 \\ &\quad + (3/1.1) \hat{\sigma}_{f(t)}^2]^{\frac{1}{2}} \quad (11) \\ &= 0.59^\circ\text{C gain in resistance} \end{aligned}$$

The expectation from Eq. 11 is analogous to that from Eq. 10, which is based on variance component estimates rather than between-time correlations of family means.

Discussion

Two main technical problems were encountered. One was the difficulty of setting frost levels to give average damage levels of around 2.5. This problem would tend to create largely spurious run \times family interactions, and to lower the resolution of family differences and thus the heritabilities. The other problem was with the transplanting in the summer, when not all seedlings had new root growth at frosting. This was reflected in extreme ‘‘noise’’ variation which, along with the smaller sample size, made the results for February very imprecise.

The analysis of variance and the derived statistics would be somewhat biased through inherent heterogeneity of variance structures between runs and, in particular, between frosting times. This bias would tend to lower individual heritabilities and create spurious interactions. It is likely to be far more important than the approximations used in the analysis.

In Eqs. 1 and 3 it is assumed that the 10 parents represented in the pollen mix contributed equally to the offspring. Unequal contributions, of the sort

reported by Moran & Griffin (1985), would tend to inflate estimates of heritability and potential genetic gains.

The likely biases from using an overall analysis of variance make gain predictions from Eq. 9 more attractive theoretically than those from Eq. 10. On this basis, the projected gains from Eq. 11 could be underestimated by a factor of 0.35/0.45, pointing to a potential figure of 0.76 instead of 0.59. Moreover, the projections disregard the possibility that, in practice, better resolution of the most resistant families and individuals may be obtainable than is indicated by repeatabilities or heritabilities for the general population.

The predictions of genetic gain assume various linear relationships, notably that the 0-5 damage ratings reflect truly equal intervals. The most obvious violation of assumptions would involve threshold effects for ratings 0 and 5. This, and other factors (e.g. random departures of temperatures from nominal values and day-to-day hardening or dehardening), would tend to bias $\hat{b}_{\text{damage.frost}}$ downwards and thus inflate predicted genetic gains. Examination of detailed statistics, however, suggested that such complications were unimportant. Moreover, the precise agreement between the estimates of $^{\circ}\text{C}_{2.5}$ and the seasonal resistance levels inferred by Menzies & Holden (1981) gives powerful support to the estimate of $\hat{b}_{\text{damage.frost}}$ and the derived gain predictions.

No evidence is available concerning the importance of non-additive gene effects which were assumed to be zero. If present, they would increase potential gains under clonal forestry (Eq. 11).

Additional gains through provenance selection seem unlikely, because of the provenance origins of the New Zealand stock (Burdon & Bannister 1973) and the comparative resistances of the different provenances (op. cit.; Chaperon & Fraysse 1986; Hood & Libby 1980).

The seasonal differences in variance component estimates indicate that genetic gains for frost resistance would be more strongly expressed outside winter. They also indicate that screening in autumn and spring might give better resolution of genetic differences. However, this seasonal variation in expression of differences is far less marked than in interior populations of *Pinus contorta* and *Pseudotsuga menziesii* (Rehfeldt 1979, 1980). Testing material at several times of the year would improve expected gain by overcoming much of the family \times time interaction. Also, it would facilitate a progressive culling that would eliminate some candidate families in the early stages of screening and allow subsequent effort to be concentrated on the more promising ones. A two-stage selection, such as is represented by Eqs. 6-11, may thus be more efficient in practice than the theoretically optimal between- and within-family selection index.

It had been postulated that seedlings from some families might be more frost-resistant in autumn if they became dormant earlier than other families,

and similarly that some seedlings from families that were late flushing in spring could be more frost resistant in spring. Dormancy and flushing times were not recorded for families, since radiata pine seedlings do not form a true dormant bud in winter, and seedlings can continue growing during the winter if conditions are suitable (Jackson 1974; Jacobs 1936). It is therefore very difficult to detect dormancy phases. While there were families that fitted this dormancy and flushing pattern (276 and 239 in autumn, and 181 in spring), most families did not change rank greatly during the year. Similarly, any tendency of some families to harden earlier and deharden later is clearly subordinate to year-round differences.

Any sacrifice in growth rate through selecting for frost resistance in radiata pine in New Zealand is likely to arise through concentrating the selection on resistance rather than through an adverse genetic correlation. This contrasts with the typical situation in species with well-defined seasonal flushes, where frost resistance tends to be associated with a short period of shoot extension and a correspondingly low growth potential.

Projecting laboratory screening results to field conditions may entail some slippage in realisable gains. For *Eucalyptus regnans* F. Mueller, however, published results (Rook et al. 1980) indicate that such slippage is negligible. Imperfect genetic correlations between tree ages should not matter because frost damage occurs just during establishment.

The significance of a given improvement in temperature tolerance is not yet quantified in New Zealand, and it would fluctuate from year to year. Experience is, however, that very modest rises in ground-surface temperature minima can dramatically affect establishment success (Washbourn 1978), which accords with the estimated value of $b_{\text{damage.frost}}$.

The culling levels assumed, and thence the gain predictions, from progeny testing, would be appropriate for systematic screening of all candidate parents in a large breeding population, rather than for direct application of the reported family means. The control-pollinated seed orchards now being established in New Zealand (New Zealand Forest Service 1985) would facilitate capture of genetic gains for a trait that is critical for special situations. Advances in vegetative propagation could both speed the capture of gains from control-pollinated orchards and allow clonal forestry which could capitalise upon within-family selection.

Conclusions

There were significant differences in frost resistance between radiata pine polycross families, such that, although there was a significant family \times time interaction, some families were consistently more frost resistant than others.

Therefore there is potential to select frost-resistant parents from within the New Zealand breeding population. Further screening would be necessary to identify suitable parent clones before seed could be produced either of appropriate half-sib families or preferably of polycross or full-sib families from controlled crosses. Within-family screening could give additional gains. Attainable gains in resistance from progeny testing appear to be around 1°C, and from within-family screening up to an additional 0.75°C. Such gains may be slightly greater outside the winter months. No direct sacrifice in growth rate is likely.

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