ESTIMATED AMONG-FAMILY AND WITHIN-FAMILY VARIANCES AND HERITABILITIES FROM THREE RADIATA PINE CLONAL TRIALS

K. J. S. Jayawickrama[1]

Forest Research, Private Bag 3020, Rotorua, New Zealand
[1)] Current address: Department of Forest Science, Oregon State University, Corvallis, OR 97331-5752, USA.

Received February 10, 2000; accepted September 5, 2001

ABSTRACT

The results from three radiata pine clonal trials, established in New Zealand between 1976 and 1990, were analysed. The first, (the "Growth and Form Factorial), had 22 parents mated in four 3 × 3 factorials giving 36 families. The second ("Long Internode Diallel") had five long internode parents mated in a diallel with reciprocals, giving 15 families. The third ("Family Block Selections") had 31 pair-crosses involving 17 parents. All three trials were raised as stem cuttings and were on one location each.

For the first two trials, data from around age nine years were analysed. Estimates for narrow-sense heritability (h^2) for diameter, straightness, branch cluster frequency score, needle retention score, malformation score and stem acceptability were lower than for broad-sense heritability (H^2). At age 15 the Family Block Selections trial showed only small differences between h^2 and H^2 for diameter, branch cluster frequency, and needle retention score. *Dothistroma* infection was assessed in the Growth and Form Factorial, spiral grain angle in the Long Internode Diallel, and wood density in the Family Block Selections trial. Differences between h^2 and H^2 were large for *Dothistroma* infection but small for spiral grain and wood density. Considering all three trials, the difference between the two types of heritability was greatest for traits with small estimates of additive variance.

Despite the information obtained from these three trials, it is proposed that more definitive dissection of clonal variances is very much needed to have clearer understanding of gains from clonal forestry. Such trials would need to have more parents and locations than the three reported here, and have efficient mating designs appropriate for estimating variance components. Radiata pine clonal trials planted recently in New Zealand will help to meet this need.

Keywords: *Pinus radiata* D. Don., heritability, clonal variance, diameter, factorial, diallel

INTRODUCTION

Full-scale clonal selection and testing for radiata pine (*Pinus radiata* D. Don.) began in 1966 (THULIN & FAULDS 1968, SHELBOURNE 1991). Early efforts at clonal forestry in New Zealand ran into serious technical difficulties, but many of the problems have been resolved (WILCOX & CARTER 1991; SWEET 1991; AIMERS-HALLIDAY *et al.* 1997; MENZIES & AIMERS-HALLIDAY 1997). New Zealand's forestry industry is gradually moving towards clonal forestry (e.g. Shareholder briefing, Half-Year Report 1995/96. Fletcher Challenge, Forests Division, Private Bag 92 114, Auckland, New Zealand; AIMERS-HALLIDAY *et al.* 1997). Quantitative studies have yielded estimates of clonal variances and broad-sense heritabilities for a variety of traits, but usually with small numbers of clones (DADSWELL *et al.* 1961, SHELBOURNE 1991, KING & JOHNSON 1991, BURDON *et al.* 1992, SHELBOURNE 1997, DONALDSON *et al.* 1997, CONCHEYRO 1998, BEAUREGARD ET AL. 1999).

One phenomenon noted during the clonal testing of radiata pine has profound implications. Several studies have shown the variation among clones within families to be more than would be expected based on standard quantitative genetic assumptions (SHELBOURNE 1991, KING & JOHNSON 1991, BURDON *et al.* 1992, CONCHEYRO 1998). Put simply, apparent non-additive genetic variances estimated from clonal trials have often been much larger than the estimated additive genetic variances. Should these apparent non-additive effects be due to propagation (e.g. C effects), they will not be recaptured in breeding the selected parents. Should they be non-repeatable in addition to being non-genetic, they will neither be recaptured in breeding or when deploying the tested clones as vegetative propagules. In either case, the gains obtained by using the selected clones will be less than predicted. The possibil-

ity of overestimating broad-sense heritability due to propagation effects was raised by LIBBY & JUND (1962).

Three datasets on radiata pine clonal tests in New Zealand were available for analysis, each of interest for different reasons. The first was from the same trial that was reported at age three by KING & JOHNSON (1991), but now with data at nearly age nine more traits. The second involved parents from the Long Internode breed (see JAYAWICKRAMA *et al.* 1997), a group for which clonal test results have not been reported previously. The third trial had age-15 data on growth, form, health and wood density. It should be noted that the trials were based on relatively few parents, and the parents had been fairly intensively selected for growth and form. Nevertheless, there was data on traits such as branch cluster frequency score, needle retention score, and spiral grain angle, for which few estimates of clonal variances and broad-sense heritability have been reported. Finally, these datasets could also shed light on the matter mentioned previously, namely the relative sizes of among- and within-family genetic variances.

These three datasets were therefore analysed to estimate among-family and within-family variances and heritabilities for commercially important traits (height, diameter at breast height (dbh), straightness, malformation, crop acceptability, needle retention, *Dothistroma* infection, wood density and spiral-grain angle) for radiata pine in New Zealand.

DESCRIPTION OF TRIALS AND ASSESSMENTS

Growth and Form Factorial

Production of planting stock

This trial had four 3 × 3 factorials formed by crossing tested first-generation parents. Since the emphasis for this group of parents was growth rate and stem quality, this will be called the "Growth and Form Factorial Trial". There were 22 parents (two of the parents were used twice) and 36 crosses. The seed were sown in October 1984 in the Forest Research Institute nursery in Rotorua; the resulting seedlings were managed as stoolbeds. In March 1986 the stoolbeds were topped to encourage lateral growth. Two types of cutting were collected from the top cluster and set in June 1986: (1) terminals and (2) sub-terminals. A third set of cuttings (from growth of fascicle shoots existing on the lower stem) were set in August 1986.

Trial Sites and Layout

The trial was planted in August 1987 in Compartment 324 of Kaingaroa Forest, in the Central North Island. The trial had six replicates, with one ramet per clone per replicate. The third set of cuttings (August setting) were allocated to replicates one and two, the second set (sub-terminals set in June) to replicates three and four, and the first set (terminals set in June) to replicates five and six. A sets-in-replicates design was used to allocate clones to eight sets (sub-replicates) within replicates. Factorial one went to sets A and E, two to B and F, three to C and G and four to D and H. Thus, five of the clones from each of the nine crosses in Factorial one went to set A, and the other five to set E. The sets-in-replicates design was introduced as the blocks-in-replicates design by SCHUTZ & COCKERHAM (1966). Some background on the use of this design in New Zealand is given by JAYAWICKRAMA & LOW (1999).

The trial site was at 570 m elevation, and had previously carried a *Pinus nigra* stand. The trees were planted at 4 × 4 m, and were pruned to a height of 6 m in 1995. Up to the time of the assessments reported no thinning took place.

Long Internode Diallel Trial

Production of planting stock

The development of the Long Internode breed of radiata pine has been described by JAYAWICKRAMA *et al.* (1997). Five highly-ranked first-generation parents from this breed were mated in a diallel design including reciprocals. These parents were chosen in 1983 after assessment of open-pollinated progeny trials at age 10 years. Four crosses did not produce enough seed, resulting in 16 of the potential 20 controlled-pollinated crosses (the 10 possible combinations and their reciprocals); no selfs were attempted.

Seed from these crosses were sown in paperpots with peat/perlite medium in November 1988, and grown to rooted plants in a glasshouse. Cuttings were lined out in the Tasman Forestry nursery at Te Teko in January 1989, and were managed to form stoolbeds. Twenty of these cloned stoolbeds were selected per cross in March 1989 to obtain cuttings. Thirty cuttings (5 cm long and 3 mm in diameter) were collected per stoolbed (clone) and rooted in paper pots over the winter of 1989. They were then transferred to root-trainers and grown over the summer of 1989. One cross did not make it through the process (leaving 15 crosses), and three clones within the others failed at the nursery stage, leaving 222 clones available for the trial. Thus the trial population was based on nine crosses and six of their reciprocals, and between 12 and 16 clones per cross.

Trial site and experimental layout

The trial was planted at Tahorakuri forest in the Central

North Island, in the winter of 1990. It had ten replicates of single-tree plots, with up to ten ramets per clone. The clones were allocated into eight sets (A–H) in a sets-in-replicates field design. Clones from a given cross were grown in the same set. The first seven sets each had 30 clones (derived from two crosses). The eighth set (H) had only 12 clones (from one cross), and was usually planted on a replicate boOundary, so that another set H could be planted alongside, the two H half-sets appearing to form one 30-tree block.

The trial site was at 360 m elevation, and had previously carried a *Pinus radiata* stand. The trees were planted at 3.5 × 4 m, and were pruned to a height of 2 m in 1997. Up to the time of the assessments reported, no thinning took place.

Family Block Selections

Production of planting stock

Ortets were selected from control-pollinated family blocks planted in 1968 in Compartment 1350, Kaingaroa Forest. These were crosses between untested first-generation parents of the "850" selection series, which were the result of an intensive programme of plus-tree selection (selecting about 1 tree in 100 hectares). The crosses planted were an unbalanced sample surviving from malfunction of a cold store.

Two sets of ortets were selected, the first at two years from planting in 1970 and the second at three years in 1971. They were selected from 31 pair-crosses involving a total of 17 parents, 13 as females and eight as males. Cuttings were taken from these ortets (in 1970 and 1971 respectively), rooted in the Forest Research Institute nursery and used to establish clonal hedges. Cuttings were then collected from the hedges in 1975, for all the ortets, and set in open nursery beds.

Trial Sites and Layout

The trial was planted in winter 1976 in Compartment 327 of Kaingaroa Forest, in the Central North Island. The trial had four replicates, with three ramets per clone in each replicate. A sets-in-replicates design was used to allocate clones to four sets (30 clones per set) within replicates. The trial site was at 560 m elevation, and had previously carried scrub and a *P. nigra* stand. The trees were planted at 3.5 × 3.5 m. The trial was pruned to a height of 2 m in 1983, to 4 m in 1984, and to 6 m in 1986. In 1986 the stand was thinned to 400 stems per hectare, removing a variable number of trees per clone.

Assessment

A number of traits are routinely assessed in radiata pine progeny trials and clonal trials in New Zealand. They are dbh, straightness, branch cluster frequency, malformation, acceptability, needle retention, spiral grain angle and wood density (Table 1). Their relevance is outlined in LOW (1991), KING & BURDON (1991), SORENSSON *et al.* (1997), JAYAWICKRAMA *et al.* (1997) and JAYAWICKRAMA & LOW (1999). Large, straight stems, free of malformation such as forks, bring more value to growers. Assessing branch cluster frequency score is a cost-effective way to rank entries for internode length, which in turn influences the yield of clearwood from unpruned stems (JAYAWICKRAMA *et al.* 1997). Needle retention is assessed mainly to rank entries for resistance to needle cast caused by *Cyclaneusma minus* (Butin) DiCosmo *et al*, since needle loss at ages 6–10 tends to affect growth later in the rotation (LOW 1991, KING & BURDON 1991). *Dothistroma* needle blight, caused by *Dothistroma pini* Hulbary, is another disease that reduces crop productivity if left unchecked; this trait is assessed on sites with a high incidence of *Dothistroma* (CARSON 1989). High spiral grain angles in radiata pine are associated with lumber distortion on drying (SORENSSON *et al.* 1997), while denser wood is usually stronger and gives higher pulp yields. The traits assessed in these three trials are given below.

Table 1. Assessment traits.

Trait	Units	Description
Diameter	millimetres	measured at 1.4 metres above ground level
Straightness	1 to 9 scale	1 = most sinuous stem at the site, 9 = straightest
Branch Habit	1 to 9 scale	1 = fewest branch clusters, 9 = most clusters
Malformation	1 to 9 scale	1 = multiple forking, 9 = no forks or ramicorns
Branching type	1 to 3 scale	1 = mainly uni-nodal, 2 = mainly bi-nodal, 3 = anything else
Acceptability	0 or 1	0 = judged not to provide an acceptable stem (too small, crooked or malformed), 1 = acceptable
Density	$kg \cdot m^{-3}$	obtained from 1 bark to bark core per tree, at breast height
Spiral grain angle	angle from stem axis	1 measurement on each of 2 opposing sides of the tree, at breast height

Growth and Form Factorial: *Dothistroma* infection was assessed at age 3, 4, 5 and 8. Diameter, straightness score, branch cluster frequency score, needle retention score, malformation score and acceptability were assessed in January 1996 when the trial was 8 ½ years old.

Long Internode Diallel: Height, diameter, branching, malformation and acceptability were assessed in March 1996 when the trial was 5¾ years old. The full set of growth and form traits were assessed in March 1999 when the trial were 8¾ years old. Spiral grain angle was assessed on all the trees in the best five replicates (those with the most clones and highest survival). This took place in October 1999 when the trees were 9¼ years old, using the bark window method and the Spiralite digital tool. The methodology for the assessment of grain spirality is explained in SORENSSON *et al.* (1997).

Family Block Selections: A full assessment took place in 1982; the data from this assessment is not reported here. All surviving trees were assessed in 1991, at age 15, in order to select trees for a processing study. Height, dbh, straightness, branch cluster frequency, needle retention, malformation and density (rings 1–5 and 10–15) were assessed.

DATA ANALYSIS

Linear Models

Growth and Form Factorial
The following linear model was used:

$$Y_{kb} = \mu + B_b + A_a + M_{m(a)} + F_{f(a)} + MF_{mf(a)} + C_{c(mf(a))} + BA_{ba} + BM_{bm(a)} + BF_{bf(a)} + e \quad [1]$$

where Y_{kb} = observation for the kth clone in the bth replicate, μ = overall mean, B_b = effect of the bth replicate, A_a = effect attributed to the ath factorial, $M_{m(a)}$ = effect attributed to mth male in the ath factorial, $F_{f(a)}$ = effect attributed to fth female in the ath factorial, $MF_{mf(a)}$ = interaction of the mth male and fth female in the ath factorial, $C_{c(mf(a))}$= effect of the kth clone derived from the mth male and fth female in the ath factorial, BA_{ba}= interaction between the ath factorial and the bth replicate, $BM_{bm(a)}$ = interaction between the bth replicate and the mth male in the ath factorial, $BF_{bf(a)}$ = interaction between the bth replicate and the fth female in the ath factorial, and e = error.

Long Internode Diallel

The following linear model was used:

$$Y_{kb} = \mu + B_b + G_i + S_j + M_m + R_r + BP_{bp} + C_{(ij)p} + e \quad [2]$$

where Y_{kb} = observation for the kth clone in the bth replicate, μ = overall mean, B_b = effect of the bth replicate, G_i = effect attributed to parental GCA, S_j = effect attributed to the SCA of the cross, M_m = maternal effect attributed to mth female, R_r = reciprocal effect attributed to rth reciprocal cross, BP_{bp}= interaction between the pth pair-cross and the bth replicate, $C_{(ij)p}$= effect of the kth clone in the pth pair-cross and e = error.

Family Block Selections
The following linear model was used:

$$Ykb = \mu + B_b + M_m + F_f + MF_{mf} + Cc_{(mf)} + e \quad [3]$$

where Y_{kb} = observation for the kth clone in the bth replicate, μ = overall mean, B_b = effect of the bth replicate, M_m = effect attributed to mth male, F_f = effect attributed to fth female, MF_{mf}= interaction of the mth male and fth female, $C_{c(mf)}$= effect of the kth clone derived from the mth male and fth female, e = error.

Estimation of Variance Components and Heritabilities

Growth and Form Factorial
The evolution of variance components over time, for Dothistroma infection in this trial, is given in CONCHEYRO (1998). Variance components for the age 8 ½ data were estimated using the linear model specified in [1]. The variance component procedure in SAS, PROC VARCOMP, was used. This method computes Type 1 sums of squares for each effect, equates each mean square involving only random effects to its expected value, and solves the resulting system of equations (SAS INSTITUTE INC. 1989). Anova-type variance component estimates have the advantage of being unbiased (HUBER *et al.* 1994). The following assumptions were made (COMSTOCK & ROBINSON 1948):

1) Equilibrium with respect to the segregation of linked genes.

2) Diploid inheritance.

It was also assumed that there were no cytoplasmic or maternal effects. The genetic expectation of the variance components were then obtained, as specified in PAUL *et al.* (1997):

σ^2_M = variance among males $= 1/4V_A + 1/16V_{AA} + \ldots$

σ^2_F = variance among females $= 1/4V_A + 1/16V_{AA} + \ldots$

σ^2_{MF}= variance due to interaction of males and females
$= 1/4\ V_D + 1/8\ V_{AA} + 1/8\ V_{AD} + 1/16\ V_{DD} \ldots$

$\sigma^2_{C(MF)}$ = variance among clones within a cross = $1/2V_A + 3/4\ V_D + 3/4\ V_{AA} + 7/8\ V_{AD} + 15/16\ V_{DD} \ldots$

Ignoring epistatic variances (V_{AA}, V_{AD}, V_{DD} and higher order terms) this led to the following estimates of genetic parameters:

Additive variance = $V_A = 2(\sigma^2_M + \sigma^2_F)$

Dominance variance = $V_D = 4\sigma^2_{MF}$

Genetic variance = $V_G = \sigma^2_M + \sigma^2_F + \sigma^2_{MF} + \sigma^2_{C(MF)}$

Phenotypic variance = $V_P = \sigma^2_M + \sigma^2_F + \sigma_{MF} + \sigma^2_{C(MF)} + \sigma^2_E$

Three types of heritability were estimated:

Narrow-sense (h^2) :

$$h^2 = \frac{2(\sigma^2_M + \sigma^2_F)}{\sigma^2_M + \sigma^2_F + \sigma^2_{MF} + \sigma^2_{C(MF)} + \sigma^2_E} \quad [4]$$

Broad-sense, based on additive and dominance variances (H^2_{FS}) :

$$H^2_{FS} = \frac{2(\sigma^2_M + \sigma^2_F) + 4\sigma^2_{MF}}{\sigma^2_M + \sigma^2_F + \sigma^2_{MF} + \sigma^2_{C(MF)} + \sigma^2_E} \quad [5]$$

Broad-sense, based on additive, dominance and clonal variances (H^2_{FS+C}) :

$$H^2_{FS-C} = \frac{\sigma^2_M + \sigma^2_F + \sigma^2_{MF} + \sigma^2_{C(MF)}}{\sigma^2_M + \sigma^2_F + \sigma^2_{MF} + \sigma^2_{C(MF)} + \sigma^2_E} \quad [6]$$

Standard errors for the heritability estimates were approximated by dividing the standard error of the variance used as the numerator by the phenotypic variance used as the denominator (BECKER 1992, MULLIN *et al* 1992). For example, the standard error for the estimate of h^2 was obtained by first obtaining the variance for σ^2_M [var = σ^2_M] and the variance for σ^2_F [= var σ^2_F]. These were each obtained using the formula :

$$var\ (\sigma^2_G) = \frac{2}{k^2} * \Sigma\left[\frac{(MS_G)^2}{df_G + 2)}\right] \quad [7]$$

where k = the coefficient of the variance component being estimated, MS_G = the gth mean square used to estimate the variance component, and df_G = the degrees of freedom for the gth mean square. Going to the final step,

$$\text{S.E.}h^2 = \frac{\sqrt{(4\ var\sigma^2_M + \sigma^2_F)}}{\sigma^2_M + \sigma^2_F + \sigma^2_{FM} + \sigma^2_{C(MF)} + \sigma^2_E} \quad [8]$$

Long Internode Diallel

The linear model in [2] was used. DIALL, a computer programme written for the analysis of diallel mating designs (SCHAFFER & USANIS 1969) was used to obtain estimates for the first five terms, and SAS for the last three. The same assumption was made as in the previous case, and the genetic expectation of the variance components used was as specified in BECKER (1992).

$\sigma^2_{GCA} = 1/4V_A + 1/16\ V_{AA} + \ldots$

$\sigma^2_{SCA} = 1/4V_D + 1/8V_{AA} + 1/8\ V_{AD} + 1/16\ V_{DD} \ldots$

$\sigma^2_{C(MF)}$ = variance among clones within a cross = $1/2V_A + 3/4V_D + 3/4V_{AA} + 7/8V_{AD} + 15/16\ V_{DD} \ldots$

Ignoring epistatic variances (V_{AA}, V_{AD}, V_{DD} and higher order terms), this led to the following estimates of genetic parameters:

Additive variance = $V_A = 4\ \sigma^2_{GCA}$

Dominance variance = $V_D = 4\ \sigma^2_{SCA}$

Genetic variance = $V_G = 2\ \sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{C(MF)}$

Phenotypic variance = $V_P = 2\ \sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{C(MF)} + \sigma^2_E$

Three types of heritability were estimated:

Narrow-sense (h^2)

$$h^2 = \frac{4\sigma^2_{GCA}}{2\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{C(MF)} + \sigma^2_E} \quad [9]$$

Broad-sense, based on additive and dominance variances (H^2_{FS})

$$H^2_{FS} = \frac{4(\sigma^2_{GCA} + \sigma^2_{SCA})}{2\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{C(MF)} + \sigma^2_E} \quad [10]$$

Broad-sense, based on additive, dominance and clonal variances (H^2_{FS+C})

$$H^2_{FS+C} = \frac{2\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{C(MF)}}{2\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_{C(MF)} + \sigma^2_E} \quad [11]$$

Standard errors were estimated for the heritability estimates using the same approach as in the Growth and Form Factorial.

Family Block Selections Variance components were estimated using the linear model specified in [3]. The variance component procedure in SAS was used. I used the same genetic expectations of the variance components as in the Growth and Form Factorial, and the same procedure for standard errors for the heritability estimates.

RESULTS

Variance component estimates are given in Table 2, Table 3 and Table 4. Clonal variances were larger (often much larger) than family variances with only two exceptions, density in rings 1 to 5 and 10 to 15. Estimates of heritability are given in Table 5. In general the estimates for narrow-sense heritability were low: 0.033 to 0.263 for the Growth and Form factorial, 0.001 to 0.857 for the Long Internode diallel, and 0.00 to 0.851 for the Family Block Selections. At the other end of the spectrum, broad-sense heritabilities which included the clonal variance were mostly higher than the narrow-sense heritabilities: 0.103 to 0.516 for the Growth and Form factorial, 0.067 to 0.669 for the Long Internode diallel, and 0.125 to 0.695 for the Family Block Selections. In several cases, the estimates of H^2_{FS+C} were greater than estimates of H^2_{FS}.

The Family Block Selections trial did not show such large discrepancies between h^2 and H^2 as the other two trials. The h^2 estimates from this trial were high as expected (0.342 to 0.851), apart from straightness and malformation. One consistent pattern in the three trials was that the discrepancy between narrow-sense and

Table 2. Variance component estimates from the Growth and Form Factorial trial.

Type of variance component	d.f.	*Dothistroma* (% infection)				Dbh	Straight-ness score	Branch cluster frequency score	Needle retention score	Malforma-tion score	Acceptabi-lity score
		Age 3	Age 4	Age 5	Age 8						
Replication	5	-1.39	11.11	6.53	35.7	-0.125	0.033	0.046	0.13050	0.256	0.00862
Factorial	3	-1.47	-5.3	-9.63	1.6	16.6	-0.0148	-0.0902	0.00334	0.0379	0.00253
Male (Factorial)	8	0.71	0.54	3.17	3.08	46.58	0.0669	0.4123	0.04160	0.0103	0.00030
Female (Factorial)	8	9.33	18.23	22.38	4.78	44.37	0.0811	0.2228	0.05460	0.0634	0.0037
Male × Female	16	2.96	4.91	2.25	3.1	-6.52	0.0442	0.0969	0.02520	0.0932	0.00795
Clone (M × F)	324	13.72	33.92	49.03	52.45	180.15	0.566	1.354	0.30570	0.512	0.02803
Rep * Factorial	15	8.38	1.5	3.85	0.51	18.67	-0.0007	-0.0105	0.01400	0.138	0.00229
Rep. × Male (Fact.)	40	0.67	1.16	-0.75	1.88	-0.88	-0.0172	-0.0161	0.00856	0.052	0.00221
Rep. × Fem. (Fact.)	40	1.14	0.78	1.9	0.46	-0.63	-0.0139	0.0267	-0.00039	-0.058	0.00186
Error	1398	58.4	103.1	177.7	101.1	427.5	1.513	1.953	0.44340	5.89	0.2022

Table 3. Variance components estimates from the Long Internode Diallel Trial.

Age	Type of variance component	d.f.	Height	Dbh increment (6 to 9)	Dbh	Straight ness score	Branch cluster frequency score	Needle retention score	Malfor-mation score	Accepta-bility score	Spiral grain angle
6	Rep	9	1.169	-	7.735	-	0.0518	0.0405	0.0979	0.00879	-
	GCA	4	3.146	-	6.281	-	0.0004	0.00034	0.0617	0.00156	-
	SCA	4	6.394	-	29.971	-	0.0134	0.00967	-0.0434	-0.000039	-
	Maternal	4	0.637	-	1.953	-	0.0011	0.00247	-0.0266	-0.00002	-
	Reciprocal	2	-0.187	-	-2.38	-	-0.0023	-0.0039	0.0462	-0.00109	-
	Rep * Cross	126	1.76	-	6.928	-	0.015	0.0517	0.1655	0.00548	-
	Clone (cross)	207	16.39	-	52.09	-	0.0617	0.1498	0.4648	0.0124	-
	Error	1393	65.09	-	659.7	-	0.457	0.894	6.826	0.217	-
9	Rep	9	-	2.52	11.48	0.0219	0.0427	-	0.0164	0.00082	0.0416
	GCA	4	-	4.37	8.16	0.016	0.0047	-	0.0013	0.001	1.6096
	SCA	4	-	6.03	32.9	0.049	0.0119	-	0.0608	0.00149	0.2889
	Maternal	4	-	0.15	2.93	0.0011	0.0003	-	0.0237	0.0014	-0.0359
	Reciprocal	2	-	2.09	0.2	0.0334	-0.0019	-	-0.0271	-0.00065	0.1519
	Rep * Cross	126	-	12.05	-6.87	0.0662	0.0333	-	0.1224	0.005	-0.00697
	Clone (cross)	207	-	38.1	136.84	0.367	0.1569	-	0.883	0.0205	1.519
	Error	1331	-	404.4	1039.1	1.958	0.5988	-	6.536	0.2209	2.4866

Table 4. Variance components estimates from the Family Block Selections trial.

Type of Variance Component	d.f.	Height	Dbh	Straightness score	Branch cluster frequency score	Needle retention score	Malformation score	Density (rings 1-5)	Density (rings 10-15)
Rep	3	2.52	-8.62	0.101	0.017	0.0052	0.0614	45.76	9.72
Male	7	42.47	961.84	0.0413	0.1322	0.1603	0.1034	178.54	429.17
Female	10	29.14	158.88	-0.0213	0.3184	0.0074	-0.1021	19.82	161.2
Male × Female	9	-3.04	67.91	-0.0227	-0.1263	0.0399	0.0572	53.15	13.66
Clone (M × F)	92	193.1	1150	0.542	0.766	0.1896	0.626	177.04	316.4
Full-sib Family	26	59.74	1036.1	0	0.27	0.1844	0.0555	224.29	525.8
Error	731	156.75	1481.9	1.097	0.899	0.17440	4.8	276.22	466.42

broad-sense heritability was greatest in the traits for which the narrow-sense heritability estimates were low – *Dothistroma* infection, malformation score and acceptability score (Table 5). In contrast, the discrepancy was far less for spiral grain angle and wood density for which heritabilities were higher.

Standard error estimates were highest for the Long Internode Diallel (0.13 to 0.95) and lowest for the Growth and Form Factorial (0.02 to 0.14) (Table 5).

DISCUSSION

The narrow-sense heritability (h^2) estimates from the Growth and Form factorial were lower than those obtained from open-pollinated trials, of long-internode parents (e.g. JAYAWICKRAMA *et al.* 1997) and mainstream landrace parents (e.g. JAYAWICKRAMA & LOW 1999). As an approximation, typical within-site h^2 estimates would be 0.7 (wood density), 0.4 (branching), 0.3 (needle retention, spiral grain angle), 0.20 (dbh, straightness, Dothistroma infection) and 0.1 (malformation, acceptability). The h^2 estimates from the Long Internode Diallel were very low, and even the H^2 estimates were low compared to the h^2 estimates from open-pollinated trials (JAYAWICKRAMA *et al.* 1997, JAYAWICKRAMA & LOW 1999).

The ratio of $h^2 : H^2$ can be taken as equivalent to the ratio of additive variance: additive variance + dominance variance. For radiata pine in New Zealand, across-site estimates for var(SCA) / var(GCA) varied from 0.0 to 0.22 for the "850" diallel (CARSON 1991), and from 0.05 to 0.79 for the "875" diallel (KING *et al.* 1998). Given this background we would not expect the ratio of $h^2 : H^2$ to greatly exceed 1 : 2. For the Growth and Form factorial, the ratio of h^2 to H^2_{FS} varied from 1.2 : 1 to 1: 5. Within the Long Internode diallel, the estimates for h^2 were much lower than for H^2_{FS} (a ratio of 1:33 in one instance) apart from malformation at age 6. This is a far greater difference than can be explained by standard quantitative genetic models.

With regards to reasons for getting such proportions, the first could be that h^2 estimates from small groups of parents are inherently imprecise. As seen from Table 5, standard errors were highest for the Long Internode Diallel, which had the smallest number of parents. The procedure for estimation results in standard errors growing larger as i) the number of degrees of freedom associated with a Mean Square gets smaller and ii) the numerator of the heritability estimate contains a variance component multiplied by a factors larger than 1 (e.g. $4(\sigma^2_{GCA} + \sigma^2_{SCA})$ in the case of H^2_{FS}). The second possibility, related to the first, is that should even one of a small number of crosses have genuinely shown specific combining ability, a misleadingly high estimate of broad-sense heritability could result. The third possibility was that the parents were highly select for some traits, reducing the genetic variation between half-sib families (and therefore the estimate of h^2). It would be no surprise, for example, that the five parents in the Long Internode all had a low branch cluster frequency given that they had previously been progeny tested. This could be one reason that the heritabilities for wood density and spiral grain angle were high compared to the other traits, since there had been little selection for either of these wood properties.

Obtaining estimates of H^2_{FS+C} greater than estimates of H^2_{FS} raises the question of whether there was epistatic variance, or whether the process of cloning had inflated the variation within families (such as through C effects) and hence H^2_{FS+C}. None of the trials were designed in a way that one could reliably differentiate between epistatic variance and C effects. The possibility of variability induced by propagation is relevant to the testing and selection of clones for deployment, and highlights the importance of using techniques which minimise non-genetic, non-repeatable variability. Should there have been significant epistatic or propagation-induced variation, this would also

Table 5. Heritability estimates from three clones-in-families trials for radiata pine in New Zealand.

Trial	Ages when assessed (years)	Type of heritability	Height	Dbh increment (6 to 9)	Dbh	Straightness score	Brunch cluster frequency score	Needle retention score
Growth and Form Factorial	8½	h^2	–	–	0.263±0.08	0.130±0.05	0.314±0.12	0.221±0.07
		H^2_{FS}	–	–	0.225±0.09	0.208±0.09	0.410±0.14	0.337±0.12
		H^2_{FS+C}	–	–	0.382±0.05	0.334±0.04	0.516±0.06	0.491±0.06
Long Internode Diallel	5¾	h^2	0.134±0.37	–	0.033±0.19	–	0.003±0.21	0.001±0.22
		H^2_{FS}	0.405±0.77	–	0.192±0.43	–	0.104±0.50	0.038±0.53
		H^2_{FS+C}	0.309±0.25	–	0.125±14	–	0.142±0.16	0.152±0.16
	8¾	h^2	–	0.038±0.18	0.027±0.22	0.027±0.22	0.024±0.30	–
		H^2_{FS}	–	0.091±0.40	0.134±0.50	0.109±0.58	0.085±0.69	–
		H^2_{FS+C}	–	0.116±0.13	0.152±0.16	0.186±0.18	0.229±0.22	–
"850" Family Block Selections	15	h^2	0.342±0.14	–	0.587±0.30	0.024±0.08	0.453±0.19	0.587±0.34
		H^2_{FS}	0.313±0.19	–	0.658±0.36	−0.031±0.20	0.279±0.23	0.673±0.45
		H^2_{FS+C}	0.625±0.18	–	0.612±0.17	0.330±0.07	0.548±0.10	0.695±0.19

Trial	Ages when assessed (years)	Type of heritability	*Dothistroma* (% infection)	Malformation score	Acceptability score	Spiral grain angle	Density (rings 1–5)	Density (rings 10–15)
Growth and Form Factorial	8½	h^2	0.096±0.05	0.022±0.02	0.033±0.02	–	–	–
		H^2_{FS}	0.171±0.10	0.079±0.06	0.164±0.05	–	–	–
		H^2_{FS+C}	0.385±0.04	0.103±0.04	0.165±0.04	–	–	–
Long Internode Diallel	5¾	h^2	–	0.033±0.14	0.027±0.13	–	–	–
		H^2_{FS}	–	0.010±0.30	0.026±0.29	–	–	–
		H^2_{FS+C}	–	0.074±0.10	0.067±0.09	–	–	–
	8¾	h^2	–	0.001±0.20	0.016±0.17	0.857±0.72	–	–
		H^2_{FS}	–	0.033±0.47	0.041±0.38	1.011±0.95	–	–
		H^2_{FS+C}	–	0.126±0.15	0.098±0.12	0.669±0.39	–	–
"850" Family Block Selections	15	h^2	–	0.000±0.06	–	–	0.563±0.32	0.851±0.36
		H^2_{FS}	–	0.020±0.14	–	–	0.662±0.42	0.804±0.39
		H^2_{FS+C}	–	0.125±0.07	–	–	0.636±0.17	0.664±0.19

[1] Types of heritability and the estimates they are based on: h^2 = narrow-sense (additive), H^2_{FS} = broad-sense (additive, dominance), H^2_{FS+C} = broad-sense (additive, dominance, clonal), ± standard error of the heritability estimate.

depress the estimates of h^2 and H^2_{FS}, since the clonal variance is contained in the denominator of both these heritabilities.

The fact that the Family Block Selections trial had less discrepancy between h^2 and H^2 than the other two ran contrary to the expectation that propagation effects would be strongest in this trial, given that the donor ortets had not been hedged for juvenility and that there was less experience with propagating radiata pine at the time. One explanation for the low heritabilities for straightness is that differences in straightness become less notable as trees age. Another is that heritabilities were reduced by physiological ageing, which improves stem form in radiata pine (MENZIES & AIMERS-HALLIDAY 1997).

BURDON *et al.* (1992) suggested that non-additive genetic variance could be more important than additive variance in less heritable traits. This could be one

explanation for the results in the three studies in this paper. The fact that broad-sense heritability theoretically has an upper bound of one, could be another factor reducing the difference from narrow-sense heritability if h^2 is also high.

These results with radiata pine can be compared with results from other forest tree species. Many publications on clonal variances do not have separate estimates of additive, dominance and epistatic variances; I will refer here to three trials in which variances were partitioned to some extent. In the first, variances were estimated for loblolly pine (*Pinus taeda* L.) using 16 parents in two 4 × 4 factorials, giving 30 full-sib families (PAUL *et al.* 1997). The clone-within-family variance ($\sigma^2_{C(MF)}$) was slightly smaller than the sum of the variances attributed to males and females ($\sigma^2_M + \sigma^2_F$) for height at age 5 years; $\sigma^2_{C(MF)}$ was considerably smaller than $\sigma^2_M + \sigma^2_F$ for dbh and individual tree volume at age 5. Thus, there was no evidence for an inflated within-family variance for these traits. The second study, on black spruce (*Picea mariana* (Mill.) B.S.P.) had 10 parents crossed in two complete five-parent diallels, without selfs, generating 40 full-sib families and 240 clones in all (MULLIN *et al.* 1992). The narrow-sense heritability estimate for 5-year height was 0.059 and the broad-sense heritability estimate was 0.093. The authors estimated that 25–40 % of the total genetic variance for height growth could be attributed to epistasis. The third study, this time on tamarack (*Larix lariciana* (Du Roi) K. Koch.) was based on open-pollinated seed, collected from 10 trees of each of three natural populations giving 30 parents in all (PARK & FOWLER 1987). For two populations additive variance far exceeded non-additive variance for height at age 5 years, but for the third it was the reverse.

The three trials reported in this paper give important new data for radiata pine in New Zealand. However, there is still a need to get more conclusive estimates of the different types of genetic variance (additive, dominance and epistatic) and non-genetic variance (propagation effects). Without them we could draw incorrect conclusions on how much gain can be obtained from clonal selection; the same is probably true for other important forestry species.

To this end, trials with many parents (50 or more) would need to be crossed in an efficient mating design resulting in 100 crosses or more, and planted on a minimum of three sites. They should be designed to partition the different types of variance adequately. The ability to test differences between separate propagation events (e.g. different stoolbed plants), for the same clone, would be an essential element of such trials. Ideas on appropriate mating designs, numbers of ramets and field designs are given by LIBBY & JUND (1962), RUSSELL & LIBBY (1986), FOSTER & SHAW (1988), RUSSELL & LOO-DINKINS (1993), WU (1996) and AIMERS-HALLIDAY *et al.* (1997). To my knowledge there are very few trials, for radiata pine or for other species, that meet the criteria mentioned above.

One view is that such studies should be based on random selections from an unimproved population. While such a structure would recreate the full variation that existed in the species, I suggest rather that crossing enough parents from the breeding population will provide parameters more relevant to breeders. The trials should be raised using good propagation techniques for the reasons mentioned previously. Radiata pine trials recently planted in New Zealand will go some way to meet these needs.

CONCLUSIONS

The studies provided evidence for substantial variance associated with clones, which could not be attributed to additive or dominance variance. This variance could be due to other forms of (epistatic) genetic variance, or could be due to propagation effects. The proportion on non-additive variance was greatest for traits with low additive variance. Propagation systems which induce minimal levels of non-genetic propagation effects are needed so that operational clonal testing can be effective.

ACKNOWLEDGMENTS

The Growth and Form Factorial was planned by C. J. A. Shelbourne. T. Faulds handled the propagation while G. R. Johnson, J. van Dorsser and T. G. Vincent directed the planting. The Long Internode diallel was planned and executed by M. J. Carson and A. Firth respectively. C. J. A. Shelbourne, J. King and D. Darling directed the raising of the clones. The Family Block Selections trial was planned by I. J. Thulin; T. Faulds and T. G. Vincent directed the propagation and planting respectively. The Growth and Form Factorial and the Long Internode Diallel were assessed by M. Miller, K. Fleet and J. Wharekura. C. B. Low helped with the analysis of the Long Internode Diallel data. Sites for these three trials were provided by New Zealand Timberlands Ltd, Tasman Forestry and the New Zealand Forest Service respectively (owners / managers of the forests at the time of planting). J. Aimers-Halliday, F. E. Bridgwater, R. D. Burdon, L. Paule and C. J. A. Shelbourne gave valuable comments on versions of this paper. This work was supported by the New Zealand government through the Foundation for Research, Science and Technology.

REFERENCES

AIMERS-HALLIDAY, J., SHELBOURNE, C. J. A. & HONG, S. O. 1997: Issues in developing clonal forestry with *Pinus radiata*. *In*: Proceedings of the NZFRI–IUFRO Confer-

ence: IUFRO'97 Genetics of Radiata Pine, 1–4 December and Workshop 5 December, Rotorua, New Zealand. Edited by R. D. Burdon and J. M Moore. pp. 264–272. FRI Bulletin 203, New Zealand Forest Research Institute, Rotorua, New Zealand.

BEAUREGARD, R., GAZO, R., KIMBERLEY, M. O., TURNER, J., MITCHELL, S. & SHELBOURNE, A. 1999: Clonal variation in the quality of radiata pine random width boards. *Wood Fiber Sci.* **31**: 222 –234.

BECKER, W. A. 1992: Manual of Quantitative Genetics. 5th ed. Academic Enterprises, Pullman, Washington, USA.

BURDON, R. D., BANNISTER, M. H. & LOW, C. B. 1992: Genetic survey of *Pinus radiata*. 4. Variance structures and heritabilities in juvenile clones. *N. Z. J. For. Sci.* **22**: 187–210.

CARSON, S. D. 1989: Selecting *Pinus radiata* for resistance to Dothistroma needle blight. *N. Z. J. For. Sci.* **19**:3–21.

CARSON, S.D. 1991: Genotype × environment interaction and optimal number of progeny test sites for improving *Pinus radiata* in New Zealand. *N. Z. J. For. Sci.* **21**: 32–49.

COMSTOCK, R. E. & ROBINSON, H. F. 1948: The components of genetic variance in populations of biparental progenies and their use in estimating the average degree of dominance. *Biometrics* **4**: 254 –266.

CONCHEYRO, S. C. 1998: Evaluation of 1986–1987 radiata pine clonal trials at Forest Research, New Zealand. Master of Applied Science thesis, Massey University, Palmerston North, New Zealand.

DADSWELL, H. E., FIELDING, J. M., NICHOLLS, J. W. P. & BROWN, A. G. 1961: Tree-to-tree variations and the gross heritability of wood characteristics of *Pinus radiata*. *Tappi* **44**: 174 – 179.

DONALDSON, L. A., CROUCHER, M. & UPRICHARD, J. M. 1997: Clonal variation of wood chemistry variables in radiata pine (*Pinus radiata* D. Don) wood. *Holzforschung* **51**: 537–542.

FOSTER, G. S. & SHAW, D. V. 1988: Using clonal replicates to explore genetic variation in a perennial plant species. *Theor. Appl. Genet.* **76**: 788 –794.

HUBER, D. A., WHITE, T. L. & HODGE, G. R. 1994: Variance component estimation techniques for two mating designs with forest genetic architecture through computer simulation. *Theor. Appl. Genet.* **88**: 236–242.

JAYAWICKRAMA, K. J. S. & LOW, C. B. 1999: *Pinus radiata* selections from different regions of New Zealand differ in branch habit, form, and growth rate. *N. Z. J. For. Sci.* **29**: 3–24.

JAYAWICKRAMA, K. J. S., SHELBOURNE, C. J. A. & CARSON, M. J. 1997: New Zealand's long internode breed of *Pinus radiata*. *N. Z. J. For. Sci.* **27**: 126–141.

KING, J. N. & BURDON, R. D. 1991: Time trends in inheritance and projected efficiencies of early selection in a large 17-year-old progeny test of *Pinus radiata*. *Can. J. For. Res.* **21**: 2100–2107.

KING, J. N. & JOHNSON, G. R. 1991: Genetic parameter estimates from a young clonal test. *In*: Proceedings of the FRI/NZFP Forests Ltd Clonal Forestry Workshop, 1–2 May 1989, Rotorua, New Zealand. Edited by John T. Miller. Forest Research Institute, Rotorua and NZFP Forests Ltd, Rotorua. FRI Bulletin No. 160. pp. 98–100.

KING, J. N., JOHNSON, G. R. & CARSON, M. J. 1998: Analysis of half diallel mating designs. II. Results from a third generation progeny test of the New Zealand radiata pine improvement programme. *Silvae Genet.* **47**: 80–87.

LIBBY, W. J. & JUND, E. 1962: Variance associated with cloning. *Heredity* **17**: 533–540.

LOW, C. B. 1991: Interaction of Cyclaneusma needle cast with early selection. *In*: Proceedings of the FRI/NZFP Forests Ltd Clonal Forestry Workshop, 1–2 May 1989, Rotorua, New Zealand. Edited by John T. Miller. pp. 113–115. FRI Bulletin No. 160, Forest Research Institute, Rotorua.

MENZIES, M. I. & AIMERS-HALLIDAY, J. 1997: Propagation options for clonal forestry with *Pinus radiata*. *In:* Proceedings of the NZFRI–IUFRO Conference: IUFRO'97 Genetics of Radiata Pine, 1–4 December and Workshop 5 December, Rotorua, New Zealand. Edited by R.D. Burdon and J.M Moore. pp. 256 –263. FRI Bulletin 203, New Zealand Forest Research Institute, Rotorua, New Zealand.

MULLIN, T. J., MORGENSTERN, E. K., PARK, Y. S. & FOWLER, D. P. 1992: Genetic parameters from a clonally replicated test of black spruce (*Picea marina*). *Can.J.For.Res.* **22**: 24–36.

PARK, Y. S. & FOWLER, D. P. 1987: Genetic variances among clonally propagated populations of tamarack and the implications for clonal forestry. *Can. J. For. Res.* **17**: 1175–1180.

PAUL, A. D., FOSTER, G. S., CALDWELL, T. & MCRAE, J. 1997: Trends in genetic and environmental parameters for height, diameter, and volume in a multilocation clonal study with loblolly pine. *For. Sci.* **43**: 87–98.

RUSSELL, J. H. & LIBBY, W. J. 1986: Clonal testing efficiency: the trade-offs between clones tested and ramets per clone. *Can. J. For. Res.* **16**: 925–930.

RUSSELL, J. H. & LOO-DINKINS, J. A. 1993: Distribution of testing effort in cloned genetic tests. *Silvae Genet.* **42**: 98–104.

SAS INSTITUTE INC. 1989: SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2, Cary, NC, USA. 846 pp.

SCHAFFER, H. G. & USANIS, R. A. 1969: General least squares analysis of diallel experiments. A computer program – DIALL. Research Report 1, Genetics Department, N.C. State University.

SCHUTZ, W. M. & COCKERHAM, C. C. 1966: The effect of field blocking on gain from selection. *Biometrics* **22**: 843–863.

SHELBOURNE, C. J. A. 1991: Clonal testing *Pinus radiata* in New Zealand. *In:* Proceedings of the FRI/NZFP Forests Ltd Clonal Forestry Workshop, 1–2 May 1989, Rotorua, New Zealand. Edited by John T. Miller. pp. 25–40. Forest Research Institute, Rotorua and NZFP Forests Ltd, Rotorua. FRI Bulletin No. 160.

SHELBOURNE, C. J. A. 1997: Genetics of adding value to the end-products of radiata pine. *In:* Proceedings of the NZFRI–IUFRO Conference: IUFRO'97 Genetics of Radiata Pine, 1–4 December and Workshop 5 December, Rotorua, New Zealand. Edited by R.D. Burdon and J.M Moore. pp. 129–141. FRI Bulletin 203, New Zealand Forest Research Institute, Rotorua, New Zealand.

SORENSSON, C. T., BURDON, R. D., COWN, D. J., JEFFERSON, P. A. & SHELBOURNE, C. J. A. 1997: Incorporating spiral grain into New Zealand's radiata pine breeding programme. *In:* Proceedings of the NZFRI–IUFRO Conference:

UFRO'97 Genetics of Radiata Pine, 1–4 December and Workshop 5 December, Rotorua, New Zealand. Edited by R.D. Burdon and J.M Moore. pp. 180 –191. FRI Bulletin 203, New Zealand Forest Research Institute, Rotorua, New Zealand.

SWEET, G. B. 1991: Summing up. *In:* Proceedings of the FRI/NZFP Forests Ltd Clonal Forestry Workshop, 1–2 May 1989, Rotorua, New Zealand. Edited by John T. Miller. Forest Research Institute, Rotorua and NZFP Forests Ltd, Rotorua. pp.164 –168. FRI Bulletin No. 160.

THULIN, I. J. & FAULDS, T. 1968: The use of cuttings in the breeding and afforestation of *Pinus radiata*. *N. Z. J. For. Sci* **13**: 66–78.

WILCOX, M. & CARTER, P. 1991: Welcome and introduction. *In:* Proceedings of the FRI/NZFP Forests Ltd Clonal Forestry Workshop, 1–2 May 1989, Rotorua, New Zealand. Edited by John T. Miller. pp. 7–9. Forest Research Institute, Rotorua and NZFP Forests Ltd, Rotorua. FRI Bulletin No. 160.

WU, R. L. 1996: Detecting epistatic genetic variance with a clonally replicated design: models for low- vs. high-order nonallelic interaction. *Theor. Appl. Genet.* **93**: 102–109.