EVALUATION OF 1986-1987 RADIATA PINE CLONAL TRIALS AT FOREST RESEARCH, NEW ZEALAND

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ABSTRACT

Clonal forestry, the establishment of plantations using tested clones, is highly sought after by the forestry industry in New Zealand and worldwide. Clonal testing is a vital element in the process leading to clonal forestry.

Two clonal trials established in 1986 and 1987 by the Forest Research Institute with juvenile ortet material have been analysed in this study. The mating design in the 1986 clones-in-family trial was single-pair crossing with amplification of the clones by fascicle cuttings. It was replicated over two sites, and the trait analysed was diameter at 1.40 m height at ages 4, 7, and 10 years.

The estimation of additive, non-additive and genetic variances showed a high proportion of non-additive variance compared with the additive variance at one of the sites, whereas the proportion was less important at the other site. The high non-additive component of variance can be due to important dominance or epistasis, or to C-effects confounded with the non-additive variance. This trend was similar for all three ages.

Realised genetic gains were obtained from selection of clones at age 10 years for clonal deployment and breeding. For clonal deployment, realised gains were high at both sites (13% and 16%). The gains were similar at both sites provided selection was based on performance values at the site, and not on indirect selection on performance of clones at the other site. Realised gains for selection at age 10 based on the performance of clones on combined sites (10% and 13%) were less than the maximum gain obtained at each individual site. Gains based on information from both sites (10% and 12% at respective sites) were more stable than those selections at any one site. For breeding, the level of gain was significantly inferior than for clonal deployment (4% and 8%), especially when the number of clones per family was restricted to one (2% and 4%). Realised gain on combined-site selection yielded less gain than direct selection at the optimum site for selection (1% and 2%).

The presence of genotype x environment interaction emphasised the need to test clones in several sites if stability of performance is desired.

It is possible to obtain gain from selections made at an early age, but selections made for breeding at the age of final assessment yielded greater expected total gain and gain per unit time.

The mating design in the 1987 clones-in-family trial was a 3 x 3 disconnected factorial. The trial was established on a single site and the trait analysed was percentage of Dothistroma needle infection at ages 3, 4 and 7years.

The mating design allowed estimation of additive, dominance and epistasis variances, which were overestimated for the lack of replication over sites. In this trial measured for Dothistroma resistance, the additive variance was the major component of the genetic variance at both ages. The evolution of components of genetic variance was confounded with the level of Dothistroma infection.

The analysis of these trials indicated the need to improve the mating and field designs to improve the accuracy in the estimation of genetic parameters, highlights the importance of annual or biennual measurements to determine trends of those parameters over time, and showed the difference in gains obtained from selection for breeding and clonal deployment for early selection and selection at the age of final assessment.

Accuracy in the estimation of genetic parameters can be achieved using factorial mating designs together with serial propagation to reduce the incidence of C effects, and with replication over several sites. Further considerations have to be made to find the most appropriate field and statistical design, but alpha designs are a possibility to explore.

Investment in a series of carefully planned clonal trials is fundamental to the future of clonal forestry in radiata pine.

A mis padres y a la Memoria de Raúl Alliani

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CHAPTER I

CLONAL FORESTRY AND CLONAL TESTING

1. INTRODUCTION

Clonal forestry is highly sought after by the forestry industry in New Zealand and worldwide. The progress made in the last 30 years in the development and refinement of vegetative propagation techniques in conifers, as well as in hardwood species, provides an adequate framework to consider clonal forestry as an option to deliver genetic gain in the forest.

Clonal forestry has been defined as the establishment of plantations using tested clones (Carson 1986). Burdon (1989) emphasised the difference between the use of tested clones and the vegetative multiplication of unidentified genotypes.

The advantages of clonal forestry have been thoroughly described in the literature (e.g. Libby and Rauter 1984, McKeand 1981) and have been reviewed for *Pinus radiata* in New Zealand by Carson (1986), and Carson and Burdon (1991).

The main advantages of using clonal forestry for radiata pine are (Carson and Burdon 1991):

- improvement of crop uniformity
- exploitation of genotype-environment (G x E) interaction
- controlling allocation of plant resources
- working with known clones
- selecting for non-conventional traits
- capturing genetic gains from non-additive genetic effects
- capturing unusual genetic segregants and correlation breakers

Quite often, there is the perception that clonal forestry is an alternative to traditional breeding. On the contrary, clonal forestry and breeding complement one another. A long-term clonal forestry programme must be supported by a long-term breeding

programme (Libby and Ahuja 1993) to generate new variability by sexual recombination for further selection. Unless new genotypes with superior characteristic are bred, the potential for added genetic gains in clonal forestry will reach a dead end (McKeand 1981, Burdon 1989). The genetic gain delivered from a breeding programme, however, can be enhanced if the production population follows a clonal deployment strategy, provided the genetic variation in the trait has a significant and repeatable non-additive component.

2. SCHEME FOR CLONAL FORESTRY

The basic process of clonal forestry is summarised in Figure I-1. It starts by the amplification of clones from crosses between selected parents, through vegetative propagation. Different methods are available to carry out the multiplication, such as fascicle cuttings, stem cuttings, somatic embryogenesis, and tissue culture.

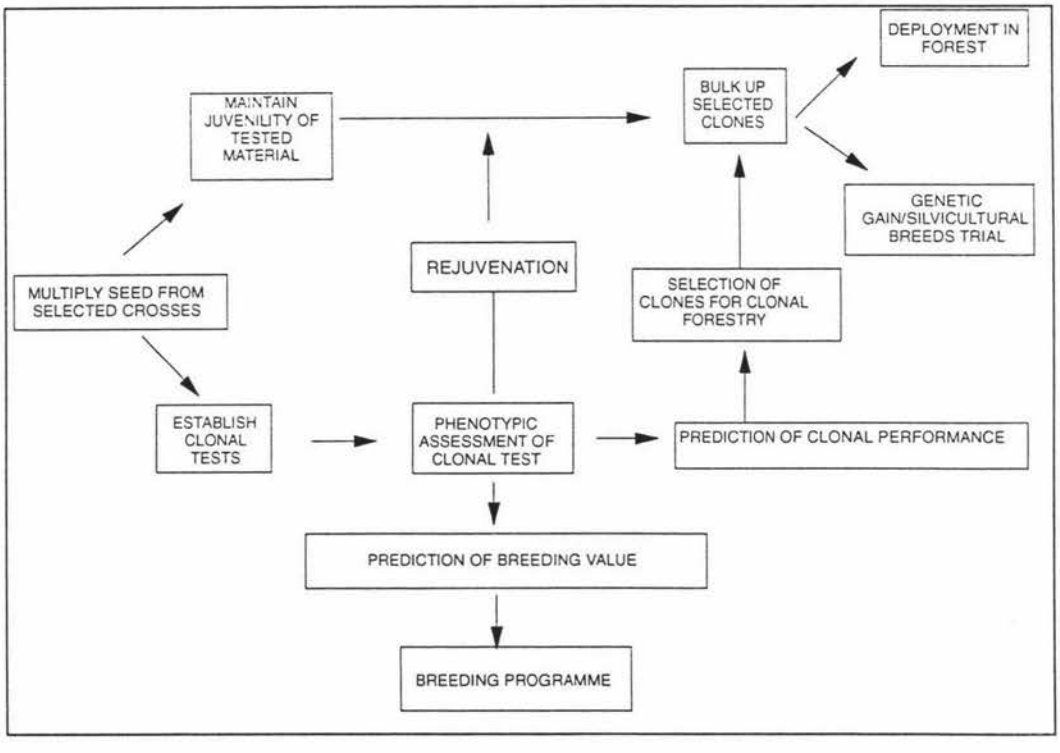

Figure I-1. Scheme for clonal forestry.

It continues with the establishment of clonal tests. Clonal testing is a vital element in determining the relative worth of clones and the stability of their behaviour under different environments. Libby and Ahuja (1993) detailed the main objectives of clonal tests as:

(1) estimate genetic parameters

(2) estimate genotype-by-environment interaction (G x E)

(3) compare the relative performance of different clones

(4) compare the performance of clones *vs* the performance of seedlings

(5) prediction of genetic gain

Trials for clonal testing are established following an appropriate experimental design. Intensive replication over sites improves the precision in the estimation of G x E interaction and avoids confounding of genotypic main effect with the interaction between the genetic component and the environmental component of variation.

Trials are measured and assessed for traits of interest. In radiata pine, this assessment for progeny trials has traditionally been carried out at age 8 years by the New Zealand Forest Research Institute, and the same protocol has been extended to the assessment of clonal trials. Fletcher Challenge Forests Ltd., have developed a successful clonal forestry system based on tissue culture. Their clonal trials are assessed at age 3 years reducing the period of clonal storage during testing.

The statistical analysis of the data from the assessment provides the elements for the estimation of genetic parameters. After the estimation of genetic parameters, the breeding value and the clonal performance are predicted for each clone. The predicted breeding value is relevant for the integration of the clone into the breeding programme. The predicted clonal performance is pertinent when the clone is used for clonal deployment.

Selections are made according to the future use, either breeding or cloning. To be able to use selected clones and successfully deploy them in the forest, it is imperative to maintain the juvenility of the tested material or to be able to rejuvenate the material once the testing phase has been completed. For the predictions on genetic gains for clonal forestry to be applicable to forest conditions, the physiological status of the material deployed has to be comparable to the tested material.

Once the clones have been selected, they are bulked up and deployed in the forest. There are several issues to address in the area of clonal deployment, but they are in themselves a separate study issue.

3. EXPERIENCE IN CLONAL FORESTRY AT FOREST RESEARCH

Radiata pine clonal trials were established by the New Zealand Forest Research Institute between 1966 and 1985. In those early trials the physiological ages of the selected ortets from which cuttings were obtained to establish clonal trials were between 5 and 17 years. Physiological ageing caused considerable reduction in diameter growth and alterations in other traits (Shelbourne 1991). Shelbourne reviewed this series of clonal trials and identified two main causes for the failure of these early attempts to institute clonal forestry:

- Inability to produce clonal material for testing due to selection of physiologically aged individuals.
- Difficulty to re-propagate enough vegetative material of the selected clones after completion of clonal testing for extensive deployment in the forest.

In an attempt to eliminate the problem of physiological ageing, a new series of clonal trials was initiated in 1986 using juvenile ortet material. Three trials were established in Kaingaroa Forest, Manawahe, and Tahorakuri with plants obtained by fascicle cuttings from 6 months old seedlings. The three trials differed in mating designs, replication over sites, traits assessed, and frequency of assessment.

4. OBJECTIVE OF THE PRESENT STUDY

The importance of clonal testing in the process leading to clonal forestry has been emphasised previously. There are several issues that have an impact on the efficiency of clonal testing and their implications in the use of selected clones for breeding or clonal deployment. This contribution intends to explore some of these issues using experimental data from two clonal trials from the new series planted in 1986:

CLONES-IN-FAMILIES TRIAL (Manawahe and Kaingaroa Forests). The genetic entries for this trial were generated by single-pair mating followed by fascicle-cutting propagation. It included 16 full-sib families and 10 clones within each family, replicated over two distinctive sites. Diameters at 1.40 m height were available at ages 4, 7, and 10 years at one site, and 7 and 10 years at the other site. This trial offered suitable data to investigate the following issues:

- Estimation of variance components and other genetic parameters (heritabilities and genotypic and phenotypic correlations).
- Evolution of components of genetic variance over time. Comparison of time trends between sites.
- Prediction of the level of genetic gain obtained under different strategies: breeding and clonal deployment.
- Comparing the realised gains from early selection with gains at the age of final assessment.
- Comparing the ranking of selected clones in different sites.

1987 CLONES-IN-FAMILES (Kaingaroa Forest, Cpt. 324). The genetic entries for this trial were generated by a 3 x 3 disconnected factorial mating design followed by fascicle cutting propagation. It comprised 4 factorials, 9 full-sib families per factorial with 10 clones within each family. The trial was established at a single site. The data available were limited to assessments of percentage of *Dothistroma pini* infection at ages; 3, 4 and 5 years, and measurements of stem diameter at 1.40 m height at age 8. This trial offered suitable data to investigate the following issues:

- Estimation of variance components and other genetic parameters. The factorial mating design offers the possibility to partition the non-additive variance into dominance and epistatic variances.
- Evolution of components of genetic variance over time for a trait different from diameter.

CHAPTER II

1986 CLONES IN FAMILIES TRIAL

1. INTRODUCTION

The 'Clones-in-Families Trial' was established by the Forest Research Institute using juvenile ortet material in 1986. At the same time tissue-cultured shoots of those clones were placed in cold storage to maintain their juvenility for vegetative propagation after selection. The cold-storage experiment was subsequently abandoned, but the field trial remained as a valuable source of information. This trial is the only clonal test replicated on more than one site, which has reached the age of final assessment, and where data from several assessments at younger ages are available at the Forest Research Institute. The 'Clone-in-Families Trial' was first analysed by King and Johnson (1991). The authors estimated genetic parameters for the trait 'height' at ages 1, 2, and 3 years old.

2. MATERIALS AND METHODS

2.1. Mating and Experimental Design

Thirty-two highly-ranked parental clones from the '268', '850'and '875' series (three of New Zealand's radiata pine growth and form series), and the '870' series (the long internode series) were crossed in a single-pair mating design producing sixteen full-sib families. Ten seedlings per full-sib family were propagated vegetatively by fascicle cuttings at the age of 6 months. The best ten ramets per clone were apportioned between two sites and planted in the field (Figure II-1). No nursery records on the performance of either the residual seedlings or the residual ramets were kept. The technique of propagation by fascicle cuttings was not fully developed at the time of establishment of this trial. As a consequence, the cuttings planted in the field were not fully homogeneous (Firth, 1997).

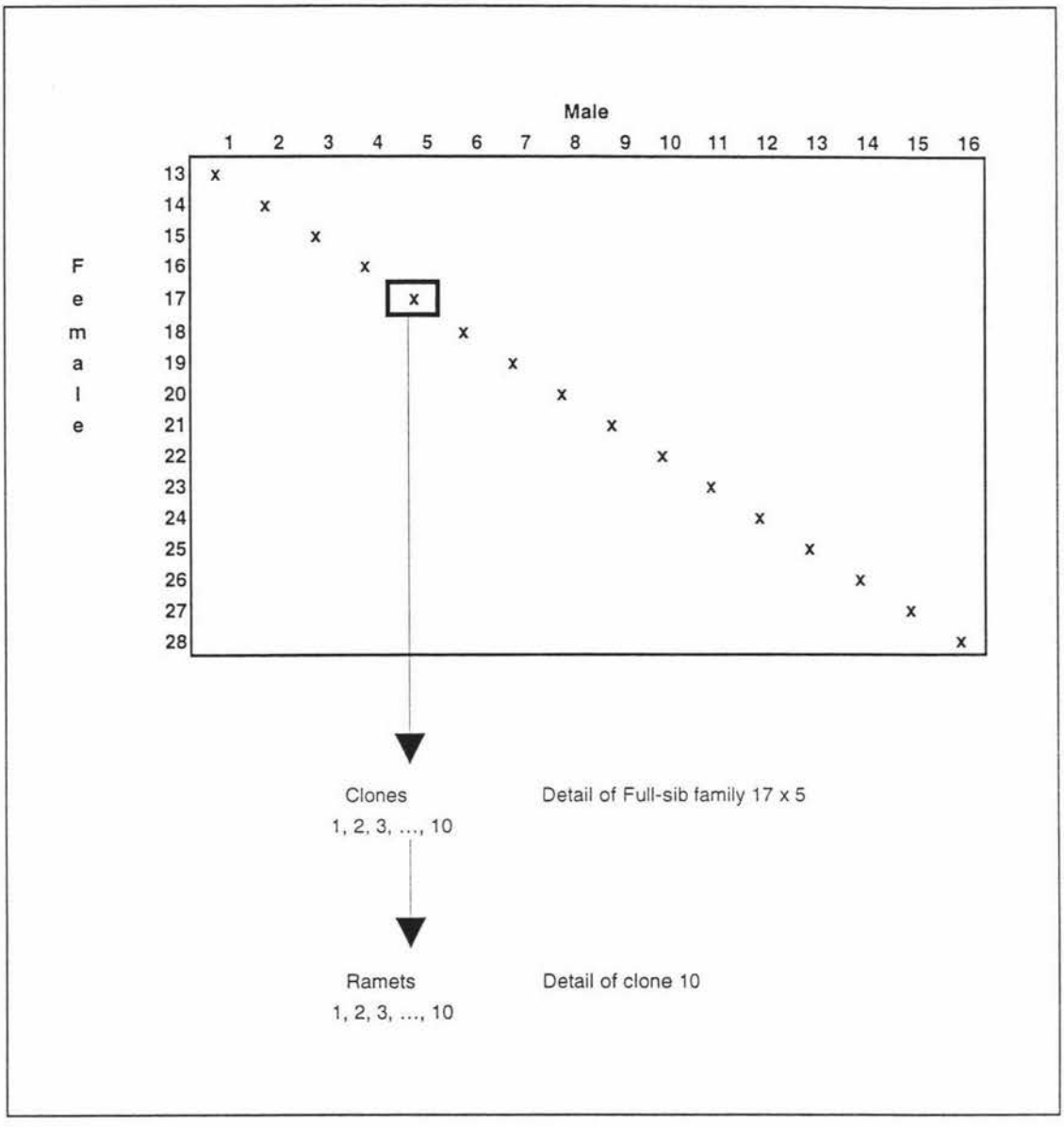

Figure II-1. Mating design of 1986 clones in families trial and amplification of clones by vegetative propagation.

The trial was established on two contrasting sites in the North Island of New Zealand; Manawahe, an ex-pasture pumice site in a warm coastal area of the Bay of Plenty, and Compartment 60 in Kaingaroa Forest, an ex-forest pumice site in a colder area on Kaingaroa Plateau.

The experimental design was Sets-in-Replications using Single-Tree-Plots (STP) with 5 replications per site. Seedlings of the same 16 full-sib families were planted in each replication as controls to compare cutting *vs* seedling performance.

The present analysis was based on measurements of 'Diameter at 1.4 m height' (DBH). Measurements were performed at age 4, 7, and 10 years at Manawahe, and at 7 and 10 years at Kaingaroa.

2.2. Data Analysis

The analysis of this trial was based on the data from the clonal material only. The comparison between seedlings *vs* cutting performance was not included as part of this study, however, preliminary results at an early age for that comparison were reported by Menzies and Carson (1991).

Analysis of variance was performed using SAS GLM Procedure (1990) for each individual site, and for combined-sites using a linear model including site, replication, family, clones within family, and interaction terms. All effects and interactions were assumed random. Sets were omitted from the analysis.

The statistical model for individual sites was :

$$Y_{ijkl} = \mu + R_i + F_j + C_{k(j)} + RF_{ij} + e_{ijkl} \qquad (1)$$

Y_{ijkl} is the *lth* ramet of the *k*th clone in the *jth* family growing in the *ith* replicate

μ is the overall mean

R_i is the effect of the *ith* replicate, $i = 1, 2, \ldots, 5$

F_j is the effect of the *jth* family, $j = 1, 2, \ldots, 16$

$C_{k(j)}$ is the effect of the *kth* clone within the *jth* family, $k = 1, 2, \ldots, 10$

RF_{ij} is the interaction effect of the *ith* replicate and the *jth* family

e_{ijkl} is the random error

The linear model for combined-sites was:

$$Y_{ijklm} = \mu + L_l + R_{i(l)} + F_j + C_{k(j)} + LF_{lj} + LC_{lk(j)} + RF_{ij} + e_{ijklm} \qquad (2)$$

Y_{ijklm} is the *mth* ramet of the *kth* clone in the *jth* family growing in the *ith* replicate in the *lth* location

μ is the overall mean

L_l is the effect of the *lth* test location, $l = 1, 2$

$R_{i(l)}$ is the effect of the *ith* replicate within the *lth* test location, i= 1, 2,..., 5

F_j is the effect of the *jth* family, j= 1, 2, ..., 16

$C_{k(j)}$ is the effect of the *kth* clone within the *jth* family, *k*=1, 2, ..., 10

LF_{jl} is the interaction effect of the *jth* family and the *lth* location

$LC_{k(j)l}$ is the interaction effect of the *kth* clone within the *jth* family and the *lth* location

RF_{ij} is the interaction effect of the *ith* replicate and the *jth* family

e_{ijklm} is the random error

REML (Restricted Maximum Likelihood) variance components and their respective standard errors were obtained using SAS Mixed Procedure (1990) for the models described above.

2.2.1. Estimation of genetic and environmental variances

The genetic model was the classical additive-dominance-epistasis linear model (Falconer 1981). The partition of the genetic variance into its components is restricted by the mating design which led to assume a simplified version. The simplified model acknowledged the fact that it is only possible to discriminate between additive and non-additive variance with the family structure generated by single-pair mating. The simplified model can be expressed as:

$$G = A + NA \qquad (3)$$

where

G =Genotypic value

A =Additive effect or breeding value

NA =Non-additive deviation

Estimates of additive and non-additive variances were obtained by equating the observational components of phenotypic variance to their causal components or

genetic expectations (Falconer, 1981) under the following assumptions (Comstock *et al.*1958)

1- regular diploid behaviour at meiosis
2- no cytoplasmic or maternal effects
3- no linkage among genes affecting any single character
4- distribution of genotypes in the parents was of the nature to be expected in a random sample from a randomly mated population

These assumptions are standard for trials established with seedling material, however, in the case of testing vegetatively propagated material an additional assumption has to be made (Foster and Shaw 1988).

5- no C-effects. C-effects have been defined as persistent non-genetic characteristics derived from the condition of the ortet common to all ramets of a clone (Burdon and Shelbourne 1974).

Single-pair mating design followed by vegetative propagation of clones within families provides a family structure with two levels of genetic relationship, full-sib families and clones within full-sib families. In this case the expectations of the genetic components are (Frampton and Foster 1993):

$$Cov_{FS} = \tfrac{1}{2}V_A + \tfrac{1}{4}V_D + \tfrac{1}{4}V_{AA} + \tfrac{1}{8}V_{AD} + \tfrac{1}{16}V_{DD} + \ldots \quad (4)$$

$$Cov_{C(FS)} = \tfrac{1}{2}V_A + \tfrac{3}{4}V_D + \tfrac{3}{4}V_{AA} + \tfrac{7}{8}V_{AD} + \tfrac{15}{16}V_{DD} + \tfrac{7}{8}V_{AAA} + \tfrac{15}{16}V_{AAD} + \tfrac{31}{32}V_{ADD} + \tfrac{63}{64}V_{DDD} + \ldots \quad (5)$$

Cov_{FS}	= Covariance of full-sib families
$Cov_{C(FS)}$	= Covariance of clones within families
V_A	= Additive variance
V_D	= Dominance variance
$V_{AA}, V_{DD}, V_{AD}\, V_{AAA}, V_{AAD}, V_{ADD}, V_{DDD}$	= Epistatic variance (additive x additive, dominance x dominance , etc)

In equations (4) and (5), all terms except for the first one ($\frac{1}{2}V_A$) have been included within the non-additive variance.

The total genetic variance was calculated as the sum of the variance among full-sib families (Eq. 4) and the variance among clones within families (Eq. 5).

There are two possible ways to estimate the additive variance. If epistatic effects are assumed negligible, the additive variance and the dominance variance can be obtained by solving for the unknowns in the system of equations (4) and (5). The system of equations can be expressed as:

$$\begin{bmatrix} \frac{1}{2} & \frac{1}{4} \\ \frac{1}{2} & \frac{3}{4} \end{bmatrix} \begin{bmatrix} V_A \\ V_D \end{bmatrix} = \begin{bmatrix} Cov_{FS} \\ Cov_{C(FS)} \end{bmatrix} \tag{6}$$

Additive variance and dominance variance are then calculated as:

$$\begin{bmatrix} V_A \\ V_D \end{bmatrix} = \begin{bmatrix} 3 & -1 \\ -2 & 2 \end{bmatrix} \begin{bmatrix} Cov_{FS} \\ Cov_{C(FS)} \end{bmatrix} \tag{7}$$

If epistatic effects are not considered to be negligible, only a crude estimate of the parameter of interest can be obtained. Under this assumption, the estimate of additive variance ($V_{A''}$) can be calculated as twice the covariance among full-sib families. It can be expressed as:

$$V_{A''} = 2\sigma^2_{FS} = V_A + \frac{1}{2}V_D + \frac{1}{2}V_{AA} + \frac{1}{4}V_{AD} + \frac{1}{8}V_{DD} \cdots \tag{8}$$

The additive variance will be overestimated (8), since its estimate ($V_{A''}$) will include half of the dominance variance, and among other epistatic variances, it will include half of the additive by additive variance

The non-additive variance can be estimated as the difference between the total genetic variance and the additive variance (Appendix II-1) and includes dominance and epistatic variances. The latter approach was used in the analysis of this trial.

References to additive variance in the remainder of this chapter refer to this crude estimate of additive variance ($V_{A^{\cdot}}$).

The nature of the data available from this trial not only allowed us to explore the relative magnitude of the different components of genetic variance, but also their evolution over time. Additive, non-additive and genetic variance were estimated for ages 4, 7 and 10 at Manawahe, and ages 7 and 10 at Kaingaroa.

2.2.2. Calculation of selection responses

One of the main reasons to establish clonal tests is to be able to predict the genetic gain obtained by selecting superior clones. As mentioned in the introductory section, the clones selected can be used in the production population or be included in the breeding population . The genetic gains obtained in each case are different, as well as the clones selected for each purpose. Both alternatives have been explored in this study.

The selection of clones is based on the analysis of data from the final assessment, which in this trial was performed at the age of 10 years. The genetic gain for breeding made on the trait of interest at age 10 can be predicted using the classical equation of prediction of genetic gain from direct selection (Falconer 1981). Genetic gain for clonal deployment can be predicted replacing narrow-sense heritability for broad-sense heritability (H^2) in equation (9).

$$R_x = i\, h^2 \sigma_P \qquad (9)$$

R_X = Response of character x directly selected

i = Intensity of selection

h^2 = Narrow sense heritability

σ_P = Phenotypic standard deviation

Another possibility is to make selections at an early age (4 or 7 years old) and predict the gain obtained at the age of the final assessment. In this case, the equation for prediction of genetic gain is the one corresponding to a correlated response (Falconer, 1981).

$$CR_Y = ih_X h_Y r_A \sigma_{P_Y} \qquad (10)$$

CR_Y = Correlated response of character y

i = Intensity of selection

h_X = Square root of the heritability of trait x

h_Y = Square root of the heritability of trait y

r_A = genetic correlation between trait x and trait y

σ_{P_Y} = Square root of the phenotypic variance of trait y

2.2.2.1. Estimation of heritabilities

The estimate of heritabilities is essential for the prediction of genetic gain. In this trial heritabilities of family and clonal means for all ages at each individual site were calculated based on the estimates of additive, and phenotypic variances (Appendix II-1).

2.2.2.2. Estimation of phenotypic and genetic correlations

To predict correlated responses it is necessary to estimate the genetic correlations between two traits. Burdon (1977) makes a distinction between *Type A* and *Type B* genetic correlations. *Type A* genetic correlations are defined as the correlations obtained when both traits are measured in the same individual. This concept is applicable for the estimation of genetic correlation in the case of early selection at individual sites.

Type B genetic correlations are defined as the genetic correlations estimated when two traits are measured on different individuals within genetic groups. The genetic correlation between environments or across sites is a special case of *Type B* genetic

correlation (Burdon 1977). This is the correlation needed if we are interested in estimating the correlated response or genetic gain obtained in site B after making selections in site A.

Another approach to estimate the genetic correlation between sites has been proposed by Notter and Diaz (1993) in animal breeding. It takes into consideration the correlation between breeding values estimated independently at each site and the accuracy of estimation of those breeding values. Van Vleck (1987) defines accuracy as the correlation between predicted and true additive genetic value ($r_{g.\hat{g}}$). Thus, the genetic correlation for a certain trait between two sites can be expressed as (Notter and Diaz 1993)

$$r_{g_{AB}} = r_{\hat{g}_A \hat{g}_B} \, a_A a_B$$

$r_{g_{AB}}$ is the true genetic correlation between sites A and B

$r_{\hat{g}_A \hat{g}_B}$ is the correlation between the estimated breeding values at site A and B

a_A is the accuracy in the estimation of breeding values at site A

a_B is the accuracy in the estimation of breeding values at site B

For the data in this study, the method to estimate *Type B* genetic correlations proposed by Burdon as well as the method proposed by Notter and Diaz to estimate genetic correlations across sites produced estimates which were out of the normal range for a correlation ($r_{G_{AB}} > 1$).

In this study two cases of indirect selection were considered, early selection and selection across sites.

- Early selection

As this clonal test was assessed at three different ages, one of the objectives of this study was to compare the genetic gain in diameter at 1.40 m at the age of 10 years obtained by selecting the best clones at age 7 with selections made at age 10. The same comparison was carried out for selections made at age 4.

In this case, genetic correlations were estimated from the relationship between the genetic covariance between ages 7 and 10, and the product of genetic standard deviation at each age. The same procedure was followed to estimate the genetic correlation between age 4 and 10 years (Appendix II-1).

- Selection across sites

In this study, we were also interested in estimating the genetic gain obtained at age 10 in Manawahe, if the best clones were selected based on data of their performance in Kaingaroa at the same age, and viceversa .

To try to get around the problem presented by inaccurate and, therefore, unreliable estimates of genetic correlations across sites needed to predict genetic gains, a different approach was considered. In clonal trials, each clone is represented in each site by the same genetic entity since they are ramets (vegetatively propagated) from the same clone. The fact that exactly the same genotype is present at each site and that there is no genetic sampling issue (as occurs when using seedlings at different sites) makes it possible to compare the performance of a certain clone over both sites.

If the best clones are selected in one site, the realised gain based on mean predicted clonal performance and breeding values on that site can be calculated, as well as the correlated realised gain at the other site. This was the method used to predict genetic gains in this study.

A selection index including family- and clone-within-family information was used to rank the clones. The phenotypic value of each clone was assumed to be a function of two independent elements: a family component or deviation of its family mean from the overall mean of the trial, and a within family component or deviation of the clone mean from its family mean. Therefore, the phenotypic value of each clone can be expressed as

$$Y_{ij} = \left(\overline{Y}_{i.} - \overline{Y}_{..}\right) + \left(\overline{Y}_{.j} - \overline{Y}_{i.}\right) \qquad (12)$$

Y_{ij} is the phenotypic value of the *ith* clone in the *jth* family

$\overline{Y}_{..}$ is the overall mean

$\overline{Y}_{i.}$ is the *ith* family mean

$\overline{Y}_{.j}$ is the *jth* clone mean

Two strategies for genetic improvement of radiata pine were considered:

- the selection of clones at age 10 for clonal deployment
- the selection of clones at age 10 for further breeding.

For the former strategy, the clonal performance of each clone was estimated, and for the latter estimates of breeding values were obtained. Clonal performance of clones and breeding values were estimated for each site separately for the trait 'Diameter at 1.4 m height'.

2.2.2.3. Estimated breeding value

The estimated breeding value for a certain clone was obtained using an index which combined family- and within-family information. The coefficients for the family- and within-family components were the narrow-sense heritability of family means and the narrow-sense heritability of clone means within families respectively.

$$EBV = h^2_{\overline{FS}} X_{FS} + h^2_{\overline{C}(FS)} X_{wFS} \qquad (13)$$

EBV	=	Estimated Breeding Value
$h^2_{\overline{FS}}$	=	Narrow-sense heritability of family means
$h^2_{\overline{C}(FS)}$	=	Narrow-sense heritability of clone means within families
X_{FS}	=	Deviation of the family mean from the overall mean, family component of phenotypic variance
X_{wFS}	=	Deviation of clonal mean from the family mean, within family component of phenotypic variance

2.2.2.4. *Estimated clonal performance*

The estimated clonal performance of each clone was obtained following the same principles as the estimated breeding values, but using appropriate coefficients to create an index. The coefficient corresponding to the family component is the same as for the estimation of clonal breeding value since only the additive component is passed to the next generation after sexual reproduction, but the coefficient affecting the within family component is, in this case, the broad-sense heritability of clonal means within families, because the additive and non-additive components of variance are passed on by vegetative propagation of the clone.

$$ECP = h^2_{\overline{FS}} X_{FS} + H^2_{\overline{C}(FS)} X_{wFS} \qquad (14)$$

ECP = Estimated Clonal Performance

$h^2_{\overline{FS}}$ = Narrow-sense heritability of family means

$H^2_{\overline{C}(FS)}$ = Broad-sense heritability of clone means within families

2.2.2.5. *Realised gain for clonal deployment and breeding*

Clones were ranked by their Estimated Clonal Performance at each selection age. The ranking was carried out separately at each site. The following analysis was based on selection of the top 10% (15) of the clones. Realised gain estimated from the mean clonal performance of the selected parents based on their performance at age 10 was expressed as a percentage of the mean of the unselected population. The same procedure was followed to calculate the realised gain for breeding, however, for this purpose clones were ranked and gains calculated on estimated clonal breeding values. As selecting more than one clone per family reduces the effective population size, which in turn could cause inbreeding, realised gains were obtained for the use of the clones for breeding with restrictions. The restriction consisted of no more than one clone per family being selected.

2.2.2.6. Predicted gain for clonal deployment and breeding

Predicted gains were calculated considering selection carried out in two stages, firstly, the best families, and secondly the best clones within each family (Appendix II-1).

For breeding without restrictions, and for clonal deployment, the intensity of selection was the same for combined family- and within-family selection and corresponded to a proportion of one clone in 143.

For breeding with restrictions, the proportion of families selected was 15 families from 16, and the proportion of clones within family selected was one clone in ten clones.

The intensity of selection (i) was obtained from tabulated values (Becker 1984).

3. RESULTS AND DISCUSSION

The overall mean for diameter at 1.4 m height (DBH) for Manawahe and Kaingaroa at age 10 were 29.2 cm and 23.7 cm respectively, with a survival of approximately 75% in both sites. Mean diameter for each age and site is shown in Table II-1. As expected, growth in diameter at Manawahe was better than growth at Kaingaroa due to more benign climatic conditions in the coastal area, and higher fertility of the ex-pasture site.

	MANAWAHE			KAINGAROA	
	4-year DBH (mm)	7-year DBH (mm)	10-year DBH (mm)	7-year DBH (mm)	10-year DBH (mm)
Mean	140.5	221.0	291.9	169.5	237.3

Table II-1. Mean diameter at each age and site (expressed in mm).

3.1. Analysis of Variance

Detailed results of the analysis of variance are shown in Appendix II-2. The analyses of variance for each individual site are summarised in Table II-2. The family effect for diameter was non-significant at Manawahe at ages 4 and 10, but significant at age 7. In Kaingaroa, the family effect was highly significant at ages 7 and 10. The interaction replication x family was non-significant in both sites and at all ages. The clones-within-family effect was highly significant at age 4, 7 and 10 years at Manawahe and at age 7 and 10 at Kaingaroa.

	MANAWAHE						KAINGAROA			
	DBH 4		DBH 7		DBH 10		DBH 7		DBH 10	
Source of variation	F	sign.	F	sign.	F	sign.	F	sign.	F	sign.
Rep	5.15	**	1.41	ns	0.93	ns	1.63	ns	0.94	ns
Family	1.58	ns	1.99	*	1.71	ns	2.91	**	2.79	**
Rep*family	0.99	ns	0.99	ns	1.10	ns	1.11	ns	1.31	ns
Clone(family)	3.57	**	4.06	**	3.57	**	2.11	**	2.64	**

(Sign.= significance) *= significant at α=0.05, **=significant at α= 0.01 ns= non-significant

Table II-2. Summary of the analysis of variance for the individual site model for diameter.

Location effect for diameter was highly significant in the combined-site analysis of variance (Table II-3). Family and clone-within-family effects followed a similar trend to the one observed in the individual-site analysis. The location x family interaction was significant at age 7, but disappeared with time. In contrast, the clone x location interaction remained highly significant at age 10. As the F test was so close to one, the interactions may while being statistically significant not be biologically significant.

	DBH 7		DBH 10	
Source of variation	F	significance	F	significance
Location	348.1	**	354.25	**
Rep(loc)	1.56	ns	0.93	ns
Family	1.91	*	2.81	**
Location*family	1.99	*	1.01	ns
Rep(loc)*family	1.09	ns	1.21	ns
Clone(family)	3.13	**	3.07	**
Location*clone(family)	1.37	**	1.47	**

*= significant at α=0.05, **=significant at α= 0.01 ns= non-significant

Table II-3. Summary of the analysis of variance for the combined-site model for diameter.

3.2. Estimates of Variance Components

For each individual site, estimates of variance components for diameter, their standard errors, and the relative importance of each variance component expressed as percentage of the phenotypic variance are shown in Table II-4.

Variance component	MANAWAHE						KAINGAROA			
	DBH4		DBH7		DBH10		DBH7		DBH10	
	Estim.	%	Estim.	%	Estim.	%	Estim.	%	Estim.	%
Replication	7.9 (6.80)	2.1	0.8 (2.7)	0.1	0	0	0	0	0	0
Family	11.7 (12.9)	3.1	42.6 (31.2)	6.5	92.5 (83.9)	4.6	70.1 (42.4)	7.8	142.0 (92.7)	7.6
Rep x Fam.	0.4 (5.5)	0.1	0	0	17.4 (34.2)	0.9	14.9 (21.5)	1.7	52.6 (44.9)	2.8
Clones-within-Family	152.6 (28.2)	40.1	287.3 (50.1)	43.7	837.0 (156.0)	41.4	187.3 (52.3)	20.8	543.7 (124.5)	29.2
Error	207.9 (15)	54.6	326.5 (23.0)	49.7	1073.0 (83.3)	53.1	626.9 (50.4)	69.7	1123.2 (92.7)	60.4
TOTAL	380.5	100	657.1	100	2019.9	100	799.2	100	1861.4	100
Clones-within-Family/Family	13.0		6.7		9.1		2.67		3.84	

Estim= Estimate

Table II-4. Estimates of variance components, standard errors (in parenthesis), and percentage of the phenotypic variance for the individual-site model.

The variance component due to family represented a small proportion of the phenotypic variance, and the replication x family variance component could be considered negligible.

The variance component due to clones-within-family was the largest genetic source of variation in both sites with a range between 20.8 and 40.1 %. It was larger in Manawahe than in Kaingaroa and remained quite constant over time in each site. The relative proportion of clone-within-family variance to family variance ranged from 3 to 13 (Table II-4) and it was greater in Manawahe than in Kaingaroa, reducing at age 7 and increasing again at age 10. It is important to mention that the clone-within-family variance was estimated with a small standard error. The largest source of phenotypic variance was due to residual error in both sites and for all ages. Variance components obtained for the combined-site analysis are shown in Table II-5.

Variance component	7-year DBH		10-year DBH	
Variance component	Estimate	%	Estimate	%
Location	1351.6 (1951.5)	63.3	1537.6 (2180.2)	43.8
Replication	1.2 (3.3)	0.05	0	0
Family	30.0 (28.9)	1.4	101.9 (74.5)	2.9
Loc. X Family	21.4 (15.3)	1.0	5.9 (25.5)	0.17
Rep X Family	4.9 (10.6)	0.2	35.9 (27.9)	1.02
Clones-within family	223.2 (43.1)	10.5	594.0 (16.8)	19.9
Loc. x clones-within-family	37.5 (22.7)	1.7	134.9 (63.4)	3.8
Error	466.0 (25.6)	21.8	1097.4 (61.9)	31.3
TOTAL	2135.8	100	3507.6	100
Clones -within-family / Family	7.44		5.24	

Table II-5. Estimates of variance components, standard error (in parentheses), and percentage of the phenotypic variance for the combined-site model.

The variance component due to location was the largest component of phenotypic variance, amounting to 68 and 43 % at ages 7 and 10 respectively. The variance component due to families was again small compared to the clone-within-family variance. The genotype x location interactions, including family x location and clone-within-family x location accounted for 2.7 and 3.87 % of the phenotypic variance at ages 7 and 10 respectively, and suggested the existence of small G x E interaction.

Under the classical genetic model for full-sib families, the family variance and the clones-within-family variance should be equal in the absence of non-additive variance (Falconer, 1981). In this study, the clone-within-family variance grately exceeded the family variance, with values between 5- and 7-fold for a combined-site analysis. In New Zealand, this apparently inflated clone-within-family variance has been reported previously in the literature (Wilcox 1982). In a review of New Zealand clonal programmes, Shelbourne (1991) detected inflated clonal variances for growth traits, particularly for diameter in old clonal trials. King and Johnson (1991), in a previous analysis of the trial of our study reported the same trend for height at ages 1, 2, and 3 years old. Studies carried out in other forest species (*Populus deltoides* Bartr.) revealed similar results (Ying and Bagley 1976; Foster and Shaw, 1988). A possible cause of this inflated clone-within family variance could be propagation effects commonly referred to as 'C-effects' since they can be a major source of interclone variation (Burdon and Shelbourne 1974). The heterogeneity of the

cuttings used for this trial indicate that propagation effects could be present. Under our model, C-effects were confounded with the clone-within-family variance. Libby and Jund (1962) proposed to isolate C-effects from the clone-within-family variance and to quantify their magnitude by serial propagation. Serial propagation involves hedging of primary cuttings to produce secondary cuttings from them. The secondary cuttings are then used to establish the trial. The primary ramets serve as a level of blocking in the experimental design. There are variable results in the literature with respect to the importance of C-effects, estimated by serial propagation. They varied from non-significant values to values of 8%. Information was available for several forest species for height and rooting traits (Wilcox and Farmer 1968; Borralho, and Kanowski 1995, Paul *et al.* 1993, Farmer *et al.* 1992; Farmer *et al.* 1989; Farmer *et al.* 1988; Cannell *et al.* 1988; Foster et al 1985, Foster *et al.* 1984). Another option is to minimise propagation effects by improving propagation techniques.

Another possible cause for the apparently inflated clone-within-family variance could be the existence of large non-additive effects.

For this particular trial, the effect of sample size should not be disregarded, as only 16 families were included.

The relative proportion of both clone-within-family and family variances for the combined sites was smaller than those obtained for the individual-site analysis. When clonal trials are not replicated over sites, or only one site is taken into account for analysis purposes, the clone-within-family and the family variances are confounded with their interactions with the environment. Replication over sites allows partitioning of the variance in such a way that interactions can be estimated, improving the accuracy in the estimation of each source of variation. More than two sites, however, are considered necessary to obtain accurate estimates of family x location and clone-within-family x location interaction, this eliminating the confounding effects.

3.3. Evolution of Additive, Non-additive, Genetic and Environmental variances

In the distribution of a trait, the variance is often related to the mean. Atchley (1984) warns of the need to be cautious when interpreting genetic variances, particularly when examining changes in genetic parameters of traits associated with growth. To avoid this problem, it has been proposed to expressed genetic variances as the genetic coefficient of variation (CV), defined as the square root of the genetic variance divided by the observed mean of the trait (Comstock *et al.* 1958).

There are few studies in the literature describing clonal variance structures in forest species and analyse the evolution of its components, additive and non-additive variances, for traits of economic interest. Better knowledge of these components and their trends over time are critical to develop efficient tree improvement programmes using clones.

Most of the studies on the evolution of the components of genetic variance in clonal trials relate to height measurements (Paul et al. 1997, Foster and Shaw 1988, Mullin *et al.* 1992, Foster 1988, Balocchi *et al.* 1993, Stonecypher and McCullough 1986). For radiata pine (*Pinus radiata*), there are no publications on the evolution of genetic parameters in clonal trials except for the study for height at ages 1, 2, and 3, based on this trial (Johnson and King 1991).

Four of the studies mentioned previously also dealt with diameter. The information for diameter was more limited due to the lack of measurement over time.

Foster and Shaw (1988) studied a clonal trial of Eastern cottonwood (*Populus deltoides* Bartr.) where diameter at 1.40 m was recorded at ages 3, 4 and 8 years. The authors found that the additive variance for diameter experienced a peak at age 3 (CV = 9%) and then decreased by age 8 (CV = 6%). Dominance variance had a peak at age 3 (CV = 6%) and dropped to 0% by age 8. Epistatic variance followed a reverse pattern, stating from 0% at age 3 and increasing to 9% at age 8 years. At age 8, the epistatic variance was more than double the additive genetic variance.

The study of Stonecypher and McCullough (1986) was based on data from a Douglas-Fir (*Pseudotsuga mensiesii* (Mirb) Franco) clonal trial with diameter measurements at ages 5 and 6 years showing that additive variance at age 5 (coefficient of variation=1.56) was greater than dominance variance (CV= 0.52). At age 6, the additive variance remained nearly at the same level as at age 5 (CV=1.33), but dominance variance trebled (CV=1.64). Epistatic variance was not present at any time.

Foster (1988) reported results from a loblolly pine clonal trial with a single diameter measurement at age 3 years. The study indicated that non-additive variance was greater than additive variance at that age, as evidenced by the ratio of non-additive/additive variance (2.0).

Recently, Paul *et al.* (1997) analysed a Loblolly pine (*Pinus taeda L.*) clonal trial. The results showed that at age 5 dominance variance exceeded additive variance for diameter. Unfortunately, this study as well as the previous one had only data for diameter at a single age.

Additional information on the importance of GCA (general combining ability) versus SCA (specific combining ability) on a trial established with seedling material has been provided by Low (1991), Carson (1991) and Li *et al.* (1996). Low (1991) and Carson (1991) found that in radiata pine for seedling material the expected trend was for GCA or additive variance to increase in relative importance over time for height and diameter.

Li *et al.* (1996) studied a loblolly pine diallel with diameter measurements at ages 5, 6 and 7 years. The study showed that additive genetic variance at the juvenile stages was more important than dominance genetic variance, but the relative proportion between them changed over time. The additive variance increased with age, while the dominance variance increased rapidly before age 5 and then levelled off. The ratios of dominance to additive variance were relatively high at age 5 (0.88), but decreased from then on.

Results from previous studies on time trends of additive and non-additive variance for diameter as well as for height were inconsistent. The only consistent element seemed to be the presence of additive and non-additive variances at most ages. Paul *et al.* (1997) suggested four main causes for such an inconsistency across studies:

- Problems in the design of the trial. The mating design used to generate the genetic entries for the trial restricts the level of partitioning of the genetic variance in all its components, additive, dominance and epistasis. Single-pair mating design, factorial and disconnected factorial mating designs, and diallel and disconnected diallel mating designs have been reported in clonal trials. The quality of field design can affect variances and their significance. A great proportion of the clonal trials described in the literature have been established on a single site, confounding genotype x environment effects with genetic variances.

- Lack of frequent assessments. It is common practice in forest trials to assess them only once when the trials are old enough to make selections from them. In the case of radiata pine measurements are carried out at age 8 years. The lack of frequent measurements at close intervals covering the whole duration of the trial is another reason why trials are not informative enough and trends are not clear.

- Different scales used to express variances. Studies present results on components of variance in two different scales, either in absolute terms or as coefficients of variation. The expression of variance as coefficients of variation would make results more comparable (Atchley 1984, Comstock *et al.* 1958, Foster and Shaw 1988; Paul *et al.* 1997, Stonecypher and McCullough 1986).

- Difference in the presence and frequency of various alleles among species, populations within species and individuals within populations. The presence of different alleles at the species, population and individual level could be investigated with molecular level studies (Paul *et al.* 1997).

Results on the evolution of estimated additive, non-additive, genetic, and environmental variances for diameter from our study are presented in Table II-6.

Genetic parameter	MANAWAHE			KAINGAROA	
	4-year DBH	7-year DBH	10-year DBH	7-year DBH	10-year DBH
$\hat{V}_A^{"}$	23.36	85.22	185.09	140.24	284.08
	(3.44)	(4.18)	(4.66)	(6.99)	(7.10)
$\hat{V}_{non-A}^{'}$	140.94	244.69	744.42	117.16	401.71
	(8.45)	(7.08)	(9.35)	(6.39)	(8.45)
$\hat{V}_G^{'}$	164.30	329.91	929.51	257.40	685.79
	(9.12)	(8.22)	(10.44)	(9.47)	(11.04)
$\hat{V}_E^{'}$	207.93	326.97	836.97	187.28	543.75
	(10.26)	(8.18)	(9.91)	(8.07)	(9.83)

Table II-6 Evolution of estimated additive ($\hat{V}_A^{"}$), non-additive ($\hat{V}_{non-A}^{'}$), genetic ($\hat{V}_G^{'}$), and environmental ($\hat{V}_E^{'}$) variances for diameter at Manawahe and Kaingaroa. Values in parenthesis are variances expressed as coefficient of variation (percent).

The time trends in the variances observed in this study are shown in Figure II-2, and they can be summarised as:

- Additive variance increased with age at Manawahe and doubled at Kaingaroa, but the coefficients of variation were relatively constant.
- Non-additive variance increased at a similar rate to additive variance on both sites. At Manawahe, the coefficients of variation for non-additive variance decreased at age 7 years, but reassumed the increasing trend at age 10.
- Genetic and environmental variance showed much the same trend as non-additive variance on both sites, also expressing a reduction at age 7 at Manawahe.

The lack of measurements at 4 years at Kaingaroa hampered the visualisation of a clear trend, since it is not possible to determine whether the drop in non-additive, genetic and environmental variances at 7 years is a consistent trend or a peculiarity of the Manawahe site.

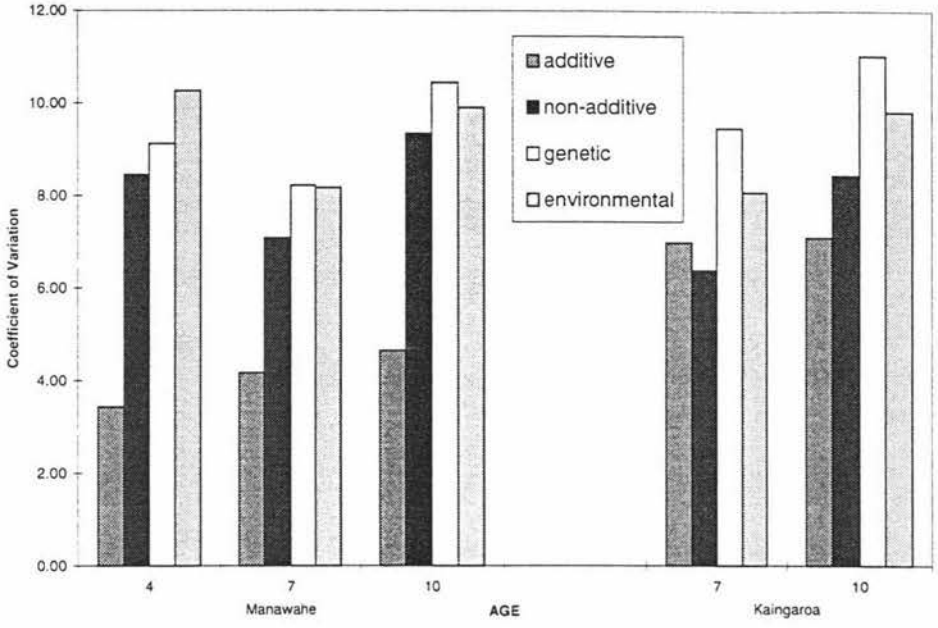

Figure II-2. Evolution of additive, non-additive, genetic, and environmental variances for diameter, expressed as coefficient of variation (percent) at Manawahe and Kaingaroa.

The ratio non-additive variance to additive variance is presented in Figure II-3. The ratio increased from age 7 to 10 years on both sites. In the case of Manawahe, the ratio was always over 1, indicating that the non-additive variance was always greater than the additive variance, particularly at age 4. In Kaingaroa, a ratio of 0.91 at age 7 years showed that the additive variance surpassed the non-additive variance.

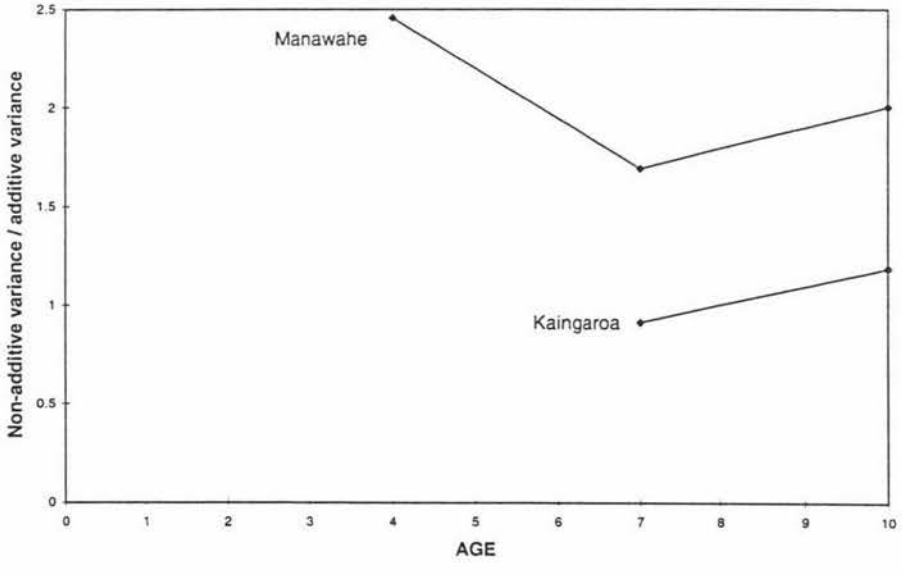

Figure II-3 Ratio between non-additive variance and additive variance.

The ratio genetic variance to environmental variance is shown in Figure II-4. In Manawahe at the age of 4 years the ratio was less than one due to a large environmental variance at that time. Between 7 and 10 years the trend seemed to be different for Manawahe and for Kaingaroa. At Manawahe, even though genetic and environmental variances increased, the increase of the genetic variance was greater than the increase of the environmental variance. The situation was opposite at Kaingaroa.

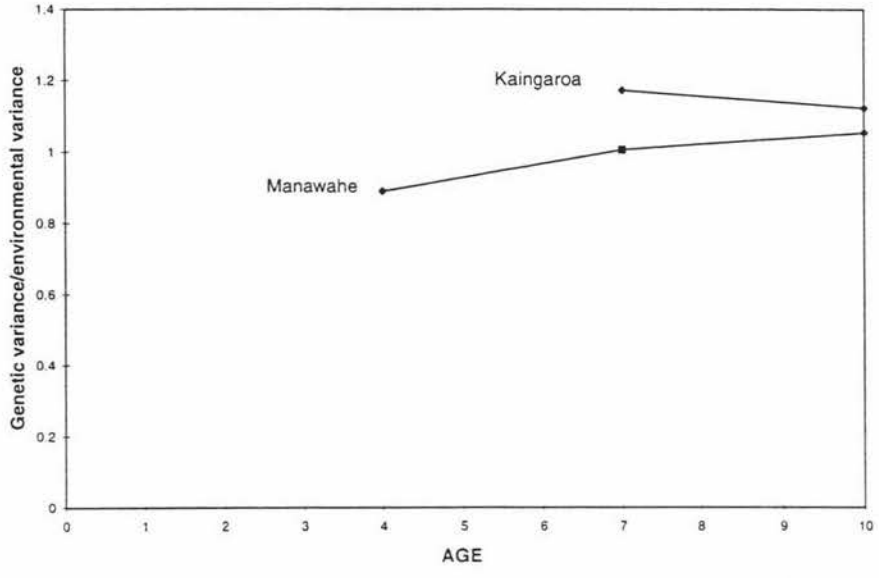

Figure II- 4 Ratio genetic variance to environmental variance.

Under the unrealistic genetic model assumed (the inclusion of C effects would affect the results), and according to the results obtained from this trial, diameter seems to be largely controlled by genes with non-additive effect, which operate at least until age 10 which corresponded to the time of the last assessment.

3.4. Heritabilities

Heritabilities are shown in Table II-7. They were used to obtain the estimated clonal performance and the estimated clonal breeding value.

In the estimation of clonal breeding values, the narrow-sense heritability of family means and the deviation of the family mean from the overall mean were the most important components, whereas the within-family component did not make an

important contribution due to the small magnitude of the narrow sense heritability of clones-within-family means. The importance of family values was demonstrated by the top-ranked clones, most of which came from the same family. In contrast, the within-family component was important in the estimation of the clonal performance, because the deviation of the clonal mean from the family mean was affected by a large broad-sense heritability of clones-within family means. The clones ranked highly on clonal performance were evenly spread over a number of families.

	MANAWAHE			KAINGAROA		COMBINED SITES	
HERITABILITY	AGE 4	AGE 7	AGE 10	AGE 7	AGE 10	AGE 7	AGE 10
$h^2_{\overline{FS}}$	0.34	0.50	0.41	0.58	0.54	0.39	0.51
$h^2_{\overline{C}(FS)}$	0.06	0.11	0.08	0.19	0.16	0.10	0.12
$H^2_{\overline{C}(FS)}$	0.74	0.77	0.74	0.50	0.61	0.72	0.71

Table II-7 Narrow-sense heritability of family means, and clone within family means and Broad-sense heritability of clone within family means for individual sites and combined sites.

Narrow-sense heritabilities were higher at Kaingaroa than at Manawahe. In contrast, broad-sense heritabilities were higher at Manawahe.

3.5. Age-age Phenotypic and Genetic Correlations for Diameter at Individual Sites

Phenotypic and genetic correlations between assessment ages were estimated at each individual site.

As the selection method considered was family selection followed by within family selection, age-age phenotypic and genetic correlations on family means (Table II-8) and separately on clone means (Table II-9) were estimated.

	Manawahe			Kaingaroa	
	Age 4	Age 7	Age 10	Age 7	Age 10
Age 4	1	0.78	0.62	—	—
Age 7	**0.85**	1	0.90	1	0.88
Age 10	**0.74**	**0.95**	1	**0.93**	1

Table II-8 Age-age phenotypic correlations on clone means(values on the top right) and genetic correlations on (values on the bottom left) for diameter.

	Manawahe			Kaingaroa	
	Age 4	Age 7	Age 10	Age 7	Age 10
Age 4	1	0.84	0.61	—	—
Age 7	—	1	0.81	1	0.93
Age 10	**0.66**	**0.64**	1	**0.95**	1

*The value of age 4-age 7 genetic correlation was excluded because it was higher than 1.

Table II-9 Age-age phenotypic correlations on family means (values on the top right) and genetic correlations (values on the bottom left) for diameter at family level.

In general, age-age genetic and phenotypic correlations on family means and clone means were high, particularly at Kaingaroa. The genetic correlations between ages 4 and 7 years at Manawahe exceeded the normal range of a correlation, which could be attributed to a very low genetic variance at age 4.

Owing to the high level of these correlations, it was expected that genetic gain from selection at age 7 years would represent a high percentage of the gain from selection at 10 years.

3.6. Realised Gain from Selection at Age 10 Years for Breeding and Clonal Deployment

Realised gains were estimated for clonal deployment and breeding. For breeding, the 15 best clones (representing the best 10% of the population) ranked on estimated clonal breeding values (ECBV) were selected at age 10 years for integration into the breeding population.

For clonal deployment, the 15 best clones (representing the best 10% of the population) were ranked on estimated clonal performance (ECP) at age 10.

3.6.1. Breeding

Table II-10 shows realised gains for breeding with and without restrictions on the number of clones per family selected. Under the model assumed, realised gains for breeding without restrictions at Kaingaroa (7.59%) were higher than at Manawahe (3.98%). These results can be explained by higher heritabilities of family means and of clones-within-family means at Kaingaroa. The difference between the gain obtained at Manawahe from direct or indirect selection (3.98 and 3.14 respectively) was negligible. The difference between gains from direct and indirect selection at Kaingaroa was more important (1.64%). Kaingaroa was a better site to select clones for breeding. However, making selections based on combined-site information produced gains at each individual site nearly as high as the gains obtained from direct selection at either site.

	Breeding - without restrictions			Breeding - one clone per family		
	Selection based on					
Gain at	Individual site. Manawahe	Individual site. Kaingaroa	Combined sites	Individual site Manawahe	Individual site Kaingaroa	Combined sites
MANAWAHE	3.98	3.14	3.85	1.60	1.19	1.0
KAINGAROA	5.95	7.59	7.22	2.06	4.25	2.52

Table II- 10 Realised gain in diameter for breeding (expressed in % gain).

When the number of clones per family selected required selection of one clone from each family, the level of realised gains reduced sharply, because several clones from poorly ranked families had to be incorporated.

For restricted selection, the realised gain from direct selection at Kaingaroa (4.25%) was higher than the gain from direct selection at Manawahe (1.60%). Selecting for a certain site, based on the ranking for that site (direct selection) always produced more gain than selecting for a certain site based on rankings obtained at another site (indirect selection). Looking at direct and indirect responses on each site, it is evident that if selection was limited to data from one site, the combined gain on both

sites would be maximised by selecting at Kaingaroa. Selections based on information from combined sites yielded less gain than the one obtained when selection was carried out at Kaingaroa.

The level of gain obtained without restrictions on the number of clones per family selected was much higher than the gain with the restriction of selecting only one clone per family. It is unrealistic not to impose restrictions on the number of clones per family selected because it could lead to a reduction in the effective population size, and consequently to inbreeding and its negative consequences. An increase in the number of clones per family, with appropriate management of the breeding population to avoid inbreeding should be considered as a possibility to increase gains.

3.6.2. Clonal deployment

For clonal deployment, the gains were much higher than for breeding (Table II-11). When clones were tested and selected on a site and used in a different site (indirect selection), the realised gain reduced considerably. The realised gain from direct selection at Manawahe was 13.08 %, whereas the gain obtained at Manawahe from indirect selection at Kaingaroa was 8.28% representing a reduction of 4.8 %. Similarly, realised gain from direct selection at Kaingaroa was 15.45%, and the gain at Kaingaroa from selection at Manawahe dropped to 5.52%, representing a reduction of 9.93%.

	CLONAL DEPLOYMENT		
	Selection based on		
% Gain at	Individual site Manawahe	Individual site Kaingaroa	Combined sites
MANAWAHE	13.08	8.28	10.31
KAINGAROA	5.52	15.45	12.87

Table II-11 Realised gain in diameter for the clonal deployment strategy (expressed as % gain).

When analysing the realised gain obtained at each individual site taking into account the combined heritabilities available from both sites, it was evident that gains at Manawahe and Kaingaroa were less than the maximum gains obtained at each site from selection at each individual site. With combined site heritabilities, gains appeared to be similar for each site indicating more stability across sites.

Considering direct and indirect responses on each site, it is evident that if selection was limited to one site, the combined gain on both sites would be maximised by selecting at Kaingaroa. Unlike in the case of using clones for breeding, selections based on information from combined sites gave gains close to the ones obtained when selection was carried out at Kaingaroa.

3.7. Genotype x Environment interaction

The analysis of this trial under the combined-site model, showed highly significant but no large clone x location interaction effects, and significant family x location interaction effects, which suggested some degree of genotype x environment interaction (G x E). G x E interaction can be explained as the phenomenon by which the performance of genotypes relative to each other varies according to the environment, so that superior genotypes in one environment may not be correspondingly superior elsewhere (Burdon, 1977). It is important to understanding G x E interaction because it has implications in the magnitude of genetic gains achieved by selection.

If the magnitude of the genotype by environment interaction is important, the breeding programme might need to be subdivided into several breeding regions, each one having a different breeding population and/or production population. This approach can only be justified when there are very large G x E interactions, because the cost of running several breeding populations is very high. Carson (1991) concluded after analysing a trial of seedling material established on 11 locations covering the North and the South Island that only one breeding programme was required to improve radiata pine in New Zealand. The results of the present study confirm this with respect to family G x E effects, and the level of G x E for clones

were low enough not to indicate a need for a different set of clones for the sites tested (although these sites would be considered similar for the Central North Island region).

For clonal deployment there are two different options, select for maximum genetic gain on each site or select for clonal stability across a planting region (or regions). Selecting for stability requires clonal testing over several sites. The gains obtained at each site might be only part of the potential gain to be obtained by matching clones with sites. Selecting clones for specific site types will maximise gains, but there will an important additional cost.

One of the objectives of this study was to compare ranking across sites. Unfortunately, since the trial was established on only two sites, further work is required before conclusions can be generalised. Figure II-5 shows the 15 best clones selected for diameter at Manawahe based on their estimated clonal performance and their respective rankings at Kaingaroa. The best clone at Kaingaroa proved to be the best one at Manawahe. Some six clones of the 15 best clones were stable, with small fluctuations in ranking, but still ranking within the best fifteen clones at both sites. The ranking of the nine remaining clones from the best 15 clones, fell at Kaingaroa changing from rankings within the first fifteen to rankings in the order of 140.

A similar trend was observed when clones were ranked at Kaingaroa (Figure II-6). Even though some clones within the best 15 at Kaingaroa performed worse at Manawahe, the ranking did not fall under 80.

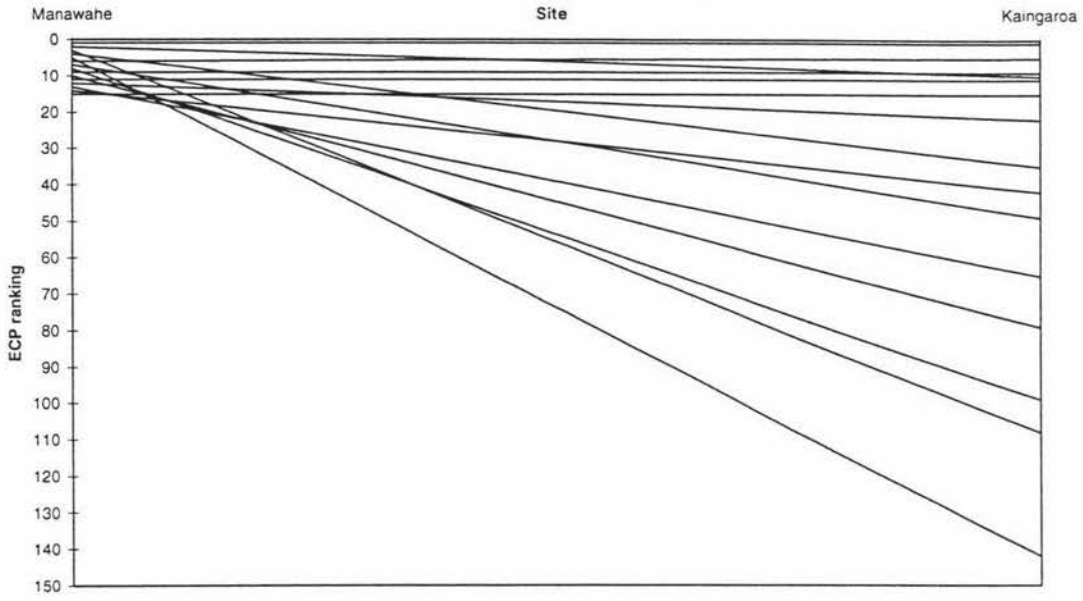

Figure II-5 15 best clones selected at Manawahe ranked on estimated clonal performance for diameter, and their respective rankings at Kaingaroa.

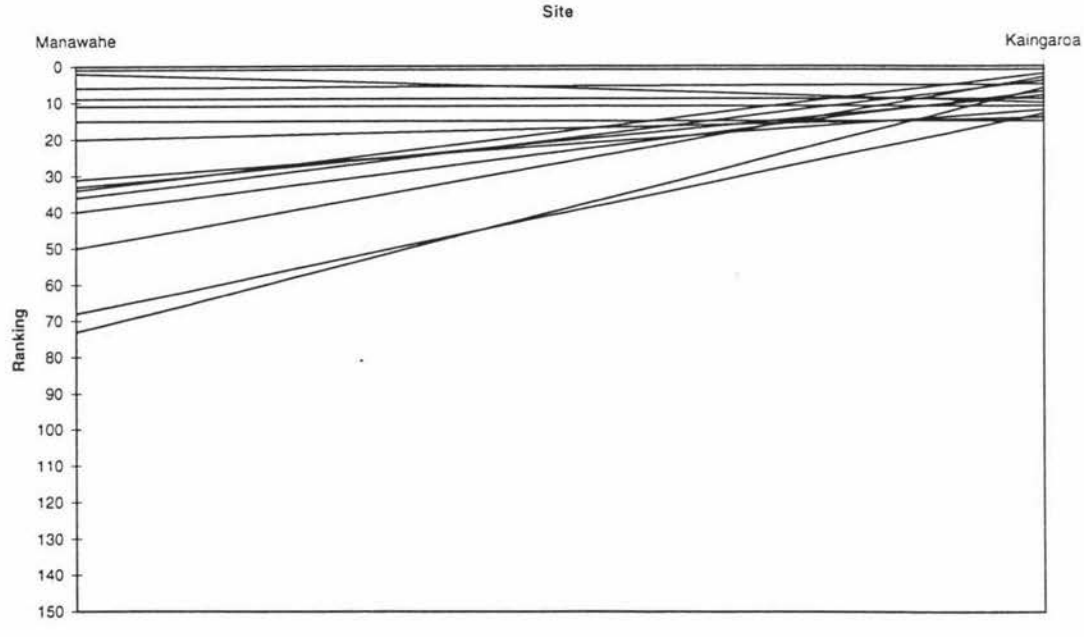

Figure II-6 15 best clones selected at Kaingaroa ranked on estimated clonal performance for diameter, and their respective rankings at Manawahe.

The selections based on combined-site information corresponded to the most stable clones across sites, but some clones still had substantial changes in ranking between sites (Figure II-7). However, the 15 best clones selected on combined site information never ranked less than 40 on either site.

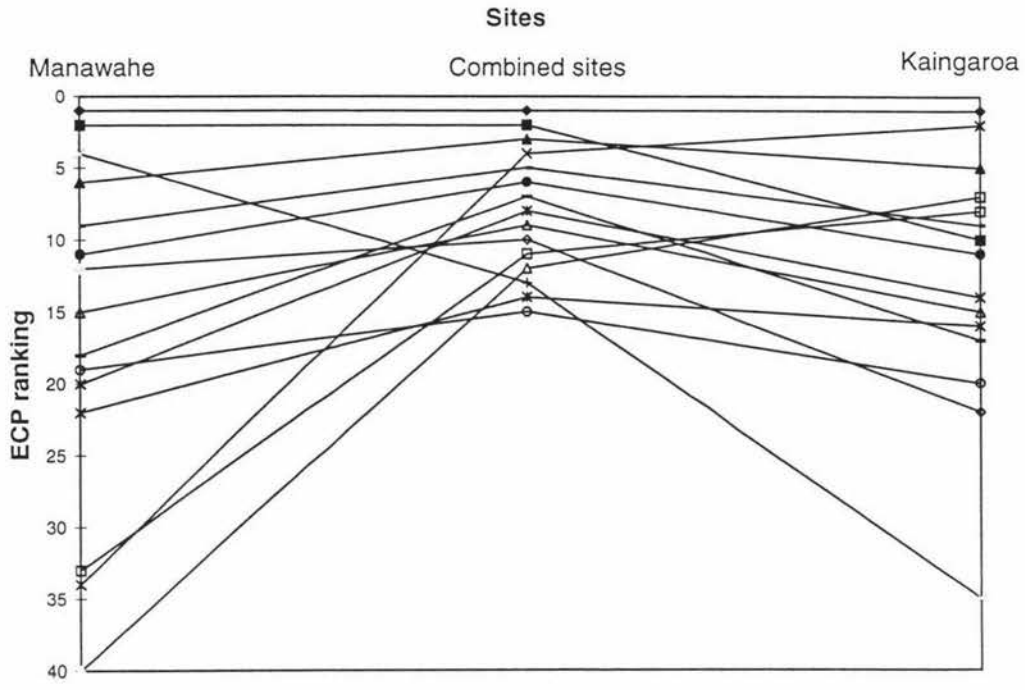

Figure II-7 15 best clones ranked on estimated clonal performance for diameter based on combined site information and their respective rankings at Manawahe and Kaingaroa.

Comparing the ranking of selected clones for breeding, changes in ranking were also observed and follow a similar trend to the one observed for clonal deployment, but the changes were more pronounced due to the restrictions on the number of clones per families.

3.8. Early Selection

3.8.1. Realised gain for early selection

Many authors have recognised the benefits of early selection (Lamberth, 1983, Bau-Jen Jiang 1985). Selecting before the time of the last assessment shortens the breeding and testing cycles, allows more rapid turn-over of generations, and consequently increases the gain per unit time. Depending on the level of genetic

control of a trait, clones that perform well at an early age could be found to perform poorly at a later age, reducing the total gain considerably. There is a trade-off between the optimal age for accurate estimation of genetic parameters conducive to higher total gains, and the age to make selections to maximise the gain per unit time. For radiata pine in New Zealand, it is common practice to select on performance at age 8 years, since there is good correlation between age 8 and harvest age (King and Burdon, 1991). In this trial, the last assessment was delayed until age 10 years for research purposes.

	MANAWAHE			KAINGAROA	
	% Gain				
STRATEGY	AGE 4	AGE 7	AGE 10	AGE 7	AGE 10
Clonal deployment	5.10	8.84	13.08	8.20	15.45
Breeding with restrictions (1clone/family)	0.51	1.16	1.60	2.84	4.25

Table II-12 Realised gains in diameter at age 10 predicted for selection at age 4 and 7 at Manawahe, and age 7 at Kaingaroa (expressed as % gain).

Realised gains at Kaingaroa for breeding and clonal deployment were higher than at Manawahe at all ages (Table II-12). The gain obtained from selections made at Manawahe on estimated clonal performance at age of 4 years was 5.1% , representing 38% of the gain obtained if selection is carried out at age 10 years. The gain from selecting clones at age 7 years was 8.9%, constituting 67.5% of the gain at age 10.

At Kaingaroa, the gain from selection at age 7 was a 54.4 % of the potential gain obtained by selecting at age 10.

Considering selection for breeding restricted to one clone per family at Manawahe, selections made at the age of 4 and 7 years represented 31.9 and 72.5 % respectively of the gain obtained by selecting at age 10. The gain obtained by selecting at age 7 at Kaingaroa corresponded to 66.8% of the gain attained selecting at age 10.

The breeding cycle in radiata pine is 13 years. The main limiting factor to shorten this cycle is flowering, because it only occurs after age 8. For that reason, early selection at age 4, 7 or 10 years does not have an impact in the gain per unit time.

There is still much to research on accelerating flowering techniques, and early flowering would make early selection a very interesting option.

The cycle for clonal deployment extends three years after selections are made. That period is needed to bulk up clonal stoolbeds to produce sufficient number of cuttings for deployment in the forest. In that case, the cycle selecting at age 4 will be 7 years, selecting at 7 years will be 10 years, and selecting at age 10 will be 13 years.

At Manawahe, when selection was carried out at age 4, the gain was 0.7% per year. Delaying selection until age 7 increased the gain to 0.9% per year. Finally, when clones were selected at age 10 the gain was 1% per years.

At Kaingaroa, selection performed at age 7 for clonal deployment produced 0.82 % gain per year. Selection at age 10, increased the gain per year to 1.19%. Based on these results, it may be justified to wait until age ten to select clones and therefore maximise gains.

3.8.2. Comparison between realised gain and predicted gain

Gains from direct selection at each individual site at age 10 were predicted for breeding and clonal deployment (Table II-13). In most cases, the predicted gain was higher than the realised gain, but the magnitude of the difference was relatively small. The differences could reflect sampling effects arising from the small size of the population. They could also be due to inaccuracy in the estimation of genetic parameters.

	% GAIN IN DBH AT AGE 10					
	Breeding no restrictions		Breeding one clone/family		Clonal deployment	
	Predicted	Realised	Predicted	Realised	Predicted	Realised
MANAWAHE	4.49	3.98	0.67	1.60	20.23	13.08
KAINGAROA	10.69	7.59	3.57	4.25	16.29	15.45

Table II-13 Comparison between predicted and realised gain in diameter.

4. CONCLUSIONS

The single-pair mating design used to create the family structure in this trial is inadequate to estimate genetic parameters. It only allows partitioning of the genetic variance into additive and non-additive variance, without further partitioning of the non-additive variance into effects resulting from dominance and epistasis.

The genetic model assumed does not come close to representing the true biological performance of clonal material and it is necessary to find a better alternative. Some of the causes are the neglect of C-effects in the model. Any future trial should include serial propagation to evaluate the magnitude of those non-genetic propagation effects and to eliminate the bias they cause in the estimation of genetic parameters.

More precise estimates of genetic parameters can be obtained by increasing the sample size, at both the family and clone-within-family level.

Study of the evolution of components of genetic variance and other genetic parameters probably requires annual or biennale assessments. The results provided by this trial indicate a large non-additive component over time, which can be due to important dominance or epistasis variance, or to C-effects confounded with the non-additive component of variance.

In this trial, low realised gains were obtained from selection of clones for breeding, particularly when restrictions on the number of clones per family to be selected were imposed. When restrictions are relaxed, the realised gain can be increased, but care must be taken to avoid excessive inbreeding or narrowing of the genetic base.

High levels of gains could be achieved by clonal deployment, provided the non-additive effects are repeatable.

Clonal tests should be repeated on several sites to contribute to accurate estimates of genetic parameters and to investigate G x E interaction. Where

there is a large amount of G x E interaction, the deployment of specific clones for specific sites can maximise genetic gains, although testing costs may be substantial.

In this trial it was possible to obtain genetic gains from early selection, but expected maximum gains for breeding were obtained from selecting at age 10.

CHAPTER III
1987 CLONES IN FAMILIES TRIAL

1. INTRODUCTION

The '1987 Clones-in-Families Trial' was established by the New Zealand Forest Research Institute using juvenile ortet material. This trial was scheduled for a full assessment at age 8, but the presence of Dothistroma needle blight infection at age 3 years offered the possibility to estimate genetic parameters for resistance to this disease and their evolution over time in a trial with adequate family structure for that purpose. Data from the first assessment were analysed by Carson (1990).

Dothistroma needle-blight is a fungal disease caused by *Dothistroma pini* Hulbary. This disease affects large areas planted in radiata pine in New Zealand (Carson, 1989). It can be overcome with aerial applications of copper oxychloride (Maclaren, 1993) and by breeding for resistance to Dothistroma (Carson and Carson, 1989).

2. MATERIALS AND METHODS

2.1. Mating and Experimental Design

Two independent mass selections of phenotypically superior trees were made in New Zealand to start the Breeding Population of Radiata Pine, the '850' series and '268' series. In 1986, 24 unrelated individuals from the '268' series, and clone 55 from the '850' series were crossed in a 3x3 Disconnected Factorial Mating Design to produce the families for the '1987 Clones in Families Trial'. Parents were selected on their good growth and form performance in a progeny trial at age 10. As a result of the mating, 9 full-sib families were produced within each factorial. Ten clones per full-sib family were selected at the age of 6 months and propagated vegetatively by fascicle cutting. The best 6 ramets per clone were planted in the field (Figure III-1). The ramets were not fully homogeneous due to insufficient experience with the propagation technique.

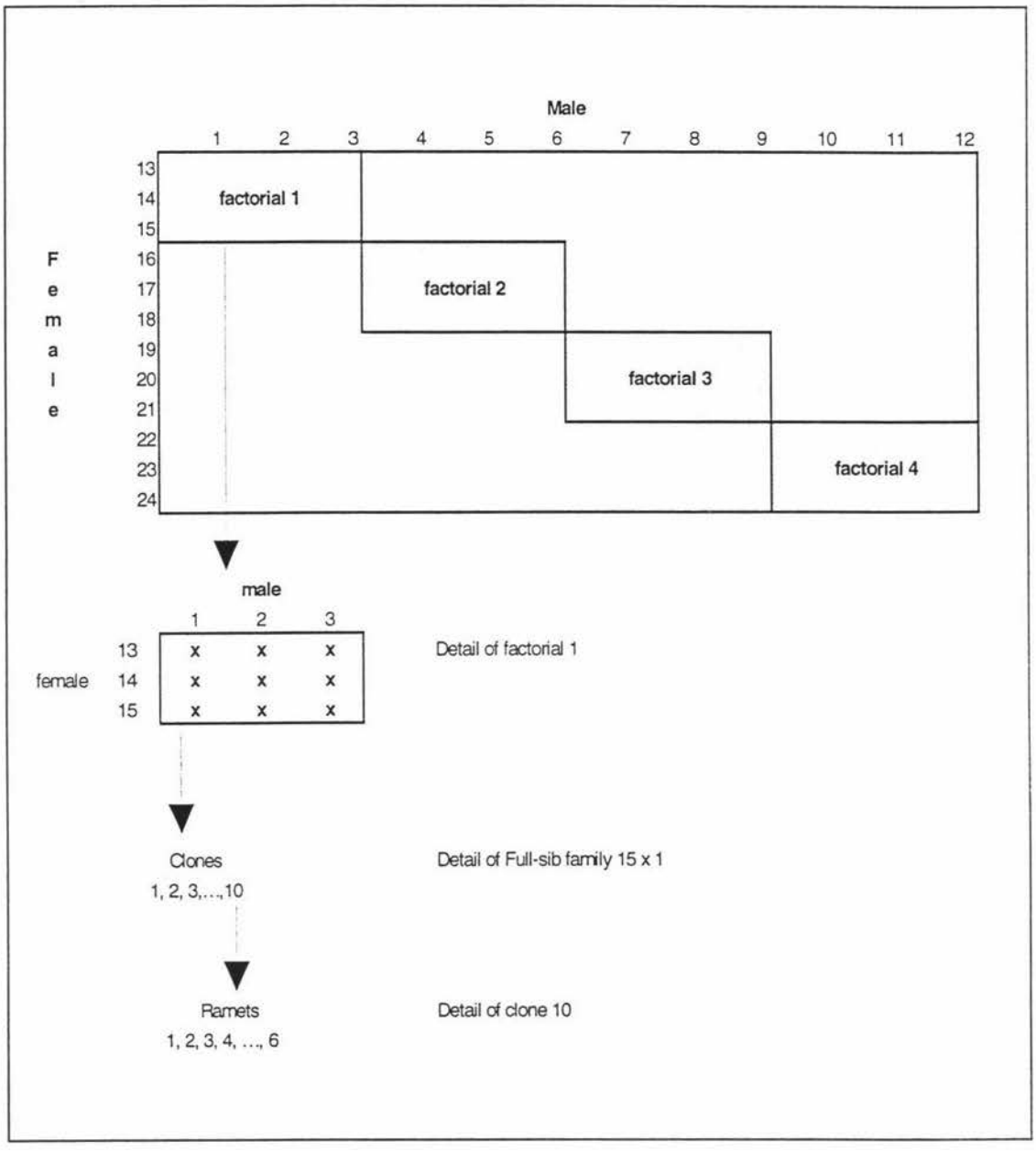

Figure III-1. Mating design of 1987 clones in families trial and amplification of clones by vegetative propagation.

The trial was established on a single site, Compartment 324, an ex-forest site in Kaingaroa Forest prone to Dothistroma needle blight.

The experimental design was Sets-in Replications using Single-Tree-Plot (STP) with 6 replications. Seedlings from control-pollinated seed from the same parents used in the factorial mating were incorporated to the trial for comparison purposes.

The present analysis was based on assessments of *Dothistroma pini* infection, expressed as percentage of needles infected (DTP) subjectively scored in 5% steps. The criteria 'Percentage of Dothistroma needle infection' or DTP was considered to indicate the degree of tree resistance to Dothistroma infection. Higher DTP indicated less tree resistance to Dothistroma infection.

Assessments were performed annually from ages 3 to 5 years.

2.2. Data Analysis

The analysis of this trial was based on data from clonal material only, since the comparison between seedlings and clones exceeded the objectives of this study.

Analysis of variance was performed using SAS GLM Procedure (1990) to test a linear model including replication, female parent, male parent, clones-within-family, female parent x male parent interaction, and interaction terms with replication. All main effects and interactions were assumed random. Sets were omitted from the analysis, because they were confounded with factorials. A certain degree of imbalance in the dataset often seen in forest trials, was present at all ages. The imbalance was due to a reduction in the survival of some clones however, all families were represented at ages 3, 4 and 5 years.

The statistical model assumed was:

$$Y_{ijklmn} = \mu + R_i + A_j + M_{k(j)} + F_{l(j)} + C_{m(kl)} + MF_{kl(j)} + RA_{ij} + RM_{ik(j)} + RF_{il(j)} + RMF_{ikl(j)} + e_{ijklmn} \quad (15)$$

Y_{ijklmn} is the nth observation of the *mth* clone in the *jth* factorial in the *ith* rep involving the *kth* male parent and the *lth* female parent

μ is the overall mean

R_i is the effect of the *ith* replication

A_j is the effect of the *jth* factorial

$M_{k(j)}$ is the effect of the *kth* male parent in the *jth* factorial

$F_{l(j)}$ is the effect of the l^{th} female parent in the *jth* factorial

$C_{m(kl)}$ is the effect of the *mth* clone in the *jth* factorial involving the *kth* male parent and the *lth* female parent

RA_{ij} is the interaction effect of the *ith* replication and the *jth* factorial

$MF_{kl(j)}$ is the interaction effect of the *kth* male parent and the *lth* female parent in the *jth* factorial

$RM_{ik(j)}$ is the interaction effect of the *ith* replicate and the *kth* male parent in the j^{th} factorial

$RF_{il(j)}$ is the interaction effect of the *ith* replicate and the *lth* female parent in the *jth* factorial

e_{ijklmn} is the random error

This model was used for 'percentage of Dothistroma needle infection (DTP)' at ages 3, 4, and 5 years.

REML (Restricted maximum likelihood) variance components and their respective standard errors were obtained using SAS Mixed Procedure (1990) for the model described above.

The genetic model assumed was the classical additive-dominance-epistasis model (Falconer 1981). The model can be expressed as:

$$G = A + D + I \qquad (16)$$

G is the genotypic value

A is the additive effect or breeding value

D is the dominance deviation

I is the epistatic deviation

The factorial mating design followed by vegetative propagation of the clones generated three-level family structure: full-sib families, half-sib families, and clones within full-sib families. This structure allows the genetic variance to be partitioned

into additive, dominance and epistatic variances. The underlying assumptions described by Cromstock *et al.* (1958) have been mentioned in the previous chapter. In this case, 'C effects' have also been considered negligible.

Estimates of additive, non-additive and epistatic variances were obtained by equating the observational components of phenotypic variance to their causal components or genetic expectations (Falconer 1981). The genetic expectations of full-sib families, half-sib families, and clones within full-sib families are (Frampton and Foster 1993):

$$\sigma^2_M = \sigma^2_F = COV_{HS} = \tfrac{1}{4}V_A + \tfrac{1}{16}V_{AA} + \ldots \quad (17)$$

$$\sigma^2_{MF} = \tfrac{1}{4}V_D + \tfrac{1}{8}V_{AA} + \tfrac{1}{8}V_{AD} + \tfrac{1}{16}V_{DD} + \ldots \quad (18)$$

$$\sigma^2_{C(MF)} = \tfrac{1}{2}V_A + \tfrac{3}{4}V_D + \tfrac{3}{4}V_{AA} + \tfrac{7}{8}V_{AD} + \tfrac{15}{16}V_{DD} + \ldots \quad (19)$$

COV_{HS}	Covariance of half-sib families
σ^2_M	Variance of male parents
σ^2_F	Variance of female parents
σ^2_{MF}	Variance of male x female parent interaction
$\sigma^2_{C(MF)}$	Clone-within-full sib family
V_A	Additive variance
V_D	Dominance variance
V_{AA}, V_{AD}, V_{DD}	Additive x additive, additive x dominance, dominance x dominance epistatic variances, ...etc

In equations (17), (18), and (19) interactions between genes at different loci (V_{AA}, V_{AD}, V_{DD}) were included under the general term epistasis variance. Epistatic variance also included interaction between more than two genes, even though they have not been included explicitly in the equations.

According to the genetic expectations mentioned previously and neglecting the interactions additive by additive, included within the variance component of male and female, the additive variance was calculated in three different ways:

$$V_A = 4(V_F) \quad (20)$$

$$V_A = 4(V_M) \quad (21)$$

$$V_A = 2(V_F + V_M) \quad (22)$$

Further conclusions in the remainder of this contribution were based on additive variance estimated according to equation (22).

The dominance variance was estimated from the variance of female-male interaction within factorials. Thus,

$$\hat{V}_D = 4(\hat{V}_{MF}) \quad (23)$$

The epistatic variance (V_I) was calculated as:

$$\hat{V}_I = \hat{V}_{C(MF)} - (\hat{V}_M + \hat{V}_F) - 3\hat{V}_{MF} \quad (24)$$

The genetic variance was calculated as the sum of variance of male, variance of females, variance of the interaction male-female, and clones-within-family.

3. RESULTS AND DISCUSSION

The mean level of Dothistroma needle infection (DTP) varied each year. It was 22.1% at the age of 3 years, reduced to14.7% at 4 years, and increased to 43.3% at 5 years.

3.1. Analysis of Variance

Results from the analysis of variance are summarised in Table III-1 and the complete analysis is shown in Appendix III-1. Factorial effect was non-significant at all ages. Female within factorial effect was highly significant at ages 3, 4 and 5 years, whereas male within factorial effect was non-significant for all three ages.

The interaction female x male was highly significant at age 3 years and significant at 4 years, but non-significant at age 5 years. Clones-within-family effect was highly significant at all three ages.

Source of variation	Age 3		Age 4		Age 5	
	F value	Sign.	F value	Sign.	F value	Sign.
Replication	0.52	ns	18.12	**	11.14	**
Factorial	0.76	ns	0.36	ns	0.14	ns
Replication*factorial	6.54	**	1.82	ns	2.19	*
Female(factorial)	5.44	**	6.92	**	10.49	**
Male(factorial)	1.27	ns	1.44	ns	2.25	ns
Female*Male(factorial)	2.35	**	1.70	*	1.26	ns
Replication*female(factorial)	1.80	*	0.91	ns	1.17	ns
Replication*male(factorial)	1.51	ns	1.12	ns	1.39	ns
Replication*female*male(factorial)	0.83	ns	1.25	ns	0.75	ns
Clone(female*male(factorial))	2.21	**	3.41	**	4.32	**

Sign.=Significance *= significant at α= 0.05, **= significant at α=0.01, ns= non-significant.

Table III-1. Summary of the analysis of variance for percentage of Dothistroma needle infection at ages 3, 4, and 5 years.

An important issue was that at the nursery there was some sort of selection when only the most vigorous ramets were chosen to be planted in the field. Measurements of some appropriate variable should be made at the nursery stage to use it afterwards as a covariate in the analysis.

3.2. Estimate of Variance Component

The phenotypic variance for percentage of Dothistroma infection increased with age (Table III-2). For ages 3, 4 and 5 years, the variance component for female within factorial was much higher than for male within factorial representing a 4.8, 8.9 and 10.7% respectively. The reason for that difference between variance of male parents and female parents is unknown, but some hypothesis can be postulated:

- mislabeling of some pollen used in the mating
- sampling error
- maternal effects
- mitochondria inheritance. In most conifers, mDNA is transmitted by the female parent (Harrison and Doyle 1990, Owens and Morris, 1991)

The variance component for clone-within female x male nested within factorial, which is the within-family variance increased with age, accounting for 14.97, 24,8 and 31.81 % of the phenotypic variance at ages 3, 4 and 5 years respectively.

Variance component	Age 3		Age 4		Age 5	
	Estimate	%	Estimate	%	Estimate	%
Replication	0	0	12.64 (8.5)	7.43	8.29 (5.7)	4.53
Factorial	0	0	0	0	0	0
Replication*factorial	6.87 (2.6)	7.36	1.07 (1.1)	0.63	1.96 (1.2)	1.071
Female(factorial)	8.56 (4.8)	9.18	16.50 (8.9)	9.69	21.86 (10.7)	11.95
Male(factorial)	0.81 (1.5)	0.87	1.06 (2.9)	0.62	3.93 (3.4)	2.15
Female*Male(factorial)	2.90 (1.9)	3.11	5.30 (4.3)	3.11	1.30 (3.2)	0.71
Replication*female(factorial)	1.20 (0.8)	1.29	0	0	0	0
Replication*male(factorial)	0.59 (0.6)	0.63	0.86 (1.3)	0.50	0.08 (0.8)	0.04
Replication*female*male(factorial)	0	0	2.86 (1.7)	1.68	0	0
Clone(female*male(factorial))	13.96 (2.0)	14.97	42.23 (4.7)	24.8	58.17 (5.9)	31.81
Error	58.34 (2.2)	62.58	87.69 (3.4)	51.52	87.28 (3.2)	47.73
TOTAL	93.23	100	170.21	100	182.87	100

Table III-2 . Estimates of variance components, standard errors (in parenthesis) and percentage of the phenotypic variance for 'Percentage of Dothistroma needle infection' at ages 3, 4 and 5 years.

3.3. Estimation of Additive, Dominance and Epistatic Variances

Estimates of additive, dominance and epistatic variances for percentage of Dothistroma needle infection are shown in Table III-3. The mating design chosen for this trial, even though better than other designs without family structure, did not provide accurate estimates of genetic variance components. The fact that the trial was established in only one site confounded the estimation of clone and family variance components with clone-by-site and family-by-site interactions respectively.

Component	Age 3 years	Age 4 years	Age 5 years
$\hat{V}_A'$ (as 4 VM)	3.24	4.28	15.76
$\hat{V}_A'$ (as 4 VF)	34.24	66.04	87.52
$\hat{V}_A'$ (as 2(VF+VM)	18.75	35.15	51.63
$\hat{V}_D'$ VD	11.60	21.22	5.21
$\hat{V}_I'$	-4.10	8.74	28.45
$\hat{V}_G'$	—	65.11	85.29

Table III-3 Estimates of additive, dominance, and epistatic variances.

The theory indicates that the estimates of additive variance obtained by equations(19), (20) and (21) should be equivalent. The variance of female parents was much larger than the variance of male parents (Table III-2), and as a consequence the estimates of additive variance varied depending on how they were calculated. The difference in the estimates depending on the equation used could be due to bias in the sampling of female and male parents, since the parents come from a selected population. This hypothesis is unlikely since the trait considered in this study was not subject to selection. At the time the '268' and '850' series were selected Dothistroma needle blight was not a problem in New Zealand. Selection was on growth traits like diameter at breast height, and for that trait the variance of females and males was not significantly different.

The negative value of the epistatic variance at an early age could be due to equation (24) representing only part of the total epistatic variance. Replacing equations (17), (18) and (19) in equation (24) it can be seen that the estimate of epistasis only included:

$$\frac{1}{4} V_{AA} + \frac{1}{2} V_{AD} + \frac{3}{4} V_{DD} + \ldots \quad (25)$$

The negative value of epistatic variance was assumed to represent zero.

3.4. Evolution of Additive, Dominance and Epistasis Variance Over Time

The evolution of the components of genetic variance expressed in absolute values (Figure III-2), showed an increase of the additive variance, an initial increase of the dominance variance at age 4 followed by a reduction at age 5, and an increase of the epistatic variance with age. When the components of genetic variance were expressed as genetic coefficient of variation (Cromstock *et al.* 1958) and therefore the variances corrected for the level of Dothistroma infection, the evolution of additive, dominance and epistatic variances changed completely (Figure III-3).

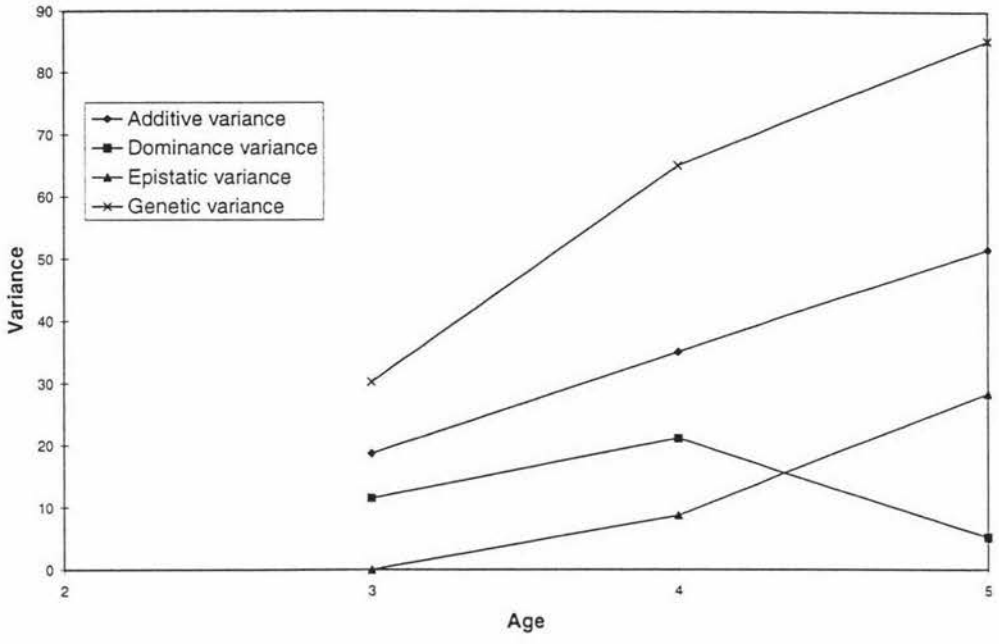

Figure III-2 Evolution of components of genetic variance (expressed as absolute values) over time.

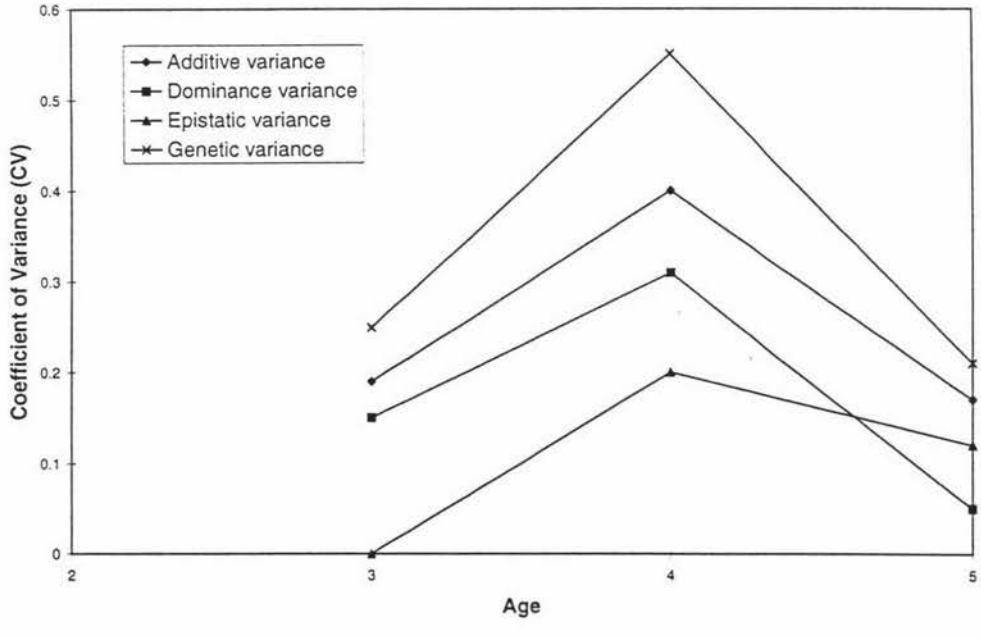

Figure III-3 Evolution of components of genetic variance (expressed as coefficient of variation) over time.

Dothistroma resistance, unlike other traits like diameter, is strongly influenced by climatic conditions and silvicultural treatments (Maclaren, 1993). Its evolution over time was confounded with the level of infection which varies with time. It is

possible however, to express the ratios between additive, dominance and epistatic variance and the genetic variance at each age (Table III-4). The ratio for age 3 years was not calculated due to the underestimation of the epistatic variance conducive to an estimate with negative value. The additive variance did not experience large changes in magnitude with age, and was the main component of the genetic variance. The ratio dominance variance to genetic variance reduced particularly at age 5, whereas the ratio epistatic variance to genetic variance increased from age 4 to age 5 years.

Ratio	Age 4 years	Age 5 years
Additive variance/genetic variance	53.9%	60.5%
Dominance variance /genetic variance	32.5%	6%
Epistatic variance/genetic variance	13.4%	34%

Table III-4 Additive, dominance and epistatic variances for percentage of Dothistroma needle infection expressed as percentage of the genetic variance.

In the model considered, 'C effects' were neglected. 'C effects' can cause significant bias in the parameter estimation (Burdon and Shelbourne, 1974). Libby and Jund (1962) proposed serial cloning to minimise this problem. Serial cloning or serial propagation consists of taking cuttings from the original clones (primary ramets), planting and hedging them. The cuttings obtained from those hedges (secondary cuttings) are then rooted and used to establish the trial. By planting several secondary ramets per clone, it is possible to estimate the variance among primary ramets within clones which is an estimate of the 'C effects' variance. The confounding influence of 'C effects' can also be minimised with some expense by using simple horticultural techniques (Foster *et al.*, 1984).

4. CONCLUSIONS

Future trials should be planted in more than one site, and special attention should be paid to having a very strong family structure, such as the one provided by a factorial or a diallel design.

The high variance between females compared with the variance between males for the trait Dothistroma resistance opens an interesting question. To better understand

the resistance to Dothistroma infection, a trial with a different approach to the problem may be required.

The estimates of additive, dominance and epistatic variances in theory are accurately estimated with the test design used in this trial. However, estimates are still suspected to be inaccurate due to potential C effects' of unknown magnitude. The assumption of negligible 'C effects' should be replaced by a good experimental design which tests the existence and quantifies the magnitude of 'C effects'. Inaccuracies in the estimates of the additive, dominance and epistatic variances generate noise, jeopardising the predictions of genetic gains.

The evolution of components of genetic variance over time for Dothistroma resistance was confounded with the level of infection. Understanding the trait resistance to Dothistroma infection is more complex than other growth traits, such as height and diameter, and may require a different approach.

CHAPTER IV

IMPLICATIONS OF THIS STUDY AND FUTURE DIRECTIONS

When the new series of clonal trials was established in 1986-1987 by the Forest Research Institute, it was expected that they would answer most of the questions raised at that time about clonal testing. After 10 years, the analysis of those trials showed that they have not been able to address all the issues and several questions still remain.

The analysis of these trials has provided a better insight into clonal testing and its deficiencies, but also offered good leads on how to go about clonal testing in the future. The new series of trials has provided a range of family structures by using different mating designs. Trials have been established on one or few sites, measurements have been done fairly frequently in some trials and very sporadically in others, and growth- and disease-resistance traits have been analysed. Developments in vegetative propagation techniques have improved the quality of the cuttings planted in the field, which clearly had an impact on the survival rates of cuttings. The use of 6-months-old seedlings as ortets hopefully removed the confounding effects of ageing.

The most valuable contributions made by this series of trials were:

- indicate the need to improve the accuracy in the estimation of genetic parameters
- highlight the importance of frequent measurements to determine trends in the genetic parameters
- show the difference in gains obtained from selection for breeding and for clonal deployment.

The improvement in the accuracy of the estimates of genetic parameters has a critical impact on the prediction of genetic gains. This improvement can be achieved by choosing an appropriate mating design to provide a strong family structure,

establishing the clonal trial according to a suitable field design, and reducing or minimising non-genetic 'C-effects'.

Results from this study clearly indicate that factorial mating design allows partitioning the estimated genetic variances in its additive, dominance, and epistasis components. In contrast, single-pair mating design offers a very limiting family structure and it is advisable not to use it.

Even with a very good factorial design, involving a large number of families and clones per family to minimise sampling error, it is indispensable to reduce or eliminate C-effects by serial propagation or improving propagation techniques. The disadvantage of serial propagation is that it will cause a delay in the establishment on the trial in the field.

The design of the clonal trials established by the Forest Research Institute was sets in replications with single-tree plots, the same as progeny tests in the breeding programme. Families and clones are confounded with sets. To avoid this problem it is necessary to explore some other designs. An interesting option is a connected incomplete block design, referred to as alpha design (Williams and Matheson 1994) currently used in Australia for clonal trials with good success. The inconvenience of alpha designs is the complexity of the trial layout, especially when a large number of families are involved.

Another important consideration is the replication of clonal trials over sites. To provide information with statistical value, it is necessary to replicate the trial over a large number of sites. The only limit to the number of sites is cost. Replication over sites permits the removal of the confounding effects of G.x.E interaction. It also contributes to a better understanding of G x E interaction and its effect on clonal performance.

Traditional methods to estimate genetic and phenotypic correlations across sites have proven to be inadequate in clonal trials and more experimental andtheoretical work needs to be done in that area.

As far as determining trends of the components of genetic variance over time, the need to have the right mating design combined with frequent trial assessments have been emphasised in this study. It is also important to express all results in a comparable scale, since most of the published information is inconsistently expressed. In the case of traits such as Dothistroma resistance where the level of infection is not constant over time, it is necessary to devise a different approach to study trends over time.

It would be desirable to have annual or biannual assessments and continue them until age of final assessment.

The information provided by these trials seems to indicate that there is an important non-additive component for diameter, which could be used advantageously if the non-additive effect is repeatable.

With respect to the gains obtained from selection of superior clones, it has been noticed in this study that gains for clonal deployment are large and can be best utilised by matching clones with sites. Gains for breeding were less important with cloning contributing to better estimates of family genetic parameters, but selections of clones within families contributing very little to the gains.

Series of carefully planned clonal trials are necessary to resolve all the issues mentioned previously. The kind of trial required at the moment will require a large investment. This investment is fundamental to the future of clonal forestry in radiata pine.

APPENDIX II-1

1. Estimation of additive, non-additive and genetic variance

$$V_G = \sigma^2_{FS} + \sigma^2_{C(FS)}$$
$$V_{A''} = 2(\sigma^2_{FS})$$
$$V_{NA} = V_G - V_{A''}$$

V_G = Total genetic variance
$V_{A''}$ = Variance of additive effects (crude estimate)
V_{NA} = Variance of non-additive deviations
σ^2_{FS} = Variance among full-sib families
$\sigma^2_{C(FS)}$ = Variance among clones within full-sib families

2. Estimation of narrow-sense heritability of family means for individual sites

$$h^2_{\overline{FS}} = \frac{\sigma^2_{FS}}{\sigma^2_{FS} + \frac{1}{n_{C(FS)}}\left(\sigma^2_{C(FS)}\right) + \frac{1}{n_{R(C)}n_{C(FS)}}\left(\sigma^2_e\right)}$$

$h^2_{\overline{FS}}$ = Heritability of family means
σ^2_{FS} = Variance among full-sib families
$\sigma^2_{C(FS)}$ = Variance among clones within full-sib families
σ^2_e = Error variance
$n_{C(FS)}$ = Harmonic mean of number of clones within family
$n_{R(C)}$ = Harmonic mean of number of ramets per clone

3. Estimate of narrow-sense heritability of clone means within family for individual sites

$$h^2_{\overline{C(FS)}} = \frac{\sigma^2_{FS}}{\sigma^2_{C(FS)} + \frac{1}{n_{R(C)}}\left(\sigma^2_e\right)}$$

$h^2_{\overline{C(FS)}}$ = Narrow-sense heritability of clonal means within families

4. Estimation of broad-sense heritability of clonal means within family

$$H^2_{\bar{C}(FS)} = \frac{\sigma^2_{C(FS)}}{\sigma^2_{C(FS)} + \frac{1}{n_{R(C)}}\left(\sigma^2_e\right)}$$

$H^2_{\bar{C}}$ = Broad-sense heritability of clone means within families

5. Phenotypic and genetic correlations between ages

$$r_{(A,B)} = \frac{Cov_{(A,B)}}{\sqrt{\sigma^2_{(A)}\sigma^2_{(B)}}}$$

$r_{(A,B)}$	Correlation between age A and age B
$Cov_{(A,B)}$	Covariance between age A and age B
$\sigma^2_{(A)}$	Variance at age A
$\sigma^2_{(B)}$	Variance at age B

Phenotypic variances and covariance are used for phenotypic correlation and genetic variances and covariance are used for genetic correlation.

6. Prediction of genetic gains

- For breeding

$$R_x = i_{FS} h^2_{F\bar{S}} \sigma_{P(F\bar{S})} + i_{\bar{C}(FS)} h^2_{\bar{C}(FS)} \sigma_{P_{\bar{C}(FS)}}$$

R_x	Response of character x directly selected
i_{FS}	Intensity of family selection
$i_{\bar{C}(FS)}$	Intensity of clone within family selection
$h^2_{F\bar{S}}$	Narrow-sense heritability of family means
$h^2_{\bar{C}(FS)}$	Narrow-sense heritability of clones within family means
$\sigma_{P(F\bar{S})}$	Phenotypic standard deviation of family means
$\sigma_{P_{\bar{C}(FS)}}$	Phenotypic standard deviation of clones within family means

For clonal deployment

$$R_x = i_{FS} h^2_{F\bar{S}} \sigma_{P(F\bar{S})} + i_{\bar{C}(FS)} H^2_{\bar{C}(FS)} \sigma_{P_{\bar{C}(FS)}}$$

R_x	Response of character x directly selected
i_{FS}	Intensity of family selection
$i_{\bar{C}(FS)}$	Intensity of clone within family selection
$h^2_{F\bar{S}}$	Narrow-sense heritability of family means
$H^2_{\bar{C}(FS)}$	Broad-sense heritability of clone within family means
$\sigma_{P(F\bar{S})}$	Phenotypic standard deviation of family means
$\sigma_{P_{\bar{C}(FS)}}$	Phenotypic standard deviation of clones within family means

APPENDIX II-2

Analysis of variance for diameter at 1.40 m height (DBH) for individual and combined sites

	DBH04			DBH07			DBH10		
Source	DF	Type III MS	F	DF	Type III MS	F	DF	Type III MS	F
Rep	4	1037.98	5.15	4	449.85	1.41	4	1067.51	0.93
Family	15	1035.97	1.58	15	2295.54	1.99	15	5814.24	1.71
Rep*family	60	201.24	0.99	60	318.73	0.99	60	1152.76	1.10
Clone(family)	134	724.65	3.57	129	1305.86	4.06	129	3741.50	3.57
Error	396	203.23		354	321.97		335	1046.82	

Analysis of variance for Diameter at 1.40 m high at age 4 (DBH4), 7 (DBH7) and 10 years (DBH10) - Manawahe.

	DBH7			DBH10		
Source	DF	Type III MS	F	DF	Type III MS	F
Rep	4	1038.53	1.63	4	1293.28	0.94
Family	15	33313.25	2.91	15	7375.12	2.79
Rep*family	60	669.97	1.11	60	1413.51	1.31
Clone(family)	132	1274.59	2.11	130	2841.93	2.64
Error	307	1274.59		299	1076.56	

Analysis of variance for Diameter at 1.40 m high at age 7 (DBH7) and 10 years (DBH10) - Kaingaroa

	DBH7			DBH10		
Source	DF	Type III MS	F value	DF	Type III MS	F value
Site	1	490273.65	348.14	1	551139.17	354.25
Rep(site)	8	766.69	1.56	8	1180.39	0.93
Family	15	4313.84	1.91	15	11747.09	2.81
Site*family	15	1241.07	1.99	15	1672.20	1.01
Rep(site)*family	120	494.35	1.09	120	1283.13	1.21
Clone(family)	133	1933.62	3.13	132	4776.25	3.07
Site*clone(family)	128	620.13	1.37	127	1561.84	1.47
Error	661	452.19		634	1060.85	

Combined-site analysis of variance for diameter at 1.40 m height at ages 7 (DBH7) and 10 years (DBH10).

APPENDIX III-1

Analysis of variance for percentage of Dothistroma infection at ages 3, 4, and 5 years

Age 3 years

Source of Variation	DF	Type III MS	F Value
replication	5	386.57	0.5245
factorial	3	1816.56	0.7602
rep*factorial	15	738.304	6.5401
Fem(factrl)	8	1689.956	5.4411
Male(factrl)	8	377.012	1.2725
Fem*Male(factrl)	16	273.222	2.3517
rep*fem(factrl)	40	87.947	1.8015
rep*male(factrl)	40	74.016	1.5154
rep*em*male(factrl)	80	48.769	0.8269
clone(fem*male(factrl))	324	130.71	2.2161
error	1318	58.982	

Age 4 years

Source of Variation		Type III MS	F Value
replication	5	3717.209	18.1197
factorial	3	1411.9249	0.3649
rep*factorial	15	205.4121	1.8242
Fem(factrl)	8	3552.8717	6.9211
Male(factrl)	8	766.5707	1.4357
Fem*Male(factrl)	16	524.0127	1.7026
rep*fem(factrl)	40	99.845	0.9072
rep*male(factrl)	40	122.8639	1.1169
rep*fem*male(factrl)	80	110.1675	1.2504
clone(fem*male(factrl))	324	300.86	3.4148
error	1317	88.106	

Age 5 years

Source of Variation		Type III MS	F Value
replication	5	2551.087	11.138
factorial	3	736.56	0.1383
rep*factorial	15	229.3791	2.1895
Fem(factrl)	8	4637.9	10.4949
Male(factrl)	8	1021.379	2.2453
Fem*Male(factrl)	16	431.39	1.2603
rep*fem(factrl)	40	78.351	1.1738
rep*male(factrl)	40	93.236	1.3957
rep*fem*male(factrl)	80	66.63	0.7505
clone(fem*male(factrl))	324	383.2454	4.3165
error	1317	88.78	

Table 2. Analysis of variance for percentage of Dothistroma needle infection at age 3 (DTP3), 4 (DTP4) and 5 years (DTP5).

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