CHAPTER 1

INTRODUCTION AND OBJECTIVES

1.1 Background

Eucalyptus grandis is a commercially grown eucalypt species in South Africa. Of the total commercial forestry area planted to eucalypts in South Africa, 73.8% (or 441 394ha) is planted to *E.grandis* and it's hybrids (Owen, 2000). *E.grandis* is used for mining timber, pulp, sawtimber, poles and firewood.

Breeding of *E.grandis* by the South African Forest Research Institute (SAFRI) (now incorporated in the CSIR) began in the early 1960's with mass phenotypic selection of 689 first generation (P_0) selections from the plantations, mostly in the summer rainfall areas of South Africa (Pierce, 1996).

A series of provenance trials of imported Australian seedlots were established during the 1970's. Family identities were retained in the trials and selections from these trials were added to the gene pool by inclusion in the main breeding population. Forward selection in the open pollinated breeding population was used to make selections for the second and third generation of breeding.

Breeding of this species in the CSIR is focussed on improvement in three traits of high economic importance and good predicted gain, namely volume, stem form and disease tolerance. The reduction of log-end splitting for solid wood products is also an important trait in some sub-populations, but the material in this study was considered too young to be reliably assessed for splitting at the time of the last assessment in November 1999 (age 5 ½ years). (Subsequent studies by the CSIR have shown that splitting can be assessed at this age, Verryn et al,2000a.)

The breeding and production strategy for *E.grandis* was reviewed and revised, prior to the establishment of the F3 generation in order to develop a breeding and production strategy that was

suited to both the biology and the economic importance of the species, and that would optimise sustainable genetic gain. There was also a need to combine the various sources of material in the *E.grandis* breeding programme into a single population to improve the efficiency of the breeding programme. Mainitaining the various sources as separate populations was proving to be too costly and insufficient manpower was available to manage all the separate sources of material to the same standard. It was also felt that combining the various sources would enable a more accurate evaluation and comparison of the different genotypes.

A cloned breeding strategy, using open pollinated families in the third generation but using control pollination to generate families for the fourth generation, was proposed (Shelbourne, 1992b). The first three sub-populations (there are 16 sub-populations in total) of the third generation breeding population were established as cloned seedlings from open pollinated selections (i.e., a cloned breeding population of half sibs).

1.2 The use of clones in forestry to estimate genetic variance components

The use of clones in forestry to obtain an estimate of the total genetic variance is not a new concept (Libby, 1964). More recently, however, tree breeders have suggested using, and others used, clonal replicates and family structure to investigate the components of genetic variation (Rosvall et al, 1998, Mullin and Park, 1992; Foster and Shaw, 1988; Park and Fowler, 1987; Foster, 1985; Matheson and Lindgren, 1985; Foster et al., 1984; Burdon and Shelbourne, 1974).

Individual performance (within-family selection) is inextricably linked to the unique environment of its specific position in the progeny test, and the confounding of genetic and environmental effects complicates individual selection and decreases the accuracy of the estimate of an individual's genetic potential (Shaw and Hood, 1985). Efficient trial design and site selection contribute to minimising the effect of environmental variation, however, individual genotypic values are still confounded with a unique environmental effect. This is possibly one of the reasons why tree breeders have tended to weight family performance strongly even when estimates of local narrow sense heritability are relatively high. If genetically identical individual genotypes (clones)

are tested in numerous environments, the environmental effect on individual performance will to some extent be limited or reduced.

The opportunity to use clonal replicates to estimate genetic variances and the potential to increase expected gain by replicating individuals in forest tree breeding populations where recurrent selection is practiced, was first discussed by Libby (1964). This method of selection was referred to as "clonal selection". Information from relatives (e.g., family performance) is frequently used to increase the accuracy with which an individual's genotype is estimated. It therefore stands to reason that the closer the relationship between the relative and the individual, the greater the value of the information from that relative because of the higher proportion of shared genes. In effect, a clone can be considered as equivalent to a family of genetically identical individuals.

The closest genetic relationship is that which exists between clones, as all genes are in common between all individuals. Cloning individuals facilitates the evaluation of a genotype in combination with numerous environments and increases the accuracy of individual rankings (Shaw and Hood, 1985). Clonal replication reduces the error variance of the mean. The accuracy of within-family selection is increased as clone means (individual means) are available to estimate the individual ranking. Cloning the seedlings in a breeding population is an innovative approach aimed at increasing the genetic gains from selection in the population by increasing the trait heritability and thus, the accuracy of within-family selection (Shelbourne,1992b).

Libby (1964) demonstrated the benefits of "clonal selection" for various quantities of ramets per clone and different levels of heritability. Selecting clones showed the greatest benefit over selection from a single expression of an individual's genotype for traits of low heritability, where a high level of selection can be done.

The use of vegetative propagation to replicate genotypes for clonal testing of individuals not only provides a means of characterising the additive and non-additive genetic variance components in the population, but also provides a means to exploit a greater proportion of the genetic variation (i.e., the non-additive variation in addition to the additive variation) in a tree improvement programme and therefore increase the genetic gain. Clones are genetically identical and therefore,

ramets of a clone give a better estimate of the whole genetic effect and not a portion of it as is the case with individuals in a family. The use of cloning to estimate non-additive genetic variance (dominance and epistasis where full sib families are cloned) is highlighted by Mullin and Park (1992) in a discussion of the methods to estimate genetic gain from "alternative" breeding strategies for clonal forestry. Formulae for the estimation of heritability and genetic gain from four different selection and deployment strategies are developed (Mullin and Park, 1992). Mullin and Park (1992) suggest that clonal selection from cloned families will produce more gains than the following three strategies considered in their study. These strategies were rogueing of the seed orchard following progeny testing (backward general combining ability selection and polycross), clonal deployement of phenotypes selected by mass selection (mass selection and cloning) and mass selection for grafting into seed orchards. In a discussion of the use of clonal replicates to estimate genetic gain in perennial plant species, Foster and Shaw (1988) note that the need for several generations or inbreeding (which is generally not practical for perennial plant species such as trees which have long generation intervals) to estimate epistatic genetic variance can be overcome by using clonally replicated individuals from full sib families.

A study of three open pollinated cloned seedling *Larix laricina* (Tamarack) populations showed that even if additive genetic variance is small in magnitude, that considerable gains are predicted for selection on clone means compared to mass selection and individual ramet (sic) selection (ortet selection) (Park and Fowler, 1987). Fowler (1986 ex Park and Fowler, 1987) described a strategy for Tamarack based on cloning the progenies of high general combining ability parents and ultimately high specific combining ability pairwise combinations of parents and suggested that notable increases in gains could be achieved if within progeny variation was exploited.

Shaw and Hood (1985) showed that the use of cloned progenies in the breeding population generally resulted in increased additive genetic gain compared to non-clonal tests as a result of an increase in the efficiency of selection and estimate of the genetic parameters. Three selection strategies were investigated in the simulation study, namely: two-stage selection on full sib families and individuals within families; three-stage selection on half sib families, full sib families selected within half sib families and individuals selected within full sib families; combined index selection. For each selection strategy the effect of a redistribution of testing effort (where there is a fixed

number of families and fixed total test size) from individuals to ramets was investigated and shown to have both beneficial (e.g., increased precision of selection when individuals are clonally replicated) and detrimental (e.g., reduction in the number of unique genotypes when effort is redistributed from individuals to ramets) consequences. Shaw and Hood (1985) highlight that for each unique situation the optimal allocation of effort (ramets versus individuals) should be determined as the relative advantage of cloning individuals compared with the non-cloned scenario was dependant on the specific scenario and the factors affecting the individual selection intensity and sources of variance in the trial.

A simulation study by Shelbourne (1992a) showed that, compared to 4 other breeding population scenarios and 10 other production population scenarios, the cloned breeding population showed the greatest gain in both the breeding and production population. The study looked at genetic gain per year for selection for a single trait at three levels of narrow sense heritability [low (0.1), medium (0.2) and high (0.4)]. Cloning the individuals increased the gain from within-family selection. Another benefit of this strategy, as illustrated by Shelbourne (1992a), is that tested clones can be supplied for rapid multiplication for deployment as selection for production (mass vegetative propagation) can take place at the same time as the selection of parents to produce the next generation.

In a comparison of the gains from a clonal seed orchard and clonal selection in a cloned breeding population, Matheson and Lindgren (1985) highlighted the substantial increases in gains that could be achieved in production through rapid deployment of clones. In situations where dominance genetic variance was zero, most of the increased gain was as a result of the shorter time lapse between selection in the breeding population and deployment in the field. In situations where the dominance variance equals the additive genetic variance, the notable increase in gain for the clonal option could be attributed equally to genetics (the use of both the additive and non-additive components of genetic variance and the increase in the accuracy of selection) and the time saved through rapid deployment of clones in production. The relative advantage of the clonal option was shown to increase as the proportion of dominance variance (assuming no epistasis) relative to the phenotypic variance increased.

Incorporating clones in the breeding population is more difficult for species which can be propagated vegetatively only from seed, embryo or juvenile seedling tissue and which do not maintain a juvenile state (Shelbourne, 1992a). Ageing effects in these species require that clones must be maintained in a juvenile state (or juvenility must be induced), in order to vegetatively multiply the clone for production once it has undergone testing and selection in a clonal program. The *Pinus* genus and other coniferous species are examples of such species. Some *Eucalyptus* species are easily rejuvenated and vegetatively propagated, by coppice for example, and do not need to be maintained in a juvenile state and therefore clonal forestry can be easily incorporated in the breeding strategy. Despite this, much of the documented work done on estimating genetic variances in cloned populations has been done on coniferous species.

No record of a cloned breeding population in any *Eucalyptus* species could be found in published literature and it is suspected that the CSIR's cloned *Eucalyptus grandis* population is, in this respect, unique at this time.

1.3 Objectives of the Study

Tree breeders are faced with many challenges in their efforts to optimise genetic gain in economically important traits in forest trees. Reliable estimates of the magnitude of the genetic variance components for traits, on which selection is to be practiced, are required in order to determine which breeding strategy will achieve the maximum gain given the practical constraints of breeding in the species.

The sub-populations B1, B2 and B3 provide the opportunity to investigate the genetic variance components in this population. The partitioning of the genetic variance in a population provides valuable information to the breeder on the relative proportion of non-additive to additive variance, which in turn impacts on the choice of breeding, production and selection strategy for that population. A better understanding of the broad and narrow sense heritability will also have an impact on our ability to quantify the benefits of cloning versus using seedlings in the CSIR's *E.grandis* breeding programme.

Juvenile selection is practiced in forestry based on the underlying assumption that the performance of a trait in a young tree provides an indication of performance at maturity or rotation age. An estimate of the age-age correlation describes the strength of the relationship between two traits or the same trait at two different ages. Juvenile-mature correlations, especially for traits related to growth, are generally not very high in magnitude in forest trees (which typically have long generation intervals) when considering a very young age compared with rotation age (Zobel and Talbert, 1984). An estimate of the genetic correlation is recognised as a more reliable predictor of future breeding values than a phenotypic correlation as the magnitude and influence of environmental effects on correlations are not usually known (Falconer, 1989). The environmental influence on the phenotypic differences may differ at different ages and will affect the accuracy of the evaluation of genotypic differences. Trials B1, B2 and B3 provide an opportunity to investigate the age-age correlation for the traits assessed between 38 and 66 months.

The objectives of this study were:

- to estimate the non-additive and additive components of the genetic variance of volume, stem form and disease tolerance in this population
- to estimate the broad and narrow sense heritabilities of volume, stem form and disease tolerance in this population
- to investigate the benefit of cloning the breeding population
- to estimate the correlation between ages 38 months and 66 months of volume, stem form and disease tolerance in this population.

CHAPTER 2

MATERIALS

2.1 Genetic Material

Selections were made in numerous progeny and provenance trials in the early 1990's and open pollinated seed was collected from these selections (Pierce, 1993). Selections were made for volume, stem form, disease tolerance and log end splitting. Open pollinated seed from more than 450 families was sown during April 1992 in petri-dishes. A family consisted of seed collected from a single open pollinated tree. Once germinated, the seed of 289 families was pricked out in the green house into bark-filled unigrow tubes, at 36 seedlings per selection (family).

Two to three weeks later the seedlings were transferred to the nursery. N.P.K 3.2.1 (25) fertiliser was applied. At 6 months the seedlings were on average 150mm tall. In October 1992 the seedlings were visually appraised and the 12 healthiest seedlings per selection (family) were transplanted in the nursery into bark-filled nursery bags and a teaspoon of 3.2.1 (25) N.P.K. fertiliser was applied to each seedling to encourage prolific shoot growth. At 9 months (January 1993) the seedlings had a mean height of approximately 500-600mm.

Eight cuttings from each of the seedlings were taken and set in January 1993. This initial setting was followed by three further settings of cuttings from the same genotypes during March, September and October 1993. The cuttings were set and raised according to standard procedures (Nel, 1991). In March 1994 the material (which differed in ages due to the multiple settings) was consolidated and sufficient material from 177 families was available for inclusion in the trials. Three trials, or sub-populations, were established namely, B1, B2 and B3. There was enough material for 6 families (AG509, AG652, AG684, AG640, BG128) to be included in two of the three trials.

The 177 families included in B1, B2 and B3 were a mixture of first and second generation families (80 select first generation families and 97 select second generation families). This was done in order to combine the various sources of material in the *E.grandis* breeding programme into a single population to improve the efficiency of the breeding programme. First generation (F1) selections were made by forward selection for volume and stem form in the best families in the best provenances from a series of provenance trials and progeny/provenance trials of material obtained from Florida. Second generation (F2) selections were made in three series of progeny trials (A1, A2 and A3). Selections for volume, stem, density and low splitting were made using a combined index of family, individual and parent information (pers. comm. Verryn, 2000). The selections combined in the third generation do, therefore, differ in the level of improvement and selection intensities also differed depending on the trial and level of improvement (details of selection intensities are not available).

2.2 Trial Design

An alpha lattice design was used for each of the three trials. The alpha lattice design is a type of incomplete block experimental design that is recommended for trials where a large number of treatments must be evaluated and where the control of experimental variation is important (Patterson and Williams, 1976). The alpha lattice design overcomes the limitations imposed by the square and rectangular lattice designs of Cochran and Cox (1957) as blocks do not have to be orthogonal with treatments and alpha lattice designs are available for a wide range of treatments, blocks and block sizes, and replications. This design endeavours to maximise the number of pairwise comparisons between treatments by limiting the number of concurrences of a pair of treatments in a block over replications and thereby achieving (or approaching) equal numbers of within block comparisons for all pairs of treatments. Another advantage of the alpha lattice design is the flexibility in analysis that the design facilitates. If there is little site variation within replications, and any variation, that does exist, is not effectively reduced by the blocks, then the trial can be analysed as a random complete block design (RCB) (Williams and Matheson, 1994).

Five ramets per clone were included in the trials. When a breeding strategy using the clonal replication of individuals is employed, the choice of the number of ramets per clone is usually constrained by limited resources that restrict the trial size (e.g., nursery facilities, manpower, time, available land). Compromises on the number of ramets per clone must be made in order to maximise genetic gain given the limited resources. The optimal number of ramets per clone has been shown to be sensitive to heritability and selection intensity (Russell and Libby, 1986; Verryn and Snedden, 2000), to the proportion of additive and non-additive variance (Shaw and Hood, 1985) and to the amount of genotype by environment interaction (Russell and Loo-Dinkins, 1993). Assuming testing is done on a single site, or that there is no genotype by environment interaction, Shaw and Hood (1985) found that the optimum number of ramets is 6 or less (for a total size of 144 trees per family) depending on the selection criteria and restrictions on family selection, as well as the heritability. A similar study, but predicting production population gains, was undertaken by Russell and Libby (1986). This study showed that except for at very low heritabilities (which were not expected in trials B1, B2 and B3) and high selection intensities, that the optimum number of ramets per clone per test site was usually 6 or less.

A single tree plot size was considered the most suitable design to provide the most accurate estimate of genetic parameters given the few entries per treatment that were available (Libby and Cockerham, 1980; Cotterill and James, 1984). Between 1 and 12 cloned individuals per family were included. A notable single exception was family BG127 that consisted of 21 individuals. An average of approximately 8 individuals per family was established. Each trial had a total of 500 treatments (clones). A summary of the trial design is detailed in Table 1.

A single breeding population was established as it was considered too costly to establish multiple populations for various end-uses. The division of the breeding population into sub-populations was purely a logistic decision. Families were allocated randomly to trials B1, B2 and B3.

Table 1. Summary of trial design for trials B1, B2 and B3.

Trial	B1	B2	B3
Design	Alpha lattice	Alpha lattice	Alpha lattice
Replications	5	5	5
Families	56	59	67
Clones	500	500	500
Plot size	single tree	single tree	single tree
Area	2.25 ha	2.25 ha	2.25 ha
Espacement	3 x 3 m	3 x 3 m	3 x 3 m

2.3 Trial Establishment

The trials were established adjacent to each other on Safcol's Port Durnford plantation near Richards Bay in Zululand, Kwa-Zulu Natal, in May 1994. The location of the trial site is illustrated in Figure 1. This site is a high growth potential site with fertile, deep soils which are well suited to *E.grandis*. *E.grandis* grows well on deep, fertile sites and the species is usually planted on good sites. The area has a high incidence of disease and exposure to natural infection is to be expected in this area. The site details are listed in Table 2.

Table 2. Details of the site location and conditions of trials B1, B2 and B3 at Port Durnford (Schulzere, 1997).

Latitude	28° 54' S
Longitude	31° 48' E
Altitude	120m
Geology	Berea sand
Soil form	Hutton (orthic A over red apedal B)
Effective Rooting Depth (ERD)	150cm
Mean Annual Precipitation (MAP)	1 441mm
Mean Precipitation in driest quarter	166mm
Mean Annual temperature (MAT)	21.1 °C
Mean maximum temperature for the hottest month	28.3 °C
Mean minimum temperature for the coldest month	12.3 °C

The trial site was pitted and 4 litres of water per tree was applied at time of planting. The trials were managed for pulp wood by Safcol and were not thinned. Weed control was inadequate as the standard prescription weeding was done after establishment but no follow up was done. No blanking was done.

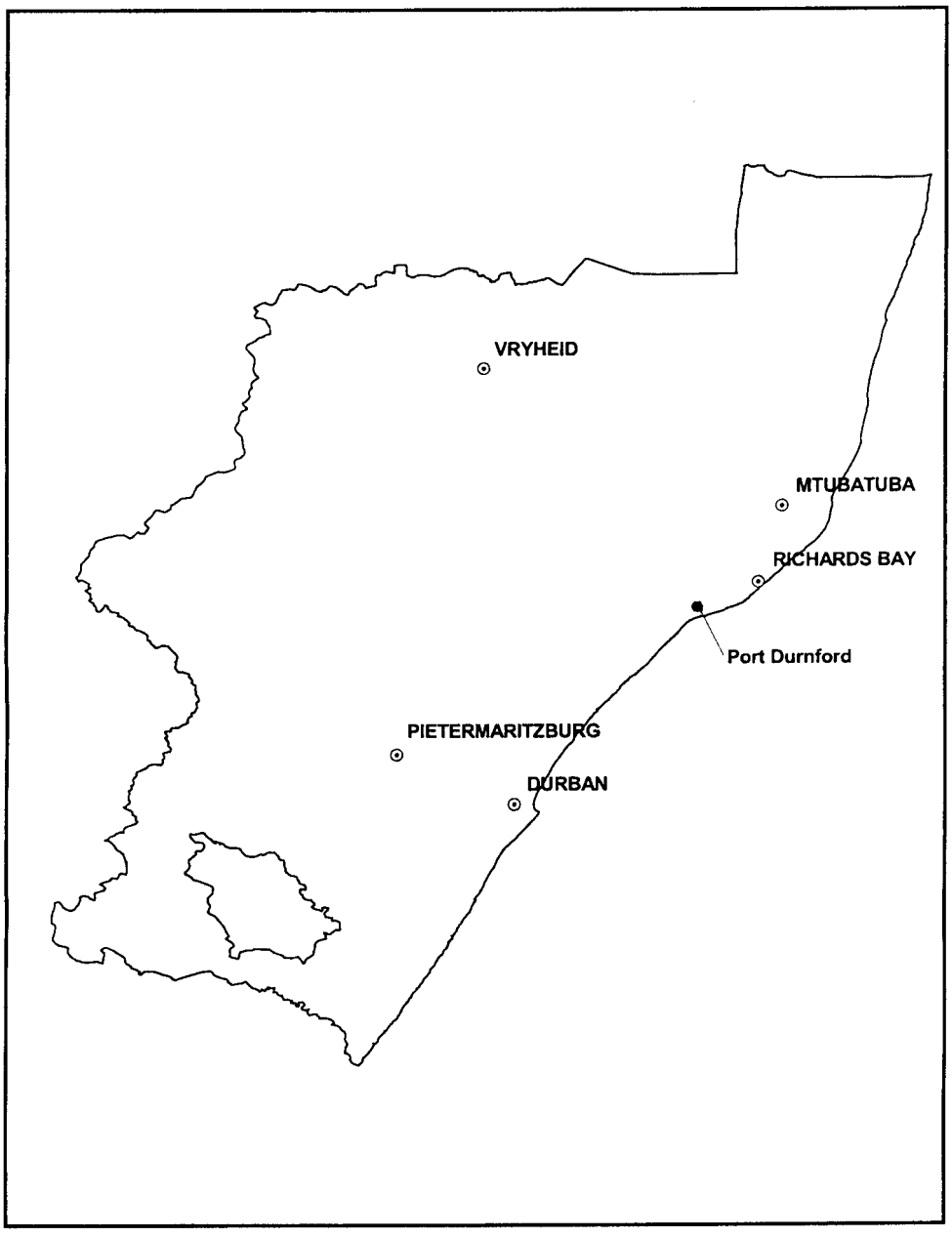

Figure 1. Map of Kwa-Zulu Natal with the location of trials B1, B2 and B3 indicated at Port Durnford.

2.4 Trial Assessment

Height growth, diameter at breast height (DBH), stem form, disease tolerance and defects were assessed at 3 years 2 months (38 months) and at 5 ½ years (66 months). It is important to note that two different measurement teams, each from a different company, were responsible for the measurement of the trials at the different ages. Height was assessed at 38 months using a height rod and at 66 months using a vertex hypsometer. DBH was assessed at a height of 1.3m height above the ground using a diameter tape. Stem was scored on an industry standard subjective 8 point scale. Disease tolerance was scored on a subjective 5 point industry standard scale. Defects, where occurring, were noted. Runts (small stunted trees) were not assessed, neither were the heights of trees with broken tops. These "runts" and trees with broken tops were noted as defects. (Appendix A details the scoring systems and mensuration techniques used in the assessment of these trials at each age.)

Individual tree volume was calculated according to the models for *E.grandis* developed by Bredenkamp and Loveday (1984). These models are detailed in Appendix A (Table A-5).

Figure 2. *E.grandis* progeny trial B1 at Port Durnford, age 66 months (November 1999).

CHAPTER 3

METHODS

3.1 Describing Variation in a Trait

The total observed variance of a quantitative trait is the phenotypic variance, and is the sum of the genotypic or genetic variance and environmental variance components (Falconer, 1989) and a genotype by environment interaction component.

$$\sigma_P^2 = \sigma_G^2 + \sigma_E^2 + \sigma_{GE}^2 \qquad (1)$$

where,

σ_P^2 is the phenotypic variance

σ_E^2 is the environmental variance component

σ_{GE}^2 is the variance attributed to genotype by environment interaction

σ_G^2 is the genotypic or genetic variance component

$= \sigma_A^2 + \sigma_{NA}^2$

σ_A^2 is the additive variance component

σ_{NA}^2 is the non-additive variance component

The genotypic variance can be further broken down into an additive and non-additive component which are influenced by gene frequencies in the population (Falconer, 1989, Namkoong, 1981). Fisher (1918, ex Cockerham 1954) first described how genetic variance could be partitioned into an additive component and a non-additive component (a dominance and epistatic component).

Partitioning the genetic variation into an epistatic component is problematic in forest trees, which have long generation intervals, as several generations and inbred lines are generally required for the

estimation of this component (Mather and Jinks, 1982). Foster and Shaw (1988), Mullin et al. (1992) and Stonecypher and McCullough (1986) discuss methods to partially separate genetic variance in full sib progeny of forest trees into additive, dominance and epistatic components. Full sib progeny are required in order to obtain an estimate of the epistatic portion of the non-additive variance.

The broad sense heritability (H^2) is the ratio of the total genetic variance to the total phenotypic variance and describes the degree to which the phenotypic differences are determined by the genotype. The ratio of the additive variance to the phenotypic variance is an estimate of the narrow sense heritability (h^2), or the degree to which the genes passed on from the parents determine the phenotypic differences (Falconer, 1989). The heritability can also be regarded as an indication of the breeding success.

$$H^2 = \frac{\sigma_G^2}{\sigma_P^2} = \frac{\sigma_A^2 + \sigma_{NA}^2}{\sigma_P^2} \qquad (2)$$

$$h^2 = \frac{\sigma_A^2}{\sigma_P^2} \qquad (3)$$

where,

H^2 is the broad sense heritability

h^2 is the narrow sense heritability

and all other parameters are as previously defined.

The heritability of a trait can, therefore, be seen to be entirely dependant on the ratio of the variance components, the estimates of which have been obtained in a specific population at a specific time in a specific environment. The heritability of a specific trait does not characterise, in absolute terms, the trait itself but is linked to the population and environment in which it was studied (Jacquard, 1983).

3.2 Analysis of Variance

The data analysis was done using the Statistical Analysis System Release 6.12 (SAS®, 1996).

For the analysis of the data for each individual sub-population (trial), replicate and block within replicate effects were considered fixed, and family and clone within-family effects random. The data was unbalanced due to mortality and design.

Each sub-population was analysed as a random complete block (see discussion in Chapter 5). The model used for the analysis of variance of the data for each individual sub-population was

$$y_{ijkl} = \mu + R_i + f_j + c_{k(j)} + e_{ijkl} \qquad (4)$$

where,

y_{ijkl} is the lth ramet or tree of the kth clone in the jth family in the ith replicate

μ is the overall mean

R_i is the effect of the ith replicate where i = 1, 2, .., 5

f_j is the effect of the jth family where j=1,2,..56 (B1), 59 (B2), 68 (B3)

$c_{k(j)}$ is the effect of the kth clone in the jth family where k=1, 2,….4 (mean number=4)

e_{ijkl} is the random error.

The analysis of variance for all traits in each of the trials is illustrated in Table 3.

Table 3. The analysis of variance and variance component estimation for volume, height, DBH, stem form and disease tolerance in trials B1, B2 and B3.

Source	Df	Expected Mean Squares
Replication	r - 1	$\sigma_e^2 + k_4\sigma_R^2$
Family	f - 1	$\sigma_e^2 + k_2\sigma_{c(f)}^2 + k_3\sigma_f^2$
Clone (family)	f(c-1)	$\sigma_e^2 + k_1\sigma_{c(f)}^2$
Error	remainder	σ_e^2

r: number of replications

f: number of families

c: mean number of clones per family

k_1, k_2, k_3, k_4 expected mean squares coefficients

Restricted Maximum Likelihood (REML) was used, as it is the recommended method of parameter estimation for mixed models (Patterson and Thompson, 1971). The option REML of SAS uses iterative MIVQUE (minimum variance quadratic estimate) estimates until there is no change in the parameter estimates. The PROC MIXED procedure of SAS was used to estimate the variance components in the individual trials.

The data were corrected for the replication effect in each trial before the data was pooled. Once pooled, the data were additively corrected for trial effect. The variance components for this pooled dataset were calculated using the SAS procedure PROC VARCOMP and the REML option.

The model used for the analysis of variance for the pooled data set was:

$$y_{ijk} = \mu + f_i + c_{j(i)} + e_{ijk} \qquad (5)$$

where,

y_{ijk} is the k[th] ramet or tree of the j[th] clone in the i[th] family

μ is the overall mean

f_i is the effect of the i[th] family where i=1,2,..177

$c_{j(i)}$ is the effect of the j[th] clone in the i[th] family where j=1, 2,….4 (mean)

e_{ijk} is the random error.

The analysis of variance for all traits in the pooled data for trials B1, B2 and B3 is illustrated in Table 4.

Table 4. The analysis of variance and variance component estimation for volume, height, DBH, stem form and disease tolerance in the pooled data for trials B1, B2 and B3.

Source	df	Expected Mean Squares
Family	f - 1	$\sigma_e^2 + k_2\sigma_{c(f)}^2 + k_3\sigma_f^2$
Clone (family)	f(c-1)	$\sigma_e^2 + k_1\sigma_{c(f)}^2$
Error	remainder	σ_e^2

f: number of families

c: mean number of clones per family

k_1, k_2, k_3 expected mean squares coefficients

3.3 Estimation of Variance Components

If the assumptions of Mendelian behaviour and equilibrium are met, then the between group variance is a measure of the covariance, and covariance of sibs can be expressed in terms of additive and non-additive components of genetic variance (Becker, 1992).

Depending on the relationship of sibs in the family, the family component of variance (σ_f^2) can be interpreted as (Becker, 1992; Falconer, 1989):

$$\text{Half sibs: } \sigma_f^2 = \frac{1}{4}\sigma_A^2 \qquad (6)$$

$$\text{Full sibs: } \sigma_f^2 = \frac{1}{2}\sigma_A^2 + \frac{1}{4}\sigma_{NA}^2 \qquad (7)$$

Open pollinated families are (assuming no selfing or related crossing has occurred and assuming no two sibs have the same parents, i.e. are half sibs) groups of half sibs as only one parent is common and the other is different and unknown.

The clone within family variance component ($\sigma_{c(f)}^2$) is a covariance of clones and is the total genetic variance minus the covariance of sibs (Park and Fowler, 1987). In half sib families the clone within family variance component can be translated into additive (σ_A^2) and non-additive (σ_{NA}^2) components of variance as follows:

$$\begin{aligned}\sigma_{c(f)}^2 &= \sigma_G^2 - \sigma_f^2 \\ &= (\sigma_A^2 + \sigma_{NA}^2) - \frac{1}{4}\sigma_A^2 - (1-k)\sigma_{NA}^2 \\ &= \frac{3}{4}\sigma_A^2 + k\sigma_{NA}^2\end{aligned} \qquad (8)$$

where,

k is the proportion of non-additive variance segregating within families.

In open pollinated families the proportion of non-additive variance segregating within families, is generally assumed to be one (Park and Fowler, 1987) and the additive (σ_A^2) and non-additive (σ_{NA}^2) components of variance can be approximated as (using equations 6 and 8):

$$\sigma_A^2 = 4\sigma_f^2 \qquad (9)$$

$$\sigma_{NA}^2 = \sigma_{c(f)}^2 - 3\sigma_f^2 \qquad (10)$$

However, inbreeding and relatedness among individuals can bias the estimate of the additive variance component in open pollinated populations of forest trees (Squillace, 1974, Namkoong, 1966). Open pollinated families in forest trees may be combinations of half sibs, full sibs and perhaps even selfs (Libby, 1992). It has, therefore, been recommended that the coefficient of relationship be increased to ⅓ in open pollinated *E.grandis* under the assumption of 20% increased "relatedness" (Verryn, 1993). The family component of variance is, therefore, estimated as follows (from equation 6):

$$\sigma_f^2 = \frac{1}{3}\sigma_A^2 \qquad (11)$$

The clonal component of variance ($\sigma_{c(f)}^2$) can, therefore, be translated into additive (σ_A^2) and non-additive (σ_{NA}^2) components of genetic variance as follows (from equation 8):

$$\begin{aligned}\sigma_{c(f)}^2 &= \sigma_G^2 - \sigma_f^2 \\ &= (\sigma_A^2 + \sigma_{NA}^2) - \frac{1}{3}\sigma_A^2 - (1-k)\sigma_{NA}^2 \\ &= \frac{2}{3}\sigma_A^2 + k\sigma_{NA}^2\end{aligned} \qquad (12)$$

where,

k is the proportion of non-additive variance segregating within families.

The additive (σ_A^2) and non-additive (σ_{NA}^2) components of variance can, therefore, be approximated as (from equations 11 and 12):

$$\sigma_A^2 = 3\sigma_f^2 \qquad (13)$$

$$\sigma_{NA}^2 = \sigma_{c(f)}^2 - 2\sigma_f^2 \qquad (14)$$

However, if assuming some increased relatedness in open pollinated families (and adjusting the coefficient of relationship to account for this relatedness), then *k* must be less than one. If open pollinated families of *E.grandis* are considered to be a 80:20 mix of half sibs and full sibs, then the family variance component can be approximated as follows (from equations 6 and 7):

$$\begin{aligned}\sigma_f^2 &= 0.8(\frac{1}{4}\sigma_A^2) + 0.2(\frac{1}{2}\sigma_A^2) + 0.2(\frac{1}{4}\sigma_{NA}^2) \\ &= \frac{3}{10}\sigma_A^2 + \frac{1}{20}\sigma_{NA}^2\end{aligned}$$

(15)

The clonal component of variance ($\sigma_{c(f)}^2$) can, therefore, be translated into additive (σ_A^2) and non-additive (σ_{NA}^2) components of variance as follows (from equation 8):

$$\begin{aligned}\sigma_{c(f)}^2 &= \sigma_G^2 - \sigma_f^2 \\ &= (\sigma_A^2 + \sigma_{NA}^2) - (\frac{3}{10}\sigma_A^2 + \frac{1}{20}\sigma_{NA}^2) \\ &= \frac{7}{10}\sigma_A^2 + \frac{19}{20}\sigma_{NA}^2\end{aligned}$$

(16)

Which is equivalent to:

$$\sigma_A^2 = \frac{10}{7}\sigma_{c(f)}^2 - \frac{19}{14}\sigma_{NA}^2 \qquad (17)$$

The additive (σ_A^2) and non-additive (σ_{NA}^2) components of variance can, therefore, be approximated as (from equations 15, 16 and 17):

$$\frac{10}{3}\sigma_f^2 - \frac{1}{6}\sigma_{NA}^2 = \frac{10}{7}\sigma_{c(f)}^2 - \frac{19}{14}\sigma_{NA}^2$$
$$\frac{19}{14}\sigma_{NA}^2 - \frac{1}{6}\sigma_{NA}^2 = \frac{10}{7}\sigma_{c(f)}^2 - \frac{10}{3}\sigma_f^2$$
$$\frac{50}{42}\sigma_{NA}^2 = \frac{10}{7}\sigma_{c(f)}^2 - \frac{10}{3}\sigma_f^2$$
$$\sigma_{NA}^2 = \frac{6}{5}\sigma_{c(f)}^2 - \frac{14}{5}\sigma_f^2$$

(18)

$$\sigma_A^2 = \frac{10}{3}\sigma_f^2 - \frac{1}{6}\sigma_{NA}^2$$
$$= \frac{10}{3}\sigma_f^2 - \frac{1}{6}\left(\frac{6}{5}\sigma_{c(f)}^2 - \frac{14}{5}\sigma_f^2\right)$$
$$= \frac{10}{3}\sigma_f^2 - \frac{1}{5}\sigma_{c(f)}^2 + \frac{14}{30}\sigma_f^2$$
$$= \frac{57}{15}\sigma_f^2 - \frac{1}{5}\sigma_{c(f)}^2$$

(19)

The narrow sense heritability (h^2) and broad sense heritability (H^2) are calculated as follows (Falconer, 1989):

$$h^2 = \frac{\sigma_A^2}{\sigma_P^2} \qquad (20)$$

$$H^2 = \frac{\left(\sigma_A^2 + \sigma_{NA}^2\right)}{\sigma_P^2} \qquad (21)$$

where,

σ_p^2 is the phenotypic variance, and

$$\sigma_p^2 = \sigma_f^2 + \sigma_{c(f)}^2 + \sigma_e^2 \qquad (22)$$

The clone mean heritabilities were calculated using the phenotypic variance of the clone mean ($\sigma^2_{\bar{c}}$) where,

$$\sigma^2_{\bar{c}} = \sigma^2_f + \sigma^2_{c(f)} + \frac{\sigma^2_e}{r} \qquad (23)$$

and r is the harmonic mean number of ramets per clone.

The broad and narrow sense heritabilities of the clone means were calculated as (using equations 20, 21 and 23):

$$H^2_{\bar{c}} = \frac{\sigma^2_A + \sigma^2_{NA}}{\sigma^2_{\bar{c}}} \qquad (24)$$

$$h^2_{\bar{c}} = \frac{\sigma^2_A}{\sigma^2_{\bar{c}}} \qquad (25)$$

The variance of the variance components and standard error of the narrow sense heritability are calculated as follows (Becker, 1992):

$$\text{var}(\sigma^2_g) = \frac{2}{k^2}\sum_g \frac{MS^2_g}{f_g + 2} \qquad (26)$$

where,

$\text{var}(\sigma^2_g)$	is the variance of the gth variance component
k	is the coefficient of the variance component being estimated
MS^2_g	is the gth mean square used to estimate the variance component
f_g	are the degrees of freedom of the gth mean square.

$$s.e.(h^2) = \frac{m \times \sqrt{\text{var}(\sigma^2_f)}}{\sigma^2_p} \qquad (27)$$

where,

$s.e.(h^2)$	is the standard error of the heritability estimate
m	is the inverse of the coefficient of relationship.

3.3.1 Scenarios for estimating genetic variation

Variance components were calculated for the individual trials (B1, B2 and B3) and for the pooled data from all three of the trials. The genetic variance components were calculated for three different scenarios that were considered feasible for the population.

Scenario 1

In scenario 1 the non-additive and additive variance components are calculated using equations 18 and 19 under the assumption that the proportion of non-additive genetic variance segregating within open pollinated families is less than one and can be approximated as set out in equation 15.

Scenario 2

In this scenario all non-additive variance is considered to be segregating within the families, but the additive variance is approximated as three times the family variance component (coefficient of relationship=⅓). In scenario 2 the non-additive and additive variance components are calculated using equations 13 and 14.

Scenario 3

In the third scenario the additive variance is approximated as four times the family variance component (coefficient of relationship=¼), and the non-additive and additive variance components are calculated using equations 9 and 10.

There is some evidence to suggest that height depression is an indication of inbreeding in *E.grandis* (Hodgson, 1975). The selection of the 12 biggest seedlings for cloning in this trial could have removed the inbred individuals and the families could, therefore, be fully half sib families. Under these assumptions scenario 3 may be appropriate.

3.3.2 Editing genetic variance component estimates

Very small negative estimates of genetic variance components were obtained and these are presented. The negative estimates reflect the lack of precision or accuracy in the estimate of the variance components. Estimates of variance components may be negative for a number of reasons such as high variability in the data, negative correlations between observations or outliers. Negative estimates may also indicate that the model being used to estimate the variance components is inappropriate. High variability was found to occur in some traits but the model was considered sound. As the negative estimates of genetic variance components were very small, a value of zero for that component was used in the calculation of heritability.

Standardising the data prior to the calculation of variance components did not notably improve the estimates and the estimates of variance components from unstandardised data is presented.

3.4 Correlations

The phenotypic age-age correlation can be calculated as (Falconer, 1989):

$$r_{p_{(age1,age2)}} = \frac{\text{cov}_{p_{(age1,age2)}}}{\sqrt{\sigma^2_{p_{age1}} \times \sigma^2_{p_{age2}}}} \qquad (28)$$

where,

$r_{p_{(age1,age2)}}$ is the estimated phenotypic correlation between the trait at age 1 and age 2

$\text{cov}_{p_{(age1,age2)}}$ is the phenotypic covariance between the trait at age 1 and age 2

$\sigma^2_{p_{age1}}, \sigma^2_{p_{age2}}$ are the phenotypic variance components from separate analysis of variance for the trait at age 1 and 2 respectively.

The genetic correlation for a trait measured at two different ages is estimated as (Becker, 1992):

$$r_{g_{(age1,age2)}} = \frac{\text{cov}_{f_{(age1,age2)}}}{\sqrt{\sigma^2_{f_{age1}} \times \sigma^2_{f_{age2}}}} \qquad (29)$$

where,

$r_{g_{(age1,age2)}}$ is the estimated genetic correlation between the trait at age 1 and age 2

$\text{cov}_{f_{(age1,age2)}}$ is the family covariance between the trait at age 1 and age 2

$\sigma^2_{f_{age1}}, \sigma^2_{f_{age2}}$ are the family variance components from separate analysis of variance for the trait at age 1 and 2 respectively.

A second method of estimating the genetic correlation using the clones and ignoring the family structure, was investigated. The use of this method relies on the assumption that the clones formed a large population of non-related individuals (which is not entirely true). The genetic correlation was estimated as:

$$\text{r}_{g_{(age1,age2)}} = \frac{\text{cov}_{c_{(age1,age2)}}}{\sqrt{\sigma^2_{c_{age1}} \times \sigma^2_{c_{age2}}}} \qquad (30)$$

where,

$r_{g_{(age1,age2)}}$ is the estimated genetic correlation between the trait at age 1 and age 2

$\text{cov}_{c_{(age1,age2)}}$ is the clonal covariance between the trait at age 1 and age 2

$\sigma^2_{c_{age1}}, \sigma^2_{c_{age2}}$ are the clonal variance components (estimated without consideration of family structure) from separate analysis of variance for the trait at age 1 and 2 respectively.

The standard errors of the estimate of genetic correlation can be calculated as (Becker, 1992):

$$SE\, r_g = \frac{1 - r^2_g}{\sqrt{2}} * \sqrt{\frac{SE\, h_1^{\,2} * SE\, h_2^{\,2}}{h_1^{\,2} * h_2^{\,2}}} \qquad (31)$$

where,

$SE\, r_g$	is the standard error of the estimated genetic correlation between two ages for a trait
r_g	is the estimated genetic correlation between two ages for a trait
$h^2_1,\ h^2_2$	is the heritability of the trait at age one and two respectively
$SE\, h^2_1, SE\, h^2_1$	is the standard error of the heritability of the trait at age one and two respectively (Becker, 1992).

The genetic correlation is estimated as the correlation of breeding values, whereas the environmental correlation includes the correlation of environmental and non-additive genetic components (Falconer, 1989).

There are two types of genetic correlations, namely Type A and Type B. Type A genetic correlations refer to estimates of correlations between traits measured on the same individuals (Burdon, 1977). Type B genetic correlations typically refer to estimates of correlation between traits measured on different individuals within genetic groups (e.g., families, clones) and do not assume a common error (Burdon, 1977). Type B correlations can, however, also be calculated where only a subset of the genetic groups is common (e.g., two different sites with 50 families at each site but only 20 common across both sites) (Kanzler and Hodge, 2000). Type B correlations were not considered in this study as it could not be assumed that there was not a common error. This study will investigate Type A genetic correlations.

3.5 Genetic Gains

Genetic gains were calculated using G-Assist version 3.0 (Verryn and Snedden, 1998). G-Assist is a deterministic tool developed to facilitate the comparison of predicted gains for different tree breeding strategies. Gain predictions are for breeding for a single trait. Formulae for gains calculations are based on published work by Shelbourne (1992a). The selection intensities are determined automatically by referencing the selection intensity tables of Becker (1992). Provision is made for some selection of the male parents by thinning the population, and thereby improving the pollen cloud, before the collection of seed from selected individuals. An adaptation for finite family sizes (i.e., a finite number of clones per family) was made in the formula for the calculation of predicted gain in the cloned breeding population (Verryn et al., 2000b).

Total predicted genetic gain was calculated as follows:

$$\Delta G_T = \Delta G_F + \Delta G_M \qquad (32)$$

where,

ΔG_T is the total predicted genetic gain

ΔG_F is the predicted genetic gain from selection of female parents (among and within families)

ΔG_M is the predicted genetic gain from selection of male parents (among and within families).

3.5.1 Breeding Population Gains

Gains were estimated assuming that the genetic correlation between the selection trait and the target trait was one.

Predicted genetic gain from selection in a cloned open pollinated breeding population was calculated by G-Assist as (Verryn et al., 2000b):

$$\Delta G_F = \frac{1}{2}\cdot\left[SI_i \cdot cr \cdot \left(\frac{\sigma_A^{\ 2}}{\sqrt{cr\cdot\sigma_A^2 + \frac{(1-cr)\cdot\sigma_A^2}{t} + \frac{\sigma_e^2}{t\cdot r}}}\right) + SI_2 \cdot \left(\frac{t-1}{t}\right)\cdot(1-cr)\cdot\left(\frac{\sigma_A^2}{\sqrt{\frac{t-1}{t}\cdot(1-cr)\cdot\sigma_A^2 + \frac{\sigma_e^2}{r}}}\right)\right] \quad (33)$$

where,

SI_1, SI_2	are the selection intensities among and within female families respectively
cr	is the coefficient of relationship
t	is the number of clones per family
r	is the number of ramets per clone.

$$\Delta G_M = \frac{1}{2}\cdot\left[SI_3 \cdot cr \cdot \left(\frac{\sigma_A^{\ 2}}{\sqrt{cr\cdot\sigma_A^2 + \frac{(1-cr)\cdot\sigma_A^2}{t} + \frac{\sigma_e^2}{t\cdot r}}}\right) + SI_4 \cdot \left(\frac{t-1}{t}\right)\cdot(1-cr)\cdot\left(\frac{\sigma_A^2}{\sqrt{\frac{t-1}{t}\cdot(1-cr)\cdot\sigma_A^2 + \frac{\sigma_e^2}{r}}}\right)\right] \quad (34)$$

where,

SI_3, SI_4	are the selection intensities among and within male families respectively.

Predicted genetic gain from selection in an open pollinated breeding population without cloning, was calculated by G-Assist as (Verryn et al., 2000b):

$$\Delta G_F = \frac{1}{2} \cdot \left[SI_1 \cdot cr \cdot \frac{\sigma_A^2}{\sigma_{fm}} + SI_2 \cdot (1 - cr) \cdot \frac{\sigma_A^2}{\sigma_w} \right] \quad (35)$$

where,

σ_{fm} is the standard deviation of open pollinated family means

σ_w is the standard deviation within families

$\sigma_w = \sqrt{((1 - cr)\sigma_A^2 + \sigma_e^2)}$

$$\Delta G_M = \frac{1}{2} \cdot \left[SI_3 \cdot cr \cdot \frac{\sigma_A^2}{\sigma_{fm}} + SI_4 \cdot (1 - cr) \cdot \frac{\sigma_A^2}{\sigma_w} \right] \quad (36)$$

where,

SI_3, SI_4 are the selection intensities among and within male families respectively.

3.5.2 Production Population Gains

Predicted genetic gains in the production population were calculated for five production population options, namely:

1. Cloned open pollinated breeding population thinned on clone means for seed production
2. Clonal orchard from forward selection on clone means in a cloned breeding population
3. Clonal orchard from forward selection in a non-cloned open pollinated breeding population
4. Clonal selection in a cloned open pollinated breeding population
5. Clonal selection in a clonal trial of forward selections in a non-cloned open pollinated breeding population.

Gains were calculated assuming that the genetic correlation with the mature trait at age of selection for the production population was one. Gains for options 1-3 were calculated in G-Assist using the generalised form (Verryn et al., 2000b) of the production population equations presented by Shelbourne (1992a) as follows:

$$\Delta G_{F_p} = \frac{1}{2} \cdot \left[SI_{1_p} \cdot cr \cdot \frac{\sigma_A^2}{\sigma_{fm}} + SI_{2_p} \cdot (1-cr) \cdot \frac{\sigma_A^2}{\sigma_w} + 2 \cdot SI_{5_p} \cdot crp \cdot \frac{\sigma_{Ap}^2}{\sigma_{fmp}} + SI_{6_p} \cdot (1-crp) \cdot \frac{\sigma_{As}^2}{\sigma_{ws}} \right] \quad (37)$$

where,

ΔG_{F_p} is the predicted genetic gain from female selection for seed production

SI_{1_p} is the selection intensity among families of the breeding population for female production parents

SI_{2_p} is the selection intensity within families of the breeding population for female production parents

SI_{5_p} is the backward selection intensity for rogueing of the seed orchard using progeny test information (equals zero if there is no backward selection)

SI_{6_p} is the selection intensity for thinning of a seedling seed orchard (equals zero if no seedling seed orchard)

crp is the coefficient of relationship in the production population

σ_{Ap}^2 is the progeny test additive genetic variance

σ_{fmp} is the standard deviation of family means in a progeny test used for backward selection

σ_{ws} is the standard deviation of the within-family variance in the seedling seed orchard

$\sigma_{ws} = \sqrt{(1-crp)\sigma_{As}^2 + \sigma_{es}^2}$

σ_{As} is the standard deviation of the additive genetic variance in the seedling seed orchard

σ_{es}^2 is the residual variance of the seedling seed orchard

and all other parameters are as previously defined.

The predicted genetic gains for male selection is calculated similarly but substituting SI_{3_p} (the selection intensity among families of the breeding population for male production parents) and SI_{4_p} (the selection intensity within families of the breeding population for male production parents) in the place of SI_{1_p} and SI_{2_p}.

The predicted genetic gain from the selection of production clones (option 4) in a cloned breeding population were calculated by G-Assist as:

$$\Delta G_c = SI_{7_p} \cdot \frac{\sigma^2_{Gc}}{\sigma_{\bar{c}}} \qquad (38)$$

where,

ΔG_C is the predicted genetic gain from selection of clones for production in a cloned breeding population

SI_{7_p} is the selection intensity among clones in the cloned breeding population

σ^2_{Gc} is the total genetic variance (additive and non-additive as estimated by the broad sense heritability)

$\sigma_{\bar{c}}$ is the standard deviation of clone means.

Predicted genetic gain from selection of production clones in a clonal trial established with forward selections made in a non-cloned open pollinated breeding population (option 5) is not an option provided by G-assist but was calculated as follows (Verryn et al, 2000c):

$$\Delta G_C = \frac{i_f \cdot cr \cdot \sigma^2_A}{\sqrt{cr \cdot \sigma^2_A + \frac{(1-cr)\cdot \sigma^2_A}{n} + \frac{\sigma^2_e}{n}}} + \frac{i_i \cdot [(1-cr)\cdot \sigma^2_A + \sigma^2_{NA}]}{\sqrt{(1-cr)\cdot \sigma^2_A + \sigma^2_e}} + \frac{i_c \cdot \sigma^2_{Gc}}{\sqrt{\sigma^2_{Gc} + \frac{\sigma^2_{e_c}}{n_r}}} \qquad (39)$$

where,

ΔG_C is the predicted genetic gain from selection of clones for production in a clonal trial following forward selection in a non-cloned breeding population (gain from selection among families and within families in the breeding population plus the gain from selection in the clonal trial)

i_f is the selection intensity among families in the breeding population

i_i is the selection intensity within families in the breeding population

i_c is the selection intensity among clones in the clonal trial

σ^2_{Gc} is the total genetic variance in the clonal trial

$\sigma^2_{e_c}$ is the error variance in the clonal trial

n is the number of genotypes per family in the breeding population

n_r is the number of ramets per clone in the clonal test

and all other parameters are as previously defined.

Normally, the narrow sense heritability would be used as a minimal estimate of the broad sense heritability for the prediction of gain from a clonal trial established with forward selections in a non-cloned breeding population, as the non-additive genetic variance component cannot be estimated. In this study, however, non-additive variance could be estimated and it is for this reason that it is included in equation 39.

3.6 Effects of selection on additive genetic variance

The effect of selection on additive genetic variance was calculated based on the formulas of Falconer (1989) but adapted to account for the effect of female and male (thinning) selection within families in the open pollinated families. A third was used as the co-efficient of relationship (see section 3.3).

The additive genetic variance in the open pollinated progeny of selected parents was calculated as:

$$\sigma^2_{A_{(t+1)}} = \frac{1}{2}\cdot\left[\frac{1}{3}\cdot\left(1-h^2_{FM\,t}\cdot k_1\right)\sigma^2_{A_t} + \frac{2}{3}\cdot\left(1-h^2_{I_t}\cdot k_2\right)\sigma^2_{A_t}\right] + \frac{1}{2}\left[\frac{1}{3}\cdot\left(1-h^2_{FM\,t}\cdot k_3\right)\sigma^2_{A_t} + \frac{2}{3}\cdot\left(1-h^2_{I_t}\cdot k_4\right)\sigma^2_{A_t}\right] \quad (40)$$

where,

$\sigma^2_{A_{t+1}}$ is the additive genetic variance in the t + 1 generation

$\sigma^2_{A_t}$ is the additive genetic variance in generation t

$h^2_{FM_t}$ is the family mean narrow sense heritability in generation t

$h^2_{I_t}$ is the individual narrow sense heritability in generation t

k_1 is the factor by which the phenotypic variance is reduced by among family selection for female parents when selection is by truncation of a normal distribution

k_2 is the factor by which the phenotypic variance is reduced by within family selection for female parents when selection is by truncation of a normal distribution

k_3 is the factor by which the phenotypic variance is reduced by among family selection for male parents when selection is by truncation of a normal distribution

k_4 is the factor by which the phenotypic variance is reduced by within family selection for male parents when selection is by truncation of a normal distribution, where

$k = i\cdot(i-x)$

i intensity of selection

x the deviation of the point of truncation from the population mean corresponding to the selection intensity.

As there was no rogueing, the factor k_3 is equal to zero, i.e., no among family selection for male parents.

CHAPTER 4

DATA EDITING

Values more than three times the inter-quartile range from the mean were omitted from the data set as outliers once the original coding sheets had been checked to exclude possible transcription errors. The position of the outliers was checked on the trial layout maps but no spatial grouping was found. The ancestry of the outliers was also considered, but no familial grouping could be found. Estimates were obtained with the 24 outliers (<1% of data) included in the data set and these compared to the estimates obtained when the outliers were excluded. Estimates were slightly improved and the error slightly reduced, when the outliers were excluded. Appendix B (Table B-1 and B-2) details the 24 observations removed from the data sets as outliers.

A small percentage of runts and dead trees (38 month assessment only) were noted but these were not assessed. No notable familial grouping of runts or dead trees was observed. Broken tops were not considered to be a common occurrence in this trial as only a small percentage of trees with broken tops were also recorded at both ages in all trials and these observations were dropped from the data set. True height values for trees with broken tops are not available and DBH measurements are considered inaccurate due to the effect of the broken crown and loss of photosynthetic capacity, on the growth. The broken tops that were noted did not appear to be restricted only to a few specific families. The percentage of broken tops, dead trees and trees described as runts, is detailed in Table 5.

For selection purposes, exclusion of the individuals from the data set reduces the number of observations for family means and family means where, possibly, more than a single runt or broken top occurred will therefore be less reliable and individuals from this family less likely to be selected using Best Linear Unbiased Prediction (BLUP).

Table 5. Percentage dead trees, runts and broken tops at 38 and 66 months in trials B1, B2 and B3.

Trial	Dead		Runts		Broken Tops	
	38 months	66 months	38 months	66 months	38 months	66 months
B1	2.68%	-	0.36%	1.12%	0.92%	0.44%
B2	3.96%	-	0%	1.08%	0.48%	0.44%
B3	n/a	-	n/a	2.20%	n/a	0.64%

The total survival in all three trials was very low as indicated in Table 6.

Table 6. Percentage survival at 38 and 66 months in trials B1, B2 and B3.

Trial	38 months	66 months
B1	62.9 %	61.8%
B2	61.4 %	60.6%
B3	60.5 %	60.0%
Mean	61.6%	60.8%

A field check of several dubious observations in an attempt to correct questionable data was done, at 72 months (May 2000), on observations where the DBH to height ratio appeared to be out of proportion (mean DBH: height at 38 months 9.8, mean DBH:height at 66 months 8.9). Trees that "shrank" either in height or DBH between the two assessments, or where missing trees at 38 months were assessed at 66 months, were also checked in field. The ratio of the height difference between the two ages, to the mean height difference in a trial, and the ratio of the DBH difference to the mean DBH difference was also scrutinized and outliers identified. These outliers could possibly have had unusual growth patterns or been influenced by competition or the lack thereof due to high mortality in the area of the specific plot. These outliers could also have been the result of assessment errors and identity mix-ups. These observations were checked in the field.

The 38 month data from trial B3 was omitted from the analyses due to a large number of errors in the assessment of this trial at this age that could not be resolved by editing. The tree breeder responsible for the assessment of the trials at 38 months suggested that the 38 month data of trials B1 and B2 be viewed cautiously as some errors (that could not be traced through editing) as a result of confused plot identities may have occurred. For this reason, the 38 month data is considered less reliable than the 66 month data.

Clone means (across all blocks and replications) were correlated with individual clone values by block as it was suspected that, within certain blocks, there may have been a confusion of clone identities. Considering that the correlation between clone means and the individual clone values by block were inflated by the inclusion of the individual value in the block in the clone mean, a phenotypic correlation of less than 0.5 for both volume and DBH, or both volume and height, was deemed indicative of a block where clonal identities were unreliable (due possibly to errors at establishment or measurement errors). Some of these correlations were not significant ($p \leq 0.05$) but the low frequencies were thought to be a contributing factor to the lack of significance. Based on these assumptions a total of 20% of the blocks were deleted at 38 months, and 11% of the blocks at 66 months in trial B1; 18% at both 38 and 66 months in B2; and 10 % at 66 months in B3. The average survival in these blocks that were removed (Table 7) was slightly lower, but consistent with the average survival in the trial.

Table 7. Percentage survival at 38 and 66 months in the blocks removed from the data sets of trials B1, B2 and B3.

Trial	38 months	66 months
B1	58.8%	59.5%
B2	55.2%	57.8%
B3	n/a	54.0%
Mean	57.0%	57.1%

Logarithmic and square root transformations to normalize the distribution of the individual disease scores were calculated (Snedecor and Cochran, 1967), but the best approximation of normality, using the Shapiro-Wilk W test statistic to evaluate normality (Shapiro and Wilk, 1965), was

obtained by pooling the various disease scores to obtain a mean disease resistance score per tree. This is a common practice and generally selection is done on a pooled score indicating tolerance of all four diseases.

Eucalypts are thought to be sensitive to competition. Estimates of genetic gains and of age-age correlations in small plot breeding trials may be inflated by competition bias (Cooper and Ferguson, 1977) and the effect on the estimation of additive and dominance variance components may be large (Hamblin and Rosielle, 1978). Incomplete block and random complete block designs have also been shown to be very senstitive to the percentage of missing trees (scenarios were tested with up to 20% missing values) and not, necessarily, the spatial arrangement of missing observations (Fu et al., 1999). For these reasons the effect of missing trees was investigated in an attempt to remove any competition bias that may have been caused by the missing trees. The number of missing trees, either adjacent to or diagonal to each tree was counted using an algorithm developed by S.D. Verryn. Individual observations were corrected for missing neighbour effects using linear regression techniques where neighbourhood effects were significant (i.e., the model was significant at $p \leq 0.05$). The effects of adjacent and diagonal neighbours were considered separately and each effect included only where significant ($p \leq 0.05$).

The following regression model was fitted:

$$\hat{y}_i = \beta_0 + \beta_1 x_{1_i} + \beta_2 x_{2i} + \varepsilon_i \qquad (41)$$

where,

$\hat{y}_i$	is the predicted value for trees (observations) i=1,2, . . n
x_{1_i}	is the number of missing trees adjacent to tree i (x_1 =1, 2, . . 4)
x_{2_i}	is the number of missing trees diagonal to tree i (x_2 =1, 2, . . 4)
β_0	is the y-axis intercept
β_1 , β_2	are the regression coefficients
ε_i	is the random error associated with observation i.

The residual (actual value minus the predicted value), which indicates whether the observed value was greater or smaller than that expected for an individual with a certain number of missing neighbours, was used to estimate the variance components for the various traits considered.

Appendix B (Table B-3 and B-4) details the models used to make the correction for missing neighbours.

Problems with the accuracy of the trial and data, such as those experienced in these trials, are not uncommon and do occur in forestry. This study has highlighted the importance of taking every precaution, from the nursery, through to the field with trial establishment, maintenance and assessment, to minimise errors and ensure the accuracy of the results that can be obtained from the trial. Careful planning and execution of a trial are essential if accurate results are to be obtained.

All results are reported to two counting figures after the decimal point.

CHAPTER 5

SEPARATE TRIAL RESULTS

The results from the separate trials (B1, B2 and B3), which were each designed as sub-populations of the breeding population, were initially considered separately in order to investigate the variance components in the separate sub-populations and to determine whether or not there were any differences in trends between sub-populations.

The trials were designed as an alpha lattice. The incomplete blocks were, however, not laid out according to the design. The treatments that were allocated by the design to the various blocks were not allocated to the blocks when the trial was laid out in the nursery due to an error in reading the trial design. The allocation of treatments to blocks in the alpha lattice design is done so that as many different pairwise comparisons between treatments as possible are made between blocks and so that pairs of treatments are found together in blocks roughly the same number of times as all other pairs. This ensures that all pairs of treatments are compared with approximately the same precision. If treatments are randomly allocated to blocks, as was effectively the case with this trial, then it is unlikely that treatments will be compared with the same precision (Williams and Matheson, 1994). High mortality resulted in small number of treatments per block and this was also thought to contribute to a reduced efficiency of the block effect by reducing the number of treatments per block. The contribution of block effect was, however, investigated (Appendix C) but was not significant ($p \leq 0.01$) in the analysis of variance (ANOVA) for the majority of traits (except for height where, apart from B3, the block effect was highly significant, $p \leq 0.0001$). It was decided, for these reasons, that block effect not be included in the model for the analysis of variance for the estimation of variance components.

Trial means, standard errors and frequencies are detailed in Table 8. Significant ($p \leq 0.05$) differences were detected between trials at both ages (38 and 66 months) for volume and DBH at

38 months, and for volume, height and disease at 66 months. Significant differences between trials were not detected for height, stem form and disease at 38 months and for DBH and stem form at 66 months. The Student-Newman-Keul and T-test showed significant (α=0.05) differences between all trials for all traits assessed (Appendix D). B3 is the most productive trial with the highest means for volume, DBH, and height at 66 months. Trial B3 is situated at the lowest point of the slope (B2 and B3 upslope) and a fertility gradient may also be present. B3 however, also seems to be the least reliable of the trials as indicated by the relatively lower F values (proportionately higher error variance relative to mean square effects) for treatment and clone within treatment effects in the analysis of variance (Appendix C, Tables C-21 to C-25).

Table 8. Trial means and descriptive statistics for the 38 and 66 months assessment of trials B1, B2 and B3. (Means are calculated over all trees and not on clone means.)

Trial	Age	Trait	Mean	Standard Error	Number of observations
B1	38 months	Volume (m^3)	0.13	0.0010	1248
		DBH (mm)	168.65	0.79	1252
		Height (m)	16.91	0.048	1249
		Stem score	5.44	0.041	1248
		Disease tolerance	0.65	0.0089	1253
	66 months	Volume (m^3)	0.32	0.0040	1303
		DBH (mm)	215.82	1.065	1304
		Height (m)	23.35	0.064	1303
		Stem score	5.49	0.023	1302
		Disease tolerance	0.59	0.0070	1304
B2	38 months	Volume (m^3)	0.14	0.0020	1245
		DBH (mm)	172.62	0.82	1253
		Height (m)	17.711	0.047	1245
		Stem score	5.88	0.036	1253
		Disease tolerance	0.56	0.0070	1255

Trial	Age	Trait	Mean	Standard Error	Number of observations
	66 months	Volume (m^3)	0.35	0.0040	1190
		DBH (mm)	218.95	1.082	1191
		Height (m)	24.30	0.068	1191
		Stem score	5.84	0.024	1192
		Disease tolerance	0.53	0.0060	1192
B3	66 months	Volume (m^3)	0.39	0.0040	1265
		DBH (mm)	222.85	1.014	1266
		Height (m)	25.62	0.063	1265
		Stem score	5.74	0.021	1266
		Disease tolerance	0.50	0.0060	1266

The number of clones per family and ramets per clone established and present in the data sets is detailed in Table 9. Due to uneven family sizes and unequal survival rates, the figures presented are averages and, therefore, recorded to two decimal places. The mean number of ramets per clone increases slightly between ages 38 and 66 months for B1 because different observations (deemed dubious, see Chapter 4) were removed from the two data sets during the editing of the data sets.

Family and clone within-family effects were significant ($p \leq 0.05$) for all traits in trials B1 and B2 at both ages (Appendix C). In trial B3, however, the family effect was significant ($p \leq 0.05$) for all traits except stem form, but the clone within-family effect was only significant ($p \leq 0.05$) for disease tolerance.

Table 9. Family and clone frequencies, both established and realised in the data for the two ages of assessment, in trials B1, B2 and B3. (Realised values are means.)

Trial	Established			Realised (means)			
				38 months		66 months	
	No. of families	Mean No. of clones/family	No. of ramets/clone	No. of clones/family	No. of ramets/clone	No. of clones/family	No. of ramets/clone
B1	56	8.93	5	8.48	2.64	8.47	2.75
B2	59	8.48	5	8.24	2.58	8.11	2.49
B3	68	7.35	5	n/a	n/a	7.05	2.64

The estimates of variance components and heritabilities obtained for the individual trials B1, B2 and B3, according to the three scenario's considered, are presented in Table 10. The three scenarios can be summarized as follows (refer to Chapter 3 for a more detailed discussion of the scenarios considered):

- Scenario 1: Coefficient of relationship= ⅓; proportion of non-additive variance segregating within open pollinated families <1
- Scenario 2: Coefficient of relationship= ⅓; proportion of non-additive variance segregating within open pollinated families= 1
- Scenario 3: Coefficient of relationship= ¼; proportion of non-additive variance segregating within open pollinated families= 1

Negative estimates of variance components were obtained and these are indicated (bold type) in Table 10. The negative estimates reflect the lack of precision or accuracy in the estimate of the variance components. The magnitudes of the negative estimates were generally small compared to the other estimates of variance components for the specific trait. For the calculation of heritabilities and percentages, the negative estimates were considered to approximate zero and standard errors were not calculated.

Table 10. Estimates of variance components and heritabilities for trials B1, B2 and B3. [The traits that are shaded have not been corrected for missing neighbours.]

Scenario	B1	Trait									
		66 months					38 months				
		Volume	Height	DBH	Stem	Disease	Volume	Height	DBH	Stem	Disease
	Var(fam)	0.0011	0.33	72.88	0.035	0.0032	0.0001	0.066	27.29	0.10	0.0031
	Std deviation (var(fam))	0.0004	0.12	29.48	0.014	0.0013	0.0001	0.069	14.75	0.044	0.0018
	Var(clone(fam))	0.0049	1.21	377.87	0.11	0.15	0.0005	0.26	129.30	0.30	0.018
	Std deviation (var(clone(fam)))	0.0006	0.19	48.42	0.02	0.0021	0.0001	0.18	30.18	0.074	0.0034
	Var(error)	0.010	3.76	888.96	0.54	0.038	0.0020	4.82	693.38	1.69	0.069
	Var(phenotypic)	0.016	5.30	1339.71	0.68	0.19	0.0026	5.15	849.96	2.09	0.091
1	Var(additive)	0.0033	1.030	201.37	0.11	**-0.017**	0.0003	0.20	77.84	0.33	0.0083
	Var(non-additive)	0.0027	0.52	249.37	0.03	0.17	0.0003	0.13	78.74	0.066	0.013
	Var(genetic)	0.0060	1.55	450.75	0.14	0.17	0.0006	0.33	156.58	0.40	0.02
	Var(A) % of var(G)	54.89	66.62	44.68	78.35	0	54.53	61.09	49.71	83.53	38.92
	Var(NA) % of var(G)	45.11	33.38	55.32	21.65	100	45.47	38.91	50.29	16.47	61.08
	h^2	0.20	0.19	0.15	0.16	0	0.12	0.039	0.092	0.16	0.092
	Standard error (h^2)	0.074	0.07	0.067	0.061	-	0.059	0.040	0.052	0.064	0.059
	H^2	0.37	0.29	0.34	0.21	0.89	0.23	0.063	0.18	0.19	0.24
	Clone mean h^2	0.31	0.32	0.24	0.29	0	0.21	0.076	0.16	0.28	0.15
	Clone mean H^2	0.57	0.48	0.54	0.37	1.0027	0.38	0.13	0.32	0.33	0.39
2	Var(additive)=3*var(fam)	0.0034	1.0042	218.64	0.10	0.0096	0.0003	0.20	81.87	0.31	0.0094
	Var(non-additive) k=1	0.0026	0.54	232.10	0.037	0.14	0.0003	0.13	74.71	0.089	0.012
	Var(genetic)	0.0060	1.55	450.75	0.14	0.15	0.0006	0.33	156.58	0.40	0.021
	Var(A) % of var(G)	56.17	64.96	48.51	73.77	6.43	55.90	60.82	52.29	77.65	44.19
	Var(NA) % of var(G)	43.83	35.04	51.49	26.23	93.57	44.10	39.18	47.71	22.35	55.81
	h^2	0.21	0.19	0.16	0.15	0.051	0.13	0.039	0.096	0.15	0.10
	Standard error (h^2)	0.07	0.07	0.067	0.061	0.021	0.059	0.040	0.05	0.064	0.059
	H^2	0.37	0.29	0.34	0.21	0.80	0.23	0.063	0.18	0.19	0.24
	Clone mean h^2	0.32	0.31	0.26	0.27	0.058	0.21	0.076	0.17	0.26	0.17
	Clone mean H^2	0.57	0.48	0.54	0.37	0.90	0.38	0.13	0.32	0.33	0.40
3	Var(additive)=4*var(fam)	0.0045	1.34	291.52	0.14	0.013	0.0004	0.26	109.16	0.41	0.013
	Var(non-additive) k=1	0.0015	0.21	159.22	0.0023	0.14	0.0001	0.062	47.42	0	0.0088
	Var(genetic)	0.0060	1.55	450.75	0.14	0.15	0.0006	0.33	156.58	0.41	0.021
	Var(A) % of var(G)	74.89	86.62	64.67	98.35	8.57	74.53	81.09	69.71	100	58.92
	Var(NA) % of var(G)	25.11	13.38	35.32	1.65	91.43	25.47	18.91	30.29	0	41.078
	h^2	0.27	0.25	0.22	0.203	0.068	0.17	0.051	0.13	0.20	0.14
	Standard error (h^2)	0.099	0.09	0.088	0.081	0.029	0.078	0.054	0.069	0.085	0.08
	H^2	0.37	0.29	0.337	0.21	0.80	0.23	0.063	0.18	0.20	0.24
	Clone mean h^2	0.43	0.42	0.35	0.36	0.077	0.28	0.101	0.23	0.35	0.23
	Clone mean H^2	0.57	0.48	0.54	0.37	0.90	0.38	0.13	0.32	0.35	0.39

Scenario	B2	Trait									
		66 months					38 months				
		Volume	Height	DBH	Stem	Diameter	Volume	Height	DBH	Stem	Diameter
	Var(fam)	0.0012	0.25	67.21	0.015	0.0035	0.0001	0.16	25.75	0	0.0004
	Std deviation (var(fam))	0.0005	0.12	34.08	0.015	0.0014	0.0001	0.085	15.66	0.020	0.0010
	Var(clone(fam))	0.0023	0.47	134.64	0.073	0.0089	0.0003	0.19	80.80	0.18	0.011
	Std deviation (var(clone(fam)))	0.0007	0.20	52.76	0.028	0.0018	0.0001	0.19	30.24	0.059	0.0022
	Var(error)	0.014	4.63	1209.10	0.64	0.035	0.0027	5.29	746.68	1.45	0.045
	Var(phenotypic)	0.018	5.35	1410.94	0.73	0.047	0.0031	5.64	853.23	1.62	0.056
1	Var(additive)	0.0039	0.86	228.46	0.042	0.011	0.0004	0.55	81.69	**-0.035**	**-0.0007**
	Var(non-additive)	**-0.0004**	**-0.14**	**-26.61**	0.046	0.0009	0.0001	**-0.20**	24.85	0.21	0.012
	Var(genetic)	0.0039	0.86	228.46	0.088	0.012	0.0005	0.55	106.55	0.21	0.012
	Var(A) % of var(G)	100	100	100	47.77	92.44	77.44	100	76.67	0	0
	Var(NA) % of var(G)	0	0	0	52.23	7.56	22.56	0	23.33	100	100
	h^2	0.22	0.16	0.16	0.058	0.24	0.11	0.098	0.096	0	0
	Standard error (h^2)	0.08	0.068	0.072	0.061	0.088	0.059	0.045	0.055	-	-
	H^2	0.22	0.16	0.16	0.12	0.26	0.15	0.098	0.12	0.13	0.22
	Clone mean h^2	0.36	0.28	0.28	0.10	0.38	0.20	0.19	0.17	0	0
	Clone mean H^2	0.36	0.28	0.28	0.21	0.41	0.26	0.19	0.23	0.24	0.37
2	Var(additive)=3*var(fam)	0.0035	0.76	201.62	0.045	0.010	0.0003	0.47	77.25	0	0.0012
	Var(non-additive) k=1	0.00004	**-0.030**	0.22	0.043	0.0019	0.0001	**-0.12**	29.30	0.18	0.010
	Var(genetic)	0.0035	0.76	201.84	0.088	0.012	0.0005	0.47	106.55	0.18	0.012
	Var(A) % of var(G)	98.97	100	99.89	50.82	84.33	73.083	100	72.50	0	10.56
	Var(NA) % of var(G)	1.031	0	0.11	49.18	15.67	26.92	0	27.50	100	89.44
	h^2	0.19	0.14	0.14	0.061	0.22	0.11	0.083	0.091	0	0.022
	Standard error (h^2)	0.08	0.068	0.07	0.061	0.088	0.059	0.045	0.055	0.036	0.054
	H^2	0.20	0.14	0.14	0.12	0.26	0.15	0.083	0.12	0.11	0.20
	Clone mean h^2	0.32	0.25	0.25	0.11	0.35	0.19	0.16	0.16	0	0.036
	Clone mean H^2	0.32	0.25	0.25	0.21	0.41	0.26	0.16	0.23	0.20	0.34
3	Var(additive)=4*var(fam)	0.0046	1.0091	268.83	0.060	0.014	0.0004	0.62	103.0028	0	0.0016
	Var(non-additive) k=1	**-0.0011**	**-0.28**	**-66.98**	0.028	**-0.0015**	0.00001	**-0.27**	3.55	0.18	0.0099
	Var(genetic)	0.0046	1.0091	268.83	0.088	0.014	0.0005	0.62	106.55	0.18	0.016
	Var(A) % of var(G)	100	100	100	67.77	100	97.44	100	96.67	0	14.08
	Var(NA) % of var(G)	0	0	0	32.23	0	2.56	0	3.33	100	85.92
	h^2	0.26	0.19	0.19	0.082	0.29	0.14	0.11	0.12	0	0.029
	Standard error (h^2)	0.11	0.09	0.097	0.081	0.12	0.079	0.06	0.073	0.049	0.072
	H^2	0.26	0.19	0.19	0.12	0.29	0.15	0.11	0.12	0.11	0.204
	Clone mean h^2	0.43	0.33	0.33	0.15	0.46	0.25	0.21	0.22	0	0.049
	Clone mean H^2	0.43	0.33	0.33	0.21	0.46	0.26	0.21	0.23	0.20	0.34

Scenario	B3	Trait				
		66 months				
		Volume	Height	DBH	Stem	[illegible]
	Var(fam)	0.0010	0.23	55.95	0.0013	0.00010
	Std deviation (var(fam))	0.00040	0.11	25.19	0.0079	0.00080
	Var(clone(fam))	0.00070	0.26	37.14	0.013	0.0074
	Std deviation (var(clone(fam)))	0.00060	0.17	38.067	0.020	0.0018
	Var(error)	0.016	4.78	1095.83	0.57	0.042
	Var(phenotypic)	0.018	5.27	1188.92	0.58	0.049
1	Var(additive)	0.0037	0.84	205.18	0.0023	**-0.0010**
	Var(non-additive)	**-0.0020**	**-0.35**	**-112.090**	0.012	0.0086
	Var(genetic)	0.0037	0.84	205.18	0.015	0.0086
	Var(A) % of var(G)	100	100	100	15.81	0
	Var(NA) % of var(G)	0	0	0	84.19	100
	h^2	0.20	0.16	0.17	0.0039	0
	Standard error (h^2)	0.068	0.063	0.085	0.054	0.065
	H^2	0.20	0.16	0.17	0.025	0.17
	Clone mean h^2	0.39	0.31	0.34	0.0081	0
	Clone mean H^2	0.39	0.31	0.34	0.051	0.32
2	Var(additive)=3*var(fam)	0.0030	0.70	167.85	0.0039	0.0004
	Var(non-additive) k=1	**-0.0013**	**-0.21**	**-74.76**	0.011	0.0072
	Var(genetic)	0.0030	0.70	167.85	0.015	0.0075
	Var(A) % of var(G)	100	100	100	26.86	4.78
	Var(NA) % of var(G)	0	0	0	73.14	95.22
	h^2	0.17	0.13	0.14	0.0067	0.0073
	Standard error (h^2)	0.07	0.063	0.085	0.054	0.065
	H^2	0.17	0.13	0.14	0.025	0.15
	Clone mean h^2	0.32	0.26	0.28	0.014	0.013
	Clone mean H^2	0.32	0.26	0.28	0.051	0.28
3	Var(additive)=4*var(fam)	0.0040	0.94	223.80	0.0052	0.0005
	Var(non-additive) k=1	**-0.0023**	**-0.45**	**-130.71**	0.0093	0.0070
	Var(genetic)	0.0040	0.94	223.80	0.015	0.0075
	Var(A) % of var(G)	100	100	100	35.81	6.37
	Var(NA) % of var(G)	0	0	0	64.19	93.63
	h^2	0.22	0.18	0.19	0.0089	0.0097
	Standard error (h^2)	0.091	0.084	0.085	0.054	0.065
	H^2	0.22	0.18	0.19	0.025	0.15
	Clone mean h^2	0.43	0.34	0.37	0.018	0.018
	Clone mean H^2	0.43	0.34	0.37	0.051	0.28

The estimates of total genetic variance differ in the same trial where negative estimates of genetic variance components were obtained for one or more of the scenarios and these estimates were

zeroed. Only a single negative estimate of a variance component (for non-additive variance for disease tolerance at 66 months under scenario) was obtained for trial B1, whereas several negative estimates were obtained in trials B2 and B3. This may possibly indicate that the estimates in trial B1 are more precise. Negative estimates of variance components were obtained under all three scenarios. (The difference in the estimates obtained under three scenarios used are presented and discussed in more detail in Chapters 6 and 10).

Environmental effects (error variance) are proportionately large (relative to the expression of genetic effects) and indicate the lack of precision of the data. The large environmental variances may also indicate that the assumption that C-effects are absent is invalid.

The estimates of the proportion of genetic variance attributable to non-additive variance for the various traits fluctuate across trials and do not show clear trends. For volume at 66 months non-additive variance ranges from 45% to 0% of total genetic variance depending on the trial and method used to estimate the variance component. For volume at 66 months estimates of the proportion of non-additive variance for volume in trials B2 and B3 are low (0-1%) whereas in trial B1, the proportion of non-additive variance ranges between 25% and 45%. Similarly, for height and DBH, the estimate of the proportion of non-additive variance in trial B1 is much higher than in B2 and B3. In contrast, the estimate of the genetic variance attributable to non-additive variance for disease tolerance at 66 months ranges from 91% to 100% in B1 and B3 but in trial B2 ranges between 0% and 16% (depending on the scenario). The proportion of non-additive variance for stem form ranges from 2% to 26% in B1, 32% to 52% in B2 and 64% to 84% in B3.

The trend in heritabilities (broad and narrow sense) is similar across all three trials and the estimates do not differ markedly from trial to trial. (The heritability estimates are summarized in Table 11 for ease of reference). However, the proportion of additive to non-additive variance does not show clear trends across all three trials. It was, therefore, decided to pool the data from all three trials and thereby increase the amount of data and number of families in order to obtain a more stable estimate of the variance components and a more stable estimate of the relative proportions of additive and non-additive variance for the traits considered.

Table 11. Estimates of heritabilities for trials B1, B2 and B3.

Scenario1							
Trait	**Age**	**B1**		**B2**		**B3**	
		h^2	**H^2**	**h^2**	**H^2**	**H^2**	**H^2**
Volume	38 months	0.12	0.23	0.11	0.15	n/a	n/a
	66 months	0.20	0.37	0.22	0.22	0.20	0.20
Height	38 months	0.039	0.063	0.098	0.098	n/a	n/a
	66 months	0.19	0.29	0.16	0.16	0.16	0.16
DBH	38 months	0.092	0.18	0.096	0.12	n/a	n/a
	66 months	0.15	0.34	0.16	0.16	0.17	0.17
Stem	38 months	0.16	0.19	0	0.13	n/a	n/a
	66 months	0.16	0.21	0.058	0.12	0.0039	0.025
Disease	38 months	0.092	0.24	0	0.22	n/a	n/a
	66 months	0	0.89	0.24	0.26	0	0.17
Scenario2							
Trait	**Age**	**B1**		**B2**		**B3**	
		h^2	**H^2**	**h^2**	**H^2**	**h^2**	**H^2**
Volume	38 months	0.13	0.23	0.11	0.15	n/a	n/a
	66 months	0.21	0.37	0.19	0.20	0.17	0.17
Height	38 months	0.039	0.063	0.083	0.083	n/a	n/a
	66 months	0.19	0.29	0.14	0.14	0.13	0.13
DBH	38 months	0.096	0.18	0.091	0.12	n/a	n/a
	66 months	0.16	0.34	0.14	0.14	0.14	0.14
Stem	38 months	0.15	0.19	0	0.11	n/a	n/a
	66 months	0.15	0.21	0.061	0.12	0.0067	0.025
Disease	38 months	0.10	0.24	0.022	0.20	n/a	n/a
	66 months	0.051	0.80	0.22	0.26	0.0073	0.15
Scenario3							
Trait	**Age**	**B1**		**B2**		**B3**	
		h^2	**H^2**	**h^2**	**H^2**	**h^2**	**H^2**
Volume	38 months	0.17	0.23	0.14	0.15	n/a	n/a
	66 months	0.27	0.37	0.26	0.26	0.22	0.22
Height	38 months	0.051	0.063	0.11	0.11	n/a	n/a
	66 months	0.25	0.29	0.19	0.12	0.18	0.18
DBH	38 months	0.13	0.18	0.12	0.19	n/a	n/a
	66 months	0.22	0.34	0.19	0.19	0.19	0.19
Stem	38 months	0.20	0.20	0	0.11	n/a	n/a
	66 months	0.20	0.21	0.082	0.12	0.0089	0.025
Disease	38 months	0.14	0.24	0.029	0.20	n/a	n/a
	66 months	0.068	0.80	0.29	0.29	0.0097	0.15