BROAD AND NARROW SENSE HERITABILITIES IN A CLONED OPEN POLLINATED *EUCALYPTUS GRANDIS* BREEDING POPULATION

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DECLARATION

I the undersigned hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part, been submitted to any other university.

Signature: ..... Date: 02/08/2001

SUMMARY

Genetic variances and heritabilities in a cloned *Eucalyptus grandis* breeding population of families derived from open pollinated selections were estimated and the results are presented. The genetic variance was partitioned into additive and non-additive genetic variance components that allowed the estimation of broad and narrow sense heritabilities. Predicted gains for breeding and production population options are discussed.

The magnitude of the coefficient of relationship between sibs was shown to have a considerable impact on the estimate of variance components and the importance of understanding the level of relatedness in the population is highlighted.

Growth traits (volume, diameter at breast height/DBH, height), stem form and disease tolerance were assessed at 38 and 66 months in each of the three separate trials established as sub-populations of the breeding population. The additive genetic variance was the largest proportion of genetic variance for the growth traits (84% for volume, 94% for height and 74% for DBH), whereas the proportion of non-additive genetic variance was notably higher for stem form and disease tolerance (37% and 46% respectively). The growth traits and stem form are, economically, the most important traits and a breeding strategy that exploits the additive genetic variation by selection to increase the frequency of the alleles causing the desirable genotypes is appropriate. The higher proportion of non-additive genetic variance for disease does, however, suggest higher gains (compared with the afore mentioned strategy of selection for general combining ability) will be achieved by exploiting the non-additive variance by for example, selection for specific combining ability, using inbred lines, clones.

The composition of the genetic variance was investigated separately in the F1 and F2 families to obtain an indication of whether or not there was a change in proportion of non-additive and additive genetic variance over the two generations. A notably larger proportion of non-additive variance was found for the growth traits and stem form among the F2 families. This is probably due to the reduction in additive variance through selection for these traits in the previous generations. No

selection for disease took place in earlier generations and the proportion of non-additive genetic variance for this trait remains approximately the same over both generations. These results may indicate that with advanced generations of breeding in this population, that gains achieved through selection for additive variance will decline compared with that achieved in previous generations. A strategy for future generations that exploits non-additive variance may be appropriate.

A high proportion of error variance was estimated and in situations such as these, cloning is particularly beneficial as is shown by the high clone mean heritabilities estimated in these trials. High mortality, resulting in fewer ramets per clone, erodes the benefit of cloning in these trials.

The predicted gains showed the benefit of the cloned breeding population both in terms of breeding population gains and production population gains. Reducing the breeding cycle by bulking up clones faster will also increase gains per year. High gains in the production population were predicted, particularly for the selection of tested clones for deployment, which can be done at the same time as selections are made for the next generation. The benefit of the cloned population was therefore shown to be twofold, namely increasing the accuracy of within family selection and increasing the gains in the rapid deployment of tested clones and therefore facilitating the faster realisation of predicted gain in the plantation.

Keywords: Broad sense heritability, narrow sense heritability, cloned breeding population, additive genetic variation, non-additive genetic variation, *Eucalyptus grandis*.

OPSOMMING

Genetiese variansies en oorerflikhede in 'n gekloonde *Eucalyptus grandis* teelpopulasie van families verkry van af oopbestuifde seleksies, is bereken en die resultate word aangebied. Die genetiese variansies is opgedeel in additiewe en nie-additiewe komponente wat die skatting van breë en eng sin oorerflikhede moontlik maak. Voorspelde vordering vir die teel- en produksiepopulasies word ook bespreek.

Die grootte van die koëffisiënt van die verwantskap tussen sibbe blyk redelike groot invloed op die skatting van die variansie komponente te hê en dit is dus belangrik om die mate van verwantskap in die populasie te verstaan.

Groei-eienskappe (volume, deursnit op borshoogte (DBH) en hoogte), stamvorm en weerstand teen siektes is op 38 en 66 maande in elkeen van die onderskeie proewe gemeet. Die additiewe genetiese variansie was die grootste proporsie van die genetiese variansie vir die groei-eienskappe (84% vir volume, 94% vir hoogte en 74% vir DBH). Die proporsie nie-additiewe genetiese variansie was merkbaar hoër vir stamvorm en siekteweerstand (37% en 46% onderskeidelik). Die groei-eienskappe en stamvorm is, ekonomies gesien, die belangrikste eienskappe en 'n teelstrategie wat die additiewe genetiese variansie ontgin deur seleksie om die frekwensie van "allele" wat die gunstige genotipes tot gevolg het te verhoog, is geskik. Die hoër proporsie van nie-additiewe genetiese variansie vir siekteweerstand wys dat 'n hoër wins gemaak sal word (in vergelyking met die bogenoemde strategie van seleksie vir algemene kombineringsvermoë) deur gebruik te maak van nie-additiewe variansie.

Die samestelling van genetiese variansie is afsonderlik in die F1 en F2 families ondersoek om 'n indikasie te kry of daar verskille tussen die proporsie nie-additiewe en additiewe variansies vir die verskillende generasies, bestaan. 'n Merkbare groter proporsie van nie-additiewe variansie is vir die groei-eienskappe en stamvorm in die F2 families gevind. Dit is moontlik te wyte aan die vermindering in additiewe variansie weens seleksie vir hierdie eienskappe in die vorige generasie. Geen seleksie vir siekteweerstand het in die vorige generasies plaasgevind nie en daarom kan dit wees dat die proporsie nie-additiewe genetiese variansie vir hierdie eienskap nie-merkbaar tussen

die twee generasies verskil nie. Hierdie resultate kan moontlik daarop dui dat, in gevorderde generasies van hierdie teelpopulasie die vordering deur seleksie vir additiewe variansie sal afneem in verhouding tot die vordering verkry deur seleksie in die vorige generasies. 'n Strategie vir die seleksie van toekomstige generasies wat die nie-additiewe variansie gebruik mag dan toepasliker wees.

'n Hoë proporsie vir die oorblywende foutvariansie was beraam en in sulke gevalle kan klonering hoogs voordelig wees, soos bewys deur die hoë erfbaarheidsyfers vir die klone verkry in hierdie proef. Die hoë mortaliteit wat tot gevolg gehad het dat minder ramette per kloon oorleef het bederf egter tot 'n mate die voordeel van klonering in hierdie proewe.

Die voorspelde vordering wys die voordeel van die gekloonde teelpopulasie in terme van beide die teel- en produksiepopulasie vordering. Verkorting van die teelsiklus deur klone vinniger te vermeerder sal ook bydra om vordering per jaar te verhoog. Hoë vordering in die produksiepopulasie is voorspel, veral vir die seleksie van getoetste klone vir aanwending wat plaas kan vind wanneer seleksie vir die volgende generasie gedoen word. Die voordeel van die gekloonde populasie is bewys tweeledig te wees, namelik verhoging van die akkuraatheid van binne familie seleksie en vermeerdering van die vordering deur die vinniger aanwending van getoetste klone en daarom die vinniger verhoging van voorspelde vordering in die plantasie deur die ontplooing van getoetste klone.

Sleutelwoorde: Breë sin oorerflikheid, eng sin oorerflikheid, gekloonde teel- populasie, additiewe genetiese variansie, nie-additiewe genetiese variansie, *Eucalyptus grandis*

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NOMENCLATURE AND DEFINITIONS

A list of abbreviations, contractions and definitions frequently used in the text is included for ease of reference; others are expanded in the text:

A	Additive genetic variance component
DBH	Diameter at breast height (1.3metres) in millimetres
Disease	Disease tolerance (Mean tolerance to *Coniothyrium, Crypohonectria, Botryosphaeria* and *Endothia* scored on a 5 point scale.)
P0	Parental generation
F1	First generation
F2	Second generation
Fam	Family
G	Genetic variance component
Ht	Height in metres
NA	Non-additive genetic variance component
SE	Standard error
Stem	Stem form (8 point scale)
VAR	Variance
h^2	Narrow sense heritability
H^2	Broad sense heritability
σ	Standard deviation
σ^2	Variance component

Subscripts:

A	Additive genetic variance component
c(f)	Clone within family
F	Family
G	Genotypic or genetic variance component
NA	Non-additive genetic variance component
P	Phenotypic

Definitions:

Clone:	a group of genetically identical individuals
Family:	Genotypes raised from the seed of a single tree
Provenance:	the original native origin (geographic) of a population
Ramet:	an individual member of a clone

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.

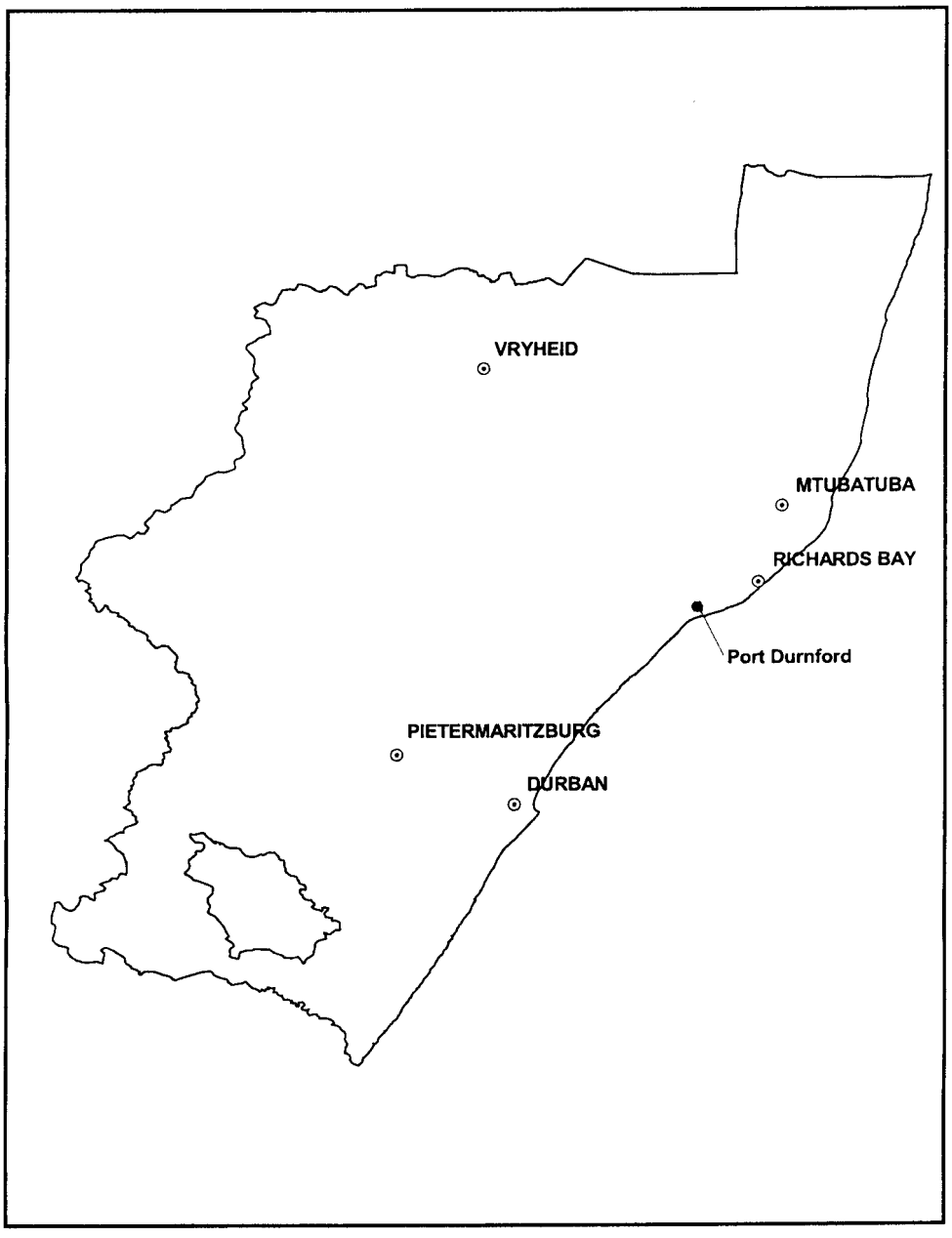

Figure 1. Map of Kwa-Zulu Natal with the location of trials B1, B2 and B3 indicated at Port Durnford.

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

CHAPTER 6

COMBINED TRIAL RESULTS

The data from all three of the trials (B1, B2 and B3) were pooled in order to obtain a more stable estimate of the variance components by increasing the amount of data available. The pooled data set consisted of 177 families and 1399 clones at 66 months.

The data from each trial was corrected for the replication effect, then pooled and corrected for the trial effect (see Chapter 5 for discussion of significant effects in the separate trials and Appendix D for the analysis of variance in the pooled data prior to correction for trial effect and the results of the T-test for significant difference between trials for each trait at each age).

Trial means and descriptive statistics are detailed in Table 12.

Table 12. Means and descriptive statistics for the pooled data (data set B123) of the 38 and 66 months assessment of trials B1, B2 and B3.

Data Set	Age	Trait	Mean	Standard Deviation	Standard Error	Number of observations
B123	38 months	Volume (m^3)	0.14	0.050	0.0010	2493
		DBH (mm)	170.63	28.53	0.57	2505
		Height (m)	17.31	1.70	0.034	2494
		Stem score	5.66	1.35	0.027	2501
		Disease tolerance	0.61	0.25	0.0050	2508

Data Set	Age	Trait	Mean	Standard Deviation	Standard Error	Number of observations
B123	66 months	Volume (m^3)	0.35	0.12	0.0020	3758
		DBH (mm)	219.18	37.29	0.61	3761
		Height (m)	24.42	2.33	0.038	3759
		Stem score	5.68	0.80	0.013	3760
		Disease tolerance	0.54	0.25	0.0040	3762

The estimates from the pooled data are considered a more stable estimate of the variance components and more informative as to the trends in additive and non-additive variance for the traits assessed. Pooling the data increases the number of observations, families and clones available for the estimate of variance components. Estimates of variance components and heritabilities for the analysis of all three trials combined (pooled data set) are presented in Table 13. As with the separate trial results, negative estimates of variance components were obtained and these are indicated (bold type) in Table 13. The negative estimates are shown but for the calculation of heritabilities and percentages, the negative estimates were considered to approximate zero.

[An analysis of survival was outside the scope of this study, however, the heritability of survival at 66 months was investigated in the separate trials and in the pooled data. Survival was found to be poorly heritable in these trials and the broad and narrow sense heritability estimates were below 0.1 in all cases.]

Table 13. Estimates of variance components and heritabilities for all three trials (B1, B2 and B3) combined. [The traits that are shaded have not been corrected for missing neighbours.]

Scenario	B123	Trait									
		66 months					38 months				
		Volume		DBH	Stem		Volume	Height	DBH	Stem	
	Var(fam)	0.0010	0.29	61.11	0.017	0.0022	0.00010	0.109	23.23	0.053	0.0018
	Std deviation (var(fam))	0.00020	0.16	16.36	0.0070	0.00080	0.000040	0.055	10.22	0.024	0.0013
	Var(clone(fam))	0.0026	0.62	188.17	0.063	0.010	0.00040	0.23	106.72	0.27	0.014
	Std deviation (var(clone(fam)))	0.00040	0.11	26.99	0.013	0.0011	0.00010	0.13	21.43	0.0025	0.0020
	Var(error)	0.014	4.41	1063.32	0.59	0.039	0.0024	5.10	725.33	1.66	0.058
	Var(phenotypic)	0.018	5.31	1312.60	0.67	0.051	0.0029	5.44	855.28	1.98	0.074
1	Var(additive)	0.0033	0.96	194.57	0.052	0.0064	0.00030	0.37	66.94	0.15	0.0038
	Var(non-additive)	0.00030	**-0.054**	54.71	0.028	0.0059	0.00020	**-0.029**	63.014	0.17	0.012
	Var(genetic)	0.0036	0.96	249.28	0.080	0.012	0.00050	0.37	129.95	0.32	0.016
	Var(A) % of var(G)	92.0006	100	78.054	64.53	52.32	60.071	100	51.51	46.16	23.77
	Var(NA) % of var(G)	8.00	0	21.95	35.47	47.68	39.93	0	48.49	53.84	76.23
	h^2	0.19	0.18	0.15	0.077	0.13	0.11	0.067	0.078	0.074	0.051
	Standard error (h^2)	0.041	0.039	0.037	0.031	0.039	0.040	0.031	0.036	0.036	0.040
	H^2	0.208	0.18	0.19	0.12	0.24	0.176	0.067	0.15	0.16	0.22
	Clone mean h^2	0.33	0.32	0.26	0.14	0.209	0.185	0.13	0.14	0.13	0.087
	Clone mean H^2	0.36	0.32	0.33	0.22	0.40	0.31	0.13	0.27	0.29	0.36
2	Var(additive)=3*var(fam)	0.0031	0.86	183.32	0.051	0.0067	0.00030	0.33	69.70	0.16	0.0053
	Var(non-additive) k=1	0.00060	0.050	65.96	0.029	0.0056	0.00020	0.012	60.26	0.16	0.011
	Var(genetic)	0.0036	0.91	249.28	0.080	0.012	0.00050	0.34	129.95	0.32	0.016
	Var(A) % of var(G)	84.00050	94.44	73.54	63.40	54.24	60.053	96.43	53.63	49.62	32.82
	Var(NA) % of var(G)	16.00	5.56	26.46	36.60	45.76	39.95	3.57	46.37	50.38	67.18
	h^2	0.17	0.16	0.14	0.076	0.13	0.11	0.060	0.082	0.080	0.071
	Standard error (h^2)	0.041	0.039	0.037	0.031	0.039	0.040	0.031	0.036	0.036	0.040
	H^2	0.21	0.17	0.19	0.12	0.24	0.18	0.062	0.15	0.16	0.22
	Clone mean h^2	0.30	0.29	0.24	0.14	0.22	0.18	0.12	0.15	0.14	0.12
	Clone mean H^2	0.36	0.30	0.33	0.22	0.40	0.31	0.12	0.27	0.29	0.36
3	Var(additive)=4*var(fam)	0.0041	1.14	244.43	0.068	0.0089	0.00040	0.43	92.93	0.21	0.0070
	Var(non-additive) k=1	**-0.00040**	**-0.24**	4.85	0.012	0.0034	0.00010	**-0.097**	37.02	0.11	0.0090
	Var(genetic)	0.0041	1.14	249.28	0.080	0.012	0.00050	0.43	129.95	0.32	0.016
	Var(A) % of var(G)	100	100	98.054	84.53	72.32	80.071	100	71.51	66.16	43.77
	Var(NA) % of var(G)	0	0	1.95	15.47	27.68	19.93	0	28.49	33.84	56.23
	h^2	0.23	0.21	0.19	0.10	0.17	0.14	0.080	0.11	0.11	0.094
	Standard error (h^2)	0.055	0.052	0.050	0.042	0.052	0.053	0.041	0.048	0.048	0.053
	H^2	0.23	0.22	0.19	0.12	0.24	0.18	0.080	0.15	0.16	0.22
	Clone mean h^2	0.40	0.38	0.32	0.19	0.29	0.25	0.16	0.19	0.19	0.16
	Clone mean H^2	0.40	0.38	0.33	0.22	0.40	0.31	0.16	0.27	0.29	0.36

Narrow sense heritability estimates were lower than expected (given the population and other heritability estimates obtained in similar material in other CSIR trials). The heritability estimates obtained indicate that the trials were not very favourable for the assessment of heritabilities and variance components. Although the site was a high growth potential site, suspected and known errors in trial layout, poor silviculture which promoted intense weed competition, high mortality and poor measurement (for example, different teams and errors in the reading of trial maps) are all factors known to have contributed to the large error effect and decrease in the accuracy of the estimate of the variance components.

Depending on the method used, the narrow sense heritabilities for volume at 66 months range between 0.17 and 0.23, and for broad sense heritability between 0.21 and 0.23, indicating a low portion of non-additive variance. The estimates of the genetic variance attributable to non-additive variance for volume at 66 months were very low (maximum 16%). Additive variance is the major component of genetic variance for height, DBH and stem form at 66 months, where the estimates of the proportion of non-additive variance range from 0% to 6%, 2% to 26%, and 15% to 37% respectively.

Although the largest portion of genetic variance in disease tolerance at 66 months was attributable to additive variance there was a substantially higher proportion of non-additive variance than observed in the other traits at this age. The proportion of genetic variance attributable to non-additive variance ranged from 28% to 48% depending on the estimation scenario.

Figures 3 to 7 illustrate the difference in the estimates of the additive and non-additive variance components for the three scenarios that were considered, and the differences in the estimates of the variance components over age (38 and 66 months). [Refer to Chapter 3 for a more detailed discussion of the scenarios used to estimate the variance components.]

Scenario 3 is generally more conservative in the estimate of the non-additive variance component. Scenario 2, however, produced no negative estimates of variance components. The effect of the coefficient of relationship on the estimate of variance components can be seen by the difference in

estimates of the variance components between scenarios 2 and 3. The estimates of narrow sense heritabilities are higher for scenario 3.

The same trends in the proportion of genetic variance attributable to additive and non-additive variance components as observed in the 66 month data, were observed in the 38 month data of the growth traits (volume, DBH and height) and stem form, although the estimates of the proportion of non-additive variance were considerably higher (e.g., for volume between 20% and 40%). At 38 months the non-additive variance accounted for most of the genetic variation in disease tolerance and ranged between 56% and 76%. The proportion of total genetic variance attributable to non-additive variance decreases from 38 months to 66 months for all traits except height, where no non-additive variance was detected.

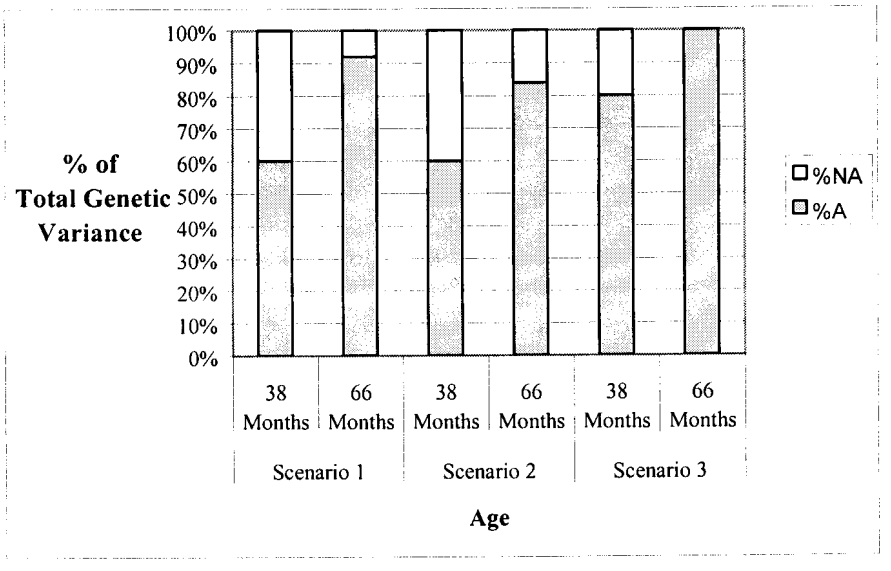

Figure 3. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance in volume at age 38 and 66 months for each of the three scenarios considered.

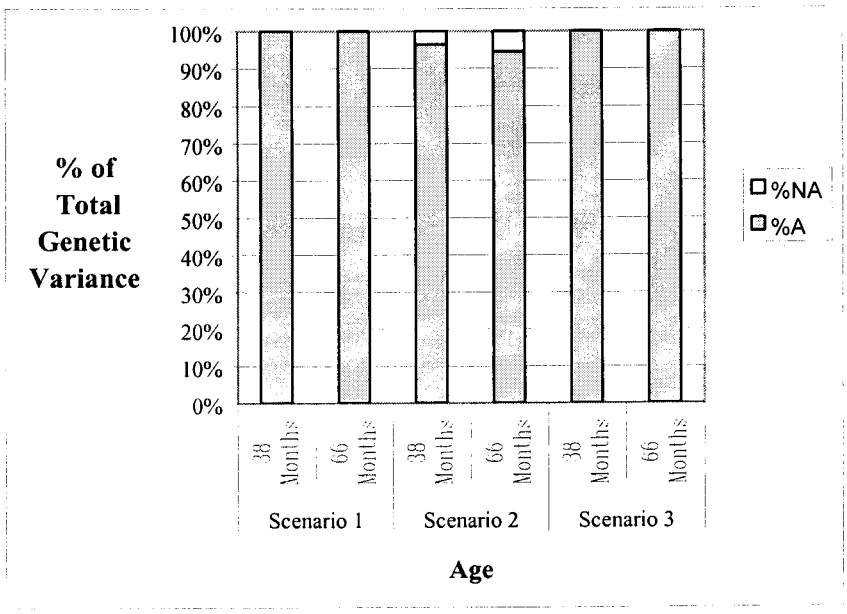

Figure 4. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance in height at age 38 and 66 months for each of the three scenarios considered.

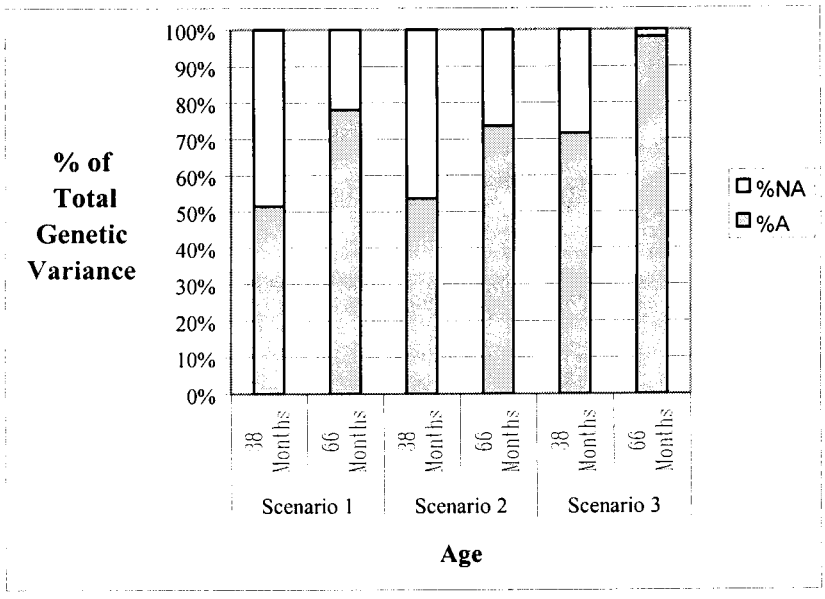

Figure 5. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance in DBH at age 38 and 66 months for each of the three scenarios considered.

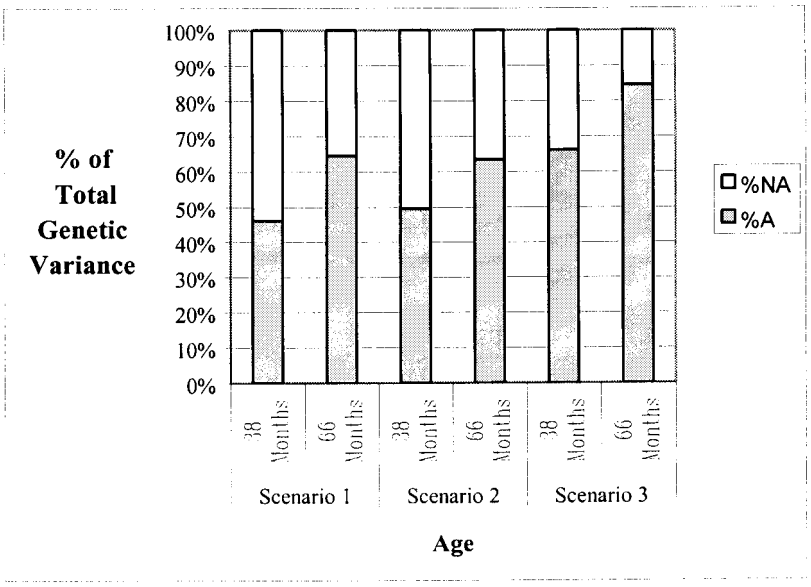

Figure 6. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance in stem form at age 38 and 66 months for each of the three scenarios considered.

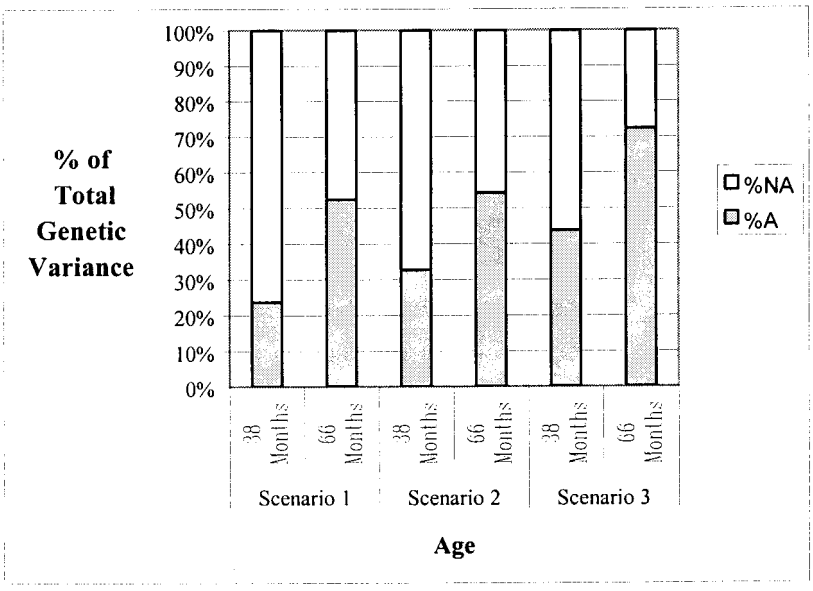

Figure 7. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance in disease tolerance at age 38 and 66 months for each of the three scenarios considered.

Despite the lack of normality in the scores for tolerance to each of four diseases scored separately, the genetic variance components were estimated, where possible, for each of the diseases. This was done in order to investigate whether or not pooling the scores for the different diseases may have caused a larger proportion of non-additive genetic variance to be detected. Summaries of the results obtained are detailed in Table 14.

Table 14. Heritability estimates and composition of genetic variance for tolerance to *Coniothtyrium, Cryphonectria, Endothia* and *Botryosphaeria* at 38 and 66 months.

Scenario		66 months				38 months			
		Coniothyrium	*Cryphonectria*	*Endothia*	*Botryosphaeria*	*Coniothyrium*	*Cryphonectria*	*Endothia*	*Botryosphaeria*
	Var(fam)	0.041	Could not be estimated	0.0027	Could not be estimated	0.0084	0.00070	Could not be estimated	0.0045
	Var(clone(fam)	0.30		0.042		0.064	0.00060		0.050
	Var(error)	1.24		0.42		0.26	0.045		0.31
1	%A	28.51		4.30		26.64	100*		12.66
	%NA	71.49		95.70		73.36	0*		87.34
	h^2	0.061		0.0042		0.058	0.054*		0.019
	H^2	0.22		0.097		0.22	0.054*		0.15
2	%A	36.39		18.23		34.98	100*		24.49
	%NA	63.61		81.77		65.020	0*		75.51
	h^2	0.078		0.018		0.077	0.044*		0.037
	H^2	0.22		0.097		0.22	0.044*		0.15
3	%A	48.51		24.30		46.64	100*		32.66
	%NA	51.49		75.70		53.36	0*		67.34
	h^2	0.10		0.024		0.10	0.059*		0.050
	H^2	0.22		0.097		0.22	0.059*		0.15

* Scenario 1: Non-additive variance:-0.0012 Additive variance: 0.0025
Scenario 2: Non-additive variance:-0.0008 Additive variance:0.0021
Scenario 3: Non-additive variance:-0.0014 Additive variance:0.0027

Family and clone within family variance components could not be estimated for *Cryphonectria* and *Botryosphaeria* at 66 months, nor for *Endothia* at 38 months. Error variance components were high and heritability estimates low (less than 0.20 for broad sense heritability and less than 0.11 for narrow sense heritability) for each of the diseases where estimates could be obtained. The trend

observed in the variance components for the pooled disease score is accentuated in the genetic variance components of each of the diseases. Non-additive variance is the major component of genetic variance for *Coniothyrium* and *Endothia* at 66 months and *Coniothyrium,* and *Botryosphaeria* at 38 months. The composition of the genetic variance for *Cryphonectria* assessed at 38 months was a notable exception as no non-additive variance was detected. The heritability estimate was very low (<0.06) and biased by the small (Scenario1: -0.0012; Scenario 2: -0.0008; Scenario 3: -0.0014) negative estimate of non-additive variance. The error was also high and the estimates are not very stable and conclusions should not be drawn from the estimates obtained for *Cryphonectria*. Figures 8 to 10 illustrate the composition of the genetic variance for tolerance to *Coniothyrium, Endothia* and *Botryosphaeria* where the variance components could be estimated.

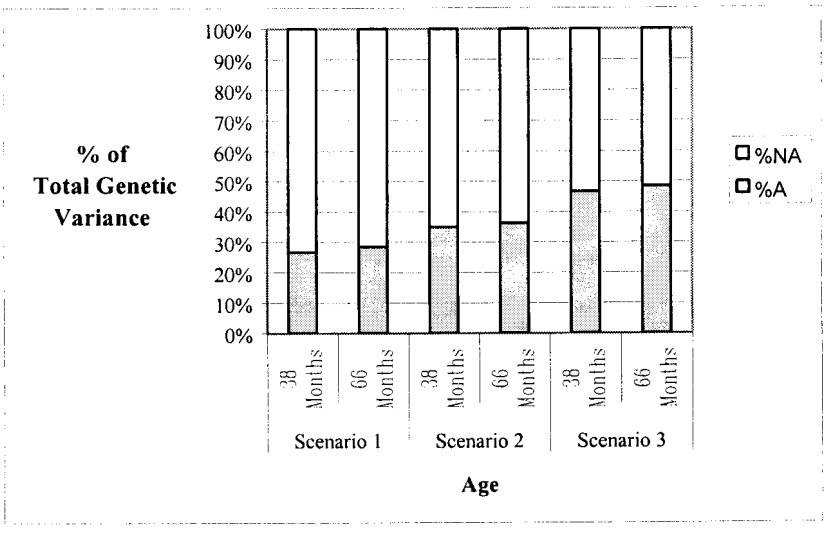

Figure 8. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance in tolerance to *Coniothyrium* at age 38 and 66 months for each of the three scenarios considered.

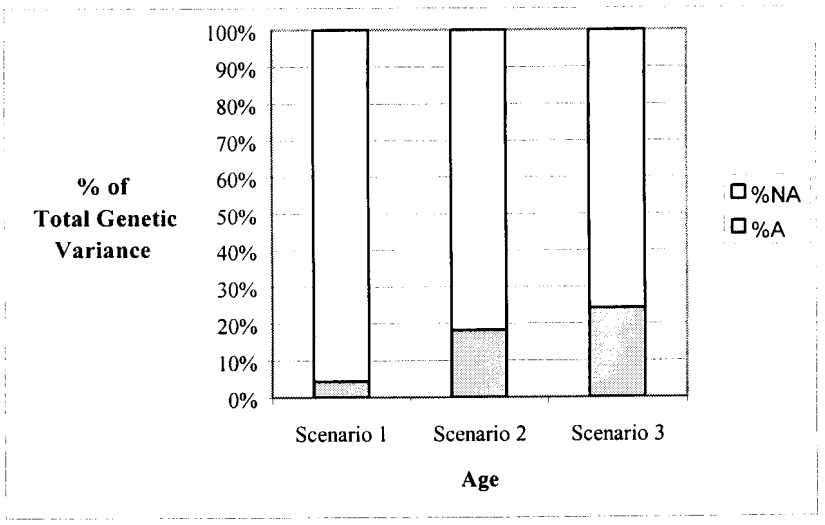

Figure 9. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance in tolerance to *Endothia* at age 66 months for each of the three scenarios considered.

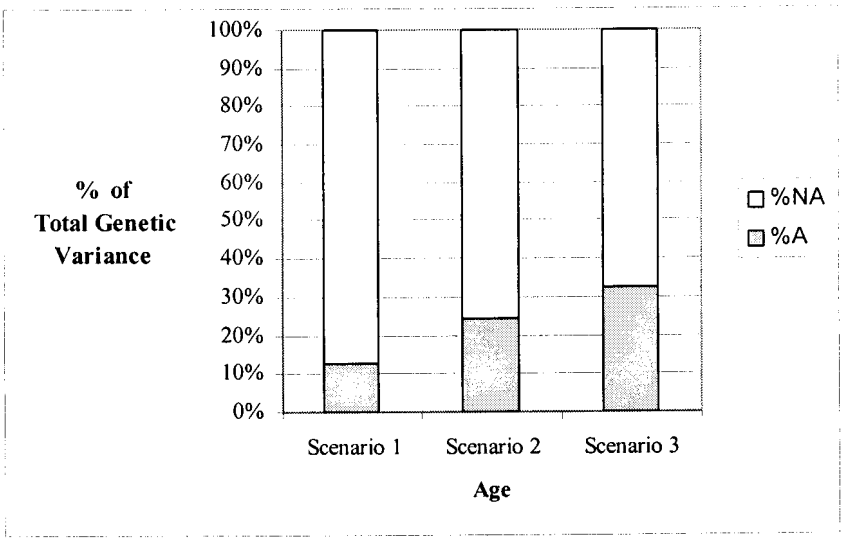

Figure 10. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance in tolerance to *Botryosphaeria* at age 38 months for each of the three scenarios considered.

CHAPTER 7

RESULTS FOR GENERATIONS F1 AND F2

When establishing the third generation breeding population, there was a need to combine the various sources and generations of material in the *E.grandis* breeding programme into a single population to improve the efficiency of the breeding programme. The "third" generation breeding population, of which trials B1, B2 and B3 are sub-populations, is in fact a combination of the progeny from first (F1) and second (F2) generation selections.

In order to investigate whether or not there was any change in the proportion of non-additive to additive genetic variance over generations, the pooled data for all three trials was divided into two sub-sets, namely the first generation and second generation selections. The number of families was approximately equal for both sets and is detailed in Table 15.

Table 15. Frequencies of first generation (F1) and second generation (F2) families and clones in the pooled data for trials B1, B2 and B3.

Age	F1		F2	
	Families	Clones	Families	Clones
38 months	53	443	61	516
66 months	80	673	97	726

Means and descriptive statistics for the F1 and F2 generation groups of families are detailed in Table 16.

Table 16. Means and descriptive statistics for F1 and F2 families in the pooled data of the 38 and 66 months assessment of trials B1, B2 and B3.

Generation	Age	Trait	Mean	Standard Error	Number of observations
F1	38 months	Volume (m^3)	0.14	0.0020	1145
		DBH (mm)	171.20	0.85	1150
		Height (m)	17.46	0.050	1145
		Stem score	5.70	0.041	1147
		Disease tolerance	0.60	0.0080	1152
	66 months	Volume (m^3)	0.37	0.0030	1801
		DBH (mm)	222.87	0.87	1802
		Height (m)	24.77	0.053	1801
		Stem score	5.703	0.019	1800
		Disease tolerance	0.53	0.0050	1802
F2	38 months	Volume (m^3)	0.13	0.0020	1348
		DBH (mm)	170.15	0.77	1355
		Height (m)	17.19	0.044	1349
		Stem score	5.64	0.036	1354
		Disease tolerance	0.61	0.0080	1356
	66 months	Volume (m^3)	0.34	0.0030	1957
		DBH (mm)	215.78	0.85	1959
		Height (m)	24.092	0.052	1958
		Stem score	5.67	0.018	1960
		Disease tolerance	0.55	0.0050	1960

The means for the F1 and F2 family groups are approximately equal at both ages, although the T-test does declare significant ($p \leq 0.05$) differences between the F1 and F2 groups for volume (38 and 66 months), height (38 and 66 months), DBH (66 months), and disease tolerance (66 months) (Appendix E). For the aforementioned traits, the F1 families were found to perform significantly ($p \leq 0.05$) better than the F2 groups although in absolute terms the difference in means between the two groups is small (Table 16). This difference is possibly explained by the higher selection intensity (to allow for the fact that these selections were "less improved" and of a previous generation to the F2) in the F1 compared with the F2. These results may possibly also indicate that it was indeed appropriate to pool F1 and F2 families in the F3 as the F1 were from a broader genetic base.

The estimates of the variance components and heritabilities for the F1 and F2 groups of families are detailed in Table 17. As with the pooled and separate trial results, negative estimates of variance components were obtained, but only for the F1 families. The negative estimates are indicated (bold type) in Table 17. The negative estimates are shown but for the calculation of heritabilities and percentages, these estimates were considered to approximate zero.

Table 17. Estimates of variance components and heritabilities for the first (F1) and second (F2) generation families from data of all three trials (B1, B2 and B3) combined. [The traits that are shaded have not been corrected for missing neighbours.]

Scenario	F1	Trait									
		66 months					38 months				
		Volume		DBH	Stem		Volume	Height	DBH	Stem	
	Var(fam)	0.0011	0.17	79.98	0.023	0.0019	0.00020	0.089	33.66	0.065	0.00070
	Std deviation (var(fam))	0.00040	0.22	27.36	0.011	0.0011	0.00010	0.080	17.18	0.037	0.0018
	Var(clone(fam))	0.0025	0.44	162.23	0.060	0.0094	0.00040	0.14	87.79	0.25	0.017
	Std deviation (var(clone(fam)))	0.00050	0.15	36.57	0.019	0.0015	0.00010	0.20	31.058	0.076	0.0030
	Var(error)	0.014	4.39	1029.58	0.59	0.037	0.0025	5.44	746.96	1.76	0.054
	Var(phenotypic)	0.018	5.0043	1271.79	0.68	0.049	0.0030	5.67	868.41	2.074	0.071
1	Var(additive)	0.0038	0.57	271.49	0.076	0.0053	0.00060	0.31	110.34	0.20	**-0.00060**
	Var(non-additive)	**-0.00020**	0.046	**-29.28**	0.0073	0.0060	**-0.00002**	**-0.082**	11.11	0.11	0.018
	Var(genetic)	0.0038	0.61	271.49	0.083	0.011	0.00060	0.31	121.45	0.31	0.018
	Var(A) % of var(G)	100	92.44	100	91.19	47.017	100	100	90.85	63.36	0
	Var(NA) % of var(G)	0	7.56	0	8.81	52.98	0	0	9.15	36.64	100
	h^2	0.22	0.11	0.21	0.11	0.11	0.18	0.055	0.13	0.095	0
	Standard error (h^2)	0.065	0.048	0.065	0.049	0.052	0.069	0.042	0.060	0.054	0.060
	H^2	0.22	0.12	0.21	0.12	0.23	0.18	0.055	0.14	0.15	0.25
	Clone mean h^2	0.37	0.21	0.37	0.21	0.18	0.32	0.11	0.23	0.17	0
	Clone mean H^2	0.37	0.23	0.37	0.23	0.39	0.32	0.11	0.25	0.27	0.41
2	Var(additive)=3*var(fam)	0.0034	0.52	239.95	0.070	0.0057	0.00050	0.27	100.97	0.19	0.0022
	Var(Non-additive) k=1	0.00020	0.096	2.26	0.014	0.0056	0.00004	**-0.039**	20.48	0.12	0.015
	Var(genetic)	0.0036	0.61	242.209	0.083	0.011	0.00050	0.27	121.45	0.31	0.017
	Var(A) % of var(G)	93.53	84.33	99.068	83.39	50.26	92.39	100	83.14	62.52	12.59
	Var(NA) % of var(G)	6.47	15.67	0.93	16.61	49.74	7.61	0	16.86	37.48	87.41
	h^2	0.19	0.10	0.19	0.10	0.12	0.16	0.047	0.12	0.094	0.031
	Standard error (h^2)	0.065	0.048	0.065	0.049	0.052	0.069	0.042	0.059	0.054	0.060
	H^2	0.21	0.12	0.19	0.12	0.23	0.18	0.047	0.14	0.15	0.24
	Clone mean h^2	0.33	0.19	0.33	0.19	0.20	0.28	0.094	0.21	0.17	0.050
	Clone mean H^2	0.35	0.23	0.33	0.23	0.39	0.31	0.094	0.25	0.26	0.40
3	Var(additive)=4*var(fam)	0.0045	0.69	319.93	0.093	0.0076	0.00080	0.35	134.63	0.26	0.0029
	Var(non-additive) k=1	**-0.00090**	**-0.076**	**-77.73**	**-0.0093**	0.0037	**-0.00010**	**-0.13**	**-13.18**	0.052	0.014
	Var(genetic)	0.0045	0.69	319.93	0.093	0.011	0.00070	0.35	134.63	0.31	0.017
	Var(A) % of var(G)	100	100	100	100	67.017	100	100	100	83.36	16.79
	Var(NA) % of var(G)	0	0	0	0	32.98	0	0	0	16.64	83.21
	h^2	0.26	0.14	0.25	0.14	0.15	0.22	0.063	0.16	0.13	0.041
	Standard error (h^2)	0.087	0.064	0.086	0.065	0.070	0.092	0.056	0.079	0.072	0.080
	H^2	0.26	0.14	0.25	0.14	0.23	0.22	0.063	0.16	0.15	0.24
	Clone mean h^2	0.44	0.26	0.44	0.25	0.26	0.38	0.12	0.28	0.22	0.067
	Clone mean H^2	0.44	0.26	0.44	0.25	0.39	0.38	0.12	0.28	0.27	0.40

Scenario	F2	Trait									
		66 months					38 months				
		Volume		DBH	Stem		Volume	Height	DBH	Stem	
	Var(fam)	0.00050	0.16	23.51	0.012	0.0023	0.00010	0.092	16.65	0.044	0.0029
	Std deviation (var(fam))	0.00020	0.18	17.043	0.0089	0.0012	0.00004	0.072	12.37	0.030	0.0018
	Var(clone(fam))	0.0028	0.80	217.48	0.067	0.011	0.00040	0.31	121.81	0.28	0.012
	Std deviation (var(clone(fam)))	0.00050	0.16	39.54	0.019	0.0016	0.00010	29.55	0.17	0.066	0.0027
	Var(error)	0.014	4.42	1092.59	0.58	0.0402	0.0023	4.82	707.027	1.57	0.062
	Var(phenotypic)	0.017	5.38	1333.59	0.66	0.053	0.0028	5.22	845.49	1.90	0.077
1	Var(additive)	0.0012	0.44	45.84	0.032	0.0067	0.00010	0.29	38.91	0.11	0.0085
	Var(non-additive)	0.0020	0.51	195.16	0.046	0.0063	0.00040	0.11	99.55	0.21	0.0068
	Var(genetic)	0.0032	0.96	240.99	0.078	0.013	0.00050	0.40	138.46	0.33	0.015
	Var(A) % of var(G)	36.99	46.13	19.02	40.78	51.82	23.86	72.19	28.10	34.30	55.59
	Var(NA) % of var(G)	63.0055	53.87	80.98	59.22	48.18	76.14	27.81	71.90	65.70	44.41
	h^2	0.071	0.082	0.034	0.048	0.13	0.042	0.056	0.046	0.059	0.11
	Standard error (h^2)	0.043	0.045	0.038	0.041	0.055	0.045	0.042	0.044	0.048	0.052
	H^2	0.19	0.18	0.18	0.12	0.24	0.18	0.077	0.16	0.17	0.20
	Clone mean h^2	0.12	0.14	0.060	0.090	0.21	0.073	0.11	0.081	0.10	0.19
	Clone mean H^2	0.33	0.31	0.32	0.22	0.41	0.31	0.15	0.29	0.30	0.34
2	Var(additive)=3*var(fam)	0.0014	0.47	70.53	0.036	0.0070	0.00020	0.28	49.96	0.13	0.0086
	Var(Non-additive) k=1	0.0019	0.48	170.47	0.043	0.0060	0.00030	0.12	88.51	0.19	0.0066
	Var(genetic)	0.0032	0.96	240.99	0.078	0.013	0.00050	0.40	138.46	0.33	0.015
	Var(A) % of var(G)	42.75	49.60	29.26	45.59	53.86	32.89	69.14	36.078	40.72	56.69
	Var(NA) % of var(G)	57.25	50.40	70.74	54.41	46.14	67.11	30.86	63.92	59.28	43.31
	h^2	0.082	0.088	0.053	0.054	0.13	0.058	0.053	0.059	0.070	0.11
	Standard error (h^2)	0.043	0.045	0.038	0.041	0.055	0.045	0.042	0.044	0.048	0.052
	H^2	0.19	0.18	0.18	0.19	0.24	0.18	0.077	0.16	0.17	0.20
	Clone mean h^2	0.14	0.16	0.093	0.10	0.22	0.10	0.10	0.10	0.12	0.19
	Clone mean H^2	0.33	0.31	0.32	0.22	0.41	0.307	0.15	0.29	0.30	0.34
3	Var(additive)=4*var(fam)	0.0018	0.63	94.035	0.048	0.0093	0.00020	0.37	66.61	0.18	0.012
	Var(non-additive) k=1	0.0014	0.32	146.96	0.031	0.0037	0.00030	0.031	71.86	0.15	0.0037
	Var(genetic)	0.0032	0.96	240.99	0.078	0.013	0.00050	0.40	138.46	0.33	0.015
	Var(A) % of var(G)	56.99	66.13	39.020	60.78	71.82	43.86	92.19	48.10	54.30	75.59
	Var(NA) % of var(G)	43.0055	33.87	60.98	39.22	28.18	56.14	7.81	51.90	45.70	24.41
	h^2	0.11	0.12	0.071	0.072	0.18	0.077	0.071	0.079	0.093	0.15
	Standard error (h^2)	0.057	0.070	0.051	0.054	0.088	0.060	0.056	0.059	0.064	0.070
	H^2	0.19	0.18	0.18	0.12	0.24	0.18	0.077	0.16	0.17	0.20
	Clone mean h^2	0.19	0.21	0.12	0.13	0.29	0.13	0.14	0.14	0.16	0.26
	Clone mean H^2	0.33	0.31	0.32	0.22	0.41	0.31	0.15	0.29	0.30	0.34

The trends across ages were fairly consistent. The estimates of the genetic variance attributable to non-additive variance for volume at 66 months in the F1 families ranged from 0 % to 6 %. Additive variance is also the dominant component of genetic variance for height and DBH at 66 months where the estimates of the proportion non-additive variance range from 0% to 16% and 0% to 1% respectively. In the F2 families, however, the proportion of non-additive variance for volume at 66 months was higher, ranging from 43% to 63%. For height at 66 months, the proportion non-additive variance ranged between 34% and 54%, and as high as between 61% and 81% for DBH.

The largest proportion of genetic variance in stem form at 66 months was attributable to additive variance in the F1 families (100% to 83%), whereas in the F2 families there was a substantially higher proportion of non-additive variance and additive variance only accounted for between 41% and 61% of the genetic variation. For disease tolerance however, the proportion of genetic variance attributable to non-additive variance was fairly consistent in both the F1 and F2 families and ranged between 33% to 53%, and 28% to 48% respectively, depending on the scenario. These trends are discussed in more detail in Chapter 10.

The proportion of total genetic variance attributable to additive and non-additive variance for each of the traits at age 66 months and for each of the scenarios considered, are illustrated in Figures 11 to 15. (Please refer to Chapter 3 for a more detailed discussion of the estimation scenarios considered.)

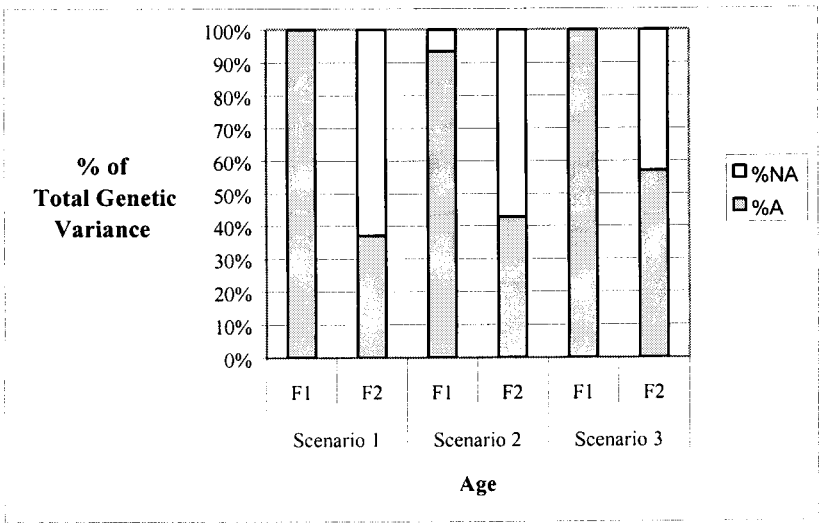

Figure 11. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance for volume at 66 months over generations (F1 and F2) for the three scenarios considered.

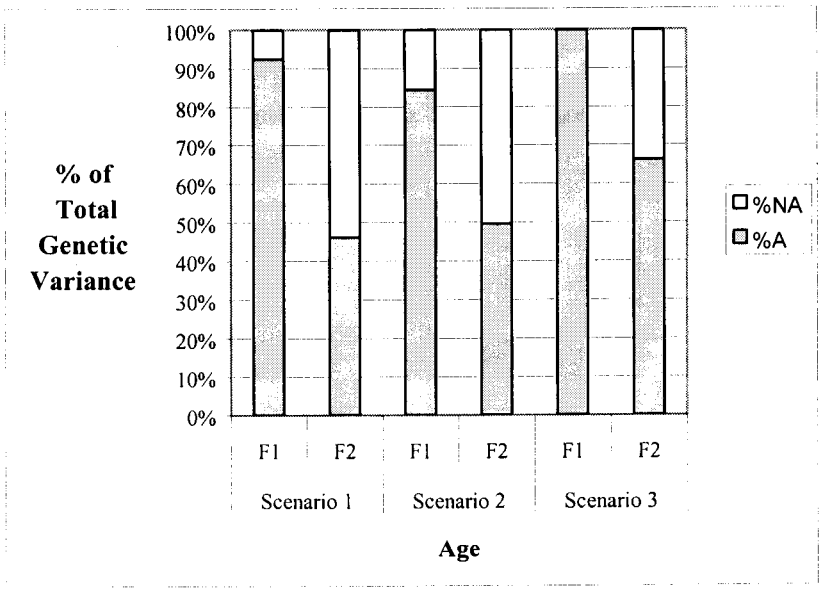

Figure 12. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance for height at 66 months over generations (F1 and F2) for the three scenarios considered.

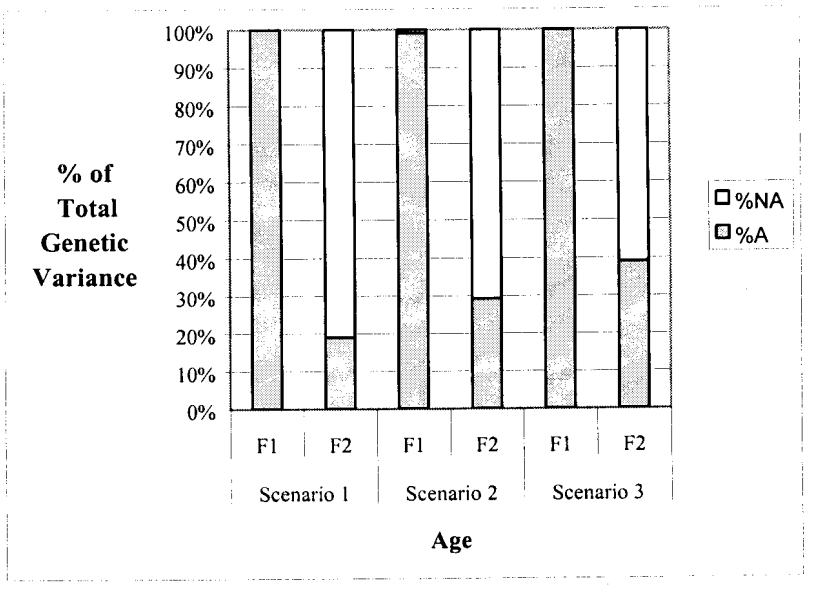

Figure 13. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance for DBH at 66 months over generations (F1 and F2) for the three scenarios considered.

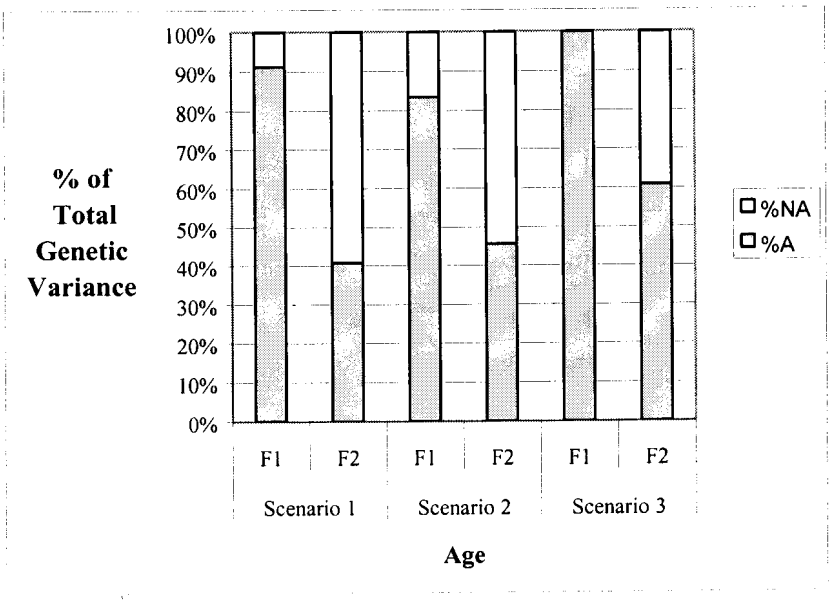

Figure 14. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance for stem form at 66 months over generations (F1 and F2) for the three scenarios considered.

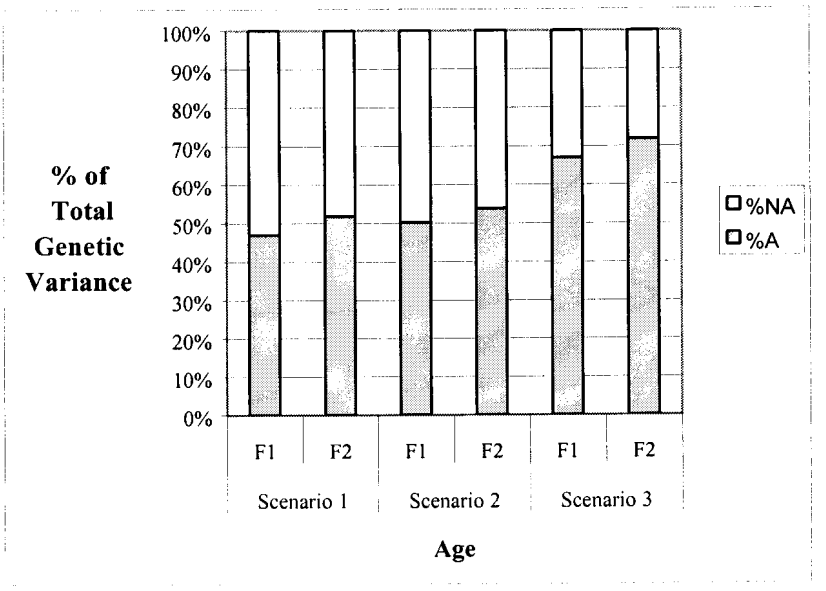

Figure 15. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance for disease tolerance at 66 months over generations (F1 and F2) for the three scenarios considered.

The change in additive variance from the F1 generation to the F2 generation was predicted for the traits selected for in the F1 generation, namely volume and stem form. The female parents (no male selection besides thinning, as families are open pollinated) of the F2 were selected in three F1 trials using information from families in these trials and information from the parents of these families (P0). Similar heritabilities were estimated in all three trials and for this reason a typical individual narrow sense heritability at time of selection, of 0.3 for volume and stem was used. The predicted additive variance in the F2 was calculated assuming the following:

- Narrow sense heritability at time of selection
- Selection intensity at time of selection
- Additive genetic variance in F1 estimated from pooled data of trials B1, B2 and B3 (as estimated by scenario 2).

The results are detailed in Table 18.

Table 18. Predicted and actual additive genetic variance for volume and stem form at 66 months in the F1 and F2.

Trait	Individual h^2	Family Mean h^2	k_1	k_2	k_4	Additive genetic variance in F1	Predicted additive genetic variance in F2	Actual additive genetic variance in F2
Volume	0.3	0.68	0.73	0.87	0.76	0.0034	0.0017	0.0014
Stem form	0.3	0.68	0.73	0.87	0.76	0.070	0.034	0.036

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 where, 61 out of 200 families are selected

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 where, an average of 1.59 individuals out of 36 individuals per family are selected

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, 9 out of 36 individuals per family are selected due to thinning

As there was no among family selection for male parents, the factor k_3 is equal to zero. The realised estimate of additive genetic variance in the F2 for volume is 18% lower than the predicted estimate for volume. For stem form however, the realised estimate of additive genetic variance is approximately equal (4% higher than the predicted estimate).

CHAPTER 8

PREDICTED GAINS

Predicted genetic gains for the breeding population were estimated in order to investigate whether or not the clonal breeding population strategy was appropriate given the additional costs and time involved. A comparison of predicted gains will help to evaluate whether or not there was benefit to cloning the seedlings.

Production population gains for various scenarios were estimated in order to compare the gains that could be made for feasible production population options based on a clonal breeding population. One of the main advantages of a cloned breeding population is that clones for production can be selected at the same time as selections are made for the next generation of breeding and this strategy is most likely to be the favoured production strategy. However, seed production options have also been considered and two options are presented (conversion of the breeding population into a seed orchard and the establishment of a forward selected clonal orchard).

Gains in the production population (clonal seed orchard) from selection in a cloned breeding population were also compared with gains for the same production strategy but where selection was done in a breeding population that had not been cloned.

Tree volume is, economically, the most important selection trait (of the traits considered in this study) and the estimates of predicted gains were calculated based on this single trait. Heritability estimates obtained under scenario 2 (coefficient of relationship=⅓, proportion of non-additive variance segregating within open pollinated families=1) were used as this scenario was found to produce the most plausible estimates (Refer to Chapter 10 for discussion of the three scenarios.)

Parameters used to predict genetic gain are based on the actual parameters (obtained from the pooled data set) in the breeding population (B1, B2 and B3) and feasible "benchmark" parameters

for selection for the next cycle of breeding that are in line with the current strategy. Attention was also paid to estimating cycle lengths that were practically feasible given the schedule of activities involved in selection and establishment of the production population, for example. Percentage gains were calculated based on the breeding population mean volume at 66months.

8.1 Predicted Breeding Population Gains

The predicted gains for selection for the next generation in a cloned breeding population were estimated assuming a 33% rogueing of families and a 25% thinning within the remaining families. As selection was for volume at 66 months and the parameter estimates obtained in the 66 month data were used to predict the gains, the genetic correlation with the "mature trait" was 1. The size of the breeding population was reduced to 140 in the next generation. The total length of the breeding cycle was 9 years (2 years to raise material + 5 ½ years growth +1 ½ years selection, thinning and rogueing and collection of seed). The predicted gains and some of the input parameters are detailed in Table 19.

Table 19. Estimate of predicted gain in the breeding population for selection at 66 months for volume in the cloned open pollinated breeding population B1, B2 and B3.

Number of families in current population	177
Average number of clones per family	8
Average number of ramets per clone	3
Coefficient of relationship	0.3
h^2 at age of selection	0.17
Phenotypic standard deviation	0.13
Number of female families selected	70
Number of male families selected	118
Number of trees selected within families for female parents	2
Number of trees selected within families for male parents	6
Predicted genetic gain	9.82%
Breeding cycle length	9 years
Predicted gain per year	1.09%

The predicted genetic gain per year was 1.09% and total predicted gain for a breeding cycle of 9 years was 9.82%.

In order to investigate the benefit of cloning the same parameters were used to simulate a population that was not cloned (seedling breeding population). Two scenarios were considered. Firstly (scenario A), where the same number of families and total number of trees were present in a non-cloned open pollinated breeding population (i.e., more individuals per family). Secondly (scenario B), where the same number of families and individuals per family were present but none of the individuals were cloned (i.e., a smaller total number of trees). The total length of the breeding cycle in both scenarios was 7 ½ years (½ year to raise material + 5 ½ years growth +1 ½ years selection, thinning and rogueing and collection of seed). The predicted genetic gain for scenario A is detailed in Table 20, and for scenario B in Table 21.

Table 20. Estimate of predicted genetic gain in the breeding population for selection for volume at 66 months in an open pollinated breeding population with the same number of families and total number of trees as the cloned population.

Number of families in current population	177
Number of individuals per family	24
Coefficient of relationship	0.3
h^2 at age of selection	0.17
Phenotypic standard deviation	0.13
Number of female families selected	70
Number of male families selected	118
Number of trees selected within families for female parents	2
Number of trees selected within families for male parents	18
Predicted genetic gain	9.94%
Breeding cycle length	7 ½ years
Predicted gain per year	1.32%

Table 21. Estimate of predicted genetic gain in the breeding population for selection for volume at 66 months in an open pollinated breeding population with the same number of families and individuals per family as the cloned population.

Number of families in current population	177
Number of individuals per family	8
Coefficient of relationship	0.3
h^2 at age of selection	0.17
Phenotypic standard deviation	0.13
Number of female families selected	70
Number of male families selected	118
Number of trees selected within families for female parents	2
Number of trees selected within families for male parents	6
Predicted genetic gain	7.17%
Breeding cycle length	7½ years
Predicted gain per year	0.96%

The total predicted genetic gain is highest (9.94%, 1.32% per year) for the non-cloned open pollinated breeding population with 12 individuals per family compared with the gains predicted for the cloned open pollinated breeding population (9.82%, 1.09% per year), and for the non-cloned open pollinated breeding population with same number of families (177) and individuals per family (8) as the cloned population (7.17%, 0.96% per year).

If, however, there had been no mortality (or blanking had been done) and the established frequencies had been realised (i.e., 5 ramets per clone) then the total predicted gains from the cloned breeding population (11.08%) would have exceeded the equivalent open pollinated breeding population with the same number of families but more (non-cloned) individuals per family (10.98%). The predicted gain per year is, however, greater for the non-cloned scenario (1.46% per year) than for the cloned scenario (1.23% per year) because of the longer cycle length due to the time required to bulk up the clones. The predicted gain for the cloned breeding population with 5 ramets per clone and the predicted gain for the equivalent non-cloned breeding population are detailed in Tables 22 and 23.

Table 22. Estimate of predicted gain in the breeding population with 5 ramets per clone, for selection at 66 months for volume in the cloned open pollinated breeding population B1, B2 and B3.

Number of families in current population	177
Average number of clones per family	8
Average number of ramets per clone	5
Coefficient of relationship	0.3
h^2 at age of selection	0.17
Phenotypic standard deviation	0.13
Number of female families selected	70
Number of male families selected	118
Number of trees selected within families for female parents	2
Number of trees selected within families for male parents	6
Predicted genetic gain	11.08%
Breeding cycle length	9 years
Predicted gain per year	1.23%

Table 23. Estimate of predicted genetic gain in the breeding population for selection for volume at 66 months in an open pollinated breeding population with the same number of families and total number of trees as the cloned population with 5 ramets per clone.

Number of families in current population	177
Number of individuals per family	40
Coefficient of relationship	0.3
h^2 at age of selection	0.17
Phenotypic standard deviation	0.13
Number of female families selected	70
Number of male families selected	118
Number of trees selected within families for female parents	2
Number of trees selected within families for male parents	18
Predicted genetic gain	10.98%
Breeding cycle length	7 ½ years
Predicted gain per year	1.46%

8.2 Predicted Production Population Gains

Predicted gains in the production population were calculated for three different types of production populations that were feasible for the cloned *E.grandis* breeding population. These options were:

A. Thinning on clone means for seed production (not considering families)
B. Clonal seed orchard from forward selection on clone means
C. Selection of cloned individuals for immediate clonal deployment.

A total of 1399 clones were available for selection in trials B1, B2 and B3 at 66 months. If the trial is heavily thinned (96%) to leave 56 clones for seed production (assuming sufficient seed production can be obtained from the remaining ramets), the predicted total gain in the production population is 21.07% with a production cycle of 2 years, i.e., 10.54% per year (Option A).

If 50 individuals (25 families and two individuals per family) are selected for a clonal seed orchard based on clone mean volume at 66 months in the breeding population, then the predicted total gain in the production population is 18.51% with a production cycle length of 5 years, i.e. 3.70% per year (Option B). A seed orchard of 50 clones is considered to be a feasible size.

Selection of individuals, which have, by nature of the cloned breeding population, already been clonally tested, is another option for the production population (Option C). Based on a broad sense heritability (H^2) of 0.208, the total predicted gain for the selection of 10 clones for production is 31.66% with a production cycle of 2 years (to allow time for coppicing and bulking up of the material, however, this time could even be shorter depending on the success of propagation), i.e., 15.83% per year.

In order to compare the predicted gains for production population scenarios where the breeding population had not been cloned, two broad scenarios were considered – a clonal seed orchard and selection of production clones from a clonal trial.

Predicted gains from a clonal orchard from forward selection in an open pollinated breeding population was compared to the gains predicted for Option B. Two open pollinated breeding

populations were considered as before, namely one population with the same total number of trees as the cloned population, but with more individuals per family (Option D), and a second population where the number of individuals per family were the same as in the cloned breeding population (i.e., therefore a smaller total population size) (Option E). The gains for Options D (177 families, 24 individuals per family) and E (177 families, 8 individuals per family) were 18.36% (3.67% per year, 5 year cycle) and 12.87% (2.57% per year, 5 year cycle) respectively. The results are summarised in Table 24.

The predicted gains from selection of 10 production clones at 5 years in a clonal trial of 70 clones (20 ramets/clone) selected from the non-cloned breeding population (35 families and 2 individuals per family selected) were estimated for the two non-cloned breeding population scenarios (namely, the same total number of individuals as the cloned population [Option F] and the same number of families and individuals per family as the cloned population [Option G]). The parameters in the clonal trial were assumed to be the same as in the cloned breeding population. The cycle length was 7 years (2 years to coppice and bulk up + selection at 5 years of age). The gains for Options F and G were 43.11% (6.16% per year) and 37.93% (5.42% per year) respectively.

Table 24. Predicted genetic gains for the production population scenarios.

Option	Description	Predicted Gains per cycle	Predicted Gains per year	Length of Production cycle
A	Thinning on clone means in the cloned breeding population (without consideration of family structure) for seed production (compare with B)	21.07%	10.54%	2 years
B	Clonal orchard from forward selection on clone means in a cloned open pollinated breeding population (compare with options A, D and E)	18.51%	3.70%	5 years

Option	Description	Predicted Gains per cycle	Predicted Gains per year	Length of Production cycle
C	Selection of cloned individuals in the cloned breeding population for immediate deployment (compare with options F and G)	31.66%	15.83%	2 years
D	Clonal orchard from forward selection in an open pollinated breeding population with the same total number of trees as the cloned population (compare with option B)	18.36%	3.67%	5 years
E	Clonal orchard from forward selection in an open pollinated breeding population with the same number of families and trees per family as the cloned breeding population (compare with option B)	12.87%	2.57%	5 years
F	Clonal selection in a clonal trial following selection in an open pollinated breeding population with the same total number of trees as the cloned population (compare with option C)	43.11%	6.16%	7 years
G	Clonal selection in a clonal trial following selection in an open pollinated breeding population with the same number of families and trees per family as the cloned breeding population (compare with option C)	37.93%	5.42%	7 years

CHAPTER 9

CORRELATIONS

The pooled data set of trials B1 and B2 was used to estimate the correlation of traits between the two ages assessed, namely 38 and 66 months. All traits, except disease tolerance, were corrected for missing neighbours at both ages.

The phenotypic individual, family mean and clone mean age-age correlations for the pooled data of trials B1 and B2 is presented in Table 25.

Table 25. Phenotypic age-age correlations estimated in the pooled data of trials B1 and B2 between 38 and 66 months, on an individual tree, family mean and clone mean basis.

Trait	Individual		Family Mean		Clone Mean	
	$r_{p_{38-66\,months}}$	p>|R|	$r_{p_{38-66\,months}}$	p>|R|	$r_{p_{38-66\,months}}$	p>|R|
Volume	0.88	0.0001***	0.78	0.0001***	0.78	0.0001***
Height	0.67	0.0001***	0.49	0.0001***	0.46	0.0001***
DBH	0.90	0.0001***	0.71	0.0001***	0.75	0.0001***
Stem	0.57	0.0001***	0.56	0.0001***	0.55	0.0001***
Disease	0.54	0.0001***	0.55	0.0001***	0.54	0.0001***

Individual: n=2231 Family Mean: n=114 Clone Mean: n=942

The age-age phenotypic correlations are generally strongest on an individual tree basis. The age-age correlation for height is notably lower (individual: r_p=0.67, family mean: r_p=0.49, clone mean: r_p=0.46) than for DBH (individual: r_p=0.90, family mean: r_p=0.71, clone mean: r_p=0.75). DBH plays a larger role in the calculation of volume than height (Appendix A Table A-5) and, this is reflected in the relatively strong age-age correlations estimated for volume between age 38 and 66 months in these trials. The age-age correlations for the subjectively assessed traits stem form and

disease tolerance, are as may be expected, poorer than the age-age correlations for the growth traits (DBH, height and volume). The effect of different measuring teams and the subjective scale used to score these traits will impact the accuracy of the correlation.

The individual tree phenotypic age-age correlations for the two separate trials are detailed in Table 26.

Table 26. Phenotypic age-age (38-66 months) correlations estimated on an individual tree basis in trials B1 and B2.

	B1		B2	
Trait	$r_{p_{38-66 months}}$	**p>|R|**	$r_{p_{38-66 months}}$	**p>|R|**
Volume	0.88	0.0001***	0.89	0.0001***
Height	0.67	0.0001***	0.66	0.0001***
DBH	0.90	0.0001***	0.89	0.0001***
Stem	0.56	0.0001***	0.58	0.0001***
Disease	0.58	0.0001***	0.50	0.0001***

B1: n=1146 B2: n=1085

The estimates of the individual phenotypic age-age correlations are approximately equal in each of the two trials and indicate the same trends as were observed for the pooled data.

Two approaches were used to investigate the genetic correlations. Family means were used in the one method and clone (within family) means in the other (See Chapter 3, equations 29 and 30). The estimates of variance components were, however, considered too inaccurate to predict reliable correlations and the estimates obtained were deemed too unreliable to report on in both cases.

CHAPTER 10

DISCUSSION

The trials were not optimal for the estimation of variance components as proportionately large error effects were detected in these trials. Trials B1, B2 and B3 were established on a high growth potential site, where relatively high heritabilities are normally expected. The low heritabilities that were realised in these trials, for traits where in other trials of similar genetic background, higher heritabilities have been commonly recorded, indicate the imprecision of the trials. Factors that contributed to the error were, errors in trial establishment (possibly including identity mix ups in the nursery and/or in the field), inaccurate trial measurement (possibly including confusion of plot identities) and poor silviculture which resulted in intense weed competition, to which eucalypts are thought to be very sensitive. The problems with suspected identity mix ups (either at trial establishment or at time of measurement) and the difficulty experienced trying to resolve these errors have highlighted the need for extra measures to prevent identity mix ups in large clonal trials such as these – especially when a single tree plot design is used (e.g., careful record keeping, duplicate labeling of individual ramets, accurate trial layout and labeling in field).

Heavy selection for survival and ease of vegetative propagation (289 out of the original 450 families sown were pricked out, of these eventually only 177 were included in the trial) took place in the nursery (refer to Chapter 2). Families with good survival and/or individuals that propagated well were better represented (more individuals per family) than other families.

The apparent large amount of error variance found in the trials necessitated the rigorous data editing. The correction for missing neighbours allowed some correction for the high mortality and the fact that certain trees' growth was favoured by less competition. Estimates obtained for the data when no correction was made for missing neighbours were less stable and had higher error than those estimates obtained when the correction was made. In order to get a reliable estimate of the nature of the additive and non-additive proportion of genetic variation in the populations,

environmental effects must be minimized as much as possible and the data was thoroughly scrutinized for this reason.

The 38 month data has been reported on, however, the fluctuations in estimates indicate instability and the heritabilities are considered too low to make reliable conclusions about the variance component estimates obtained in these trials. The 38 month data was considered less reliable than the 66 month data (Chapter 4) and further discussion of the results is based on the 66 month data.

The estimate of the variance components obtained from the separate populations do not readily show clear trends. The fluctuations observed might be an artifact of the high mortality, small family numbers, intense weed competition, differences in environmental conditions that may have caused different genetic responses in individuals, and differences in the gene pool among other factors. Pooling the three sub-populations increased the number of families and degrees of freedom and provided a more stable estimate of the variance components and the heritabilities.

One observation that is of particular interest is that the proportion of genetic variance for disease tolerance attributable to non-additive variance shows particularly large fluctuations (at 66 months B1: 91% - 100%, B2: 0% - 16%, B3: 94% - 100%) [Table 10]. This apparently anomalous trend is not readily explained but may be due to the notably higher occurrence of *Cryphonectria* and lower occurrence of *Endothia* in trial B2, compared to B1 and B3. This may have triggered different responses in the genotypes. Although strong clonal differences are observed for growth traits in trial B1 (indicated by the high proportion of non-additive variance), reliable conclusions cannot be drawn due to the generally low heritabilities and inaccurate estimates of variance components.

C-effects, or non-random environmental effects, were assumed absent or negligible. C-effects are described as environmental (i.e., non-genetic) effects common to a clone and may arise because of the condition of the ortet or the particular part of the ortet, from which a specific cutting is taken. (Libby and Jund, 1962; Burdon and Shelbourne, 1974). C-effects may bias the estimate of variance components and may inflate or deflate the heritability. Libby (1976 ex Park and Fowler, 1987) described 3 types of C-effects, namely a maternal effect common to all ramets of an ortet, an effect due to the condition of ramets from a single ortet, which causes variance among these ramets, and

an environmental covariance due to the positioning of the plants in the nursery. The large environmental effect may indicate that the assumption of negligible C-effects might be invalid. Possible effects (such as nursery conditions) common to ramets of several ortets may have contributed to the large environmental effects detected.

Of the three scenarios for which variance components were calculated, scenario one is considered, theoretically, to be the most probable and least likely to bias the estimates of the variance components. In scenario 1 the variance components are estimated using a coefficient of relationship of ⅓ to account for increased relatedness in the open pollinated families, and assuming that the proportion of non-additive variance segregating within open pollinated families is less than one. Using the second scenario, however, to estimate variance components resulted in only a single negative estimate (non-additive variance component for height at 38 months in the F1 families) of a variance component. In scenario 2 the variance components are estimated using a coefficient of relationship of ⅓ and the proportion of non-additive variance segregating within half sib families is assumed to be one. Negative estimates of variance components were small but indicate the error of the estimate.

The differences in estimates under the different scenarios highlight the considerable effect that the coefficient of relationship has on the estimate of variance components and the importance of an accurate estimate of this parameter in the specific population. In the CSIR's population of *E.grandis* a coefficient of ⅓ is used to account for relatedness in the open pollinated families, in order to prevent an underestimate of the additive variance. Comparison of the estimates obtained under scenario 2 and scenario 3, where the only difference in the methodology is the value of the coefficient of relationship (in scenario 2 it is ⅓, whereas in scenario 3 it is ¼ assuming that the more related individuals had been removed in the nursery through selection for height), illustrates the effect of the coefficient of relationship and the importance of accurately estimating the coefficient in the population. Estimates of additive variance were greater for scenario 3 than for scenario 2 illustrating how an underestimation of the coefficient of relationship could lead to an overestimate of heritability.

Further discussion of the additive and non-additive genetic variance components and heritabilities will be based on the results obtained using scenario 2 [coefficient of relationship=⅓ assuming increased relatedness among half sibs in *E.grandis* families (Verryn, 1993), proportion of non-additive variance segregating within open pollinated families=1]. This methodology and these assumptions were used by Park and Fowler (1987) and Foster (1985), to calculate variance components in open pollinated populations of tamarack and eastern cottonwood, respectively. The trends observed were, however, the same under all three scenarios.

The pooled results [Table 13] showed that most of the genetic variance for the growth traits, namely volume (84%), DBH (74%) and height (94%), was additive. The expression of DBH was under notably higher non-additive genetic control and this, rather than height, can be seen as the source of the non-additive variance for volume. These findings are in contrast to the findings of van Wyk (1990) who reported that the level of additive variance for volume production in *E.grandis* (36, 60 and 105 months) was slightly less than that of dominance variance as estimated by variance due to general combining ability (σ^2_{GCA}) and variance due to specific combining ability (σ^2_{SCA}) in a partial diallel progeny test of 20 P0 selected parents. The parents were not considered to be inbred (van Wyk, 1975) and the difference in findings between these studies could, therefore, not be attributed to inbreeding in the parents used in the partial diallel which may inflate estimates of specific combining ability. The data set used by van Wyk (1990) was also smaller than the data set used for the current study and the number of times some of the parents were used as males or females was low (0 or 1) in several cases and this may have affected the reliability of specific combining estimates. The differences may also be due to the difference in the genetic background of the two populations or to problems with the data sets from trials B1, B2 and B3.

Non-additive genetic variation was strongly expressed in disease tolerance (46%) and stem form (37%) [Table 13]. This confirms trends observed by Verryn (2000) for disease and stem form and by van Wyk (1990) for stem form.

An industry standard, subjective, scale, which has been in use for many years, was used for the assessment of stem form. The low heritabilities and relatively high error may indicate that the scale

used to assess stem form may have to be refined to obtain a more accurate measure of this trait. Much progress has been made in past generations to improve stem form and these results could be indicating that a refinement of the measurement scale is needed for future generations in order to measure this trait accurately. Although the full range of the scale was scored in all trials at both ages, the distributions of the scores had heavy tails and the high frequency with which 6's and 7's were scored may support the need for a refinement of the scale or an investigation of an alternative non-subjective method to score stem form (e.g., image analysis).

The high clone mean heritabilities observed in the pooled data for traits are notable. Clone mean heritabilities are useful when clones are compared. For volume at 66 months the broad sense clone mean heritability is 0.36, for stem form, 0.22 and for disease tolerance 0.40 [Table 13]. The narrow sense clone mean heritabilities are also considerably higher compared to the narrow sense individual heritabilities. For volume the clone mean narrow sense heritability was 0.30 compared to 0.17 for the individual, for stem form 0.14 compared to 0.076, and for disease tolerance 0.22 compared to 0.13 [Table 13]. In situations where high environmental variation is expected in a trial (be it due to factors in the nursery, trial design, site factors, imprecision in the assessment of traits, or other possible causes of experimental error) and low heritabilities are expected, cloning is particularly beneficial as can be seen by high clone mean heritabilities estimated in this trial. The benefit of selection in the cloned population (where selection can be done on clone means) is illustrated by the gains predicted for this scenario, compared with a non-cloned breeding population. This is discussed in detail later.

Dividing the pooled data into F1 and F2 groups facilitated an investigation of the changes in the proportion of additive versus non-additive variance for traits in the two groups. A small proportion of non-additive variance was estimated in the growth traits (volume, DBH, height) and stem form of the F1 families. The vast majority of genetic variance was attributable to additive variance for the growth traits and stem form in the F1 families. At 66 months the proportion of genetic variance attributable to additive variance was 94% for volume, 84% for height, 99% for DBH and 83% for stem form [Table 17]. In contrast non-additive variance was the major proportion of genetic variance in these traits in the F2 families. At 66 months the proportion of genetic variance attributable to non-additive variance was 57% for volume, 50% for height, 71% for DBH and 54%

for stem form in the F2 [Table 17]. Low individual heritabilities were found for all traits for both the F1 and F2 family groups and therefore, caution is advised in the interpretation of the variance components. The trends in the distribution of the genetic variance in the generations are fairly consistent over the two ages assessed.

Relic provenance effects may cause the additive component to be overestimated in the F1 families, whereas a higher potential proportion of relatedness in the F2 families could also bias the estimate of the variance components, causing the additive variance to be underestimated. There is also some evidence to suggest that eucalypt land races are somewhat inbred (Eldridge, 1995) and this may have influenced the proportion of non-additive genetic variance estimated in the F2. Never-the-less a classical explanation for the increase in the proportion non-additive variance in the F2 families would be the reduction in additive variance as a result of the selection in forming the F2. This would also explain why the percentage of non-additive variance did not change over generations in the case of disease score, as the resistance to these diseases was not actively selected for in previous generations, and both groupings would have been subject to the same selection intensity.

A simulation study over 10 generations of Scots pine where the breeding strategy was based on within-family clonal selection, showed that the majority of the 50% loss of additive genetic variance in the breeding population, took place in the first three generations after which the additive genetic variance stabilised (Rosvall et al., 1998). The rapid reduction in additive variance in the first few generations of selection is attributed to an increase in gametic phase linkage disequilibrium subsequent to truncation selection (Rosvall et al, 1998; Bulmer, 1980).

The available data from the *E.grandis* breeding population facilitates the prediction of the reduction in additive variance in the F2 and a comparison with the realised additive genetic variance in the F2. The large drop in additive genetic variance for volume and stem form (selection traits in the F1) that is observed from the F1 to the F2 agrees with the findings of Rosvall et al. (1998).
It is, however, notable that the actual additive variance for volume in the F2 is 18% less than is predicted, and this may indicate that a higher selection efficiency and intensity was realised. The predicted additive variance in the F2 for volume and stem form was 0.0017 and 0.034 respectively, compared with the realised estimate of 0.0014 and 0.036 for volume and stem respectively [Table

18]. The prediction of the additive variance in the F2 was complicated by the selection in the F1, which was done using information from the P0 and F1 generation and was done over three separate trials (sub-populations A1, A2 and A3). The difference in the genetic base of the two groups of F1 families may also reduce the accuracy of the prediction of additive variance in the next generation. The F1 families in trials B1, B2 and B3 were selected in provenance trials whereas the F1 families in trials A1, A2 and A3 were selected in SA unimproved landrace material.

The trends observed in this *E.grandis* population (sharp reduction in additive variance in F2 where truncation selection has taken place in the previous generation) may indicate that with advanced generations of breeding in this population of *E.grandis*, that gains achieved through selection for additive variance will decline compared with that achieved in previous generations. (The lower narrow sense heritabilities in the F2 support this indication.) In this case, a strategy that makes use of for example, specific crosses among particular combinations of parents with high specific combining ability to produce families that perform better than the parents, which may not be parents with the highest breeding value (general combining ability) may be appropriate (Cotterill, 1997). The use of cloning, which exploits the total genetic variance (additive and non-additive) and the estimate of broad sense heritability may also become more important, particularly in the production population. Further studies, making use of data from the other sub-populations and the P0 generation, need to be undertaken to model and benchmark the change in additive variance in the *E.grandis* breeding population.

Where selection is practiced at an age other than rotation age in an improvement programme, it is essential that the choice of selection age be based on accurate and sound estimates of the genetic correlation. Caution is advised when obtaining estimates of age-age correlations from small samples as inaccurate estimates of genetic parameters and environmental effects may result in under- and over estimates, or falsely negative estimates, of the age-age correlations (Magnussen, 1991). The magnitude of the heritability and the standard error of the heritability estimate also play a role in the stability of the estimate of the genetic correlation (Verryn et al., 1997). Higher heritability estimates (and relatively low standard error estimates) at the ages for which the correlation is estimated, are more likely to show more stable estimates of the genetic correlation

(but not necessarily higher estimates.) Genetic correlations are more sensitive to error than estimates of heritability and, therefore, require large samples and accurate values.

Reports on age-age correlations in tropical eucalypts generally indicate that there is potential for early selection in these species. In *E.cloeziana*, selection at 29 months was found to be more efficient in terms of gain per unit time, than any of the other selection strategies considered (namely selection at 42, 56, 67, and 80 months) (Marques et al., 1996). In eucalypt hybrid clones in the Congo, high (>0.8) genetic correlations for height and DBH between the "juvenile" age of 35 months and the "mature" age of 67 and 80 months (Bouvet, 1992).

Results of unpublished CSIR studies in three second generation *E.grandis* progeny trials indicate the potential for early selection as early as 30 months for DBH and stem form at 5 years (Pandoy et al., 1998). The merit of indirect selection showed the benefit of early selection and the resultant reduction in breeding cycle length.

The individual phenotypic correlations are high for volume (0.88), but poorer for the two traits that were subjectively assessed, namely stem form and disease tolerance. Two different measurement teams were responsible for the measurement of the trials at the two ages assessed and the accuracy and consistency of the teams, especially with the subjective assessment of stem and disease score, may have affected the accuracy of the age-age correlations. The family and clone mean phenotypic correlations for volume, economically the most important trait, are relatively high (>0.75). The estimates of the family and clone mean phenotypic age-age correlations are generally weaker than the individual correlations.

The strong phenotypic correlations may suggest that early selection for phenotypic differences can be done (i.e., at 38 months rather than 66 months). Accurate early selection for breeding and progress does, however, depend on accurate early prediction of the breeding value of the individuals. The inaccuracy of the estimate of the genetic variance components and the low heritabilities precluded the estimate of accurate, stable and reliable genetic age-age correlations (Franklin, 1979, Gill 1987).

A comparison of predicted genetic gains from between and within-family selection for a trait of low (0.1), medium (0.2) and high (0.4) narrow sense heritability under five different breeding population options, showed the highest predicted gains for the cloned breeding population option (Shelbourne, 1992a). Variances used in the calculations were estimated from a 7 ½ year *Pinus radiata* progeny test. The breeding strategies evaluated were: recurrent mass selection, breeding population of open pollinated progenies, breeding population of open pollinated progenies from archive, breeding population of full sibs and a cloned open pollinated and control pollinated breeding population. Shelbourne (1992a) assumed that dominance or epistatic variance was absent, and that there were neither C-effects nor ageing effects. Gains may be reduced if these assumptions are not valid. Gains were calculated independently for both male and female parents and in the case of open pollinated progenies it was assumed that some degree of selection could be applied to the male parents through selectively thinning the population to improve the pollen cloud. Despite the superior gains, Shelbourne (1992a) highlighted that this strategy may not be the most favourable where the species does not readily rejuvenate from mature material and large numbers of clones must be maintained in a juvenile state.

Park and Fowler (1987) conclude that substantial additional gains could be made in Tamarack (*Larix laricina*) by cloning the individuals in a breeding population. Their conclusions were drawn from a study of three 5 year cloned Tamarack populations. Comparisons in genetic gains in height were made based on 3 different selection strategies (mass selection, ramet selection and clone mean selection based on 30 ramets per clone) in each of the 3 different populations. As Tamarack is not readily rejuvenated, recommendations were made to first select high GCA and SCA parents from a large base and then clone the seedlings from these families and retain some ramets of each clone in juvenile phase (e.g., by hedging). Cryopreservation is also presented as possible method of maintaining juvenility. Rejuvenation and ability to vegetatively propagate is not a constraint in *Eucalyptus* and increases in gains from cloning the individuals can be expected.

Using data from this study, the predicted genetic gains for the breeding population show that cloning the breeding population can (even with only 3 ramets per clone) substantially increase the total gains 7.17% to 9.82% (i.e., by 37% for this scenario, Tables 19 and 21) compared to a non-cloned open pollinated breeding population with the same number of families and individuals per

family. This increase in gain agrees with the findings in the literature and can be attributed to the increase in accuracy of within-family selection through the reduction in the error associated with the estimate of the individual values. Clone means facilitate a far more stable estimate of an individuals genotype. Relatively low selection intensities were considered, however, due to the small number of ramets per clone after mortality. The low selection intensities eroded the gain and an even greater benefit could have been illustrated if the established frequencies had been realised. Gain for all scenarios considered was also reduced by the low heritabilities realised in this trial (compared to the heritabilities that were expected considering estimates obtained in other trials of similar material) (pers. comm. Pierce, 2000).

The non-cloned open pollinated breeding population with the same total number of individuals and families but more individuals per family (24 individuals per family compared with 8 for the cloned population) showed only a 0.12% increase in total gain (0.23% increase in gain per year due to the shorter breeding cycle) (Tables 19 and 20). The comparison assumes that there was no mortality in the seedling families (i.e., 40 seedlings per family). The increase in selection intensity where families consisted of 24 individuals (even though these individuals were not cloned) contributed to the prediction of higher gains for the non-cloned scenario. If, however, these two scenarios are considered with 5 ramets per clone (i.e., as if blanking had been done), then the total gain predicted for the cloned breeding population (11.08%) exceeds the total gain predicted for the non-cloned breeding population (10.98%) with 40 individuals per family (Tables 22 and 23). The benefit of the large number of individual genotypes in the families in the non-cloned scenario did not out weigh the benefit of increasing the accuracy of within family selection by cloning the individuals and using clone means for selection. Gain per year is, however, greater for the non-cloned breeding population scenario due to the shorter cycle. The length of the breeding cycle is extended by 18 months in the case of the cloned breeding population due to the time required to bulk up the clones and this reduces the predicted gains per year for the cloned strategy. The predicted gains per year for the cloned breeding population can be increased if the time required to bulk up the material can be shortened by nursery practices that will improve success. The use of microcuttings may also reduce the time needed to bulk up individual clones.

The option to deploy tested clones into production at the same time as selection for the next breeding generation is the most exciting option (in terms of potential gains and time savings) for production offered by the cloned breeding population strategy.

The option of clonal selection for production in a cloned full sib breeding population was considered in a simulation study conducted by Matheson and Lindgren (1985). The cloned full sib breeding population option was shown to produce more gains in the production population compared with the seed orchard option even when there was no dominance variance (the magnitude of this gain differed depending on the proportion selected). This was mainly due to the reduction in time between selection in the breeding population and deployment in the field. The advantage of the clonal option over the seed orchard increased with increasing proportions of dominance variation. The advantage of the cloned breeding population was shown to be two fold, namely an advantage due to genetic factors (increased accuracy of selection, exploitation of additive and non-additive genetic variance) and an advantage due to the time saved by deploying improved genotypes more rapidly into the plantations.

Mullin et al. (1992) showed that clonal selection in a cloned full sib black spruce (*Picea mariana*) progeny trial could substantially increase gain, compared with selection in a progeny trial where the individuals had not been cloned. The large increases in gain were attributed to the capture of genetic variance due to epsistasis and a greater portion of the additive variation, through increased accuracy in selection.

A study of a clonal diallel of Douglas fir (*Pseudotsuga mensiesii*) showed that broad sense heritabilities were approximately double the narrow sense heritabilities for height (2–6 years of age) and DBH (5 and 6 years of age) and Stonecypher and McCullough (1986), therefore, concluded that cloning was a promising method of increasing the gains in height and DBH growth for Douglas fir. Predictions of genetic gains are inextricably linked through the estimation of genetic and environmental variances, to the population, environment and age of the test material but Stonecypher and McCullough (1986) suggest that given adequate sample sizes and test environments that broader inferences about the suitability of deploying clones within a species, can be made.

The results from the this study show that the predicted gain from the deployment of select clones, which facilitates the capture of both the additive and non-additive components of genetic variation, far exceeds the predicted gain per year and total gains for the other production populations considered [15.83% per year with a production cycle of 2 years for clonal deployment (31.66%) compared with 3.70% per year with a production cycle of 5 years for a forward selected clonal orchard (18.51%) and 10.54% per year with a production cycle of 2 years for conversion of breeding population to a seed orchard (21.07%)] [Table 24]. The effective length of the breeding and production strategy is reduced as clonally tested clones can be selected directly from the breeding population based on clone means and there is no need for further clonal testing. If wood property selection criteria, such as log-end splitting, are considered then the selection age may have to be increased to accommodate selection in these traits. The option to deploy tested clones compared to untested clones (which then require a subsequent testing phase which reduces the gains made per year) or untested seedling progenies, is a distinct advantage of the cloned breeding population strategy. The trend in the composition of genetic variance over generations (much higher proportion of non-additive variance in traits which have undergone selection in previous generations) also suggests that deploying clones (which exploits both the additive and non-additive genetic variance) is the most promising option to maximise gains in the production population.

The comparison of the predicted gains from the deployment of clones selected in the cloned breeding population, to clones selected in a clonal trial following selection in a non-cloned breeding population, showed that although in both cases the deployment of clones exploited both the non-additive and additive genetic variance, that the additional time required to clonally test selections from the breeding population notably reduced the gains per year. Predicted gains from deployment of clones from cloned breeding population where 15.83% per year (31.66% total) compared with predicted gains of 5.42%-6.16% (depending on whether the breeding population had the same number of total individuals as the cloned breeding population, or whether the same number of families and trees per family were considered) and 37.93%-43.11% total predicted gains, for selection of clones in a clonal trial following selection in the breeding population [Table 24]. The increased total predicted gains for clonal deployment following clonal testing of selections

made in a non-cloned breeding population may in part be due to the increased number of ramets per clone that can be tested.

Conversion of the breeding population to a seed orchard (Option A, Chapter 8; thinning based on clone means) is also a relatively low cost option to obtain improved open pollinated seed (which exploits only the additive genetic variation) rather than deploy clones. In certain circumstances, clonal deployment may not be an option due, for example, to the resources and expertise (which may not be available) required to establish and run a clonal nursery. The progenies are untested and gains could be increased (but the time extended and gains per year decreased) by progeny testing and rogueing the orchard. The gains per year from conversion to a seed orchard (10.54%) compare favourably to the gains per year predicted for the clonal deployment option (15.83%) considering the increased cost of this option. There may, however, be conflicts in the requirements for the management of the breeding population and production population if the breeding population is to be thinned on clone means regardless of family structure for the conversion to a seed orchard. A low intensity thinning could be done in the breeding population and once seed for the next generation had been collected a heavier thinning on clone means for conversion to a seed orchard (production population) could be done. Careful attention would have to be paid to timing when planning the thinning and seed collection operations.

Gains in the production population for the same production strategy following different breeding populations were also compared. A production population consisting of a clonal orchard from forward selection was considered for the three breeding population scenarios (cloned breeding population, non-cloned breeding population with the same total number of individuals – i.e., more individuals per family, and a non-cloned breeding population with the same number of individuals per family as the cloned population). Total predicted gains were highest (18.51%) for the clonal orchard from forward selection on clone means in a cloned open pollinated breeding population (Option B, Chapter 8). Predicted gains for the clonal orchard from forward selection in a non-cloned open pollinated breeding population (177 families, 24 individuals per family Option D) were less than 1% lower than for Option B. The lowest gains were predicted for selection in an open pollinated breeding population with the same number of families and individuals per family (8) as the cloned population (12.87%) [Option E]. In all instances, progeny testing and rogueing

could improve gains from the forward selected clonal orchard, but this has not been considered in these scenarios. The clonal orchard from forward selection in the cloned population may not be the most promising option for seed production when compared with the conversion of the breeding population to a seed orchard. However, these comparisons of predicted gain have shown that the predicted gains for selection form a cloned breeding population exceed the gains predicted from selection in non-cloned populations – even when the same total number of trees in the population are considered.

CHAPTER 11

CONCLUSION

The trials were not optimal for the estimation of variance components due to large environmental effects, high mortality and the low heritabilities that were realised.

A knowledge of the degree of relatedness and selfing in open pollinated families is required for improved estimates of the coefficient of relationship. This coefficient has a notable effect on the estimate of variance components, as illustrated by the different estimates obtained in scenarios 2 and 3, and an inaccurate estimate of the amount of relatedness may bias the heritability estimates.

Growth traits were found to be under predominantly additive genetic control (in the pooled data). The current selection strategy, which is based on general combining ability, is appropriate in circumstances where the selection traits are under strong additive control and cloning, and the resultant increase in cost and time, may not be necessary if other measures are taken to reduce the experimental error (e.g., reduce weed competition, accurate trial layout in nursery and field, clearly labeled plots in field etc). Disease tolerance and stem form were, however, found to be under relatively strong non-additive control and a different selection strategy may be required.

One of the main advantages of the cloned breeding strategy is that cloning facilitates the more precise assessment of the genotypic differences between individuals within families, as ramets of clones do not have the genetic variation that exists among seedlings. Clone means are available to assess the genotypic differences between individuals within a family. The cloned breeding population strategy is, however, an expensive strategy and the increase in cost and time, may not be necessary if other measures are taken to reduce experimental error (e.g., reduce weed competition, accurate trial layout in nursery and field, clearly labeled plots in field etc) and increase the heritability of selection traits. The economic importance of the traits and the benefit in terms of

genetic gain (realizing the gain faster in the plantations) and cost (no additional clonal trials need be established) afforded by the reduction in the time to deployment of select clones from the cloned breeding population, will also influence the choice of strategy. Deploying clones exploits all the genetic variation (additive and non-additive). Broad sense heritability was generally notably higher than the narrow sense heritability for stem form and disease and indicated the potential for increased gain for these traits through selection of tested clones for production.

The trend in the distribution of genetic variance in the F1 and F2 families indicates a higher proportion of non-additive variance in the F2 families for all traits except disease, which had not been selected for in previous generations. This may indicate that with advanced generations of breeding in this population of *E.grandis*, that gains achieved through selection for additive variance will decline compared with that achieved in previous generations. (The lower narrow sense heritabilities in the F2 support this indication.) A strategy for future generations that exploits the non-additive variance may be appropriate.

Far higher gains were predicted for the cloned breeding population compared with a non-cloned population of the same number of families and individuals per family thereby indicating the benefit of using clone means to assess individual genotypic differences within families. If planted as planned and blanking is done to maintain the frequencies at those that were established, the cloned breeding population also showed more gain than the non-cloned breeding population with the same total number of trees (as the cloned population) but with more individuals per family. Predicted gains for the production population demonstrated the benefit of exploiting the total genetic variance by deploying tested clones into production only 2 years (possibly even less) after selection in the cloned breeding population. Predicted gains for the conversion of the cloned breeding population into a seed orchard were only slightly lower than the predicted gains for a forward selected clonal orchard, but the time saved (and therefore, increased gain per year) make this an attractive option for the production of improved seed (even though at this stage the seed is not progeny tested).

This study has shown and discussed the benefit of a cloned breeding population and the advantage of the rapid deployment of clonally tested material for the production population. The benefits afforded by the cloned population are mainly two-fold, namely an increase in the accuracy of

selection, and the time saving and increase in gains through the selection of tested clones for deployment at the same time as selection the next generation. This study has, however, also shown the importance of minimising the experimental error at all stages of the trial's lifetime (e.g., accurate labeling and identity control in the nursery and at trial layout and establishment, blanking in the event of high mortality, several weedings should weed competition be a problem, accurate reading of trial maps during trial assessment).

Ultimately the choice of strategy must be decided by a combination of factors. These include the nature of the genetic control of the selection trait(s), the available financial resources (which in turn, also determine available manpower and facilities), the time constraints and the predicted gain.

Further investigations should be done, in this population, to investigate the genetic control of economically important wood properties. Global trends are towards higher quality and quantity timber (as opposed to only quantity) where a premium will be paid by processors for timber with certain wood qualities. The CSIR has, and will continue to position itself to meet the demand for this material and appropriate breeding, production and selection strategies will have to be put in place and knowledge of the genetic control of these traits will be invaluable.

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APPENDIX A

ASSESSMENT TECHNIQUES

Table A-1. Procedure for the 38 month assessment of trials B1, B2 and B3.

Trait	Instrument	Method of assessment	Unit
Height	Height rods	from base to tip of tree	dm
DBH	Diameter tape	over bark at 1,3 m from ground level	mm
Stem form	Visual	on total bole from base to tip of tree	1 - 8
Coniothyrium	Visual	on total bole from base to live crown	0 - 4
Cryphonectria	Visual	on total bole from base to live crown	0 - 4
Endothia	Visual	on total bole from base to live crown	0 - 4
Botryosphaeria	Visual	on total bole from base to live crown	0 - 4
Defects	Visual	missing tree broken top forked tree runt dead tree leaning tree	M B F S D L

Table A-2. Procedure for the 66 month assessment of trials B1, B2 and B3.

Trait	Instrument	Method of assessment	Unit
Height	Vertex Hypsometer	from base to tip of tree	m
DBH	Diameter tape	over bark at 1,3 m from ground level	mm
Stem form	Visual	on total bole from base to tip of tree	1 - 8
Coniothyrium	Visual	on total bole from base to live crown	0 - 4
Cryphonectria	Visual	on total bole from base to live crown	0 - 4
Botryosphaeria	Visual	on total bole from base to live crown	0 - 4
Endothia	Visual	on total bole from base to live crown	0 - 4
Defects	Visual	missing tree broken top forked tree runt	G B F R

Table A-3. Industry standard subjective scale for stem form assessment in trials B1, B2 and B3.

Score	Description	Summary of defects
8	Straight stem - pole quality	STRAIGHT no defects
7	Slight sweep and/or 1 minor bend	NEARLY STRAIGHT 1 - 2 minor defects
6	One slight sweep + >1 minor bend OR More than 1 slight sweep + 1 minor bend OR More than 2 minor bends	VERY SLIGHTLY CROOKED 3 - 4 minor defects
5	Moderate sweep + 1 moderate bend OR Two moderate sweeps + minor defect OR Two moderate bends + minor defect	SLIGHTLY CROOKED 2 moderate defects OR 2 moderate + 1 minor defects
4	Moderate sweep + major bend OR More than two moderate sweeps OR More than two moderate bends OR Two major bends + minor defects	MODERATELY CROOKED 1 moderate + 1 major defect OR > 2 moderate defects OR 2 major + 2 minor defects
3	Obvious sinuosity or major crooks	CROOKED > 2 major defects OR 2 major + 2 moderate defects
2	Presence of multiple severe straightness defects	VERY CROOKED several major and moderate defects
1	Unmerchantable as a short log cork screw)	MALFORMED major defects

Table A-4. Industry standard subjective scale for the visual assessment of the diseases *Cryphonectria, Coniothyrium, Botryosphaeria* and *Endothia* in trials B1, B2 and B3.

Score	Description	Summary of infestation
0	No visual sign of any disease infestation	NIL
1	Some visual disease infestation	25% COVERAGE
2	Mild visual disease infestation	50% COVERAGE
3	Moderate visual disease infestation	75% COVERAGE
4	Chronic visual disease infestation	100% COVERAGE

Table A-5. Model used for the estimation of volume for *Eucalyptus grandis* (Bredenkamp and Loveday, 1984).

$$\log V = b_0 + b_1 \log(DBH+d) + b_2 \log Ht$$

where,

log is the common logarithm to the base 10
V is the stem volume (m^3), to 75 mm tip diameter
DBH is the diameter at breast height (mm)
d is the correction factor (mm)
Ht is the tree height (m).

Coefficients where diameter at breast-height (DBH) is measured in millimetres:

DBH (mm)	b_0	b_1	d	b_2
< 200	-11.16217	3.65167	100	1.14760
200 < DBH < 400	- 4.98199	1.32829	- 70	1.17827
> 400	- 5.39010	1.41460	- 60	1.29911

APPENDIX B

DATA EDITING

Table B-1. Observations deemed outliers (more than three times the Inter Quartile Range from the mean) and removed from the data sets for trials B1, B2 and B3, sorted by trial, replication and block.

Clone	Trait		Family	Trial	Replication	Block
	Height at age (months)	DBH at age (months)				
AG596/9	38	38	AG596	B1	1	5
BG22/8	38	-	BG22	B1	1	7
AG535/3	66	-	AG535	B1	1	19
BG165/8	-	38	BG165	B1	2	14
AG647/9	38	-	AG647	B1	3	5
AG489/8	38	-	AG489	B1	4	1
BG128/5	38	-	BG128	B1	4	13
BG110/7	38	-	BG110	B2	1	2
AG689/1	38	38	AG689	B2	2	1
BG61/3	38	-	BG61	B2	2	2
AG643/4	38	-	AG643	B2	2	16
BG39/3	38	-	BG39	B2	2	18
AG519/8	38	-	AG519	B2	3	1
AG611/6	38	-	AG611	B2	3	5
BG111/1	66	-	BG111	B2	3	7
BG39/6	38	-	BG39	B2	3	7
AG599/8	38	38	AG599	B2	3	11
BG39/7	38	38	BG39	B2	3	20
BG152/9	38	-	BG152	B2	4	8
AG649/9	38	38	AG649	B2	4	12
AG649/8	38	38	AG649	B2	5	14
AG465/2	38	-	AG465	B2	5	17
BG71/6	66	-	BG71	B3	4	2
BG75/1	66	-	BG75	B3	4	3

Table B-2. Observations deemed outliers (more than three times the Inter Quartile Range from the mean) and removed from the data sets for trials B1, B2 and B3, sorted by family.

Clone	Trait		Family	Trial	Replication	Block
	Height at age (months)	DBH at age (months)				
AG465/2	38	-	AG465	B2	5	17
AG489/8	38	-	AG489	B1	4	1
AG519/8	38	-	AG519	B2	3	1
AG535/3	66	-	AG535	B1	1	19
AG596/9	38	38	AG596	B1	1	5
AG599/8	38	38	AG599	B2	3	11
AG611/6	38	-	AG611	B2	3	5
AG643/4	38	-	AG643	B2	2	16
AG647/9	38	-	AG647	B1	3	5
AG649/8	38	38	AG649	B2	5	14
AG649/9	38	38		B2	4	12
AG689/1	38	38	AG689	B2	2	1
BG110/7	38	-	BG110	B2	1	2
BG111/1	66	-	BG111	B2	3	7
BG128/5	38	-	BG128	B1	4	13
BG152/9	38	-	BG152	B2	4	8
BG165/8	-	38	BG165	B1	2	14
BG22/8	38	-	BG22	B1	1	7
BG39/3	38	-	BG39	B2	2	18
BG39/6	38	-		B2	3	7
BG39/7	38	38		B2	3	20
BG61/3	38	-	BG61	B2	2	2
BG71/6	66	-	BG71	B3	4	2
BG75/1	66	-	BG75	B3	4	3
24			**21**			

Table B-3. Regression models used for the correction for missing trees in trials B1, B2 and B3.

Trial	Trait	Age	Model	Pr>F
B1	Volume	38 months	$\hat{y}=0.00467x_1+0.00280x_2+0.12$	0.0001***
		66 months	$\hat{y}=0.02245x_1+0.01281x_2+0.27$	0.0001***
	Height	38 months	$\hat{y}=-0.20028x_1+17.03$	0.0011**
		66 months	Model non-significant $(p \le 0.05)$	
	DBH	38 months	$\hat{y}=4.03029x_1+1.86632x_2+159.89$	0.0001***
		66 months	$\hat{y}=8.17575x_1+4.20196x_2+198.47$	0.0001***
	Stem Score	38 months	Model non-significant $(p \le 0.05)$	
		66 months	$\hat{y}=0.11880x_1+5.31$	0.0001***
	Disease Tolerance	38 months	Model non-significant $(p \le 0.05)$	
		66 months	Model non-significant $(p \le 0.05)$	
B2	Volume	38 months	$\hat{y}=0.00879x_1+0.13$	0.0001***
		66 months	$\hat{y}=0.03041x_1+0.00774x_2+0.29$	0.0001***
	Height	38 months	$\hat{y}=-0.25893x_2+17.94$	0.0001***
		66 months	Model non-significant $(p \le 0.05)$	
	DBH	38 months	$\hat{y}=5.93902x_1+163.50$	0.0001***
		66 months	$\hat{y}=10.53912x_1+2.71715x_2+198.05$	0.0001***
	Stem Score	38 months	$\hat{y}=0.10973x_1+5.71$	0.0024**
		66 months	$\hat{y}=0.15987x_1+5.59$	0.0001***
	Disease Tolerance	38 months	Model non-significant $(p \le 0.05)$	
		66 months	Model non-significant $(p \le 0.05)$	
B3	Volume	66 months	$\hat{y}=0.03247x_1+0.02175x_2+0.30$	0.0001***
	Height	66 months	$\hat{y}=0.21660x_2+25.26$	0.0004**
	DBH	66 months	$\hat{y}=9.36506x_1+5.56324x_2+200.24$	0.0001***
	Stem Score	66 months	$\hat{y}=0.09329x_1+0.05793x_2+5.51$	0.0001***
	Disease Tolerance	66 months	Model non-significant $(p \le 0.05)$	

$\hat{y}_i$ 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)

* Significant at p≤ 0.05

** Significant at p≤ 0.01

*** Significant at p≤0.0001

Table B-4. Regression models used for the correction for missing trees in the pooled data of trials B1, B2 and B3.

Trait	Age	Model	Pr>F
Volume	38 months	$\hat{y}=0.00754x_1 + 0.12$	0.0001***
	66 months	$\hat{y}=0.02976x_1 +0.01607x_2 +0.28$	0.0001***
Height	38 months	$\hat{y}=-0.14701x_1 -0.14570x_2 +17.57$	0.0001***
	66 months	Model non-significant $(p \leq 0.05)$	
DBH	38 months	$\hat{y}=5.11523x_1 +1.32590x_2 +160.91$	0.0001***
	66 months	$\hat{y}=9.79673x_1 +4.72259x_2 +197.44$	0.0001***
Stem Score	38 months	$\hat{y}=0.06888x_1 +0.54$	0.0120*
	66 months	$\hat{y}=0.12672x_1 +0.04689x_2 +5.42$	0.0001***
Disease Tolerance	38 months	Model non-significant $(p \leq 0.05)$	
	66 months	Model non-significant $(p \leq 0.05)$	

$\hat{y}_i$ 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)

* Significant at p≤ 0.05
** Significant at p≤ 0.01
*** Significant at p≤0.0001

APPENDIX C

ANALYSIS OF VARIANCE IN THE SEPARATE TRIALS (B1, B2 AND B3)

Table C-1. Analysis of variance for volume in trial B1 at age 38 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	0.021	0.0054	2.83	0.0239*
Block within replication	75	0.19	0.0026	1.38	0.0239*
Family	55	0.25	0.0045	2.40	0.0001***
Clone within-family	419	1.30	0.0031	1.64	0.0001***
Error	694	1.31	0.0019		

Table C-2. Analysis of variance for DBH in trial B1 at age 38 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	3454.37	863.59	1.42	0.2264 ^{NS}
Block within replication	75	61050.90	814.012	1.34	0.0361*
Family	55	71670.50	1303.10	2.14	0.0001***
Clone within-family	419	395705.28	944.40	1.55	0.0001***
Error	698	425206.80	609.18		

Table C-3. Analysis of variance for height in trial B1 at age 38 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	100.18	25.044	12.20	0.0001***
Block within replication	75	323.079	4.31	2.10	0.0001***
Family	55	236.76	4.31	2.10	0.0001***
Clone within-family	419	1262.68	3.014	1.47	0.0001***
Error	695	1426.39	2.052		

Table C-4. Analysis of variance for stem form in trial B1 at age 38 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	17.90	4.47	2.71	0.0295*
Block within replication	75	139.91	1.87	1.13	0.2242 ^{NS}
Family	55	220.34	4.0061	2.42	0.0001***
Clone within-family	419	997.76	2.38	1.44	0.0001***
Error	694	1147.57	1.65		

Table C-5. Analysis of variance for disease tolerance in trial B1 at age 38 months.

Source	DF	SS	MS	F V alue	Pr > F
Replication	4	1.23	0.31	4.72	0.0009**
Block within replication	75	8.54	0.11	1.75	0.0002**
Family	55	8.46	0.15	2.37	0.0001***
Clone within-family	419	43.63	0.10	1.60	0.0001***
Error	699	45.44	0.065		

Table C-6. Analysis of variance for volume in trial B2 at age 38 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	0.044	0.011	4.45	0.0015**
Block within replication	77	0.23	0.0030	1.22	0.1112 ^{NS}
Family	58	0.34	0.0058	2.35	0.0001***
Clone within-family	428	1.50	0.0035	1.42	0.0001***
Error	677	1.68	0.0025		

Table C-7. Analysis of variance for DBH in trial B2 at age 38 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	13413.67	3353.42	4.85	0.0007***
Block within replication	77	65065.23	845.0030	1.22	0.1043 ^{NS}
Family	58	94007.92	1620.83	2.34	0.0001***
Clone within-family	428	409987.31	957.91	1.39	0.0001***
Error	685	473492.51	691.23		

Table C-8. Analysis of variance for height in trial B2 at age 38 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	21.87	5.47	2.72	0.0288*
Block within replication	77	373.00	4.84	2.41	0.0001***
Family	58	240.11	4.14	2.06	0.0001***
Clone within-family	428	1318.36	3.08	1.53	0.0001***
Error	677	1361.56	2.011		

Table C-9. Analysis of variance for stem form in trial B2 at age 38 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	28.55	7.14	5.35	0.0003**
Block within replication	77	129.18	1.68	1.26	0.0764 ^{NS}
Family	58	107.71	1.86	1.39	0.0328*
Clone within-family	428	789.66	1.85	1.38	0.0001***
Error	685	914.44	1.33		

Table C-10. Analysis of variance for disease tolerance in trial B2 at age 38 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	1.22	0.30	7.00	0.0001***
Block within replication	77	3.39	0.044	1.01	0.4534 ^{NS}
Family	58	5.015	0.086	1.99	0.0001***
Clone within-family	428	30.42	0.071	1.64	0.0001***
Error	687	29.84	0.043		

Table C-11. Analysis of variance for volume in trial B1 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	0.16	0.040	3.70	0.0054**
Block within replication	84	1.17	0.014	1.28	0.0542 ^{NS}
Family	55	2.16	0.039	3.60	0.0001***
Clone within-family	418	9.82	0.024	2.15	0.0001***
Error	741	8.089	0.011		

Table C-12. Analysis of variance for DBH in trial B1 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	21999.30	5499.83	5.76	0.0001***
Block within replication	84	118348.79	1408.91	1.48	0.0052**
Family	55	160154.52	2911.90	3.05	0.0001***
Clone within-family	418	813209.65	1945.48	2.04	0.0001***
Error	742	708155.071	954.39		

Table C-13. Analysis of variance for height in trial B1 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	25.67	6.42	1.84	0.1198 ^{NS}
Block within replication	84	515.62	6.14	1.76	0.0001***
Family	55	622.37	11.32	3.24	0.0001***
Clone within-family	418	2690.24	6.44	1.84	0.0001***
Error	741	2589.13	3.49		

Table C-14. Analysis of variance for stem form in trial B1 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	6.52	1.63	3.21	0.0125*
Block within replication	84	47.045	0.56	1.10	0.2565 ^{NS}
Family	55	69.72	1.27	2.50	0.0001***
Clone within-family	418	330.85	0.79	1.56	0.0001***
Error	740	375.64	0.51		

Table C-15. Analysis of variance for disease tolerance in trial B1 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	0.23	0.057	1.57	0.1800 ^{NS}
Block within replication	84	4.14	0.049	1.37	0.0209*
Family	55	5.92	0.11	2.98	0.0001***
Clone within-family	418	30.66	0.073	2.03	0.0001***
Error	742	26.77	0.036		

Table C-16. Analysis of variance for volume in trial B2 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	0.15	0.038	2.80	0.0253*
Block within replication	77	1.42	0.018	1.34	0.0346*
Family	58	2.20	0.038	2.75	0.0001***
Clone within-family	420	9.16	0.022	1.59	0.0001***
Error	630	8.66	0.014		

Table C-17. Analysis of variance for DBH in trial B2 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	9909.71	2477.43	2.38	0.0506 ^{NS}
Block within replication	77	122045.95	1585.012	1.52	0.0041**
Family	58	147231.058	2538.47	2.44	0.0001***
Clone within-family	420	672872.80	1602.078	1.54	0.0001***
Error	631	657127.39	1041.41		

Table C-18. Analysis of variance for height in trial B2 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	148.26	37.064	9.04	0.0001***
Block within replication	77	597.00	7.75	1.89	0.0001***
Family	58	521.22	8.99	2.19	0.0001***
Clone within-family	420	2446.57	5.83	1.42	0.0001***
Error	631	2588.16	4.10		

Table C-19. Analysis of variance for stem form in trial B2 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	4.23	1.057	1.97	0.0972 ^{NS}
Block within replication	77	57.25	0.74	1.39	0.0204*
Family	58	56.96	0.98	1.83	0.0003**
Clone within-family	420	327.65	0.78	1.46	0.0001***
Error	632	338.65	0.54		

Table C-20. Analysis of variance for disease tolerance in trial B2 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	0.32	0.081	2.36	0.0526 ^{NS}
Block within replication	77	2.89	0.038	1.09	0.2855 ^{NS}
Family	58	6.46	0.11	3.24	0.0001***
Clone within-family	420	23.11	0.055	1.60	0.0001***
Error	632	21.70	0.034		

Table C-21. Analysis of variance for volume in trial B3 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	0.26	0.064	3.69	0.0055**
Block within replication	85	2.0082	0.024	1.37	0.0206*
Family	67	2.37	0.035	2.05	0.0001***
Clone within-family	411	7.75	0.019	1.09	0.1586 ^{NS}
Error	697	12.052	0.017		

Table C-22. Analysis of variance for DBH in trial B3 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	8947.00046	2236.75	1.96	0.0990 ^{NS}
Block within replication	85	126168.13	1484.33	1.30	0.0433*
Family	67	146920.70	2192.85	1.92	0.0001***
Clone within-family	411	510951.015	1243.19	1.09	0.1631 ^{NS}
Error	698	796709.94	1141.42		

Table C-23. Analysis of variance for height in trial B3 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	381.17	95.29	23.93	0.0001***
Block within replication	85	443.73	5.22	1.31	0.0387*
Family	67	605.30	9.034	2.27	0.0001***
Clone within-family	411	1887.83	4.59	1.15	0.0508 ^{NS}
Error	697	2776.12	3.98		

Table C-24. Analysis of variance for stem form in trial B3 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	8.33	2.082	3.85	0.0042**
Block within replication	85	45.36	0.53	0.99	0.5127 ^{NS}
Family	67	44.94	0.67	1.24	0.1000 ^{NS}
Clone within-family	411	242.13	0.59	1.09	0.1595 ^{NS}
Error	698	377.054	0.54		

Table C-25. Analysis of variance for disease tolerance in trial B3 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Replication	4	0.36	0.091	2.31	0.0568 NS
Block within replication	85	4.96	0.058	1.48	0.0050**
Family	67	4.75	0.071	1.80	0.0002**
Clone within-family	411	22.73	0.055	1.40	0.0001***
Error	698	27.52	0.039		

SS	Type III Sum of Squares
NS	Non-significant
*	Significant at p≤ 0.05
**	Significant at p≤ 0.01
***	Significant at p≤0.0001

APPENDIX D

ANALYSIS OF VARIANCE IN POOLED DATA WITH TRIAL EFFECT

Table D-1. Analysis of variance for volume (m^3) in the pooled data of trials B1 and B2 at age 38 months.

Source	DF	SS	MS	F Value	Pr > F
Trial	1	0.015	0.015	6.70	0.0098**
Family	113	0.62	0.0055	2.43	0.0001***
Clone within-family	844	2.84	0.0034	1.48	0.0001***
Error	1534	3.49	0.0023		

Table D-2. Analysis of variance for height (m) in the pooled data of trials B1 and B2 at age 38 months.

Source	DF	SS	MS	F Value	Pr > F
Trial	1	0.018	0.018	0.01	0.9303 ^{NS}
Family	113	514.77	4.56	1.94	0.0001***
Clone within-family	844	2807.92	3.33	1.42	0.0001***
Error	1535	3601.43	2.35		

Table D-3. Analysis of variance for DBH (mm) in the pooled data of trials B1 and B2 at age 38 months.

Source	DF	SS	MS	F Value	Pr > F
Trial	1	5295.00	5295.00	7.84	0.0052**
Family	113	171446.018	1517.22	2.25	0.0001***
Clone within-family	844	812006.57	962.093	1.42	0.0001***
Error	1546	1043912.39	675.23		

Table D-4. Analysis of variance for stem form (1-8 point scale) in the pooled data of trials B1 and B2 at age 38 months.

Source	DF	SS	MS	F Value	Pr > F
Trial	1	0.011	0.011	0.01	0.9339 ^{NS}
Family	113	341.85	3.025	1.95	0.0001***
Clone within-family	844	1847.99	2.19	1.41	0.0001***
Error	1542	2388.53	1.55		

Table D-5. Analysis of variance for disease tolerance (0-4 point scale for each disease) in the pooled data of trials B1 and B2 at age 38 months.

Source	DF	SS	MS	F Value	Pr > F
Trial	1	0.017	0.017	0.29	0.5906 ^{NS}
Family	113	15.0049	0.13	2.29	0.0001***
Clone within-family	844	80.72	0.096	1.65	0.0001***
Error	1549	89.89	0.058		

Table D-6. Analysis of variance for volume (m^3) in the pooled data of trials B1, B2 and B3 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Trial	2	0.093	0.047	3.17	0.0422*
Family	176	7.28	0.041	2.81	0.0001***
Clone within-family	1222	27.49	0.023	1.53	0.0001***
Error	2357	34.68	0.015		

Table D-7. Analysis of variance for height (m) in the pooled data of trials B1, B2 and B3 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Trial	2	129.0051	64.50	14.84	0.0001***
Family	176	2009.72	11.42	2.63	0.0001***
Clone within-family	1222	7523.51	6.16	1.42	0.0001***
Error	2358	10249.68	4.35		

Table D-8. Analysis of variance for DBH (mm) in the pooled data of trials B1, B2 and B3 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Trial	2	3925.46	1962.73	1.77	0.1711 ^{NS}
Family	176	480888.45	2732.32	2.46	0.0001***
Clone within-family	1222	2072873.74	1696.30	1.53	0.0001***
Error	2360	2622036.63	1111.03		

Table D-9. Analysis of variance for stem form (1-8 point scale) in the pooled data of trials B1, B2 and B3 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Trial	2	2.56	1.28	2.35	0.0955 ^{NS}
Family	176	185.51	1.054	1.94	0.0001***
Clone within-family	1222	925.38	0.76	1.39	0.0001***
Error	2359	1282.083	0.54		

Table D-10. Analysis of variance for disease tolerance (0-4 point scale for each disease) in the pooled data of trials B1, B2 and B3 at age 66 months.

Source	DF	SS	MS	F Value	Pr > F
Trial	2	0.27	0.13	3.46	0.0316*
Family	176	19.22	0.11	2.84	0.0001***
Clone within-family	1222	80.32	0.066	1.71	0.0001***
Error	2361	90.75	0.038		

SS	Type III Sum of Squares	*	Significant at p≤ 0.05
NS	Non-significant	**	Significant at p≤ 0.01
		***	Significant at p≤0.0001

Table D-11. T-test for volume in the pooled data of trials B1 and B2 at age 38 months.

Trial	Mean	n	T-Test Grouping
B2	0.14	1245	A
B1	0.13	1248	B

Means grouped with the same letter are not significantly different (α=0.05)

Table D-12. T-test for height in the pooled data of trials B1 and B2 at age 38 months.

Trial	Mean	n	T-Test Grouping
B2	17.71	1245	A
B1	16.91	1249	B

Means grouped with the same letter are not significantly different (α=0.05)

Table D-13. T-test for DBH in the pooled data of trials B1 and B2 at age 38 months.

Trial	Mean	n	T-Test Grouping
B2	172.62	1253	A
B1	168.65	1252	B

Means grouped with the same letter are not significantly different (α=0.05)

Table D-14. T-test for stem form in the pooled data of trials B1 and B2 at age 38 months.

Trial	Mean	n	T-Test Grouping
B2	5.88	1253	A
B1	5.44	1248	B

Means grouped with the same letter are not significantly different (α=0.05)

Table D-15. T-test for disease tolerance in the pooled data of trials B1 and B2 at age 38 months.

Trial	Mean	n	T-Test Grouping
B1	0.65	1253	A
B2	0.56	1255	B

Means grouped with the same letter are not significantly different (α=0.05)

Table D-16. Student-Newman-Keuls test for volume in the pooled data of trials B1, B2 and B3 at age 66 months.

Trial	Mean	n	SNK Grouping
B3	0.39	1265	A
B2	0.35	1190	B
B1	0.32	1303	C

Means grouped with the same letter are not significantly different (α=0.05)

Table D-17. Student-Newman-Keuls test for height in the pooled data of trials B1, B2 and B3 at age 66 months.

Trial	Mean	n	SNK Grouping
B3	25.62	1265	A
B2	24.30	1191	B
B1	23.35	1303	C

Means grouped with the same letter are not significantly different (α=0.05)

Table D-18. Student-Newman-Keuls test for DBH in the pooled data of trials B1, B2 and B3 at age 66 months.

Trial	Mean	n	SNK Grouping
B3	222.85	1266	A
B2	218.95	1191	B
B1	215.82	1304	C

Means grouped with the same letter are not significantly different (α=0.05)

Table D-19. Student-Newman-Keuls test for stem form in the pooled data of trials B1, B2 and B3 at age 66 months.

Trial	Mean	n	SNK Grouping
B2	5.84	1192	A
B3	5.74	1266	B
B1	5.49	1302	C

Means grouped with the same letter are not significantly different (α=0.05)

Table D-20. Student-Newman-Keuls test for disease tolerance in the pooled data of trials B1, B2 and B3 at age 66 months.

Trial	Mean	n	SNK Grouping
B1	0.59	1304	A
B2	0.53	1192	B
B3	0.50	1266	C

Means grouped with the same letter are not significantly different (α=0.05)

APPENDIX E

T-TEST FOR GENERATIONS F1 AND F2

Table E-1. T-test for volume (corrected for missing trees) at age 38 months for the F1 and F2 groups of families (subsets of the pooled data of trials B1 and B2).

Generation	Mean	n	T-Test Grouping
F1	0.0024	1156	A
F2	-0.0020	1361	B

Table E-2. T-test for height (corrected for missing trees) at age 38 months for the F1 and F2 groups of families (subsets of the pooled data of trials B1 and B2).

Generation	Mean	n	T-Test Grouping
F1	0.14	1156	A
F2	-0.12	1361	B

Table E-3. T-test for DBH (corrected for missing trees) at age 38 months for the F1 and F2 groups of families (subsets of the pooled data of trials B1 and B2).

Generation	Mean	n	T-Test Grouping
F1	0.46	1156	A
F2	-0.39	1361	A

Table E-4. T-test for stem form (corrected for missing trees) at age 38 months for the F1 and F2 groups of families (subsets of the pooled data of trials B1 and B2).

Generation	Mean	N	T-Test Grouping
F1	0.025	1156	A
F2	-0.021	1361	A

Table E-5. T-test for disease tolerance (not corrected for missing trees) at age 38 months for the F1 and F2 groups of families (subsets of the pooled data of trials B1 and B2).

Generation	Mean	n	T-Test Grouping
F2	0.61	1356	A
F1	0.60	1152	A

Table E-6. T-test for volume (corrected for missing trees) at age 66 months for the F1 and F2 groups of families (subsets of the pooled data of trials B1 B2, and B3).

Generation	Mean	n	T-Test Grouping
F1	0.016	1803	A
F2	-0.015	1962	B

Table E-7. T-test for height (not corrected for missing trees) at age 66 months for the F1 and F2 groups of families (subsets of the pooled data of trials B1, B2 and B3).

Generation	Mean	N	T-Test Grouping
F1	24.77	1801	A
F2	24.092	1958	B

Table E-8. T-test for DBH (corrected for missing trees) at age 66 months for the F1 and F2 groups of families (subsets of the pooled data of trials B1, B2 and B3).

Generation	Mean	N	T-Test Grouping
F1	3.37	1803	A
F2	-3.10	1962	B

Table E-9. T-test for stem form (corrected for missing trees) at age 66 months for the F1 and F2 groups of families (subsets of the pooled data of trials B1, B2 and B3).

Generation	Mean	N	T-Test Grouping
F1	0.011	1803	A
F2	-0.010	1962	A

Table E-10. T-test for disease tolerance (not corrected for missing trees) at age 66 months for the F1 and F2 groups of families (subsets of the pooled data of trials B1, B2 and B3).

Generation	Mean	N	T-Test Grouping
F2	0.55	1960	A
F1	0.53	1802	B

Means grouped with the same letter are not significantly different (α=0.05)

Note: Due to the correction for missing trees where applicable, the "means" are in fact effects and although as such they should sum to zero, the two groups do not as the subsequent grouping into F1 and F2 is imbalanced. Without the grouping of families into F1 and F2 generation categories, the sum for each of the corrected traits is zero.