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	
1	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
	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.

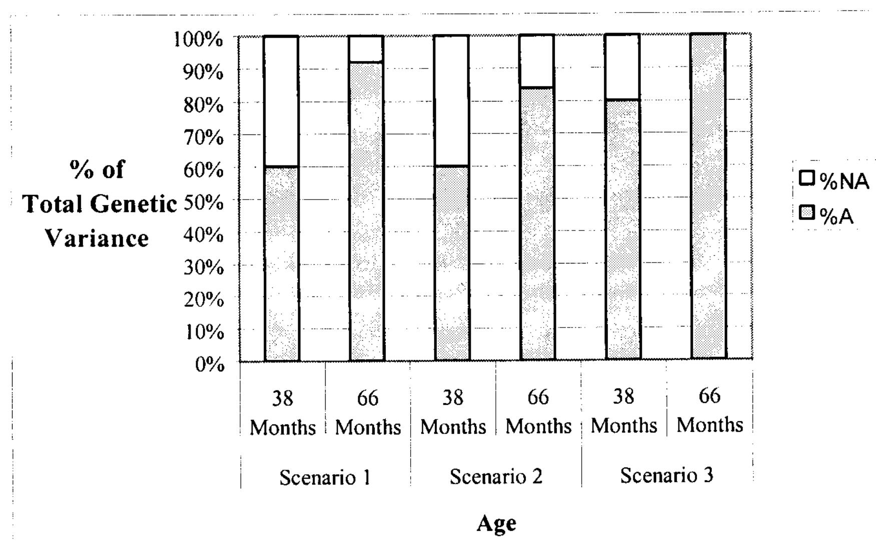

Figure 3. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance in volume at age 38 and 66 months for each of the three scenarios considered.

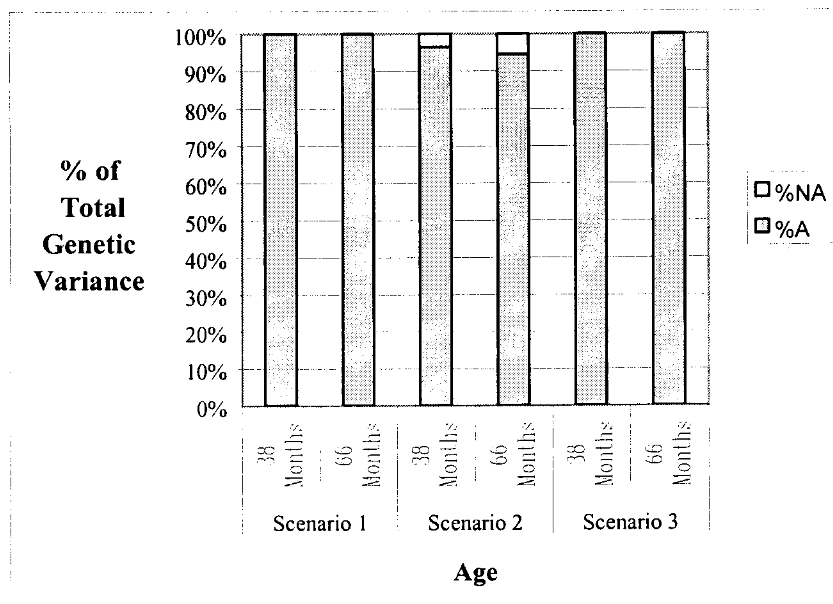

Figure 4. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance in height at age 38 and 66 months for each of the three scenarios considered.

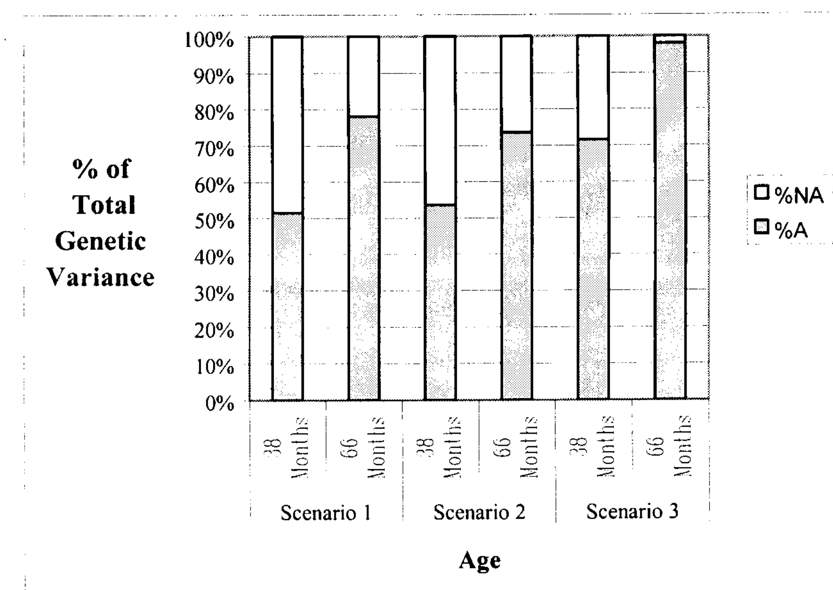

Figure 5. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance in DBH at age 38 and 66 months for each of the three scenarios considered.

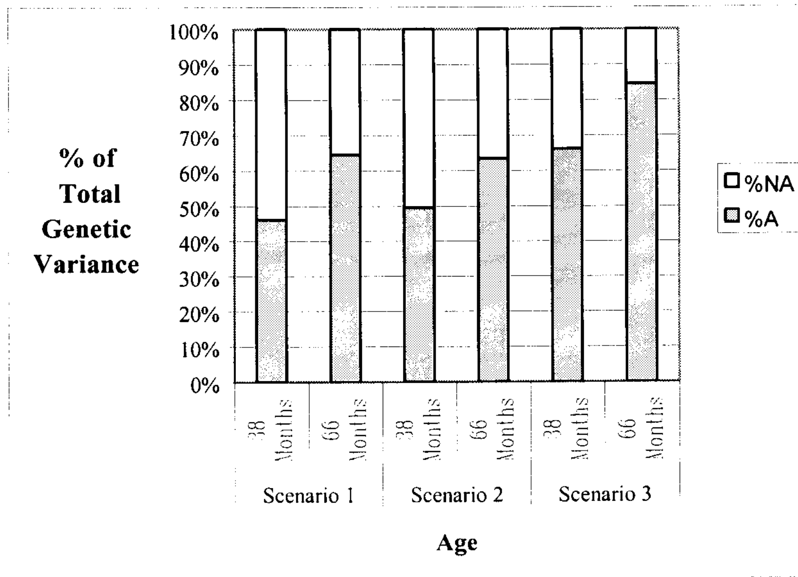

Figure 6. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance in stem form at age 38 and 66 months for each of the three scenarios considered.

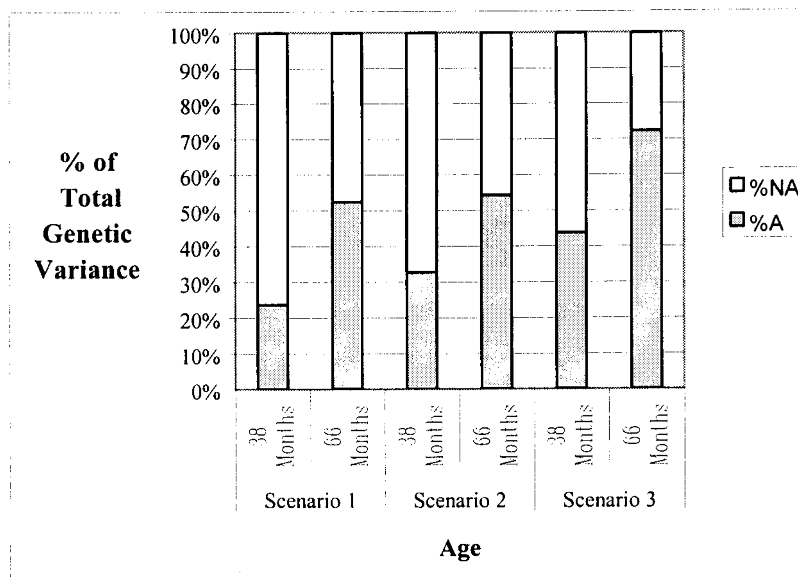

Figure 7. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance in disease tolerance at age 38 and 66 months for each of the three scenarios considered.

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.

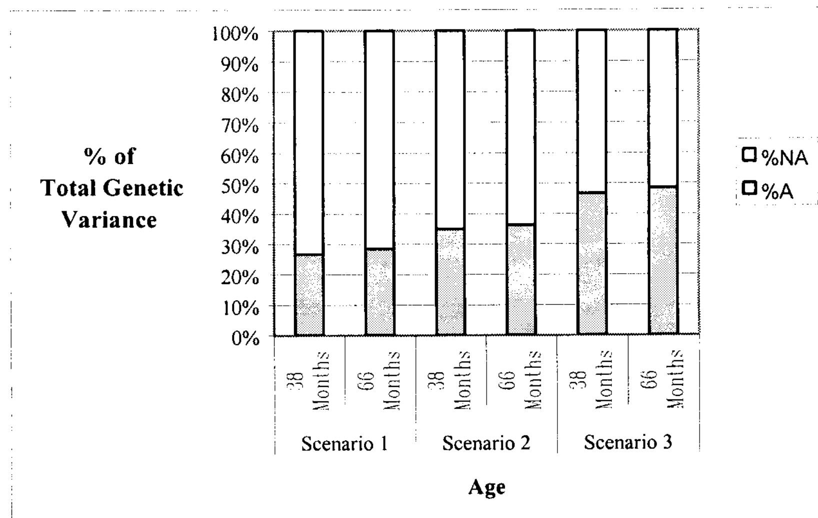

Figure 8. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance in tolerance to *Coniothyrium* at age 38 and 66 months for each of the three scenarios considered.

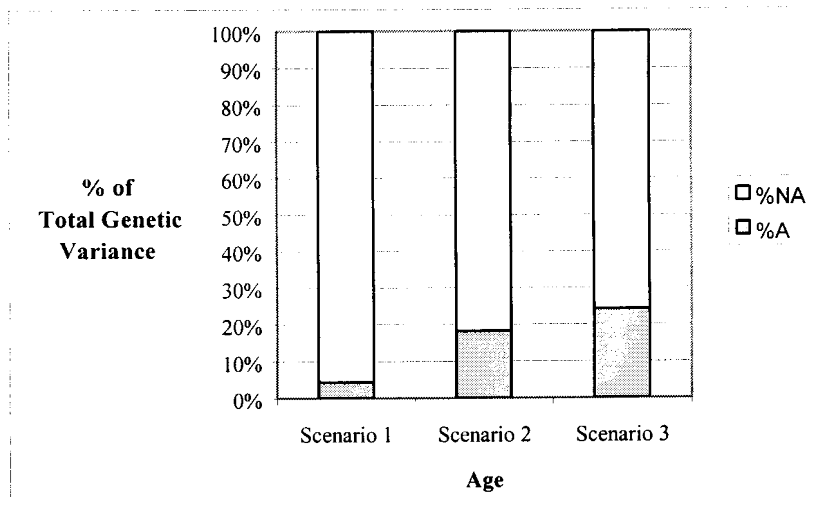

Figure 9. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance in tolerance to *Endothia* at age 66 months for each of the three scenarios considered.

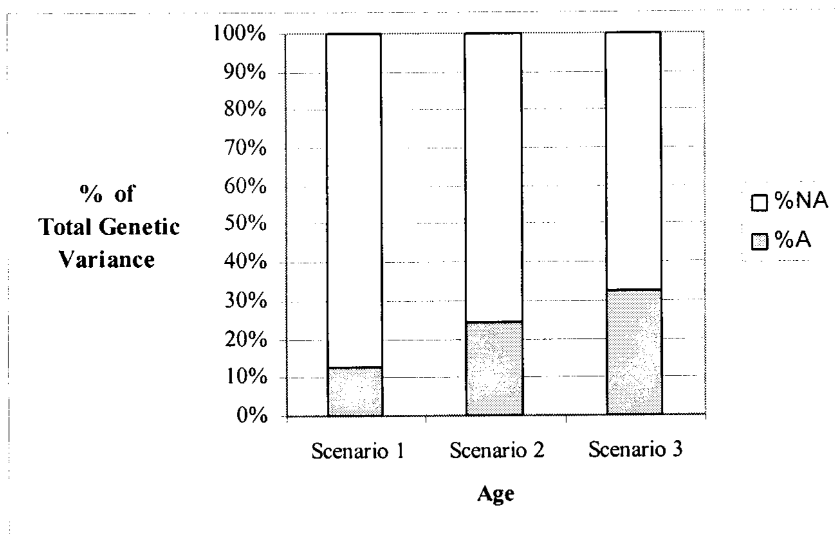

Figure 10. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance in tolerance to *Botryosphaeria* at age 38 months for each of the three scenarios considered.

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 score	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.)

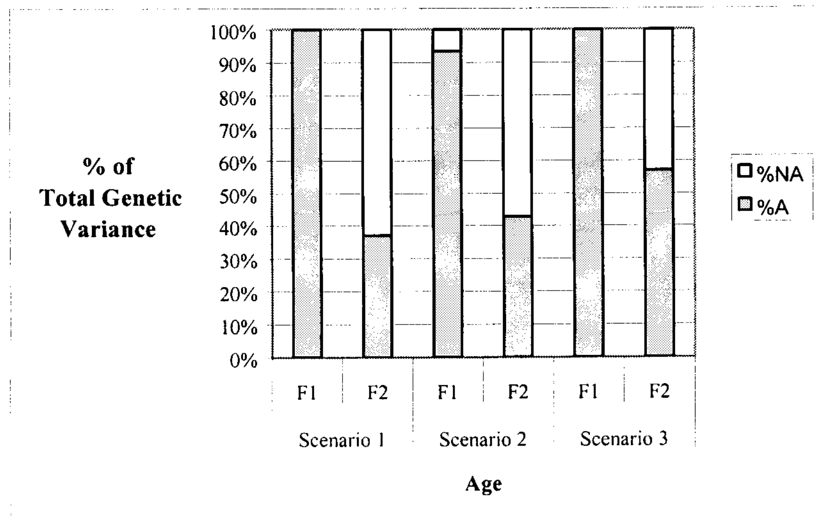

Figure 11. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance for volume at 66 months over generations (F1 and F2) for the three scenarios considered.

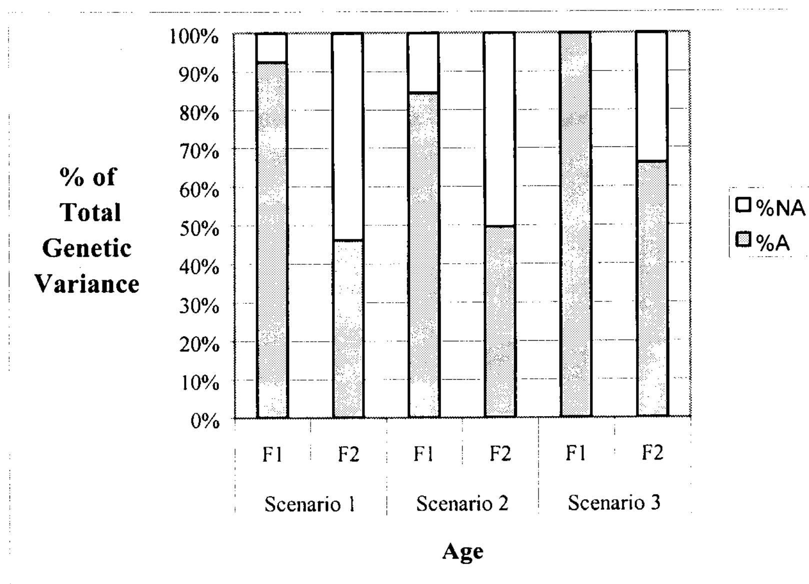

Figure 12. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance for height at 66 months over generations (F1 and F2) for the three scenarios considered.

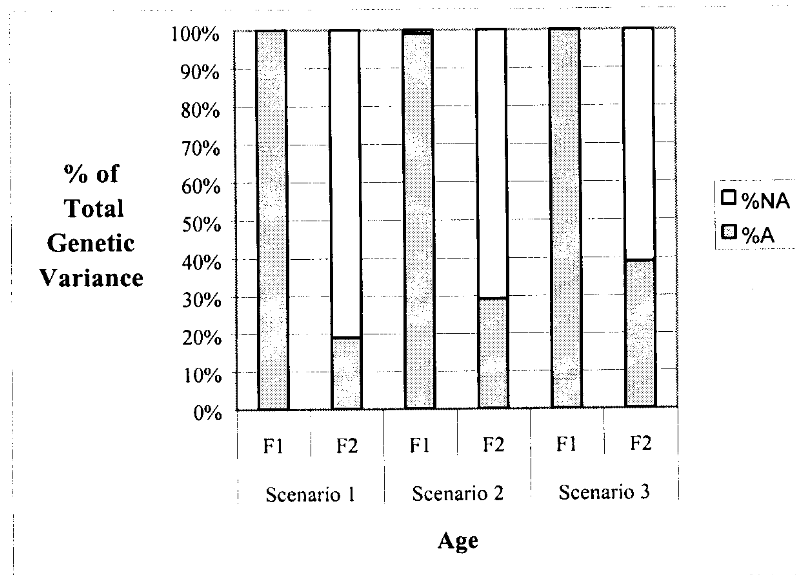

Figure 13. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance for DBH at 66 months over generations (F1 and F2) for the three scenarios considered.

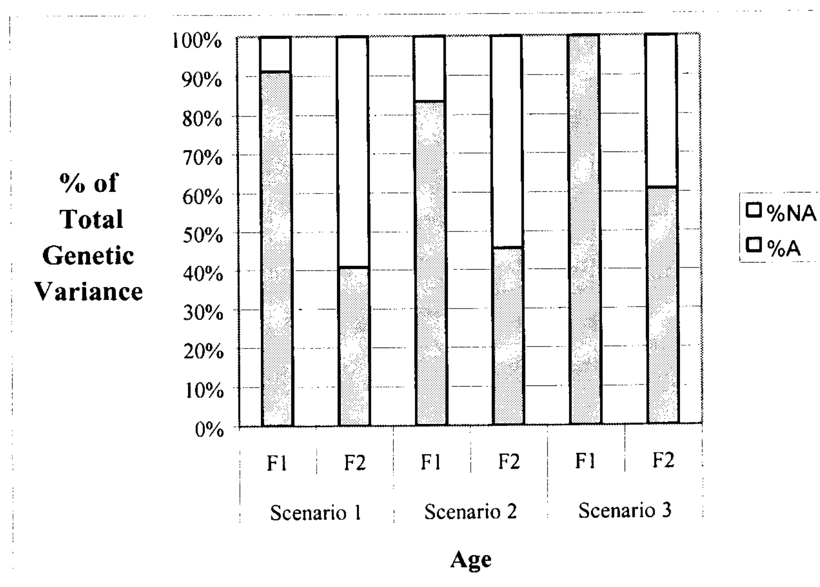

Figure 14. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance for stem form at 66 months over generations (F1 and F2) for the three scenarios considered.

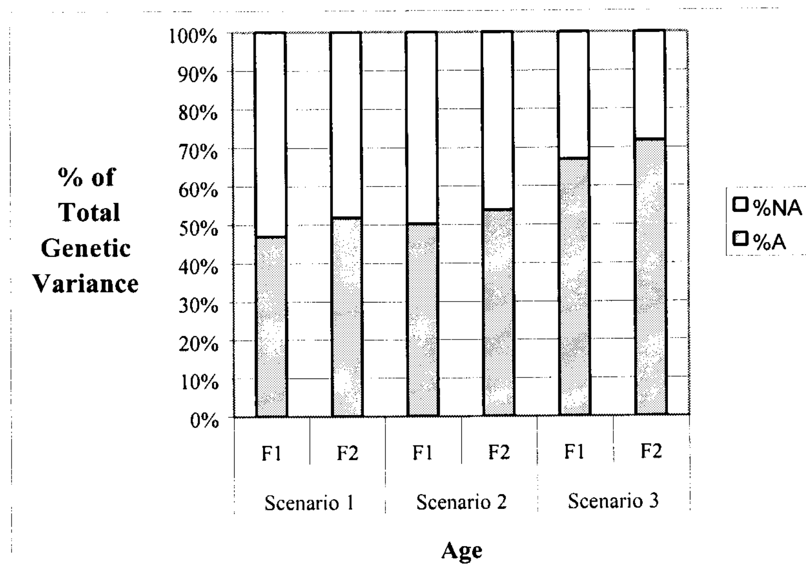

Figure 15. Estimated additive (A) and non-additive (NA) variances as a percentage of total genetic variance for disease tolerance at 66 months over generations (F1 and F2) for the three scenarios considered.

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.