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## Theme: Diversified Species

**Task No: F30105**
**Milestone Number: 1.05.33**

**Report No. FFR- DS062**

# Genetic Parameters, Breeding Values and Gain for Growth, Form and Density from *Eucalyptus nitens* Progeny Test at FR491 Keens Block – Southland Plantation Forests Ltd

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Date: June 2013

# TABLE OF CONTENTS





# EXECUTIVE SUMMARY

*Eucalyptus nitens* plantations totalling 12,000 ha and growing at above 20 $m^3$/ha/year mean annual increment (MAI) are grown on short rotations in Southland. These plantations are harvested to produce export woodchips for kraft pulping. The firm involved seeks to increase the profitability of the enterprise by creating a breeding population composed of individuals that will increase the amount of kraft pulp produced per hectare. This is a function of three traits: growth rate, wood basic density and pulp yield of each tree.

The potential of *E. Nitens* as a source of pulp wood in New Zealand has given the impetus to several cycles of breeding, starting with the testing of native provenances, Australian seed orchard material and selections from provenance/progeny trials. The resulting selected genotypes have been maintained in clonal archives that are also managed as seed production areas. The current breeding programme aims to

(i) confirm the breeding value of selections in the archive, and
(ii) confirm the breeding value of forwards selections from a trial of these offspring.

This interim report details the results of the age six year measurement of the FR491 *Eucalyptus nitens* progeny test at Keens Block in Southland. Selections from this trial will be used to form part of the next generation of *E. nitens* to be tested in New Zealand, while providing further information on the genetic value of the parents.

Four *E. Nitens* seed orchards in New Zealand supply the current seed requirements of *E. nitens* growers. The current breeding strategy requires progeny testing of selections made within these orchards. The present progeny test was established in 2007, and 3600 trees were measured for diameter at breast height (DBH), straightness (STR),stem malformation (MAL), acceptability (ACC) and basic density (DEN) at age six. Height was derived from a regression equation on DBH, but really is the same trait as DBH, i.e. transformed. Volume was derived using a conical volume equation. Basic density was measured from increment cores that were collected at the time growth and form traits were assessed. Genetic parameters and breeding values were estimated for all traits. Several selection scenarios were explored to estimate genetic gain.

There were significant differences among seed orchard sources for all form traits and density, but not for growth traits. Waiouru sources tended to be superior for form traits, while Tinkers was best for density. The ATSC control generally performed poorly in this trial relative to the other orchard material. The fixed effect of Set was significant only for density, but was left in the model for all traits. Perhaps this significance was merely by chance, as Set was not significant for any other trait.

The analysis demonstrated exploitable genetic variation in all traits, although the variation was lower than expected. Narrow-sense individual-tree heritability estimates were low for all traits, but was greatest for density (0.23 ± 0.05), followed by straightness (0.11 ± 0.02), DBH/height (0.10 ± 0.02), volume (0.09± 0.03), acceptability (0.07 ± 0.02) and malformation (0.06 ± 0.02). Moderate to high positive significant genetic correlations were observed between DBH and acceptability, straightness and malformation, straightness and acceptability, and malformation and acceptability. Correlations between traits involving density were generally low and non-significant.

With the completion of this round of testing, FFR members with seed orchards will be able to use data from this trial to make decisions about which parents to remove from the orchard to increase the genetic gain of future seed collections. Maximum genetic gains could be achieved by deploying only the top 5% of families, giving increases in stem acceptability (34.7%), followed by volume (19.2%), malformation (13.4%), straightness (10.8%), DBH (8.3%), density (5.3%) and height (4.8%) , but the question of how much genetic diversity to retain (increasing diversity reduces gain) would be up to the individual companies to decide.

Selection of new genotypes for the next generation of breeding is likely to favour retaining diversity, and limit the number of selections to one or two from the top 34 families. Under this scenario gains are predicted for stem acceptability (22.3%), followed by volume (15%), malformation (6.2%), straightness (6.9%), DBH (5.9%), density (3.9%) and height (3.4%)

Grafting of new genotypes for inclusion in archives and seed orchards could begin as early as spring 2013, subject to funding.

# INTRODUCTION

This project sits in the eucalypt section of Diversified Species Theme in Future Forests Research (FFR). The project aims to identify growth, form and density selections that will contribute to the selection of the next generation of *E. nitens* in New Zealand.

The initial breeding strategy for New Zealand *E. nitens*[1] established an open-pollinated, breeding population with 305 families divided into 10 sublines. The first meeting of the technical steering committee of FFR (2008) voted that:

- the status of *E. nitens breeding* trials, selections, orchards and archives in New Zealand be documented, and
- a draft formal breeding plan[2] for the species be produced.

This breeding plan provides direction, goals and a measure of progress for future genetic improvement. Current goals of the programme are to:

- maintain long-term viability of the breeding population (using parental reconstruction),
- select families with elevated pulp production, and
- identify selections for use in improved commercial seed production (including health traits).

*Eucalyptus nitens* plantations totalling 12,000 ha are grown in southern New Zealand for pulpwood export. At present, the genetic material deployed in these stands is open-pollinated (OP) seed collected from second generation seed orchards located in the central and southern South Island of New Zealand. These include the Tinkers, Waikuku, Alexandra and Drumfern sites. Forestry Tasmania also operates *E. nitens* seed orchards in Tasmania. OP seeds collected from outstanding individuals in all of these orchards were established in new progeny tests in southern New Zealand in 2005. A second phase established progeny from 115 selections from Tinkers and Waiouru orchards in 2007 and provides the basis for this report. The purpose of these tests was to confirm the genetic worth of the parents in the seed orchards as a basis for their retention in the breeding population (backwards selection) and to provide selections from within the new trials (forwards selection).

The owner of the land on which the trial grows is the major grower of *E. nitens* in New Zealand, and also owns and operates pulping operations offshore. Harvested trees are chipped at the dockside and exported to kraft pulping facilities. This business structure is vertically integrated from the growing of trees through to their processing to pulp. Traits relevant to this owner's objective are volume, wood density and pulp yield. Each of these traits has a significant impact on the profitability of a breeding programme that aims to maximise profit per hectare of land[3].

This interim report details the results of the age 6 measurements (growth, form, density) of the FR491 *Eucalyptus nitens* progeny test at Keens Block. Selections from this trial will be used to form part of the next generation of *E. nitens* selections to be tested in New Zealand, while providing further information of the genetic value of the parents. Genetic parameters and breeding values were estimated for all traits. Several selection scenarios were explored to provide general recommendations for advancing the *Eucalyptus nitens* breeding population in New Zealand.

# METHODS

## Genetic Material

A total of 115 outstanding mother trees were selected from two second-generation New Zealand seed orchards, from which OP seed was collected (Table 1). This New Zealand material could be traced back to the original Australian collection regions in central-east Victoria, Australia (Appendix 1). Additionally, one Australian Tree Seed Centre (ATSC) bulk collection from OP parent trees (Blue Range provenance) was nominated as the control. The 25 selections from Tinkers were also tested in FR481-Fortification Block progeny trial.

**Table 1. Seed orchard origin of 115 mother trees used in FR491**

| ATSC (1) | Tinkers (25) | Waiouru (90) | | | |
|---|---|---|---|---|---|
| 18075 | 896_408 | 898_004 | 898_037 | 898_072 | 898_105 |
| | 896_423 | 898_005 | 898_038 | 898_075 | 898_108 |
| | 897_101 | 898_006 | 898_039 | 898_076 | 898_109 |
| | 897_109 | 898_007 | 898_040 | 898_077 | 898_110 |
| | 897_110 | 898_008 | 898_041 | 898_078 | 898_111 |
| | 897_119 | 898_009 | 898_042 | 898_079 | 898_112 |
| | 897_129 | 898_010 | 898_046 | 898_080 | 898_113 |
| | 897_134 | 898_011 | 898_047 | 898_081 | 898_114 |
| | 897_135 | 898_012 | 898_049 | 898_082 | 898_115 |
| | 897_141 | 898_013 | 898_050 | 898_083 | 898_116 |
| | 897_142 | 898_014 | 898_051 | 898_084 | 898_117 |
| | 897_143 | 898_016 | 898_052 | 898_086 | 898_118 |
| | 897_144 | 898_017 | 898_053 | 898_087 | 898_119 |
| | 897_145 | 898_018 | 898_054 | 898_088 | 898_120 |
| | 897_148 | 898_021 | 898_055 | 898_089 | 898_122 |
| | 897_153 | 898_022 | 898_057 | 898_091 | |
| | 897_155 | 898_024 | 898_060 | 898_092 | |
| | 897_156 | 898_028 | 898_063 | 898_093 | |
| | 897_161 | 898_029 | 898_064 | 898_094 | |
| | 897_163 | 898_030 | 898_065 | 898_095 | |
| | 897_164 | 898_031 | 898_066 | 898_096 | |
| | 897_168 | 898_033 | 898_067 | 898_098 | |
| | 897_169 | 898_034 | 898_068 | 898_100 | |
| | 897_173 | 898_035 | 898_070 | 898_101 | |
| | 897_177 | 898_036 | 898_071 | 898_104 | |

## Trial Design

The trial comprised 30 replicates, each made up of three sets-within-replicates, of 40 planting spaces (Figure 1). The sets were used to simplify trial preparation, while equal numbers of families from each seed orchard were allocated to each set within replicate to minimise the likelihood of a set effect. The trial was raised and planted out according to McConnochie[4, 5] (2005, 2006); location was Keens Block – Southlands Plantation Forest Ltd (46° 0'39.72"S 167°58'8.41"E). Following a previous rotation, slash was windrowed in north-south lines, approximately 20 m apart. Containerised *E.nitens* planting stock (Figure 1) were planted in August 2007 at 3.0 x 2.8 metre spacing. Each replicate spanned two windrows, with one set planted in six rows across one windrow. A line of edge trees around the entire trial was planted later, but not consistently at 2.8 metres between the trial trees; nor did they consistently establish with the same vigour as the trial trees.

**Figure 1. FR491 *Eucalyptus nitens* progeny trial at Southlands one year after planting.**

## Trial Measurements

A total of 3600 trees were measured for diameter at breast height (DBH, mm), stem straightness (STR; on a scale of 1 worst to 9 best; Figure 2), stem malformation (MAL; Figure 3), and acceptability (ACC; 0 is not acceptable, 1 is acceptable, 2 is very acceptable) at age six (Figure 4). Height (HGT; m) was derived by measuring a select number of trees to form regression equations based on DBH (Figure 5); and volume (VOL) was calculated based on DBH (m) and derived heights using a conical volume equation:

$$VOL = \frac{(DBH^2) \times HGT \times \pi}{4}$$, where DBH is converted to metres and VOL is in cubic metres.

Cambium-to-cambium wood cores 6 mm diameter were obtained from all trees of >6 cm DBH in replicates 15-29, and then frozen (Figure 6). Density of wood cores was then measured in the laboratory.

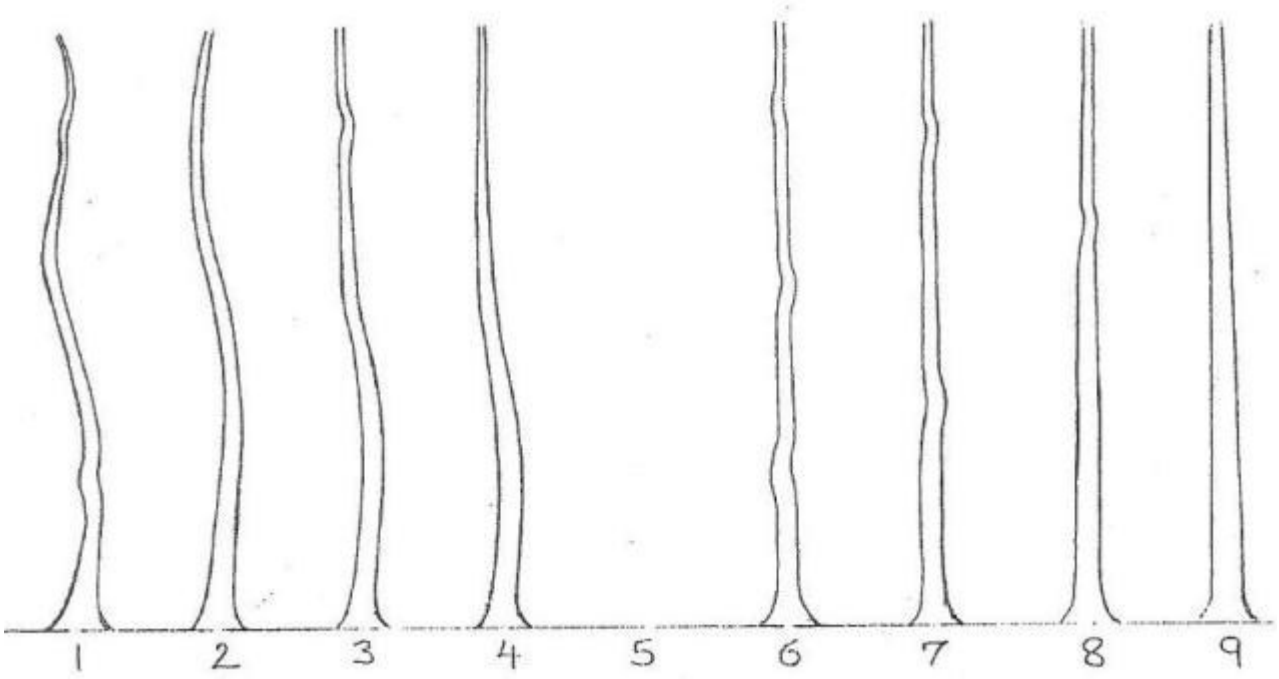



**Figure 2. Stem straightness score for *Pinus radiata* stem quality[6] as applied in the 2011 Fortification Road *E. nitens* assessment. Class 5 is not scored to avoid central bias.**

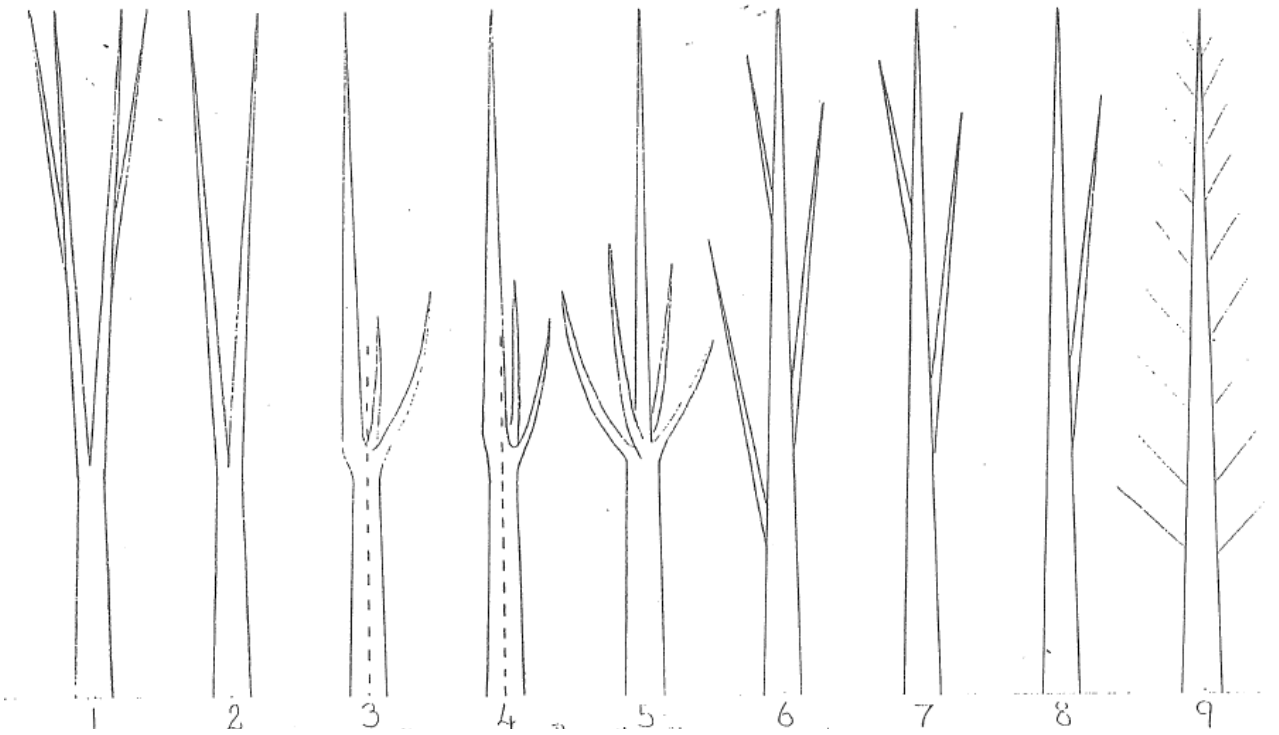



**Figure 3. Malformation score for *Pinus radiata* stem quality (NZRPBC 1997) as applied in the 2011 Fortification Road *E. nitens* assessment.**

**Figure 4. Straightness, malformation and acceptability being assessed at FR491 *Eucalyptus nitens* progeny trial by M. Miller.**

## Statistical Analyses

See Appendix 2.

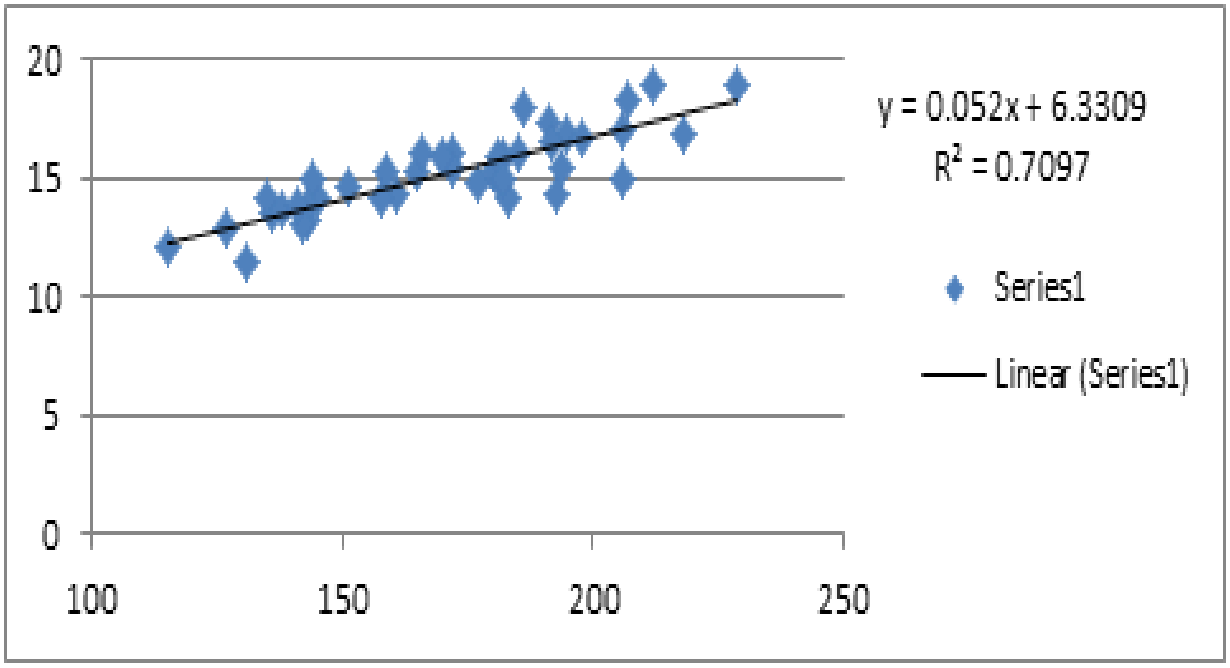



**Figure 5. Regression equation relating DBH (x-axis) to HGT (y-axis) used to derive heights for population at FR491.**

**Figure 6. Cores being collected from FR491 Keens Block Southland**

## Selection andGenetic Gain

The FFR *E. nitens* breeding population is to be managed as a rolling front cycle, whereby forwards and backwards selections from progeny tests are made as each progeny test matures. The breeding values for all individuals and parents (= family) for diameter, height, volume, straightness, malformation, acceptability and density were predicted. A couple of selection scenarios were explored in order to estimate the genetic gain.

First, a backwards selection scenario was simulated in which families were sorted from highest to lowest based on the EBVs for each trait. The genetic gain (%) compared with the population mean was estimated from direct selection of each trait. Gains were calculated for selection levels at 5% (top 6 families), 10% (top 12 families), 15% (top 17 families), 20% (top 23 families), and 25% (top 29 families).

Second, a forwards selection scenario was simulated in which all progeny being tested were sorted from highest to lowest based on the EBVs for each trait. The genetic gain (%) compared with the predicted population mean was estimated from selection of the top 1-10% of the population with no regard to relatedness. These selections could provide the next generation of genotypes for breeding and testing.

Third, another forwards selection approach explored within-family selection to provide some control over genetic diversity in the next generation breeding population. The genetic gain associated with selecting the single best and two best progeny in the 34 highest ranking families (top 30% of families) was estimated for each trait. Gains from this approach could be compared with gains from 1% and 2% selection from the second approach above where relatedness was not considered.

In all cases of predicting genetic gain,

$$\text{Gain} = \frac{\bar{x}_{BV\_SEL}}{\bar{x}_{Pred}} \times 100\%$$

where $\bar{x}_{BV\_SEL}$ is the mean predicted breeding value of selected population and

$\bar{x}_{Pred}$ is the predicted mean using fixed-effect solutions from the model and

$= \mu + \frac{\sum \text{Orchard}_i}{m} + \frac{\sum \text{Set}_j}{n}$, where m and n are the number of Orchard sources (3) and Sets (3), respectively.

# RESULTS AND DISCUSSION

## Trial Statistics

At age six years, the FR491 progeny trial had an overall phenotypic mean for:

- DBH 165.54 mm,
- height 14.94 m,
- volume 0.34 $m^3$,
- straightness 7.11,
- malformation of 7.24,
- acceptability of 0.67 and
- density of 487.09 kg $m^{-3}$ (
- Table 2).
- Mean survival was 94.5% (data not shown).

## Significance of Seed Orchard and Set

Seed orchard source was not statistically significant for growth, but was significant for density and form traits (

Table 2). Waiouru seed orchard source material had significantly better straightness, malformation and acceptability scores than either Tinkers or the ATSC control (Table 2). Tinkers seed orchard source material had a significantly better malformation score than the ATSC control, and although not significant, Tinkers orchard material had lower mean straightness and acceptability than the ATSC control. For density, all comparisons between the three orchards were significantly different with Tinkers >Waiouru> ATSC (Table 2). The results for density are interesting in that Tinkers was also the best source for density in FR481 progeny trial. The fixed effect of Set was significant only for density (Table 3), Set B having greater density than the other two sets. This may be due just to chance as all other traits had non-significant Set effects.

**Table 2. Trial statistics for Seed Orchard Source: Means followed by different letters within a column are statistically different ($p < 0.05$) based on pairwise-comparisons of means (t-statistics). A significant Wald F-stat or (p-value) suggests that seed orchard source is a significant source of variation for that trait.**

a) DBH (mm)

| Source | Mean | StDev | Minimum | Maximum | n |
|---|---|---|---|---|---|
| **Tinkers** | 166.73a | 30.48 | 41.0 | 274.0 | 704 |
| **Waiouru** | 165.33a | 27.92 | 39.0 | 242.0 | 2529 |
| **ATSC** | 163.70a | 27.79 | 87.0 | 231.0 | 168 |
| **OVERALL** | 165.54 | 28.46 | 39.0 | 274.0 | 3401 |
| **Wald F-Stat (p-value)** | 0.62 (0.541) | | | | |

b) Height (m)

| Source | Mean | StDev | Minimum | Maximum | n |
|---|---|---|---|---|---|
| **Tinkers** | 15.00a | 1.58 | 8.46 | 20.58 | 704 |
| **Waiouru** | 14.93a | 1.45 | 8.36 | 18.91 | 2529 |
| **ATSC** | 14.84a | 1.44 | 10.85 | 18.34 | 168 |

| | | | | | |
|---|---|---|---|---|---|
| **OVERALL** | 14.94 | 1.48 | 8.36 | 20.58 | 3401 |
| **Wald F-Stat (p-value)** | 0.62 (0.541) | | | | |

c) Volume ($m^3$)

| Source | Mean | StDev | Minimum | Maximum | n |
|---|---|---|---|---|---|
| **Tinkers** | 0.3504 | 0.1507 | 0.0111 | 1.2134 | 704 |
| **Waiouru** | 0.3397 | 0.1357 | 0.0100 | 0.8700 | 2529 |
| **ATSC** | 0.3315 | 0.1386 | 0.0645 | 0.7687 | 168 |
| **OVERALL** | 0.3415 | 0.1391 | 0.0100 | 1.2134 | 3401 |
| **Wald F-Stat (p-value)** | 1.21 (0.301) | | | | |

d) Straightness (1-9 with no 5)

| Source | Mean | StDev | Minimum | Maximum | n |
|---|---|---|---|---|---|
| **Waiouru** | 7.19a | 1.33 | 1 | 9 | 2529 |
| **Tinkers** | 6.98b | 1.37 | 2 | 9 | 704 |
| **ATSC** | 6.55c | 1.67 | 2 | 9 | 168 |
| **OVERALL** | 7.11 | 1.36 | 1 | 9 | 3401 |
| **Wald F-Stat (p-value)** | 20.46 (< 0.001) | | | | |

e) Malformation (1-9)

| Source | Mean | StDev | Minimum | Maximum | n |
|---|---|---|---|---|---|
| **Waiouru** | 7.36a | 2.55 | 1 | 9 | 2529 |
| **ATSC** | 7.17ab | 2.66 | 1 | 9 | 168 |
| **Tinkers** | 6.81b | 2.83 | 1 | 9 | 704 |
| **OVERALL** | 7.24 | 2.62 | 1 | 9 | 3401 |
| **Wald F-Stat (p-value)** | 7.34 (< 0.001) | | | | |

f) Aceptability (0-2)

| Source | Mean | StDev | Minimum | Maximum | n |
|---|---|---|---|---|---|
| **Waiouru** | 0.70a | 0.51 | 0 | 2 | 2529 |
| **Tinkers** | 0.62b | 0.53 | 0 | 2 | 704 |
| **ATSC** | 0.56b | 0.52 | 0 | 2 | 167 |
| **OVERALL** | 0.67 | 0.52 | 0 | 2 | 3400 |
| **Wald F-Stat (p-value)** | 8.23 (< 0.001) | | | | |

g) Density (kg $m^{-3}$)

| Source | Mean | StDev | Minimum | Maximum | n |
|---|---|---|---|---|---|
| **Tinkers** | 497.91a | 28.42 | 428.0 | 600.0 | 222 |
| **Waiouru** | 486.09b | 30.20 | 375.0 | 593.0 | 841 |
| **ATSC** | 454.59c | 26.30 | 406.0 | 537.0 | 49 |
| **OVERALL** | 487.09 | 30.84 | 375.0 | 600.0 | 1112 |
| **Wald F-Stat (p-value)** | 49.93 (< 0.0001) | | | | |

**Table 3. Trial statistics for Set for density (kg $m^{-3}$): Means followed by different letters are statistically different (p < 0.05) based on pairwise-comparisons of means (t-statistics). Note: Set was not statistically significant for all other traits but left in model.**

| Set | Mean | StDev | Minimum | Maximum | n |
|---|---|---|---|---|---|
| **B** | 494.04a | 31.30 | 375.0 | 600.0 | 407 |
| **C** | 484.52b | 29.39 | 406.0 | 582.0 | 358 |
| **A** | 481.50b | 30.30 | 416.0 | 571.0 | 347 |
| **Wald F-Stat (p-value)** | 7.17 (< 0.001) | | | | |

## Variance Components, Heritability and Genetic Correlations

The observed variances associated with replicates ($\widehat{\sigma}^2_{\mathrm{REP}}$) and individual trees ($\widehat{\sigma}^2_{\mathrm{TREE}}$) were always significant (p < 0.001). Narrow-sense individual-tree heritability estimates were low for all traits, but were greatest for density (0.23 ± 0.05), followed by straightness (0.11 ± 0.02), DBH/height (0.10 ± 0.02), volume (0.09± 0.03), acceptability (0.07 ± 0.02) and malformation (0.06 ± 0.02) (Table 4).

Heritability estimates for the majority of these traits were similar to those estimated at the Fortification Block trial (FR481) and typical for those of *E. nitens* from other trials[7]. However, $\widehat{\mathrm{h}}^2$ for density in this trial was half the estimate for density at FR481 (0.48).

Family mean repeatability followed a similar trend with $\hat{h}^2_{HS}$ranging from 0.42 for malformation to 0.56 for straightness (Table 4). Coefficient of additive genetic variation ($CV_{\hat{A}}$) followed a slightly different trend (Table 4). $CV_{\hat{A}}$ was greatest for malformation, acceptability, straightness, and volume, while $CV_{\hat{A}}$ for DBH, height and density were lower (Table 4). Caution is necessary in that $CV_{\hat{A}}$ for volume was estimated at the individual-tree level, and probably differs from$CV_{\hat{A}}$ at the per-hectare crop level[8]. Further, both height and volume are derived measurements based on actual DBH. Therefore the validity of height and volume data in this trial may be suspect.

In fact, DBH, height and volume are really the same trait. That is, height and volume are transformed DBH measurements. As a result bivariate models to estimate the covariance among these three traits failed to run. Pearson genetic correlations between DBH and height breeding values, and between DBH and volume breeding values were 1 and 0.98 respectively, again indicating that these are the same traits. Perhaps a better approach to estimate volume of trees would be to use actual height measurements for each tree, or from half the replicates.

The only statistically significant genetic correlations were between DBH and acceptability (0.67), straightness and malformation (0.51), straightness and acceptability (0.76), and between malformation and acceptability (0.74) (Table 5).Genetic correlations between growth or form traits with density were relatively low but not significantly different from zero (

Table 5). Correlations near zero indicate that traits are independent of one another.

**Table 4. Estimates of observed variance components (estimate ± approximate standard error, SE); causal variances ($\hat{V}_A$ and $\hat{V}_P$), narrow-sense heritability ($\hat{h}^2$ ), family mean heritability ($\hat{h}^2_{HS}$), and coefficient of additive genetic variation ($CV_{\hat{A}}$). For all traits, $\hat{\sigma}^2_{REP}$ and $\hat{\sigma}^2_{TREE}$ were statistically significant (p < 0.001).**

| Estimate | DBH | Height | Volume | Straightness | Malformation | Acceptability | Density |
|---|---|---|---|---|---|---|---|
| $\hat{\sigma}^2_{REP}$ | 89.31 ± 25.1 | 0.242 ± 0.068 | 0.0022 ± 0.0006 | 0.079 ± 0.025 | 0.093 ± 0.040 | 0.003 ± 0.001 | 27.12 ± 14.49 |
| $\hat{\sigma}^2_{TREE}$ | 112.48 ± 26.8 | 0.304 ± 0.072 | 0.0024 ± 0.0006 | 0.301 ± 0.058 | 0.681 ± 0.210 | 0.029 ± 0.008 | 309.76 ± 69.78 |
| $\hat{\sigma}^2_{ERR}$ | 616.99 ± 26.5 | 1.668 ± 0.072 | 0.0149 ± 0.0006 | 1.458 ± 0.059 | 6.092 ± 0.231 | 0.238 ± 0.009 | 533.64 ± 61.04 |
| $\hat{V}_A$ | 70.30 ± 16.7 | 0.190 ± 0.045 | 0.0015 ± 0.0004 | 0.188 ± 0.036 | 0.426 ± 0.132 | 0.018 ± 0.005 | 193.60 ± 43.62 |
| $\hat{V}_P$ | 729.47 ± 18.4 | 1.973 ± 0.050 | 0.0173 ± 0.0004 | 1.760 ± 0.044 | 6.773 ± 0.168 | 0.266 ± 0.007 | 843.39 ± 37.98 |
| $\hat{h}^2$ | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.09 ± 0.03 | 0.11 ± 0.02 | 0.06 ± 0.02 | 0.07 ± 0.02 | 0.23 ± 0.05 |
| $\hat{h}^2_{HS}$ | 0.53 ± 0.06 | 0.53 ± 0.06 | 0.50 ± 0.06 | 0.56 ± 0.05 | 0.42 ± 0.08 | 0.44 ± 0.07 | 0.48 ± 0.06 |
| $CV_{\hat{A}}$ | 5.06% | 2.92% | 11.30% | 12.77% | 19.69% | 14.16% | 2.86% |

**Table 5. Estimated additive genetic (upper diagonal) and phenotypic correlations (below diagonal) among DBH, Straightness, Malformation, Acceptability and Density at trial FR491. Significant genetic correlations are in bold. Note: statistical significance of genetic correlations was tested with 2-tail likelihood ratio test (LRT).**

| | DBH | Straightness | Malformation | Acceptability | Density |
|---|---|---|---|---|---|
| **DBH** | | 0.21 | 0.08 | **0.67** | -0.05 |
| **Straightness** | 0.13 | | **0.51** | **0.76** | 0.12 |
| **Malformation** | 0.04 | 0.33 | | **0.74** | 0.08 |
| **Acceptability** | 0.37 | 0.53 | 0.60 | | 0 |
| **Density** | 0.10 | 0.08 | 0.06 | 0.08 | |

## BackwardsSelection and Genetic Gain

The FFR *Eucalyptus nitens* breeding population is to be managed as a rolling front cycle. A selection scenario of backwards selection of parents is useful for making culling decisions in seed orchards, and to confirm the genetic merit of parents in the current breeding programme. The greatest gain over the predicted population mean for a trait occurred where the selection intensity was greatest (i.e. 5%) and steadily declined as the number of families selected increased. In other words there is a trade-off between achieving gain and increasing genetic diversity.

The greatest gain was demonstrated for acceptability (34.7%), followed by volume (19.2%), malformation (13.4%), straightness (10.8%), DBH (8.3%), density (5.3%) and height (4.8%) when selecting 5% of the best families (not including the ATSC bulk seedlot) (Figure 7).

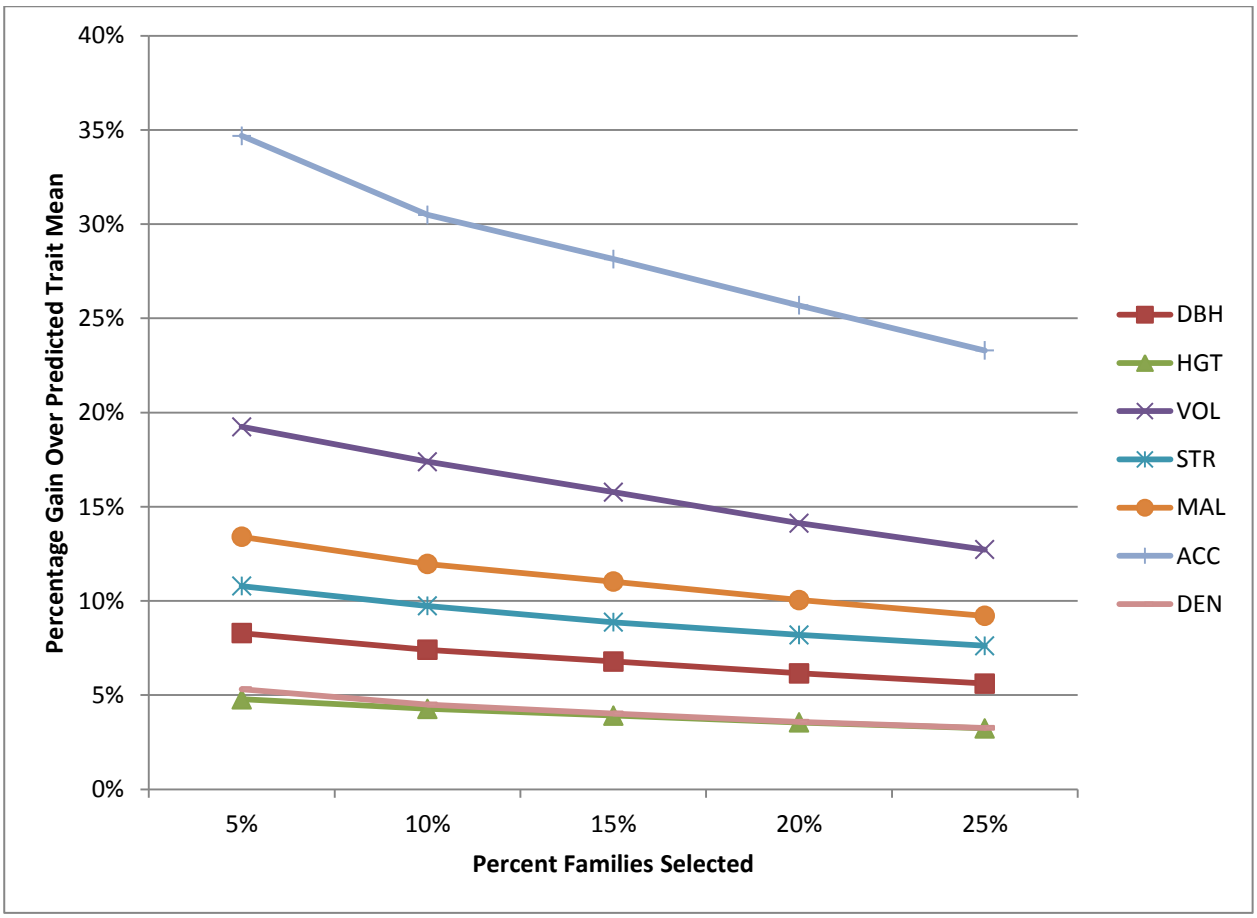



**Figure 7. Relative gain for backwards selection of 5%, 10% 15%, 20%, and 25% of families. Genetic gains for each trait are shown relative to the predicted trait mean.**

## Forwards Selection Scenarios

By considering only offspring tested in FR491, genetic gain was predicted for a forwards selection scenario. Selections for the next generation *E. nitens* breeding population were simulated at varying levels of selection intensity (1-10%) (Table 6). For example, by selecting 1% of the population (34 genotypes), predicted gain over the population mean was: acceptability (30.0%), volume (19.0%), malformation (9.3%), straightness (8.6%), DBH (7.3%), density (5.6%) and height (4.2%). The gain depicted in Table 6 ignores the fact that multiple selections from the same family were selected. For example, sixteen families (out of 115) are represented in the best 1% selected population for DBH. Nevertheless, the superior performance of family 898_120 for growth was demonstrated by having 12 progeny in top 1% of the population for DBH (Figure 8).

**Table 6. Predicted genetic gain (in percentage terms) for forwards selection by selecting top 1-10% of progeny ignoring relatedness of selections.**

| Selection | DBH | Height | Volume | Straightness | Malformation | Acceptability | Density |
|---|---|---|---|---|---|---|---|
| **1%** | 7.27% | 4.19% | 18.97% | 8.55% | 9.25% | 30.04% | 5.64% |
| **2%** | 6.70% | 3.86% | 16.92% | 7.94% | 8.99% | 26.79% | 4.81% |
| **3%** | 6.33% | 3.65% | 15.77% | 7.57% | 8.66% | 25.05% | 4.33% |
| **4%** | 6.06% | 3.49% | 14.96% | 7.31% | 8.37% | 23.80% | 4.03% |
| **5%** | 5.83% | 3.36% | 14.28% | 7.08% | 8.13% | 22.72% | 3.78% |
| **6%** | 5.63% | 3.25% | 13.70% | 6.89% | 7.92% | 21.89% | 3.61% |
| **7%** | 5.46% | 3.15% | 13.19% | 6.73% | 7.73% | 21.21% | 3.46% |
| **8%** | 5.30% | 3.05% | 12.73% | 6.57% | 7.55% | 20.62% | 3.32% |
| **9%** | 5.15% | 2.97% | 12.33% | 6.42% | 7.39% | 20.10% | 3.20% |
| **10%** | 5.02% | 2.89% | 11.99% | 6.29% | 7.24% | 19.66% | 3.09% |

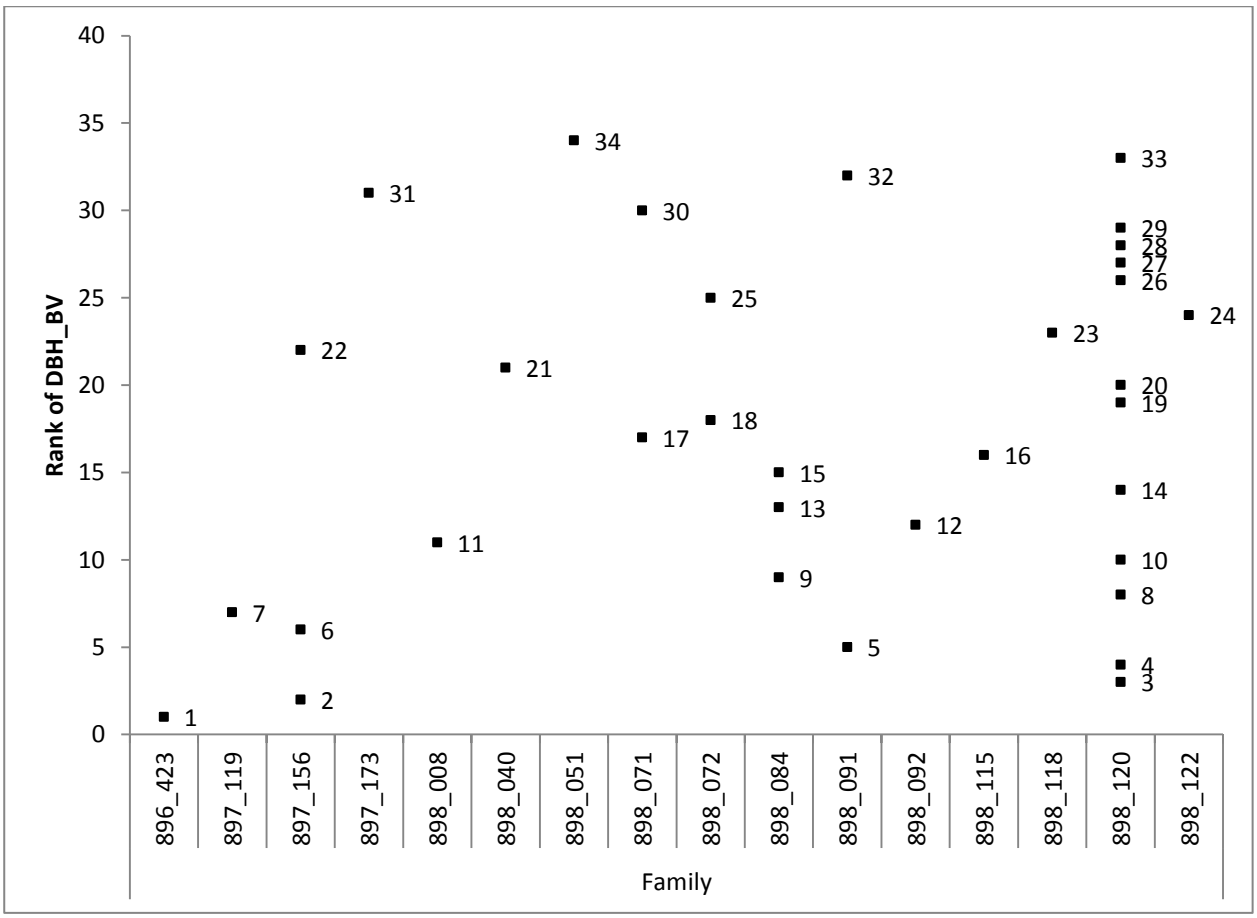



**Figure 8. Rank of selections by family for forwards selection based on selecting the best 1% of progeny for BV_DBH. Number adjacent to marker represents the rank of the selection out of the population. Note: family 898_120 has 12 progeny represented in this top 1%.**

## Within-family Forwards Selection

Genetic gain will be maximised if selection is based on the genotypes with the best breeding values, ignoring relatedness. However, to manage the build-up of inbreeding in future generations, restricting the relatedness of selections will increase genetic diversity of advanced breeding populations. Limiting selection to the single best one or two best progeny from the top 34 performing families allows some control over genetic diversity of the next generation of *E. nitens* breeding population without severely affecting potential genetic gain. For example, 6.3% and 5.9% genetic gain was predicted for DBH by selecting the best and two best progeny, respectively (Figure 9). Comparing this to predicted gains ignoring relatedness (Table 6), there would only be a 1% reduction in gain (7.3-6.3%) when selecting 1% of the population. But the control over relatedness in advanced breeding may off-set this reduction. Similar trends were seen with other traits (Figure 9).

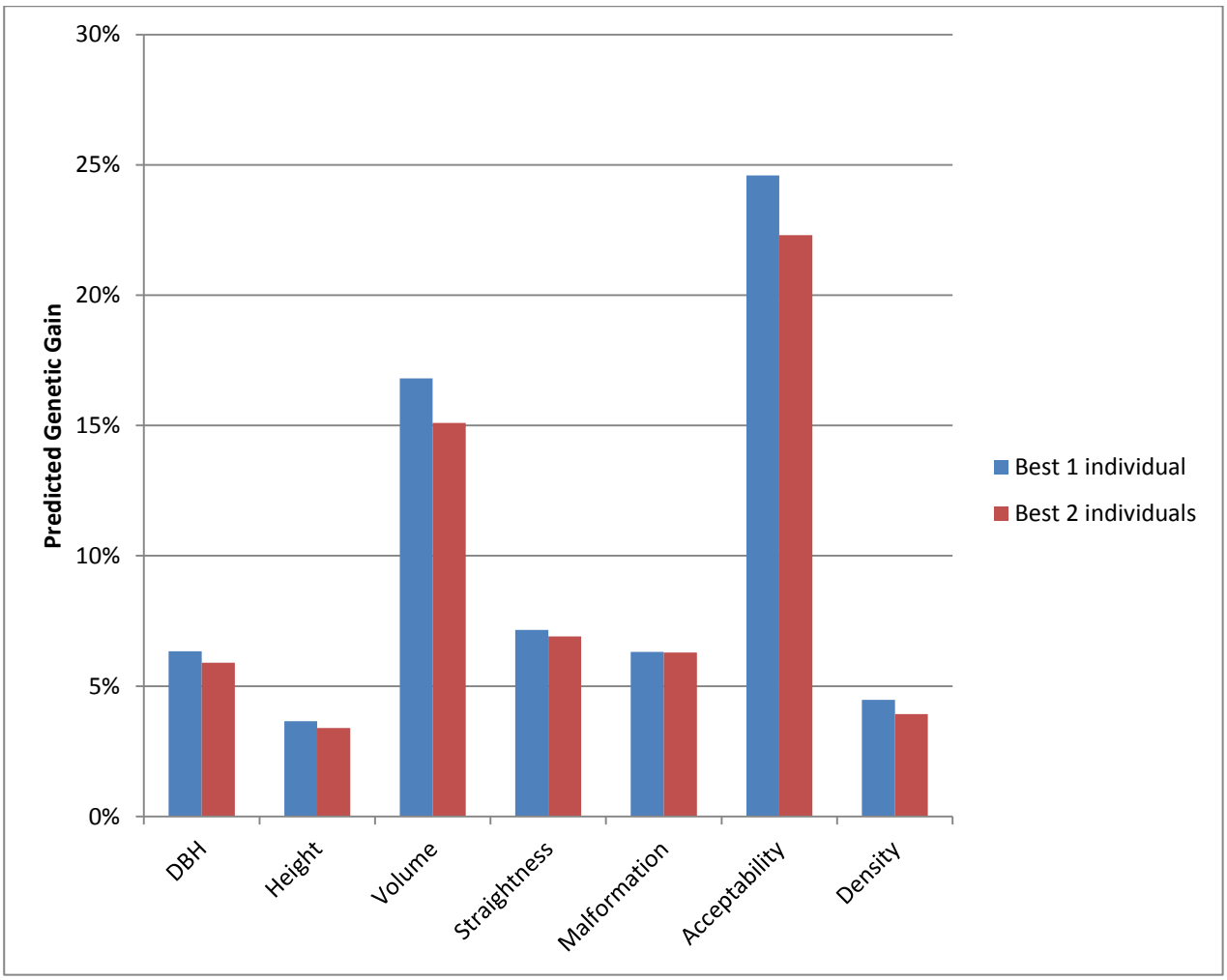



**Figure 9. Within-family forwards selection: predicted genetic gain (in percentage terms) by selecting best one or two progeny from the top 34 families**

# CONCLUSION

There were significant differences among seed orchard sources for all form traits and density, but not for growth traits. Waiouru sources tended to be superior for form traits, while Tinkers was best for density. The ATSC control generally performed poorly in this trial relative to the other orchard material. The fixed effect of Set was significant only for density, but was left in the model for all traits. Perhaps this significance was merely by chance as Set was not significant for any other trait.

The analysis demonstrated exploitable genetic variation in all traits, although this was lower than expected. Narrow-sense individual-tree heritability estimates were low for all traits, but were greatest for density (0.23 ± 0.05), followed by straightness (0.11 ± 0.02), DBH/height (0.10 ± 0.02), volume (0.09± 0.03), acceptability (0.07 ± 0.02) and malformation (0.06 ± 0.02). Moderate to high positive significant genetic correlations were observed between DBH and acceptability, straightness and malformation, straightness and acceptability, and malformation and acceptability. Correlations between traits involving density were generally low and non-significant.

A rolling front selection scheme allows selection of the best performing genotypes regardless of generation based on breeding values. Three selection scenarios were explored: backwards selection, forwards selection, and within-family forwards selection, to provide control over genetic diversity. A selection scenario of backwards selection of parents is useful for making culling decisions in seed orchards and to confirm the genetic merit of parents in the current breeding programme. The greatest gain over the predicted population mean for a trait occurred where the selection intensity was greatest (i.e. 1%), and steadily declined as the number of families selected increased.

The trade-off between maximising genetic gain and maximising genetic diversity was demonstrated. Individual selection with no restriction on relatedness results in a loss of genetic diversity. The alternative of maximising the number of families retained reduces genetic gain but retains genetic diversity. The situations where (i) excessive uncompetitive families are retained in the name of maintaining genetic diversity, or (ii) where excessive diversity is lost through the emphasis on gain, are normally readily detected in operational breeding programs, and an intermediate strategy can be implemented. Forwards selection within the current trial would require pedigree reconstruction to separate maternally or paternally related individuals within the selections[9]. Pedigree reconstruction is feasible for *E. nitens* in New Zealand, as most of the parental DNA lineages are available from previous studies[10]. Pedigree reconstruction would also be required whether propagation is via collection of open-pollinated seed, or in possible scenarios that clones or grafts can be taken from the field material.

The selection scenarios presented in this study are meant to serve as general guidelines to assist in advancing the *Eucalyptus nitens* breeding population in New Zealand. Traits relevant to this owner's objective are volume, wood density and pulp yield. Therefore a selection index involving volume, density and pulp yield would be ideal. This study used derived heights and consequently volume measurements. Caution is needed in that volume measurements may not be accurate. A general recommendation would be to measure heights at least on a sample of replicates, similarly to what was done with density. Additionally, NIR-based measurements of pulp yield are required to develop a kraft pulp index. Before turning over the next generation, the breeding population will also be tested for resistance to browsing by *Paropsis charybdis.* This, as well as the kraft pulp yield index will determine the final selections for turning over the next generation of *E. nitens* in New Zealand.

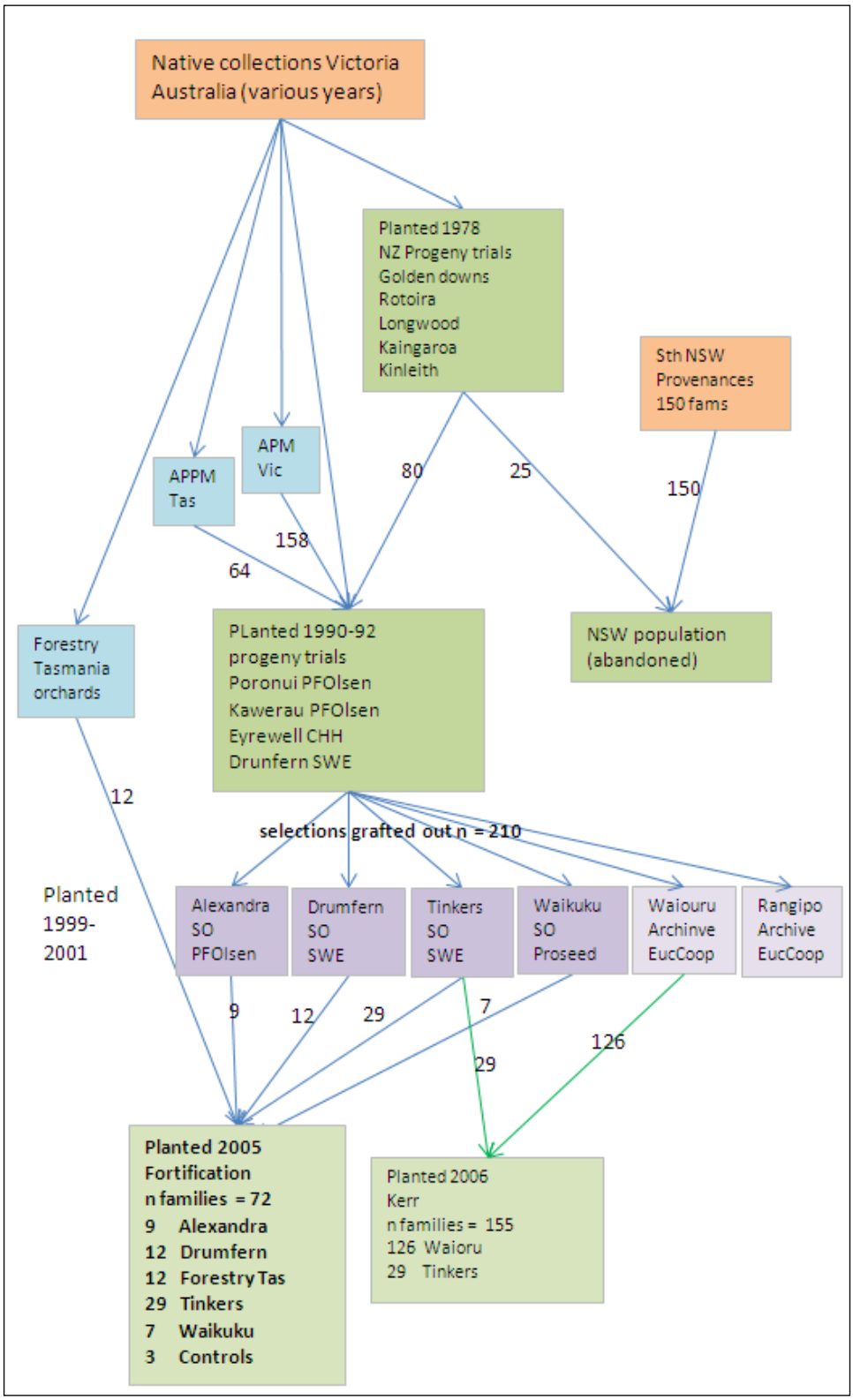
# ACKNOWLEDGEMENTS

We wish to acknowledge Southwood exports for their interest and provision of field sites; also FFR for their ongoing financial support. Ruth McConnochie is acknowledged for setting up the trial, and for maintaining liaison with the landowner.

# APPENDICES

## Appendix 1. Outline of the populations involved in the history of breeding Eucalyptus nitens in New Zealand.

Native Australian sources are shown in Orange; Australian seed orchards (SO) in blue; New Zealand progeny tests in green, New Zealand clonal archives (some used for seed production) in purple. Known numbers of families carried forwards (usually OP) shown on the connecting arrows



## Appendix 2. Statistical Analyses

A series of analyses were conducted in ASReml[11] in order to estimate the variance components and derive the associated genetic parameters and breeding values for DBH, height, volume, straightness, malformation, acceptability and density. First, a univariate mixed-effects individual-tree model was used.

[2] $$\mathbf{y} = \mathbf{Xb} + \mathbf{Z_1 r} + \mathbf{Z_2 a} + \mathbf{e}$$

where, $\mathbf{y}$ is a vector of observations on a trait, $\mathbf{b}$ is the vector of fixed effects (mean, seed orchard, set), $\mathbf{X}$ is the known incidence matrix relating observations in $\mathbf{y}$ to the fixed effects in $\mathbf{b}$,

$\mathbf{r}$ is the vector of random effects of replicate ~N(0, $\hat{\sigma}^2_{\mathrm{REP}}$),

$\mathbf{a}$ is the vector of random effects of individual tree ~N(0, $\hat{\sigma}^2_{\mathrm{TREE}}$) based on the numerator relationship matrix in ASReml,

$\mathbf{e}$ is the vector of random residual terms ~N(0,$\hat{\sigma}^2_{\mathrm{ERR}}$),

$\mathbf{Z_1}$ and $\mathbf{Z_2}$ are the known incidence matrices relating the observations in $\mathbf{y}$ to effects in $\mathbf{r}$ and $\mathbf{a}$, respectively.

The significance of fixed effects, Orchard and Set, was tested with Wald-type F-statistics[12] in ASReml. Statistical significance from pairwise comparisons of means for both Orchard and Set was tested with t-statistics in Microsoft Excel. The significance of random effects was tested using one-tail likelihood ratio tests (LRT)[13].

Observed variance components were used to estimate causal components of variance:

$\hat{\mathrm{V}}_{\mathrm{A}} = 0.625 \times \hat{\sigma}^2_{\mathrm{TREE}}$ = additive genetic variance, and

$\hat{\mathrm{V}}_{\mathrm{P}} = \hat{\sigma}^2_{\mathrm{TREE}} + \hat{\sigma}^2_{\mathrm{ERR}}$ = phenotypic variance.

Individual-tree narrow-sense heritability was estimated:

$$\hat{\mathrm{h}}^2 = \frac{\hat{\mathrm{V}}_{\mathrm{A}}}{\hat{\mathrm{V}}_{\mathrm{P}}}$$

In addition family mean repeatability was estimated as

$$\hat{\mathrm{h}}^2_{\mathrm{HS}} = \frac{\hat{\sigma}^2_{\mathrm{fam}}}{\mathrm{V}_{\mathrm{Pfam}}} = \frac{\hat{\sigma}^2_{\mathrm{TREE}}/4}{\hat{\sigma}^2_{\mathrm{TREE}}/4 + \frac{(\hat{\sigma}^2_{\mathrm{TREE}} \times 0.75 + \hat{\sigma}^2_{\mathrm{ERR}})}{n}}$$

where $\mathrm{V}_{\mathrm{Pfam}}$ is the phenotypic family variance, $(\hat{\sigma}^2_{\mathrm{TREE}} \times 0.75 + \hat{\sigma}^2_{\mathrm{ERR}})$ is the residual variance from family model, and *n* = harmonic mean number of observations per family.

Variances are not independent of the scale and the mean of the respective traits[14]. Therefore the coefficient of additive genetic variation ($\mathrm{CV}_{\hat{\mathrm{A}}}$) was estimated in order to compare the genetic variance across traits. The $\mathrm{CV}_{\hat{\mathrm{A}}}$ expresses the additive genetic variance relative to the mean of the trait of interest, and gives a standardized measure of the genetic variance relative to the mean. The higher the $\mathrm{CV}_{\hat{\mathrm{A}}}$, the higher is its relative variation.

$$\mathrm{CV}_{\hat{\mathrm{A}}} = \frac{\sqrt{\hat{\mathrm{V}}_{\mathrm{A}}}}{\bar{\mathrm{x}}} \times 100\%$$

where $\bar{x}$ is the population mean. An alternative approach was used to estimate $CV_{\hat{A}}$ for stem straightness, malformation and acceptability which are subjectively measured traits and bounded by a scale[15].

$$CV_{\hat{A}} = \frac{\sqrt{\hat{V}_A}}{\sqrt{(X_{mean} - X_{min})(X_{max} - X_{mean})}} \times 100\%$$

where $X_{mean}$ is the mean, and $X_{min}$ and $X_{max}$ are the lower and upper bounds of the scale, respectively.

Breeding values were predicted for all individuals in the trial as well as for parents. The accuracy (ACC) of breeding values was also calculated for each trait:

$$ACC = \sqrt{1 - \frac{PEV}{\hat{V}_A}}.$$

Accuracy is the correlation between the true and predicted breeding values, where PEV is the predicted error variance (equivalent to the squared standard error of prediction in ASReml *.sln file). The higher the accuracy the more confident one can be about the predicted breeding values.

Genetic and phenotypic correlations between pairs of traits were estimated by using a bivariate individual-tree mixed-effects model:

[3] $$\mathbf{y_i} = \mathbf{X_i b_i} + \mathbf{Z_{r_i} r_i} + \mathbf{Z_{a_i} a_i} + \mathbf{e_i} \quad ,$$

where $\mathbf{y_i}$ is the vector of observations indexed (i) by trait;

$\mathbf{b_i}$ is the vector of fixed effects (mean, seed orchard, and set) and $\mathbf{X_i}$ is the known incidence matrix relating observations in $\mathbf{y_i}$ to the fixed effects in $\mathbf{b_i}$, where

$$\mathbf{X_i b_i} = \begin{bmatrix} \mathbf{X_1} & \mathbf{0} \\ \mathbf{0} & \mathbf{X_2} \end{bmatrix} \begin{bmatrix} \mathbf{b_1} \\ \mathbf{b_2} \end{bmatrix};$$

$\mathbf{r_i}$ is the vector of random effect of replicates ~ $MVN(\mathbf{0}, \mathbf{P} \otimes \mathbf{I_r})$ where,

$\mathbf{P} = \begin{bmatrix} \hat{\sigma}^2_{REP_1} & \hat{\sigma}_{REP_1 REP_2} \\ \hat{\sigma}_{REP_1 REP_2} & \hat{\sigma}^2_{REP_2} \end{bmatrix}$ and $\mathbf{I_r}$ is an identity matrix equal to the number of replicates;

$\mathbf{a_i}$ is the vector of random additive effects of individual trees ~$MVN(\mathbf{0}, \mathbf{G} \otimes \mathbf{A})$, where

$\mathbf{G} = \begin{bmatrix} \hat{\sigma}^2_{A_1} & \hat{\sigma}_{A_1 A_2} \\ \hat{\sigma}_{A_1 A_2} & \hat{\sigma}^2_{A_2} \end{bmatrix}$ and $\mathbf{A}$ = numerator relationship matrix generated from the pedigree in ASReml,

$\hat{\sigma}_{A_1 A_2}$ is the additive genetic covariance between traits 1 and 2, $\hat{\sigma}^2_{A_1}$ and $\hat{\sigma}^2_{A_2}$ are the additive genetic variances of traits 1 and 2, respectively;

$\mathbf{e_i}$ is the random vector of residual terms ~ $MVN(\mathbf{0}, \mathbf{R} \otimes \mathbf{I})$, where $\mathbf{R} = \begin{bmatrix} \hat{\sigma}^2_{E_1} & \hat{\sigma}_{E_1 E_2} \\ \hat{\sigma}_{E_1 E_2} & \hat{\sigma}^2_{E_1} \end{bmatrix}$;

$\mathbf{0}$ is the null matrix; $\mathbf{I}$ is the identity matrix equal to the number of observations; $\mathbf{I_r}$ is an identity matrix equal to the number of replicates; $\mathbf{Z_{r_i}}$ and $\mathbf{Z_{a_i}}$ are the known incidence matrices relating observations in $\mathbf{y_i}$ to random effects in $\mathbf{p_i}$, and $\mathbf{a_i}$, respectively.

The additive genetic correlation ($\hat{r}_A$) between pairs of traits was estimated as:

$$r_A = \frac{\hat{\sigma}_{A_1A_2}}{\sqrt{\hat{\sigma}^2_{A_1}\hat{\sigma}^2_{A_2}}}.$$

The phenotypic correlation ($\hat{r}_P$) between pairs of traits was estimated as:

$$r_P = \frac{\hat{\sigma}_{A_1A_2}+\hat{\sigma}_{E_1E_2}}{\sqrt{(\hat{\sigma}^2_{A_1}+\hat{\sigma}^2_{E_1})(\hat{\sigma}^2_{A_2}+\hat{\sigma}^2_{E_1})}}.$$

Statistical significance of genetic correlations was tested using two-tail LRT.

For all variance components, correlations, and heritability estimates, approximated standard errors were calculated using Taylor Series expansion method in ASReml[11].

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