

Genetic analysis of *Eucalyptus fastigata* progeny trials and implications to selection

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EXECUTIVE SUMMARY

Progeny trials for *Eucalyptus fastigata* in the third cycle, planted in 2009 and 2010 in Kaingaroa and Waihaha were assessed in 2012-2013 for growth, form and adaptability. These trials are now advanced to the age where selections for the next generation can be made and due to forest management requirements in Kaingaroa, it is necessary to collect measurements for selection purposes prior to the thinning.

The objective of this milestone was to first assess trees at Kaingaroa for growth, form and wood stiffness and at Waihaha for growth, form, and foliar health. Following phenotypic assessment, genetic analyses were conducted for estimation of genetic variation and covariation, and to identify any genotype by environment interaction across sites. Finally a list of breeding values for selection of next generation seed orchard and breeding population individuals were produced.

Results from this study indicate that there is great potential for genetic improvement of productivity and tree form in the *E. fastigata* breeding population. Wood stiffness was measured for the first time in this breeding population, with an average modulus of elasticity of ~12 GPA (MOE in gigapascal) and a moderate estimate of heritability. Genetic correlations between the traits and between the different ages showed that some highly correlated traits can be replaced by one another if there is a requirement for more efficient assessment scheme. Due to the seed introductions from South African and Australian seed sources along each breeding cycle, provenance/seed source variation is still considerable for form traits and should be taken into account in selections.

Selection and marking of trees to remain post-thinning can now be progressed during 2018-19.

INTRODUCTION

In New Zealand, the breeding programme of *Eucalyptus fastigata* is currently in its third cycle of selections. The breeding population comprises of Australian provenances and seed sources from South Africa and New Zealand (Figure 1). New seed introductions have been made to the breeding programme in each cycle. Breeding objectives along the cycles have been for improved growth and form as well as adaptation traits such as frost tolerance (Wilcox 1980, Wilcox 1982, Kennedy et al. 2011). Substantial genetic gains were estimated for growth and form (15%) in the first generation and for form (12%) in the second generation (Kennedy et al. 2011).

Eucalyptus fastigata is recognised as one of the steadiest eucalypts across a variety of environments in New Zealand (Cannon and Shelbourne 1991) and can tolerate frosts of -10°C (Miller et al. 2000), but is sensitive to out-of-season frosts (Menzies et al. 1981). Good form is one of the most important breeding objectives, as this species has a tendency for large, persistent branches especially in open stands (Boland et al. 1984). *Eucalyptus fastigata* has many valuable end uses as sawn timber, fine papers or pulp production (Low et al. 2009). Its wood properties are described as moderately hard, of moderate strength and durability (Boland et al. 1984). Wood properties have not been measured in the breeding population previously, however, other studies in New Zealand reported wood density of 450 to 600 kg m³ and wood stiffness 11 to 26 GPa (gigapascal) modulus of elasticity (MOE) (Jones et al. 2010).

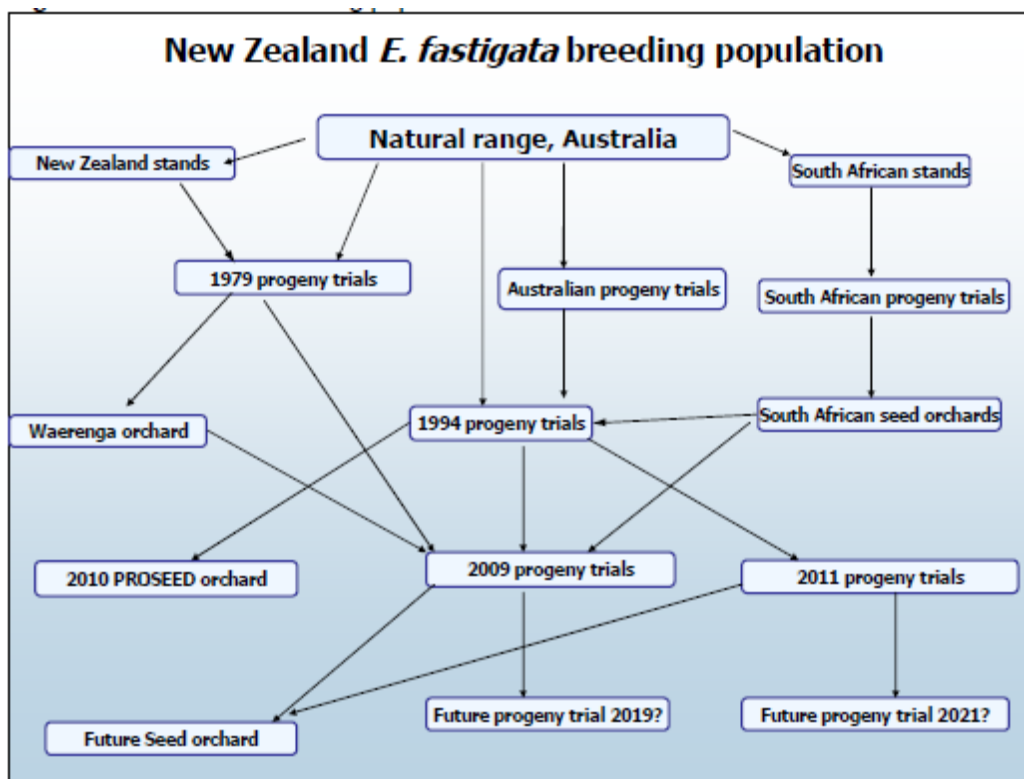


Figure 1. *Eucalyptus fastigata* breeding programme. (Figure from Low et al. 2009).

Progeny trials in the third cycle planted in 2009 and 2010 at Kaingaroa and Waihaha (Figure 2) were assessed in 2012-2013 for growth, form and adaptability. Substantial genetic gains were reported for the early age measurements (Suontama et al. 2014). These trials are now advanced to the age where selections for the next generation can be made and due to forest management requirements at the site in Kaingaroa, it is necessary to collect measurements for selection purposes prior to the thinning.

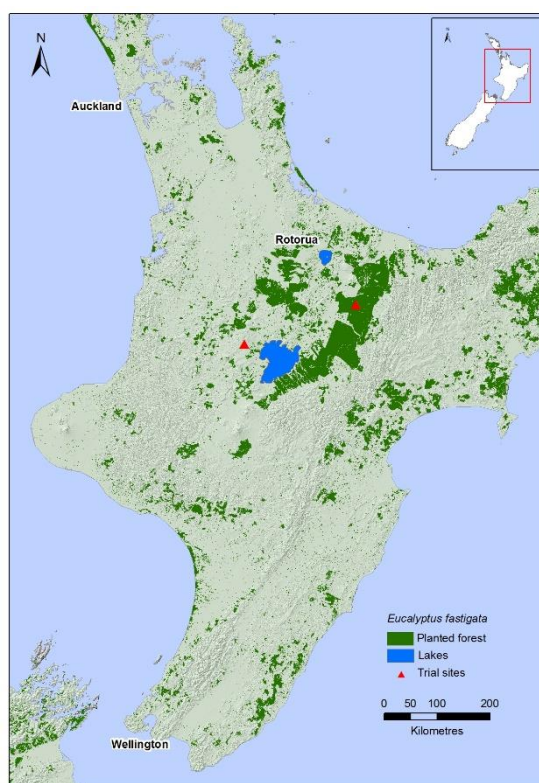


Figure 2. *Eucalyptus fastigata* progeny trials planted at Kaingaroa and Waihaha.

The objective of this milestone was to assess trees at Kaingaroa for growth, form and wood stiffness and at Waihaha for growth, form, and foliar health. Following phenotypic assessment, genetic analyses were conducted for estimation of genetic variation and covariation, and to identify any genotype by environment interaction across sites. Finally a list of breeding values for selection of next generation seed orchard and breeding population individuals was produced.

METHODS

Assessment traits for growth and form were defined as following:

Diameter at breast height (DBH), stem straightness scored on a 1 to 9 scale where 1 is very sinuous and 9 is perfectly straight (STR), malformation on a 1-9 scale where 1 = multiple forks and 9 = no forks (MAL), branching on a 1-9 scale where 1=heavy branching and 9=light, fine branching (BRA), acceptability on a 0-1 scale where 0 is unacceptable on any of poor growth, straightness, malformation or health, and 1 = acceptable scores in all of the traits (AC).

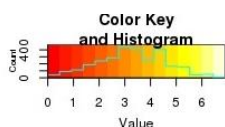
Wood stiffness was measured using ST300 for acoustic wave velocity (AVEL) as a surrogate trait for wood stiffness.

Data was analysed using linear mixed models in ASReml-R (Butler et al. 2009). The data set for Kaingaroa was analysed using spatial models to correct for micro-site environmental effects. Data set for Waihaha was analysed fitting the trial design effects of replicate and replicate incomplete-block interaction as the random terms in the model. The approach of fitting the trial design effects instead of spatial models was due to a large number of missing trees as a result of environmental stresses (Figures 3 & 4). For any multivariate analyses undertaken for trait-trait, age-age or across-sites analyses, trial effects were corrected by using the replicate incomplete-block interaction term in the models. The fixed effects of intercept and group of control vs. trial trees, the random effect of seed-source (Australian provenances, South African seed-sources, and New Zealand landraces) and the additive genetic effect of individual tree were fitted in the models. Due to the tendency of

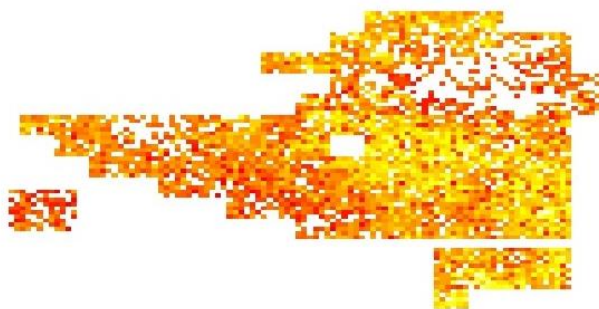
eucalypt species for self-fertilization (Griffin and Cotterill 1988), a proportion of 15% for selfing was fitted in the numerator relationship matrix in ASReml -R. This proportion was assumed as a compromise based on several literature references reporting a higher proportion of selfing in natural stands than in seed orchards (Griffin and Cotterill 1988, Moran et al. 1989, Gaiotto et al. 1997, Burczyk et al. 2002).

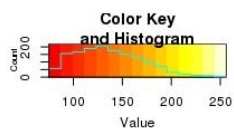
First, the statistical description of trait distributions with population parameters was conducted (Tables 1 & 2). Univariate analyses to estimate heritability for each trait were then undertaken. Heritability estimate for a binary trait of acceptability was estimated using generalised linear models with a logit link function, assuming the residual variance of 3.28987 (Butler et al. 2009). Multivariate analyses between growth, form and wood stiffness (wood stiffness at Kaingaroa only) were conducted to estimate genetic correlations between the assessment traits. These genetic correlations will direct the future assessment scheme with a possibility to include a smaller number of measurements if high genetic correlations between the traits are found. Genetic correlations between the early age (age 3 to 4) and the current measurements were estimated using bivariate models. Genotype by environment interaction (G x E) was investigated by estimating genetic correlations between the two sites for the traits with bivariate models. The magnitude of G x E will indicate if there is any instability of genotypes between these two sites.

Figure 3. Spatial plot for height at age 3 at Waihaha.



Height at age 3 (m) at Waihaha





DBH at age 8 (mm) at Waihaha



Figure 4. Spatial plot for DBH at age 8 at Waihaha.

RESULTS

Trees at Kaingaroa were larger for DBH as a result of being planted almost one year earlier than the trees at Waihaha (Tables 1 & 2). The average tree form in terms of stem straightness, branching and malformation were also considerably better at Kaingaroa than at Waihaha, where the mean scores for these traits were unacceptable for breeding purposes. As reported previously for the age 3 assessment at Waihaha (Suontama et al. 2014), this site was affected by pest/foliar disease problems as well as probably by out-of-season frosts. The current assessment proved that these problems have accumulated with time at this site. Only 3% of the trees were acceptable at Waihaha compared to Kaingaroa where 34% of the trees were identified as acceptable for breeding purposes. The maximum score at Waihaha was 9 for all form traits, indicating that there are some promising trees at this site, too. Stem straightness had the highest average value at Kaingaroa (~7). The mean scores for branching and malformation, around 6 at Kaingaroa, show that there is need for genetic improvement of branching and form. Acoustic wave velocity using the ST300 was measured at Kaingaroa for the first time in this breeding population. The average wood stiffness when transferred to modulus of elasticity was ~12 GPA assuming a green density of 1000 kg/m³.

Heritability of all traits ranged from low to moderate (Table 1 & Table 2). Heritability at Kaingaroa was moderate for all other traits except for malformation and acceptability. Stem straightness and branching had a heritability of 0.15 whereas DBH showed a heritability estimate of 0.24 and wood stiffness of 0.35 at Kaingaroa. Provenance variation was considerable for all form traits as well as for wood stiffness and acceptability (Table 1). A moderate heritability of 0.21 was estimated for DBH at Waihaha whereas all other traits had low heritability estimates (Table 2). A considerable provenance variation was, however, found for stem straightness and branching as well as for acceptability at Waihaha (Table 2). Malformation had a low overall variation and provenance variation was small as well. In general, heritability estimates of the traits indicate that considerable genetic improvement is possible to be achieved for growth and form at Kaingaroa, whilst an attention should also be paid on the existing provenance differences especially when it comes to the tree form.

Table 1. Statistical description and heritability of assessment traits at age 8 at Kaingaroa.

Trait	No. obs.	Mean	Min	Max	Standard deviation	Additive genetic h ²	Provenance h ²
Diameter at breast height (DBH) (mm)	3397	169	96	328	37	0.242±0.04	0.295±0.07
Stem straightness (STR) (score)	3397	7.06	1	9	1.5	0.153±0.03	0.396±0.15
Branching (BRA) (score)	3397	6.61	1	9	1.92	0.149±0.02	0.383±0.15
Malformation (MAL) (score)	3396	6.3	1	9	2.62	0.054±0.02	0.364±0.17
Wood stiffness (AVEL) (km/s)	3376	3.43	2.54	4.39	0.27	0.354±0.06	0.597±0.16
Acceptability (AC) (0/1)	3397	0.34	0	1	0.47	0.043±0.02	0.27±0.12

Table 2. Statistical description and heritability of assessment traits at age 7.5 at Waihaha.

Trait	No. obs.	Mean	Min	Max	Standard deviation	Additive genetic h ²	Provenance h ²
Diameter at breast height (DBH) (mm)	1472	137	136	256	33.68	0.209±0.06	0.209±0.06
Stem straightness (STR) (score)	1472	5.06	1	9	1.68	0.077±0.04	0.218±0.08
Branching (BRA)(score)	1471	3.53	1	9	1.78	0.056±0.04	0.180±0.07
Malformation (MAL) (score)	1472	2.22	1	9	1.57	0.033±0.037	0.077±0.05
Acceptability (AC) (0/1)	1472	0.033	0	1	0.18	NA	0.340±0.29

NA= Additive genetic variation for individual tree term in the model was zero.

Additive genetic correlations between the assessment traits ranged from low to moderate at both sites (Table 3 & Table 4). There were only two statistically significant correlations between growth and tree form, which were between DBH and branching at Kaingaroa and between DBH and stem straightness at Waihaha. Genetic correlation between DBH and branching at Kaingaroa was highly unfavourable, indicating selection for improved growth favours heavier branches. On the other hand, at Waihaha the other significant genetic correlation implies that when selecting for improved growth, stem straightness will improve, too. Form traits were moderately favourably genetically correlated at both sites although these genetic correlations were not statistically significant at Waihaha. A genetic correlation of 0.68 at Kaingaroa between stem straightness and malformation implies that either of these traits could be replaced by the other trait in the assessment scheme. Wood stiffness had a favourable genetic correlation with DBH of 0.29 which is different to earlier research in eucalypts (Kube 2005). This genetic correlation is of similar direction as in *E. nitens* wood property measurements at age 7 (Suontama et al. 2016). The positive genetic correlation between DBH and wood stiffness can be a consequence of the early assessment age. Acoustic wave velocity had an unfavourable genetic correlation with branching, indicating that selection for wood stiffness at age 8 will favour trees with heavier branches.

Genetic correlation between the early assessment traits and the current assessment traits were interesting (Table 5 & Table 6). Genetic correlation between age 4 and 8 measurements for DBH at Kaingaroa was 0.86, stating that the earlier measurement predicts relatively well the later growth. Somewhat lower but still a moderate genetic correlation of 0.62 was estimated between height at age 4 and DBH at age 8 at Kaingaroa. Malformation at age 4 and 8 are principally genetically the same traits, therefore, if more than one assessment will be undertaken in the

breeding programme, any further assessment for malformation is not essential. A genetic correlation between age 4 DBH and branching at age 8 indicates that selection for faster early growth will favour trees with heavy branching habits at Kaingaroa. Similar genetic correlation at this site was also found between height and branching, although it was somewhat lower (Table 5). At Waihaha, estimated genetic correlation between early height at age 3 and DBH at age 7.5 showed that these growth traits are genetically very similar at these different ages. Forking at age 3 was favourably highly genetically correlated with stem straightness, branching and malformation at age 7.5 at Waihaha.

Table 3. Estimates of additive genetic correlations between the assessments traits at Kaingaroa.

	STR	BRA	MAL	AVEL
DBH	-0.094±0.148	-0.759±0.070	-0.14±0.198	0.288±0.121
STR		0.233±0.151	0.682±0.162	0.131±0.138
BRA			0.291±0.203	-0.470±0.119
MAL				-0.033±0.170

Table 4. Estimates of additive genetic correlations between the assessments traits at Waihaha.

	STR	BRA	MAL
DBH	0.635±0.279	-0.042±0.336	0.304±0.419
STR		0.314±0.437	0.347±0.559
BRA			0.479±0.607

Only a small genotype by environment interaction (G x E) was identified across sites (Table 7). This GxE was identified for branching but it had a large standard error. It can be concluded that there is no important G x E for any of the traits based on moderately high genetic correlations across the two sites. Consequently, selection can be undertaken by ranking the best genotypes regardless of the site. Furthermore, there is a possibility to select any good performing individuals at Waihaha as improved pest/frost resistant stock.

Table 5. Estimates of additive genetic correlations between age 3 and 8 the assessment traits at Kaingaroa.

	HT	DBH	Mal
DBH	0.619±0.09	0.859±0.050	0.034±0.168
STR	-0.203±0.164	-0.273±0.168	0.393±0.161
BRA	-0.576±0.116	-0.748±0.094	0.058±0.185
MAL	-0.171±0.224	-0.364±0.212	0.986±0.137
AVEL	0.433±0.125	NA	0.179±0.155

NA = LogL not converged

Table 6. Estimates of additive genetic correlations between age 3 and age 7.5 the assessment traits at Waihaha.

	HT	FOR	HLTH
DBH	0.737±0.08	NA	-0.764±0.125
STR	0.496±0.216	-0.687±0.324	-0.558±0.265
BRA	-0.239±0.321	-0.782±0.422	-0.558±0.265
MAL	-0.049±0.420	-0.948±0.374	-0.029±0.397

NA = *LogL* not converged
 HLTH = Health at age 3

Table 7. Additive genetic correlations with their standard errors estimated across the sites.

	r_G
DBH	0.687±0.182
STR	0.971±0.264
BRA	0.583±0.304
MAL	0.759±0.535

CONCLUSIONS

Results from this study indicate that there is great potential for genetic improvement of productivity and tree form in the *E. fastigata* breeding population. Due to the seed introductions from South African and Australian seed sources along each breeding cycle, provenance variation is still considerable for form traits and should be taken into account in selections. Genetic correlations between the traits and between the different ages showed that some highly correlated traits can be replaced by one another if there is a requirement for more efficient assessment scheme. No relevant genotype by environment interaction was found across the two sites. The badly damaged site at Waihaha may offer an opportunity to select for highly robust pest/frost resistant stock which can be expected perform relatively well in demanding environments. Nevertheless, if such selections will be made, it is recommended to test these selections in permanent samples plots. Wood stiffness was measured for the first time in this breeding population, showing MOE of ~12 GPA and moderate estimate of heritability. Further testing of wood properties at later age is recommended for this species to obtain more confidence in these initial results.

Selection and marking of trees to remain post-thinning can now be progressed during 2018-19.

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