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Results of assessments in FR375, Beaumont controlled-pollinated 1999 progeny trial

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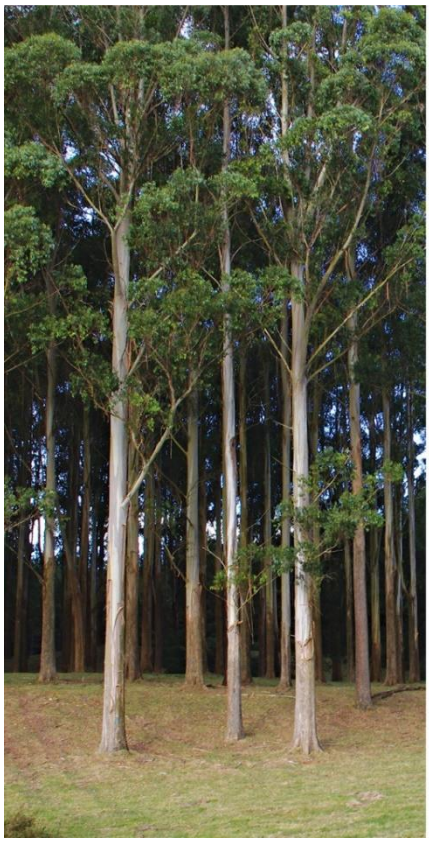

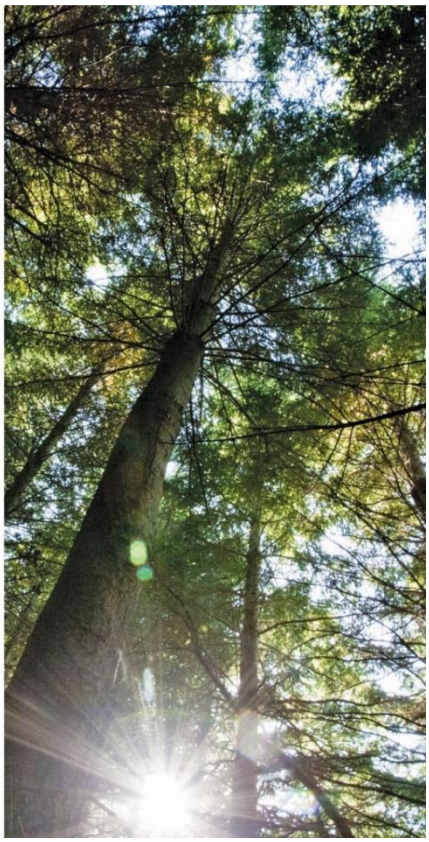

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EXECUTIVE SUMMARY

The 1999 controlled-pollinated Douglas-fir trial at Beaumont (FR375) was designed to compare selections from the first Douglas-fir breeding population. They had been selected as the best individuals in the best-adapted provenances in the 1957 & 1959 provenance trials, grafted up in 1989 and planted into the Waikuku archive in 1990. Flowers on grafts in the Waikuku archive were bagged, then pollinated with a pollen mix of 10 genotypes from 1994 to 1996, but difficulties in obtaining pollen meant that several different pollen mixes were used for pollinations in different years.

Seed-set was generally low, so it was decided to sow the seed that had been collected in 1997, but to also include some open-pollinated seed that had been collected from some of the same genotypes by Proseed. Germination was also low for most families; but eventually fifty selections were available to be tested in a trial. Scientists in British Columbia had enjoyed success with early assessments of trials on fertile soils (farm-field trials), so this trial was planted at 2500 stems per hectare on a good site, with the expectation of an early assessment and ranking.

Some wet areas developed in the trial, leading to mortality and some toppling, but the majority of the trees grew well. The trees were first assessed when they were three years old. At that time it was noted that some of the trees in the wetter areas appeared to not have grown at all.

A comprehensive assessment was performed in 2015, and the following traits were assessed: breast-height diameter, stem straightness, branching frequency, stem malformation, acceptability, acoustic stiffness, and pith-to-bark increment core wood density.

Heritability was estimated to be 0.31 for diameter and 0.37 for acoustic stiffness, with a lower heritability of around 0.20 for straightness, malformation and acceptability. Heritability of density was 0.26 for rings 1 to 5, but lower heritability's were estimated for rings 6 to 10 (0.17) and rings 11 to 15 (0.12). The branching frequency score had the lowest heritability of 0.08. In this trial, provenance had no significant effect on diameter growth.

Genetic correlations estimated are only indicative as most of them have large standard errors. Genetic correlation between diameter and acoustic stiffness was negative, but not significant at -0.14. Genetic correlations for density rings 1 to 5 with rings 6 to 10 and 11 to 15 were high. Because heartwood density is determined by similar genes in different rings, and rings 1 to 5 have a higher heritability for density than other rings, selection can be undertaken based on rings 1 to 5. Negative, but low genetic correlations were estimated between density rings 1 to 5 and 6 to 10 with diameter, whereas a high (-0.98) unfavourable genetic correlation was estimated between density for rings 11 to 15 and diameter.

Some genotypes had good breeding values for all traits, so will be obvious selections for seed production. In a trial like this, the poly-cross mating design normally limits the number of forwards selections that can be made. It is the intention of this programme to use DNA-based techniques to identify male parentage and limit any inbreeding in forward selections of interest.

INTRODUCTION

The formation of the Douglas-fir Research Co-operative in 1993 injected more funding into Douglas-fir research, than had been assigned previously. Prior to the formation of the Cooperative, Proseed had commissioned the selection and grafting of superior Douglas-fir trees in the 1957 and 1959 provenance trials. Proseed made these selections available to the Cooperative, as the start of a breeding programme.

The Cooperative decided on a breeding strategy of controlled crossing on the 189 selections that had been made[1]. Genotypes would be tested in a poly-cross trial, where all genotypes would be pollinated by a 10-genotype pollen mix.

Double-pair mating was also initiated for forward selections, where each genotype would be mated with one geographically close neighbour and one genotype from a more distant provenance. This would set up many unrelated families for selection of candidates for the next breeding population.

The first year of crossing (1995) produced good crops of cones, but many cones contained no seed. Subsequent years produced fewer cones and many of these had only empty seeds. Flowers on grafts in the Waikuku archive were pollinated with a pollen mix of 10 genotypes, but difficulties in obtaining pollen meant that several different pollen mixes were used.

By 1997, there were over 70 seedlots in storage, so it was decided to sow the available seed to set up the first controlled-pollinated trial, FR375.

FR375 seedlot code numbers are shown in Table 1. The trial was planted as 25 replicates of single-tree plots. Some families had less than 25 plants, so the shortfall was made up using more plants from families with more than 25 plants. Some 5-tree row plots were planted as family rows on the east side of the single-tree plot trial. The row plots were planted at 2.5 metres by 3 metres. Ernslaw One staff were asked to provide a site and they supplied what was considered to be an excellent site. The site is a relatively flat, river terrace site where the old Beaumont forest buildings had stood within 100 metres of the Clutha River. They arranged for the site to be ripped, but the contractor ripped lines parallel to the river, rather than down the gentle slope to the river. This later hindered water drainage and there was some early mortality caused by waterlogging.

The site was very fertile, as evidenced by the growth of the nearby 1977 *Sequoiadendron* trial, so a short term, farm field trial at close spacing was planned. The trial (FR375) was planted on the ripped lines at a spacing of 2.5 metres between ripped lines and 1.5 metres within lines (2500 stems per hectare) in 1999.

The trial was first measured in 2002 at age three years. Best trees were over three metres tall, but many trees had grown little since planting. No significant family differences were found.

The trial was measured again in 2009, but family differences were obscured by the large within-family variation. Some trees whose roots were in boggy ground, suffered from toppling early in life, but many managed to recover with a large stem displacement (butt-sweep). All trees appeared to have healthy foliage, so needle retention was not assessed.

Hindrance from inter-woven branches at the close spacing in the trial was a concern. However, Ernslaw One staff trimmed the branches of the trees by chainsaw facilitating navigation and allowing the use of the ST300 tool to assess acoustic stiffness (Figure 1).

Figure 1. **Interior of the stand after pruning**

Trial Assessment

The following traits were measured or assessed in May 2015: 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); branching (internode length) on a 1-9 scale where 1 = branches distributed evenly over entire year's growth, 9 = a clear internode of one metre between branch whorls (BR); malformation on a 1-9 scale where 1 = multiple forks and 9 = no forks or ramicorn branches (MAL); 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 (ACC); and acoustic velocity in kilometres per second as measured by the ST300 tool (a surrogate for timber stiffness).

Increment cores were taken from all trees that were reasonably straight and greater than 100 mm in diameter. Cores were taken from 636 trees out of the 932 trees that had diameters measured. The cores were cut into three segments: rings 1-5, 6-10 and 11-15. All cores could be cut into rings 1-5 and 6-10, but some trees were becoming suppressed and outer segments were small and latewood. Segments containing rings 11-15 that were less than one cm in length were discarded because it was impossible to mark with a sample identifier and in general small samples give unreliable results. A total of 393 trees for this ring group were used in the analysis.

Data Analysis

Data were analysed using ASReml-R[2].

The data were analysed using a model with the parameters of the trial layout (trees, sets within replicates, a spatial tree model with rows and columns[3]). Breeding values were estimated for each trait and each mother whose progeny were in the trial.

RESULTS

Tree survival was 66%. Most mortality was caused by waterlogging where pools of water would form after rain and the soil would remain saturated for long periods. Some mortality was caused by trees toppling in strong winds because their roots were unable to hold the tree up in wet soil.

The analysis estimated basic statistics of mean, minimum, maximum and standard deviation (Table 2). Mean diameter of 191 millimetres appears low for Douglas-fir on a good site like this, but is the result of the close spacing of 2500 stems per hectare. Mean diameter of the trees in the row plots was 240 mm at a lower stocking of 1250 stems per hectare. Density measurements for rings 1 to 5 had an effect of compression wood resulting outliers in the distribution of density cores. Values above 450 kg/m^3 were clearly defined as outliers based on phenotypic distribution and therefore, discarded from the analysis. This data modification resulted in significantly higher heritability's as a random noise caused by the outliers was removed. Density values above 440 for rings 6 to 10 were regarded as outliers and removed. However, this did not have a considerable effect on heritability estimates.
Provenance did not have a significant effect on growth rate as found in the 1959 and 1996 trials raised from seed imported from USA[4]. However, provenance did have an effect on stem straightness, branching and density for rings 1 to 5. The use of mainly Californian provenance pollen mixes is a likely reason for the absence of provenance differences in growth rate.

Variance components were generated by the analysis and these were used to estimate heritability (Table 3). The heritability (h^2) of the important traits of diameter, velocity (wood stiffness) and straightness were all moderate-to-high and adequate for good discrimination of families ($h^2 < 0.31$, 0.37 and 0.25, respectively). The heritability of wood density was moderate for rings 1 to 5 (0.26), but low for rings 6 to 10 (0.17) and 11 to 15 (0.12). However, the relative imbalance of plant numbers per family and the small data set, resulted in large standard error for most genetic parameters. Therefore, these genetic parameters are only indicative.

Genetic correlations between traits were estimated (Table 4 and Table 5). The expected negative correlation between diameter growth and wood stiffness was lower than was found in the assessments of the 1972 Douglas-fir progeny trial[5]. This may be because the 1972 progeny trial had been thinned to 350 stems per hectare while the Beaumont site received no thinning. Wood density for rings 1 to 5 was highly genetically correlated with density for rings 6 to 10 and 11 to 15. This means that density of different rings is mainly determined by the similar genetic effects. This is why selection in this population can be done on the basis of rings 1 to 5 due to the higher heritability than for other rings. A negative genetic correlation for diameter was particularly high with density of rings 11 to 15, but low with other rings.

Breeding values were estimated for each trait and each tree and their mothers in the trial. Estimates of BLUP (Best Linear Unbiased Prediction) breeding values have a mean around zero. First, breeding values were estimated separating the provenance effect as in the model description for genetic parameters), and second, including the provenance in the breeding values. First, the difference between these two sets of breeding values is that values for ten of the fifty clones were estimated at exactly the average breeding value for all traits when separating the provenance effect. Including the provenance effect in the EBVs added this provenance variation to these zero breeding values. Secondly, selections based on the breeding values where provenance effect is separated off, are comparable between provenances if tree grower would like to ignore the provenance variation. On the other hand, the breeding values including provenance variation, take into account for a part of the differences between families arising from the provenance variation. There are three families relatively good for all traits in both sets of breeding values.

CONCLUSION

The original decision to test the 888 and 889 series clones by controlled-pollination did not work out as was hoped. Obtaining seed using controlled-pollination proved much more difficult than anticipated, and certainly much more difficult than for radiata pine.

A heritability of 0.31 was estimated for diameter, 0.37 for wood stiffness and 0.26 for density in rings 1 to 5. A heritability of 0.25 was estimated for straightness and 0.21 for malformation, which should favour good improvement in these traits. Because wood density for different rings can be regarded as the same trait and as the rings 1 to 5 has the highest heritability for density, selection can be based on this ring group. Unfavourable genetic correlations between diameter and acoustic velocity, and diameter and density can cause some difficulties in making selection decisions and tree grower may have to do few compromises.

However, three of the 50 genotypes tested in this trial performed well for growth, form and wood stiffness and are good candidates for a production population, and for continuing in the breeding population. There are some other genotypes that were average for diameter growth, but good for form and wood stiffness and some genotypes good for diameter and average for other traits but weak for density. These aspects may also be considered in a production population.

The best genotypes will be grafted over the next few years for archiving and inclusion in seed orchards, as appropriate.

It appears that the waterlogging, wind throw and other imbalance in this trial would not make it the first candidate for use in a genomics programme with Douglas-fir.

ACKNOWLEDGEMENTS

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APPENDICES

Table 1. Code and genotype numbers allocated to sets.

Set A					Set B				
Code	Mother	Provenance	Latitude	Pollen	Code	Mother	Provenance	Latitude	Pollen
1	889589	Mt Tamalpais	37.88	FB	101	888431	Fort Bragg	39.35	OP
2	888498	Snohomish	48.25	FB	102	888452	Santa Cruz	37.08	BT
3	889545	Bandon	43.08	FB	103	888452	Santa Cruz	37.08	OP
4	889598	Olympic	47.08	FB	104	889538	Stewart Point	38.65	OP
5	888406	Stewart Point	38.65	FB	105	889554	Ashley, NZ	44.00	OP
6	888404	Coos Bay	43.42	FB	106	889555	Ashley, NZ	44.00	OP
7	888468	Wiskah	47.10	FB	107	889557	Ashley, NZ	44.00	DH
8	889558	Ashley	44.00	FB	108	889559	Ashley, NZ	44.00	OP
9	889605	Olny	46.08	FB	109	889559	Ashley, NZ	44.00	DH
10	888467	Snoqualmie	47.00	FB	110	889575	Stewart Point	38.65	OP
11	888426	Olympic	47.08	FB	111	889576	Santa Cruz	37.08	OP
12	889529	Olny	46.08	FB	112	889580	Coos Bay	43.42	OP
14	889547	Tahkenitch	43.83	FB	113	889586	Fort Bragg	39.35	OP
16	888420	Fort Bragg	39.35	FB	114	889592	Olny	46.08	OP
17	889546	Langlois	42.95	FB	115	889595	Wiskah	47.10	AS
18	888499	Fourmile	43.03	FB	116	889596	Wiskah	47.10	AS
19	888495	Olny	46.08	FB	118	889598	Olympic	47.08	AS
20	889599	Olympic	47.08	FB	119	889600	Siuslaw	44.00	AS
21	889563	Olympic	47.08	FB	120	889603	Coos Bay	43.42	AS
22	889532	Deadwood	44.10	FB	122	889613	Mad River	40.92	OP
23	889609	Deadwood	44.10	FB	123	889614	Mad River	40.92	OP
24	888447	Snohomish	48.25	FB	125	889617	Fort Bragg	39.35	OP
25	889592	Olny	46.08	FB	126	889617	Fort Bragg	39.35	BT
26	889633	Ashley	44.00	FB	127	889618	Stewart Point	38.65	OR
27	889618	Stewart Point	38.65	FB	129	889624	Fort Bragg	39.35	OP
28	889615	Dehaven	39.60	FB	130	888424	Granite Falls	48.08	OP
29	889621	Mt Tamalpais	37.88	FB	201	889589	Mt Tamalpais	37.88	FB
30	889611	Berteleda	41.80	FB	202	888498	Snohomish	48.25	FB
31	889604	Coos Bay	43.42	FB	207	888468	Wiskah	47.10	FB
32	889622	Santa Cruz	37.08	FB	209	889605	Olny	46.08	FB
100	94/32	Fort Bragg	39.35	OP	212	889529	Olny	46.08	FB
					217	889546	Langlois	42.95	FB
					220	889599	Olympic	47.08	FB
					221	889563	Olympic	47.08	FB
					222	889532	Deadwood	44.10	FB
					223	889609	Deadwood	44.10	FB
					224	888447	Snohomish	48.25	FB
					228	889615	Dehaven	39.60	FB
					229	889621	Mt Tamalpais	37.88	FB
					231	889604	Coos Bay	43.42	FB
					232	889622	Santa Cruz	37.08	FB
					100	94/32	Fort Bragg	39.35	OP

Pollen codes are as follows: AS = Ashley clones, BT = Berteleda clones, FB = Fort Bragg clones, DH = Dehaven clones, OP = open-pollinated

Table 2. Statistical description of age 16 traits assessed at the Beaumont controlled-cross progeny trial.

Trait	*Mean*	*Min*	*Max*	*SD*
Dbh16	191.16	48.00	345.00	48.49
Vel16	4.50	3.03	5.56	0.37
Bra16	5.20	1.00	9.00	1.93
Mal16	7.13	1.00	9.00	2.63
Str16	5.89	1.00	9.00	1.88
Acc16	0.42	0.00	1.00	n.a.
D15	385.19	314	450	26.65
D610	369.55	295	440	28.26
D1115	387.66	291	508	36.28

Dbh16 = age 16 diameter (mm)
Vel16 = Acoustic velocity in kilometres / second used as a surrogate for wood stiffness
Bra16 = branching score (1-9)
Mal16 = Malformation score (1-9)
Acc16 = Acceptability score (0 or 1)
D15= Density rings 1 to 5 (kg/m^3)
D610= Density rings 6 to 10 (kg/m^3)
D1115=Density rings 11 to 15 (kg/m^3)

Table 3. Estimates of variance components and heritability for age 16 traits assessed at the Beaumont controlled-cross progeny trial.

	σ^2_a	σ^2_e	Row	Col	h^2
Dbh16	728.45	1650.21	-0.15	-0.06	$0.21_{0.12}$
Vel16	0.06	0.09	0.13	-0.07	$0.37_{0.14}$
Bra16	0.3	3.36	0.22	0.05	$0.08_{0.07}$
Mal16	1.49	5.61	0.1	-0.02	$0.21_{0.11}$
Str16	0.91	2.68	0.07	-0.005	$0.25_{0.11}$
Acc16	0.04	0.2	0.07	0.01	$0.19_{0.10}$
D15	191.08	546.06	-0.01	0.01	$0.26_{0.14}$
D610	131.68	665.62	0.04	0.02	$0.17_{0.11}$
D1115	145.36	1037.81	0.14	-0.17	$0.12_{0.15}$

σ^2_a additive genetic variance
σ^2_e error variance
row variance attributed to rows
col variance attributed to columns
h^2 narrow sense heritability

Table 4. Estimates of genetic correlations between age 16 traits assessed at the Beaumont controlled-cross progeny trial.

	Vel16	Bra16	Mal16	Str16	Acc16
Dbh16	$-0.14_{0.31}$	$0.37_{0.43}$	$0.79_{0.22}$	$-0.02_{0.32}$	$0.51_{0.26}$
Vel16		$-0.64_{0.38}$	$-0.14_{0.36}$	$-0.22_{0.34}$	$-0.20_{0.35}$
Bra16			$0.24_{0.52}$	$0.31_{0.48}$	$0.86_{0.53}$
Mal16				$0.80_{0.20}$	$0.92_{0.21}$
Str16					$0.98_{0.12}$

Table 5. Estimates of genetic correlations between density measurements and density with DBH16.

	D610	D1115	DBH16
D15	$0.75_{0.24}$	$0.93_{0.21}$	$-0.26_{0.34}$
D610		[1]	$-0.11_{0.41}$
D1115			$-0.98_{0.07}$

1) Non-estimable