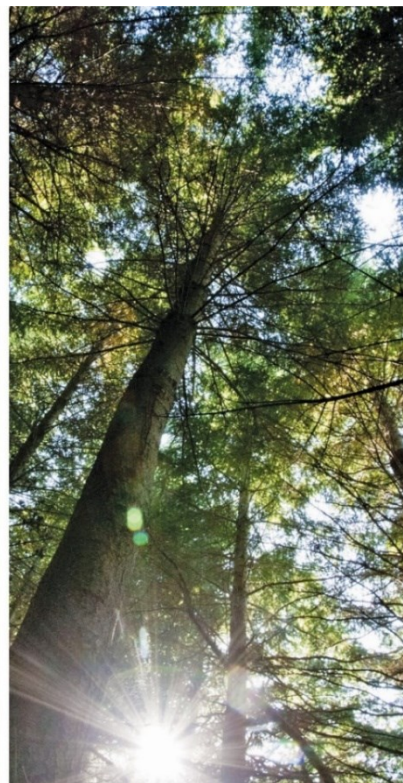


Assessment of NZDFI's 2011 *Eucalyptus quadrangulata* breeding populations

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EXECUTIVE SUMMARY

This report concerns the phenotyping of NZDFI's 2011 *E. quadrangulata* breeding populations at McNeil and Cuddon for heartwood. Genetic analysis of the trials was hampered by the small number of families (~20). Larger *E. quadrangulata* breeding trials including ~80 additional families were established in 2016 and will be phenotyped in due course.

Analysis indicate that heartwood diameter and sapwood diameter were genetically correlated between sites. Heartwood diameter was not genetically correlated to tree diameter (DBH or core length) but negatively to sapwood diameter. This would suggest that neither DBH nor core length can be used as proxy to select for heartwood quantity.

Genetic control of heartwood quality (i.e. predicted extractive content) was inconclusive. The small number of families and the generally low heartwood extractive content contributed to the larger uncertainty in these values.

Families from these trials were previously ranked for form, growth and growth strain based on assessments in the Cuddon, NZRC Paparua and Woodville trials (SWP-T120). Some families recommended for propagation in that report matched families performing well for heartwood traits. In particular family 518 seems to be performing well in all traits.

The data has been made available to the industry to select appropriate genotypes for commercial production.

INTRODUCTION

The New Zealand Dryland forests Initiative (NZDFI), aims to establish a domestic, sustainable, natural durable hardwood industry (Millen et al., 2018). To achieve this the NZDFI has established a breeding program to deliver well performing planting stock. In addition to fast growth and improved form, favourable wood properties are key objectives in the breeding program to produce high-value timber. The eucalyptus species in the NZDFI program were chosen preliminarily for their natural durability and their fast growth potential under the climatic conditions in the drier parts of New Zealand. *E. quadrangulata* is a class 2 ground durable timber (Bootle 2005) and one of several Eucalyptus species developed by NZDFI.

Heartwood quality and quantity are key wood properties for the envisaged utilisation of *E. quadrangulata* and are therefore included in a breeding program. Heartwood quantity is partly under genetic control (Hillis, 1987) and varies within a species. Heartwood extractives are a main factor providing natural durability (Hawley et al., 1924). Previous SWP funded research showed that the extractive content (EC) can be predicted with NIR spectroscopy (RMSE ~1%) (Technical report SWP-T040, Li & Altaner, 2019) and that EC is highly variable (1 to 20%) in other NZDFI eucalypt species.

The NZDFI *E. quadrangulata* breeding populations consists of several trial series (SWP-T120). In 2011, the first open-pollinated breeding population of *E. quadrangulata* was established in a series of progeny trials to evaluate the differences between individual families. NZDFI's aim is for each breeding population to test a minimum of 100 families per species, however poor flowering in Australia restricted the number of seedlots available at this time. NZDFI purchased open-pollinated families from CSIRO's tree seed centre and 24 families were successfully raised in 2011. This breeding population of *E. quadrangulata* was extended in 2015 when Proseed obtained a further 88 families from ATSC and Forestry Corporation. 83 Families produced seedlings for field testing in 2016. Additionally the University of Canterbury deployed 64 seedlings per family for the UC SFF growth strain testing programme based at Murrays Nursery, Woodville (Altaner 2019).

This report refers to milestones 8 and 12 of the SWP work plan 130. The objective of this work was to phenotype the *E. quadrangulata* breeding populations planted at McNeil and Cuddon in 2011 for heartwood quality and quantity.

METHODS

Trial

Two open-pollinated *E. quadrangulata* family progeny trials were established in 2011 at McNeil, Wairarapa, and Cuddon, Marlborough, New Zealand. The seed was purchased from CSIRO's tree seed centre.

At the McNeil site single-tree plots were established in 80 blocks with 20 trees in each block. The trial consists of 21 families with different number of individuals per family ranging from 34 to 80, totalling 1600 trees. The trial was assessed for height at age 1.6 years in April 2013 and again at the age of 4.4 years in February 2016 for form and DBH. Recently, the trial was reassessed for DBH at the age of 9.5 years in March 2021.

At the Cuddon site, single-tree plots were established in 70 blocks with 24 trees in each block. The trial consists of 23 families with different number of individuals per family ranging from 30 to 71, totalling 1680 trees. The trial was assessed for height at age 1.6 years in April 2013 and again at the age of 2.3 years in December 2013 for form and DBH. Recently, the trial was reassessed for DBH at the age of 8.9 years in August 2020.

Sampling strategy

For the Cuddon site, a maximum of 20 surviving trees with a diameter (DBH) above 50 mm were randomly selected from each family for sampling. 455 cores were cored in total at the Cuddon site in October 2020. For the McNeil site, 15 trees with a diameter (DBH) above 50 mm were randomly selected from each family for sampling. 315 cores were cored in total at the McNeil site in May 2021.

Coring

A bark to bark 14 mm diameter core including the pith was extracted using a purpose-built corer at ~0.5 m stem height. The cores were labelled and packed into plastic bags to avoid drying during the day.

Heartwood quantity (heartwood diameter)

The heartwood diameter in the stem was assessed by measuring the heartwood length with a ruler on the core samples in the green state on the day of coring. The heartwood was highlighted by immersing cores into an aqueous 0.1% solution of methyl orange that changed heartwood colour to pink while the sapwood remained yellow. Additionally, the length of the core (without bark) was measured.

Heartwood quality (extractive content)

The core samples were then oven-dried at 60°C for a week. Extractive content was predicted from Near Infrared (NIR) spectra taken on the sanded tangential-radial surface of the oven-dried cores using a fibre optics probe. A maximum of six measurements along the heartwood every 0.5 cm cantered on the pith were collected. Heartwood extractive content of each NIR measurement was predicted with a previously developed calibration (Li & Altaner, 2016) and the average heartwood extractive content for the tree was calculated by averaging the radial values.

Data analysis

Data was analysed with the R software (R Core Team 2019). Univariate analyses were simplified from a general model including a fixed overall mean and random replicate, plot and additive effects to the following model

$$y = Xb + Zf + r$$

Where y is the vector of phenotypic observations for a single site, b is the vector of the fixed effect of the (overall mean), f the vector of additive genetic effects and r is the vector of the residuals. X and Z are incidence matrices linking the phenotypes to the overall mean and additive genetic values vector respectively (Apiolaza 2012).

The model was fitted with the ASReml package (Gilmour et al., 2009) to generate the correlation between the traits' phenotypic and genotypic variation. The phenotypic and genotypic variation was estimated to compute the narrow sense half-sib heritability (h^2) of each trait according to

$$h^2 = \frac{\text{var}(A)}{\text{var}(Y)} = \frac{4\sigma_f^2}{\sigma_f^2 + \sigma_b^2 + \sigma_r^2}$$

Where σ_f^2 is the additive genetic variance for the family; σ_b^2 is the variance for the block and σ_r^2 is the residual variance. The heritability estimated in this study assumed a relationship coefficient among families of one quarter, i.e. true half-sibling progeny.

The coefficient of genetic variation (CGV) for each trait was determined using the equation below.

$$CGV = \frac{\sqrt{4x\sigma_f^2}}{\text{population mean}}$$

RESULTS

The summary statistics of the measurements in the NZDFI *E. quadrangulata* breeding population at McNeil at age 9.6 years old and at Cuddon at age 9.1 years old are given in Table 1. The main traits of interest are natural durability (i.e. extractive content) and heartwood quantity (i.e. heartwood diameter). Table 2 lists the correlation for the traits between the two sites.

Table 1: Descriptive statistics and heritability (h^2) (95% confidence interval in brackets - 95% CI spanning 0 are coloured grey) for *E. quadrangulata* wood properties for McNeil at age 9.6 years and for Cuddon at age 9.1 years; Coefficient of phenotypic variation (CPV) and Coefficient of genetic variation (CGV). *For the Cuddon site DBH was measured at age 8.9 years and for the McNeil site at age 9.1 years.

Trait	Site	Mean	Standard Deviation	Min	Max	CPV (%)	CGV (%)	h^2 ($r_c = 0.25$)
DBH (mm)*	McNeil	113.28	29.92	51	204	26.41	10.15	0.15 (-0.03, 0.32)
	Cuddon	105.12	20.15	51	167	19.16	14.01	0.53 (0.16, 0.86)
Core length (mm)	McNeil	99.93	24.91	40	183	24.93	9.08	0.14 (-0.07, 0.32)
	Cuddon	95.64	17.96	42	151	18.78	11.83	0.39 (0.08, 0.70)
Heartwood diameter (mm)	McNeil	45.97	26.97	0	115	58.67	39.80	0.46 (0.08, 0.78)
	Cuddon	33.52	26.64	0	110	79.45	40.90	0.27 (0.02, 0.51)
Sapwood diameter (mm)	McNeil	53.95	16.88	0	125	31.30	30.87	0.96 (0.33, 1.44)
	Cuddon	62.04	23.49	13	141	37.87	31.98	0.70 (0.21, 1.10)
Extractives content (%)	McNeil	2.79	1.53	0.77	12.59	55.05	23.58	0.19 (-0.06, 0.42)
	Cuddon	5.44	2.96	0.65	22.68	54.30	NA	NA

Table 2: Genetic correlation (r_g) between 9.6 year old *E. quadrangulata* at McNeill and 9.1 year old *E. quadrangulata* at Cuddon for different traits (95% CI in brackets – 95% CI spanning 0 are coloured grey).

Traits	DBH	Core length	Heartwood diameter	Sapwood diameter	Extractive content
Correlation between sites (r_g)	-0.18 (-0.89, 0.49)	0.32 (-0.48, 1.09)	0.62 (0.11, 1.13)	0.57 (0.18, 0.99)	ND

Tree diameter

The trees in the two trials were of similar diameter, but diameter was more variable in the McNeil site. This can be deduced from DBH (over bark diameter at 1.3 m stem height) and core length (diameter under bark at ~0.5 m stem height) (Table 1). The phenotypic correlation ($r^2 = 0.82$) between core length and DBH was very similar for the two *E. quadrangulata* sites (Figure 1). This is consistent with what was found for other NZDFI breeding populations.

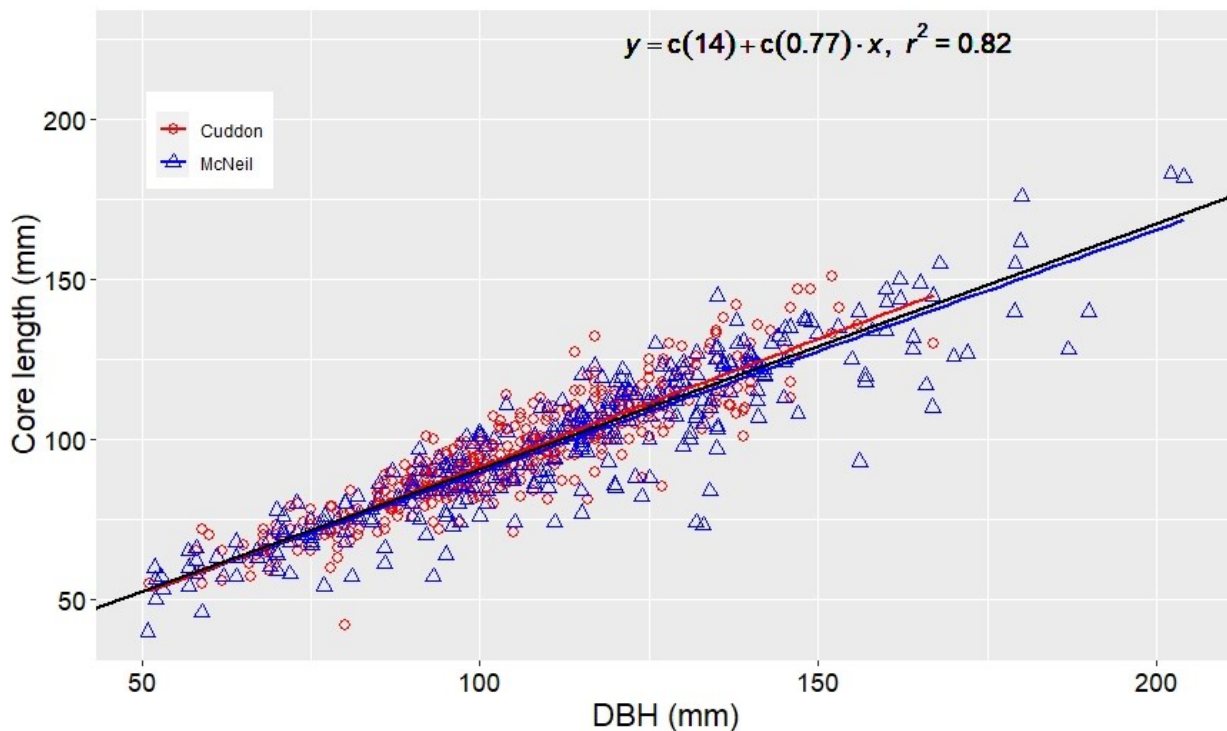


Figure 1; Relationship between core length and DBH for *E. quadrangulata* at McNeil (blue triangles) and Cuddon (red circles).

The heritability estimates for tree diameter were higher for the Cuddon site (DBH $h^2 = 0.53$; core length $h^2 = 0.39$) than for the McNeil site (DBH $h^2 = 0.15$; core length $h^2 = 0.14$) but with overlapping 95% confidence intervals (Table 1). The small number of families (~20) in these trials contributed to the wide confidence intervals. Heritability estimated of this magnitude are not uncommon for tree diameter. No correlations (CI_{95} including 0) were found for tree diameter between the two sites (DBH -0.18 ; $CI_{95} -0.89, 0.49$ and core length 0.32 ; $CI_{95} -0.48, 1.09$) (Table 2). This can be also seen from the different rankings of family medians shown in Figure 2.

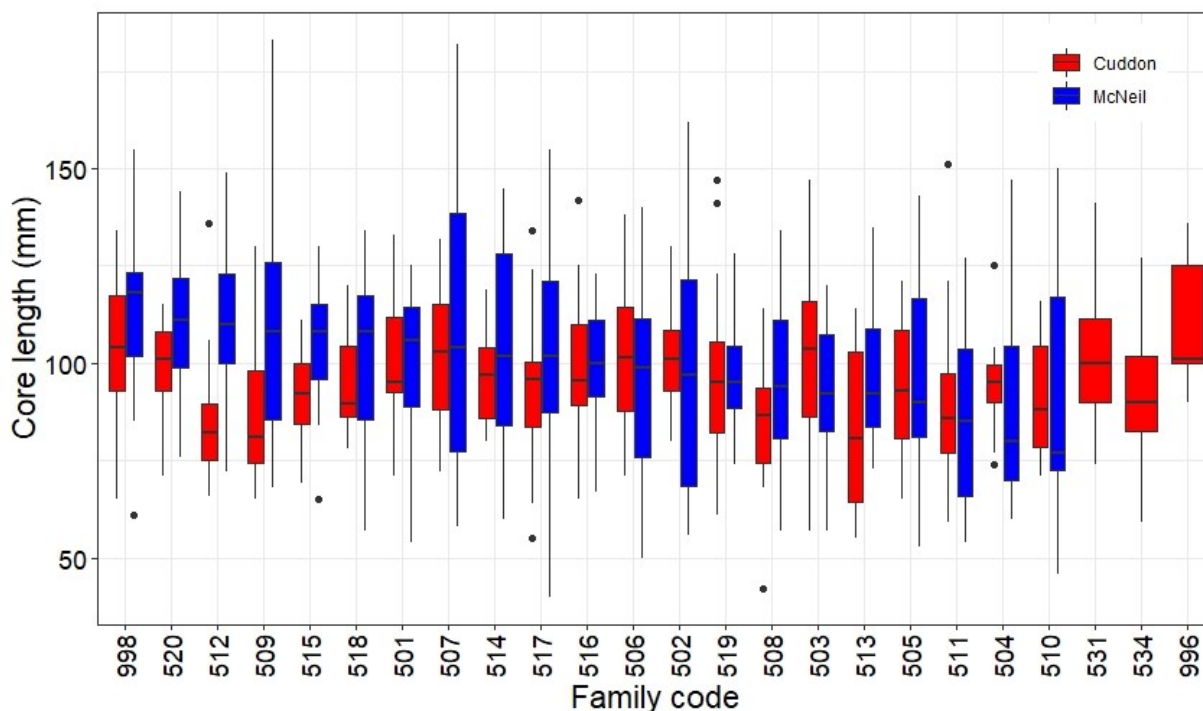


Figure 2: Boxplots for core length of *E. quadrangulata* families planted at McNeil (9.6 years old) (blue) and Cuddon (9.1 years old) (red) ranked by family median of the McNeil site.

Heartwood quantity

Site seemed to have an effect on heartwood formation. While the trees which were sampled on the two sites were of similar diameter (mean core length 99.9 mm for McNeil and 95.6 mm for Cuddon) trees at McNeil (46.0 mm) had a larger heartwood diameter than those at Cuddon (33.5 mm) (Table 1). Consequently the sapwood was wider at the Cuddon (62.0 mm) than the McNeil site (54.0 mm). Site effects on heartwood formation have been found for other NZDFI species. However, the nature of relevant site variables is unknown and subject to investigation.

Heartwood diameter as well as sapwood depth were heritable. While heritability for heartwood diameter was comparable to that of core length or DBH, sapwood depth was under stronger genetic control (Table 1). Previously assessed *E. bosistoana* (Li, Apiolaza et al. 2018) and *E. globoidea* (SWP-T092) populations, did not show this marked difference in heritability of heartwood and sapwood diameter, but it needs to be kept in mind that the confidence intervals were overlapping in the present study. The limited number of families (~20) in these *E. quadrangulata* trials contributed to the larger confidence intervals and need to be confirmed.

Heartwood diameter ($r_g = 0.62$) as well as sapwood diameter ($r_g = 0.57$) were correlated between the 2 sites suggesting that families selected in one site were also performing well in the other (Table 2, Figure 3). Strong genetic correlation for these traits between two sites were also reported for *E. bosistoana* (Li, Apiolaza et al. 2018).

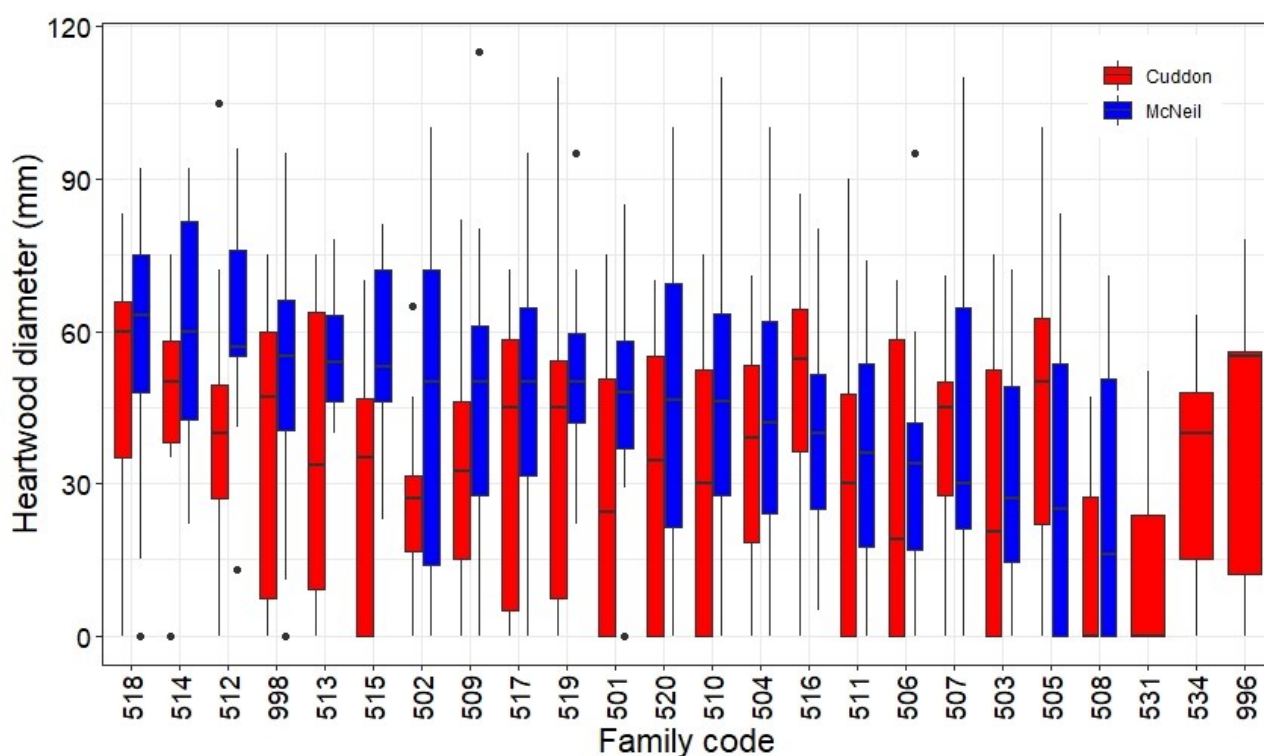


Figure 3: Boxplots for heartwood diameter of *E. quadrangulata* families planted at McNeil (9.6 years old) (blue) and Cuddon (9.1 years old) (red) ranked by family median of the McNeil site.

Heartwood quality

The mean extractive content predicted from NIR spectra heartwood (Table 1) was with 2.79% (McNeil) and 5.44% (Cuddon) low compared to that of *E. bosistoana* (7.5-9.6% at age 7 years old (Li, Apiolaza et al. 2018)) and *E. globoidea* (9% at age 8 years old SWP-T092) trials. The low extractive contents from NIR predictions were confirmed by ASE ethanol extraction of 13 cores from the Cuddon and 12 cores from the McNeil site, averaging 5.45% (Figure 4).

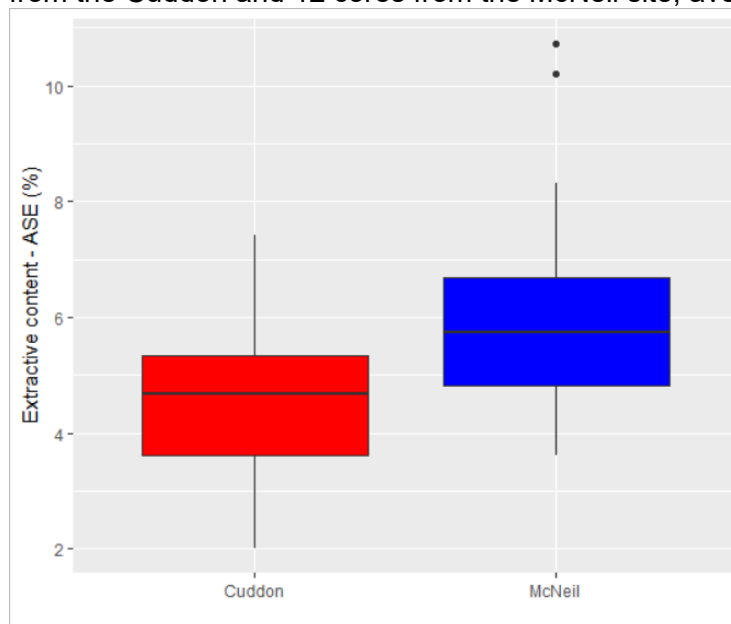


Figure 4: Extractive content determined by ASE ethanol extraction of randomly chosen cores from the Cuddon ($n = 13$) and McNeil ($n=12$) sites.

The low extractive content as well as the small number of families in in these *E. quadrangulata* trials were likely contributing to the uncertainty about the heritability estimated of predicted extractive content (0.19 McNeil; ND Cuddon) (Table 1). This was in contrast to previous assessments of *E. globoidea*, where genetic control of heartwood extractive content was high (e.g. SWP-T092), but lower heritability was reported for predicted extractive content in *E. bosistoana* (Li, Apiolaza et al. 2018). The low heritability contributed also to different rankings between the two sites (Table 2 and Figure 5).

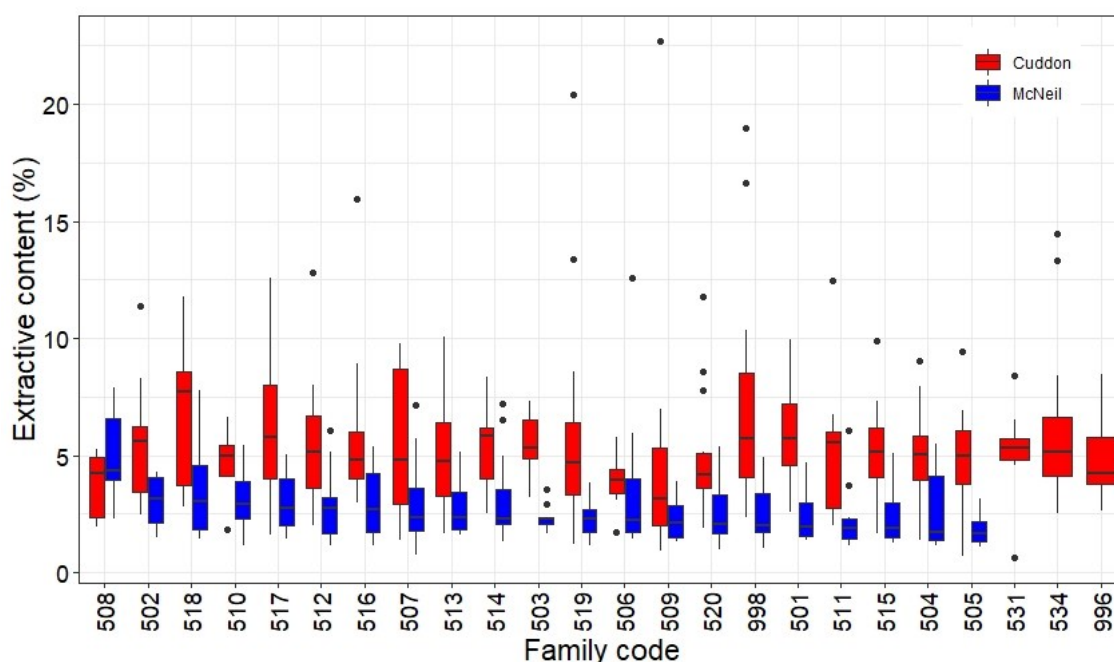


Figure 5: Boxplot for extractive content of *E. quadrangulata* families planted at McNeil (9.6 years old) (blue) and Cuddon (9.1 years old) (red) ranked by family median of the McNeil site.

Genetic correlation between traits

Genetic correlations between the traits have been determined (Table 3). As expected core length was strongly correlated to DBH on both sites (0.95 McNeil; 0.99 Cuddon), giving confidence in the data.

Strong negative correlations were found between sapwood diameter and heartwood diameter (-0.86 McNeil; -0.85 Cuddon). As genetic correlations between heartwood diameter and core length or DBH were uncertain, the results suggest that selection for heartwood quantity cannot be done through proxy measurements of DBH for *E. quadrangulata* at age ~9 years old. It also follows that more heartwood is produced by trees with less sapwood not bigger trees. These results differed to that reported for *E. bosistoana* (Li, Apiolaza et al. 2018) and *E. globoidea* (SWP-T092) where sapwood diameter as well as tree diameter were correlated to positively to heartwood diameter. The genetic correlations between predicted extractive content and the other traits were negative but uncertain (i.e. had a wide 95% CI spanning 0). However an unfavourable negative genetic correlation between heartwood diameter and predicted extractive content was also reported for other NZDFI species *E. bosistoana* (Li, Apiolaza et al. 2018) and *E. globoidea* (SWP-T092).

Table 3: Genetic correlation between heartwood traits for 9.6 year old *E. quadrangulata* at McNeil (above diagonal) and for 9.1 year old *E. quadrangulata* at Cuddon (below diagonal). 95% CI in brackets – values with a 95% CI spanning 0 are coloured grey.

<i>Traits</i>	<i>DBH</i>	<i>Core length</i>	<i>Heartwood diameter</i>	<i>Sapwood diameter</i>	<i>Extractive content</i>
DBH		0.95	0.68	-0.22	-0.34
		(0.81, 1.08)	(0.26, 1.07)	(-0.89, 0.42)	(-1.23, 0.59)
Core length	0.99		0.40	0.10	-0.36
	(NA)		(-0.22, 1.06)	(-0.63, 0.80)	(-1.38, 0.64)
Heartwood diameter	-0.40	-0.30		-0.86	-0.45
	(-1.05, 0.21)	(-0.98, 0.38)		(-1.06, -0.65)	(-1.19, 0.30)
Sapwood diameter	0.80	0.77	-0.85		0.35
	(0.53, 1.08)	(0.46, 1.07)	(-1.06, -0.63)		(-0.27, 0.98)
Extractive content	NA	NA	NA	NA	

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