



# **Technical Report**

# Title: Genetic variation in collapse and other wood properties of *Eucalyptus quadrangulata* at mid-rotation age

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## EXECUTIVE SUMMARY

This report is a draft chapter of Vikash Ghildiyal's PhD thesis exploring genetic and technological solutions to reduce collapse in eucalyptus timbers. In this chapter 2 NZDFI *E. quadrangulata* breeding trials have been assessed for collapse on cores and other wood properties. This work was financially supported by SWP under SWP-WP131 Milestone 4.

Literature reports of *E. quadrangulata* timber featuring collapse were confirmed. Heartwood collapse was under genetic control and could be reduced in a future plantation resource by appropriate selections.

However, these trials planted in 2011 included only ~20 families, and therefore there is limited selection potential. The majority of NZDFI's *E. quadrangulata* breeding population has been planted in 2016 and is awaiting phenotyping in the near future.

## **3.1 Introduction**

*E. quadrangulata* known as white-topped box, is a medium to large hardwood growing on the better soils of the coast and adjacent ranges in New South Wales and southern Queensland. It is considered a common commercial species from native stands in its areas of occurrence (Bootle, 2005). *E. quadrangulata* timber has with a stiffness of 18 GPa and a strength of 163 MPa good mechanical properties (Bootle, 2005). Its heartwood is rated class 2 for in-ground durability, i.e. lasting 15 to 25 years in service (AS5604, 2005). It has uses in heavy construction, for poles and posts, crossarms and railway sleepers.

*E. quadrangulata* was selected as one of five species for the NZDFI breeding programme based on its good in-ground durability and fast early growth in trial plantings (Nicholas & Millen, 2012). Apart from growth and health, the breeding programme includes favourable wood properties as key traits to ensure high-value of the timber (Millen et al., 2018). For *E. quadrangulata* these are natural durability and drying collapse, which has been observed for the species (Bootle, 2005; Poynton, 1979).

Checking and collapse have been found to vary within trees, between trees and also between species (Chafe et al., 1992; Kube & Raymond, 2002). Problems appear to be most severe near the stump and decrease along the length of the stem (Chafe, 1985; Pankevicius, 1961; Purnell, 1988). Sapwood is less prone to collapse than heartwood, for which a sharp radial decline towards the pith was reported (Chafe, 1986). Collapse was also found to be negatively correlated with basic density (Chafe, 1985), and positively to extractive content for several eucalypt species (Chafe, 1987).

Technical solutions to mitigate drying collapse have been developed (Ananías et al., 2020; Chafe, 1992; Dawson et al., 2020). However, strategies such as appropriate sawing techniques (Campbell & Hartley, 1978; Chafe, 1992; Jacobs, 1979), drying pre-treatments (Ellwood, 1953; Haslett & Kininmonth, 1986; Kong et al., 2018; Lee & Jung, 1985; Vermass & Bariska, 1995; Zhang et al., 2011) or reconditioning (Chafe et al., 1992; Jacobs, 1979) are not necessarily economical (Blakemore & Northway, 2009; Chafe et al., 1992) and occur ongoing costs. Tree breeding, exploiting the genetic control of within species variation has the potential to eliminate the checking and collapse problem in the longer term (Blakemore & Northway, 2009; Kube & Raymond, 2005).

This study evaluates tree breeding as a tool for controlling collapse in *E. quadrangulata*. The objectives of this study were: 1) assessing genetic parameters and amount of genotype by environment interactions for tangential collapse, extractive content, basic density, core length and heartwood diameter in two ~9-year-old *E. quadrangulata* breeding trials as well as 2) evaluating the relationships between collapse and other traits relevant to the NZDFI breeding program.

## 3.2 Material and methods

## 3.2.1 Trials

Two open-pollinated *E. quadrangulata* family progeny trials were established in 2011 at McNeil, Wairarapa, and Cuddon, Marlborough, New Zealand. 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. The trial was reassessed for DBH at the age of 9.5 years in March 2021. Core samples were extracted from 315 trees at the age of 9.6 years in April 2021.

The description of the Cuddon trial (located in Marlborough, Blenheim), sampling strategy and coring technique were described elsewhere.

## 3.2.2 Core length, 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 in an aqueous 0.1% solution of methyl orange that changed heartwood color to pink while the sapwood remained yellowish (Figure 1). Additionally, the length of the core (without bark) was measured.



Figure 1. E. quadrangulata cores with heartwood dyed pink after application of methyl orange

## **3.2.3 Drying core samples**

After measuring core length and heartwood diameter, the core samples were then oven-dried at 60°C for a week before the NIR measurements.

## 3.2.4 Extractive content

Extractive content was measured using Near Infrared (NIR) spectroscopy. NIR spectra were taken on the sanded tangential-radial surface of the oven-dried cores using a fibreoptics probe connected to a Bruker Tensor 37 spectrometer. A maximum of six measurements spaced 0.5 cm apart along the heartwood and centred around the pith were acquired for each core. Heartwood extractive content of each NIR measurement was predicted with an updated version, augmented with *E. quadrangulata* samples, of a previously developed method for other (Li & Altaner, 2016) and the average heartwood extractive content for the tree was calculated by averaging the radial values per core weighted by the representative heartwood area.

## 3.2.5 Maximum tangential shrinkage

After the NIR measurements, the cores were equilibrated to a stable moisture content at 60% relative humidity and 25°C. The maximum tangential shrinkage in the core, separately for heartwood and sapwood, was calculated according to the method described elsewhere.

## 3.2.6 Basic density

The basic density of the cores was calculated from dry-wood mass and green volume. The green volume was measured using water-displacement.

Basic density = 
$$\frac{W_{dry}}{V_{green}}$$

Where,

W = Weight (g), V = Volume (cm<sup>3</sup>)

## **3.2.7** Statistical analysis

Data were analysed with the R software (R Core Team 2021). Descriptive statistics including the coefficient of phenotypic variation (CPV) were calculated for each trait. The reported as Pearson correlation coefficient was reported as phenotypic correlation between traits.

## **Genetic analysis**

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 (assumed to be identically and independently normally distributed) residuals. X and Z are incidence matrices linking the phenotypes to the overall mean and additive genetic values vector, respectively (Apiolaza, 2012).

Subsequently the correlation between the two trials were determined by expanding the above equation by stacking up the vectors, in such a way that b, f and r contain the values for both trials (Apiolaza, 2012).

The models were fitted with the ASReml package (Gilmour et al., 2009) in the R software (R Core Team 2021). Heritability and genetic correlations between traits were determined according to the standard formulas described elsewhere.

## 3.3 Results and discussion

The summary statistics of the measurements in the NZDFI *E. quadrangulata* breeding populations at McNeil at age 9.6-year-old and at Cuddon at age 9.1-year-old are given in Table

1.

Table 1. Descriptive statistics and heritability $(h^2)$ (95% confidence interval (CI <sub>95</sub> ) in brackets) for E.
quadrangulata wood properties at McNeil aged 9.6 years ( $n = 315$ ) and at Cuddon aged 9.1 years ( $n$

Trait	Sites	Mean	Standard Deviation	Min	Max	CPV (%)	CGV (%)	$h^2 (rc = 0.25)^{**}$
Tangential collapse HW (%)*	Cuddon	19.61	5.80	0.62	33.05	29.58	16.38	0.31 (-0.01, 0.61)
	McNeil	17.34	4.40	4.61	29.60	25.37	15.38	0.37 (-0.01, 0.73)
Tangential collapse SW (%) *	Cuddon	16.80	5.84	0.14	31.31	34.76	8.27	0.06 (-0.09, 0.20)
	McNeil	13.49	3.52	5.95	24.06	26.09	0.03	Not heritable
	Cuddon	95.32	17.86	42	151	18.74	11.20	0.35 (0.05, 0.63)
Core length (mm)	McNeil	99.93	24.91	40	183	24.93	9.08	0.14 (-0.07, 0.32)
Heartwood	Cuddon	33.34	26.62	0	110	79.84	41.97	0.27 (0.02, 0.52)
diameter (mm)	McNeil	45.97	26.97	0	115	58.67	39.80	0.46 (0.08, 0.78)
Sapwood diameter (mm)	Cuddon	61.76	23.69	0	141	38.36	32.64	0.72 (0.25, 1.12)
()	McNeil	53.95	16.88	0	125	31.30	30.87	0.96 (0.33, 1.44)
Future atimes contant	Cuddon	5.48	3.00	0.65	19.82	54.74	0.03	Not heritable
Extractives content (%)	McNeil	2.79	1.53	0.77	12.59	55.05	23.58	0.19 (-0.06, 0.42)
Basic density (g/cm <sup>3</sup> )	Cuddon	0.66	0.04	0.53	0.97	6.06	4.93	0.54 (0.14, 0.89)
	McNeil	NA	NA	NA	NA	NA	NA	NA

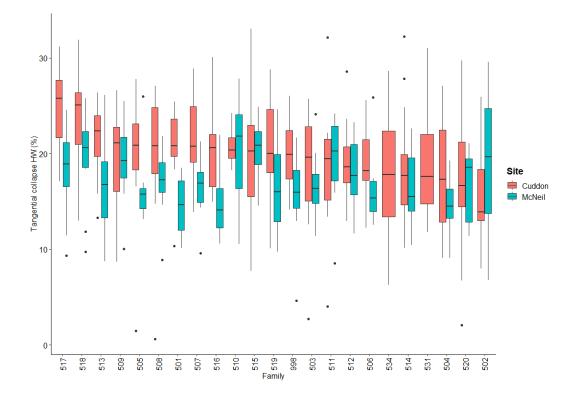
= 445); grey if  $CI_{95}$  includes 0; CPV: coefficient of phenotypic variation and CGV: coefficient of genetic variation.

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\*maximum tangential shrinkage, \*\*rc = 0.25 is the relatedness coefficient for assumed half siblings

#### 3.3.1a. Collapse

The heritability estimates and coefficients of genetic variation for tangential collapse in the heartwood were similar in both trials (Cuddon:  $h^2 = 0.31$ ; CGV = 16.38%, McNeil:  $h^2 = 0.37$ ; CGV = 15.38%) (Table 1). The heritability of tangential collapse in the heartwood fell into the range of most reported estimates for other eucalypts such as for *E. nitens* (0.11-0.61) (Hamilton et al., 2004; Kube, 2005), *E. dunnii* (0.63) (Harwood et al., 2005), *E. grandis* (0.29) (Bandara, 2006) or *E. globoidea* (0.30) (Iyiola et al., 2022). However, care must be taken when comparing these results as the applied relatedness coefficient (rc) differed and is typically unknown. The heritability estimates in conjunction with the coefficient of genetic variability indicated that heartwood collapse can potentially by reduced through selection in *E. quadrangulata*. Family rankings for tangential collapse in heartwood are displayed in Figure 2.



*Figure 2. Boxplots for tangential collapse in heartwood of E. quadrangulata families planted at McNeil (9.6-year-old - blue) and Cuddon (9.1-year-old - red) ranked by family median of the Cuddon site* 

Collapse in the sapwood was not as pronounced as in heartwood and not heritable (Table 1). Collapse in the sapwood is of minor concern as the target product is heartwood and little sapwood can be recovered during solid wood processing.

#### 3.3.1b Tree size

Sapwood has no natural durability. Consequently, if *E. quadrangulata* is grown for its durable wood, heartwood, not the whole stem, is a more meaningful growth measure. Heartwood diameter was under genetic control in both trials (Cuddon:  $h^2 = 0.27$ , McNeil:  $h^2 = 0.46$ ) (Table 1). This fell into the range of reported heritability estimates for heartwood diameter for other tree species ranging from 0.27 to 0.71 (Table 2).

The coefficient of genetic variation (CGV) for heartwood diameter was similar in both trials (Cuddon: 41.97%; McNeil: 39.80%) (Table 1) and fell into the range of reported CGVs for other tree species ranging from 14% to 45% (Table 2). Higher CGVs were reported for *E. bosistoana* (51-61%) (Li et al., 2018). A low CGV of 7.58% was reported for heartwood to sapwood ratio in 8-year-old *E. grandis* (Santos et al., 2004).

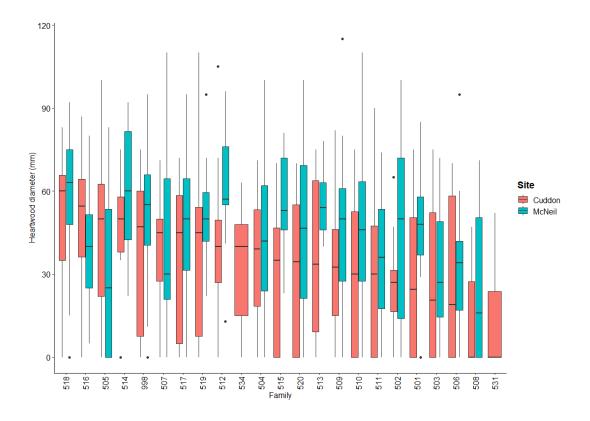
The sapwood band of *E. quadrangulata* was broader than that of the stringybark *E. globoidea* (Bootle, 2005; Iyiola et al., 2022), but similar to what was reported for the more closely related box *E. bosistoana* at age ~7-years (Li et al., 2018).

The estimated heritabilities for heartwood diameter (Cuddon:  $h^2 = 0.27$ ; McNeil:  $h^2 = 0.46$ ) and core length (Cuddon:  $h^2 = 0.35$ ; McNeil:  $h^2 = 0.14$ ) were comparable and lower than for sapwood depth (Cuddon:  $h^2 = 0.70$ ; McNeil:  $h^2 = 0.96$ ) (Table 1). Previously assessed *E*. *bosistoana* (Li et al., 2018) and *E. globoidea* 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 wide and overlapping with the present study. The limited number of families (~20) in these *E. quadrangulata* trials contributed to the larger confidence intervals.

Family rankings for heartwood quantity are displayed in Figure 3.

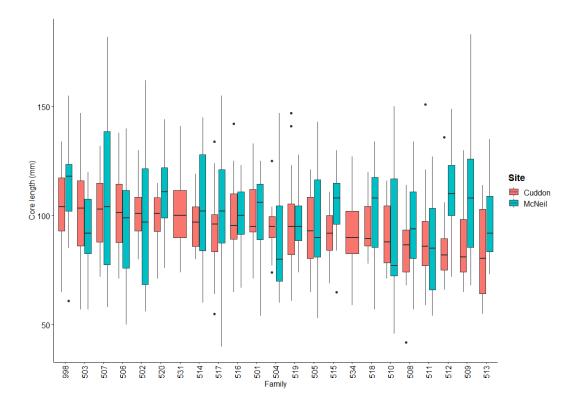
Tree species	<i>h</i> <sup>2</sup>	CGV (%)	rc	Source
E. globoidea	0.52	20.7	0.25	Iyiola et al. (2022)
E. grandis	0.39	7.58	0.25	Santos et al. (2004)
E. bosistoana	0.66 to 0.71	51-61	0.4	Li et al. (2018)
Tectona grandis	0.27	14.5	1	Naranjo et al. (2012)
Pinus sylvestris	0.3 to 0.5	17-20	0.5	Fries and Ericsson (1998);
				Ericsson and Fries (1999)
Sequoia sempervirens	0.4 to 0.5	39-48	1	Meason et al. (2016)
P. radiata	0.49			Cown et al. (1992)
E. cladocalyx	0.3 to 0.38		0.4	Bush et al. (2011)
Larix eurolepis	0.68	10	0.5	Pâques and Charpentier
				(2015)

Table 2. Heritability  $(h^2)$  values and CGV (%) reported for heartwood diameter in different tree species



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

Heritability estimates for core length were higher for the Cuddon site ( $h^2 = 0.35$ ) than for the McNeil site ( $h^2 = 0.14$ ) but with overlapping 95% confidence intervals (Table 1). The heritability estimates for core length reported in this study was similar to what was reported for *E. globoidea* at age 8-year-old (Iyiola et al., 2022). Family rankings for core length are displayed in Figure 4.



*Figure 4. Boxplots for core length of E. quadrangulata families planted at McNeil (9.6-year-old - blue) and Cuddon (9.1-year-old - red) ranked by family median of the Cuddon site* 

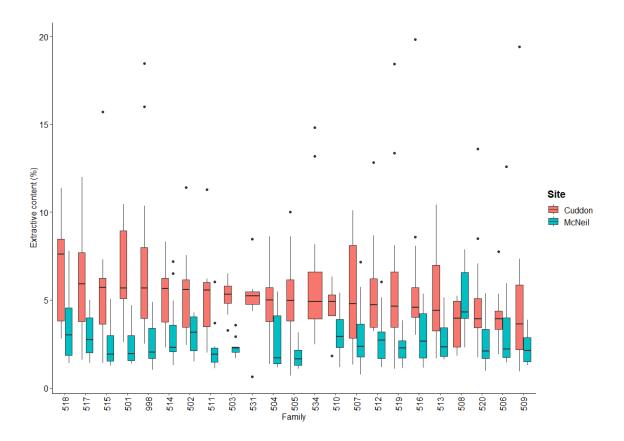
## 3.3.1c. Heartwood quality

For the intended use of *E. quadrangulata* timber, natural durability is a key trait. Like all properties of a natural material, natural durability is variable. A more consistently performing product can be obtained by exploiting genetic control of that variation. However, natural durability is resource and time consuming to measure directly. It has been shown that natural durability is related to heartwood extractives (Li et al., 2020) and that heartwood extractives can be measured efficiently by NIR spectroscopy (Li et al., 2018).

The mean predicted extractive contents in *E. quadrangulata* heartwood at age ~9-year-old was 2.79% (McNeil) and 5.48% (Cuddon) (Table 1). The statistically significant difference (p < 2e-16) in mean values between sites could be attributed by the site effects on heartwood quality. The mean values were lower compared to that of *E. bosistoana* (7.5-9.6%) at age 7-year-old

(Li et al., 2018) and comparable to *E. globoidea* (2.6-3.6 %) at age 8-9-years-old. Family rankings for heartwood quality are displayed in Figure 5.

The low extractive content as well as the small number of families in these *E. quadrangulata* trials were likely contributing to the low heritability for this trait in these trials (Cuddon:  $h^2 =$  NA, McNeil:  $h^2 = 0.19$ ) (Table 1). Heritability estimates for heartwood extractives in the class 1 ground-durable *E. bosistoana* and *E. cladocalyx* were reported to be  $h^2 = 0.2$  to 0.4 at age 7-year-old (Li et al., 2018) and  $h^2 = 0.25$  at age 8-year-old (Bush et al., 2011), respectively. Interestingly, the heritability estimate for extractive content of the class 2 durable *E. globoidea* was reported to be greater than 1 (Iyiola et al., 2022).



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

## 3.3.1d Wood density

Basic density is an essential trait for pulpwood quality as well as for carbon sequestration (Kube, 2005) and also related to properties relevant for structural applications. While basic density was with a heritability estimate of  $h^2 = 0.54$  (CI<sub>95</sub> 0.14, 0.89) (Table 1) under genetic control, the limited genetic variation (CGV = 4.93%) indicated that substantial genetic gain cannot be expected. This was consistent with literature on other eucalypt species, reporting heritability estimates ranging from 0.40 to 0.95 (Apiolaza et al., 2005; Borralho et al., 1992; Davies et al., 2017; Dean et al., 1990; Harrand & López, 2007; Kube et al., 2001; Muneri & Raymond, 2000; Poke et al., 2006; Salas et al., 2014; Van Deventer, 2008) and CGVs ranging from 1.5% to 9.6% (Blackburn et al., 2010; Denis et al., 2013; Hamilton & Potts, 2008; Hamilton et al., 2009; Kien et al., 2008; Stackpole et al., 2010) for basic density. Family rankings for basic density are displayed in Figure 6.

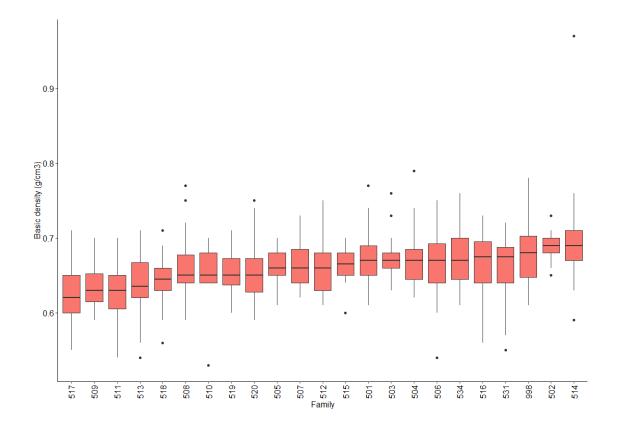


Figure 6. Boxplot of basic density of E. quadrangulata families grown at Cuddon at 9.1-year-old

## **3.3.2** Phenotypic and genetic correlations between traits

The phenotypic and genetic correlations between traits are displayed separately for each site in Table 3 and Table 4. The genetic correlations (ranking correlation of breeding values) for the *E. quadrangulata* families between the traits are visualised in Figure 7 for the Cudden site and Figure 8 for the McNeil site.

Table 3. Phenotypic correlation between traits for 9.1-year-old E. quadrangulata at Cuddon (above diagonal) and for 9.6-year-old E. quadrangulata at McNeil (below diagonal). CI<sub>95</sub> in brackets – values with a CI<sub>95</sub> including 0 are coloured grey)

Traits	Tangential	Tangential	Core length	Heartwood	Sapwood	Extractive	Basic density
	collapse HW	collapse SW		diameter	diameter	content	
Tangential		0.57	0.11	0.29	-0.20	0.16	-0.22
collapse HW		(0.49, 0.64)	(0.00, 0.22)	(0.18, 0.38)	(-0.30, -0.09)	(0.05, 0.27)	(-0.32, -0.11)
Tangential	0.47		0.05	-0.02	0.06	-0.03	-0.19
collapse SW	(0.37, 0.56)		(-0.04, 0.15)	(-0.11, 0.08)	(-0.04, 0.15)	(-0.14, 0.08)	(-0.28, -0.10)
Core length	0.21	-0.02		0.50	0.20	0.10	0.23
	(0.09, 0.32)	(-0.13, 0.09)		(0.43, 0.57)	(0.10, 0.29)	(-0.01, 0.21)	(0.14, 0.32)
Heartwood	0.24	0.01	0.79		-0.75	0.22	0.14
diameter	(0.12, 0.35)	(-0.11, 0.12)	(0.75, 0.83)		(-0.79, -0.71)	(0.11, 0.32)	(0.05, 0.23)
Sapwood diameter	-0.03	-0.04	0.21	-0.43		-0.13	0.02
	(-0.15, 0.09)	(-0.15, 0.07)	(0.10, 0.32)	(-0.52, -0.34)		(-0.24, -0.02)	(-0.07, 0.11)
Extractive content	0.12	0.02	0.18	0.16	0.04		0.15
	(0.00, 0.24)	(-0.10, 0.14)	(0.06, 0.29)	(0.04, 0.28)	(-0.09, 0.16)		(0.04, 0.26)
Basic density	NA	NA	NA	NA	NA	NA	

Traits	Tangential	Tangential	Core length	Heartwood	Sapwood	Extractive	Basic density
	collapse HW	collapse SW		diameter	diameter	content	
Tangential collapse		0.52	-0.33	0.61	-0.52	NA	-0.79
HW		(0.01, 1.05)	(-1.04, 0.41)	(0.03, 1.19)	(-1.03, -0.02)		(-1.22, -0.42)
Tangential collapse	NA		0.33	-0.26	0.38	NA	-0.51
SW			(-0.69, 1.46)	(-1.45, 0.92)	(-0.61, 1.46)		(-1.01, -0.01)
Core length	0.19	NA		-0.37	0.77	NA	0.64
	(-0.66, 0.97)			(-1.05, 0.38)	(0.44, 1.10)		(0.21, 1.08)
Heartwood	0.68	NA	0.40		-0.87	NA	0.03
diameter	(0.25, 1.13)		(-0.22, 1.06)		(-1.05, -0.68)		(-0.62, 0.63)
Sapwood diameter	-0.50	NA	0.10	-0.86		NA	0.33
	(-1.02, 0.04)		(-0.63, 0.80)	(-1.06, -0.65)			(-0.13, 0.81)
Extractive content	0.04	NA	-0.36	-0.45	0.35		NA
	(-0.79, 0.91)		(-1.38, 0.64)	(-1.19, 0.30)	(-0.27, 0.98)		
Basic density	NA	NA	NA	NA	NA	NA	

Table 4. Genetic correlation between traits for 9.1-year-old E. quadrangulata at Cuddon (above diagonal) and for 9.6-year-old E. quadrangulata at McNeil (below diagonal). CI<sub>95</sub> in brackets – values with a CI<sub>95</sub> including 0 are coloured grey)

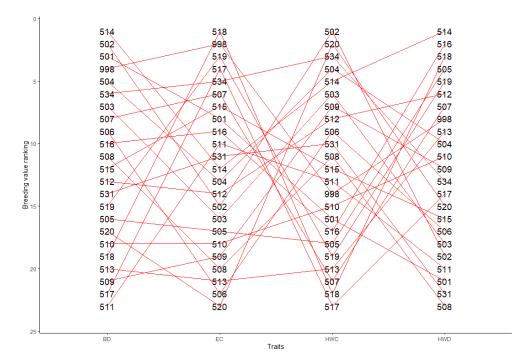


Figure 7. Rankings of family breeding values for basic density (BD), extractive content (EC), tangential collapse in heartwood (HWC) and heartwood diameter (HWD) of 24 E. quadrangulata families at Cuddon at age ~9.1-year-old. Note HWC ranked low to high, while BD, EC and HWD were ranked high to low.

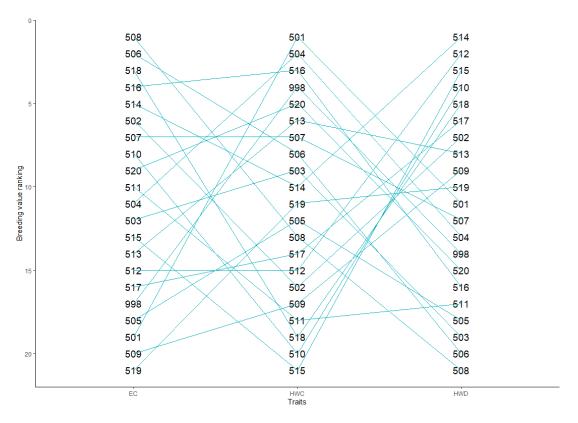


Figure 8. Rankings of family breeding values for extractive content (EC), tangential collapse in heartwood (HWC) and heartwood diameter (HWD) of 21 E. quadrangulata families at McNeil at age ~9.6-year-old. Note HWC ranked low to high, while EC and HWD were ranked high to low.

## 3.3.2a Tree growth

The positive phenotypic correlations between core length and heartwood (Cuddon:  $r_p = 0.50$ ; McNeil:  $r_p = 0.79$ ) as well as sapwood diameter (Cuddon:  $r_p = 0.20$ ; McNeil:  $r_p = 0.21$ ) indicated that in general larger trees have not only more heartwood but also a wider sapwood band (Table 3). Similar positive phenotypic correlation between core length and heartwood diameter was reported for 8-year-old *E. globoidea* ( $r_p = 0.88$ ) (Iyiola et al., 2022), 22.5-yearold *E. tereticornis* ( $r_p = 0.79$ ) (Kumar & Dhillon, 2014), *Acacia melanoxylon* ( $r_p = 0.875$ ) (Knapic et al., 2006), 30 to 37-year-old plantation grown *P. radiata* ( $r_p = 0.71$ ) (Wilkes, 1991); also between DBH and heartwood diameter for 7-year-old *E. bosistoana* ( $r_p = 0.59$ ) (Li et al., 2018). However, core length (tree diameter) was correlated more strongly to heartwood (Cuddon:  $r_p = 0.50$ ; McNeil:  $r_p = 0.79$ ) than sapwood diameter (Cuddon:  $r_p = 0.20$ ; McNeil:  $r_p = 0.21$ ) in both trials.

The genetic correlations between heartwood diameter and core length in the two trials was uncertain (i.e. CI<sub>95</sub> including 0) (Table 4), what was probably a result for the small number of families in the trial. In other studies, strong positive genetic correlations between these traits were reported:  $r_g = 0.99$  for 9-year-old *E. globulus* (Miranda et al., 2014),  $r_g = 0.89$  to 0.98 for 7-year-old *E. bosistoana* (Li et al., 2018),  $r_g = 0.44$  for *E. cladocalyx* (Bush et al., 2011),  $r_g = 0.87$  to 0.92 for *L. kaempferi* (Pâques, 2001), and  $r_g = 0.98$  for 35-year-old *Juglans nigra* (Woeste, 2002). A lower genetic correlation ( $r_g = 0.022$ ) was reported between these two traits for 25-year-old *P. sylvestris* (Fries & Ericsson, 1998). The not perfect genetic correlation between core length and heartwood diameter (Cuddon:  $r_g = -0.37$ ; McNeil:  $r_g = 0.40$  (Table 4) is caused by variation in the sapwood depth (Figure 9).

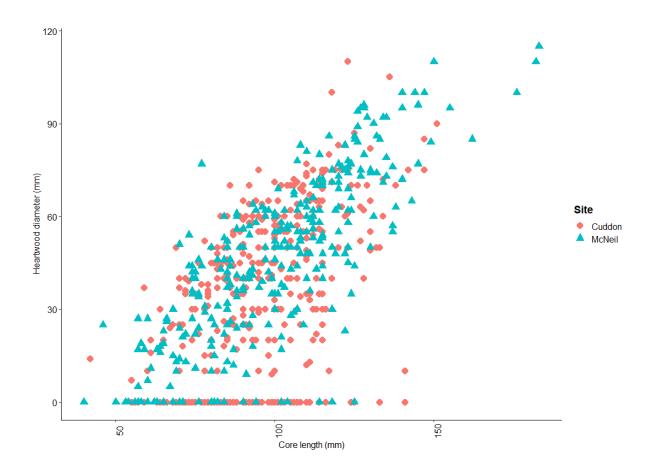


Figure 9. Relationship between core length and heartwood diameter for E. quadrangulata grown at the Cuddon (red circles) and McNeil (blue triangles) sites

The negative correlation between heartwood and sapwood diameter (Cuddon:  $r_p = -0.75$ ; McNeil:  $r_p = -0.43$ ) implied that trees with more heartwood had a narrower sapwood band. The associated stronger genetic correlations (Cuddon:  $r_g = -0.87$ ; McNeil:  $r_g = -0.86$ ) (Table 3) also suggested that genotypes with more heartwood had a narrower sapwood band rather than larger diameter. Yet, positive phenotypic ( $r_p = 0.20$ ) and genotypic correlations ( $r_g = 0.53$ ) between these traits were reported for 8-year *E. globoidea* (Iyiola et al., 2022). A moderate positive phenotypic correlation between sapwood and heartwood diameter was also reported for *Picea mariana* (Hazenberg & Yang, 1991).

#### **3.3.2b Heartwood quality**

The weak (Cuddon:  $r_p = 0.22$ ; McNeil:  $r_p = 0.16$ ) but significant (CI<sub>95</sub> Cuddon: 0.11, 0.32; McNeil: 0.04, 0.28) phenotypic correlation between the heartwood diameter and extractive content (Table 3) indicated that trees with more heartwood also tended to have higher amounts of heartwood extractives. This was different to *E. globoidea*, for which a weak but negative phenotypic correlation ( $r_p = -0.14$ , CI<sub>95</sub>; -0.18, -0.10) was reported between these traits (Iyiola et al., 2022).

No significant genetic correlations were found between these traits, probably due to the small number of families in these trials (Table 4). Significant negative genetic correlations between have been reported for other species:  $r_g = -0.45$  (CI<sub>95</sub>: -0.62, -0.28) for 8-year-old *E. globoidea* (Iyiola et al., 2022) or  $r_g = -0.86 \pm 0.18$  s.e. for 7-year-old *E. bosistoana* (Li et al., 2018), indicating genotypes with more heartwood tend to have lower amounts of extractives in the heartwood. However, a positive genetic correlation between the heartwood diameter and the extractive content ( $r_g = 0.32$  for *L. eurolepis*) were also reported (Pâques & Charpentier, 2015). Ranking correlations, however, between extractive content and other traits were displayed in Figure 7 and **Error! Reference source not found.** but it doesn't seem substantial in the Cuddon site (Figure 7) as no heritability was found for this trait.

## **3.3.2c** Tangential collapse (maximum tangential shrinkage)

Heartwood extractives had a weak positive phenotypic correlation to heartwood collapse (Cuddon:  $r_p = 0.16$ ; McNeil:  $r_p = 0.12$ ), while they did not affect sapwood collapse (Table 3). This was consistent with the argument that extractives close pores in the cell walls, reducing their permeability and consequently resulting in higher negative pressure during drying. It is also consistent with reports that the two traits were positively correlated at the phenotypic level in several eucalypts (Chafe, 1987). However, while significant, these correlations were not

strong indicating that other factors, for example density, contributed to collapse. No genetic correlation was found between these traits (Table 4) indicating that genotypes with high extractive content, i.e. good durability, and low collapse can be found.

Collapse in heartwood and sapwood was negatively correlated with basic density, both at the phenotypic ( $r_p = -0.22$ ) and genetic ( $r_g = -0.79$ ) level (Table 3 and Table 4), suggesting that basic density had a bigger effect on heartwood collapse in *E. quadrangulata* then extractive content. The observed correlation between basic density and heartwood collapse was consistent with previous observations (Chafe, 1986; Chafe, 1985; Kube & Raymond, 2002) and the explanation that denser timber has thicker cell walls which can resist larger negative pressures during drying. While it was suggested that collapse could be included in a breeding program at little additional cost if basic density is measured (Kube & Raymond, 2005), Yi-qiang et al. (2005) pointed out that, although there is a significantly negatively linear relationship between residual collapse and basic density, only less than 50% of the variation in residual collapse was explained by basic density, and consequently reducing achievable genetic gains.

Heartwood collapse was not significantly correlated to tree diameter, i.e. core length (Table 4), consistent with what was reported for *E. globoidea*. In contrast, positive phenotypic and genetic correlations ( $r_p = 0.47$ ,  $r_g = 0.75 \pm 0.10$  s.e.) were reported between the two traits in 12-year-old *E. nitens* (Kube, 2005), implying that trees with bigger diameter were more prone to collapse. On the other hand, heartwood collapse was positively correlated to heartwood diameter on the genetic (Cuddon:  $r_g = 0.61$ ; McNeil:  $r_g = 0.68$ ) and phenotypic (Cuddon:  $r_p = 0.29$ ; McNeil:  $r_p = 0.24$ ) level while it was negatively correlated to sapwood diameter (Table 3 and Table 4). This was not consistent with the independence of these traits in *E. globoidea* (Iyiola et al., 2022). Collapse is associated with wood anatomy and amplified by the

permeability of the tissue. Variation in the occurrence of collapse prone cells between species could contribute to these differences.

Sapwood and heartwood collapse were positively correlated at the phenotypic (Cuddon:  $r_p = 0.57$ ; McNeil:  $r_p = 0.47$ ) (Table 3) and the genetic level (Cuddon:  $r_g = 0.52$ ) (Table 4), implying that trees selected for low collapse in heartwood will also have low collapse in sapwood. Similar correlations ( $r_p = 0.44$ , CI<sub>95</sub>; 0.41, 0.48,  $r_g = 0.64$ , CI<sub>95</sub>; 0.36, 0.90,) were reported between these traits for *E. globoidea* (Iyiola et al., 2022).

#### 3.3.2d Basic density

The phenotypic and genotypic correlations between basic density and core length were positive ( $r_p = 0.23$ ,  $r_g = 0.64$ ) (Table 3 and Table 4), implying the faster growing trees were producing more biomass not only because of a) higher volume but also b) more cell wall material per volume. The genetic correlation between DBH and wood density is well-studied in *E. globulus* and other eucalypts. Correlations vary considerably in both sign and magnitude but tend to be negatively correlated (Downes et al., 2006; Kube et al., 2001; McDonald et al., 1997; Nickolas et al., 2020; Salas et al., 2014; Stackpole et al., 2010). The sign of the correlation may be site dependent and change from negative on wet sites to positive on dry sites (McDonald et al., 1997). This could explain the positive association in this study as the *E. quadrangulata* trees were grown in dryland environments. However, the significant positive correlations of basic density were not observed with heartwood and sapwood diameter.

The weak phenotypic correlation between basic density and extractive content ( $r_p = 0.15$ ) (Table 3) was consistent with the theoretical consideration that additional extractive mass deposited into the wood tissue increases its mass but not its volume. However, in practice the increase in oven-dry mass caused by a higher extractive content is typically small because of the small amounts of extractives normally occurring in wood (Vermaas, 1975) and, specifically in this trial, being less than 5% (Table 1).

#### **3.3.3 Site effects on wood traits**

Phenotypic variation (*P*) can be expressed as the sum of genetic (*G*) and environmentally (*E*) controlled variation as well as the genotype by environment ( $G \times E$ ) interaction:

## $P = G + E + G \times E.$

Differences in the population means for different sites are a measure of *E* and environmental variables that correlate to *E* can inform the choice of future plantings. Genetic correlations between the same trait in different sites can be interpreted as a measure of  $G \times E$  interaction and are relevant for selecting genotypes which perform well in a range of environmental conditions. Consistent rankings across sites (low  $G \times E$  interaction) are reflected as high genetic correlations.

Site seemed to influence heartwood formation in this species. While the sampled trees on the two sites were of similar diameter (mean core length 99.9 mm for McNeil and 95.6 mm for Cuddon), heartwood diameter was larger at McNeil (46.0 mm) than 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 and sapwood diameters were also reported for ~7 year-old *E. bosistoana* (Li et al., 2018) and ~9-year-old *E. globoidea*.

While the trees at McNeil had a larger heartwood diameter, they deposited less extractive in their heartwood (2.79%) compared to those at Cuddon (5.48%) (Table 1). An analogous site effect on heartwood diameter and extractive content was reported for ~7-year-old *E. bosistoana* (Li et al., 2018). Heartwood collapse was affected by site (Cuddon 19.61%, McNeil 17.34%) (Table 1), with the site showing a larger mean for heartwood collapse also having higher

extractive content. This observation matches the understanding of the causes for collapse, i.e. that heartwood extractives reduce the permeability of the cell walls and therefore increase the negative pressure in the cells. Data from more sites is needed to investigate the nature of the site effects on wood properties and make recommendations on appropriate siting of new plantations.

The genetic correlations between the two *E. quadrangulata* breeding trial sites were displayed in Table 5. The comparison of the family breeding value rankings between the two sites were visualized for different traits in Figure 10.

Table 5. 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. CI<sub>95</sub> in brackets; CI<sub>95</sub> spanning 0 are coloured grey

(-0.46, 1.12)
(0.08, 1.13)
(0.23, 0.99)
(-0.82, 0.77)

No genetic correlation was observed for heartwood collapse between the two sites (heartwood collapse 0.03; CI<sub>95</sub> -0.73, 0.85) (Table 5, Figure 10). However, strong genetic correlation was reported for tangential collapse between three sites in *E. nitens* (Kube and Raymond, 2005).

Ranking was also not consistent across the sites for core length (Figure 10), indicated by no or weak genetic correlation (core length 0.36; CI<sub>95</sub> -0.46, 1.12) (Table 5). Nevertheless, heartwood

diameter ( $r_g = 0.62$ ) as well as sapwood diameter ( $r_g = 0.61$ ) were moderately correlated between the two sites suggesting that families selected in one site were also performing well in the other (Table 5, Figure 10). Strong genetic correlation for these traits between two sites were also reported for *E. bosistoana* (Li et al., 2018). Rankings for extractive content were not sustained across the sites (**Error! Reference source not found.**) as no heritability was found for this trait in the Cuddon site resulting in the breeding values close to zero.

Genetic correlations for the combined site analysis are shown in Table 6. Most cross correlations were not significant (CI<sub>95</sub> including 0). It should be noted that the limited number of families (~20) in these *E. quadrangulata* trials contributed to the larger confidence intervals. The observed negative correlation between heartwood collapse (McNeil) and core length (Cudden) across sites (Table 6) was similar to what was observed between these two traits within each site and also consistent with what was observed for these traits for *E. globoidea* between three sites.

Table 6. Genetic cross correlation between traits from 9.6-year-old E. quadrangulata at McNeil and
9.1-year-old E. quadrangulata at Cuddon (95% confidence intervals in brackets-95% CI spanning 0
are coloured grey)

Traits	Site	Core length	HW diameter	Extractive content
HW	Cuddon & McNeil	0.07 (-0.86, 0.98)	0.21 (-0.53, 0.85)	-0.04 (-0.88, 0.88)
Collapse				
	McNeil & Cuddon	-0.71 (-1.23, -0.17)	-0.16 (-0.96, 0.62)	NA
Core length	Cuddon & McNeil		-0.28 (-0.86, 0.26)	-0.03 (-0.82, 0.74)
	McNeil & Cuddon		0.62 (-0.13, 1.37)	NA
HW	Cuddon & McNeil			-0.42 (-1.22, 0.33)
diameter	McNeil & Cuddon			NA

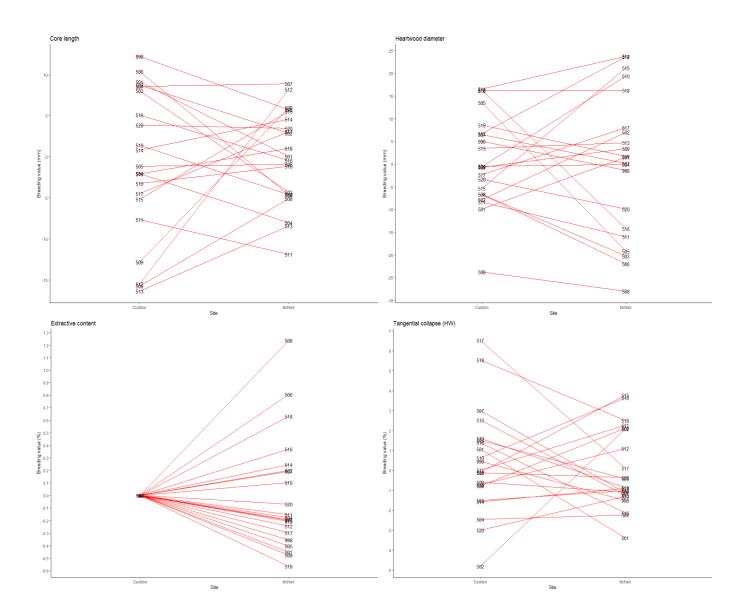


Figure 10. Ranking correlation between family breeding values of two different sites (Cuddon and McNeil) for core length, heartwood diameter, extractive content and tangential collapse heartwood (HW). Note as extractive content was not heritable at the Cuddon site, the related breeding values were 0.

## 3.3.4 Breeding values and multiple trait selection

In general, breeding programmes aim is to improve multiple traits, either by selecting the best or culling the worst families. Selection for one trait has consequences on the genetic gain of others as traits are usually not independent of each other (Table 4). Therefore, selection for multiple traits requires breeding goals, which need to be set by the industry and are ideally based on economic weights (Candy & Gerrand, 1997; Evison & Apiolaza, 2015; Jansson et al., 2017). In the absence of economic weights, superior genetics can be identified by weighing all traits equally and selecting families with above average breeding values in several traits. The main wood property traits of interest in NZDFI's *E. quadrangulata* breeding programme are heartwood collapse (HWC), extractive content (EC), heartwood diameter (HWD) and potentially basic density (BD). The families with superior breeding values for these traits were identified in Table 7. Tallying above average performance of a family for target traits allowed to identify superior genotypes. The relationship between family breeding values of key traits is visualised separately from both sites in Figure 11.

9 Families produced timber with less than average heartwood collapse on both sites (Table 7). However, 13 families met the criteria if selected from each site separately. If additionally, to low collapse also large heartwood diameter is desired the number of families to be selected from both sites reduced to 2. 3 Families in the Cuddon trial and 5 families in the McNeil trial met these criteria. No family met the criteria of good heartwood features i.e. extractives, heartwood diameter and low collapse on both sites. However, two families in the McNeil, which in contrast to the Cuddon trial showed heritability for this trait, trial met these criteria. This is illustrated that a meaningful breeding programme needs to include more than the ~20 families established in these trials to find families with good overall performance.

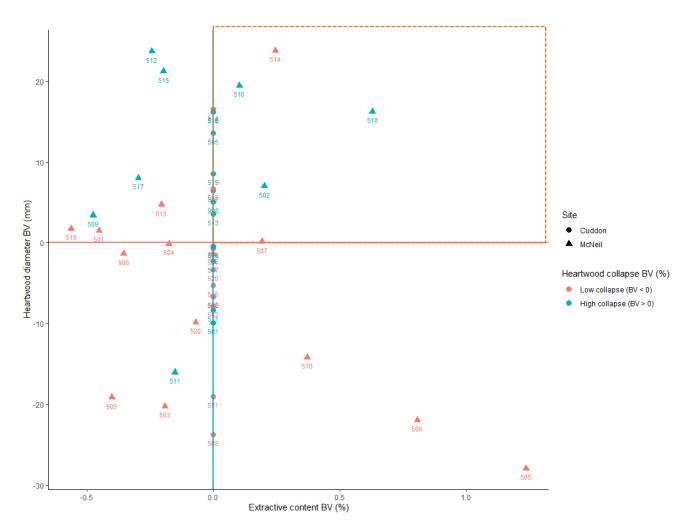


Figure 11. Relationship between family breeding values of heartwood diameter and extractive content for 23 E. quadrangulata families (Cuddon) at age ~9-year-old (circles) and 21 E. quadrangulata families (McNeil) at age ~9.5-year-old (triangles). Families performing above average are located in the orange dashedlined right corner of the quadrant. Families with good (red - low collapse) and bad (blue - high collapse) performance for collapse (HWC) are highlighted.

Table 7. Family breeding values for E. quadrangulata (Cuddon and McNeil) classified for superior (0) and inferior (1) to the population mean

Family		Cuddon site McNeil site											Cuddon all traits	McNeil all traits	Combined all traits		
	BD		EC		HWC		HWD		EC HWC						-		
	BV (%)	Selection	BV (%)	Selection	BV (%)	Selection	BV (mm)	Selection	BV (%)	Selection	BV (%)	Selection	BV (mm)	Selection			
507	0.00	0	9E- 07	0	3.01	1	6.48	0	0.19	0	-1.11	0	0.13	0	1	0	1
514	0.06	0	-1E- 07	1	-1.58	0	16.54	0	0.25	0	-0.90	0	23.77	0	1	0	1
531	- 0.01	1	7E- 08	0	-0.36	0	-19.01	1	NA		NA		NA	NA	2	NA	2
534	0.01	0	1E- 06	0	-2.75	0	-1.35	1	NA		NA		NA	NA	2	NA	2
501	0.02	0	4E- 07	0	1.08	1	-9.91	1	-0.45	1	-3.39	0	1.53	0	2	1	3
502	0.04	0	-5E- 07	1	-4.80	0	-7.80	1	0.20	0	2.08	1	7.01	0	2	1	3
516	0.00	0	4E- 07	0	1.38	1	16.28	0	0.37	0	-2.14	0	-14.20	1	1	2	3
998	0.02	0	3E- 06	0	0.46	1	5.01	0	-0.36	1	-1.50	0	-1.37	1	1	2	3
506	0.00	0	-2E- 06	1	-0.60	0	-6.63	1	0.81	0	-1.08	0	-21.94	1	2	2	4
518	-	1	4E-	0	5.53	1	16.14	0	0.63	0	2.51	1	16.21	0	3	1	4
519	0.04	1	06 2E-	0	1.61	1	8.57	0	-0.56	1	-0.86	0	1.72	0	2	2	4
503	0.01 0.01	0	06 -6E- 07	1	-1.51	0	-6.66	1	-0.19	1	-1.02	0	-20.25	1	2	3	5
508	0.00	1	-1E- 06	1	-0.11	0	-23.74	1	1.24	0	-0.36	0	-27.94	1	4	1	5

512	- 0.01	1	-4E- 07	1	-0.73	0	6.70	0	-0.24	1	1.10	1	23.67	0	3	2	5
515	0.00	1	5E- 07	0	-0.10	0	-5.30	1	-0.20	1	3.79	1	21.22	0	3	2	5
520	-0.02	1	-2E- 06	1	-2.99	0	-3.32	1	-0.07	1	-1.30	0	-9.86	1	3	2	5
504	0.02	0	-2E- 07	1	-2.45	0	-0.44	1	-0.18	1	-2.24	0	-0.11	1	3	3	6
509	- 0.06	1	-1E- 06	1	-0.77	0	-0.69	1	-0.48	1	2.12	1	3.41	0	4	2	6
513		1	-2E- 06	1	2.52	1	3.51	0	-0.21	1	-1.26	0	4.70	0	4	2	6
517	- 0.07	1	1E- 06	0	6.53	1	-2.30	1	-0.30	1	0.10	1	8.03	0	4	2	6
505	- 0.02	1	-7E- 07	1	1.52	1	13.55	0	-0.40	1	-0.42	0	-19.14	1	4	3	7
510	- 0.03	1	-1E- 06	1	0.62	1	-0.51	1	0.10	0	3.64	1	19.44	0	5	2	7
511	- 0.07	1	2E- 07	0	0.02	1	-8.36	1	-0.15	1	2.26	1	-16.02	1	4	4	8

The breeder might not want to consider collapse (or weight the trait less) as *E. quadrangulata* is less prone to collapse compared to *E. globoidea*. In that case, to simultaneously improve the durability (i.e. extractive content) and heartwood diameter in the species, 2 families met the criteria at both sites. However, as extractive content was not heritable for the Cuddon site (Figure 11), the 5 families in McNeil site which met the criteria for good extractives and heartwood diameter could be selected.

Furthermore, *E. quadrangulata* is categorized as a class 2 ground durable wood, i.e. not as durable as *E. bosistoana* (class 1) (AS5604, 2005). In a situation where the wood users are interested in highly ground durable products, intense selection for extractive content would be necessary. As stated earlier, *E. quadrangulata* is prone to collapse but culling the worst families from the breeding population might be sufficient to avoid most processing problems.

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