Specialty Wood Products Research Partnership

UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

Technical Report

Recommended sampling intensity for phenotyping durable eucalypt breeding trials for heartwood quality

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Date: March 2021

Report: SWP-T121

FOREST GROWERS RESEARCH

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EXECUTIVE SUMMARY

The available heartwood quantity (diameter at 0.5 m stem height) and heartwood quality (NIR predicted extractive content) for ten NZDFI breeding trials was analysed with the intention to optimise sampling intensity and consequently reduce resource demands. Heartwood phenotyping is resource intensive and requires older/bigger trees delaying selection decisions and extending breeding cycles.

The analysis concluded that accurate family and individual tree breeding values for heartwood quality can be obtained from the assessment of the heartwood obtained from 8 individuals per family and collecting 6 NIR spectra on each heartwood sample.

Trees suitable for heartwood phenotyping have developed at least 30 mm of heartwood diameter, in order to acquire 6 NIR spectra. Heartwood formation was dependent on tree size, not tree age, with noticeable differences between species and sites.

The proportion of trees in a trial having developed sufficient heartwood for phenotyping (i.e. 30 mm) depended on their size. This proportion was calculated by combining the likelihood of a tree having developed heartwood depending on its diameter and the distribution of diameters in the trial. In turn, this allowed the determination of the number of trees which need to be cored to obtain in average the required number of heartwood samples (i.e. 8).

As corelength (under-bark diameter at 0.5 m stem height) is an impractical measure to decide the timing for heartwood phenotyping, the linear correlations to diameter at breast height (DBH) were established for the species. This allowed the determination of the required number of trees, which need to be cored to obtain the necessary heartwood samples from the most recent growth (i.e. DBH) assessments.

Heartwood phenotyping was costed. Field sampling of a stem core required at least NZ$ 12 and the price of obtaining the extractive content from 6 NIR spectra was NZ$ 4. The phenotyping costs per family in a trial under the above determined conditions can be as low as NZ$ 125 but increase significantly if smaller trees are sampled or the coring equipment is not well maintained.

INTRODUCTION

This report summarises the findings of the work contracted under work plan SWP-WP109 Milestone 1.

NZDFI aims to develop a sustainable domestic resource of naturally ground durable hardwood. There is large variation in durability within a species (Bush et al., 2011; Haupt et al., 2003). As durability is partly under genetic control NZDFI has established a breeding programme to select superior planting stock and ensure a consistently high-quality product. Details of the NZDFI project (www.nzdfi.org.nz), its breeding strategy and heartwood quality assessment have been published elsewhere (Altaner et al., 2017; Millen et al., 2019; Millen et al., 2018). The number and size of trials in the NZDFI breeding programme were summarised in Table 1.

The NZDFI breeding trials are not only assessed for growth and form but also for wood quality including heartwood quantity and heartwood quality. Heartwood quality is the trait in the breeding programme which currently requires the most resources for phenotyping. Heartwood samples were extracted from a tree with a purpose-build corer (Figure 1). Obtaining an intact full diameter core of a tree took a skilled person about 1 min with a good corer. In practice, however, a team of 6 were able to core, label and collect more than 300 cores a day. Once the cores were available, they needed to be assessed for heartwood diameter and corelength in the green state with a ruler. After drying the heartwood quality was assessed by NIR spectroscopy. NIR spectra were acquired every 10 mm along the heartwood of the core with a fibre-optics probe. The time it took to assess a core depended on its heartwood diameter. From experience an average 6 to 8 cores can be assessed per hour by NIR. Previously developed calibrations (chemometric partial least squares regression (PLSR) models) were used to quickly predict extractive content (Li and Altaner, 2018; Li and Altaner, 2019a; Li and Altaner, 2019b; Li et al., 2018), which was shown to relate to mass loss measurements of durability (Li et al., 2020).

*Figure 1: **Top**: corer developed by Callaghan Innovation to extract bark-to-bark stem cores with a diameter of ~14 mm from high density eucalypts. **Bottom left**: Coring an E. globoidea tree using a light-weight battery powered corer. **Bottom right**: labelled E. bosistoana cores with heartwood highlighted pink through staining with the methyl orange pH indicator.*

Table 1: Assessment status of NZDFI breeding populations for heartwood as of February 2021

Species	Trial established	No of families	Age cored (y)
E. bosistoana	Martin 2009	50	8.9
	Lawson 2009	60	6.1
	Cravens 2009	60	6.7
	Avery 2010	40	8.1
	Martin 2010	39	6.6
	Cravens 2010	33	6.8
	Dillon 2012	84	Not assessed
	McNeil 2012	80	Not assessed
	Ngaumu 2012	81	7.8
E. argophloia	Avery 2010	15	Not assessed
	Cuddon 2011	18	Not assessed
	Dillon 2011	18	Not assessed
	Ngaumu 2011	18	Not assessed
E. tricarpa	Avery 2011	18	Not assessed
	Dillon 2011	14	Not assessed
	Trimble 2011	24	Not assessed
	Dillon 2017	15	Not assessed
	Liassaman 2017	16	Not assessed
E. quadrangulata	Martin 2011	-	Not assessed
	Cuddon 2011	22	9.1
	McNeil 2011	20	Not assessed
	Trimble 2011	20	Not assessed
	NZRC Paparoa 2016	72	Not assessed
	Bradshaw 2016	72	Not assessed
E. globoidea	Ngaumu 2011	115	Scheduled 2021
	Atkinson 2011	141	7.6
	Avery 2011	161	9.2

With the availability of heartwood data from ten breeding trials, it was now possible to review the sampling strategy for heartwood phenotyping, with the objective to reduce the required resources, while still obtaining accurate genetic information. The sampling strategy varied between the trials. For some trials all suitable surviving trees were cored, while in others only a maximum of 30 randomly selected suitable trees were selected from each family. As small trees will not survive the extraction of a 14 mm diameter stem core, suitable trees were defined as having a DBH greater than 30 or 50 mm (depending on the trial).

A prerequisite for heartwood assessments is that the trees have formed heartwood. Heartwood formation depends on species, tree size, tree age and site (Hillis, 1987; Taylor et al., 2002). For the NZDFI eucalypts *E. bosistoana*, *E. quadrangulata* and *E. globoidea* stem sections with an under-bark diameter of less than 50 mm typically had no heartwood and stem sections with an under-bark diameter of more than 100 to 150 mm always had heartwood. The latter was more species dependent and indicated differences in sapwood width. Depending on growth rate and site, a suitable proportion of trees with heartwood were present in the breeding trials from age 6 to 10 years old.

The objective of this work was to optimise the sampling strategy for phenotyping the NZDFI breeding trials for heartwood quality. This work aimed to find a balance between the required resources and the accuracy of the selection, which improves (however with diminishing returns) with sample numbers. Specifically the following questions were addressed:

- How many individuals per family need to be sampled to obtain genetic parameters of sufficient accuracy?
- How many NIR spectra need to be acquired per core to obtain genetic parameters of sufficient accuracy?
- What is the earliest timing for heartwood phenotyping?
- What are the costs for heartwood phenotyping?

METHODS

Trials

Analysis of timing of heartwood phenotyping of durable eucalypts considered ten NZDFI breeding trials indicated in Table 1. These trials were of similar design (sets-in-replicates, single tree plot) and scattered throughout Marlborough and Wairarapa. More detailed information is stored in NZDFI's database and as an example the trial which was used for reviewing NIR is described below. Trials were sampled (cored) and phenotyped (heartwood quantity and quality) according to the methodology as described below.

Modelling sampling intensity was based on the intensively sampled open pollinated progeny trial of 141 *E. globoidea* families established in 2011 at Atkinson (Wairarapa) New Zealand. The seed was collected from across the natural range of the species in Australia and from three NZ plantation sites with a known seedlot. The Atkinson trial is a sets-in-replicates, single tree plot design with each family replicated 40 to 80 times across the site. There were 36 trees per plot at a spacing of 2.4 m between rows and 1.8 m within the rows, totalling 8640 trees. There was a total of 240 blocks planted with a one row surround. There were springs in various locations throughout the site. While some of these were avoided when laying out the trial, other wet areas resulted in early losses due the wet soil conditions and 23 plots have been abandoned due to high mortality.
The trial had been assessed in 2015 for growth, form and DBH and was also thinned to 6491 remaining trees. The trial was thinned again in April 2019, removing another 1171 trees. At that time, cores were taken for heartwood assessments.

Coring

All living selected trees with a diameter larger than 30 or 50 mm, depending on the trial, were sampled with a purpose-built corer. A bark to bark 14 mm diameter core including the pith was extracted 50 cm above the ground. The cores were labelled and packed into a cooler box to avoid drying during the day.

Heartwood quantity and quality

The heartwood was highlighted with an aqueous 0.1% solution of methyl orange, which changed to pink on heartwood while it remained yellow on the sapwood. The heartwood diameter in the stem was assessed by measuring the heartwood length with a ruler on the core samples in the green state at the day of coring. The core samples were oven-dried at 60°C for a week.
The cores were then sanded to expose a radial-tangential wood surface. Heartwood quality was assessed by predicting ethanol soluble extractive content from Near Infrared (NIR) spectra. NIR spectra were taken using a fibre optics probe on the sanded surface of the cores every 1 cm along the heartwood. The spectra were acquired from 9,000 to 4,000 cm^{-1} at 4 cm^{-1} intervals and 32 scans were averaged for each spectrum. Heartwood extractive content for individual spectra were predicted with a previously developed chemometric model (Li and Altaner, 2019a).

Costing

The costs of coring the last three NZDFI breeding trials have been used to calculate an average price for each extracted core. This cost included assessment for heartwood quantity but did not include costs for development and replacement of the purpose-built corers. Significant progress in the design of the corers has been made over that period, resulting in halving coring costs. The manufacturing of a new batch of corers has been contracted in February 2021 (Appendix 1). These coring costs of NZ$ 12 to 25 were significantly higher than the published AS$ 2.13 (Downes et al., 1997), but deemed reasonable by RPBC (personal communication). The Australian figure was

estimated from an hourly rate (AS$ 20, crew of 2, 8 h/d) for coring 150 cores a day and therefor did not include selection, traveling, accommodation and green core measurements.
The costs of extractive content predictions by NIR (heartwood quality) on a core depending on its heartwood diameter has been calculated by timing individual steps in the process and estimating labour and equipment costs (Appendix 2). The NIR costs of NZ$ 2 to 12 per core were significantly lower than those reported by Downes et al. (1997) of AS$ 50, which included milling of the sample into a powder.

The following costs were used to calculate the phenotyping costs of a family for heartwood:

- coring a tree: NZ$ 12,
- acquiring one NIR spectra: NZ$ 0.48
- sample preparation per core for NIR: NZ$ 1.11.

These costs were optimistic, accounting already for recent advances in productivity for both, coring and NIR spectroscopy.

Sampling intensity

The family and individual tree breeding values for extractive content were calculated after fitting a random mixed-effects model considering the factors family, block and tree using the lmer function of the lme4 package in R (Bates et al., 2015). The breeding values using all data (trees and spectra per tree) were used as reference when comparing to breeding values obtained for a subset of the available data.

The whole dataset was randomly sampled (without replacement) to investigate the effects of a) trees per family and b) spectra per core on the accuracy of breeding values. Family and individual tree breeding values were computed 25-times for each combination of 2, 4, 8 and 16 trees per family and 2, 4, 6, 8, 10 and 22 NIR spectra per tree. The Spearman ranking correlation between these averages and the whole data reference were reported.

Two strategies for locating the sampling points of NIR spectra on the core were tested; random location of the sampling points within the heartwood or sequentially from the pith outwards (Figure 2). The latter will focus tree selection on the area likely to have the lowest amount of extractives in a stem (AS5604, 2005; Hillis, 1987; Sherrard and Kurth, 1933), i.e. assessing the worst wood close to the pith. Additionally it appears to be suitable for small trees i.e. early selection.

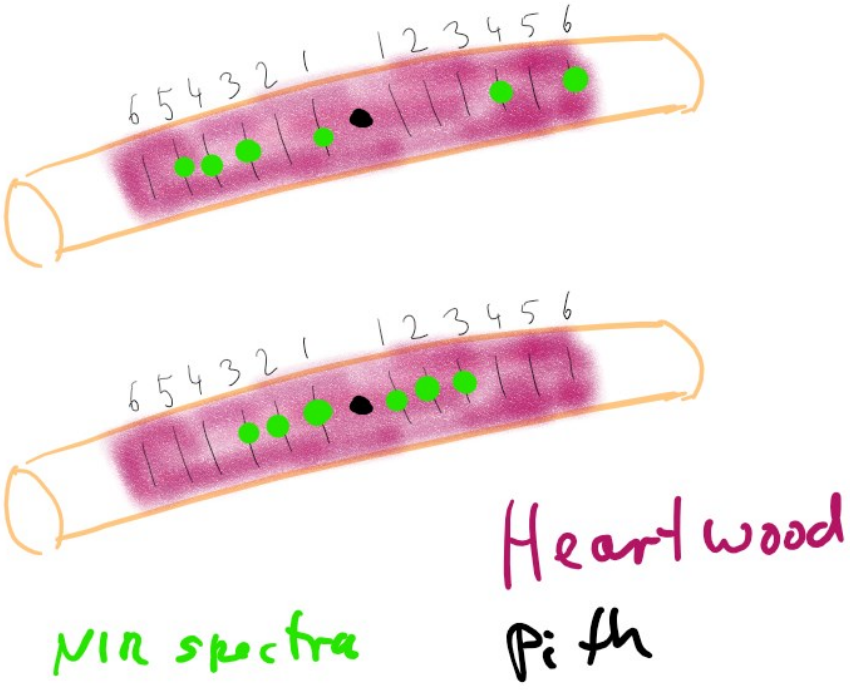

Figure 2: NIR spectra location on cores predicting heartwood extractive content. ***Top****: random placement of 6 spectra in the heartwood of the core.* ***Bottom****: 6 spectra on sequential radial position outwards from the pith.*

Timing of heartwood assessments

A binomial model predicting the likelihood of a tree having formed at least 30 mm diameter heartwood (L_{HW30}) depending on the corelength (CL) was calculated for each trial and each species using the glm function in R (Team, 2020). The constant a determined the 'intercept' and the factor b the rise of the curve.

$$L_{HW30} = \frac{1}{1 + e^{a+b\times CL}}$$

L_{HW30} gives the likelihood of a single tree with a certain diameter to have a heartwood diameter of more than 30 mm. In any stand/trial the trees will have a distribution of diameters, with smaller trees less likely having formed heartwood than bigger trees. For early heartwood phenotyping the number or proportion of trees in a trial of a given mean diameter with the necessary amount of heartwood is of interest. This was modelled as a Gaussian normal distribution (ND) around a trial mean corelength ($\overline{TrialCL}$) and the species dependent coefficient of variation calculated by averaging the coefficient of variations for all available trials of a species ($CVCL_{Species}$).

$$ND_{\overline{TrialCL},CVCL_{Species}}(CL) = e^{\frac{CL - \overline{TrialCL}}{\overline{TrialCL} \times CVCL_{Species}}}$$

The proportion of trees having formed at least 30 mm heartwood (P_{HW30}) in a trial with a normal distribution of sizes (ND) depending on the average corelength ($\overline{TrialCL}$) was calculated by integrating the product of ND and L_{HW30} over corelength (CL) (Appendix 3).

$$P_{HW30}(\overline{TrialCL}) = \frac{\int [ND_{(\overline{TrialCL})}(CL) \times L_{HW30}(CL)]\, dCL}{\int ND_{(\overline{TrialCL})}\,(CL)\, dCL}$$

The average number of trees per family need to be cored ($n_{Trees\ to\ core}$) in a trial with an average corelength ($\overline{TrialCL}$) to obtain in average 8 heartwood trees per family could then be calculated.

$$n_{Trees\ to\ core}(\overline{TrialCL}) = \frac{8}{P_{HW30}(\overline{TrialCL})}$$

Linear regressions between corelength (CL) (and the most recent DBH measurement for each species were obtained. The constant c determined the intercept and the factor d the slope of the relationship.

$$CL = c + d * DBH$$

This allowed to use the more practical measurement of DBH to decide on the timing for heartwood phenotyping by coring at 0.5 m stem height.

RESULTS

Number of individuals per family and number of spectra per core

To optimise the sampling effort for heartwood quality measurements, the effect of the number of trees/individuals per family (coring) as well the number of extractive content predictions per tree/core (NIR spectroscopy) is of interest. Resources are needed for extracting core samples from trees in the field and subsequently acquiring NIR spectra from the cores to assess heartwood quality.

The 2011 *E. globoidea* Atkinson trial was intensively sampled with up to 47 trees containing heartwood per family and up to 23 spectra per core. The full dataset was used to calculate breeding values for NIR predicted extractive content and used as 'true' reference. Subsequently the full dataset was randomly sampled 25 times for each combination of 2, 4, 8 or 16 trees per family and 2, 4, 6, 8 or 22 spectra per core. The ranking correlation coefficients between the breeding values of the full data set and each subsample was used as measure of accuracy of the sampling intensity.

Currently the NZDFI breeding programme identifies families (i.e. seed of a mother tree with unknown pollinators) with favourable wood quality. Within family selection, i.e. identifying the top individuals rather than top families is of interest considering the establishment of seed orchards (Millen et al., 2018) and collecting genetic information on their pedigree (Altaner and Kim, 2020). Within family selections should require a more intensive sampling regime. Therefore this simulation considered both, the ranking correlations of breeding values for families and individual trees.

Describing data

The 2011 *E. globoidea* Atkinson dataset used for this analysis included 141 families with a median of 19 individuals per family and 9 spectra per core. The distribution of individuals per family and number of spectra per core was shown in Figure 3.

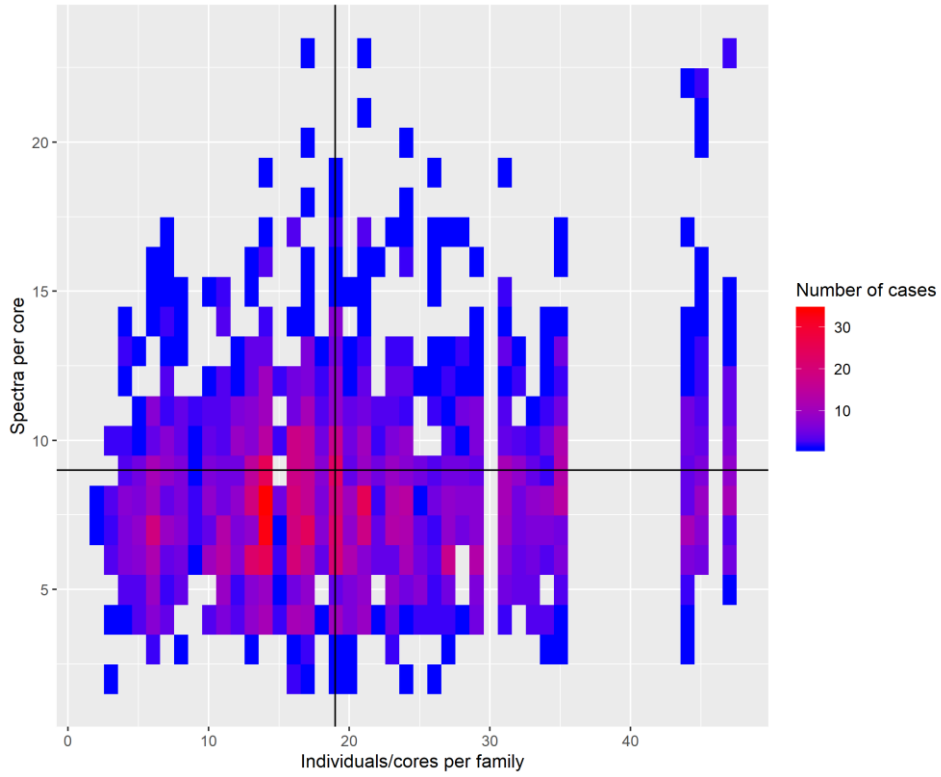

Figure 3: Distribution of heartwood data for the 2011 E. globoidea Atkinson trial including. A median of 19 trees per family had developed heartwood (vertical line). A median of 9 spectra were taken from each core (horizontal line).

The mean NIR predicted extractive content was 9.5%. No difference was detected between the two radii of the full diameter core (Figure 4). This was expected as the two sides were randomly assigned during NIR spectra acquisition.

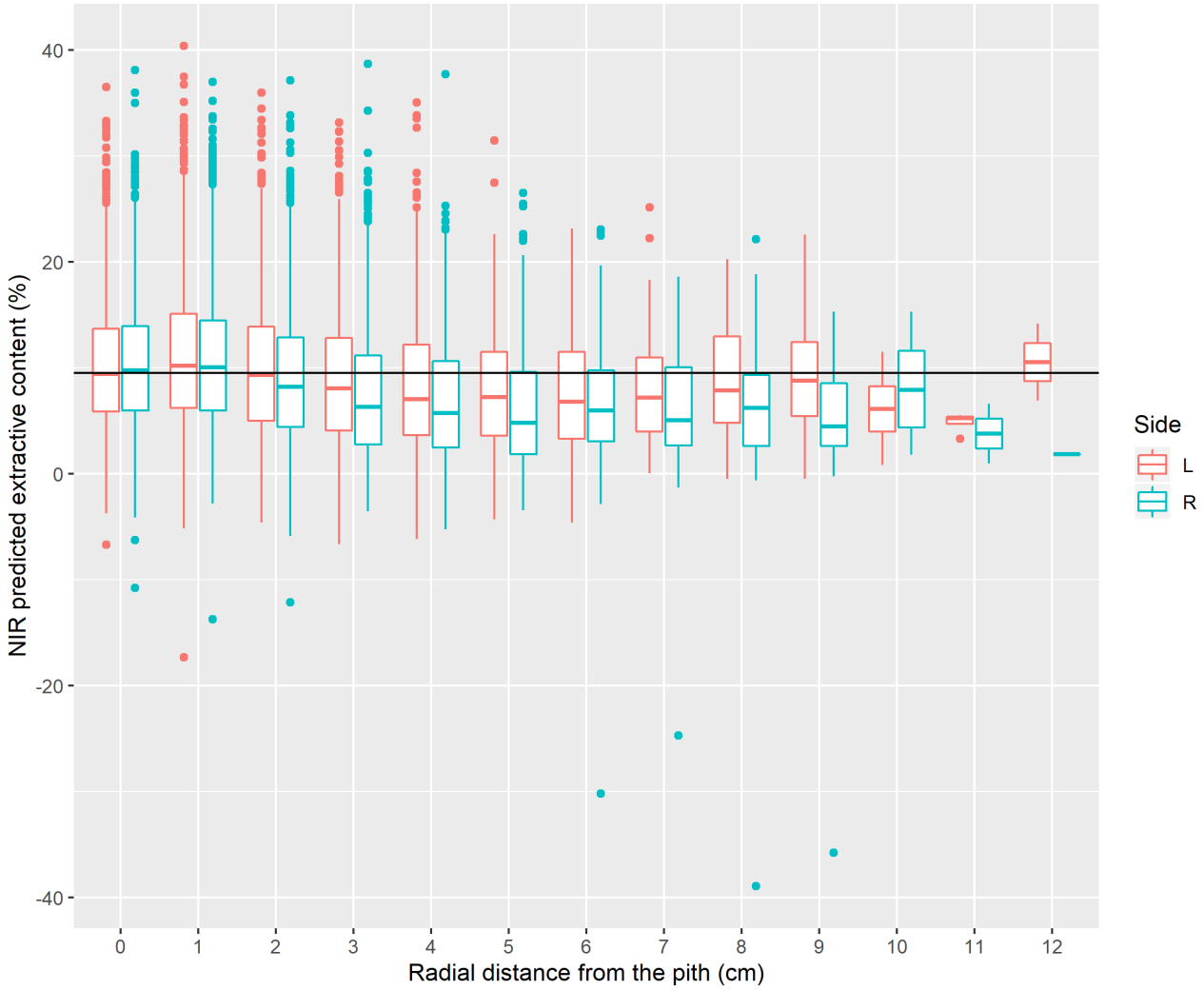

Figure 4: NIR predicted extractive content (%) in the heartwood of the E. globoidea breeding population grown at Atkinson depending on radial distance from the pith. Horizontal black line: overall mean. Differentiated by the randomly assigned left (L) and right (R) radius of the full diameter cores.

Radial sampling strategy for NIR

Figure 5 displays the accuracy of rankings of family breeding values for heartwood quality depending on the number of spectra taken from a core and the number of trees sampled from a family. Two strategies for locating spectra positions on a core were investigated, random position in the heartwood (left) and radial positions outwards from the pith (right) (Figure 2). In all cases improvement in ranking correlations waned noticeably beyond 6 spectra per core. The benefit of increasing the number of individuals per family diminished between 8 and 16. Sampling heartwood quality randomly across the core gave more accurate rankings than sequentially measuring outwards from the pith at the same sampling intensity. However, the difference was small once more than 2 trees per family and 4 spectra per tree were sampled.

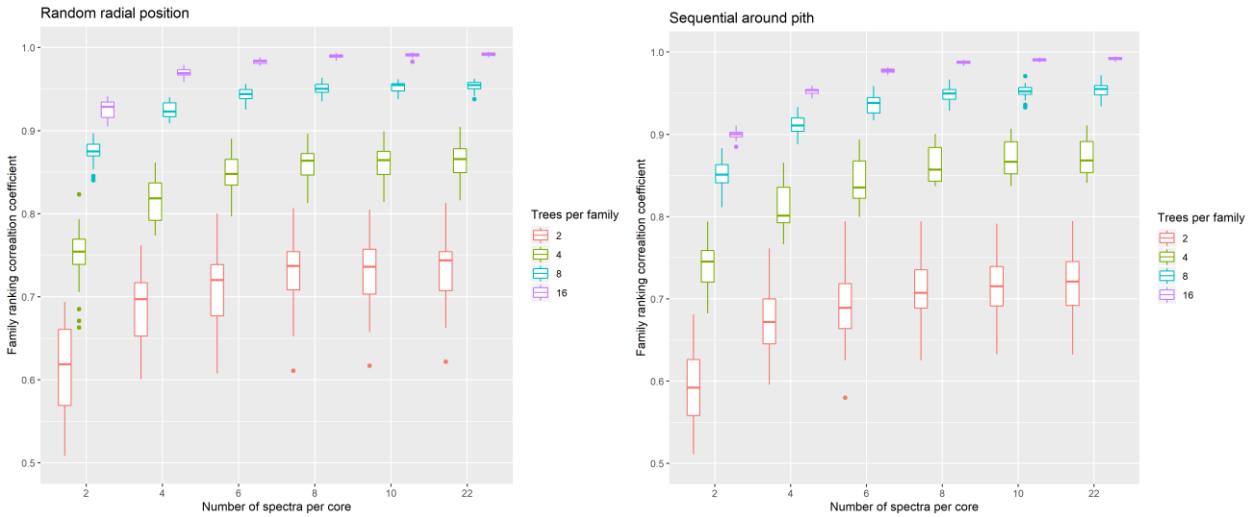

Figure 5: Ranking correlation of family breeding values for NIR predicted extractive content of the full 2011 E. globoidea Atkinson data set analysis to random subsamples with increasing numbers of individuals per family and spectra per core. Left: random radial position in a core; right: sequential radial positions around the core (Figure 2).

Figure 6 displays the accuracy of rankings of individual tree breeding values for heartwood quality depending on the number of spectra taken from a core and the number of trees sampled from a family. Similar to the average family breeding values (Figure 5), the difference between the two strategies became small for individual tree breeding values once more than 4 individuals per family and 6 spectra were sampled. Gains in ranking accuracy diminished noticeably for individual tree breeding values one more than 6 spectra per core were acquired and more than 8 trees per family sampled.

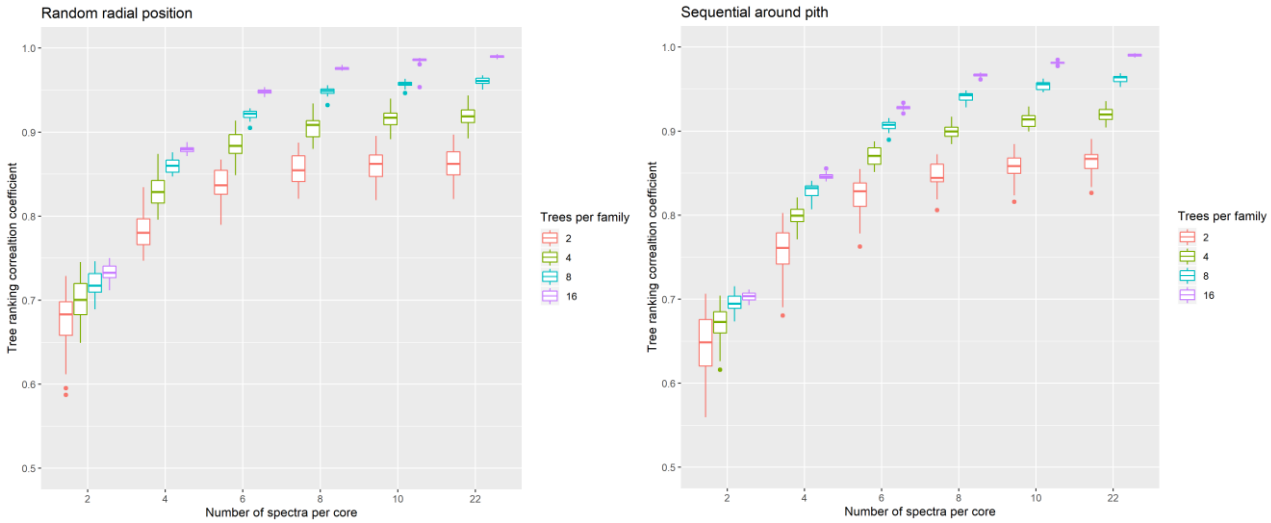

Figure 6: Ranking correlation of individual tree breeding values for NIR predicted extractive content of the full 2011 E. globoidea Atkinson data set analysis to random subsamples with increasing numbers of individuals per family and spectra per core. Left: random radial position in a core; right: sequential radial positions around the core (Figure 2).

A good sampling strategy appeared to target 8 individuals per family with heartwood and obtaining 6 spectra from each core. Considering early screening, needing to accommodate small trees, it seemed preferable to target cores with more than 30 mm heartwood diameter and obtain three spectra on each side of the core at 0.5, 1 and 1.5 cm from the pith.

Sampling effort

The considerations discussed above (Figure 5 and Figure 6) did not consider the amount of information (the product of spectra per core and trees per family) contained in the individual cases. The amount of data is directly related to the resources required to obtain it. Figure 7 displays the

ranking correlations for family and tree NIR predicted extractive content ordered by the number data points per family for sequential radial positions around the pith. Correlation coefficients of around 0.9 were obtainable with 32 data points per family. 32 data points per family can be obtained from 4, 8 or 16 individuals per family and acquiring 8, 4 or 2 spectra per core, respectively. Considering family rankings, 32 data points obtained from 8 individuals with 4 spectra each resulted in higher family ranking correlations than the other two combinations.
As expected, the accuracy of individual tree breeding value rankings benefited more from an increased number of spectra per core than individuals per family. Correlation coefficients of around 0.9 were obtainable with 32, 40 and 48 data points per family, representing combinations of 4 and 8, 4 and 10 as well as 8 and 6 trees per family and spectra per tree, respectively.
At least 48 data points per family were needed, a combination of 8 trees per family and 6 spectra per core, to obtain correlation coefficients greater than 0.9 for both, family as well as individual tree rankings. Similarly accurate family rankings could be obtained with fewer spectra per core, while similarly accurate individual tree rankings could be obtained with fewer trees per family. The latter can be misleading as individual tree selections also benefits from phenotyping more individuals, i.e. an increased chance to find an exceptional individual.

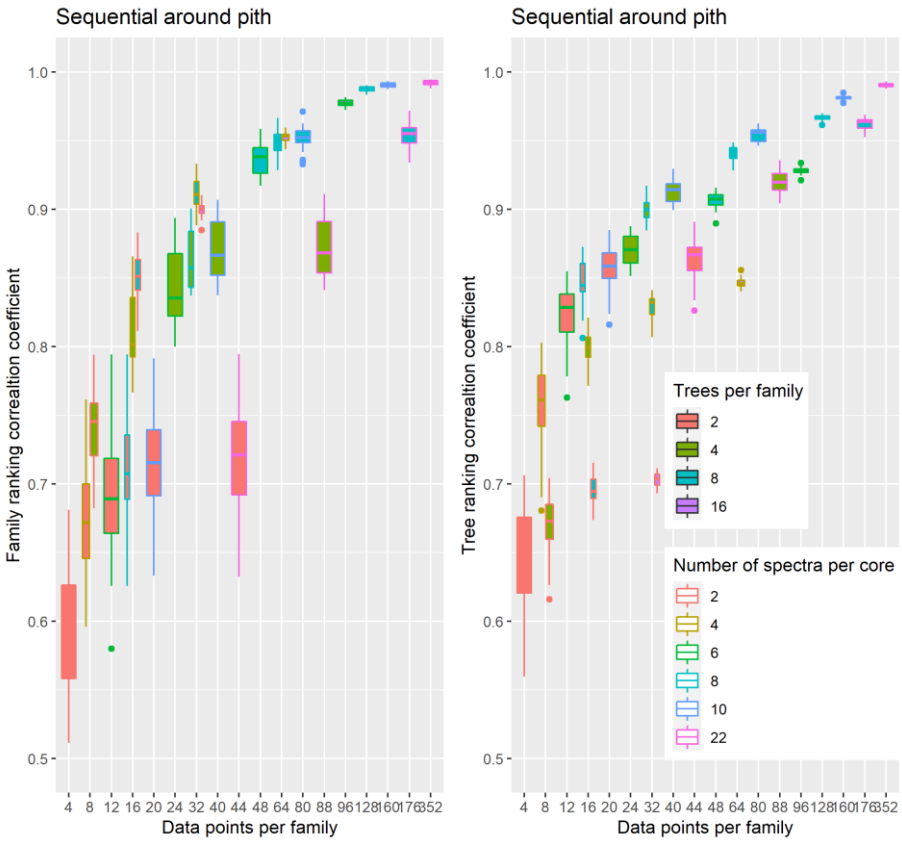

Figure 7: Ranking correlation between family averages (left) and individual tree (right) breeding values for NIR predicted extractive content of the full 2011 E. globoidea Atkinson data set and random sub-samples ordered by the number of data points per family. NIR spectra collection sequentially outwards from the pith.

Planning the phenotyping of breeding trials requires not only to have information on the necessary sampling intensity, but also the associated costs. Coring a tree and obtaining an extractive content by NIR have been costed using the in-house experience. Details can be found in Appendix 1.
The coring and NIR costs can be used to compare the accuracy of breeding values for different sampling intensities on a cost per family basis.
Considering the above determined sampling intensity of 8 cores per family and capturing 6 spectra each, heartwood phenotyping costs of NZ$128 were estimated (Figure 8). This figure assumeed that all of the cored trees had developed enough heartwood for assessments. This, however, is not necessarily the case if trials are assessed early. More trees need to be cored if not all trees have developed sufficient heartwood, increasing the phenotyping costs.

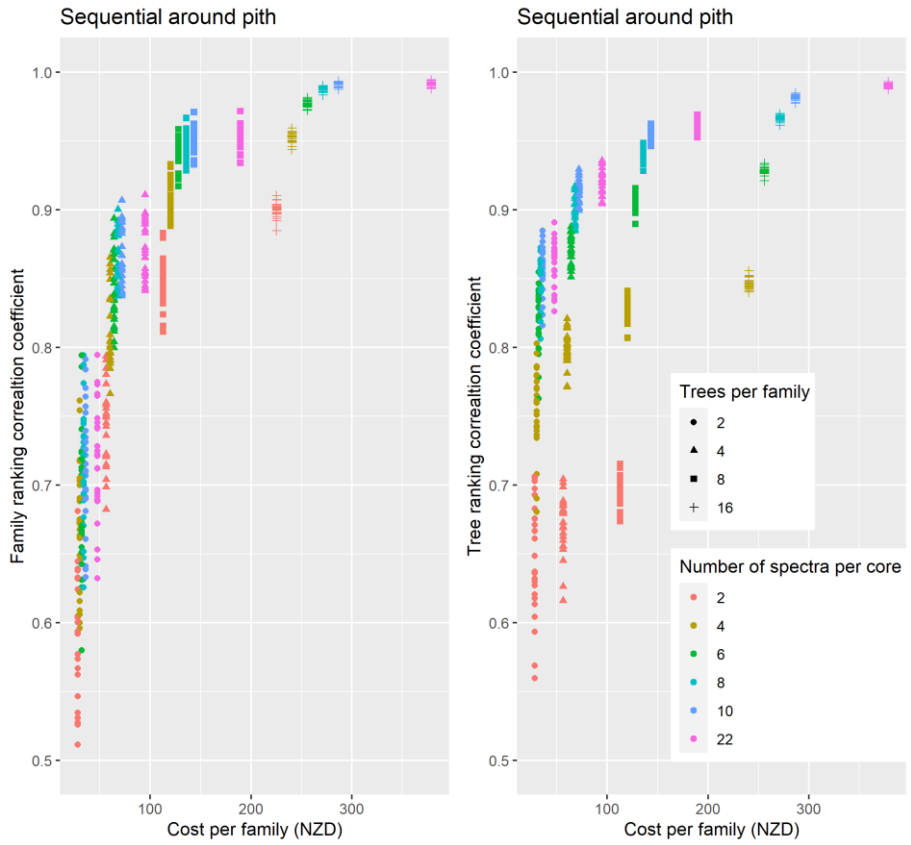

Figure 8: Ranking correlation between family averages (left) and individual tree (right) breeding values for NIR predicted extractive content of the full 2011 E. globoidea Atkinson data set and random sub-samples with increasing phenotyping costs per family. NIR spectra collection sequentially outwards from the pith.

Heartwood formation

To directly assess heartwood properties, the tree needs to have formed heartwood. In the NZDFI breeding programme heartwood traits, i.e. extractive content as a proxy for natural durability and heartwood diameter for quantity, are assessed on 14 mm diameter bark-to-bark tree cores. In the interest of timely selection and consequently timely delivery of improved genetic planting stock, phenotyping should be conducted as early as possible. The age when heartwood development commences differs widely between species (Hillis, 1987), and heartwood was recorded to appear in *E. vimilialis* before the age of 5 years old (Nicholls, 1970). However, this is dependent on tree size, i.e. its growth rate and site conditions. The direct influence of site on heartwood formation is poorly understood (Hillis, 1987; Taylor et al., 2002). For heartwood phenotyping not necessarily the presence of heartwood but the time when enough trees have formed sufficient heartwood for assessment is of interest. Considering the accuracy of breeding value rankings above, a 30 mm heartwood diameter will allow collection of the required 6 NIR spectra spaced at 5 mm intervals from the pith. Figure 9 shows the *E. bosistoana* trees separated by the presence of more or less than 30 mm of heartwood in 7 breeding trials as well as the modelled likelihood of the two groups depending on the corelength. The graphs for considering any amount of heartwood (i.e. heartwood diameter greater than 0 mm) were similar, shifted to smaller trees, but less useful in the heartwood phenotyping context as a reasonable amount of heartwood is needed for the measurements. The smallest *E. bosistoana* trees having 30 mm heartwood diameter were ~60 mm under-bark at 0.5 m stem height. Therefore smaller trees do not need to be sampled for heartwood properties. Once under-bark diameter at 0.5 m stem height reached ~150 mm all trees had a 30 mm heartwood in diameter.

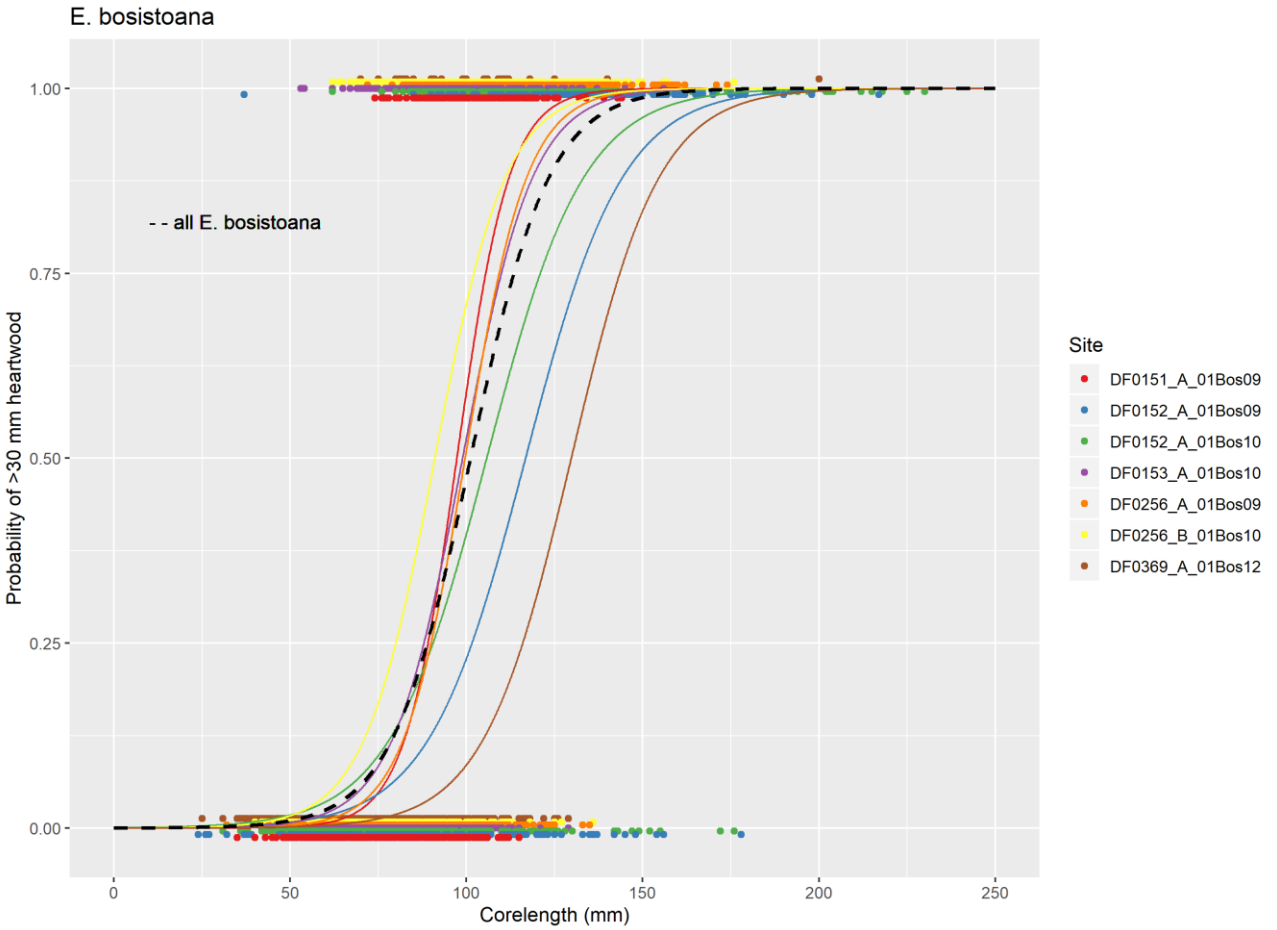

*Figure 9: **Points**: E. bosistoana trees with less (0) and more than (1) 30 mm heartwood at 0.5 m stem height in 7 breeding trials aged 6.6 to 8.9 years old. **Lines**: likelihood of trees with 30 mm heartwood at 0.5 m stem height in the trials (solid lines) as well as the overall data (black dashed line).*

A noticeable difference between the sites was apparent. This difference was not explained by tree age (data not shown) and genetics as the same families were present in each, the 2009, 2010 and 2012 sites, respectively. The environmental factors controlling this site effect are unknown and currently investigated (Morgenroth and Mason, 2020).
Considering the unknown site differences and considerable sampling costs, it is advisable before embarking on tree coring for early heartwood phenotyping to check a few trees in a trial for the presence of heartwood. For *E. bosistoana* this is the case when the average trial under-bark diameter at 0.5 m stem height is between 75 to 125 mm. *E. bosistoana* trials with smaller trees are unlikely to have formed enough heartwood, while trials with larger trees have likely enough heartwood and could be sampled without probing.

Under-bark stem diameter at 0.5 m stem (corelength) is an impractical measure to decide on the timing for heartwood phenotyping. Tree size is commonly measured as diameter at breast height (DBH), assessed above-bark and at 1.3 m stem height, and available for breeding trials. Quantifying the relationship between the two measures would allow the use of a more practical measurement of DBH to decide on the timing for heartwood phenotyping by coring at 0.5 m stem height. Figure 10 shows the linear relationship between these two measures for the 7 *E. bosistoana* trials. It should be noted that not only stem taper and bark thickness but also the time between the two measurements effected the relationships. The available DBH measurements for *E. bosistoana* preceded the coring operation by up to 1.2 years, but typically less than 0.5 years (Table 2).

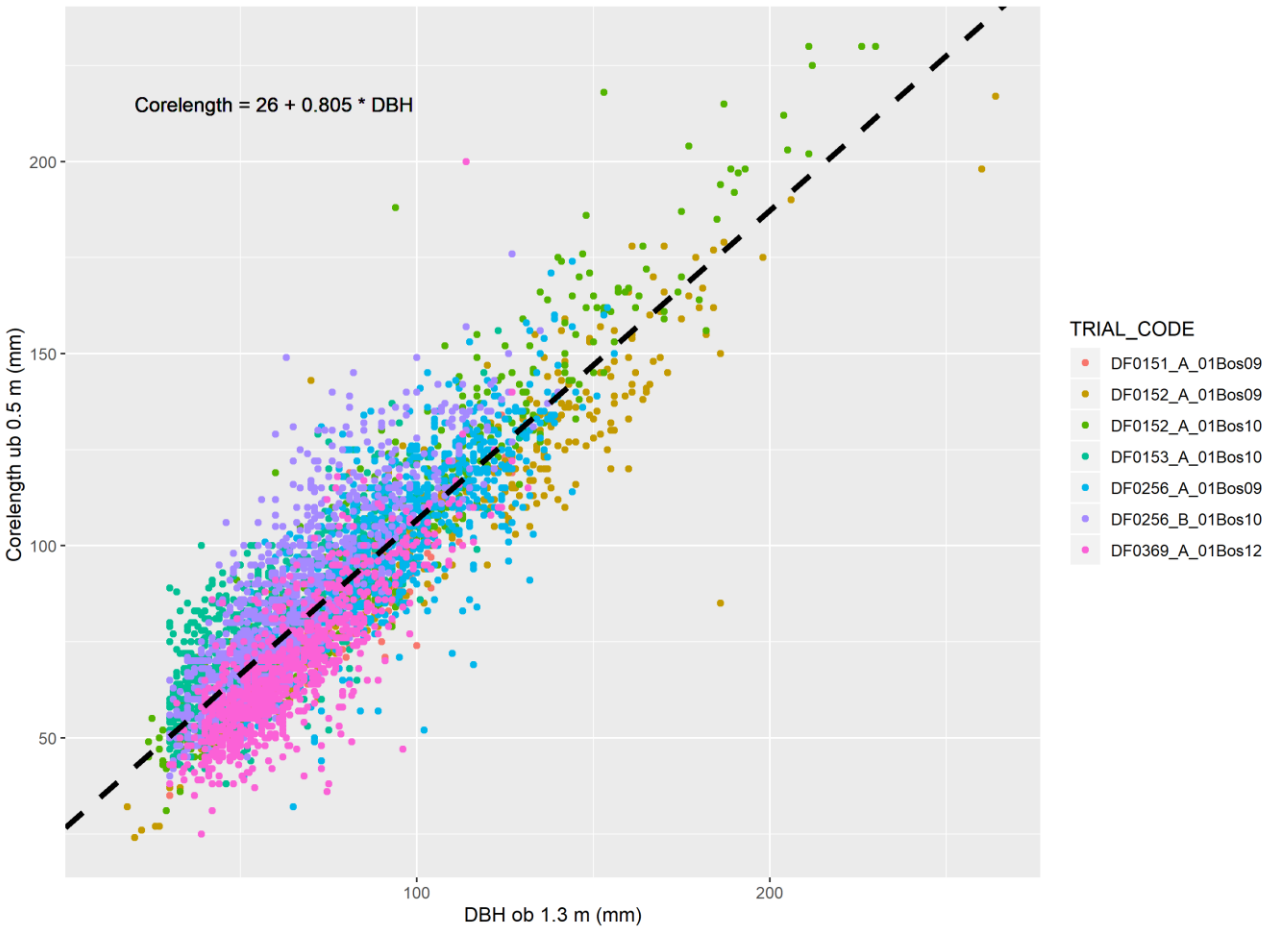

Figure 10: Relationship between corelength (under-bark diameter at 0.5 m stem height) to DBH (over-bark diameter at 1.3 m stem height) for E. bosistoana. Linear relationship for all data shown in black. Note: the time between the DBH assessment and the varied between 0 and 1.2 years for the E. bosistoana trials (Table 2).

Table 2: Summary statistics of the analysed breeding trials.

Species	Trial	Year planted	Age DBH (y)	Age cored (y)	Mean core length (mm)	Trees with >30 mm heartwood (%)
E. bosistoana	DF0151_A_01Bos09	2009	6.2	6.7	80.8	175 : 582 = 23%
E. bosistoana	DF0152_A_01Bos09	2009	6.7	6.7	100.3	139 : 230 = 38%
E. bosistoana	DF0152_A_01Bos10	2010	6.3	6.8	98.7	261 : 389 = 40%
E. bosistoana	DF0153_A_01Bos10	2010	6.9	8.1	76.6	208 : 897 = 20%
E. bosistoana	DF0256_A_01Bos09	2009	8.5	8.9	101.0	484 : 443 = 52%
E. bosistoana	DF0256_B_01Bos10	2010	6.5	6.6	84.7	430 : 685 = 39%
E. bosistoana	DF0369_A_01Bos12	2012	7.2	7.8	67.7	22 : 978 = 2%
E. quadrangulata	DF0155_A_01Qua11	2011	8.9	9.1	95.6	238 : 199 = 54%
E. globoidea	DF0362_A_01Glo11	2011	9.0	9.2	82.1	2058 : 601 = 77%
E. globoidea	DF0362_A_01Glo11	2011	3.4	7.6	141.8	2173 : 21 = 99%

The probability of having formed at least 30 mm heartwood depending on stem diameter for *E. globoidea* and *E. quadrangulata* are shown in Figure 11 and Figure 12, respectively. The relationship between DBH and under-bark diameter at 0.5 m stem height (corelength) for *E. globoidea* and *E. quadrangulata* are shown in Figure 13 and Figure 14, respectively.

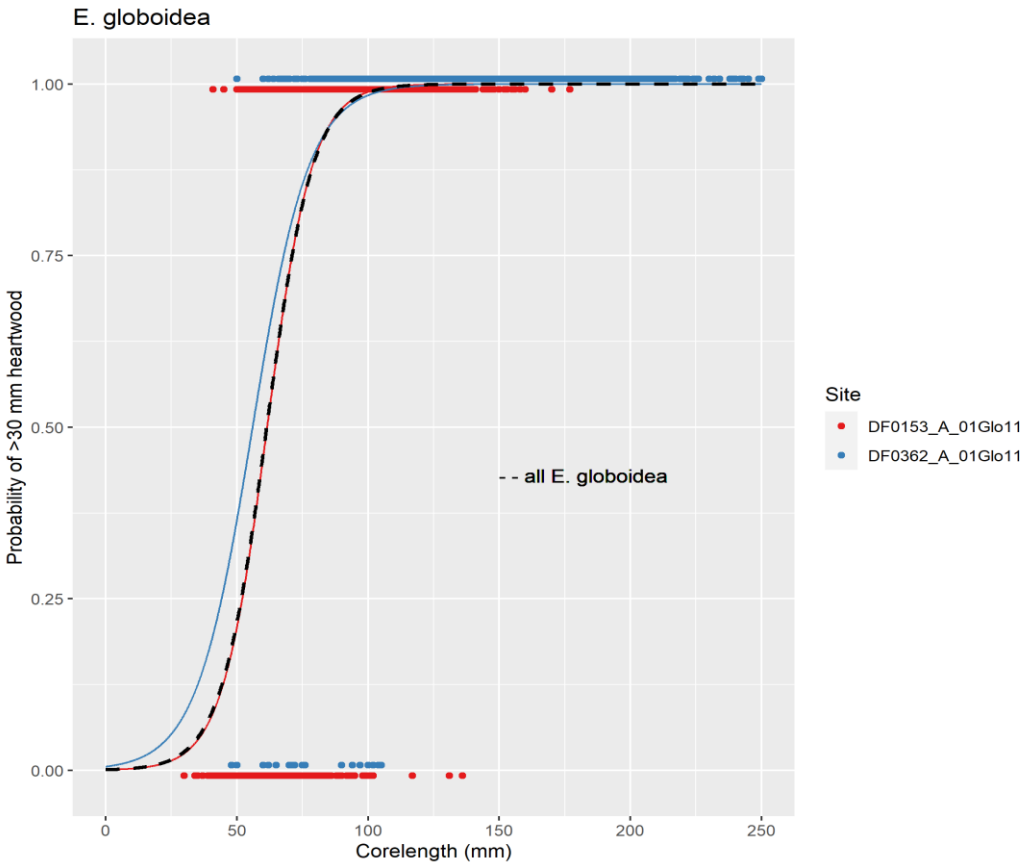

*Figure 11: **Points**: E. globoidea trees with less (0) and more than (1) 30 mm heartwood at 0.5 m stem height in 2 breeding trials aged 7.6 and 9.2 years old. **Lines**: likelihood of trees with 30 mm heartwood at 0.5 m stem height in the trials (solid lines) as well as the overall data (black dashed line).*

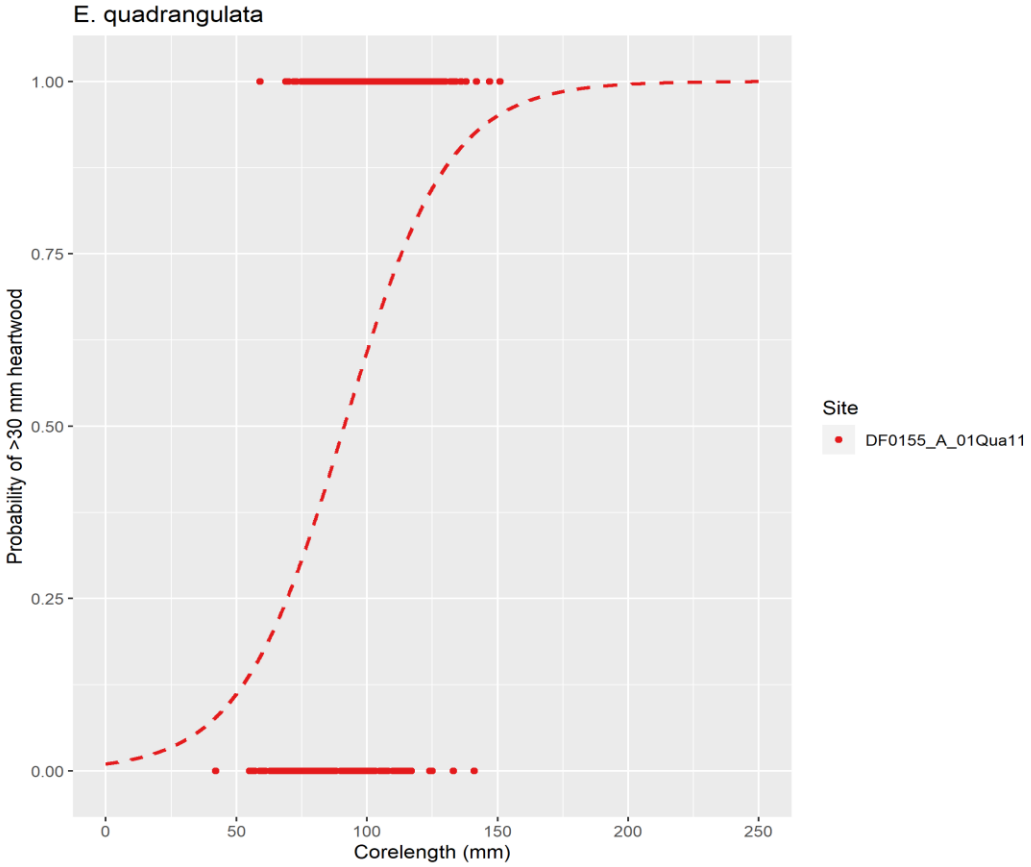

*Figure 12: **Points**: E. quadrangulata trees with less (0) and more than (1) 30 mm heartwood at 0.5 m stem height in a breeding trial aged 9.1 years old. **Dashed line**: likelihood of trees with 30 mm heartwood at 0.5 m stem height in the trial.*

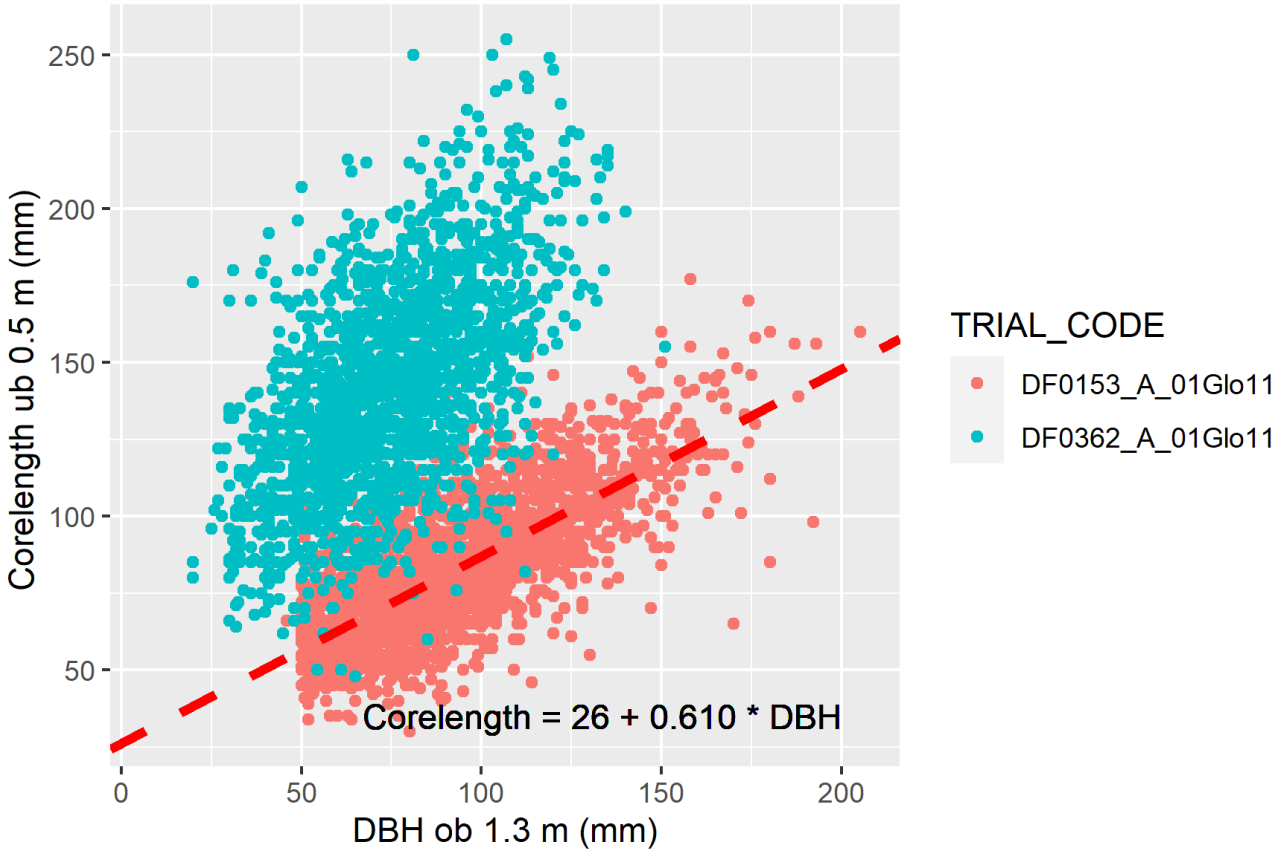

*Figure 13: Relationship between corelength (under-bark diameter at 0.5 m stem height) to DBH (over-bark diameter at 1.3 m stem height) for E. globoidea. **Dashed red line**: Linear relationship for DF0153_A_01Glo11 trial. Note: the DF0362_A_01Glo11 trial data was not used as the DBH assessment preceded coring by 4.2 years (Table 2).*

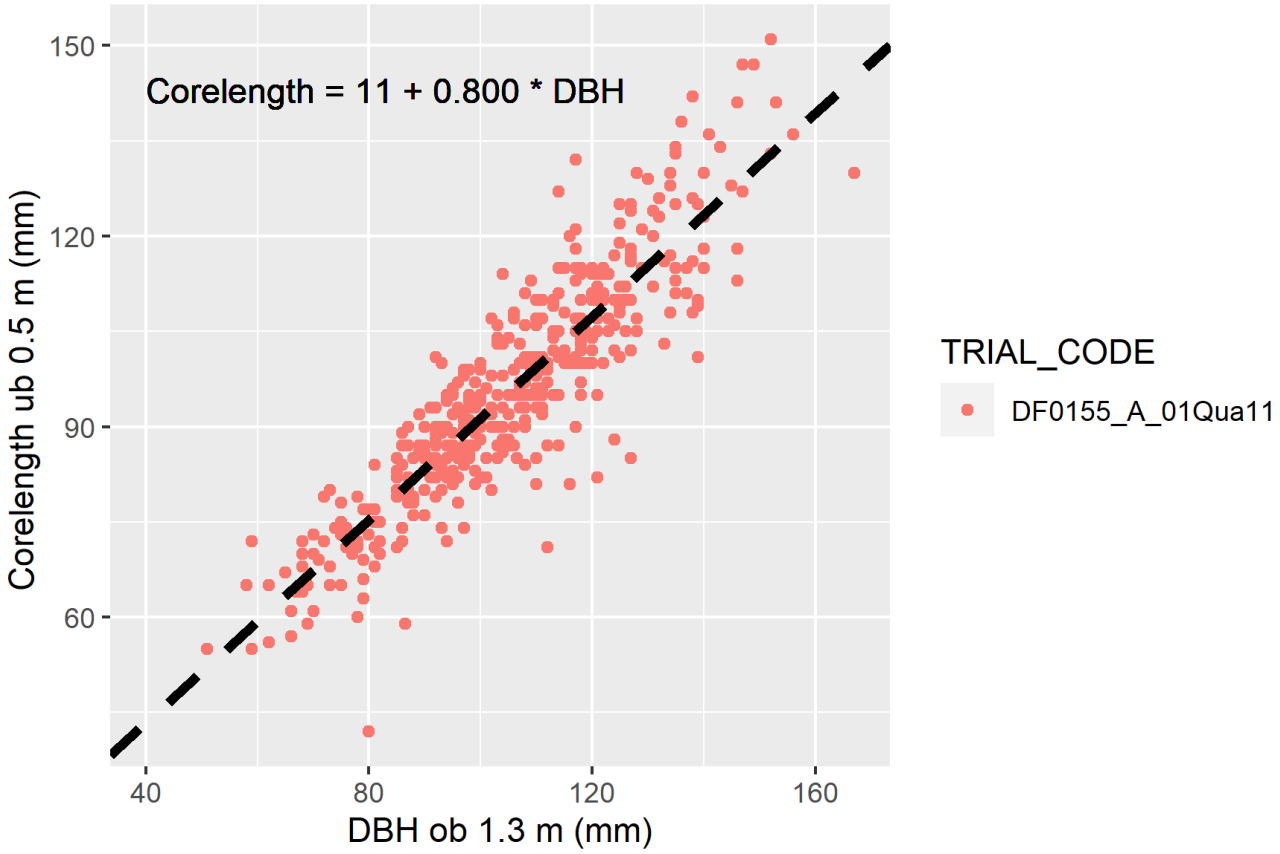

Figure 14: Linear relationship between corelength (under-bark diameter at 0.5 m stem height to DBH (over-bark diameter at 1.3 m stem height) for E. quadrangulata. The time between the DBH and coring assessment was 0.2 years (Table 2).

Timing of heartwood assessments

It was possible to calculate the number of trees which need to be cored per family depending on the average tree size in the trial, knowing a) the likelihood of a tree with a given diameter to have formed heartwood, b) the distribution of diameters in a trial and c) the necessary number of trees with heartwood per family for accurate estimations of genetic parameters. Figure 15 shows the number of *E. bosistoana* trees per family which needed to be cored depending on the average trial diameter, for both DBH and corelength, to obtain in average 8 trees with more than 30 mm heartwood per family. For example if the trial DBH trial mean is 100 mm, approximately 20 trees per family need to be cored to obtain suitable heartwood samples from 8 trees per family in average. This number is halved to 10 if the trial would be cored at a DBH of ~140 mm. It should be noted that a) smaller families will have fewer trees with heartwood and b) that these curves describes the typical behaviour of *E. bosistoana* across sites and vary in detail between sites.

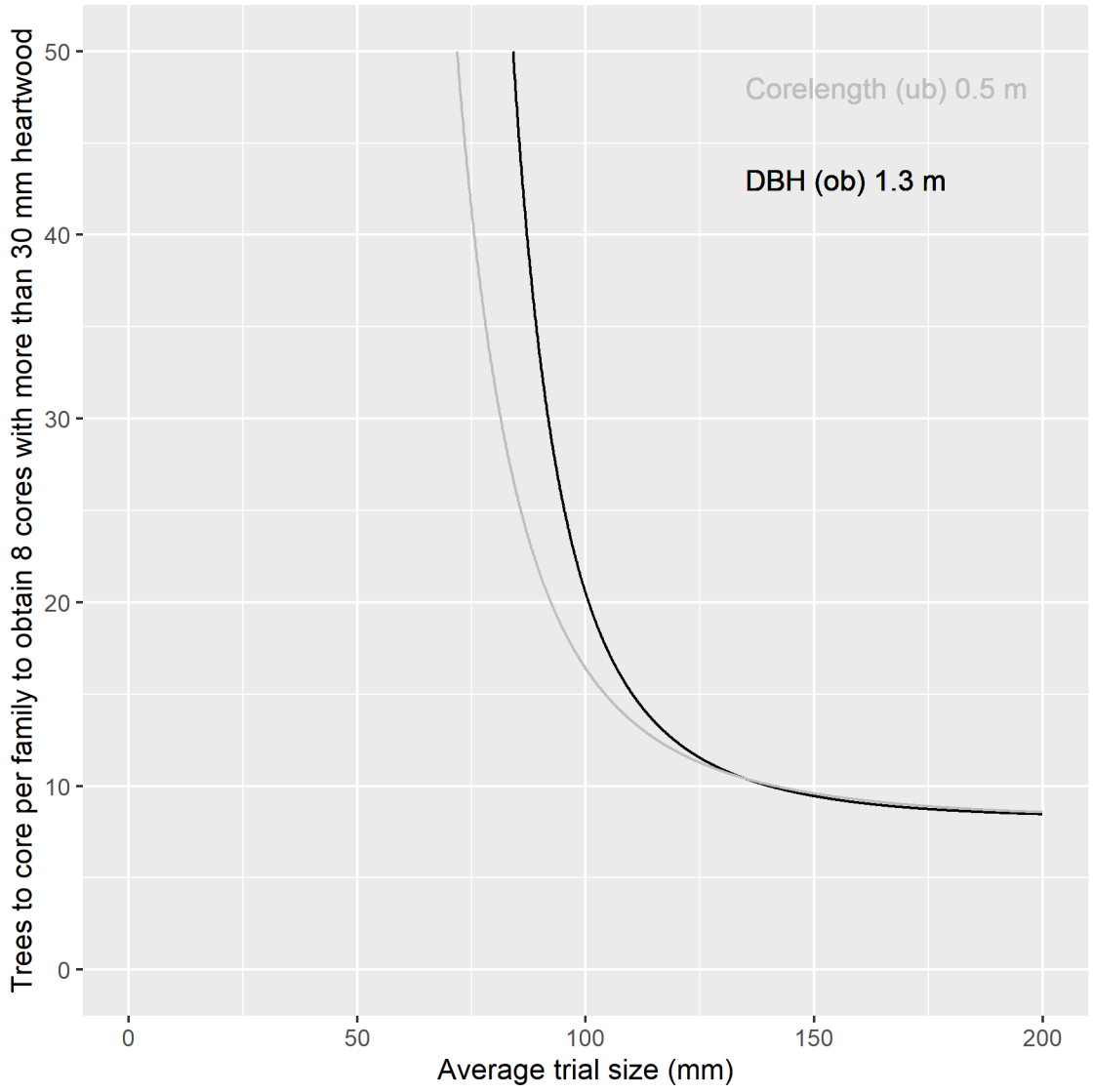

Figure 15: Number of trees which need to be cored to obtain in average 8 cores with more than 30 mm heartwood depending the average tree size in a E. bosistoana trial (black: DBH; red: core length).

Similar curves were calculated for *E. globoidea* and *E. quadrangulata* (Figure 16). *E. globoidea* can be assessed for heartwood features at smaller size, reflecting the narrower sapwood width of this species. The key parameters relating to early heartwood phenotyping for each of the three NZDFI species, *E. bosistoana*, *E. globoidea* and *E. quadrangulata*, were summarised in Table 3. It should be noted that these models were based on the data of only 1 *E. quadrangulata* trial.

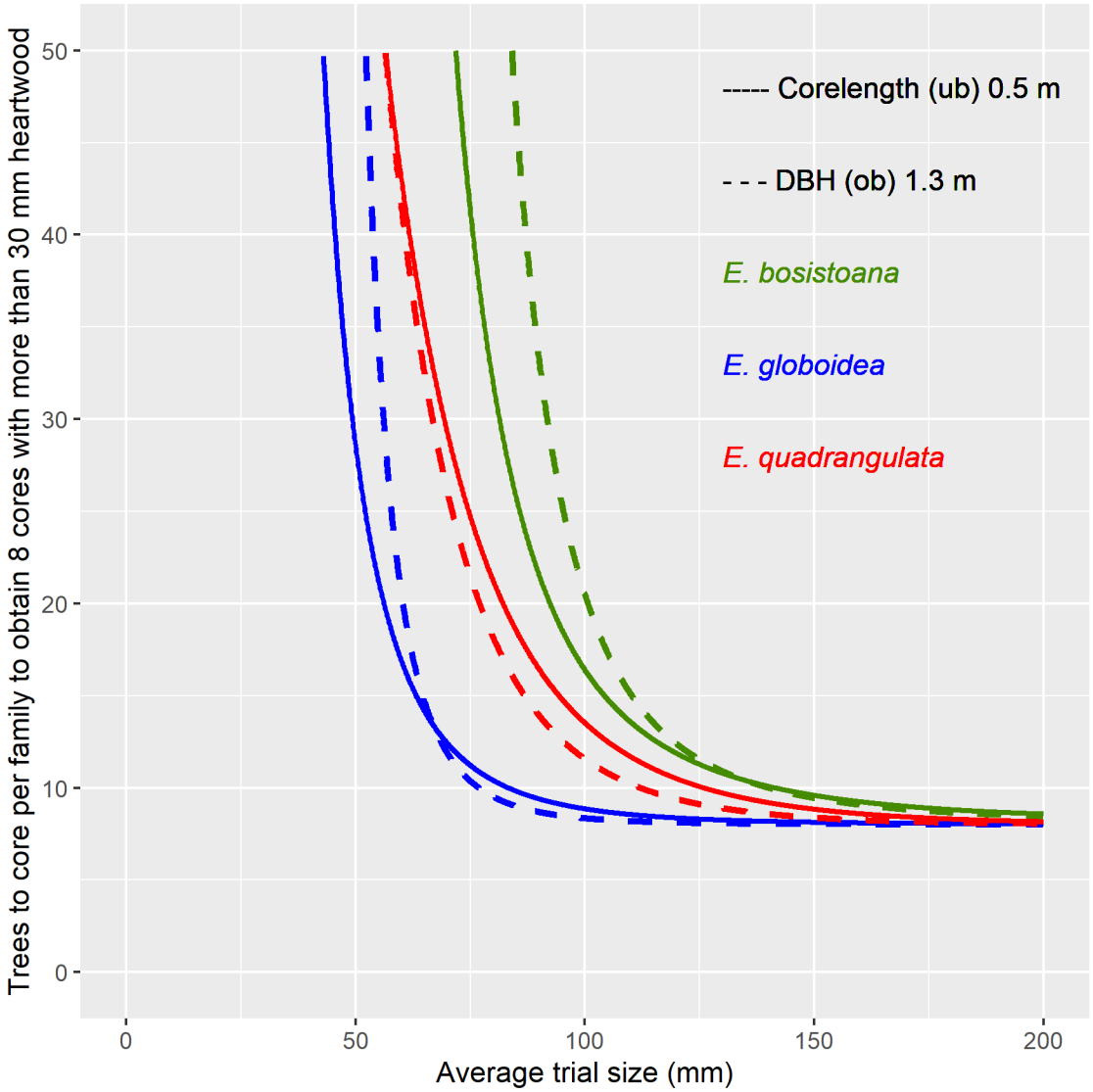

Figure 16: Number of E. bosistoana (green), E. globoidea (blue) and E. quadrangulata (red) trees, which need to be cored to obtain in average 8 cores with more than 30 mm heartwood depending the average tree size in a trial (dashed: DBH; solid: core length).

Table 3: Summary of early heartwood phenotyping data and model parameters for E. bosistoana, E. globoidea and E. quadrangulata. CV: coefficient of variation

Species	No. of trials	Mean core length CV (%)	Trees with >30 mm heartwood (%)	Model parameters		Mean trial DBH for 15 trees to core (mm)
				CL ~ DBH	L_{HW30} ~ CL	
E. bosistoana	7	27.2	1718 : 4204 = 29%	c: 26 d: 0.805	a: -9.05 b: 0.089	110
E. quadrangulata	1	18.0	238 : 199 = 54%	c: 11 d: 0.800	a: -4.59 b: 0.050	87
E. globoidea	2[a]	25.3	4231 : 622 = 87%	c: 26 d: 0.610	a: -6.98 b: 0.114	65

[a] Only 1 trial was used for the CL ~ DBH relationship as the two assessments were separated by 4.3 years (Table 2).

Finally, it was possible to estimate the heartwood phenotyping costs per family using the required amount of heartwood data and the known phenotyping costs (Figure 17). Heartwood phenotyping costs steeply increased for trials with smaller trees and levelled at approximately NZ$ 125 per family. However, this did not consider the effect of increasing coring costs for larger diameter trees. Increased coring costs with tree diameter would result in a trial mean DBH with a minimum cost per family. Also not considered were increased trial maintenance cost, risk of trial damage/loss, and forgone gains by early selection, all demanding earlier selection.

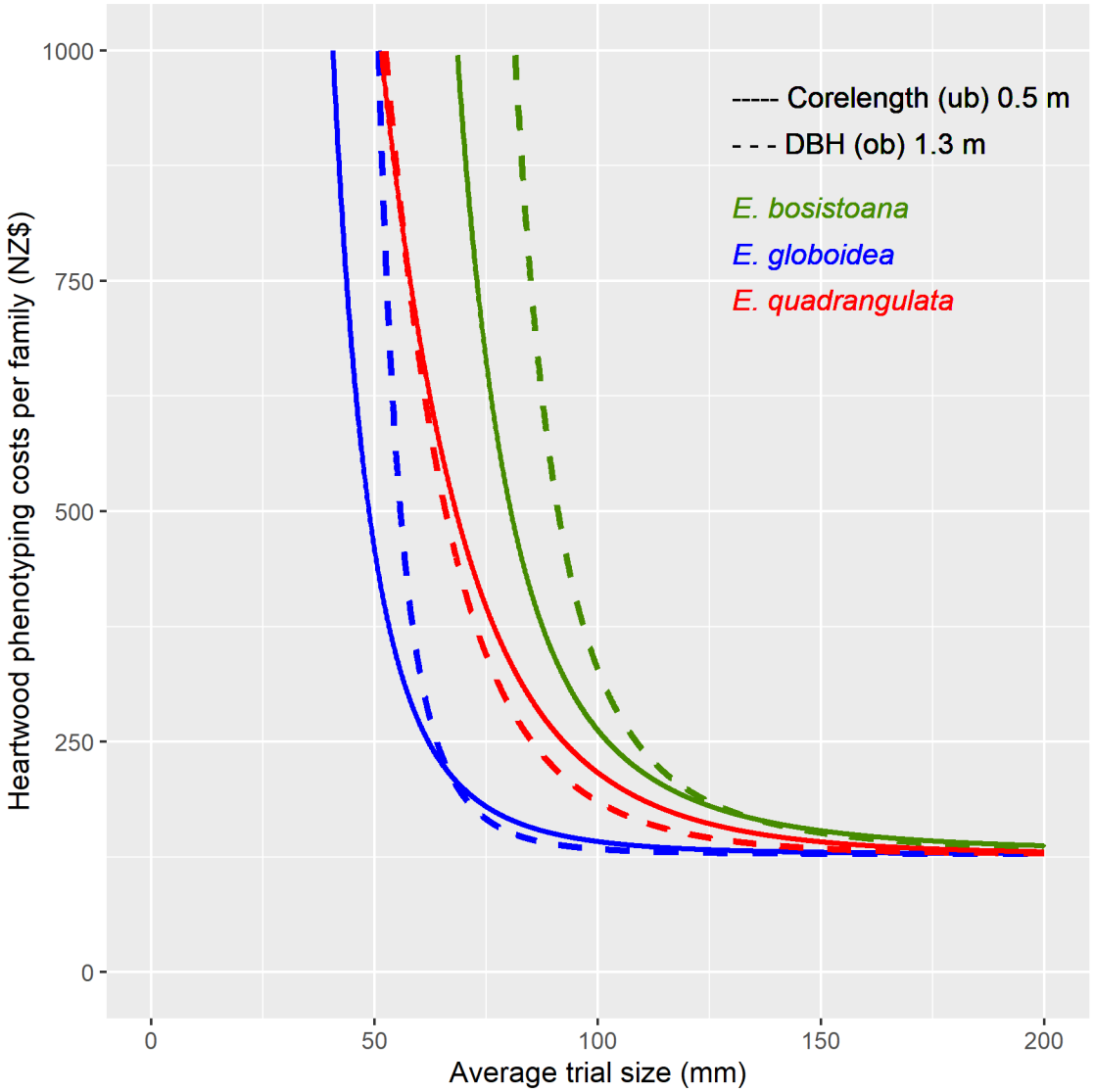

Figure 17: Estimated heartwood phenotyping costs for E. bosistoana, E. globoidea and E. quadrangulata depending average tree size in a trial aiming for an accuracy of family and individual tree breeding values of ~0.9 (requiring 8 trees with more than 30 mm heartwood per family and acquiring 6 NIR spectra per core).

ACKNOWLEDGEMENTS

This work would not have been possible without the NZDFI and the numerous students, collaborators and colleagues working in the field, collecting data in the lab and discussing ideas.

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APPENDICES

Appendix 1: Coring costs

	Travel costs (NZ$)	Rate (NZ$/h)	Worked hours (h)	Cost (incl. GST) (NZ$)	Cost per core (NZ$)
2011 Avery *E. globoidea*		**Number of cores: 2752**		**Cores per h: 33.8**	
Selection		100	2	200.00	0.07
Marking				4,894.46	1.78
1 Supervisor			81.5	6,749.02	2.45
3 Contractors				15,396.10	5.59
2 Students	1884.03	25	2 x 81.5	5,959.03	2.17
Total				**33,198.61**	**12.06**
2011 Cuddon *E. quadrangulata*		**Number of cores: 455**		**Cores per h: 26.8**	
Selection		100	2	200.00	0.44
Marking				1,633.58	3.59
1 Supervisor			17	1,347.40	2.96
3 Contractors				3,914.32	8.60
2 Students	1076.69	25	2 x 17	1,926.69	4.23
Total				**9,021.99**	**19.83**
2012 Ngaumu *E. bosistoana*		**Number of cores: 1000**		**Cores per h: 21.1**	
Selection		100	2	200.00	0.20
Marking				5,466.07	5.45
1 Supervisor			47.5	5,279.40	5.26
3 Contractors				11,864.93	11.83
2 Students	1150.94	25	2 x 47.5	3,525.94	3.52
Total				**26,336.34**	**26.26**

Note: Ongoing improvements of the corer design resulted in increasing productivity and reducing costs of coring over time. Therefore NZ$ 12 per core were used for the calculations in this report.

Appendix 2: NIR spectroscopy costs

Fixed costs per core				
	Rate (NZ$/h)	**Time per core (s)**	**Time per core (h)**	**Cost per core (NZ$)**
Drying[a]	5[b]		0.0480	0.24
Labour sanding	25	25	0.0069	0.17
Labour labelling	25	25	0.0069	0.17
Labour EC prediction	100	5	0.0014	0.14
Facilities sample preparation	25	55[c]	0.0153	0.38
Sum				**1.11**

Variable costs per core				
	Rate (NZ$/h)	**Time per spectra (s)**	**Time per spectra (h)**	**Cost per spectra (NZ$)**
Labour marking	25	1	0.0003	0.01
Labour NIR spectra	25	18	0.0050	0.13
Facilities NIR	69.8[d]	18	0.0050	0.35
Sum				**0.48**

[a] Oven holds 1000 cores; 48 h drying time
[b] NZ$120 per day
[c] Total core preparation time
[d] The university of Utah, Materials Characterisation Lab, https://mcl.mse.utah.edu/lab-rates/ accessed February 2021

NIR spectroscopy costs per core depending on number of spectra were calculated by adding the fixed cost per core to the product of the variable costs per spectra and the number of spectra.

Number of spectra on core	**Cost per core (NZ$)**
1	1.59
2	2.07
3	2.55
4	3.03
5	3.51
6	3.99
7	4.47
8	4.96
9	5.44
10	5.92
11	6.40
12	6.88
13	7.36
14	7.84
15	8.32
16	8.80
17	9.28
18	9.77
19	10.25
20	10.73
21	11.21
22	11.69
23	12.17
24	12.65
25	13.13

Appendix 3: Modelling the occurrence of heartwood

Orange: L_{HW30} - likelihood of *E. bosistoana* trees having developed more than 30 mm heartwood diameter depending on corelength (stem under-bark diameter at 0.5 m stem height) (see Figure 9).

Black solid line: ND - Modelled normal distribution of corelength using the mean and standard deviation of *E. bosistoana* data.
Black dotted line: density function of *E. bosistoana* corelength data.

Red solid line: P_{HW30} - Product of probability (orange) and normal distribution (black solid line), i.e. its integral is the proportion of trees with more than 30 mm heartwood diameter in the trial.
Red dotted: density function of corelength data of *E. bosistoana* with more than 30 mm heartwood diameter.

Blue solid line: Product of (1 - P_{HW30}) and normal distribution, i.e. its integral is the proportion of trees with less than 30 mm heartwood diameter in the trial.
Blue dotted line: density function of corelength data of *E. bosistoana* with less than 30 mm heartwood diameter.