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Improving Heartwood of Durable Eucalypts - Manuscript

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IMPROVING HEARTWOOD OF DURABLE EUCALYPTS

Abstract The New Zealand Drylands Forests Initiative (NZDFI) aims to establish a new hardwood forest industry based on naturally durable eucalypts. As key a product NZDFI has identified sustainably-grown naturally durable posts and poles for the agricultural industry as an alternative to CCA treated pine (Millen, 2009). For these products natural durability is essential. But natural durability is also highly variable in the resource. To ensure a quality product the variability can be reduced by genetic selection. NZDFI's wood quality research programme addresses the variable performance of the timber through a breeding programme. However, incorporating heartwood quality into a breeding programme is not straight forward and requires a novel approach which is able to cope with the necessary large sample numbers. NZDFI has developed a novel sampling system as well as heartwood quality assessment. This will facilitate a viable hardwood forest industry based on naturally durable eucalypts.

1. INTRODUCTION

Wood is a bio-material and biodegradable. Biodegradability is a positive attribute when considering the disposal at the end of a product's life. However, susceptibility of wood to decay by organisms can result in premature product failure. Biodegradation of wood is particularly rapid in moist conditions with ground contact – e.g. for wooden poles. The environment in which wood is used is described by Hazard Classes (e.g NZS3640:2003)(NZS3640:2003, 2003). The natural resistance of timbers against biodegradation is highly variable, while some timbers decay quickly others can withstand moist inground conditions for a considerable time (Bootle, 2005, Scheffer and Morell, 1998). This property is referred to as (natural) durability and assessed by various national standards (ASTM, 2005, EN350-1, 1994, AWPC, 2007). Unfortunately high natural durability is uncommon among tree species and those which are utilised are mostly rare and/or unsustainably harvested (UNEP, 2012). As an alternative, the resistance to biological decay of non-durable timber can be improved technologically by modifying the chemical structure of the wood (Hill, 2006) or by impregnation with preservative of which there are numerous (Eaton and Hale, 1993, Goodell et al., 2003, Richardson, 1993). On an industrial scale the former is a more recent approach and achieves either only lower resistance (thermal modification) or is costly (acetylation). The latter is used extensively for decades but converts the bio-degradable wood into a toxic waste for which often no acceptable disposal option has been found (Graham, 2009, Read, 2003, Townsend and Solo-Gabriele, 2006) .

Biodegradation of wood is caused by fungi, bacteria, insects and marine organisms.

- Fungal decay (Schmidt, 2006)

Numerous fungi are able to enzymatically decompose the chemical constituents (hemicelluloses, cellulose and lignin) from which wood is made. These fungi use the wood constituents as their energy source. Different types of fungal decay have been described (whit-rot, brown-rot, or soft-rot). These differ in appearance and in the type of enzymes that decompose wood. But in all cases the result is a complete loss of structural integrity, i.e. strength. A prerequisite for fungal decay is the presence of free water (and oxygen). This implies that all wood in air-dry (and water logged) conditions is safe from fungal decay. Fungal decay needs to be considered especially when wood is used in ground contact, for example as posts and poles.

- Sap stain (Zabel and Morrell, 1992)

Some wood-colonising fungi do not have the enzymes required to break down the structural wood cell wall components (i.e. hemicelluloses, cellulose and lignin) but can feed on reserve materials, (mostly starch), present in sapwood. Therefore, heartwood is not affected by sap stain. These fungi do not compromise the structural properties of wood but do pose a hygienic issue and discolour the wood.

- Bacteria (Clausen, 1996, Greaves, 1971)

Bacteria have also been reported to enzymatically break-down wood components. This can occur in oxygen deficient environments. However, the process is slow and does not pose a significant threat to timber.

- Wood-degrading beetles (Peters et al., 2002)

Some beetles, like house borer's (e.g. lyctids or anobiids) destroy wood mechanically by chewing. They are not able to feed directly on the cell wall components (lignin, cellulose and hemicelluloses) like wood decaying fungi. They usually digest the tree's reserves (mainly starch) which are present in sapwood only. Some (e.g. ambrosia beetles) rely on symbiotic fungi to break down wood components. Insects do not require the presence of free water and therefore sapwood is prone to attack in dry conditions. As a consequence usees of sapwood inside buildings need to consider this threat. Some ants and other insects can cause similar damage.

- Termites (Ahmed et al., 2004, Shelton and Grace, 2003)

Termites also destroy wood mechanically. They, however, can also use wood cell walls as an energy source with the help of symbiotic bacteria present inside their body. Termites usually need soil contact but some species can build tunnels to reach wooden structures above ground. Termites occur in tropical/subtropical regions and termite resistant woods are in high demand for construction in these parts of the world.

- Wood-degrading marine organisms (Cragg et al., 1999, Eaton et al., 1989, Nishimoto et al., 2015)

Some molluscs (e.g. Teredinidae - shipworms) and crustaceans (e.g. Limnoriidae - wood lice) use wood as habitat and food. These species need to be considered when wood is to be used in marine constructions like piers. Only few timbers last a significant time in marine conditions.

The natural durability according to the Australian standard "Timber – Natural durability ratings" (AS5604, 2005) for NZDFI species is listed in Table 1. This refers to timber harvested from old-growth natural forests in Australia, and timber from other sources especially when grown in short-rotation plantations can compare unfavourably.

Table 1. Natural durability ratings of NZDFI species according to (AS5604, 2005) and heartwood colour (Bootle, 2005)

Species	Lyctid susceptibility of sapwood	Termite resistance of heartwood	In-ground life expectancy (years)	Above-ground life expectancy (years)	Life expectancy in southern waters (years)	Colour
Eucalyptus bosistoana	*Susceptible*	*Resistant*	*>25*[a]	*>40*	*21 to 40*	*Pinkish pale brown*
Eucalyptus argophloia	*Susceptible*[b]	*ND*	*ND*	*ND*	*ND*	*Orange-brown to deep red-brown*[c]
Eucalyptus quadrangulata	*Not susceptible*	*Resistant*	*15 to 25*	*15 to 40*	*ND*	*Pale yellow*
Eucalyptus sideroxylon	*Susceptible*	*Resistant*	*>25*	*>40*	*41 to 60*	*Dark red*
Eucalyptus globoidea	*Not susceptible*	*ND*	*15 to 25*	*ND*	*21 to 40*	*Pinkish pale brown*

[a] See (Cookson, 2004)
[b] See (Cookson et al., 2009)
[c] See (AWPC, 2007)

1.1. Basis for natural durability

The large differences in resistance to fugal decay between timber species is predominately caused by its chemical composition, i.e. the secondary metabolites deposited in heartwood (Schultz et al., 1995, Rudman, 1963, Hawley et al., 1924). The effect of wood density on fungal decay is less clear as higher density wood often coincides with a higher extractive content. But it has been reported that density has a positive effect on decay resistance (Bush, 2011, Cookson and McCarthy, 2013, Edlund, 1998, Plaschkies et al., 2014, Sehlstedt-Persson and Karlsson, 2010, Wong et al., 1983, Yu et al., 2003). For mechanically caused damage from insects and marine organisms, wood density has been shown to have a significant effect (Peters et al., 2014). However, these results were based on observations between species and such large variation is not necessarily present within a species (Cragg et al., 2007). Other factors contribute to the natural resistance of wood to biological decay, for example pore size (Peters et al., 2002) or permeability (Bush, 2011).

In most trees the wood in a stem changes from sapwood to heartwood some years after formation (Hillis, 1987, Beakbane et al., 1971, Rowe, 1989, Taylor et al., 2002). The first year rings, closest to the bark, are young and contain some living cells, which are referred to as parenchyma. This wood is defined as sapwood and contains reserve materials like starch. After a species specific period of years the reserve materials are removed from the older parenchyma and the parenchyma cells die.

Following this transition the tissue is called heartwood and it starts forming at the centre of the stem. During heartwood formation some species synthesise numerous smaller organic compounds, which are deposited into the wood. These compounds are called heartwood extractives. Heartwood extractives are not a structural part of the wood cell walls, but some of the compounds are coloured or have bioactive properties. Therefore, heartwood of some species is of attractive colour and can be resistant against biodegradation. Sapwood is never regarded as naturally durable (e.g. AS5604)(AS5604, 2005) or is coloured other than the typical pale brown (Kohl, 2012).

Another important change in the wood properties with time is a decrease in permeability. The transport function of the tissue is removed to reduce the risk of embolism by pit aspiration, tylosis or deposition of extractives. This can precede heartwood formation (Ziegler, 1968) and is thought to reduce the susceptibility for wood to biodegradation.

1.2. Variability and control of heartwood traits

Wood properties, including heartwood associated features, are variable within a species. This is of economic importance as timber products and wood processing benefit from consistent and adequate properties (Walker, 2006). Some of the variation in wood properties is under genetic control, enabling the selection of superior trees, i.e. healthy trees which produce larger quantities of good quality timber. On top of variability between individuals, variation of heartwood extractives

and natural durability within a stem was reported. Within stem variation exists as characteristic spatial pattern as well as random local variations. Environmental parameters affect heartwood features highlighting the need to address heartwood in site-species matching and growth and yield modelling (Sharma et al., 2014).

1.2.1. Within tree variability of heartwood features

The natural durability of heartwood decreases towards the pith (Sherrard and Kurth, 1933, Taylor et al., 2002). This is recognised in relevant standards, for example (AS5604, 2005) stating that:

> "… the inner heartwood (the first few growth rings around the pith), generally, has a lower natural durability than the rest of the heartwood."

The typical radial pattern of heartwood durability is mirrored by the amount of extractives in the stem (Sherrard and Kurth, 1933, Taylor et al., 2002). Additionally slight increases in heartwood durability were reported with stem height. This is in analogy to wood 'quality' of juvenile pine corewood (Burdon et al., 2004), which generally has unfavourable properties for most applications.

Additionally heartwood extractives can vary considerably in their abundance on a micro scale. For a durable product a homogeneous distribution within cells walls, between cell types (e.g. fibres, parenchyma) and within year rings is desirable (Taylor et al., 2002). On the other hand a variable extractive content can be the defining feature in the appearance of timbers like *Microberlinia brazzavillensis* (Kohl, 2012) or *Dacrydium cupressinum*.

Preliminary results on the distribution of extractives in *E. bosistoana* confirm these general statements for the macroscopic (within stems) and microscopic (within year rings) distribution of extractives.

1.2.2. Genetic control of heartwood features

Previous studies have reported on the variability and the degree of genetic control of heartwood features in various tree species e.g. (Bush et al., 2011, dos Santos et al., 2004, Miranda et al., 2014, Poke et al., 2006, Stackpole et al., 2011, Denis et al., 2013). The research on within tree variation is limited. However, substantial within species variation in durability has been demonstrated. Although the durability of heartwood from young trees is generally lower than that of old trees, individuals having class 1 durable heartwood at young age have been reported (Bush, 2011, Palanti et al., 2010). Therefore the production of ground-durable posts from young, short-rotation plantations should be possible by selecting a genetically superior resource.

Preliminary results have found a wide range of extractive contents in heartwood samples of *E. bosistoana*. Ethanol soluble extractives varied between 1.5% to 15% (wt/wt basis) at age 4½ (McLaughlin, 2013, Sharma et al., 2014), indicating that trees of good durability at a young age could be found.

A recent study on the variability in heartwood traits of hybrid larches reported heritabilities of >0.65 for heartwood diameter and extractive content (total phenolics) (Paques and Charpentier, 2015). Genetic gains of 10% in heartwood diameter and phenolics content were predicted. Furthermore, it was reported that the influence of female (European larch) and male (Japanese larch) parents differed between the traits. A good prospect for genetic improvement of *E. cladocalyx* heartwood quantity (%), methanol extractive content and durability was reported (Bush, 2011). The data did not indicate unfavourable genetic correlations between growth and durability. Similar results were found for other species.

Another heartwood feature determining the value of the timber is its colour. The genetic control of heartwood colour has been studied for several species (Gierlinger et al., 2004, Mosedale et al., 1996, Moya et al., 2013, Rink and McBride, 1993).

1.3. Chemistry of heartwood extractives

Little to nothing is known about the identity of the chemical compounds in heartwood of NZDFI's eucalypts (*E. bosistoana*, *E. argophloia*, *E. tricarpa*, *E. quadrangulata* and *E. globoidea*). In former decades research on the chemical heartwood compounds of some durable eucalypts has been conducted (Hillis, 1991). In more recent times research in this area focused on non-durable eucalypts, the species grown for pulp and paper, where low extractive contents are desirable (Swan and Akerblom, 1967). Chemotaxonomy of eucalypts by heartwood (Hathway, 1962) and leaf (Hillis, 1966, Hillis and Inoue, 1967, Padovan and Manzan, 2014) compounds has been explored. Bark and root extractives of eucalypts were also studied (Cadahia et al., 1997a, Cadahia et al., 1997b, Dayal, 1987, da Seca and Domingues, 2006).

The heartwood compounds in a species are numerous (Hillis, 1987, Rowe and Conner, 1979), each molecule having different properties e.g. in bioactivity (Morris and Stirling, 2012, Ohtani et al., 2009), colour (Balogh and Anderson, 1965, Takahashi and Mori, 2006, Zavarin and Smith, 1962) or interaction with glues (Wang, 1992). Furthermore the relative proportion of the individual compounds varies within a species (Daniels and Russell, 2007, Niamke et al., 2014). Therefore not only the absolute amount of extractives but also the extractive composition is important in respect to wood properties like durability and colour. As the biosynthesis of the individual compounds is also partly under genetic control (Fries et al., 2000) there are possibilities to further improve wood quality by breeding. A requirement is the identification of the key compounds in the heartwood for the NZDFI species.

Individual extractives from heartwood or other tree tissues can also be of value themselves. Numerous reports are published on natural compounds to be used as natural wood preservative (Singh and Singh, 2012), in medicine or for other applications. For example a compound isolated from *E. globoidea* buds (Globoidnan A) has been found to be an inhibitor of HIV integrase (Ovenden et al., 2004) Recently the variability in the essential oils extracted from *E. bosistoana* leaves has

been reported (Bouzabata et al., 2014). Tannins from eucalypts are other compounds of interest (Cadahia et al., 1997b).

1.4. Measuring heartwood

The amount of sapwood and heartwood in a tree can have a marked impact on its value. In the case of naturally durable NZDFI species high heartwood content is desirable. However, sapwood is the desired part of the stem for pulp production and other wood manufacturing processes as heartwood can drastically increase processing costs (Morais and Pereira, 2007). Sapwood width is also a key factor for understanding water balances of forest plantations (Kumagai et al., 2005), which are increasingly in the spotlight with climate change and intensified agriculture. Therefore measuring the sapwood width in standing trees is not only of interest for research purposes but also for quality control in production forestry in a variety of settings. Unfortunately assessing sapwood depth in standing trees is not trivial.

It is possible to detect heartwood and sapwood on extracted cores. This can be difficult for some species which do not have marked colour or moisture content differences between sapwood and corewood. Colour stains are available to highlight heartwood or sapwood (Hillis, 1987). Alternatively microscopy, X-ray tomography or NIR (near infrared spectroscopy) have been used to detect the heartwood sapwood boundary in cores (Pfautsch et al., 2012).

A transportable magnetic resonance imaging system has been developed, which can measure sap flow in trees non-destructively (Jones et al., 2012). In its current form portability (size and weight of the equipment) and the limitations to stem diameter (~10 cm) prevent this tool to be used more widely.

Recently electrical resistivity tomography has been found useful to detect sapwood in standing trees non-destructively (Wang et al., 2016). The reported accuracy of the method has been questioned for eucalypts (Pfautsch et al., 2016) and the need of multiple sensors limits its usefulness for fast assessment of many trees for quality control of plantations or breeding trials.

1.5. Measuring durability

The methodology set by standards to assess the durability of timber is by measuring the mass loss of the samples after exposure to wood degrading organisms either in field or under laboratory conditions. The details differing significantly depending on the hazard class tested for (AWPC, 2007). These require numerous (>6) reasonable sized (>20 mm) samples and testing for a considerable time frame (>12 weeks) (AWPC, 2007). The effort of measuring natural durability by mass loss was recognised as prohibitive for inclusion in large scale screening programmes (Bush et al., 2011, Paques and Charpentier, 2015).

Experimental alternatives to accelerate durability assessments have been proposed. The required exposure time can be shortened by accelerating the decay by

creating more favourable environmental conditions (Cookson and McCarthy, 2013). Furthermore it is possible to use more sensitive techniques to assess decay in the early stages, before significant mass loss occurs. In the early stage of decay remarkable changes in the physical properties and the chemical composition of the wood can be observed. Acoustics, which are associated with the stiffness of a material, have been found useful to detect decay early (Machek et al., 2001). Several studies report that NIR was useful to predict the severity of decay in wood, based on changes in the chemical composition of the woody cell walls. Therefore NIR is removing the need to measure mass loss (Fackler and Schwanninger, 2012). However, these are still difficult to realise in a sizable but lean breeding programme.

As outlined above (1.1) the durability of wood is related to the extractive content in heartwood. Again, the resources needed for the conventional test method to determine the extractive content by solvent extraction of milled wood (TAPPI, 2007) is prohibitive to be useful in a screening programme (Takashima et al., 2015). Harju and Venäläinen (2006) proposed to use a more efficient Folin-Ciocalteu assay for total phenolics to more rapidly assess durability in *Pinus sylvestris*. Alternatively, NIR is a technique able to obtain information on the chemical composition of a material (Osborne et al., 1993) and is used for this purpose for agricultural products. Several studies have found NIR being able to accurately assess the extractive content of heartwood e.g. (Bush et al., 2011, Alves et al., 2012, Poke et al., 2004, Stackpole et al., 2011, Giordanengo et al., 2008). However, the prediction of mass loss by decay fungi was not always reported to be accurate enough to support a breeding programme e.g. (Bush et al., 2011, Gierlinger et al., 2003).

Wounding was suggested as an early testing method to breed for heartwood (Harju et al., 2009). The wound reaction in stems after injury is not identical but somewhat related to heartwood formation (Blanchette et al., 1992).

1.6. NZDFI's approach for assessing heartwood in breeding populations

NZDFI has established numerous breeding trials since 2009. Details of the breeding trials can be found in (Luis' paper for this publication). As the trees age and start to form heartwood we will assess our families on a) the amount of heartwood in the stem and b) the extractive content in the heartwood. Having characterised our genetic resource we will be able to select trees with abundant heartwood rich in extractives for propagation.

The trees with high extractive content are more likely to produce more naturally durable timber. The resources necessary to test natural durability in a breeding programme are prohibitive. Additionally sample size (one 12 mm diameter core of each individual) is limited. Therefore a potentially more durable resource is selected through the proxy of high heartwood extractive content. The natural durability rating of the selected resource needs to be determined on a later stage according to standards by independent organisations. As this is only done for the selected trees the resources required are reduced significantly compared to assessing natural

durability for the entire breeding population. Standards vary depending on the envisaged export marked.

Fort the breeding population the extractive content in the heartwood will be assessed by NIR spectroscopy on cores with a fibre optics probe as milling and subsequent extraction is resource demanding. In order to assess the extractive content in heartwood in this way we need to calibrate the system for each NZDFI species.

2. METHODS

2.1. Tree corer

The assessment of heartwood requires a heartwood sample. As we do not want to fell trees in our breeding population we need to non-destructively extract a wood core for analysis. This is not a trivial task. The NZDFI species are of very high density making the use of the conventional hand corers impossible especially as we require larger diameter cores for the further measurements. CSIRO in Australia had designed a tree corer in the 1990's (Downes et al., 1997) to extract 12 mm diameter cores from eucalypts leaving a 22 mm hole. But its manufacturing had stopped in the 2000's and no tree corer was available internationally when NZDFI embarked on its wood quality programme. Only recently a version of TRECOR was made available again by Forest Quality (Australia). We therefore needed to design a new tree corer. As TRECOR is driven by a petrol engine it makes the design heavy and raised concern regarding fire safety with land owners.

In collaboration with Callaghan Innovation, we have recently developed a battery-powered, light-weight tree corer which allows the easy and quick extraction of 14 mm diameter cores from a 21 mm hole, ideal for younger smaller diameter trees (Figure 1). Extraction of cores is quick, taking only ~30 s for a 15 cm diameter tree. A battery back lasts for 20-40 cores depending on tree diameter. A team of 2 was able to core >1000 trees in less than 2 weeks including the assessment of heartwood quantity. The corer can be made available through the New Zealand School of Forestry, University of Canterbury.

Figure 1. Extracting a core from an E. bosistoana tree using a light-weight, battery-powered tree corer developed for the New Zealand School of Forestry.

2.2. Calibration of NIR for extractive content in E. argophloia

In order to quickly predict the extractive content in heartwood, NIR needs to be calibrated with samples of known extractive content. Discs were collected from 37 *E. argophloia* trees aged 7 at the base of the stems. Samples were air-dried and subsequently equilibrated at 20 ℃ and 65% relative humidity resulting in a equilibrium moisture content of ~9%.

NIR spectroscopy

NIR spectra were taken from the sanded cross-section of the discs with a fibre optics probe (Bruker) at wavelengths from 9000 to 4000 cm^{-1} at 4 cm^{-1} intervals. 32 Scans were averaged for each spectrum. For each discs NIR spectra were collected every 5 mm from one side of the heartwood-sapwood boundary to the other across the pith. For each wood disc the spectra were weight-averaged to represent the cross-sectional area according to their radial position.

Extractive content (EC)

Heartwood from each disc was isolated by drilling into the cross-section with a 12 mm drill. The drill frass was milled with a Wiley mill fitted with a 2 mm screen into a finer powder. The powder was extracted with ethanol using an Accelerated Solvent Extractor (ASE – Thermo Fisher) using the following extraction conditions: static time 15 min, temperature 70℃, 100% rinse volume and 2 extraction cycles. Extractive content was calculated from the known oven dry wood powder mass and the oven dry extractive content determined after evaporation of the solvent.

NIR spectra processing

Data was analysed in R (Team, 2014). The prospectr package (Stevens and Ramirez–Lopez, 2014) was used for spectra pre-processing. The first and second derivatives were calculated using the Savitzky-Golay algorithm with 2nd order polynomial and a window size of 15 data points. Significant Multivariate Correlation (sMC) was done using the plsVarSel package (Mehmood et al., 2012). Calibration models were developed using the pls package (Mevik et al., 2015) with leave-one-out cross-validation after dividing the samples randomly into a calibration (n = 30) and validation (n = 7) data set.

3. RESULTS

To achieve a good calibration it is necessary to cover the full range of extractive contents (EC) in the resource. The available *E. argophloia* heartwood samples had a wide range of extractive content varying between 4.64 and 18.85% (Table 2), similar to what was reported for *E. bosistoana* (Sharma et al., 2014). NIR calibrations also benefit from larger numbers and more than the available 37 *E. argophloia* samples would have been desirable.

Table 2. Summary statistics of E. argophloia heartwood ethanol extracts used for NIR calibration

	Mean Extractive content (%)	Min Extractive content (%)	Max Extractive content (%)	CV
Calibration n=30	*10.76*	*4.64*	*15.80*	*0.25*
Validation n=7	*11.24*	*7.85*	*18.85*	*0.35*
All n=37	*10.59*	*4.64*	*18.85*	*0.27*

3.1. Pre-processing methods

Table 3 shows the quality of PLS regression models to predict the amount of ethanol soluble heartwood extractives in *E. argophloia* from NIR spectra collected from cross-sections of discs after various spectra manipulations. Regardless of the pre-processing method the PLS models were of reasonable accuracy with residual mean square errors (RSME) of ~2% when considering that the extractive content varied between 4.6% and 18.9%. However, models based on the 1st derivative of the raw spectra yielded the best results with $RMSE_C$ = 1.91% and $RMSE_V$ = 1.11%. The 1st derivative of the spectra were used in the subsequent analysis.

Table 3. PLS regression models for calibration and validation of EC with and without pre-treatment methods. (SNV: Standard Normal Variate; RMSE: Residual Mean Square Error)

	Calibration			Validation	
Pre-treatment	R^2_C	$RMSE_C$ (%)	Number of components	R^2_V	$RMSE_V$ (%)
Raw spectra	*0.51*	*2.14*	*5*	*0.73*	*1.31*
SNV	*0.52*	*2.13*	*6*	*0.67*	*1.46*
1st derivative	*0.61*	*1.91*	*5*	*0.81*	*1.11*
2nd derivative	*0.65*	*1.82*	*6*	*0.40*	*1.98*
SNV + 1st derivative	*0.59*	*1.96*	*7*	*0.75*	*1.27*
SNV + 2nd derivative	*0.68*	*1.72*	*6*	*0.21*	*2.27*

3.2. Variable selection

Not all signals in NIR spectra are correlated to the investigated property. Elimination of unimportant wavenumbers often results in more reliable and robust models. Significant Multivariate Correlation (sMC) was chosen for variable selection (Tran et al., 2014). Numerous significant signals were identified by the sMC algorithm (Figure 2). Signals which explain most of the variance in extractive content were located between 6000 cm^{-1} to 4000 cm^{-1} with two additional peaks at 8330 cm^{-1} and 6880 cm^{-1}. The bands near 6000 cm^{-1} and 4680 cm^{-1} have been assigned to the 1st overtone of C-H stretching vibrations of methyl, methylene and ethylene groups (Schwanninger et al., 2011, Schimleck et al., 1996) in extractives. The band around 5793 cm^{-1} is the stretching vibrations of CH bands that correlated

to lignin. Two strong and board signals in the average 1st derivative spectra of *E. argophloia* heartwood at ~7070 cm^{-1} and ~5100 cm^{-1} assigned to adsorbed water were more or less rejected by the model.

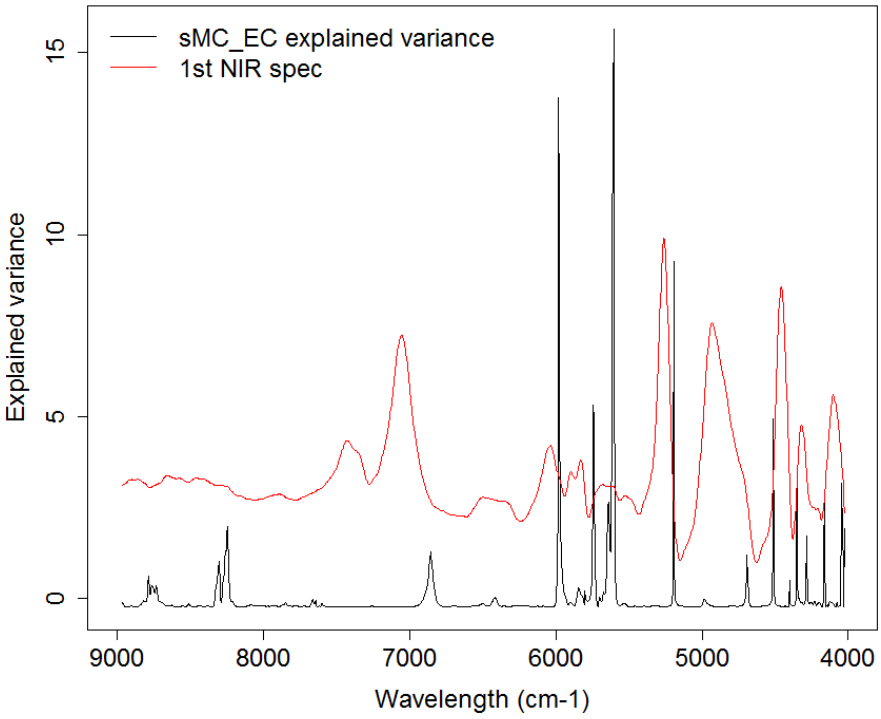

Figure 2. Average 1st derivative NIR spectra for E. argophloia heartwood (red) and the explained variance in EC for each wavenumber (black).

The 173 wavenumbers (~15% of the total) of the NIR spectra identified by the sMC algorithm (significance level = 0.05) were chosen to build an improved PLS model. This improved the accuracy of the model, reducing the $RMSE_v$ from 1.11% to 0.92% (Table 4).

Table 4. PLS regression models for calibration and validation of EC with selected variables on 1st derivative NIR spectra of E. argophloia

	Calibration			Validation	
Variables used	R^2_C	$RMSE_C$ (%)	Number of components	R^2_V	$RMSE_V$ (%)
173 selected by sMC	*0.81*	*1.33*	*6*	*0.91*	*0.92*
1296 (all)	*0.61*	*1.91*	*5*	*0.81*	*1.11*

3.3. Cross species calibration of NIR for EC

As the available number of *E. argophloia* samples was at the lower limit of what is usually required for robust NIR calibrations the possibility of building a cross species calibration was investigated. Previously a NIR calibration for heartwood EC of *E. bosistoana* based on 126 samples was created. *E. bosistoana* is a species closely related to *E. argophloia* (Brooker, 2000). The *E. bosistoana* model was giving reasonable predictions of the EC in the *E. argophloia* data. The RMSE was with 1.43% still small compared to the variation in the sample (Table 2). No obvious bias between the measured and predicted EC values was observed (Figure 3). This indicted that it could be feasible to build a multiple eucalyptus species NIR calibration for EC.

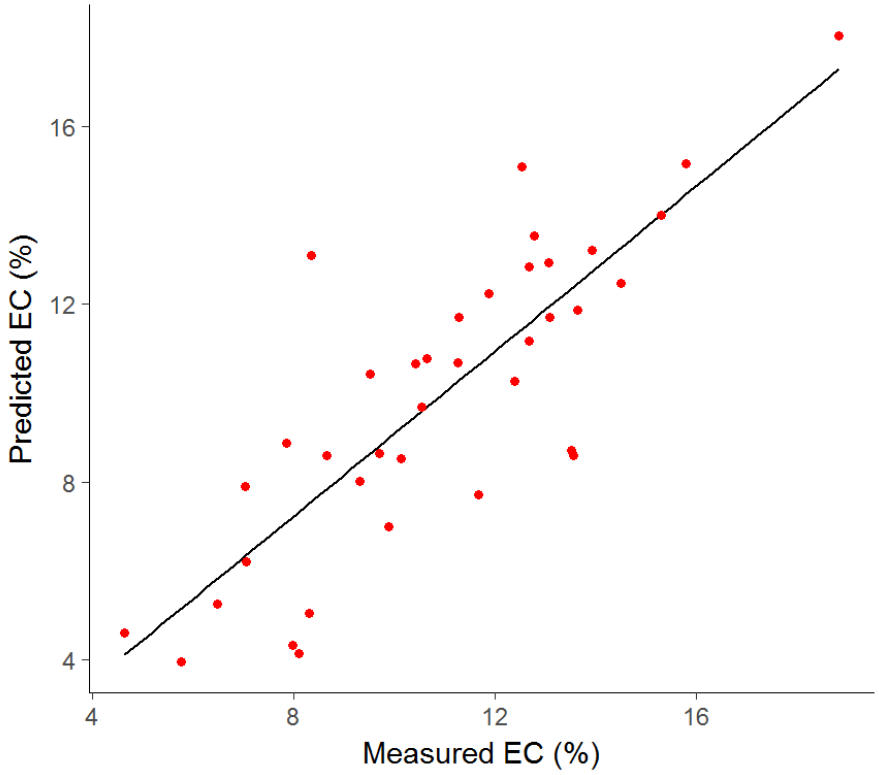

Figure 1. Correlation of measured and predicted EC from NIR spectra of discs of E. argophloia using a calibration for E. bosistoana.

4. CONCLUSION

Reviewing literature on naturally durable wood it appears necessary and possible to ensure consistent high quality timber through a breeding programme. A tree corer has been developed to quickly extract 12 mm cores from high density trees, as such a tool was not commercially available. Furthermore, assessing natural durability directly is resource consuming and impractical to include directly in a breeding programme due to the high numbers of sample required. But it has been shown that it was possible to predict ethanol soluble extracts in heartwood of *E. argophloia* quickly by NIR. This required the development of a PLS regression model from solid wood samples. Using the sMC algorithm a robust model was method obtained to predict the ethanol soluble extracts in heartwood of *E. argophloia*. To compensate for the rather small number of samples used for calibration a model for *E. bosistoana* was also tested for *E. argophloia*. This also gave good predictions, indication that a cross species calibration could be feasible.

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