An approach to quantify natural durability of *Eucalyptus bosistoana* by near infrared spectroscopy for genetic selection

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Abstract

Natural durability within a timber species can be variable. Hence efficient assessments of natural durability are required to ensure quality either through tree breeding or segregation during production. In this study, first the relationship between extractive content and mass loss of *Eucalyptus bosistoana* heartwood caused by a white and a brown-rot fungus was validated. Then the ability of NIR spectroscopy as a high-throughput method to evaluate heartwood decay resistance was examined. Finally the NIR method was applied to a tree breeding trial. A correlation between extractive content and mass loss against the white-rot fungus (*Perenniporia tephropora*) and the brown-rot fungus (*Coniophora olivacea*) ~~fungus~~ were found. Analysis of NIR spectra indicated that this relationship is causal with shared bands for mass loss and extractive content models at 6650, 6017, 5265 and 4659 cm^{-1}. Partial least squares regression (PLSR), supplemented with spectra normalisation and variable selection, allowed prediction of mass loss with a residual mean square error (RMSE) of 7.48~~% and 5.76~~ % and 5.76 % for the white-rot and brown-rot, respectively. This level of precision allowed the characterisation of a *E. bosistoana* resource which showed a range of mass loss from 0 to 60 %. Genetic control was found for mass loss by the white-rot (h^2 = 0.70 and 0.24) and the brown-rot (h^2 = 0.15 and 0.13) at two sites in New Zealand. The rankings were correlated between sites, with genetic correlations (R_g^2) of 0.69 and 0.63 for white-rot and brown-rot, respectively, as well as to the predicted extractive content (0.82 to 0.92). However, the study indicated a significant site effect on the decay resistance of the *E. bosistoana* heartwood. In summary, this study has shown that the decay resistance could be assessed rapidly and efficiently using NIR technology for genetic selection.

Keywords: *Coniophora olivacea*; Decay resistance; Heartwood; Mass loss; *Perenniporia tephropora*; Tree breeding

1 Introduction

Wood is a biodegradable material. To ensure a long service life in unfavourable environments timber is often treated with toxic preservatives (NZS3640, 2003; Treu et al., 2019) creating hazardous waste (Townsend and Solo-Gabriele, 2006). However, the heartwood of some species is highly resistant against biodegradation, able to substitute preservative treated timber for in-ground or marine applications (AS5604, 2005; Scheffer and Morell, 1998). Unfortunately such highly naturally durable timber is not common and often sourced from unsustainably or illegally harvested tropical forests (Nellemann, 2012). As such resources are dwindling, there is an opportunity to sustainably grow naturally durable timber species in fast-growing, short-rotation plantations (Bhat et al., 2005; Bush et al., 2011; Dünisch et al., 2010; Stirling et al., 2015).

Eucalyptus bosistoana heartwood is listed as class 1 ground-durable, able to last more than 25 years in the ground according to the Australian Standard (AS5604, 2005) and has many other favorable properties such as fast growth (Poynton, 1979), some frost tolerance (Hamilton, 1983) and high stiffness (Bootle, 2005). *E. bosistoana* has been chosen by the New Zealand Dryland Forests Initiative (NZDFI) to establish a sustainable plantation resource of naturally durable timber to, in the first instance, supply posts for agricultural industries (Millen et al., 2018).

The natural durability of heartwood is variable, both within (Sherrard and Kurth, 1933) and between trees (Bush et al., 2011; Hillis, 1987). Durability standards, which have been developed largely for old-growth native forest

resources, recommend the use of the generally better performing outer heartwood of mature trees (AS5604, 2005). Therefore, good resistance to biodegradation of the timber, which is grown in short-rotation plantations needs to be ensured. A breeding programme can ensure quality, as the variation in decay resistance between individual trees of a species is partially under genetic control (Bush et al., 2011; Harju and Venalainen, 2002; Yu et al., 2003).

A major impediment for the inclusion of natural durability into tree breeding programmes, which rely on large sample numbers, is the laborious and time consuming nature of the available methods for assessing durability (AWPC, 2007). While field tests are not only affected by environmental factors such as soil, temperature and rainfall, they also, in particular for class 1 durable timber which needs to last more than 25 years in ground, carry on for decades. Alternatively, accelerated laboratory tests can be used for screening, but suffer limited transferability to ~~even~~ real-life conditions as they are specific to the limited number of organisms included in the test (Jacobs et al., 2019). Laboratory test results are more comparable between laboratories but still take several months to complete. Consequently, a rapid and efficient method for durability assessments is needed.

Apart from wood anatomy (Meyer-Veltrup et al., 2017), a key factor determining the natural durability of heartwood is the presence of secondary low molecular weight organic compounds, the so called extractives (Hawley et al., 1924; Rudman, 1964). Therefore in a first instance, the extractive content (EC) in heartwood may provide an indirect measure of relative decay resistance and therefore could be used as an alternative way to assess natural durability within ~~a~~ species (Morris and Stirling, 2012). Again solvent extraction methods are costly and laborious, requiring thorough sample preparation (TAPPI, 2012) and hence are unsuitable when large tree breeding ~~plantations~~populations are concerned. However, Near Infrared Reflectance (NIR) spectra, contain information of the chemical composition of materials. NIR spectra are quickly acquired with affordable instrumentation from solid wood samples, reducing demand on sample preparation and measurement (Schimleck et al., 2005). NIR spectroscopy has been successfully used to accurately predict the EC in wood (Li and Altaner, 2018; Ribeiro da Silva et al., 2013; Schimleck et al., 2009) and subsequently employed in a tree breeding programme (Li et al., 2018).

NIR is also able to successfully predict non-chemical wood properties such as stiffness or density by multivariate statistical analysis (Tsuchikawa and Kobori, 2015). Not surprisingly some attempts were made with varying success to use the technology to predict fungal decay resistance directly rather than the correlated EC (Bush et al., 2011; Gierlinger et al., 2003; Jones et al., 2011).

The objectives of this study were: firstly to verify the current procedure of selecting *E. bosistoana* of high EC in a breeding programme to improve durability. Secondly use this dataset to determine how precisely mass loss by fungi can be ~~directly~~ predicted from NIR spectra and thirdly to explore the potential of applying the NIR calibrations for decay resistance to a tree breeding trial.

2 Materials and methods

2.1 Materials

Origin of the 1765 *E. bosistoana* heartwood samples has been described in detail (Li and Altaner, 2018; Li et al., 2018). In brief, 41 open-pollinated *E. bosistoana* families were grown at two different sites in the ~~s~~South Island of New Zealand in 2010, including the Craven Road (Longitude: 173°56′, Latitude: 41°26′) and Martin (Longitude: 172°39′, Latitude: 43°11′) sites studied in detail here (Li et al., 2018). The sites were on alluvial soils and experienced an average annual rainfall of around 1000 mm. When ~7 years old, a 14-mm diameter core was extracted at ~50 cm trunk height. In total, 1115 trees from 40 families and 650 trees from 35 families were sampled from the Martin and Craven Road sites, respectively. Detailed information on genetic variation of the growth traits diameter at breast height (DBH), heartwood diameter, sapwood diameter and sapwood area were reported previously (Li et al., 2018).

2.2 NIR spectra and predicted extractive content (pEC)

Acquisition of NIR data and the procedure to establish a partial least squares regression (PLSR) calibration to predict the amount of ethanol soluble material (EC) from NIR spectra was reported earlier (Li and Altaner, 2018). Briefly, NIR spectra with a resolution of 4 cm^{-1} between 9000 and 4000 cm^{-1} were recorded with a NIR fibre-optical probe (Model N-500, Bruker Optics, Germany) every 5 mm along the heartwood of air-dried stem cores and used to predict EC with a PLSR model (Li and Altaner, 2018). The effect of grain direction and moisture content on the NIR spectra was reduced by using the External Parameter Orthogonalisation (EPO) algorithm (Roger et al., 2003). The pEC of a tree was calculated as the mean of the ~~weighted~~ core measurements. ~~The pEC of a radial position was weighted by the area of the ring in the radial position.~~

EC was determined by Accelerated Solvent Extraction with ethanol from dried and milled (20-mesh screen) wood.

2.3 Decay testing

After NIR spectra collection, a subset of the cores from section 2.1 and two additional similar sites was used for fungal decay tests. Stratified sampling was applied to ensure this subset represented equal numbers of low (<5 %), medium (5~~-10%) and high (>10~~-10 %) and high (>10 %) EC. In total 421, 20 mm long, ~~421~~ sections of fully developed heartwood were cut from 273 cores next to the pith. The mass loss test was a modification of the Australasian Wood Preservation Committee procedures (AWPC, 2007; Cookson, 2017). Prior to exposure to the brown-rot fungus (*Coniophora olivacea*) and the white-rot fungus (*Perenniporia tephropora*) the samples were leached after vacuum-impregnation with water (30 min. at −95 kPa) in jars with at least three times their volume of water on a shaking water bath at 35 °C for five days. Water was changed daily. Samples were then adjusted in the laboratory until constant mass was achieved (at about 11 % moisture content) and the initial air-dry masses were recorded. Test blocks were placed in plastic bags and sterilised by gamma-irradiation at 25 kGy.

Samples were exposed to decay fungi in an agar-tray bioassay. Stainless steel trays with dimensions of 370 × 225 ×× 95 mm were sterilized by gamma-irradiation and filled with 1.2 L of autoclaved 2 % agar solution containing 1 % malt extract under sterile conditions and left to solidify. Six trays were inoculated with about 15 plugs of one of the test fungi, while one tray was left un-inoculated as the sterile control to which later 6 samples were added, and incubated at 25 °C. After 10 days a plastic mesh, sterilised by gamma-irradiation, was placed in each tray and the sterilised heartwood samples were then placed on top with the grain oriented horizontally. After 12 weeks at 25 °C, the samples were removed from the trays, gently wiped to remove excess surface mycelium, air-dried in the laboratory to constant mass, and then weighed to obtain the final masses. The percentage of mass loss (ML) caused by the fungus in each block was determined by the following equation:

$$\mathrm{ML}\ (\%) = \frac{m_0 - m_1}{m_0} \times 100 + M_c$$

Where m_0 was the initial air-dry mass, m_1 was the final air-dry mass of the sample after exposure to the fungus and M_c was the average mass change (%) of the six sterile controls.

Non-durable *Pinus radiata* and *E. obliqua* specimens were included in the tests to verify the vitality of the cultures. These samples averaged >60% and >50 % and >50 % ML by *C. olivacea* and ~20% and >50 % and >50 % ML by *P. tephropora*, respectively.

2.4 NIR modeling of ML by the brown-rot and white-rot fungus

NIR spectra and ML data were used to builat PLSR calibration models separately for brown-rot and white-rot using the 238 and 183 samples, respectively. Different spectra normalization algorithms were tested for their effect on the precision of the PLSR models, including standard normal variate (SNV) as well as first and second derivatives using Savitzky-Golay smoothing with a second-order polynomial and a window size of 15 data points. The stability of the PLSR models was analysed from the data which was randomly split 100 times into a calibration data set, comprising 80 % of the total number of samples, and a validation data set, made up of the remaining 20 % of the total number of samples. For each data set the number of PLSR components was optimised based on the lowest residual mean square error (RMSE).

The significance multivariate correlation (sMC) filter method (Mehmood et al., 2012; Tran et al., 2014) was used to assess the effect of variable selection and identify NIR frequencies correlated to ML.

2.5 Statistical analysis

The calculation of genetic parameters has been previously described in details (Change 'details' to 'detail') (Li et al., 2018). Statistical analysis was conducted with the R software (R Core Team, 2017). The *pls* package (Mevik et al., 2015) was used for developing the PLSR calibration models and optimal components selection with leave-one-out cross-validation. NIR spectra processing were done using the *prospectr* package (Stevens and Ramirez–Lopez, 2014). The important variationvariable selection by using the sMC (alpha = 0.05) algorithm was implemented by the *plsVarSel* package (Mehmood et al., 2012). All genetic models were fitted using the *ASReml-R* package (Gilmour et al., 2009).

3 Results

3.1 Decay tests of *E. bosistoana* heartwood

The ML data ranged from -0.3 % to 59.9% with an average of 9.62 59.9% with an average of 9.62 (add space after 59.9 but do not replace to by -) (Why no space here? What are your rules regarding space before %? There are more instances without a space in the manuscript.)% for the white-rot fungus *Perenniporia tephropora* and from -0.50% to 48.7% with an average of 7.82 % to 48.7 % with an average of 7.82 % for the brown-rot fungus *Coniophora olivacea* (Table 1). The extractive content of the heartwood in the trees was predicted by NIR (pEC) and varied from 0.63% to 17.2% with a mean of 7.68 % to 17.2 % with a mean of 7.68 (replace 17.2 with 17.20)%. Negative correlations between pEC and ML for white-rot (r = -0.32, $P < 0.01$) and brown-rot (r = -0.42, $P < 0.01$) were observed. Samples with a pEC above ~10 % seemed to be mostly decay resistant under the test conditions (Fig. 1).

Table 1 Summary statistics for mass loss (ML) data of *E. bosistoana* heartwood exposed to the white-rot fungus *P. tephropora* and the brown-rot fungus *C. olivacea* used for multivariate calibration of NIR spectra, as well as their predicted extractive content (pEC). CV: coefficient of variation; SD: standard deviation; n: number of samples.

alt-text: Table 1

	ML		pEC
	White-rot (*Perenniporia tephropora*)	Brown-rot (*Coniophora olivacea*)	
n	183	238	421
Max (%)	59.90	48.70	17.20

Mean (%)(Change column width to accommodate (%) in same row)	9.62	7.82	7.68
Min (%)	~~-0.30~~ −0.30	−0.50	0.63
CV	1.05	1.01	0.56
SD (%)	10.14	7.91	4.26

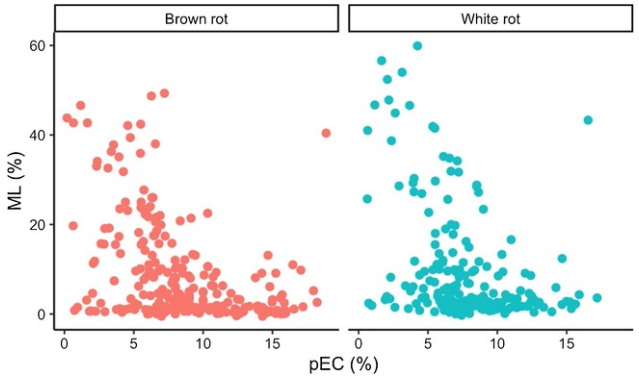

Fig. 1 Correlation between the predicted extractive content (pEC) of *E. bosistoana* heartwood and mass loss (ML) caused by the white-rot fungus *P. tephropora* and the brown-rot fungus *C. olivacea*.

alt-text: Fig. 1

3.2 Calibrating NIR for mass loss in *E. bosistoana* heartwood

Six different NIR spectra processing methods (including no processing) were assessed for their effect on the precision of PLSR calibration models for ML of *E. bosistoana* heartwood exposed to the white-rot fungus *P. tephropora* or the brown-rot fungus *C. olivacea*. When using all variables, spectra processing did only show small effects on the residual mean square error (RMSE) of the models for both, the ML caused by the white-rot and the brown-rot (Fig. 2). The mean coefficient of determination for the calibrations (R^2_{cal}), ranging between 0.36 and 0.58, for the ML prediction of white-rot was higher than for brown-rot with R^2_{cal} ranging between 0.22 and 0.48.

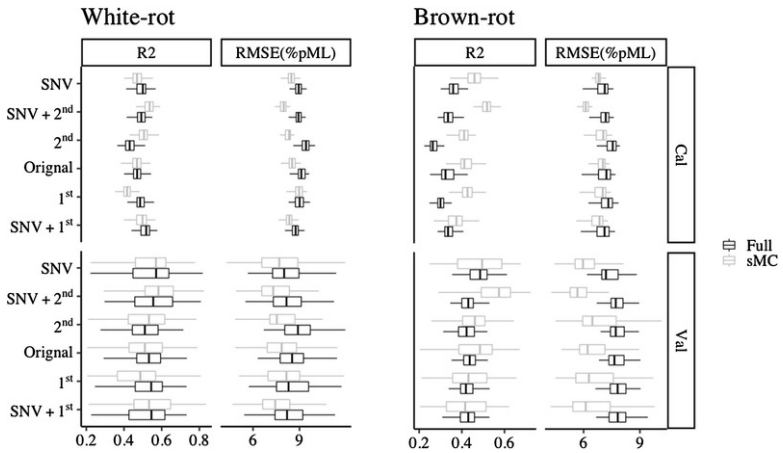

Fig. 2 Effect of spectra processing and variable selection ~~(sMC)~~ on the performance PLSR models to predict mass loss in *E. bosistoana* heartwood caused by the brown-rot fungus *C. olivacea* and the white-rot fungus *P. tephropora*. PLSR models were calculated from 100 random samples of calibration and validation data. Full spectra (1296 frequencies) in black and models after sMC variable selection in grey. R^2: coefficient of determination; RMSE: root-mean-square error of prediction; Cal: calibration data; Val: validation data; SNV: standard normal variate spectra, Original: unmodified spectra, 1~~st: first derivative spectra, 2nd~~st: first derivative spectra, 2nd : second derivative spectra.

alt-text: Fig. 2

The sMC algorithm identified only ~10% of the total variables (i.e. frequencies) as being useful for ML prediction for both fungi. These models were generally performing better than those based on the full spectra (Fig. 2). PLSR models from SNV and SNV+2ndnd normalised spectra showed smallest errors in ML prediction for both, white- and brown-rot (Fig. 2). For the white-rot and brown-rot RMSE reduced from 8.17% to 7.48% and 7.79% to 5.76% for the SNV+2nd % to 7.48 % and 7.79 % to 5.76 % for the SNV+2nd transformed spectra, respectively. The optimal number of PLSR components in the models from 100 randomly chosen calibration data sets was reduced and less variable after sMC variable selection (Fig. 3). Together withBecause of the smaller RMSE and the low and stable number of PLSR components, SNV+2ndnd spectra treatment with sMC variable selection waiis the best choice to predict both, ML by the white-rot (*P. tephropora*) or the brown-rot (*C. olivacea*) (Fig. 3). These predictions, with an average $RMSE_V$ for ML by the white-rot wereof 7.48 %, were based on only 56 of the 1296 frequencies and in the case of the brown-rot 5.76 % usingon 173 frequencies, achieving and $RMSE_V$ of 5.76 %, and only requiring only 7 and 2 PLSR components, respectively.

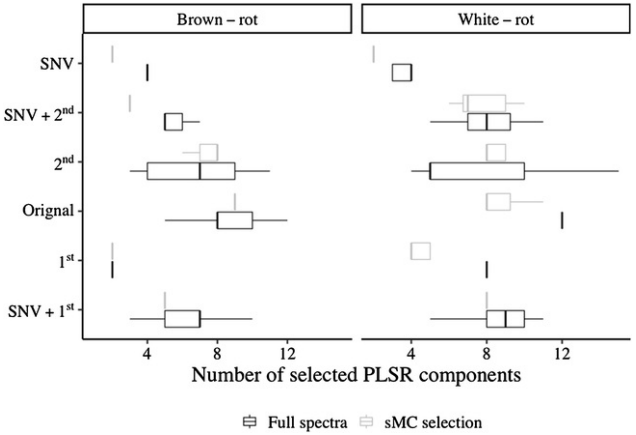

Fig. 3 Optimal number of PLSR components in the brown- and white-rot models to predict mass loss in *E. bosistoana*. Boxplots represent 100 randomly chosen calibration data sets for each spectra processing method, each without (full spectra) and with sMC variable selection. SNV standard normal variate spectra, Original: unmodified spectra, 1stst: first derivative spectra, 2nd: second derivative spectra.

alt-text: Fig. 3

The positive effect of sMC variable selection on the PLSR models can be seen from the smaller standard deviations of 100 simulated pML predictions of the models based on SNV+2ndnd spectra (Fig. 4 top). The examination of residuals revealed that both, the optimizsed white- and brown-rot models, tended to under-predict at high ML values caused (Fig. 4 bottom).

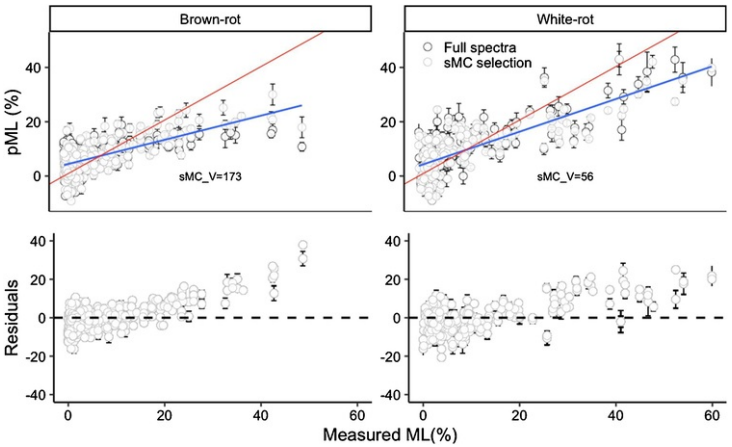

Fig. 4 Measured vs. predicted mass loss (pML) in *E. bosistoana* heartwood caused by the brown-rot fungus *C. olivacea* and the white-rot fungus *P. tephropora* data using SNV+2ndnd processed spectra based on full and sMC selected EPO and SNV+2nd transformed spectra (top). Bars for predicted values of individual samples represent the standard deviations obtained from the 100 simulated models. Regression line of the model in blue; equality of measured and predicted ML in red. Residuals from 100 simulated models dependent on measured ML (%) in

the brown- and white-rot models ~~using of sMC selection and SNV+2nd~~nd ~~processed EPO transformed spectra~~ (bottom). ~~pML: predicted mass loss,~~ sMC_V: number of selected variables by sMC.

alt-text: Fig. 4

Similar frequencies contributed to the white- and brown-rot ML models, indicating that the two fungi were responding to the same chemical features in the heartwood (Fig. 5). While the relationship of ML to wood chemistry represented in NIR spectra was more complex ~~than that to EC~~, with more signals in the region from 7000 to 4000 cm^{-1}, than that to EC, they shared four strong bands at 6650, 6017, 5265 and 4659 cm^{-1}. This indicated a close relationship between the ethanol soluble extractives in *E. bosistoana* heartwood and the mass loss by wood decaying fungi.

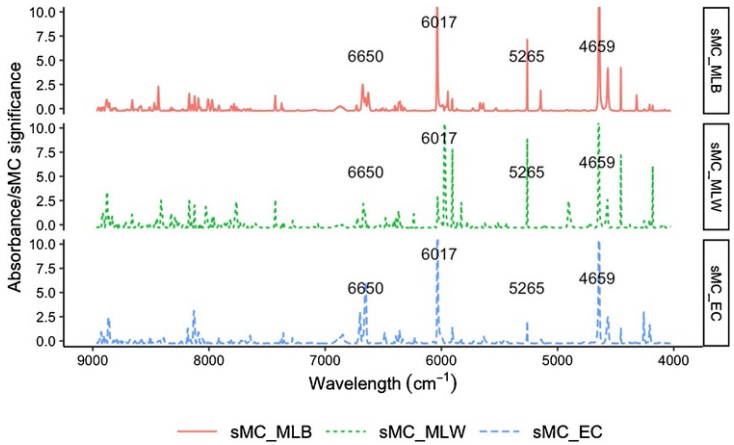

Fig. 5 Contribution of individual NIR frequencies on PLSR models for predicting ethanol soluble extractives (EC) and mass loss of *E. bosistoana* heartwood by a white-rot (MLW) and a brown-rot (MLB) fungus ~~of *E. bosistoana* heartwood~~ as identified by the sMC algorithm.

alt-text: Fig. 5

3.3 Predicting mass loss in *E. bosistoana* breeding trials

The average ~~of the~~ pML by the white- and brown-rot fungus from the 100 simulated models, using SNV+2~~nd~~nd transformation with sMC, were used for genetic analysis. The pEC had a significant negative correlation with the predicted mass losses (pML) (Fig. 6). The linear model correlation between the pEC and the pML were similar for the brown-rot (r = -0.47) and the white-rot (r = -0.55).

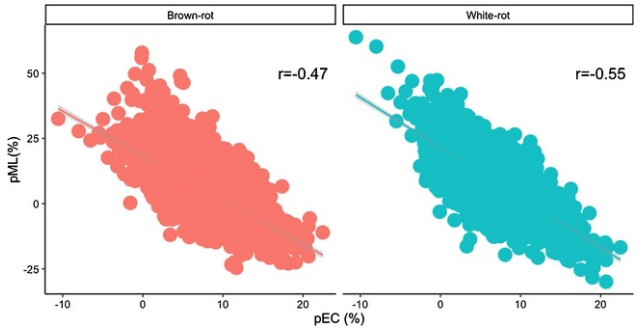

Fig. 6 Correlation between the predicted EC (pEC) and the predicted ML (pML) by the white-rot fungus *P. tephropora* and the brown-rot fungus *C. olivacea* in heartwood samples from 1765 *E. bosistoana* trees.

alt-text: Fig. 6

While the average pEC in the heartwood of *E. bosistoana* was of similar magnitude in both sites (7.67~~% and 9.63~~ % and 9.63 %), the pML by both fungi was ~~14.86~~% and 16.98 ~~% and 16.98 % (delete (as entered in brackets later).)~~ (delete here)higher for trees from Craven Road (14.86 % and 16.89 %) than for those grown at Martin (1.86~~% and 2.01~~ % and 2.01 %), indicating a site-level effect (~~Table 4~~2 (reference should be to Table 2 not 4)). The CV for the pEC (42~~% and 46~~ % and 46 %) was lower than for the pML (58 %~~ to~~ –74 %) in both sites (Table 2).

Table 2 Descriptive statistics of heartwood traits of 7-year-old *E. bosistoana* grown in Canterbury (Martin) and Marlborough (Craven Road), New Zealand. pEC: predicted heartwood extractive content, pML: predicted mass loss, CV: coefficient of variation, h^2: narrow sense heritability (standard error).

alt-text: Table 2

Site	Trait	Min	Max	Mean	CV (%)	h^2
Martin	pEC (%)	1.83	22.38	9.63	42	0.16 (0.05)
	pML (%) brown-rot	−24.59	55.86	1.86	63	0.13 (0.02)
	pML (%) white-rot	−29.92	38.26	2.01	74	0.24 (0.04)
Craven Road	pEC (%)	0.77	18.02	7.67	46	0.25 (0.08)
	pML (%) brown-rot	−10.45	57.83	14.86	58	0.15 (0.02)
	pML (%) white-rot	−8.93	47.33	16.98	69	0.70 (0.11)

Estimated narrow sense heritabilities for pEC and pMLs were higher at the Craven Road than the Martin site (Table 2), with pML for white-rot resistance ($h^2 = 0.70$ and 0.24) being under stronger genetic control than pML for brown-rot resistance ($h^2 = 0.15$ and 0.13) and pEC ($h^2 = 0.25$ and 0.16).

Strong negative genetic (-0.82 to -0.92) and phenotypic (-0.72 and -0.83) correlations were found between the pEC and the pMLs by the two wood decaying fungi (Table 3). The correlations were similar between the sites. Moderate positive correlations ranging from 0.67 to 0.72 were found between pML of the two fungi. Genetic correlations (r_g) between the two sites for pEC as well as pML for the white-rot and brown-rot were 0.60, 0.63 and 0.69, respectively (Fig. 7).

Table 3 Phenotypic (above diagonal) and genetic correlations (below diagonal) between heartwood traits of 7-year-old *E. bosistoana*. Standard error in parentheses. pML: predicted mass loss; pEC: predicted extractive content.

alt-text: Table 3

Martin	Trait	pML brown-rot	pML white-rot	pEC
	pML brown-rot		0.71 (0.02)	−0.73 (0.01)
	pML white-rot	0.72 (0.09)		~~0.72 (0.02)pEC 0.92 (0.09)~~ −0.72 (0.02)
	pEC	−0.92 (0.09)	−0.84 (0.12)	
Craven Road	pML brown-rot		0.69 (0.01)	−0.78 (0.03)
	pML white-rot	0.67 (0.12)		~~0.83 (0.02)pEC 0.82 (0.11)~~ −0.83 (0.02)
	pEC	−0.82 (0.11)	−0.89 (0.05)	

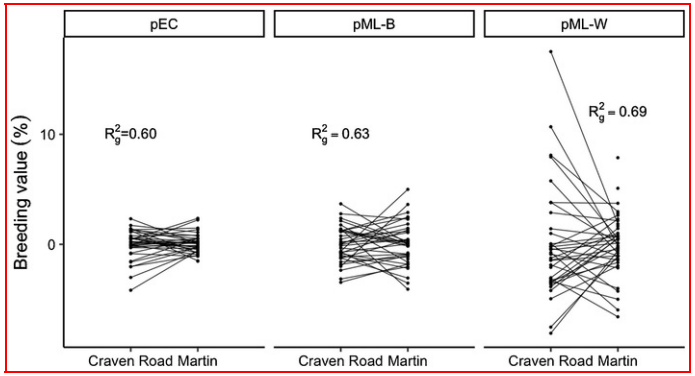

Fig. 7 Family rankings for predicted extractive content (pEC) as well as predicted mass loss by the white-rot fungus *P. tephropora* (pML-W) and the brown-rot fungus *C olivacea* (pML-B) in heartwood of *E. bosistoana* families grown on two sites, Craven Road and Martin. Family breeding values are expressed as deviation from the trait mean.

alt-text: Fig. 7

The family level breeding values of pML by both fungi were plotted against each other for both sites (Fig. 8). The below average breeding values for the pML correlated with those for above average pEC.

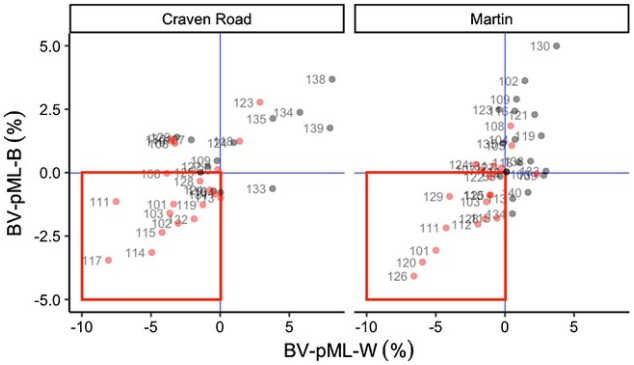

Fig. 8 Relationship between breeding values for predicted mass loss of 56 *E. bosistoana* families at age 7 across 2 sites for the white-rot fungus *P. tephropora* (BV pML-W) and the brown-rot fungus *C olivacea* (BV pML-B). Breeding values for predicted extractive content (pEC) above average (red) and below average (black). Red square: families with below average pML for both fungi. Blue lines separate below and above average pML breeding values.

alt-text: Fig. 8

The realised genetic gains of pML both by the white-rot and the brown-rot fungus were presented in Table 4 for selection of the top 10~~%, 20% and 30~~ %, 20 % and 30 % of the families. The pML by the brown-rot improved by 18~~.06~~ % to -32~~.12~~ % relative to the population mean, depending on site and selection intensity. Larger genetic gains ranging from 30~~.38~~ % to -62~~1.86~~ % were achievable for the white-rot.

Table 4 Potential genetic gain of predicted mass loss by the white-rot fungus *P. tephropora* (pML-W) and the brown-rot fungus *C. olivacea* (pML-B) of *E. bosistoana* families at age 7-years from two sites depending on selection intensity. Improvement relative to the population mean.

alt-text: Table 4

Selection intensity	Martin			Craven Road		
	10~~%20%30%10%20%30~~ %	20 %	30 %	10 %	20 %	30 %
pML-W (%)	54.48	39~~.43~~	30~~.38~~	61~~.86~~2	49~~.01~~	43~~.33~~
pML-B (%)	32.12	25~~.14~~	21~~.48~~	27~~.40~~	21~~.11~~	18~~.06~~

4 Discussion

4.1 Decay tests of *E. bosistoana* heartwood

A stratified sample from more than 1700 cores was used to obtain a broad range of pEC for the assessment of ML. The broad range in ML benefited the multivariate statistics used for NIR calibration (Martens and Næs, 1984). Large variation in ML by wood rots is commonly reported (AWPC, 2007; Brischke et al., 2013; Bush et al., 2011; Harju and Venäläinen, 2006; Jones et al., 2011). The higher ML by the white-rot ($P = 0.013$) indicated that it ~~is to be~~ a bigger threat to *E. bosistoana* heartwood and a more rigorous test for its durability assessment.

Like for the *E. bosistoana* heartwood, significant negative relationships between ML due to wood decay fungi and heartwood extractives (Hawley et al., 1924) have been reported for other species such as *Thuja plicata*, *Chamaecyparis nootkatensis* (Taylor et al., 2006), *Larix* sp. (Gierlinger et al., 2004), *E. cladocalyx* (Bush et al., 2011) or *Tectona grandis* L. (Haupt et al., 2003).

4.2 Calibrating NIR for mass loss in *E. bosistoana* heartwood

The PLSR models were stable over 100 random calibration data selections, and had reasonable ability for ML prediction by the white- and the brown-rot fungus in *E. bosistoana* heartwood (Fig. 4). Spectra conversion by SNV ~~and~~ followed 2~~nd~~nd derivative transformation reduced the optimal number of PLS components. Variable selection with the sMC algorithm reduced the $RMSE_V$ of the brown-rot model by ~17 %, using only ~8 % of the frequencies (Fig. 4). A positive effect of 2~~nd~~nd derivative transformation of NIR spectra was also reported ~~by Sykacek et al. (2006),~~ for PLSR models predicting ML in larch by the brown-rot *Gloeophyllum trabeum*~~.~~ (Sykacek et al. 2006).(Please link reference) The precision of the ML models for *E. bosistoana* by the two fungi were with coefficients of determination between 0.55 to 0.58 (Fig. 2) not as good as the EC model ($R^2 = 0.87$) (Li and Altaner, 2018). Successful use of NIR to predict ML by decay fungi were also reported for *Sequoia sempervirens* (Jones et al., 2011) as well as *Cupressus lusitanica* and *C. macrocarpa* (Jones et al., 2013). Factors contributing to the lower performance of the ML models were the fact that ML measurements were likely~~are~~ less precise than EC measurements and that mass loss by decay fungi was~~is~~ not only affected by chemical wood features like heartwood extractives (Hawley et al., 1924) but also by physical and anatomical features like density and porosity (Taylor et al., 2002), which ~~are~~were not directly represented in NIR spectra. Furthermore, small quantities of toxic heartwood compounds may have had a strong effect on ML (Taylor et al., 2006) but ~~are~~were difficult to quantify in NIR, which has a reasonably high detection threshold. In addition, the measured ML values were not normally distributed but skewed towards zero, resulting in a nonlinear relationship between pEC and ML. The linear nature of PLSR models therefore resulted in many predicted negative ML values, reducing the model precision (Minasny and McBratney, 2008). This should not influence the usability in a tree breeding program as selections can be based on relative values.

The sMC algorithm allowed the identification of relevant frequencies in the PLSR models (Tran et al., 2014). The sMC algorithm identified numerous frequencies related to ML in *E. bosistoana*, mainly located in the regions from 9000 to 8000 cm^{-1}~~, 7000 to 5500 cm^{-1}~~, 7000 to 5500 cm^{-1} and 5000 to 4000 cm^{-1} (Fig. 5). The four signals at 6650, 6017, 5265 and 4659 cm^{-1}, which not only dominated the ML models but were also prominent in the EC model, were associated with extractives and lignin (Li and Altaner, 2019; Schwanninger et al., 2011). The fact that these four signals were relevant for ML and EC models supported the conclusion that heartwood extractives inhibit decay fungi is not only statistical (r of -0.47 to -0.55; Fig. 6) but causal (Hawley et al., 1924). However, as more NIR signals are correlated to ML, other compounds than those in dried ethanol extracts, e.g. those which were not extracted or destroyed during the extraction process (~~Ellwood and Ecklund, 1961~~Hillis 1987(Please link to Hillis 1987 reference (and delete Ellwood reference from reference list)), could have contributed to decay resistance of *E. bosistoana* heartwood.

4.3 Predicting mass loss in *E. bosistoana* breeding trials

The moderate heritability of pML by the two fungi found at both sites, with h^2 ranging from 0.13 to 0.70 spanned the reported range for ML of other species; 0.21 to 0.27 for *Picea glauca* (Yu et al., 2003), 0.52 to 0.58 for *E. cladocalyx* (Bush et al., 2011) or 0.49 for yellow-poplar (*Liriodendron tulipifera* L.) (Lowerts and Kellison, 1981).

Between the two fungi, the level in pML were similar at a site and showed moderate genetic correlation (0.72/0.67). The significant genetic correlation between pEC and pML for the white-rot (-0.84/-0.89) as well as brown-rot (-0.92/-0.82) indicated that selection for pEC is a workable alternative to increase decay resistance in *E. bosistoana* (Harju and Venäläinen, 2006). However, selection for natural durability should be combined with growth traits, which have been shown to be unfavourably correlated to pEC (Li et al., 2018).

A site effect on pEC has been reported for *E. bosistoana* (Li et al., 2018). The site effect seemed to be even more pronounced for pML, with a ~8-fold difference between the 2 sites (Table 2). Despite this difference in absolute values the rankings were moderately correlated with R_g^2 of 0.63 and 0.69 for brown-rot and white-rot, respectively.

5 Conclusion

Fungal decay tests validated that on average the resistance of *E. bosistoana* heartwood against wood decay increased with pEC. This confirmed that the use of a quick assessment of extractive content as a proxy measurement

for natural durability, which is currently used in a~~n~~ (change the''an'' to ''a'') commercial *E. bosistoana* breeding programme (Li et al., 2018; Millen et al., 2018), should result in a more durable resource.

Further, it was possible to predict mass loss of *E. bosistoana* heartwood from NIR spectra combined with PLSR modelling, with a precision that allows ranking of individuals in a breeding trial. NIR spectra analysis indicated that heartwood extractives control a significant part of the variation in natural durability of *E. bosistoana*. It is conceivable that in future NIR spectroscopy can be used for quality, i.e. natural durability, control of ~~future~~ wood products, as NIR cameras could be installed in sawmills.

Lastly, while ML of *E. bosistoana* heartwood against *C. olivacea* and *P. tephropora* was found to be under genetic control, the analysis indicated a strong site effect which warrants further research.~~Declaration of Competing InterestThe authors declare no conflicts of interest.~~ (Please check CRediT format - does not appear to have the correct headline format in the PDF) (CRediT - is that spelled correctly?)

CRediT authorship contribution statement

Yanjie Li: Writing - original draft. (Please update according to Q4) **Clemens Altaner:** ~~Supervision, Writing - review & editing.~~

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Highlights

- A near-infrared spectroscopy calibration was developed, which were able to quickly distinguish between more and less decay resistant *E. bosistoana* heartwood.More and less decay resistant *E. bosistoana* heartwood was quickly and non-destructively distinguished by near-infrared spectroscopy.

- This method allowed the identification of genetics with superior decay resistance in an *E. bosistoana* breeding program.~~This method allowed the identification of genetics with superior decay resistance in a tree breeding program, enhancing the quality of naturally durable *E. bosistoana* timber.~~ (se insert bullet point)Near-infrared spectroscopy also has potential for quality control of natural durability of timber by segregation.

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This method allowed the identification of genetics with superior decay resistance in an *E. bosistoana* breeding program.

Near-infrared spectroscopy also has potential for quality control of natural durability of timber by segregation.

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