Specialty Wood Products Research Partnership

UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

Natural durability: Correlation between extractive content and fungal assay

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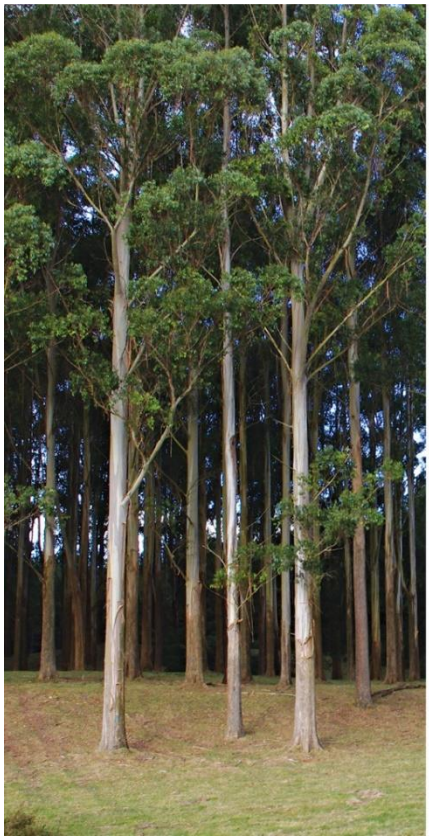

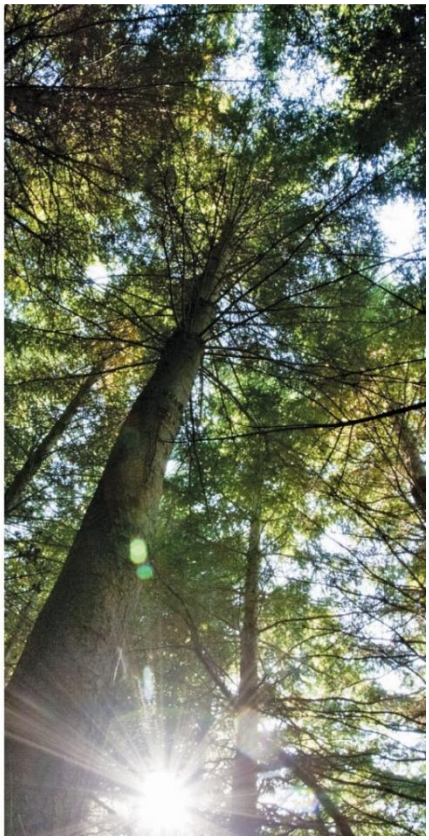

Date: 8th May 2018
Publication No: SWP-T045

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EXECUTIVE SUMMARY

The main objective of this work was to validate if extractive content (EC) predicted by NIR spectroscopy correlated with mass-loss durability measurements. 7 year-old *Eucalyptus bosistoana* heartwood samples with known EC (NIR) grown in New Zealand on four sites were tested for durability (mass-loss) against two basidiomycete fungi, a brown-rot and white-rot.

- The result suggested that high EC predicted by NIR identified samples with low mass-loss and can therefore be used to select durable genotypes. However, some samples with low mass-loss (i.e. durable) had a low EC (NIR) and therefore would have been missed. Note: this does not compromise the identification of the durable individuals.
- The average mass loss for the 7 year-old *E. bosistoana* NZ grown unimproved trees met at least the performance of a class 1 (above-ground) rated species (*Corymbia maculata*) (AS 5604 2005).
- Further, some 7 year-old *E. bosistoana* trees produced heartwood, which showed no significant (<3%) mass-loss.
- Large variation was found between trees and between sites, highlighting the need to select durable genotypes as well as investigating site factors contributing to durability of heartwood.

It needs to be noted that the stratified sampling strategy optimised to aid the 1st objective (correlation between EC and mass-loss) amplified the extremes, i.e. more high and low EC samples than would be expected in a random sample.

INTRODUCTION

A target product of NZDFI is ground-durable timber. NZDFI has established a series of breeding trials to deliver growers healthy plants, which produce good amounts of quality timber. The key wood property is natural durability. Natural durability describes the resistance of wood to decay by fungi and insects. Only heartwood, which contains bioactive extractive compounds, has natural durability (AS 5604 2005). Coast grey box or *E. bosistoana* is one of the main species planted in a number of trials throughout New Zealand by NZDFI. The mature heartwood of this species is known to be of class 1 natural durability in Australia (the highest rating).

Heartwood quantity

The heartwood diameter varies within a species. Heartwood quantity is partly under genetic control (Hillis, 1987). To maximise value of NZDFI plantations, trees which have a propensity to produce a large volume of heartwood should be selected in a breeding programme.

Heartwood quality

The measurement of natural durability is resource intensive (Harju & Venäläinen, 2006; Li & Altaner, 2016). High resource demands prevent this trait from being included in breeding programmes. Heartwood extractives are a main factor providing natural durability (Hawley, Fleck, & Richards, 1924). EC is highly variable within *E. bosistoana*, varying at least 10-fold between trees (Sharma, McLaughlin, Altaner, Chauhan, & Walker, 2014; Van Lierde, 2013). A NIR prediction model has been developed (Li & Altaner, 2018) to predict EC using NIR spectral measurements. As the EC can be efficiently measured, NZDFI is selecting genotypes of high EC to increase the chance of ground-durable timber in the future deployment population.

The objective of this study was to validate selection via EC prediction using NIR measurements. To do this mass-loss of samples with known EC (NIR) were measured using durability test. Durability test was carried against two basidiomycete fungi, a brown-rot and white-rot on 440 *E. bosistoana* samples from four different sites in New Zealand by Laurie J. Cookson (LJC) in Australia.

METHODS

Material

Eucalyptus bosistoana samples discussed in this report were collected from four different breeding trials planted by NZDFI. 35 Families were planted in 2009 in single tree plot design at Cravens Road and Lawson in Marlborough. Another 40 families were planted in 2010 at Martins in Canterbury and Cravens Road in Marlborough. A battery powered 14 mm inner-diameter increment corer was used to sample the trees. In total 370 trees were cored at Craven Road (2009) and 760 trees at Lawson in 2016. 1,115 trees were cored at Martin and 650 at Cravens Road (2010) in 2017. For these core samples the heartwood and sapwood diameters (heartwood quantity) have been measured as well as the EC (NIR) (heartwood quality).

A subset of these samples was used for the decay test. Stratified sampling (low (<5%), medium (5-10%) and high (>10%) EC) was applied to ensure that this subset represented the whole range of EC at each site. A limiting factor was the need to ensure that a 20 mm long (i.e. radial) heartwood piece could be cut from the cores, restricting the choice to larger trees. From each core, one or two samples were supplied, to a total of 440 samples. In order to avoid the transition zone (with not fully developed heartwood) the samples were cut close to the pith. Table 1 summarises the number of cores and their characteristics for each site. The cores were 12-15 mm diameter due to anisotropic

shrinkage after drying at 100˚C prior to shipment from New Zealand, there was some variation in shapes. Some cores yielded only one sample, and these were all exposed to brown-rot. Where heartwood was of sufficient length, two samples were taken from each core so that one could be exposed to the brown-rot and the other to the white-rot fungus.

The samples contained not only heartwood from *E. bosistoana* but also from control seed-lots of *E. globoidea* and *E. quadrangulata*. These were marked with (997, 998 and 999) and excluded from the analysis.

A range of comparative timbers, 20 x 20 x 10 mm (10 mm in grain direction) were also used and were mostly supplied by LJC. The timbers in test were:

1. *E. bosistoana* heartwood cores (University of Canterbury), unknown durability.
2. *P. radiata* sapwood cores (University of Canterbury), non-durable.
3. Sawn *P. radiata* from 2 trees (LJC), non-durable.
4. Sawn *E. obliqua* heartwood from 3 trees (LJC), class 3 in-ground; class 3 above-ground.
5. Sawn *Corymbia maculata* heartwood from 3 trees (LJC), class 2 in-ground; class 1 above-ground
6. Sawn *Intsia bijuga* (merbau) heartwood from 3 trees (LJC), class 3 in-ground; class 1 above-ground

This decay test is most relatable to above ground conditions where soft rot is less prevalent.

Methodology:

Full diameter cores were taken at the bottom of the tree trunk (i.e. ~50 cm height) through the pith.

Heartwood quantity

Heartwood was highlighted by applying a pH indicator (methyl orange) to the core surface in the green state. Heartwood changed colour to pink while no colour change occurred when applied to sapwood (Figure 1). The total length of the core samples without bark as well as the length of the heartwood was measured in the green state with a ruler. Sapwood depth was calculated as the difference between the 2 measurements. The surface of the cores was sanded (P 100) to expose clean wood before NIR spectra were collected with a fibre optics probe (Bruker) on the ~radial-tangential surface every 5 mm along the heartwood. The EC was predicted for each spectra using the previously developed model (Li & Altaner, 2018). Heartwood EC for a tree was than calculated as weight average (representing cross sectional area) of the individual spectra.

Figure 1 E. bosistoana cores stained with methyl orange. Heartwood is highlighted pink.

Decay test

The heartwood region with identification number written on every test sample was supplied to Laurie J. Cookson (LJC) in Australia. Test cores were further numbered by LJC on their ends, along with other test specimens, using a Sharpie pen. Test specimens were leached prior to exposure. For leaching, the test specimens were vacuum-impregnated with water (30 mins at -95 kPa) and then placed in jars with at least three times their volume of water on a shaking water bath at 35˚C for five days. Water in jars was changed daily. Timber species were placed in separate jars. A set of *E. obliqua* and *C. maculata* blocks were left unleached for comparison.

Test specimens were then arranged on wire mesh trays and left to condition in the laboratory until constant mass was achieved (at about 11% moisture content) and weighed on a 3-figure balance to obtain initial air-dry masses. Test blocks were placed in plastic bags according to decay tray destination, packaged and sent for sterilisation by gamma-irradiation at 25 kGy.

Test specimens were exposed to decay fungi in an agar-tray bioassay. Stainless steel trays 370 x 225 x 95 mm high were wrapped in autoclave bags and sterilized by gamma-irradiation. Under sterile conditions, 1.2 L of an autoclaved solution of 2% agar and 1% malt extract was poured into each tray and left to cool and solidify in the sterile air bench. Each tray was inoculated with about 15 plugs of the appropriate test fungus, while one tray was left un-inoculated as the sterile control. After 10 days incubation at 25˚C, a plastic mesh sterilised by gamma-irradiation was placed in each tray. Sterilised test specimens were then placed upon the plastic meshes within the trays. There were three trays for each fungus. No special effort was made to arrange test specimens into rows, thereby reducing the risk of contamination. However, all cores were arranged so that the grain direction was horizontal. Sawn blocks were placed with grain direction vertical (Figure 4 and 5).

The following test fungi were used:

Coniophora olivacea DFP 1779, brown rot fungus.

Perenniporia tephropora DFP 7904, white rot fungus.

The trays were placed in a 25˚C incubation room for 12 weeks. At the completion of the bioassay, the test specimens were removed from the trays, gently wiped of excess surface mycelium, and air dried in the laboratory to constant mass, when they were weighed once more to obtain final masses. The percentage mass loss in each block was determined, and adjustments made according to the slight mass gains that occurred in the sterile controls.

Data analysis

Data was analysed in R (RTeam, 2013)

RESULTS

Only data for cores which were sent for durability test is discussed here. As stratified sampling was used heartwood data (Table 1) differed from those reported for the whole breeding populations reported earlier.

The mean along with the coefficient of variation for different variables is given in Table 1. There was a negative correlation (r = 0.30) between heartwood diameter and extractive content. Different sites differed significantly for both heartwood diameter and extractive content. Martin 2009 had the highest average EC (10.2%). Cravens Road 2010 trees had the largest heartwood diameter in conjunction with having the largest diameters.. The box plots for heartwood diameter and EC are given in Figure 2 and Figure 3, respectively. These show that the sampling strategy ensured a wide range of values in the samples for the 2010 samples.

Table 1 Mean and (coefficient of variation %) of different variables at four different sites. D stands for 2 samples per core and S stands for single sample per core.

Site	No. of cores	Heartwood diameter (mm) (CV%)	Core length (mm) (CV%)	Ratio core length to heartwood diameter (CV%)	Extractive content (NIR) (%) (CV%)
Cravens Road 2009	60 (50D, 10S i.e. 110 samples)	52.4 (32)	132.5 (21)	2.75 (37)	6.4 (31)
Cravens Road 2010	58 (52D, 6S i.e. 110 samples)	63.8 (41)	134.5 (29)	2.21 (20)	8.4 (60)
Lawson 2009	68 (42D, 26S i.e. 110 samples)	45.0 (31)	101.9 (16)	2.46 (31)	8.5 (24)
Martins 2009	67 (43D, 24S i.e. 110 samples)	48.0 (29)	105.5 (21)	2.29 (24)	10.2 (49)

Figure 2 Boxplots for heartwood diameter at four different sites

Figure 3 Boxplots for extractive content for four different sites

Decay test

The decay fungi employed were the brown-rot fungus *Coniophora olivacea*, and the white-rot *Perenniporia tephropora.* Test specimens were equally divided amongst three decay trays for each fungal species. All fungi grew well in the trays, and there was no contamination (Figure 4 and 5). A summary of the decay results is provided in Table 2. The mean mass-loss was adjusted for changes

to sterile controls, which had minor mass gain of 2.4 to 3.0%, most likely due to conditioning under slightly different temperature and humidity environments within the laboratory. It is usual in fungal bioassays to consider that significant decay has occurred when adjusted mass-loss is more than 3%. The appearance of the test blocks from brown-rot tray 3, air-dried after 12 weeks incubation, is shown in Figure 6.

C. olivacea caused heavy decay to both comparative timbers with low natural durability. There was 65.7% (cores) and 68.8% (sawn) mean mass-loss of *Pinus radiata* sapwood. There was also heavy decay of *E. obliqua* heartwood with 54.7% mean mass-loss. *C. olivacea* caused moderate decay to *Corymbia maculata* heartwood with a mean mass-loss for leached blocks of 13.8% (mostly due to decay in tree 3). The heartwood of merbau, *Intsia bijuga*, was most resistant to decay with only 0.1% mean mass-loss (below significant levels).

P. tephropora caused heavy decay to *E. obliqua* heartwood with mean mass-loss of 56.6%. White-rot tends to be more active on hardwood than softwood, and it was less destructive than *C. olivacea* to *P. radiata* cores with 24.2% mean mass-loss, and *P. radiata* sawn blocks with 18.6% mean mass-loss. *P. tephropora* caused minor decay to *C. maculata* heartwood with a mean mass-loss for leached blocks of 8.4% (mostly due to decay in tree 3). There was also minor decay in the heartwood of *I. bijuga*, with 4.1% mean mass-loss.

The effect of leaching prior to decay was examined for *E. obliqua* and *C. maculata*. For *C. olivacea* there was little difference in mean mass-loss between unleached (52.0%) and leached (54.7%) blocks of *E. obliqua*. Similarly, there was little difference between unleached (14.5%) and leached (13.8%) blocks of *C. maculata*. Pre-leaching was more beneficial for *P. tephropora* as there was no significant decay of unleached *C. maculata* blocks (1.6%) compared to leached blocks (8.4%). Also, there was more decay in leached *E. obliqua* blocks (56.6%) than unleached blocks (44.3%).

Figure 4 Brown-rot (C. olivacea) tray 2 after 12 week incubation period, showing extensive growth over test specimens

Figure 5 White-rot (P. tephropora) tray 2 after 12 week incubation period, showing extensive growth over test specimens.

Figure 6 Cleaned and reconditioned test specimens after exposure to C. olivacea. Note shrinkage in some of the sawn test blocks.

*Table 2 Percentage mean mass-loss (standard deviations) by brown-rot and white-rot after adjustment for sterile controls. * excluding control seed-lots 997, 998 and 999.*

Timber	Leached	Adjusted mean mass loss (standard deviation)	
		C. olivacea **(brown-rot)**	***P. tephropora*** **(white-rot)**
P. radiata cores	Y	65.7 (2.9)	24.2 (10.1)
P. radiata sap	Y	68.8 (2.3)	18.6 (12.9)
E. obliqua heart	Y	54.7 (5.5)	56.6 (7.9)
E. obliqua heart	N	52.0 (5.6)	44.3 (10.3)
C. maculata heart	Y	13.8 (13.4)	8.4 (15.5)
C. maculata heart	N	14.5 (22.4)	1.6 (1.4)
I. bijuga heart	Y	0.1 (0.2)	4.1 (4.6)
Lawson 2009	Y	5.4 (7.4)	4.9 (5.7)
Cravens 2009	Y	17.9 (11.9)	15.5 (13.2)
Cravens 2010	Y	9.6 (12.7)	16.0 (17.4)
Martins 2010	Y	4.0 (7.2)	2.7 (2.1)
All sites	Y	9.0 (11.3)	10.35 (13.2)
E. bosistoana age 7 All sites*	Y	8.8 (11.3)	8.5 (11.0)
Lawson 2009*	Y	5.6 (7.5)	5.1 (5.8)
Cravens 2009*	Y	18.4 (12.1)	12.4 (10.8)
Cravens 2010*	Y	9.1 (13.2)	13.1 (15.4)
Martins 2010*	Y	4.0 (7.15)	2.7 (2.1)

Of the *E. bosistoana* cores, 43% proved to be resistant to decay with 3.0% or less mass-loss. There appeared to be an important site difference where 79 of the 110 cores from Martin were resistant compared to only 14 of the 110 cores from Craven Road 2009. Similarly, of 110 cores from each 2010 trial, 38 from Cravens Road and 58 from Lawson were resistant to decay. The box plot for adjusted mass-loss for *E. bosistoana* for 4 different sites is given in Figure 7.

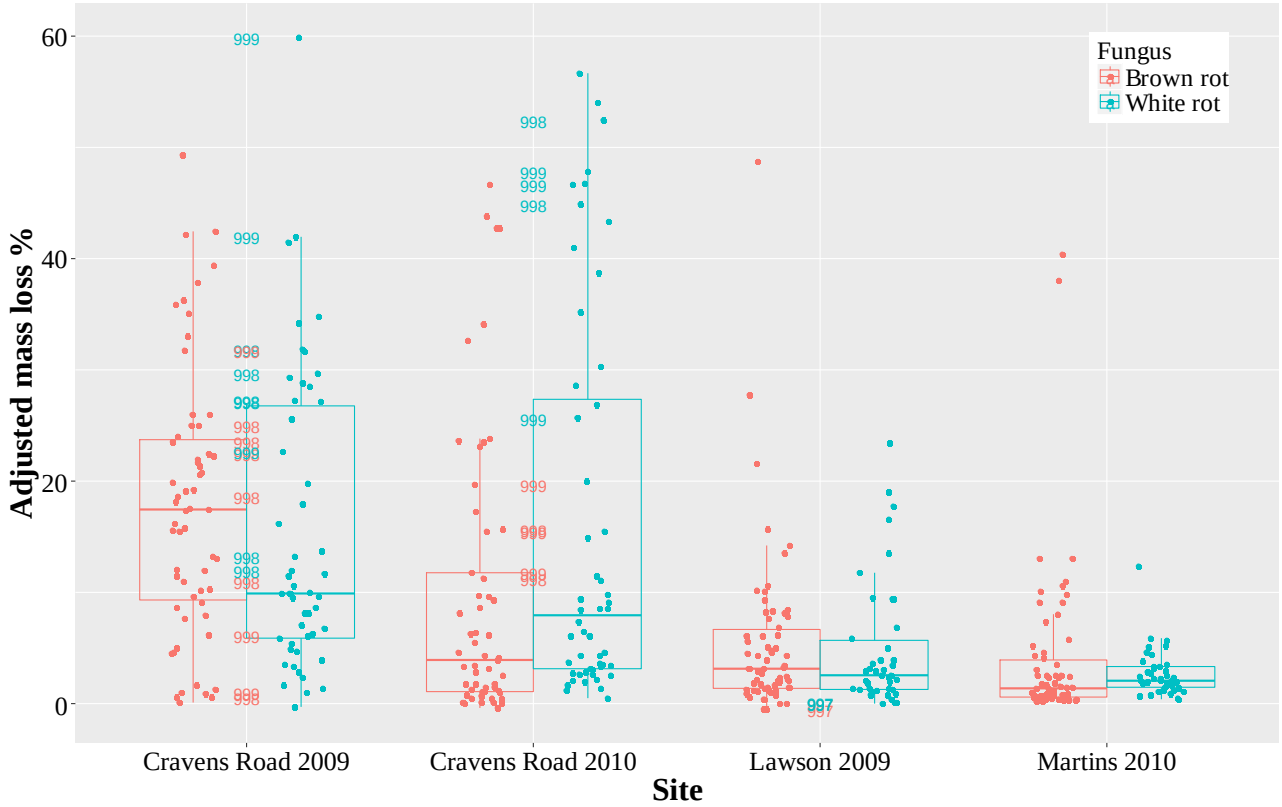

Figure 7 Boxplot for adjusted mass-loss for four different sites. Note: 997, 998, 999 indicate samples from control seed-lots of E. globoidea and E. quadrangulata.

A t-test suggested no significant difference in adjusted mass-loss between the two fungi for these *E. bosistoana* samples. However, the following analysis also considers the each fungi individually. Large variation between samples was found and therefore potential for genetic selection for low mass-loss (if selection for high EC would be deemed unsatisfactory). The degree of genetic control (heritability) would need to be determined, but has been shown to be significant in other species including eucalypts (Bush, 2011).

Correlation between NIR EC and mass loss

A negative correlation between EC and adjusted mass-loss for white-rot (r = -0.32) and brown-rot (r = -0.42) was observed (Figure 8 and 9). For individual sites, correlation coefficients (R) along with p-values are given in Table 3. There was a significant negative correlation for three trials except for Martin, where irrespective of EC adjusted mass-loss was less than 3% for 72% of the samples. Strongest correlations were found for the trials with the lowest average EC, i.e. Craven Road.

The data showed (Figure 7 and 8) that samples with NIR predicted EC greater than ~10% were resistant to the decay fungi. However, samples with NIR predicted EC lower than ~10% were not necessarily non-durable. Therefore, selection for high EC should identify individuals of higher durability. However, the cost is to reject some individuals of good durability. This makes breeding less efficient but should ensure improved durability.

Another important finding of this study is the large difference between the sites. Sites where trees exhibit low EC (like Carven Road) seemed to be more suitable for durability screening purposes than sites which provide samples with consistently low mass-loss (like Martin). It is of interest to investigate the drivers for these site differences in durability (to improve heartwood quality by siting) as well as the chemical difference in the heartwood extractives.

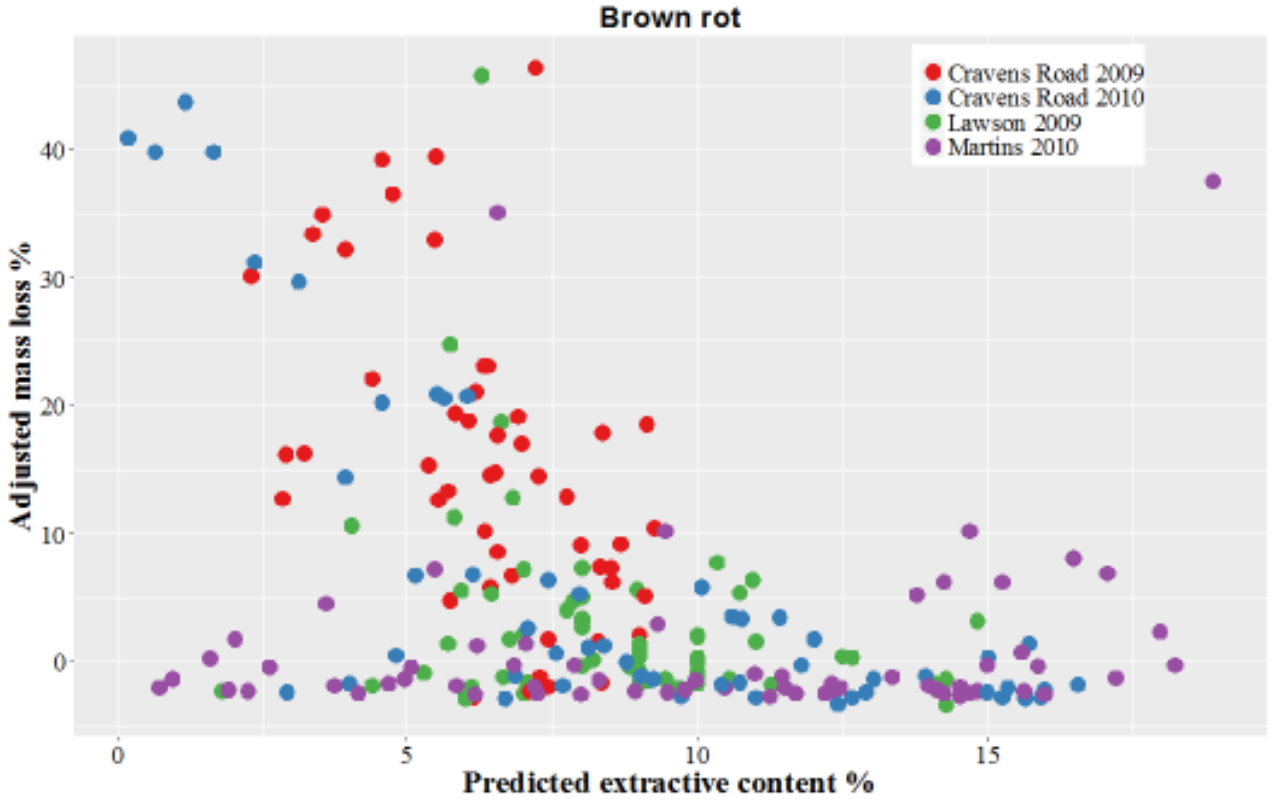

Figure 8 Correlation between adjusted mass loss and predicted extractive content for white rot

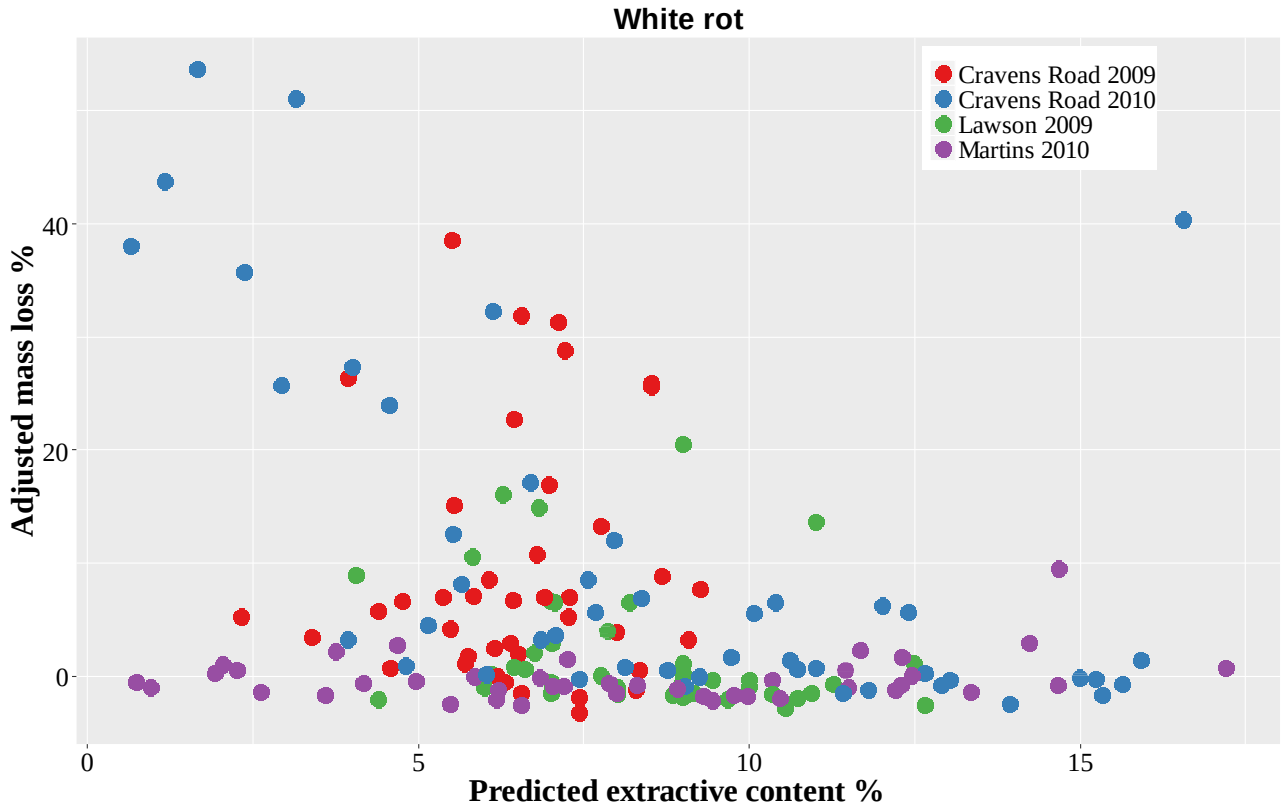

Figure 9 Correlation between adjusted mass-loss and predicted extractive content for white rot

Table 3 Coefficient correlation between extractive content and adjusted mass-loss along with P value for four individual sites excluding control seed-lot samples 999,998 and 997

			Brown-rot		White-rot	
Site	R	p-value	R	p-value	R	p-value
Cravens 2009	-0.31	0.003	-0.53	$7.8X10^{-5}$	-0.04	0.81
Cravens 2010	-0.65	$1.95x10^{-13}$	-0.72	$1.02 X10^{-9}$	-0.59	$1.04 X10^{-5}$
Lawson 2009	-0.25	0.009	-0.26	0.03	-0.27	0.09
Martins 2010	0.17	0.07	0.15	0.22	0.22	0.15

Durability

Heartwood of 7 year-old NZ grown *E. bosistoana* in average at least met the performance of a class 2 (above-ground) rated species (Table 2). This can be interpreted as supporting data to include *E. bosistoana* into the new NZ 3602 standard for applications like decking. Additionally some trees had no significant mass-loss (<3%) indicating that by genetic selection, potentially paired with appropriate site choice, class 1 durable timber from short rotations should be possible (Figure 10 and 11).

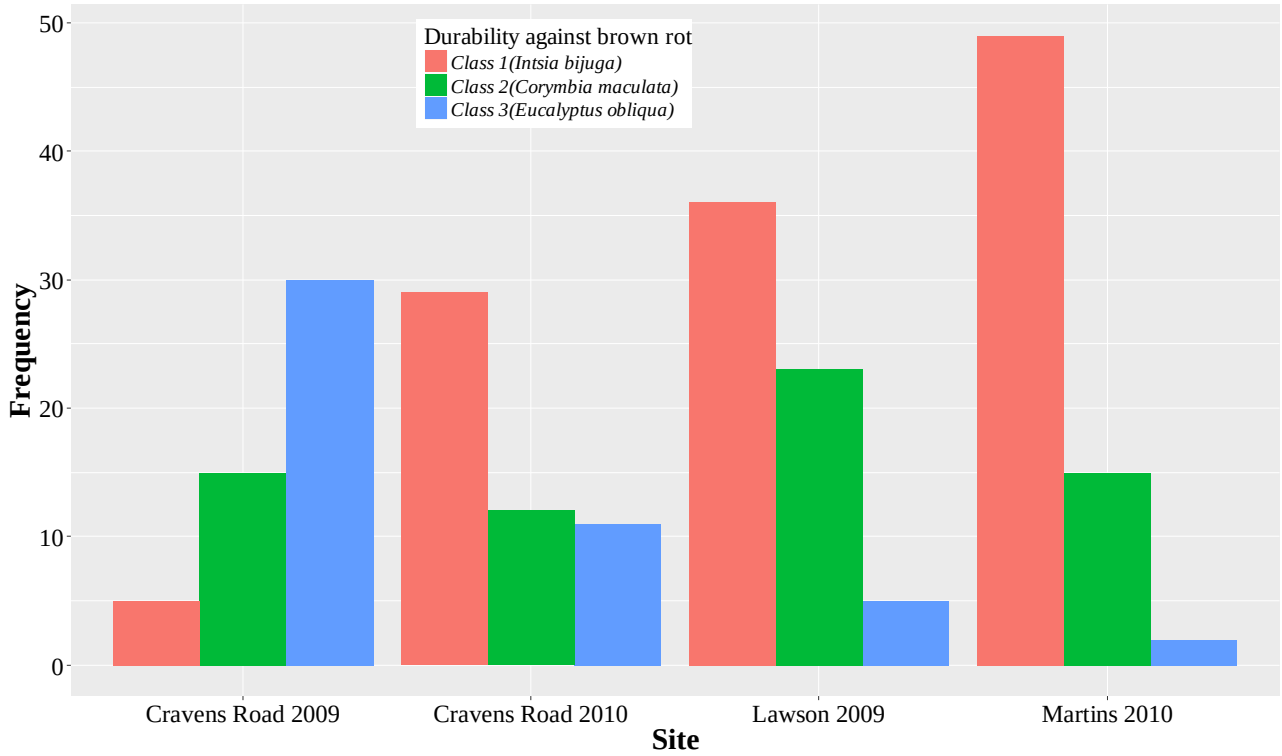

Figure 10 Frequency of durability of 7 year-old E. bosistoana heartwood samples by site deduced from performance of control timbers of known durability when tested against C. olivacea (brown-rot).

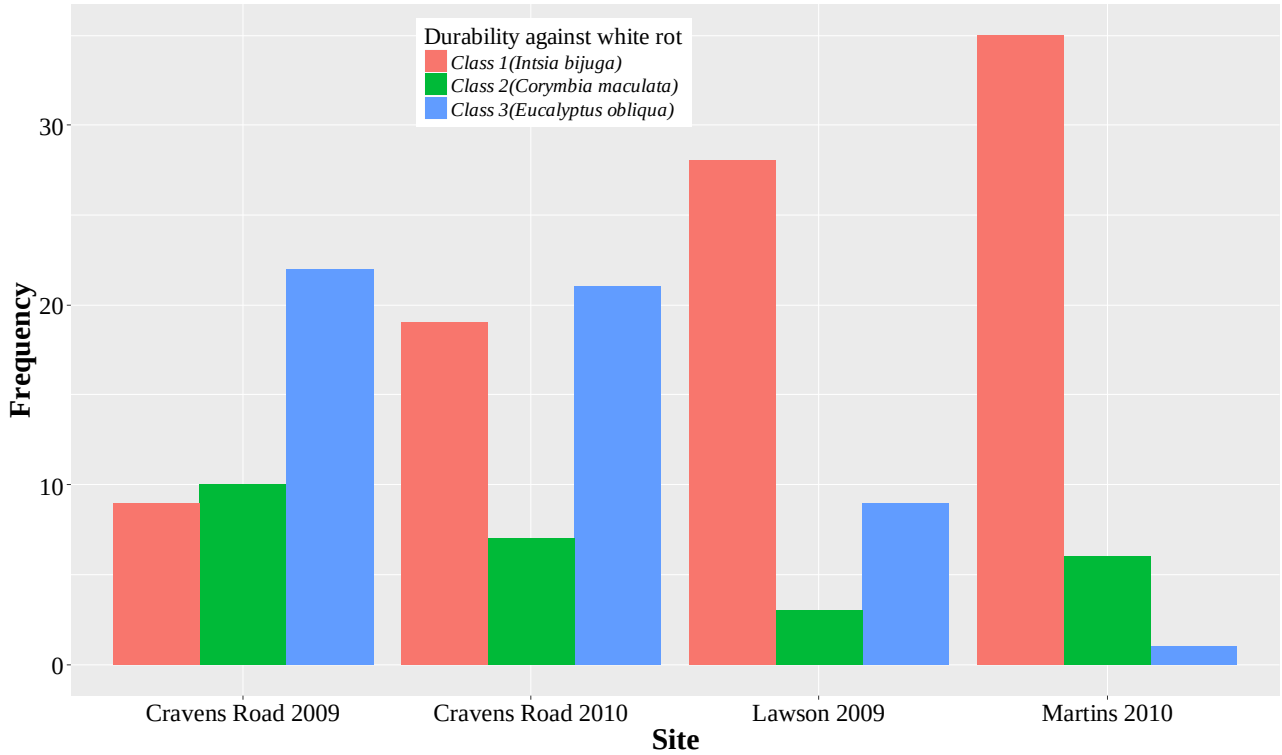

Figure 11 Frequency of durability of 7 year-old E. bosistoana heartwood samples by site deduced from performance of control timbers of known durability when tested against P. tephropora (white-rot).

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