Early Heartwood Screening by Wounding

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TABLE OF CONTENTS

INTRODUCTION

Trees can be screened at age (1-2 years) for wood properties such as growth stress, collapse, density or stiffness (Chauhan and Entwistle, 2010; Chauhan et al., 2013). Early selection reduces trial costs, shortens breeding cycles and ensures timely deployment of improved material. Early selection for heartwood is challenging because heartwood formation in eucalypts only starts at age ~5. Early selection for heartwood would be possible if a strong correlation of heartwood to another tree feature is found.

The wound response of trees has similarities to heartwood formation. Extractives deposited in wound wood originate from a similar metabolic pathway as heartwood. Therefore, the wound response could be correlated to heartwood formation. A strong correlation would allow screening eucalypts for heartwood at age 1-2, i.e. before heartwood is formed. The correlation between heartwood features and the wound reaction of the offspring has been investigated for *Pinus sylvestris* (Harju et al., 2009). They reported a heritability of 0.31 between the pinosylvin, a heartwood compound, concentration in the wound wood of the offspring and that in the heartwood of the mother trees. These results suggested that early testing for heartwood durability may be possible in a breeding programme.

Compartmentalization in trees and heartwood formation (CODIT)

Trees have a defence mechanism which is triggered by injury or infection. The concept of a tree's response to wounding was described as 'compartmentalisation', which is also known under the acronym CODIT (Shigo, 1984). Compartmentalization may be considered as a conceptual model related to the process of boundary formation in trees, which prevents the spread of infection in trees. The model describes the defence of trees as two parts, consisting of four different types of static barriers called "walls" that resists the spread of infection. The first part consists of three walls, which are formed prior to infection. Wall 1 resists vertical (axial) spread, wall 2 resists inward (radial) spread, and wall 3 resists lateral (tangential) spread. Among these defence walls wall 1 (axial) is the weakest, whereas, wall 2 is moderately strong. When the injuries approach deeper sheaths of xylem, the resisting power of wall 2 decreases. Wall 3 is the strongest wall of compartmentalization.

The second part (or wall 4) is formed after injury. Wall 4, the wound reaction, is relevant to this project. Two types of tissue can be distinguished in the response to wounding. First the formation of new callus-like tissue with which trees close the wound. Secondly xylem tissue which was exiting prior wounding and is undergoing anatomical and histochemical changes (Eyles et al., 2003).

Wound wood

Wound wood is formed in response to mechanical or the microbiological injury. Several terms are used for this dynamic mechanism in trees such as pathological heartwood, protection wood, discoloured wood and reaction zone. Wound wood and heartwood formation have similarities including the presence of secondary metabolites (extractives), reduced permeability and often water content. Both wound wood and heartwood are protective reactions and follow similar metabolic pathways defending plants from microorganisms (Harju et al., 2009). However, wound wood is triggered by external events, whereas heartwood is initiated in response to internal factors (Taylor et al., 2002). The extent of discolouration in wound wood is greatest in the axial direction as compared with radial and tangential directions as the axial compartmentation wall is the weakest (Wardell and Hart, 1970). Both wound wood and heartwood contain a wide range of bioactive compounds (Eyles et al., 2003; Harju et al., 2009). However, the chemical composition of extractives formed in wound wood also differs from those in heartwood (Eyles et al., 2003). No information on the identity of wound wood or heartwood extractives is available for *E. bosistoana*.

METHODS

Heartwood data

Data on heartwood diameter and extractives content were available for *E. bosistoana* from breeding trials planted in 2009. These were established at 3 sites (Craven Road, Lawson East and Lawson North) and assessed in 2016 by taking a ~14 mm diameter core from the base of the stem. The sapwood and heartwood diameter were measured on the green cores after highlighting the heartwood with a pH indicator (methyl orange). The ethanol soluble extractives content was predicted from NIR spectra taken from the cross-section of dried cores. Coring and calibration of NIR for extractive content is reported in another report to SWP.

Woundwood data

In 2015 another *E. bosistoana* breeding trial was established at a nursery in Woodville. Trees were planted in family blocks of 8 individuals, with blocks at least 3 times replicated. This trial included 27 families which were also present in the 2009 plantings assessed for heartwood. Only families existing in both trials were considered in this experiment. The surviving individuals with a stem diameter >3 cm in 3 blocks (i.e. up to 24 individual per family) were wounded by drilling a 5 mm hole through the stem at ~50 cm height with a battery powered drill (Figure 1). Trees were wounded on the 29th September 2016. Wounds on the trees were marked with spray paint to assist harvest in November 2016. The wounded trees were harvested on the 10th November 2016. The wounded section of the stem was recovered and the wound was exposed by splitting through the pith perpendicular to the drilled hole. The wound reaction was assessed with and without staining with a pH indicator (methyl orange). The discoloured zone (wound wood) was measured with a calliper in axial direction. Table 1 lists the number of trees per family for which wound reaction data was obtained.

Figure 1: Wounding of 1.6 year old *E. bosistoana* by drilling a hole through the stem at ~50 cm height with a battery powered drill (a, b, c). Wounds marked with a box on the trees (d, e, f).
Table 1: Wound reaction data aggregated for each family.

Family	Number of samples	Wound reaction (mm)	CV (%)
A	18	1.02	66
B	22	0.91	34
C	22	0.85	49
D	14	0.75	61
E	17	1.21	47
F	21	0.77	74
G	20	1.23	42
H	20	0.95	33
I	19	0.95	33
J	21	1.17	36
K	17	0.91	57
L	19	1.17	48
M	12	1.14	30
N	17	1.17	36
O	21	1.05	42
P	17	1.17	73
Q	18	0.91	53
R	21	0.86	37
S	20	1.10	33
T	21	1.11	36
U	15	1.00	51
V	11	0.85	51
W	15	0.84	44
X	13	1.21	46
Y	18	0.94	45
Z	13	1.05	41
Aa	8	1.64	66
All	470	1.02	50

RESULTS

Wound reaction

The wounded sections of the stem recovered by splitting showed variation in the extent of wound reaction between trees but were identical for the 2 stem halves (Figure 2). A large within family variation in the wound reaction was observed (Table 1; Figure 3). This could have been partly due to the inaccurate assessment of the wound reaction or due to a low genetic control of the trait. The area of wounding often contained knots and these might have added noise to the wound reaction data.

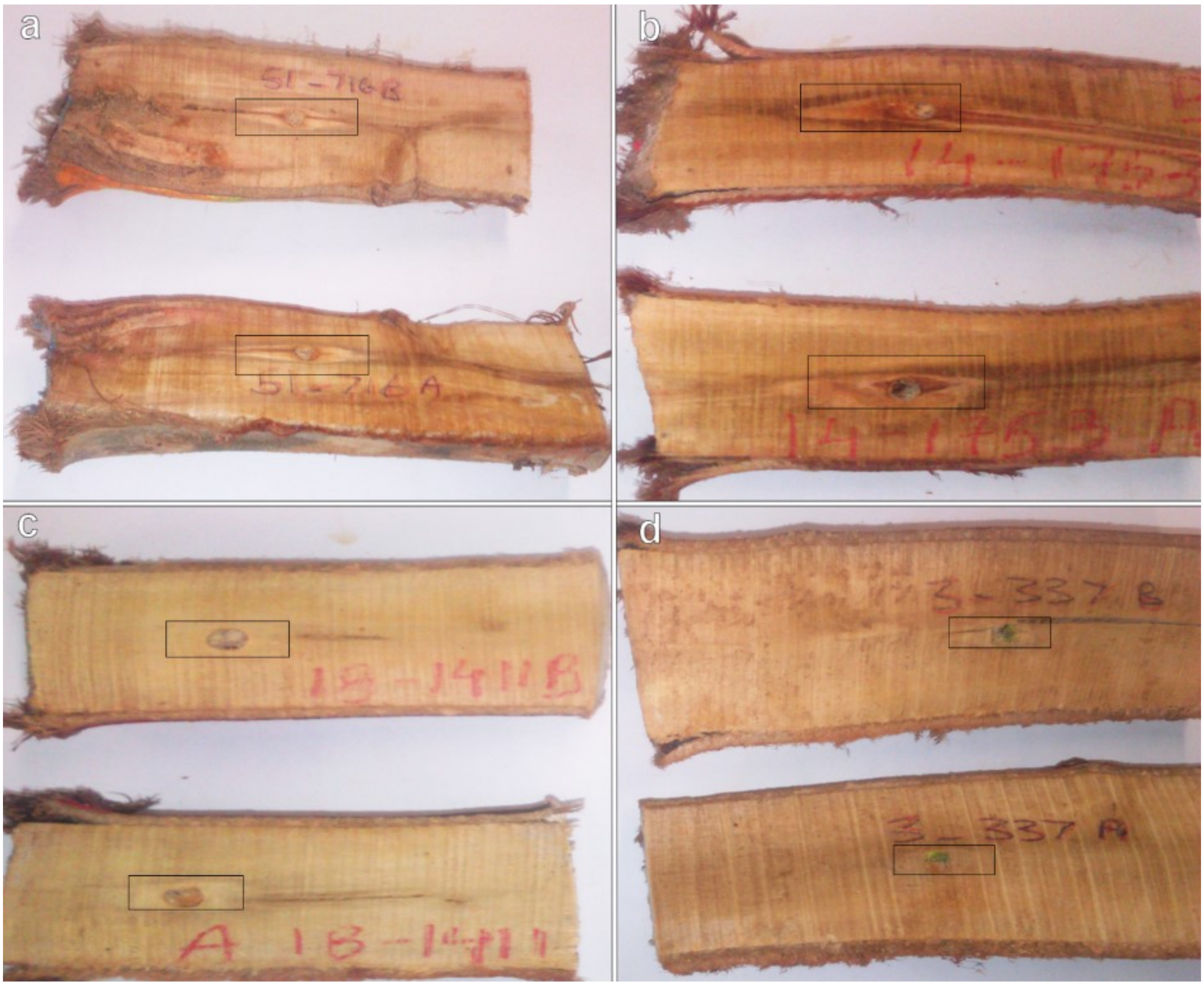

Figure 2: Representative wound reaction in *E. bosistoana* 6 weeks after wounding. Discolouration (marked with a box) was accessed with (a, b) and without (c, d) pH indicator (methyl orange).

Heritability of wound reaction

Figure 3 shows the 27 *E. bosistoana* families ranked for wound response 6 weeks after wounding. The heritability of the wound reaction was estimated from the data. The heritability of the wound reaction was 0.26 with a 95% credibility interval of 0.00 to 0.53. The genetic control was similar to that of growth-strain but lower than those of other physical wood properties for trees in this trial. A heritability of 0.26 would allow for genetic selection, however selection for wound reaction is not desirable in itself as the amount of wound reaction is of no commercial benefit. However, if the wound reaction is correlated to a trait of commercial interest it can be used as a proxy for genetic selection. This can be useful if the wound reaction is more efficient (cost, time) to analyse than the target trait.

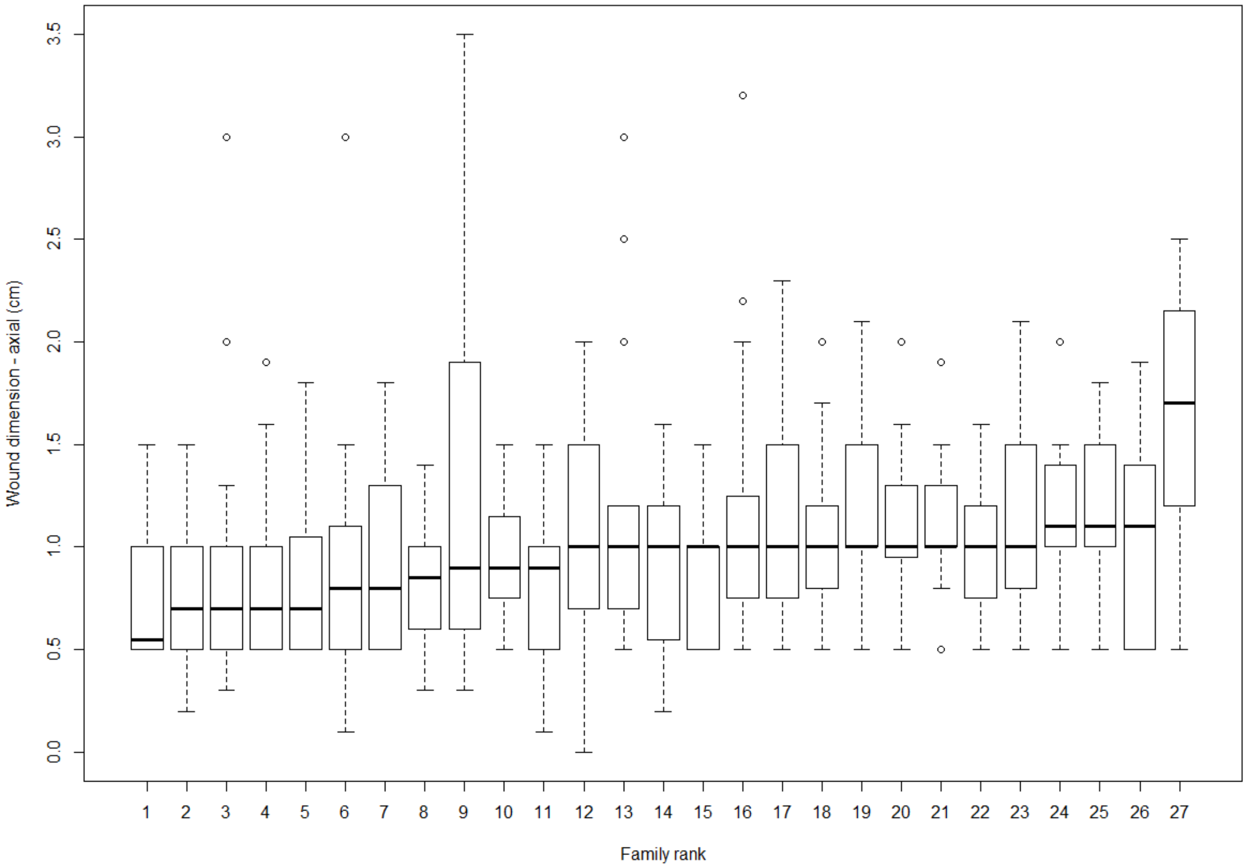

Figure 3: 27 *E. bosistoana* ranked for the size of their wound response 6 weeks after wounding.

Genetic correlation of wound reaction to other wood properties

A range of wood properties had been measured for the wounded trees in conjunction with the SFF project 407602 "*Minimising growth-strain in eucalypts to transform processing*". Genetic correlation are given in Table 2. No strong correlations between the axial length of the wound reaction and other wood properties or tree size were observed. This implies that the wound reaction cannot be used as a proxy measure for those traits. In particular the independence of the wound reaction from tree size is noteworthy as larger trees with large and small wound reaction can be found. This might indicate that the available physiological resources for a tree are not a restricting factor for wound response.

Table 2: Genetic correlations between length of axial wound reaction and other wood properties and tree size in *E. bosistoana* at age ~2. 95% credibility intervals are given in parentheses.

Trait	**Genetic correlation to wound reaction**
Growth-strain	-0.02 (-0.64, 0.60)
Acoustic velocity	0.15 (-0.25, 0.55)
Density	0.27 (-0.17, 0.70)
Stiffness	0.22 (-0.20, 0.64)
Volumetric shrinkage	0.26 (-0.25, 0.76)
Tree height	0.04 (-0.65, 0.73)
Diameter	0.08 (-0.68, 0.84)

Correlation of wound reaction to heartwood features

Heartwood features were not assessed on the same trees which were wounded. However, heartwood data was available for the same families from another trial. Therefore correlation between heartwood and wound reaction traits was possible on a family basis. The amount of heartwood as well as the extractive content within the heartwood differed between the sites. Differences were observed for the population means as well as the individual family means. This indicated an environmental as well as a G x E interaction for heartwood traits. Further details can be found in a separate SWP report (SWP-T012).

No strong correlations were found between the axial dimensions of the wound reaction and heartwood diameter or extractive content for the individual sites or the pooled data (Table 3). The weak correlations between wound reaction and heartwood diameter for Lawson East and the pooled data as well as the extractive content at Lawson North were not significant considering the large confidence intervals.

Table 3: Correlations between family means of length of axial wound reaction (cm) and heartwood diameter (cm) as well as extractive content (%). 95% credibility intervals are given in parentheses.

Site	Heartwood diameter	Extractive content
Lawson North	-0.18 (-0.57, 0.27)	-0.11 (-0.52, 0.34)
Lawson East	-0.30 (-0.61, 0.95)	0.12 (-0.28, 0.48)
Craven Road	-0.15 (-0.50, 0.25)	-0.07 (-0.44, 0.32)
All	-0.28 (-0.60, 0.11)	0.00 (-0.38, 0.38)

CONCLUSION

The extension of the axial wound response of ~2-year old *E. bosistoana* seedlings was found to be heritable (h^2=0.26 with a 95% credibility interval of 0.00 to 0.53). However, no correlation between the axial wound response in the ~2-year old seedlings and heartwood diameter or extractive content in 7-year old trees was found. Therefore it is not possible to assess heartwood formation early in a breeding programme by measuring the axial wound response in 2-year old seedlings.

On a first glance this was somewhat different to a report which found that the amount of individual heartwood compounds in mother trees was genetically related to the amount of those compounds in wound wood of young offspring (Harju et al., 2009). This report suggested that it could be possibility to screen *P. sylvestris* early for natural durability. However, a key difference between the work on *P. sylvestris* and *E. bosistoana* is the former assessed individual chemical extractive compounds while the latter assessed the size of the wound reaction, the size of the heartwood and the total amount of ethanol soluble compounds. It also should be noted that the strength of genetic correlations differed between the individual compounds in *P. sylvestris*.

Therefore it might still be possible to screen *E. bosistoana* early (at age ~2 years) for natural durability but with a different assessment of the wound reaction. Quantifying individual compounds in the wound wood for breeding purposes is not as attractive as assessing the dimensions of the wound reaction as the analysis is labour intensive and costly.

Finally, axial wound dimensions were not significantly correlated to any other wood properties in the 2-year old seedling. This might suggest that wounding for example by pruning has no significant effect on wood formation of the stem apart from that in close proximity of the wound.

ACKNOWLEDGEMENTS

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REFERENCES

Chauhan, S., and Entwistle, K. (2010). Measurement of surface growth stress in *Eucalyptus nitens* Maiden by splitting a log along its axis. *Holzforschung* **64**, 267-272.

Chauhan, S. S., Sharma, M., Thomas, J., Apiolaza, L. A., Collings, D. A., and Walker, J. C. F. (2013). Methods for the very early selection of *Pinus radiata* D. Don. for solid wood products. *Annals of Forest Science* **70**, 439-449.

Eyles, A., Davies, N. W., and Mohammed, C. (2003). Wound wood formation in Eucalyptus globulus and Eucalyptus nitens: anatomy and chemistry. *Canadian Journal of Forest Research* **33**, 2331-2339.

Harju, A. M., Venalainen, M., Laakso, T., and Saranpaa, P. (2009). Wounding response in xylem of Scots pine seedlings shows wide genetic variation and connection with the constitutive defence of heartwood. *Tree Physiology* **29**, 19-25.

Shigo, A. L. (1984). Compartmentalization - a conceptual framework for understanding how trees grow and defend themselves. *Annual Review of Phytopathology* **22**, 189-214.

Taylor, A. M., Gartner, B. L., and Morrell, J. J. (2002). Heartwood formation and natural durability - a review. *Wood and Fiber Science* **34**, 587-611.

Wardell, J. F., and Hart, J. H. (1970). Early Responses of Sapwood of *Quercus bicolor* to Mechanical Injury. *Canadian Journal of Botany* **48**, 683-686.