Analysis of Heartwood –Sapwood Demarcation Methods and Variation of Sapwood and Heartwood within and Between 15 Year Old Plantation Grown *Eucalyptus Regnans*

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Abstract

This study was carried out using 15-year old Eucalyptus regnans F. Muell trees from three seedlots in a progeny trial in Norbethong, Victoria in Australia. Colour, stain and light table methods were used to demarcate the boundary between the sapwood and heartwood on the discs cross sectional surface. The main objective of this study was to examine the variation within and between tree in heartwood and sapwood in plantation grown Eucalyptus regnans material and also to compare the three techniques used in demarcating the boundary between sapwood and heartwood. Wood discs were removed from the base, breast height, 20%, 40%, 60% and 80% of the total tree height. Measurements were made on cross sectional surfaces of the discs to determine the sapwood width and heartwood percentage. The results showed that there was no significant difference in heartwood-sapwood demarcation methods using natural colour and stain. However sapwood-heartwood demarcation method using light table method had significant difference with both colour and stain methods with P = 0.000. The mean sapwood and heartwood width varied significantly between trees and along the height. The lowest mean sapwood width of 21.0 mm was found at breast height which increased with height to 33.9mm at 80% tree height it also increased from breast height to 29.9mm at the base of the tree. The largest heart wood percentage of 69.51% was found at breast height and decreased with height to zero at 80% height and it also decreased from breast height to 65.75% at the base. A very high correlation was found between the heartwood diameter and disc diameter.

Keywords: Sapwood-heartwood, Eucalyptus regnans, wood characteristics, heartwood-sapwood boundary

1) Introduction

This study was aimed at evaluating three separate techniques used to demarcate the boundary between sapwood and heartwood. The study also analyses the within and between variation of heartwood and sapwood. The amount of sapwood may influence wood properties and utilization and it is an important selection criteria to forest industries involved in timber drying, wood pulping, preservation and furniture manufacturing (Yang et al. 1985; Mirand et.al 2009). In some high value hardwood species, a tree with narrow sapwood is highly desirable for aesthetic reasons (Hillis, 1968). Sapwood may be heavier than heartwood at the time of cutting the tree due to higher moisture content. However, the basic density may be less than that of heartwood because of the absence of materials which may have been infiltrated into heartwood. Within the species the width of the sapwood is directly related to dominance of tree in stand, with more vigorous trees having wider sapwood (Panshin and De Zeew, 1980; Welwood and Juraz, 1968; Maria 2007). The age of the tree at which sapwood is transformed to heartwood is reported to be the same for both slow and fast grown trees (Hillis 1972). Other external factors influencing sapwood width include site, and tree diameter, (Hillis and Ditchburne, 1974, Smith, et al., 1966). Other factors include elevation (Lassens okkonen, 1969) tree vigor (wellwood & juvazs 1968) and climate (Chalk 1951).

Sapwood is reported to be widest at the upper part of the stem and decreases in width towards the base (Panshin de Zeeuw, 1980).

Most mature tree contain a central core of heartwood usually darker in colour than the sapwood surrounding it. In *E. regnans* the heartwood appears a light colour and in some Instances may be slightly pink (Nicholls and Griffins, 1978). The presence of heartwood may affects utilisation of some wood as the extractives in heartwood as well as features such as tyloses may make the wood less permeable and therefore harder to treat with preservatives or pulp with pulping liquors (Hillis, 1972; Mirand 2009). However, the heartwood more durable because of the presence of extractives and also the coloured heartwoods add value in the appearance of the product. Eucalypts from plantation have a smaller proportion of heartwood and lower amount of extractives which make them more suitable for pulp and paper (Hillis, 1972).

In most trees two types of wood can be recognized, sapwood and heartwood. Sapwood is the outer, pale-colored wood and heartwood the inner, mostly darker wood. sapwood is generally considered inferior to heartwood as it is susceptibility to fungi and wood destroying insects

However, by use of the appropriate preservative treatment, sapwood can be made equal if not superior to heartwood of the same species in durability. To assist in the best utilization of sapwood, the properties of sapwood and heartwood are reviewed, with emphasis being given to the determination of sapwood and heartwood, relative strengths, durability and utilization (Bamber, 1987).

Sapwood becomes heartwood with the death of the parenchyma (Bamber, 1987). It is difficult however to show, even with the most sophisticated techniques, the precise zone where this takes place. It is possible by using some of the other properties which change from sapwood to heartwood to obtain a fairly reliable estimate of the sapwood-heartwood boundary (Bamber, 1987). Sapwood can be demarcated from heartwood using various techniques; physical method which is based on natural colour difference between sapwood and heartwood and is referred in this text as 'colour method'. The other second method is a chemical technique which use stain to differentiate between the two based on the differences in PH for both sapwood and heartwood and is referred to as the 'stain method'. The third technique is an anatomical technique based on the presence or absence of tyloses and is referred to as the 'light table method'.

Sapwood and heartwood differ in many characteristics and in some species they may be separated on the basis of colour. For some eucalypts, separation by colour is possible and is the method used in heartwood studies (Nicholls and Pederick 1979). In many species however, sapwood and heartwood are of similar colour or the contrast in colour between the sapwood and heartwood is not sufficient enough to enable reliable measurements to be made. In these instances the difference in PH between the sapwood and heartwood form the basis for stain to be used. Stains used on eucalypt wood include 0.2 percent solution dimethyl yellow in ethanol (Hillis, 1987) and methyl orange at 0.1 percent concentration (McKimm, 1985). An anatomical feature such as tyloses may also be used to distinguish heartwood from sapwood but only in species where such features are closely associated with heartwood formation (Bamber and Fukazawa, 1985). Tyloses are formed as part of transformation of sapwood to heartwood in many tree species. Tyloses amount and frequency contribute to a general reduction of permeability of the wood to pulping liquor (Hills 1972) as well as preservertives. It is likely that their presence affects water movement in living trees as well. (Sano & Fukazawa, 1991). However, depending on the end use, the presence of tylosses may also be advantageous as they increase the durability of the wood (Meyer, 1967).

2. Materials and Methods

The material used in this study was taken from 15 year old progeny trial of *E. Regnans* located at Siberia Range near Narberthong in the central highlands of Victoria in Australia. The progeny trial contained 21 families of *E. Regnans*, each derived from seed collected from outstanding trees selected in certain areas of natural forests. The trial was one of a series of experiments designed to identify genetically superior *E. Regnans* genotypes, on this and other sites, as a basis for a future breeding program.

A general examination was made for trees in the sample population which established that the dominant trees in the site had a diameter at breast height of between 240mm to 250mm. This diameter class was chosen for sampling of six trees in order to minimise the effect of differences in growth between trees. Six trees with straight bole and no visible defect were sampled.

The six trees selected for felling were cut and Northern direction marked on each. 15mm discs were cut from each tree at various heights; base, breast height, 20% and 60% and 80% of the total tree height. These discs were used for the work on the demarcation of sapwood from heartwood using various methods.   Lines were drawn on the upper surface across each disc in the North-south, East-West, Northwest-Southwest and Northeast-Southeast directions as shown in Figure 1. At each sapwood-heartwood boundary a pencil dot was marked directly on the line (Figure 1). This point was determined on the basis of the natural colour difference between the sapwood and the heartwood in the green condition. Pencil dots were also marked on each line at the boundary of the sapwood and the cambium as shown in Figure 1 in order to provide a reference point for the measurements to follow. Four linear measurements were made to the nearest 1mm using a ruler. The first measurement was the distance along each line from the cambium-sapwood boundary to the opposite cambium-sapwood boundary. This distance represented the underbark disc diameter. The second measurement was the distance along each line from the sapwood-heartwood boundary to the opposite sapwood-heartwood boundary.   This distance represented the heartwood diameter. The third and fourth measurements were of the two sapwood zones present at opposite ends of the North-South and East-West lines.

Each sapwood zone was defined as the distance between the cambium-sapwood boundary and the sapwood-heartwood boundary. This measurement represented the width of the sapwood.
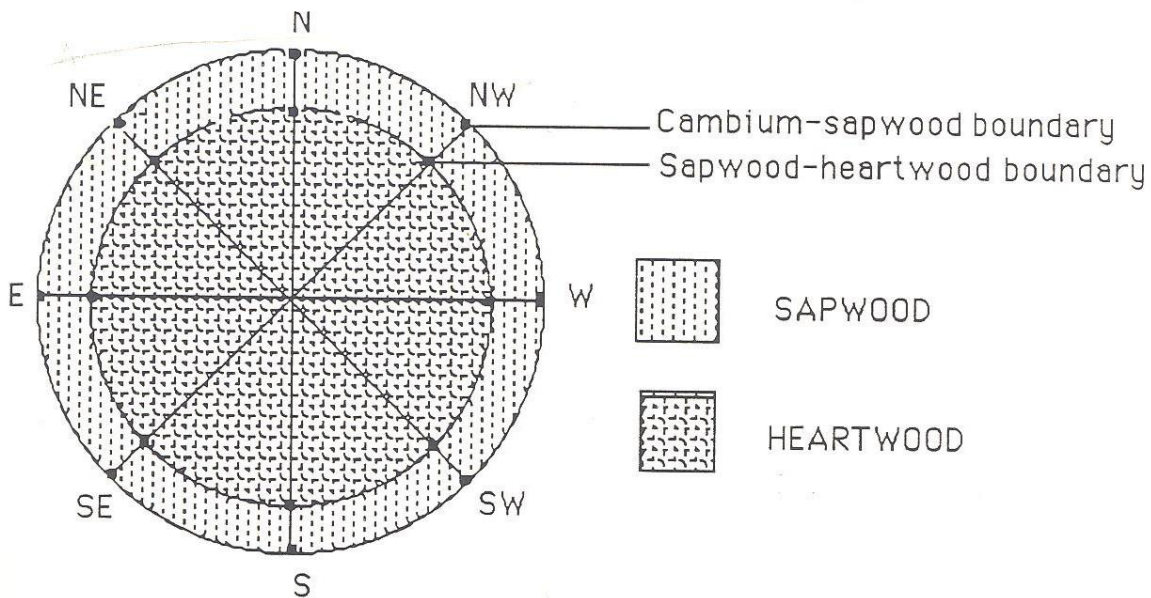

Figure 1: Illustration showing the four axial lines across the disc surface used for linear measurement of sapwood, heartwood and disc diameter (bark zone not shown).

Using the same 15mm discs that were used for the colour described above, methyl orange indicator at 0.1% concentration was brushed on the upper surface marked with the four lines. The brushing was done carefully by moving the brush from the bark to the pith in order to avoid any remaining sawdust or cellular debris being drawn from the heartwood to the sapwood-heartwood boundary. Application of this stain resulted in the heartwood being coloured red and the sapwood yellow. At each sapwood-heartwood boundary, as indicated by the change from yellow to red colour, a pencil dot was marked directly on the same lines previously used for the colour method. Using this new set of points to demarcate sapwood from heartwood, measurements were made of the heartwood diameter and sapwood widths in a similar fashion as that described earlier for the stain method. In addition, the number of growth rings present within the sapwood at the North, South, East and West positions were counted and recorded.

The same 15mm disc used for the colour and stain methods as described earlier were then used for light table method. Previously stained discs were cut using a table saw to produce three strips approximately 30mm wide as shown in Fig. 2. One strip (b, Figure 2) extended from bark to bark along the North-South axis and included the pith. The other two strips (a and c, Figure 2) were from the East and West positions in the disc.

All three strips per disc were cut in such a way as to include the pencil lines and pencil dots previously used for the colour and stain methods.

The strips were then reduced in thickness along the grain to 5mm for light table examination. Each 5 mm thick strip was placed on a light table in such a way as to allow the light to pass through the open vessels present in the sapwood and the clearly visible to the observer. Vessels occluded due to the presence of tyloses blocked the light and appeared dark. A pencil dot was marked on the same lines previously used for the colour and stain methods at the point where light could no longer be seen coming through the vessels. This point was assumed to be sapwood-heartwood boundary. Using this new set of points to demarcate sapwood from heartwood, measurements were made of the sapwood width on the north, south, east and west axes in a similar fashion as described for the colour and stain methods. Due to the loss of Kerf in preparing the strips of wood for the light table method, the heartwood diameter along each axis could not be directly measured. Instead, the heartwood diameter was obtained by subtracting the width of the two sapwood zones from the previously measured disc diameter on the same axis. The disc removed at 80% of the total tree height was not included in this method due to the absence of heartwood based on the colour and stain methods previously employed.
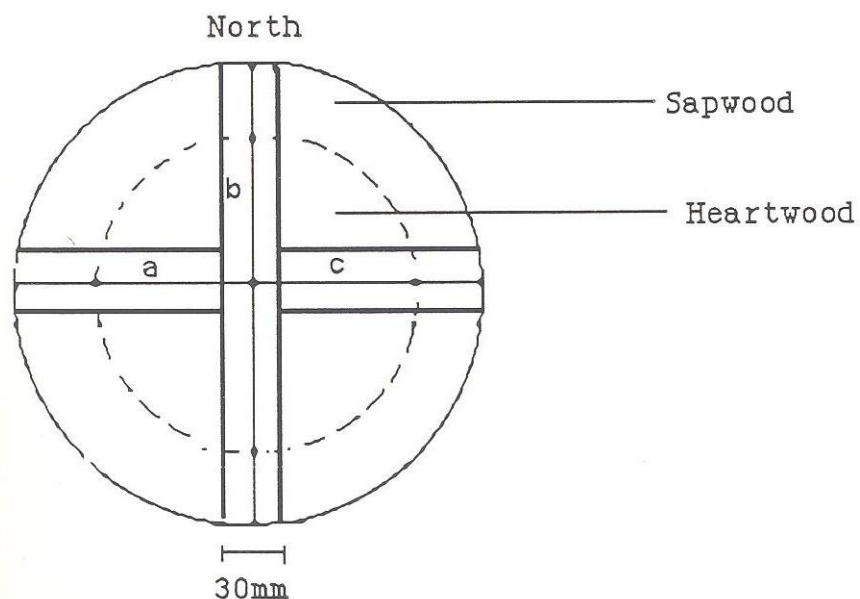

Figure 2: Diagram showing the three strips (a, b and c) removed from each disc and used in the light table method.

3.0 Results and Discussion

3.1Comparison of Sapwood-heartwood Demarcation Methods

Students t test result for comparisons between mean sapwood widths for the three methods are presented in Table

1. The t test statistics in table 1 indicates that the sapwood–heartwood demarcation methods using colour and stain are not significantly different. P =0.654 while the comparison of both colour to light table had significant difference with p=0.000. This is an indication that colour and stain can be used interchangeably in demarcating sapwood-heartwood boundary in *Eucalyptus regnans*

The mean sapwood widths and standards deviations from the colour, stain and light table methods are presented in Table 2.

Table 1: Students t test results for comparisons of mean sapwood widths for the three methods used to demarcate the sapwood-heartwood boundary.

Comparisons	T-Statistics	DF[1]	P Value
Colour and Stain	0.4	119	0.654ns[2]
Colour and Light table	-11.53	119	0.000**
Stain and Light table	-11.52	119	0.000* *

> ➤ 'DF[1]' degrees of freedom.
> ➤ 'ns[2]' is not significant at 5%
> ➤ '**'is significant at 1.0%.

Table 2: Mean Sapwood width (X) and standard deviation (SD) for three methods used to demarcate the sapwood-heartwood boundary.

Method	X (mm)	SD
Colour	25.2	6.4
Stain	25.0	6.3
Light Table	51.0	22.2

The mean sapwood widths of 25.2mm and 25.0mm (Table 2) for the colour and stain methods respectively were found not be to significantly different with a very slight variation in standard deviation of 6.4 and 6.3 respectively. However, the mean sapwood width of 51.0mm determined using the light table method was significantly higher than the mean sapwood widths of the colour and stain methods respectively. This result indicates that the light table method which relies on the presence or absence of tyloses results is a substantially different to the colour and stain methods. It can also be seen in Table 2 that there is a relatively large degree of variation associated with the light table method as indicated by the standard deviation of 22.2 as opposed to colour and stain methods with standard deviation of 6.4 and 6.3.

At all the five height (examined, no significant differences were found between sapwood widths for the colour and stain methods. It may be seen in Figure 3 that the sapwood widths for the colour and stain methods were very similar at each of the five heights. At four of the five heights the mean sapwood width obtained by light table method significantly differed from mean sapwood widths as obtained by either the colour or stain methods. At breast height (BH), the mean sapwood width of 72.7 mm for the light table method (Figure 3) was over three times as large as the mean sapwood width of about 20mm for the other two methods. The greatest difference between the light table method and the other two methods was found at this height. At the 20%, 40% and 60% heights the mean sapwood widths for the light table method were all significantly higher than the mean sapwood widths for the other two methods.

These results show that the colour and stain methods are essentially the same in defining heartwood and that they may be used interchangeably in demarcating the sapwood-heartwood boundary in 15-year old *E. regnans*. It appears that all three methods may be used interchangeably at the base of the tree but all other heights the light table method results in a demarcation of the sapwood-heartwood boundary which is considerably different from the other two methods. However the light table method which relies on the presence or absence of tyloses in the vessels may in some way have additional significance in relation to sap flow measurements.
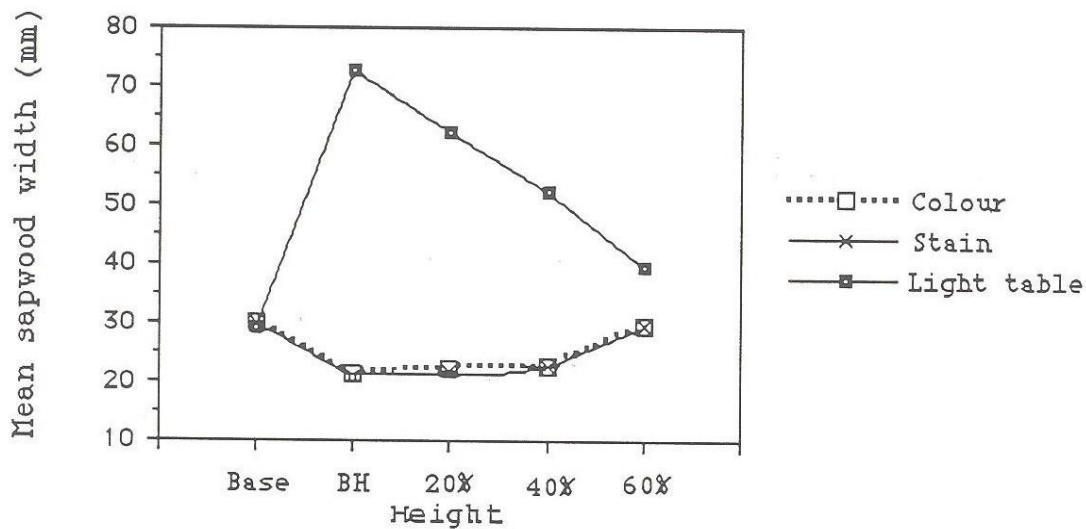
Figure 3. variation of mean sapwood width with height for three methods used to demarcate the sapwood heartwood boundary.

3.2 Sapwood width and variation

The analysis of variance of sapwood width between trees and between six heights and between four axis in six 15 year old. *E. Regnans* trees is presented in Table 5

Table 5. Analysis of variance of sapwood width for six trees, six heights and four axes of 15 year old *E. Regnans*

Source	DF[1]	Sum Square	Mean Square	F	P value
Trees	5	3.476	0.6952	3.39	0.008 **[2]
Heights	5	33.291	0.6582	3.39	0.008 **
Axes	3	1.402	0.4673	2.28	0.086 ns
Trees * heights	25	5.027	0.2011	0.98	0.501 ns
Trees * axes	15	3.920	0.2613	1.28	0.239 ns
Heights * axes	15	3.919	0.2612	1.28	0.239 ns
Error	75	15.362	0.2048		

[1] 'DF' is degrees of freedom
[2] '**' is significant at 1.0%; 'ns' is not significant at 5%.,

The variation of sapwood width between trees and between heights are highly significant at 1.0% level. The interaction of trees and heights, trees and axes are not significant indicating that variation of sapwood width with height and axes in the tree follows similar pattern in the sampled trees. The mean sapwood width, standard deviation and average number of growth rings present in the sapwood of each height for six trees are presented in Table 4. The trees had an overall mean sapwood value of 25mm which was within the general Eucalyptus species sapwood width range (Dadswell 1972; and Hillis 1987). The average number of growth rings within the sapwood of the sampled trees was 4.7 which was similar to 5 growth rings reported by Hillis (1987) on the same species. The individual sapwood width ranged from 12mm to 45mm which was measured in four positions per disc and six discs per tree (each disc representing a height) which represent a relatively substantial within tree sampling. From the base the sapwood increased with the increase in height which was a similar trend observed in Douglas fir grown in United states (Lassen et. al 1969).

Table 5: Mean sapwood width at six heights for six 15-year old *E. Regnans* trees.

Height	Mean (mm)	SD[1]	Average growth rings
Base	29.9	7.1	5.0
BH	21.0	3.2	4.7
20%	21.9	4.4	4.4
40%	22.8	3.6	4.2
60%	29.2	5.8	5.0
80%	33.9	4.4	4.7

3.3 Heartwood Percentage and Variation

The results of the analysis of variance of the heartwood percentage between trees and between heights is six 15 year old *Eucalyptus regnans* trees is presented in **Table 6**

Table 6: Analysis of variance of heartwood percent between trees and between heights for 15 year old *E. Regnans* trees**.**

Source	DF[1]	Sum Square	Mean Square	F	P value
Trees	5	289.7	57.95	4.65	0.004 **[2]
Heights	5	19897.5	3979.50	319.13	0.000 **
Error	25	311.7	12.47		

The analysis showed significant difference both between the trees (p=0.004), and with heights (p=0.000) at 1% level. The mean heartwood percent for the six trees ranged from 41.44% to 48.68% while the standard deviation within each tree heights ranged from 22.09 to 27.13. The relatively high standard deviation for each tree is primarily due to the large variation in heartwood percent within heights in the trees. The mean heartwood percentage, range of values, standard deviation and coefficient of variation at each height of the six heights for the six trees are shown in Table 7

Table 7: Mean Heartwood percent, range of values, standard deviation and coefficieient of variation for six heights of six-15 year old *E. Regnans* trees**.**

Height	Mean %	Maximum value (%)	Minimum value (%)	SD[1]	Coeff var[2]
Base	60.76	65.75	53.95	4.03	6.63
BH	67.41	69.51	63.31	2.33	3.45
20%	60.52	66.02	54.61	4.87	8.04
40%	54.15	58.14	50.46	3.11	5.74
60%	30.24	42.1	18.60	8.08	26.71
80%	0	0	0	0	0

4.2 LSD (PR = 0.05)
1 'sd' is standard deviation
2 'Coeff var' is coefficient of variation.

The largest mean heartwood percentage of 67.41 was found at the breast heights. Based on LSD test (Pv=0.05) the mean heart percentage of 60.76 at the base of the tree was significantly lower than mean heartwood percent of 67.41 at breast height. There was a relatively large amount of variation between trees in the mean heartwood percentage at 60% of the total tree height as evidenced by standard deviation of 8.08 and coefficient of variation of 26.71. The least amount of variation between trees in the mean heartwood percentage was found at breast height as indicated by standard deviation of 2.33 and coefficient of variation of 3.45. This results appear consistent with the work reported on plantation grown *Eucalyptus globulus* where the heartwood area decreased from the breast height upwards (Maria, 2007). The decrease of heartwood from breast height to base had also been reported in a study on *Melia azandarach* trees grown in Saudi Arabia (Loutfy 2009). The reason for high percentage of heartwood at breast height could be due to the fact that the heartwood formation starts from the breast height.

The results showed a relationship between heartwood diameters at each of the six tree heights with the disc diameter of similar height and a correlation coefficient of 0.967 was found for relationship between these two variables. Similar high correlation was reported in a study on Eucalyptus globulous (Maria et al 2007 and Miranda et al., 2009). Other studies on *Acacia melonoxylon* and *Quercus castaneaefolia* on heartwood variation by Sofia et al., 2005 and Ayobi et al., 2011 respectively also found heartwood to be highly correlated to stem diameter. This high correlation indicates that tree diameter can effectively be used to estimate the heartwood percentage of standing tree.

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