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Investigating wood anatomical factors influencing the treatability of refractory southern pine- an imaging approach

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ABSTRACT

Southern pine sapwood is non-durable, and preservative treatment is necessary to protect it against biological degradation. According to AS 1604.1 2021, timber products must have their sapwood fully treated with preservatives for various applications and hazard classes. However, there is a growing industry concern over the refractory sections of sapwood, which are untreated pockets of wood that do not absorb preservatives effectively after vacuum pressure treatment. This study investigated some wood anatomical differences between treated and refractory southern pine sapwood subjected to light organic solvent preservative (LOSP) treatment. The study utilised light microscopy, safranin solution uptake, and microcomputer tomography (µCT) to identify any differences between the two types of sapwood. Light microscopy results showed wood rays and horizontal resin canals were sapwood's main lateral liquid movement path.

Furthermore, in refractory sapwood, the movement of liquid from rays to the tracheid was interrupted. µCT imaging results showed interrupted pathways within refractory samples. Safranin uptake comparison between treated and refractory sapwood showed that treated sapwood had higher liquid uptake capacity. The assessment using µCT images showed that the treated sections of sapwood had a higher proportion of voids in resin canal areas and a greater percentage of uninterrupted voids within the sample volumes than the untreated sections. Comparison between refractory sections with and without resin canals showed that the presence of resin canals increased the void connectivity in refractory sections.

Keywords: Treatability, LOSP preservatives, southern pine, refractory sapwood, microcomputer tomography (µCT), light microscopy, image analysis

1. INTRODUCTION

Timber is one of the world's most used building materials; more than 27 million square meters of timber were harvested in 2021 only in Australia (2022). The sapwood of any timber species can be considered non-durable (2021), and depending on the application, different preservative treatments are required. Timber can be pressure treated with chemical preservatives to protect it against insect and microbial agent attacks and wood-boring invertebrates (Khademibami *et al.* 2022). Sapwood is the primary target for the preservative treatment, as heartwood in most species cannot be effectively treated mainly due to low permeability, the higher concentration of extractives, and other anatomical features . The sapwood of major softwood plantations in Australia, such as radiata pine (*Pinus radiata*), southern pine *Pinus caribaea* var. hondurensis, *Pinus elliottii* var. elliottii and their hybrid), hoop pine (*Araucaria cunninghamii*), and others, is expected to be effectively treated with preservatives due to its high permeability (Greaves 1980). However, several reports of unexplained variability in treatability for southern and radiata pine sapwood material have recently surfaced in Australia. It is still unknown why parts of sapwood become impermeable to the pressurised preservative treatment. These parts or sections of sapwood will be referred to in this paper as refractory sapwood.

Previous studies have reported that the difference in genetic provenances, growth locations, growing conditions of pine trees, and processing-induced variations such as drying of wood may result in changes in permeability and ultimately lead to variations in sapwood's treatability (Winandy *et al.* 2001, Winandy *et al.* 2022). The anatomy of the wood is considered paramount in what defines the permeability of the wood. Different structures have been suggested to be influential in the treatability of the wood, such as pits (Wardrop *et al.* 1961, Usta *et al.* 2006) (Usta *et al.* 2003), ray parenchyma cells as well as ray tracheids (Banks 1970, Comstock 1970, Liese 2022). It has been reported that bordered pits of coniferous wood become aspirated during drying (Messner *et al.* 2003); further studies have been done on investigating the effects of drying temperatures and schedule (Usta *et al.* 2006). However, in the case of refractory pine wood, various parameters affecting the preservative treatment penetration including the anatomical features, cell structure and chemistry, need to be investigated. Effective treatment of wood is important for the timber industry. While the exact reason for the refractory sapwood is unclear, it is understood that the anatomy, extractives contents (including pine resin), growing conditions and wood drying may affect the sapwood permeability (Leggate *et al.* 2019, Leggate *et al.* 2020, Leggate *et al.* 2021) knowing the cause of refractory sapwood could help with employing practices by the industry to reduce its impact.

In this paper, southern pine represents Caribbean pine (*Pinus caribaea* var. hondurensis), slash pine (*Pinus elliottii* var. elliottii) and their hybrid. In Australia southern pine is the dominant exotic softwood species; in Queensland it is grown in around 148,000 ha of plantations. This timber supports a diverse processing sector such as engineered wood products, sawn timber, reconstituted panels, landscaping and lower-grade end uses (Lee 2015). This highlights the importance of an effective preservative treatment of southern pine for the industry.

Previous studies by Leggate *et al*. (2019) on 19-year-old southern pine from Queensland plantations showed that regardless of the genotype or stocking rate in the plantation, the gas and liquid radial permeability of the timber increased from the pith to bark; while conversely, resin content decreased. A further complementary study by Leggate *et al*. (2020) focused on some anatomical traits involved in permeability in the same timbers. This study investigated the frequency and diameter of tracheids, resin canals, sapwood and heartwood, as well as the location and concentration of resin and the radial permeability of the southern pine wood (Leggate *et al.* 2020).

There is a gap in developing systematic practical efforts to determine the factors defining the differential treatability of treated and refractory sapwood. The current study is part of a larger investigation being undertaken by the Forest Products Innovation team, Queensland Department of Agriculture and Fisheries (QDAF), into finding the cause of refractory wood in some Australian softwood pine species. The study investigated the differences in wood structure and voids in treated and refractory southern pine sapwood using microscopy, safranin uptake and micro-computed tomography (µCT). Micro-computed tomography (µCT) and innovative image processing techniques provide an opportunity for alternative, rapid, accurate approaches to explore wood structural characteristics. µCT has previously been explored to study the anatomical features of wood, such as porosity, pore size, pore numbers, vessel density, pore conductivity, different preservation conditions, and many other applications in the wood industry (Steppe *et al.* 2004, Whitau *et al.* 2016, Leggate *et al.* 2021). This paper uses 3D imaging and µCT to investigate the anatomical differences between refractory and treated sapwood of southern pine.

2. EXPERIMENTAL METHODS

Refractory (to treatment) and treated sections of plantation-grown southern pine boards were used to study the anatomical differences between the two using µCT and light microscopy. For this, southern pine boards treated with light organic solvent preservative (LOSP) according to AS 1604.1-2021 (2021) were collected from industry representatives in Queensland, Australia. Sample boards were cut at both ends, and cross-section biscuits were used for spot tests i.e., determination of heartwood/sapwood and treated/refractory sections and a section for scanning (resin canals, earlywood (EW)/latewood (LW) ratio determination) (Figure *1*(A)). PAN (1-(2-pyridylazo)-2-naphthol) solution and methyl orange were sprayed on transverse surfaces to identify the preservative-treated and heartwood/sapwood areas, respectively (Figure *1*(B)). Samples were then scanned on the transverse surface using an Epson perfection dual lens system V850 Scanner (EPSON, Suwa, Japan) at high resolution (9600 dpi) for further imaging and analysis (Figure *1*(C&D)). According to the spot test results, only sapwood sections were chosen for further microscopic work and the determination of anatomical features.

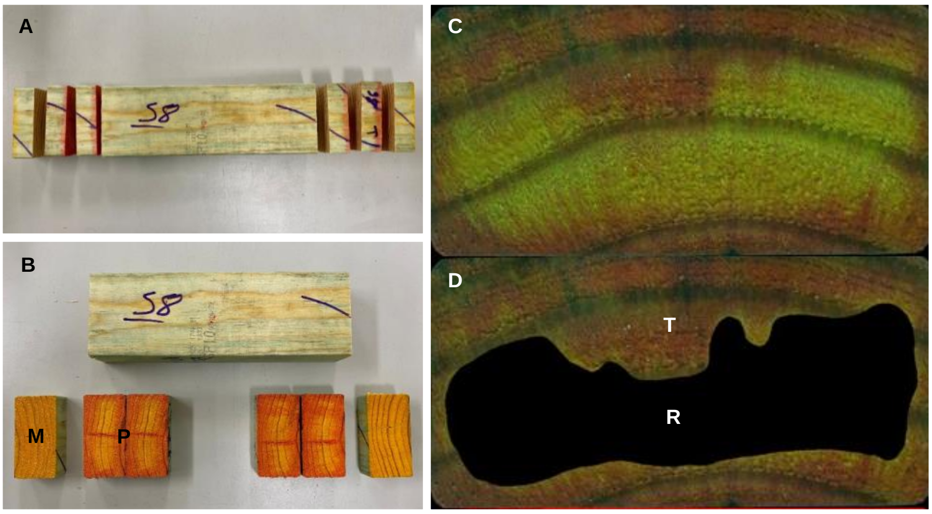


Figure 1: (A) Sample preparation for imaging studies, including thin sections from each end of boards for spot tests and scanning, (B) thin sections used for spot tests and (C and D) process of imaging sections of selecting treated (T) and refractory (R) using ImageJ.

A Nikon Eclipse LV100ND microscope (Tokyo, Japan) was used for microscopy imaging. The fluorescence light option (FL2) was used for samples stained with safranin. The data generated from scanned samples of six tested boards, including the number of resin canals, were analysed using an ImageJ program. From the spot tested samples, two blocks of treated and refractory sections were cut with the dimensions of 11 x 11 x 11 mm selected for µCT imaging.

Using the sapwood samples' ability to uptake safranin solution, a novel technique was used to quantify the wood's treatability. The experiment was done on two southern pine boards with more treated areas and two boards with more refractory areas. 10 mm x 10 mm x 20 mm blocks of treated sapwood samples were made from each end of the boards from treated sections of the boards. Similarly, refractory sapwood samples were made from two ends of refractory selected boards.

The main path of liquid preservatives into the wood during preservative treatment is via radial and tangential rather than longitudinal movement. In order to replicate the wood treatment condition, the transverse faces of blocks, which were the smallest face on the blocks, were coated with nail varnish to block the longitudinal flow of liquid into the wood. Then the samples were submerged in 1% safranin overnight. Samples were then rinsed with water and surface dried. Then the surface of the samples to the depth of ~1.5 mm were removed using a blade. This was to remove any excess stain on the surface and only have the stain absorbed by the wood inside.

Samples were oven-dried at 40°C overnight and then ground separately using a wood grinder. 1.2 g of ground material for each board was mixed with 20 ml 50% ethanol solution and left overnight. The solution's optical density (OD) at 519 nm was measured using a spectrophotometer after being diluted three-fold in 50% ethanol.

Samples were µCT-scanned using a SKYSCAN 1272 CMOS Edition. The analysis of 3D images was done in Skyscan software Bruker packages (SKYSCAN 1272 CMOS Edition). The scanning was conducted at 40kV and 200µA at the resolution of 2µm. The reconstructed images (Nrecon-Bruker microCT) generate a 3D structure from 2D slice images taken from the sample. The 3D imaging stacks the 2D scans of the sample and builds the volume of wood and air in greyscale from 0-255. The analysis was done by re-creating volumes of voids within the structures using the greyscale range. The pathways (voids) within the timber structures were analysed by defining a region of interest (ROI) starting on one side of the block in the tangential face travelling through the volume (in the radial direction), and recreating the connected pathways within the structure. The uninterrupted reconstructed voids that followed each other were analysed as potential pathways available for chemical penetration into the structure. Pathways were selected after microscopic imaging which had showed the differences in safranin uptakes between different parts of the treated and refractory wood. The generated bulks were then analysed using SkyScan 1272 CMOS software and ImageJ.

3. RESULTS AND DISCUSSION

A summary of the treated area of each sample determined through spot testing, scanning and image analysis is presented in Figure 2. The standard deviation in each bar represents the differences between the thin section cut from each side of the board end.

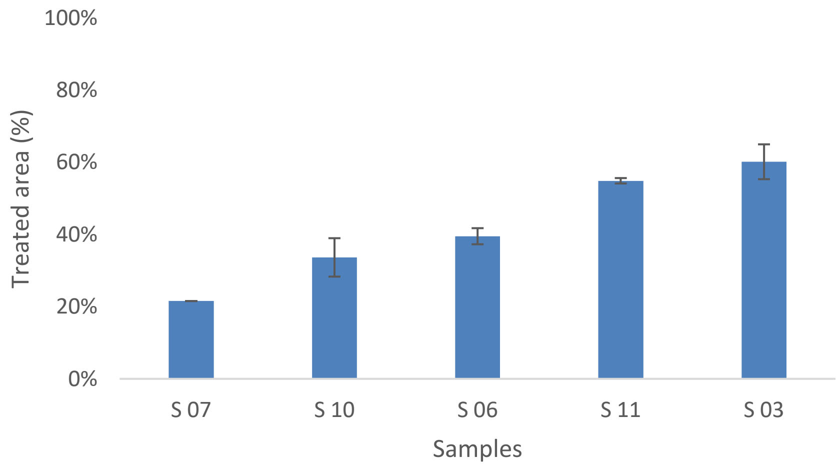


Figure 2: The percentage of the treated area in the cross-section of samples after spot testing and scanning.

The general anatomical observation of southern pine sapwood under a fluorescence microscope after being stained with safranin is shown in **Fel! Hittar inte referenskälla.**. The safranin-stained area was more evident in the treated section than in the refractory sections. While rays and horizontal resin canals in both types of samples were stained, staining was more significant in treated compared to refractory samples. Furthermore, tracheid cells of treated sapwood were obviously more stained than refractory wood. Safranin uptake rate of treated sapwood was significantly higher than refractory wood (Figure 4). The images from µCT (Figure 5) showed a higher percentage of voids within the wood block in treated sections than in refractory sections.

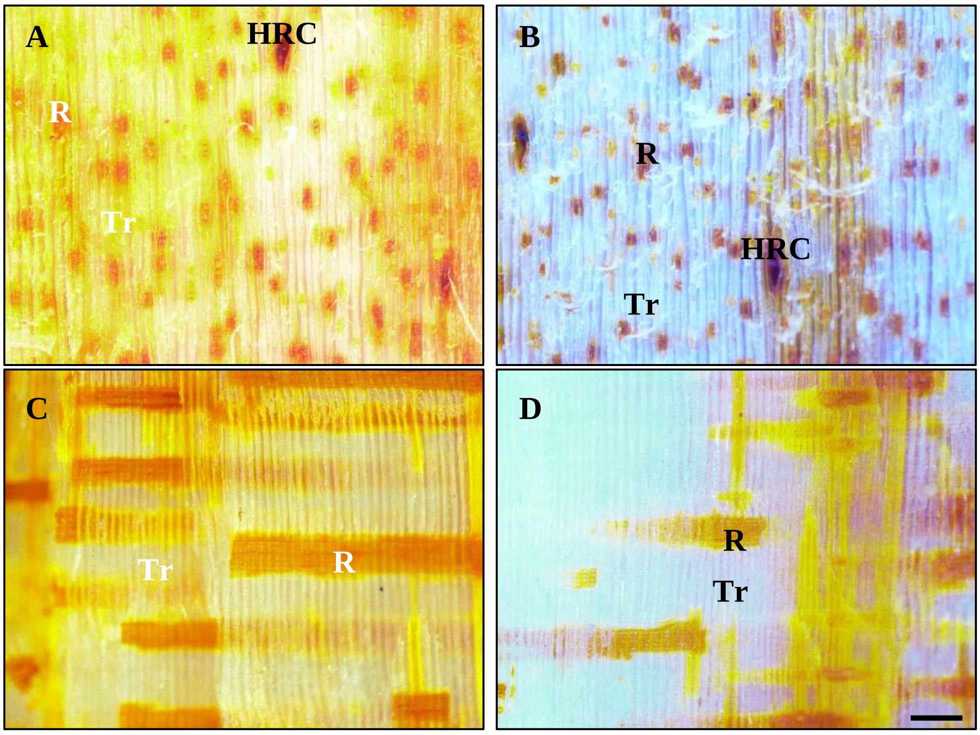


Figure 3: Fluorescent light microscopy of treated (A and C) and refractory wood (B and D) softwood of southern pine. A and B show tangential, and C and D show radial sections of samples. Horizontal resin canal (HRC); tracheid (Tr); ray (Ra). The scale-bar represents 200 µm for all images.

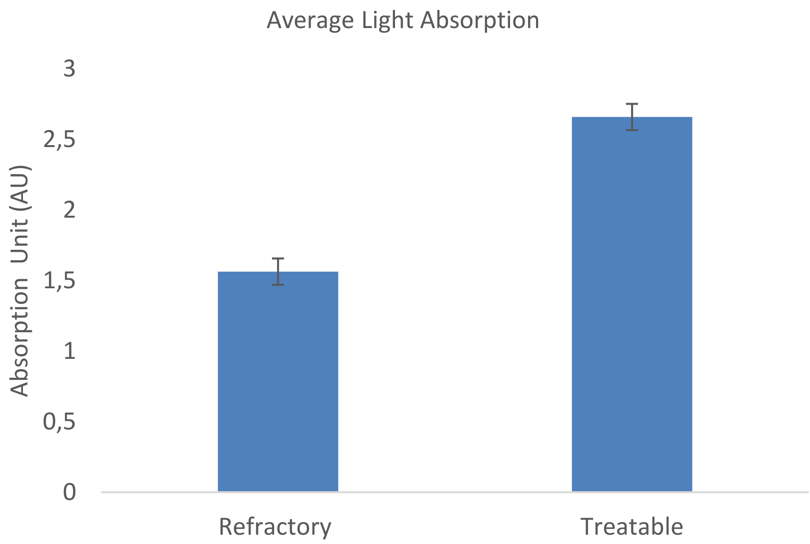


Figure 4: Treatability of treated and refractory southern pine sapwood based on the safranin solution uptake. The light absorption of safranin solution uptake extracted from stained southern pine sapwood. Error bars represent the standard error for two replicates.

In the radial direction, as shown in Figure *5*, the green colour shows the voids within the structure, and the brown colour shows the wood component. As observed in the microscopic images, most of the rays were stained with safranin in treated sections, while in the refractory sections, only parts of the sections were stained. The µCT images also showed interrupted pathways inside refractory sections, while the treated sections had connected pathways within the sample volume. In the radial direction, the role of rays in fluid transport and treatability has been reported previously, where a lower number of parenchyma cells in rays has been linked to decreased fluid flow within timber structure (Zimmer *et al.* 2014, Zimmer *et al.* 2014). A study of copper penetration in southern pine (Feng et al. 2019) showed similar effects of rays as pathways for increasing the flow of preservatives in the timber structure.

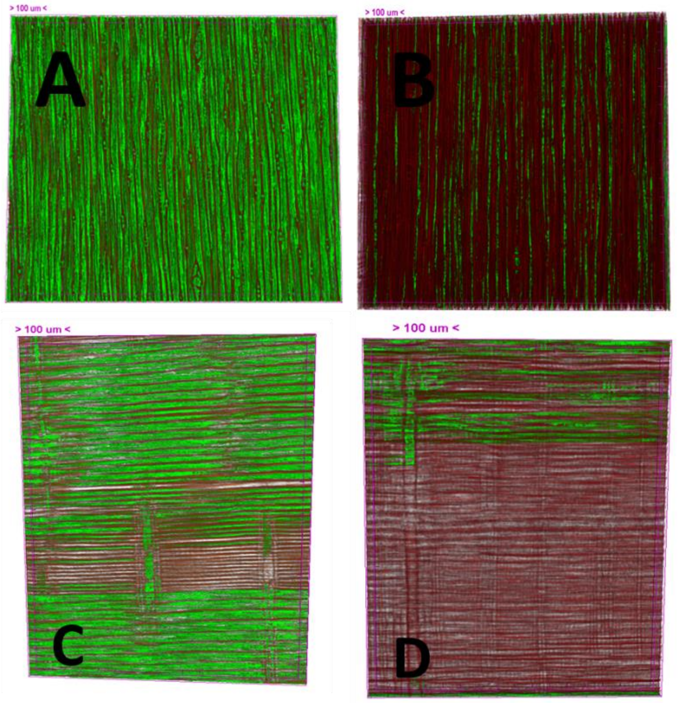
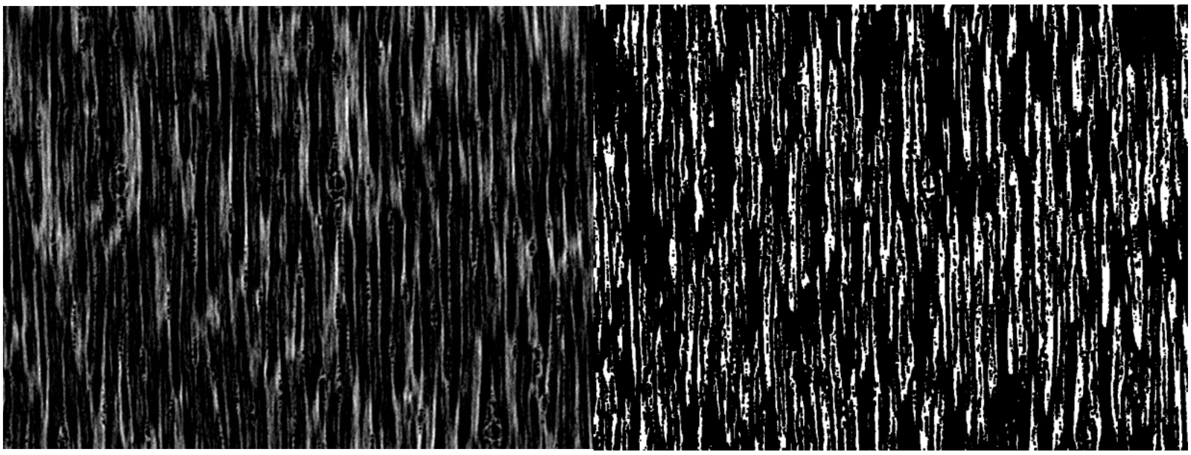
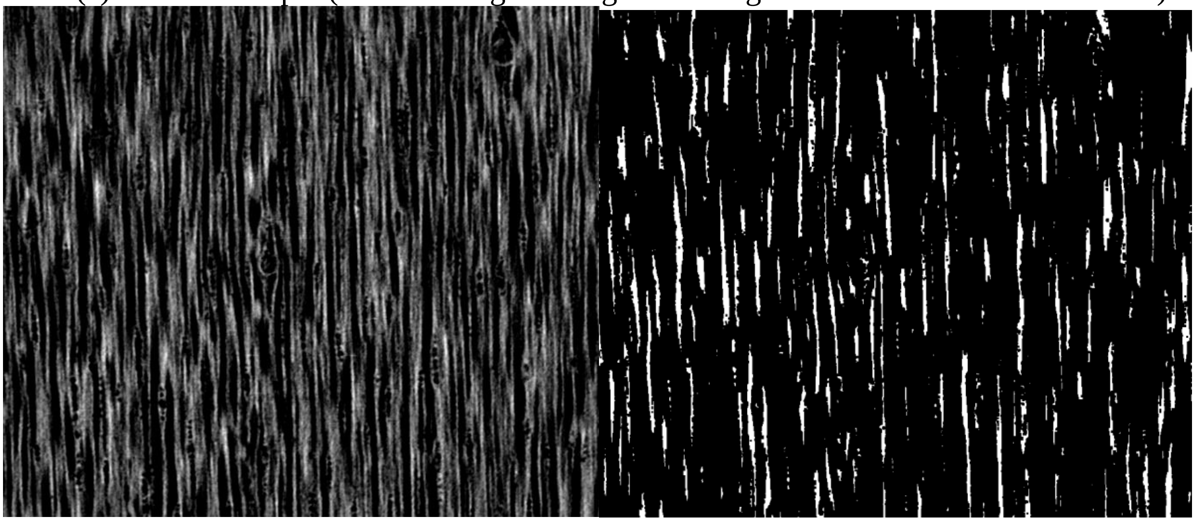


Figure 5: the µCT images of southern pine sapwood for treated (A and C) and refractory (B and D), A and B show tangential, and C and D show radial sections of samples. Green sections show the voids in the structure and brown sections show wood.

ImageJ was used to analyse the 2D images obtained from µCT scans in batches. The thresholding process followed the guidelines presented in Figure 6. The resulting white regions correspond to voids within the structure, defined as cavities that run continuously from the block's edge without interruption.



(a) Treated sample (left full image and right the image of cavities within the volume)



(b) Refractory sample (left full image and right the image of cavities within the volume)

Figure 6: Images of tangential view of the scanned samples.

The percentage of void spaces in refractory sections was lower than in the treated sections. Figure *8* summarises the average void percentages for treated (a) and refractory (b) sample sections. The presence of continuous voids in treated and refractory samples was determined using a region of interest (ROI) on the tangential surface, with the criterion that the voids extend uninterruptedly throughout the block. In two refractory samples where there were no resin canals, the voids were observed to terminate rapidly after reconstruction began (Figure 7). In the section with resin canals, however, the connected voids were found to extend much further into the structure, as shown in Figure 7 as section with and without resin canals. Similar observations were reported by Ahmed *et al*. (2012) that the frequency of axial resin canals affects the preservative penetration and percentage of treatability of Scots pine. Ahmed *et al*. (2012) further reported that the dimensions of radial and axial resin canals were significantly higher in treated than untreated sections of Scots pine. A study of visualising copper penetration in southern pine using µCT showed an accumulation of copper within the rays and resin canals (Evans *et al.* 2013). The study of copper penetration in southern pine samples showed that the location of copper in timber structures was coincidental with rays in connection with resin canals (Feng *et al.* 2019. Additionally). This study showed that parenchyma cells surrounding vertical resin canals had copper presence. Overall a similar observation was reported as a connected structure was observed between the resin canals (axial and radial), suggesting a 3D penetration of copper in the sample structure (Feng *et al.* 2019).

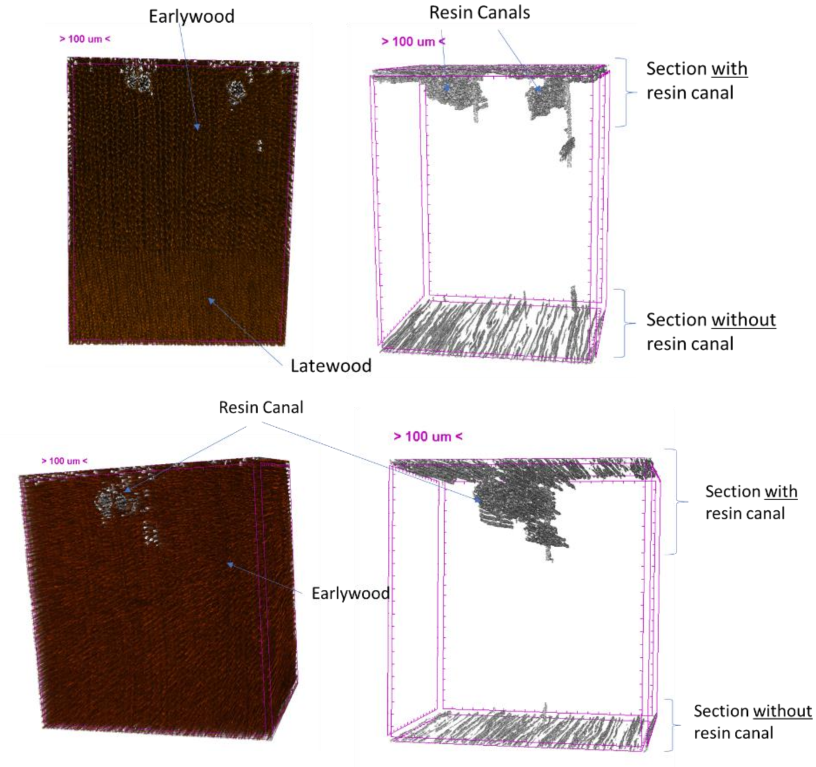


Figure 7: The µCT images of refractory wood sections and the uninterrupted voids from each direction.

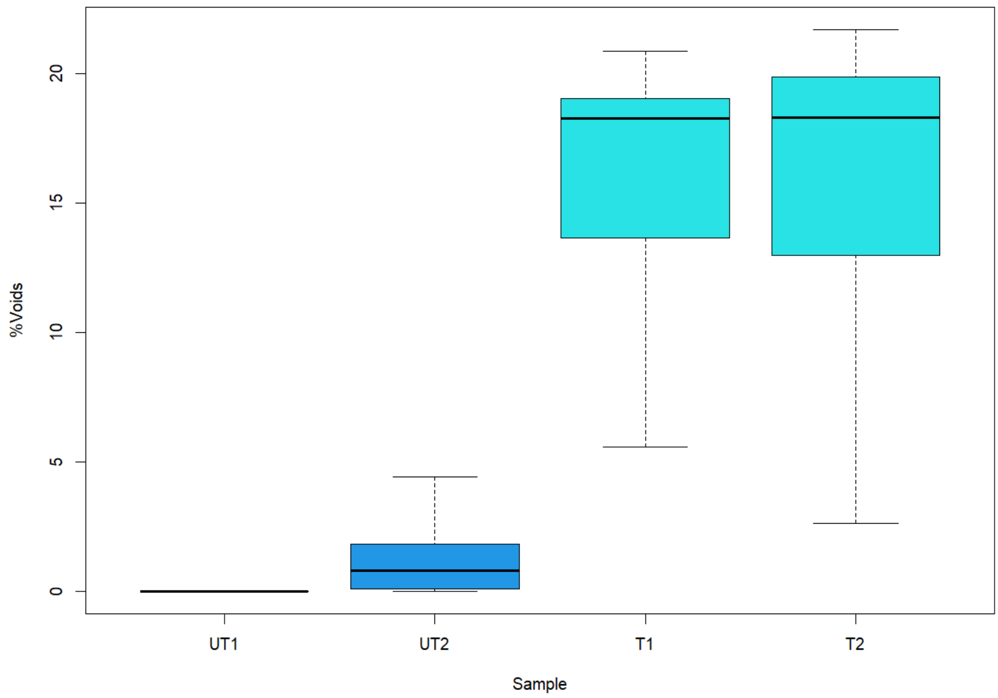


Figure 8: Percentage of voids in samples determined by selecting the tangential face and travelling into the block. The values show the overall percentage area determined for each sample (T: treated; UT: untreated refractory). In the boxplot maximum, minimum and median of each dataset is shown.

Figure 9 shows the portion of connected voids within treated sample blocks. The percentage of voids is much higher in earlywood sections than in latewood sections. However, the resin canals and rays worked as the connectors to continue the pathways within the structure in the latewood sections of treated wood, as shown in Figure 9. The effects of resin canals and rays as pathways for treatment penetration into timber have been reported previously (Zimmer *et al.* 2012, Leggate *et al.* 2019, Leggate *et al.* 2020, Wood *et al.* 2020). Similarly, the differences observed in earlywood and latewood bands of southern pine when treated with copper were reported by Evans et al. (Evans *et al.* 2012). This study showed that lower copper was visualised in latewood bands, possibly due to a lower number of resin canals in latewood sections (Evans *et al.* 2012)

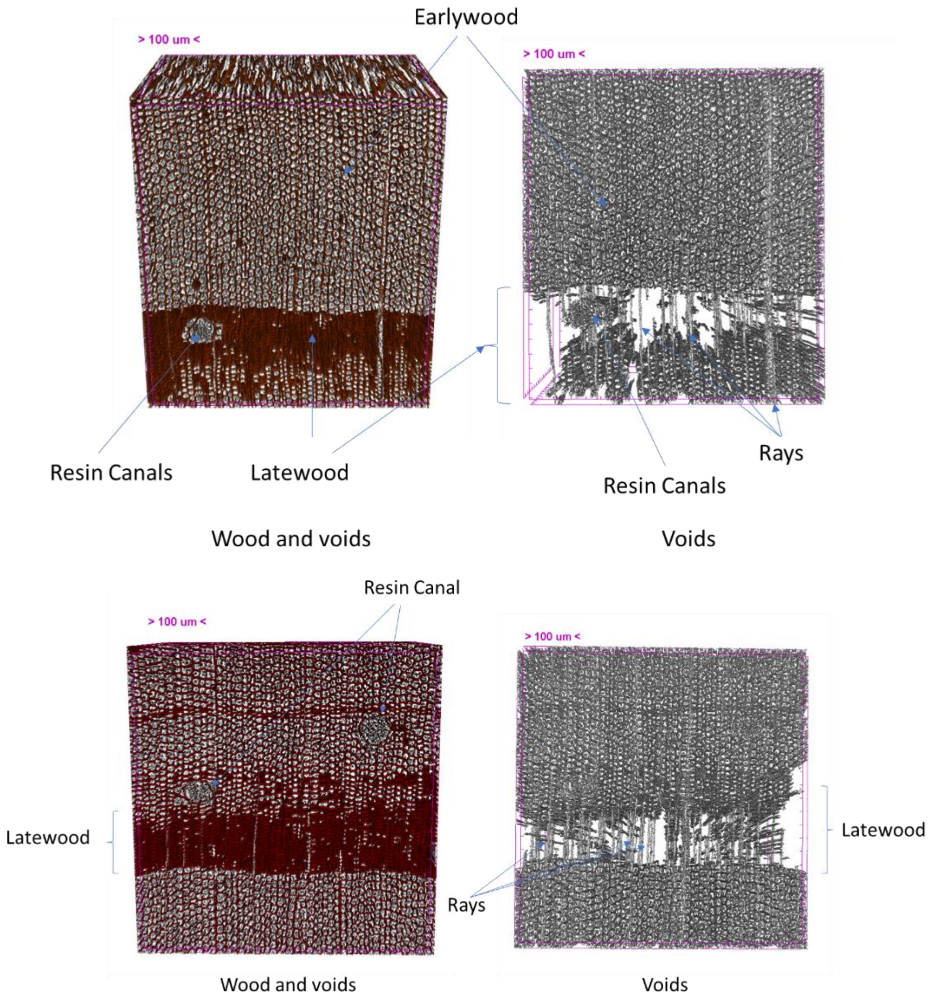


Figure 9: 3D images of treated pine, image on the right shows the void sections only (wood component has been subtracted) within the treated samples and image on the left showed voids (grey colour) and wood (brown colour).

Figure *10* shows the percentage of EW portions and the percentage of treated area in each sample section. The preliminary data showed higher treated area percentage in samples with higher EW percentage. Further research on larger sample set and different ratios of EW and LW and resin canals is required to investigate the possible differences between treatment penetration in EW and LW bands.

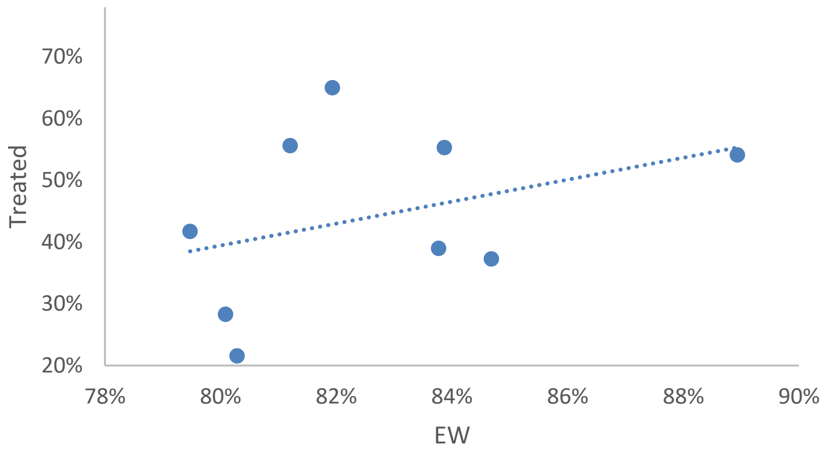
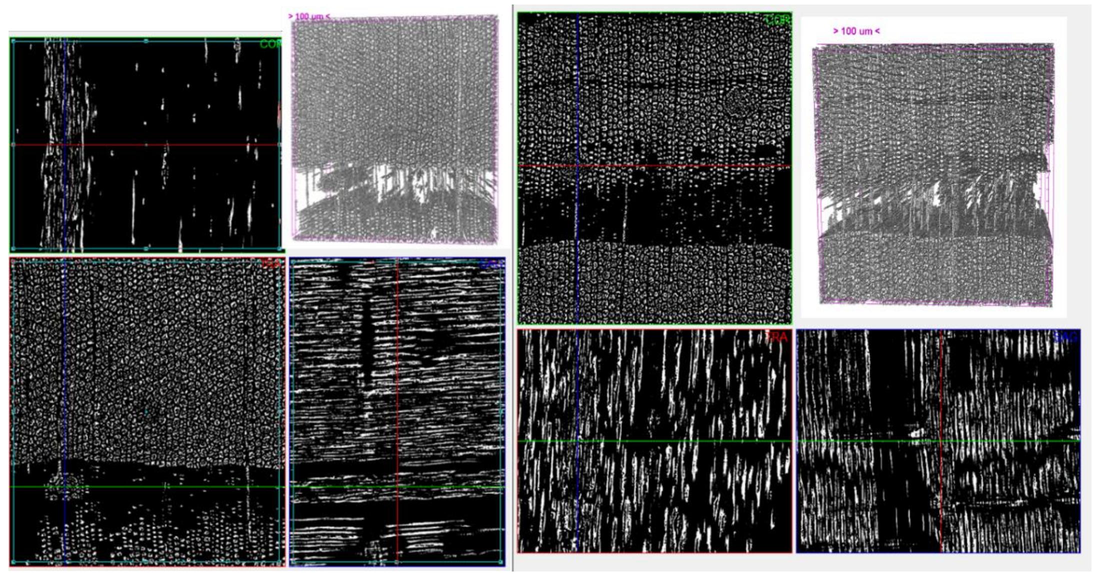
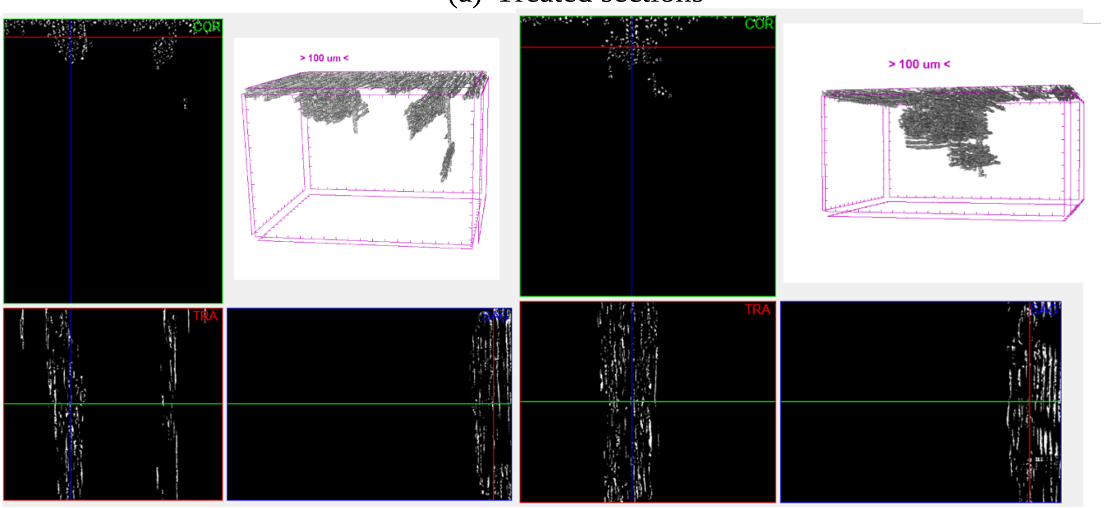


Figure 10: Percentage of the treated area versus the percentage of EW in tested sections.

Images of treated and refractory sections with reference to resin canal effects are presented in Figure *11*.



(a) Treated sections



(b) Refractory sections

Figure 11: Continuation of voids within the sample block illustrating differences between treated and refractory wood.

The resin canals within EW and LW sections of treated samples showed a higher percentage of connected voids than the refractory sections. The resin canal number in the sample biscuits was also determined using the scanned faces and Image J as presented in Figure *12*. A positive relationship was observed in the limited number of samples studied between the percentage of treated wood versus the total number of resin canals.

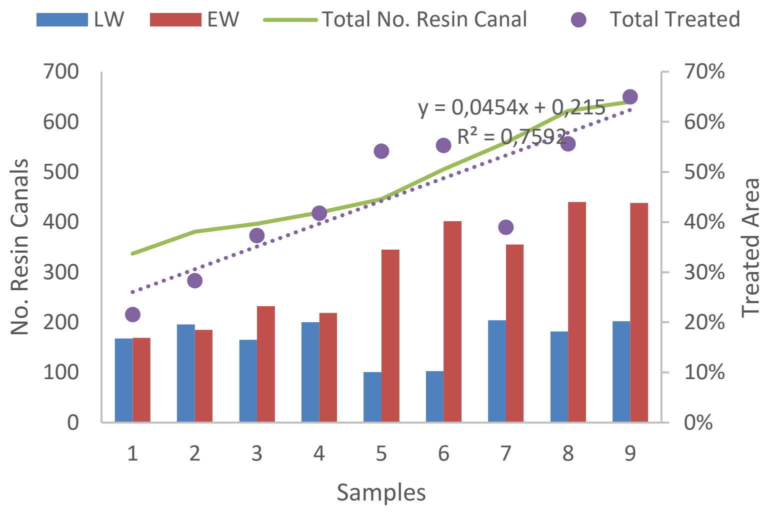


Figure 12: Summary of the number of resin canals in EW and LW sections and percentage of treated sections in the samples tested.

Resin canals are considered one of the factors influencing wood permeability and treatability (Yörür *et al.* 2018, Leggate *et al.* 2020). The 2D slices collected from µCT scans were used to determine the void percentage in each resin canal present in treated and refractory samples. Figure *13* shows a higher percentage of voids in the treated resin canals than in the resin canals in refractory sections. The role of resin canals in the treatability of Scots pine has been studied, and a higher number of resin canals/mm^2 was reported in treated sections than in untreated sections. It is also reported that blocked resin canals had a major influence on the lower distribution of preservatives in untreated sections (Ahmed *et al.* 2012).

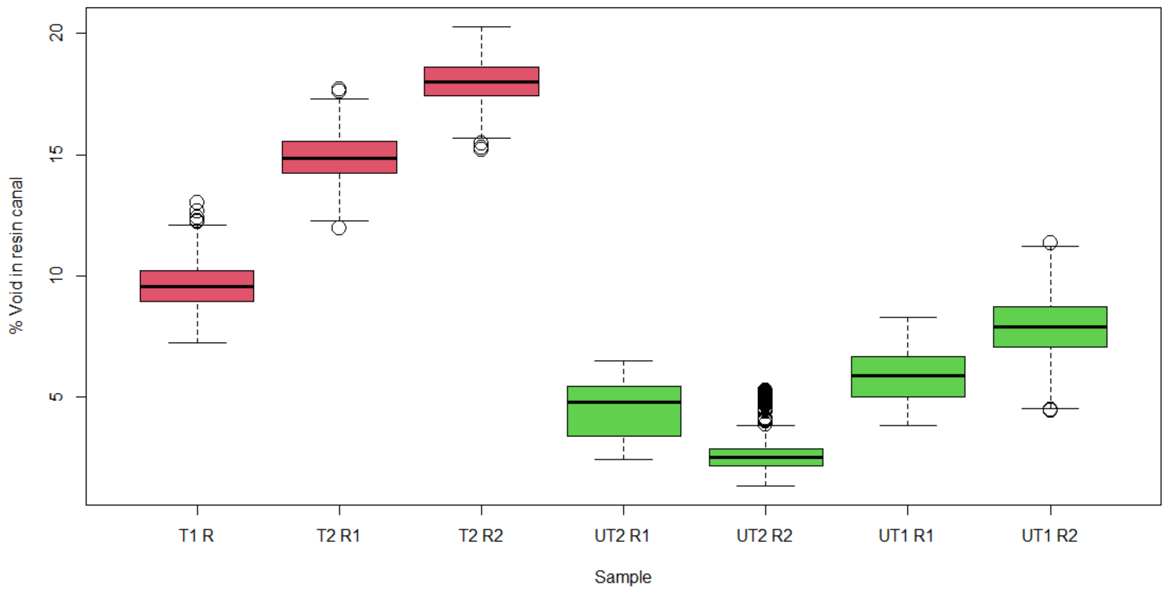


Figure 13: Percentage of continuous voids in resin canal (R) sections of treated (T1 and T2) and refractory (UT1 and UT2) samples determined using batch processing in ImageJ.

4. CONCLUSIONS

This project investigated the anatomical differences between treated and refractory sapwood of plantation southern pine timber from Queensland, Australia using light microscopy and microcomputer tomography (µCT) imaging methods. Novel staining and quantifying techniques were used to investigate the liquid uptake of samples.

The results showed that the lateral path of safranin stain in the sapwood is mainly through rays and, to some extent horizontal resin canals (fusiform rays). This has been previously reported by Feng *et al.* (2019) on southern pine samples treated with micronised and amine-copper preservatives (Feng *et al.* 2019). While wood permeability is much higher longitudinally, the wood preservative treatment relies mainly on lateral liquid flow due to the greater exposed area during treatment (Banks 1972, Leggate *et al.* 2019). This highlights the role of horizontal resin canals and rays in particular, which may be key elements in this species' preservative treatability of the sapwood. µCT results showed similar results with a higher percentage of voids observed in treated sections than in refractory sections in the radial direction.

There was an interrupted safranin stain in the refractory sections, while in treated sections, visible signs of safranin uptake were observed from rays and horizontal resin canals into tracheid cells. The µCT imaging comparison showed that the treated sections had continuous uninterrupted voids in the structure of the samples from both sides of the tangential face and in the radial direction. In the refractory sections, the voids were discontinued from each edge of the tangential face.

This study's novel liquid uptake technique was reliable and showed that treated sapwood absorbed significantly more stain than refractory sapwood (Figure 4). This could be due to less liquid being absorbed by the tracheid cells. This method could be considered a quantitative approach for determining the liquid uptake capacity of the sapwood.

The existence of a resin canal increased the void connectivity in the refractory samples. However, the voids were discontinued just after each resin canal.

5. ACKNOWLEDGEMENTS

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