IMPREGNATION OF RADIATA PINE WOOD BY VACUUM TREATMENT: IDENTIFICATION OF FLOW PATHS USING FLUORESCENT DYE AND CONFOCAL MICROSCOPY

by

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SUMMARY

Radiata pine sapwood and heartwood were dried with or without pre-steaming and then impregnated by vacuum treatment with water, toluidine blue and fluorescein. Sapwood uptake was 0.571 g/cm³ and was not affected by pre-steaming. As expected, the uptake by heartwood that had not been pre-steamed was very low. Pre-steaming increased liquid uptake from 0.113 g/cm³ to 0.438 g/cm³. When the uptake by pre-steamed heartwood from radial, tangential and transverse surfaces was compared, the greatest increase was from the radial surfaces, suggesting that pre-steaming of heartwood resulted in changes to the tangential liquid flow pathways. The liquid flow pathways in sapwood consisted of axial and radial resin canals, ray parenchyma cells in both fusiform and uniseriate rays. Penetration into tracheids was also observed. Without pre-steaming, there was limited liquid flow into heartwood, and this was generally confined to resin canals and ray parenchyma. Pre-steaming of heartwood increased penetration of dye into the resin canal network, presumably due to removal or redistribution of resin. Fluorescein was also evident in bordered pits between tracheids, suggesting that one of the ways that pre-steaming increased heartwood treatability was by altering the condition of bordered pits to allow greater conduction. The combination of fluorescein dye and confocal microscopy was found to be a particularly effective way of visualising flow patterns, as it was possible to examine thick sections, which avoided microtome damage at the section surface. Examination of dry wood also minimised the possibility of dye redistribution.

Key words: Radiata pine, heartwood, sapwood, impregnation, fluorescein dye, pre-steaming, confocal microscopy, flow path, resin canal, ray parenchyma, bordered pit.

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INTRODUCTION

Dry radiata pine (*Pinus radiata* D. Don) heartwood is difficult to impregnate with preservatives, whereas dry sapwood can be pressure-treated without difficulty. According to McQuire (1970), the flow pathway of liquid preservative during pressure treatment is via the ray parenchyma. Tracheids become filled with preservative solution when the pit membranes of half-bordered pit pairs between ray parenchyma and tracheids become ruptured by the pressure treatment.

Bamber and Burley (1983) described other radial and axial flow pathways in dry radiata pine sapwood where impregnation occurs along interstitial spaces created when thin-walled ray parenchyma cells collapse during drying. Olsen (1987) observed dry radiata pine wood that had been soaked in dye. Flow occurred along the ray parenchyma cells, although the stained rays were often in contact with a resin canal. Booker (1990) developed an impregnation model for long boards of radiata pine sapwood where liquid enters the wood via the radial resin canals which are interconnected with axial resin canals. The interstitial spaces created by collapse of ray parenchyma cells were considered as a secondary flow path, with liquid then entering tracheids via half-bordered pit pairs. Booker (1990) did not find conduction in uniseriate rays without radial resin canals.

The above studies typically apply to liquid flow paths in the dry sapwood of radiata pine which can be treated readily. Impregnation of heartwood is usually much more difficult and pre-steaming of green lumber and high-temperature kiln drying are techniques that have been used to improve treatability. Although these techniques are practised widely, the wood anatomy of the flow paths has not been described. Matsumura et al. (1996) found that the removal of resin by methanol extraction increased the specific permeability of Japanese larch heartwood, and that there was a significant correlation between the increase of permeability and the loss of extractives. A linear relationship was also found between increase of permeability after resin extraction and the number of resin canals. According to Cown (1992), the methanol soluble resin content of radiata pine sapwood is usually about 1.5% by weight. However, the resin content of heartwood is quite variable and ranges from 2 to 10%. From these reports, one gets the impression that the resin canals may also be the primary flow paths in heartwood.

Bamber and Burley (1983) reported that the bordered pits of latewood tracheids and ray tracheids were rarely closed. Aspiration of earlywood bordered pits could therefore explain the reduced liquid flow in earlywood compared to latewood. However, Kishima (1965) indicated that aspiration only restricts the liquid flow through a pit rather than prevents it. Booker (1990) obtained such extremely low tangential liquid permeability values for radiata pine sapwood that had been resaturated with water that he considered that liquid flow through a series of aspirated pits is negligible. Matsumura et al. (1995) could not explain the variation in specific permeability of Japanese larch heartwood solely by bordered pit aspiration and suggested that pit encrustation and occlusion may also be important factors. Such pit encrustation and occlusion may occur in non-aspirated pits as well as aspirated pits.
The objective of this study was to observe liquid flow in radiata pine under a confocal microscope using a fluorescein dye. Confocal microscopy allows clear visualisation of the dye flow path with a three-dimensional perspective and can be carried out on dry wood avoiding redistribution of the dye during specimen preparation (Knebel & Schnepf 1991). In particular, the liquid flow paths were compared in heartwood that had been dried with and without pre-steaming of the green wood. The flow paths were also studied by comparing uptake from radial, tangential and transverse surfaces.

MATERIALS AND METHODS

Wood samples

Three 30-years-old radiata pine trees with a DBH of approximately 50 cm were felled in Kaingaroa forest in the Central North Island of New Zealand. From each tree a 1 metre long log was obtained from below breast height. Four 5 cm thick boards were sawn from each log symmetrically about the pith. The heartwood had an average moisture content of 39% and a basic density of 497 kg/m³ and the sapwood had a moisture content of 130% and a basic density of 473 kg/m³. The boards were cross-cut to a length of 60 cm and the ends were coated with paint, after which some of these green boards were pressure-steamed for 105 minutes at 122–126 °C and 140–150 kPa followed by 30 minutes at –80 kPa. All boards were then kiln dried using a conventional kiln schedule (dry bulb 70 °C / wet bulb 60 °C for 7 days, followed by 4 hours of reconditioning at 100/100 °C). From each dry board, end matched wood samples (4 x 4 x 10 cm long) were prepared from sapwood and heartwood so that the annual rings were either parallel or perpendicular to the surfaces. These samples were then stored in a controlled environment room to maintain 12% moisture content.

Impregnation

To study the direction of liquid uptake, the surfaces of some of the above samples were selectively sealed with silicone. These treatments included sealing all surfaces except the radial faces (tangential uptake) and all surfaces except the transverse ends (longitudinal uptake). Other treatments included sealing all surfaces except the tangential faces (radial uptake), the outer tangential surface (radial uptake from the direction of the bark) and the inner tangential surface (radial uptake from the direction of the pith).

A vacuum treatment method was used because high pressure treatments may be capable of causing changes in the wood structure, such as the rupturing of bordered pit membranes (McQuire 1970). For heartwood, impregnation involved immersion in the liquid for 60 minutes at –80 kPa and then 40 minutes at atmospheric pressure. For sapwood, the periods were 30 and 20 minutes respectively. As a result of a preliminary experiment, the rupturing of pit membranes by the vacuum treatment at –80 kPa was not confirmed. The solutions used for impregnation were de-aerated, double distilled water (ddH₂O), 0.5% toluidine blue in ddH₂O, 0.01% fluorescein (C₂₀H₁₂O₅) in ddH₂O and a mixture of 0.01% fluorescein and 0.5% toluidine blue in ddH₂O. Uptake (g/cm³) was calculated by dividing the deference in weight before and after impregnation by the specimen volume.
Microscopy

The impregnated samples were sliced in 1 cm long specimens and then divided into sixteen after dye impregnation and drying. The blocks (1 x 1 x 1 cm) were examined by conventional light microscopy, and by confocal microscopy using a Leica TCN/NT laser scanning confocal microscope. Transverse, tangential and radial sections 40–70 μm thick were prepared from the dry blocks using a sledge microtome. Sections were infiltrated with immersion oil and mounted on a microscope slide with a coverslip. Sections were observed by confocal microscopy using oil immersion lenses. A series of 16 optical sections acquired at 1024 x 1024 pixel resolution with eight sequential frame averages, were recorded using a Krypton/Argon mixed gas laser with excitation wavelengths at 488, 568 and 647 nm and fluorescence at 530, 600 and 665 nm. Unstained areas of wood were observed by natural autofluorescence of the lignified cell walls (Kutschka & McOrmond 1972).

A projection image was created from either a two- or a three-colour overlay image and was saved as a .tif file (24-bit red, green blue format). Subsequent image processing was carried out using Micrografx Picture Publisher software. Each image was subjected to a series of filters including gaussian blurring followed by a sharpening filter to reduce speckling in the image, and an image scale was added. Images were combined into a montage and printed on a Kodak dye sublimation printer.

RESULTS AND DISCUSSION

Uptake of liquid by sapwood and heartwood

Liquid uptake by sapwood averaged 0.571 g/cm³ and was not significantly increased by pre-steaming of the green wood. The moisture content of the treated sapwood was 130.8 ± 3.7% (SE: Standard Error) which was almost the same as the green moisture content (130.3 ± 2.2%). Pre-steaming of the heartwood increased liquid uptake from 0.113 ± 0.013 g/cm³ to 0.438 ± 0.009 g/cm³. Liquid uptake by the pre-steamed heartwood was therefore about 77% of the uptake by the sapwood. This result is not surprising because pre-steaming of green wood is a common industry practice used to improve treatability of heartwood. Having determined the liquid uptake levels for the sapwood and heartwood with and without pre-steaming, liquid flow patterns were studied with further impregnation studies combining coloured and fluorescein dyes and selective sealing of the surfaces of the wood samples.

Uptake in samples with sealed surfaces

Uptake by sapwood occurred mainly through the transverse and tangential surfaces (Fig. 1). More than 90% of the uptake by unsealed sapwood samples was achieved when only the tangential faces were unsealed and when only the transverse faces were unsealed. This suggests that the primary flow paths in sapwood are in the longitudinal and radial directions. Penetration of liquid in the radial direction was also found to be greater in the direction from the bark than in the direction from the pith. On the other hand, uptake from a radial surface was only 21% of the uptake from unsealed samples, suggesting that tangential flow pathways in sapwood are comparatively minor.
Fig. 1. Liquid uptake by radiata pine sapwood using vacuum treatment. Selected surfaces were sealed with liquefied silicone and the uptake was compared to samples with unsealed surfaces. Specimens were prepared with only the transverse surfaces unsealed (L), tangential surfaces unsealed (R), the outer tangential surface unsealed (Rp), the inner tangential surface unsealed (Rb) or a radial surface unsealed (T). L, R, Rp, Rb and T refer to the direction of liquid flow through the wood.

Fig. 2. Liquid uptake by radiata pine heartwood using vacuum treatment. – a: Samples without pre-steaming before drying. – b: Samples with pre-steaming at 122–126 °C for 105 minutes and 140–150 kPa followed by 30 minutes at −80 kPa. Selected surfaces were sealed with liquefied silicone and the uptake was compared to samples with unsealed surfaces. Specimens were prepared with only transverse surfaces unsealed (L), tangential surfaces unsealed (R), or the radial surfaces unsealed (T). L, R, and T refer to the direction of flow through the wood.
Uptake by heartwood without pre-steaming was also mainly from the transverse and tangential surfaces (Fig. 2a). Compared to samples with unsealed surfaces, about 50% of the uptake was achieved from the tangential surfaces alone or the transverse surfaces alone. Like the sapwood samples, no more than 30% of the uptake for unsealed samples was achieved from radial surfaces.

Heartwood samples with increased uptake due to pre-steaming also had the greatest uptake from tangential and transverse surfaces (Fig. 2b). Compared to samples with unsealed surfaces, more than 80% of the uptake was achieved when only the tangential surfaces were unsealed and when only the transverse surfaces were unsealed. However, the uptake from radial surfaces of pre-steamed heartwood was also high (70% of uptake for unsealed pre-steamed heartwood samples). The pre-steaming of heartwood increased penetration from all surfaces, but the increase was especially great for the radial surfaces, suggesting that pre-steaming of heartwood resulted in changes to the tangential liquid flow pathways.

**Observation of flow paths in sapwood**

When sapwood samples were impregnated with toluidine blue, most axial and radial resin canals and rays were stained clearly. Some tracheids in the earlywood and the latewood were also stained, indicating some conduction not only from rays to tracheids via half-bordered pit pairs, but also between adjacent tracheids via bordered pits. The resin canal results agree with those of Booker (1990), who claimed that resin canals are the primary conductive pathway into radiata pine sapwood. In this study, we found that the uptake from tangential surfaces was essentially the same as the uptake from transverse surfaces (Fig. 1). This suggests that radial resin canals are equally conductive as axial resin canals, although the number of axial and radial resin canals at the surface of a wood sample will necessarily depend on the shape and particularly the length of the specimen. Booker (1990) proposed that uptake in a full length board occurs via radial resin canals that then interconnect with axial resin canals. Bosshard and Hug (1980) confirmed the existence of anastomoses between axial and radial resin canals in *Pinus sylvestris*, which makes it probable that resin canals will also form the main pathways to impregnation of the wood of *Pinus sylvestris* and other *Pinus* species. In this study, we also confirmed conduction by uniseriate rays as well as the fusiform rays of radiata pine sapwood.

Most of the conductive tracheids were located adjacent to rays. However, the observed blue dye in some earlywood tracheids is also an indication that some tangential flow is possible without high pressure. According to Booker (1990), about two thirds of bordered pits are aspirated in dry radiata pine sapwood and liquid flow between earlywood tracheids is difficult without high pressure treatment. High pressure treatment processes may be able to rupture bordered pit membranes and a vacuum treatment process used in this study avoided this. Liquid flow into earlywood tracheids could have been via bordered pits that were not aspirated or it could have been that the bordered pits were only loosely aspirated and therefore able to conduct.
Observation of flow paths in heartwood

When heartwood that had not been pre-steamed was impregnated with toluidine blue, the uptake was very low. In the lowest cases, toluidine blue was seen only on the surfaces. Typically, toluidine blue was also observed in some of the resin canals. The dye was occasionally present in rays and the tracheids surrounding rays and resin canals. In contrast, the distribution of toluidine blue in pre-steamed heartwood samples was similar to that in sapwood.

The flow pathways in heartwood were also studied using fluorescein and confocal microscopy. These results were consistent with the observations using toluidine blue; however, the combination of fluorescein and confocal microscopy was found to be a particularly effective way of visualising flow patterns. First, it was possible to examine thick sections, which avoided microtome damage at the section surface. Second, examination of dry wood also minimised the possibility of dye redistribution. Third, the fluorescein could be clearly observed at varying depths by using the confocal microscope, which made it possible to study the distribution of dye through several cell layers.

Figure 3a–f are images obtained by confocal microscopy of fluorescein in radiata pine heartwood. Figure 3a shows fluorescein in ray parenchyma cells of heartwood that had not been pre-steamed. However, the dye was not present in the ray tracheids. This suggests that without pre-steaming, liquid flow through rays is via ray parenchyma only, and that ray tracheids are usually non-conductive. Figure 3b is also of heartwood without pre-steaming and shows that although resin canals and ray parenchyma can be conductive, there is limited tangential penetration of tracheids.

Pre-steaming of heartwood resulted in greater penetration of dye into the resin canal network and ray parenchyma. This is likely to be due to removal or redistribution of resin (Matsumura et al. 1996). The fluorescein dye was also evident in the bordered pits between tracheids, indicating that when heartwood is steamed before drying, the conduction of liquid between tracheids increases (Fig. 3c–f). The upper part of Figure 3c shows blue dye in rays and tracheids and the bottom part shows fluorescein in the same wood section, indicating that there is little difference in the distribution of these dyes. Both dyes were visible in some bordered pits of tracheids in pre-steamed heartwood. Figure 3d and 3e are tangential and transverse sections that show fluorescein in tracheids. It is evident that the tracheids were conductive after pre-steaming. This is consistent with the increased uptake of liquid from the...
radial surfaces of pre-steamed heartwood (Fig. 2b). Figure 3f also shows evidence of conductive bordered pits, indicating that conduction between tracheids occurs via bordered pits damaged by pre-steaming. An arrow in Figure 3f points to a space between pit border and pit membrane produced by pre-steaming. This is probably due to physical damage or extraction of the materials that encrust heartwood pits. These results suggest that pre-steaming changes the condition of the bordered pits in radiata pine heartwood and that the increased treatability of pre-steamed heartwood is due, at least in part, to increased conduction through the bordered pits.

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REFERENCES


