THREE-DIMENSIONAL VISUALIZATION OF SPIRAL GRAIN AND COMPRESSION WOOD IN *PINUS RADIATA* IMAGED BY CIRCULAR POLARIZED LIGHT AND FLUORESCENCE

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Abstract. To visualize the development of spiral grain in young pine trees, a novel technique was developed that is based on tracking the orientation of axial resin canals. Serial transverse sections covering a stem length of more than 5 mm were imaged at high resolution with a professional flatbed scanner using circular-polarized transmitted light. ImageJ was used to fully align the section images and to identify resin canals. Canal locations were used to generate a three-dimensional (3D) view of resin canal organization, and thus spiral grain, using the plug-in 3D viewer in ImageJ. Imaging confirmed the rapid onset of spiral grain, with a near vertical grain adjacent to the pith reorienting to a strong left-handed spiral within the first year of growth. Although vertical trees had a symmetrical grain pattern, trees tilted to generate compression wood showed grain changes on their lower sides. Lignin autofluorescence from these sections was imaged with a stereo-fluorescence microscope, with the combination of blue excitation and the collection of green fluorescence highlighting compression wood. 3D reconstructions were made from overlays of the fluorescence and the processed resin canal images. There were fewer canals in the compression wood, and these appeared to be straighter than the twisted canals found elsewhere. This new method provides new insights to our understanding of the formation of spiral grain and compression wood and a possible link between their occurrences.

Keywords: Circular polarized light, compression wood, radiata pine, resin canals, spiral grain, 3D visualization.

INTRODUCTION

Spiral grain and compression wood are common in radiata pine trees, and these wood quality issues devalue the wood because of decreased strength of sawn timber (Cown et al 1991). These issues negatively affect surface smoothness (Sepúlveda 2001), which may downgrade the quality and cause the rejection of large proportions of sawn boards (Johansson et al 2001). Furthermore, most drying problems in *Pinus radiata* are related to twist, which depends on both diameter and spiral grain (Cown et al 1996). Tarvainen (2005) estimated the annual loss from distortion caused by spiral grain in dried timber in Europe to be nearly €1 billion per annum. Compression wood, however, contributes to the development of warping. The interrelationship between compression wood and spiral grain in radiata pine and other species has only rarely been investigated and is not understood. In this study, resin canals, which are formed from the

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same cambial initials as the tracheids and align with the grain (Bannan 1936), were used as a proxy to demonstrate grain changes.

Researchers have used various modern techniques to study the internal structure of wood. Impressive progress has been made in digital imaging and, in particular, in three-dimensional (3D) visualization and analysis of objects (Pirard 2012). These modern approaches include x-ray microtomography (Steppe et al 2004), confocal microscopy (Donaldson and Lausberg 1998), fast Fourier transformations and image analysis (Moëll and Fujita 2004), and magnetic resonance imaging (Müller et al 2001). These techniques, which are mainly nondestructive and noninvasive, have been useful for detecting cell elements in wood section images and for locating defects, knots, heartwood, and sapwood in logs and converted timber (Wei et al 2008, 2009). They have also been used for determining density (Fromm et al 2001) and for anatomical characterization (Trtik et al 2007; van den Bulcke et al 2009). Bucur (2003a, 2003b) provides an overview of existing imaging methods for investigations of wood structure. Various attempts have been made to measure compression wood on disk surfaces using image-processing techniques, based on the darker color of compression wood (Nyström and Kline 2000; Warensjö et al 2002; Pont et al 2007; Duncker and Spiecker 2009). Accurate detection of compression wood has not, however, been fully successful because of the gradation between normal, mild, and severe compression wood. Furthermore, these modern imaging methods have not yet been extensively applied to investigations of spiral grain.

To evaluate young radiata pine trees for spiral grain and compression wood, a quick, easy, reliable, and robust method was required. None of the existing techniques was either suitable or available for this study. Therefore, a new methodology based on image analysis was developed. This new imaging and 3D visualization technique provides a better tool to understand the grain orientation at various positions inside the stem and a possible link between spiral grain and compression wood.

MATERIALS AND METHODS

Sectioning and Imaging

One-year-old radiata pine stem segments (25 mm long and up to 10 mm in diameter) were cut from trees that were grown either vertically or had been tilted to 30° from vertical. The segments were preserved in formaldehyde acetic acid (10% [v/v] formaldehyde, 5% [v/v] acetic acid, and 50% [v/v] ethanol). Segments were washed in warm water (30°C, 15 min), and 60-μm-thick serial transverse sections were cut with a sliding microtome (HM400, Microm, Walldorf, Germany). These serial sections were transferred to a microscope slide and mounted in glycerol. A sequence of 72 or more serial sections corresponding to more than 5 mm of stem was cut. Setting section thickness to 60 μm allowed even sections to be cut across the complete stem from pith to bark. Imaging was completed with a professional flatbed scanner (Perfection V700, Epson, Suwa, Nagano, Japan) at a scan resolution of 2400 dpi and in 24-bit color. For scanning, nearly 20 slides (four sections per slide) at a time were placed between two sheets of quarter wave-retarder films (catalogue number NT27-344, Edmund Optics, Woodlands, Singapore) oriented at 90° to each other, with these placed between two sheets of linear polarizing film (catalogue number NT38-491, Edmund Optics) that were also oriented at 90° to each other and at 45° to the quarter wave-retarder films. These optics meant that the wood sections were imaged with circular polarized light (Arpin et al 2002; Higgins 2010; Thomas and Collings 2014; Fig 1a). Although these sheets were loose, optimal positions and angles were marked such that correct scanning positions could be rapidly reset. Lignin autofluorescence was imaged with a Leica DFC 310 FX digital camera connected to a stereo-fluorescence microscope (model MZ10F Fluo, Leica Microsystems, Heerbrugg, Switzerland), using blue excitation and observing green fluorescence.

Image Processing

To generate 3D reconstructions, the background in each serial section image was filled with

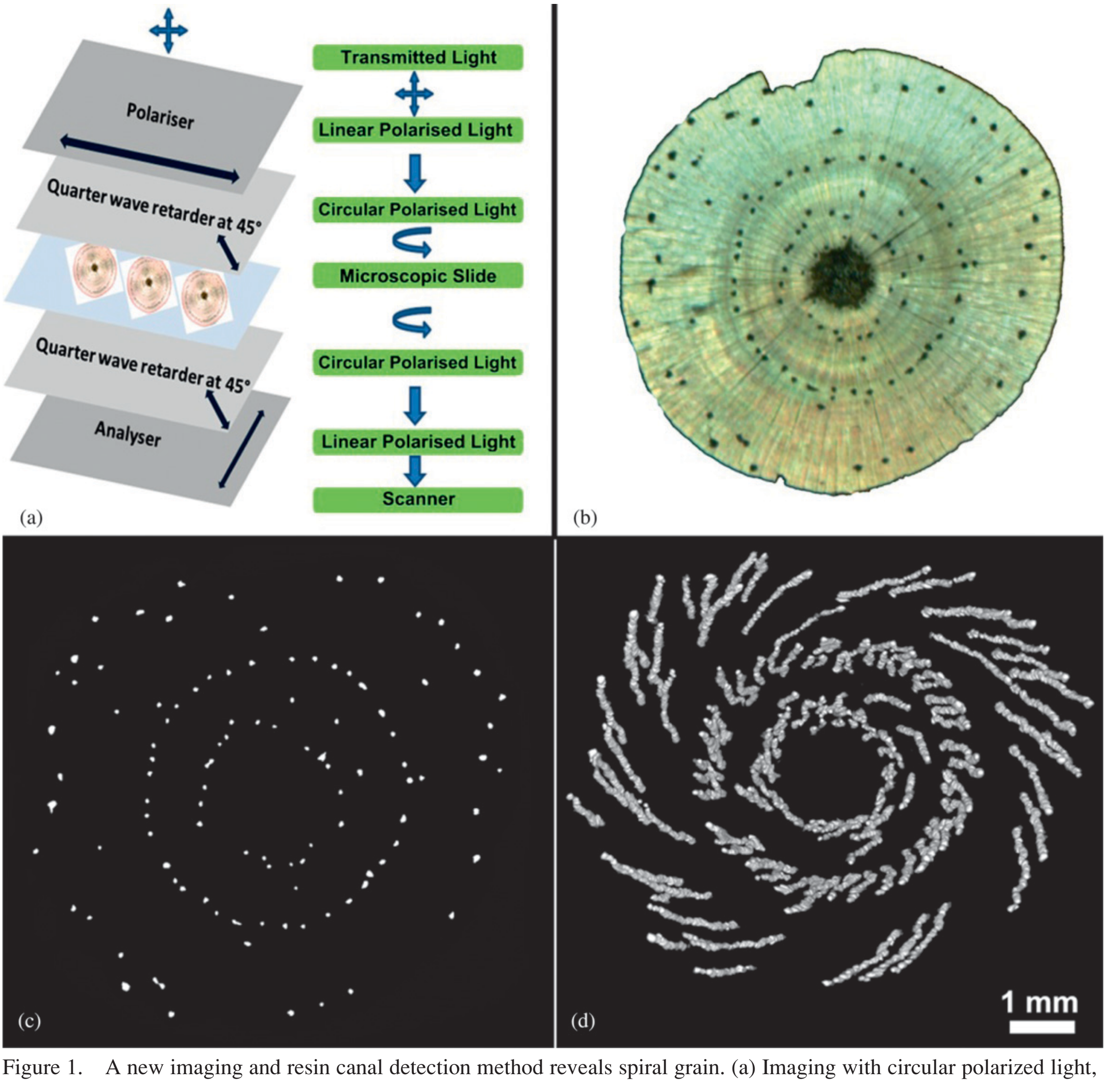

Figure 1. A new imaging and resin canal detection method reveals spiral grain. (a) Imaging with circular polarized light, (b) a transverse section imaged with circular polarized transmitted light, (c) resin canals (white dots) as detected with ImageJ, and (d) 3D visualization of resin canals and symmetric spiral grain in a vertical tree.

white (Fig 1b) and the images were aligned manually in Adobe Photoshop CS4 (version 11.0.1, Adobe Systems, San Jose, CA). Subsequent image processing was completed using ImageJ (version 1.46m, National Institutes of Health, Bethesda, MD). The stack of images of partially aligned serial sections was fully aligned with the plug-ins "StackReg" and a custom-written plug-in called "Cumulative Rotation." Uniform thresholding was applied, and the "Analyze particles" function was run to detect the resin canals with restrictions (size = 25-500 pixels, circularity = 0.5-1.0). This function recorded measurements for each canal including area, stack position, and the centroid (the location of the canal's center). An output file generated by the "Show Masks" option showed the location of the canals (Fig 1c) and was used to generate a 3D image of spiral grain using the plug-in "3D Viewer" (Fig 1d; Thomas and Collings 2014).

Another 3D image reconstruction was made to visualize both spiral grain from the scanned images and compression wood from stereo-fluorescence microscope images. The fluorescence microscope images had their background adjusted to black to enable uniform processing and were partially aligned with Adobe Photoshop (Fig 2a). The blue and green channels of these RGB color images were removed, and the brightness–contrast was adjusted such that only the compression wood parts were visible (Fig 2b). The images were then aligned using "StackReg" in ImageJ. Overlays of the fluorescence and scanned images of resin canals were made in Adobe Photoshop (Fig 2c), with subsequent image processing completed in ImageJ. An image stack of the overlays was made, and a 3D projection of this image stack was made to visualize compression wood and spiral grain using the "3D viewer" plug-in (Fig 2d; Thomas and Collings 2014). Side (Fig 2e) and bottom views (Fig 2f) of the 3D visualization were captured to show the organization of compression wood and spiral grain from a different angle.

RESULTS AND DISCUSSION

Imaging Stem Transverse Sections with a Flatbed Scanner

Our new imaging technique, using a combination of the polarizer and quarter wave-retarder film (Foster 1997; Arpin et al 2002; Higgins 2010), is a novel approach to create high-quality images suitable for image analysis (Thomas and Collings 2014). It is an easy and economic way of replicating polarized light microscopy and is particularly useful for imaging large areas that can be difficult with conventional microscopy. Circular polarized light has previously been suggested as a way to overcome image asymmetries such as the Maltese cross effect present with linear polarized light (Higgins 2010). However, the use of circular polarized light with automatic image analysis is novel, and this new imaging technique is simple to create, is convenient to operate, and provides better quality images. Also, this new method is quick and easy to operate and gives reproducible results.

3D Image Reconstructions and Analysis of Grain Orientation

3D reconstructions of the circular polarized light images revealed the organization of resin canals. In vertical trees, the canals were arranged in concentric bands around the pith with the canals nearest to the pith nearly straight and those in the outer regions twisted leftward (Fig 1d). Because the resin canals follow the tracheids in radiata pine, this twisting of the resin canals demonstrated the rapid and early onset of left-handed spiral grain in radiata pine trees, a pattern that is typical of pines (Fielding 1967; Harris 1989; Cown et al 1991). There were, however, fewer canals in the zones of compression wood formed in tilted trees (Fig 2c). This is consistent with Cown et al (2003), who observed few or no canals in radiata pine compression wood. Significantly, the canals formed outside the band of compression wood were straighter that the twisted canals in the opposite wood (Fig 2d).

The Unknown Link between Compression Wood and Spiral Grain

Fluorescence imaging demonstrated its capability to detect compression wood in wood cross section images. 3D reconstruction of serial sections collected from more than 5 mm revealed that the pattern of compression wood looked similar at different depths (Fig 2e). By combining circular polarized light scanning and stereo-fluorescence microscope images, links between compression wood and spiral grain were visible for the first time. The relatively straight orientation of the resin canals in compression wood areas suggested a possible link between the formation of compression wood and spiral grain development in radiata pine. Previously, researchers have looked at the incidence of compression wood and spiral grain separately and have made only very limited efforts to approach these issues. In this

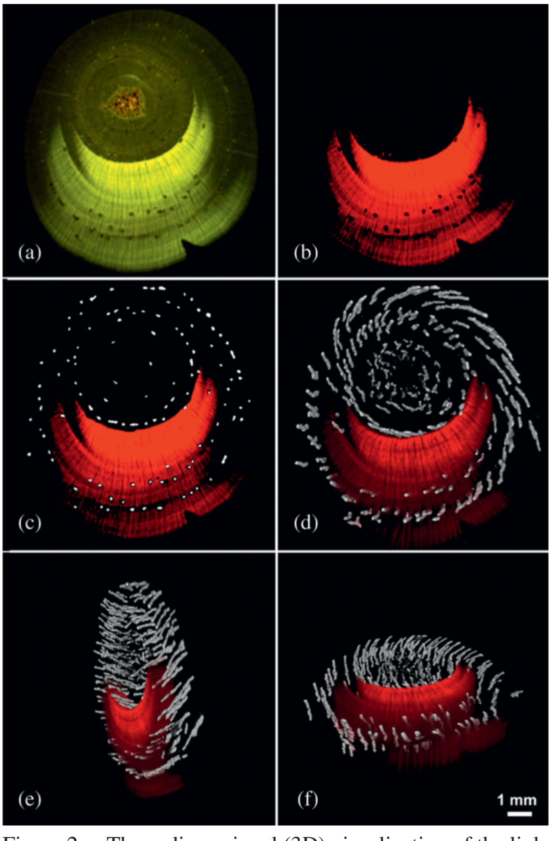

Figure 2. Three-dimensional (3D) visualization of the link between compression wood and spiral grain. (a) Fluorescence image of a transverse section from a tilted tree, with compression wood on the lower side, (b) compression wood segregated by thresholding, (c) overlay of the fluorescence image "b" and the corresponding scanner images in which resin canals were detected, (d) 3D visualization of compression wood and spiral grain, (e) side view of compression wood and spiral grain, and (f) bottom view of compression wood and spiral grain.

study, visualization of the internal structure of full cross sections of young pine tree stems was successfully made for the first time. The orientation of the resin canals has shown the grain deviation along the length of the stem, and hence, this 3D reconstruction technique gives a great opportunity to study the generation and progression of spiral grain and compression wood in wood. Also, this study is the first attempt to look at grain across an entire stem and to consider events at a cellular level.

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