OCCURRENCES OF MILD COMPRESSION WOOD IN *AGATHIS BORNEENSIS* AND *DACRYDIUM ELATUM*

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ABSTRACT

Studies on the compression wood in tropical gymnosperms are uncommon due to their limited distribution and over-exploitation. Microscopic examination of the heartwood of two tropical gymnosperms, *Agathis borneensis* (local name: bindang, damar minyak) and *Dacrydium elatum* (local name: sempilor) growing on higher elevations in Sarawak, Malaysia showed the occurrence of mild compression wood. Intercellular spaces were present in the compression wood of *A. borneensis*, but not in *D. elatum*. Rounded shapes of tracheids, typical of severe compression wood, were not observed in any of the samples examined. In *D. elatum* helical cavities were present, which corresponded in location to cell wall checks seen in cross-sectional views. The S₁ layer was relatively thick in both wood species but a distinct S₃ layer was observable only in the mild compression wood of *D. elatum*. Although the main feature of the mild compression wood tracheids of both wood species was greater lignification of the outer S₂ region, autofluorescence and KMnO₄ staining showed the fluorescence and staining intensity in the corner middle lamella in some cases to be much stronger than that in the outer part of S₂ layer.

Keywords: Tropical gymnosperms, helical cavities, intercellular spaces, cell wall ultrastructure, lignin concentration.

[In the online version of this paper Figure 1, 2, 3, and 6 are reproduced in colour.]

INTRODUCTION

Compression wood anatomy has mainly been investigated in conifers growing in the temperate zones where compression wood is known to occur in all coniferous species as well as *Ginkgo* (Timell 1978, 1986; Yoshizawa & Idei 1987). In contrast, studies on the wood anatomy of compression wood in the tropical gymnosperms are rare. This may be because native conifer species in many tropical countries are not easily available due to their restricted distribution, usually at cooler elevations (Lemmens *et al.* 1995), and over-exploitation of tropical gymnosperms, such as the genus *Agathis* (Whitmore 1977).

Comprehensive data and knowledge on the wood quality of tropical gymnosperms are lacking (Lim *et al.* 2003; Sharma & Altaner 2014). Macroscopic features of Malayan softwoods based on a 10x magnification lens have been described (Menon 1993). The IAWA Softwood List (IAWA Committee 2004) contains detailed information on the diagnostic wood anatomical features of *Agathis* and *Dacrydium*. *Agathis borneensis* and *Dacrydium elatum*, among the limited native gymnosperms of Sarawak, are highly fancied for decorative veneer and plywood manufacture because of straight grain and the very fine texture of their wood. The heartwood stakes of sempilor last between 2 and 5 years in-ground contact, while that of bindang cannot last more than 2 years under similar exposures (Wong & Ling 2009). The wood is also highly prized for interior finishing and paneling and for use in making decorative furniture in Malaysia (Menon 1986; Forest Department Sarawak 1999; Wong 2002). During the evaluation of the wood quality of tropical softwoods growing in Sarawak, Malaysia, we observed frequent occurrences of mild compression wood (MCW) under the microscope in these tropical gymnosperms.

The anatomical and chemical characteristics of compression wood and their influence on wood processing and utilization have been well documented (Timell 1986; Donaldson & Singh 2013). Compression wood can occur in a range of severity types from mild to severe. The major anatomical characteristics of severe compression wood (SCW) are: rounded cell shape, helical cavities in the S_2 layer, intercellular spaces, and high lignification in the outer part of S_2 layer. With their distinctive features, SCW is readily detected in transversely cut greenwood discs due to a darker color wood. However, MCW is not easily detected because cell forms and the color of wood are often similar to those of normal wood. MCW has been most commonly found in the juvenile wood of radiata pine (Harris 1977) and in the transition zone between normal wood and SCW (Nanayakkara *et al.* 2009). In MCW tracheids are slightly rounded to normal in appearance, and the presence of intercellular spaces is not a consistent feature. However, all forms of compression wood share the single most distinguishing feature which is greater lignification of the outer S_2 region; only the extent varies with the severity of compression wood. This characteristic (greater lignification of the outer S_2) alone serves as an important diagnostic feature for distinguishing compression wood from normal wood. Here we report the presence of mild compression wood in two tropical gymnosperms. We examined anatomical characteristics and lignin distribution of MCW of these wood species by confocal laser scanning microscopy (CLSM) and transmission electron microscopy (TEM).

MATERIALS AND METHODS

The heartwood wood samples of *Agathis borneensis* and *Dacrydium elatum* were obtained from the Sarawak Forestry Corporation (SFC), Kuching, Malaysia. Bindang logs were obtained from a sawmill in the Baram region, Sarawak. Sempilor trees were felled in Mount Pueh Permanent Forest near Lundu, Sarawak, located on the border between Sarawak and the Indonesian Province of West Kalimantan and about 100 km from Kuching City. Past Sarawak forest records showed that the genus *Agathis* is

gregarious on coastal kerangas terraces in West and North Sarawak and also abundant in submontane and montane forests above 700 m altitude, while the species *D. elatum* thrives in the submontane and montane kerangas forests above 850 m altitude in Central and North Sarawak (Anderson 1980). Small pieces of the wood, suspected to contain compression wood on the basis of stem excentricity from the fastest growing portion of excentric stem discs, were fixed in a solution of 2% paraformaldehyde and 2% glutaraldehyde (in 0.05M cacodylate buffer, pH 7.2). The samples were dehydrated in acetone and embedded in Spurr's resin (Spurr 1969). Semi-thin sections were observed with an optical microscope (Zeiss) after staining with toluidine blue. For lignin staining by acriflavine the sections were examined with a confocal laser scanning microscope (CLSM) (Leica, TCS SP5/AOBS) after staining with 0.0001% acriflavine for 10 min (Donaldson 2001). Confocal images were acquired using an argon laser with an excitation wavelength of 488 nm and an emission wavelength of 505–550 nm. Densitometric analyses of fluorescence intensities in the confocal images were done by line profile analysis using the software program Image-Pro Plus 7.0, Media Cybernetics, Bethesda, MD, USA. Ultrathin sections cut on an ultramicrotome using a diamond knife, were stained with 1% KMnO_4 (prepared in 0.1% sodium citrate), to

Figure 1. Light micrographs of *Dacrydium elatum* (A, B) and *Agathis borneensis* (C). Note that *Dacrydium* does not display distinct growth rings (A) and tracheids appear rounded in shape (B). Stained with safranin. — Scale bars = 200 μm .

contrast lignin in wood cell walls (Bland *et al.* 1971; Mauer & Fengel 1990). Stained sections were examined with a JEM-1400 transmission electron microscope (TEM). Some samples were also examined with a scanning electron microscope (SEM: Hitachi S-2400) after gold coating.

RESULTS AND DISCUSSION

The eccentricity and darker color of the secondary xylem in transversely cut discs, typical features of SCW, were difficult to find in the wood of both tropical gymnosperms. Under light microscopy *Dacrydium elatum* did not display distinct growth rings (Fig. 1A). The morphological appearance of tracheids in *D. elatum* was relatively homogeneous and axial parenchyma was filled with extractives. Interestingly, in *D. elatum* two distinct types of tracheids were present, one type being narrower in diameter than the other (Fig. 1B). In contrast, *Agathis borneensis* displayed recognizable growth rings and the tracheid lumens contained resinous materials (Fig. 1C). While it was not easy to differentiate MCW tracheids from normal tracheids under light microscopy (Fig. 1), CLSM clearly revealed the presence of MCW in both species (Fig. 2 & 3).

Figure 2. Confocal fluorescence micrographs of *Dacrydium* (A) and *Agathis* (B). Note the presence of mild compression wood in *Agathis* (arrows). — Scale bars = 50 μm .

Figure 3. Confocal fluorescence micrographs of mild compression wood in *Dacrydium elatum* (A) and *Agathis borneensis* (B). — Scale bars = 20 μm .

Figure 4. Helical cavities are sparse in the mild compression wood of *Dacrydium* spp. SEM (A) and TEM (B) micrographs (in the TEM view they appear as notches). Note the presence of checks in the S_2 layer in (A) (arrows), but less distinct in *Agathis borneensis* (C: arrows, likely cell wall checks). — Scale bars in A = 20 μm , in B = 10 μm , in C = 100 μm .

MCW tracheids of the two Sarawak gymnosperms differed somewhat in their microscopic features. Inter cellular spaces in cell corners were common in *A. borneensis*, but were rare in *D. elatum* (Fig. 2). Helical cell wall cavities in *D. elatum* corresponded in location to checks present in the S_2 layer (Fig. 4A). Their presence however was variable and they were absent in some tracheids (Fig. 4B). Helical cavities were not present in *A. borneensis* (Fig. 4C). Radial striations similar in appearance to those described by Singh *et al.* (1998) in radiata pine were found in the S_2 layer of MCW; they were particularly striking in *D. elatum* (Fig. 5A). TEM examination demonstrated the presence of a distinct, but extremely thin, S_3 layer in the MCW of *D. elatum* (Fig. 5B). The S_3 layer in the MCW of *A. borneensis* was less distinct (Fig. 5C). Previously, Singh *et al.* (2003) had confirmed the presence of an S_3 layer in the MCW of radiata pine. The S_1 layer in the MCW of both *A. borneensis* and *D. elatum* was relatively thick in proportion to the entire thickness of the secondary wall but appeared to be less lignified compared to the S_2 layer (Fig. 5A, B). The thickness of the S_1 layer in the MCW of *A. borneensis* and *D. elatum* ranged from 0.4 to 1.5 μm , covering about

Figure 5. A distinct, albeit extremely thin, S_3 layer (B: arrows) is present in the mild compression wood of *Dacrydium elatum* (A), but in *Agathis borneensis* this layer is not distinct (C). Note the presence of a relatively thick S_1 layer (arrows) in both wood species. Staining with KMnO_4 . — Scale bars in A & B = 2 μm , in C = 5 μm .

20% (range 15~30%) of the width of the secondary wall. Timell (1986) reported that the S₁ layer in the SCW was 25–30% of the width of the secondary wall compared to 10% in normal wood.

Combined use of CLSM and TEM, based on lignin staining by acriflavine and potassium permanganate, respectively, provided information on the lignin concentration in the secondary wall layers of MCW. Both TEM and CLSM images of *A. borneensis* and *D. elatum* MCW showed a greater concentration of lignin in the outer part of the S₂ layer, most prominently in the cell corner regions (Fig. 5, 6). Although the main feature of the MCW was increased lignification of the outer S₂ region, in some cases this layer was only marginally more lignified compared to the remainder of the S₂ wall. The fluorescence of the middle lamella (ML), particularly in cell corner regions, in MCW tracheids was generally stronger than the outer part of the S₂ layer (Fig. 6), indicating a higher concentration of lignin in the ML.

Figure 6. Confocal fluorescence micrograph (A) and the line scan (B) indicate variable lignin concentration across the S₂ layer in *Agathis borneensis*. Staining with acriflavine. ML: middle lamella; S2L: outer part of the S₂ layer. — Scale bar = 20 μ m.

Lignin distribution is one of the most intensely studied features of both SCW and MCW tracheids. Some workers reported that lignin concentration of compression wood was higher in the outer part of the secondary wall than in the middle lamella (Lange 1958; Donaldson *et al.* 1999; Singh & Donaldson 1999); however, others have indicated that most of the lignin in compression wood is located in the middle lamella (Casperson 1965; Côté *et al.* 1968; Fukazawa 1974). Wi *et al.* (2000) found some parts of the middle lamella in *Ginkgo* compression wood to display greater intensity of UV absorption than the outer part of S₂ layer. Considering that there is a continuum between normal and severe compression wood, with the moderate and mild forms present in between, this continuum reflects variability in lignin concentration across the cell wall, with the highest concentration of lignin present in the outer S₂ layer in SCW tracheids. Lignin concentration is only marginally higher (compared to the rest of the S₂ wall) in the mild forms of compression wood. Donaldson *et al.* (2004) found that in the S₂ layer of the tracheids of very mild forms of radiata pine compression wood lignin was

most highly concentrated in the cell corner regions. The present work shows the lignin concentration in the MCW to be variable depending on the degree and/or intensity of compression wood.

The present work showed the occurrence of MCW in the two tropical softwood species. However, factors inducing and regulating MCW in tropical gymnosperms remain to be investigated. Silvicultural practices, such as thinning and pruning leading to accelerated growth rates, have been shown to contribute to formation of large amounts of MCW in temperate zones (Cown & McConchie 1981, 1982). There is a need to extend the observations to tropical gymnosperms because the factors responsible for MCW formation in the tropics may not always be the same as in temperate zones. Understanding of hormonal regulation of MCW is important (Funada *et al.* 1990), and so is the effect of MCW on the wood quality (Xu *et al.* 2009). The relationship of the presence of cell wall checks and helical cavities in mild compression wood tracheids to cell wall aspects, such as extent of lignification of the outer S₂ wall, thickness of the S₂ layer and the presence/absence of an S₃ layer, would be an interesting topic for further exploration. In addition, the durability tests of MCW in *A. borneensis* and *D. elatum* against various wood decaying microorganisms will expand the horizon of our knowledge on compression wood, as marked differences in the degradation of the S₂ layer by bacteria have been observed for compression wood (Singh 1997; Kim & Singh 1999).

CONCLUSIONS

Two tropical gymnosperms, *Agathis borneensis* and *Dacrydium elatum*, growing in Sarawak, Malaysia, showed frequent occurrences of mild compression wood. The typical rounded shape of tracheids in SCW was not seen in the MCW of both wood species examined. Intercellular spaces were present only in the SCW of *A. borneensis*. Helical cavities corresponding to the location of cell wall checks were present in the MCW of *D. elatum* but not in *A. borneensis*. Greater lignification of the outer part of the S₂ layer was found in both cases. The corner middle lamella in some cases showed higher lignin concentration than the outer part of the S₂ layer.

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