Mokchae Konghak 34(2) : 68~77, 2006

Micromorphological and Chemical Characteristics of Cengal (*Neobalanocarpus heimii*) Heartwood Decayed by Soft Rot Fungi*1

Yoon Soo Kim*2†, Adya P. Singh*3, Andrew H. H. Wong*4, Tae-Jin Eom*5, and Kwang Ho Lee*2

ABSTRACT

The heartwood of cengal (*Neobalanocarpus heimii*) is known to have a high degree of decay resistance by virtue of its high extractive content. After 30 years in ground contact an utility pole of this tropical hardwood was found to be degraded only in the surface layers by cavity-forming soft rot fungi. The present work was undertaken 1) to characterize the degradation of cengal heartwood from the aspect of ultrastructure and chemistry and 2) to investigate the correlation between soft rot decay and its extractive microdistribution in wood tissues. The chemical analysis of cengal heartwood revealed the presence of a high amount of extractives as well as lignin. The wood contained a relatively high amount of condensed lignin and the guaiacyl units. Microscopic observations revealed that vessels, fibers and parenchyma cells (both ray and axial parenchyma) all contained extractives in their lumina, but in variable amounts. The lumina of fibers and most axial parenchyma were completely or almost completely filled with the extractives. TEM micrographs showed that cell walls were also impregnated with extractives and that pit membranes connecting parenchyma cells were well coated and impregnated with extractives. However, fungal hyphae were present in the extractive masses localized in cell lumina, and indications were that the extractives did not completely inhibit fungal growth. The extent of cell wall degradation varied with tissue types. The fibers appeared to be more susceptible to decay than vessels and parenchyma. Middle lamella was the only cell wall region which remained intact in all cell types which were severely degraded. The microscopic observations suggested a close correlation between extractive microdistribution and the pattern and extent of cell wall degradation. In addition to the toxicity to fungi, the physical constraint of the extractive material present in cengal heartwood cells is likely to have a profound effect on the growth and path of invasion of colonizing fungi, thus conferring protection to wood by restricting

*1 Received on May 31, 2005; accepted on September 20, 2005.
This work was supported by the grant from Regional Research Centers Program of Korea Research Foundation through Bio-Housing Research Institute at Chonnam National University.
*2 Department of Forest Products and Technology, Chonnam National University, Gwangju 500-757, Korea
*3 New Zealand Forest Research Institute Ltd. Private Bag 3020, Rotorua, New Zealand
*4 Faculty of Resource Science and Technology, Universiti Malaysia Sarawak (UNIMAS), 94300 Kota Samarahan, Sarawak, Malaysia
*5 Department of Wood Science and Technology, Kyungpook National University, Daegu, Korea
† Corresponding author : Yoon Soo Kim (kimys@chonnam.ac.kr)

fungal entry into cell walls. The presence of relatively high amount of condensed lignin is also likely to be a factor in the resistance of cengal heartwood to soft rot decay.

Keywords : cengal heartwood, *Neobalanocarpus heimii*, natural durability, soft rot, extractive microdistribution, microscopy

1. INTRODUCTION

Heartwoods of many tree species, particularly those growing in the tropics, are known to have a high degree of decay resistance by virtue of their high extractive content (Schultz and Nicholas, 2000). Analytical and bioassay studies have shown many extractive components of such woods to be toxic to wood degrading microorganisms, and therefore potential exists to harvest these components as future "wood preservatives" (Smith *et al.*, 1989).

The heartwood of the Malaysian timber cengal (*Neobalanocarpus heimii*) has high natural durability (Mohd. Dahlan and Tam, 1985), and Basidiomycete resistance property of its extractives has been ascertained (Yamamoto and Hong, 1988). However, cengal heartwood is not completely immune to fungal decay. Soft rot fungi, which belong to Ascomycetes and Deuteromycetes, can cause substantial decay of wood under conditions where competition with wood decaying Basiodiomycetes is not present or minimal. Soft rot fungi are widespread in nature, being tolerant to a wide range of moisture, pH and temperature (Daniel and Nilsson, 1998).

The cengal heartwood examined in this study was degraded only in the surface layers by soft rot fungi after 30 years in ground contact. The work was undertaken to understand why the cengal heartwood was so durable, with a focus to obtain information on the following features: 1) the content and microdistribution of extractives and 2) the type and content of lignin.

2. MATERIALS and METHODS

2.1. Cengal Wooden Pole Sampling

The ground line surface of a 30-yr-old square-sawn (120 × 120 mm cross-sectional area) utility pole of cengal (*Neobalanocarpus heimii*) located in the rural area in Kuala Selangor, Selangor, Malaysia, was found to be softened, with suspected premature failure, by the national telecommunication company (Telekom Malaysia Berhad). The pole was excavated and a 500 mm long wooden pole sample at the ground line position was probed for extent of biodegradation. The cross-section of the pole at this region revealed that the wood material was essentially heartwood, and that dark brown discoloration of surface degradation (suspecting fungal decay) on all sides of the wood extended 10~15 mm deep from the surface, accompanied with isolated occurrence of surface subterranean termite attacks on the discolored zone. The boundary between this discolored, degraded zone and the adjacent sound heartwood was rather abrupt.

2.2. Determination of Extractives, Lignin and Sugar Content

For the chemical analysis, well-known methods were applied to the woodmeal of cengal heartwood, such as extraction with ethanol-benzene, hot water and 1% NaOH. The wood meals of sound and decayed cengal heartwood were hydrolyzed with 3% H_2SO_4 and the alditol-acetate of neutral sugar was analyzed with gas

chromatography. Lignin content was determined by Klason method. The composition of lignin was characterized by the oxidation of lignin in an alkaline nitrobenzene solution (Chen, 1992). FT-IR spectra were taken with KBr pellets (1mg extractives-free wood powder in 300 mg KBr) using a Perkin Elmar 282 IR-spectrophotometer.

2.3. Microscopy

Pieces of cengal heartwood obtained from sound (nondecayed) and decayed regions from the pole were sectioned with a sliding microtome, and were examined with a confocal laser scanning microscope for cellular distribution of the extractive material. For transmission electron microscopy (TEM), small pieces of wood taken from moderately and heavily decayed regions were dehydrated in acetone and embedded in Spurr's low viscosity resin (Spurr, 1969). Transverse ultrathin sections were cut with a ultramicrotome using a diamond knife. Sections were stained with 1% potassium permanganate (prepared in 0.1% sodium citrate) and then examined with a JEOL 1010 TEM.

3. RESULTS and DISCUSSION

Examination of hand-cut sections under polarization microscopy indicated presence of soft rot cavities in wood cell walls (not illustrated). No other form of microbial decay was present. However, termites were found associated with the degraded regions of the pole.

3.1. Chemical Characteristics

The chemical composition of sound and decayed cengal heartwood is summarized in Table 1. It is apparent that cengal heartwood contains high amounts of lignin and extractives. The percentage of extractive soluble in organic solvent (15%) is much greater than the percentage of water soluble extractives. In the degraded cengal wood, the values of hot water (16.2%) and 1% alkali (56.1%) soluble extractives are much greater as compared to sound wood, suggesting that a good proportion of low-molecular polysaccharides is degraded. In contrast, the amount of organic solvent soluble extractives decreased in decayed cengal wood. The analysis with thin layer chromatography showed that some spots which were present in the organic solvent-extractives of sound wood were absent in the decayed wood (figures not shown). Chemical analysis indicated some extractives (mainly phenolics) were degraded over a long period of exposure of the pole in ground contact. In comparison with sound wood, the decayed cengal wood contains higher amount of lignin and less holocellulose. Only about half of the total polysaccharides remains in the highly degraded cengal heartwood (Table 1), reflecting the extent of polysaccharide losses due to degradation.

The lignin content of sound (34.5%) (Table 1) cengal heartwood is much higher than that of softwoods growing in temperate zones and also many hardwoods. The structural information obtained on the lignin of sound cengal heartwood from the nitrobenzene oxidation products (Table

Table 1. Chemical composition of sound and decayed cengal heartwood (%)

	Hot water Extractives	1% alkaline extractives	Ethanol/benzene extractives	Holocellulose	Lignin	Ash
Sound	3.2	22.3	15.0	61.8	34.5	2.1
Decayed	16.2	56.1	9.7	38.2	67.5	4.4

Table 2. Lignin monomer structures of sound and decayed cengal heartwood (%)

	p-Hydroxybenzaldehyde	Vanillin	Syringaldehyde	Total
Sound	1.5	7.0	12.1	20.6
Decayed	0.2	2.1	3.7	6.0

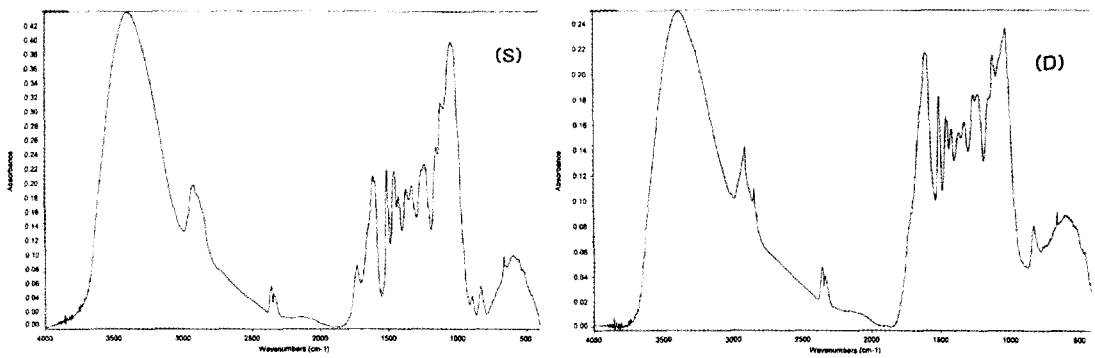

Fig. 1. FT-IR spectra of sound (S) and decayed (D) cengal heartwood.

2) provides evidence of a much lower content of p-hydroxylbenzaldehydes in this wood as compared to the majority of hardwoods growing in temperate zones. Lower amount of phenolic aldehydes indicates that cengal heartwood contains a high proportion of condensed lignin. The alkaline nitrobenzene oxidation gave a ratio of vanillin to syringaldehyde 1:1.7, suggesting that cengal heartwood has a relatively high amount of guaiacyl lignin as compared to most temperate hardwoods. The reaction products of nitrobenzene oxidation showed that lignin in cengal heartwood has more condensed structure and more guaiacyl lignin than that in the hardwoods growing in temperate zones.

Chemical differences between sound and decayed woods are illustrated in the FT-IR spectra. The most notable differences in the IR spectra between the sound and the decayed cengal heartwood samples are: 1) A total loss of absorption band 1730 cm^{-1}. This might be due to hemicellulose degradation (Kuo *et al.*, 1988). 2) Increased absorbance at 1620, 1420, 1270 and 1130 cm^{-1} due to lignin. A significant increase in the absorption around 1620 cm^{-1} in the decayed cengal wood reflects a high lignin and phenolics content of this wood. 3) Decreased absorbance at 1060, 1040 and 890 cm^{-1} in degraded cengal wood might be due to the degradation of cellulose. Additionally, X-ray diffractograms indicated a loss in cellulose crystallinity in the degraded wood (figures not shown), suggesting that even cellulose crystallinity suffers from soft rot attack.

3.2. Anatomical and Micromorphological Characteristics

The patterns of extractive microdistribution in degraded and sound cengal heartwood are shown in Figs. 2 and 3. The bulk of extractives are associated with rays and axial parenchyma cells. Rays are almost completely filled with extractives. Although the amount of extractives in axial parenchyma cells appears to be variable, these cells also contain large masses of extractives (Fig. 2). The lumina of a large proportion of parenchyma cells are completely filled with ex-

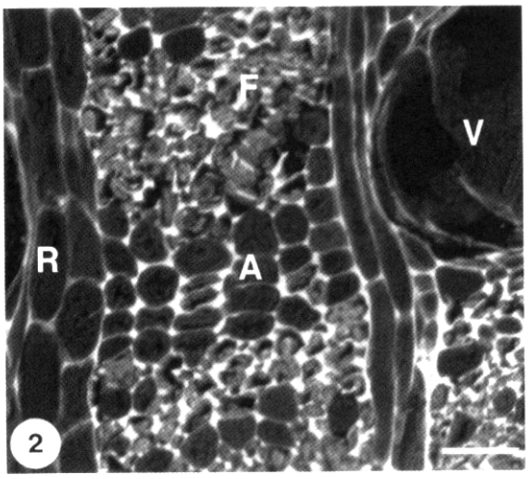

Fig. 2. Transverse section of decayed heartwood showing the distribution of extractives and the degradation in various tissues. Ray cells are almost completely filled with extractives. Extractives are also widely present axial parenchyma cells. Fiber cell walls are heavily degraded. V, vessel; R, ray; F, fibers and A, axial parenchyma. Confocal micrograph. Bar = 50 μm.

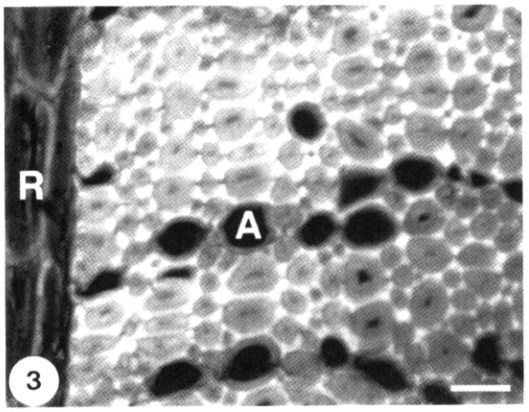

Fig. 3. Transverse section of sound heartwood fiber cells. Fiber lumens are variously filled with extractives. Extractives appear to be present in the walls of all tissues. Confocal micrograph. Bar = 25 μm.

tractives, but in some cells the lumina are only partially filled. Extractives are also widely present in the lumina of fibers (Fig. 3). The fibre walls are impregnated with extractives, although their distribution in the cell walls appears patchy (Fig. 3). In vessels, extractives appear to be mainly associated with cell walls, including the walls of tyloses where present.

In order to obtain the information on the pattern of extractive microdistribution at cellular and subcellular levels in relation to the extent and pattern of degradation of various tissues, sections obtained from heartwood regions varying in the extent of degradation were observed under TEM. In the present work, we focused mainly on sections taken through moderately degraded regions, which provided useful comparisons of the differences among cell types with regard to their resistance/susceptibility to degradation.

Fig. 3 shows that vessel and parenchyma cells are intact, while the cell wall of fiber is heavily degraded, leaving only the middle lamella. Several fungal hyphae are present in the lumina of parenchyma cells completely filled with extractives. Middle lamella and primary pit-field regions are very dense; the secondary wall is less dense and also inhomogeneous, some parts appearing denser than others. In vessel, the warty layer appears to be heavily impregnated with extractives. The warty layer has separated from the secondary wall. The vessel wall also appears to have a high concentration of extractives, and appears striated. Fig. 4 also shows largely intact vessel and parenchyma cell walls. However, localized degradation has occurred only in small parts of the double cell wall, where fungal hyphae are traversing this common wall between adjoining parenchyma cells. Fungal hyphae are also present in the extractive masses, which fill the lumina of parenchyma cells, with a preference for the peripheral regions.

Fig. 5 shows parenchyma-fiber and fiber-fiber entry by fungal hyphae, resulting from direct

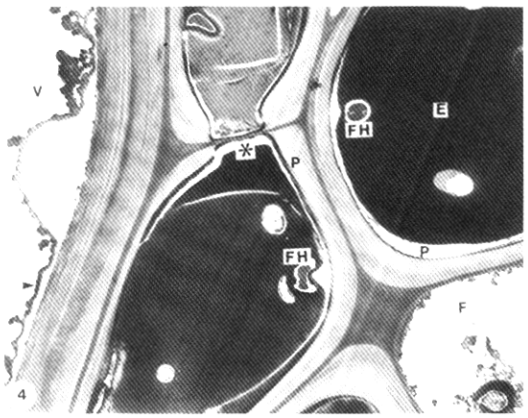

Fig. 4. Transverse section through a degraded region of heartwood. Fiber (F) is heavily degraded but parenchyma (P) and vessel (V) walls are intact. The lumens of parenchyma cells are completely filled with extractives (E), which appear as a dense, compact mass, and contains fungal hyphae (FH). Vessel and parenchyma cell walls appear to be impregnated with extractives, and the extractive material is closely applied to parenchyma cell walls, including that of the primary pit-field region (asterisk). The thin warty layer, which has detached from the vessel wall, appears very dense (arrowhead). TEM micrograph.

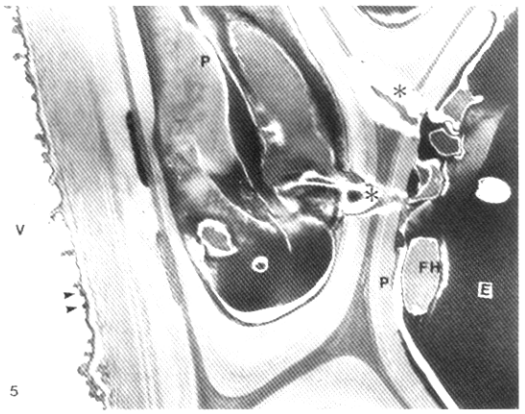

Fig. 5. Transverse section through a degraded region of heartwood. Vessel (V) wall is intact, and parenchyma cell walls show localized degradation. Extractives completely fill the lumens of parenchyma cells and are also present in the walls of these cells and vessels. A thin, dense warty layer present in the vessel is closely applied to the secondary wall (arrowheads). Fungal hyphae (FH) are present throughout the extractive masses (E) contained in the lumens of parenchyma cells (P) and also traverse the common walls between these cells (asterisks). TEM micrograph.

penetration of the shared walls. Much of the secondary walls in fibers is degraded, and the coarse granular material which remains is most likely lignin residue. The middle lamella is not degraded, and so is the innermost part of the secondary wall, which surrounds extractive-filled lumina. Parenchyma cell walls are largely intact, although extractive masses have separated from the wall, and thus no longer form a tight seal with it, but this may be a fracture artifact.

Advanced decay is shown in Fig. 6. The walls of both fibers and parenchyma are heavily degraded. In the parenchyma cell, fungal hyphae are present in the extractive masses which fill the cell lumen, being localized mainly in the peripheral regions near the degraded cell wall.

Fig. 7 also shows an advanced stage of decay. The secondary walls of both fibers and parenchyma are completely degraded, although the lumina of parenchyma cells are completely filled with extractives. Only the middle lamella remains intact. Fungal hyphae are mainly located in peripheral regions of the extractive masses present in cell lumina. Fig. 8 shows a region containing heavily degraded fibers. Secondary walls have largely disappeared except in cell corners, where the S_1 layer remains. However, in one of the cell corners even this wall layer is no longer present, and only middle lamella remains intact. The circular bodies which are present throughout the degraded regions of the fiber secondary wall are reminiscent of soft rot cavities.

The information presented here using a range of chemical and microscopic techniques suggests

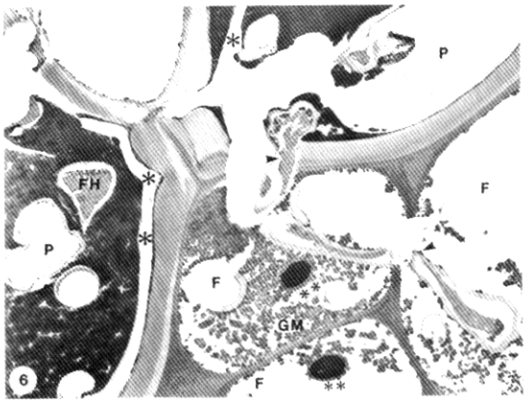

Fig. 6. Transverse section through a degraded region of heartwood. The arrowheads indicate direct hyphal penetration of common walls between a parenchyma cell (P) and a fiber (F) and between two fibers. Much of the secondary wall in fibers is degraded; the remaining coarse granular material (GM) is most probably lignin residue. The middle lamella is not degraded and so is the innermost part of the secondary wall around the extractive-filled lumens (paired asterisks). Parenchyma cell walls are largely intact, although fungal hyphae (FH) colonize the extractive masses present within the cell lumen. Asterisks indicate cracks within the extractive masses. TEM micrograph.

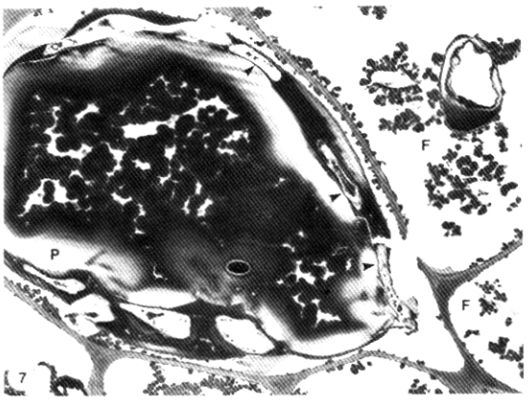

Fig. 7. Transverse section through a degraded region of heartwood. Fungal hyphae (arrowheads) are present within peripheral regions of the extractive mass which fills the lumen of a parenchyma cell (P). The secondary walls of both parenchyma and fibers (F) are heavily degraded. TEM micrograph.

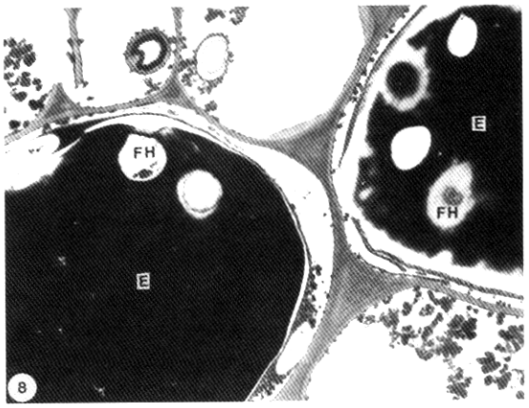

Fig. 8. Transverse section through a degraded region of heartwood. Although parenchyma cell lumens contain abundant extractives (E), the secondary walls of these cells are heavily degraded. FH, fungal hyphae. TEM micrograph.

that a combination of factors, including high content of extractives and lignin, lignin structure, and the pattern of extractive microdistribution, may have contributed to the long service life of cengal heartwood pole, where only surface decay due to soft rot was detected. It is well known that woods which contain high amounts of extractives and lignin have high natural durability (Singh *et al.*, 1987; Nilsson *et al.*, 1988, 1992; Schultz *et al.*, 1995; Taylor *et al.*, 2002). Considerable information is also available on the effect of lignin concentration in various regions of wood cell walls and their resistance/susceptibility to microbial decay (Kim and Singh, 2000; Singh *et al.*, 2003). However, much less is known about the relationship between microdistribution of extractives in wood cells and their biodegradation, and therefore the discussion will be focused mainly on this aspect.

It is evident from the microscopic observations that fibers were least resistant to soft rot decay.

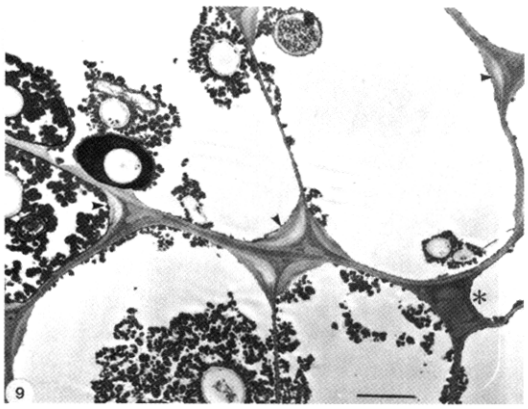

Fig. 9. Transverse section through a degraded region of heartwood containing fibers. The secondary walls of fibers are heavily degraded, except cell corner regions of the S_1 layer, which appear intact (arrowheads). However, in one cell corner even this wall is absent (asterisk). Middle lamella is not degraded. TEM micrograph. Bar = 2 μm for Figs. 3~8.

Parenchyma cells showed remarkably high degree of resistance and vessels appeared to be most resistant. Judging by the observed pattern of extractive microdistribution, it is clear that cellular location of extractives had an important influence on the decay characteristics of various cengal heartwood tissues.

It is apparent that cengal heartwood parenchyma cells, which were highly resistant to soft rot decay, contained abundant extractives. Although fungal hyphae were present in the extractive masses filling the cell lumina, fungal growth is likely to have greatly slowed down due to the physical blockage of the cell lumina by the extractive masses and the fungistatic effect of extractives. Phenolic type extractives are among the most potent chemicals which inhibit fungal growth (Aloui *et al.*, 2004) and the chemical analysis showed that they were abundant in cengal heartwood.

Extractive impregnation of the cell walls of parenchyma and vessels, including the membranes of primary pit-field regions and the warty layer in vessels, may also be an important factor restricting cell wall degradation and also intercellular movement of fungal hyphae. Pit membranes are the cell wall regions most susceptible to fungal decay, enabling hyphae to readily grow and multiply within wood tissues. However, in the parenchyma of cengal heartwood, the membrane of pit-field regions appeared to be highly resistant to invasion by fungal hyphae because these cell wall regions were completely covered with extractives and were also impregnated with this material.

The distribution of extractives in fiber lumina was more variable as compared to parenchyma cells. The lumina of the majority of fibers were incompletely filled with extractives; only small proportions were completely filled, containing compact masses of extractives. This would in part explain the greater susceptibility of fibers to soft rot as compared to parenchyma cells. Also, fiber walls appeared to be less heavily impregnated with extractives as compared to parenchyma cells and vessels, judging by their higher natural color, and fluorescence under the confocal microscope. The type and amount of lignin present in fiber walls may be another factor, although this was not determined at the cell wall level in the present work.

A discussion of the relationship of total extractive and lignin content of cengal heartwood to the high durability of this wood in ground contact is also relevant. The analytical data (Table 1 and 2) indicate that cengal heartwood is highly rich in extractives, phenolic extractives being the likely major component. Additionally, the lignin content of cengal wood (Table 2) appears to be among the highest recorded for hardwood species (Fengal and Wegener, 1984), particularly among tropical hardwoods (Wong, 1993; Nilsson *et al.*, 1988). Lignin structure of cengal heartwood would also be an important

factor in the high durability of this tropical hardwood against various wood decaying microorganisms. It is well known that both the amount and type of lignin and extractives in wood are important factors in relation to biodegradation of wood (Eriksson *et al.*, 1990), and the work presented here shows that cengal heartwood is rich in both lignin and extractives containing high proportions of the type of lignin (condensed) and extractives (phenolic type) which are known to be highly resistant to degradation by wood decay organisms.

Natural durable wood species are characteristically rich both in lignin and extractives, and the work presented here on cengal heartwood in addition to confirming this provides additional new information on the characteristics of lignin and extractives present in cengal heartwood and the microdistribution of these components at cellular level, which forms the basis for understanding why such timbers are naturally durable. The presence of surface decay in cengal heartwood by soft rot after several years in ground contact was not surprising. The cengal heartwood surface at the ground line would have been susceptible to prolonged leaching of water-soluble extractives which may be one reason for the presence of soft rot attack. However, sufficiently high amounts of extractives would still have remained in the wood, as the TEM observations indicate, restricting decay to surface layers. Soft rot fungi can adapt to a wide range of environmental conditions, high degree of tolerance to copper compounds and other toxic preservatives (Daniel and Nilsson, 1998). Nevertheless, slow rate of degradation suggests that cengal heartwood have high resistance also to soft rot for the reasons discussed above.

In conclusion, the work using a range of chemical and microscopic techniques presented here provides evidence that in addition to the amount and toxicity of heartwood extractives, the pattern of their cellular distribution, both within lumina and cell walls (Kuo and Arganbright, 1980; Streit and Fengel, 1994; Kleist and Schmitt, 1999; Kleist and Bauch, 2001), is an important factor in relation to microbial decay of wood. A combination of factors, including high extractive content, the presence of proportionally higher phenolic extractives, the microdistribution of extractives, the high lignin content, and the presence of substantial amount of condensed lignin, is likely to have contributed to the observed high decay resistance of cengal heartwood.

REFERENCES

1. Aloui, F., N. Ayadi, F. Charrier, and B. Charier. 2004. Durability of European oak (*Quercus petraea and Quercus robur*) against white rot fungi (*Coriolus versicolor*): relations with phenol extractives. Holz Roh- und Werkstoff 62: 286~290.
2. Chen, C.-L. 1992. Nitrobenzene and cupric oxide oxidations, In: Lin, S. Y. and Dence, C. W. (Eds) Methods in Lignin Chemistry. Springer-Verlag, Berlin.
3. Daniel, G. and T. Nilsson. 1998. Developments in the study of soft rot and bacterial decay. In: Bruce, A and Palfreyman, J.W. (Eds.) Forest Products Biotechnology. Taylor and Francis Ltd., London.
4. Eriksson, K.-E. L., R. A. Blanchette, and P. Ander. 1990. Microbial and Enzymatic Degradation of Wood and Wood Components. Springer-Verlag, Berlin.
5. Fengel, D. and G. Wegener. 1984. Wood: Chemistry, Ultrastructure, Reactions. Walter de Gruyter, Berlin.
6. Kim, Y. S. and A. P. Singh. 2000. Micromorphological characteristics of wood biodegradation in wet environments: a review. IAWA Journal 21: 135~155.
7. Kleist, G. and U. Schmitt. 1999. Evidence of accessory compounds in vessel walls of Sapelli heartwood (*Entrandrophragma cylindricum*) obtained by transmission electron microscopy. Holz Roh-

und Werkstoff 57: 93~95.

8. Kleist, G. and J. Bauch. 2001. Cellular UV microspectrophotometric investigation on Sapelli heartwood (*Entrandrophragma cylindricum* Sprague) from natural provenances in Africa. Holzforschung 55: 117~122.
9. Kuo, M. and D. F. Arganbright. 1980. Cellular distribution of extractives in redwood and incense cedar. Holzforschung 34: 17~22.
10. Kuo, M. I., J. F. McClelland, S. Luo, P.-L. Chien, R. D. Walker, and C.-Y. Hse. 1988. Applications of infrared photoacoustic spectroscopy for wood samples. Wood Fiber Science 20: 132~145.
11. Mohd. Dahlan J. and M. K. Tam. 1985. Natural durability of some Malaysian timbers by stake tests. Malaysian Forester 48: 154~159.
12. Nilsson, T., J. R. Obst, and G. Daniel. 1988. The possible significance of the lignin content and lignin type on the performance of CCA-treated timber in ground contact. International Research Group on Wood Preservation, Document IRG/WP/1357.
13. Nilsson, T., A. P. Singh, and G. Daniel. 1992. Ultrastructure of the attack of *Eusideroxylon zwageri* wood by tunneling bacteria. Holzforschung 46: 361~367.
14. Schultz, T. P., W. B. Harms, T. H. Fisher, K. D. Mcmurtrey, J. Minn, and D. D. Nicholas. 1995. Durability of an angiosperm heartwood: the importance of extractives. Holzforschung 49: 29~34.
15. Schultz, T. P. and D. D. Nicholas. 2000. Naturally durable heartwood: evidence for a proposed dual defensive function of the extractives. Phytochemistry 54: 47~52.
16. Singh, A. P., T. Nilsson, and G. Daniel. 1987. Ultrastructure of the attack of wood of two high lignin tropical hardwood species, *Alstonia scholaris and Homalium foetidum*, by tunneling bacteria. Journal of Institute of Wood Science 11: 26~42.
17. Singh, A. P., Y. S. Kim, S. G. Wi, K. H. Lee, and I. J. Kim. 2003. Evidence of the degradation of middle lamella in a waterlogged archaeological wood. Holzforschung 57: 115~119.
18. Smith, A. L., C. L. Campbell, D. B. Walker, and J. W. Hanover. 1989. Extracts from black locust as wood preservatives: extraction of decay resistance from black locust heartwood. Holzforschung 43: 293~296.
19. Spurr, A. R. 1969. A low viscosity embedding medium for electron microscopy. Journal of Ultrastructure Research 26: 31~43.
20. Streit, W. and D. Fengel. 1994. Heartwood formation in *Quebracho colorado*: Tannin distribution and penetration of extractives into the cell walls. Holzforschung 48: 361~367.
21. Taylor, A. M., B. L. Gartner, and J. J. Morrell. 2002. Heartwood formation and natural durability-a review. Wood and Fiber Science 34: 587~611.
22. Wong, A. H. H., 1993. Susceptibility to soft rot in untreated and copper-chrome-arsenic treated Malaysian hardwoods. Ph. D. Thesis, Univ. Oxford.
23. Yamamoto, K. and L. T. Hong. 1988. Decay resistance of extractives from cengal (*Neobalanocarpus heimii*). Journal of Tropical Forest Science 1: 51~55.