Vol. 43 No. 3, 1980

THE DECAY OF TROPICAL HARDWOODS I. APPLICATION OF SCANNING ELECTRON MICROSCOPE†

by

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Summary

White-rot decay caused by *Coriolus versicolor* and brown-rot decay caused by *Piptoporus betulinus* of 3 Malaysian hardwoods, Meranti tembaga (*Shorea leprosula*), Punah (*Tetramerista glabra*) and Jelutong (*Dyera costulata*) were observed using the scanning electron miscroscope. Erosion canals and cell-wall thinning were seen in white-rotted samples of all the 3 woods. Cell-wall thinning was not obvious in the early stages of decay of brown-rotted samples. *C. versicolor* degraded the S_3, S_2 and S_1 layers. The S_3 layer was resistant to *P. betulinus*. Complete disintegration of ray parenchyma was observed in some white-rotted samples.

Introduction

The study of wood decay by specific microorganisms is well documented and comprehensively reviewed by Levi (1964), Seifert (1968) and Wilcox (1970). The majority of these studies were confined to temperate hardwoods and softwoods, and the loss of wood substances (mainly cellulose and lignin) was determined during progressive stages of decay (Richards, 1962; Savory and Pinion, 1958).

During the past decade the study of the morphology of wood decay has gained importance and has contributed much to the understanding of the decay process by miscroorganisms, especially decay-fungi. Findlay and Levy (1969) were among the first to indicate the potentials of the scanning electron microscope (SEM) in the study of timber decay. Liese (1970) has comprehensively reviewed the micromorphological aspects of wood decay caused by different groups of microorganisms. The application of the scanning electron microscope has brought to light many distinct features of the different types of decayed wood which were not observed previously under the light microscope. One distinct advantage is the ability of the SEM to produce 3-dimensional images from (even) relatively large (up to 1 cu. cm.) specimens. This together with the high resolution provided by the SEM which can give a range of magnifications from x 20 to x 50,000 makes it ideal for timber decay studies. Sample preparation is relatively easy and the material undergoes very little distortion (Findlay and Levy, 1969).

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† Part of the work submitted to the University of Oxford for an M.Sc., degree.

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The present paper describes some observations on the micro-morphological changes of the cell walls of 3 Malaysian hardwoods attacked by the white-rot fungus, *Coriolus versicolor* and the brown-rot fungus, Piptoporus betulinus.

Materials and Methods

The respective fungus was inoculated onto sterilized wood blocks using the technique described by Cowling (1961). After different periods of incubation, the wood blocks were removed and fixed following the method of Bravery (1971). Air-dried blocks without fixation were also used. Samples were critical-point dried, mounted on to aluminium stubs with carbon-UHU mixture, then coated with 300 – 500A gold for observation. Samples without critical-point drying were also used for observation after gold coating. All observations were made in a Cambridge Stereoscan 150 using an accelerating voltage of 5 KV, 10 KV or 20 KV.

The 3 woods examined were Meranti tembaga, Jelutong and Punah.

Observations

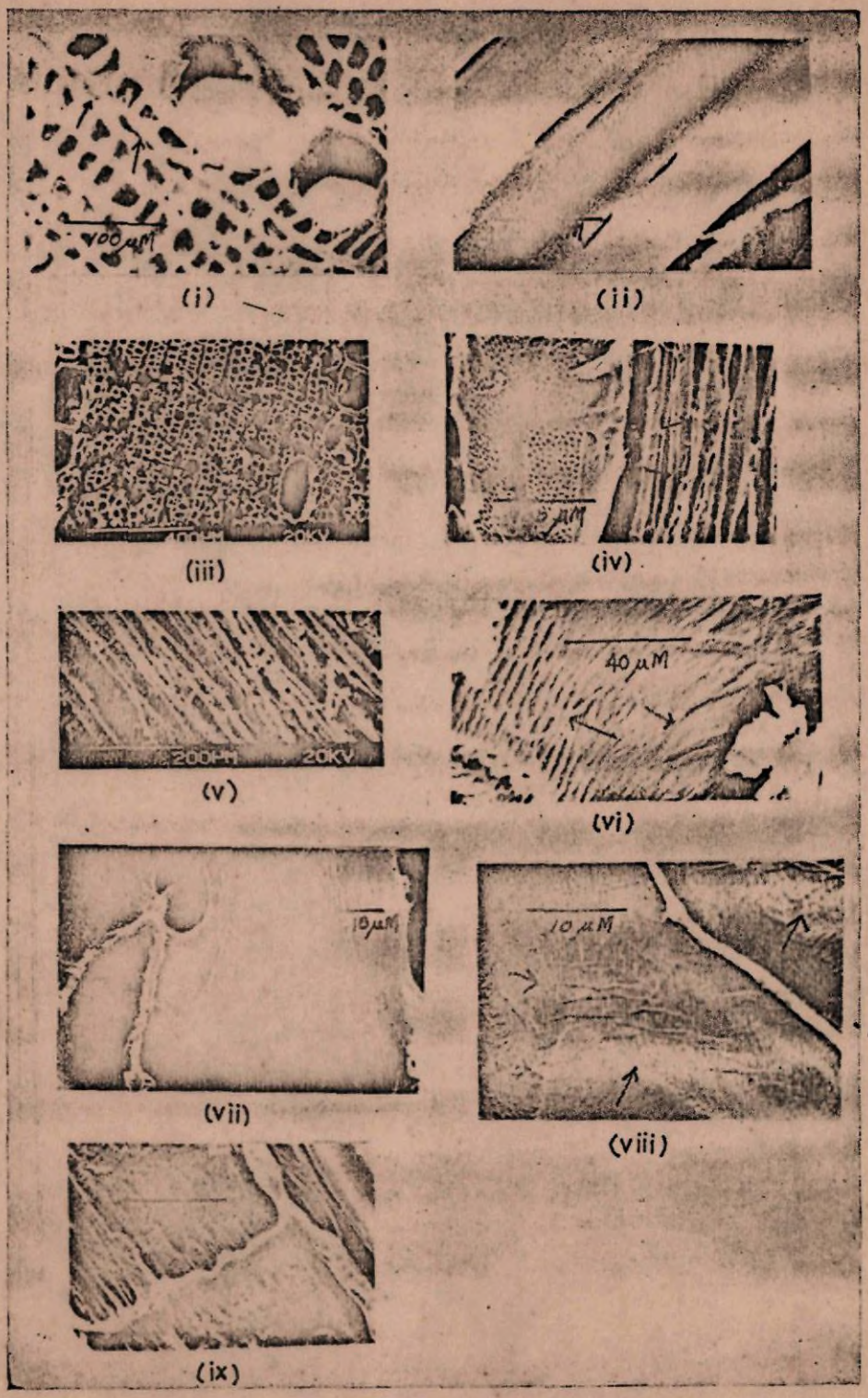
(a) Attack by *Coriolus versicolor*

Cell-wall degradation was characteristic and typical of a white rot attack. The erosion canals or erosion troughs (Figure 1) immediately beneath the fungal hyphae were seen in all the 3 woods examined. These erosion canals were much wider than deep and the fungal hypha was often found in the centre (Figure 1(viii)). Very often the presence of an erosion canal without hypha was also observed, especially in Meranti tembaga samples (Figure 1(vi)). In some samples a ridge (presumably where the hypha was once situated) was seen in the centre of such canals.

Cell-wall degradation by *C. versicolor* in all the 3 woods, was from the lumen outwards towards the middle lamella. The S_3 layer of the secondary wall was the first to be attacked followed by the S_2, S_1 and the primary cell wall. The middle lamella seemed to be resistant, especially areas around the cell corners (Figure 1(iii)). In cross-section, the thinning and disintegration of the cell walls were very obvious (Figure 1(iii)).

Figure 1. Scanning electron micrographs of white-rotted Jelutong wood.

(i) and (ii) Sound wood. Arrows indicate ray parenchyma. (iii) Cross section of decayed wood showing disappearance of medullary rays and disintigration of fibres. (iv) and (v) Decayed fibres and vessels showing enlargement of pit apertures. (vi) Erosion canals (arrow) in vessel. (vii) Hypha penetration of a degraded pit. (viii) and (ix) Erosion canals with hypha. The orientation of the microfibrils of the S_1 and S_2 layers are visible (arrow).


100 μM
(i)
(ii)
(iii)
(iv)
200H
20KV
(v)
40 μM
(vi)
10 μM
(vii)
10 μM
(viii)
(ix)

In many samples the degradation and removal of the S_3 layer revealed the orientation of the micro-fibrils of the underlying layers (S_2 and S_1). This was clearly visible in the erosion canals. Figure 1(viii) shows the orientation of the microfibrils of the S_2 and S_1 layers of the cell-wall. The microfibrils of the S_2 layer lie parallel to each other, but are orientated with a sharper angle towards the cell axis than the lower S_1 microfibrils (Figures 1(viii) and 2(iv)).

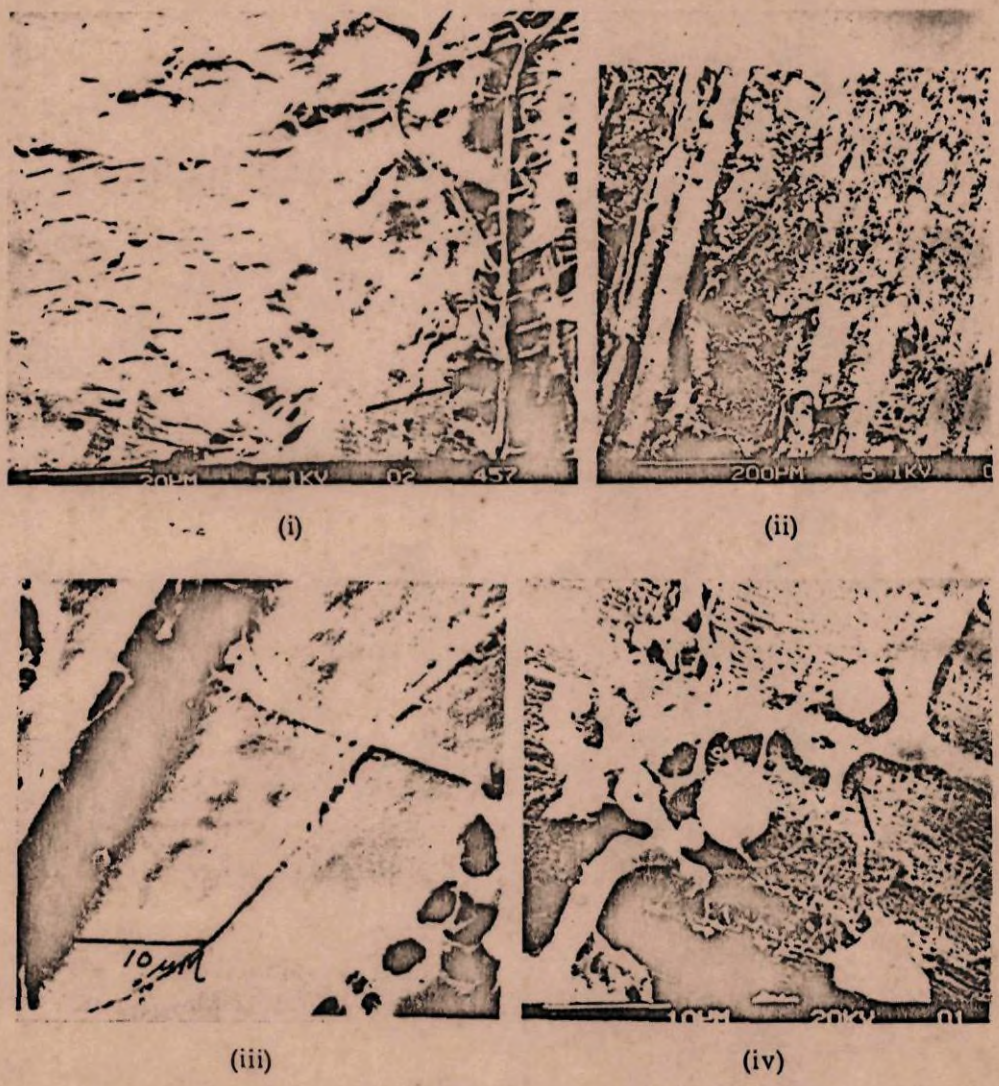


Figure 2. Scanning electron micrographs of white-rotted Punah wood.

(i) Degradation of vessel wall showing erosion canal (arrow) with a hypha in the centre. (ii) and (iii) Hyphae in fibres and vessel. (iv) Hypha on fibre cell wall showing clamp connections and erosion canal (arrow) clearly visible. Near parallel orientation of the micro-fibrils of the outer cell wall layer also visible.

Hyphae were more prevalent in vessels and fibres of Jelutong, Meranti tembaga, and in vessels and medullary rays of Punah. Intercellular penetration was mainly through pit apertures (Figure 1(vii)). Only in very few cases were bore-holes seen. At advanced stages of decay pit apertures were much enlarged, presumably due to the dissolving actions of ectoenzymes secreted by the fungal hyphae. These enlarged pit apertures often merged to give complete degradation of the cell wall. The ray parenchyma cells were the first to be attacked as indicated by their complete disappearance in intermediate stages of decay (Figure 1(iii)).

(b) *Attack by Piptoporus betulinus*

SEM shows no visible cell-wall thinning (Figure 3(ii)) of woods attacked by this brown-rot fungus except in very advanced stages of decay. No erosion canals were seen in any of the 3 woods decayed by *P. betulinus.*

The S_3 layer of all the 3 woods was not degraded at early stages of decay. Cell-wall degradation seemed to begin from the outer layers (S_2 and S_1). At advanced stages there was a tendency for distortion and collapse of cells although the form of the cells was maintained. Scanning electron micrographs show the presence of cracks and cavities in the S_2 layer of the fibre walls (Figure 3(iii)). These cracks sometimes occurred just beneath the hypha, which ran along the cell wall on the side of the lumen (Figure 4(iv)). These cracks presumably reflect the mechanism of decay of this brown-rot fungus compared to *C. versicolor.* As the S_3 layer of all the 3 woods was not affected, and as this fungus is not known to metabolise lignin (Meier, 1955), the carbohydrate decomposing enzymes were presumably able to diffuse into the outer layers (S_2 and S_1) of the cell wall and selectively remove the carbohydrate components.

Hyphae distribution in all the 3 woods was essentially similar to that of white-rotted wood. However, more were present in the vessels of Jelutong and Meranti tembaga and in the vessels and ray parenchyma cells of Punah in early stages of decay. Inter-cellular penetration was through pit apertures, but bore-holes (Figure 3 (v)) were more frequently seen than in white-rotted woods. No complete destruction of the ray parenchyma similar to that of white-rotted samples was seen, even in advanced stages of decay. The amount and intensity of hyphae were generally less than that found in samples decayed by *C. versicolor.*

Discussion and Conclusions

The SEM observations revealed some distinct differences in the decay of the 3 species of wood by *C. versicolor* and *P. betulinus.* In samples decayed by *C. versicolor,* there were variations in the distribution of the

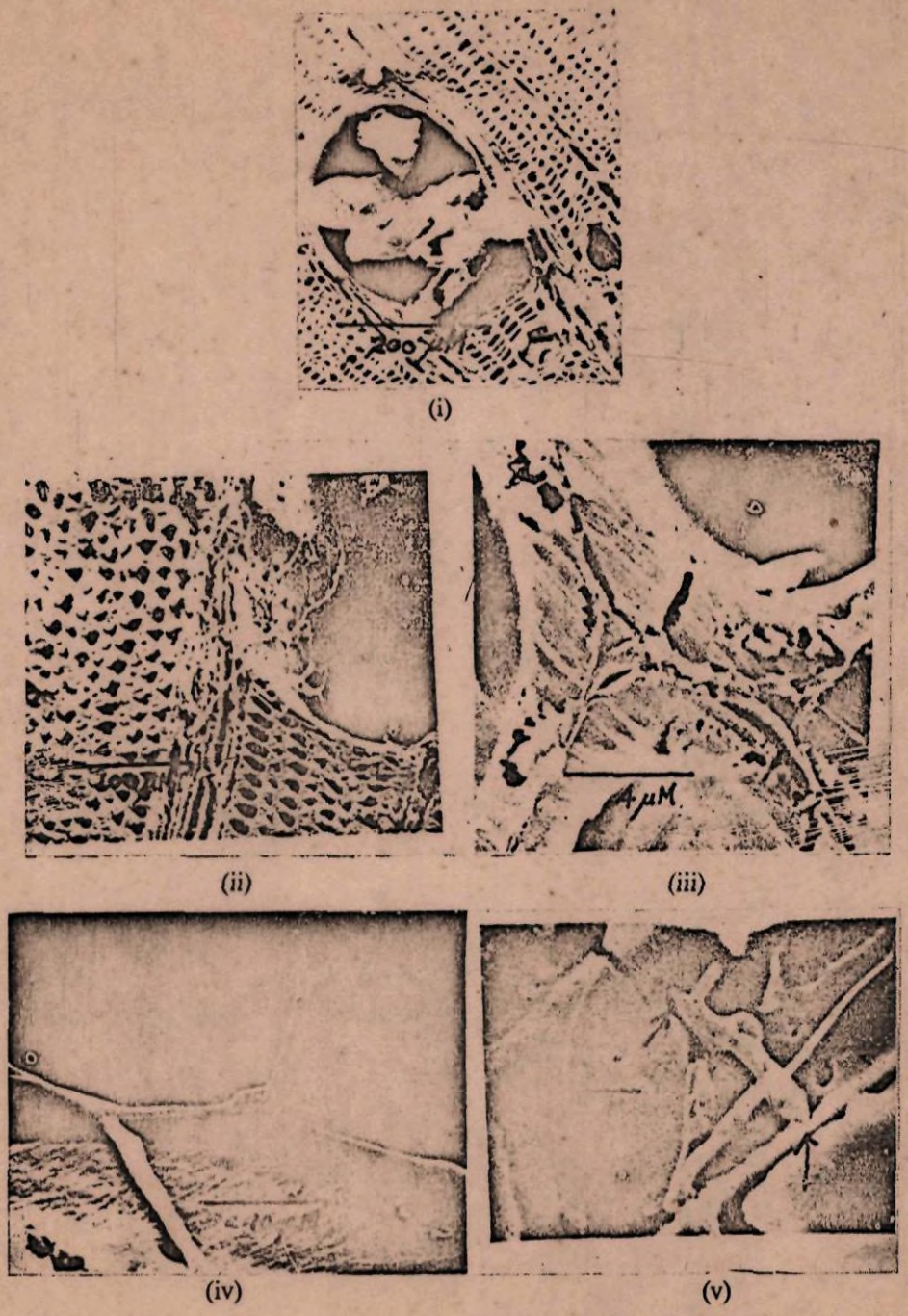
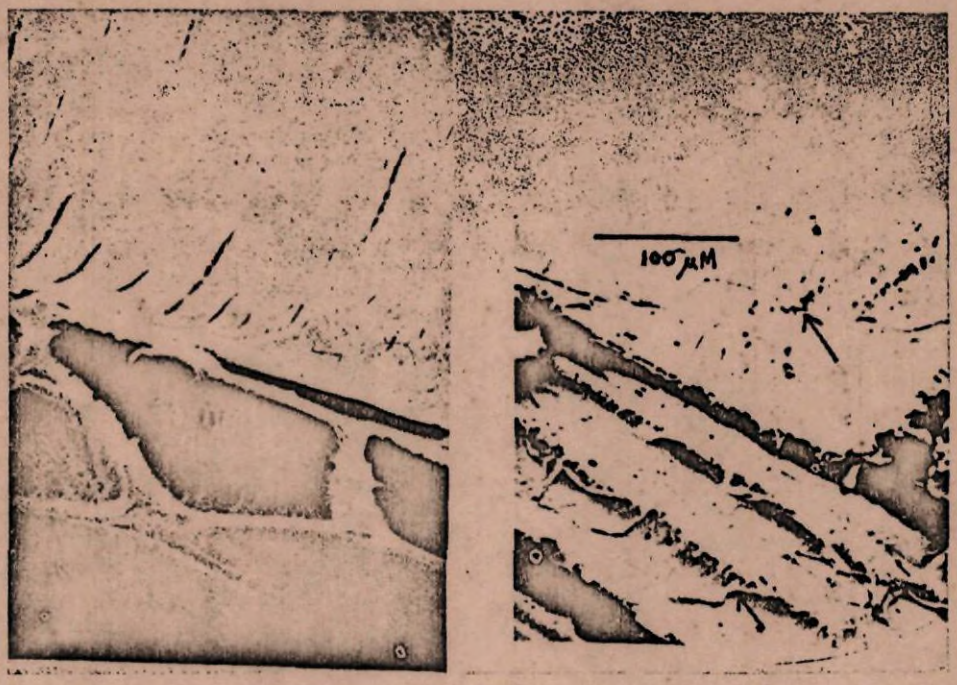
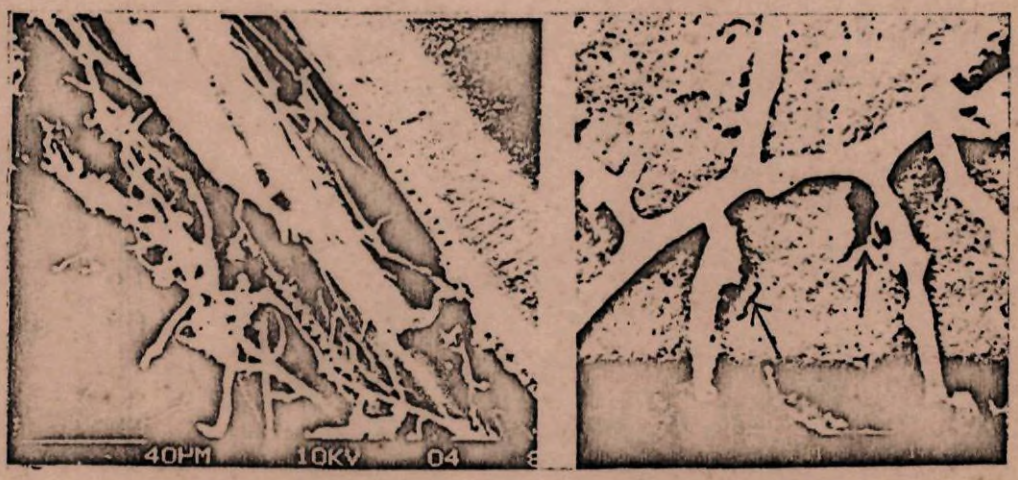


Figure 3. Scanning electron micrographs of brown-rotted Meranti tembaga wood.
(i) Sound wood. (ii) Hyphae penetrating a vessel. (iii) Cracks and cavities in secondary walls of decayed fibre cells. (iv) Hypha penetrating a pit in vessel. (v) Bore-hole penetration by hypha (arrow) in ray cell.



(i) (ii)



(iii) (iv)

Figure 4. Scanning electron micrographs of brown-rotted Punah wood.

(i) Sound wood. (ii) Hypha penetrating pit in ray cell (lower arrow) and cracks in vessel wall (upper arrow). (iii) Abundant hyphae in ray cells. (iv) Cracks and fissures in vessel wall (arrows) beneath hyphae.

amount of hyphae in the different cell types. Generally hyphae were present mainly in the vessels and parenchyma cells. However, in Punah, few hyphae were seen in the fibres and in Meranti tembaga few were seen in the ray parenchyma, in the early stages of decay. This variation in hyphal distribution has been reported by Cowling (1961) and Greaves and Levy (1965).

Bore-hole formation in samples decayed by *C. versicolor* was less frequently observed than in samples decayed by *P. betulinus*. However, there were difficulties in distinguishing bore-holes and enlarged pit-apertures caused by hyphal penetration. Only at the initial stages of hyphal penetration can bore-holes be positively identified because the degradation of bore-holes after hyphal penetration was very often similar to degraded pit-apertures. Cowling (1961) and Waterman and Hansbrough (1957) have reported similar observations on temperate hardwoods and softwoods.

The progressive thinning of the cell walls caused by *C. versicolor* was in contrast to that caused by *P. betulinus*. This progressive thinning was caused by the successive degradation of the cell-wall layers from the lumen outwards. In advanced stages of decay, the widening of the pit apertures and coalescence of these degraded pits together with the erosion canals lead to the typical appearance of white-rotted wood. In contrast *P. betulinus* has no apparent effect on the S_3 layer. Cowling (1961), Meier (1955) and Wilcox (1970) have reported that the S_3 layer was very resistant to brown-rot attack. However, in advanced stages of brown-rot decay of sweetgum sapwood the eroded appearance of the fibres was thought to be due to a decomposed S_3 layer (Cowling, 1961).

The SEM observations on these 3 tropical Malaysian woods have indicated that the effects and micromorphology of wood-decay by fungi (white rot and brown rot) are universal. Characteristic differences of white rot and brown rot are much more easily seen through the SEM because of the high power of resolution. Another advantage is the ease with which samples could be prepared for observation. Air-dried fresh samples or critical-point dried samples can be used. Even samples in very advanced stages of decay can be observed after critical-point drying.

Acknowledgements

I wish to thank the following for making this study possible: (i) The Malaysian Government for the award of a scholarship; (ii) The Director-General of Forestry, Peninsular Malaysia for the period of study leave; (iii) The Director, F.R.I. Kepong, for support and leave from the Institute; (iv) The wood anatomist F.R.I. Kepong for the wood samples; and (v) Professor J.H. Burnett for advice and encouragement and M. Wyatt for the electron photo-micrographs.

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