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# Observation of Soft-Rot Wood Degradation Caused by Higher Ascomyceteous fungi

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**The capability of higher ascomyceteous fungi to cause typical soft-rot decay for wood under laboratory conditions is reviewed and discussed. Fungi tested were extremely active in the decomposition of timbers. Scanning electron micrographs illustrated typical soft-rot decay pattern of higher wood decay ascomycetes, with the exception of *H. trugodes* that caused white-rot decay. Most of the fungi tested could be grouped as soft-rot fungi that showed typical soft-rot type II. Hypha confined primarily to the resin canals in softwoods or vessel elements in hardwoods and spread tracheid to tracheid via pits of cell wall to cell wall with mechanical force.**

**KEYWORDS: Ascomyceteous fungi, Soft-rot decay, White-rot decay, Softwood, Hardwood**

Three major groups of wood inhabiting fungi are the Ascomycota, Mitosporic fungi and Basidiomycota, Fungi colonize wood and degrade cell wall components to form brown rot, white rot, or soft rot. Ascomycetes and Fungi Imperfecti generally cause soft-rot decay of wood (Nilsson, 1973; Nilsson *et al.*, 1989), with the exception of some of the higher ascomycetes such as the Xylariaceae (Rogers, 1979; Eaton and Hale, 1993). Since Hartig (1894) grouped two main groups of white and brown rot, based on residual wood color, the term, sort-rot, was first defined anatomically as formation in chains of cavities with conical ends with the spiraling growth of a hypha within the S2 layer of a wood cell wall (Savory, 1954). The value of the scanning electron microscope for the study of wood decay processes in now well established (Nicole *et al.*, 1995). However in comparison with the basidiomycetes causing wood decay, higher wood decay ascomycetes have been poorly studied at the electron microscopy level, although they have been examined at the light microscopy level (Nilsson *et al.*, 1989).

The purpose of the present study is to observe and determine the mechanism of soft-rot degradation of wood cell wall by selected higher ascomyceteous fungi. This will provide new concept of sort-rot decay in two different sort-rot type I and type II.

## Materials and Methods

The main higher ascomyceteous fungi of wood decay, *Biscogniauxia nummularium* var. *exutans* (Cke.) Mill, *B. uniapiculatum* (Pens. & Sacc.) Mill, *Hypoxylon trugodes* Berk. & Broome, *H. truncatum* (Schw. : Fr.) J. H. Miller, *Nemania terricola* J.H. Miller, *N. subluteum* Ellis & Everh, *Rosellinia subiculata* (Schw. : Fr.) Sacc., *Xylaria multiplex* (Kunze) Fr., were tested for their capacity to attack of various tropical hardwoods. The fungi tested were collected on 12~24th October 1998 from the University of Malay in Malaysia. Most timbers had been attacked by higher ascomycetes fungi from the natural conditions examined. However, they could not be identified in macro and microscopic level. Hand sectioned samples taken from the areas of stroma of fungi and visible sign of whitish mycelium were examined under a microscope using bright field illumination and polarized. Samples were kept in refrigerator and examined immediately in order to avoid drying of the mycelium and wood samples. Sections by razor blades were unstained or stained with safranin-picro-aniline blue.

Sections with 0.5 to 1.0 cm thickness were cut by a freezing fracture method, fixed in 2% $OsO_4$ solution overnight at 4C, and then washed in distilled water. Sections were dehydrated in a grade ethanol series and the ethanol was then gradually substitute by chemically dry mounted on aluminum SEM stubs. The stubs samples were then gold coated and examined under a scanning electron microscope (JEOL JSM-T330A).

## Results

The decaying capacities of higher ascomycetous fungi unidentified tropical hardwoods are in presented in Table 1. Most of the species caused soft-rot decay of wood under laboratory conditions, even though *H. trugodes* showed white-rot decay type of pits enlargement. Plate 1 illustrate unusual white-rot decay on cross section and longitudinal section. Plate 3 presents typical soft-rot cavities on unidentified hardwood sections inoculated with *B. uniapiculatum*. Plate 1-A shows S2 layer of secondary wall to middle lamella in wood cell wall was eroded in th cross section. A few Hypha are also present in the degraded areas of cell lumen. Erosion has started from S3 layer and penetrated through S2 and S1 layers, and then actively progresses along the S1 layer and

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**Table 1.** Wood decay fungi tested for their ability to cause soft-rot cavity on various decayed hardwoods in natural conditions

| Fungi | Observation | |
|---|---|---|
| | Stroma | Cavity formation |
| *Biscogniauxia nummularium* var. *exutans* (Cke.) Mill | Stroma only on bark | cylindrical cavities on fibre |
| *B. uniapiculatum* (Penz. & Sacc.) Mill | Stroma only on bark | cubical cavities on fiber |
| *Hypoxylon trugodes* Berk. & Broome. | Stroma only on bark | diamond shape pits enlarged on fibre |
| *H. truncatum* (Schw. : Fr.) J. H. Miller | Stroma only on bark and decorticated wood | cylindrical cavities |
| *N. terricola* J. H. Miller. | Stroma only on bark and decorticated wood | cubical and cylindrical cavities on heavily wood. |
| *N. subluteum* Ellis & Everh. | Stroma on bark and decorticated wood | cylindrical cavities on wood. |
| *Rosellinia subiculata* (Schw. : Fr.) Sacc. | Stroma dispersed on bark | cylindrical cavities on wood |
| *Xylaria multiplex* (Kunze) Fr. | Stroma only on bark but heavily decayed wood | whitish mycelium. |

middle lamella. However, some of cell shows S3 layer has been eroded. Plate 1-C and 1-D show the pit enlargement of wood fiber as well as early stage of pit formation from cell lumen. *Biscogniauxia uniapiculatum, N. terricola, N. subluteum* and *X. multiplex* showed significant decay but *H. trugodes* and *R. subiculata* did not attack timber in tropical hardwoods in natural conditions,. Although there are some degrees in wood decay, most of hardwood were infected by fungi. *Nemania terricola* caused significant cavities of both cubical and cylindrical cavities. However, softwood was more resistant to fungal attack than hardwood, even though pine was decayed by higher ascomycetes in some degrees.

Plate 2-E shows the relationship of fungal colonization to the wood fiber wall undergoing cell wall penetration cell to cell. Hypha within rays parenchyma is constricted at contacting point which is connected with next cell one by one. However, hypha on cell lumen of fiber after early stage becomes massive and produces mucilage around hypha. *Xylaria multiplex* absolutely degraded S3 layer and S2 layer of secondary wall in the fiber in the level of the scanning electron microscopy (Plate 3-G and 3-I). Heavily occupied and advanced degradation of wood elements by hypha was observed on he cross section. Plate 3-I and 3-J show the secretion of enzyme around penetration area, showing dissolution of S3 layer of cell wall surface. Hypha within the cavities is constricted at the point where a penetration hypha passes into an adjacent cavity.

## Discussion

Seven out of eight species higher ascomyceteous fungi tested so far can decay timber in natural conditions. It is not surprising that all of fungi are bark or wood habitant. Recently, Lee (1997) reported a selected list of higher ascomyceteous fungi, showing their habitants and the type of wood decay caused. More than 90% caused soft-rot or white-rot decay in timber. Mouzours (1986, 1989a, b) demonstrated the white-rot decay activity of manglicolous ascomyceteous fungi and Lee (1997) also reported that a few *Xylaria* species could degrade wood cell wall in a manner of white-rot decay. This is also confirmed that a few higher ascomycetes cause white-rot wood decay type on pine, beech and balsa. He also demonstrated the processing of two different sort-rot decay type. so called soft-rot I and soft-rot II, with cavity formation, pit enlargement.

Stages of sort-rot and white-rot decay are easily confused, but the differences in their method of cell wall degradation were reveled by the light microscopy. To supplement this information, the process of wood decay has been investigated at the SEM level. The mechanisms involved in wood decay in a range of timbers and different strains of xylariaceous fungi are reported and discussed. Soft-rot fungi have been reported to cause lignin degradation (Levi and Preston, 1965; Eslyn *et al.*, 1975), even though cavity formation was a general symptom of decay by cellulolytic fungi (Liese, 1970). At the early stage, the cavity formation by white-rot decay organism is easily confused with sort-rot diamond shape cavity. Form the initial cavity, further cavities may occur by lateral branches from the mature cavity or by a fine probosic hypha which grows vertically from the tips of existing cavities. Diamond shape cavity was made by enlargement of pits, when a hypha lying in the wood cell lumen and contacting to pit. Observation of cell wall penetration by hypha is presented in Plate 2-E and 3-I. Recently Lee (1997) described that hypha confined primarily to the resin canals in softwood or vessel elements in hardwood. However, at early fungal colonization of hypha in resin canals, vessel elements and ray parenchymas, hypha spread from tracheid to tracheid via pits (white-rot and soft-rot II). As already indicated, higher ascomycetes are natural inhabitant of the

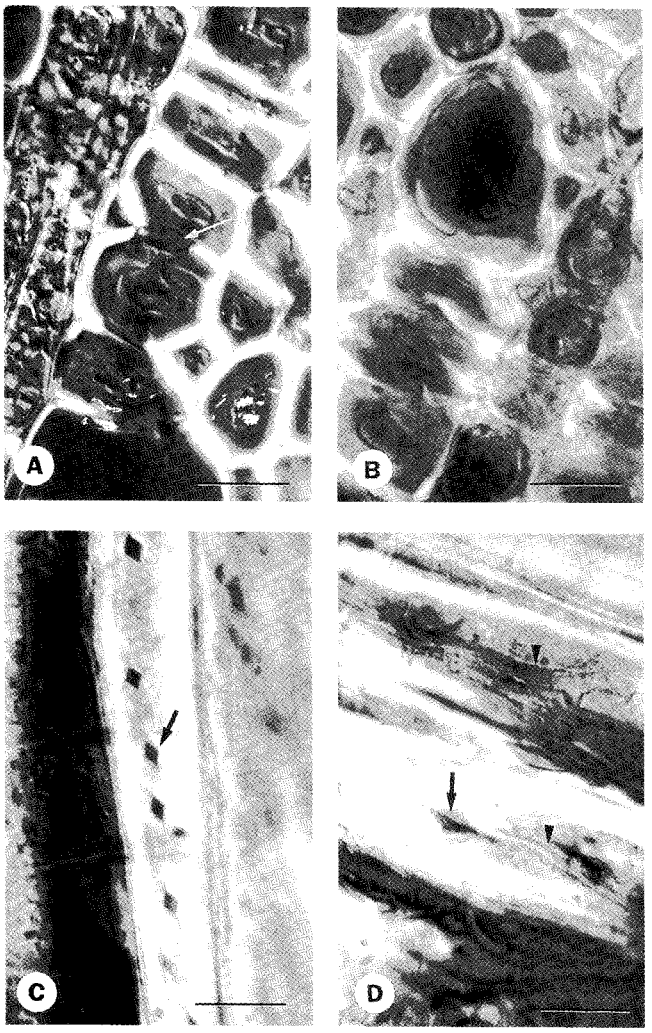


**Plate 1.** A-D. Polarized light micrographs of unclear white rot decay of *Biscogniauxia uniapiculatum*. A. Cross section showing erosion of cell wall of fibers fro S2 layer of the secondary wall to middle lamella. Arrow shows absolutely degraded middle lamella. Bar lines: 20 μm. B. Cross section showing erosion of cell wall from S3 layer of the secondary wall to middle lamella. Bar lines: 20 μm. C. Diamond type of pit enlargement of fiber in decay process in redial section. Arrow indicate pit enlargement. Bar lines: 20 μm. D. Longitudinal growth of old stages hypha in tangential section and pit enlargement. Arrow indicate pit enlargement with hypha. Arrowhead indicate hypha on cell wall within cell lumen. Bar lines: 50 μm.

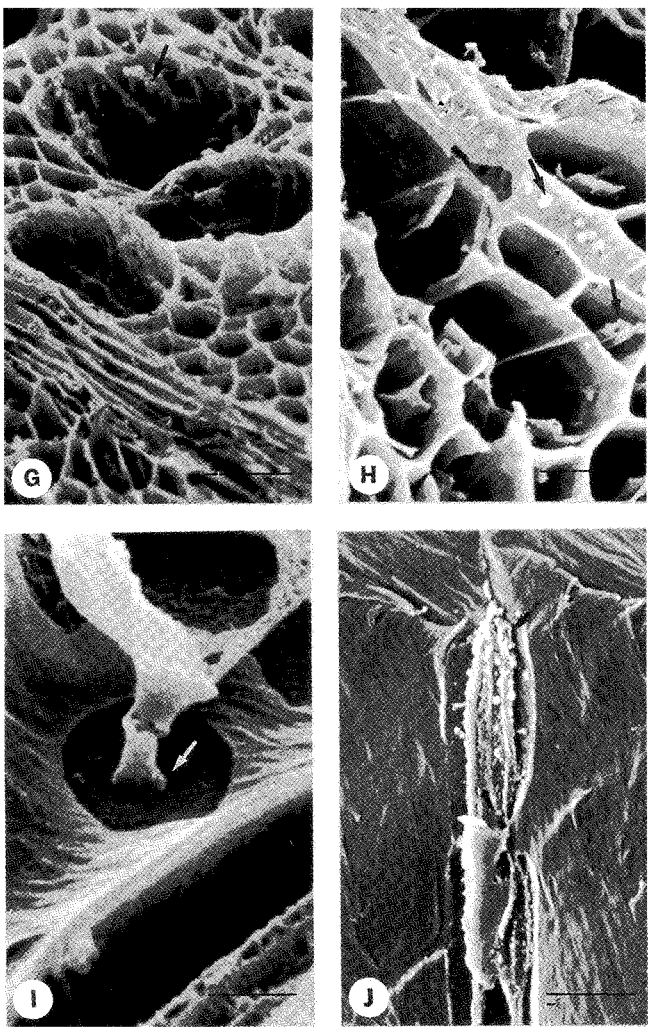


**Plate 2.** E-F. Scanning electron micrographs of soft-rot decayed timber by *Hypoxylon trugodes*. E. Hypha in fibers penetrated ray parenchyma and advanced degradation of rays. Black arrow indicate absolute degradation of S2 layer and white arrow points hypha penetration of cell wall. F. Heavily colonized rays and fibers. Note mucilage on S3 layer of cell lumen. Arrow indicate hypha, degrading of S3 layer.

wood degradation ecosystem. This paper has shown that higher ascomycetes are capable of decay wood under laboratory conditions, which reflects their importance in the recycling of lignocellulosic substrates and application to industrial uses.

Decay occurs primarily from the enzymatic activities of a few groups of specialized fungi (Lee, 1997). In order to understand the decay process, the chemical changes of the wood and screening of extracellular enzymes are main subjects of importance. SEM studies reveled the presence of extracellular degradative enzymes, which would account for the localized attack of lignocellulose by sort-rot or white-rot fungi. Plate 1 demonstrated that secretion of enzyme around penetration hypha, with dissolution of cell wall surface, as hypha penetrated cell wall.

In conclusion, this study has shown that higher ascomycetous fungi can degrade softwoods and hardwoods with cavities formation on secondary cell wall. Further studies should include a comparison of soft-rot decay (type I and II) formation with white-rot in the mechanism of cell wall degradation.

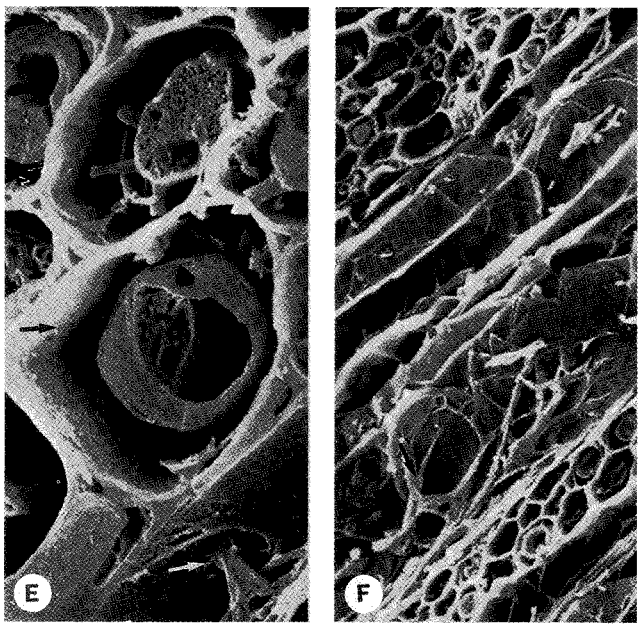


**Plate 3.** G-J. Scanning electron micrographs of soft-rot decayed by *Xylaria multiplex*. G. Heavily occupied degraded vessel by hypha. Note absolutely degraded S3 layer and S2 layer of fiber. Arrow indicate hypha within vessel. Bar lines: 20 μm. H. Advanced degradation of wood elements of the cross section. Arrow indicate hypha. Bar lines: 10 μm. I. Secretion of enzyme around penetration hypha (white arrow), showing dissolution of cell wall surface. Bar lines: 1 μm. J. Hypha within the cavities is constricted at the point where a penetration hypha passes into an adjacent cavity (arrow). Bar lines: 2 μm.

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