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Weight Loss and Cell Wall Degradation in Rubberwood Caused by Sapstain Fungus *Botryodiplodia theobromae*

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Keywords

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Summary

Botryodiplodia theobromae is the predominant fungus causing sapstain in rubberwood in Kerala, India. The fungus causes up to 12.2 percent weight loss in rubberwood over a period of sixteen weeks. Transmission Electron Microscopy (TEM) of sapstained rubberwood provided evidence on hyphal invasion of cells by *B. theobromae* through the pit region, facilitated by its ability to degrade pit membranes. The study also revealed that *B. theobromae* caused degradation of lignified cell walls by erosion of the cell wall surfaces of wood elements.

Introduction

With the rapid increase in population, demand for timber by various sectors has increased considerably. Also, the availability of conventional timbers has decreased due to deforestation. The growing demand for timber can be met to some extent by utilising alternative species and increasing the timber production through intensive management. Recently, in tropical countries like India and Malaysia, rubberwood (*Hevea brasiliensis* (Willd. ex A. Juss.) Mull. Arg.) has gained importance as a substitute for conventional timber. Studies have established the suitability of rubberwood for furniture and panel products. However, the growth of sapstain and surface moulds on rubberwood poses serious problems for its utilisation.

Botryodiplodia theobromae Pat. is the predominant fungus causing sapstain on rubberwood. The warm-humid tropical climatic conditions of Kerala State (India) aggravate the stain problem in rubberwood. The staining caused by *B. theobromae* has also been reported in various other timbers of tropical and temperate countries (Umezurike 1969; Pinheiro 1971; Thapa 1971; Olofinboba 1974; Hong 1976; Florence and Sharma 1990; Encinas 1996). But there have been few studies designed toexamine the effect of the sapstain fungus *B. theobromae* on qualities of rubberwood other than discolouration. This paper confirms our earlier observations of weight loss caused by the growth of B. *theobromae* in rubberwood based on Transmission Electron Microscopy (TEM) and reports the nature of degradation caused to cells/tissues, as well as describing cell to cell hyphal penetration.

Materials and Methods

Weight loss was selected as a representable factor to measure the biodegradation of rubberwood caused by *B. theobromae.* Weight loss was determined in rubberwood blocks over a period of 16 weeks following the procedure described by Stranks (1976). Eighty test blocks (15 × 5 × 50 mm) of rubberwood were cut from fresh healthy and defect free sapwood, oven dried at 103 ± 2 °C until the weight became constant, cooled in desiccator and initial weight recorded. The sample blocks were then placed over wet filter paper in a damp chamber to bring them back to equilibrium moisture content. Following steam sterilisation at 100 kPa for 15 min, 40 control blocks were inoculated with plain agar discs and the remaining 40 blocks with 8 mm diameter mycelial discs taken from the edge of a 7-day-old actively growing culture of *B. theobromae.* All the blocks were incubated in round bottom tubes (140 × 25 mm) containing 15 ml of sterile water. The blocks were kept out of direct contact with water by glass supports projecting above the water level in the tube. The atmosphere around the inoculated block in the tube was humidified by keeping a sterile filter paper wick (100 × 25 mm) covering the entire length of the test block before closing the tubes with cotton plugs; the lower end of the wick was immersed in sterile water. All 80 blocks were incubated at 28 ± 2 °C. Each month, 10 blocks each from inoculated and control sets were taken out from the tubes. After carefully scraping off the mycelial growth using a scalpel, the blocks were dried in an oven at 103 ± 2 °C to constant weight to record the final weight. The percentage of weight loss was calculated based on the original ovendry weight of the block. The same blocks were used for the study of hyphal penetration and cell wall degradation.

Morphological changes in cell walls caused by the penetration of hyphae were examined using Transmission Electron Microscopy (TEM). Uninfected control and *B. theobromae* infected rubberwood samples were cut into small pieces and processed for TEM. Wood samples were fixed in 3% glutaraldehyde (in 0.05 M sodium cacodylate buffer) for 5 h at room temperature and subsequently in 2% osmium tetroxide (OsO_4) (in 0.05 M sodium cacodylate buffer) overnight at 4 °C. Samples were dehydrated in acetone and embedded in Spurr's lowviscosity resin. Ultrathin

sections were cut with a diamond knife on an ultramicrotome, stained with 1% $KMnO_4$ (in 1% citrate buffer) and examined with a Philips 300 TEM.

Results

The weight loss of rubberwood over a period of 16 weeks is given in Table 1. The mean weight loss for the first four weeks was 8%. It increased gradually during the next 12 weeks and reached up to 12.2% by the end of 16 weeks. The rate of biodegradation in terms of weight loss during the first 12 weeks was almost identical and only at the end of 16 weeks did it show a significant increase.

Table 1. Weight loss in rubberwood infected with *Botryodiplodia theobromae* for a period of 16 weeks

Incubation period (weeks)	Weight loss percentage Mean ± SE
4	8.0 ± 0.25
8	8.3 ± 0.36
12	8.5 ± 0.55
16	12.2 ± 1.09

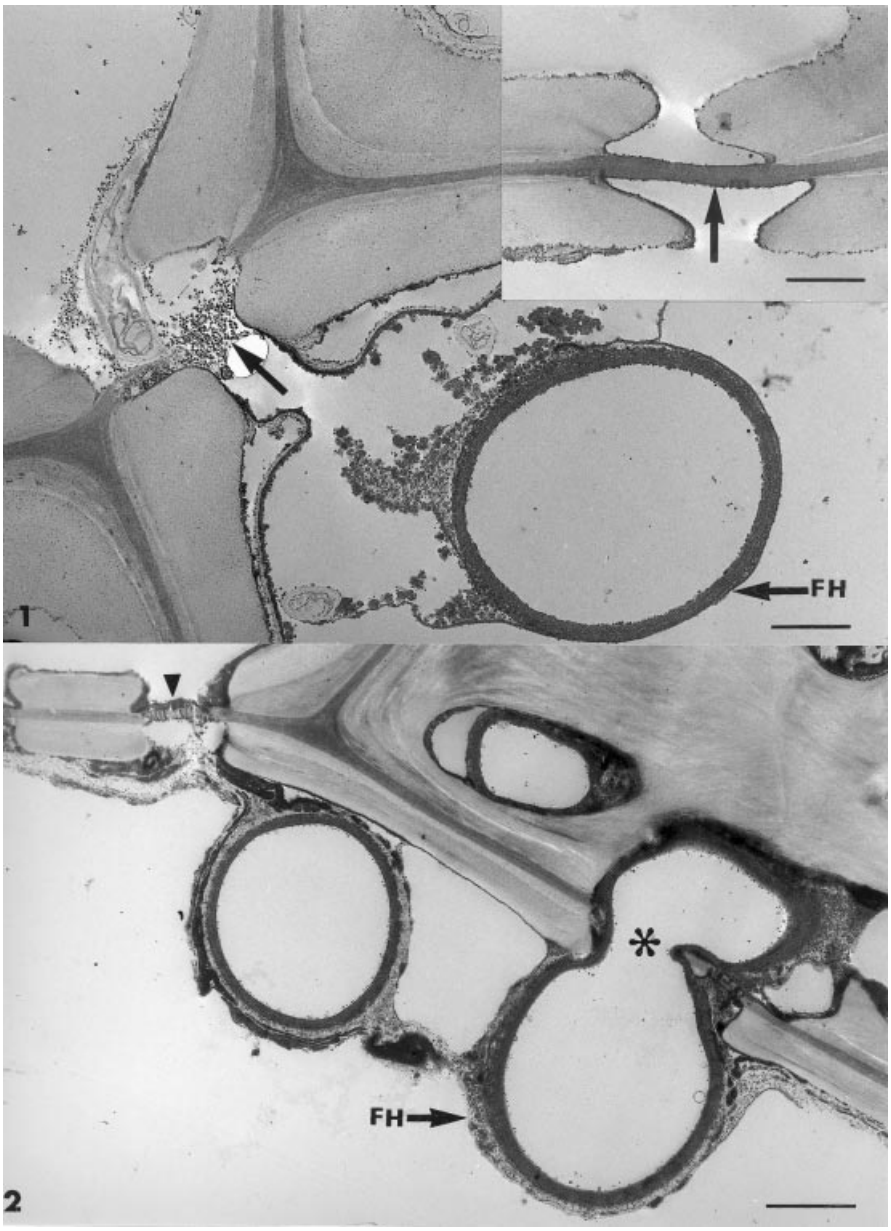

Fig. 1. Transverse sections through parts of adjoining fibres. The pit membrane in the common wall between the fibres (arrow) is almost completely degraded. Scale bar = 1 µm TEM. The inset shows an intact pit membrane between adjoining fibres. FH, fungal hypha, scale bar = 1 µm TEM. (Rubberwood exposed to fungal infection for 16 weeks).

Fig. 2. Transverse section through parts of adjoining parenchyma cells. A fungal hypha (FH) has traversed a common cell wall through a pit region (asterisk). The arrowhead indicates an undegraded pit membrane. Scale bar = 2 µm TEM.

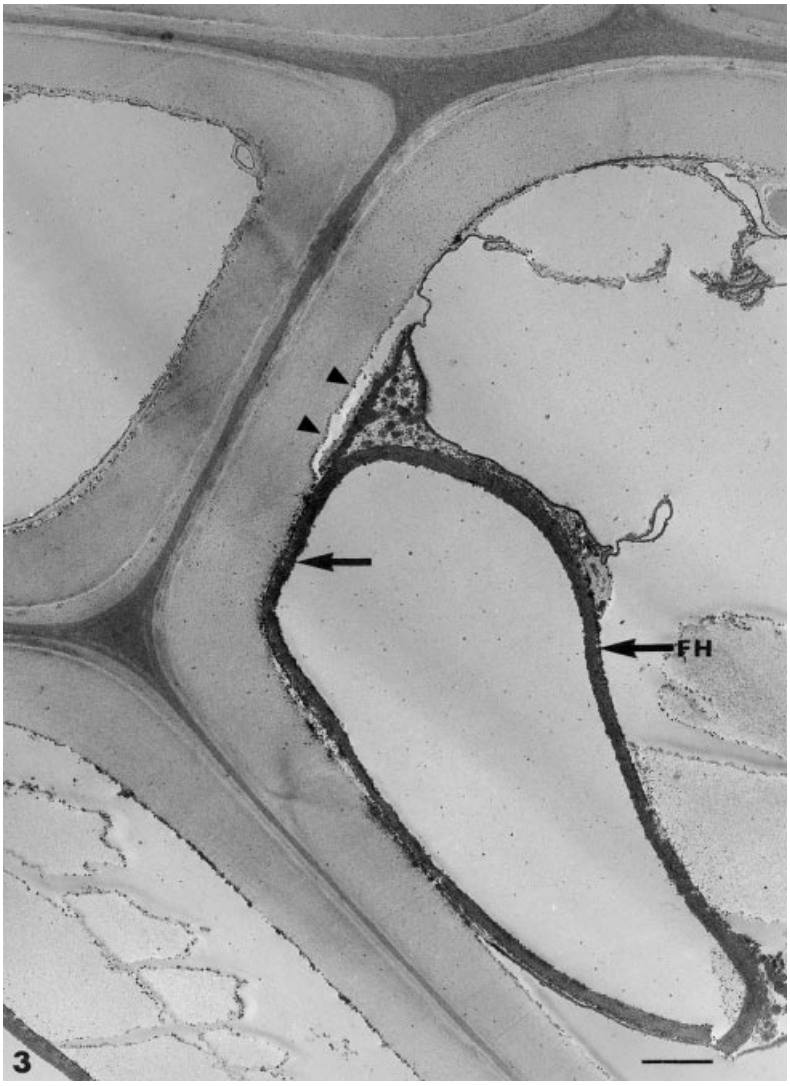

Fig. 3. Transverse section through parts of adjoining fibres. Part of the wall in one of the fibres appears to be eroded (arrowheads) and a fungal hypha (FH) is closely applied to an adjoining part of this wall (arrow). Scale bar = 1 μm TEM.

Although all the exposed samples were examined by TEM, the illustrations provided are only from the samples which had been exposed for 16 weeks, because other samples showed little or no degradation of lignified cell walls.

After initial colonisation on the surface of rubberwood, the hyphae of *B. theobromae* penetrate the inner tissues. Cell to cell spread of hyphae is mainly *via* pits present in the cell walls. TEM provided evidence that hyphal penetration through pits is facilitated by the ability of *B. theobromae* to degrade pit membranes. The illustrations provided here show a pit membrane present in the common wall between adjoining fibres (Fig. 1 and Inset) and complete penetration of a hypha through a degenerated pit between two adjoining parenchyma cells (Fig. 2). The intact pit membrane (Fig. 1, Inset) is continuous across the pit and appears rather compact. The degraded pit membrane (Fig. 1) is seen as granular material in the pit chamber in the region of the original membrane. Presence of a fungal hypha can be seen near the granular material. Figure 2 shows the presence of a fungal hypha which has not yet penetrated a neighbouring pit. The pit membrane appears to be intact; it is traversed by plasmodesmata. The hypha has penetrated the wall through a nearby pit and in doing so has become constricted to occupy the available space of the pit aperture within the confines of the lignified wall material.

Evidence of degradation of lignified cell walls in rubberwood due to the presence of fungal hyphae of *B. theobromae* is provided in Figure 3. A hypha can be seen adhering to the wall in one of the fibres. Part of the fibre wall near the hypha appears to be eroded, as is evident from the following micromorphological features; the fibre wall in this region is depressed, the cell wall in the hyphal region is irregular and it is not covered by a thin dense material which covers the intact faces of fibres that are not degraded (*e. g.* bottom left cell in Fig. 3).

Discussion

The weight loss recorded in rubberwood due to the infection of *B. theobromae* is consistent with that reported by Encinas and Daniel (1996) in aspen, rubberwood and birch; Hong (1976) in rubberwood; Tabirih and Seehann (1984) in *Triplochiton scleroxylon*; K. Schum and Umezurike (1978) in *Gossweilerodendron balsamiferum*. The weight loss of about 8.5% recorded during 12 weeks may possibly be due to the utilisation of sugar and starch content in the parenchymatous cells. The presence of a high amount of carbohydrate in rubberwood has been reported by Kadir and Sudin (1989).

Tabirih and Seehann (1984) reported the weight loss caused by *B. theobromae* in *Triplochiton scleroxylon* and *Fagus sylvatica*. In *T. scleroxylon*, the weight loss recorded was 8.6% at the end of 16 weeks, whereas in *F. sylvatica* it was 3.6%. This difference was attributed to the presence of differential amounts of parenchyma in *T. scleroxylon* (43%) and *F. sylvatica* (20%). In this study, the observed high weight loss of 12.2% at the end of 16 weeks can in part be attributed to the degradation of cell wall by *B. theobromae* as evidenced from TEM studies. These observations are in agreement with the results for birch by Encinas and Daniel (1996), who reported that *B. theobromae* not only degraded the secondary cell walls of fibres and parenchymatous cells, but also caused incipient wood decay. The ability of *B. theobromae* to cause wood decay has also been reported by other workers (Krapivina 1960; Levy 1967; Umezurike 1978; Encinas and Daniel 1996). Cell to cell penetration of *B. theobromae* hyphae during hyphal proliferation and colonisation in rubberwood tissues, as well as the ability of this fungus to degrade rubberwood are clearly demonstrated in the present study. Although it has long been known that sapstain fungi, including *B. theobromae*, colonise wood tissues by spreading from cell to cell primarily through pits, and in some instances also by penetrating across cell walls, the question remains as to whether the fungi accomplish this by physical (e. g. hydrostatic pressure at hyphal tip) or chemical (enzymatic) means (Eaton and Hale 1993). However, TEM has provided evidence that pit membranes are degraded and not ruptured, suggesting that enzymes rather than hydrostatic pressure may be involved in the breakdown of pit membranes. The presence of hyphae in contact with the residual membrane material strongly suggests a role for hyphae in membrane degradation. Future work using a cytochemical approach may help to understand the nature of enzymes involved in cell wall degradation.

It is generally accepted that sapstain fungi do not adversely affect the strength of wood they infect, and the weight losses are attributable to the utilisation of sugars and nitrogenous materials present in parenchyma cells. The use of TEM in this study has, however, revealed that *B. theobromae* hyphae may cause degradation of lignified cell walls of rubberwood in the form of erosion of the walls exposed to the cell lumen. Although the erosion did not appear to be extensive, the observation does help to explain the basis for additional weight losses in the rubberwood exposed to *B. theobromae* for longer than twelve weeks. The study indicates the possibility of strength loss due to degradation of cell wall materials in infected rubberwood.

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