

ANATOMICAL CHARACTERISATION OF TEAK (*TECTONA GRANDIS*) WOOD DECAYED BY FUNGUS *CHRYSOSPORIUM ASPERATUM*

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SANGHVI GV, KOYANI RD & RAJPUT KS. 2013. Anatomical characterisation of teak (*Tectona grandis*) wood decayed by fungus *Chrysosporium asperatum*. Teak wood logs are most often invaded by *Chrysosporium asperatum*. The extent of damage caused by it within a given period was investigated by in-vitro decay test. Sound wood blocks of *Tectona grandis* inoculated with *C. asperatum* showed no appreciable weight loss in the early phase of fungal colonisation but registered a 34–38% weight loss within three months. At the outset, fungal mycelia entered into wood tissue through vessels and xylem rays, invading all cell types by ramifying through pits on lateral walls. Fungal invasion commenced from the cell corners and the middle lamellae of the fibre wall, without any pronounced effect on the primary and secondary wall layers. Xylem cells were separated due to dissolution of middle lamella in the early stage, but in the advanced stages of decay all cell types showed formation of erosion channels and bore holes. In the advanced stage of infection, vessels were deformed due to explicit degeneration and eventually collapsed due to loss of rigidity. Xylem rays were more vulnerable to degradation than axial elements. Structural alterations induced in response to fungal invasion are described in the paper.

Keywords: Fungal decay, selective delignification, simultaneous rots, soft rot

SANGHVI GV, KOYANI RD & RAJPUT KS. 2013. Ciri-ciri anatomi kayu jati (*Tectona grandis*) yang direput oleh kulat *Chrysosporium asperatum*. Balak pokok jati sering diserang oleh *Chrysosporium asperatum*. Tahap kerosakan akibatnya dalam tempoh tertentu disiasat secara ujian pereputan in-vitro. Blok kayu *Tectona grandis* yang berkeadaan baik diinokulasi dengan *C. asperatum* tidak menunjukkan sebarang kehilangan berat yang ketara pada awal fasa pengkolonian kulat. Namun, setelah tiga bulan, kehilangan berat sebanyak 34% hingga 38% diperhatikan. Pada permulaannya, miselium memasuki tisu kayu melalui salur vesel serta salur xilem lalu menyerang semua jenis sel secara bercabang-cabang melalui lubang-lubang halus pada dinding lateral. Serangan kulat bermula dari sudut sel hingga ke lamela tengah dinding gentian tanpa memberi kesan kepada lapisan dinding primer serta sekunder. Sel xilem terasing akibat penguraian lamela tengah pada peringkat awal. Namun, pada peringkat pereputan yang lebih lewat, semua jenis sel menunjukkan pembentukan alur hakisan dan lubang penebukan. Pada peringkat ini, vesel berubah bentuk akibat kemerosotan yang nyata dan akhirnya runtuh kerana kehilangan ketegaran. Salur xilem lebih terkesan terhadap kemerosotan berbanding dengan unsur aksial. Perubahan struktur akibat tindak balas serangan kulat dihuraikan dalam kertas kerja ini.

INTRODUCTION

Wood deterioration is an essential process in the environment that recycles complex organic matter but it also leads to destruction of wood. In recent years, microbial degradation of lignin has received considerable attention, particularly on selective delignification due to its effects on biopulping of wood in the paper industry, bioremediation of chemical wastes in soil and water, and digestibility of lignocellulosic food sources for animal consumption (Blanchette 2000, Schwarze 2007, Koyani et al. 2010, 2013,

Koyani 2011). Tree decay is the major worldwide cause of damage to timber in living as well as in fallen wood logs stored in saw mills and timber yards in the forests. Mechanical injuries due to horticultural or silvicultural practices, pruning of branches and stem borers act as a source of fungal infection (Koyani et al. 2010, Metzler et al. 2012). In living trees, not only injuries but their size and position of injury, thickness of bark, tree age and season also have significant contribution to the extent of wood damage (Deflorio et al.

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2007). Nevertheless, dead trees and wood logs are either attacked by fungi already present on wound scars and dead branches before felling of the trees or during rainy season by saprophytic fungi present in soil and windblown spores.

Among the wood degrading microbes, white rot fungi are the most important agents in forest ecosystem which infect dead wood bores and living trees under favourable conditions (Maloy & Murray 2001). High moisture content limits aeration and inhibits degradation of fallen timber (Boddy & Rayner 1983). In the rainy season, though moisture content is higher, occurrence of several strains of different fungi is common in tropical forest. *Chrysosporium asperatum* is not only common on dead trunks and dead branches of living trees in the forest but also seen very frequently on wood logs lying in saw mills and timber depot. When moisture content of wood is higher than fibre saturation point, it promotes fungal propagation. However, growth of fungi declines with the reduction in moisture content of wood and is completely inhibited when moisture content falls below 20% (Mohebbi 2003). During rainy season, climatic conditions are moderate with high moisture content which favours fungal growth. Moreover, the wood surface provides suitable condition to establish *C. asperatum* infection. On the contrary, in dry seasons *C. asperatum* may be present inside the xylem but not observed on the outer surface of wood logs. *Chrysosporium asperatum* exists in two forms viz. anamorph and telomorph. Anamorphs reproduce asexually and do not produce fruiting bodies while telomorphs form fruiting bodies and reproduce by sexual means.

The main aim of the present investigation was anatomical characterisation of decay pattern and extent of wood damage caused by *C. asperatum*. This has not been investigated for teak (*Tectona grandis*).

MATERIALS AND METHODS

Fungus

Teak wood samples infected with *C. asperatum* were collected from logs of trees growing in Girnar Forest of Gujarat state, western India. Samples were excised from the wood logs using chisel and hammer, immediately packed in sterile polyethylene bags and brought to the laboratory. These samples were cut into small pieces (5 mm

× 5 mm × 5 mm) and were then surface sterilised using 0.1% HgCl₂ for 40–45 s with intermediate washing by sterile distilled water followed by a treatment of 70% ethanol by dipping the samples for 5 to 6 s. Thereafter, these samples were inoculated on 4% malt extract agar media. Pure cultures, verified by the Agarkar Research Institute Pune, were established by routine methods and maintained at 4 °C.

Wood samples and decay test

Sapwood portion of sound wood of *T. grandis*, obtained from a saw mill, was used for in-vitro decay test. Cubic wood blocks measuring 2 cm × 2 cm × 2 cm were prepared from the stem disc which was free from knots. For every incubation period, nine wood blocks were incubated, from which three blocks were weighed after marking and then soaked in water for 24 hours for re-wetting to obtain optimal humidity of wood to develop the fungus. After autoclaving at 120 °C for 30 min, the blocks were surface sterilised with 70% ethanol, kept in autoclaved Petri dish containing malt extract agar and inoculated with 15-day-old pure culture of *C. asperatum*. The samples were incubated for 30, 60, 90 and 120 days at 27 ± 1 °C and 70% relative humidity. For every incubation period, three wood blocks without fungus inoculation were also maintained as control. After each incubation period, the three marked test blocks were removed and cleaned to remove the mycelia. The blocks were oven dried and weighed to determine per cent weight loss, while the rest of the blocks were fixed in formaldehyde acetic acid alcohol (Berlyn & Miksche 1976) and transferred after 2 hours to 70% ethanol for storage and paraffin embedding. Experiment was performed in triplicates for each incubation period.

Transverse, radial and longitudinal sections of 12–15µm thickness were cut using sliding microtome and stained with safranin-astra blue combination (Srebotnik & Messner 1994). After dehydration in ethanol-xylene series, the sections were mounted in dibutyl phthalate xylene. Three wood blocks from each incubation period were dehydrated with tertiary butyl alcohol series (30, 50, 70 and 90% followed by 3 × 100% pure tertiary butyl alcohol) and processed by routine method of paraffin embedding. Important results were microphotographed with a trinocular research microscope.

RESULTS

Anatomical characterisation of wood

Secondary xylem of *T. grandis* is ring porous with distinct growth rings. Sapwood is pale yellow while heartwood is light golden brown in fresh and brown to dark brown in dry condition. In early wood, vessels are large, oval to circular in outline. They are chiefly solitary but sometimes occur in radial multiples of two to three vessels.

Parenchyma cells form thin sheath around the vessels which are distinct in early wood. Rays are uni-multiseriate with oval to polygonal ray cells.

Test wood block weight loss

Under natural conditions, fungal mycelia grow on the surface of dead wood logs as powdery mass (Figure 1a). Although *C. asperatum* was found frequently on wood logs, no fruiting was observed in any of the samples collected. At the end of

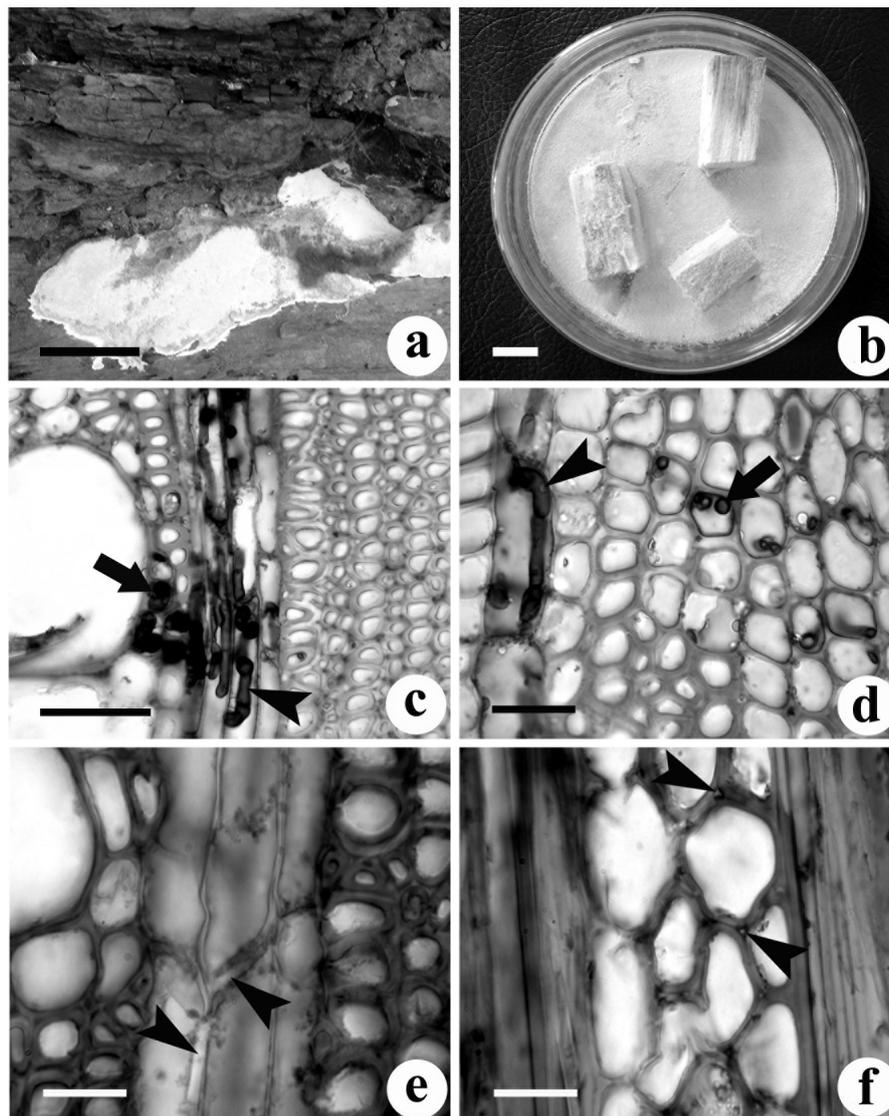


Figure 1 Transverse (c, d, e) and tangential longitudinal (f) views of secondary xylem of *Tectona grandis* showing features of decay by *Chrysosporium asperatum*: (a) naturally-infected teak wood showing fungal growth on outer surface, (b) wood blocks inoculated with *C. asperatum* after 10 days of inoculation, (c) movement of fungal mycelia from vessel and vessel associated axial parenchyma into ray cells (arrowhead), (d) movement of fungal mycelium from a ray cell into adjacent ray cell (arrowhead), (e) dissolution of middle lamella and separation of xylem rays in the early part of the fungal invasion (arrowheads), (f) initiation of degradation at the cell corners along the middle lamella of ray cells (arrowheads); scale bars a and b = 5 mm, c = 100 µm, d–f = 50 µm

first week of inoculation, fungal mycelia began to ramify on wood blocks (Figure 1b) which were completely covered with mycelia mat after 12–15 days of inoculation. At the end of third week, mycelial mat appeared as uniform mass of fungi. Thus, it became difficult to make out the presence of wood block in the Petri dish. There was minimal weight loss even when wood blocks were completely covered with fungal mycelia after 30 days of incubation. However, weight loss was rapid (Table 1) thereafter and showed a 34.38% weight loss after 120 days of inoculation.

Table 1 Average weight loss of teak (*Tectona grandis*) wood blocks* inoculated with *Chrysosporium asperatum*

Incubation period (days)	Weight loss (%)
60	15.28 ± 7.43
90	23.09 ± 10.29
120	34.38 ± 9.69

*n = 30, including three control blocks for each treatment

Wood colonisation and cell wall degradation

After 10–12 days of inoculation, fungal mycelia ramified completely over wood blocks and after 30 days of incubation, it invaded all cell types of secondary xylem through vessels and vessel-associated axial parenchyma (Figure 1c). From the vessels, hyphae traversed into neighbouring rays and gradually extended in all directions covering xylem fibres and adjacent axial parenchyma cells (Figures 1c and d). At this stage, no visual damage in cell walls was observed within 15 days of incubation. Fungal mycelia moved from one cell to the next through pits present on the cell wall. However, initiation of cell wall separations at cell corners was observed occasionally in some of the sections (Figures 1e and f).

After 60 days of inoculation, degradation pattern of *C. asperatum* resembled selective delignification, i.e. separation of cell walls commenced in the fibre walls along the middle lamellae without any pronounced effect on primary and secondary wall layers (Figure 2a). Separation of fibres and axial parenchyma cells was first observed adjacent to rays. Later, fibre walls showed formation of small cavities due to localised degradation of hemicellulose and cellulose. These cavities ran parallel to the

cellulose microfibrils of fibre walls (Figures 2b and c), while ray cells showed development of bore holes (Figure 2d).

After 90 days of fungal inoculation, 'U' shaped erosion troughs across the cell walls of xylem fibres became distinct (Figure 2e); they were irregular in shape and size. In some instances, the erosion reached the middle lamella by completely removing the cell wall in a localised area (Figure 2e). At this stage, most of the fibres and ray cells lost rigidity which consequently led to cell-wall collapse. Presence of fungal mycelia was observed frequently in the lumen of the collapsed cells (Figure 2e). Wood blocks exposed up to 120 days showed more pronounced effect on all cell types of xylem. Xylem fibres were deformed and lost their rigidity due to erosion of cell walls (Figure 2f). Bore holes on ray-cell walls were more distinct, irregular in shape and had varying diameters.

DISCUSSION

Initially, infected wood blocks exhibited separation of xylem cells by dissolution of middle lamella, which was characteristic of white rot. Under condition of nitrogen starvation as well as sulphur and carbon deprivation, rot fungi produce lignolytic enzymes which promote the oxidation of lignin to free radicals. These free radicals undergo spontaneous reaction with water or oxygen and depolymerise (also referred as enzymatic combustion) the lignin (Bennet et al. 2002).

Fungal mycelia invade wood cells through vessel elements while access to other cell types of xylem is facilitated by ray cells. Further access to adjacent cells occurs through pits or direct penetration takes place through the bore holes. In the present study, as the mycelial invasion progressed further, formation of longitudinal cavities was observed on the lateral walls of fibres, which is considered as an important characteristic of soft rot (Eriksson et al. 1990, Zabel & Morrell 1992). Similar cavities have been observed in beech wood inoculated with *Meripilus giganteus* (Schwarze & Fink 1998), Norway spruce wood inoculated with *Physisporinus vitreus* (Lehringer et al. 2010) and *Ailanthus excelsa* infected with *Inonotus hispidus* (Koyani et al. 2010). In teak, cavities developed along cellulose microfibrils in the secondary walls of xylem fibres in response to *C. asperatum* invasion.

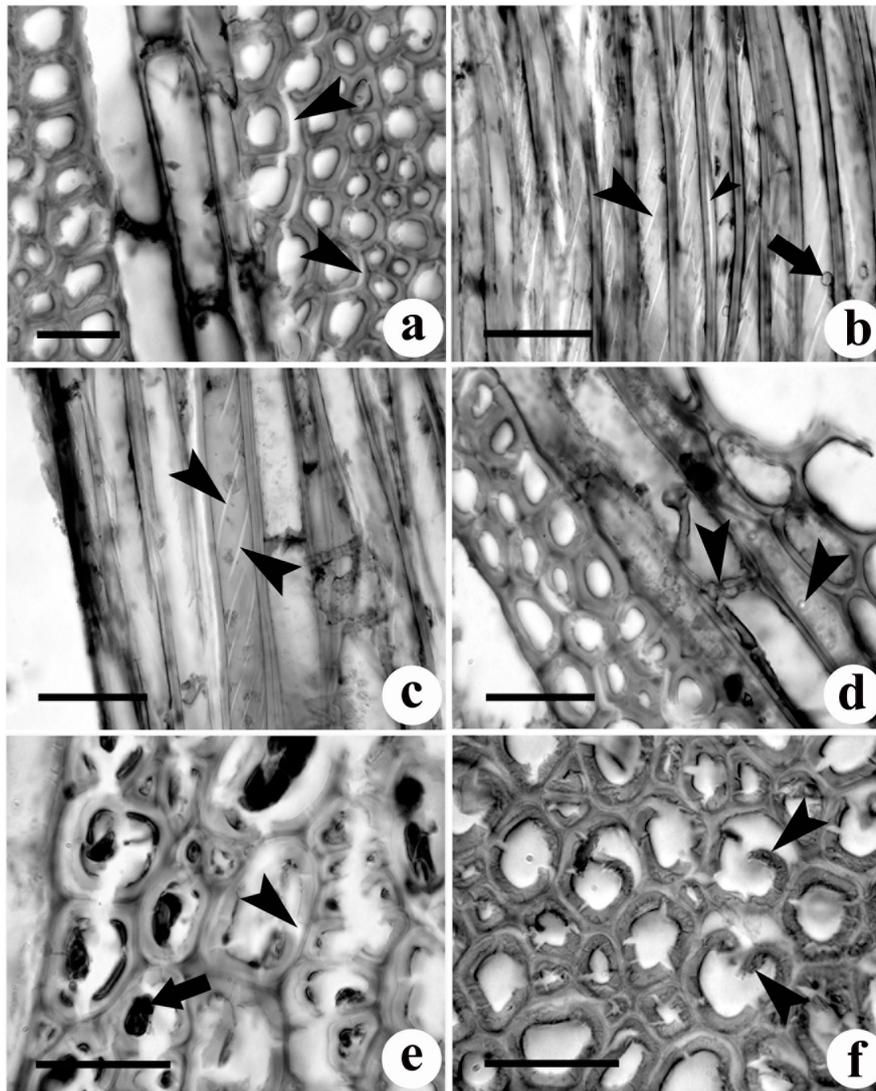


Figure 2 Tangential longitudinal (b, c) and transverse (a, d, e, f) views of *Tectona grandis* xylem wood degradation caused by *Chrysosporium asperatum*: (a) fibres adjacent to rays showing separation of cell walls along the middle lamella of walls (arrowheads), (b) xylem fibres showing erosion troughs oriented parallel to the cellulose microfibril angle of the fibre wall (arrowheads, small arrowhead show separation of fibre wall while arrow indicate; bore holes), (c) axial parenchyma showing erosion troughs oriented parallel to the cellulose microfibril angle of the cell wall (arrowheads), (d) movement of fungal mycelium from one of the ray cells into neighbouring cell through the pit present on the lateral wall and formation of bore holes on ray cell wall (arrowheads indicate fungal hyphae), (e) separation and breakdown of the fibre walls (arrowhead, arrow showing fungal mycelia in fibre lumen), (f) collapse of fibre walls after 120 days of fungal inoculation (arrowheads) showing erosion troughs and eroded walls; scale bars (a, c–f) = 50 μm , (b) = 100 μm

Soft rots can occur in dry environments and may be macroscopically similar to brown rot (Blanchette 2000). In the present study, patterns of decay were slightly different—starting with separation of cells (i.e. defibration) but later forming longitudinal cavities characteristic of soft rot decay type 1. In soft rot type 1, cavities are formed in the S_2 layer and hyphae traverse from one cell to the next by forming bore holes

(Schwarze 2007). After 90 days of inoculation, formation of bore holes was observed only in ray cells. Similar results are also reported by earlier workers (Nilsson et al. 1989, Blanchette 2000, Fazio et al. 2010). Strength loss of the wood is also associated with the soft rot attack. Soft rot cavities formed in the wood as well as extensive cellulose degradation can result in extremely poor strength characteristics (Hoffmeyer 1976).

As decay progresses, extensive carbohydrate loss occurs and lignin concentrations increase in the residual wood.

Axial alignment of tracheids, vessels and fibres and the radial arrangement of xylem ray parenchyma facilitate fungal access into the wood and allow widespread distribution of hyphae within the xylem (Schwarze et al. 2004, 2008). In the present study, hyphal entry into adjacent cells occurs via pit apertures by degradation of pit membranes or by direct penetration through the cell wall. The feature of pit membrane degradation can be significantly utilised in the process of wood preservation for improving permeability of water-borne wood preservatives (Schwarze et al. 2008). Degradation ability of secondary xylem is considered to be associated with lignin composition of the individual cell type (Schwarze et al. 2000) and the degree of lignin deposition (Schwarze et al. 2008). Libriform fibres and xylem ray parenchyma have relatively high syringyl monomer content (Iiyama & Pant 1988) which shows peak ultraviolet absorbance at short wave length (Fergus & Goring 1970a, b). In contrast, fibre tracheids appear to have high guaiacyl monomer content and show their peak ultraviolet absorbance at a longer wavelength. In the present investigation, vessels were more resistant to decay compared with xylem fibres, and narrow vessels were more resistant to fungal attack than wide ones. Collapse of wider vessels was reported in earlier studies of *Azadirachta* (Koyani 2011) and *Ailanthus excelsa* (Koyani et al. 2010) wood. The persistence of lignin-rich vessel elements in teak wood inoculated with *C. asperatum* may be due to high percentage of guaiacyl monomer content (Schwarze et al. 2000, Koyani et al. 2010, Koyani 2011).

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