Pattern of delignification in *Ailanthus excelsa* Roxb. wood by *Inonotus hispidus* (Bull.: Fr.) Karst.

Rina D. Koyani, Gaurav V. Sanghvi, Isha M. Bhatt and Kishore S. Rajput*

Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara – 390002, India

(Received 27 March 2010; final version received 13 August 2010)

The pattern of delignification in *Ailanthus excelsa* Roxb. wood, naturally infected by *Inonotus hispidus* (Bull.: Fr.) Karst., was studied by light microscopy. *Inonotus hispidus* produced a typical pattern of soft rot decay even though it is grouped with white rot basidiomycetes. Fungal hyphae colonised all cell types of the secondary xylem but more damage was observed in xylem fibres. In the early stage, infection commenced on the cell wall corners and middle lamellae of the fibre wall without any pronounced effect on the primary and secondary wall layers. Delignification of fibre wall became apparent when all cell types became completely invaded by fungal hyphae. It started from within the lumina towards the middle lamellae, occurring initially in the immediate vicinity of hyphae growing on the luminal surface by forming an erosion trough. At an advanced stage of decay, localised degradation of lignin, cellulose and hemicellulose resulted in the formation of small cavities within the secondary walls (S2) of fibres. These cavities were never observed to contain any fungal hyphae. Though the vessels were resistant to infection, xylem rays and fibres were relatively less resistant to attack by *I. hispidus*. In severe infection, vessel lumens were found to be filled with sclerotic tissue which blocked them, resulting in complete collapse. The formation of cavities and the extent of cell wall damage are described in detail.

**Keywords:** delignification; wood decay; soft rot; white rot basidiomycetes; *Ailanthus excelsa*; *Inonotus hispidus*

**Introduction**

Micro-organisms degrade wood under various environmental conditions. Some, such as fungi, can tolerate a wide range of temperature, humidity and pH conditions, while others have limited tolerance (Eaton and Halle 1993; Eriksson et al. 1990). Although both fungi and bacteria can degrade wood, white rot basidiomycetes are considered to be more aggressive than bacteria. Wood-inhabiting fungi include species of moulds, staining and decay fungi, many of which can cause economic losses (Scheffer and Cowling 1966). Decay fungi include many taxa from Basidiomycota and relatively few from the Ascomycota. These fungi cause brown, white and soft rots in affected wood tissues. Therefore, they are considered to be the most economically important as lignocellulose degradation is a central step for carbon recycling in terrestrial ecosystems. However, white rot fungi are of particular interest because they are one of the few groups of micro-organisms that can selectively degrade lignin (Otjen and Blanchette 1985). Some of them can simultaneously degrade all wood components, viz. lignin, cellulose and hemicellulose (Blanchette and Reid 1986), while others are capable of both types of decay in the same wood or in different woody species (Blanchette 1984a,b).

Mechanical injury or bark beetles and stem borer insects are the main sources of fungal infection. Mechanical injuries may be due to lopping, pruning, or debarking of trees with medicinal value. The present investigation studied *Ailanthus excelsa* Roxb. of the family Simaroubaceae infected with *Inonotus hispidus* (Bull.: Fr.) Karst. It is commonly known as “Mahaninha” due to its resemblance to neem trees (*Azadirachta indica* A. Juss.) and is highly valued in the Ayurveda, Chinese and Australian medicinal systems (Kirtikar and Basu 1995; Chevellier 1996; Dash and Padhya 2006). The bark of *A. excelsa* is used to treat diarrhoea and dysentery, especially when there is blood in the stool (Chopra et al. 1958; Dash and Padhya 2006). The bark has been used in Asian and Australian medicine to counteract worms, excessive vaginal discharge, malaria and asthma (Kirtikar and Basu 1995; Chevellier 1996). It has marked antispasmodic and cardiac-depressant properties (Nadkarni, 1976). In Ayurveda, it is used to counteract halitosis (Kirtikar and Basu, 1995) and it strengthens the body’s natural rejuvenative processes if given along with *Arjuna myrobalsan*. Debarking of trees, beetles and stem borer insects are the primary causes for fungal attack in *Ailanthus excelsa* Roxb. The present study aimed to provide an insight into the fungal pathogenesis within the host and ascertain the mechanism of action via histological methods. An anatomical approach was used to identify the mode of fungal movement, infection in xylem tissue and the extent of cell wall damage to various wood elements.

*Corresponding author. Email: ks.rajput15@yahoo.com*
Materials and methods
Wood samples of *Alianthus excelsa* infected with *Inonotus hispidus* were collected from trees growing in the Maharaja Sayajirao University hostel campus and from the Gir forest of Junagadh, Gujarat State (India). Only trees with fruiting bodies of *Inonotus hispidus* coming out from the stems and branches were sampled. Sample disks measuring approximately 60×60×60 mm (length, width and depth) were excised from the main stems and thick branches using a chisel and hacksaw. Half of the samples were fixed immediately in Formalin-Acetic acid-Alcohol (FAA) (Berlyn and Miksche 1976) and the other half unfixed samples were inoculated in potato dextrose agar (PDA) and malt extract media for isolation and identification of the fungal species. Transverse, radial and longitudinal sections of 12–15 μm in thickness were cut on a sliding microtome and stained with a safranin and astra-blue combination (Srebotnik and Messner 1994). After dehydration in an ethanol-xylene series, the sections were mounted in DPX. Some sections were also treated with potassium iodide, coomassie brilliant blue (CBB), Sudan Black B and ferric chloride for the localisation of starch, proteins, lipids and tannins, respectively (Krishnamurthy 1999).

To isolate and identify the fungal pathogen, small wood blocks were surface-sterilized with 95% ethanol for 10–15 s followed by 1% sodium hypochlorite for 45–50 s. These wood pieces were then aseptically inoculated on PDA medium. Cultures were incubated in an environmental incubator at 28°C for complete growth. Pure culture was obtained after successive transfer on PDA medium, and preserved at 8°C.

Results
Appearance of decay
In humid conditions, the outer dead bark becomes exposed due to mechanical injury, such as pruning or cut branch stubs, which along with wood boring insects and beetles are the main source of infection. Insects make holes/tunnels into the sapwood through the bark of healthy or stressed trees (Figure 1A, B). When such infected stems and branches are debarked, they usually exhibit areas showing infected and healthy portions of wood. Naturally infected wood can be easily recognized macroscopically by the black lines or bands produced by the fungi in the decayed wood, while fungal infection due to beetle attack shows dark rings encircling the tunnels (Figure 1A, B). Naturally infected wood is stained a dirty whitish-grey to yellowish-brown colour. However, variations in wood colour or pattern of lines/patches are characteristic to individual fungal species. When these samples were carefully cut within the same wood block and inoculated on PDA media, it was found that these demarcating lines/patches were colonies of individual fungal species, such as *Inonotus hispidus*, *Fusarium* sp., *Chaetomium* sp., *Alternaria alternata* or *Penicillium* sp.

Histology of decay
As mentioned earlier, fungal infection by *I. hispidus* is associated with branch stubs, pruning wounds or tunnels made by wood borer insects. Fungal hyphae were most abundant within the vessels lumen, xylem rays and fibres (Figure 1C, D), and were 1–2 μm in diameter and septate. Fungal hyphae attacked all cell types of the secondary xylem, but xylem rays and vessel members were found to be relatively resistant to fungal attack (Figure 1F); preferential degradation of xylem fibres resulted in a distinct degradation pattern (Figures 1F and 2A).

Sections through the borer holes, branch stubs and pruning wounds showed that mycelia travelled through vessel lumina via rays and infected all cell types. Fungal hyphae invaded neighbouring cells through the pits present on the lateral walls (Figure 1F). In advanced stage of decay, wood became completely eroded, light in weight and lost its original colour. At cellular level, cell walls also lost their integrity and cavities of various shapes and size developed in the cell walls (Figure 2A). In the wood fibres, hyphae entered from adjacent vessels via rays (Figure 2B and C) by cell wall erosion (Figure 2D). Hyphal growth was mostly confined to the secondary walls of fibres, where the majority of hyphae and their associated bore hole were ~1 μm in diameter (Figure 2D). Hyphae frequently grew transversely through the cell walls of the fibres and were often diverted within secondary walls around the lumina of the fibres (Figures 1F and 2A). Cell walls of xylem rays and vessels showed no visible signs of fungal attack (Figure 2E), but fibres showed the first sign of structural alterations, which were typical of soft rot attack. Although, hyphae were also visible within the fibre lumina, cell wall degradation was only apparent where hyphal tunnelling had occurred within the cell walls (Figure 2F).

The degradation pattern of *I. hispidus* resembled selective delignification i.e. at an early stage of decay, degradation commenced in the corner of fibres, along the middle lamellae, without any pronounced effect on the primary or secondary wall layers (Figure 3A). Sections stained with safranin and astra blue showed that the discoloured inner secondary wall was delignified and stained blue due to the absence of lignin. As the decay progressed, localized degradation of lignin, hemicellulose and cellulose resulted in the formation of small cavities within the S2 layer of the secondary wall of libriform fibres (Figures 2F and 3B). Single cavities were 2–4 μm in tangential diameter but, most of the time, two or three cavities fused to form a relatively larger one (Figure 3C, D). These cavities were consistently separated by a radial structure (Figure 2F).
Transverse sections showed hyphal growth and formation of tunnels within the $S_2$ layers with branches or hyphal extensions penetrating into xylem fibres (Figures 2A and 3D). These hyphae changed their direction to grow inside the cell wall after crossing the $S_2$ layer. This type of attack by *I. hispidus* is characteristic of soft rot with a typical ‘L-bending’ of the hyphae (Figure 3E). In advanced stages of decay, several small cavities merged to form tunnels of indefinite length and shape (Figure 2A, F). These tunnels may be oval, circular (Figure 3C, E, F), irregular (Figures 2D and 3D), C- or half-moon-shaped (Figures 2A and 4D) and L- or T-shaped (Figure 3C, E). In addition to cavities formed by hyphae within the cell wall, discrete notches of cell wall erosion by hyphae lying within the lumina were also observed frequently in the cell walls of the xylem fibres (Figure 3E).
Compared to fibres, vessels were relatively resistant to fungal attack and degradation of their cell walls was very slow. Although, several fungal hyphae ramified on the inner walls through the vessel lumina (Figures 1C, F and 2E), it was markedly delayed and structural alterations commenced only after complete degradation of adjacent fibres (Figures 2F and 4D). Unlike xylem fibres, vessel walls showed progressive delignification in the secondary wall from the lumen to middle lamellae rather than formation of cavities. Delignification of middle lamellae eventually resulted in the separation of cell walls (Figure 4D).
At an advanced stage of decay, xylem fibres became completely degraded (Figure 1G) and lost their integrity and rigidity (Figure 4A, B) whereas vessel lumina were filled with sclerotic tissue formed by fungal hyphae (Figure 4E, F). These sclerotic tissues are the main source of fruiting bodies that emerge from the injured stem and branch stubs.

**Discussion**

*Inonotus hispidus* is one of the most frequently occurring fungi affecting trees such as ash, apple, London plane, walnut, elm, sycamore, lime, etc. It is often known as heart rot fungus (Nutman 1929) but, in the present study, fruiting bodies were also observed on relatively young branches of
Mycology 209

5–7 inch in diameter. Earlier studies had shown that *I. hispidus* has the capacity to attack young sapwood (McCraen and Toole 1974). In the early stages of infection, initial signs of decay are yellowish brown and delimited by a brown reaction zone. Such reaction zones commonly contained tyloses and result in blocked lumina of the vessels. Tyloses are an outgrowth of adjacent ray or axial parenchyma cells into the lumen which block the vessel partially or completely. It is a common feature and reported in several earlier studies (Shain 1967, 1979; Pearce 1996).

Light microscopic studies have revealed that *I. hispidus* causes soft rot, in addition or instead of a white rot pattern (Schwarze and Fink 1997; Schwarze et al. 1995). Such soft rot activity may commonly precede white rot, when the fungus invades previously unaffected zones of xylem in which moisture content is supra-optimal (Schwarze 1995; Schwarze et al. 1995). In *Ailanthus*,

Figure 4. Transverse (A–F) view of infected wood of *Ailanthus excelsa* showing different stages of wood degradation. (A) Portion of severely infected wood showing complete loss of cell wall integrity (arrows). (B) Portion of severely infected wood showing complete loss of cell wall compounds. (C) Vessel lumen occluded with fungal hyphae (arrowheads). Note the middle lamella of the vessel wall is stained with astra blue (arrow). (D) Separation of vessel wall from the adjacent xylem derivatives (arrowhead). (E) Initiation of sclerotic tissue (pseudoparenchyma) formation in one of the vessel element (arrow). (F) In the advanced stage of decay, the vessel lumen is completely occluded by the pseudoparenchyma formation of fungal filaments (arrowheads). Scale bar (A–C, E, F) = 100 μm; D = 50 μm.
infection by *I. hispidus* is initiated during the monsoon when moisture levels are very high and the cut branch stubs are exposed to rain.

Based on degradation pattern, infection is usually separated into three types: white rot, brown rot and soft rot (Liese 1970; Blanchette 1991; Eaton and Halle 1993; Schwarze and Fink 1998). In white rot, all cell constituents are degraded and broadly classified into: (a) selective delignification and (b) simultaneous rot. In selective delignification, hemicellulose and lignin are preferentially degraded first, especially in the early stages. The most remarkable anatomical effect is separation of fibres by dissolution of the middle lamellae. In simultaneous rot, lignin and structural polysaccharides are attacked in more or less similar fashion (Worrall et al. 1997; Schwarze and Fink 1998). Wood degradation by *I. hispidus in Ailanthus* has both a white and soft rot pattern. In the initial stages, it resembles selective delignification, especially at an early stage of decay. Degradation commences at the corners of the middle lamellae within the xylem fibres without any distinct effect on the primary and secondary wall layers. A similar pattern of wood degradation has been reported for London plane by *I. hispidus* (Schwarze et al. 1995; Schwarze and Fink 1997). It is well established that various species of *Inonotus* cause selective delignification. However, it appears to be a temporary phase, because the structure of the cavities and the formation of multiple L-bending by the associated hyphae were typical of soft rot type. Such a pattern of intra-wall branching and associated cavity formation within the cell walls by *I. hispidus* is reminiscent of soft rot fungi. This particular pattern of cell wall degradation was assigned to form group 13 by Courtois (1963). It is characteristic of soft rot, in which cavities resembling tunnels are formed along the orientation of the cellulose microfibrils in the secondary wall (Courtois 1963). In the present investigation, *I. hispidus* made similar cavities along the cellulose microfibrils in the secondary walls of the xylem fibres. It was generally not recognised in the past that soft rot is a common feature which has been described for a range of wood-decaying basidiomycetes (Nilsson and Daniel 1988; Daniel et al. 1992; Schwarze and Fink 1997, 1998; Worrall et al. 1997).

Compared to xylem fibres, vessels are more resistant to decay caused by *I. hispidus*. In hardwoods, vessel walls are considered to be resistant to degradation by white rot basidiomycetes, as described in detail (Blanchette et al. 1987). The persistence of lignin-rich vessel elements in *Ailanthus excelsa* wood decayed by *I. hispidus* may be due to the high percentage of guaiacyl lignin.

*Ailanthus* is a fast-growing soft wooded dicot timber tree and is given priority in certain districts of Gujarat State. It is one of the most important species cultivated under agroforestry and social forestry programmes. Besides *I. hispidus*, *Polyporus* sp., *Ganoderma* sp., *Fomes* sp., etc. are other common rot fungi that attack this tree and require further study to understand the pattern of delignification by these species. In vitro studies are also required on other species to understand the extent of cell wall damage caused by these rot fungi.

**Acknowledgement**

The authors thank the anonymous referees for their valuable suggestions and the Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India, for the financial support.

**References**

Berlyn GP, Milsche JP. 1976. Botanical microtechnique and cytochemistry. Ames (IA): Iowa State University Press.

Blanchette RA. 1984a. Screening of wood decayed by white rot fungi for preferential lignin degradation. Appl Environ Microbiol. 48: 647–653.

Blanchette RA. 1984b. Selective delignification of eastern hemlock by *Ganoderma tsugae*. Phytopathology 74: 153–160.

Blanchette RA. 1991. Delignification by wood decay fungi. Annu Rev Phytopathol. 29: 381–398.

Blanchette RA, Reid ID. 1986. Ultrastructural aspects of wood delignification by *Phlebia (Meralius) tremellosus*. Appl Environ Microbiol. 53: 239–243.

Blanchette RA, Obst JR, Hedges JI, Weliiky K. 1987. Resistance of hardwood vessels to degradation by white rot basidiomycetes. Can J Bot. 66: 1841–1847.

Chevillier A. 1996. The Encyclopedia of Medicinal Plants: a Practical Reference Guide. London: Dorling Kindersley; p. 74–80.

Chopra RN, Handa LK, Kapoor LD. 1958. Indigenous Drugs of India. Calcutta: UN Dhur and Sons; p. 493.

Courtois H. 1963. Mikromorphologische Befallsymptome beim Holzabbau durch Moderfäulepilze. Holzforschung und Holzvertretung 15: 88–101.

Daniel G, Volc J, Nilsson T. 1992. Soft rot and multiple T branching by the basidiomycete *Oudemansiella macida*. Mycol Res. 96: 49–54.

Das SK, Padhya S. 2006. Review on ethnomedicines for diarrhoea diseases from Orissa. J Hum Ecol. 20: 59–64.

Eaton RA, Halle MDC. 1993: Wood: decay, pests and protection. London: Chapman and Hall; p. 546.

Eriksson KE, Blanchette RA, Ander P. 1990. Microbial and enzymatic degradation of wood and wood components. Berlin: Springer.

Kirtikar KR, Basu BD. 1995. Indian Medicinal Plants: Dehradun. Bangalore: International Books Distributor; vol. 1, p. 505–507.

Krishnamurthy KV. 1999. Methods in cell wall chemistry. Boca Raton (FL): CRC Press.

Liese W. 1970. Ultrastructural aspects of woody tissue disintegration. Ann Rev Phytopathol. 8: 231–257.

McCraen FI, Toole ER. 1974. Felling infected oaks in natural stands reduces dissemination of *Inonotus hispidus*. Phytopathology 64: 269–266.

Nadkarni KM. 1976. Indian Materia Medica, Bombay: Popular Prakashan; p. 56.

Nilsson T, Daniel G. 1988. Micromorphology of the decay caused by *Chondrostereum purpureum* (Pers.: Fr.) Pouzar and *Flammulina velutipes* (Curt.: Fr.) Singer. International Group on Wood Preservation, Document No. IRG/WP/1358.

Nutman FJ. 1929. Studies of wood destroying fungi. I. *Polyporus hispidus* (Fr.). Ann Appl Biol. 16: 40–64.
Otjen L, Blanchette RA. 1985. Selective delignification of birch wood (Betula papyrifera) by Hirschioporu pargamenus in the field and laboratory. Holzforschung 40: 183–189.

Pearce RB. 1996. Antimicrobial defences in the wood of living trees. Tansley Review No. 87. New Phytol. 132: 203–233.

Schwarze FMWR. 1995. Entwicklung und biomechanische Auswirkungen von holzersetzenden Pilzen in lebenden Bäumen und in vitro. Dissertation Universität Freiburg; Erndtebrück: SVK Verlag; p. 163.

Schwarze FMWR, Fink S. 1997. Reaction zone penetration and prolonged persistence of xylem rays in London plane wood degraded by the basidiomycetes Inonotus hispidus. Mycol Res. 101: 1207–1214.

Schwarze FMWR, Fink S. 1998: Host and cell type affects the mode of degradation by Meripilus giganteus. New Phytol. 139: 721–731.

Schwarze FMWR, Lonsdale D, Fink S. 1995. Soft rot and multiple T-branching by the basidiomycete Inonotus hispidus in ash and London plane. Mycol Res. 99: 813–820.

Scheffer TC, Cowling EB. 1966. Natural resistance of wood to microbial breakdown. Annu Rev Phytopathol. 4: 147–170.

Shain L. 1967. Resistance of sapwood in loblolly pine to infection by Fomes annosus. Phytopathology 57: 1034–1045.

Shain L. 1979. Dynamic responses of differentiated sapwood to injury and infection. Phytopathology 69: 1143–1147.

Srebotnik E, Messener K. 1994. A simple method that uses differential staining and light microscopy to assess the selectivity of wood delignification by white rot fungi. Appl Environ Microbiol. 60: 1383–1386.

Worrall JJ, Anagnost SE, Zabel RA. 1997. Comparison of wood decay among diverse lignicolous fungi. Mycologia 89: 199–219.